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Diversity and dynamics of bacterial populations in marine bioaerosols

by

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General introduction

Aerosols and their formation

Aerosols are defined as 'a dispersion of solid and liquid particles suspended in gas' (Saltzman 2007) and can be separated in primary and secondary aerosols. Primary aerosols are particles directly emitted or injected into the atmosphere. Secondary aerosols are atmospheric particles created by in situ aggregation or nucleation from gas phase molecules.

The definition of the term 'primary biological aerosol particle' (PBAP) by the ACGIH (1999) comprises airborne particles, large molecules or volatile compounds that are living contain living organisms or are released from living organisms.

Therefore, every particle of biological origin would count to the category PBAP: bacteria, archaea, fungal spores/fragments, pollen, viruses, algae, cyanobacteria, biological crusts, lichens, plant debris, biopolymers (e.g. humic-like substances, cellulose), epicuticular waxes, animal dander and fur fibers. In the literature, the terms 'PBAP' and 'bioaerosol' are widely used synonymously.

The size ranges of bioaerosols can, thus, be wide, as their composition can be highly divers and depends on a variety of factors. This includes the type of microorganism or toxin and the type of particles they are associated with (e.g. mist, dust and gases). Sizes usually range from 0.02 µm to 100 µm in diameter and follow a defined classification: Particles smaller than 0.1 µm are considered to be nuclei mode; particles from 0.1 µm to 2 µm are in the accumulation mode; and even larger particles are considered to be in coarse mode (Figure 1; Dowd & Maier 2000). As bioaerosols largely depend on their medium and its prevailing environmental parameters, they accordingly react to air currents by moving quickly or slowly, and are impacted by gravity, depending on their size, air density and air currents.

The atmosphere is divided in different layers e.g. troposphere (up to 10 km), stratosphere (up to 50 km) and mesosphere (50-80 km). Most interesting for the dispersal of aerosols are the lower parts of the troposphere. The boundary layer extends to a height of 0.1 km above the ground. It is largely responsible for the transport (short and long distances) of microorganisms. It can be separated in three parts. The laminar boundary layer is the still air associated with the earth and projecting surfaces. Its thickness varies from 1 µm with strong winds up to several meters with stillness of air. So it is strongly influenced by weather conditions. The turbulent boundary layer is considered to be always in motion and responsible for the horizontal

transport (wind dispersion) which occurs when microorganism associated with particles are launched. In the lower layers the linear flow is interrupted by surface projections and their associated laminar boundary layer. This results in a friction against the airflow and the creation of swirling turbulences. This area of interaction between the two described layers is considered the third layer, the local eddy layer.

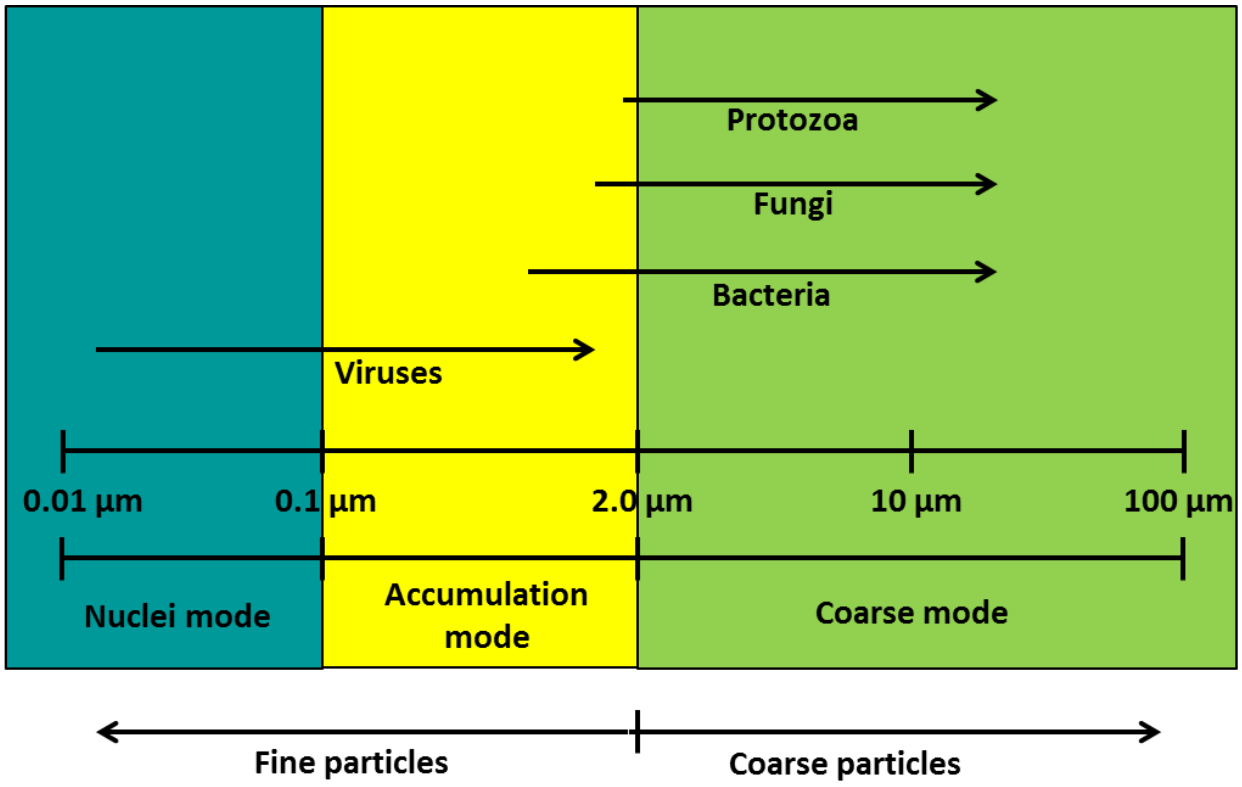

Figure 1 Diagrammatic representation of the relative sizes of bioaerosols. The depictions of the various kinds of organisms are indicative of their potential sizes when associated with airborne particles (rafts) The terminologies used to describe the various sizes of the bioaerosols are also indicated (Source: Dowd & Maier 2000).

