



The biology of fog: results from coastal Maine and Namib Desert reveal common drivers of fog microbial composition

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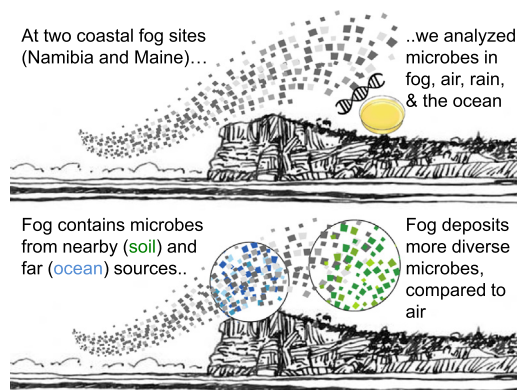
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HIGHLIGHTS

- Coastal fogs from Namibia and Maine both contained diverse microbial communities.
- Fog contains more ocean microbes (compared to soil) when fog is near the coast.
- Fog results in more microbial species to be deposited onto land than air alone.
- A fog event changes the composition of microbes in the air.

GRAPHICAL ABSTRACT



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ABSTRACT

Fog supplies water and nutrients to systems ranging from coastal forests to inland deserts. Fog droplets can also contain bacterial and fungal aerosols, but our understanding of fog biology is limited. Using metagenomic tools and culturing, we provide a unique look at fungal and bacterial communities in fog at two fog-dominated sites: coastal Maine (USA) and the Namib Desert (Namibia). Microbial communities in the fog at both sites were diverse, distinct from clear aerosols, and influenced by both soil and marine sources. Fog from both sites contained Actinobacteria and Firmicutes, commonly soil- and air-associated phyla, but also contained bacterial taxa associated with marine environments including Cyanobacteria, Oceanospirillales, Novosphingobium, Pseudoalteromonas, and Bradyrhizobiaceae. Marine influence on fog communities was greatest near the coast, but still evident in Namib fogs 50 km inland. In both systems, differences between pre- and post-fog aerosol communities suggest that fog events can significantly alter microbial aerosol diversity and composition. Fog is likely to enhance viability of transported microbes and facilitate their deposition, making fog biology ecologically important in fog-dominated environments. Fog may introduce novel species to terrestrial ecosystems, including human and plant pathogens, warranting further work on the drivers of this important and underrecognized aerobiological transfer between marine and terrestrial systems.

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1. Introduction

In ecosystems ranging from temperate coastal forests (Dawson, 1998; del-Val et al., 2006; Ewing et al., 2009) to hyperarid deserts (Norgaard et al., 2012; Seely and Hamilton, 1976), fog supports high levels of biodiversity and productivity (Azua-Bustos et al., 2010; Jacobson et al., 2015; Namibia National Committee for World Heritage, 2012; Warren-Rhodes et al., 2007; Warren-Rhodes et al., 2013), and in many regions it is the only source of water. Fog water can provide vital support to microbial communities, driving the majority of plant litter decomposition in some water-limited systems (Dirks et al., 2017; Jacobson et al., 2015). Fog also potentially modulates nutrient delivery between marine and terrestrial systems, as seen in fogs of Southern Chile (Weathers et al., 2000; Weathers and Likens, 1997), Northern California, USA (Ewing et al., 2009) and Northeastern USA (Dueker et al., 2011; Jordan and Talbot, 2000; Jordan et al., 2000; Weathers et al., 1988; Weathers et al., 1986; Weathers et al., 1995). However, to date, the biology of fog – the microbial community that is transported and may make a living in fog – has been little explored.

The few researchers who have examined fog biology have focused primarily on bacteria and fungi using culture-based methods. While these methods give an indication of viability of fog-incorporated microbes, they are also known to greatly underestimate concentrations and diversity of environmental microbes (e.g. (Amann et al., 1995; Hugenholtz et al., 1998)). Using culture-based methods, Fuzzi et al. (1997) found that radiation fog in Italy's polluted Po Valley increased bacteria and yeast aerosol concentrations, but not mold, by up to two orders of magnitude when compared to clear (non-foggy) aerosol conditions. Fuzzi et al. (1997) further posited that fog droplets may serve as “culture media,” supporting microbial aerosol ecology. Dueker et al. (2011) later found that coastal advective fogs in Maine, USA significantly increased culturable bacterial aerosol deposition and viability, and that bacterial communities in fog were reflective of the marine environment (Dueker et al., 2012b). These findings strongly suggest that microbes transported in fog droplets maintain viability over longer periods than in dry aerosols (Amato et al., 2005; Dueker et al., 2012b; Fuzzi et al., 1997), which could allow them to contribute functionally to the ecosystems where they are introduced. Thus, fog and its microbiome may play an important role in “seeding” terrestrial ecosystems with bacteria, fungi, and viruses from adjacent marine and freshwater ecosystems. Fog microbes, like cloud microbes, may also be capable of metabolism during transport (Amato et al., 2017), meaning that the biology of fog may transform itself and fog chemistry before contact with terrestrial systems.

Culture independent studies of bacteria in non-stratus clouds, which can be seen as a close analog to ground-based fogs, have demonstrated strong evidence of bacterial metabolism in cloud droplets (Amato et al., 2017; Amato et al., 2005; Amato et al., 2007a; Delort et al., 2010). Indeed, Delort et al. (2010) posited that non-stratus clouds may serve as structure for microbially-dominated, near-surface atmospheric ecosystems. Ground-based fogs could well be functioning similarly. In fact, we posit that fog is a novel ecosystem in and of itself that is structured by the microphysics of droplets, moisture source locations, aerosol content, and long-distance transport across ecosystems.

