Air mass source determines airborne microbial diversity at the ocean-atmosphere interface of the Great Barrier Reef marine ecosystem

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Abstract

The aerosphere is the least understood biome on Earth despite its critical role as a microbial transport medium. The influence of surface cover on composition of airborne microbial communities above marine systems is unclear. Here we report evidence for a dynamic microbial presence at the ocean-atmosphere interface of a major marine ecosystem, the Great Barrier Reef, and identify that an oceanic or continental trajectory for aerosols correlates with observed shifts in bacterial and fungal diversity. A putative airborne source for coral symbionts and pathogens was also demonstrated thus highlighting biological connectivity between atmosphere and ocean.

Main text

Airborne microbial transport is central to dispersal outcomes [1] and several studies have demonstrated diverse microbial biosignatures are recoverable from the aerosphere. Microbial transport has been shown to occur across inter-continental distances above terrestrial habitats [2–4]. Variation has been recorded seasonally [5, 6], with underlying land use [7], and due to stochastic weather events such as dust storms [8] . Above marine systems abundance of recoverable microorganisms decreases exponentially with distance from land [9] and relatively little is known about potential biogeography of airborne microorganisms above the oceans. Some studies have shown that aerosolization of marine microorganisms to the aerosphere is an important process, e.g. [10], but the reverse deposition of airborne taxa to the ocean is poorly understood. This deposition is, however, a potential source of marine pathogens [11] and symbionts which are crucial to the health of coral reefs [12]. Here we test the hypothesis that persistent microbial inputs to the ocean-atmosphere interface of the

Great Barrier Reef ecosystem vary according to surface cover during transit of the air-mass and that an atmospheric source of putative coral-associated microbial taxa occurs.

The Great Barrier Reef is an ideal model system for research on bio-aerosols because incoming air mass during the average residence time for microorganisms in air [13] arises from two distinct sources: a terrestrial continental source in Australia transported across east and northeast dust paths and an oceanic source in the Coral Sea (Fig. 1a). Our study took advantage of a persistent flat-calm sea state during September-October 2016 that minimised interference from microorganisms that are aerosolised by marine spray in heavier sea states at the Great Barrier Reef [14]. We recovered massive bulk-phase air samples 25m above sea level using a high-volume liquid impinger apparatus (Coriolis μ, Bertin Technologies, France) [15] during a voyage of the *RV Investigator* to circumnavigate the reef and recovered approximately 3,000m^3 air during daily from which environmental DNA was recovered (n = 53) [15] (Supplementary Information, Supplementary Methods). We used the National Oceanic and Atmospheric Administration (NOAA) HYSPLIT-WEB model (https://ready.arl.noaa.gov/HYSPLIT.php) to identify back trajectories of air mass during the average residence time for microbial cells in air [13] and this could be delineated very clearly into those with recent transit over continental or oceanic surfaces (Fig. 1a). The concurrent concentration of atmospheric radon gas measured in real time was higher from back trajectories originating over continental Australia compared with those that originated from the ocean (Mann-Whitney U Test, P = 0.003) thus further verifying the binning of air mass into a continental or oceanic origin. High-throughput sequencing of the bacterial 16S rRNA gene and fungal Internal Transcribed Spacer region loci were performed as previously described [15] and phylogenetic analysis of amplicon sequence variants (ASVs) were used to estimate diversity [16]

(Supplementary Information, Supplementary Methods). Sequencing of control samples revealed very low recovery of putative contaminant microbial signatures, a total of only 17 out of 1403 bacterial and 5 out of 3775 fungal sequences were statistically classified as putative contaminants (Supplementary Information, Supplementary Methods). With few exceptions all samples were sequenced to asymptote thus allowing meaningful ecological inference from these data (Supplementary Information Fig. 1a).

All air samples supported ultra-low biomass as inferred indirectly from DNA yield (mean recovery 0.23 ng/m^3, SD 0.21 ng/m^3) with no significant difference between sample groups (Mann-Whitney U Test, P = 0.37). Terrestrial or oceanic transit significantly determined bacterial community structure at the ocean-atmosphere interface above the great Barrier Reef (P = 0.001). In terms of overall taxa richness, bacteria displayed similar richness in terrestrial and ocean-derived samples whereas fungi were markedly more speciose in continent-derived samples (Supplementary Information, Fig. S1b).

