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

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ABSTRACT

Parts of the Antarctic are experiencing dramatic ecosystem change due to rapid and record warming, which may weaken biogeographic boundaries and modify dispersal barriers, increasing the risk 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 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.

1. Introduction

Parts of Antarctica are warming at record rates, affecting both ecosystems and associated biota and processes (Convey and 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 and 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, 1996; Marshall, 1997; Marshall and Convey, 1997; 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.,

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2020). Whether this is the case and the implications 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 microorganisms may circulate around the continent several times before moving further south.

For decades, the focus has been on microorganisms (Lonsdale, 1999) with only recent interest in microbial invasions (Mallon et al., 2018; Kinnunen et al., 2016; Malard and 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 (Tedersoo 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 (Womack 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 (Seifried 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 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., 2020a,b). They participate in cloud formation, impacting precipitation patterns through ice nucleation (Fröhlich-Nowoisky et al., 2016, Šantl-Temkiv et al., 2019); therefore, airborne microorganisms can have an influence on cloud formation, radiation and precipitation (Sato and 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 the intensification and latitudinal shift of westerly winds (Perren et al., 2020, Liang et al., 2021). Thus, understanding the direct link between climatological conditions and biological 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 with 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.

2. Results and discussion

2.1. 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) [Fig. 1, Supplementary Data 1]. A total of 107 samples (±19,032 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., 2019a,b, Tignat-Perrier et al., 2019, Tignat-Perrier et al., 2020a,b, 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., 2019a,b), 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., 2019a,b, Archer et al., 2020, Tignat-Perrier et al., 2020a,b). 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

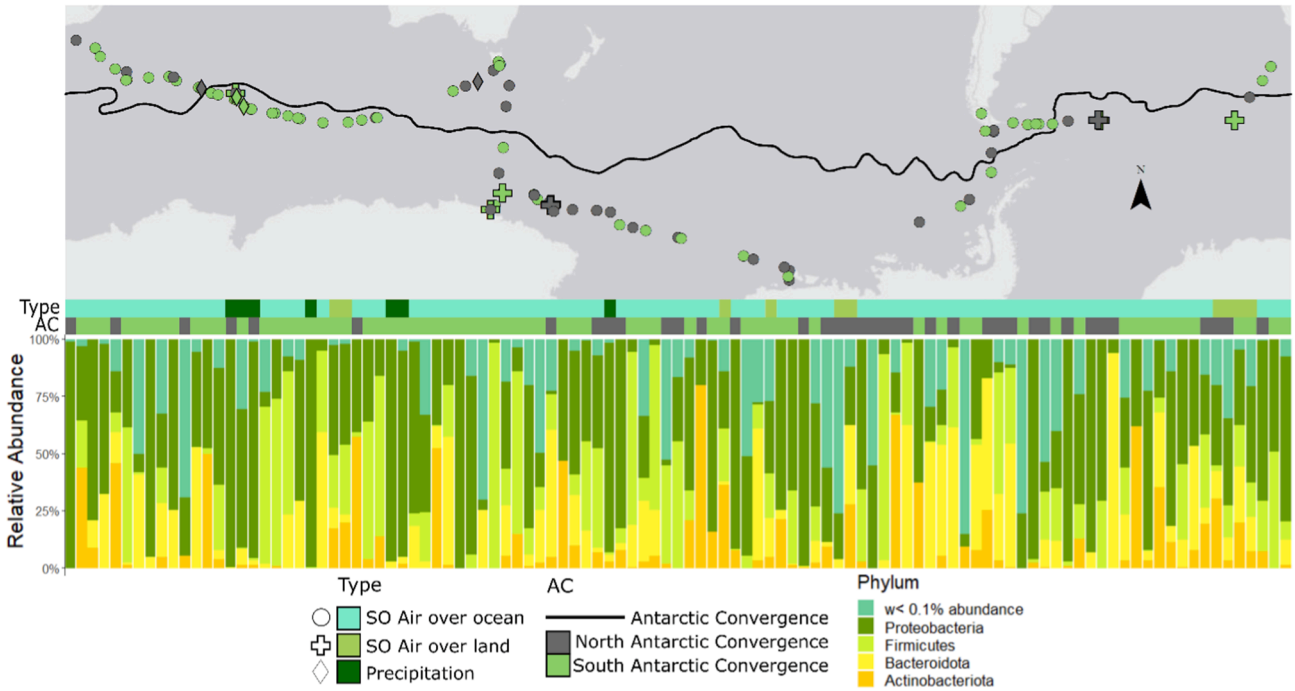

Fig. 1. Map of the air sampling locations, shaped by sample type and coloured by the origin of the air mass (back trajectory) relative to the Antarctic Convergence Zone. The relative abundance of bacterial taxa is given at the phylum level in the order of sampling. The microbial taxa dominating Southern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

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).