The primary forming mechanism for marine bioaerosols is assumed to be a sea-to-air transfer of bacteria by bursting of small bubbles at the ocean surface (Figure 2), generated by breaking waves (whitecaps), impacting rain on the sea surface as well as boat traffic (Blanchard 1963; Duce & Hoffmann 1976; Monahan et al. 1983; Klassen & Roberge 1999). They can be dispersed to depths of several meters (Grammatika & Zimmerman 2001). When the bubbles rise through the water column, small particles such as viruses and bacteria are collected and accumulate on their surfaces with an enrichment factor of up to 15-25 (Aller et al. 2005). The bubbles then pass through the marine surface micro-layer (SML), which also contains high

concentrations of microorganisms and organic matter (Burrows et al. 2009; Després et al. 2012). The SML is located within the air-sea interface and represents the uppermost top of the water column (< 1 mm). It is best described as a gelatinous biofilm (Sieburth 1983; Cunliffe & Murrell 2009) in which the chemical, physical, and biological features greatly differ from the sub-surface water just a few centimeters beneath. Thickness and composition of the SML are strongly influenced by wind speed and the resulting turbulences at the water surface. Accordingly, higher wind speeds induce bigger turbulences (waves) and result in a thinner SML. The SML is inhabited by a typical bacterial community, the so called bacterioneuston which is a unique biocoenosis at the water surface (Naumann 1917). All domains of life (bacteria: Maki 1993; archaea: Cunliffe et al. 2008; eucaryota: Cunliffe & Murrell 2009) have been detected in the SML. Nevertheless, the SML is subject to extreme environmental conditions such as the accumulation of organic material, enhanced UV radiation as well as the mentioned interplay of wind, waves and turbulences, representing a very dynamic habitat for bacteria.

Reaching the sea surface the bubbles burst and material is ejected into the atmosphere. Sea salt and organic compounds are simultaneously transported across the air-sea interface (Pósfai et al. 2003; Aller et al. 2005) and form PBAP (Blanchard & Syzdek 1982; Smith et al. 1993; Cavalli et al. 2004) which are easily suspended and transported into the lower atmosphere (Woodcock 1953; Gustafsson & Franzen 2000; Grammatika & Zimmerman 2001). In summary it can be stated that the process of marine aerosols forming is strongly wind-driven. As oceans cover more than 70% of the earth surface, housing bacterial concentrations of 3×10^5 cm^{-3} to 5×10^6 cm^{-3} in surface waters, it is assumed that most PBAP aerosols are emitted from the oceans.

