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

Elena Barbaro [a,b*], Roberta Zangrando[b], Marco Vecchiato[b,c], Rossano Piazza[a,b], Warren R. L. Cairns[b], Gabriele Capodaglio[a,b], Carlo Barbante[b], Andrea Gambaro[a,b]

[a] Department of Environmental Sciences, Informatics and Statistics, University of Venice, Ca' Foscari, Calle Larga Santa Marta 2137, 30123, Venice, Italy

[b] Institute for the Dynamics of Environmental Processes CNR, Dorsoduro 2137, 30123, Venice, Italy.

[c] University of Siena, Department of Physical Sciences, Earth and Environment, Strada Laterina,8 53100 Siena, Italy

Corresponding author. Elena Barbaro, University of Venice, 30123 Venice, Italy

Phone: +39 041 2348545.Fax +39 041 2348549. E-mail: barbaro@unive.it

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Abstract

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

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

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

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

1. Introduction

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

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

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

1987; Mace et al., 2003a, b), fog (Zhang and Anastasio, 2001), and in dew water (Scheller, 2001). They can be present as dissolved combined amino acids (proteins and peptides) (Kuznetsova et al., 2005; Ge et al., 2011), dissolved free amino acids from the hydrolysis of the combined amino acids (Mopper and Zika 1987; Milne and Zika, 1993), and particulate amino acids (from solid microorganisms and debris particles inside the liquid aerosol phase) (Kuznetsova et al., 2005).

Several emission sources can affect not only the total concentration of dissolved free amino acids in the atmosphere, but also the amino acid composition of the aerosol. Amino acids have been detected in volcanic emissions (Mukhin et al., 1978; Scalabrin et al., 2012), biomass burning has also been suggested as a possible source of amino acids as part of the WSOC content (Mace et al., 2003a; Chan et al., 2005). The different amino acids found in continental particles are thought to have been originally produced by plants, pollens and algae, as well as fungi and bacterial spores (Milne and Zika, 1993; Scheller, 2001; Zhang and Anastasio, 2003; Mace et al., 2003a) and can be found in high concentrations in soil and desert dust. The continental contribution was evaluated by Mace et al. (2003b), who found that biogenic amino acids were present in the fine particles and that coarse particles contained amino acids from mainly anthropogenic sources. The anthropogenic sources currently identified are tobacco smoke (Ge et al., 2011), incinerators, waste collection centers and sewage treatment plants (Leach et al., 1999). Zhang and Anastasio (2002) identified livestock farming as the main source of amino acid ornithine in Californian aerosols. Matsumoto and Uematsu (2005) describe how long-range transport influences the concentration of amino acids in the North Pacific Ocean, while an evident marine source was verified by Weydan and Preston (2008) in the South Atlantic Ocean. Several studies investigated the free dissolved amino acids in marine aerosols (Gorzelska and Galloway, 1990; McCarthy et al., 1998; Mace et al., 2003; Matsumoto and Uematsu, 2005; Kuznetsova et al., 2005; Wedyan and Preston; 2008; Mandalakis et al., 2011) but few studies have been conducted in the polar regions. Schmale et al. (2013) conducted a complete study on the characterization of Sub-Antarctic marine aerosols and they identified hatching penguins as a source of amino acids in the aerosol of Bird Island in the Southern Atlantic

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

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

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

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

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

2. Experimental section

2.1 Sample collection

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

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

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

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

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

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

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

2.2 Sample processing

To avoid contamination from laboratory air particles and from the operator, samples were handled under a clean laminar flow bench (class 100). The pre-analytical and sample extraction protocol has 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 µL of ^{13}C
175 isotopically-labelled amino acid standard solutions (with concentrations ranging between 2 and 3
176 µg 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 µm 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 µL 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^{-1}: 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^{-1}) and 100 µL of a solution containing all the isotopically-labeled ^{13}C amino acids
211 (concentrations ranging between 2 and 3 µg mL^{-1}). 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 ×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**

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

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

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

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

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

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

3. Results and Discussion

3.1 Free amino acid determination in the coastal area

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

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

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

In this work, we found that the predominant compounds were Gly and Arg, which together constituted 66-85% of the total amino acid content. Gly and Arg had different proportions in the five samples, and the other compounds were present in similar proportions in all the samples, with average percentages of 9% for Glu, 7% for Ala, 5% for Thr, 4% for Asp, 2% for Val while 1% for other amino acids (Phe, Tyr and Pro). In Fig. 2 it can be seen that the first sample collected between 29 November and 09 December had a high proportion of Arg (74%), compared to Gly (11%). In contrast to this, in the other samples, Gly was the predominant compound, with a percentage between 48 to 56%, while Arg was present as 18% of the total.

Scheller (2001) demonstrated that high quantities of Arg were closely linked with plant growth, but the cluster means backward trajectories (Fig. 3) calculated for our samples show that 1 % of the air masses come from open-ocean areas whilst the major part (99 %) principally come from the interior of the Antarctic continent, areas that are characterized by a lack of vegetation. This suggests that the local marine influence was probably the main source of amino acids in the aerosol collected at MZS and that the concentration of coastal atmospheric amino acids is probably linked to local primary production in the Ross Sea, as suggested by studies in other areas (Meskhidze and Nenes, 2006;Vignati et al., 2010; Yoon et al., 2007; Müller et al., 2009). We hypothesize that the main source of Arg in the aerosols collected at the coastal Antarctic station MZS was probably a diatom bloom as Arg is involved in their urea cycle (Bromke, 2013). The MODIS data (Fig. 4) show higher chlorophyll concentrations during the period covered by the first sampling period, while a strong decrease in the biomass production index was observed in the other sampling times. This relationship between marine primary production and Arg concentration suggests that this amino acid may have a marine biological origin and that its concentration is closely linked to algae growth.

