# VIBRIO RESPONSE TO SAHARAN DUST DEPOSITION VARIES BY DELIVERY METHOD

By

#### MADISON PAIGE O'MALLEY

(Under the Direction of Erin K. Lipp)

#### **ABSTRACT**

Deposition of dust aerosols from the Saharan Desert is a major contributor to micro and macronutrients across marine habitats. In marine systems, dust can affect biogeochemistry of surface waters, driving autotrophic and heterotrophic microbial processes. Our understanding of biological responses to dust comes from studies on the soluble fraction of material leached from dust particles; however, work on *in situ* responses to dust events suggest that leached materials may drive responses differently from whole dust. Here we show that dust delivery method has a significant effect on microbial community composition, especially among early responders (24 h or less). We found that with the addition of whole dust aerosols, natural seawater bacterial community composition shifted significantly after 16 h of incubation. Furthermore, the primary taxa driving the change in composition were in the family Vibrionaceae, which increased significantly in whole dust treatments compared to both leachate and controls (p = 0.02).

INDEX WORDS: Vibrio, Vibrionaceae, Iron, Saharan Dust, Whole Dust, Leachate, Climate

Change

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## CHAPTER 1 LITERATURE REVIEW AND INTRODUCTION

#### Introduction

The bacterial family Vibrionaceae is a common marine taxon that includes multiple *Vibrio* species pathogenic to humans, causing cholera, gastroenteritis and septicemia. These opportunistic bacteria can grow rapidly at warm temperatures, are adept at accessing limited nutrients, and are expected to benefit from climate change, with clinical infections increasing in numbers, intensity, and range (Baker-Austin et al. 2017). *Vibrio* and Vibrionaceae populations are also known to undergo blooms at a local scales – where they rapidly grow to high concentrations over short time periods in response to environmental stimuli (Westrich et al. 2016). Blooms may be facilitated by local marine 'heat waves' (Frolicher and Laufkotter 2018), the influx of dissolved organic matter from algae (Boyer et al. 2006), or the deposition of desert dust and associated iron (Westrich et al. 2016). Conditions in which blooms are likely could be exacerbated by longer term increases in regional and global sea surface temperatures.

#### Vibrio and disease

Vibrio are a widely distributed group of marine bacteria, but typically make up only a very small fraction, roughly 1%, of surface water communities (Westrich et al. 2016). They live an opportunistic lifestyle that make them adaptable pathogens of both marine organisms and humans. They are often found in association with marine particulates including plankton (algae and zooplankton) and marine snow, where they are capable of degrading substrates with extracellular enzymes (Thompson and Polz, 2006). They can also survive in a free-living state or

hosted within higher organisms. According to the Centers for Disease Control and Prevention (CDC) all species of *Vibrio* (and all species within the broader Vibrionaceae family) are considered to be potentially pathogenic to humans (CDC, 2019). *Vibrio* related illnesses primarily affect humans through foodborne (shellfish) exposure, transmission through skin, especially through open wounds, and through ears and eyes (CDC, 2019).

In the U.S., the species of greatest public health concern are *V. parahaemolyticus*, *V. alginolyticus*, and *V. vulnificus*. Infections are typically spread through the ingestion of undercooked or raw shellfish or through wound exposure and can be fatal for immunocompromised (Marques et al. 2010). *Vibrio* are considered a leading cause seafoodborne gastroenteritis, worldwide (Marques et al. 2010). Most reported cases in the U.S. are caused by *V. parahaemolyticus*, which accounts for 50-60% of case reports (Greenfield et al. 2017; Jacobs et al. 2017). An infection from *V. parahaemolyticus* causes mild to severe symptoms of nausea, vomiting, diarrhea, fever and headaches. According to the Scallan et al. (2011), symptoms typically last a couple of days and cases are underreported by up to 142-fold.

Vibrio alginolyticus is the second most common Vibrio in U.S. case reports and rates of infection have risen dramatically over the last 20 years (Jacobs Slifka et al. 2015). V. alginolyticus infections are usually transmitted through direct exposure to marine water resulting in wound infections, as well as eye and ear infections. V. alginolyticus infections in children are common (Jacobs Slifka et al. 2015).

The other *Vibrio* of high concern is *V. vulnificus*. *V. vulnificus* is commonly transmitted by shellfish, raw seafood, or wound infections similar; however, infections from *V. vulnificus* can have a mortality rate of 50% and accounts for roughly 95% of all seafood related deaths, making it the deadliest foodborne pathogen in the US (Marques et al. 2010; Froelich and Noble,

2015). When not fatal, infections can still last for weeks and cause illness, diarrhea, nausea, vomiting, headaches and a fever. Severe cases of *V. vulnificus* can cause septicemia, hypotension, and secondary lesions on extremities (Froelich and Noble, 2015). In cases of septicemia and severe illness, death can occur as early as one to three days after infection (Greenfield et al. 2017). *V. vulnificus* is also known as a marine "flesh eating bacteria," because these infections can lead to necrotizing fasciitis (Froelich and Noble, 2015). Similar to fatal cases of foodborne infections, wound infections are typically fatal when there is an underlying disease, such as a compromised immune system or liver disease, and if not fatal often require skin grafts or limb amputation after infection (Williams et al. 2015). In recent years the number of wound infections has increased making this form of infection the most common by *V. vulnificus* with fatalities in up to 25% of cases (Williams et al. 2015). Due to the high rate of mortality, infections from *V. vulnificus* have great economic implications, often shutting down shellfish harvesting areas for one to three weeks at a time (Greenfield et al. 2017, Froelich and Noble, 2015) and affecting tourism due to high media coverage of severe infections and mortalities.

Vibrio are often associated with particles or plankton in the ocean; therefore, filter feeders such as mollusks, oysters, muscles, and other bivalves concentrate them during feeding (Froelich and Noble, 2015). Often bivalves and shellfish can filter and ingest concentrations of Vibrio greater than 100 times that in the surrounding waters (Froelich and Noble, 2015). Due to this bioaccumulation, consumption of raw oysters and shellfish pose one of the greatest pathogenic risks to human health (Froelich and Noble, 2015).

#### Vibrio Ecology

Due to human health and economic implications of *Vibrio* related infections, the ecology and physiology of *Vibrio* have been highly researched. Since *Vibrio* are easily cultured, this

genus can be used as a model organism for ecological and evolutionary bacterial population responses to nutrients, temperature, and salinity fluxes (Takemura et al. 2014). *Vibrio* species are ubiquitous across marine habitats and can occasionally be found in freshwater ecosystems (Materna et al. 2012). They can be found free-living in water, or more commonly associated with biofilms or colonized on marine organisms and biological surfaces (Takemura et al. 2014). They are capable of rapid growth over short time periods due to their ability to utilize many different sources of carbon and nitrogen (Takemura et al. 2014).

Vibrio are considered generalists or opportunists and can occupy a variety of niches within marine habitats. Vibrio only make up a very small proportion of the cells in a bacterial community; however, Vibrio cells are relatively large, allowing them to comprise a larger biomass despite fewer cells (Takemura et al. 2014). The implication of this is that when Vibrio populations grow (or bloom), their corresponding increase in biomass could have an outsized effect on carbon and nutrient processing. Vibrio are known to bloom to high concentration over short time intervals with input of substrate or limiting nutrients (Westrich et al. 2016). For example, Saharan Dust events can trigger a bloom due to delivery of iron and other potential nutrients (Westrich et al. 2016, 2018). Blooms occur quickly, last only 24 to 48 hours, and are difficult to predict and observe (Takemura et al. 2014).

Vibrio have adapted many advantageous traits for survival, including persistence under nutrient limited conditions (Takemura et al. 2014). By reducing cell volume in periods of starvation and maintaining elevated levels of rRNA copies, Vibrio are capable of enhanced protein synthesis when conditions return to a more favorable state (Eilers et al. 2000). This metabolic flexibility could be ecologically advantageous in the open ocean environment with varying nutrient regimes. Another advantage of Vibrio that helps them compete in a variety of habitats is their

large genomes and propensity for gene exchange. *Vibrio* can use all three forms of horizontal gene transfer (HGT) – conjugation through pili, transduction (they have many prophages in their genomes), and transformation (they are naturally competent in the presence of chitin) (Seitz and Blokesch, 2014).

#### Vibrio and Climate Change

As anthropogenic greenhouse gases increase in the atmosphere, more than just air temperature is affected. Climate change is projected to alter the physical and chemical properties of the global oceans (Poloczanska et al. 2016). The oceans, covering roughly 71% of Earth's surface, act as the greatest heat sink on the planet (Faizal and Rafiuddin Ahmed, 2011). Physical and chemical changes expected with climate change include rising sea surface levels, increased sea surface temperatures, decreased ocean pH (ocean acidification), increased stratification, loss of sea ice and altered ocean circulation (Burge et al. 2014, Hoegh-Guldberg and Bruno, 2010; Poloczanska et al. 2016).

These changes can all affect marine systems, including microbial communities, which are the basis for food webs in oceans. *Vibrio*, in particular, are sensitive to climate shifts, especially due to rising sea surface temperatures. Specific *Vibrio* species each have an optimal temperature and salinity range for growth, but most proliferate at warmer temperatures (Eiler et al. 2006). Recent work by Jacobs et al. (2015) estimate significant regional expansion of optimal *Vibrio* habitat, as well as expanded seasonal windows for growth, based on projected changes in coastal water temperatures. In one of the few long-term data sets available, Vezzulli et al. (2016) found that *Vibrio* had increased significantly as a proportion of the bacterial community in the North Sea as water temperatures increased over the last half-century.

#### The Vibrio and Iron Relationship

In addition to temperature, *Vibrio* populations can also respond to influx of iron.

Acquisition of iron is important in both *Vibrio* pathogenesis and ecology. Iron is an essential nutrient for all life on earth. In aerobic conditions, iron is needed for many cellular processes such as the reduction of oxygen for the synthesis of ATP, the formation of heme, the production of chlorophyll and assimilation of nitrogen (Neilands 1995; Bergquist et al. 2007).

Although iron is one of the most abundant elements on the planet, its concentration in a bioavailable form in the ocean is extremely low due to the low solubility of iron under oxidizing conditions (Bergquist et al. 2007). Iron in the ocean can be found in the surface from deposited dust, in the sediment, or hydrothermal vents (Payne et al. 2016). Reduced iron at the surface is oxidized in a matter of seconds to minutes when present in oxygenated ocean water (Payne et al. 2016; Baker and Croot 2010). Iron is found in such low concentrations that it can be considered a limiting nutrient for primary production in many regions of the ocean (Bergquist et al. 2007; Baker and Croot 2010).

