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

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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 have higher concentrations of amino acids compared to the coastal site. The amino acid composition in the aerosol collected at Dome C had also changed compared to the coastal site, 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 world-wide 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 extent 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). Recently Kristensoon et al., (2010) investigated the ability of someamino acids (e.g. glycine or leucine) to act as cloud condensation nuclei (CCN), they found that particles containing amino acids at "atmospherically relevant mixture ratios" are good CCN. These compounds can also serve as a source of nutrients for marine ecosystems due 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 concentrations of airborne amino acids, which may be related to aerosol growth in Antarctica

in some circumstances. 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, obtained using Google Earth maps.

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 circular quartz fiber filters (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 >1µm. 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 aswell 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

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

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

The larger high volume back-up filters were spiked with 400 µL of internal standard solution and were extracted with 25 mL then 5 mL of ultrapure water in an ultrasonic ice bath as described above.

2.3 Instrumental analysis

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

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

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

194 A binary gradient elution program was followed at a flow rate of 0.2 mL min^{-1}: 0-15 min, an
195 isocratic step with 30% of eluent B; 15-20 min, a gradient from 30 to 100% B; 20-25 min an
196 isocratic washing step with 100% of eluent B; 27-30 min, re-equilibration to30% eluent B. The
197 injection volume was 10 µL.
198 In this work the amino acids were quantified using the isotope dilution method where an
199 isotopically labeled standard was available. For other amino acids, where a labeled standard was
200 unavailable, an internal standard was used to quantify the analytes. A detailed description of which
201 analytes are quantified with which method can be found in Barbaro et al. (2014). In both cases, the
202 results were corrected for daily instrumental sensitivity variations by evaluating the instrumental
203 response factors.
204 Reagents and materials used for this study are reported in the Supplement.

205 **2.4 Quality control**

206 The entire analytical procedure was validated by estimation of trueness, repeatability and efficiency
207 (yield%) of the sample treatment process as described by Bliesner (2006). To ensure that it was fit
208 for purpose for the enantiomeric determination of amino acids in Antarctic aerosol, the validation
209 was carried out by spiking five cleaned quartz filters (for each type of filter) with 100 µL of a
210 solution containing all the native L and D amino acids (with concentrations ranging between 2 and
211 4 µg mL^{-1}) and 100 µL of a solution containing all the isotopically-labeled ^{13}C amino acids
212 (concentrations ranging between 2 and 3 µg mL^{-1}). The filters were subsequently extracted as
213 described above in section 2.2 “Sample processing”.
214 Tables S1, S2 and S3 report a summary of the yields, trueness and relative standard deviations
215 (n=5) for each type of filter used in this study. Average yields of 61%, 56% and 56% were obtained
216 from the circular, slotted and backup filters, respectively. In some cases, these values are lower than
217 those reported in the literature (Mandalakis et al., 2010; Barbaro et al., 2011).Trueness is the most
218 important parameter to determine during a method validation; it refers to the degree of closeness of

219 the determined value to the known "true" value. It is expressed as an error, calculated as (Q −T)/T
220 ×100, where Q is the determined value and T is the "true value".
221 For the circular filters, all D- and L-amino acids considered in this work were validated with an
222 error percentage ranging from -13% (D-Leu/D-Ile) to+8% (L-Tyr).
223 In the backup filters, only D- and L-Hys produced unacceptable percent errors,for this reason these
224 compounds were excluded from the quantification. The other amino acids considered in this study
225 were quantified with an accuracy ranging from-9% (D-Met) to+9% (D-Ala, L-Thr).
226 Some amino acids (D-Ala, L-Asn, D-Asn, D-Glu, D-Phe, L-Ser, D-Ser, and D-Val) were excluded
227 from the quantification using the slotted quartz fiber filters as very high percent errors were
228 calculated. We believe that this behavior is probably due to the different mode of use of this
229 sampling support: the slotted quartz fiber filters were used as impact supports while the other
230 supports were used as filters. The other amino acids studied in this work had percent error values
231 between -13% (D-Tyr) and +13% (D-Leu/D-Ile) and so the method was fit for purpose for their
232 quantification.
233 The repeatability is determined as the relative standard deviation of the analytical results for the 5
234 spiked filters. For each type of filter used in this study, the repeatability was always below 10%.
235 The method detection limit (MDL) for the analytical procedure is defined as three times the
236 standard deviation of the average values of the field blank (n=3). Tables S1, S2 and S3 report the
237 relative MDLs for each quantified amino acid in the three different sampling supports, the absolute
238 mean blank values (n=3) in these tables are subtracted from the analytical results. All discussions in
239 the following sections below are based upon blank corrected values.
240 A comparison between previously published data (Barbaro et al.,2011; Matsumoto and Uematsu,
241 2005) and the MDLs obtained for each type of filter in this work shows that we obtained lower
242 blank values than those previously reported.

