

1 **Free amino acids in Antarctic aerosol: potential markers for the evolution and**  
2 **fate of marine aerosol**

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22 **Abstract**

23 To investigate the impact of marine aerosols on global climate change it is important to study their  
24 chemical composition and size distribution. Amino acids are a component of the organic nitrogen in  
25 aerosols and particles containing amino acids have been found to be efficient ice nuclei.

26 The main aim of this study was to investigate the L- and D- free amino acid composition as possible  
27 tracers of primary biological production in Antarctic aerosols from three different areas: two  
28 continental bases, Mario Zucchelli Station (MZS) on the coast of the Ross Sea, Concordia Station at  
29 Dome C on the Antarctic Plateau, and the Southern Ocean near the Antarctic continent. Studying  
30 the size distribution of amino acids in aerosols allowed us to characterize this component of the  
31 water-soluble organic carbon (WSOC) in marine aerosols near their source and after long-range  
32 transport. The presence of only free L- amino acids in our samples is indicative of the prevalence of  
33 phytoplanktonic material. Sampling at these three points allowed us to study the reactivity of these  
34 compounds during long-range transport.

35 The mean total amino acid concentration detected at MZS was  $11 \text{ pmol m}^{-3}$ , a higher percentage of  
36 amino acids were found in the fine fraction. The aerosol samples collected at Dome C had the  
37 lowest amino acid values ( $0.7$  and  $0.8 \text{ pmol m}^{-3}$ ) and the coarse particles were found to be enriched  
38 with amino acids compared to the coastal site. The amino acid composition had also changed  
39 suggesting that physical and chemical transformations had occurred during long range transport.

40 During the sampling cruise on the R/V Itlica on the Southern Ocean, high concentrations of amino  
41 acids were found in the total suspended particles, this we attribute to the presence of intact  
42 biological material (as microorganisms or plant material) in the sample. *from what?*

43

World-wide,  
you mean?

44 **1. Introduction**

45 The organic composition of marine aerosols is particularly interesting as it contributes a substantial  
46 portion of the aerosol mass, especially in the submicron size fraction (Bigg, 2007). The study of  
47 marine aerosols is of interest as anything that can change their size, composition or concentration in  
48 the atmosphere may have an impact on the Earth's climate, since as noted by O'Dowd et al., (2004)  
49 "Marine aerosol contributes significantly to the global aerosol load and consequently has an  
50 important impact on both the Earth's albedo and climate". This is because, the sheer ~~extension~~<sup>extent</sup> of  
51 the ocean means that marine aerosol is one of the most important natural aerosol sources on a global  
52 scale (O'Dowd and De Leeuw, 2007, Rinaldi et al. 2010). Several studies (Facchini et al., 2008a, b;  
53 Rinaldi et al., 2010) have demonstrated that the organic chemical composition of marine aerosols  
54 depends on a combination of different factors, such as primary emission via bubble bursting and the  
55 subsequent transformation into secondary aerosol. During the primary emission *via* bubble bursting  
56 processes, the presence of phytoplankton can further alter the organic chemical composition and  
57 physical proprieties of marine aerosols (Kuznetsova et al., 2005).

58 The organic fraction of marine aerosols contains water-soluble organic compounds (WSOC), which  
59 include numerous species of organic acids, amines, carbonyl compounds and amino acids (Saxena  
60 and Hildemann, 1996). Amino acids are ubiquitous compounds, and are an active component of the  
61 organic nitrogen content of aerosols because some of them have been shown to enhance the ice  
62 nucleating ability of atmospheric particles (Szyrmer and Zawadzki, 1997). These compounds can  
63 also serve as a source of nutrients for marine ecosystems ~~thanks~~<sup>due</sup> to their high bioavailability (Zhang  
64 et al., 2002).


65 A large number of studies have confirmed the presence of amino acids in the condensed phase of  
66 aerosols (Gorzelska and Galloway, 1990; Spitzzy, 1990; Milne and Zika, 1993; Saxena and  
67 Hildemann, 1996; Zhang et al., 2002; Zhang and Anastasio, 2003; Mandalakis et al.,  
68 2010;Mandalakis et al., 2011; Ge et al., 2011 and its references), in rainwater (Mopper and Zika,

69 1987; Mace et al., 2003a, b), fog (Zhang and Anastasio, 2001), and in dew water (Scheller, 2001).  
70 They can be present as dissolved combined amino acids (proteins and peptides) (Kuznetsova et al.,  
71 2005; Ge et al., 2011), dissolved free amino acids from the hydrolysis of the combined amino acids  
72 (Mopper and Zika 1987; Milne and Zika, 1993), and particulate amino acids (from solid  
73 microorganisms and debris particles inside the liquid aerosol phase) (Kuznetsova et al., 2005).  
74 Several emission sources can affect not only the total concentration of dissolved free amino acids in  
75 the atmosphere, but also the amino acid composition of the aerosol. Amino acids have been  
76 detected in volcanic emissions (Mukhin et al., 1978; Scalabrin et al., 2012), biomass burning has  
77 also been suggested as a possible source of amino acids as part of the WSOC content (Mace et al.,  
78 2003a; Chan et al., 2005). The different amino acids found in continental particles are thought to  
79 have been originally produced by plants, pollens and algae, as well as fungi and bacterial spores  
80 (Milne and Zika, 1993; Scheller, 2001; Zhang and Anastasio, 2003; Mace et al., 2003a) and can be  
81 found in high concentrations in soil and desert dust. The continental contribution was evaluated by  
82 Mace et al. (2003b), who found that biogenic amino acids were present in the fine particles and that  
83 coarse particles contained amino acids from mainly anthropogenic sources. The anthropogenic  
84 sources currently identified are tobacco smoke (Ge et al., 2011), incinerators, waste collection  
85 centers and sewage treatment plants (Leach et al., 1999). Zhang and Anastasio (2002) identified  
86 livestock farming as the main source of amino acid ornithine in Californian aerosols. Matsumoto  
87 and Uematsu (2005) describe how long-range transport influences the concentration of amino acids  
88 in the North Pacific Ocean, while an evident marine source was verified by Weydan and Preston  
89 (2008) in the South Atlantic Ocean. Several studies investigated the free dissolved amino acids in  
90 marine aerosols (Gorzelska and Galloway, 1990; McCarthy et al., 1998; Mace et al., 2003;  
91 Matsumoto and Uematsu, 2005; Kuznetsova et al., 2005; Weydan and Preston; 2008; Mandalakis et  
92 al., 2011) but few studies have been conducted in the polar regions. Schmale et al. (2013) conducted  
93 a complete study on the characterization of Sub-Antarctic marine aerosols and they identified  
94 hatching penguins as a source of amino acids in the aerosol of Bird Island in the Southern Atlantic

95 Ocean. To our knowledge, this paper is the first to investigate the different compositions and  
96 particle-size distributions of amino acids in Antarctic aerosols.

97 Chirality is an important feature of amino acids and the homochirality of life on Earth occurs  
98 because L-amino acids are the only enantiomers used during the biosynthesis of proteins and  
99 peptides (Cronin and Pizzarello, 1997). The principal biochemical source of D-amino acids are  
100 peptidoglycans, the main structural components of bacterial cell walls (Voet and Voet, 1999).  
101 Chiral information can be useful in revealing the primary and secondary origins of aerosol  
102 components as demonstrated by several recent studies (Kuznetsova et al, 2005; Wedyan and  
103 Preston, 2008; Nozière et al., 2011; González et al., 2011; González et al., 2014). Amino acid  
104 enantiomeric ratios can be powerful markers for characterizing nitrogenous materials (McCarthy et  
105 al., 1998). Kuznetsova et al. (2005) indicated that the relative enrichment in L-amino acids may  
106 result from planktonic particles that concentrate at the sea surface while D-enantiomers come  
107 predominantly from bacteria (Wedyan and Preston, 2008). Therefore the presence of free D-isomers  
108 is indicative of a larger proportion of bacteria in aerosols (Wedyan and Preston, 2008).

109 The aims of this study are to investigate the occurrence and concentration levels of dissolved free L-  
110 and D-amino acids in the Antarctic aerosols, to determine how these compounds produced from the  
111 seawater surface are distributed in size-segregated aerosols, and to study their compositional and  
112 distribution changes after long-range atmospheric transport.

113 Due to their long distance from anthropogenic and continental emission sources, polar regions are  
114 excellent natural laboratories for conducting studies on the behavior, evolution and fate of marine  
115 aerosols. In Antarctica, long-range atmospheric transport of anthropogenic pollutants is minimal  
116 because the continent is surrounded by the Southern Ocean. This means that natural sources are the  
117 main contributors to atmospheric aerosols (Bargagli, 2008, Bourcier et al., 2010). Our aim is to  
118 study aerosol particle formation and growth in Antarctica because there is minimal interference  
119 from confounding anthropogenic sources. 