One of the major sources of iron to the world's oceans is atmospheric deposition. In particular, the Saharan desert provides among the greatest amount of nutrient rich dust to the atmosphere, which is later deposited in marine and terrestrial areas downwind in the tropical Atlantic. Originating as desert dust storms in northern Africa, dust, along with trace metals and nutrients, is moved into the troposphere and carried across the Atlantic Ocean with the easterly trade winds annually. The dust is deposited along from Africa to the Caribbean with the greatest amounts of deposition close to the original source (Figure 2). Deposition of dust provides an episodic source of otherwise limiting nutrients to surface waters, which can be used by microbial communities (Duarte et al. 2006; Payne et al. 2016).

Like all aquatic bacteria, *Vibrio* require iron to survive (Payne et al. 2016). Iron found in the open ocean, can be as low as 20-30 pM, which well below the required amount for most *Vibrio* growth (Payne et al. 2016). To utilize the scarce amount of iron in the marine ecosystem, microorganisms have developed specialized mechanisms for iron uptake. One mechanism is the use of siderophores, an iron-scavenging complex secreted by *Vibrio* and other bacteria (Payne et al. 2016). When secreted, siderophores scavenge and bind to iron where they can now be recognized by a specific receptor on the outer membrane and transferred into the cell (Payne et al. 2016). When iron is limiting, many species of *Vibrio* are able to produce one or more siderophores to help scavenge the little iron available (Baker and Croot, 2010). Apart from siderophores, many *Vibrio* also can take up siderophores produced by other bacteria and also contain iron transport genes (Payne et al. 2016; Thode et al. 2018).

Additionally, through horizontal gene transfer, *Vibrio* have obtained genes that encode for ferric and ferrous iron transport, specific proteins that aid in the synthesis and transport of high-affinity iron chelators and different types of iron receptors which can lead to distinct selective advantages for the adapted *Vibrio* (Thode et al. 2018). The role of iron transport mechanisms is best understood through research on *V. cholerae*, where 1% of the entire genome is dedicated to the transport of iron (Payne et al. 2016).

#### The Role of Desert Dust in Tropical Vibrio Ecology

When wind sweeps across a desert, it picks up "desert dust" - a mixture of minerals (e.g., iron), particles, bacteria and other microorganisms (Prospero 2007). The dust can then travel thousands of miles before being deposited either on land or in a body of water. Each year, approximately 800 Tg of Sahara dust particles are lifted into the air (Prospero et al. 2014). Within one week, as much as 25% of this dust can travel across the Atlantic Ocean where it is

deposited on land or in the upper mixed layer of the surface ocean (Pan et al. 2018) (See example, Figure 1). During the summer months, dust from the Sahara Desert travels northward reaching the Caribbean, whereas in the winter months, the dust primarily travels west towards northeastern South America (Moulin et al. 1997). One of the expected impacts of climate changes is increased desertification in already arid regions that may experience drought and increasing water stress (IPCC 2018). Recent studies suggest that the effects of climate and environmental change on arid conditions in northern Africa are increasing (Popov et al. 2011), resulting in a greater frequency and intensity of dust events.

In addition to direct human exposure, which can cause serious respiratory illnesses (e.g., Kanatani et al. 2010), dust events also impact downwind ecology (and potentially human health, indirectly). When dust is deposited into the ocean surface, a microbial response is triggered by the influx of limiting nutrients and trace metals (especially iron). Newly available iron, and other nutrients, including phosphorous can stimulate growth in phytoplankton, including potentially harmful algae (Lenes et al. 2001). More recently studies have shown that heterotrophic bacteria can also grow following dust events (Duarte et al. 2006), including *Vibrio*, which can quickly bloom in response to dust deposition (Westrich et al. 2016, 2018; Borchardt et al. in press).

Iron deposited in the North Atlantic by Saharan dust, provides up to 87% of the dissolved iron needed for marine surface water communities (Westrich et al. 2018). *Vibrio* have been shown to be highly responsive to the addition of Saharan dust delivered to coastal surface waters in the Caribbean and Gulf of Mexico, exploiting the iron and other nutrients and trace metals, to increase growth rates (Westrich et al. 2016). These short, 48-hour, blooms peak by 24 hours post dust, and are followed by sequential blooms of other heterotrophic bacteria and finally cyanobacteria (Borchardt et al. in press). The temporary release from nutrient-limitation with

dust arrival could directly drive the *Vibrio* blooms with a resulting crash after exhaustion of organic carbon sources (Westrich et al. 2018).

#### **Study Introduction**

There remain open questions about the nature of aerosols that are deposited during dust events and how the nutrients (iron) are delivered to the surface water. Prior work has suggested that bioavailability (solubility) of iron increases in dust aerosols as they are transported long distances (e.g., across the Atlantic) (Mahowald et al. 2009, Westrich et al. 2016). But work by Zhang et al. (2019) suggests that soluble inorganic iron, as may be expected from leached aerosols, may be insufficient to stimulate *Vibrio* growth, leading to the question posed in this thesis: how does the mode of dust delivery (whole dust versus leached fractions only) affect the surface water community response?

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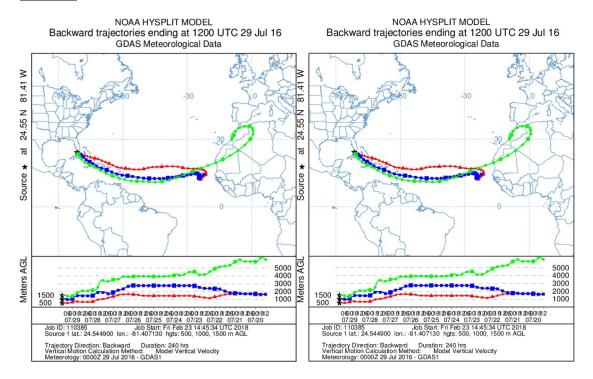
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### **Figures:**



**Figure 1.1.** The back trajectory shows Sahara Dust arriving in the Florida Keys on the 29<sup>th</sup> of July 2016. The dust can be back tracked to Saharan Africa, where it is seen beginning its journey on the 20<sup>th</sup> of July 2016.

#### **CHAPTER 2**

# VIBRIO RESPONSE TO SAHARAN DUST DEPOSITION VARIES BY DELIVERY METHOD

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#### **Abstract:**

The tropical and subtropical Atlantic Ocean receives dust aerosols, arriving from the Sahara and Sahel, which can drive microbial processes. Of particular interest is the early biological response to dust arrival, which can have a dramatic effect on processing and bioavailability of nutrients and trace metals, especially dissolved or bioavailable forms of iron. The addition of whole dust aerosols causes the natural seawater bacterial community composition to shift significantly, with the family Vibrionaceae as the primary driver. This was further supported by direct measurements of *Vibrio* in these microcosms, which also showed both significantly higher levels of *Vibrio* (p = 0.03) and increased growth rates in whole-dust treatments. The addition of leached dust alone did not result in any change among Vibrionaceae. These findings suggest that factors associated with delivery of particulates as well as dissolved fractions of dust are critical in how the microbial community responds to dust.

#### **Introduction:**

Several times annually fine particulates (dust) from the Sahara Desert are picked up and transported into the atmosphere with easterly trade winds and later deposited in surface waters and terrestrial environments across the Atlantic Ocean, from the Amazon to Texas (Prospero 2006). In marine waters, dust deposition provides an episodic source of nutrients and trace metals, such as iron, to the ocean surface where they typically occur in extremely low concentrations in a bioavailable form (Lenes et al. 2001, Duarte et al. 2006). In particular, these dust events provide a critical subsidy of iron that can affect both phytoplankton and bacterial growth (Mahowald et al. 2009, Westrich et al. 2016). Saharan dust is also an important source of other external nutrients, such as carbon, nitrogen and phosphorus, which are critical to supporting heterotrophic bacterial growth (Duarte et al. 2006). Roughly 43% of the total aeolian

dust deposited in the oceans worldwide is received by the North Atlantic alone, with more than half of it coming from the Sahara-Sahel (Moxim et al. 2011). These dust events occur annually, with pulses lasting 3-5 days between June and August (Perry et al. 1997, Prospero 2007).

Many areas of the tropical Atlantic are less iron-limited than other oligotrophic regions because of the repeated renewal from episodic dust events (Fitzsimmons and Boyle 2014), but dust deposition still stimulates local biological responses in this region (Reche et al. 2009). One study recorded an increase of 16-fold in the amount of iron in the Gulf of Mexico during a single dust event, and a subsequent bloom of the N-fixing cyanobacteria *Trichodesmium* (Lenes et al. 2001). In part because of the importance of iron-limitation among primary producers, much of our knowledge about biological responses to dust has focused on autotrophs and their uptake of soluble iron (Sedwick et al. 2007; Westrich et al. 2016). However, a growing body of research suggests that heterotrophic bacteria may play a role in processing iron-associated dust (Pulido Villena et al. 2008; Pitta et al. 2017). Dust-deposited iron can be rapidly processed in marine surface waters, with soluble forms considered to be the most readily bioavailable. However, much of the dissolved fraction (operationally defined as able to pass through a 0.2 µm pore size filter) may consist of colloidal iron, in an organic or inorganic state, rather than as soluble iron (Fitzsimmons et al. 2015), which can affect how the iron is processed and by whom. Siderophore production enhances uptake among bacteria (Granger and Price 1999 Hassler et al. 2011), but picoeukaryotes may also be able to rapidly take up iron through binding with saccharides, which are readily available and help to solubilize iron (Hassler et al. 2011).

Several studies have specifically examined dust events and their effect on microbial communities in the tropical Atlantic and suggest that heterotrophs are among the earliest responders (Mahowald et al. 2009; Marañón et al. 2010; Pulido Villena et al. 2014) with recent

evidence also suggesting rapid stimulation of picoeukaryotic populations (Borchardt et al. in revision). These early responders likely include taxa that are able to use dust-associated iron in a variety of states. *Vibrio*, a normally rare marine taxonomic group that can opportunistically bloom to high levels, can produce several different siderophores and take up exogenous siderophores through multiple receptors (Thode et al. 2018), suggesting a metabolic capacity to respond to iron in dust, as well as other associated nutrients. Additionally, *Vibrio* cells are relatively large compared to more common oligotrophic heterotrophs (Cermak et al. 2017), which suggests that blooms of this group could have an important effect on local biogeochemical processing (Westrich et al. 2016 and 2018; Polz et al. 2006) and potentially prime the community for successional responses (Borchardt et al. in press).

