Airborne bacteria confirm the pristine nature of the Southern Ocean boundary layer

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Microorganisms are ubiquitous and highly diverse in the atmosphere. Despite the potential impacts of airborne bacteria found in the lower atmosphere over the Southern Ocean (SO) on the ecology of Antarctica and on marine cloud phase, no previous region-wide assessment of bioaerosols over the SO has been reported. We conducted bacterial profiling of boundary layer shipboard aerosol samples obtained during an Austral summer research voyage, spanning 42.8 to 66.5°S. Contrary to findings over global subtropical regions and the Northern Hemisphere, where transport of microorganisms from continents often controls airborne communities, the great majority of the bacteria detected in our samples were marine, based on taxonomy, back trajectories, and source tracking analysis. Further, the beta diversity of airborne bacterial communities varied with latitude and temperature, but not with other meteorological variables. Limited meridional airborne transport restricts southward community dispersal, isolating Antarctica and inhibiting microorganism and nutrient deposition from lower latitudes to these same regions. A consequence and implication for this region's marine boundary layer and the clouds that overtop it is that it is truly pristine, free from continental and anthropogenic influences, with the ocean as the dominant source controlling low-level concentrations of cloud condensation nuclei and ice nucleating particles.

bioaerosol | marine aerosol | Southern Ocean

The atmosphere is a highly diverse microbiome (1–4). Microorganisms are ubiquitous throughout it, often at surprisingly high cell concentrations given the volume of air in which they are typically diluted. These airborne microorganisms may be transported thousands of kilometers by atmospheric winds due to the long residence time of typical cell sizes (5). The potential for intercontinental microbial transport is now appreciated (6) and specifically observed with Saharan (7–10) and Asian dust events (11). A proportion of airborne bacteria (16 to 40%) has been shown to remain viable after continental-scale transport (12), and deposition of airborne bacterial communities plays a crucial role in microbial dispersion (13, 14). With regard to atmospheric processes, certain microorganisms, or their by-products, also act as ice nucleating particles (INPs) (15, 16). INPs trigger the freezing of supercooled cloud droplets, which changes cloud reflectivity, affects the amount of energy in different atmospheric layers, and modifies the amount of radiation reaching the surface (17).

Complete characterization of the microbial assemblage in an aerosol sample, including spores (e.g., from moss) and pollen, is now readily achievable using high-throughput sequencing. This approach has been used by various teams to ascribe sources to atmospheric aerosols, but mostly in terrestrial air masses (2, 3, 11, 18–24). Knowledge of the abundance, distributions, and atmospheric relevance of marine bioaerosol remains more limited (6, 25–27).

The atmosphere over the Southern Ocean (SO), strictly considered to be the region south of 60°S but more loosely encompassing the region south of the seasonally fluctuating Antarctic Convergence Zone, is considered as pristine, particularly in Austral summer (28–30). The Antarctic circumpolar current and the atmospheric circumpolar vortex in the SO serve to form a major barrier to potential colonizers of Antarctica (31, 32).

In terms of cloud formation processes, oceanic emissions are therefore thought to be the main source of aerosol particles feeding low cloud formation over the SO region (33). While sea spray is clearly the dominant aerosol type by number and mass, bacteria may play a role if they serve as INPs or as giant cloud condensation nuclei. McCluskey et al. (34) noted that larger-diameter (>0.2-μm) marine INPs comprised heat-labile particulate organic carbon, potentially indicative of intact or fragmented microorganisms. Despite the potentially important role of SO bioaerosols, no previous region-wide assessment of bioaerosols and their sources in the high-latitude marine atmospheric boundary layer has been reported, limiting understanding of potential impacts on both ecology and cloud processes in this region.

In this study, we conducted bacterial profiling from air filter samples taken in the SO summertime atmospheric boundary layer during the Clouds, Aerosols, Precipitation, Radiation, and atmospherIc Composition Over the southeRn oceaN study. Samples of ambient aerosol (*SI Appendix*, Table S1) were collected between 12 January and 19 February 2018, during the research voyage of the Australian Marine National Facility (MNF) research vessel (R/V) *Investigator* that departed from

Significance

We found that the summer airborne bacterial community in the marine boundary layer over the Southern Ocean directly south of Australia is dominated by marine bacteria emitted in sea spray, originating primarily from the west in a zonal band at the latitude of collection. We found that airborne communities were more diverse to the north, and much less so toward Antarctica. These results imply that sea spray sources largely control the number concentrations of nuclei for liquid cloud droplets and limit ice nucleating particle concentrations to the low values expected in nascent sea spray. In the sampled region, the sources of summer cloud-active particles therefore are unlikely to have changed in direct response to perturbations in continental anthropogenic emissions.

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The authors declare no competing interest.

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Data deposition: Raw sequence data are available from BioProject (accession no. PRJNA577148) in the Sequence Read Archive of the National Center for Biotechnology Information.