120

121 Our investigation was carried out over three different Antarctic summer campaigns, including two  
122 consecutive field campaigns (2011-2012 and 2012-2013) on the Antarctic plateau at the Italian-  
123 French base of Concordia Station (DC). One sampling period (2010-2011) was carried out at the  
124 Italian coastal base MZS and finally, aerosols were sampled from the R/V Italice on the Southern  
125 Ocean, between Antarctica and New Zealand (2012).

## 126 **2. Experimental section**

### 127 **2.1 Sample collection**

128 Aerosol sampling was carried out over three different Antarctic expeditions during the austral  
129 summer period, in the framework of the “Progetto Nazionale di Ricerche in Antartide” (PNRA).  
130 The sampling sites are shown in Fig. 1.

131 During the first expedition one sampling campaign collected five aerosol samples from the Italian  
132 base MZS from 29<sup>th</sup> November 2010 to 18<sup>th</sup> January 2011. The sampling site was at the Faraglione  
133 Camp (74° 42' S – 164° 06' E), about 3 km south of MZS in Victoria Land. The site is a promontory  
134 at 57 m asl. It was chosen because it is located in a valley that is physically separated from the main  
135 station area by a hill, to reduce as much as possible eventual pollution from the research station. ~~g~~

136 During the second expedition four aerosol samples were collected from the 19<sup>th</sup> December 2011 to  
137 28<sup>th</sup> January 2012 at the Italian-French base Concordia Station located at Dome C (DC) on the East  
138 Antarctic plateau (75° 06' S – 123° 20' E), ~~g~~ and seven other samples retrieved from the Ross Sea  
139 (Antarctica) on the R/V Italice during the oceanographic sampling campaign from 13 January to 19  
140 February 2012 (Fig 1).

141 In the third expedition, five aerosol samples were obtained from 07<sup>th</sup> December 2012 to 26<sup>th</sup>  
142 January 2013 at Dome C. The sampling site at Dome C during both expeditions was located about 1  
143 km south-west of the Concordia Station buildings, upwind of the dominant wind direction (from the  
144 south-west). Aerosol samples from the terrestrial bases (MZS and DC) were collected using a TE-

145 6070, PM10 high-volume air sampler (average flow  $1.21 \text{ m}^3 \text{ min}^{-1}$ ) equipped with a Model TE-235  
146 five-stage high-volume cascade impactor (Tisch Environmental Inc.) fitted with a high-volume  
147 back-up filter (quartz fiber filter Media 8" x 10") and a 5.625" x 5.375" slotted quartz fiber filter for  
148 collecting particle size fractions in the following ranges:  $10.0 - 7.2 \mu\text{m}$ ,  $7.2 - 3.0 \mu\text{m}$ ,  $3.0 - 1.5 \mu\text{m}$ ,  
149  $1.5 - 0.95 \mu\text{m}$ ,  $0.95 - 0.49 \mu\text{m}$ ,  $< 0.49 \mu\text{m}$ . The sampling period for each sample was 10 days, for a  
150 total air volume of  $\sim 15,000 \text{ m}^3$  per sample.

151 During the oceanographic cruise, airborne aerosols were collected onto ~~two~~ circular quartz fiber filter ~~S~~  
152 (SKC Inc., Eighty Four, To-13 model) using a TE 5000 High Volume Air Sampler (Tisch  
153 Environmental Inc.) to determine the TSP (total suspended particulate) fraction, defined as particles  
154 with a diameter  $> 1 \mu\text{m}$ . To avoid contamination from the ship's exhaust, air samples were  
155 automatically taken under wind sector control. The sampler was located at the bow and sampling  
156 only took place when the wind came from between  $-135^\circ$  to  $135^\circ$  relative to the bow and ship  
157 direction and when the relative wind speed was  $> 1 \text{ m s}^{-1}$ . The sample collection was set to five  
158 days, but the actual sampling time varied, subject to wind sector and speed control as well as cruise  
159 events. Due to these events the actual aerosol sampling volumes varied from between 511 and 2156  
160  $\text{m}^3$ . The sea voyage track chart is reported in Fig. 1.

161 All filters were pre-combusted (4 h at  $400^\circ\text{C}$  in a muffle furnace), to avoid contamination they were  
162 wrapped in two aluminum foils, after sampling they were re-wrapped in clean double aluminum foil  
163 and were stored at  $-20^\circ\text{C}$  prior to analysis. Field blank samples were collected by loading, carrying  
164 and installing the filter holder into the instrument with the air pump closed.

## 165 2.2 Sample processing

166 To avoid contamination from laboratory air particles and from the operator, samples were handled  
167 under a clean laminar flow bench (class 100). The pre-analytical and sample extraction protocol has  
168 been previously described in detail by Zangrando et al. (2013) for other compounds. The same

169 protocol is summarized below and was applied to the identification of amino acids in Antarctic  
170 samples.

171 Each quartz fiber filter was cut in half using stainless steel scissors that were previously washed  
172 with methanol. Filters were broken into small pieces using clean tweezers, and were placed into  
173 50mL conical flasks. Slotted quartz fiber filters from the cascade impactor and circular quartz fiber  
174 filters from the TSP samplers were treated in the same way. They were spiked with 100  $\mu\text{L}$  of  $^{13}\text{C}$   
175 isotopically-labelled amino acid standard solutions (with concentrations ranging between 2 and 3  
176  $\mu\text{g mL}^{-1}$ ), they were then ultrasonically extracted twice for 15 minutes in an ice bath with 5 mL and  
177 then 2 mL of ultrapure water. The extracts were combined and filtered through a 0.45  $\mu\text{m}$  PTFE  
178 filter in order to remove particulate and filter traces before instrumental analysis.

179 The larger high volume back-up filters were spiked with 400  $\mu\text{L}$  of internal standard solution and  
180 were extracted with 25 mL then 5 mL of ultrapure water in an ultrasonic ice bath as described  
181 above.

### 182 **2.3 Instrumental analysis**

183 The enantiomeric determination of free L- and D-amino acids by HPLC-MS/MS has been described  
184 in detail by Barbaro et al. (2014). This instrumental method has been applied to the aqueous extracts  
185 of the aerosol samples collected during this study.

186 An Agilent 1100 Series HPLC Systems (Waldbronn, Germany; with a binary pump, vacuum  
187 degasser, autosampler) was coupled with an API 4000 Triple Quadrupole Mass Spectrometer  
188 (Applied Biosystem/MSD SCIEX, Concord, Ontario, Canada) using a TurboV electrospray source  
189 that operated in positive mode by multiple reaction monitoring (MRM).

190 Chromatographic separation was performed using a 2.1x 250 mm CHIROBIOTIC TAG column  
191 (Advanced Separation Technologies Inc, USA) with a two mobile eluents. Eluent A is ultrapure  
192 water with 0.1% v/v formic acid and eluent B is ultra pure methanol with 0.1% v/v formic acid.



193 A binary gradient elution program was followed at a flow rate of 0.2 mL min<sup>-1</sup>: 0-15 min, an  
194 isocratic step with 30% of eluent B; 15-20 min, a gradient from 30 to 100% B; 20-25 min an  
195 isocratic washing step with 100% of eluent B; 27-30 min, re-equilibration to 30% eluent B. The  
196 injection volume was 10 µL.

197 In this work the amino acids were quantified using the isotope dilution method where an  
198 isotopically labeled standard was available. For other amino acids, where a labeled standard was  
199 unavailable, an internal standard was used to quantify the analytes. A detailed description of which  
200 analytes are quantified with which method can be found in Barbaro et al. (2014). In both cases, the  
201 results were corrected for daily instrumental sensitivity variations by evaluating the instrumental  
202 response factors.

203 Reagents and materials used for this study are reported in the Supplement.

#### 204 **2.4 Quality control**

205 The entire analytical procedure was validated by estimation of trueness, repeatability and efficiency  
206 (yield%) of the sample treatment process as described by Bliesner (2006). To ensure that it was fit  
207 for purpose for the enantiomeric determination of amino acids in Antarctic aerosol, the validation  
208 was carried out by spiking five cleaned quartz filters (for each type of filter) with 100 µL of a  
209 solution containing all the native L and D amino acids (with concentrations ranging between 2 and  
210 4 µg mL<sup>-1</sup>) and 100 µL of a solution containing all the isotopically-labeled <sup>13</sup>C amino acids  
211 (concentrations ranging between 2 and 3 µg mL<sup>-1</sup>). The filters were subsequently extracted as  
212 described above in section 2.2 “Sample processing”.