Previous work evaluating the early response to dust events, especially among *Vibrio*, has revealed several important findings, including; 1) specific *Vibrio* species can use processed dust as their sole source of iron (Westrich et al. 2016), 2) *Vibrio* populations in oligotrophic marine surface waters increase significantly during dust events (Westrich et al. 2016 and 2018), and 3) *Vibrio* are one part of an early response to a longer successional pattern among microbial community members following dust events (Borchardt et al. in press), but 4) *Vibrio* may not be able to bloom in response to inorganic iron inputs alone (Zhang et al. 2019). Given the discrepancy between observations during *in situ* dust events (Westrich et al. 2016 and 2018, Borchardt et al. in revision) and additions of inorganic iron or leachate from desert soils (Zhang et al. 2019), we examined the potential effects of adding 'whole' dust to the system versus a leachate of dust. This is based on the hypothesis that small particulates, particulate iron, or other factors, including the direct introduction of bacteria from aerosols (Pitta et al. 2017) may be essential in the marine microbial community response. Our aim was to determine if particulate

dust plays an important role in how the microbial community responds to dust events. This step is especially important for understanding how heterotrophic bacteria may be utilizing particulate and dissolved iron, as well as other limiting factors deposited during a dust event. Therefore, this study addressed four different goals; 1) to determine if the response of *Vibrio* changes based on dust delivery method, 2) observe and assess microbial community compositional changes based on dust delivery method, 3) determine if aerosol introduced bacteria play a role in the change in microbial composition, and 4) determine if there are other culturable bacteria that respond to a particular dust delivery methods besides *Vibrio*.

#### **Methods:**

Boundary layer aerosols were collected daily (24 h composite samples) on acid washed 47 mm Whatman-41 cellulose filter paper during the Saharan dust season in the Lower Florida Keys, USA (24°38′4.16″ N, 81°21′17.02″ W) in July 2016. Twelve replicate aerosol samples were collected each day using a high-volume aerosol sampler (model 5170-VBL, Tisch Environmental) located at the shoreline and situated a height of 1 m above sea level, and then stored at -80°C (Seim et al., in prep). The water column depth near the collection site was <2 m. For this study we selected two dates, 28 and 29 July 2016, with high levels of Saharan dust (confirmed by aerosol optical thickness (AOT) measures of 0.2225 and 0.1875 on July 28<sup>th</sup> and 29<sup>th</sup> respectively, and analysis of trace metals from the aerosol samples). For each collection date, one replicate filter was used for whole dust additions and the second was leached by passing 100 ml of slightly acidified (pH = 5.6) ultra-pure water through the filter and then further filtered through a 0.2 μm pore size membrane (Buck et al. 2010) for leachate additions. Total and dissolved iron concentrations were measured in aerosol leachates from replicate Whatman-41

filters using ICPMS as described by Morton et al. (2013). Nutrients were measured in the leachate using a Seal QuAAtro autoanalyzer and organic matter in the leachate (sample minus blank filter control) was determined using the High Temperature Catalytic Oxidation method on a Shimadzu TOC-Vs analyzer with nitrogen module (Borchardt et al. in press).

Microcosm experiments were conducted at the Florida Keys Marine Lab in August 2017 and did not coincide with any dust events. Water for microcosms was collected using trace metal clean procedures from an offshore oligotrophic reef site (24.825857 N, 80.814060 W) into individual acid-washed 4 L cubitainer vessels, which were kept shaded and cool until arrival at the marine lab (~2 h). Cubitainers were placed in running seawater raceways to maintain ambient temperatures. Raceways were shaded with neutral density mesh. Temperature and light levels were monitored over the course of the experiments using HOBO pendant loggers (Onset Corp.). Cubitainers were randomly assigned to a treatment group: no addition control (n=3), blank Whatman-41 filter control (n=3), whole dust aerosol from July 28 (n=3), whole dust aerosol from July 29 (n=2), aerosol leachate from July 28 (n=3), and aerosol leachate from July 29 (n=3). For the whole aerosol additions, the filter was cut into quarters using Teflon-coated scissors in a trace-metal free environment. Immediately prior to initiation of the experiment, 1/4 filters were shaken for ~2 min in 25 ml of collected seawater and slowly added (with the ¼ filter) to respective treatment cubitainers. Likewise, 25 ml (1/4) of the 100 ml leachate produced for each filter was added to the appropriate cubitainers, ensuring a similar quantity delivered between each method.

Water samples were collected (aseptically and using trace metal clean techniques) from each cubitainer into 500 ml sterile polypropylene bottles at a series of time points (0h, 16h, 24h, and 48h) and analyzed for: 1) total culturable *Vibrio*, 2) total culturable heterotrophic bacteria,

and 3) bacterial community composition. Additionally, 16S rRNA gene amplicons from ¼ aerosol filters from July 28 and 29 were also sequenced to evaluate the potential role of exogenous bacteria introduced into the microcosms (see below).

#### **Culturable Bacteria**

To determine the concentration of *Vibrio* spp. 100 μL of each sample was spread-plated in triplicate onto TCBS (thio-citrate bile salts) agar plates (Oxoid Brand) for all time points. Plates were incubated overnight at 30 °C. All yellow and green colonies were counted and reported as colony forming units (CFU) ml<sup>-1</sup> for each time point and treatment.

Total culturable heterotrophic bacteria concentrations were determined by spread plating  $100 \,\mu\text{L}$  samples onto half strength Marine Agar plates (Zobell 2216) and incubated overnight at  $30\,^{\circ}\text{C}$ . All colonies were counted and CFU ml<sup>-1</sup> were recorded per time point and treatment. Once colony counts were recorded, plates were flooded with 1 mL of PBS and colony growth was collected into a 2 ml microfuge tube using a sterile scraper. A total of  $100\,\mu\text{L}$  of the scraped community was collected and centrifuged at  $11,200\,\text{x}$  g for 8-10 min. The supernatant liquid was decanted, and the pellet saved at  $-20\,^{\circ}\text{C}$  until the DNA was extracted according to the Qiagen DNeasy Blood and Tissue Pretreatment for Gram-Positive Bacteria protocol (Qiagen, Germantown, MD).

#### **Bacterial Community Composition**

To evaluate changes in the total bacterial community composition during experimental manipulations, ~500 mL of sampled water was passed through a 0.2 μm pore-size Sterivex filter (Millipore Sigma, Burlington, MA) using a peristaltic pump. Sterivex filters were pumped dry, capped and held at -80° C prior to processing. DNA was extracted from frozen samples using a phenol:chloroform extraction method described by Tinker and Ottesen (2016). Additionally,

DNA was extracted from replicate ¼ aerosol filters collected on 28 and 29 July. Samples were extracted using the Qiagen Power Soil kit following manufacturer's protocol (these samples are also part of a separate study on the aerosol community; Seim et al. in preparation)

For Sterivex obtained DNA, DNA from growth on marine agar plates, and aerosol DNA, the 16S rRNA gene V4 hypervariable region was amplified using the 515F and 806R primer set (Caporaso et al. 2011). PCR reactions were carried out in a master mix containing 2 µL of 5x Q5 Buffer (New England BioLabs (NEB), Ipswich, MA), 0.5 μL each of 10μM forward (5'-GTGCCAGCMGCCGCGGTAA-3') and reverse (5'-GGACTACHVGGTWTCTAAT-3') primers (Table S1), 0.2 µL of 10 mM dNTPs mix (NEB), 0.1 µL of Q5 Hot Start High-Fidelity DNA polymerase (NEB), and 2 µL of DNA template. Molecular grade water was added for a final volume of 10 µL per reaction. The following thermocycler protocol was followed: 98°C for 30 sec, 10 sec at 98°C, 20-25 cycles of 52°C for 30 sec, 72°C for 30 sec, and a final extension time of 2 min at 72°C. A second round of PCR was performed to add custom multiplex adaptor barcodes (Tinker and Ottesen, 2016). This reaction contained 6.0 µL 5x Q5 Buffer (NEB), 1.5 μL of 10 μM of barcode primers (Tinker and Ottesen 2016), 0.6 μL of 10 mM dNTPs mix (NEB), and 0.3 µL of Q5 Hot Start High-Fidelity DNA polymerase (NEB). Molecular grade water and 9 µL of pooled PCR technical replicate product were added for a final volume of 30 μL per reaction All reactions were conducted in duplicate. A thermocycler protocol using the subsequent parameters was followed to add barcodes: 98°C for 30 sec, 98°C for 10 sec, 4 cycles of 30 sec at 52°C, 30 sec at 72°C, 6 cycles of 10 sec at 98°C, 72°C for 1 min, and final extension at 72°C for 2 min. Following barcoding, technical replicates were pooled and PCR amplified DNA was purified using 1:1 volume SPRI magnetic beads (Sera-Mag SpeedBeads, GE Healthcare, Little Chalfont, United Kingdom), re-eluted in a 1:1 volume with 10 mM of Qiagen

PowerSoil C6 solution (Qiagen, Germantown, MD), and was quantified on a Qubit dsDNA HS Assay Kit (Thermo Scientific, Wilmington, DE) (Rohland & Reich, 2012).

Samples were checked for quality using a fragment analyzer (Advanced Analytical Technologies) and concentrations normalized through qPCR by the Georgia Genomics and Bioinformatics Center (GGBC, Athens, GA). Samples were then pooled into a single run containing a total of 170 samples with 50% PhiX using v2 chemistry on an Illumina MiSeq to obtain PE250 reads (Illumina, Inc. San Diego, CA). Because of the very low sequence diversity expected from culturable heterotrophic bacteria growth compared to whole microbial community, amplified DNA derived from marine agar plates was diluted to 1:1000 the concentration of other samples in the pool (0.0005ng/µL total DNA concentration) in 10 mM Tris pH 8.5 as recommended by GGBC.

Microbial community composition was compared for unique amplicon sequence variants (ASVs) and diversity estimates within and between treatments. The DADA2 pipeline version 1.11.1 (Callahan et al. 2016) was followed in R Studio. The first step of the pipeline was to upload sequences before trimming and filtering. Sequences were truncated to read lengths between 160 and 240 nucleotides and trimmed following the standard filtering parameters in DADA2 to identify true sequence variants. Paired-end reads were merged and any sequences that did not align by 12 identical bases in the overlap region were screened and removed. A total of 11,996 ASVs were identified from the merged samples before chimera removal. Chimeras were removed if identified sequences could be exactly reconstructed by combining a left-segment and right-segment from two more abundant "parents". After the removal of chimeras, 3,140 ASVs (81.7% of the reads) remained. To assign taxonomy, RDP (Cole et al. 2014) was used as a reference database (given that Greengenes has been shown to mis-assign taxa associated with

Vibrio and Vibrionales (Lydon and Lipp 2018)). Using the Phyloseq package (McMurdie and Holmes 2013) in RStudio, chloroplasts and mitochondria-assigned ASVs were removed from our taxonomy tables, while unknowns were kept and assigned to the "unknown" category for barplots. With the taxonomies assigned to family level, Phyloseq was used to create stacked bar plots of relative abundance as well as perform all statistical analyses. After comparing the number of reads per sample, 9 samples with low reads (below 12,000) were removed from data analysis, including all statistics and plots.