243 **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 of 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 a121 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 aerosol samples above 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 aerosol 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 as18% 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 theAntarctic continent, areas that are characterized by alack 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 is consistent with 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

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

Fig. 5 presents the concentrations of free amino acids collected during both field campaigns, and shows a similarity between the trends and compositions of the analyzed compounds between the various size fractions. Ten amino acids (L-Ala, L-Arg, L-Asp, L-Glu, L-Leu, Gly, L-Phe, L-Thr, L-Tyr, L-Val) had concentrations above MDLs (Supplementary Tables S2 and S3) in all samples collected in both field campaigns. The concentrations of D-amino acids were always below MDLs, as seen in our coastal results. It was observed that Gly, L-Asp and L-Ala together accounted for about 80% of the total amino acid content. The total mean free amino acid concentrations, as the sum of the free amino acid concentrations in all the sample stages , were 0.8 pmol m^{-3} for the 2011-2012 campaign and 0.7 pmol m^{-3}for2012-2013 campaign (Fig. 5). To our knowledge, these mean concentrations areas are lower than those reported in the literature (Gorzelska and Galloway, 1990; Milne and Zika, 1993; Mace et al., 2003b; Kuznetsova et al., 2005; Matsumoto and Uematsu, 2005; Wedyan and Preston, 2008; Mandalakis et al., 2010; Barbaro et al., 2011; Mandalakis et al., 2011; Scalabrin et al., 2012), suggesting that this aerosol composition may describe the amino acid global background concentration .

In Fig. 5B, the sample collected from 27 December 2012 to 06 January 2013 shows an altered concentration profile, with the highest concentrations in one of the coarse fractions(S4 stage 1.5-0.95 µm). After evaluating the wind rose plots and activity at the base for each sample in the two summer campaigns, we believe that these samples were contaminated by human activity at Concordia station (Supplementary Fig. S7).

The meanconcentrations of free amino acids in the coarse aerosolparticles collected at DC for the two field campaigns were 407 and 421fmol m^{-3} (see Fig. 5)..At our coastal site, the mean free amino acids concentration in the coarse fraction was264 fmol m^{-3} (Fig. 2). At DC, the free amino acid concentration in the coarse aerosol, expressed as a fraction percent of the total free amino acids

concentration was found to be 13% in 2011-12 and 23% in the 2012-13 campaign. Conversely, during our 2010-2011 sampling campaign at MZS, which is located near the marine aerosol source, we found that only 2% of the total free amino acid concentration was present in the coarse fraction.

During the Antarctic summer, the surface inversion over the polar ice cap is relatively weak and aerosols produced on the ocean's surface can be transported through the upper troposphere to the Antarctic plateau where they are easily mixed down to the surface(Cunningham and Zoller, 1981). There are also transfer mechanisms from the lower stratosphere to the upper troposphere that occur near the coast of the Antarctic continent. Aerosol from different sources mixes into the upper troposphere, and this air descends uniformly over the Antarctic plateau due to surface cooling flows off the plateau causing the katabatic wind. This means that during the summer, there is a continuous flux of relatively clean air from the upper troposphere with aerosol from high altitude inputs and long range transport (Cunningham and Zoller, 1981;Stohl and Sodemann, 2010).

Cluster means backward trajectories analysis of all the samples collected during both summer campaigns at DC revealed a prominent marine source (Fig. 3). Fig. 3 shows that the 10-days backward trajectories came from the Southern Ocean where there are no land based man made influences.

Fig. 5 shows that the concentration of amino acids for the 2011-2012 summer Antarctic campaign was higher than the values reported for the 2012-2013 Antarctic campaign, and underlines that the main difference between the two campaigns is mainly in the percentages of amino acids in the coarse fraction. We suggest that the transport processes of the air masses were the main cause of these variations as the time spent inland by the air masses in the 2011-2012 summer was about 36 hours (Fig. 3) whilst in 2012-2013 the time range was between 4 and 7 days (Fig. 3).

The analysis of the size distribution of the free amino acids (Fig 5)combined with the air mass back trajectories (Fig. 3) allowed us to suggest that the amino acids in the aerosol collected at DC can have two possible sources. The first hypothesis is thatthey were present in primary emitted coarse

mode aerosol particles, which come from phytoplanktonic sea spray coarse mode particles (Matsumoto and Ueamatsu, 2005), or from soil dust coarse mode particles (Mace et al., 2003). Particles and their chemical constituents can travel for many weeks in the upper troposphere without being lost, provided they are not subject to wet deposition, or that the compounds are reacting in the aerosol phase. The second hypothesis is that amino acids hada marine source and these aerosols underwent several physico-chemical transformations during long-range transport. Our results suggest that amino acids were present in the fine particles over the surface of the Southern Ocean from bubble bursting processes. The air masses subsequently passed into the upper troposphere and then over the continent where they remained for several days before descending onto the ice sheet. These fine aerosol particles could either grow during long-range transport, due to condensation of molecules from the gas phase or by collision of small and large particles (coagulation) (Petzold and Karcher, 2012;Roiger et al., 2012). However, these processes are unlikely in Antarctica due to the very clean conditions. The most likely explanation is that the fine fraction has been subjected to other processes that increased the particle size of the aerosol. The most likely remaining process is ice nucleation during long-range transport promoted by the intense cold over the plateau and presence of amino acids in the aerosol particles (Szyrmer and Zawadzki, 1997). The specific reason for the increase of amino acids percentage in the coarse particles is not clear, based on the available data. In our future investigations, we will also evaluate the aerosol mass, which is probably a key parameter to measure that will help explain this increase of concentration in the coarse particles.

