

## Article

# Characterization of the Cultivable Microbiota Components of Marine Bioaerosols in the North Tropical Atlantic

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**Abstract:** Microorganisms are key to balancing marine ecosystems and have complex interactions at the ocean–atmosphere interface, affecting global climate and human health. This research investigated the diversity of cultivable bacteria and fungi in marine bioaerosols in the North Tropical Atlantic Ocean. Using the technique of spontaneous sedimentation in selective culture media, samples were collected during oceanographic expeditions. After isolation and purification, microbial strains were identified by phenotypic and genetic analyses. Fungi isolated included *Acrophialophora*, *Aspergillus*, *Chrysosporium*, *Cladosporium*, *Fonsecaea*, *Mucor*, *Rhodotorula*, *Schizophyllum*, *Stemphylium*, *Candida*, *Curvularia*, *Cystobasidium*, *Exophiala*, *Neotestudina*, *Penicillium*, *Pestalotiopsis*, and *Preussia*. The bacterial isolates belonged to the *Bacillota*, *Pseudomonadota*, *Enterobacteriaceae* family, *Bacillus* genus, and *Serratia liquefaciens* groups. About 40% of bacteria and 42% of fungi were identified as potential human pathogens, suggesting a relationship between human actions and the microbiota present in bioaerosols on the high seas. Sea surface temperature (SST) and wind speed influenced microorganisms. More studies and analyses in different scenarios should be conducted considering environmental and climate variables in order to deepen knowledge and generate information on the subject, so that standards can be established, and quality parameters determined.

**Keywords:** bioaerosols; meteoceanographic parameters; tropical Atlantic Ocean; PIRATE buoys



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## 1. Introduction

Microorganisms are present in all environments on planet Earth, although in some of these, in loco sampling is impossible due to difficult access and/or the current absence of compatible technologies. The oceans, until the last century, were included in this group. The presence of marine microorganisms in the air over the Atlantic Ocean had not been documented until the mid-twentieth century, with the first articles published on the subject in the 1950s [1,2]. Research efforts in an attempt to fill these gaps on oceanic microorganisms in the atmosphere, diversity, biogeography, interactions, and participation in global biogeochemistry are evident from the increasing number of papers published in the following decades. We performed a quick search on a free research platform using a combination of keywords: marine microbial, atmospheric communities, aerosol; this search resulted in a finding increasing numbers of articles produced on the subject throughout the 1960s until the early 2020s.

Bioaerosols are conceptualized as solid particles carried by the air originating from biological organisms [3], which are aerosolized from the marine environment to the atmosphere through the continuous generation of bubbles caused by the friction of the wind on

the surface of the water [4,5]. The Sea Surface Microlayer (SML) is the main contributor of organic matter and inorganic salts to the atmosphere [6], including microorganisms, such as bacteria and fungi [3,6,7], viruses, pollen, and their derivatives, such as endotoxins and mycotoxins [7].

This particulate biological material suspended in the atmosphere has functions for the Earth system, the climate and public health at the local, regional, and global scales, as they act directly in the dispersion and absorption of solar radiation or, even indirectly, in the formation of cloud condensation nuclei, influencing droplet sizes, cloud cover, and albedo [4,8,9]. Its distribution, abundance, and diversity are affected by different meteorological factors such as temperature, precipitation, relative humidity, and wind speed, as well as climate change and global warming [10], thus making it essential to collect abiotic data complementary for the thematic discussion, on a relatively long-time scale and at the time of sampling.

Among the main factors that impact the microbiological diversity in the atmosphere, there is the air temperature [11]. In the Tropical Atlantic Ocean, this factor oscillates meridionally, leading to the Intertropical Convergence Zone (ITCZ), which migrates seasonally, being more north in June, July, and August and reaching its southernmost position in December, January, and February. The trade winds are associated with the migration of sea surface temperature (SST) and ITCZ. The SE trade winds move towards the NH (SH) as the ITCZ is further north (south) [12].

In addition, high air temperatures and low air humidity values favor the transport of bioaerosols, due to the atmospheric water vapor absorbed by the particles, favoring their increase in weight and, consequently, their gravitational deposition [13,14].

As well as the dispersion of material from the ocean–atmosphere interface by wind, in combination with surface tension gradients, it represents a unique mechanism that ensures microbiological transport. Additionally, changes in physical forcings such as wind speed and water circulation affect the composition and molecular arrangement of biofilms in the ocean surface microlayer and, consequently, the composition of bioaerosols.

The increase in pressure, as it reflects the increase in the amount of cold air, which alone can facilitate the release and transport of microorganisms, which can lead to a high abundance in the atmosphere [13]. Finally, climate change implies a change in the global atmospheric circulation, which contains not only chemical compounds but also bioaerosols, mainly fungal spores and fungal hyphae clefts [10]. These climate changes can change the seasonal and characteristic systems of each location, changing the patterns of meteorological variables and, consequently, affecting bioaerosols.