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

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

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

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

Concordia Station at Dome C is an ideal site for studying the chemical composition of remote Antarctic aerosol. Several studies (Fattori et al., 2005; Jourdain et al., 2008; Becagli et al., 2012; 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 pmol m^{-3} for the 2011-
352 2012 campaign and 0.7 pmol m^{-3} for 2012-2013 campaign (Fig. 5). To our knowledge, these mean
353 concentrations areas 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 µm). 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 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 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^{-3} (see Fig. 5) for the two field campaigns, while our
387 coastal data had a mean free amino acids concentration of 264 fmol m^{-3} (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; Noziére 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 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 µm. 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 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°C and 18°C
462 (sample 1), in contrast, temperatures during the remaining sampling periods were always below
463 -1°C, with a lowest value of -8°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 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 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°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 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 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 µm, whereas the data collected during the
486 cruise was for aerosols with a particle diameter above 1 µm. 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 µm and
488 10 µm (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 pmol m^{-3} and this concentration was lower than that measured in the cruise's aerosols (3.5
491 pmol m^{-3}). So we suspect that the aerosols with a diameter above 10 µm, 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 µm includes pollens which typically vary between 17-58 µm, fungal

497 spores between 1-30 µm, and algal spores between 15-120 µm. Instead bacteria have a diameter
498 between 0.25-8 µm, and viruses have diameters that are typically less than 0.3 µm (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 > 10µm. 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 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 µm (446 e 382 fmol m^{-3} respectively for the l
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.

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

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

The study of aerosols with diameters>10 µm indicated that bubble bursting processes can also emit microorganisms that are composed of a higher number of neutral amino acids.

Author contributor

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

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References

Argentini, S., Viola, A., Sempreviva, M., Petenko, I.: Summer boundary-layer height at the plateau site of Dome C, Antarctica, Boundary-Layer Meteorology, 115, 409-422, doi: 10.1007/s10546-004-5643-6, 2005.

Barbaro, E., Zangrando, R., Moret, I., Barbante, C., Cescon, P., and Gambaro, A.: Free amino acids in atmospheric particulate matter of Venice, Italy, Atmos. Environ., 45, 5050-5057, doi:10.1016/j.atmosenv.2011.01.068, 2011.

Barbaro, E., Zangrando, R., Vecchiato, M., Turetta, C., Barbante, C. and Gambaro, A.: D- and L- amino acids in Antarctic lakes: assessment of a very sensitive HPLC-MS method, Anal Bioanal Chem, 406, 5259-5270, doi: 10.1007/s00216-014-7961-y, 2014.

Bargagli, R.: Environmental contamination in Antarctic ecosystems, Sci Total Environ., 400, 212-226, doi:10.1016/j.scitotenv.2008.06.062, 2008.

Becagli, S., Scarchilli, C., Traversi, R., Dayan, U., Severi, M., Frosini, D., Vitale, V., Mazzola, M., Lupi, A., Nava, S., and Udisti, R.: Study of present-day sources and transport processes affecting oxidised sulphur compounds in atmospheric aerosols at Dome C (Antarctica) from year-round sampling campaigns, Atmos. Environ., 52, 98-108, doi:10.1016/j.atmosenv.2011.07.053, 2012.

Bliesner, D.M.: Validating chromatographic methods a practicalguide. edited by John Wiley & Sons, Inc., Hoboken, 2006.

Bigg, E. K.: Sources, nature and influence on climate of marine airborne particles, Environ. Chem., 4, 155-161, doi:10.1071/en07001, 2007.

Bourcier, L., Sellegri, K., Masson, O., Zangrando, R., Barbante, C., Gambaro, A., Pichon, J. M., Boulon, J., and Laj, P.: Experimental evidence of biomass burning as a source of atmospheric Cs-137, puy de Dome (1465 m a.s.l.), France, Atmos. Environ., 44, 2280-2286, doi:10.1016/j.atmosenv.2010.04.017, 2010.

Bromke, M. A.: Amino Acid Biosynthesis Pathways in Diatoms, Metabolites, 3, 294-311, 2013.

Chan, M. N., Choi, M. Y., Ng, N. L., and Chan, C. K.: Hygroscopicity of water-soluble organic compounds in atmospheric aerosols: Amino acids and biomass burning derived organic species, Environ. Sci. & Technol., 39, 1555-1562, doi:10.1021/es049584l, 2005.

Cronin, J. R., and Pizzarello S.: Enantiomeric excesses in meteoriticamino acids, Science, 275, 951–955, doi:10.1126/science.275.5302.951, 1997.

Cunningham, W. C., and Zoller, W. H.: The Chemical Composition of Remote Area Aerosols, J. Aerosol Sci., 12, 367-384, 1981.

De Haan, D. O., Corrigan, A. L., Smith, K. W., Stroik, D. R., Turley, J. J., Lee, F. E., Tolbert, M. A., Jimenez, J. L., Cordova, K. E., and Ferrell, G. R.: Secondary Organic Aerosol-Forming Reactions of Glyoxal with Amino Acids, Environ. Sci. Technol., 43, 2818-2824, doi:10.1021/es803534f, 2009.

Draxler, R. R., and Rolph, G. D.: HYSPLIT (HYbrid Single-Particle Lagrangian Integrated Trajectory) Model access via NOAA ARL READY Website, avaialable at: http://www.arl.noaa.gov/HYSPLIT.php (last access: May 2013), NOAA Air Resources Laboratory, College Park, MD, 2013.

Facchini, M. C., Decesari, S., Rinaldi, M., Carbone, C., Finessi, E., Mircea, M., Fuzzi, S., Moretti, F., Tagliavini, E., Ceburnis, D., and O'Dowd, C. D.: Important Source of Marine Secondary Organic Aerosol from Biogenic Amines, Environ. Sci. Technol., 42, 9116-9121, doi:10.1021/es8018385, 2008a.

Facchini, M. C., Rinaldi, M., Decesari, S., Carbone, C., Finessi, E., Mircea, M., Fuzzi, S., Ceburnis, D., Flanagan, R., Nilsson, E. D., de Leeuw, G., Martino, M., Woeltjen, J., and O'Dowd, C. D.: Primary submicron marine aerosol

602 dominated by insoluble organic colloids and aggregates, Geophys. Res. Lett., 35, L17814, doi:10.1029/2008gl034210,
603 2008b.