#### **Statistical Analyses**

Data for culturable *Vibrio* and heterotrophic bacteria on marine agar, concentrations (CFU ml<sup>-1</sup>) were normally distributed based on the Shapiro-Wilk test. Outliers in culture concentrations (*Vibrio* and marine agar) were identified using Grubb's test. After outliers were removed, a two-way ANOVA was performed to determine the main effect each treatment. If results were found to be significant (at  $\alpha = 0.05$ ), Tukey multiple comparison procedures were followed to determine the differences between treatments at each time point. For culturable *Vibrio* specific growth rate at  $T_{16}$  and  $T_{48}$  was determined (growth rate  $\mu = [(\log T_n - \log T_0) \times 2.303]$ /time (h)) (with outliers removed as identified above) and distribution of data was confirmed as normal based on the Shapiro-Wilk test. One-way ANOVA followed by Tukey's post-hoc test was used to compare growth rates between treatments at each time point. Analyses were conducted in GraphPad Version 8.0.2.

For community analyses based on 16S rRNA gene amplicon sequencing (total and culturable [growth on marine agar]), data were stratified by treatment type and time and analyses were conducted in RStudio Version 3.5.1 Alpha diversity (Shannon) for both community types was calculated to determine within sample diversity. Diversity measures were determined to be

normally distributed; therefore, ANOVA followed by Tukey's multiple comparison post-hoc test, were performed to determine significant differences in mean alpha diversity by treatment and time point. For beta diversity measures, a weighted Bray Curtis distance matrix was calculated to create non-metric multidimensional scaling plots (NMDS) for both the microcosm total community and the culturable community. The vegan package (Oksanen et al., 2016) in R was used to perform permutation-based multivariate analysis (PERMANOVA) using the adonis function, which used the weighted Bray Curtis metric distance matrix to determine if the treatments had different beta diversity centroids. The betadisper function (vegan package) was also used to calculate whether treatments had different dispersions. Finally, changes in the relative abundances of specific ASVs binned at the Family level were analyzed with the DESeq2 function in R (Love, Huber, & Anders, 2014). Specific changes in relative abundance of all ASVs identified as Vibrionaceae in total community samples were determined by calculating reads per sample divided by total reads, tested for normality using the Shapiro-Wilk test, and analyzed with two-way ANOVA, to determine the main effect of treatments. If results were found to be significant (at  $\alpha = 0.05$ ), Tukey's multiple comparison post-hoc test was followed to determine the differences between treatments and time points.

Geneious Version 11.1.2 was used to identify matching ASVs between the aerosol samples and the total and culturable microcosm communities. Microcosm ASVs and their respective sequences were used to create a custom BLAST reference. The reference was then used to compare overlapping ASVs at >99% identity.

Initial analyses revealed that both culturable bacteria (total and *Vibrio*) concentrations and community composition data did not differ within treatment types (i.e., no addition control and blank filter control; whole dust from July 28 and July 29; and leachate from July 28 and 29).

Therefore, results by treatment type were collapsed into three groups: control (n = 6), whole dust (n = 5), and leachate (n = 6) for all analyses.

### **Results:**

### **Dust Aerosol Chemistry**

Total iron in the ¼ aerosol filters was similar between the two dates, 24,682 ng (July 28) and 26,144 ng (July 29). The fraction of soluble iron, however, was 1.5% on July 28 (~376 ng) but was only 0.6% on July 29 (~164 ng). It is estimated that these amendments provided an additional 0.63 (July 29) to 1.42 (July 28) soluble nM Fe to the vessels in both the leachate and whole dust treatments. In addition to iron, aerosols also contributed additional dissolved organic carbon (+1.31 μmol for July 28 and +1.70 μmol for July 29), dissolved organic nitrogen (+0.08 μmol for July 28 and +0.12 μmol for July 29), nitrate (+0.37 μmol for July 28 and +0.52 μmol for July 29), ammonium (+0.24 μmol for July 28 and +0.48 μmol for July 29), and orthophosphate (+0.003 μmol for July 28 and +0.002 μmol for July 29) to each treatment vessel.

### **Culturable Bacteria**

Culturable *Vibrio* were present across all treatments at the initiation of the experiment  $(T_0)$  at levels ranging from an average of 6 to 17 CFU ml<sup>-1</sup>. *Vibrio* counts increased by  $T_{16}$  and  $T_{24}$  in the whole dust treatment, from a mean of 11 at  $T_0$  to 739 and 1,049 CFU ml<sup>-1</sup>, respectively (p = 0.061 and p = 0.003) (Figure 2.1). While *Vibrio* concentrations also increased over time in both the control and leachate treatments, the level of increase was moderate and not significantly different from time  $T_0$ . At  $T_{48}$ , control samples increased to 26 CFU ml<sup>-1</sup>, while whole dust and leachate concentrations decreased to 863 and 31 CFU ml<sup>-1</sup>, respectively. At  $T_{16}$ , whole dust samples were significantly higher than leachate samples (p = 0.039). Significant differences

between treatments were noted at  $T_{24}$ , with concentrations in the control and leachate treatments significantly lower (30 CFU ml<sup>-1</sup> and 66 CFU ml<sup>-1</sup>, respectively) than that in the whole dust treatment (1,049 CFU ml<sup>-1</sup>) (p < 0.002, for both) (Figure 2.1).

Specific growth rate  $\mu$  among whole dust treatments averaged 0.178 h<sup>-1</sup> at T<sub>16</sub> which was greater but not significantly different than that found for leachate (0.071 h<sup>-1</sup>) or control (0.017 h<sup>-1</sup>). By T<sub>24</sub>, mean *Vibrio* growth rate in whole dust treatments reached 0.231 h<sup>-1</sup> and was significantly greater than either the leachate treatment (0.089 h<sup>-1</sup>) or control (0.073 h<sup>-1</sup>) (p = 0.041) (Figure 2.2).

Total culturable heterotrophic bacteria (i.e., capable of growth on marine agar) were present across all treatments at the initiation of the experiment ( $T_0$ ) at levels ranging from 68 to 76 CFU ml<sup>-1</sup>. Total bacterial counts in whole dust treatments at  $T_{16}$  and  $T_{24}$  increased from a mean of 74 at  $T_0$  to 968 at  $T_{16}$  and 1,493 CFU ml<sup>-1</sup>  $T_{24}$ , but increases were only significant between  $T_0$  to  $T_{24}$  (p = 0.032). In control samples, mean concentrations increased from 70 at  $T_0$  to 467 at  $T_{16}$  and 768 CFU ml<sup>-1</sup>  $T_{24}$ , with the only significant increase from  $T_0$  to  $T_{24}$  (p = 0.004). Lastly, among leachate treated samples, mean concentration increased from 68 at  $T_0$ , 284 at  $T_{16}$  and a maximum of 592 CFU ml<sup>-1</sup> at  $T_{24}$ , however not significantly (Figure 2.1).

Sequencing of bacteria growing on marine agar plates was used to determine taxonomic composition of the culturable fraction of the community. Of the top 100 taxa across all treatments and time points, Vibrionaceae, Rhodobacteraceae, Pseudoalteromonadaceae, and Flavobacteriaceae were the most abundant. Vibrionaceae relative abundance increased by  $T_{16}$  and  $T_{24}$  in the whole dust treated samples, but the difference was not significant (Figure 2.3). Between  $T_0$  and  $T_{48}$  only Alteromonadaceae increased significantly (p < 0.000).

### **Microbial Community Composition**

After removal of 9 samples with extremely low reads, the remaining 59 samples for the total microbial community analysis included 1,434,544 total reads and approximately 1,394 ASVs following trimming, filtering, and removal of chimeras and non-bacterial sequences. At  $T_0$ , both community composition and alpha diversity were similar across all three treatments (Shannon index 4.1 to 4.2, Figure S2), but by  $T_{24}$  alpha diversity in the whole dust samples declined compared to control and leachate (p = 0.01). At  $T_{48}$  alpha diversity appeared similar to  $T_0$  and did not show any significant changes by treatment type (Shannon, 4.1 to 4.2). Similarly, beta diversity among the treatment groups was similar at the start of the experiments (Bray-Curtis, 0.1 to 0.3), but diverged significantly by  $T_{16}$  and  $T_{24}$  (p = 0.023 and 0.013, respectively, Figure 4). NMDS plots showed that whole-dust treatments in particular, were well-clustered at these time points while there was more overlap between leachate treatments and control.

At the start of the experiment, the most abundant families across treatments were Gplla (*Synechococcus*), SAR11 (*Candidatus Pelagibacter*), Flavobacteriaceae, Rhodobacteraceae, and Rhodospirillaceae. Over time, the most abundant taxa changed depending on the treatment type (Figure 2.5). At  $T_{16}$ , there were few significant changes in relative abundance of taxa between the treatments. Between control and whole dust, the DESeq2 analysis showed that the only ASV to increase significantly in relative abundance in the whole dust treated samples by  $T_{16}$  was in the Family Puniceicoccaceae (p = 0.042). At  $T_{16}$  ASVs from Gplla (*Synechococcus*) (p = 0.047), SAR11 (p = 0.047) and Rhodobacteraceae (p = 0.047) all decreased in leachate samples and Hyphomicrobiaceae (p = 0.047) increased compared to control samples. Lastly, the relative abundance of ASVs from three Families were significantly higher in the whole dust samples compared to leachate at  $T_{16}$ , including Gplla (*Synechococcus*) (p = 0.040), SAR11 (p = 0.039)

and Puniceicoccaceae (p = 002). At  $T_{24}$ , the only ASV to increase in relative abundance in the whole dust treatment compared to control was Vibrionaceae (p = 0.001). Between control and leachate treated samples at  $T_{24}$ , the relative abundance of Gplla (*Synechococcus*) (p = 0.013), Rhodobacteraceae (p = 0.026), Rhodospirillaceae (p = 0.026), and SAR11 (p = 0.050) ASVs were all significantly lower among leachate treatments, while Flavobacteriaceae (p = 0.026), Planctomycetaceae (p = 0.026), Saprospiraceae (p = 0.026), and Verrucomicrobiaceae (p = 0.026) all increased in leachate treatments. Lastly, at  $T_{24}$ , Vibrionaceae relative abundance increased significantly (p = 0.022) in whole dust treatments compared to leachate, while Flavobacteriaceae (p < 0.001) was higher in leachate (Figure 2.6). Vibrionaceae was the primary taxon driving changes in the whole dust treatment community. The relative abundance of Vibrionaceae increased from a mean of 0.78% at  $T_0$  to 6.27% at  $T_{16}$  (p < 0.002) and reached its highest relative abundance of 14.13% at  $T_{24}$  (p < 0.032) before declining to 9.00% by  $T_{48}$  (Figure 2.7).