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

2.2.1. 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]. Regionally, Uetake 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).

2.2.2. 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. Uetake et al. (2020) looking at global patterns of bacterial diversity in the air found a latitudinal differentiation of composition at the phylum level. Here, we observed more Proteobacteria in global 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, $R^2 = 0.08$, $p < 0.001$).

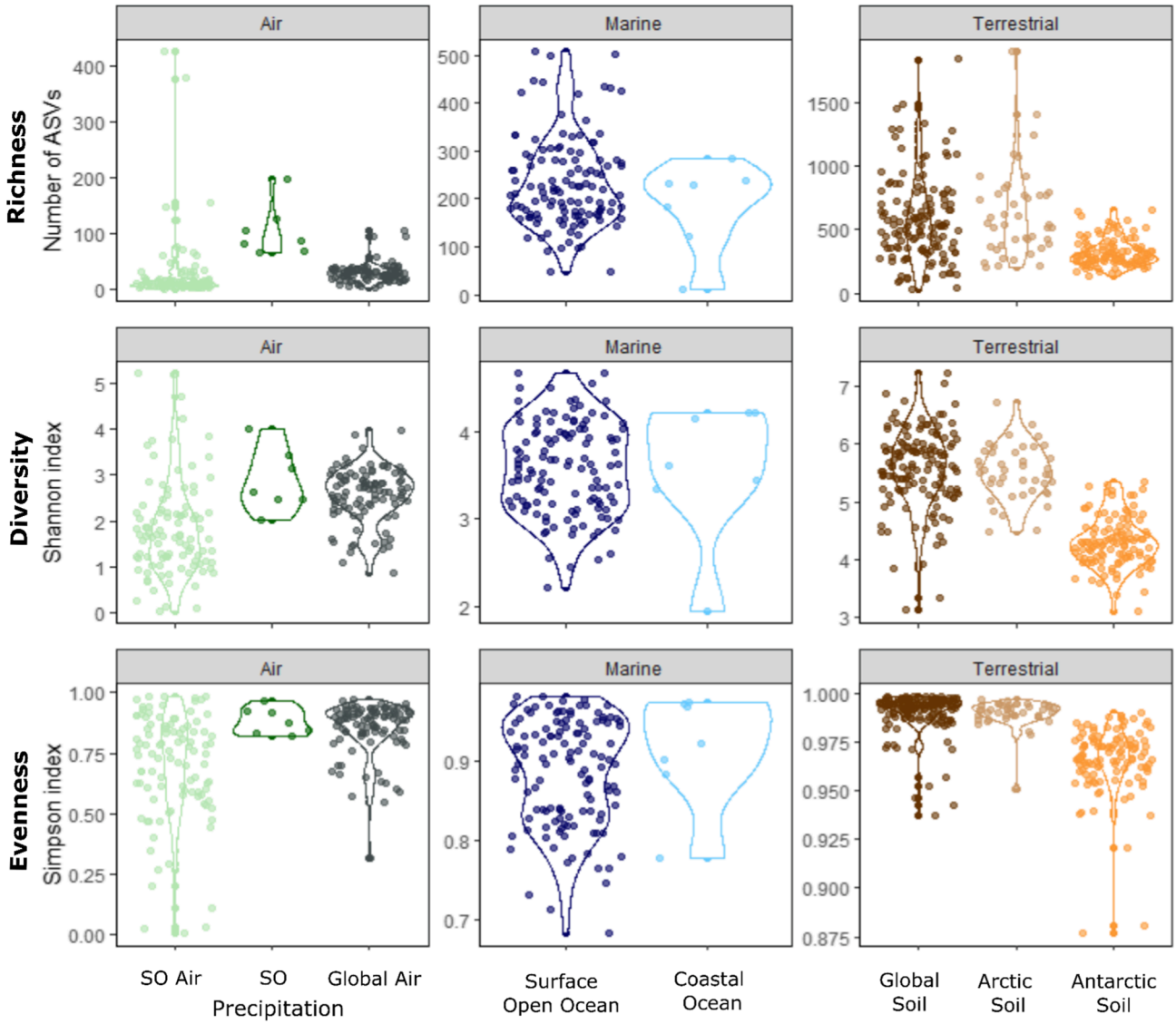

Fig. 2. Comparison of the alpha diversity of Southern Ocean air (SO Air, n = 100) and precipitation (SO Precipitation, n = 7) to other global air samples (global air, n = 98) and other non-air ecosystems including surface open ocean (n = 123), surface coastal ocean (n = 7), global soils (n = 146), Arctic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