The chemical composition of aerosols may change during long-range transport due to photochemical, chemical and ionic reactions (Milne and Zika, 1993; Noziére and Còrdova, 2008; De Haan et al., 2009). Milne and Zika (1993)verified that amino acids are destroyed*via* reactions with photochemically formed oxidants such as hydroxyl radicals, to form products such as the ammonium ion, amides and keto-acids. However, in the upper atmosphere, the chemical processes

take place at slower rates than in the boundary layer (Roiger et al., 2012). In aqueous-phase aerosols, glyoxal can react with amino acids, leading to scavenging processes (De Haan et al., 2009). Recent studies on organic aerosol growth mechanisms (Maria et al., 2004) underlined that oxidation processes that remove hydrophobic organic compounds, are slower in large carbonaceous aerosols.

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

A comparison between the concentrations of hydrophobic Ala at the two sampling sites(MZS and DC) shows a very similar average concentration (70 fmol m^{-3}) in the coarse particles. This is an interesting behavior that confirms the hypothesis of limited atmospheric reactivity as proposed by Maria et al. (2004),who suggested a longer hydrophobic aerosol lifetime as a result of the slower oxidation rates. Thanks to this phenomenon, Ala significantly contributes to the amino acid content in these "remote aerosols" as it does not degrade during long range transport.

Fig. 6 shows that the main difference between the two campaigns is mainly in the percentage of hydrophilic and neutral amino acids present. A longer transportation time from the source to the sampling site would allow chemical transformation through photochemical reactions to take place, decreasing the concentration of hydrophilic amino acids thus modifying the composition so that the

448 more stable Gly (a neutral component) becomes the main compound (Fig. 6). In the 2012-2013
449 summer, the time spent inland by the air masses ranged from between 4 and 7 days whist in the
450 2011-2012 summer it was only 36 hours.
451 Looking at the acid-base proprieties of the amino acids, some differences can be observed between
452 two different types of aerosol. As described above, the predominant amino acid in the MZS aerosols
453 was Arg, which contributed considerably to the percentage of basic compounds (53%). The pH
454 neutral components represented an important percentage (40% and 68% for coastal and inland
455 aerosols respectively). Gly is mainly present in large quantities in these aerosols because of its very
456 low atmospheric reactivity (half life of 19 days) (McGregor and Anastasio, 2001) and its presence is
457 usually considered an indicator of long-range aerosol transport (Milne and Zika, 1993; Barbaro et
458 al., 2011). The acid compounds (Asp and Glu) contribution was quite different in the aerosols from
459 the two different stations: with a low percentage in the coastal samples at MZS (7%) that was in
460 contrast with the higher content in the aerosols from DC (33% and 26% respectively for the two
461 consecutive field campaigns). This result can be explained by a study conducted by Fattori et al.
462 (2005) on the DC aerosol, where high acid content was found. High concentrations of hydrochloric,
463 nitric and sulfuric acids were found in the aerosol fine fraction, promoting numerous series of acid-
464 base atmospheric reactions that neutralize the basic compounds. In the atmosphere, amino acids are
465 present in very low quantities so it is thought that they do not influence the pH of aerosols.
466 However, the pH of aerosols, can influence the chemical form of the amino acids present.

467 **3.3 Free amino acids during an oceanographic cruise**

468 Measurements of free amino acids were carried out on aerosol samples collected on the Southern
469 Ocean onboard the R/V Italica from 13th January to 19th February 2012. Aerosols were sampled
470 using a TSP sampler that collects particles with a diameter above 1 µm. The first and second
471 samples covered the track between New Zealand (from Lyttelton harbor) and MZS (Antarctica),
472 and the sixth and last samples were collected during the return journey between Antarctica and New

Zealand. Samples 3, 4 and 5 were collected on the Ross Sea near the Antarctic continent (Fig. 1). Five L-amino acids (L-Asp, L-Arg, L-Glu, L-Phe, L-Pro) and Gly were present in the samples, while other L- and D-amino acids had concentrations below MDLs (Supplementary Table S1). The total concentrations of free amino acids varied between 2 and 12 pmol m^{-3}.

