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Aerobiology over the Southern Ocean – implications for bacterial colonization of Antarctica

Lucie A. Malard[1]*, Maria-Luisa Avila-Jimenez[2], Julia Schmale[3], Lewis Cuthbertson[4], Luke Cockerton[4] & David A. Pearce [4,5]*

[1] Department of Ecology and Evolution, University of Lausanne, 1015 Lausanne, Switzerland.

[2] NatureMetrics, Surrey Research Park, Guildford, GU2 7HJ, United Kingdom.

[3] Extreme Environments Research Laboratory, École Polytechnique Fédérale de Lausanne, Sion, Switzerland.

[4] Faculty of Health and Life Sciences, Northumbria University, Newcastle-upon-Tyne NE1 8ST, United Kingdom.

[5] British Antarctic Survey, High Cross, Madingley Road, Cambridge CB3 OET, United Kingdom.

*Corresponding authors: david.pearce@northumbria.ac.uk and lucie.malard@unil.ch

Abstract

Parts of the Antarctic are experiencing dramatic ecosystem change due to rapid and record warming, which may weaken biogeographic boundaries and dispersal barriers, increasing the risks of biological invasions. In this study, we collected air samples from 100 locations around the Southern Ocean to analyze bacterial biodiversity in the circumpolar air around the Antarctic continent as understanding dispersal processes is paramount to assessing the risks of microbiological invasions. We also compared the Southern Ocean air bacterial biodiversity to other non-polar ecosystems to identify the potential origin of these Southern Ocean air microorganisms. The bacterial diversity in the air had both local and global origins and presented low richness overall but high heterogeneity, compatible with a scenario whereby samples are composed of a suite of different species in very low relative abundances. Only 4% of Amplicon Sequence Variants (ASVs) were identified in both polar and non-polar air masses, suggesting that the polar air mass over the Southern Ocean can act as a selective dispersal filter. Furthermore, both microbial diversity and community structure both varied significantly with meteorological data, suggesting that regional bacterial biodiversity could be sensitive to changes in weather conditions, potentially altering the existing pattern of microbial deposition in the Antarctic.

Keywords

Antarctica, Aerobiology, Dispersal, Bacteria, Biodiversity, Invasion, Climate Change

Introduction

Parts of Antarctica are warming at record rates, affecting both ecosystems and associated biota and processes (Convey & Peck, 2019, Pörtner et al., 2019, Clem et al., 2020). The resulting changes are difficult to predict as the impact of climate change on these local communities and their ecosystem functions are still not well defined. It has been predicted that anthropogenic activities and climate change will weaken both biogeographic boundaries and dispersal barriers of terrestrial and marine ecosystems, increasing the risks of biological invasions (Convey & Peck, 2019). Although humans participate in the dispersal of non-indigenous microorganisms, the impact of anthropogenic activities is still limited in Antarctica (Cowan et al., 2011) and aerial transport remains the primary source of new biological input such as moss and lichen spores, near-microscopic fauna and microorganisms (Marshall et al., 1996; Smith et al., 2013, Barberán et al., 2014, Barberán et al., 2015, Smets et al., 2016, Maki et al., 2019). However, the contribution of aerial dispersal in shaping the overall pattern of biodiversity and ecosystem function remains poorly understood.

For Antarctic invasions (and ultimately colonisation), access might represent a particular challenge as the continent is both geographically remote and air and water currents in the region form a potential dispersal barrier around the continent. Indeed, it has been suggested that the Antarctic Convergence zone within the Southern Ocean, and the resulting Antarctic circumpolar vortex, as well as the 'cyclone belt' surrounding Antarctica (with commonly four or five distinct cyclones effectively 'following' each other around the continent) can act as a dispersal barrier for airborne organisms (Pearce et al., 2009, Womack et al., 2010, Archer et al., 2019, King-Miaow et al., 2019, Uetake et al., 2020). Whether that is the case and the implication of a changing climate on this potential dispersal barrier are yet to be determined (Womack et al., 2010). It is also worthy of note that the large-scale atmospheric patterns around the continent are from west to east. As a result, airborne organisms may circulate around the continent several times before moving further south.

For decades, the focus has been on macro-organisms (Lonsdale, 1999) with only recent interest for microbial invasions (Mallon *et al.*, 2015, Kinnunen *et al.*, 2016, Malard & Pearce, 2022). This is largely because for a long time, 'everything is everywhere, but the environment selects'

(Bass Becking, 1934) led to the assumption that microorganisms lack the biogeographic patterns necessary for differential distribution. Only the development of high throughput sequencing has been able to provide overwhelming evidence that microorganisms do display specific biogeographic distribution patterns (Foulds et al., 2014, Bahram et al., 2018, Delgado-Baquerizo *et al.*, 2018) suggesting the potential for dispersal limitation and that microbial invasions are likely to be ecologically important, impacting the diversity and function of resident communities.

Whether microbial biogeography in the atmosphere exists at all is still open to question and requires further research. Indeed, little attention has been given to microbial diversity patterns in the atmosphere as the environment has traditionally been regarded as a conduit rather than a habitat (Wommack et al. 2010). Recent studies have begun addressing these issues in the atmosphere, showing for example that marine bioaerosol communities can be distinct from those found in adjacent terrestrial locations (Siefried et al. 2015). Overall, studies in other regions have shown that patterns of microbial dispersal are predominantly local (Herbold et al., 2014) interspersed with sporadic long-range events (Smith et al., 2013, Barberán et al., 2014, Barberán et al., 2015, Crawford et al., 2017, Maki et al., 2019). If the aerial microbiology around the Antarctic follows this pattern, it will have very important implications in terms of conservation and the maintenance of the microbial biodiversity in the region. Indeed, lower environmental filtering due to the weakening of dispersal barriers induced by climate change may support the transport and establishment of cosmopolitan taxa, capable of long-distance dispersal, over more specialized and endemic taxa and may support the invasion of non-indigenous taxa to the Antarctic. Therefore, identifying the origin of incoming airborne microbes is essential to predict the potential impact of their integration into local ecosystems.

Although it is now well established that microorganisms spread through the air, the process is limited by survival (Cowan et al., 2011), which is likely to have represented a significant natural barrier against this type of invasions in the past. However, whether microbial survival in air is to change, or can change following rapid climate warming, remains to be determined. Microorganisms are known to be metabolically active in the atmosphere (Tignat-Perrier et al., 2020). They participate in cloud formation, impacting precipitation patterns through ice nucleation (Fröhlich-Nowoisky et al., 2016, Šantl-Temkiv et al., 2019); therefore, airborne microorganisms have influence on cloud formation, radiation and precipitation (Sato & Inoue, 2021). The changing climate leads to changes in the frequency, intensity, spatial extent, duration, and timing of extreme weather and climate events in general (Seneviratne et al., 2021) and in particular also over the Southern Ocean (Meucci et al., 2020, Hepworth et al., 2022). Moreover, persistent features that

strongly influence the atmospheric dynamics of the Southern Ocean change with a changing climate, such as the El Niño Southern Oscillation (Cai et al., 2022) and intensification and latitudinal shift of the westerly winds (Perren et al., 2020, Liang et al., 2021). Thus, understanding the direct link between climatological conditions and ecological dispersal is essential to determine the rate of climate-driven ecological change worldwide and for the Antarctic, over the Southern Ocean. Studies have already suggested that temperature, UV radiation, humidity and weather-related factors may impact atmospheric diversity elsewhere (Šantl-Temkiv et al., 2018, Tignat-Perrier et al., 2019) while others have found little to no correlation (Uetake et al., 2020), illustrating the need for more global and standardised research in the field.

To answer these questions, the first step is to investigate the biodiversity of the atmosphere, the factors that influence this aerial diversity and to identify potential dispersal barriers. To date, the overwhelming picture emerging from the air sampled across the Antarctic is one of relatively low biomass but high diversity across all samples (Busse et al., 2003, Pearce et al., 2009, Van Houdt et al., 2009, Bottos et al., 2014, Herbold et al., 2014, Archer et al., 2019). However, the drivers of this diversity and its origins remain elusive.

In this study, we collected air samples from 100 locations around the Southern Ocean and its islands as well as seven concurrent precipitation samples. This included unique samples from marine areas that are normally inaccessible as well as the sub- and peri-Antarctic islands. Focusing on bacterial communities, we used this data to make two core comparisons: a) Southern Ocean samples derived in this study with existing published sequences derived from different habitats around the globe, and b) sequences obtained in this study combined to air sequences from other studies and separated North and South of the Antarctic Convergence zone. The primary aim of this study was to test the hypothesis that the Antarctic Convergence zone acts as a dispersal barrier, limiting the risk of microbiological invasions to continental Antarctica.

Results and Discussion

1. Which bacterial taxa dominate Southern Ocean Air?

Southern Ocean air samples were collected between December 2016, and March 2017, whilst the R/V Akademik Tryoshnikov circumnavigated the Antarctic continent on the Antarctic Circumpolar Expedition (ACE) (Landwehr et al., 2021) [Figure 1, Supplementary Data 1]. A total of 107 samples (± 19032 reads/sample) with 1013 assigned amplicon sequence variants were identified in Antarctic air and precipitation samples. Overall, Proteobacteria, Firmicutes,

Bacteroidetes and Actinobacteria were the most abundant phyla across all samples [Fig. 1]. These phyla are consistent with other studies of global aerobiological biodiversity (DeLeon-Rodriguez *et al.*, 2013, Smith *et al.*, 2013, Cuthbertson *et al.*, 2017, Mayol *et al.*, 2017, Els *et al.*, 2019, Tignat-Perrier *et al.*, 2019, Tignat-Perrier *et al.*, 2020, Lang-Yona *et al.*, 2022), including Antarctica (Archer *et al.*, 2019, Uetake *et al.*, 2020). While these four phyla have been shown to dominate the atmospheric diversity globally, many others have been identified, and across this study twenty-seven bacterial phyla were identified [Supplementary Data 2]. These included, for example, Nitrospirota, Verrucomicrobia, Plantomycetes and Acidobacteria, which are important members of most airborne communities (Bowers *et al.*, 2009, Bowers *et al.*, 2011, Šantl-Temkiv *et al.*, 2018, Els *et al.*, 2019), including in Antarctica (Bottos *et al.*, 2014, Uetake *et al.*, 2020). The presence of these phyla may also indicate the importance of the contribution from aquatic or soil bacterial sources (DeLeon-Rodriguez *et al.*, 2013, Uetake *et al.*, 2020).

We also identified a total of 378 genera [Supplementary Data 2], notably *Enhydrobacter* (Gammaproteobacteria, relative abundance: 4.8%), *Psychrobacter* (Gammaproteobacteria, relative abundance: 3.7%), *Staphylococcus* (Firmicutes, relative abundance: 1.2%), *Mesorhizobium* (Alphaproteobacteria, relative abundance: 1.2%) and *Acinetobacter* (Gammaproteobacteria, relative abundance: 0.9%). These have all been identified in Antarctic air samples (Archer *et al.*, 2019) and elsewhere (Els *et al.*, 2019, Archer *et al.*, 2020, Tignat-Perrier *et al.*, 2020). While the relative abundance of each genus is variable, consistent genera are identified in air samples globally. For example, *Enhydrobacter* has recently been identified in the air over the Atlantic and Pacific Oceans but not in the associated oceanic waters. *Enhydrobacter* has been proposed for a reclassification to the family Rhodospirillaceae (purple non-sulfur bacteria, in the Alphaproteobacteria), a family of phototrophic organisms. Lang-Yona *et al.* (2022) also found that *Massilia*, *Acinetobacter* and *Mesorhizobium* were associated with oceanic air communities. Across all aerobiology studies to date, it appears that there are consistent dominant phyla and genera, suggesting that there may be a common aerial microbiome, but that abundance and diversity may vary significantly by region (Archer *et al.*, 2021 Preprint).