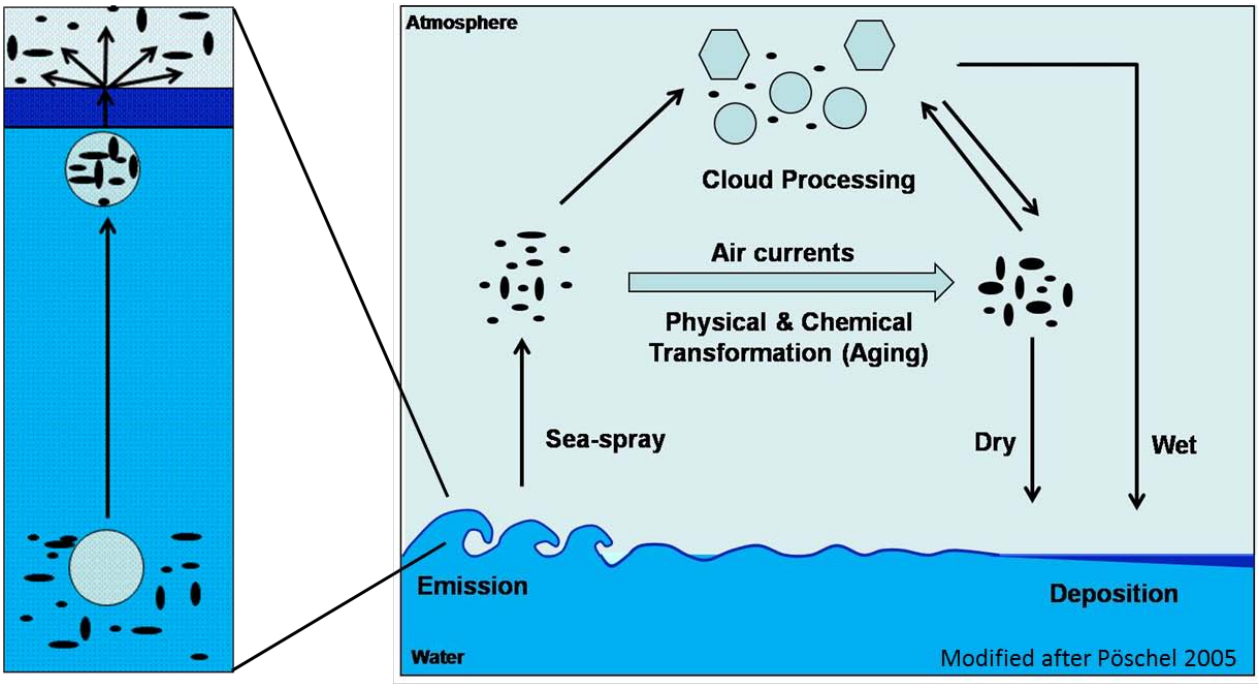

Figure 2 Bubble bursting process and the distribution process of marine primary biological aerosols particles (modified after Pöschl 2005).

Historical background

There is a long history of investigating airborne microorganisms. Already in the 17th century Antony van Leeuwenhoek mentioned airborne bacteria (Gregory 1971), but not until the middle of the 19th century the presence and importance of aerolised biological particles were recognised and, since then, have repeatedly been addressed in several investigations (e.g. Ehrenberg 1847; Pasteur 1861; Carnelly et al. 1887; De Bary 1887). Following studies in the multidisciplinary field of aerobiology were only sporadic of nature, proofing the existence of viable bacteria in the atmosphere in altitudes of up to 80 km in exemplary and qualitative approaches (e.g. Meier 1935; Timmons et al. 1966; Imshenetsky et al. 1978). The term 'aerobiology' was first utilised in the 1930ies for the 'study of live in the air' (Bovallius & Roffey 1986). Technological advances in recent years allowed for studies with systematic, quantitative and long-term sampling approaches but, unfortunately, the field of aerobiology is still characterised by a lack of knowledge (Burrows et al. 2009; Després et al. 2012; Gandolfi et al. 2013). This is particularly due to the small number of environmental studies published to date. In addition, a theoretical overall comparison is complicated by a lack of standardised methods, thus aggravating meaningful conclusions. These non-standardised methods comprise

different sampling techniques as well as analyses for quantification and identification of species and/or communities.

Bacterial communities in bioaerosols

The airborne microbial ecosystem is rather complex and, to date, only little is known about its composition (Burrows et al. 2009) as most studies concerning airborne bacteria focus on indoor environments e.g. hospitals (Bauer et al. 1990) and homes and offices (Pastuszka et al. 2000) for health aspects. To date several investigations were performed to find out which organisms are in the air, their sojourn time in the air, whether they are harmful or not, and which anthropogenic, environmental, and climatic parameters have an impact on the community composition.

What is known is that various microbial species occur in the air and that their communities change over time and those environmental parameters play a vital role in their formation and composition. Current investigations in terrestrial environments showed that bioaerosols often feature similar bacterial communities as the underlying ecosystems (Brodie et al. 2007; Bowers et al. 2011a; Bowers et al. 2012). Furthermore, microbial compositions of airborne communities underlie spatial fluctuations, most likely induced by meteorological conditions (Maron et al. 2006; Fierer et al. 2008) and varying among different scales of time (daily, weekly, monthly and seasonal; Maron et al. 2005). Brodie et al. (2007) detected the consistent presence of bacterial families with pathogenic members in urban aerosols. Genetic relatives of selected agents of bioterrorism (e.g. *Bacillus* sp.) can, thus, be detected in the native airborne bacterial communities.

The microorganisms can be suspended in airstreams and may have long sojourn times of up to several days in the atmosphere, undergoing physical and chemical transformation processes (e.g. surface coating, reaction with photooxidants, coagulation; Pöschl 2005). Bacteria in aerosols have potential influence on clouds and precipitation. Some species have been found to be efficient ice nucleating agents (Schnell & Vali 1972; Maki et al. 1974) and waterdroplet nuclei (Bauer et al. 2002). This characteristic to catalyse freezing and the formation of ice crystals can trigger precipitation (Christner et al. 2008; Pratt et al. 2009) and water droplet trigger cloud formation and therefore can play an important role in the world climate formation.

The transport duration of those particles is directly correlated with particle size and weight. Accordingly, bacteria with a size of 1.0-5.0 µm stay longer in the air then bigger particles, which drop down faster (Després et al. 2012). Prospero et al. (2005) showed that even the transport of viable bacteria and fungi from Africa to the Caribbean with soil dust is possible. Various other studies proved that bacterial transport over long distances is highly likely, enabling microorganisms for intercontinental transport (e.g. Prospero 1999; Kellogg & Griffin 2006; Polymenakou et al. 2008). As this also applies to potential pathogens, composition and dynamics of these communities are of particular interest for security and health reasons, urgently needing further investigation. Most outdoor areas surveyed are urban (e.g. Brodie et al. 2007; Fierer et al. 2008; Franzetti et al. 2011; Bowers et al. 2011b; Bertolini et al. 2013) and rural (e.g. Bauer et al. 2002; Harrison et al. 2005; Maron et al. 2005; Bowers et al. 2011a). Only three studies about marine bioaerosols (a one year observation at the Baltic Sea with samples taken every two weeks, a study conducted at the coast of San Diego with eight samples and a study with three samples taken during a ship cruise in the East Sea, Korea) were published in the last four years.