The first and last samples had the highest concentrations of free amino acids (Fig. 7), and their relative sampling periods were characterized by temperatures ranging between -1°C and 18°C (sample 1), in contrast, temperatures during the remaining sampling periods were always below -1°C, with a lowest value of -8°C (sample 4). Higher temperatures can facilitate metabolic processes and accelerate atmospheric chemical reactions, as well as promote bubble bursting from the sea surface. This is probably the main source of amino acids in our on-ship samples. This is also supported by the back-trajectory analysis (Supplementary Fig. S8a-g), that demonstrate only a marine influence for that period. The concentration of amino acids was strongly influenced by sea conditions during sampling. The field report (Rapporto sulla campagna Antartica, 2012), noted that during navigation from New Zealand to the ice-pack region, the winds were always above 30 knots, with maximum values of 60 knots with wave heightsof12 meters. This probably explains the higher total concentration of free amino acids in the first two samples (12 pmol m^{-3}). Along the same track, but under calmer sea conditions (sample 7), we observed a slight reduction in the total concentration of free amino acids (8 pmol m^{-3}).These values were very similar to those reported by Matsumoto and Uematsu (2005) in the Pacific Ocean and to those reported by Gorzelska and Galloway (1990)and Wedyan and Preston (2008) in the Atlantic Ocean. The lowest concentrations were observed in samples 2 and 6, probably due to the fact that they were collected far from Oceania and from the Antarctic coast, in an area characterized by expansive pack ice and by temperatures below -1°C, where the bubble bursting process was reduced.

The samples collected near the Antarctic coast (samples 3,4 and 5) were the most interesting ones because the results could be compared with the amino acid values detected in the coastal station MZS. The mean total concentration in the samples collected on the Ross Sea was 3.5 pmol m^{-3},

about half of the values detected in our Southern Ocean samples. Such values are similar to the concentrations observed in the aerosols collected at MZS station (median 5 pmol m^{-3}). However, this is not a true comparison: for the sampling campaign at MZS, a cascade impactor was used to collect aerosol samples with a particle-size below 10 µm, whereas the data collected during the cruise was for aerosols with a particle diameter above 1 µm. However, if we exclude data from the back-up and the fifth slotted filters, the cascade sampler covers a particle size between 0.95 µm and 10 µm (stages 1 to 4), making a comparison between the two data sets more feasible. In the MZS aerosols, the median value of the amino acids concentration in the aerosols collected on stages 1 to 4 was 1 pmol m^{-3} and this concentration was lower than that measured in the cruise's aerosols (3.5 pmol m^{-3}). So we suspect that the aerosols with a diameter above 10 µm, that were collected with theTSP sampler but not the cascade impactor, could be the main source of the difference in amino acid concentration values in the samples collected on the R/V Italica.

The back-trajectory analysis (Supplementary Fig. S8C-E) demonstrated that the air masses came from inland Antarctica, where no vegetation is present. The biological material present in the atmosphere with a size > 10 µm includes pollens which typically vary between 17-58 µm, fungal spores between 1-30 µm, and algal spores between 15-120 µm. Instead bacteria have a diameter between 0.25-8 µm, and viruses have diameters that are typically less than 0.3 µm (Jones and Harrison, 2004). For this reason, we propose that the biological materials influenced the concentration of the total free amino acids in the shipboard aerosols.

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

524 Asp was detected in only one sample (sample 5), with a concentration of 502 fmol m^{-3}. This value is
525 very similar to those measured in the two field campaigns on the Antarctic plateau (DC),
526 considering only the slotted filter stages above 1 µm (446 e 382 fmol m^{-3} respectively for the l
527 summer field campaigns of 2011-12 and 2012-13). The back-trajectory analysis (Supplementary
528 Fig. S8E) demonstrated that this air mass came from the plateau, where aspartic acid was a
529 predominant component of the amino acid content.
530 In the aerosols collected during the cruise, the Arg concentration was very low because the
531 sampling conducted on board R/V Italica during the summer of 2012 excluded fine particles,
532 whereas Arg was one of the most abundant compounds observed in the coastal station found in the
533 fine fraction.
534

535 **4. Conclusions**

536 This first study on the size distribution of amino acids in Antarctica has identified possible sources
537 of marine aerosols in this region and has characterized some chemical and physical transformations
538 that take place during transport to the interior of the Antarctic continent.
539 Marine emissions of fine particles occurred *via* bubble bursting processes on the surface of the
540 Southern Ocean. The mean total amino acid concentration detected at MZS was 11 pmol m^{-3}, with a
541 higher percentage of amino acids found in the fine fraction. The aerosol samples collected at Dome
542 C had the lowest amino acid values (0.7 and 0.8 pmol m^{-3}) and the coarse particles were found to be
543 enriched with amino acids compared to the coastal site. Numerous chemical and photochemical
544 events may have contributed to a decrease in the concentration in amino acids in the fine fraction,
545 and the chemical reactions were faster for hydrophilic compounds than for hydrophobic ones, as
546 suggested by an observed Ala enrichment.
547 The presence of only the L-enantiomers of free amino acids in Antarctic aerosols suggests that
548 marine particles were the main sources of free amino acids in this area and that these compounds

549 can be modified when transported to the interior of the continent. Gly and Ala, are the most stable
550 compounds, and may be used as biogenic markers of long-range marine aerosols. The back-
551 trajectory analysis demonstrated that the differences in the transport time of air masses inside
552 Antarctica can result in modifications to the percentage of amino acids in the coarse particles.
553 The study of aerosols with diameters>10 µm indicated that bubble bursting processes can also emit
554 microorganisms that are composed of a higher number of neutral amino acids.
555

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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748 **Figure captions**

749 Figure 1. The sampling sites: the Italian base “Mario Zucchelli Station” (MZS) (74° 42’S – 164°
750 06’ E), the Italian-French base “Concordia Station” (Dome C) (75° 06’ S – 123° 20’ E) and the
751 track chart of the R/V Italica.