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Hobart, Tasmania and sailed to within 40 km of the Antarctic ice edge (latitude 66.46°S) (Fig. 1), before returning. Herein, we use DNA sequencing and source tracking, complemented by atmospheric back trajectory analyses, to determine the origin of the boundary layer SO airborne bacteria and the broader implications of their composition and source.

Results

Taxonomy. Taxonomical results indicate a dominance of marine bacteria in the aerosol as well as a latitudinal differentiation of composition at the phylum level (35) (Fig. 2*B*). Samples were clearly separated by taxonomy into three regions: 1) North (from 44.2 to 50.7°S: SA1, SA2, SA23, SA3, SA4); 2) Middle (from 54.1 to 62.0°S: SA7, SA21, SA20, SA19, SA18, SA9, SA13, SA15, SA16, SA14); and 3) South (from 64.6 to 65.4°S: SA12, SA11, SA10). The North region was dominated by *Bacteroidetes* (45.5%) and included, at relatively higher abundances than in other regions, *Planctomycetes* (mean 19.7% of relative abundance) and *Verrucomicrobia* (2.8%), and *Euryarchaeota* in the domain Archaea (2.5%). The Middle region was dominated by both *Proteobacteria* (50.9%) and *Bacteroidetes* (46.3%), while the South region was dominated by *Proteobacteria* (90.1%) and *Patescibacteria* (7.8%), both occurring at much higher percentages than in the North and Middle regions. More than half of the genera were identifiable as probable marine bacteria (e.g., SAR92 clade, NS marine group, *Polaribactor*, *Aureispira*, *Altererythrobacter*, *Flavicella*) (*SI Appendix*, Fig. S1), while 45 of the 50 major amplicon sequence variants (ASVs) (*Materials and Methods*), which accounted for the majority of the sequences (average 75% relative abundance) (*SI Appendix*, Table S2), were identified by BLAST as having a marine origin (>99.2% sequence similarity with an isolate or sequence from a marine environment). The remaining five ASVs were associated with soil or freshwater sources. Indicator ASVs in each sample region are shown in *SI Appendix*, Fig. S2. The number of indicator ASVs is highest in the North ($n = 19$), followed by the Middle region ($n = 2$). Potential indicator ASVs in the South and cross-categories (e.g., common to multiple regions) were not found by the criteria used (IndVal value >0.5, P value <0.003). A BLAST search against the 19 North indicator ASVs found that 17 were marine bacteria from temperate regions such as Mediterranean Sea, East China Sea, and North and South Pacific Ocean and that 2 were chloroplasts of marine species of the green algae, *Chlorophyta* (*Prasinoderma singularis*: ASV 143; *Chloroparvula pacifica*: ASV 342, both with 100% BLAST pairwise identity). The two ASVs in the Middle latitude region were identical to polar marine bacteria (bipolar: ASV2; Antarctica: ASV5).

Diversity Measures. We found that species richness measures decreased to the south (farthest from rich continental sources) and confirm the latitudinal clustering of communities inferred by taxonomy alone (Fig. 2*A* and *SI Appendix*, Fig. S3). Alpha diversity measures provide overall indices of species richness for each sample (i.e., over the region traversed by the ship during each sampling period) and were characterized using the Chao1 and Shannon indices. Chao1 predicts the total ASV richness, while the Shannon index is a general diversity measure that is positively correlated with both overall richness and evenness, and is disproportionally sensitive to differences in abundance of rare ASVs. Alpha diversities were significantly different among latitudinal regions (ANOVA Chao1: $F = 13.34$, $P < 0.0001$; Shannon: $F = 34.91$, $P < 0.0001$). Both measures were significantly higher for the North region (Kruskal–Wallis test, $P < 0.05$) than in the other two zones. Further, both were significantly lower in the South region than in the other two more northerly regions (Kruskal–Wallis test, $P < 0.05$).

Beta diversity is used to assess the heterogeneity of communities and was characterized for our samples using the Bray–Curtis index. Ordination analysis of bacterial beta diversity (Fig. 2*C*) supports the clustering of communities into three distinct latitudinal bands (analysis of similarities $R = 0.74$, $P = 0.001$), similar to those proposed by taxonomical differences.

Statistical Analysis with Meteorological Data. Latitude, air temperature, and water temperature were strongly positively