Since fogs form through activation of ambient ground-level aerosol particles, sources for these aerosols should play a key role in determining the microbial composition of fog. Previous aerosol studies have outlined that ground-level microbial aerosols have both local and remote sources, determined primarily by dominant wind patterns and local aerosol production mechanisms (e.g. (Bowers et al., 2012; Bowers et al., 2011; Burrows et al., 2009)). The ocean surface can be a dominant source of aerosols to the coastal environment (de Leeuw et al., 2000; Vignati et al., 1999), and unsurprisingly, ocean bacteria are present in aerosols (Aller et al., 2005; Cho and Hwang, 2011), non-stratus clouds (Amato et al., 2007b) and advective coastal fog (Dueker et al., 2012b). If coastal waters are polluted, coastal aerosols will contain

bacteria associated with that pollution, which may include pathogens (Cao et al., 2014; Dueker and O'Mullan, 2014; Dueker et al., 2012a). The atmospheric transport and viability of human pathogens in polluted environments is understudied, however (Lai et al., 2009). Fog has the potential to both transport and maintain pathogen viability.

While past non-stratus cloud and culture-based fog studies have provided a view into the potential for the microbial ecology of fog, scarce data have limited our ability to identify generalities in fog biology, including dominant sources and functional significance. We compared fog microbial communities in two fog-dominated ecosystems, Coastal Maine (Northeastern USA) and the Namib Desert (western coast of southern Africa) to provide a first look at the full microbiology of fog. Using both culture-based and culture-independent tools, we characterized microbial aerosols under clear and foggy conditions to assess the impact of fog on the viability and transport of microbes from the Atlantic Ocean into terrestrial ecosystems. Our findings underscore the potential for fog to serve as an important and understudied mechanism of microbial dispersal and interecosystem connection, and elucidate novel patterns that can serve as a foundation for future work in fog biology.

2. Materials and methods

2.1. Sites

The geography of coastal Maine is conducive to the creation of marine aerosols and frequent fog formation (Davis, 1966). Here, sampling occurred on a south-facing rocky shore of Southport Island (Heron Ledge), Maine (N43.80261 W69.66841), where dominant winds from the south lead to coastal advection fogs in summer and fall (Davis, 1966; Dueker et al., 2011). Sampling of fog, clear air, and fog/rain events occurred within 30 m of the ocean during two field campaigns: June 28–July 7, 2008, and Sept. 8–Sept. 14, 2008, as detailed in Dueker et al. (2012b); Dueker et al. (2011) (Table S1). One-minute wind speed, wind direction, humidity, temperature and precipitation data were measured by a Vantage Pro2 Plus Weather Station (Davis Instruments, Hayward, CA). Fog presence/absence was determined using a combination of field observations and time-lapse photography. The chemistry of these coastal fogs, as outlined in Dueker et al. (2011), demonstrated marine influence and contained Nitrogen and Phosphorus (both inorganic and organic fractions).

The Namib Desert is a hyperarid coastal fog desert that extends from southern Angola to northern South Africa, and is one of the oldest and driest deserts in the world (Eckardt et al., 2013). Fog commonly reaches 50 km inland (Jacobson et al., 2015), and is often formed when low level stratus clouds from the Atlantic move inland and intersect with the land surface. Two dominant winds at the Namib come mainly from the S to SW (primarily Sep–Nov) and the E to NE (primarily May–Aug) (Lancaster et al., 1984). Fog, rain, and clear air were sampled at Gobabeb Research and Training Center (southern site, S23.56 E15.04, 55 km from coast) from June 5–13, 2016, and near the Uniab River Canyon (northern site, S 20.0215 E 13.634, 50 km from the coast) on June 20, 2016 (Table S1). Total ion concentration of fog water in the Namib is about 14.5 ppm (seawater is ~35,000 ppm) and chemistry of fog water in the Namib is comparable to that from other coastal deserts (Eckardt and Schemenauer, 1998). Meteorological data were calculated from minute averages recorded by the Gobabeb SASSCAL FogNet station (<http://www.sasscalweathernet.org/>).

2.2. Fog, rain, aerosol, and ocean sampling

In Coastal Maine, ocean samples (1 l) were gathered from the ocean surface adjacent to the sampling site at 0.25 m depth, 2–10 m from shore. Active aerosol sampling (under both foggy and clear conditions) was conducted using duplicate SKC Bioaerosol Samplers (SKC, Eighty Four, PA) according to Fierer et al. (2008) and previously outlined in Dueker et al. (2011). Fog samples were also captured using sterilized

passive fog collectors (PFC) (Falconer and Falconer, 1980; Weathers et al., 1995) consisting of circular Teflon string (0.5 mm diameter) arrays mounted at 2 m on PVC frames (see Dueker et al. (2011)). Rain samples were gathered using sterile buckets and funnels deployed upon the advent of a rain event. Ocean, fog, rain, and aerosol samples (both passively and actively collected) were passed through a 0.22 µm Sterivex filter to capture bacterial cells and frozen at -80°C until analysis.

In Namibia, ocean water was sampled on June 15th, 2016 near Swakopmund Pier, approximately 20 m from the beach surf. We used sterile plastic bottles to collect 500 ml of water from the top 20 cm of the ocean surface. Fog was sampled passively by collecting and filtering water that impacted on a sterile surface. At the onset of fog, a smooth metal surface facing into the fog at a 45° angle was covered with Mylar sheeting, the bottom of which was shaped into a trough. Before collection of 30–60 ml of fog, we washed the sheet with 1% bleach, 95% EtOH, then sterile water, and collected a sterile diH_2O fog blank. At the southern site, the Mylar collection occurred on a 1×1 m thick aluminum sheet on a stand (“dew collector”), but when this was not accessible at the northern site, we secured a sheet to a car windshield and performed the same sterilization, blank, and sample collection procedure. Air deposition was sampled passively using settle plates. Empty and sterile media plates (10 mm diameter) were exposed for 10–24 h near southern site’s meteorological station. We washed plates with sterile diH_2O in the lab, and filtered the water with a 0.22 µm syringe filter. No air samples were collected at the northern site. Filters were allowed to air dry and then transported at room temperature back to Michigan, where they were frozen at -80°C until analysis.

Culturing methods in the Namib were similar to those for whole-community air and rain sampling, and only occurred at the southern site. We set plates with media (malt with streptomycin, malt, R2A with cyclohexamide) out for 2–12 h, often overnight to avoid drying of the agar. After exposure, cultures grew for up to 12 days in the lab while we performed daily photographs and colony counts of morphotypes. Subcultures were isolated from the original plates and allowed to grow onto filter paper discs once they were grown as pure cultures on a new plate. Discs were then desiccated for transport to Michigan, US in 96-well plates covered with AirPore paper. We lost many R2A plates in transport and therefore sampling with bacteria-targeted media was limited. At Kellogg Biological Station, we revived isolates, extracted DNA, and sequenced 16S (27F and 1492R) and ITS (ITS1 and ITS4) regions using Sanger technology at MSU Genomics Core Facility.