A total of 38 ASVs were common (at >99 % identity) between the total community and the culture community across all treatments and time points, including Alteromonadaceae (2 ASVs), Micrococcaceae (3 ASVs), Pseudoalteromonadaceae, (6 ASVs), Rhodobacteraceae (10 ASVs) and Vibrionaceae (5 ASVs) (Table S2, S3, S4).

### **Aerosol Community Composition**

To determine taxa that may have been introduced to the whole dust treatments via the aerosols themselves, the aerosol community composition was analyzed (also see Seim et al., in prep). Between the aerosol communities (from filters collected on July 28 and 29 2016) and the corresponding Cubitainers in which whole dust was added, there were 33 ASVs in common at greater than 99% similarity, comprising primarily Gplla (*Synechococcus*) (9 ASVs),

Vibrionaceae (5 ASVs), Rhodobacteriaceae (5 ASVs), and Oceanospirillaceae, Staphylococcaceae, and Alteromonadaceae (2 ASV each) (Table S5 and S6). Because we had a specific interest in the *Vibrio* response in the microcosm experiments, we further investigated taxa from the Vibrionaceae family in the aerosols. All five of the Vibrionaceae ASVs that were common between the aerosols and the microcosms were detected across all treatments and timepoints but at levels < 0.5% relative abundance. Of note, there was one Vibrionaceae ASV that was in relatively high abundance on both July 28 (5%) and 29 (6%) aerosols but was never observed in the experimental microcosm treatments.

### **Discussion:**

This study was conducted to examine the specific effects of the addition of whole versus leached aeolian dust on heterotrophic microbes in a tropical marine system. The primary finding of this study was that dust delivery method has a significant impact on bacterial responses to dust additions, with Vibrionaceae acting as the primary responder to whole dust additions. The results of the microcosm study indicate that the bacterial community composition changes as early as 16 hours following addition of whole dust but was indistinguishable from control or leachate treatments by 48 hours. This finding is consistent with observations *in situ* where *Vibrio* abundances increase during the first 14 – 24 hours following the onset of a dust event and return to baseline within in 48 h (Westrich et al. 2016, Borchardt et al. in press). Although the composition of members of the community changed at least modestly in all treatments, only Vibrionaceae composition increased significantly due to whole dust additions compared to the control and leachate by 24 h post addition. This is also supported by culturable counts showing that *Vibrio* concentrations similarly increased by 2 orders of magnitude within 16 – 24 h, but

only with the addition of whole dust. By 24 h post dust addition the growth rate of *Vibrio* in the whole dust treatment exceeded 0.23 h<sup>-1</sup> (or >5.5 day<sup>-1</sup>). *Vibrio* are known to be fast growing in response to favorable conditions (including addition of carbon and/or nutrients) (Eiler et al. 2007). Worden et al. (2006) reported *V. cholerae* growth rates in seawater of 2-3 day<sup>-1</sup> in the presence of organic carbon or algal blooms. Growth rates in response to dust suggest that the addition of whole dust alone provides enough nutrients for the *Vibrio* to grow, supported by studies from Westrich et al. (2016 and 2018). Furthermore, the growth rate observed would be sufficient to result in *Vibrio* bloom concentrations as observed previously (Westrich et al. 2016, Borchardt et al. in press).

Whole dust and leachates added  $\sim 0.6$  to 1.4 nM of additional soluble iron to the experimental treatments. Typically, soluble Fe in Florida Keys is generally 2 nM or less, outside of dust events, but can be highly variable (Borchardt et al. in press). Similarly, for organics (DOC and DON) and nutrients, additions provided modest increases over background levels. Borchardt et al. (in press) found DOC levels <90  $\mu$ M, DON levels <9  $\mu$ M, nitrate <0.3  $\mu$ M, ammonium <2  $\mu$ M, and orthophosphate <0.05  $\mu$ M (outside of dust events). The addition of the leachates alone (soluble fraction) had little effect on the Vibrionaceae response and stimulated different bacterial groups than whole dust treatments. This is in contrast to the expectation that soluble iron is more bioavailable and more likely to stimulate a clear response (Reche et al. 2009) but is consistent with recent work indicating that only very high levels of dust leachate or direct addition of inorganic iron can stimulate *Vibrio* growth (Zhang et al 2019).

Here we show that the delivery of whole dust may be required to mimic *in situ* dust events (Westrich et al. 2016, 2018, Borchardt et al. in press). Our results suggest that aerosols were not directly contributing rapidly growing bacteria to the treatments, including

Vibrionaceae. Rather, results suggest that other factors associated with whole dust were driving the Vibrionaceae and community response. This may be due to addition of iron in other forms beyond dissolved iron, for example, particulate Fe (rather than only dissolved Fe), complex bound Fe (including fungal oxalate bound Fe), colloidal Fe, or the direct contribution of ligands or siderophores from aerosols, including sea spray (Payne et al. 2016; Sullivan et al. 2012; Wozniak et al. 2015, Vinateir et al. 2016). Vibrio contain several genes on their two chromosomes that aid in the transport of Fe into the cell (Payne et al. 2016). These genes, making up > 1% of the *Vibrio* genome, enable *Vibrio* to uptake Fe with the use of siderophores and other iron chelating complexes (Payne et al. 2016). Siderophores are most often excreted from the cell but can also be found bound to the cell surface as a receptor for Fe molecules (Granger and Price 1999). Vibrio are able to both produce siderophores to facilitate iron uptake as well 'cheat' to use exogenous siderophores (Cordero et al. 2012). Given the propensity for Vibrio to utilize scarce iron sources, previous studies suggested that iron was the primary driver in observed Vibrio blooms following Saharan dust influx in the tropical Atlantic (Westrich et al. 2016 and 2018). However, results from this study suggest that soluble iron alone provided by dust may be insufficient to promote Vibrio growth (also noted by Zhang et al. 2019). Rather, this work suggests that factors associated with whole dust and/or non-leached aerosols are critical to the early microbial response to dust. According to Fitzsimmons and Boyle (2013), an average of 30-70% of the dissolved Fe in the ocean can be classified as part of the colloidal size fraction. Commonly referred to as Fe bound to soluble, siderophore-like organic ligands, dissolved Fe defined as the fraction <0.2 μm, is actually composed of many small particles (colloids) (Fitzsimmons and Boyle 2013). However, the process of leaching aerosols through acidified water may affect the structure of some very weak organic ligands, potentially higher levels of

ligand-bound iron could be associated with whole dust aerosols compared to that found in leached material. However, at pH 5.6, used to leach aerosols, it is unlikely that many ligands would be dissociated. Given that *Vibrio* are commonly able to use exogenous siderophores (Odero et al. 2012), any increase in abundance of ligands in the whole dust fraction could help to explain their differential response between whole dust and dust leachate, despite similar levels of total and dissolved iron.

The effect of whole dust may also be due to the addition of small particles themselves. Certain populations of *Vibrio* are likely to be particle associated and the presence of particulates from dust may have enhanced these groups (e.g., Lyons et al. 2007, Takemura et al. 2014). However, previous studies suggest that free-living, rather than particle-attached, *Vibrio* are more likely to bloom (Takemura et al. 2014).

Finally, ASVs of Vibrionaceae transferred from aerosols did not appear to be an important part of the responses noted in our experiments, this does not negate the potential that other aerosol-derived bacteria could affect the community. For example, it has been suggested that dust aerosols may seed surface waters with metabolically active bacteria (Rahav et al. 2016a), C- and N-fixing bacteria (Rahav et al. 2016b), or bacteria capable of other processes that may stimulate growth, including production of novel siderophores (Sullivan et al. 2012). Some of the Vibrionaceae physically added to the treatments from dust aerosols may be capable of nitrogen fixation in marine environments (Urdaci et al. 1988). N-fixation is energy intensive and required large amounts of iron present in the environment. When dust is deposited, the influx of iron, the co-factor for nitrogen fixation, may allow *Vibrio* access to higher levels of nitrogen and therefore increase growth rate.

### **Conclusion:**

In a typical nutrient limited ecosystem, the addition of Saharan Dust has been known to cause rapid microbial responses (Duarte et al. 2006; Westrich et al. 2016). The hypothesis, based on these observations, was that communities would change based on the addition of dissolved and particulate trace metals in the dust. Because leached dust contains the soluble fraction of iron, which is generally assumed to be the most bioavailable fraction, experimental studies of dust effects have generally relied on leachates. However, recent work suggests that the composition of the whole dust may elicit different responses in the microbial community. This was recently noted in the seemingly contradictory findings between Westrich et al. (2016 and 2016) where Vibrio were observed to bloom during natural in situ dust events, but the same response could not be replicated with inorganic iron or leached desert dusts (Zhang et al. 2019). The results of this study show that *Vibrio* responds differently to the method of dust delivery. The culturable *Vibrio* and heterotrophic bacteria, as well as the total marine community, show that members of the Vibrio spp. respond more positively to the addition of whole dust compared to leached dust. Taxa that were common in both the aerosol and water samples show that some bacteria are being introduced in the dust, while others are only increasing due to the addition of nutrients. The capabilities of Vibrio to utilize iron in many forms likely allows them to bloom when dust storms occur and potentially respond to other factors in dust that have not been previously assessed, including colloidal and ligand bound sources of iron as well as specific forms of carbon that may derive from dust and/or aerosol transport.

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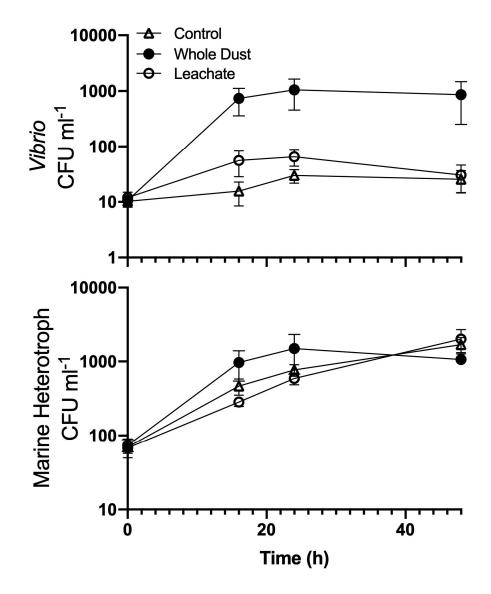
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## **Figures:**



**Figure 2.1.** Mean culturable *Vibrio* (top) and total heterotrophic bacteria (bottom) concentrations (CFU ml<sup>-1</sup>+ SE) by time point and treatment (control, whole dust, and dust leachate ['leachate']). *Vibrio* concentrations increased significantly between  $T_0$  and  $T_{16}$  and  $T_{24}$  in whole dust treatments. At  $T_{24}$  *Vibrio* concentrations in whole dust treatments were significantly greater than those in leachate or control.