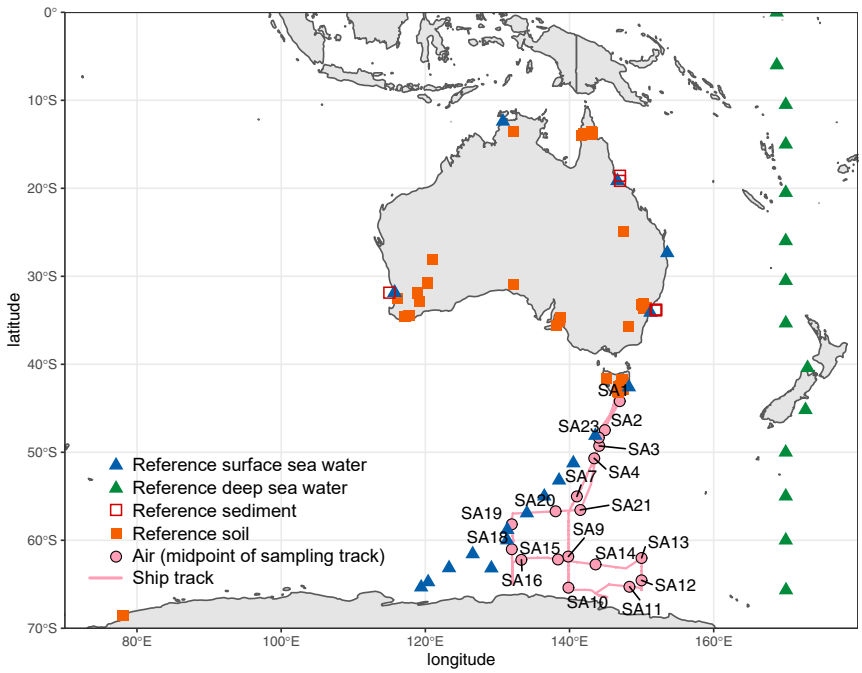

Fig. 1. Ship track, air sampling sites (SA#), and locations of collection of reference samples: Australian and Antarctic soils, ocean sediments, surface sea water, and deep sea water. Further details on samples and references are in *SI Appendix*, Table S1.

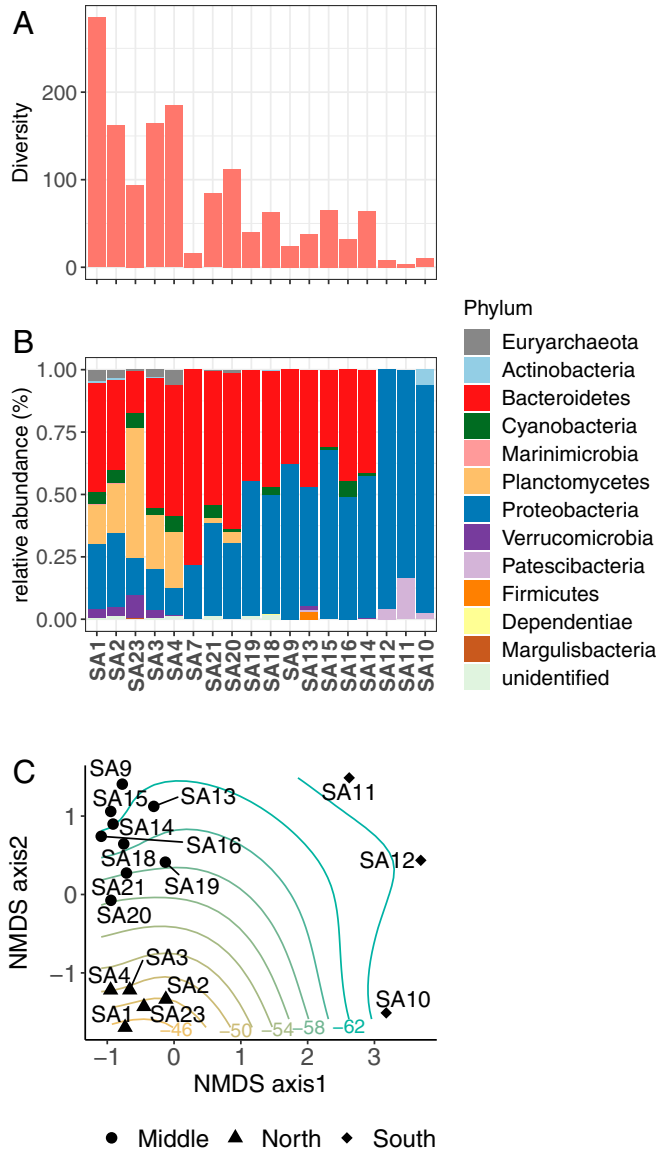

Fig. 2. (*A*) Sample-to-sample variability of alpha diversities (Chao1) of airborne bacteria. Samples have been ordered from lower to higher latitude (left to right). (*B*) Sample-to-sample variability of bacterial communities at the phylum level. Samples have been ordered as in *A*. (*C*) NMDS ordination of the samples shown in *A* and *B*, based on Bray–Curtis dissimilarities, with latitude contours (degrees South) overlain.

correlated (*SI Appendix*, Fig. S4) with both alpha diversity measures ($P < 0.0001$); other meteorological factors were not significantly correlated ($P > 0.01$). Pairwise correlations among latitude, air temperature, and water temperature were strong and positive (latitude–air temperature: $r = 0.98$; latitude–water temperature: $r = 0.99$; air temperature–water temperature: $r = 0.97$). Since both air and water temperatures decrease as latitude to the south increases, due to solar radiative effects, we selected each of these factors for separate multivariate analysis to avoid cross-correlation effects. Results of redundancy discriminate analysis (RDA) (*Materials and Methods*) to evaluate differences between bacterial communities (i.e., beta diversity) on the basis of seven meteorological factors (*SI Appendix*, Table S3 *A–C*) showed that latitude was the most significant factor (RDA $F = 1.82$, $P = 0.024$ in *SI Appendix*, Table S3*A*) followed by air temperature (RDA $F = 1.71$, $P = 0.046$ in *SI Appendix*, Table S3*B*).