2.3. Molecular methods

At both sites, bacterial and fungal DNA was extracted from filters using the PowerWater DNA isolation kit (MoBio Laboratories, Carlsbad, CA), according to the manufacturer’s instructions. Coastal Maine bacterial community composition was determined using amplicon pyrosequencing performed at Molecular Research DNA labs (www.mrdnlab.com, MRDNA, Shallowater, TX, USA), following Dowd et al. (2008). Briefly, samples were amplified using the universal eubacterial primer 27F using HotStarTaq Plus Master Mix Kit (Qiagen, Valencia, CA) at 94°C for 3 min, 28 cycles of 94°C for 30 s, 53°C for 40 s, 72°C for 1 min, elongation at 72°C for 5 min. Samples were purified using Agencourt Ampure beads (Agencourt Bioscience Corporation, MA, USA) and sequenced with Roche 454 FLX titanium instruments and reagents according to manufacturer’s guidelines.

Maine bacterial sequences were processed on the Mothur platform according to Schloss et al. (2011). Briefly, we used PyroNoise, allowing for 1 mismatch to the barcode and 2 mismatches to the forward primer. Sequences were trimmed to remove barcodes and primer sequences, and those with <200 bp and/or homopolymers >8 bp were removed. Remaining sequences were aligned using SILVA (v128) reference alignments (Pruesse et al., 2007). Sequences outside of the desired sequence

alignment space were removed. Chimeras were detected and removed using UCHIME (Edgar et al., 2011). Sequences were then classified using the Wang et al. (2007) taxonomy at 80% cutoff, then clustered using the average neighbor analysis to assign operational taxonomic units (OTUs) at a 97% similarity cutoff.

We characterized Namib fungal and bacterial communities by amplifying the ITS and 16S rRNA gene regions using ITS1-F/ITS2 and 515f/806r primers, respectively, including three sterile water extraction kit blanks. These small-subunit ribosomal genes are commonly used to identify bacteria and fungi from environmental samples because they contain both conserved and variable regions useful for comparing across a wide taxonomic range (Schoch et al., 2012). Libraries were submitted for 250-bp paired-end sequencing on the Illumina MiSeq platform at Michigan State University Genomics Core Facility, who provided standard quality control for the Illumina platform, including base calling (Illumina Real Time Analysis (RTA) v1.18.61), demultiplexing, adaptor and barcode removal, and RTA conversion to FastQ format.

Bacterial reads were first chimera checked and quality filtered (Trimmomatic 0.33), then contigs were created (Pandaseq20150212). OTUs were picked at the 97% identity level using UCLUST6.1, and phylogenetically identified using the most recent Greengenes database (gg_otus_13_8 release). Singletons were removed and contigs were screened using the QIIME 1.9.1 (Caporaso et al., 2010b) with the following parameters: quality score >30 , sequence length >200 and <275 , maximum homopolymer of 6, 0 maximum ambiguous bases, and 0 mismatched bases in the primers and barcodes. These DNA sequences were aligned using PyNAST (Caporaso et al., 2010a).

Fungal contigs were created using default settings in fastq_mergepairs implemented in the USEARCHv8.1 pipeline (<http://drive5.com/usearch/>). Merged sequences were quality filtered to an expected error threshold of 1.0 fastq_filter (Edgar and Flyvbjerg, 2015). Sequences were then truncated to 380 bp with shorter sequences padded to reach the 380 bp because ITS region length is highly variable (Nilsson et al., 2008). Combined reads were dereplicated and OTUs were picked at the 97% identity level using UPARSE (Edgar, 2013) then chimera checked using reference based UCHIME2 (Edgar, 2016) against the UNITE 7.1 ITS1 chimera database (Kojal et al., 2013) within the USEARCHv9.1 pipeline. Representative sequences were classified against a reference database by extracting ITS1 sequences from the UNITE 7.1 reference database (20.11.2016 release) using ITSx (Bengtsson-Palme et al., 2017).

2.4. Statistical analyses

Because we used different sequencing technology, primers, and bioinformatics pipelines for Maine and Namib samples, we avoided testing hypotheses that required direct comparison of the relative abundance of certain groups of taxa. Instead, we identified trends within each data set (e.g. abundances of fog taxa relative to ocean samples) using identical statistical analyses, and compared trends across sites. First, filtered and aligned FASTA files from the above pipelines were imported as phyloseq objects into R for filtering and statistical analysis (McMurdie and Holmes, 2013). Distribution of sample groups, sequence depth, and replication are summarized in Table S2. Chlorophyta, Streptophyta (Chloroplasts), and Mitochondria sequences were removed from bacterial datasets. OTUs resulting from extraction kit blanks and passive fog collection control samples were removed from all relevant samples to eliminate extraction contamination and sequencing artifacts. Since the presence of aerosol taxa in fog blanks (and subsequent removal from samples) could overestimate aerosol-fog compositional differences, we also performed an identical analysis on samples without fog blank taxa removed (Fig. S1).

To assess betadiversity, we chose to filter samples and relativize abundances instead of rarefying because we had a large range of sequences per sample in our dataset (Table S2), and we did not want to

unnecessarily throw away reads or samples (McMurdie and Holmes, 2014). All samples with <200 sequences (both bacterial and fungal) and all taxa with <5 sequences across all samples were removed. We created a Bray-Curtis dissimilarity matrix based on the relative abundances of OTUs, and of aggregated Phyla (to obtain a range of dissimilarities to ocean samples), in each sample. We used this matrix to calculate dissimilarity between ocean samples and other groups, and tested whether this similarity was significantly related to air mass history (determined by wind back trajectories, see SI). We also tested for differences among groups using PerMANOVA (R vegan package) and visualized sample similarity using Nonmetric Multidimensional Scaling (phyloseq) (Oksanen et al., 2017). We calculated alpha diversity by first rarefying all samples with >1000 sequences to an even depth (Table S2). We chose Shannon-Weiner index of species diversity because this index considers species evenness, but has not reported to be sensitive to differences in primer choice, like Chao1 (Fredriksson et al., 2013).