Fahlgren et al. (2010) published a year survey of airborne Bacteria sampled at a Tower 200 m inland at the Swedish coastline of the Baltic Sea deploying culture-dependent and independent techniques. They described a highly diverse community with a few abundant operational taxonomic units (OTUs) and a long tail of rare OTUs. The dominant genera were *Sphingomonas* sp. and *Pseudomonas* sp. and potentially pathogenic strains as well as sequences closely resembling bacteria known to act as ice nuclei were detected. The origin of the sampled air mass was estimated using backward trajectories, indicating a predominant marine source. Urbano et al. (2011) combined also culture-dependent and culture-independent techniques to investigate four marine bioaerosol samples taken at a pier on the US Pacific Coastline. Sequence analysis revealed that Firmicutes and Proteobacteria were predominant among bacteria. The culture-dependent study verified the viability of microbes from all phyla detected through the culture-independent study. Contrary to their expectations and despite oceanic air mass sources they found a high abundance of terrestrial and marine sediment-associated microorganisms. The latest publication concerning marine bioaerosols is a study were bioaerosol as well as surface seawater samples were collected at three sites in the East Sea (Cho & Hwang 2011). They analysed the samples with culture-dependent and independent techniques. They found that the 16S rRNA gene from the aerosol samples were

dominated by Gammaproteobacteria and Bacteroidetes. The numbers of phylotypes of airborne bacteria were comparable to those of seawater bacteria in coastal and remote offshore sites. Over half of the bacterial OTUs detected in the aerosols were of marine origin. Half of the OTUs found in the surface waters occurred in the aerosol samples. Thus, surface seawater seems to be a main source of marine airborne bacteria. However, in the intermediate offshore site, airborne bacterial OTUs were all of terrestrial origin (Cho & Hwang, 2011).

Disadvantages of culture-dependent methods

For a long time, culture-dependent studies were typically used for the investigation of PBAPs and are still used today (e.g. ZoBell & Mathews 1936; Kelly & Pady 1954; Shahamat et al. 1997; Griffin et al. 2006; Fahlgren et al. 2010; Cho & Hwang 2011; Griffin et al. 2011; Urbano et al. 2011). However, as only a small fraction of environmental bacteria is culturable (around 0.001-0.01% of seawater bacteria; Colwell 2000) and culturability rapidly declines after aerolisation (Heidelberg et al. 1997), culture-dependent methods always underestimate the actual abundances and diversity of bacteria. This is mainly because culture-dependent methods introduce several strong biases due to the selective nature of media. This is a particular weakness for investigations on airborne bacteria as their origin and respective influences can be strongly heterogeneous (soil, marine, plants) and thus hardly predictable. Further problems relate to the choice of incubation temperature and incubation time, selecting for fast/slow growing bacteria and/or for thermotolerant/mesophilic bacteria. For example, marine bacteria require lower temperatures for growth compared to specialised bacteria such as pathogens which can grow at 25 °C or higher. Furthermore, sampling efficiency and culturability not only depends on the bacterial strains but is also strongly affected by experimental and environmental factors: e.g. filtration vs. impingement sampling, air sample volume and relative humidity. In addition, the growth of fungi on the media may also interfere with the counting. Recent studies showed that, in direct comparison, culture-dependent and -independent methods produce different results for the regarded bacterial communities (Cho & Hwang 2011; Ravva et al. 2012), although other studies also found overlapping results (Fahlgren et al. 2010; Urbano et al. 2011). Considering the obvious short comings of culture-dependent methods for characterisation of airborne bacterial populations, the current studies employed non-culture methods for the investigation of marine bioaerosols.

Influence of environmental parameters

Several studies, so far, detected direct correlations of environmental parameters with bacterial concentration in the atmosphere. For example, air temperature was found to be positively correlated with total bacteria concentration (Bovallius et al. 1978; Harrison et al. 2005). This could be due to a correlation of temperature with important meteorological and climatological variables that, in turn, may have effects on bacteria concentrations via the mentioned boundary layer turbulence, daytime and/or season. However, a study conducted by Rosas et al. (1994) in Mexico City showed that bacterial concentration is not always correlated with the temperature but also with the daily range of temperature. This could be an indication for the importance of seasonal effects. This is confirmed by the findings of Maron et al. (2006) and Kaarakainen et al. (2008) who observed that seasonal changes in airborne bacterial communities are more pronounced than weekly or daily variation.

Relative humidity was frequently identified to correlate with the number of bacteria (Rosas et al. 1994), although this could not be confirmed in all studies (Mouli et al. 2005). As higher wind speeds facilitate increasing aerosol generation by the uplift of bacteria, wind speed generally was found to correlate positively with bacterial concentrations (Bovallius et al. 1978; Lighthart et al. 2004; Mouli et al. 2005).

Meteorological parameters most likely also influence the metabolism of airborne bacteria, thereby directly affecting the composition of the airborne bacterial community (Brodie et al. 2007; Fierer et al. 2008). This underlines once more the importance to correlate all gathered data with as many environmental parameters as possible.

Research aims

The main aim of the current thesis was to gather basic knowledge about bacterial communities in marine bioaerosols considering their composition and abundance as well as the influence of environmental factors on spatial and temporal dynamics on airborne bacterial communities. In the light of absence of standardised methodologies, a complementing evaluation of quantification methods was performed. Furthermore, culture-independent methods (pyrosequencing, ARISA, q-PCR) were chosen in order to eliminate known biases which would have been introduced by culture-dependent methods.