Source Tracking. Seasonality will strongly influence bacterial communities. Since our methodology precluded testing seasonality as an environmental variable, we have used mainly summertime reference samples for source tracking. Five samples (SA1, SA23, SA4, SA18, and SA19) had sufficient DNA for amplification with the 27F/519R primers. SA1, SA23, and SA4 were from the North region, while SA18 and SA19 were from the Middle. Fig. 3 shows the results from SourceTracker2 analysis, indicating the relatedness of each air sample to the diverse reference samples (*SI Appendix*, Table S1: nearby surface sea water, deep sea water, ocean sediment, Australian and Antarctic soils, freshwater lake, sludge, human stool). The results suggest that emissions from surface sea water from the same latitude band strongly contributed to the bacterial communities in all five samples (33.4 to 91.0%). Further, contributions often were associated with a broader latitude band than that in which the sample was obtained (Fig. 3, gray shaded regions), reflecting the natural range, across space and time, over which the species occur. Most of the remaining portions (9.6 to 69.2% of the detected ASVs) were classified as "Unknown" because they did not occur within our selected references. However, all ASVs classified as Unknown—except from SA23, a northerly air sample—were identified as marine origin by using a BLAST search against GenBank. The Unknown portion of SA23 contained four ASVs (6.6% of the total) attributed to moss and two ASVs (1.3%) associated with freshwater bacteria. All other 46 ASVs in SA23

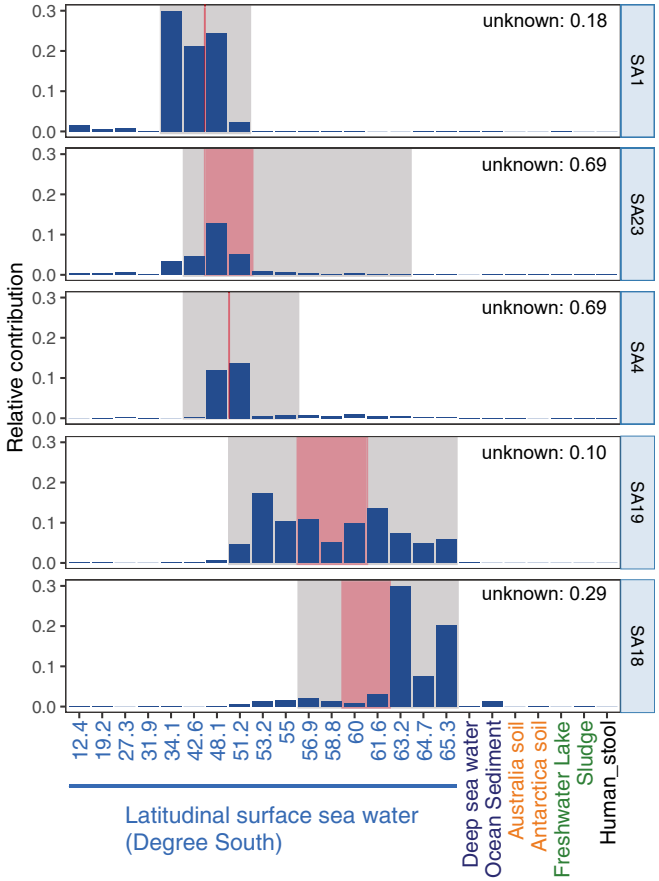

Fig. 3. Relative contribution from possible sources to air samples using the V1 to V3 region of the 16S rRNA gene. Red shading/lines show the ranges of ship latitudes during filter collections, and gray shading shows the latitude ranges spanned by 72-h back trajectories from each sampling site. The fractions of unknown sources are displayed in the upper right of each panel. All of these unaccounted for ASVs were confirmed as marine bacteria using BLAST searches.

were marine sequences. All of our samples had very low contributions of bacteria typically occurring in soil (0 to 0.06%), in freshwater (0 to 0.03%), and from humans (0 to 0.03%).

Air Mass Back Trajectories. The 3- and 10-d Hybrid Single Particle Lagrangian Integrated Trajectory Model (HYSPLIT) back trajectories for air masses arriving at the surface at the ship latitude and longitude are similar for two different input meteorological datasets (Fig. 4) and show distinct spatiotemporal differences along the ship sampling track (Figs. 1 and 4 and *SI Appendix*, Fig. S5). Air masses sampled in the North and Middle regions were transported from the western open ocean, remaining mostly within the marine boundary layer west of the sampling location, while air masses in the South region were transported from over or close to Antarctica.