604 Fattori, I., Becagli, S., Bellandi, S., Castellano, E., Innocenti, M., Mannini, A., Severi, M., Vitale, V., and Udisti, R.:
605 Chemical composition and physical features of summer aerosol at Terra Nova Bay and Dome C, Antarctica, J. Environ.
606 Monitor., 7, 1265-1274, doi:10.1039/b507327h, 2005.

607 Fisher, M., Cox, J., Davis, D. J., Wagner, A., Taylor, R., Huerta, A., and Money, N. P.: New information on the
608 mecanism of forcible ascospore discharge from *Ascobolus immersus*, Fungal Genet. Biol., 41, 698-707, 2004.

609 Ge, X., Wexler, A. S., and Clegg, S. L.: Atmospheric amines - Part I. A review, Atmos. Environ., 45, 524-546,
610 doi:10.1016/j.atmosenv.2010.10.012, 2011.

611 Gorzelska, K., and Galloway, J. N.: Amine nitrogen in the atmospheric environment over the North Atlantic Ocean,
612 Global Biogeochem. Cy., 4, 309-333, 1990.

613 Grammatika, M., and Zimmerman, W. B.: Microhydrodynamics of flotation processes in the sea surface layer, Dynam.
614 Atmos. Oceans, 34, 327-348, doi:10.1016/s0377-0265(01)00073-2, 2001.

615 Handorf, D., Foken, T., Kottmeier C., The stable atmospheric boundary layer over an Antarctic ice Sheet. Boundary-
616 Layer Meteorology, 91, 165-189, doi:10.1023/A:1001889423449, 1999.

617 Jones, A. M., Harrison, R. M.: The effects of meteorological factors on atmopheric bioaerosol concentrations-a review,
618 Sci Total Environ., 326, 151-180, doi:10.1016/j.scitoenv.2003.11.021, 2004.

619 Jourdain, B., Preunkert, S., Cerri, O., Castebrunet, H., Udisti, R., and Legrand, M.: Year-round record of size-
620 segregated aerosol composition in central Antarctica (Concordia station): implications for the degree of fractionation of
621 sea-salt particles, J. Geophys. Res.-Atmos., 113, D14308, doi:10.1029/2007jd009584, 2008.

622 Keene, W. C., Maring, H., Maben, J. R., Kieber, D. J., Pszenny, A. A. P., Dahl, E. E., Izaguirre, M. A., Davis, A. J.,
623 Long, M. S., Zhou, X., Smoydzin, L., and Sander, R.: Chemical and physical characteristics of nascent aerosols
624 produced by bursting bubbles at a model air-sea interface, J. Geophys. Res.-Atmos., 112, D21202,
625 doi:10.1029/2007jd008464, 2007.

626 Knulst, J. C., Rosenberger, D., Thompson, B., and Paatero, J.: Intensive sea surface microlayer investigations of open
627 leads in the pack ice during Arctic Ocean 2001 expedition, Langmuir, 19, 10194-10199, doi:10.1021/la035069+, 2003.

628 Kuznetsova, M., Lee, C., Aller, J., and Frew, N.: Enrichment of amino acids in the sea surface microlayer at coastal and
629 open ocean sites in the North Atlantic Ocean, Limnol. Oceanogr., 49, 1605-1619, 2004.

630 Kuznetsova, M., Lee, C., and Aller, J.: Characterization of the proteinaceous matter in marine aerosols, Mar. Chem., 96,
631 doi:359-377, 10.1016/j.marchem.2005.03.007, 2005.

632 Leach, J., Blanch, A.C.: Volatile organic compounds in an urban airborne environment adjacent to a municipal
633 incinetor, waste collection centre and sewage treatment plant, Atmos. Environ., 33, 4309-4325, doi: 10.1016/S1352-
634 2310(99)00115-6, 1999.

635 Leck, C., and Bigg, E. K.: Source and evolution of the marine aerosol - A new perspective, Geophys. Res. Lett., 32,
636 L19803, doi:10.1029/2005gl023651, 2005.

637 Mace, K. A., Artaxo, P., and Duce, R. A.: Water-soluble organic nitrogen in Amazon Basin aerosols during the dry
638 (biomass burning) and wet seasons, J. Geophys. Res.-Atmos., 108, 4512, doi:10.1029/2003jd003557, 2003a.

639 Mace, K. A., Duce, R. A., and Tindale, N. W.: Organic nitrogen in rain and aerosol at Cape Grim, Tasmania, Australia,
640 J. Geophys. Res.-Atmos., 108, 4338, doi:10.1029/2002jd003051, 2003b.

641 Mandalakis, M., Apostolaki, M., and Stephanou, E. G.: Trace analysis of free and combined amino acids in atmospheric
642 aerosols by gas chromatography-mass spectrometry, J. Chromatogr. A, 1217, 143-150,
643 doi:10.1016/j.chroma.2009.11.021, 2010.

644 Mandalakis, M., Apostolaki, M., Tziaras, T., Polymenakou, P., and Stephanou, E. G.: Free and combined amino acids
645 in marine background atmospheric aerosols over the Eastern Mediterranean, Atmos. Environ., 45, 1003-1009,
646 doi:10.1016/j.atmosenv.2010.10.046, 2011.

647 Maria, S. F., Russell, L. M., Gilles, M. K., and Myneni, S. C. B.: Organic aerosol growth mechanisms and their climate-
648 forcing implications, Science, 306, 1921-1924, doi:10.1126/science.1103491, 2004.

649 Matsumoto, K., and Uematsu, M.: Free amino acids in marine aerosols over the western North Pacific Ocean, Atmos.
650 Environ., 39, 2163-2170, doi:10.1016/j.atmosenv.2004.12.022, 2005.