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

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

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

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

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

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

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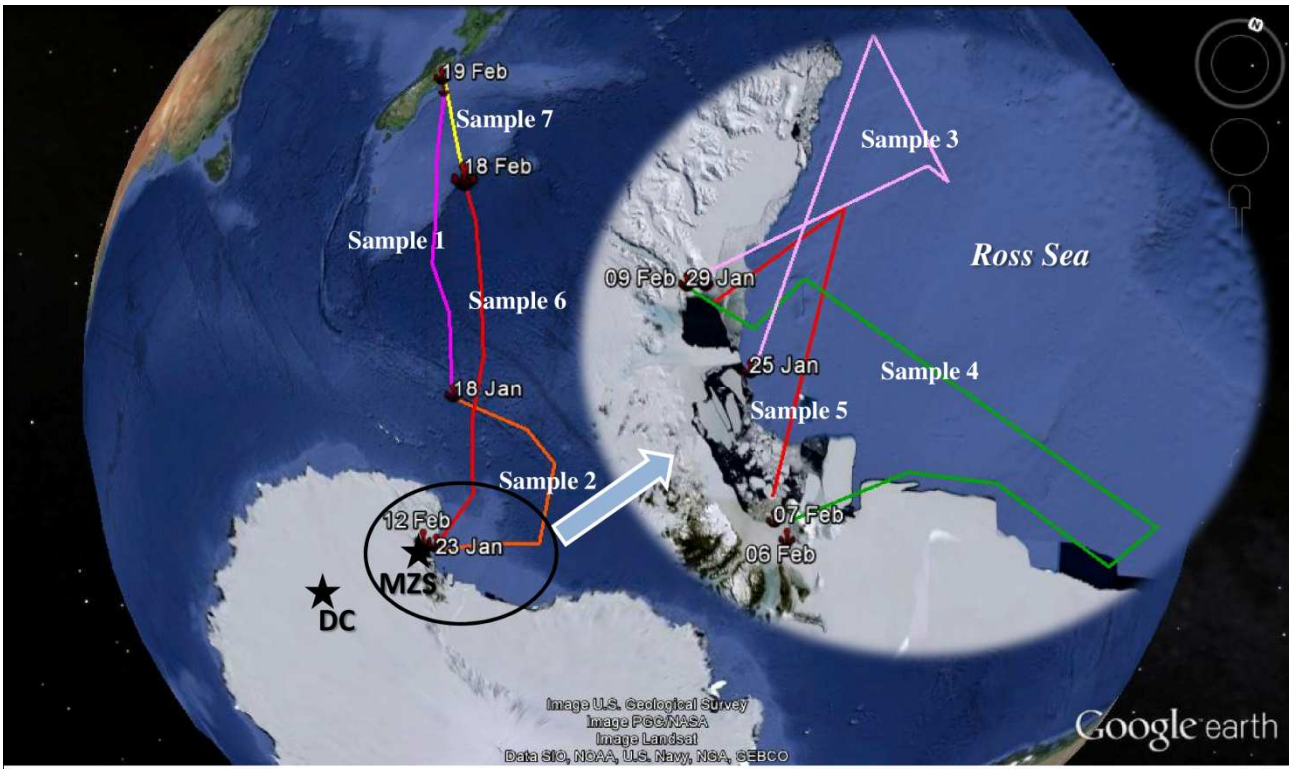

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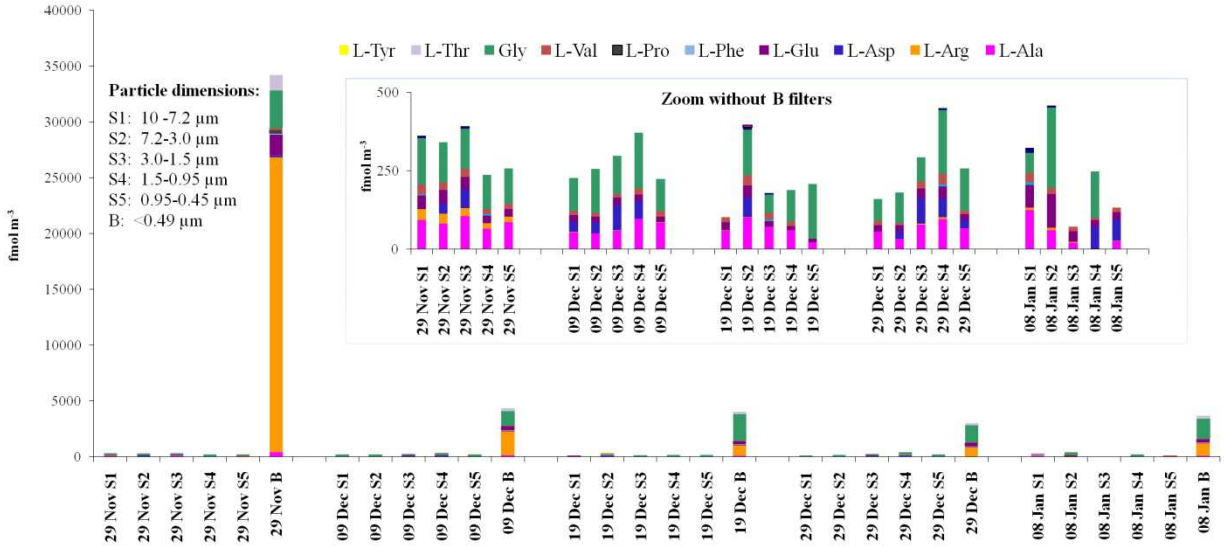