We interrogated the phylogenetic diversity of terrestrial and marine-sourced air by generating heatmaps of distribution for the 100 most abundant taxa. This analysis captured 98.4% bacterial and 66.9% fungal overall diversity in the libraries. Clear taxonomic differences in bacterial (Fig. 1b) and fungal (Fig. 1c) assemblages between ocean and continent-derived air mass occurred. The robust nature of these data was further highlighted by the lack of recoverable taxa in experimental controls (Supplementary Information, Fig. S2) and this is particularly important for interrogation of low biomass environments such as the aerosphere. Overall the number of shared bacterial taxa was high amongst samples derived from ocean (87.9%) and terrestrial (89.6%) sources. Fungi displayed fewer shared taxa between ocean (64.9%) and continental (69.1%) sources. Nonetheless a high number of taxa specific to either

ocean (538 bacteria, 1,335 fungi) or continental (395 bacteria, 1,810 fungi) aerosols were recorded (Figs. 1b, 1c). We screened bacterial taxa from our sequence libraries at genus level to known coral-associated taxa including symbionts and pathogens (Supplementary Information, Supplementary Methods) [17]. The ocean derived air mass supported 8.4% putative coral associates whilst for continental sources this value was lower at 2.9% (Supplementary Information, Table S1). There were few fungal sequences with which to make comparisons but at the genus level there were 8.7% of ocean samples and 6.2% continental taxa that have been recorded as coral associates (Supplementary Information, Table S2). The most differentially abundant ASV from our study (*Ralstonia* sp.) showed high similarity to a previously identified coral endosymbiont (Supplementary Information, Table S3).

Shifts in relative abundance of bacteria mainly occurred within the Phylum Proteobacteria and followed a temporal pattern during the voyage where the dominant genus *Alistipes* was gradually replaced by *Bradyrhizobium* and *Ralstonia*, and then in turn by *Acinetobacter* (Fig. 2a, Supplementary Information, Fig. S3). All have been recovered as isolates or environmental rRNA gene sequences from both marine and terrestrial sources, thus making any attempt at source tracking challenging. For the Fungi terrestrial air sources supported higher diversity than marine sources and this reflected the large number of taxa associated with terrestrial habitats. A striking observation was that 56% of recovered genera supported yeast-like taxa, and this provides support for a marine origin of aerosols since oceanic waters are thought to support elevated abundance of yeasts over filamentous fungi [18]. Shifts in diversity were less pronounced overall for the Fungi (Fig. 2b, Supplementary Information, Fig. S4), and were partially obscured by the high diversity relative to Bacteria at the genus level. Major shifts in relative abundance occurred for *Aureobasidium, Cladosporium,*

Coprinopsis, *Rhodosporidiobolus* and *Rhodotorula*, and all of these genera have known terrestrial and marine records, although it should be noted that terrestrial fungal spores have been recorded in many marine microbial diversity assessments but are unlikely to be active components of an ocean surface water microbiome.

We further interrogated the phylogenetic diversity of terrestrial and marine-sourced air using Net Relatedness Index (NRI) analysis to estimate the level of phylogenetic structuring and putative recruitment from local and regional pools [15]. The NRI analysis revealed that bacterial assemblages from both continental and marine origin displayed positive NRI values with effect sizes indicative of non-random assembly. Communities were thus phylogenetically highly structured, which is possibly due to environmental filtering of traits during transit over the different surface covers (Supplementary Information Fig. S5). The fungi from continental sources displayed a similar though more variable trend of phylogenetic structuring although in ocean-derived samples this clustering was relatively weak indicating they were more randomly assembled (Supplementary Information Fig. S5). When considered in tandem with our low diversity estimates for fungi in ocean derived samples these findings suggest that fungi are severely depleted in marine aerosols. Community phylogenetics and all our analyses support the hypothesis that long-range transport of microbial taxa in air results in selection during transit over different surface covers [15, 19, 20].

Overall our findings demonstrate that the aerosphere provides connectivity between the Great Barrier Reef marine ecosystem and exogenous microbial input from terrestrial and marine sources. This may be important to coral symbioses and also to pathogen input via persistent airborne deposition. Our study provided a unique insight on variability of airborne microbial inputs to the largest coral reef ecosystem on Earth.

Data availability

All sequence data generated by this study has been submitted to the EMBL European Nucleotide Archive (ENL) under BioProject PRJEB31630 with sample accession numbers ERS3215240 to ERS3215312.

Author contribution statement

S.D.J.A, K.C.L, T.C., M.H. and S.B.P. conceived the study; S.D.J.A. conducted ship-board fieldwork; S.D.J.A and K.C.L. performed laboratory experiments; S.D.J.A., K.C.L., T.C., K.K-M. and S.B.P. performed data analysis; S.D.J.A, K.C.L, T.C., K.K-M., M.H. and S.B.P. analysed and interpreted the findings; S.B.P. wrote the manuscript.