213 Tables S1, S2 and S3 report a summary of the yields, trueness and relative standard deviations  
214 (n=5) for each type of filter used in this study. Average yields of 61%, 56% and 56% were obtained  
215 from the circular, slotted and backup filters, respectively. In some cases, these values are lower than  
216 those reported in the literature (Mandalakis et al., 2010; Barbaro et al., 2011). Trueness is the most  
217 important parameter to determine during a method validation; it refers to the degree of closeness of

218 the determined value to the known "true" value. It is expressed as an error, calculated as  $(Q - T)/T$   
219  $\times 100$ , where Q is the determined value and T is the "true value".

220 For the circular filters, all D- and L-amino acids considered in this work were validated with an  
221 error percentage ranging from -13% (D-Leu/D-Ile) to +8% (L-Tyr).

222 In the backup filters, only D- and L-Hys produced unacceptable errors %, for this reason these  
223 compounds were excluded from the quantification. The other amino acids considered in this study  
224 were quantified with an accuracy ranging from -9% (D-Met) to +9% (D-Ala, L-Thr).

225 Some amino acids (D-Ala, L-Asn, D-Asn, D-Glu, D-Phe, L-Ser, D-Ser, and D-Val) were excluded  
226 from the quantification using the slotted quartz fiber filters as very high error percentages were  
227 calculated. We believe that this behavior is probably due to the different mode of use of this  
228 sampling support: the slotted quartz fiber filters were used as impact supports while the other  
229 supports were used as filters. The other amino acids studied in this work had error % values  
230 between -13% (D-Tyr) and +13% (D-Leu/D-Ile) and so the method was fit for purpose for their  
231 quantification.

232 The repeatability is determined as the relative standard deviation of the analytical results for the 5  
233 spiked filters. For each type of filter used in this study, the repeatability was always below 10%.

234 The method detection limit (MDL) for the analytical procedure is defined as three times the  
235 standard deviation of the average values of the field blank (n=3). Tables S1, S2 and S3 report the  
236 relative MDLs for each quantified amino acid in the three different sampling supports, the absolute  
237 mean blank values (n=3) in these tables are subtracted from the analytical results. All ~~the~~  
238 discussions in the following sections below are based upon blank corrected values.

239 A comparison between previously published data (Barbaro et al., 2011; Matsumoto and Uematsu,  
240 2005) and the MDLs obtained for each type of filter in this work shows that we obtained lower  
241 blank values than those previously reported.

## 242 2.5 Back-trajectory calculation and satellite imagery

write as "percent error"

e.g.  
And  
all  
places

243 Backward air trajectories arriving at MZS, Dome C and R/V Italice were computed using a Hybrid  
244 Single Particle Lagrangian Integrated Trajectory (HYSPLIT) transport and dispersion models  
245 (Draxler and Rolph, 2013). The meteorological data used for computing all the backward  
246 trajectories were the NCEP/NCAR Global Reanalysis Data. For MZS data, a vertical velocity  
247 model was used while an isentropic model was employed for the analysis of DC air masses, as  
248 suggested by Stohl et al (2010).

249 240 hours <sup>of</sup> back-trajectories beginning at MZS and DC were calculated for each sampling campaign  
250 period. Four runs were computed for every sampling day at six hour intervals and the resulting  
251 multiple trajectories were “mean-clustered aggregated” into 6 groups, based on the scree-plot  
252 analyses of total spatial variance.

253 A sensitivity study has been performed to verify the stability of the HYSPLIT back trajectory  
254 calculations. We calculated the back-trajectories beginning at 10 m agl (above ground level), 100  
255 m, 500 m and 1000 m at MZS and DC to evaluate how the trajectories varied with height. The  
256 results are shown in Supplementary Fig. S1-S3. It can be seen that the clusters of simulated air  
257 masses have similar trajectories although with different percentages of the total number of  
258 calculated back trajectories. For this study we used the 500 m back trajectories because we want to  
259 evaluate long range transport. This is because the mean mixed-layer height is 250–400 m agl at DC  
260 (Argentini et al., 2005) while the boundary-layer height is usually below 50 m at the Antarctic coast  
261 (Handorf et al., 1999).

262 We have also estimated the stability of the HYSPLIT model by varying the position of source at  
263 MZS as well as DC using a 121 point matrix built by adding or subtracting one degree of latitude or  
264 longitude from the real source for each sampling day. These back-trajectories calculated from the  
265 121 simulated sources have the same behavior (Supplement Fig. S4-S6), thus confirming the  
266 stability of the HYSPLIT calculations.

267 For the oceanographic cruise, trajectory matrices were performed in order to simulate the ship’s  
268 itinerary. In this case, for each 24-h sampling event, 5-day backward trajectories were computed.

269 The data related to chlorophyll were obtained *via* an Aqua/MODIS NASA satellite continually  
270 orbiting the globe (<http://neo.sci.gsfc.nasa.gov/>).

### 271 3. Results and Discussion

#### 272 3.1 Free amino acid determination in the coastal area

273 Nine L-amino acids (L-Ala, L-Asp, L-Arg, L-Glu, L-Phe, L-Pro, L-Tyr, L-Thr) and Gly had blank  
274 corrected concentrations higher than the MDLs (Supplementary Tables S2 and S3), while all D-  
275 amino acids had values below the MDLs, probably due to a negligible presence of bacteria in the  
276 aerosol source (Kuznetsova et al., 2005; Wedyan and Preston, 2008). The total concentration of  
277 amino acids, calculated as the sum of their six size distributions in all aerosol samples, has a median  
278 value of 5 pmol m<sup>-3</sup> and a mean value of 11 pmol m<sup>-3</sup>, due to the higher amino acid concentrations  
279 in the first sample (29 November-9 December), as shown in Fig. 2.

280 The mean total concentration of free amino acids determined in this study was very similar to those  
281 found in the literature for marine aerosols in remote areas. Matsumoto and Uematsu (2005) reported  
282 a mean free amino acid concentration of 10.7 pmol m<sup>-3</sup> in the Pacific Ocean, while Gorzelska and  
283 Galloway (1990) and Wedyan and Preston (2008) observed means of 3 pmol m<sup>-3</sup> and 20 pmol m<sup>-3</sup>  
284 respectively in the Atlantic Ocean. Scalabrin et al. (2012) determined a mean concentration of 2.8  
285 pmol m<sup>-3</sup> using the same sampling method reported here at an Arctic coastal station.

286 Higher mean concentrations of amino acids were found in the Mediterranean. Barbaro et al. (2011)  
287 determined a mean value of 334 pmol m<sup>-3</sup> in the Venice Lagoon (Italy); Mandalakis et al. (2010,  
288 2011) found 166 pmol m<sup>-3</sup> and 172 pmol m<sup>-3</sup> in two studies in the Eastern Mediterranean around  
289 Greece, respectively. In the Southern hemisphere, Mace et al. (2003b) performed several studies on  
290 the coast of Tasmania (Australia), and found mean free total amino acid concentrations that ranged  
291 from between 15 and 160 pmol m<sup>-3</sup>.

Water samples from

→ more water samples?

Beclear

292 In this work, we found that the predominant compounds were Gly and Arg, which together  
293 constituted 66-85% of the total amino acid content. Gly and Arg had different proportions in the  
294 five samples, and the other compounds were present in similar proportions in all the samples, with  
295 average percentages of 9% for Glu, 7% for Ala, 5% for Thr, 4% for Asp, 2% for Val while 1% for  
296 other amino acids (Phe, Tyr and Pro). In Fig. 2 it can be seen that the first sample collected between  
297 29 November and 09 December had a high proportion of Arg (74%), compared to Gly (11%). In  
298 contrast to this, in the other samples, Gly was the predominant compound, with a percentage  
299 between 48 to 56%, while Arg was present as 18% of the total. Be consistent with  
300 Scheller (2001) demonstrated that high quantities of Arg were closely linked with plant growth, but spacing before units  
301 the cluster means backward trajectories (Fig. 3) calculated for our samples show that 1 % of the air  
302 masses come from open-ocean areas whilst the major part (99 %) principally come from the  
303 interior of the Antarctic continent, areas that are characterized by a lack of vegetation. This suggests  
304 that the local marine influence was probably the main source of amino acids in the aerosol collected  
305 at MZS and that the concentration of coastal atmospheric amino acids is probably linked to local  
306 primary production in the Ross Sea, as suggested by studies in other areas (Meskhidze and Nenes,  
307 2006; Vignati et al., 2010; Yoon et al., 2007; Müller et al., 2009). We hypothesize that the main  
308 source of Arg in the aerosols collected at the coastal Antarctic station MZS was probably a diatom  
309 bloom as Arg is involved in their urea cycle (Bromke, 2013). The MODIS data (Fig. 4) show higher  
310 chlorophyll concentrations during the period covered by the first sampling period, while a strong  
311 decrease in the biomass production index was observed in the other sampling times. This  
312 relationship between marine primary production and Arg concentration suggests that this amino  
313 acid may have a marine biological origin and that its concentration is closely linked to algae  
314 growth.