The investigation was structured in three parts. (1) A spatial aspect of marine bioaerosols was studied in the course of a sampling campaign with a ship cruise from the North Sea to the Baltic Sea. (2) A temporal aspect was investigated in a yearlong survey where daily samples were collected at the remote island of Helgoland (German Bight, North Sea). (3) The latter sampling campaign was additionally used for a comparison of two quantification methods (q-PCR vs. FLAPS) advancing towards the utilisation of standardised methods for the quantification of marine PBAPs.

All results were analysed in context of meteorological parameters (e.g. temperature, humidity, radiation, wind speed) for possible correlation. Back-tracking of air masses were performed in order to determine the areas of origin of sampled air masses (e.g. marine, coastal and continental).

Outline

The present thesis consists of a general introduction, three chapters (each representing one manuscript), and a general discussion.

Manuscript I (Submitted to MikrobiologyOpen)
Seifried, J. S.,Wichels, A., and Gerdts G.
Spatial distribution of marine airborne bacterial communities
The Manuscript represents the investigation of the spatial aspect of bacterial community in marine bioaerols in the North and Baltic Sea area including Skagerrak and Kattegat. The composition of bacterial populations is investigated and analysed in context of environmental parameters/factors to check for possible influencing variables. The planning, laboratory work, analyses and manuscript writing were carried out by Jasmin Seifried under the guidance of Antje Wichels and Gunnar Gerdts. Jörg Peplies was in charge of the analyses of pyrosequencing data (LGC genomics, Germany, Berlin) via the SILVAngs pipeline.

Manuscript II (to be submitted to Aerobiologia)
Seifried, J. S., Wichels, A., and Gerdts G.
Temporal variability of airborne bacterial communities at a marine sampling site
This manuscript represents the investigation of temporal aspect in bacterial populations in marine bioaerosols with an annual sampling survey conducted at Helgoland, German Bight. The planning, laboratory work, analyses and manuscript writing were carried out by Jasmin Seifried under the guidance of Antje Wichels and Gunnar Gerdts.

Manuscript III (to be submitted to Aerobiologia)
Seifried, J. S., Wichels, A., and Gerdts G.
Quantification of marine airborne bacteria: comparative study on C-FLPAS vs. q-PCR
This manuscript represents the comparison and evaluation of quantification of atmospheric bacteria with q-PCR and direct fluorescent measurements with a FLAPS II system including possible implications for the comparability of studies using different sampling devices and possible implications for future work. The planning, laboratory work, analyses and manuscript writing were carried out by Jasmin Seifried under the guidance of Antje Wichels and Gunnar Gerdts.

Chapter I

Spatial distribution of marine airborne bacterial communities

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Running Head: Bacterial communities in marine bioaerosols

Keywords: Bioaerosols, pyrosequencing, impinger, marine, trajectories

Abstract

The spatial distribution of bacterial populations in marine bioaerosol samples was investigated during a cruise from the North Sea to the Baltic Sea via Skagerrak and Kattegat. The analysis of the sampled bacterial communities with a pyrosequencing approach revealed that the most abundant phyla were represented by the Proteobacteria (49.3%), Bacteroidetes (22.9%) Actinobacteria (16.3%) and Firmicutes (8.3%). Cyanobacteria were assigned to 1.5% of all bacterial reads. A core of 37 bacterial OTUs made up more than 75% of all bacterial sequences with the most abundant OTU, *Sphingomonas sp.*, comprised 17% of all bacterial sequences. The most abundant bacterial genera were attributed to distinctly different areas of origin, suggesting highly heterogeneous sources for bioaerosols of marine and coastal environments. Furthermore, the bacterial community was clearly affected by two environmental parameters - temperature as a function of wind direction and the sampling location itself. However, a comparison of the wind directions during the sampling and calculated backward trajectories underlined the need for more detailed information on environmental parameters for bioaerosol investigations. The current findings support the assumption of a bacterial core community in the atmosphere. They may be emitted from strong aerolising sources, probably being mixed and dispersed over long distances.

Introduction

The presence of bacteria in the atmosphere and their relevance for atmospheric processes have been the subject of investigations since the 19th century (see Després et al. 2012 and references therein). Although inorganic particles such as sulfate, sea salt, mineral dust and vulcanic ashes are comparably well studied, biological particles received only little attention which is also due to a former underestimation of their occurrence in the atmosphere (see Matthias-Maser & Jaenicke 1995; Jaenicke 2005; Després et al. 2012 and references therein). Primary biological aerosol particles (PBAP) are defined as solid airborne particles originating from biological organisms including microorganisms, plant debris, pollen, fungi spores and cells as well as plant wax released by living organisms (IGAP 1992). Their release into the atmosphere can be active and/or passive, emitting from nearly all kind of surfaces. Marine PBAP mainly emerge from a bubble bursting process (Blanchard & Woodcock 1980; Blanchard & Syzdek 1982) by which small air bubbles (formed by breaking waves/whitecaps) get suspended to the depth of several meters into the water column. While resurfacing, small particles get collected and accumulated on the bubbles' surfaces with an enrichment factor of 15-25 (Aller et al. 2005), thus exceeding the natural concentration of bacteria in the surrounding waters. When the bubbles reach the water surface, collected material is ejected into the atmosphere. The same process creates the concentration of sea salt in marine air (Burrows et al. 2009). As oceans cover more than 70% of the global surface with a bacterial concentration of 3×10^5 cm^{-3} to 5×10^6 cm^{-3} in surface waters, it is assumed that many PBAP are emitted from the oceans. Nevertheless, still little is known about bioaerosols from marine environments. Most PBAP studies focused on public health concerns in hospitals and workplaces (e.g.: Eames et al. 2009; Tang 2009; D'Arcy et al. 2012; Eduard et al. 2012), and the few outdoor studies were mainly conducted in urban and rural environments (e.g Maron et al. 2005; Després et al. 2007; Li et al. 2010). To date, the lack of standardised sampling methods and/or analyses in PBAP investigations remains one of the main problems in bioaerosol investigation (Burrows et al. 2009; Després et al. 2012; Gandolfi et al. 2013). Interpretation and comparison of findings from different studies is, thus, impeded by the employment of different sampling techniques.