Discussion

The taxonomic composition of air samples from the Middle region was very similar at the phylum level to that found in the reference database seawater sequences (*SI Appendix*, Fig. S6). However, the North region air samples were distinguished by a high abundance of *Planctomycetes*. This phylum is generally found in high-productivity open ocean and is also relatively more abundant in the mesopelagic zone (200- to 2,000-m depth) (36). All 44 major *Planctomycete* ASVs were closely related to uncultured marine species, such as from studies of the northeast subarctic Pacific Ocean, a Vancouver Island inlet, the California coast, and the SO (37–40). Among these, the subarctic samples were taken from the bathypelagic zone (>2,000 m), while other samples were taken from the epipelagic zone (10 to 100 m). That is, all of the *Planctomycete* ASVs were most closely related to isolates/sequences obtained below surface seawater. It is possible that their relatively high occurrence is associated with an upwelling event that occurs around Tasmania (41) between January and March. The *Bacteroidetes* were notably absent from any South region samples (*SI Appendix*, Fig. S1). This phylum, and especially its *Flavobacteriia* class, is one of three phylogenetic groups found to consistently dominate bloom-associated bacterial communities (42); they specialize in decomposing complex organic matter (43). While their absence could be partly attributed to a reduced abundance in the region's waters, it is likely that it is also strongly related to the trajectories of the air masses sampled, as discussed below.

Taxonomic composition at the genus level, detailed ASV analyses, and source tracking analysis all suggested that the bacteria in our air samples were predominantly from marine sources. The majority of the 50 most abundant ASVs were most closely related to bacteria isolated or obtained from seawater, while source tracking analysis indicated that 91.8 to 99.9% were marine (30.5 to 90.3% from colocated SO surface waters and 9.6 to 69.2% from more distant sources or different years). Although a large marine influence was also found by Archer et al. (44), who studied airborne bacteria and fungi over the Great Barrier Reef, other marine bioaerosol studies typically show a large influence of terrestrial bacteria. For example, air samples taken over the North Sea and the Baltic Sea (26) showed an influence from both marine and coastal environments, whereas over the northern Chinese marginal seas and the northwestern Pacific Ocean, Ma et al. (25) demonstrated that bioaerosol sources were mostly continental. In both studies, the sampling locations were much closer to terrestrial sources than in our SO study, and HYSPLIT analysis indicated that back trajectories had passed over terrestrial areas within 72 h prior to sampling. It has been estimated that around 10% of prokaryotes would remain suspended after 4 d aloft (5). Mayol et al. (6) showed that terrestrial bacteria typically dominated the boundary layer marine aerosol in remote regions of the western Pacific and Indian Oceans and ascribed this to long-distance microbial transport. Because their

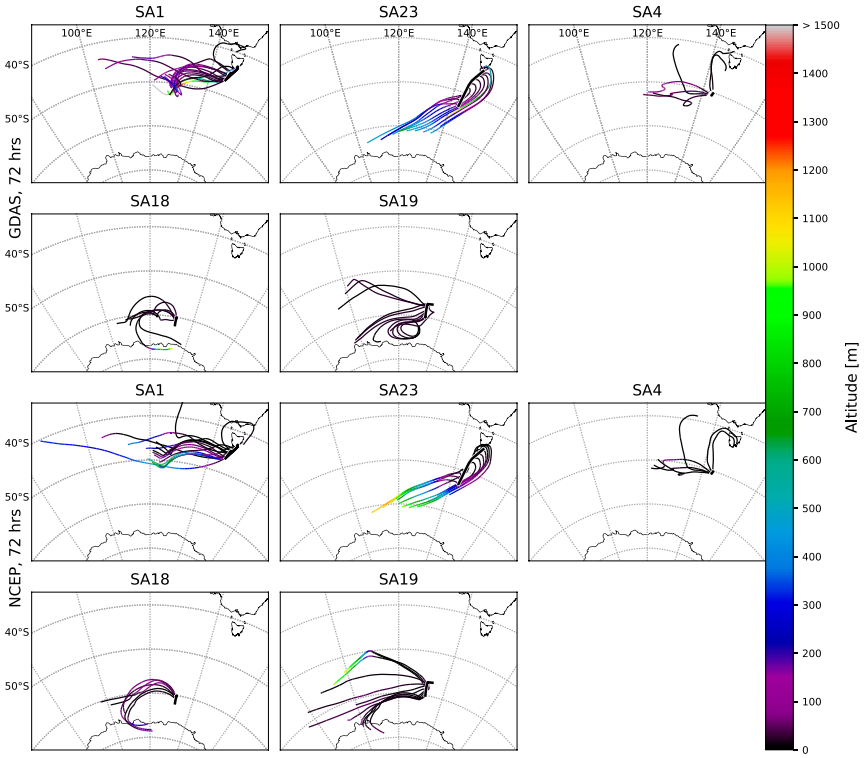

Fig. 4. Three-day back trajectories calculated using HYSPLIT and two different meteorological datasets (GDAS 1° and NCEP/NCAR reanalysis), initiated at each sampling date and location, and 25-m altitude.

study was conducted at lower latitudes, a higher continental influence would be expected.