315 Meteorological conditions play an important role in aerosol formation processes. The first sampling  
316 period (29 November-09 December) was characterized by temperatures ranging between -10°C and  
317 -1.5°C, while in the successive sampling periods, the air temperature was always above -2°C

318 (PNRA-ENEA, 2014). Studies conducted on the sea surface microlayer (Grammatika and  
319 Zimmerman, 2001; Knulst et al., 2003) established that air temperatures  $<-5^{\circ}\text{C}$  create surface  
320 slurries which may result in the expulsion of salts and particulate organic matter. Under such  
321 conditions, near-surface turbulence was increased, leading to an increase of material in the  
322 microlayer, where bubble formation and bursting actively contributed to the transport mechanisms.  
323 Leck and Bigg (2005) showed that the main occurrences of fine aerosol formation in the arctic  
324 atmosphere were observed when the ice pack is cracking forming leads that melt and refreeze. Our  
325 first sample was collected when the pack ice was melting and refreezing, and we did in fact observe  
326 the highest concentration of total amino acids in the fine aerosols during this period.

327 The hypothesis of a local marine source for the aerosols collected at the coastal station MZS was  
328 also confirmed by the distribution of the amino acids in the different particle size fractions. Fig. 2  
329 shows that 98% of the total free amino acids are generally found in the fine particles ( $<1\mu\text{m}$ ,  
330 combined S5 and B filters). While the remaining 2% is evenly distributed over the other coarser  
331 fractions  $>1\mu\text{m}$  (filter stages S1 to S4). Our experimental data <sup>is consistent with</sup> ~~supports~~ the observations of O'Dowd  
332 et al. (2004) and Keene et al.(2007) who showed that WSOC in sea spray submicron particles are  
333 mostly associated with the smallest size fraction (0.1-0.25  $\mu\text{m}$ ). Other authors (Facchini et al.,  
334 2008b; Modini et al., 2010) have shown that WSOC were present in all aerosol size fractions and  
335 confirm that the greatest enrichment was in the fine fraction. Our observations are in line with this  
336 literature data as amino acids are part of the WSOC family of compounds and so should have the  
337 same behavior in sea spray submicron particles.

### 338 **3.2 The determination of free amino acids at a remote continental area.**

339 Concordia Station at Dome C is an ideal site for studying the chemical composition of remote  
340 Antarctic aerosol. Several studies (Fattori et al., 2005; Jourdain et al., 2008; Becagli et al., 2012;  
341 Udisti et al., 2012) have investigated the distribution of inorganic compounds and of a few organic

342 molecules (e.g., methanesulfonic acid) in aerosol, but the free amino acid concentration and  
343 composition has not yet been studied.

344 Fig. 5 presents the concentrations of free amino acids collected during both field campaigns, and  
345 shows a similarity between the trends and compositions of the analyzed compounds between the  
346 various size fractions. Ten amino acids (L-Ala, L-Arg, L-Asp, L-Glu, L-Leu, Gly, L-Phe, L-Thr, L-  
347 Tyr, L-Val) had concentrations above MDLs (Supplementary Tables S2 and S3) in all samples  
348 collected in both field campaigns. The concentrations of D-amino acids were always below MDLs,  
349 as seen in our coastal results. It was observed that Gly, L-Asp and L-Ala together accounted for  
350 about 80% of the total amino acid content. The total mean free amino acid concentrations, as the  
351 sum of the free amino acid concentrations in all the sample stages, were  $0.8 \text{ pmol m}^{-3}$  for the 2011-  
352 2012 campaign and  $0.7 \text{ pmol m}^{-3}$  for 2012-2013 campaign (Fig. 5). To our knowledge, these mean  
353 concentrations are lower than those reported in the literature (Gorzelska and Galloway, 1990;  
354 Milne and Zika, 1993; Mace et al., 2003b; Kuznetsova et al., 2005; Matsumoto and Uematsu, 2005;  
355 Wedyan and Preston, 2008; Mandalakis et al., 2010; Barbaro et al., 2011; Mandalakis et al., 2011;  
356 Scalabrin et al., 2012), suggesting that this aerosol composition may describe the amino acid global  
357 background concentration.

358 In Fig. 5B, the sample collected from 27 December 2012 to 06 January 2013 shows an altered  
359 concentration profile, with the highest concentrations in one of the coarse fractions (S4 stage 1.5-  
360  $0.95 \mu\text{m}$ ). After evaluating the wind roses and activity at the base for each sample in the two  
361 summer campaigns, we believe that these samples were contaminated by human activity at  
362 Concordia station (Supplementary Fig. S7).

363 Cluster means backward trajectories analysis of all the samples collected during both summer  
364 campaigns revealed a prominent marine source (Fig. 3). During the Antarctic summer, the surface  
365 inversion over the polar ice cap is relatively weak and aerosols produced on the ocean's surface can  
366 be transported through the upper troposphere to the Antarctic plateau where they are easily mixed  
367 down to the surface (Cunningham and Zoller, 1981). There are also some transfer mechanisms from

368 the lower stratosphere to the upper troposphere that occur near the coast of the Antarctic continent.  
369 Aerosol from different sources mixes into the upper troposphere, and this air descends uniformly  
370 over the Antarctic plateau due to surface cooling flows off the plateau causing the katabatic wind.  
371 This means during the summer, there is a continuous flux of relatively clean air from the upper  
372 troposphere with aerosol from high altitude input and long range transport (Cunningham and Zoller,  
373 1981; Stohl and Sodemann, 2010).

374 The analysis of the size distribution of the free amino acids (Fig 5) combined with the air mass back  
375 trajectories (Fig. 3) allowed us to ~~identify~~ **suggest** (still just an idea here) the aerosol sources and the transformation mechanisms  
376 that these aerosols undergo during long-range transport. Our results suggest that amino acids were  
377 present in the fine particles over the surface of the Southern Ocean from bubble bursting processes.  
378 The air masses subsequently passed into the upper troposphere and then over the continent where  
379 they remained for several days before descending onto the ice sheet. These fine aerosol particles can  
380 grow during long-range transport, due to condensation of molecules from the gas phase or by  
381 collision of small and large particles (coagulation) (Petzold and Karcher, 2012; Roiger et al., 2012).  
382 However, ~~this is~~ **these processes are** unlikely in Antarctica due to the very clean conditions. The specific reason for this

383 **enrichment** is not clear based on the available data. In our future investigations, we will also  
384 evaluate the **aerosols mass**, which is probably a key parameter to measure that will help explain this  
385 enrichment. The concentration of free amino acids in the coarse particles of aerosols collected at  
386 DC had mean values of 407 and 421 fmol m<sup>-3</sup> (see Fig. 5) for the two field campaigns, while our  
387 coastal data had a mean free amino acids concentration of 264 fmol m<sup>-3</sup> (Fig. 2). The aerosols  
388 collected at DC were characterized by a prevalence of free amino acids in the fine fraction, with a  
389 notable enrichment of amino acids in the coarse particles (13% of the total in 2011-12 and 23% of  
390 the total in 2012-13) compared to coastal aerosol. In fact, during our 2010-2011 sampling  
391 campaign at MZS, which is located near the aerosol source, we observed only 2% of total free  
392 amino acids in the coarse particles. The most likely explanation for this enrichment of amino acids  
393 in the coarse fraction, is that the fine fraction has been subjected to processes that increased the



394 particle size of the aerosol. The most likely process is ice nucleation during long-range transport  
395 promoted by the intense cold over the plateau and presence of amino acids in the aerosol particles  
396 (Szyrmer and Zawadzki, 1997).

397 The chemical composition of aerosols may change during long-range transport due to  
398 photochemical, chemical and ionic reactions (Milne and Zika, 1993; Nozière and Còrdova, 2008;  
399 De Haan et al., 2009). Milne and Zika (1993) verified that amino acids are destroyed *via* reactions  
400 with photochemically formed oxidants such as hydroxyl radicals, to form products such as the  
401 ammonium ion, amides and keto-acids. However, in the upper atmosphere, the chemical processes  
402 take place at slower rates than in the boundary layer (Roiger et al., 2012). In aqueous-phase  
403 aerosols, glyoxal can react with amino acids, leading to scavenging processes (De Haan et al.,  
404 2009). Recent studies on organic aerosol growth mechanisms (Maria et al., 2004) underlined that  
405 oxidation processes that remove hydrophobic organic compounds, are slower in large carbonaceous  
406 aerosols.