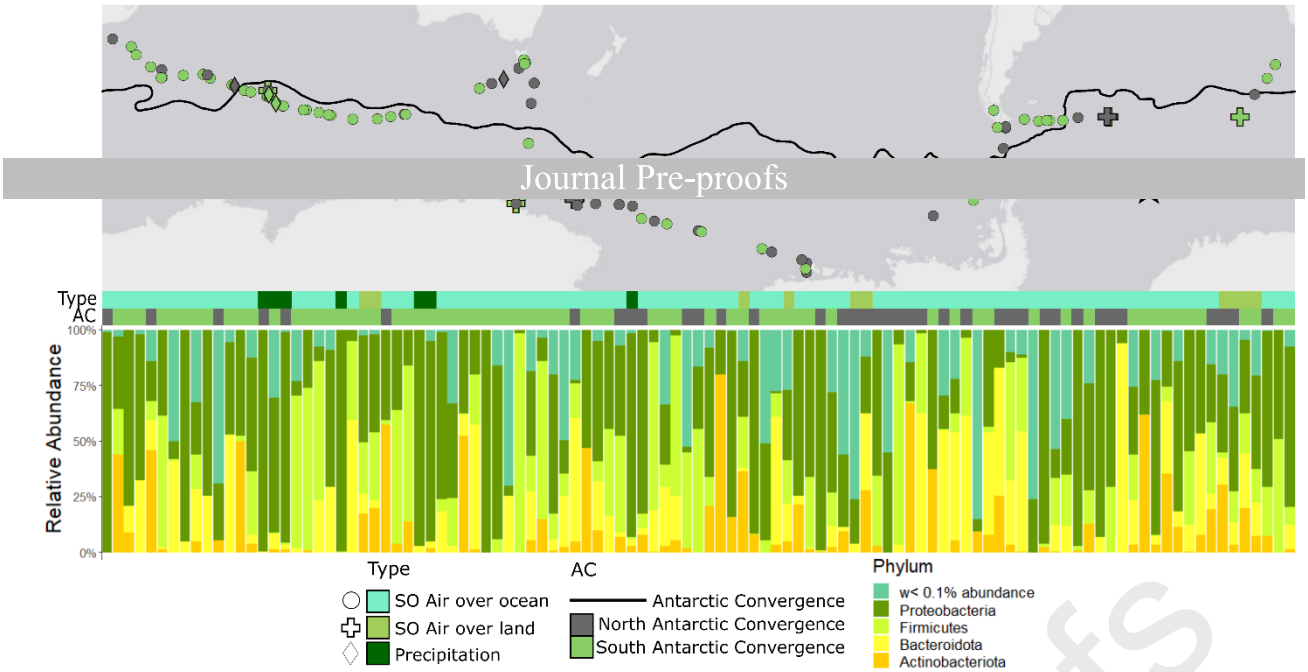

Figure 1: Map of the air sampling locations, shaped by sample type and coloured by the origin of the air mass (back trajectory) relative to the Antarctic Convergence. The relative abundance of bacterial taxa at the phylum level in the order of sampling. The microbial taxa dominating Southern Ocean (SO) air are highly variable with geographic location, even for consecutive samples and at the Phylum level.

2. How does the Southern Ocean air biodiversity compare globally with other ecosystems?

a. Alpha diversity

We compared the alpha diversity of the Southern Ocean air (SO air) and precipitation samples to published sequences from air sampled from other locations (referred to as 'global air') and both marine and terrestrial (ie. non-air) ecosystems [Fig. 2, Fig. S1]. Overall, the richness and diversity were much lower in all air samples (mean air richness/diversity: 27.5-30.6/1.83-2.57) when compared to soil and marine ecosystems (mean global soil richness/diversity: 560/5.5, mean open ocean richness/diversity: 233/3.58). The Southern Ocean air diversity also showed a higher heterogeneity (mean SO air Simpson index: 0.70) [Table S1] than other ecosystems. Globally, we found that soil microbial richness and diversity tended to a maximum in temperate regions,

confirming a previously observed pattern (Bahram *et al.*, 2018). Marine microbial biodiversity tended to increase towards the poles, also a pattern that had previously been observed (Ladau *et al.*, 2013). However, while air-borne microbial richness was relatively stable with latitude across the globe, diversity decreased significantly with increasing latitude [Fig. 3]. Interestingly, Bottos et al. (2020) demonstrated a decrease in alpha diversity of airborne bacteria communities with increasing latitude, from Australia to the Antarctic continent. We should note that the Arctic is not represented in this comparison due to a lack of data availability from that region, and, as such, high latitude air samples correspond only to the Southern Ocean air samples collected in our study. Whether this pattern holds for the Arctic region is still to be determined. Overall, the decrease in diversity at high latitudes was driven by the high unevenness of the airborne communities [Fig. 3]. Indeed, the Simpson index indicated that some communities were very uneven, especially at the highest latitudes, with one or few species largely dominating the communities [Fig. 3]. This variability of SO air communities was also reflected in the community composition [Fig. 1, Fig. 4A] and is in line with other highly variable airborne communities elsewhere (Lang-Yona *et al.*, 2022)

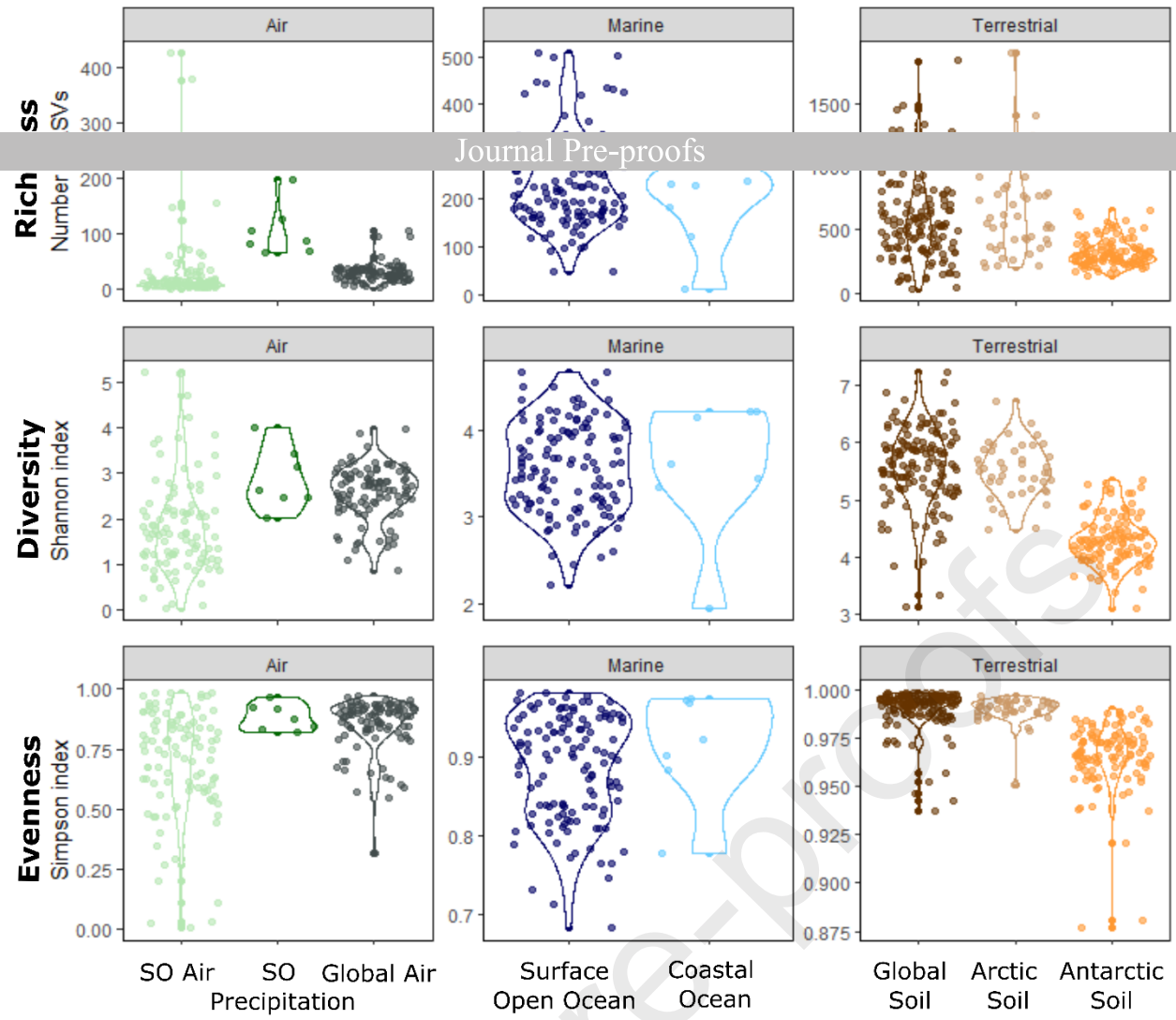

Figure 2. Comparison of the alpha diversity of Southern Ocean air (SO Air, n=100) and precipitation (SO Precipitation, n=7) to other global air samples (global air, n=98) and other non-air ecosystems including surface open ocean (n=123), surface coastal ocean (n= 7), global soils (n=146), Arctic soils (n=43) and Antarctic soils (n=113)

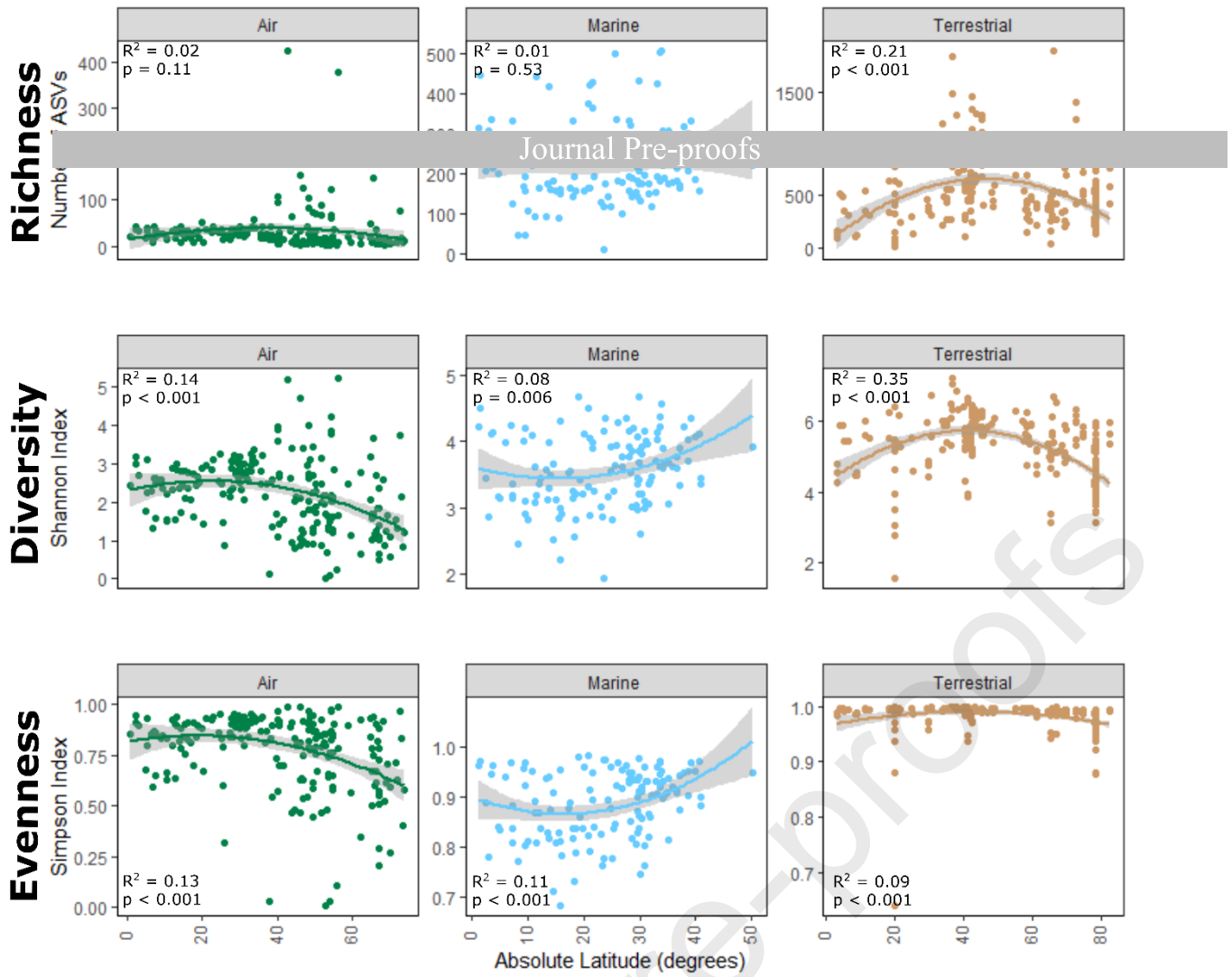

Figure 3: Latitudinal distribution of the richness and diversity of air (n = 198), marine (n = 130) and soil (n = 302) bacterial communities. The absolute latitude was used, merging northern and southern latitudes. Second order polynomials fits are shown with R^2 and associated p-values.