651 McCarthy, M. D., Hedges, J. I., Benner, R.: Major Bacterial Contribution to Marine Dissolved Organic Nitrogen,
652 Science, 281, 231-234, doi:10.1126/science.281.5374.231, 1998.

653 McGregor, K. G., and Anastasio, C.: Chemistry of fog waters in California's Central Valley: 2. Photochemical
654 transformations of amino acids and alkyl amines, Atmos. Environ., 35, 1091-1104, doi:10.1016/s1352-2310(00)00282-
655 x, 2001.

656 Meskhidze, N., and Nenes, A.: Phytoplankton and cloudiness in the Southern Ocean, Science, 314, 1419-1423,
657 doi:10.1126/science.1131779, 2006.

658 Milne, P. J., and Zika, R. G.: Amino-acid nitrogen in atmospheric aerosols -occurance, sources and photochemical
659 modification, J.Atmos. Chem., 16, 361-398, doi:10.1007/bf01032631, 1993.

660 Modini, R. L., Harris, B., and Ristovski, Z. D.: The organic fraction of bubble-generated, accumulation mode Sea Spray
661 Aerosol (SSA), Atmos. Chem. and Phys., 10, 2867-2877, 2010, http://www.atmos-chem-phys.net/10/2867/2010/.

662 Mopper, K., and Zika, R.G.: Free amino acids in marine rains: evidence for oxidation and potential role in nitrogen
663 cycling, Nature, 325, 246-249, doi:10.1038/325246a0, 1987.

664 Mukhin, C., Ilinuma, Y., Bondarev, V.B., Safonova, E. N.: The role of volcanic processes in the evolution of organic
665 compounds on the primitive earth, Modern Geology, 6, 119-122, 1978.

666 Müller, C., Iinuma, Y., Karstensen, J., van Pinxteren, D., Lehmann, S., Gnauk, T., and Herrmann, H.: Seasonal
667 variation of aliphatic amines in marine sub-micrometer particles at the Cape Verde islands, Atmos. Chem. Phys., 9,
668 9587-9597, doi:10.5194/acp-9-9587-2009, 2009.

669 Noziére, B., Dziedzic, P., and Còrdova, A.: Formation of secondary light-absorbing ‘‘fulvic-like’’ oligomers: A
670 common process in aqueous and ionic atmospheric particles?, J. Geophys. Res. Lett., 34, L21812,
671 doi:1029/2007GL031300, 2007.

672 Noziére, B., and Còrdova, A.: A Kinetic and Mechanistic Study of the Amino Acid Catalyzed Aldol Condensation of
673 Acetaldehyde in Aqueous and Salt Solutions, J. Phys. Chem., 112, 2827-2837, doi:10.1021/jp7096845, 2008.

674 O'Dowd, C. D., Facchini, M. C., Cavalli, F., Ceburnis, D., Mircea, M., Decesari, S., Fuzzi, S., Yoon, Y. J., and Putaud,
675 J. P.: Biogenically driven organic contribution to marine aerosol, Nature, 431, 676-680, doi:10.1038/nature02959, 2004.

676 O'Dowd, C. D., and De Leeuw, G.: Marine aerosol production: a review of the current knowledge, Philos. T. Roy. Soc.
677 A, 365, 1753-1774, doi:10.1098/rsta.2007.2043, 2007.

678 Petzold, A., and Karcher, B.: Atmospheric Physics - Aerosols in the Atmosphere, Springer-Verlag Berlin Heidelberg,
679 Germany, 2012.

680 Pommie, C., Levadoux, S., Sabatier, R., Lefranc, G., and Lefranc, M. P.: IMGT standardized criteria for statistical
681 analysis of immunoglobulin V-REGION amino acid properties, J. Mol.Recognit., 17, 17-32, doi:10.1002/jmr.647, 2004.

682 PNRA-ENEA Osservatorio meto-climatico: http://www.climantartide.it, last access: May 2014.

683 Rapporto sulla campagna Antartica, Estate Australe 2011-2012 - Ventisettesima Spedizione,
684 http://www.italiantartide.it/spedizioni/xxvii/documentazione/RapFin2012bis.pdf (last access: May 2014), 2012.

685 Rinaldi, M., Decesari, S., Finessi, E., Giulianelli, L., Carbone, C., Fuzzi, S., O'Dowd, C. D., Ceburnis, D., and Facchini,
686 M. C.: Primary and Secondary Organic Marine Aerosol and Oceanic Biological Activity: Recent Results and New
687 Perspectives for Future Studies, Adv. Meteorol., 2010, 310682, doi:10.1155/2010/310682, 2010.

688 Roiger, A., Huntrieser, H., and Schlager, H.: Long range trasport of air pollutans, in: Atmospheric Physics, edited by:
689 Schumann, U., Springer, London, 2012.

690 Saxena, P., and Hildemann, L. M.: Water-soluble organics in atmospheric particles: A critical review of the literature
691 and application of thermodynamics to identify candidate compounds, J. Atmos. Chem., 24, 57-109,
692 doi:10.1007/bf00053823, 1996.

693 Scalabrin, E., Zangrando, R., Barbaro, E., Kehrwald, N. M., Gabrieli, J., Barbante, C., and Gambaro, A.: Amino acids
694 in Arctic aerosols, Atmos. Chem. Phys., 12, 10453-10463, doi:10.5194/acp-12-10453-2012, 2012.

695 Scheller, E.: Amino acids in dew - origin and seasonal variation, Atmos. Environ., 35, 2179-2192, doi:10.1016/s1352-
696 2310(00)00477-5, 2001.

697 Spitzy, A.: Amino acids in marine aerosol and rain, in: Facets of modern Biogeochemistry, edited by: Ittekkot, V.;
698 Kempe, S.; Michaelis, W.; Spitzy, A., Springer, Berlin, 313-317, 1990.

699 Stohl, A., and Sodemann, H.: Characteristics of atmospheric transport into the Antarctic troposphere, J. Geophys. Res.-
700 Atmos., 115, D02305, doi:10.1029/2009jd012536, 2010.