407 From the physicochemical proprieties of amino acids, a “hydropathy” index can be made, as  
408 suggested by Pommie et al. (2004). This classifies the amino acids as hydrophilic (Asp, Hyp, Glu,  
409 Asn, Lys, Gln, Arg), hydrophobic (Ala, Val, Leu, Ile, Met, Phe) or neutral (Gly, Pro, Ser, Thr, Tyr,  
410 Hys). This helps in evaluating the contribution of each kind of amino to each class of aerosols  
411 collected over the three different field campaigns. Fig. 6 shows that the hydrophilic components  
412 were predominant in the locally produced marine aerosols released into the atmosphere near MZS,  
413 while hydrophobic compounds were dominant in the aerosols collected at the continental station  
414 (DC). The low abundance of hydrophobic amino acids in coastal aerosols was also observed by  
415 Mandalakis et al. (2011), and is probably caused by their lower tendency to dissolve in the aqueous  
416 particles contained in coastal aerosols. This classification allows us to hypothesize that a higher  
417 proportion of hydrophilic amino acids reflects a higher water content in the aerosol.

418 A comparison between the concentrations of hydrophobic Ala at the two sampling sites (MZS and  
419 DC) shows a very similar average concentration ( $70 \text{ fmol m}^{-3}$ ) in the coarse particles. This is an

420 interesting behavior that confirms the hypothesis of limited atmospheric reactivity as proposed by  
421 Maria et al. (2004), who suggested a longer hydrophobic aerosol lifetime as a result of the slower  
422 oxidation rates. Thanks to this phenomenon, Ala significantly contributes to the amino acid content  
423 in these “remote aerosols” as it does not degrade during long range transport.

424 Fig. 5 shows that the concentration of amino acids for the 2011-2012 summer Antarctic campaign  
425 was higher than the values reported for the 2012-2013 Antarctic campaign, and underlines that the  
426 main difference between the two campaigns is mainly in the percentages of hydrophilic and neutral  
427 amino acids present. We suggest that the transport processes of the air masses were the main cause  
428 of these variations as the time spent inland by the air masses in the 2011-2012 summer was about  
429 36 hours (Fig. 3) whilst in 2012-2013 the time range was between 4 and 7 days (Fig. 3). A longer  
430 transportation time from the source to the sampling site allows chemical transformation through  
431 photochemical reactions to take place, decreasing the concentration of hydrophilic amino acids thus  
432 modifying the composition so that the more stable Gly (a neutral component) becomes the main  
433 compound (Fig. 6).

434 Looking at the acid-base proprieties of the amino acids, some differences can be observed between  
435 two different types of aerosol. As described above, the predominant amino acid in the MZS aerosols  
436 was Arg, which contributed considerably to the percentage of basic compounds (53%). The pH  
437 neutral components represented an important percentage (40% and 68% for coastal and inland  
438 aerosols respectively). Gly is mainly present in large quantities in these aerosols because of its very  
439 low atmospheric reactivity (half life of 19 days) (McGregor and Anastasio, 2001) and its presence is  
440 usually considered an indicator of long-range aerosol transport (Milne and Zika, 1993; Barbaro et  
441 al., 2011). The acidic compounds (Asp and Glu) contribution was quite different in the aerosols  
442 from the two different stations: with a low percentage in the coastal samples at MZS (7%) that was  
443 in contrast with the higher content in the aerosols from DC (33% and 26% respectively for the two  
444 consecutive field campaigns). This result can be explained by a study conducted by Fattori et al.  
445 (2005) on the DC aerosol, where high acid content was found. High concentrations of hydrochloric,

446 nitric and sulfuric acids were found in the aerosol fine fraction, promoting numerous series of acid-  
447 base atmospheric reactions that neutralize the basic compounds. In the atmosphere, amino acids are  
448 present in very low quantities so it is thought that they do not influence the pH of aerosols.  
449 However, the pH of aerosols, can influence the chemical form of the amino acids present.

### 450 **3.3 Free amino acids during an oceanographic cruise**

451 Measurements of free amino acids were carried out on aerosol samples collected on the Southern  
452 Ocean onboard the R/V *Italica* from 13th January to 19th February 2012. Aerosols were sampled  
453 using a TSP sampler that collects particles with a diameter above 1  $\mu\text{m}$ . The first and second  
454 samples covered the track between New Zealand (from Lyttelton harbor) and MZS (Antarctica),  
455 and the sixth and last samples were collected during the return journey between Antarctica and New  
456 Zealand. Samples 3,4 and 5 were collected on the Ross Sea near the Antarctic continent (Fig. 1).  
457 Five L-amino acids (L-Asp, L-Arg, L-Glu, L-Phe, L-Pro) and Gly were present in the samples,  
458 while other L- and D-amino acids had concentrations below MDLs (Supplementary Table S1). The  
459 total concentrations of free amino acids varied between 2 and 12  $\text{pmol m}^{-3}$ .

460 The first and last samples had the highest concentrations of free amino acids (Fig. 7), and their  
461 relative sampling periods were characterized by temperatures ranging between  $-1^{\circ}\text{C}$  and  $18^{\circ}\text{C}$   
462 (sample 1), in contrast, temperatures during the remaining sampling periods were always below  
463  $-1^{\circ}\text{C}$ , with a lowest value of  $-8^{\circ}\text{C}$  (sample 4). Higher temperatures can facilitate metabolic  
464 processes and accelerate atmospheric chemical reactions, as well as promote bubble bursting from  
465 the sea surface. This is probably the main source of amino acids in our on-ship samples. This is also  
466 supported by the back-trajectory analysis (Supplementary Fig. S8a-g), that demonstrate only a  
467 marine influence for that period. The concentration of amino acids was strongly influenced by sea  
468 conditions during sampling. The field report (Rapporto sulla campagna Antartica, 2012), noted that  
469 during navigation from New Zealand to the ice-pack region, the winds were always above 30 knots,  
470 with maximum values of 60 knots with wave heights of 12 meters. This probably explains the

471 higher total concentration of free amino acids in the first two samples ( $12 \text{ pmol m}^{-3}$ ). Along the  
472 same track, but under calmer sea conditions (sample 7), we observed a slight reduction in the total  
473 concentration of free amino acids ( $8 \text{ pmol m}^{-3}$ ). These values were very similar to those reported by  
474 Matsumoto and Uematsu (2005) in the Pacific Ocean and to those reported by Gorzelska and  
475 Galloway (1990) and Wedyan and Preston (2008) in the Atlantic Ocean. The lowest concentrations  
476 were observed in samples 2 and 6, probably due to the fact that they were collected far from  
477 Oceania and from the Antarctic coast, in an area characterized by expansive pack ice and by  
478 temperatures below  $-1^\circ\text{C}$ , where the bubble bursting process was reduced.

479 The samples collected near the Antarctic coast (samples 3,4 and 5) were the most interesting ones  
480 because the results could be compared with the amino acid values detected in the coastal station  
481 MZS. The mean total concentration in the samples collected on the Ross Sea was  $3.5 \text{ pmol m}^{-3}$ ,  
482 about half of the values detected in our Southern Ocean samples. Such values are similar to the  
483 concentrations observed in the aerosols collected at MZS station (median  $5 \text{ pmol m}^{-3}$ ). However,  
484 this is not a true comparison: for the sampling campaign at MZS, a cascade impactor was used to  
485 collect aerosol samples with a particle-size below  $10 \mu\text{m}$ , whereas the data collected during the  
486 cruise was for aerosols with a particle diameter above  $1 \mu\text{m}$ . However, if we exclude data from the  
487 back-up and the fifth slotted filters, the cascade sampler covers a particle size between  $0.95 \mu\text{m}$  and  
488  $10 \mu\text{m}$  (stages 1 to 4), making a comparison between the two data sets more feasible. In the MZS  
489 aerosols, the median value of the amino acids concentration in the aerosols collected on stages 1 to  
490 4 was  $1 \text{ pmol m}^{-3}$  and this concentration was lower than that measured in the cruise's aerosols ( $3.5$   
491  $\text{pmol m}^{-3}$ ). So we suspect that the aerosols with a diameter above  $10 \mu\text{m}$ , that were collected with  
492 the TSP sampler but not the cascade impactor, could be the main source of the difference in amino  
493 acid concentration values in the samples collected on the R/V *Italica*.

494 The back-trajectory analysis (Supplementary Fig. S8C-E) demonstrated that the air masses came  
495 from inland Antarctica, where no vegetation is present. The biological material present in the  
496 atmosphere with a size  $> 10 \mu\text{m}$  includes pollens which typically vary between  $17\text{-}58 \mu\text{m}$ , fungal

497 spores between 1-30  $\mu\text{m}$ , and algal spores between 15-120  $\mu\text{m}$ . Instead bacteria have a diameter  
498 between 0.25-8  $\mu\text{m}$ , and viruses have diameters that are typically less than 0.3  $\mu\text{m}$  (Jones and  
499 Harrison, 2004). For this reason, we propose that the biological materials influenced the  
500 concentration of the total free amino acids in the shipboard aerosols.