b. Beta diversity

We compared the beta diversity of global bacterial communities using non-metric multidimensional scaling (NMDS) to view differences in community composition between sample types [Fig. 4A]. The Antarctic soil communities were different from other soil communities and presented lower richness and diversity than other soil samples [Fig. 2]. This was further observed at the phylum level, with Antarctic soils harbouring more Actinobacteria and Bacteroidetes [Fig. 4B]. Surface open ocean marine communities clustered away from coastal ocean marine communities, primarily due to the high relative abundance of Cyanobacteria, which represented

over 50 % of the surface ocean communities. Global air communities clustered away from Southern Ocean air communities, with Proteobacteria representing 75 % of the global air communities. Utake et al. (2020) looking at global patterns of bacterial diversity in the air found a latitudinal diffe[illegible] air community samples while Southern Ocean air communities harboured more Firmicutes and Bacteroidetes, known to form endospores protecting the genetic material of the bacteria (Martiny *et al.*, 2006, Filippidou *et al.*, 2016). However, we observed a decrease in Firmicutes but increase in Bacteroidetes with increasing latitude. While the difference in communities may reflect the selection pressure of local environmental conditions, we cannot exclude the influence of different sampling protocols and sequencing approaches on the community composition, despite the use of the same primers. Overall, global air, marine and terrestrial communities were different from each other (PERMANOVA, $R^2 = 0.14$, $p = 0.001$), and Southern Ocean air communities were unique, highly heterogeneous, and different from other airborne communities elsewhere PERMANOVA, $R^2 = 0.08$, $p < 0.001$).

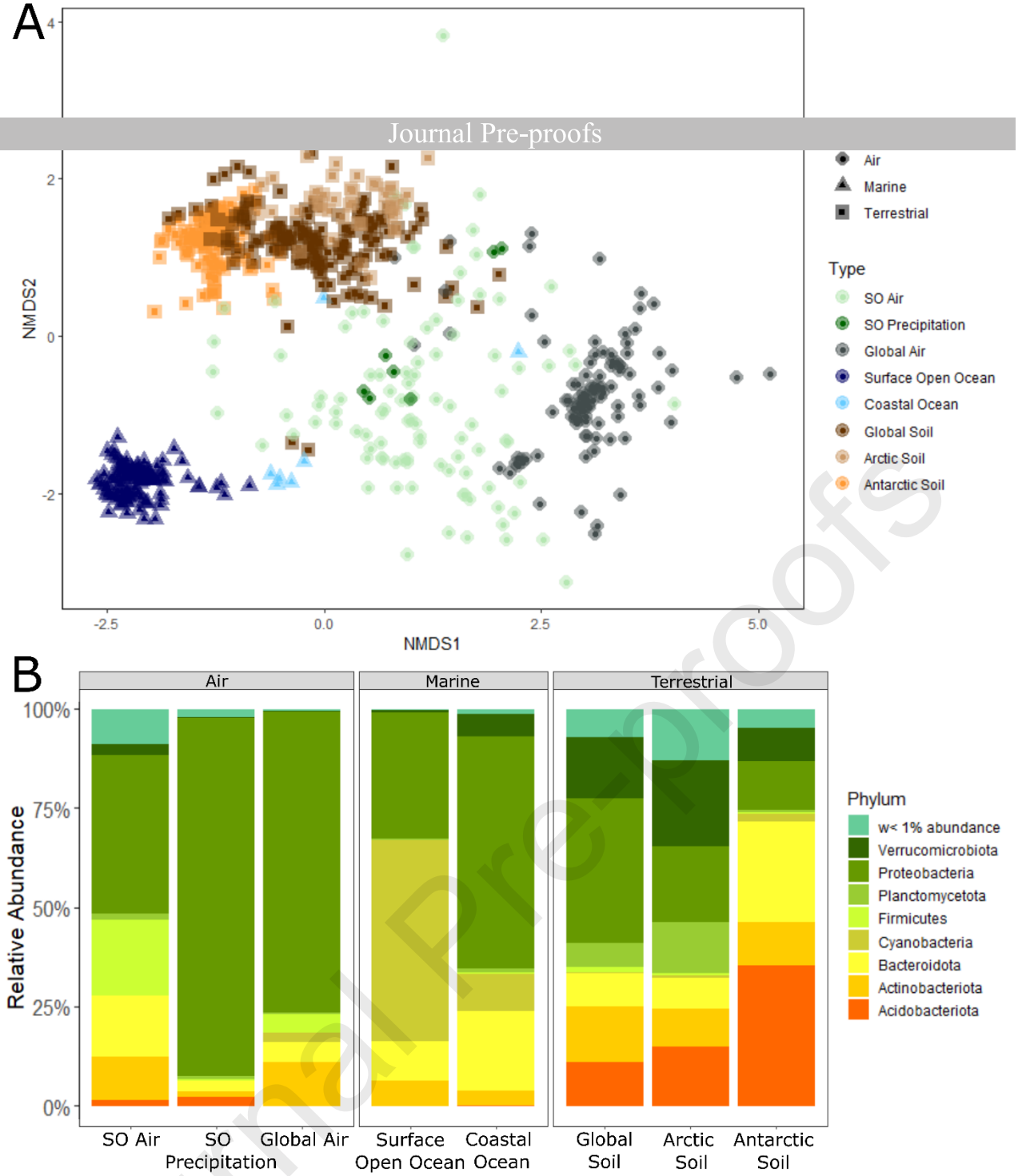

Figure 4. A. Visualization of community dissimilarity using non-metric multidimensional scaling (NMDS) of the Bray-Curtis distance between air, marine, and soil bacterial communities. B. Relative abundance of bacteria at the phylum level.

c. Biomass

In this study, 3,322,968 L of air was collected in total across an 85-day period. It contained a total cell density as estimated by qPCR of the 16S rRNA gene of 7.5 x 10^2 cells m^{-3} and above. Despite the comparative hostility of the environment, remoteness from traditionally recognised sources of air-borne bacteria, apparent low levels of nutrients and desiccation, significant numbers of bacteria were consistently found across the region. This is much lower than has been cited for other locations around the globe. Indeed, studies have shown that global concentrations of bacteria in the atmosphere generally range from 10^4 to 10^6 cells m^{-3} although these studies have showed that the range of airborne microbial concentrations is far wider than this and is highly variable (Gandolfi et al., 2013, Šantl-Temkiv et al., 2018, Maki et al., 2019, Tignat-Perrier et al., 2019). For example, across nine global sampling sites, bacterial concentrations varied from 9.2 x 10^1 to 1.3 x 10^8 cells m^{-3} with the lowest concentration recoded in Station Nord at the Villum research station (Greenland) and the highest bacterial concentration recorded on the semi-arid plateau of Namco (China) at over 4700m elevation (Tignat-Perrier et al., 2019). In Nuuk (Greenland), bacterial concentrations of $1.3 \times 10^3 \pm 1.0 \times 10^3$ cells m^{-3} have been recorded (Šantl-Temkiv et al., 2018) while bacterial concentration in free tropospheric air above the Alps ranges from 3.4 x 10^4 cells to 2.67 x 10^5 m^{-3} (Xia et al., 2014). Of particular relevance to this study, the average microbial abundances in the atmospheric boundary layer (ABL) are quoted as $\sim 1.9 \times 10^4$ bacteria m^{-3} (Mayol et al. 2017). Hence, in common with broad scale observed patterns in the higher animals and plants, we observed a significant decrease in biomass with latitude. We can therefore conclude that the total biomass in the atmosphere over the Southern Ocean is low, and between one to two orders of magnitude lower than the biomass found elsewhere. This observation was consistent with the abundance of fluorescent particles 0.00017-0.1201 m^3, a previously used best estimate of biomass in the air above the Southern Ocean (Moallemi et al. 2021).

3. How unique are Southern Ocean airborne communities?

The Southern Ocean air and global air communities shared 4% of the total number of ASVs combined totaling 139 shared ASVs [Fig. 5A], primarily Proteobacteria, Firmicutes and Actinobacteria [Fig. 5B]. This result alone suggested that the air over the Southern Ocean does not act as a strict dispersal barrier but a rather selective barrier to microorganisms entering the Antarctic (since these common ASVs were identified in non-polar ecosystems). However, the relatively low number in common strongly suggests that it could be acting as a selective dispersal filter. Furthermore, although this is a relatively low number of ASVs, their mean relative abundance across all samples was equivalent to 36 % of the communities. In comparison, the 788 ASVs unique

to the Southern Ocean air represented 34 % of the communities while the 2270 ASVs unique to the global air also represented 30 % [Supplementary data 3]. In other words, about 1/3 appear potentially restricted to the Southern Ocean, 1/3 appear potentially common to the rest of the globe (except the Southern Ocean) and 1/3 appear cosmopolitan in terms of relative abundance. This result highlights the presence of a low number of highly abundant taxa, and a high number of very low abundance taxa in each community and confirms the very high variability in diversity discussed previously in this study and observed by other studies and is important in terms of why microbial biodiversity is important ie though environmental function and community resilience (via factors such as functional gene redundancy).

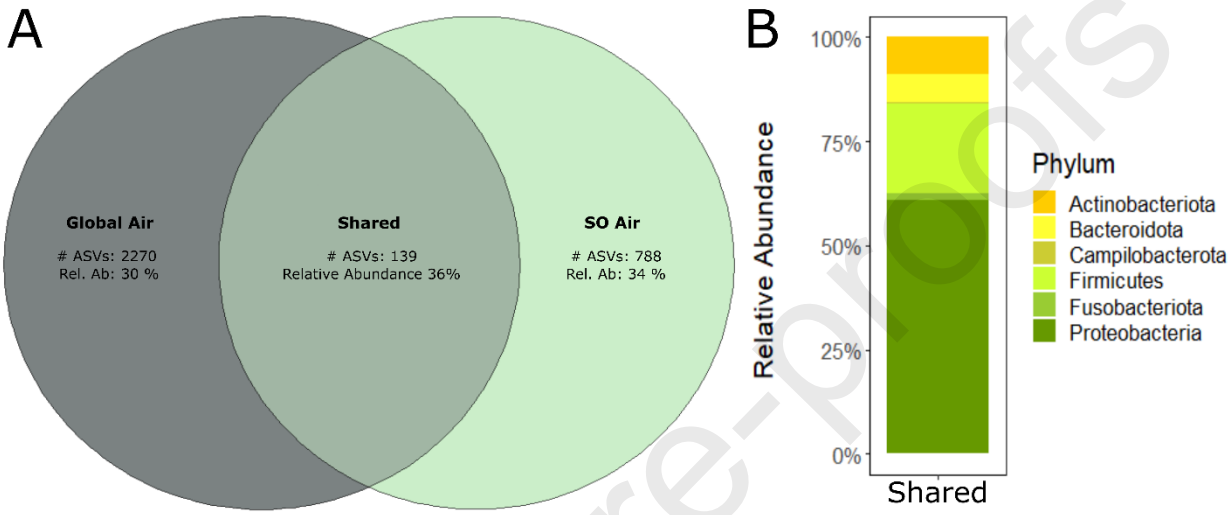

Figure 5. A: number of ASVs (# ASVs) shared between global air and Southern Ocean air samples. The percentage indicates the relative abundance (Rel. Ab.) represented by the number of ASVs across all samples. B. Relative abundance of shared bacteria at the phylum level.