Dust from inland Australia is a major source of airborne bacteria in Australian terrestrial regions (45), and therefore, samples along the northern edge of our sampling tracks (SA1 and SA23, which passed very close to Tasmania, as well as SA4) were shown in HYSPLIT analyses to have periods influenced by air masses that had passed over Tasmania or southeast Australia (Fig. 4). These were expected to be most influenced by such dust sources. For this reason, we included Australian soils as reference samples (Fig. 1). However, SA1 and SA23 were also dominated by marine bacteria and had very low contributions from Australian and Antarctic soil bacteria (0 to 0.06%). Prior studies concluded that the strongest transport from Australia to high latitudes and lower altitudes occurs in wintertime (46), consistent with our findings that suggest very little impact in the marine boundary layer during summertime. Our findings are also consistent with a summertime minimum in frequency of arrival of continental air masses at Macquarie Island (160°W) (47), and with very little transport of New Zealand dust west of 150°W longitude (48). For sample SA23, moss sequences accounted for 6.6% of the total. Moss spores are readily lofted with winds and are better adapted by shape for airborne transport than bacteria. This dispersal ability likely underlies the close floristic affinities shared by mosses in the Southern Hemisphere (49). Overall, these results suggest that samples collected on the ship during the period of this study were typically influenced by westerly winds passing over the SO, with no notable land influence.

The community structure of airborne bacteria is driven by meteorology, location, time (time of the day and season), and atmospheric composition (4, 50). In marine environments, wind direction, wind speed, and temperature influence organismal diversity (25, 26). Among these factors, wind speed is correlated with aerosol production. Hu et al. (51) showed that both viable and total bacterial concentrations in surface air correlated strongly with wind speed, and that correlations were significantly higher for winds above 5.4 m s^{-1} (i.e, at which wave breaking occurs). Alpha diversities in this study did not, however, correlate with wind speed and wind direction at the sampling location but were correlated with latitude and its correlated factors (air temperature and water temperature) (*SI Appendix*, Fig. S4). The RDA for beta diversity also showed that latitude and air temperature are the most influential environmental factors, whereas wind speed and wind direction again were not significant (*SI Appendix*, Table S3). Therefore, latitude and air temperature were the most important controls on airborne bacterial composition and diversity, possibly due to their association with ocean productivity. This finding is consistent with those of Seifried et al. (26), who found that sampling location (i.e., latitude, longitude, and temperature) was the most influential environmental factor controlling composition. The weak relation with other local meteorological data suggests that the cumulative emissions over wider upwind regions were often strong contributors to the sampled populations.

Our source tracking analysis of five samples spanning a range of latitudes showed very high contributions (91.8 to 99.9%) from marine environments, especially from nearby regions and those ±3° away from the sampling track latitude. This was most evident with two of these, samples SA18 and SA19, both of which were in the Middle region. In general, the best match between air mass histories and the latitudes of the identified seawater sources was in 72-h back trajectories rather than for shorter (24-h) or longer (240-h) periods (Fig. 4). This optimal 72-h time range roughly corresponds to the estimated bacterial atmospheric residence time reported in previous studies (e.g., 1 to 5 d in ref. 52 and 4 d in ref. 5). The upwind source regions predicted by both source tracking and 72-h HYSPLIT analysis extended to more than 1,000 km from the sampling sites, much farther than the source estimates of Tignat-Perrier et al. (4), who found that airborne bacteria and fungi were affected by land within 50 km at nine different meteorological stations surrounded by land and ocean. Also, the cumulative time that the air mass spends over different potential emission areas and the height of the air mass along its back trajectory impact the influence of surface sources on the aerosol composition. Most of the air masses associated with the low-alpha diversity samples in the South region had trajectories that had spent considerable time over ice-covered areas of the Antarctic continent, where one might expect emission rates of bacteria to be quite low (*SI Appendix*, Fig. S5). Ice on the Antarctic continent contains few microorganisms, contributing only minimally to airborne bacteria observed in a downstream environment (53). Although some trajectories (SA18 and SA19) may have passed over ice-free regions, the estimated contributions from highly diverse Antarctic soil (19) were either undetectable or very low (0.03%) based on source tracking analysis. Therefore, based on the air mass trajectories, the bacterial input into samples in the South region is expected to be much smaller than for other regions; accordingly, we found low alpha diversities in those samples.

This study reports data for airborne bacterial communities across a wide latitude range over the SO and for longitudes with low inputs of dust during summer. Our evidence that the source of the bioaerosol is overwhelmingly marine contrasts with findings from other ocean regions, suggesting that the circumpolar circulation in the SO creates distinct conditions. We found that the bacteria in marine boundary layer aerosol were dominated by sea spray sources, and with minimal contributions from other potential sources, such as Australian and Antarctic soils. Beta diversity of airborne bacteria was affected primarily by latitude and air temperature at the ship. The typically westerly origin of airmass locations (within the boundary layer) 72 h upwind of the sampling site thus had a strong, cumulative effect on the airborne bacteria.