501 In these samples, the presence of algal spores was also confirmed by the detection of Pro at 4%  
502 (mean value) of the total concentration of amino acids. Fisher et al. (2004) measured the relevant  
503 concentration of Pro in ascospores, demonstrating that this amino acid can be used to identify the  
504 presence of spores in aerosols. In the MZS aerosols, the presence of spores could not be evaluated  
505 because the sampler did not sample the particles  $> 10\mu\text{m}$ . This is probably the reason why the Pro  
506 concentration was always below MDLs at MZS.

507 Asp was detected in only one sample (sample 5), with a concentration of  $502 \text{ fmol m}^{-3}$ . This value is  
508 very similar to those measured in the two field campaigns on the Antarctic plateau (DC),  
509 considering only the slotted filter stages above 1  $\mu\text{m}$  ( $446$  e  $382 \text{ fmol m}^{-3}$  respectively for the 1  
510 summer field campaigns of 2011-12 and 2012-13). The back-trajectory analysis (Supplementary  
511 Fig. S8E) demonstrated that this air mass came from the plateau, where aspartic acid was a  
512 predominant component of the amino acid content.

513 In the aerosols collected during the cruise, the Arg concentration was very low because the  
514 sampling conducted on board R/V *Italica* during the summer of 2012 excluded fine particles,  
515 whereas Arg was one of the most abundant compounds observed in the coastal station found in the  
516 fine fraction.

517

#### 518 **4. Conclusions**

519 This first study on the size distribution of amino acids in Antarctica has identified possible sources  
520 of marine aerosols in this region and has characterized some chemical and physical transformations  
521 that take place during transport to the interior of the Antarctic continent.

522 Marine emissions of fine particles occurred *via* bubble bursting processes on the surface of the  
523 Southern Ocean. The mean total amino acid concentration detected at MZS was  $11 \text{ pmol m}^{-3}$ , with a  
524 higher percentage of amino acids found in the fine fraction. The aerosol samples collected at Dome  
525 C had the lowest amino acid values ( $0.7$  and  $0.8 \text{ pmol m}^{-3}$ ) and the coarse particles were found to be  
526 enriched with amino acids compared to the coastal site. Numerous chemical and photochemical  
527 events may have contributed to a decrease in the concentration in amino acids in the fine fraction,  
528 and the chemical reactions were faster for hydrophilic compounds than for hydrophobic ones, as  
529 suggested by an observed Ala enrichment.

530 The presence of only the L-enantiomers of free amino acids in Antarctic aerosols suggests that  
531 planktonic particles were the main sources of free amino acids in this area and that these  
532 compounds can be modified when transported to the interior of the continent. Gly and Ala, are the  
533 most stable compounds, and may be used as biogenic markers of long-range marine aerosols. The  
534 back-trajectory analysis demonstrated that the differences in the transport time of air masses inside  
535 Antarctica can result in modifications to the percentage of amino acids in the coarse particles.

536 The study of aerosols with diameters  $>10 \text{ }\mu\text{m}$  indicated that bubble bursting processes can also emit  
537 microorganisms that are composed of a higher number of neutral amino acids.

538

Why not  
other marine?

539 **Author contributor**

540 E. Barbaro, M. Vecchiato and R. Zangrando designed the experiments, performed the HPLC-MS  
541 analyses, and elaborated the data. A. Gambaro and C. Barbante were the principal investigators of  
542 the project that supported this work. All the authors have helped in the discussion of the results and  
543 collaborated in writing the article.

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560

561

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729 **Figure captions**

730 Figure 1. The sampling sites: the Italian base “Mario Zucchelli Station” (MZS) ( $74^{\circ} 42' S - 164^{\circ}$   
731  $06' E$ ), the Italian-French base “Concordia Station” (Dome C) ( $75^{\circ} 06' S - 123^{\circ} 20' E$ ) and the  
732 track chart of the R/V *Italica*.

733 Figure 2. Amino acid size distribution in the samples collected during the summer of 2010-11 at  
734 Mario Zucchelli Station (Antarctica).

735 Figure 3. Cluster means backward trajectories analyses at 500 m agl at the coastal base “Mario  
736 Zucchelli Station” (MZS) during the summer of 2010-2011 and cluster means backward trajectories  
737 at the Italian-French base Dome C (DC) during the summers of 2011-2012 and 2012-2013.

738 Figure 4. Distribution of chlorophyll concentrations in the Ross Sea for each sampling period  
739 obtained through the Aqua/MODIS NASA satellite.

740 Figure 5. Size distributions of amino acid concentrations in the samples collected during the  
741 summer of 2011-12 (A) and during the summer of 2012-13 (B) at the Italian French base  
742 “Concordia Station” (Dome C).

743 Figure 6. Comparison between percentages of hydrophilic, neutral and hydrophobic amino acid  
744 contributions of the aerosols sampled at the Mario Zucchelli Station and at Dome C.

745 Figure 7. Amino acid distribution in the aerosols sampled on the R/V *Italica* during the  
746 oceanographic cruise on the Southern Ocean during the summer of 2012.

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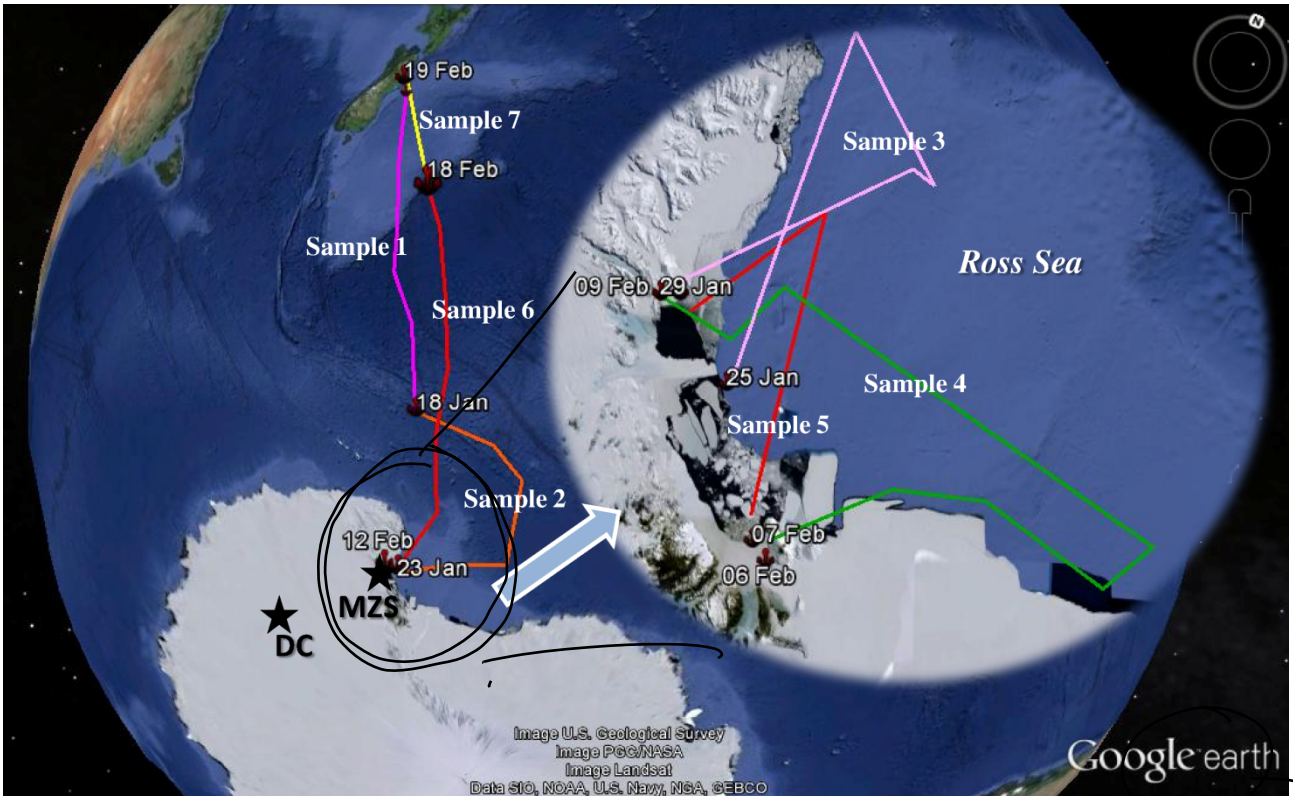
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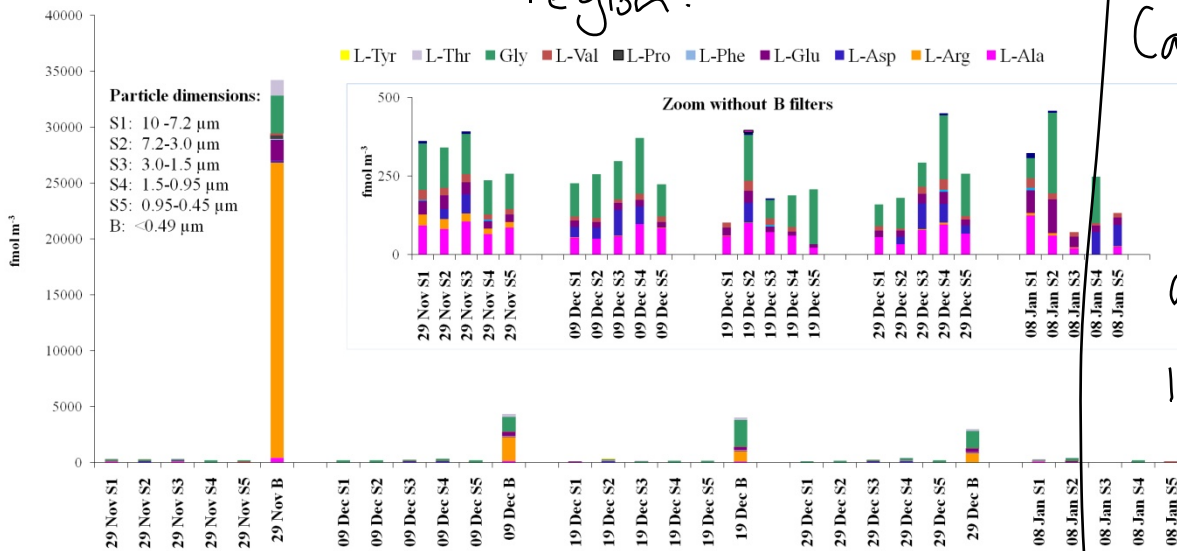
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754 Figure 1. The sampling sites: the Italian base “Mario Zucchelli Station” (MZS) (74° 42’ S – 164°  
 755 06’ E), the Italian-French base “Concordia Station” (Dome C) ( 75° 06’ S – 123° 20’ E) and the  
 756 track chart of the R/V Italica. Draw a circle to show expanded