3. Results

Despite vastly different local conditions and geography, fog bacterial communities in both systems were dominated by Proteobacteria, Bacteroidetes and Actinobacteria (Fig. 1), and include bacteria known to inhabit both terrestrial and aquatic environments (dominant genera in Tables 1, S3). We identified a total of 545 bacterial OTUs and 168 fungal OTUs (grouped at 97% similarity) when summed across four fog events in the Namib, and 474 bacterial OTUs across eight fog events from coastal Maine (fungi were not measured in Maine). We were able to store and revive 10 fungal isolates from Namib fog, representing six species, two bacterial species (*Bacillus*), and an additional five fungal isolates from clear air samples (Table S3). Sequence data are available under SRA accession number SRP155760. Bacterial strains isolated from Maine fog are reported in Dueker et al. (2012b). Seven of the ten fungal isolates from the Namib are or are closely related to (con-generic with) extremophilic fungi as reported elsewhere (Cantrell, 2017). Our study did not quantify pathogenicity of bacterial and fungal communities, but many of the dominant microbial genera we detected in fog in both sites are also known to contain plant, animal, and human pathogens (Tables 1, S3). Although several of these pathogens were also present in air, it is worth noting that several of them (e.g. *Erwinia*, *Pantoea*, *Massilia*) were among the most dominant taxa found in fog from both Maine and Namib sites.

Fog communities, as a whole, were not significantly different from communities sampled in rainy or clear conditions (Fig. 2, Table S4). Community composition of both fog and clear aerosols varied over

time and across meteorological conditions (Fig. 2). Inclusion of taxa found in fog collection blanks did not change statistical trends or overall community patterns (Fig. S1). Microbes associated with fog were influenced by local and remote microbial sources, including soil and water. For example, fog from both Namibia and Maine contained Actinobacteria and Firmicutes (Fig. 1), commonly soil- and air-associated phyla, but also contained bacterial taxa associated with marine environments including Cyanobacteria, Oceanospirillales, *Novosphingobium*, *Pseudoalteromonas*, and Bradyrhizobiaceae.

The marine signature in fog communities varied widely across samples (Figs. 2, 3); the percent of ocean OTUs in fog ranged from 1 to 75% (Table S5). In the Namib, similarity of fog communities to ocean communities (Fig. 3) was not related to the fog's association with the ocean (based on HYSPLIT model analysis, Table S6). However, coastal Maine samples, which were sampled within 10 m of the coastline, were on average more similar to ocean samples than Namib samples measured further inland (~50 km from the coast) (Fig. 3).

Community alpha diversity and deposition was higher in fog microbial communities than non-fog aerosol communities immediately preceding or following fog events (Figs. 4, 5). Culture-based examinations show that plates exposed to fog had almost three times the growth and twice the richness as plates exposed during clear air conditions preceding fog events, even though fog plates were deployed for half as long (3 vs. 6 h.). This pattern of increased deposition under fog is supported by previous culture-based observations, including in coastal Maine (Dueker et al., 2012b; Dueker et al., 2011; Fuzzi et al., 1997). Although we could not measure absolute deposition (i.e. abundance) using culture-independent techniques, sequencing results also showed that diversity was greater under fog conditions than clear conditions (Fig. 5). In addition, aerosol microbial communities changed over the course of specific fog events (Fig. 2, arrows). Namibia fog fungal communities were enriched with Ascomycota (20.1% of total sequences), compared to pre-fog clear conditions (0.47%) (Table S7). In coastal Maine, fog generally had a lower relative abundance of Bacteroidetes (11.3%) compared to clear conditions (20.1%). *Pseudomonas* and Bacteroidetes seemed to “drop out” of aerosols after fog events in coastal Maine, as did Proteobacteria, Acidobacteria, and *Ophiocordyceps* after fog events in the Namib (Table S7). Rhodospirillales were present in both fog and clear samples, at both sites. On average, relative abundance of taxa in the order Rhodospirillales was greater in fog samples than in clear samples in both sites (0.2% vs. 7% in Maine; 2% vs. 4% in Namib). However, the difference was not statistically significant in either site, largely due to high variation among samples (including some Maine samples in which this order was not present).

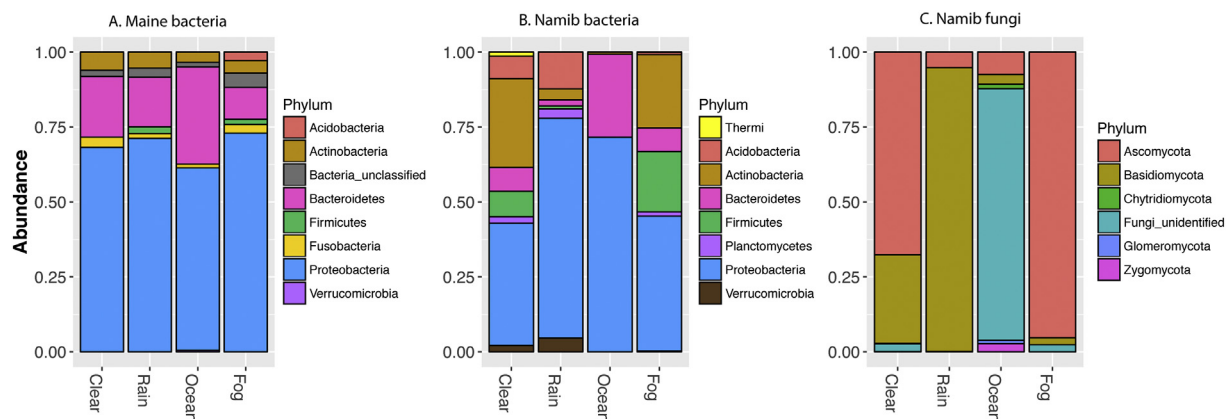


Fig. 1. Taxonomic abundance of Maine bacteria (A), Namib bacteria (B) and Namib fungi (C) across sample types. Graphs show the 8 most abundant bacterial phyla across all samples within each site. We caution direct comparison of taxa relative abundance across sites since different sequencing platforms and primer sets were used. Samples of the same type collected over time were merged, rarefied to adjust for differences in sampling effort, then reported as relative abundances.