This result is perhaps unsurprising, as core microbiomes are starting to emerge as a common feature of microbiological biodiversity and biogeography studies worldwide. Els *et al.* (2019) identified a core microbiome in free tropospheric air microbial community over Mount Sonnblick in the Austrian Alps (3106 m above sea level) which consisted of 61 OTUs (11% of all the OTUs they detected). Archer *et al.* (2019) found in a direct comparison that Antarctic non-native assemblages shared only 5.7% of bacterial ASVs with markedly more diverse bioaerosols found in

New Zealand. In contrast, in this study, while 139 ASVs were shared between Southern Ocean air and global air, we did not identify any ASV present in over 50 % of all air samples. The most prevalent ASV shared by 25 % of all samples was classified as a *Psychrobacter* (Gammaproteobacteria). They are aerobic, Gram-negative coccobacilli and most *Psychrobacter* strains are psychrotrophic and can grow at 5 °C. Many strains are also radiation resistant, making them well adapted to live in the air (Juni, 2015).

The 4% ASV similarity in polar and non-polar air were diverse in taxonomy including both cosmopolitan species and extremophiles, most represented by only one sequence variant (where more than one ASV, the number is indicated in parentheses). Of the 139 ASVs identified as present in both Antarctic and non-Antarctic air, about 50% were attributable to specific environmental species [Table 1].

Notable characteristics	Genus
Ubiquitous to all ecosystems	*Pseudomonas* (4), *Brevundimonas* (2)
Cosmopolitan/ubiquitous in soils and/or water	*Acinetobacter (6), Actinomyces, Flavobacterium* (3) and related *Sphingobacterium*, *Sphingobacteriaceae, Empedobacter, Massilia (3), Methylobacterium-Methylorubrum* (3), *Rhodococcus*, *Variovorax* and *Bacteroides* (4)
Identified in Freshwater	*Aeromonas*, *Brevundimonas* (a Gram negative bacterium widely distributed in nature), Candidatus *Limnoluna* affiliated with the Phylum Actinobacteria, Candidatus *Planktophila* an actinobacterium representing one of the most important taxa in freshwater bacterioplankton, *Caulobacter* an aquatic bacterium that thrives in nutrient poor environments, *Chryseobacterium* (2), *Enhydrobacter*, *Aquabacterium* (2) and *Rhodoferax* (purple non-sulphur bacteria)
Identified in air	*Enhydrobacter* and *Aerococcus*

Extremophiles	*Shewanella, Psychrobacter, Tepidomonas*
Gram positive	*Blastococcus, Blastomonas, Brachybacterium*, *Actinomyces*
Functional (involved in the Nitrogen cycle)	Ellin6067, *Lentimicrobium, Noviherbaspirillum, Paracoccus, Burkholderia-Caballeronia-Paraburkholderia, Bradyrhizobium*
Functional (Methylotrophic)	*Methylotenera*
Other notable taxa (as not marine)	Betaproteobacterium *Malikia* and *Cornamonadaceae* (3)

Table 1: A non-exhaustive list of the genera shared across all air samples and their ecosystem or notable characteristics

4. What is the role of the sub- and peri-Antarctic islands?

In this study, while most samples were collected aboard the ship (n=90) in the Southern Ocean (SO air over ocean), samples (n=10) were also collected on land, on the Antarctic and sub- and peri-Antarctic islands (SO air over land). Despite the species richness of air over terrestrial and marine environments being similar (ANOVA, $p = 0.58$), a significant difference was found in both the Shannon and Simpson diversity indices between Southern Ocean air samples taken over marine sites when compared to those taken over terrestrial sites [Fig. S2A]. Air communities were equally rich but communities over oceans were very uneven compared to those taken over islands. The difference in community composition between air samples was driven by the high variability of communities over the Ocean [Fig. S2B]. In total, 689 ASVs were uniquely identified in SO air over ocean while only 87 ASVs were unique to the air over land [Fig. S2C]. Furthermore, we observed that each of the Islands had a distinct pattern of biodiversity in the air above them, suggesting that each of the Islands in the Southern Ocean is unique [Fig. S3]. The pattern of aerial biodiversity above the Islands of the Southern Ocean suggests that for the Southern Ocean at least, we can probably discount the 'air-bridge' or 'stepping-stone' hypothesis, by which microbial biodiversity does not reach the Antarctic continent by using islands as stepping-stones to reduce the effective distance it is necessary to travel. It does, however, argue for the importance of conservation and

biosecurity measures tailored to each island location, since each island has its own unique biodiversity (and hence influence on the environment around it).

5. Which environmental factors affect Southern Ocean air bacterial communities?

a. Pressure and cyclones

We tested the influence of several key environmental and meteorological variables on the diversity and community composition. Of the environmental parameters recorded at the time of sampling (latitude, longitude, pressure, air temperature, relative humidity, average wind direction, average wind speed, maximum wind speed, minimum wind speed, dew point, cloud level and solar irradiance) and the atmospheric state over the five previous days of each sample (cold or warm advected air, maximum potential temperature (theta), median latitude of air mass trajectory and the presence or absence of a cyclone), none correlated with the aerial richness, diversity and evenness of communities over the Southern Ocean [Table S2]. The random forest models did not explain any of the variance observed in alpha diversity of these communities. We can therefore tentatively conclude that, at least in the immediate to short-term, the atmospheric environment itself is not the primary determinant of the bacterial diversity of the air above the Southern Ocean.

However, when investigating the effect of environmental parameters on community composition, we found that the average maximum potential temperature (max theta indicates the altitude of the originating air mass) and the air pressure resulted in a significant relationship with community composition based on the permanova test [Table S2] and that the presence of cyclones might increase the dissimilarity of communities [Figure 6A].

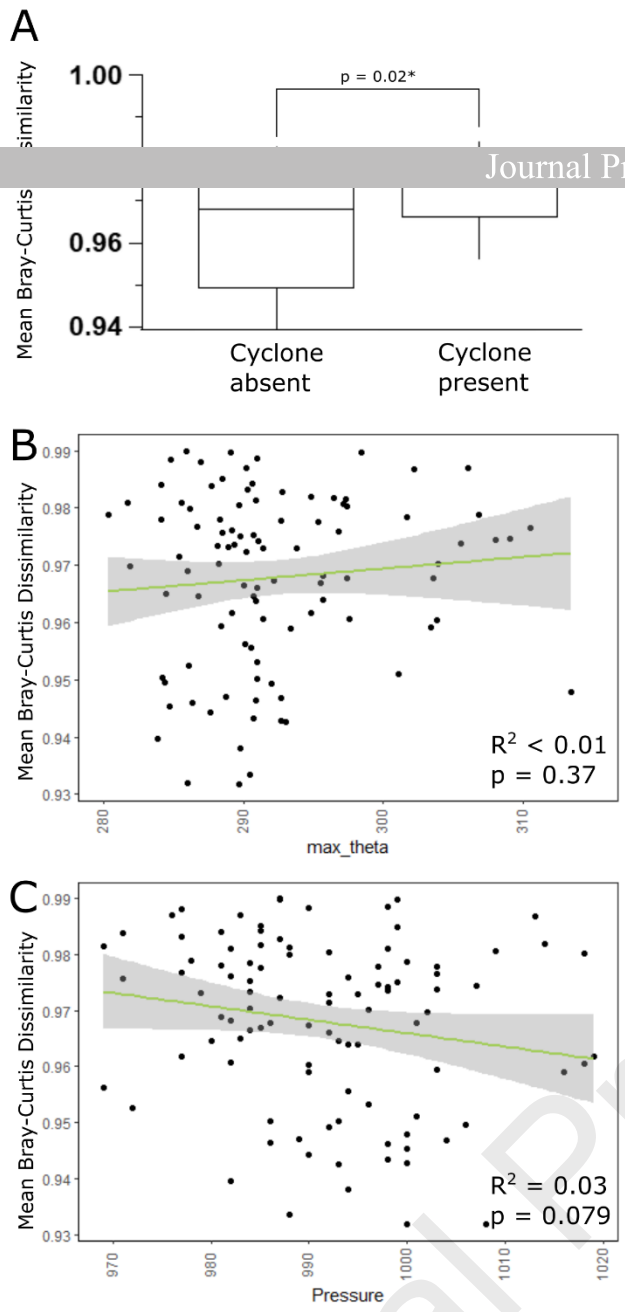

Figure 6. A. Differences in community dissimilarity (Bray-Curtis) with the presence and absence of cyclones. B. Linear model of community dissimilarity (Bray-Curtis) along the maximum potential temperature of five-day back trajectories. C. Linear model of community dissimilarity (Bray-Curtis) along the pressure gradient.

High maximum potential temperature displayed somewhat higher community dissimilarity [Fig. 6B]. As the potential temperature is a surrogate for the air mass origin, a high value suggests that air masses have descended diabatically from the atmosphere further aloft, likely indicating their

origin from further north, perhaps the rest of the globe, hence the high variability in community composition (although the data itself does not validate this interpretation). During low pressure system situations, the community composition is more dissimilar and less stable [Fig. 6C]. Low pressure systems are associated with cyclones, a frequent and highly characteristic feature of the Southern Ocean (Papritz *et al.*, 2014), and indeed we do see, albeit weak, a relationship between higher community composition dissimilarity and cyclone presence [Fig. 6A].

Clearly, an in-depth interpretation of this relationship is not possible with the observations available. Indeed, we are still a very long way from an understanding of exactly how physical parameters would mechanistically influence communities in the air. However, it is conceivable that the cyclogenesis mixes air masses from different sources, leading to more diverse and variable communities.

b. Precipitation

In this study, we collected some precipitation samples during the rare events (n=7 precipitation events but only four days of precipitation in total) as well as air samples before or after the event. Precipitation samples presented higher richness and communities were significantly different from Southern Ocean air sampled on the same day [Fig. S4]. Precipitation samples were largely dominated by Proteobacteria (Gammaproteobacteria and Alphaproteobacteria) while concurrent air samples were more diverse with Firmicutes, Alpha- and Gamma- Proteobacteria and Actinobacteria [Fig. S4]. Interestingly, 15 of the ASVs shared with the precipitation samples were also identified in the 4% of shared ASVs between the polar and global air. These included *Enhydrobacter*, *Bradyrhizobium*, *Aquabacterium* and *Rhodoferax* (Table 1).

Recent studies have shown that precipitation communities differ significantly from the air communities at the time of precipitation events. For example, a study showed an increase in bacterial concentrations in the air preceding a storm (Xia *et al.*, 2013) and others have suggested that microorganisms precipitated with fog, cloud water, snow, hail or rain differ in their species composition from free tropospheric air masses and thus, do not mirror the air community structure (Amato *et al.*, 2017, Els *et al.*, 2019, Evans *et al.*, 2019). These studies suggest that snow or cloud samples are not suitable proxies for free troposphere air microbiome composition.