Materials & Correspondence

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Conflict of Interest

The authors declare no competing interests.

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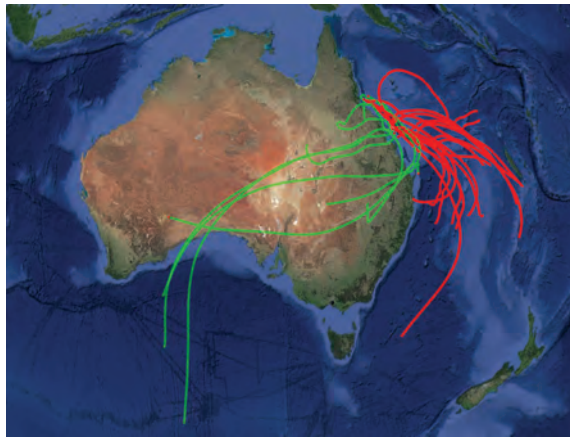
Figure Legends

Figure 1. (a) HYSPLIT back trajectory analysis for modelled transit routes (3 d residence time [12]) with colours representing air mass origin over continent (green) or ocean (red) surface; (b) distribution and relative abundance for the 1,000 most abundant bacterial amplicon sequence variants (ASVs), Continent n = 8, Ocean n = 19; (c) distribution and relative abundance for the 1,000 most abundant fungal ASVs, Continent n = 8, Ocean n = 17.

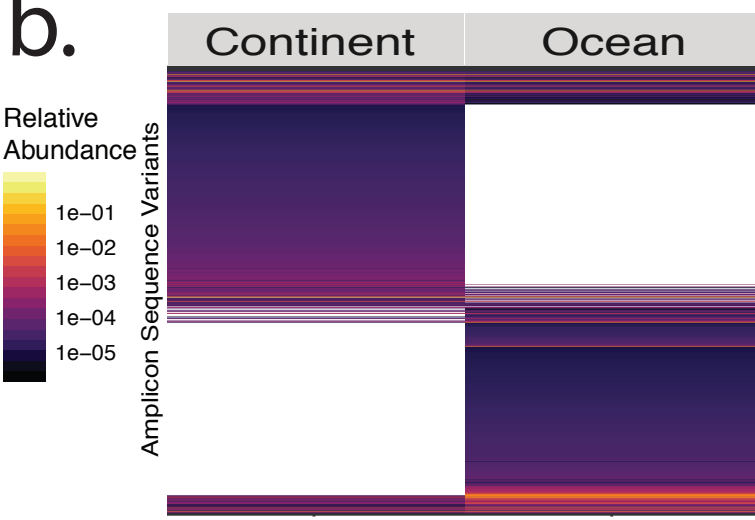
Figure 2. (a) Distribution and relative abundance of the 100 most abundant bacterial amplicon sequence variants (ASVs) with genus identified, Continent n = 8, Ocean

n = 19; (b) Distribution and relative abundance of the 100 most abundant fungal ASVs with genus identified, Continent n = 8, Ocean n = 17.

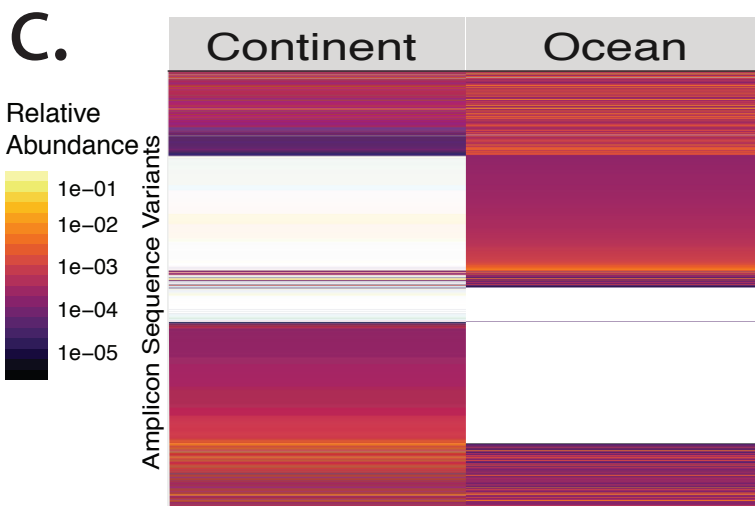
a.