2.2.3. 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 7.5×10^2 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 also 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 9.2×10^1 to 1.3×10^8 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 4700 m 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 3.4×10^4 cells to 2.67×10^5 m^{-3} (Xia et al., 2013). 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).

2.3. 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

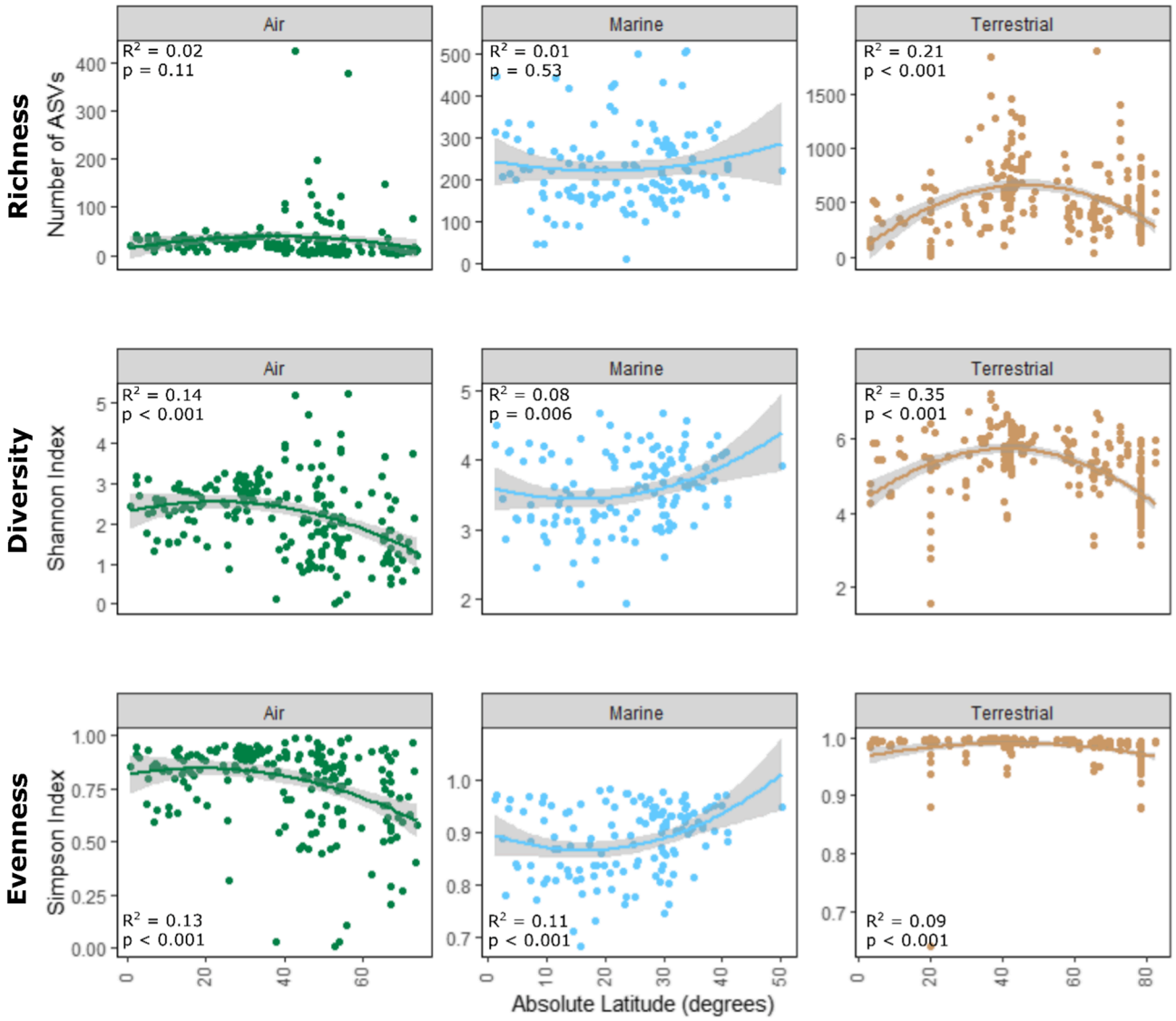

Fig. 3. Latitudinal distribution of the richness and diversity of air (n = 198), marine (n = 130) and soil (n = 302) bacterial communities. The absolute latitude was used, merging northern and southern latitudes. The fits of second order ploynomials are shown with R^2 and associated p-values.