The implication of this observation is that precipitation provides increased biodiversity input. As a result, precipitation patterns could influence microbial biodiversity in Southern Ocean air through the addition of new diversity or through a change in the pattern of dominant groups. This lends support to the idea that high altitude transfer is more important for biodiversity and

colonisation than low altitude transfer. Precipitation is formed mostly via the ice phase in clouds (Korolev & Field, 2008), which may be the reason behind those biodiversity differences. The ice phase in clouds relies on ice nucleating particles, and bioaerosols are prime ice nucleating particles (INP) (Pratt et al., 2009). Hence, precipitation samples might be better at detecting microorganisms that are good INP, for example, Gammaproteobacteria which are well known for their ice nucleation activity (Failor *et al.*, 2017). Given the formation level of clouds, they can both originate from a source in the marine boundary layer and from aloft, the latter indicating long-range transport. Although precipitation events are still relatively infrequent in this region, models suggest that precipitations will increase, especially in the higher latitudes of the Southern Ocean (Liu & Curry, 2010, Bracegirdle *et al.*, 2020).

c. Other factors influencing communities

Of particular significance, given the availability and use of back-track trajectory data, and the idea that wind moves biodiversity from one location to another, was the apparent lack of influence of either wind speed or, more importantly, wind direction. The impact of the wind on aerial communities is still unclear as some studies have found some impacts of wind speed and direction on communities (Tignat-Perrier *et al.*, 2019, Tignat-Perrier *et al.*, 2020), while others have not (Uetake *et al.*, 2020). Furthermore, when considering the impact of wind speed and direction, we must also consider temporal variables and spatial variables (as the vessel was moving) and therefore, the situation is likely complex. Other environmental factors such as air temperature, UV radiation or humidity have been suggested to have some influence on airborne communities (Šantl-Temkiv *et al.*, 2018, Tignat-Perrier *et al.*, 2019, Tignat-Perrier *et al.*, 2020, Archer *et al.*, 2021 Preprint) but this was not observed in the Southern Ocean. Overall, observations tend to vary by study, likely reflecting the importance of local conditions at the time of sampling.

6. Where do Southern Ocean airborne bacteria likely originate?

Here, we used source-tracking to determine the potential ecosystem of origin of the ASVs identified in the Southern Ocean air and precipitation samples. Of the 1013 Southern Ocean ASVs identified, 584 were identified in the global database produced and therefore, had a potential origin [Fig. 7A]. These contributed to 41 % of the total relative abundance across the whole database, suggesting these were rather dominant taxa. Interestingly, we could not explain more than 30% of the origin of the different Southern Ocean sample types [Fig. 7B], further highlighting the unique ASVs identified in each group of samples. More taxa from other airborne sources were identified

in the precipitation samples while more taxa of terrestrial origin, especially Antarctic soils, were identified in the sub- and peri-Antarctic islands' air [Fig. 7C]. This result is an indication of the stronger influence of local inputs in these samples, suggesting that islands have a strong influence on th[illegible] marine origin [Fig. 7C-F], despite most of the sampling being conducted above the ocean. The overall low input of ocean-associated microorganisms into the air globally (Archer *et al.*, 2021 Preprint) may explain the limited influence of local ecosystems as sources of airborne microorganisms over the oceans, as shown in this study and over the Pacific and Atlantic Oceans (Mayol *et al.*, 2017, Lang-Yona *et al.*, 2022).

In the literature, the consensus about the origins of aerobiological diversity is a combination of both, aerosolization of local material and long-distance transport, and differential source regions and transport have been shown to influence microbial composition of the atmosphere (DeLeon-Rodriguez *et al.*, 2013, Šantl-Temkiv *et al.*, 2018). In oceanic air masses, microorganisms appear to originate primarily from long-distance transport of terrestrial microorganisms, although large uncertainties remain on the origins of ASVs. Only a standardised global investigation of microbial communities in all ecosystems, as was started with the Earth Microbiome Project (Thompson *et al.*, 2017), and the creation of an open-source database could shed light on the origin and dispersal patterns of microorganisms in the air.

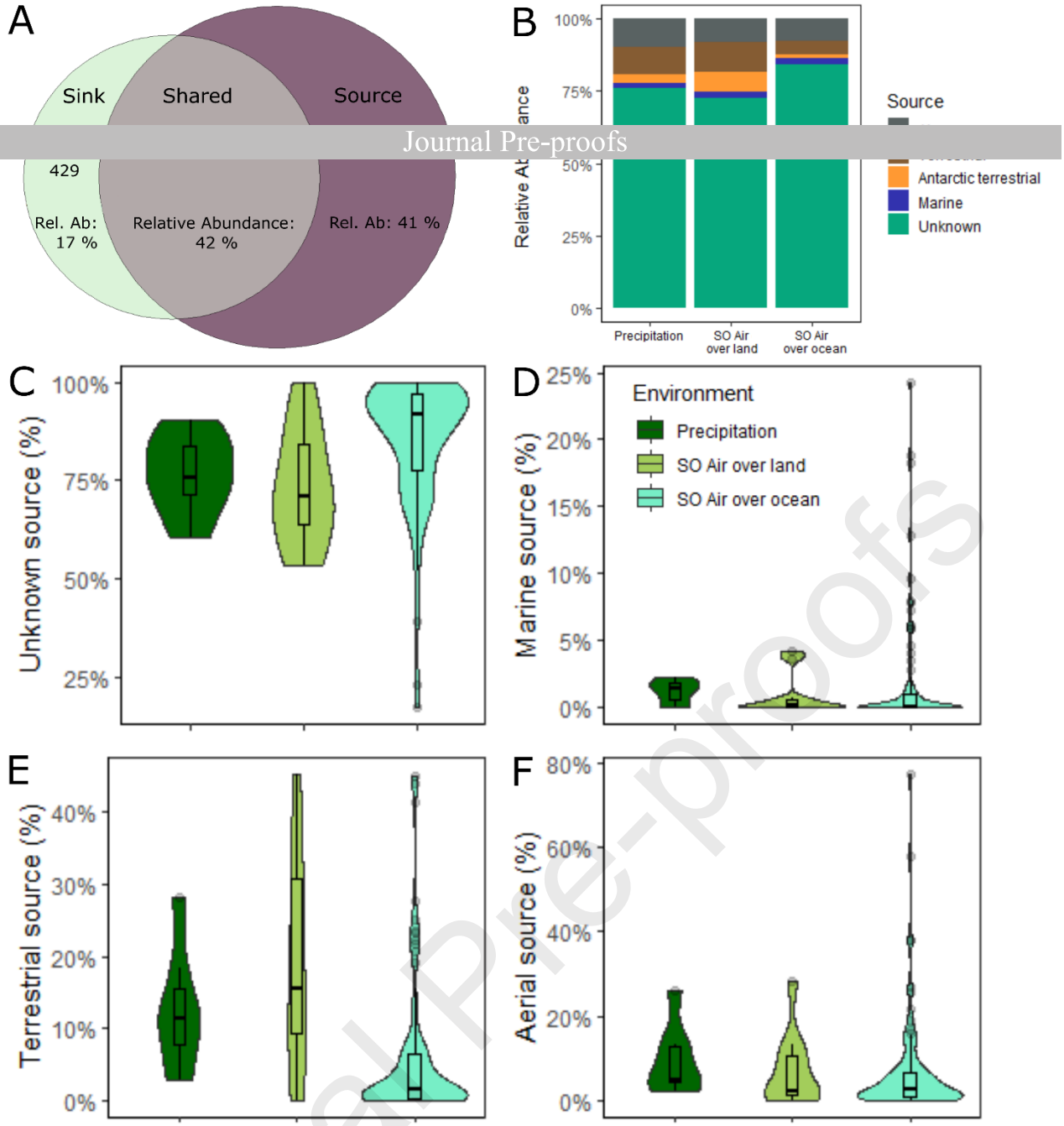

Figure 7: A. Euler diagram showing the number of ASVs (# ASVs) with an origin identified from the global database and the relative abundance (Rel. Ab.). B. Mean potential origin of ASVs across each Southern Ocean sample type. C-F. Mean origin of ASVs per sample type.

7. Is the Antarctic Convergence Zone limiting bacterial dispersal to the Antarctic continent?

Finally, we tested the hypothesis that the Antarctic Convergence might act as a dispersal barrier (Pearce *et al.*, 2009, Archer *et al.*, 2019, King-Miaow *et al.*, 2019, Uetake *et al.*, 2020). To this end, and to consider the dynamic movements of air masses rather than consider the convergence as a static barrier, we identified the origin of the air mass using 5-day back trajectories and compared the median latitude of the air mass to the latitude of the Antarctic Convergence at the relevant longitude. We compared global air samples (always considered north) and Southern Ocean air samples north of the Antarctic Convergence to the Southern Ocean air samples collected south of the convergence [Fig. 8]. We found differences in evenness and community composition, mainly driven by the global air communities [Fig. 8B] as these differences were not apparent when comparing only Southern Ocean air sample north and south of the convergence. The majority of ASVs were unique to samples either north or south of the convergence, with 308 ASVs (9 % of the total ASVs) shared between both [Fig. 8C]. Hence, the Antarctic Convergence does not appear to be acting as a strict dispersal barrier but rather, the Southern Ocean may itself act as a selective dispersal filter. The size of the Southern Ocean and remoteness of the continent might, in themselves, be major filters of microorganisms unable to survive in the air longer than the minimum time required to attain suitable ecosystems. Therefore, the Southern Ocean itself may be limiting the dispersal of global airborne microorganisms to the Antarctic continent.

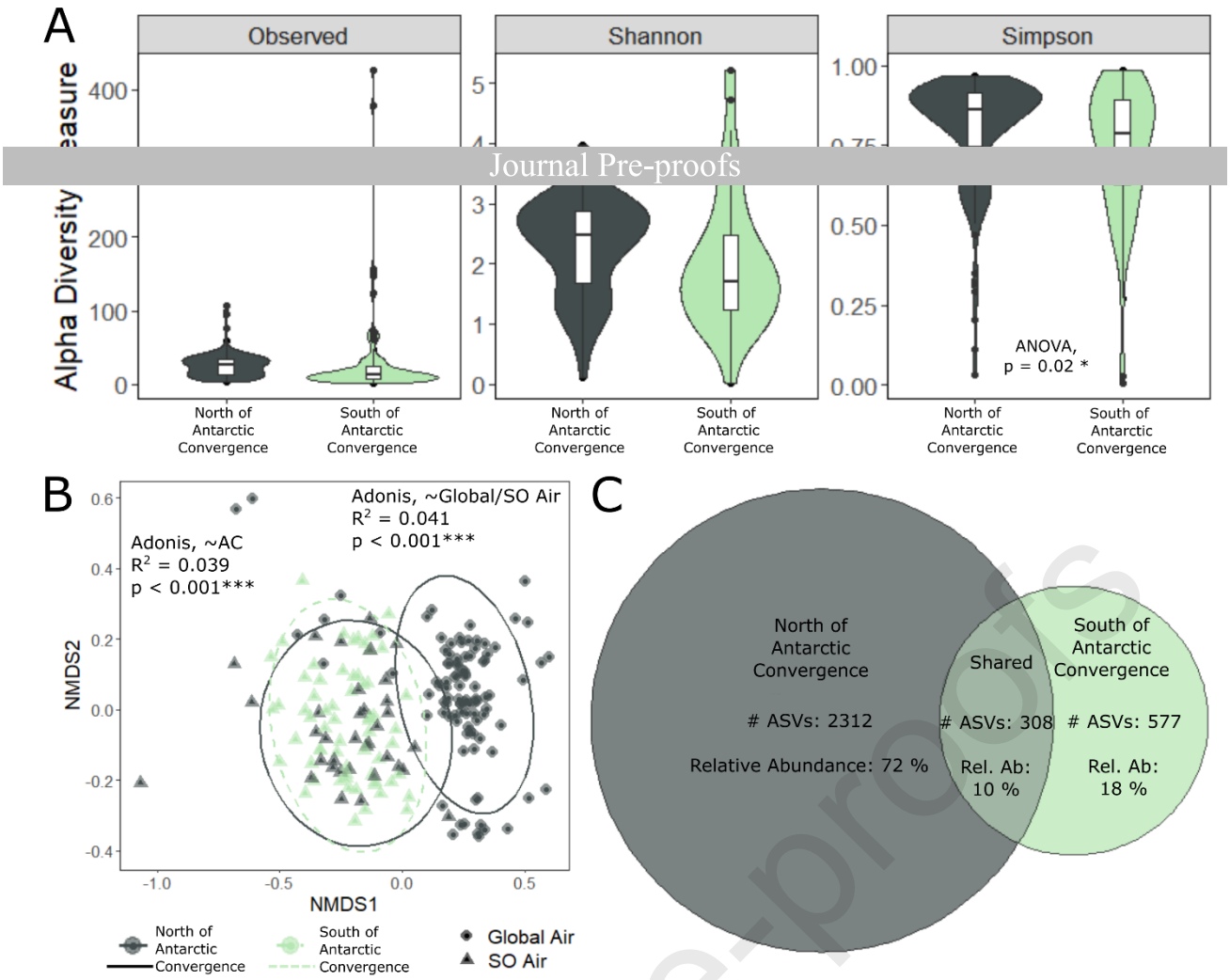

Figure 8: Influence of the Antarctic Convergence Zone on microbial diversity above the Southern Ocean, using global air samples as well as the samples collected during the ACE expedition around the Southern Ocean. A. Alpha diversity comparisons B. NMDS of communities based on Bray-Curtis dissimilarity illustrating the differences between Southern Ocean air and Global air communities C. Euler diagram showing the number of shared ASVs north and south of the Antarctic Convergence ASVs (# ASVs), weighted by the relative abundance (Rel. Ab.). of ASVs in percentages.