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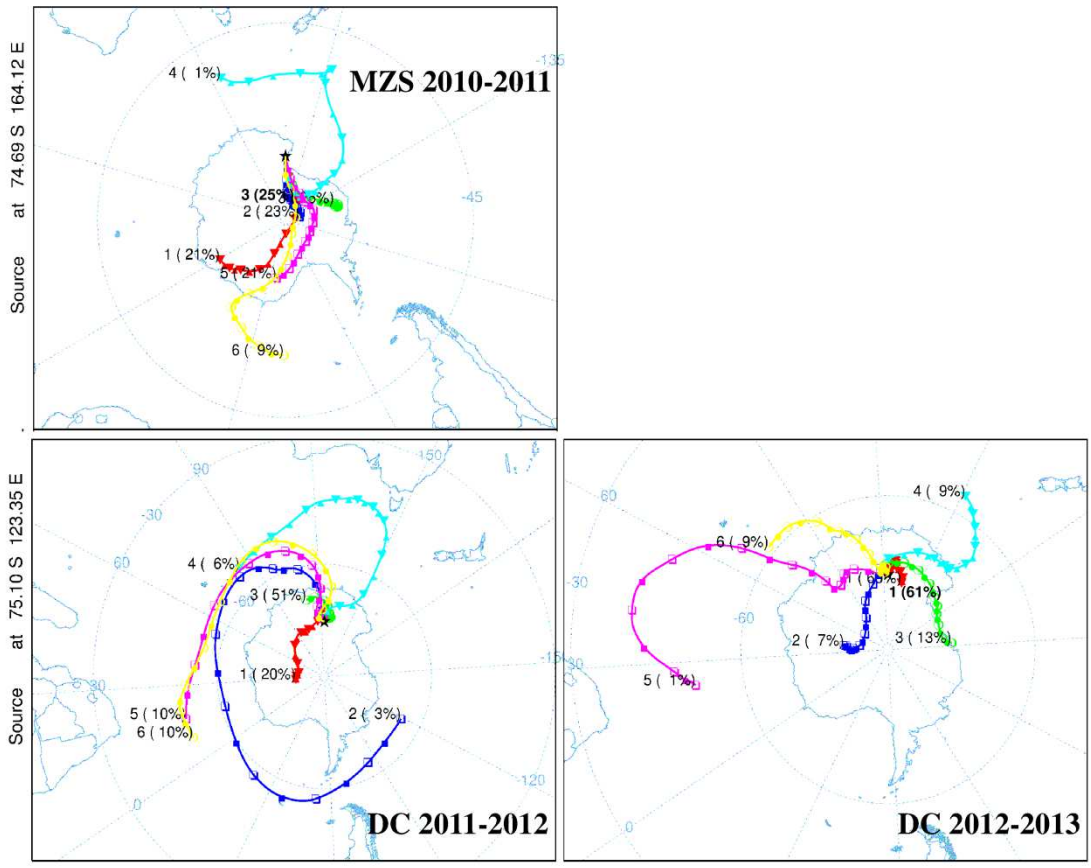