Table 1
Dominant bacterial genera across all fog events in Maine and Namibia and associated traits, when available.

| Higher taxonomic level | Genus | Relative abund | Dominant metabolism | Motility | Gram stain | Spore forming | Common habitats |
|---|---------------------------|----------------|--|---|------------|---------------|---|
| Maine bacteria | | | | | | | |
| Bacteria (Domain) | Unclassified | 0.068 | Varies | Varies | Varies | Varies | |
| Acetobacteraceae (Family) | Unclassified | 0.058 | Aerobic | Varies | G- | | Plants, alcoholic drinks, bees, wastewater |
| Oxalobacteraceae (Family) | <i>Massilia</i> * | 0.056 | Aerobic | Yes | G- | No | Soil, marine sediment |
| Flavobacteriaceae (Family) | Unclassified | 0.050 | Aerobic/microaerobic | Varies | G- | No | Soil, freshwater, ocean, food, hospitals, fish, insects, plants |
| Pseudoalteromonadaceae (Family) | <i>Pseudoalteromonas</i> | 0.032 | Aerobic | Yes | G- | No | Seawater, marine sediment |
| Oxalobacteraceae (Family) | <i>Duganella</i> * | 0.032 | Aerobic | Yes | G- | No | Plant associated, soil |
| Acidobacteria Gp1 (Family <i>incertae sedis</i>) | unclassified* | 0.031 | | | | | |
| Enterobacteriaceae (Family) | <i>Pantoea</i> | 0.029 | Fac Anaerobic | Yes | G- | No | Plant associated, plant endophytes |
| Gammaproteobacteria (Class) | Unclassified | 0.026 | Varies | | G- | | |
| Oceanospirillales (Order) | Unclassified | 0.022 | Varies | Yes | G- | | Freshwater, ocean, oil spills |
| Namib bacteria | | | | | | | |
| Geodermatophilaceae (Family) | <i>Geodermatophilus</i> * | 0.043 | Aerobic | Yes | G+ | Yes | Soil, plant associated, freshwater sediment |
| Enterobacteriaceae (Family) | <i>Erwinia</i> | 0.043 | Fac anaerobic | Yes | G- | No | Plant associated |
| Acetobacteraceae (Family) | Unclassified | 0.035 | Aerobic | Varies | G- | No | Food, acidic environments, plant associated |
| Micrococcaceae (Family) | Unclassified | 0.031 | Aerobic/Fac Anaerobic | No | G+ | No | Soil, plant associated, other |
| Bacillaceae (Family) | <i>Bacillus</i> * | 0.028 | Aerobic/Fac Anaerobic | Yes | G+ | Yes | Soil, plant associated, hypersaline environment, built environment, food, marine sediment |
| Prevotellaceae (Family) | <i>Prevotella</i> | 0.027 | Anaerobe | No | G- | No | Human fecal, human mouth, soil |
| Enterobacteriaceae (Family) | Unclassified | 0.025 | Fac anaerobic | Yes | G- | No | Human intestines, soil, freshwater, sewerage |
| Porphyromonadaceae (Family) | <i>Porphyromonas</i> | 0.021 | Anaerobic | | G- | No | Human skin |
| Oceanospirillaceae (Family) | <i>Marinomonas</i> | 0.018 | Aerobic | Yes | G- | No | Seawater, marine sediments, hypersaline environment |
| Actinomycetaceae (Family) | <i>Actinomyces</i> | 0.018 | Anaerobic | No | G+ | No | Soil, human microbiome, compost, plant litter |
| Higher taxonomic level | Genus | Rel abun | Description | Common habitats | | | |
| Namib fungi | | | | | | | |
| Lasiosphaeriaceae (Family) | <i>Fimetariella</i> | 0.354 | Contains endophytes, some plant pathogens | | | | |
| Pleosporaceae (Family) | Unclassified | 0.092 | Contains allergens, plant pathogens, saprotrophs, agricultural pests | Plants, alcoholic drinks, bees, wastewater | | | |
| Dothideomycetes (Class) | Unclassified | 0.084 | Contains lichen symbionts, endophytes, saprotrophs | Soil, marine sediment | | | |
| Pleosporales (Order) | Unclassified | 0.074 | Contains lichen symbionts, endophytes, plant pathogens | Soil, freshwater, ocean, food, hospitals, fish, insects, plants | | | |
| Apiosporaceae (Family) | Arthrinium | 0.051 | Contains endophytes, plant pathogens; produces antibiotics | Seawater, marine sediment | | | |
| Pleosporales (Order) | Periconia | 0.049 | Contains endophytes, saprophytes | Plant associated, soil | | | |
| Ophiocordycipitaceae (Family) | Ophiocordyceps | 0.035 | Global insect parasite | | | | |
| Fungi (Kingdom) | Unclassified | 0.024 | | Plant associated, plant endophytes | | | |
| Sordariomycetes (Class) | Unclassified | 0.018 | Contains plant pathogens, endophytes; medically important | | | | |
| Montagnulaceae (Family) | Unclassified | 0.016 | Contains saprotrophs, human pathogens | Freshwater, ocean, oil spills | | | |

* Because aerosol samples are often low-biomass, there is greater concern that contamination to bias results. We took many measures to prevent and correct for contamination, but in full disclosure, indicated those bacterial groups that we identified in fog that also contain taxa commonly identified as lab contaminants (as reported in Salter et al. (2014)). It is worth noting that many so-called 'contaminants' are also highly ubiquitous in the environment, which would also make their presence unsurprising in air and fog.