701 Szyrmer, W., and Zawadzki, I.: Biogenic and anthropogenic sources of ice-forming nuclei: A review, B. Am. Meteorol.
702 Soc., 78, 209-228, doi:10.1175/1520-0477(1997)078<0209:baasoi>2.0.co;2, 1997.

703 Udisti, R., Dayan, U., Becagli, S., Busetto, M., Frosini, D., Legrand, M., Lucarelli, F., Preunkert, S., Severi, M.,
704 Traversi, R., and Vitale, V.: Sea spray aerosol in central Antarctica. Present atmospheric behaviour and implications for
705 paleoclimatic reconstructions, Atmos. Environ., 52, 109-120, doi:10.1016/j.atmosenv.2011.10.018, 2012.

706 Vignati, E., Facchini, M. C., Rinaldi, M., Scannell, C., Ceburnis, D., Sciare, J., Kanakidou, M., Myriokefalitakis, S.,
707 Dentener, F., and O'Dowd, C. D.: Global scale emission and distribution of sea-spray aerosol: Sea-salt and organic
708 enrichment, Atmos. Environ., 44, 670-677, doi:10.1016/j.atmosenv.2009.11.013, 2010.

709 Voet, D., Voet, J.G.: Biochemistry,edited by John Wiley and Sons, New York, 1999.

710 Wedyan, M. A., and Preston, M. R.: The coupling of surface seawater organic nitrogen and the marine aerosol as
711 inferred from enantiomer-specific amino acid analysis, Atmos. Environ., 42, 8698-8705,
712 doi:10.1016/j.atmosenv.2008.04.038, 2008.

713 Yoon, Y. J., Ceburnis, D., Cavalli, F., Jourdan, O., Putaud, J. P., Facchini, M. C., Decesari, S., Fuzzi, S., Sellegri, K.,
714 Jennings, S. G., and O'Dowd, C. D.: Seasonal characteristics of the physicochemical properties of North Atlantic
715 marine atmospheric aerosols, J. Geophys. Res.-Atmos., 112, D04206, doi:10.1029/2005jd007044, 2007.

716 Zangrando, R., Barbaro, E., Zennaro, P., Rossi, S., Kehrwald, N. M., Gabrieli, J., Barbante, C., and Gambaro, A.:
717 Molecular Markers of Biomass Burning in Arctic Aerosols, Environ. Sci. Technol., 47, 8565-8574,
718 doi:10.1021/es400125r, 2013.

719 Zhang, Q., and Anastasio, C.: Chemistry of fog waters in California's Central Valley - Part 3: Concentrations and
720 speciation of organic and inorganic nitrogen, Atmos. Environ., 35, 5629-5643, doi:10.1016/s1352-2310(01)00337-5,
721 2001.

722 Zhang, Q., and Anastasio, C.: Free and combined amino compounds in atmospheric fine particles (PM2.5) and fog
723 waters from Northern California, Atmos. Environ., 37, 2247-2258, doi:10.1016/s1352-2310(03)00127-4, 2003.

724 Zhang, Q., Anastasio, C., and Jimemez-Cruz, M.: Water-soluble organic nitrogen in atmospheric fine particles (PM2.5)
725 from northern California, J. Geophys. Res.-Atmos., 107, 4112, doi:10.1029/2001jd000870, 2002.

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729 **Figure captions**

730 Figure 1. The sampling sites: the Italian base “Mario Zucchelli Station” (MZS) (74° 42’S – 164°
731 06’ E), the Italian-French base “Concordia Station” (Dome C) (75° 06’ S – 123° 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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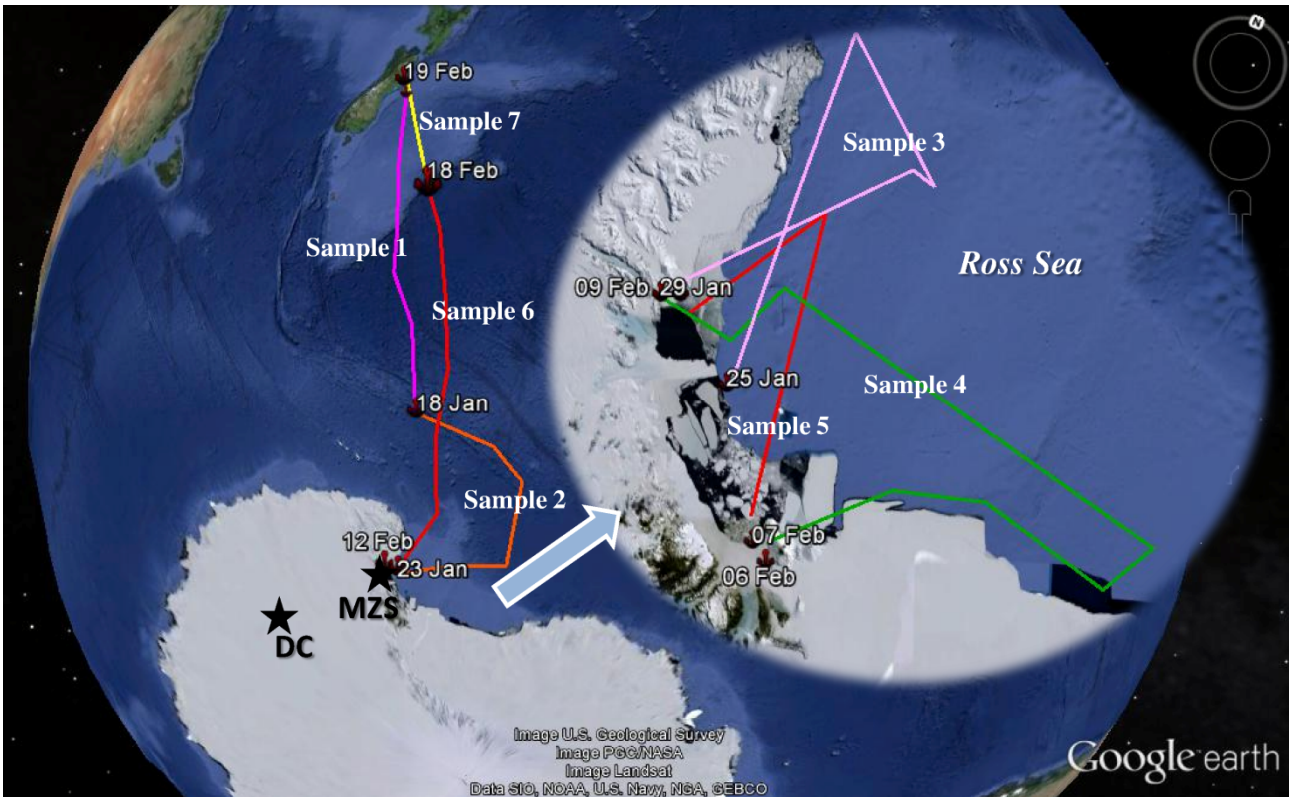