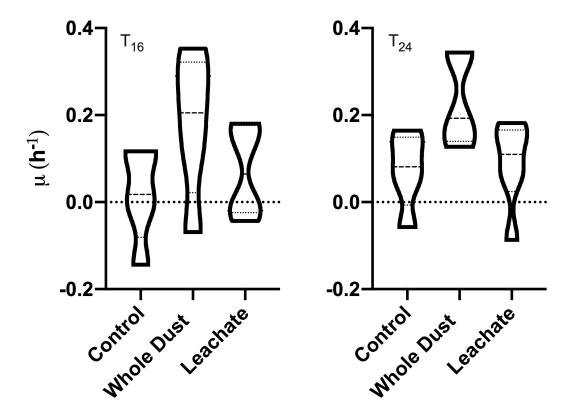
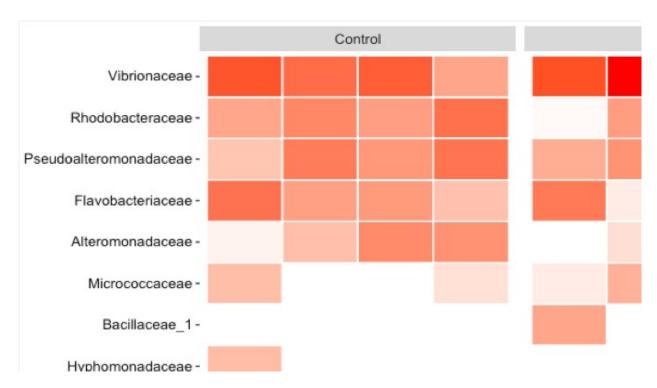
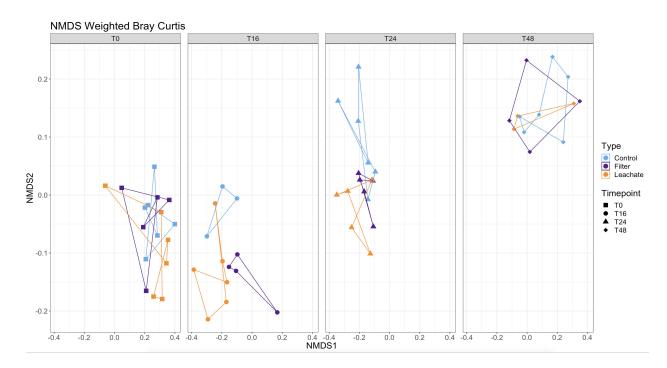


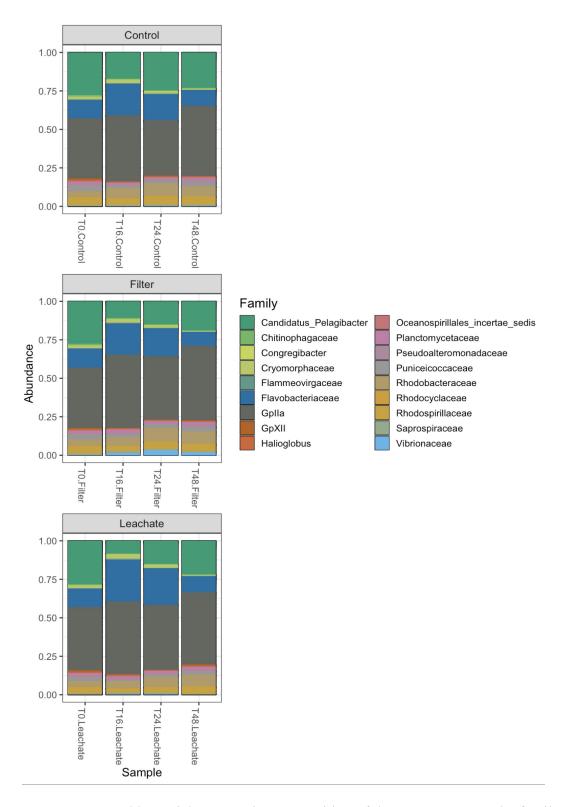
Figure 2.2. Growth rate ( $\mu$ ) for culturable *Vibrio* at T<sub>16</sub> (left) and T<sub>24</sub> (right). Mean growth rates were not significantly different between treatments at T<sub>16</sub>. At T<sub>24</sub> growth rate was significantly different between treatments (p = 0.0431), with whole dust treatments demonstrating the highest growth rates.



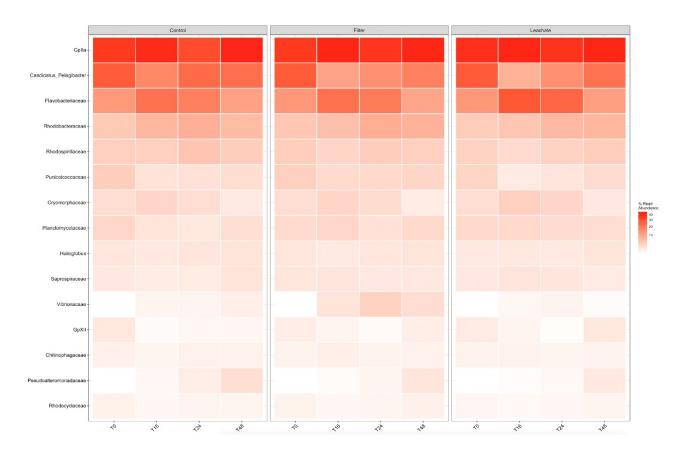
**Figure 2.3.** Heat map of relative abundance of the top 15 bacterial families among culturable bacteria on marine agar across treatment (control, whole dust ['filter'], and dust leachate ['leachate']) and time point. Vibrionaceae show a significant increase at time 16h and 24h samples.



**Figure 2.4.** Non-metric multidimensional scaling plot (NMDS) shows the grouping of treatments (control, whole dust ['filter'], and dust leachate ['leachate']) by beta diversity. At time 0h all of the samples overlap, showing similarity of the community in each sample. Time 16h and 24h are both separated from T0, so their communities are similar, but significantly different from time 0h (p=0.02) and p=0.0002 respectively). By T<sub>48</sub> the samples are overlapping.

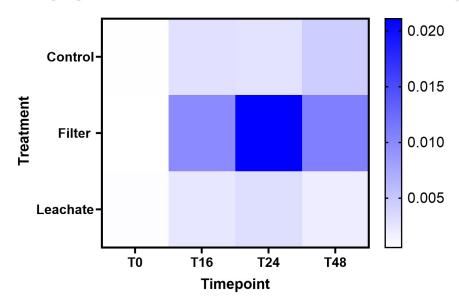


**Figure 2.5.** Total bacterial community composition of the top 100 taxa at the family level by treatment (control, whole dust ['filter'], and dust leachate ['leachate']) and time point.



**Figure 2.6.** Heat map of average relative abundance of the top 15 bacterial taxa present per treatment (control, whole dust ['filter'], and dust leachate ['leachate']) by time point across all samples. Vibrionaceae show a significant increase at time 16h and 24h in the whole dust treatment.

# **Changing Vibrio Relative Abundance in Total Community**



**Figure 2.7.** Heat map focusing on change in relative abundance of Vibrionaceae treatment (control, whole dust ['filter'], and dust leachate ['leachate']) and time point across all samples. Vibrionaceae show a significant increase within 16 h and was significantly different than leachate treatment or control.

### CHAPTER 3

#### CONCLUSION

Dust from the Saharan desert travels across the Atlantic Ocean in pulses several times annually and is deposited in surface waters and terrestrial environments from the Amazon to Texas. In marine waters, dust deposition can deliver important limiting nutrients and trace metals, especially iron, that may occur in a more bioavailable form following chemical processing in the upper atmosphere. We know from previous studies that iron from these dust events is likely one of the significant limiting factors that can affect both phytoplankton and bacterial growth (Westrich, 2016). Saharan dust is also a great source of other external nutrients, such as carbon, nitrogen and phosphorus, that are critical to supporting heterotrophic bacterial growth (Duarte et al. 2006).

The results of this study show that *Vibrio* responds differently to dust delivery method. Culturable *Vibrio* and heterotrophic bacteria show that ASVs of the *Vibrio* spp. respond significantly to the addition of whole dust compared to leached dust. The common taxa in the aerosol and water samples show that certain bacteria are in fact being introduced in the dust, but others are only increasing due to the increase in added nutrients.

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# Appendix:

# **Tables:**

Table S1. V4 PCR and Illumina Sequencing Primers.

Primer	V4 PCR primer (5'-3')	Adaptor (5'-3')	Linker	Illumina Sequencing Primer (5'-3')
F	AATGATACGGCGACCACCG AGATCTACAC	TATGG TAATT	GT	GTGCCAGCM GCCGCGGTAA
R	CAAGCAGAAGACGGCATAC GAGAT	AGTCA GTCAG	CC	GGACTACHV GGGTWTCTA AT

**Table S2.** Taxonomy of ASVs that overlap at >99% similarity between total community and culturable community (ASVs from total community).

ASV	Class	Order	Family	Genus	Species
4	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	NA	NA
49	Bacilli	Bacillales	NA	NA	NA
92	Gammaproteobacteria	Alteromonadales	Pseudoalteromonadaceae	Pseudoalteromonas	NA
129	Gammaproteobacteria	Vibrionales	Vibrionaceae	Vibrio	NA
189	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Tropicibacter	NA
212	Gammaproteobacteria	Alteromonadales	Pseudoalteromonadaceae	Pseudoalteromonas	NA
243	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Roseibium	NA
337	Alphaproteobacteria	Caulobacterales	Hyphomonadaceae	Oceanicaulis	NA
348	Gammaproteobacteria	Oceanospirillales	Oceanospirillaceae	Marinomonas	NA
476	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Celeribacter	NA
496	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	Alteromonas	NA
502	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Labrenzia	NA
524	Cytophagia	Cytophagales	Flammeovirgaceae	Persicobacter	diffluens
548	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Celeribacter	NA
720	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Tenacibaculum	NA
801	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	Aestuariispira	NA
883	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Cloacibacterium	NA
916	Gammaproteobacteria	Vibrionales	Vibrionaceae	Vibrio	NA
984	Actinobacteria	Actinomycetales	Micrococcaceae	Kocuria	NA
1119	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	Aestuariibacter	NA
1209	Bacilli	Bacillales	Staphylococcaceae	Staphylococcus	NA
1324	NA	NA	NA	NA	NA
1347	Gammaproteobacteria	Alteromonadales	Pseudoalteromonadaceae	Psychrosphaera	NA
1556	Gammaproteobacteria	Vibrionales	Vibrionaceae	Vibrio	NA
1624	Gammaproteobacteria	Vibrionales	Vibrionaceae	Vibrio	NA
1631	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Tenacibaculum	NA
1659	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Leisingera	NA
1677	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Tenacibaculum	xiamenense
1907	Actinobacteria	Actinomycetales	Micrococcaceae	Micrococcus	NA
2040	Gammaproteobacteria	Vibrionales	Vibrionaceae	Photobacterium	NA
2059	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Roseibium	NA
2294	Gammaproteobacteria	Alteromonadales	Pseudoalteromonadaceae	Pseudoalteromonas	NA
2427	Gammaproteobacteria	Alteromonadales	Pseudoalteromonadaceae	Pseudoalteromonas	phenolica
2437	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	NA	NA

**Table S3.** The overlapping ASVs and number of reads per samples between total and culturable communities with respect to whole community.