4. Discussion

We conducted a whole-community assessment of fog microbial communities in two iconic fog systems. Although studies from the two systems were not standardized a priori, they presented a unique opportunity to assess generalities in controls on fog biology due to the remarkably similar questions and sampling approaches (ocean, air, and fog collection). Still, we must first acknowledge the limitations due to our use of different methods, particularly molecular methods. Although environmental samples can be surprisingly insensitive to sequencing platform (Luo et al., 2012; Tremblay et al., 2015), there is also evidence that reference database (Tremblay et al., 2015) and primer choice (Fredriksson et al., 2013) can alter reported values of relative abundance (and even dominance) of different taxa. We did not find specific evidence for this based on patterns in previous studies (e.g. primers

used in Maine (27f) can inflate Firmicutes and Betaproteobacteria abundance (Fredriksson et al. 2013), but these groups were more abundant in Namibia), but took care to avoid direct comparisons of taxa abundance and alpha diversity across sites. Instead we analyzed within-site trends and then identified common trends in the two sites. Although not without caveats, we think having observations from two sites strengthens our study and importantly, allowed us to identify several broad drivers of fog biology, the generality of which could be tested for by more strictly standardized future studies.

4.1. Fog mediates microbial linkages across marine and terrestrial systems

Coastal fogs appear to be strongly influenced by the aerosolization of ocean microbial communities, much like coastal aerosols (de Leeuw et al., 2000; Dueker et al., 2012b; Dueker et al., 2017; Dueker et al.,

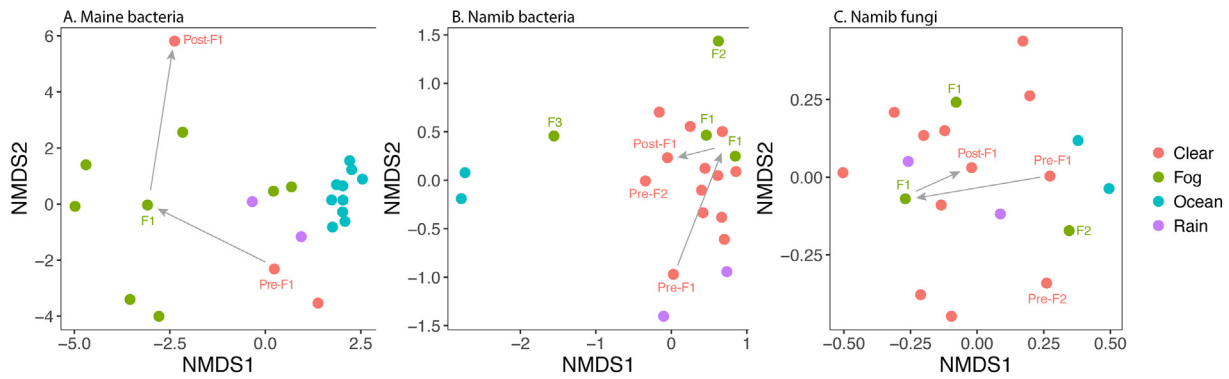


Fig. 2. Nonmetric multidimensional scaling plots of Maine bacteria (A), Namib bacteria (B) and Namib fungi (C). Labels describe more specific conditions within sample type categories. Some fog events are labeled to show corresponding clear conditions before and after. F3 in the Namib is the Northern site (fungal F4 filtered out due to low sequence number per sample), while F1 and F2 are from the southern site. PerMANOVA statistical tests are reported in Table S4. All ocean samples were collected within 0.25 m of the surface.

2011; Vignati et al., 2001). The microbial communities in fogs in the Namib and Maine were clearly dominated by gram-negative bacteria (primarily Proteobacteria and Bacteroides), just as found in marine-originating cloud droplets (Amato et al., 2017), marine bacterial aerosols under dry conditions (e.g. (Cho and Hwang, 2011; Fahlgren et al., 2010)), and culture-based coastal fog and ocean surface bacterial community analyses (Dueker et al., 2012b). While ocean influence was detected at both sites, the magnitude of this influence was modulated by distance from the source. In Maine, where samples were collected nearer to the coast, fog samples were more similar to the ocean compared to the fogs of the Namib (sampled ~50 km inland), where soil-associated bacteria had a greater influence (Fig. 1).

Of note is the high variability among individual fog events. In both Maine and Namibia, microbial communities associated with fog and clear air samples showed greater variability than those from ocean water and rain (Fig. 1). Different wind patterns during and preceding sampling could have resulted in different source areas for microbes, resulting in different communities in turn. The Namibian sites for instance are located in the middle of a strong rainfall transition zone with precipitation much higher to the east and considerably lower in the west. Soil microbial communities can vary considerably across precipitation gradients (Bachar et al., 2010), providing different potential source communities in fog and air based on current wind patterns.

Ocean proximity may also explain differences in cultured fog communities observed in previous studies. Dueker et al. (2012b) found several genera of cultivatable marine bacteria in coastal fog from Maine, while in the Po River Valley of Italy (an inland site surrounded by

mountains), Fuzzi et al. (1997) did not find specific evidence of marine-associated microbes in fog. Although coastal proximity is one clear driver of fog community composition, there was also large variation in ocean influence across time in both sites. This variation could be explained by meteorological differences among fog events, but in the Namib we found no relationship between the meteorological metrics we assessed (time over ocean) and ocean similarity (Table S6). Our ability to detect a relationship between fog attribute and marine influence could have been obscured by the low sample size or inability to sample fog at the same stage of the fog's lifetime. Future work could examine high-resolution sampling over the course of a single fog event to assess what attributes drive the prevalence and persistence of ocean taxa.

Although fungi are known to make significant contributions to atmospheric aerosols overall (Elbert et al., 2007), we found that marine contributions were weaker for fungi than for bacteria (Figs. 1, 5). Ocean surface waters harbor low concentrations and diversity of fungi (Richards et al., 2015), and therefore may not emit high levels of marine fungal aerosols. Alternatively, morphological differences between fungal spores and bacteria might lead to differential dispersal through fog itself. Still, in one Namibia fog, 8% of ITS reads in the community consisted of ocean fungal taxa, suggesting that the ocean influence is not entirely absent, and warrants further study.