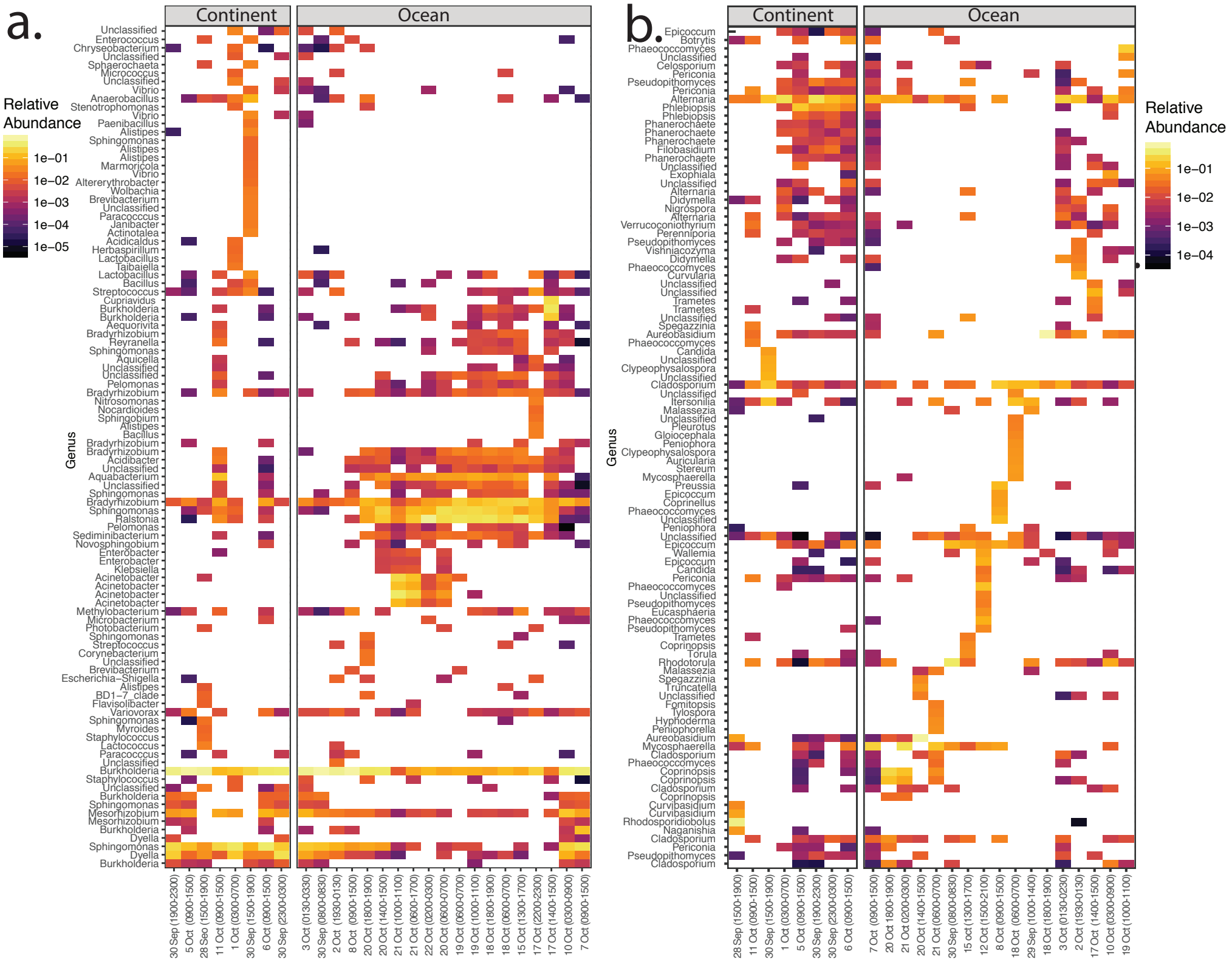


b.


Continent
Ocean
Relative Abundance
1e−01
1e−02
1e−03
1e−04
1e−05
Amplicon Sequence Variants

c.