region.

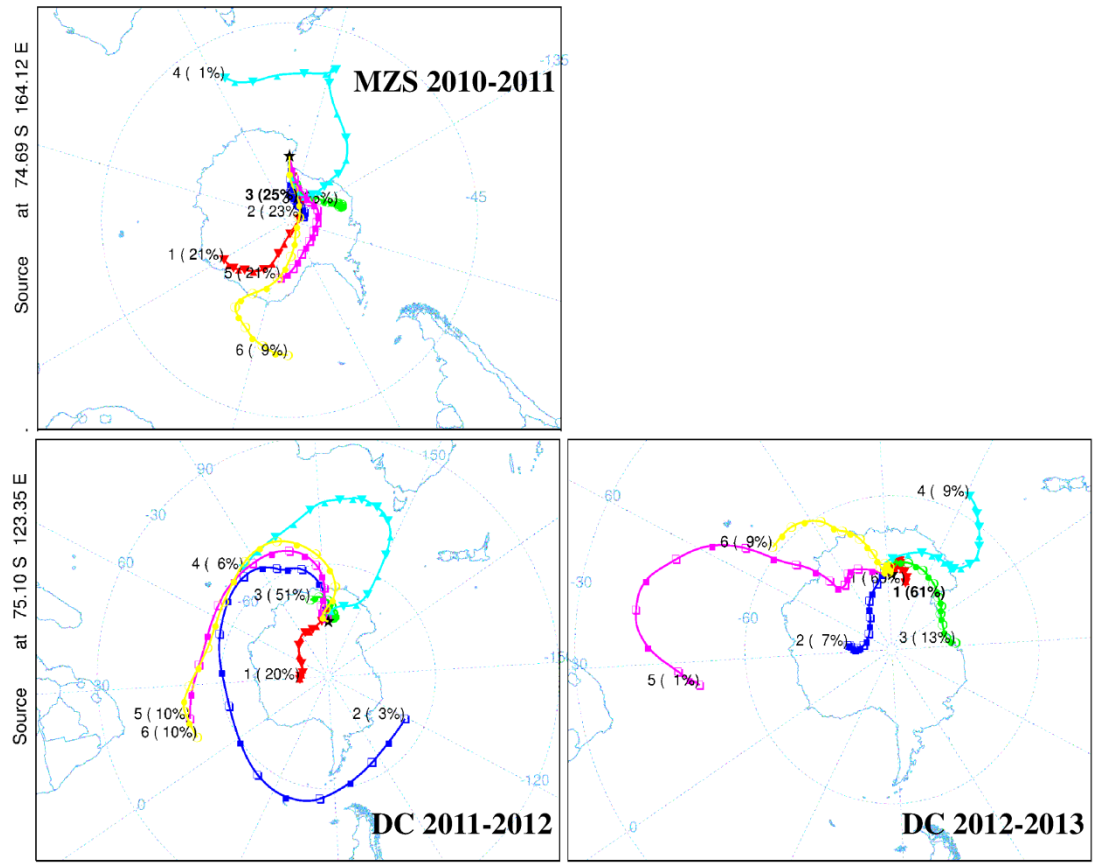


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758 Figure 2. Amino acid size distribution in the samples collected during the summer of 2010-11 at  
 759 Mario Zucchelli Station (Antarctica).

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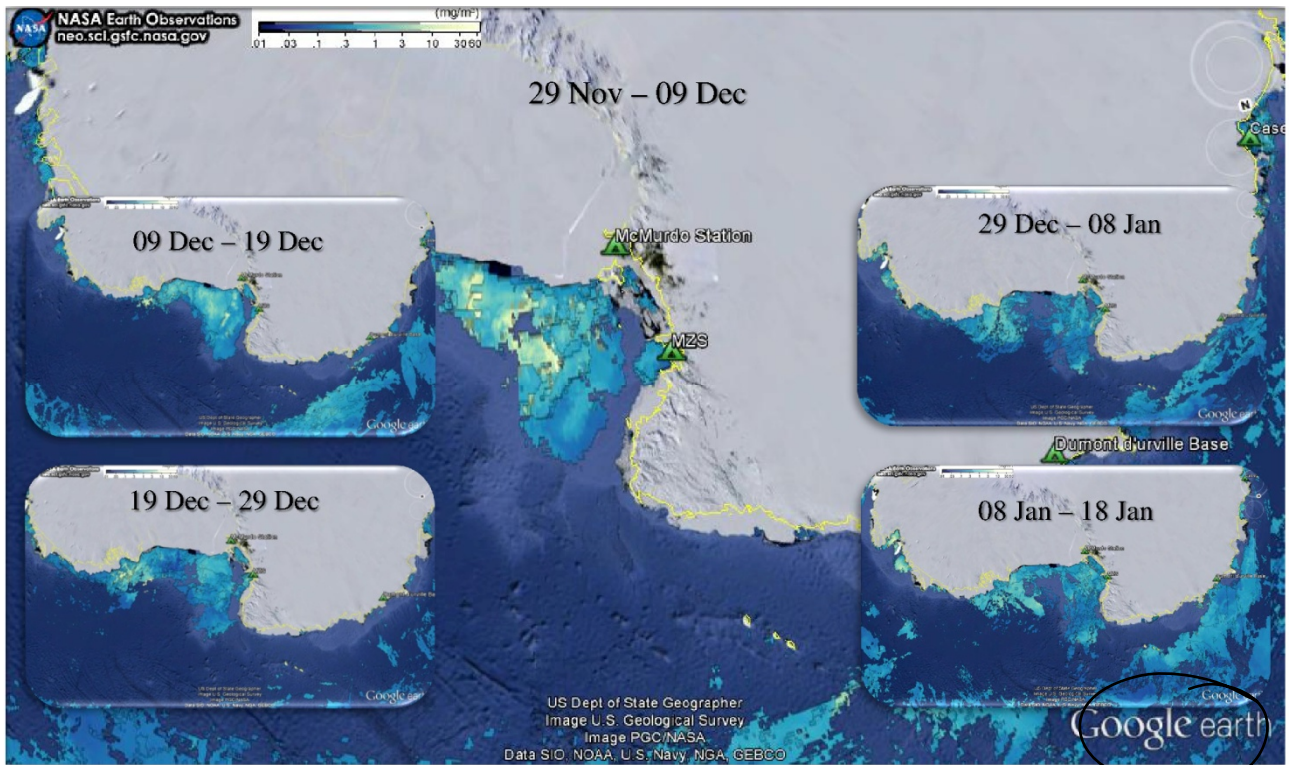
Can google earth be used without acknowledgment? I think it needs a comment in the caption.