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).

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. (2019a,b) 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 living 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 locations [Table 1].

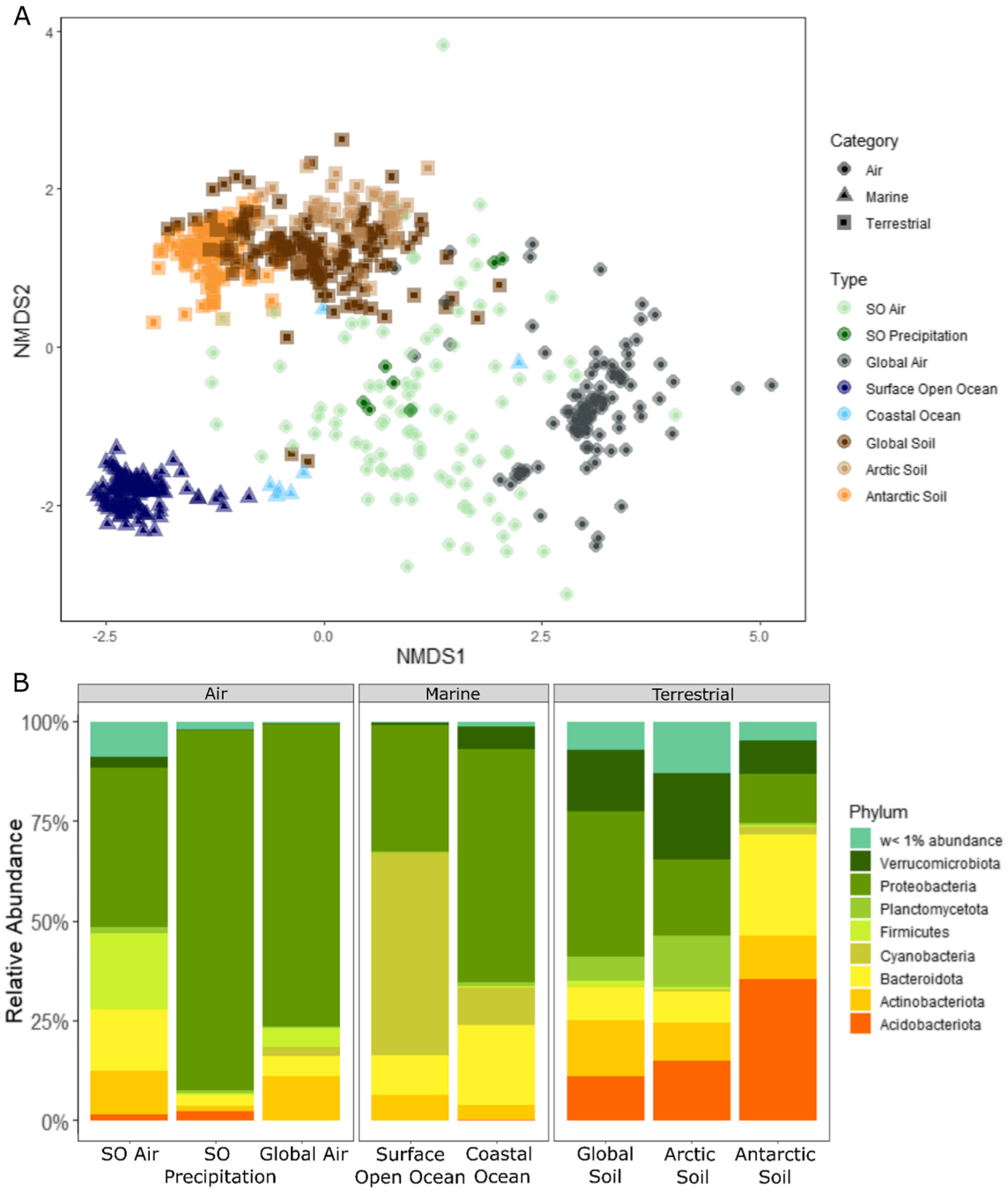

Fig. 4. A. Visualization of community dissimilarity using non-metric multidimensional scaling (NMDS) of the Bray-Curtis distance between air, marine, and soil bacterial communities. B. Relative abundance of bacteria at the phylum level.