Summary

We investigated patterns of bacterial biodiversity in the air above the Southern Ocean and provided evidence of low biomass, high diversity, heterogeneous, and unique communities from both local and global origin. We found that air communities over the Southern Ocean and its islands were significantly different from other ecosystems. However, these differences were not due to the Antarctic Convergence Zone acting as a dispersal barrier, but rather the Southern Ocean acting as a selective dispersal filter. We identified 139 ASVs that were previously identified in air samples elsewhere, suggesting that these taxa may be pre-adapted to life in the atmosphere, efficient dispersers and therefore, may form part of a potential aerobiome.

Significant differences in microbial air diversity following meteorological patterns (air pressure, maximum potential temperature and the presence or absence of cyclones) and differences in communities from precipitation events suggests that bacterial biodiversity may be sensitive to changes in weather patterns that may result from climate change. These observations have important implications as climate change is known to increase precipitation in the Arctic (Pörtner *et al.*, 2019). If they also increase in the Antarctic, it will lead to increased rates of microbial input and potentially higher diversity and increased risks of biological invasions. In addition, as the region warms, there will be more ice-free areas and free niches to colonise, potentially disrupting ecosystem function. Therefore, changing weather patterns through climate change may increase the frequency or the ability of microorganisms to reach Antarctica, illustrating the key role of the atmosphere the biogeography of microorganisms in Antarctica.

Materials and Methods

ACE expedition and environmental data

Antarctic air samples were collected aboard the R/V Akademik Tryoshnikov over an 85-day period between December 22nd, 2016, and March 16th, 2017, whilst the ship circumnavigated the Antarctic continent. The circumnavigation began and ended at Cape Town with stops at Hobart, Australia and Punta Arenas, Chile during the voyage [Figure 1A] (Landwehr *et al.*, 2021). Location via GPS co-ordinates and weather data were collected continuously throughout the voyage via a Vaisala weather station aboard the ship, and included Latitude, Longitude, Average wind direction, Average wind speed, Minimum wind speed, Maximum wind speed, Cloud level, Sky coverage, Relative humidity, Temperature, Dew point, Pressure, Solar radiance and UV.

We used back trajectories (Thurnherr *et al.*, 2020) calculated with the Lagrangian analysis tool LAGRANTO (Sprenger & Wernli, 2015) based on wind fields from the operational analysis data of the European Centre for Medium-Range Weather Forecasts (ECMWF) to derive the maximum potential temperature and median latitude within the five days prior to the air mass arrival to the sampling location (see details in Schmale *et al.* (2019). We released 20 trajectories from within the boundary layer around the ship position each hour and averaged those for the duration of the sample collection. To classify whether a sample experienced air masses coming primarily from north or south of the polar front, we compared the median latitude per sample to the latitude of the Antarctic Convergence at the relevant longitude. The presence or absence of cyclones and the location of the sampling in the warm or cold sector of a cyclone were derived from published data sets (Thurnherr *et al.*, 2020, Thurnherr & Wernli, 2020) and as described in Thurnherr *et al.* (2021).

Air and precipitation sample collection

Southern Ocean air samples were collected with sampling units set around the vessel to reduce the influence of sea spray and potential human bacterial sources [Figure 1B]. Air samples from terrestrial locations were collected at the sub-Antarctic islands of Kerguelen, Balleny, Crozet, Bouvet and South Georgia and occasionally over the Antarctic continent. For terrestrial sites, sampling units were positioned at a height of 1.5m to reduce the impact of local turbulence.

Dry samples were collected via a membrane filtration apparatus set up, whereby a Welch WOB-L vacuum pump at a flow rate of 20L min^{-1} (Welch, Mt. Prospect, IL, USA) was connected by tubing to a Sartorius filtration unit (Göttingen, Germany) containing a 47 mm × 0.2 μm pore size cellulose nitrate membrane filter (GE Healthcare Life Sciences, Chicago, IL, USA). Samples were collected opportunistically for between one and 36 hours. Dry samples were supplemented with a surface air system (SAS) sampler as backup. Wet samples were collected via a Bertin Coriolis μ (Bertin Technologies, Montigny-le-Bretonneux, France), where the collection cones were filled with sterile DNase and RNase free H_2O (Thermo Fisher Scientific), and the sampler run at a flow rate of 300 L m^{-1} for a duration of 50 minutes. Wet samples were supplemented with an SKC sampler as backup. Precipitation samples were collected using a sterile funnel and filtered onto nitrocellulose 0.22 μm filters (Merck Millipore, Germany) using a sterile filtration unit (Sartorius, Groningen, Germany). All samples were stored at -80 °C for the duration of the expedition.

DNA extraction and 16S amplicon sequencing

In total, 100 air samples and 7 precipitation samples were used in this study. Samples collected on filter substrates were first dissected into quarters using an ethanol and flame sterilised scalpel and a sterile petri dish in a Class II Microbiological safety cabinet. The dissected quarter filter was then placed directly into a labelled bead tube for extraction. Water based (Sterile) samples stored either in collection cones or falcon tubes were transferred to sterile 15 mL falcon tubes and centrifuged for a duration of 20 minutes at 5000 g. Following centrifugation, the supernatant was removed leaving 1 mL, within which the formed pellet was re-suspended. This 1 mL was then loaded directly into a labelled bead tube for extraction. Where samples contained more than 15 mL liquid, they were combined after centrifugation, and the previous steps were repeated.

DNA was extracted from each sample using the Qiagen PowerSoil kit (Qiagen, Hilden, Germany) following the manufacturer's instructions and DNA extracts were stored at -20 °C. 16S rRNA gene libraries were constructed using the universal primers 515F and 806R (Kozich *et al.*, 2013) to amplify the V4 region. Amplicons were generated using a high-fidelity Accuprime DNA polymerase (Invitrogen, Carlsbad, CA, USA), purified using the AMPure magnetic bead capture kit (Agencourt, Beckman Coulter, MA, USA), and quantified using a QuantIT PicoGreen fluorometric kit (Invitrogen). The purified amplicons were then pooled in equimolar concentrations using a SequalPrep plate normalization kit (Invitrogen), and the final concentration of the library was determined using a SYBR green quantitative PCR (qPCR) assay. Libraries were mixed with Illumina-generated PhiX control libraries and our own genomic libraries and denatured using fresh NaOH. The resulting amplicons were sequenced on the Illumina MiSeq V2 (500 cycles).

Quantitative-PCR

DNA extraction of membrane filters was performed in a class II microbiological cabinet. Filters were first cut in half (using a heat and UV sterilised scalpel) and sliced into thin ribbons to avoid clustering. DNA extraction was performed using the DNAeasy Powersoil Pro kit (Qiagen, Hilden, Germany) Amplification of the 16S rRNA gene was carried out using the primer pair 27fmod (AGRGTTTGATCMTGGCTCAG) and 519Rmodbio (GWATTACCGCGGCKGCTG), (Kozich et al. 2013) using a Step One Plus Real Time PCR System (Applied Biosystems, Massachusetts, United States). Each 20 µl qPCR reaction contained: 10 µl of 2X SYBR green master mix, 2 µl ROX reference dye, 0.2 µM forward primer, 0.2 µM reverse primer, 5.6 µl DNA and PCR grade water to 20 µl. The amplification method was as follows: initial 95°C for 5 mins, then 40 cycles of (94°C for 15 seconds and 53°C for 30 seconds), followed by a melt curve. To facilitate absolute quantification of the 16S rRNA gene, DNA extracts from *E. coli* k12 cells were PCR amplified and ran on an agarose gel, with product bands cut out and weighed for DNA

extraction, using the Monarch Gel Extraction kit (New England Biolabs, Massachusetts, United States). DNA concentration (ng μl^{-1}) and purity (A260/280nm) were determined via the use of a nanodrop spectrophotometer (Thermo Fisher Scientific, Massachusetts, United States). Gene copy number was determined using the following equation: (Amount of DNA (ng) x 6.022 x10^{23})/(Gene Length (bp)x 1 x 10^9 x 660), where 6.022 x1023 represents Avogadro's Constant, 1 x10^9 a conversion factor, and 660 is the average mass of 1 base pair (bp). A 1 in 10 serial dilution was then performed to generate 16S rRNA standards containing known gene copy numbers ranging from 1x10^7 to 1x10^1, which were run alongside air sample DNA and plotted to generate a standard curve, facilitating the quantification of 16S rRNA gene copies in each sample.

Bioinformatic processing

The resulting amplicons were processed using the DADA2 pipeline (Callahan *et al.*, 2016). Forward and reverse read pairs were trimmed and filtered, with forward reads truncated at 230 bp and reverse reads at 200 bp, no ambiguous bases allowed, and each read required to have <2 expected errors based on their quality scores. Amplicon sequence variants (ASVs) were independently inferred from the forward and reverse reads of each sample using the run-specific error rates. Reads were dereplicated, pairs were merged, and chimeras were removed from each sample. Taxonomic assignment was performed against the SILVA v138 database (Quast *et al.*, 2012, Yilmaz *et al.*, 2014) using the implementation of the RDP (ribosomal database project) naive Bayesian classifier (Wang *et al.*, 2007). The decontam package (Davis *et al.*, 2018) was used to identify potential contaminants using the prevalence function. The ASV table was also manually curated to discard ASVs present in the kit and MiSeq controls in higher abundance than in other samples, leaving 107 samples (± 19032 reads/sample) with 1013 assigned ASVs.

Global database

We produced a global 16S rRNA database of marine, soil and air samples using data extracted from NCBI and based on studies using the primer set 515F-806R sequenced on Miseq (Fig. S1, Table S3, ASV and taxonomy tables are available on FigShare). Each dataset from individual studies was analysed separately using the DADA2 pipeline to independently calculate the error rate and infer ASVs. Three types of datasets were encountered but all were processed with the same criteria as the ACE samples, unless specified. Datasets with paired end reads (forward and reverse) or datasets with forward reads only but with the same amplicon length as paired-end reads were treated with the same criteria as the ACE samples. A few of the older datasets had forward reads only with smaller amplicons and were truncated to 100 bp before proceeding with the DADA2 pipeline.

The unique ASV tables and the final ACE table were merged using the mergeSequenceTables function in DADA2 and identical ASVs were merged using the collapseNoMismatch function in DADA2 with a minimum overlap of 90 bp, to ensure merging of ASVs with different amplicon lengths. Only ASVs present in at least two samples were retained. Taxonomic assignment was performed against the SILVA v138 database (Quast *et al.*, 2012, Yilmaz *et al.*, 2014) using the implementation of the RDP naive Bayesian classifier (Wang *et al.*, 2007).

Statistical analyses

All statistical analyses were performed in the R environment using primarily a combination of the phyloseq (McMurdie & Holmes, 2013) and vegan (Dixon, 2003), and visualised using ggplot2 (Wickham, 2016).