Our results provide evidence that latitudinal and longitudinal (from 100 to 150°E, the approximate range encompassed by the 72-h back trajectories) oceanographic zones constrain bacterial composition, suggesting that biogenic INP composition and abundance in the summertime boundary layer over this region of the SO will be similarly stratified. Furthermore, the low probabilities of transport of continental air and dust to the marine boundary layer in this study region during summer suggest minimal direct anthropogenic impacts (pollution or soil emissions driven by land use change) on cloud properties. Any such impacts must occur through free tropospheric transport and exert an influence on clouds from above, yet the relative absence of continental microorganisms likewise implies that such mixing and entrainment are limited over larger scales. Rather, marine aerosols likely provide the main source of cloud-active particles. Our findings therefore imply latitudinal variability in aerosols controlling the microphysical properties of SO clouds (34), suggesting corresponding variations in cloud properties that are strongly linked to ocean biological processes. While longitudinal coverage was limited in this study, we might also expect longitudinal changes in aerosol due to, for example, transport downstream of land masses. These results provide observational support for the suggestion by Hamilton et al. (29) that this region of the SO represents one of very few marine boundary layer regions across the globe that is unlikely to have changed due to anthropogenic activities.

Materials and Methods

Filter Preparation and Air Sampling. We used Whatman Nuclepore track-etched polycarbonate membrane filters (0.4-μm pore size collection membrane overlying an 8-μm support membrane, 47-mm diameter; MilliporeSigma) fitted within sterile open-faced filter holders (Nalgene sterile analytical filter units, 130–4020; Thermo Fisher Scientific) to collect aerosols. We sterilized sampling and support filters by soaking in 10% H_2O_2 for

10 min, followed by three rinses in 0.1-μm pore-filtered deionized water, and drying on aluminum foil in an ultraviolet (UV)-sterilized laminar flow hood. We immediately loaded filters into the filter units and then repackaged the filter units in sealed plastic bags. We opened filter units on deck and mounted them beneath a rain shield during sampling for ∼24 or 48 h from the uppermost deck (∼23 m above sea level) of the R/V *Investigator*. Initial flow rates averaged 39 standard liters per minute (0 °C, 1,013 millibar), decreasing over time according to particle loading conditions. Average accumulated volume was 50.5 m^3. To reduce contamination from the ship stack and other scientific activities, we employed an automated sector sampler to turn the pump off if the wind speed was less than 10 kn, greater than 80 kn, or coming from the rear 270° of the ship. After sampling, we removed filters from the filter units in a laminar flow hood with sterile forceps, placed them in sterile petri dishes sealed with Parafilm, and stored them at −20 °C until processing.

Laboratory Contamination Removal Strategies. Because the SO air is exceptionally clean and its bacterial concentration low, we took great care to minimize DNA contamination during processing (54, 55). We conducted all processes prior to PCR inside a horizontal laminar flow hood (Table top work station) in the aerosol laboratory of Colorado State University's Department of Atmospheric Science (i.e., in a building not used for molecular biology studies). We irradiated the work area with UV light overnight and wiped handled equipment with DNA AWAY (Thermo Fisher Scientific) just before extractions. We sprayed the rotor of the minicentrifuge (Centrifuge 5424; Eppendorf), placed permanently within the laminar flow hood, with hypochlorite solution before use. We decontaminated all tube racks and pipettes by UV irradiation within a UV box (M-2009), and individuals handling the samples wore clean room laboratory coats (KIMTECH PURE A7; Kimberly-Clark) and nitrile gloves (MK-296; Microflex). Nitrile gloves also were carefully wiped with DNA AWAY and then deionized with a static remover (SJ-H036A; Keyence). Tubes (e.g., 1.5-mL tubes and PCR tubes) were deionized before use. Each PCR included PCR-negative controls, and amplicons were not detectable with High Sensitivity D1000 ScreenTape (Agilent Technology) used for electrophoresis. We compared all sequences with potential contamination sequence data in our laboratory obtained from prior projects that used the same methods and equipment (SI nega: DNA extraction controls, TO-nega: PCR control in PRJNA577148), but none matched any in this study.