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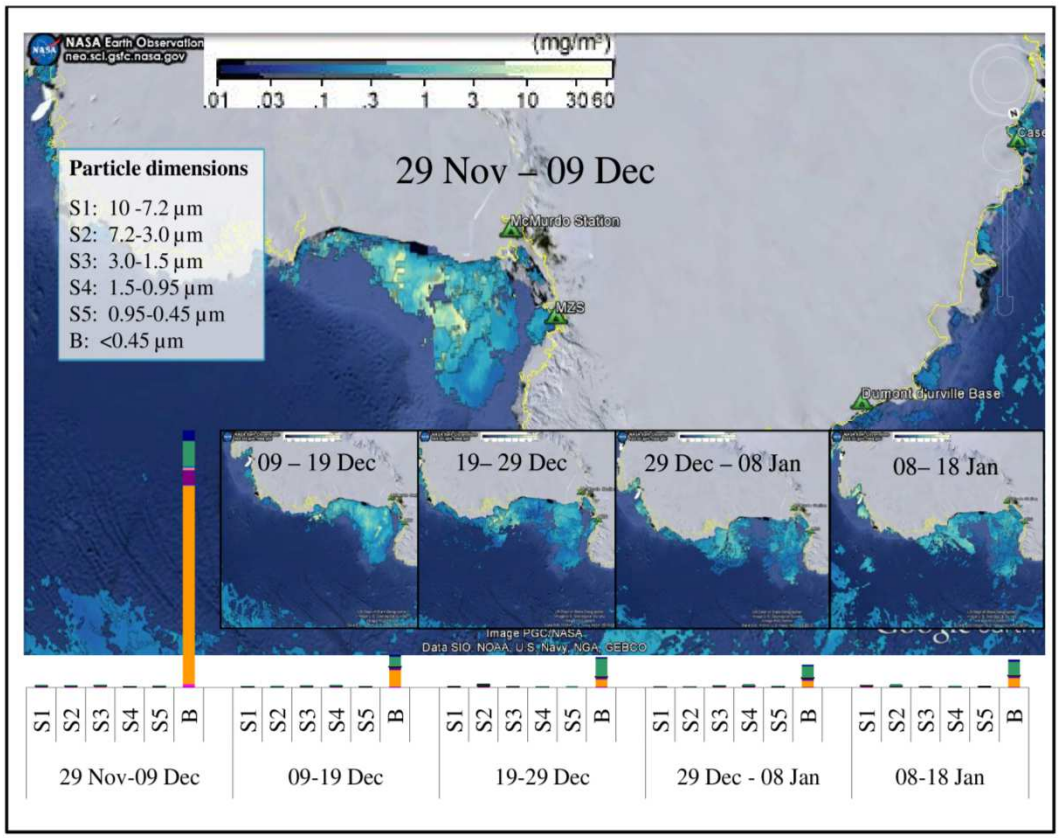

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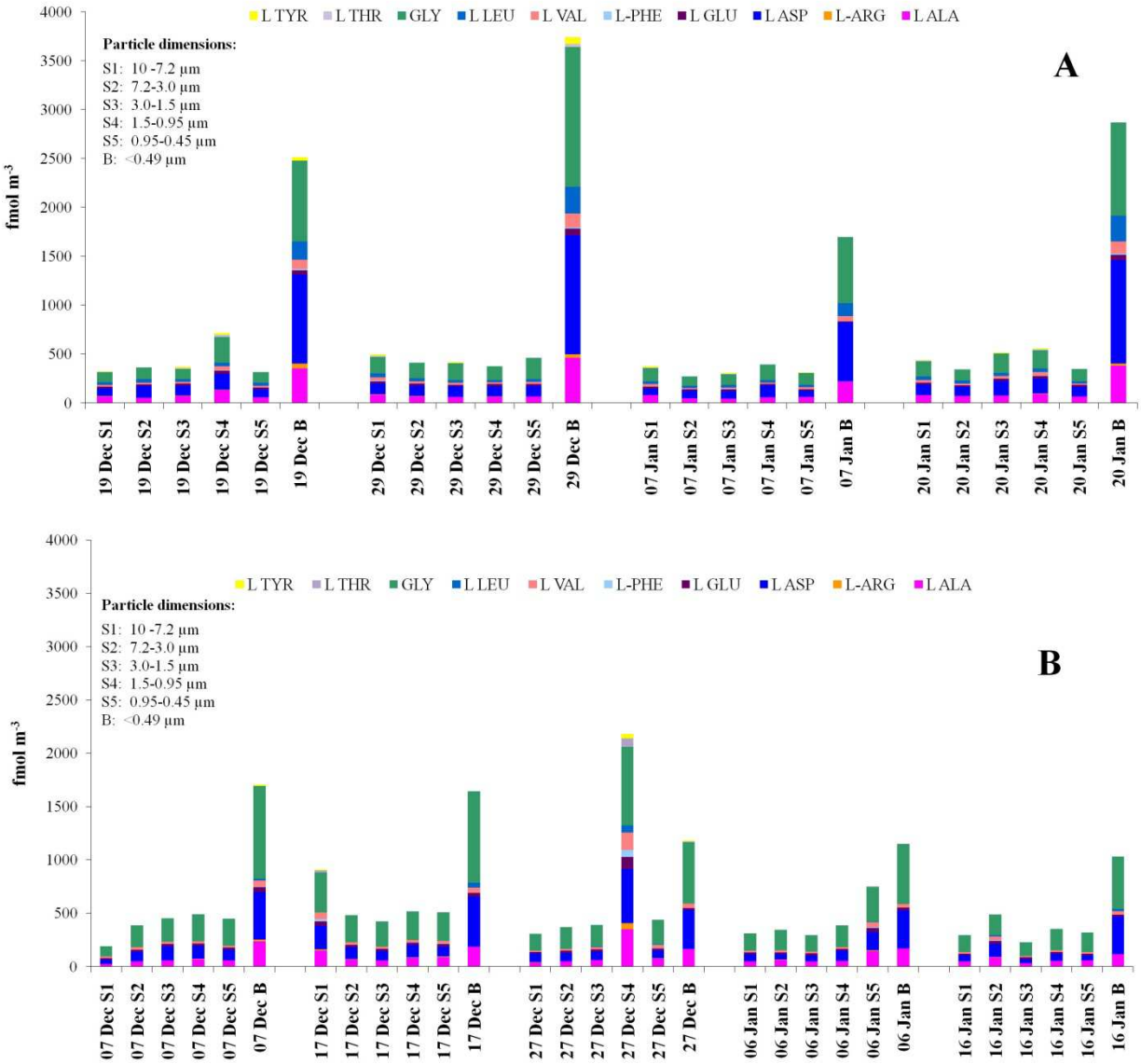