In this sense, despite efforts, information on the diversity and distribution of these organisms throughout the oceans, especially the Atlantic Ocean, is still scarce. Thus, this article aimed to analyze and identify the diversity of cultivable bacteria and fungi present in marine bioaerosols collected along the North Tropical Atlantic Ocean (NTA) and also to collect oceanographic and meteorological variables and possibly associating them with the diversity found, thus evidencing possible determining relationships between oceanographic and meteorological variables and microbial diversity.

## 2. Materials and Methods

### 2.1. Study Area

The study area comprised the North Tropical Atlantic, from 0° to 15° N (Figure 1a). Samplings were carried out during an expedition by the Prediction and Research Moored Array in the Tropical Atlantic Program (PIRATA) around meteorological–oceanographic buoys (Figure 1b) and corresponded to the following points: P1 (15° N 38° W), P2 (12° N 38° W), P3 (8° N 38° W), P4 (4° N 38° W), and P5 (0° 35° W). The samples were collected in November and December of 2017, on board the Hydro-oceanographic (NPqHo) ship “Vital de Oliveira” of the Brazilian Navy, and in June 2022, on board the oceanographic ship “Antares” of the Brazilian Navy.



**Figure 1.** (a) Location map of the study area; (b) standard meteo-oceanographic buoy referring to the PIRATA Project.

## 2.2. Samplings

Microbial particles were collected using the spontaneous sedimentation technique on the surface of selective culture media in Petri dishes [15,16]. Briefly, selective culture media were exposed to air for 30 min with the opening of the Petri dishes, then closed and conditioned. Sets, in duplicate, of selective medium for bacteria [Plate Count Agar (PCA, Difco®) diluted in sea water at 10 ppm] and fungi [Potato Dextrose Agar (PDA) added with 10 µg/mL of Ampicillin] were used. The collected material was then transported to the Laboratório de Microbiologia Ambiental e do Pescado (LAMAP–UFC).

## 2.3. Sample Processing: Isolation and Identification of the Microbiota

In the laboratory, the grown bacterial colonies were selected and isolated. Cultures were checked for purity, morphology, and cell wall characteristics by Gram-staining technique [17,18]. Then, the cultures were submitted to biochemical tests according to the Bergey's Manual [17] arranged as a dichotomous key in the following phenotypes: production of catalase and oxidase enzymes, motility, production of hydrogen sulfide (H<sub>2</sub>S), production of indole, test of methyl red (VM) and Voges–Proskauer (VP), urea broth, Simmons citrate [19,20]. Due to identification by biochemical tests, genetic identification by PCR and sequencing was not performed on the isolated bacteria.

For the isolation of fungal cultures, plates with growth were rehydrated (10 ppm NaCl saline solutions) and diluted. Inoculated plates were incubated at 28 °C for 7 days [14]. After isolation, the samples were purified and, when isolated colonies were obtained, DNA extraction was performed following an existing protocol with adaptations for the samples [21], the polymerase chain reaction (PCR) using the ITS-1 primers (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') and, finally, the sequencing of the amplicons achieved.

The amplicons obtained were sent to ACTGene Analises Moleculares, for sequencing, using an automatic sequencer ABI 3500 Genetic Analyzer (Applied Biosystems, Waltham, MA, USA). The sequences obtained were analyzed using the 3500 Data Collection software and compared to sequences from the GenBank database of the National Center for Biotechnology Information (NCBI).

#### 2.4. Oceanographic and Meteorological Variables

Meteo-oceanographic data were collected together at the time of collection and were obtained through equipment present in the facilities of the ships of each shipment. The variables obtained were wind speed, relative humidity, atmospheric pressure, and air temperature. During the expeditions, no CTD stations were carried out so, seeking to complement the abiotic data, SST data from satellite databases available online was included. In this work, 20 years of monthly SST and surface winds (10 m) from January 2003 to December 2022 were analyzed. The SST data had a 9 Km spatial resolution and were available in the MODIS Aqua Level 3 product, derived from the MODIS sensor from NASA aboard the Aqua satellite <http://apdrc.soest.hawaii.edu/> (accessed on 20 June 2023). The surface wind database was from the ERA5 reanalysis model, with 0.25 degrees of resolution. ERA5 is the fifth generation European Center for Medium-Range Weather Forecasts (ECMWF) reanalysis for global climate. The SST data were used to analyze the climatology of the study area and to estimate the SST anomalies during the collection period. The SST anomaly was obtained by removing the monthly climatological average. The anomaly values made it possible to identify whether the SST was above or below the historical average during the collection analysis period. In addition, the satellite data allowed for extracting the values at the same points as the collections analyzed here.

#### 2.5. Statistical Analysis

Statistical analyses were performed using the Rstudio software, which uses the R statistical programming language, (version 4.2.3, The R Foundation, Vienna, Austria) readxl, RVAideMemoire, car and the tidyverse packages were used. Initially, the Shapiro–Wilk test was performed to verify the possible normality of the meteo-oceanographic data. Since these data did not present a normal distribution and due to the sample size, non-parametric tests were chosen. The Levene test was also performed for homogeneity of median variances for the 2017 and 2022 groups. The Mann–Whitney test was performed for two independent groups of meteo-oceanographic variables between the years 2017 and 2022. The Kruskal–Wallis test (ANOVA one-way for non-parametric data) with the chi-square value for groups 2017 and 2022.