Despite their inevitable biases (selectivity of media, incubation time, incubation temperature), most investigations on PBAPs used culture-dependent techniques. This pre-selection for special types of bacteria is a considerable disadvantage for investigations on bioaerosols as

their origin can be strongly heterogeneous. Recent studies showed that culture-dependent and -independent methods produce different results for the same bacterial communities (Cho & Hwang 2011; Ravva et al. 2012), although some findings may overlap (Fahlgren et al. 2010; Urbano et al. 2011). Furthermore, even if viable, only a small fraction of environmental bacteria are culturable (around 0.001-0.01% of seawater bacteria; Colwell 2000) and culturability rapidly declines after aerolisation (Heidelberg et al. 1997). Studies on marine/coastal bioaerosols, so far, used a combination of culture-dependent and culture-independent methods. Bacteria were identified by cloning (Fahlgren et al. 2010; Urbano et al. 2011) or Denaturing Gradient Gel Electrophoresis (DGGE) with subsequently conducted sequencing of bands (Cho & Hwang 2011). Both resulted in only few sequences which most likely reflected only the most abundant taxa. As these methods do not allow for covering the bacterial communities as a whole, the current study employed a high-throughput pyrosequencing approach which has been successfully used before (Bowers et al. 2009; Bowers et al. 2011a). Pyrosequencing provides a detailed description of the microbial community including rare taxa and facilitates robust statistical analyses.

Considering the advantages of pyrosequencing, the current study aimed to characterise the bacterial community of marine bioaerosols without the restrictions of culture-dependent approaches and to test for the influences of spatial heterogeneity and selected environmental parameters.

Material and Methods

Sample collection

Bioaerosol samples were collected in August 2011 during a ship cruise from the North Sea to the Baltic Sea via Kattegat and Skagerrak (Figure 1). Samples were only collected when the ship was moving in order to eliminate possible biases which could be introduced by the ship chimney. In order to cover a wide spatial range in distribution, samples were collected in between stations - two in the morning and two in the afternoon. An impingement aerosol sampler (Dycor XMX/2L MIL; Edmonton, Canada) was employed to gather the total number of 36 samples. The impingement aerosol sampler was positioned on the top deck of the research vessel, 10 m above sea level (a.s.l.). This sampling device is qualified for processing high volumes of air, stripping away larger dust particles and accumulating the very small micro debris and aerosols within a diameter of 1 to 10 µm. Particles were washed out in collection vials (50 ml falcon tubes) with 5 ml phosphate buffered saline (PBS). Each sample consisted of six subsamples (10 minutes sampling time each) which were pooled for further analysis. In total, 0.72 m^3 air were collected per sample. The sample was then filtered through 0.2 µm Isopore™ membrane filters (GTTP-type, diameter 13 mm; Millipore, Eschborn, Germany) and stored at -20°C for later processing.

Meteorological/environmental parameters (temperature, absolute wind speed and wind direction, global radiation, longwave radiation, air pressure and humidity) were recorded with the DAVIS-Ship FS on board. Measurements were recorded in five-minute intervals and arithmetically averaged for the period of one hour.