					_		_					_	_	_	_	_	_		ASVS															_	_
Timepoint	Treatment	4	49	92	129	189	212	243	337	348	476	496	502	524	548	720	801	883	916		1119	1209	1324	1347	1556	1624	1631	1659	1677	1907	2040	2059	2294	2427	2437
TO	Control Filter	640	1	1	0	0	0	1	2	0	0	0	0	1			0	3	4	0	1	0	1	0	0	0	0	1	0	1	0	0	0	0	0
TO	Control Filter	400	6	1	0	0	0	2	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TO	Control Filter	439	4	0	1	0	0	2	2	2	0	0	0	3	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TO TO	Control No Addition	970	5	1	1	0	0	1	1	0	0	0	0	0		0	0	3	1	0	1	4	1	0	0	0	0	0	0	0	0	0	0	1	0
TO TO	Control No Addition Control No Addition	462 696	6	1	0	0	0	10	0	0	0	0	0	0	2	0	0	1	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0
TO	Filter 28th	911	3	1	0	1	0	1	3	0	0	0	0	2	2	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TO	Filter 28th	382	10	3	1	1	0	2	3	4	1	1	1	0	1	2	0	2	0	0	1	0	0	0	0	0	0	2	0	0	0	0	0	0	0
TO	Filter 28th	1025	10	2	4	1	1	3	2	0	2	0	0	3	1	0	0	0	0	0	1	0	1	0	0	0	0	4	0	0	0	0	0	0	0
TO TO	Filter 29th	571	7	3	0	0	0	6	3	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	3	0
TO TO	Filter 29th Leachate 28th	589 605	2	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TO	Leachate 28th	448	5	0	0	1	0	3	2	0	0	2	0	0			0	2	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
TO	Leachate 28th	871	5	0	0	1	0	7	1	1	0	1	0	2	2	0	0	2	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0
T0	Leachate 29th	670	5	1	0	0	0	2	2	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TO	Leachate 29th	389	3	1	0	0	0	3	0	0	0	1	0	2	0	0	2	1	0	0	0	0	0	0	0	7	0	1	0	0	0	0	0	0	1
T16	Leachate 29th	391	34	3	5	1	44	32	0	0	2	5	0	0	3	0	1	3	0	0	2	0	0	2	0	0	0	0	0	0	0	0	0	0	0
T16 T16	Control Filter Control Filter	1119 610	39	53	14	6	0	0	10	0	0	0	0	0	2	0	0	0	5	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T16	Control Filter	563	14	87	21	28	0	9	5	0	0	3	1	3	1	0	7	2	12	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0
T16	Control No Addition	890	0	0	0	0	0	0	0	181	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T16	Control No Addition	359	129	41	8	13	0	8	0	0	2	1	0	3	14	0	3	0	0	0	4	1	4	2	0	0	0	0	0	0	0	0	1	0	1
T16	Control No Addition	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T16	Filter 28th Filter 28th	924	688	20	12	3	0	5	0	0	0	0	0	0	2	128	0	5	0	0	0	0	6	2	0	0	0	0	0	0	0	0	0	0	0
T16	Filter 28th	885	125	_	9	9	0	11	2	1	0	0	5	12	_	0	1	3	0	7	0	1	0	0	0	1	0	0	0	0	0	1	0	0	0
T16	Filter 29th	868	441			13	1	11	5	1	19	1	5	5	9	0	0	0	1	0	3	0	0	5	1	0	1	1	0	0	0	0	0	0	0
T16	Filter 29th	978	6	4	1	4	0	2	4	0	0	0	2	1	1	0	0	1	1	1	0	0	2	0	0	0	0	1	0	0	0	0	0	0	0
T16	Leachate 28th	573	28	13	24	1	0	6	2	0	4	2	2	6	1	0	0	0	0	0	0	0	0	1	7	1	0	0	0	0	0	0	0	0	0
T16	Leachate 28th	553	29	19 41	16	0	0	11	2	0	0	0	2	2	0	0	7	3	0	0	6	0	0	2	0	0	0	5	0	0	0	0	0	0	3
T16	Leachate 28th Leachate 29th	666 865	56 115	2	10	14	0	16 6	3	0	0	1	15	2	3	0	1	0	1	0	1	0	0	1	0	1	0	0	0	0	0	1	0	1	0
T16	Leachate 29th	812	145	16	25	5	0	16	1	0	1	88	6	4	3	0	0	3	1	0	5	11	0	2	1	0	0	2	0	0	0	2	0	0	0
T16	Leachate 29th	577	23	45	25	7	571	13	1	5	1	1	0	4	2	0	1	4	2	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
T24	Control Filter	463	30	42	54		1	4	0	0	0	5	1	3	1	0	0	0	0	1	1	0	0	0	0	0	0	2	0	0	0	0	0	0	0
T24 T24	Control Filter Control Filter	546 432	18 63	143 128		9	0	7	12 16	2	5	2	0	10	7	0	7	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T24	Control No Addition	135	3	50	8	3	0	1	1	0	0	2	0	2	0		0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
T24	Control No Addition	308	35	128	-	22	0	0	0	1	6	15	3	4	29	0	0	3	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T24	Control No Addition	231	171	110	17	18	0	10	0	0	5	3	0	1	11	0	10	0	0	1	0	0	0	0	3	1	0	0	0	0	0	0	0	0	0
T24	Filter 28th	995		126			0	6	2	0	1	0	7	4		0	8	3	0	1	0	0	0	4	0	0	0	0	0	0	0	1	0	0	0
T24 T24	Filter 28th Filter 28th	881 455	436 665	104 40		13	0	47 14	1	0	1	0	10	9	7	0	9	0	0	0	0	0	0	0	0	0	0	0	8	0	0	0	0	0	0
T24	Filter 29th	882	2639	_	_	14	1	14	19	0	6	4	3	11	3	1	1	5	1	4	0	0	3	0	2	1	5	0	0	2	2	0	0	0	0
T24	Filter 29th	380	36	23	18	15	0	6	7	0	1	1	8	2	13	0	0	0	2	5	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0
T24	Leachate 28th	1010	17	70	17	9	0	14	9	1	2	0	8	4	4	0	3	1	0	1	0	0	0	2	0	1	0	0	0	1	0	1	0	0	0
T24	Leachate 28th	533	60	113		10	0	7	2	0	0	0	10	14		0	2	1	0	2	2	0	0	1	0	0	0	0	2	0	0	0	0	0	0
T24 T24	Leachate 28th Leachate 29th	707 857	8 46	3 132	5 37	19	0 572	5 17	19	0	5	0	0	6	7	0	7	3	1	0	0	0	0	0	0	0	5	0	0	2	0	0	3	0	0
T24	Leachate 29th	421	189	19	9	12	0	21	0	4	0	94	22	2	9	0	0	2	0	4	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
T24	Leachate 29th	428	161	_	21	16	0	25	4	11	6	5	10	7	12	0	2	3	3	0	1	10	0	0	0	0	0	0	0	0	0	0	0	0	0
T48	Control Filter	2598	39	309	83	114	0	9	60	0	15	8	2	7	0	0	1	1	2	0	1	0	0	0	0	0	0	0	1	1	0	1	0	0	0
T48		2048	_		419		0	6	17	0	7	2	6	6	1	0	0	0	1	1	1	0	0	1	0	1	0	0	1	1	0	0	0	0	0
T48	Control No Addition	972			149	205 79	0	0	21	0	22	4	7	6	6	0	0	3	26	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T48	Control No Addition Control No Addition	1693	5 429	82 174	_	94	0	483	7	0	7	11	6	7	9	0	0	0	0	6	2	0	8	0	0	0	3	0	0	0	0	0	0	0	0
T48		1132	15		251		0	5	10	0	15	5	6	4	12	0	1	3	0	3	0	0	0	1	0	2	2	0	0	0	0	0	0	0	0
T48	Filter 28th	436	61	0	120		0	0	0	188		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
T48	Filter 28th	1820	53	265			0	7	8	1	6	5	24	8	7	0	4	4	0	8	0	0	0	2	0	0	0	0	0	1	0	0	0	0	0
T48	Filter 28th Filter 29th	819 2360	134	131 46		40	0	1	12	0	5	0	17	3	23		1	0	2	4	0	0	0	0	0	2	0	0	1	0	0	0	0	0	0
T48 T48	Filter 29th Filter 29th	1500		175	_	227	0	166	25	1	143	7	19	6 23		0	0	4	0	3	3	0	7	0	0	0	1	0	1	0	0	0	2	0	0
T48		3268		0	549		0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T48	Leachate 28th	935	0	104		0	0	0	0	154		0	0	1	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0
T48	Leachate 28th	1722	82	563		133	0	8	22	0	11	8	39	30		0	1	0	0	2	2	0	0	0	6	0	2	0	1	2	8	1	0	0	0
T48	Leachate 29th	1	134		0	0	0	0	0	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T48		2665	46	29	50	68	283	4	15	0	3	2	3 19	12		0	0	2	0	2	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0
T48	Leachate 29th	1401	13	181	69	53	203	4	51	0	22	2	13	8	2	0	0	3	5	2	1	1 4	U	U	U	0	0	0	0	0	0	0	0	0	U

**Table S4.** Overlapping ASVs and number of reads per samples between culturable and whole community with respect to culturable community