2.4. 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 both 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 might 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).

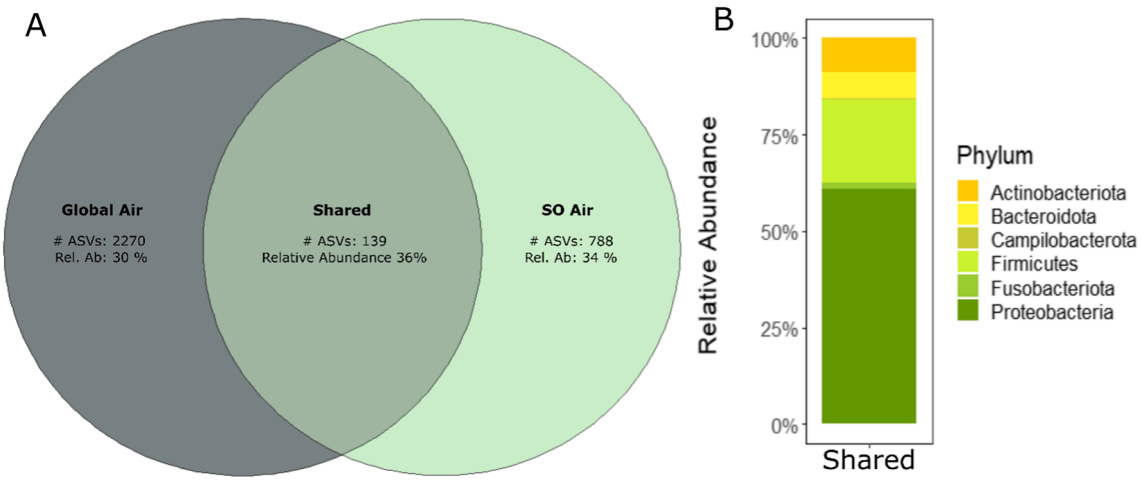

Fig. 5. A: number of ASVs (# ASVs) shared between global air and Southern Ocean air samples. The percentage indicates the relative abundance (Rel. Ab.) represented by the number of ASVs across all samples. B. Relative abundance of shared bacteria at the phylum level.

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

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, Tepidimonas*
Gram positive	*Blastococcus, Blastomonas, Brachybacterium, Carnobacterium, Corynebacterium* (7) and *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)

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

2.5.1. Pressure and cyclones

We tested the influence of several key environmental and meteorological variables on both 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 [Fig. 6A].

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.