Quality assurance and control (QA/QC) was performed throughout the field and laboratory sampling and analysis process with the use of certified inputs (culture media and reagents), manually calibrated equipment (following the manufacturers' instructions) and internationally validated methodologies.

### 3. Results

#### 3.1. Microbiota Cultivable in Marine Bioaerosols

According to the morphological characteristics of the isolated fungal colonies, 19 colonies belonging to 9 different genera were identified: *Acrophialophora* sp. (5%), *Aspergillus* sp. (27%), *Chrysosporium* sp. (5%), *Cladosporium* sp. (16%), *Fonsecaea* sp. (27%), *Mucor* sp. (5%), *Rhodotorula* sp. (5%), *Schizophyllum* sp. (5%), and *Stemphylium* sp. (5%). Of the bacterial

isolates, 56 isolates representing the phyla *Bacillota* (35%) and *Pseudomonadota* (20%) were characterized; family *Enterobacteriaceae* (25%); genera *Bacillus* (5%); and *Serratia liquefaciens* (15%) (Table 1).

**Table 1.** Operational Taxonomic Units (OTUs) of bacteria and fungi detected in bioaerosols collected at points along the coast of Brazil in the NTA ocean and the Equator line phenotypically identified.

		P1	P2	P3	P4	P5
Bacteria	<i>Pseudomonadota</i>	X			X	
	<i>Enterobacteriaceae</i>	X	*	*	X	X
	<i>Bacillota</i>	X			X	X
	<i>S. liquefaciens</i>				X	X
	<i>Bacillus</i>				X	X
Fungi	<i>Aspergillus</i> sp.	X	X	X		
	<i>Fonsecaea</i> sp.		X	X		
	<i>Rhodotorula</i> sp.		X			
	<i>Stemphylium</i> sp.			X		
	<i>Acrophialophora</i> sp.		X			
	<i>Chrysosporium</i> sp.			X		
	<i>Schizophyllum</i> sp.			X		
	<i>Cladosporium</i> sp.		X	X		
	<i>Preussia</i> sp.			X		
	<i>Neotestudina</i> sp.	X	X			
	<i>Preussia</i> sp.		X			
	<i>Curvularia</i> sp.		X	X		
	<i>Penicillium</i> sp.		X	X		
	<i>Exophiala dermatitidis</i>					X
	<i>Candida</i> sp.			X		X
	<i>Pestalotiopsis</i> sp.			X		
	<i>Cystobasidium</i> sp.				X	
	<i>Mucor</i> sp.				X	

\* It was not possible to isolate strains.

At the first collection point (P1), bacterial strains belonging to the Phyla *Pseudomonadota* and *Bacillota*, and the *Enterobacteriaceae* family were found, as for fungal strains, only the genus *Aspergillus* was identified.

At the second (P2) and third (P3) collection points, it was not possible to isolate bacterial strains in the collected samples. In the selective media, there was growth of fungal colonies with characteristics of the genera: *Aspergillus* sp., *Fonsecaea* sp., *Rhodotorula* sp., and *Acrophialophora* sp. (P2), and *Fonsecaea* sp., *Schizophyllum* sp., *Stemphylium* sp., *Cladosporium* sp., *Chrysosporium* sp., and *Aspergillus* sp. (P3).

At the fourth collection point (P4), different qualities of bacteria were found, including: *Serratia liquefaciens*, phylum *Bacillota*, phylum *Pseudomonadota*, *Enterobacteriaceae* family, and *Bacillus* genus. As for the fungal samples, only the genus *Mucor* was possibly identified.

At the last collection point (P5), only bacterial microorganisms corresponding to the *Enterobacteriaceae* family, phylum *Bacillota*, and *Serratia liquefaciens* were found.

As for the results of fungal sequencing, 19 colonies were genetically identified, these data were compiled in the table below (Table 2):

**Table 2.** Microbiological diversity results according to each collection point.

Collection Point	Code Sample	Identification	Similarity	Access (GenBank)	Nucleotide Coverage (%)	E-Value
P1	1	<i>Aspergillus</i> sp.	95%	MN788648.1	100	0
	12	<i>Neotestudina</i> sp.	97%	OW983104.1	100	0
	06.1	<i>Rhodotorula sphaerocarpa</i>	86%	MT355634.1	100	0
P2	7	<i>Aspergillus</i> sp.	89%	MK725871.1	100	0
	5	<i>Preussia</i> sp.	95%	KC013967.1	100	0
	7	<i>Cladosporium</i> sp.	84%	KX788174.1	100	0
	16	<i>Curvularia</i> sp.	86%	MN215656.1	100	0
	23	<i>Penicillium</i> sp.	82%	MN634532.1	100	0
	06.2	<i>Neotestudina</i> sp.	92%	OW983104.1	100	0
	8	<i>Penicillium citrinum</i>	87%	OP163524.1	100	0
P3	9	<i>Aspergillus</i> sp.	93%	KY203991.1	100	0
	11	<i>Candida</i> sp.	95%	KY911171.1	100	0
	14	<i>Aspergillus</i> sp.	92%	MN700120.1	100	0
	15	<i>Aspergillus japonicus</i>	83%	GQ359413.1	100	0
	16	<i>Curvularia</i> sp.	73%	MN540246.1	100	0
	18	<i>Pestalotiopsis</i> sp.	81%	ON681710.1	100	0
P4	26	<i>Cystobasidium</i> sp.	87%	LC424143.1	100	0
P5	29	<i>Candida</i> sp.	88%	KP131656.1	100	0
	30	<i>Exophiala dermatitidis</i>	86%	MN410630.1	100	0