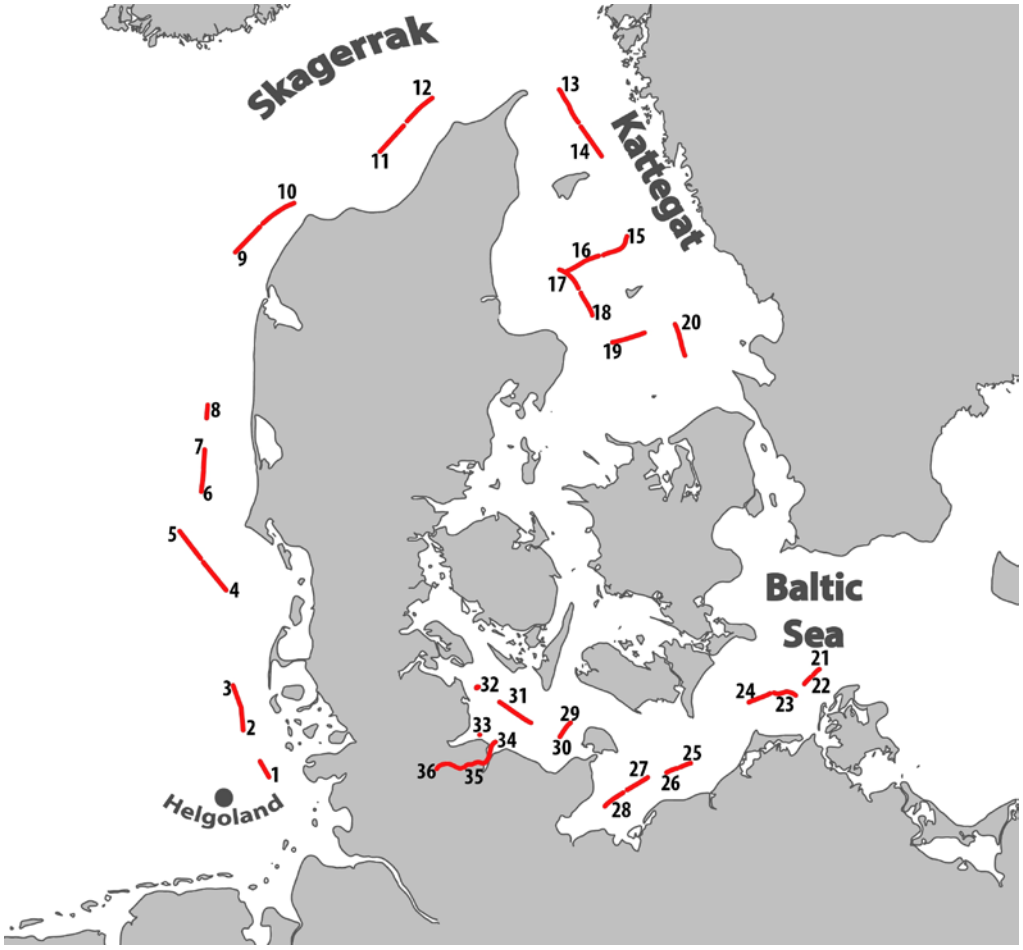

Figure 1 Sampling transects in chronological order, conducted in August 2011. Each transect represents the total sampling time of one hour.

DNA extraction

Following the procedure of Sapp et al. (2006), a chemical cell lysis with an additional freeze-thaw-step (Maron et al. 2005) and a subsequent phenol-chloroform-extraction was performed. The total volume of 20 µl of DNA extract per sample was obtained. Concentration and pureness of the extracted DNA was then determined photometrically using the microplate reader Tecan Infinite© 200 from NanoQuant photometry (Tecan, Männedorf, Switzerland). All DNA was stored at -20°C for later use.

16S rRNA gene amplicon pyrosequencing

All 36 samples were applied to pyrosequencing of the 16S rRNA gene. The primers which were used to construct an amplicon library were already described elsewhere (Krause et al. 2012). Due to the small yield of PCR products with the pyrosequencing tagged primer, a reamplification PCR for the prokaryotic 16S rRNA gene was conducted. In a first PCR reaction, the eubacterial forward primer GM3 and the reverse primer 907 (Muyzer et al. 1995) were applied with each 5 ng DNA as template. Subsequently, 1 µl of the reaction was used for a PCR with pyrosequencing tagged primers. PCR reactions were run with 2.5 µl taq buffer (10x), 5 µl TaqMaster PCR Enhancer® (5x), 0.7 µl of each primer (20 mM), 0.75 µl dNTPs (2.5 mM each), and 3 U of taq DNA polymerase (5 Prime, Hamburg, Germany), and were adjusted with ultrapure water to a reaction volume of 25 µl. The conditions for both PCR reactions (with and without pyrosequencing tagged primers) were the following: 94°C for 10 min, followed by 30 cycles with 94°C for 1 min, 44°C for 1:30 min and 68°C for 2 min; final elongation at 68°C for 5 min. PCR products were obtained for 31 out of the 36 collected samples. PCR products were then verified by gel electrophoreses and by the absence of amplifications in DNA-free controls. PCR products were purified with the peqGOLD Gel Extraction Kit (peqlab, Erlangen, Germany). Sequencing was done on a Roche 454 GS-FLX Titanium platform (LGC Genomics, Berlin, Germany).

All sequence reads were processed on the NGS analysis pipeline of the SILVA rRNA gene database project (SILVAngs; Quast et al. 2013). Each read was aligned against the SILVA SSU rRNA SEED and quality controlled, using the SILVA Incremental Aligner (SINA) (Pruesse et al. 2012); (Quast et al. 2013): reads shorter than 50 aligned nucleotides and reads with more than 2% of ambiguities, or 2% of homopolymers, were excluded from further processing. Suspected contaminations and artefacts as well as reads with a low alignment quality (50 alignment identity, 40 alignment score reported by SINA) were also identified and excluded from downstream analysis.

After these initial steps of quality control, identical reads were identified in a dereplication-step. Unique reads were clustered on a per sample basis (OTUs), and the reference read of each OTU was classified. Dereplication and clustering was conducted using cd-hit-est (version 3.1.2; http://www.bioinformatics.org/cd-hit; Li & Godzik 2006) with identity criteria of 1.00 and 0.98. For classification, local nucleotide BLAST were compared to the non-redundant version of the SILVA SSU Ref dataset (release 111; http://www.arb-silva.de) using blastn

(version 2.2.22+; http://blast.ncbi.nlm.nih.gov/Blast.cgi; Camacho et al. 2009). The obtained classification of the OTU reference reads were each mapped onto all reads that had been assigned to the respective OTU. Reads without any BLAST hits and reads with only weak BLAST hits function ' (% sequence identity + % alignment coverage)/2' not exceeding a value of 93) remained unclassified. These reads were assigned to the meta group “No Relative" in the SILVAngs fingerprint and Krona charts (Ondov et al. 2011). The remaining classified sequences formed an OTU table and singletons were excluded from the analyses. Although non-bacterial sequences, chloroplast and mitochondrial sequences were recorded; they were not further analysed. Only phyla, classes and families with an occurrence of 1% or higher were considered for the heatmap construction. All sequence data were deposited at the NCBI Sequence read archive (Accessionnumber: SRP043406).