For the global comparison, alpha diversity of all samples was computed in phyloseq with the plot_richness function and ANOVA with Tukey's honest significant difference (HSD) tests were used to compare differences between sample types. Linear models with second order polynomials were used to evaluate latitudinal associations with alpha diversity. For beta diversity, sample counts were transformed to proportions, the Bray-Curtis dissimilarity matrix was computed and visualised using non-metric multidimensional scaling (NMDS). A PERMANOVA with the adonis function was used compare beta diversity between sample types.

Focusing on the Southern Ocean air (ACE expedition) samples, we compared marine air, terrestrial air and precipitation samples. Alpha diversity was computed with the plot_richness function and was compared between sample types using ANOVA with Tukey's honest significant difference (HSD) tests. Linear regressions were computed to evaluate relationships between environmental variables and alpha diversity. We also used random forest models to identify the most important variables associated with the alpha diversity of air communities. The random forest (RF) models were computed using the rfPermute function with 5000 permutations and 5000 trees in the rfPermute package (Archer & Archer, 2020). For beta diversity, sample counts were transformed to proportions, Bray-Curtis dissimilarity matrix was computed and visualised using non-metric multidimensional scaling (NMDS). A PERMANOVA with the adonis function was used compare beta diversity between sample types and to identify associations with environmental variables. The ps_euler function from the MicEco (Russel, 2020) package was used to identify shared ASVs between groups of interests and produce venn diagrams.

Finally, we used the FEAST package (Shenhav *et al.*, 2019) for the source tracking analysis. To identify the potential origin of Antarctic air ASVs, we used the global database as sources of ASVs and the ACE samples as sink. Differences between source origin and Southern Ocean sample types were tested with ANOVA and Tukey's HSD tests. Finally, the ps_euler function was used to identify the potential aerobiome shared between the Southern Ocean and global air samples.

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Data and materials availability: The DNA sequences from this project are deposited at the European Nucleotide Archive under the BioProject accession PRJNA697829. The global ASV table and associated taxonomy and metadata is available on FIGSHARE https://figshare.com/projects/Aerobiology_of_the_Southern_Ocean/140588 and includes the Southern Ocean samples.

Supplementary Data1: Southern Ocean metadata table including the environmental variables.

Supplementary Data2: Abundance and prevalence of ASVs with the associated taxonomy.

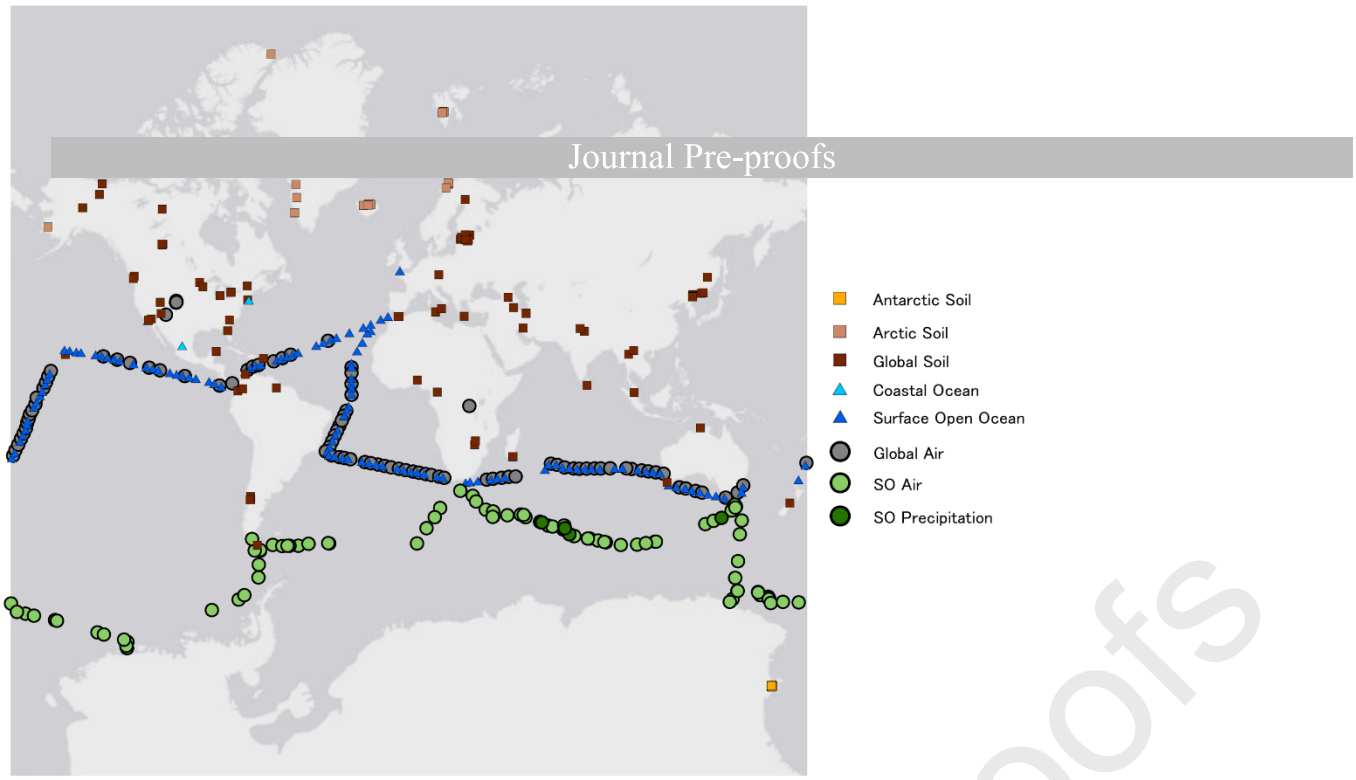
Supplementary Data3: Shared ASVs of Southern Ocean and global air samples.

Supplementary Materials for

Aerobiology over the Southern Ocean – implications for bacterial colonization of Antarctica

Lucie A. Malard*, Maria-Luisa Avila-Jimenez, Julia Schmale, Lewis Cuthbertson, Luke Cockerton & David A. Pearce*

*Corresponding authors: david.pearce@northumbria.ac.uk and lucie.malard@unil.ch



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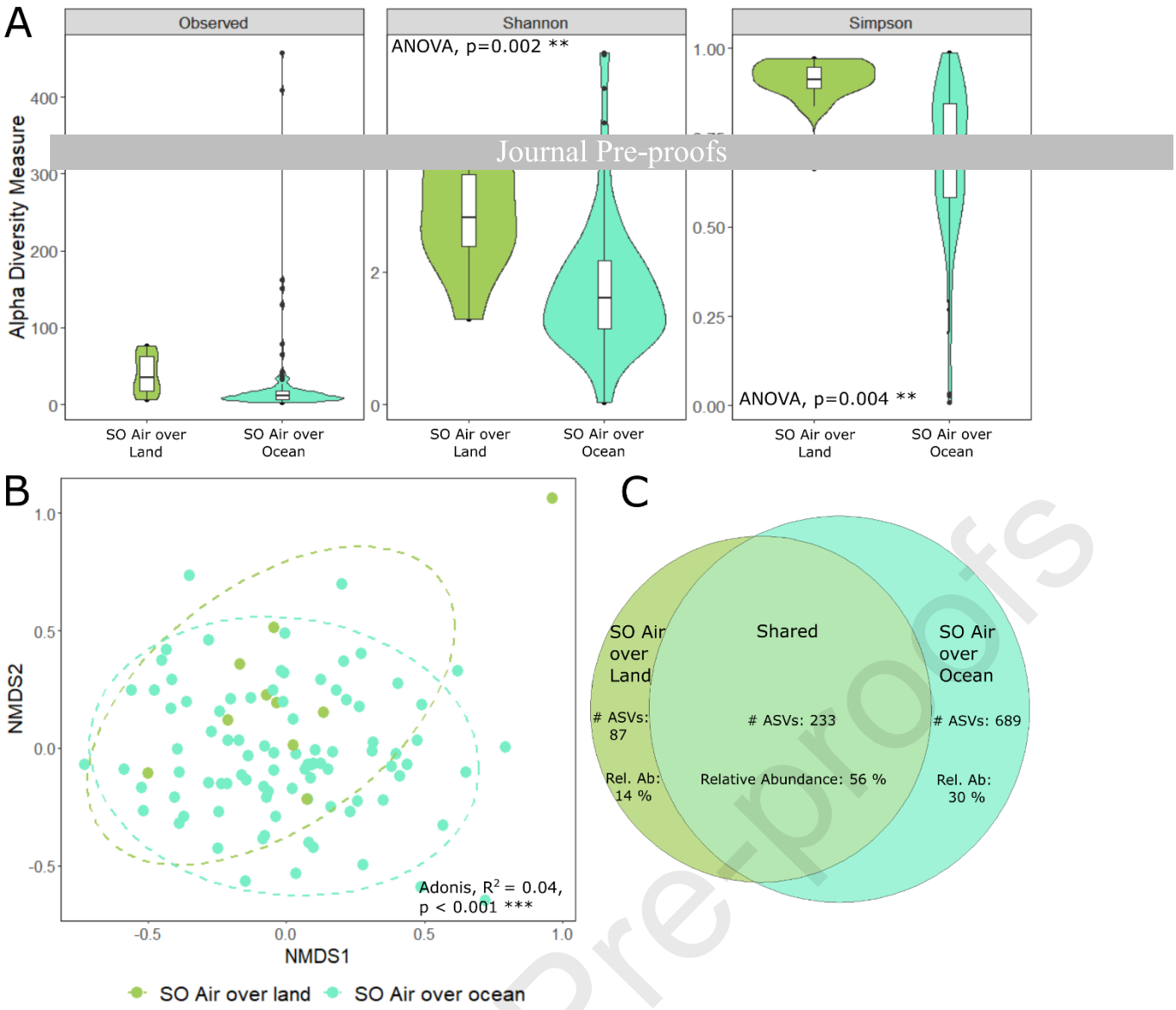
931 **Figure S1:** *Map of the global dataset*

932



933

934 **Figure S2:** *The difference in microbial diversity of the air above terrestrial and marine locations around the*
935 *Southern Ocean. A. Alpha diversity comparisons. B. NMDS of communities based on Bray Curtis*
936 *dissimilarity. C. Euler diagram illustrating the number of shared ASVs (#ASVs) between sample types,*
937 *weighted by the relative abundance of ASVs in percentages (Rel. Ab).*