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762 Figure 3. Cluster means backward trajectories analyses at 500 m agl at the coastal base “Mario  
 763 Zucchelli Station” (MZS) during the summer of 2010-2011 and cluster means backward trajectories  
 764 at the Italian-French base Dome C (DC) during the summers of 2011-2012 and 2012-2013.

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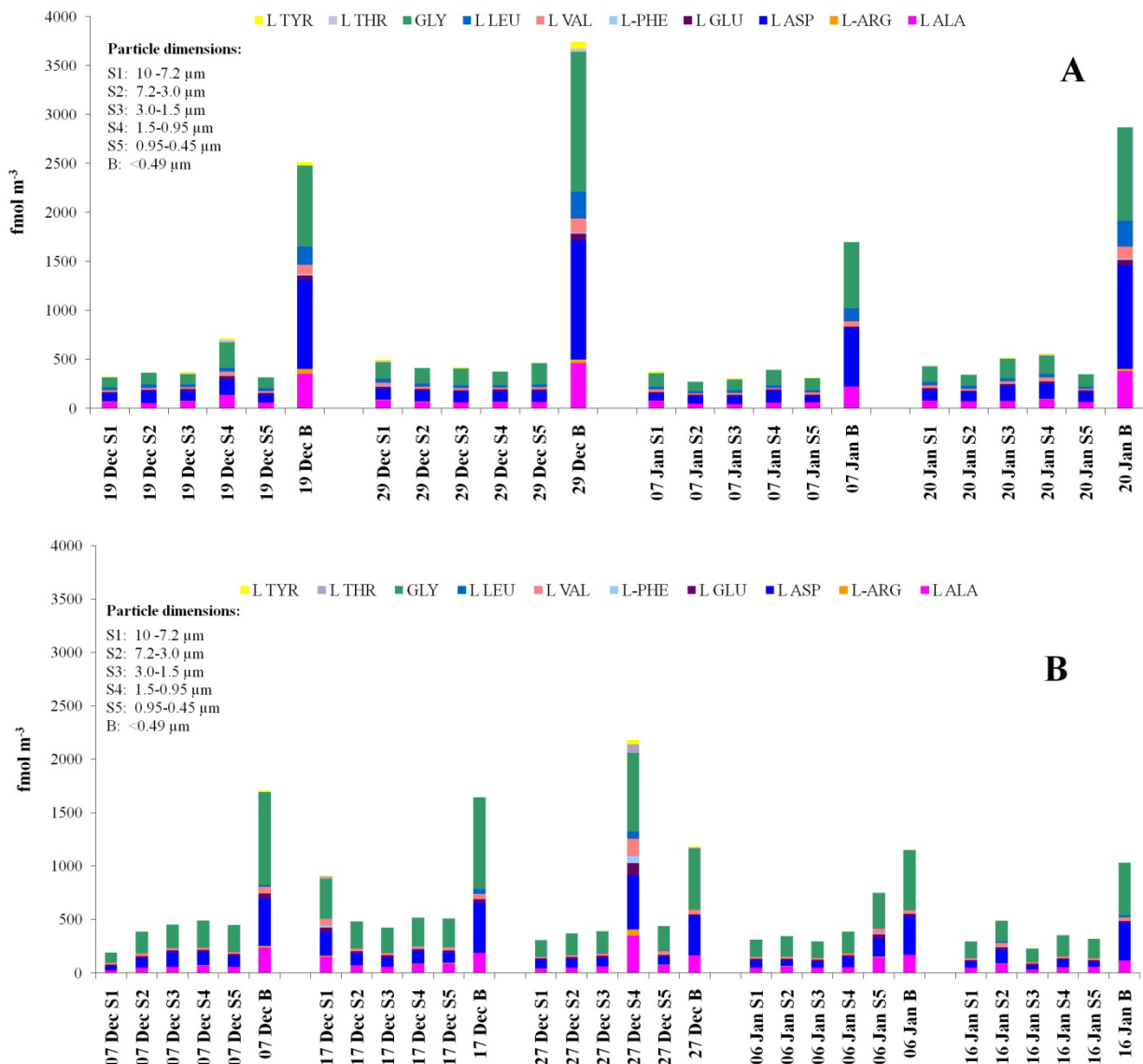


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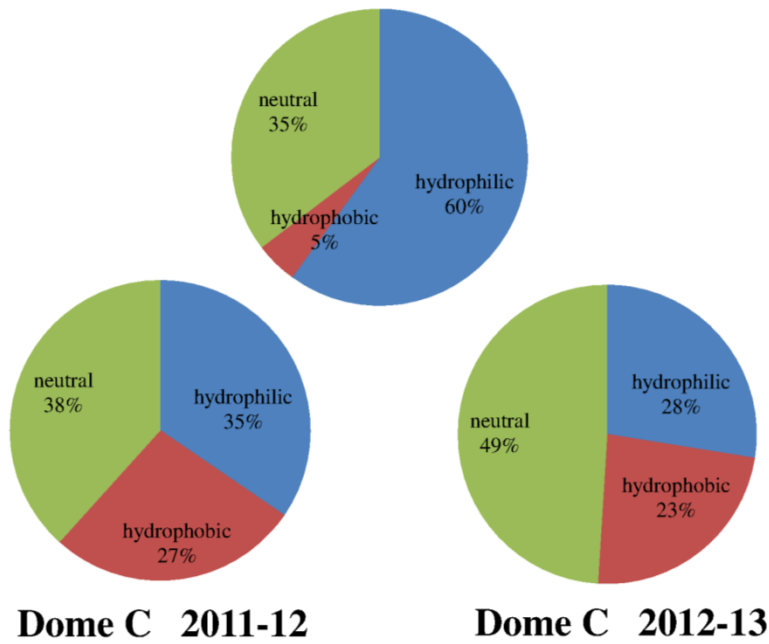




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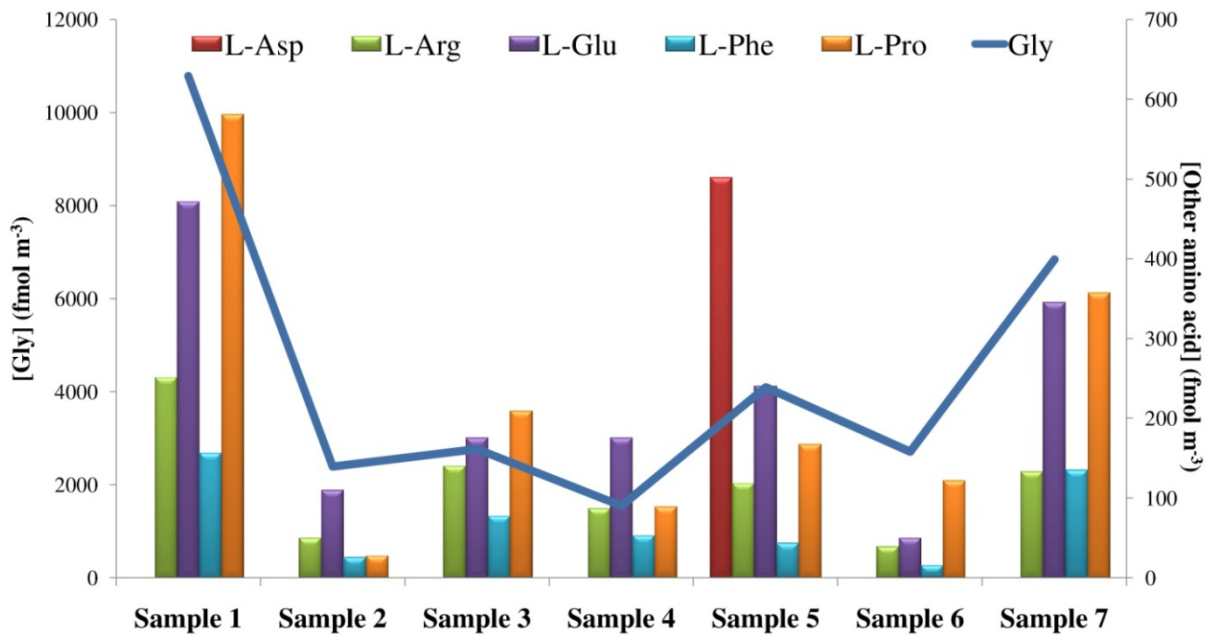
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**MZS 2010-11**



773

774 Figure 6. Comparison between percentages of hydrophilic, neutral and hydrophobic amino acid  
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777 Figure 7. Amino acid distribution in the aerosols sampled on the R/V Italia during the  
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