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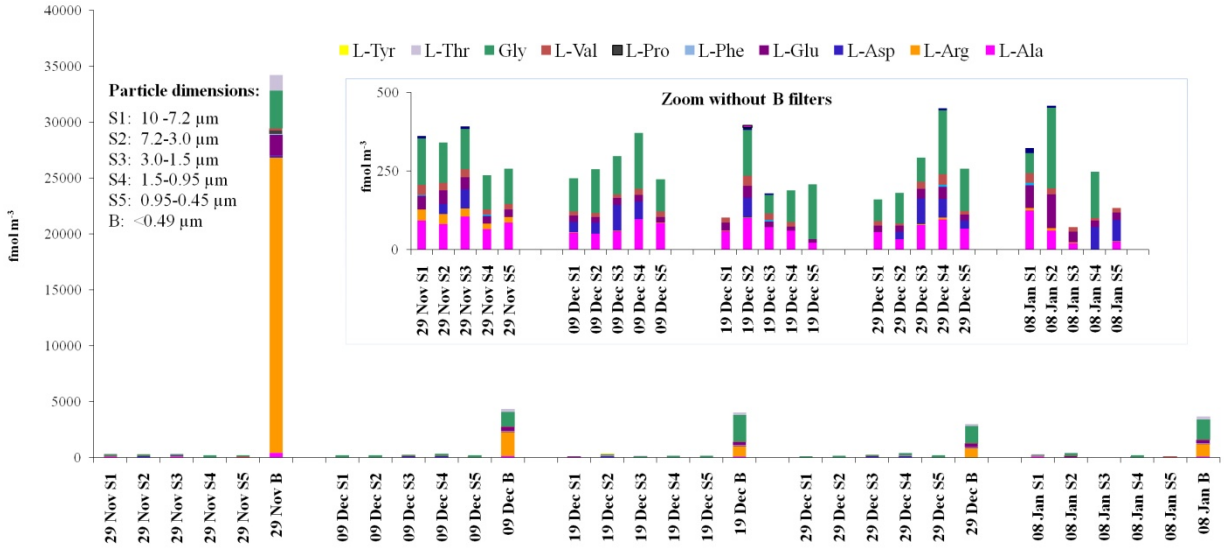

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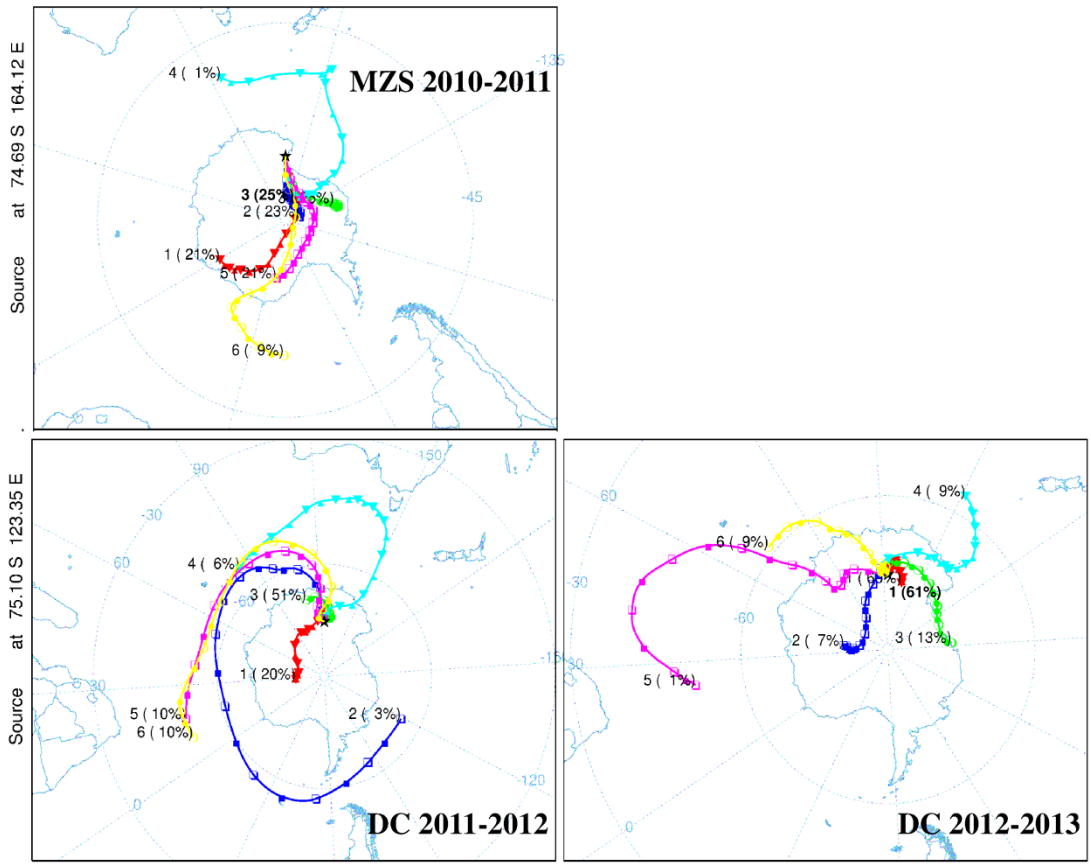