DNA Extraction, PCR, and DNA Sequencing. For DNA extraction, we first cut a filter into strips with flame-sterilized stainless steel scissors and transferred the strips into sterile 5-mL tubes with 2 mL of Nuclease-Free water (AM9937; Thermo Fisher Scientific). Particles on filters were resuspended with a minishaker (MS1 Minishaker; IKA) for 2 min after a 30-s sonication. We then concentrated the 2 mL of suspension into ∼200 μL with precleaned Microcon DNA Fast Flow Centrifugal Filter Units (MilliporeSigma) and used the DNeasy PowerLyzer Microbial Kit (Qiagen) to extract DNA; for DNA extraction, we used a high-recovery modification to the standard protocol (56). For initial PCR, we used a low DNA-containing enzyme (AmpliTaq Gold DNA polymerase, Low DNA; Applied Biosystems), which is essential to minimize false positives. PCR targeted the V1 to V3 and V4 to V5 regions of the 16S ribosomal RNA (rRNA) gene with primers 27F/519R (57) and 515yF/926pfR (58) with barcodes and Illumina adapters. We used the 27F/519R sequence only for source tracking analysis with reference samples (details in *ASV Source Tracking*), whereas we used the more universal primers 515yF/926pfR for all other bioinformatic analyses. PCR conditions comprised 37 cycles of denaturation at 95 °C for 15 s, annealing at 50 °C for 15 s, extension at 72 °C for 40 s, and an additional final extension at 72 °C for 7 min for 27F/519R; we used a GeneAmp PCR System 9700 (Applied Biosystems) for PCR. We used the same PCR conditions with the 515yF/926pfR primers, apart from annealing at 46 °C. We sent all initial PCR products to RTL Genomics for library preparation and sequencing by MiSeq (Illumina).

ASV Analysis and Alpha and Beta Diversity. All sequence libraries from samples, negative control filters, and PCR negatives as well as reference sequences for source tracking (only for the V1 to V3 region) were clustered together into ASVs with the R package "DADA2" (59). On average, 13,376 sequences per sample (maximum: 26,694, minimum: 4,371) were used for analyses.

Taxonomy was assigned using The Ribosomal Database Project Classifier tool (60), implemented using DADA2 accessing the Silva 132 rRNA database for sequence identification (61). We used DADA2 to remove all potential chimeric sequences and Qiime2 (62) to analyze alpha diversities. We analyzed beta diversity, nonmetric multidimensional scaling (NMDS), and RDA with the "metaMDS" and "adonis" functions of the R package Vegan (63). NMDS visualizes beta diversity by mapping nonparametric monotonic relations between the dissimilarities (Bray–Curtis in this study) in the sample–sample matrix and the Euclidean distances between samples in two-dimensional ordination space (64). We used Geneious R10 (https://www.geneious.com) to check the closest relatives of our ASVs by basic local alignment search tool (BLAST) against the National Center for Biotechnology Information (NCBI) database. Raw sequence data (fastq file) are available from BioProject (accession no. PRJNA577148) in the Sequence Read Archive of the NCBI (https://www.ncbi.nlm.nih.gov/sra/) (35).

ASV Source Tracking. We used SourceTracker2 (65) to estimate possible sources in the regions of our voyage sampling tracks. Seasonality is expected to be an important control over bacterial abundance and type, and therefore, surface sea water reference samples (78 samples) taken from the literature were from studies occurring in the same season [from 5 January to 28 February in 2017; PRJNA385736 (66)]. Twenty-eight deep sea water and 12 ocean sediment samples were also taken from PRJNA385736. We included 61 and 12 soil samples from the Australian continent, Tasmania and Antarctica (PRJNA317932 and PRJNA417164) (67–69), 4 bioreactor sludge samples [PRJNA317604 (70)], 10 freshwater lake samples [PRJNA356946 (71)], and 11 human stool samples [PRJNA298846 (72)] as source tracking reference samples. BioProject and accession numbers for these source tracking reference samples are listed in *SI Appendix*, Table S1.

Meteorological Data. Meteorological data (air temperature, water temperature, precipitation, humidity, atmospheric pressure, wind speed, and wind direction) were measured primarily from the vessel's foremast at a height of 24 m from the waterline (73). We used the R package "htmltools" for time series visualization of these data (*SI Appendix*, Fig. S7). The MNF ship underway data (e.g., ship navigation, meteorology) are available for download from the MNF data archive (https://mnf.csiro.au/en/MNF-Data; R/V *Investigator* voyage IN2018_v01).

Backward Trajectories. We calculated the three-dimensional trajectories of air masses arriving at the ship along its sampling tracks using the HYSPLIT (74) using gridded meteorological data from both the Global Data Assimilation System (GDAS 1; 1° grid) and the National Center for Environmental Prediction (NCEP)/National Center for Atmospheric Research (NCAR) reanalysis (NCEP/NCAR; 2.5° grid). Three- and 10-d back trajectories were initiated every 4 h after the start of each filter sampling period and initialized with the corresponding location of the ship. Trajectory arrival height at the ship was set as 25 m, the approximate elevation of the filter sampler.

Data Analysis and Visualization. We used the R packages "stats" and "dunn.test" for ANOVA and Kruskal–Wallis tests, respectively. We used the R package "indicspecies" for indicator species analysis and the R package "ggcorrplot" to evaluate Pearson correlations between two measures of alpha diversities (Chao1 and Shannon) and eight meteorological factors. We used the R Package "superheat" to create a taxonomy heat map.

Data Availability. All data supporting the findings of this study are available within this article and *SI Appendix*; BioProject PRJNA577148, containing all amplicon libraries (SAMN13020512-SAMN13020530 and SAMN13286348-SAMN13286354) is accessible at https://www.ncbi.nlm.nih.gov/Traces/study/?acc=PRJNA577148&o=acc_s%3Aa.

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