Of the microorganisms identified, 10 divergent identifications were found, among which 4 were identified at the species level, the genus *Aspergillus* sp. was the most represented (26%), followed by *Candida* sp. (11%) and *Curvularia* sp. (11%) and the remaining *Cladosporium* sp. (5%), *Cystobasidium* sp. (5%), *Exophiala dermatitidis* (5%), *Neotestudina* sp. (5%), *Penicillium* sp. (5%), *Pestalotiopsis* sp. (5%), *Preussia* sp. (5%), and *Rhodotorula sphaerocarpa* (5%).

### 3.2. Oceanographic and Meteorological Variables

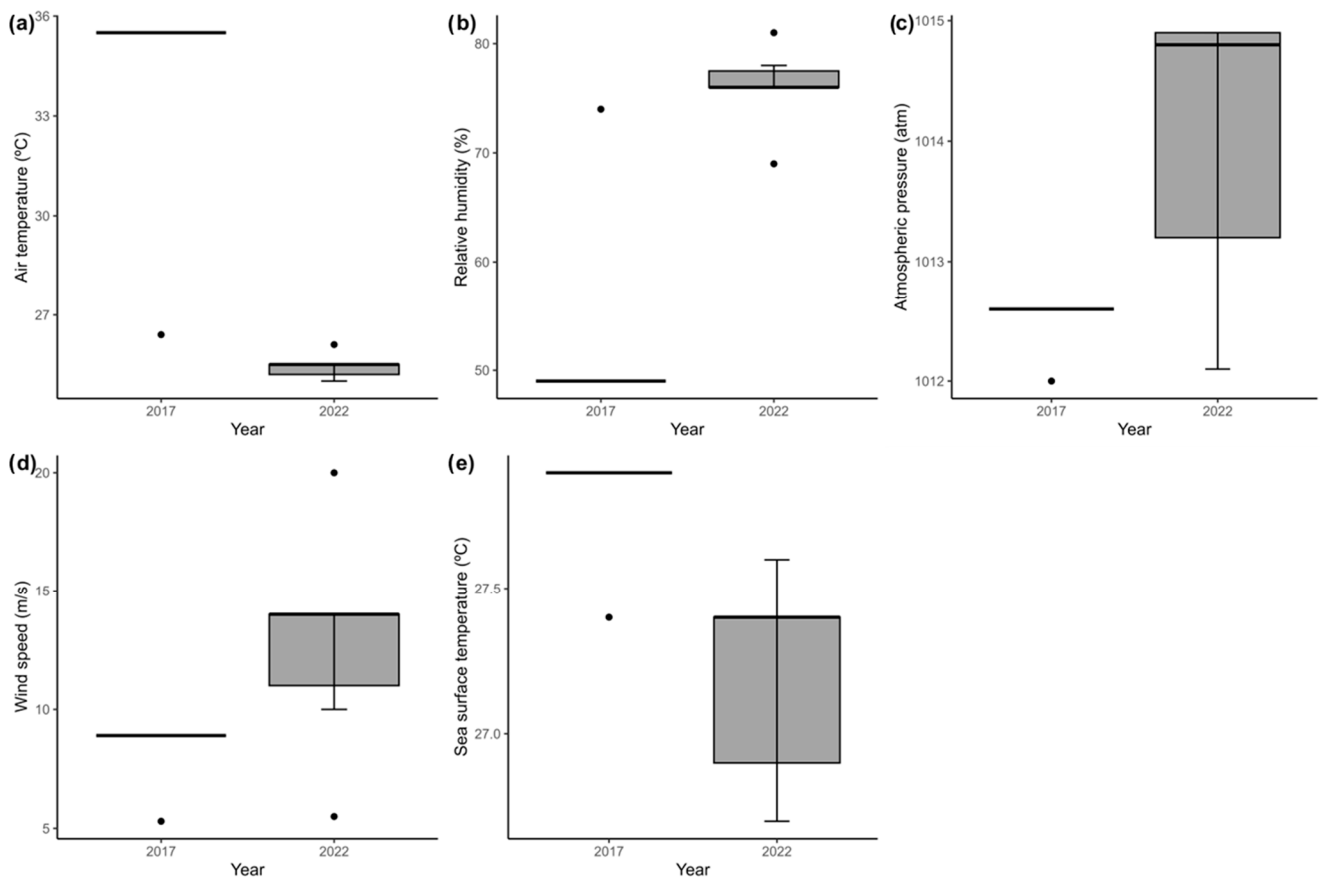
The parameters that were obtained were organized in Table 3:

**Table 3.** Meteorological and oceanographic parameters of the moments of microbiological collections.

Environmental Parameters	Collect Points 2017					Collect Points 2022				
	P1	P2	P3	P4	P5	P1	P2	P3	P4	P5
Air Temperature (°C)	28.4	26.4	35.5	25.7	26.5	25.2	25.5	25.1	26.1	25
Relative humidity (%)	63	74	49	82	78	69.0	76.0	85.0	78.0	81.0
Atmospheric Pressure (hPa)	1013	1012	1012.6	1008.6	1008.5	1014.7	1014.9	1012.9	1012.1	1012.7
Wind Speed (knots)	2.4	5.3	8.9	15.6	18	20	14	9	5.5	10
Sea Surface Temperature (°C)	26.9	27.4	27.9	27.6	26.7	25.4	25.95	27.35	28.16	27.8

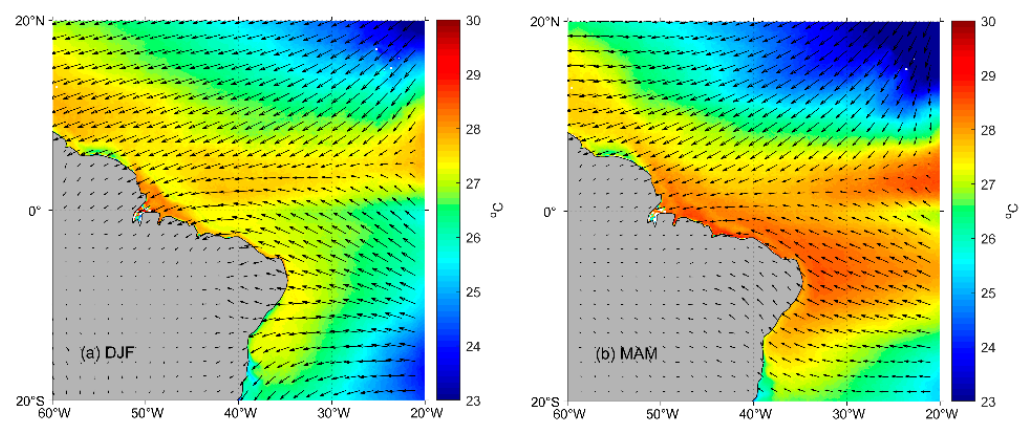
Statistical tests were performed to verify the presence of significant differences (Figure 2). Thus, the results indicate significant differences in the abiotic variables ( $p < 0.05$ ) between the collection years (2017 and 2022), which results in possible significant differences in the organisms found between the collection years (2017 and 2022).



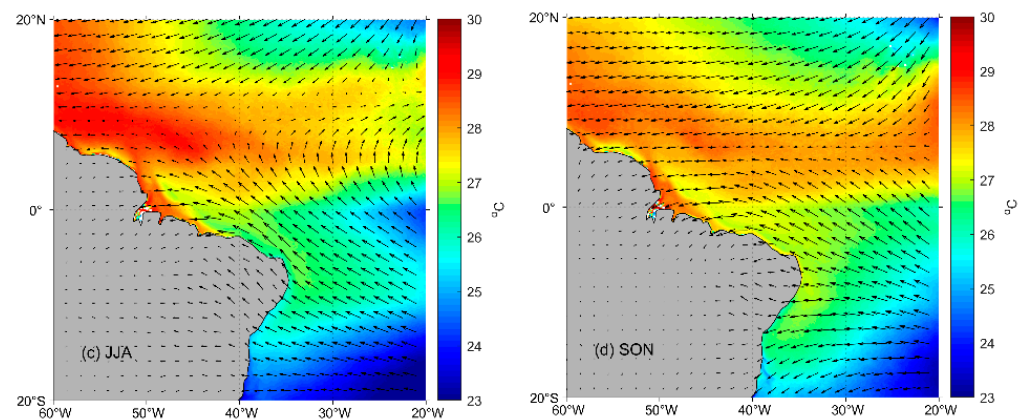


**Figure 2.** Boxplot comparing abiotic data annually (2017 and 2022). (a) Air Temperature, (b) relative humidity, (c) atmospheric pressure, (d) wind speed, and (e) sea surface temperature.