2.5.2. 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

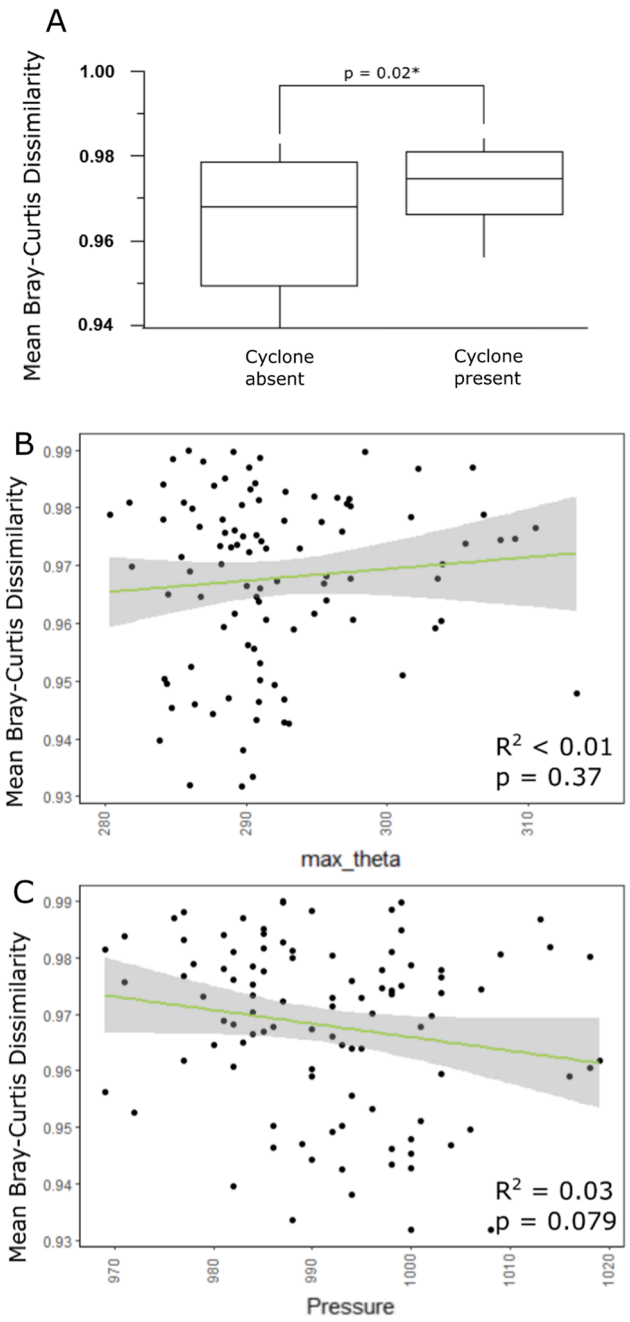

Fig. 6. A. Differences in community dissimilarity (Bray-Curtis) with the presence and absence of cyclones. B. Linear model of community dissimilarity (Bray-Curtis) along the maximum potential temperature of five-day back trajectories. C. Linear model of community dissimilarity (Bray-Curtis) along the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

events. For example, a study showed an increase in bacterial concentration 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., 2019a,b, Evans et al., 2019). Hence, 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 and 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) (Petters and Wright, 2015) Hence, precipitation samples might sub-select airborne 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 and Curry, 2010, Bracegirdle et al., 2020).

2.5.3. *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., 2020a,b), 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., 2020a,b, Archer et al., 2021 Preprint) but this was not observed for the Southern Ocean. Overall, observations tend to vary by study, likely reflecting the importance of local conditions at the time of sampling.

2.6. *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 the biodiversity of the air above them. Across the study, we found low numbers of ASVs of 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