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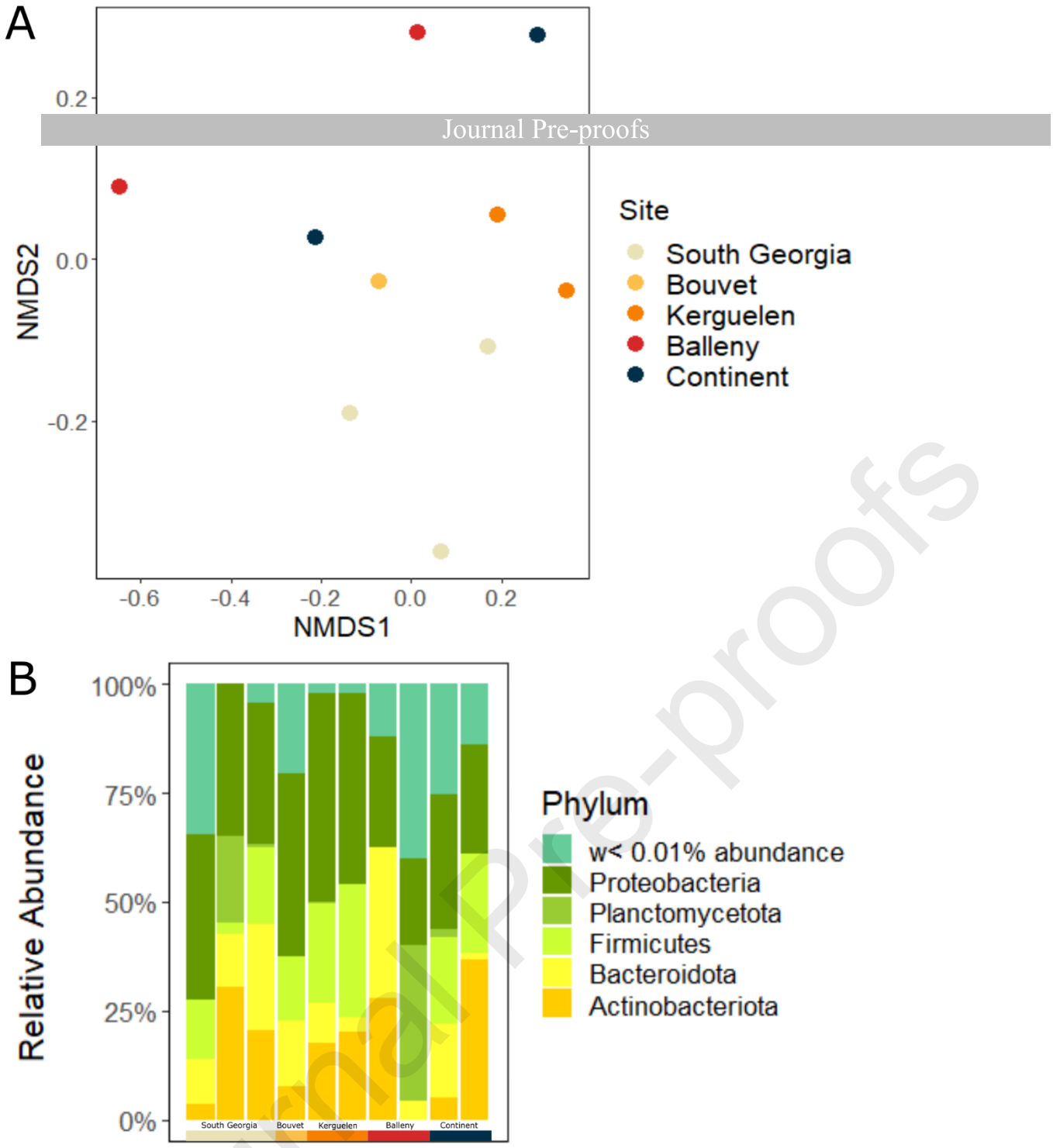

939

940 **Figure S3:** *The difference in microbial diversity of the air above terrestrial locations around the Southern*
941 *Ocean. A. NMDS of communities based on Bray Curtis dissimilarity. B. Phylum level diversity of Southern*
942 *Ocean Air communities above Southern Ocean Islands.*

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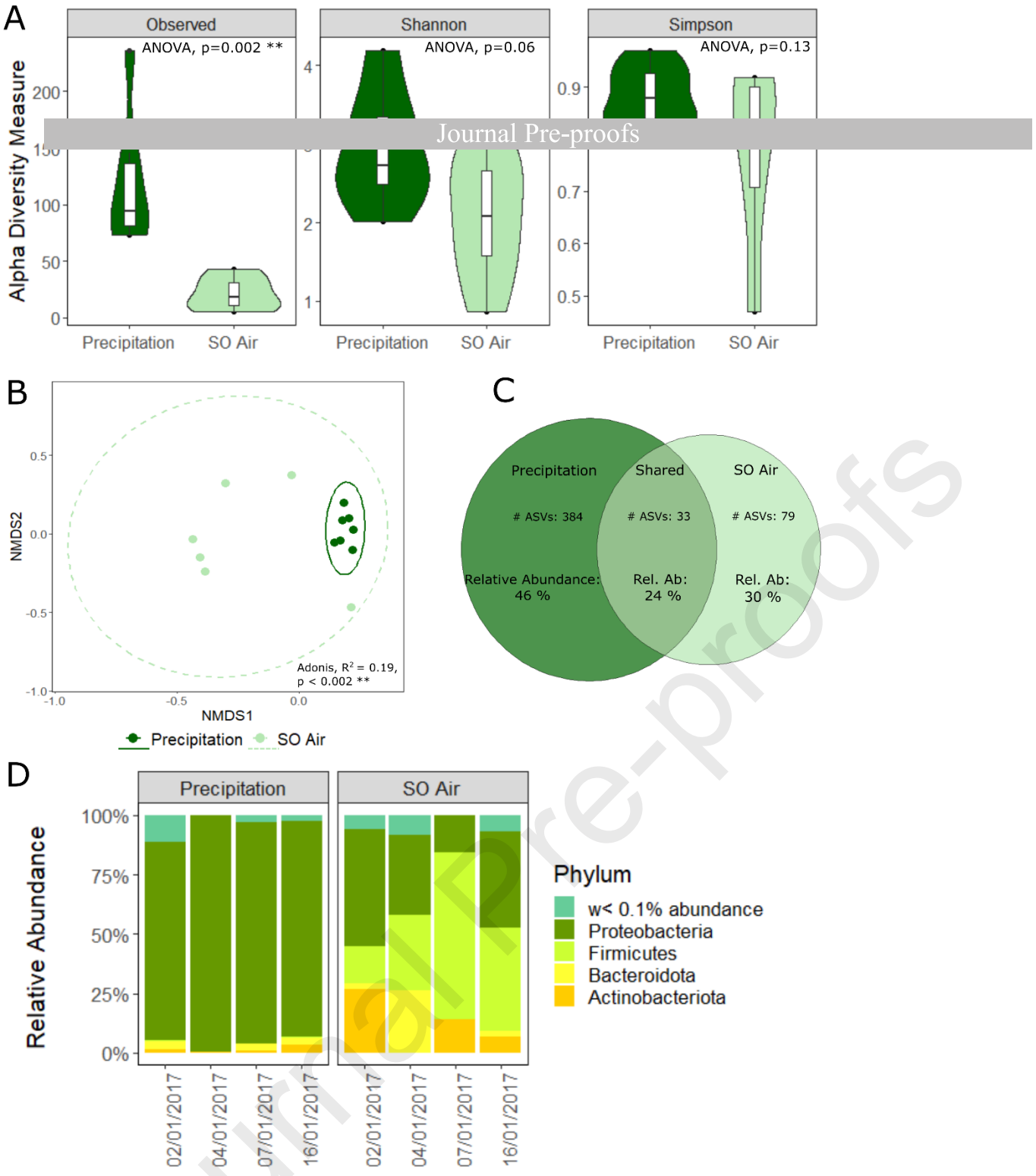

Figure S4: *The difference between microbial diversity in precipitation samples and air sampled on the same day as the precipitation. A. Alpha diversity comparisons. B. NMDS of communities based on Bray Curtis dissimilarity. C. Euler diagram illustrating the number of shared ASVs (# ASVs) between sample types, weighted by the relative abundance of ASVs in percentages (Rel. Ab). D. Relative abundance of taxa at the phylum level. Same day precipitation samples are aggregated.*

Supplementary Table 1: *Differences in microbial alpha diversity. For each column, subscript characters with the same letters (a,b,c,d,e) indicate no statistically significant difference (p > 0.05) with the Tukey HSD test.*

	Observed (mean ± SD)	Shannon index (mean ± SD)	Simpson index (mean ± SD)
SO Air (ACE)	27.5 ± 60.1 [a]	1.83 ± 1.04 [a]	0.70 ± 0.22 [a]
SO Precipitation	106 ± 94.3 [abc]	2.88 ± 0.68 [b]	0.88 ± 0.05 [b]
Global Air	30.6 ± 16 [a]	2.57 ± 0.59 [b]	0.85 ± 0.11 [bc]
Surface Open Ocean	233 ± 94.3 [b]	3.58 ± 0.53 [c]	0.89 ± 0.05 [bcd]
Coastal Ocean	185 ± 91.7 [abd]	3.56 ± 0.81 [bcd]	0.91 ± 0.07 [bcde]
Global Soil	560 ± 334 [e]	5.50 ± 0.83 [e]	0.99 ± 0.03 [be]
Arctic Soil	627 ± 347 [e]	5.52 ± 0.47 [e]	0.99 ± 0.008 [be]
Antarctic Soil	319 ± 108 [cd]	4.31 ± 0.43 [d]	0.97 ± 0.17 [be]

Supplementary Table 2: *Linear regressions of environmental variables against Southern Ocean air alpha diversity using observed ASVs (richness), Shannon index and Simpson index and PERMANOVA on beta diversity. * indicates statistical significance while ° indicates close to significance.*

	Richness		**Diversity**		**Evenness**		**Community composition**	
	R^2	P-value	R^2	P-value	R^2	P-value	R^2	P-value
Latitude	0.007	0.19	0.07	0.24	0	0.41	0.009	0.66
Longitude	0	0.63	0	0.38	0	0.85	0.008	0.94
Pressure	0.027	0.06°	0	0.70	0	0.70	0.012	0.049*
Temperature	0.027	0.06°	0	0.92	0	0.62	0.009	0.69
Relative humidity	0.009	0.18	0.03	0.29	0	0.88	0.010	0.35
Average wind direction	0	0.83	0.6	0.24	0	0.63	0.008	0.93
Average wind speed	0	0.71	0	0.93	0	0.58	0.010	0.35
Max wind speed	0	0.80	0.31	0.06°	0	0.79	0.010	0.38
Min wind speed	0	0.70	0	0.67	0.002	0.27	0.009	0.62
Dew point	0	0.51	0.03	0.29	0	0.63	0.011	0.22
Cloud level L1	0	0.97	0.13	0.17	0	0.51	0.010	0.28
Solar radiance	0	0.83	0.002	0.34	0	0.57	0.009	0.81
Cold/warm air	0	0.83	0	0.53	0.01	0.17	0.01	0.27
Max theta	0	0.89	0	0.65	0	0.36	0.01	0.038*
Median latitude	0	0.67	0.02	0.31	0	0.48	0.01	0.53

Supplementary Table 3: *Studies used to build the global database of 16S data*

STUDY	ACCESSION	TITLE
(GIL	Journal Pre-proofs	
		dynamics
(THOMPSON *ET AL.*, 2017)	PRJEB15217	Earth Microbiome Project
(THOMPSON *ET AL.*, 2017)	PRJEB18099	Earth Microbiome Project
(THOMPSON *ET AL.*, 2017)	PRJEB18643	Earth Microbiome Project
(THOMPSON *ET AL.*, 2017)	PRJEB19798	Earth Microbiome Project
(THOMPSON *ET AL.*, 2017)	PRJEB5714	Earth Microbiome Project
(MAYOL *ET AL.*, 2017)	PRJNA319484	Long-range transport of airborne microbes over the global tropical and subtropical ocean
(RUIZ-GONZÁLEZ *ET AL.*, 2019)	PRJEB25224	Higher contribution of globally rare bacterial taxa reflects environmental transitions across the surface ocean
(BAHRAM *ET AL.*, 2018)	PRJEB19856	16S metabarcoding data of global soil samples
(MALARD *ET AL.*, 2019)	PRJEB29109	Bacterial diversity and biogeographical patterns in Arctic soils

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Dear Environment International,

Please find attached a resubmitted manuscript addressing each of the points raised by all three reviewers in full (as outlined in the response to reviewer comment and track changes versions). We are aware that the reference list format still needs addressing and are happy to follow your specific guidelines on this.

With best wishes

David

Author contributions: Conceptualization: DAP, JS; Methodology: DAP, JS, LC, LC, LAM ; Data analysis and visualisation: LAM; Writing draft: LAM, DAP; Writing review and editing: JS, MLAJ

Competing interests: All other authors declare they have no competing interests

Data and materials availability: The DNA sequences from this project are deposited at the European Nucleotide Archive under the BioProject accession PRJNA697829. The global ASV table and associated taxonomy and metadata is available on FIGSHARE https://figshare.com/projects/Aerobiology_of_the_Southern_Ocean/140588 and includes the Southern Ocean samples.
Supplementary Data1: Southern Ocean metadata table including the environmental variables.
Supplementary Data2: Abundance and prevalence of ASVs with the associated taxonomy.
Supplementary Data3: Shared ASVs of Southern Ocean and global air samples.