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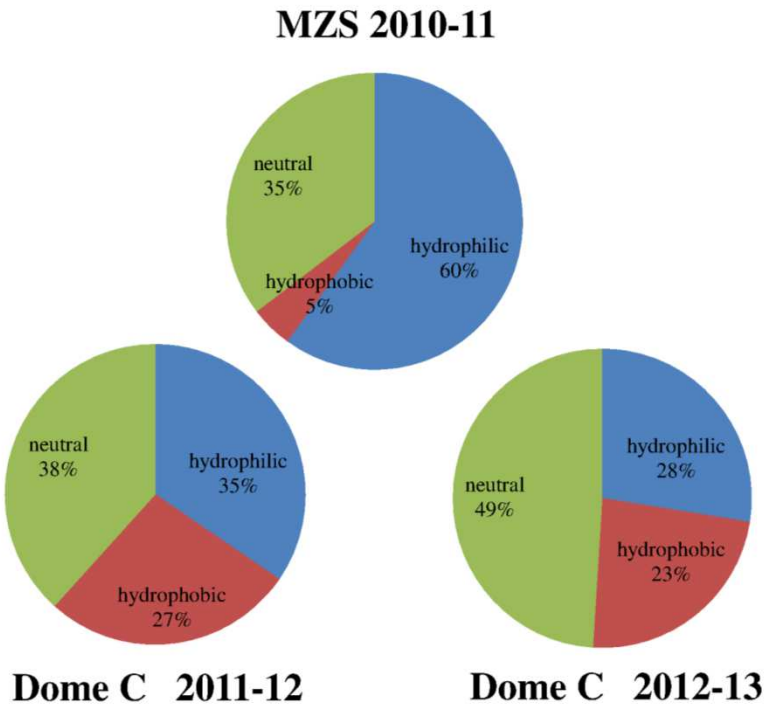

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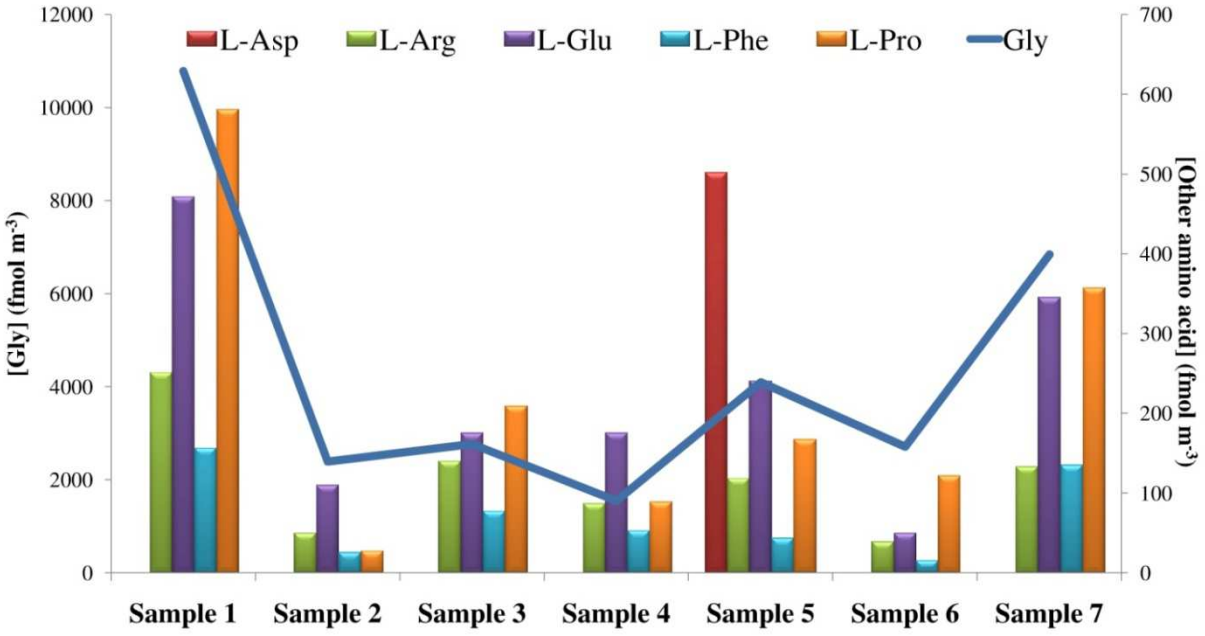

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