Continent
Ocean
Relative Abundance
1e−01
1e−02
1e−03
1e−04
1e−05
Amplicon Sequence Variants


a.
Continent
Ocean
Relative Abundance
1e−01
1e−02
1e−03
1e−04
1e−05
Genus
Unclassified
Enterococcus
Chryseobacterium
Unclassified
Sphaerochaeta
Micrococcus
Unclassified
Vibrio
Anaerobacillus
Stenotrophomonas
Vibrio
Paenibacillus
Alistipes
Sphingomonas
Alistipes
Alistipes
Marmoricola
Vibrio
Altererythrobacter
Wolbachia
Brevibacterium
Unclassified
Paracocccus
Janibacter
Actinotalea
Acidicaldus
Herbaspirillum
Lactobacillus
Taibaiella
Lactobacillus
Bacillus
Streptococcus
Cupriavidus
Burkholderia
Burkholderia
Aequorivita
Bradyrhizobium
Reyranella
Sphingomonas
Aquicella
Unclassified
Unclassified
Pelomonas
Bradyrhizobium
Nitrosomonas
Nocardioides
Sphingobium
Alistipes
Bacillus
Bradyrhizobium
Bradyrhizobium
Acidibacter
Unclassified
Aquabacterium
Unclassified
Sphingomonas
Bradyrhizobium
Sphingomonas
Ralstonia
Pelomonas
Sediminibacterium
Novosphingobium
Enterobacter
Enterobacter
Klebsiella
Acinetobacter
Acinetobacter
Acinetobacter
Acinetobacter
Methylobacterium
Microbacterium
Photobacterium
Sphingomonas
Streptococcus
Corynebacterium
Unclassified
Brevibacterium
Escherichia−Shigella
Alistipes
BD1−7_clade
Flavisolibacter
Variovorax
Sphingomonas
Myroides
Staphylococcus
Lactococcus
Paracocccus
Unclassified
Burkholderia
Staphylococcus
Unclassified
Burkholderia
Sphingomonas
Mesorhizobium
Mesorhizobium
Burkholderia
Dyella
Sphingomonas
Dyella
Burkholderia
30 Sep (1900-2300)
5 Oct (0900-1500)
28 Seo (1500-1900)
11 Oct (0900-1500)
1 Oct (0300-0700)
30 Sep (1500-1900)
6 Oct (0900-1500)
30 Sep (2300-0300)
3 Oct (0130-0330)
30 Sep (0800-0830)
2 Oct (1930-0130)
8 Oct (0900-1500)
20 Oct (1800-1900)
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21 Oct (0600-1700)
22 Oct (0200-0300)
20 Oct (0600-0700)
19 Oct (0600-0700)
19 Oct (1000-1100)
18 Oct (1800-1900)
18 Oct (0600-0700)
15 Oct (1300-1700)
17 Oct (2200-2300)
17 Oct (1400-1500)
10 Oct (0300-0900)
7 Oct (0900-1500)
b.
Continent
Ocean
Relative Abundance
1e−01
1e−02
1e−03
1e−04
Genus
Epicoccum
Botrytis
Phaeococcomyces
Unclassified
Celosporium
Periconia
Pseudopithomyces
Periconia
Alternaria
Phlebiopsis
Phlebiopsis
Phanerochaete
Phanerochaete
Phanerochaete
Filobasidium
Phanerochaete
Unclassified
Exophiala
Unclassified
Alternaria
Didymella
Nigrospora
Alternaria
Verrucoconiothyrium
Perenniporia
Pseudopithomyces
Vishniacozyma
Didymella
Phaeococcomyces
Curvularia
Unclassified
Unclassified
Trametes
Trametes
Unclassified
Spegazzinia
Aureobasidium
Phaeococcomyces
Candida
Unclassified
Clypeophysalospora
Unclassified
Cladosporium
Unclassified
Itersonilia
Malassezia
Unclassified
Pleurotus
Gloiocephala
Peniophora
Clypeophysalospora
Auricularia
Stereum
Mycosphaerella
Preussia
Epicoccum
Coprinellus
Phaeococcomyces
Unclassified
Peniophora
Unclassified
Epicoccum
Wallemia
Epicoccum
Candida
Periconia
Phaeococcomyces
Unclassified
Pseudopithomyces
Eucasphaeria
Phaeococcomyces
Pseudopithomyces
Trametes
Coprinopsis
Torula
Rhodotorula
Malassezia
Spegazzinia
Truncatella
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Hyphoderma
Peniophorella
Aureobasidium
Mycosphaerella
Cladosporium
Phaeococcomyces
Coprinopsis
Coprinopsis
Cladosporium
Coprinopsis
Curvibasidium
Curvibasidium
Rhodosporidiobolus
Naganishia
Cladosporium
Periconia
Pseudopithomyces
Cladosporium
28 Sep (1500-1900)
11 Oct (0900-1500)
30 Sep (1500-1900)
1 Oct (0300-0700)
5 Oct (0900-1500)
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30 Sep (0800-0830)
15 oct (1300-1700)
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18 Oct (1800-1900)
3 Oct (0130-0230)
2 Oct (1930-0130)
17 Oct (1400-1500)
10 Oct (0300-0900)
19 Oct (1000-1100)