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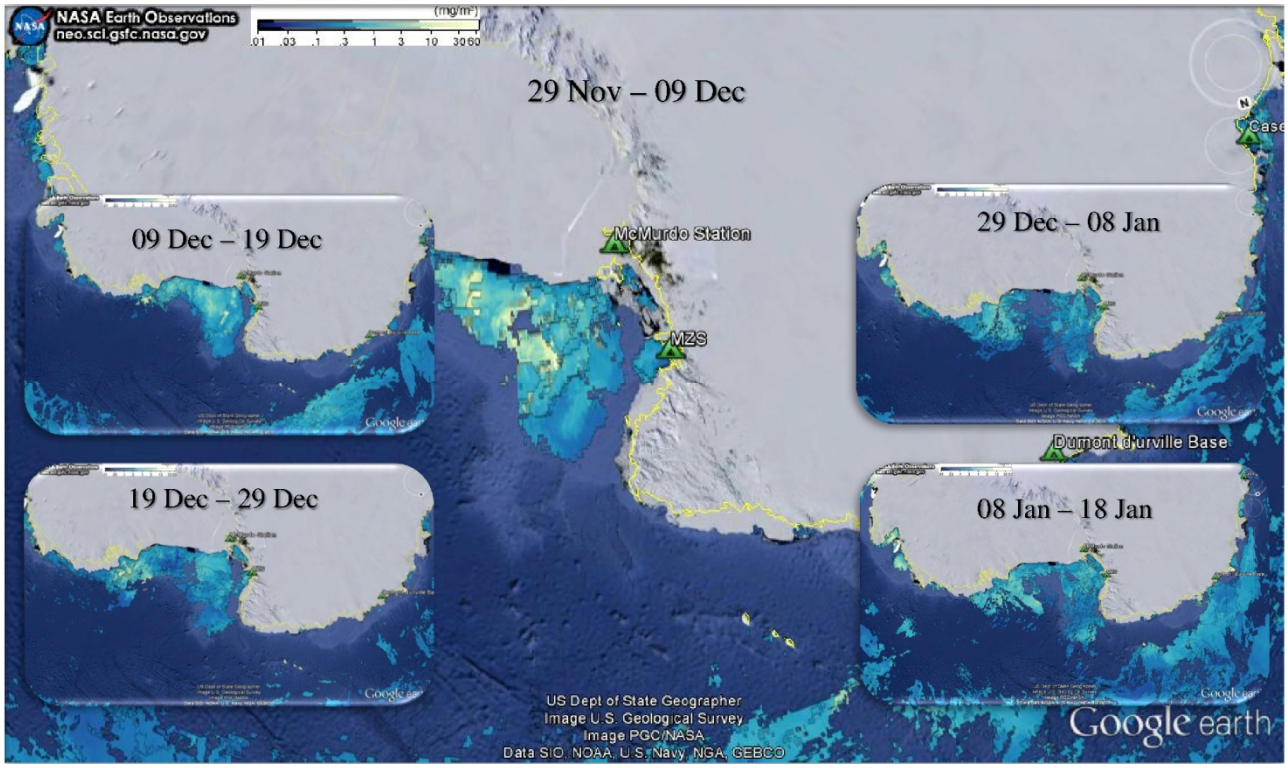

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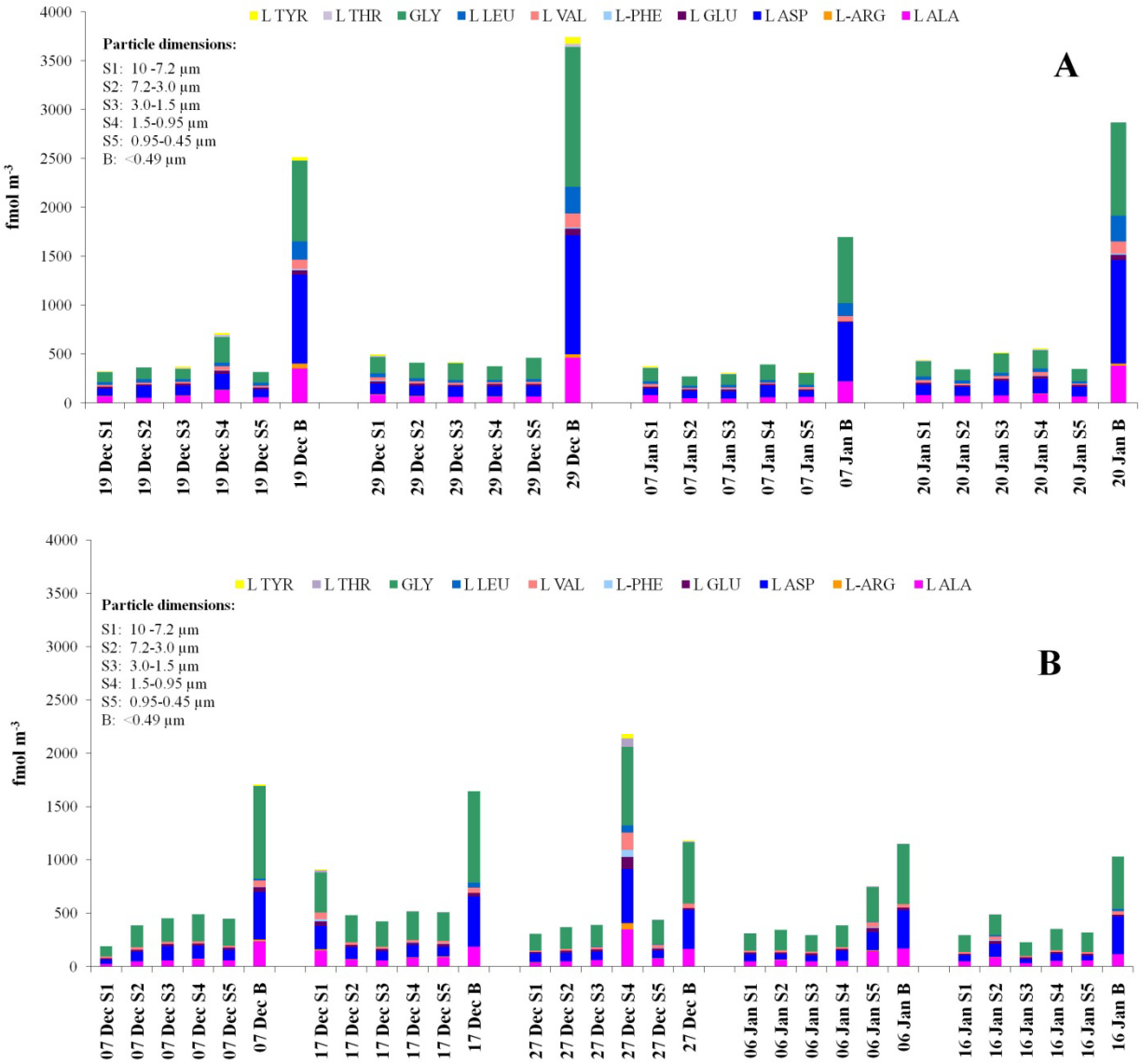