		_																		ASVs																_
Timepoint	Treatment	Δ	49	97	12	20 1	180	212	243	337	3/18	476	496	502	524	548	720	801	883		984	1119	1209	1324	1347	1556	1624	1631	1659	1677	1907	2040	2059	2294	2427	2437
ТО		0	9	2			0	2	21	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	5	38	0	0	0	0	0	0	0	0
то	Electrical Control of the Control	-	13				0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11	0	0	0	0	0	0	0	0	0	0	0
ТО	Control No Addition	-	48	+	-	_	0	0	2	0	0	0	7	0	0	0	0	2	0	21	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	39
ТО	Control No Addition	0	70	-	-	_	0	0	58	16	0	0	16	169	0	0	0	0	6	0	0	0	4	0	0	0	11	0	0	0	0	0	0	0	0	47
ТО	Control No Addition	1	82	21	1 4	4	19	292	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	12	0	0	0	0	0	0	19	0	2	0
TO	Filter 28th	0	37	0	(	)	0	0	30	0	0	0	1	31	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0
TO	Filter 28th	1	45	21	1 (	)	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TO	Filter 28th	0	15	0	(	)	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	15	0	0	0	0	0
TO	Filter 29th	0	3	0	(	)	0	0	4	0	7	0	0	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T0			15				0	0	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TO	15-0-0-0-0-0-0-0-0-0-0-0-0-0-0-0-0-0-0-0		92		-	-	0	0	0	0	0	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	50	0	0	0	0	0	0	0
TO			47			_	0	0	53	0	0	0	1	0	29	0	0	7	0	0	0	0	104	7	4	8	0	0	0	0	0	0	0	0	0	0
TO						_	0	0	0	0	0	0	1	27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T0			160				0	0	0	1	0	0	14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ТО			31	+			1	0	1	0	0	0	2	0	59	0	0	2	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
TO TO		0	7	0		-	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0
T16			34		_	_	7	0	0	0	0	0	0	0	0	0	0	1	0 16	0	0	0	0	3	2	0	0	0	0	0	0	0	0	0	0	0
T16		0	13	+	+	_	1	0	0	0	0	0	0	0	16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T16	Control No Addition	$\rightarrow$	34		-	-	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0
T16		0	30	-	-	-	0	1	5	0	0	0	0	0	0	0	88	1	0	0	0	0	0	10	0	0	12	0	0	0	0	0	0	0	0	0
T16	Control No Addition	0	4	0	-	_	13	0	0	0	0	0	6	0	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T16		0	205	+	_		5	0	6	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	63	0	0	0	0	0	0	0	0	11	0	0
T16		0	5	0			0	0	0	0	0	0	0	13	39	0	0	15	0	0	0	0	0	1	0	3	0	0	0	0	0	0	0	0	0	0
T16		0	22				0	0	3	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T16	Filter 29th	0	23	14	1 1	2	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	31	0	0	0	0	0
T16	Filter 29th	0	15	0	2	9	2	3	0	0	0	0	0	8	0	50	0	0	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T16	Leachate 28th	0	11	0	1	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11	0	10	0	0	0	0
T16					-	_	7	0	0	0	0	0	0	41	0	0	0	0	0	0	3	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
T16							0	0	0	0	0	0	0	54	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T16			25		-	_	1	0	4	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T16		$\rightarrow$		+-	-	_	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T16		0	7	3			2	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	17
T24 T24		$\rightarrow$	49	-	_	_	6	0	5	0	0	0	1	7	0	0	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0	8	0	0
T24		0	0	2	_	-	4	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	9	0	0	0	0	0	0	0	0	0
T24	Control No Addition	$\rightarrow$	18				3	0	0	0	0	0	0	13	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T24		$\rightarrow$	13	-	_	_	3	0	0	0	0	0	1	1	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T24		0	9	5			2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
T24		0	40				20	0	0	0	0	0	0	0	0	0	0	0	0	0	53	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0
T24							4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16	0	0	0	0	0	0
T24	Leachate 28th	0	1	4	2	2	13	0	5	0	0	0	0	0	0	0	0	0	0	0	6	0	0	3	0	0	0	6	0	0	0	0	0	0	0	0
T24		0	0	17	7 1	L	34	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
T24		0	_	0	_	-	7	13	0	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T24			12				4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T24		0	9	2			13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T24		0		4			25	0	0	0	0	0	0	1	7	0	0	0	0	0	0	0	0	1	0	0	19	0	0	14	1	0	0	0	0	10
T48						_	3	0	0	0	0	0	0	11	16	0	0	0	0	0	3	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0
T48		0	78 13	_	_	_	21	0	1	0	0	0	0	7	0	0	0	0	10	0	0	0	0	0	0	0	33	0	0	0	0	0	0	0	0	2
T48		0	33	-	-		6 24	0	6	0	0	0	0	16	34	0	0	0	0	0	5	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T48		$\rightarrow$	20	+	_		5	0	0	0	0	0	0	10	0	0	0	0	1	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T48			46				11	0	7	0	0	0	0	0	0	0	0	0	0	0	1	5	0	3	0	0	0	0	0	11	0	0	0	0	0	0
T48		-	15				18	0	2	0	0	0	0	0	0	0	0	0	0	0	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T48		0	0	5			9	50	0	0	0	0	0	2	0	0	0	0	0	0	2	19	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T48		0	1	6	_		33	4	0	1	0	0	2	23	0	0	0	0	19	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0
T48		-	44				99	0	0	0	0	0	0	61	0	0	0	0	0	0	6	0	0	4	0	0	2	1	0	0	0	0	0	0	0	0
T48		$\rightarrow$		-	_	_	18	0	0	0	0	0	2	6	0	0	0	1	0	0	0	0	0	0	11	0	0	0	0	0	0	0	0	0	0	0
T48	Leachate 29th	0	16	3	1	6	6	0	9	0	0	0	0	9	0	0	0	0	0	0	7	0	0	0	0	1	0	0	0	0	56	0	0	0	0	0
T48	Leachate 29th	0	24	0	4	1	4	0	1	0	0	0	0	0	0	0	0	0	0	0	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

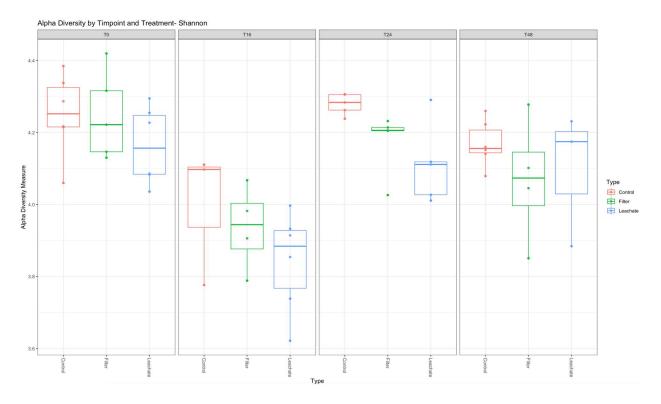
**Table S5.** Overlapping ASVs between the aerosol and total marine community at greater than 99% similarity. The table is sorted alphabetically by Family.

Sterivex ASV	Query coverage	Kingdom	Phylum	Class	Order	Family	Genus	Species
M1753	100.00%	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Corynebacteriaceae	Corynebacterium	NA
M774	100.00%	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	Curtobacterium	NA
M189	100.00%	Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	Alteromonas	NA
M1146	100.00%	Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	Alteromonas	NA
M2439	100.00%	Bacteria	Firmicutes	Bacilli	Bacillales	Bacillales_Incertae_Sedis_XII	Exiguobacterium	NA
M846	100.00%	Bacteria	Firmicutes	Bacilli	Bacillales	Staphylococcaceae	Staphylococcus	NA
M348	100.00%	Bacteria	Firmicutes	Bacilli	Bacillales	Staphylococcaceae	Staphylococcus	NA
M1	100.00%	Bacteria	Cyanobacteria/Chloroplast	Cyanobacteria	Family_II	GpIIa	NA	NA
M11	100.00%	Bacteria	Cyanobacteria/Chloroplast	Cyanobacteria	Family_II	GpIIa	NA	NA
M53	100.00%	Bacteria	Cyanobacteria/Chloroplast	Cyanobacteria	Family_II	GpIIa	NA	NA
M6	100.00%	Bacteria	Cyanobacteria/Chloroplast	Cyanobacteria	Family_II	GpIIa	NA	NA
M1226	100.00%	Bacteria	Cyanobacteria/Chloroplast	Cyanobacteria	Family_II	GpIIa	NA	NA
M17	100.00%	Bacteria	Cyanobacteria/Chloroplast	Cyanobacteria	Family_II	Gplla	NA	NA
M172	100.00%	Bacteria	Cyanobacteria/Chloroplast	Cyanobacteria	Family_II	Gplla	NA	NA
M176	100.00%	Bacteria	Cyanobacteria/Chloroplast	Cyanobacteria	Family_II	GpIIa	NA	NA
M71	100.00%	Bacteria	Cyanobacteria/Chloroplast	Cyanobacteria	Family_II	GpIIa	NA	NA
M913	100.00%	Bacteria	Proteobacteria	Gammaproteobacteria	Gammaproteobacteria_incertae_sedis	Congregibacter	NA	NA
M800	100.00%	Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Oceanospirillaceae	Thalassolituus	NA
M1122	100.00%	Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Oceanospirillaceae	Thalassolituus	NA
M199	100.00%	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	NA
M2225	100.00%	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Enhydrobacter	aerosaccus
M852	100.00%	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	NA
M499	100.00%	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteriaceae	Methylobacterium	NA
M1677	100.00%	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Celeribacter	NA
M1114	100.00%	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Paracoccus	NA
M1119	100.00%	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	NA	NA
M453	100.00%	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	NA	NA
M142	100.00%	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	NA	NA
M1562	100.00%	Bacteria	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	Vibrio	NA
M49	100.00%	Bacteria	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	Vibrio	NA
M243	100.00%	Bacteria	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	Vibrio	NA
M496	100.00%	Bacteria	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	Vibrio	NA
M574	100.00%	Bacteria	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	Vibrio	NA

**Table S6.** Overlapping Vibrionaceae ASVs between aerosol and total community with respect to total community.

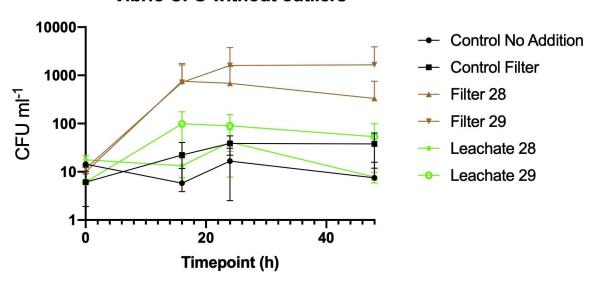
ASV	% Identical Sites	Phylum	Class	Order	Family	Genus	Species	# Samples	Treatments	Timepoint	Total Reads
49	100.00%	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	Vibrio	NA	21	All	All	9360
243	100.00%	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	Vibrio	NA	19	All	All	1117
496	99.60%	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	Vibrio	NA	17	All	All	303
574	99.60%	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	Vibrio	NA	8	All	All	226
1562	99.20%	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	Vibrio	NA	8	All	All	26

# **Figures:**



**Figure S1.** Comparison of alpha diversity (Shannon Index) across each time point by treatment (control, whole dust ['filter'], and dust leachate ['leachate']).

# **Vibrio CFU without outliers**



**Figure S2.** Culturable *Vibrio* growth (CFU  $ml^{-1} \pm SE$ ) based on timepoint and treatment. Based on similar results per treatment, replicates were collapsed and referred to as "Control," "Whole Dust," and "Leachate."