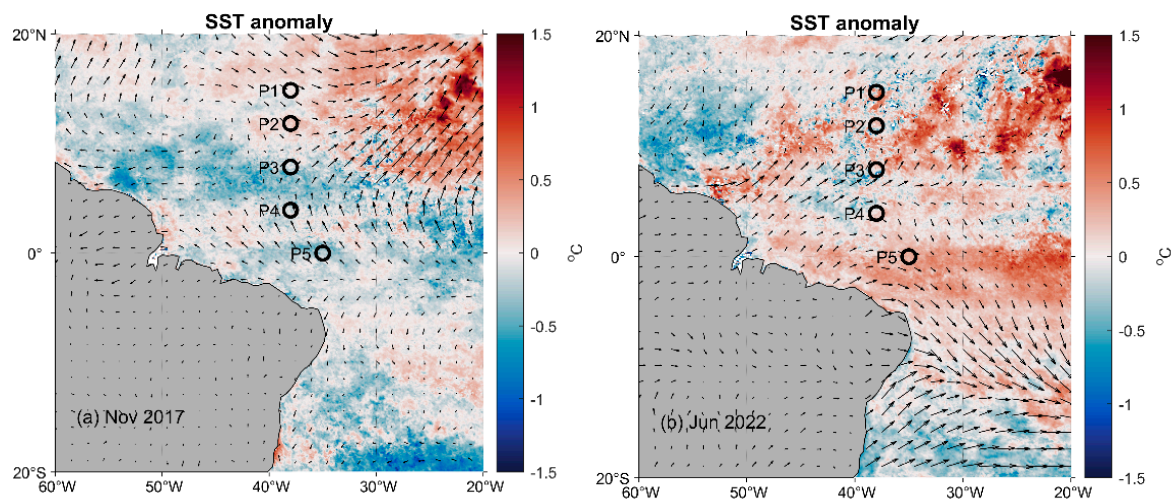
SST and surface wind data allow to analyze the climatology and anomalies of the study area (Figures 3 and 4). Figure 3 shows the seasonal climatology of SST and surface winds. As expected, in the NTA, the SST is lower during the DJF and higher during the JJA, oscillating around 2 °C throughout the year. This migration drives the trade winds and the ITCZ, which is north in JJA and south in DJF.



**Figure 3.** Cont.



**Figure 3.** Seasonal SST climatology for (a) DJF, (b) MAM, (c) JJA, and (d) SON.



**Figure 4.** Sea surface temperature (MODIS Aqua) and wind (ERA5) anomalies for (a) November 2017 and (b) June 2022. Positive (red) and negative (blue) anomalies indicate oscillations in monthly climatology. Positions P1 to P5 are indicated by circles.

SST and wind anomalies were calculated for the period of the oceanographic campaigns, removing the monthly climatological average for the specific months and years of the oceanographic campaigns analyzed in this study. It would show us if the oceanic and atmospheric conditions were close to the climatology or presented atypical conditions. Figure 4 shows the SST anomalies and surface winds values for November 2017 and June 2022.

During the 2017 shipment, SST varied little concerning the historical average. However, it showed negative anomalies mainly at points P3 to P5. During the 2022 shipment, the same region presented positive SST anomalies. This characteristic agrees with the statistical results that bring significant annual differences between the variables. Therefore, there were significant yearly variations of SST, which were below the climatological averages in 2017 and above the climatological average in 2022, indicating once again that differences in this parameter can influence and cause changes in the microbiota found.

#### 4. Discussion

Multiple microbiological genera were found in the study area, evidencing a plurality that, until now, is at least little known and studied. In addition, the relationship between specimens and anthropogenic presence or action is remarkable.

As for the fungal genera, *Fonsecaea*, *Rhodotorula*, *Mucor*, and *Acrophialophora* are widely associated in the literature with human pathogens and are present in the air of different



environments, especially in urban areas and coastal cities [22–25]. The presence of these representatives that are potentially pathogenic for humans in marine bioaerosols along the ocean can be caused due to different reasons, including the intense ship traffic and its relationship with tourist activities, industrialization, and global warming [26,27]. These mentioned anthropogenic activities impact and interfere with the sustainability of the marine environment due to changes in symbiotic relationships and microorganisms in the marine environment [28].

*Chrysosporium* is a genus present in different marine ecosystems including algae, sponges, and fish, making its presence in the study area consistent and expected. It is also important to emphasize the biotechnological capacity that the genus presents through the production of basic structures for the production of new drugs [29].

From pioneering research into atmospheric microbiota diversity to current research into the marine environment, the genus *Aspergillus* is found [30]. In addition to the amplitude in its presence, *Aspergillus* sporulation capacity and its association with atmospheric CO<sub>2</sub> concentrations are known, increasing the sporulation of organisms and, consequently, their presence in the environment [10]. Historically, species of the genus *Cladosporium* are often found in oceanic air masses and marine environments, especially in mangrove sediments [30]. The presence of *Cladosporium* can be explained due to the tendency of these spores to thrive in environments with a hot climate and air currents, parameters that are remarkable in the collected sites; in addition, their spores are mainly affected by variations in average temperature and pressure [10,31,32]. Fungal organisms of the *Cladosporium* and *Aspergillus* genera have previously been found in bioaerosol samples from Antarctica [33], evidencing, however, the lack of recent studies on the subject throughout the study area, or even possible relationships between the study area and the places where they are already present.

Species of the genus *Stemphylium* have a long record of detection in bioaerosols in different regions and altitudes, including along the Atlantic Ocean [34]. Despite the higher frequency in polar air masses, exposure to different temperatures and pressure can modify sporulation, possibly increasing it, which prolongs and favors its permanence and transport of fungal organisms to other locations [31,32].