Together, findings from both fog sites suggest that fog does not simply serve as a refuge to local bioaerosols, but as an effective microbial transport mechanism connecting marine and terrestrial ecosystems. Although this transport is possible in dry aerosols as found in this study

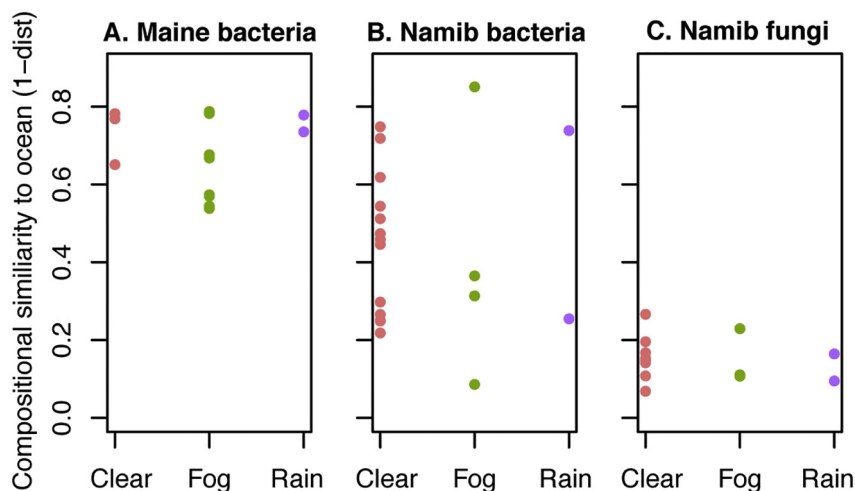


Fig. 3. Compositional similarity (1 – Bray-Curtis distance, calculated at the Phylum level) of clear, fog, and rain communities to composition of ocean communities collected at that site in Maine bacteria (A), Namib bacteria (B) and Namib fungi (C).

| | Air preceding fog | Fog | Rain | High Wind |
|-------------------------------|-------------------|-------------|-------------|-------------|
| Gr rate (cm ² /d)* | 0.21 | 0.61 | 0.74 | 0.77 |
| Observed spp | 8 | 16 | 10 | 12 |

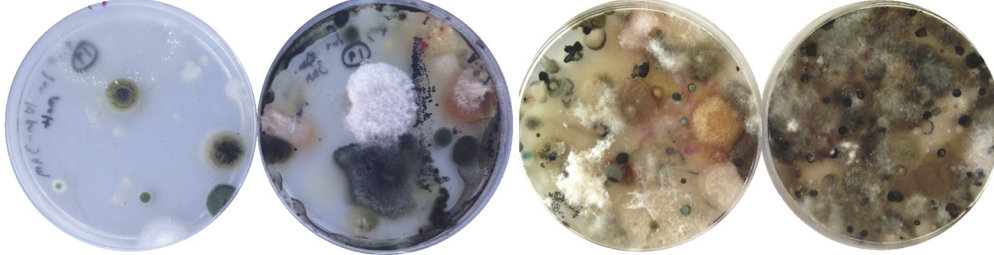


Fig. 4. Microbial cultures grown directly from exposure to air preceding fog (3 h exposure), inland fog (1 h), rain (1 min), and high wind (<1 min) in the Namib Desert, Namibia. Images show malt media plates after 6 days of growth, and majority fungal morphotypes. Growth rate was measured daily using image analysis of plate coverage, and observed species shows number of morphotypes on day 8 of growth. Note many of the morphotypes shown here were sequenced but some did not revive after transport and are not shown in Table S3.

and others (e.g. (Aller et al., 2005; Blanchard, 1989; Dueker et al., 2017; Fahlgren et al., 2010)), fog increases the viability and likelihood of deposition of these cells (Dueker et al., 2012b; Fuzzi et al., 1997), increasing their impact on local ecosystems and hosts. Bacterial aerosols are known to be metabolically active, including marine bacteria in dry air (Klein et al., 2016) and bacteria in lower atmospheric cloud droplets (Amato et al., 2017). Incorporation into a fog droplet may further promote metabolic activity in gram-negative bacteria including Rhodospirillales (Klein et al., 2016), *Pseudomonas* and *Sphingomonas* (Amato et al., 2017), which were all detected in Maine and Namib fogs.

Although so far we only document this trend at two sites, this marine-terrestrial connection could have important implications for biodiversity and public health. Dispersal is increasingly recognized as a significant driver of microbial diversity and community composition (Hanson et al., 2012; Martiny et al., 2006). Fog could act as a vector of viable marine microbes to terrestrial systems, or increase preferential deposition of viable bioaerosols that would not otherwise be deposited (this is further discussed in the following section). The colonization of ecosystems by these fog microbes could have important ecological impacts, as in the case of the colonization of rocks in the Atacama Desert by cyanobacteria, which may have arrived via marine fog (Azua-Bustos et al., 2012; Warren-Rhodes et al., 2007), and may also be supported by the delivery of water and nutrients of that fog (Azua-Bustos et al., 2012; Azua-Bustos et al., 2010). Both Maine and Namib fogs are known to contain ecologically-relevant concentrations of nutrients (Dueker et al., 2011; Eckardt and Schemenauer, 1998).

In addition to its effect on ecological dynamics in the receiving ecosystem, the role of fog in linking marine and terrestrial systems has implications for transport of pathogenic microbes in polluted coastal and terrestrial environments. Previous studies have shown that bacterial and viral aerosols can originate from sewage contaminated waterways (Dueker and O'Mullan, 2014; Dueker et al., 2012a; Dueker et al., 2017; Montero et al., 2016). These pathogens could also be incorporated into urban fogs, further increasing their viability, deposition, and threat to plants, animals, and humans. Castello et al. (1995) confirmed the transfer of the Tomato Mosaic Tobamovirus in coastal Maine fog, and a microbial study of a severe Beijing smog event confirmed the presence of human respiratory pathogens in the atmosphere that increased with smog density (Cao et al., 2014). Although we did not assess microbial pathogenicity in our study, we did find that fog transports many taxonomic groups that contain pathogenic fungal species, including suspected plant pathogens and those causing respiratory infections in immunocompromised people (Table S3). If fog acts as a significant biological vector, any changes in fog frequency, for example due to warming coastal waters in the Namib (Haensler et al., 2011), could then have implications for public health and biodiversity.