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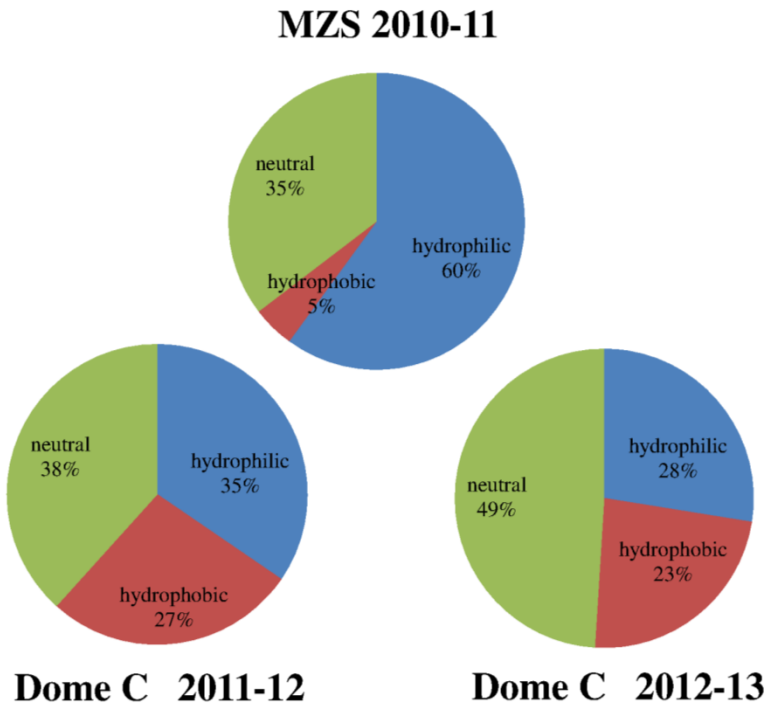

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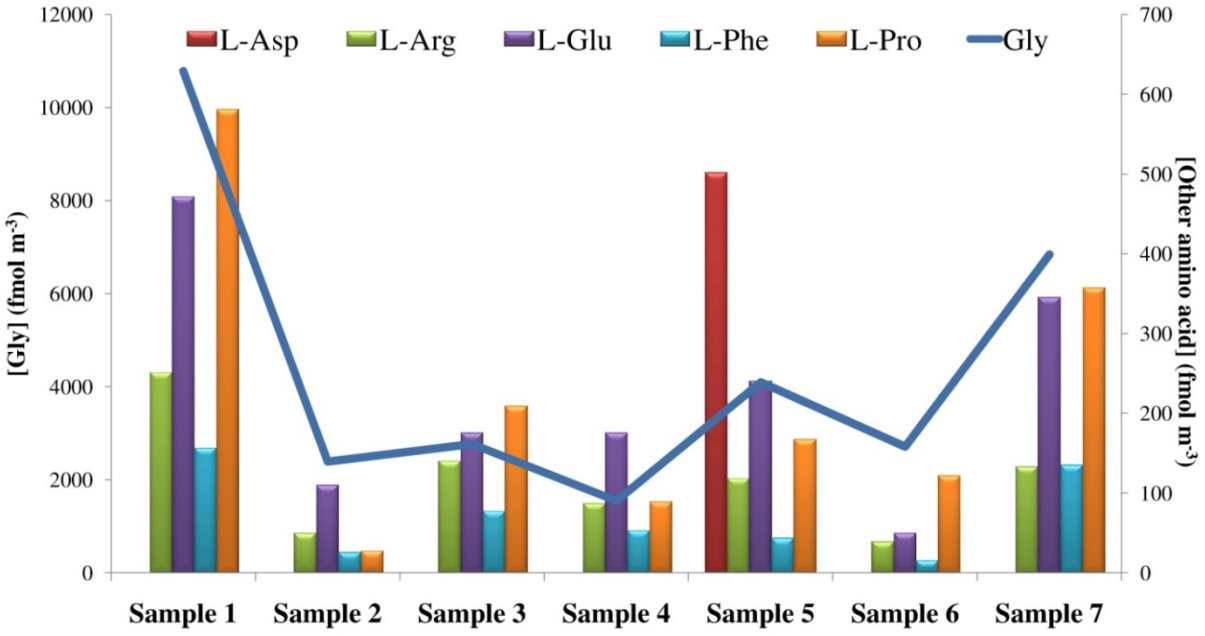

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