Species of the genus *Schizophyllum* are cosmopolitan in occurrence and different concentrations of carbon dioxide can affect the reproduction of species of this genus, and low concentrations of this gas allow the formation of more complex fungal structures [35].

Regarding the bacterial material found, it was composed of bacteria that have significant pathogenic potential, such as those of the *Enterobacteriaceae* family, which, as they are not considered inhabitants of marine environments, are associated with pollution from the mainland, or even through ship traffic in the region associated with tourist and industrial activities [27]. In some of the older studies, the genus *Bacillus* appears in abundance [5], however, according to our results, in the NTA, only one isolate corresponded to this genus.

This could possibly be explained due to the fact that Gram-negative bacteria are more commonly found in the marine environment, in agreement with works by other authors in relation to the marine environment [36]. The *Bacillota* phylum was the most abundant in strains isolated from marine bioaerosols (35%), according to [3] in their research on the spatial distribution of bacterial populations in marine bioaerosol samples during a cruise from the North Sea to the Baltic Sea, identified representatives of the phylum *Bacillota* in 8.3% of the isolates. According to [37], in their study on bacterioplankton in estuaries, the phylum *Bacillota* did not appear to be an abundant component, with the exception of the estuary which had significant values of suspended particulate matter, indicating that they are more represented in waters of great turbidity, as well as the surface waters of the study area, which have high values of wind speed that carry the microbiota from the waters and transport it to the air. The phylum *Pseudomonadota* represented 20% of the isolates, the second most abundant in the results, corresponding with the results of pioneering studies such as [38], in which most of the isolates belonged to the Phylum *Bacillota* and *Pseudomonadota*, converging with the results obtained and also more recent studies [36,37].

The *Serratia liquefaciens* species is characterized by inhabiting different environments, such as sediment, water, and has already been found in marine organisms [39], in addition to being associated with the production of histamine that causes contamination in humans [40]. Therefore, given its presence in the microbiota of marine organisms, it is possible that it was carried and, therefore, was found in the bioaerosols collected in this study.

The biogeographical patterns of microorganisms are little known and are controlled by multiple factors (selection, drift, dispersion, and mutation) [41]. When focusing on bioaerosols, the most important of them becomes dispersal, which may depend on the properties of each taxon such as initial abundance in the community of origin. The dispersion of bioaerosols is directly linked to the atmospheric dispersion capacity that works as a limiting factor, considering environmental variables such as the rate of UV radiation [42]. That is, the richness found in this work is directly associated with the ability of this rate to survive and adapt to dispersion in the atmospheric environment.

When we compare the characterization data with the sequencing data, it is possible to notice that there was an overlap of some genera, among them *Aspergillus* sp., *Cladosporium* sp., *Rhodotorula* sp. This overlap corresponds to approximately 48% of the samples that were characterized. Indicating that, despite the divergences between the samples that were identified and gives greater security of genetic identification, the characterization is able to provide some significant idea of the local microbiota.

In addition, we also obtained a coincidence in the genera that were found according to each buoy when we compared the data obtained in relation to characterization and sequencing. In P1, P2, and P3, in both types of identification, it was possible to identify the genus *Aspergillus* sp., and *Rhodotorula* was identified in P2 in both identification methodologies.

*Candida* sp. is a pathogenic fungal genus in which some species are considered multi-resistant pathogens [43] and have already been found in the marine environment forming association with sponges [44] after the discharge of effluents at the site [45] opening up the possibility of human interference in the aerial microbiome of the study area.

*Curvularia* sp. Is considered a genus of worldwide distribution and some species have already been found in aquatic environments and in the air [46,47]; however, it is widely known to be a pathogenic fungus among different species of animals and plants, including humans. It is worth noting that this genus, in addition to being cosmopolitan, has been described through the recent discovery of new species [48] and may in the future become a risk in view of its pathogenic capacity and its identification in the study area.

Yeasts of the genus *Cystobasidium* sp. Were recently found in association with marine animals such as sponges [44] and also attached to thermal insulation boards present in marine environments [49], that is, these yeasts are present in the marine air close, at least, to the region of the North Atlantic Ocean and North Pacific.

*Exophiala dermatitidis* is a fungal species that is associated with skin and lung infections. Organisms of this genus are present in different types of environments and have the ability to withstand extreme conditions such as high temperatures or salinity and variable pH [50,51]. In addition, this organism is often found in tropical regions, natural conditions that are consistent with the study area, justifying its occurrence and bringing the problem of pathogenic organisms overlapping in relation to others due to changes in climatic conditions such as increased temperature [52].

We believe that it is the first time that *Neotestudina* sp. was isolated and cultured from marine samples, especially in the air of the study region (12° N and 15° N). Bearing in mind that this genus can be isolated from soils in tropical regions [53] and has historically been associated with skin diseases [54–56], it is suggested that its transport from land to marine aerosols is due to human influence in the study area, probably through the maritime flow of vessels, modifying the local microbiota. Another important point is that this species was only found in the 2022 collection, suggesting that its presence is seasonal, or that climate anomalies may be associated with changes in the microbiota of the area, since, in 2020, another species of the same genus was also found for the first time in soil samples in Nigeria [53].