4.2. Fog increases microbial deposition and alters aerosol communities

In addition to providing a novel linkage between marine and terrestrial systems, fog also alters the diversity and structure of local microbial aerosol communities. In time series from both continents, aerosol

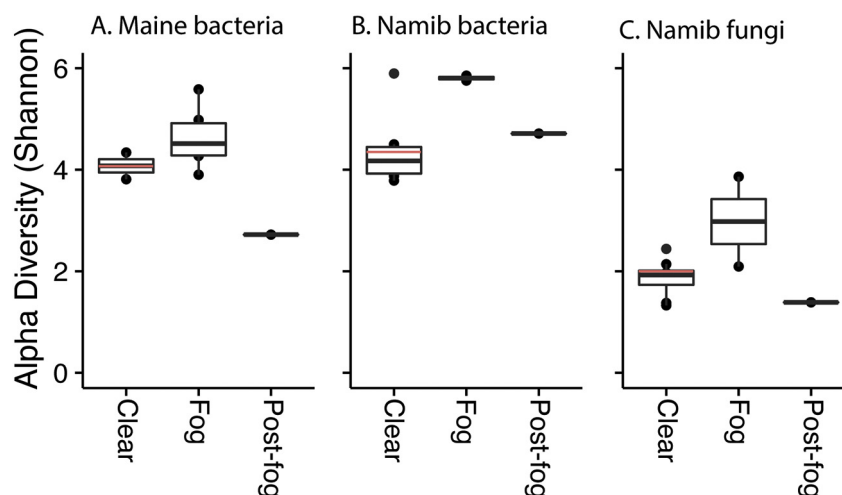


Fig. 5. Changes in alpha diversity during and after fog in Maine bacteria (A), Namib bacteria (B) and Namib fungi (C). “Clear” category includes all clear air samples; the red bar shows the alpha diversity of the clear air sample immediately preceding the fog.

community composition shifted with the onset of fog, and changed further in the period after the fog event (Fig. 2, arrows, Table S7). In the Namib, we also found that fog led to greater deposition of viable fungi (Figs. 2, 4), as previously reported for bacteria in coastal Maine (Dueker et al., 2012b). This finding, combined with the temporal shifts in composition, suggests that changes in aerosol communities before and after fog may be driven by deposition of certain microbes during fog events (Dueker et al., 2012b). Future studies should look at changes in bacterial and fungal abundance throughout the course of individual fog events with finer (~1 h) resolution to understand how deposition changes over time.

Microbial deposition from fog is likely to depend on certain traits like particle size, specific cell shape, and external membrane structure. For instance, organisms known to be efficient cloud condensation nuclei and ice nucleators (Schnell, 1977), particularly certain *Pseudomonas* species (Morris et al., 2014), may be preferentially scavenged from the atmosphere and gravitationally deposited, or impacted on vertical surfaces, by fog. Some of our findings suggest that deposition during fog relates to microbial traits, but our data did not allow us to identify general patterns that predict microbial deposition. In Maine, *Dechloromonas*, *Pseudomonas* and *Sphingomonas* showed the biggest changes in relative abundance pre- and post-fog. These genera are gram-negative and rod-shaped, but the relative abundance of *Pseudomonas* in the atmosphere decreased with fog, whereas *Dechloromonas* and *Sphingomonas* increased. This may be explained by certain *Pseudomonas* spp. having unique cell membrane structures (Morris et al., 2008), making them more likely to serve as fog nuclei and deposit during fog. Although we cannot systematically identify microbial traits likely to facilitate fog deposition, we do show that the fungal community deposited by fog is unique from that associated with dry aerosol deposition, and is larger and more diverse. This was also found in culture-based bacterial deposition measurements in Dueker et al. (2012b); Dueker et al. (2011). Future studies should consider other microbial traits such as spore size, cell morphology, particle association, or aggregation behavior which may affect a microbe's likelihood to deposit during fog events.

Mechanistic studies and more standardized cross-site examinations could help predict the microbial changes in dry aerosol communities that occur after fog events. We might expect taxa that decrease in aerosols following a fog to be those taxa prevalent in (or deposited by) fog. It seemed that fog deposition explained the decreased relative abundance of *Ophiocordyceps*, a fungal genus that parasitizes insects. *Ophiocordyceps* was one of the most abundant taxa in fog, and also one of the groups that showed the biggest reduction from pre- to post-fog conditions. However, most of the taxa that had lower abundance after fog were also those rare or even absent during fog, suggesting that many processes determine the effect of fog on bioaerosols, including fog introducing previously-absent taxa as transported droplets evaporate. The mechanisms and in particular the trait-mediated shifts in microbial communities under and after foggy conditions need further investigation to identify how this feature could alter microbial aerosol composition.

4.3. Conclusions and implications for future research

Here we present a whole-community characterization of fog microbial communities from two iconic fog systems, coastal Maine, USA, and the Namib Desert, Namibia. The patterns and functional implications derived from these iconic systems can serve as a foundation for future work in fog biology and its role in terrestrial ecosystem dynamics. We found that local sources strongly shape fog microbial communities, resulting in more marine species in fogs near the coast. But we also detected strong marine signatures 50 km inland, suggesting that fog droplets are likely to provide the “culture media” for long-term survival and transport of microbes to areas further inland (Fuzzi et al., 1997). This marine-to-terrestrial direction of materials exchange complicates current models of terrestrial influences on marine systems, since it shows

that the microbial composition of sea water and adjacent ecosystems can be redistributed back to land through aerosols and fog-facilitated deposition. These microbial communities deposited under fog are likely to be more diverse, more viable, and compositionally distinct from dry aerosols, warranting further study of the functional role these organisms play on land, and their implications for public health. Furthermore, warming sea surface temperatures and altered wind regimes are likely to affect the distribution of fog in many coastal systems (Haensler et al., 2011). In addition to the more commonly foreseen effects of reduced water availability, a change in microbial dispersal that may accompany changes in fog dynamics could have additional consequences for terrestrial ecosystems that we are just beginning to understand.

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Conflict of interest

The authors declare they have no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2018.08.045>.

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