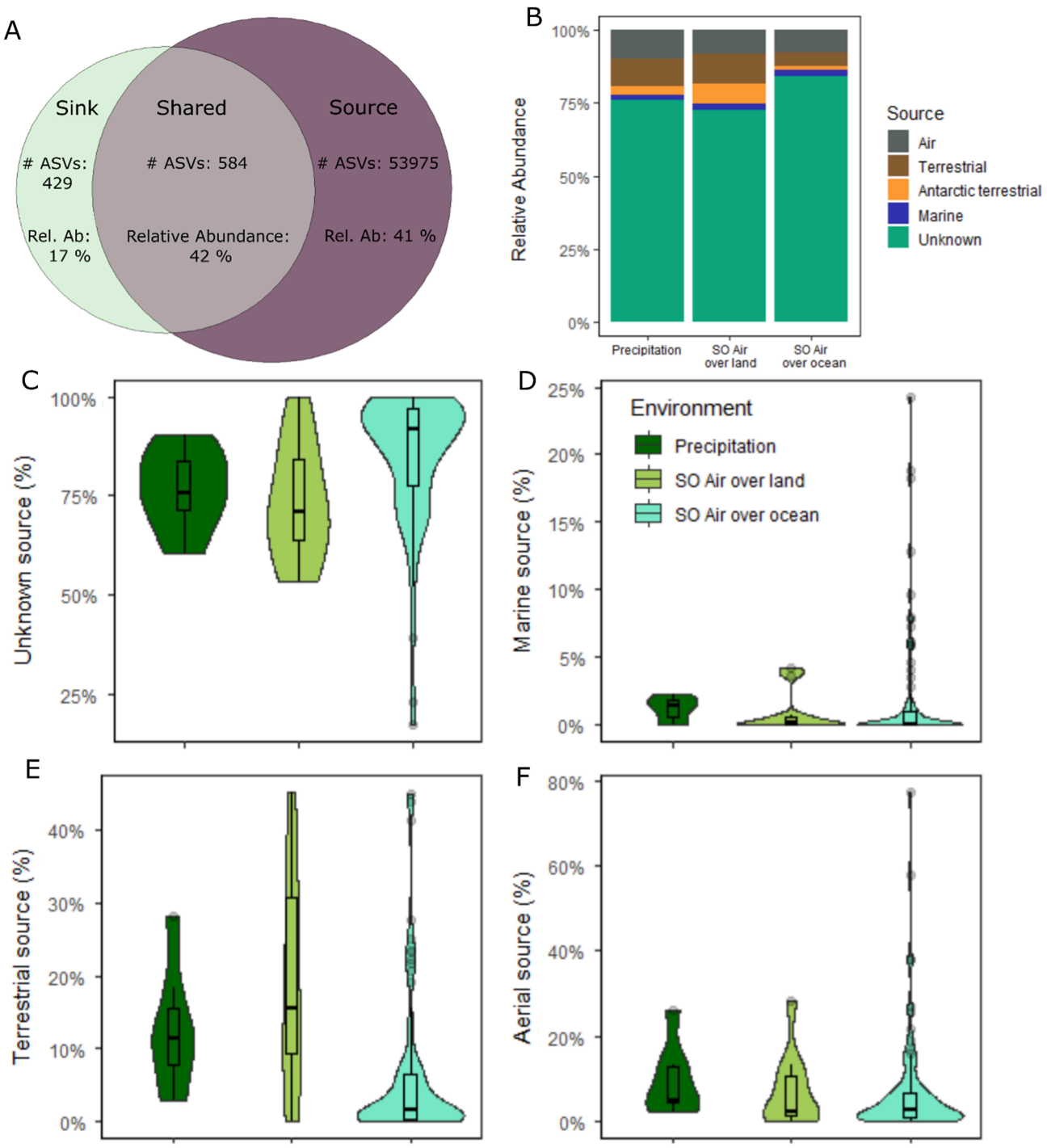

Fig. 7. A. Euler diagram showing the number of ASVs (# ASVs) with an origin identified from the global database and the relative abundance (Rel. Ab.). B. Mean potential origin of ASVs across each Southern Ocean sample type. C-F. Mean origin of ASVs per sample type.

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.

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

Finally, we tested the hypothesis that the Antarctic Convergence Zone 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 track 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 zone [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 zone. The majority of ASVs