*Penicillium* sp. Is a genus widely studied in the literature and, consequently, already isolated several times from the marine environment; as a recent example, it was found in seawater, macroalgae, and tree trunks resulting in 16 different species of *Penicillium* sp. Isolated beyond the description of a new species [57]. This genus was also found in several urban and coastal regions and is considered a pollutant in the region's aerosols, which may indicate, with its presence in the study area, that the air quality needs to be better evaluated [25].

As for the genus *Pestalotiopsis* sp., it is known how this is present in mangrove environments and is currently shown to be an advancing tool for biotechnology research since endophytic fungi are prolific resources for secondary bioactive metabolites and, as a result of its structural complexity, this genus has proven to be efficient [58,59]. With that, several studies bring the research and identification of new species of this genus, which encourages the possibility of using this isolate for future biotechnological tests [60].

*Preussia* sp., like other genera found, is used in biotechnology both due to its anti-inflammatory effects and in enhancing the growth of agricultural products [61,62]. Therefore, like *Pestalotiopsis* sp., it results in a new possibility of obtaining biotechnological products. *Rhodotorula sphaerocarpa* is a species of a fungal genus marked by being associated with human pathogens and is easily present in the environment. Studies indicate that this species increases its respiration in environments with higher temperature values, even though its reproduction is dependent on this environmental variable [63]. In addition, authors indicate that species of this genus, derived from marine water samples, may present biotechnological activity as antioxidants and bioconverter of marine residues, allowing, once again, the use of isolates obtained as possible biotechnological products [64,65].

It is necessary to emphasize how, within this richness found throughout the NTA, the most significant part of the results suggest microorganisms that are associated with the presence and actions of human beings. That is, in addition to the indirect anthropic impact associated with the increase in emissions of global warming gases, such as carbon dioxide, and how these interfere both in environmental variables that interfere in the local microbiota and in the reproduction and viability of microorganisms, there is also the impact direct that occurs through the ship traffic that historically occurs in the area and that directly deposits exotic species in the region, altering the microbiome of the area.

Microorganisms are directly impacted by different environmental variables (air temperature, wind speed, UV radiation, etc.) that control both the survival and permanence as well as the ability to reproduce and viability of microbiological spores, which varies according to each species [66].

With that, we have the association between the fungal diversity found and the climatic parameters, where according to the means and distributions found (Figure 2), it is possible to notice differences in the values of the two years collected ( $p < 0.05$ ), these significant differences of abiotic data can influence and result in changes in the microbiota present in the study area. The average temperature in 2017 was 28.5 °C, while in 2022, it was 25.3 °C. Initially, it was expected that this difference could represent a possible greater fungal diversity, since high temperatures are generally associated with the reproduction of microorganisms; however, the sample that presented the greatest diversity was that of 2022 (nine different identifications) in which, despite the average being lower, presented much more homogeneous values ( $sd = 0.44$ ), which was different from the first sample ( $sd = 4.03$ ). The Relative Humidity of the Air values were also higher and more homogeneous in the 2022 collections, agreeing with the diversity results, since due to the atmospheric water vapor being absorbed by the particles, weight gain is favored and, consequently, the gravitational deposition of the particles, making it possible, through the collection of spontaneous sedimentation, to obtain a greater number of isolates and, consequently, fungal diversity. The wind speed in the year 2022 was more intense, which also corroborates the greater diversity found in this year. Taking into account the importance that winds have in the formation of bioaerosols through the bursting of bubbles in the oceanic surface

microlayer, therefore, higher values of wind speed may be associated with the generation of more particles of microorganisms present in the ocean and, consequently, a greater diversity.

The availability of long-term data from satellites and models allowed an assessment of SST and wind conditions for November 2017 and June 2022 (Figure 3). The maps of SST anomalies indicate that in 2017, the temperatures were slightly below average (weak negative anomalies), and in 2022, the same region had above-average temperature values (positive anomaly).

When the SST was above the climatological average in 2022, the diversity was greater, highlighting a possible relationship between the two variables. Therefore, when we group these data, the SST may significantly influence the microbiological diversity more than the air temperature, considering the diversity data and its relationship with the meteorological data.

The results represent the existing microbiota in the NTA ocean but admit the limitations of culture and microbiological survival.

## 5. Conclusions

Different microbiological taxonomic groups were present in the air of the North Tropical Atlantic Ocean, among them a great variety of fungi and bacteria, corresponding to nine and five types, respectively. These findings shed light on the airborne microbiome present in the study area, as well as the possibility of studying the origin and fate of these microorganisms. A considerable part of the microorganisms found are associated with human pathogens or related to anthropic actions, evidencing possible impacts of human actions in the region since changes in microbiomes can lead to serious changes in the entire food chain of ecosystems.

It is also noteworthy that the results show the need for regional studies to obtain complete data that can compile quantifications and non-cultivable diversities, given the importance of microorganisms for different biogeochemical cycles and, therefore, their global relevance. Finally, these studies have been carried out and continued in search of a complete volume of data by the production team of this work.

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