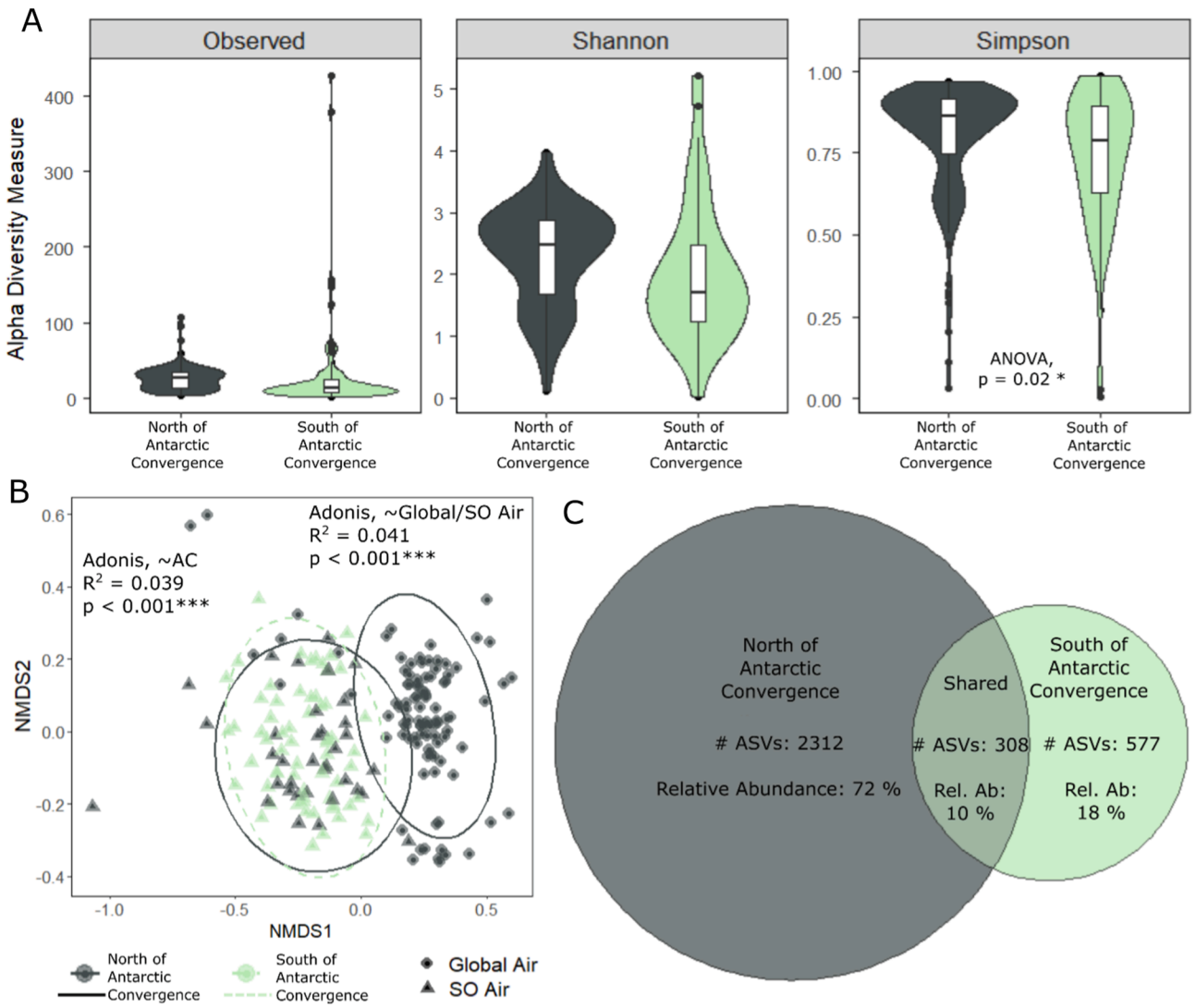

Fig. 8. Influence of the Antarctic Convergence Zone on microbial diversity above the Southern Ocean, using global air samples as well as the samples collected during the ACE expedition around the Southern Ocean. A. Alpha diversity comparisons B. NMDS of communities based on Bray-Curtis dissimilarity 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 Zone ASVs (# ASVs), weighted by the relative abundance (Rel. Ab.). of ASVs in percentages.

were unique to samples either north or south of the convergence zone, with 308 ASVs (9 % of the total ASVs) shared between both [Fig. 8C]. Hence, the Antarctic Convergence Zone 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.

3. 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 air above 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.

4. Materials and methods

4.1. 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, South Africa with stops at Hobart, Australia and Punta Arenas, Chile during the voyage [Fig. 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 radiation.

We used back trajectories (Thurnherr et al., 2020a,b) calculated with the Lagrangian analysis tool LAGRANTO (Sprenger and 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 at 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., 2020a,b, Thurnherr and Wernli, 2020) and as described in Thurnherr et al. (2021).

4.2. 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 [Fig. 1B]. Air samples from terrestrial locations were collected at the sub- and peri-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 1.5 m 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 h. 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 min. 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.

4.3. DNA extraction and 16S rRNA gene 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 (Coriolis) samples stored either in collection cones or falcon tubes were transferred to sterile 15 mL falcon tubes and centrifuged for a duration of 20 min 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 (Caporaso et al., 2011) 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).

4.4. 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 s and 53 °C for 30 s), 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 run 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 (A260/280 nm) 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) × 6.022 × 10^{23})/(Gene Length (bp) × 1 × 10^9 × 660), where 6.022 × 10^{23} represents Avogadro's Constant, 1 × 10^9 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 1 × 10^7 to 1 × 10^1, 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.

4.5. 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 were 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 (±19,032 reads/sample) with 1013 assigned ASVs.

4.6. Global database

We produced a global 16S rRNA gene database of marine, soil and air samples using data extracted from the NCBI database 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 conserved. 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).

4.7. Statistical analyses

All statistical analyses were performed in the R environment using primarily a combination of phyloseq (McMurdie and 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 and 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 to 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 interest 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.

CRediT authorship contribution statement

Lucie A. Malard: Methodology, Visualization, Formal analysis, Writing - original draft. **Maria-Luisa Avila-Jimenez:** Writing - review & editing. **Julia Schmale:** Co-Conceptualization, Methodology, Formal analysis. **Lewis Cuthbertson:** Methodology. **Luke Cockerton:** Methodology. **David A. Pearce:** Conceptualization, Methodology, Funding acquisition, Investigation, Project administration, Resources, Supervision, Writing - original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available upon request.

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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.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2022.107492.

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