Air mass backward trajectories

In order to determine the origin of a given air parcel, backward trajectories of all sampled air masses were created using the HYSPLIT model (HYbrid single-particle Lagrangian integrated trajectory model; Draxler & Rolph 2013). Five-day backward trajectories (commonly used in bioaerosol studies) were calculated for air parcels arriving at three different arriving heights above sea level (a.s.l.), each representing a different air layer: 10 m (sampling height, ground influence), 500 m (the upper part within the boundary layer) and 1500 m (above boundary layer) a.s.l.. This was done for each sampling period, detecting possible mixing events between different air layers. The midway of each transect was chosen as the starting point and the start time for the corresponding trajectory.

Statistical analyses

All statistical analyses were performed with the computer software packages PRIMER 6 (with PERMANOVA+ ad-on; PRIMER-E Ltd) and GraphPad Prism 5.04 (GraphPad Software, Inc.). Alpha diversity for each sample was expressed by Simpson's diversity index with $1-\lambda' = 1-\sum(n_i*(n_i-1)/(N*(N-1))$ as it provides good estimate of diversity at relatively small sample sizes without assumptions about underlying distribution of species abundance (Magurran 2004). Obtained alpha diversity values were compared with regard to different environmental factors (see Table 1 for abbreviation), being the following: 'sampling location' (factor levels: 'NS', 'SK', 'KT', 'BS' and 'KC'), 'wind direction' (factor levels: 'N', 'E', 'SE', 'S',

'SW', 'W', 'NW'), 'backward trajectory influence' (BWT Influence factor levels: marine, more marine (75%), mixed, more continental (75%), continental), 'backward trajectory' (BWT factor levels: no crossing, no height; crossing, no height; crossing, height; no crossing, height), 'cardinal direction' (factor levels: 'N', 'E', 'S', 'W'), 'height' (factor levels: low; 500 m a.s.l.; 1000 m a.s.l; 1500 m a.s.l; 2000 m a.s.l), and 'rain' (factor levels: no rain (N); rain during sampling (0); rain 6 h before sampling (6); rain 60 h before sampling (60)) (see Table S1). Due to the different amount of samples within each factor level, the data did not fulfill the requirements of an ANOVA (Kolmogorov-Smirnov test, Bartlett's test). Therefore, the non-parametric Kruskal-Wallis test was performed followed by Dunn's post hoc procedure.

Bray-Curtis similarities were calculated for the square root transformed OTU abundance data (equalisation of different sample size) and visualised by principal co-ordinate analysis (PCO; Krause et al. 2012). Beta diversity was then analysed with a permutational multivariate ANOVA (PERMANOVA; Anderson 2001) with the same factors as for alpha diversity (see above).

A distance based linear model (DistLM) with distance based redundancy analysis (dbRDA) visualisation was performed on the Bray-Curtis similarities of the OTU abundances with Euclidean distances of the environmental parameters (predictor valuables). This allowed for modeling the relationship of the OTU abundance data and environmental parameters in order to test for possible influences of the environmental parameters. Environmental parameters (global radiation; longwave radiation; absolute wind direction; absolute wind speed; air pressure; air temperature; humidity) were log(x+1) transformed, normalised, and subjected to a principal component analysis (PCA) to visualise patterns of the relationship of the different environmental parameters.

Table 1 Abbreviations

Group	Abbreviation
North	N
East	E
Southeast	SE
South	S
Southwest	SW
West	W
Northwest	NW
North Sea	NS
Skagerrak	SK
Kattegat	KT
Baltic Sea	BS
Kiel Canal	KC

Results

Weather conditions and backward trajectories

Weather conditions during the sampling period were mild (13-21°C), humid (69-96%) and wind speed varied from 1 to 15 m/s (Table S2).

Five-day backward trajectories were calculated for each sample with three transects which were characterised by different arriving heights (10 m, 500 m, 1500 m). In all sampled air parcels, the origins of the backward trajectories were highly variable, but western and southern directions clearly dominated. Based on these backward trajectories, there were no direct eastern winds. Samples with strong marine influence (e.g. sample 14; Figure 2B) were always characterised by air masses with projected trajectories which were close to the sea surface. However, also samples with air masses of mixed origin (50 % continental influence, 50 % marine influence) (e.g. sample 1) were observed (Figure 2A). For the majority of samples, the origin of air parcels samples was consistent with the general wind direction recorded by the DSHIP system. In few samples, the origin differed from the recorded wind direction (e.g. 17 and 31; Figure 2C,D). For example, the eastern wind direction during the sampling at location 31 was in clear contrast to the backward trajectories which showed a strong southern/continental influence. The five-days back-calculation of air parcel movements showed a consistent circling close to the ground over the German mainland. The air parcel of sample 17 started in the western direction over the North Atlantic and crossed Scotland, the North Sea and Denmark before the sampling in the Kattegat from a southern direction.

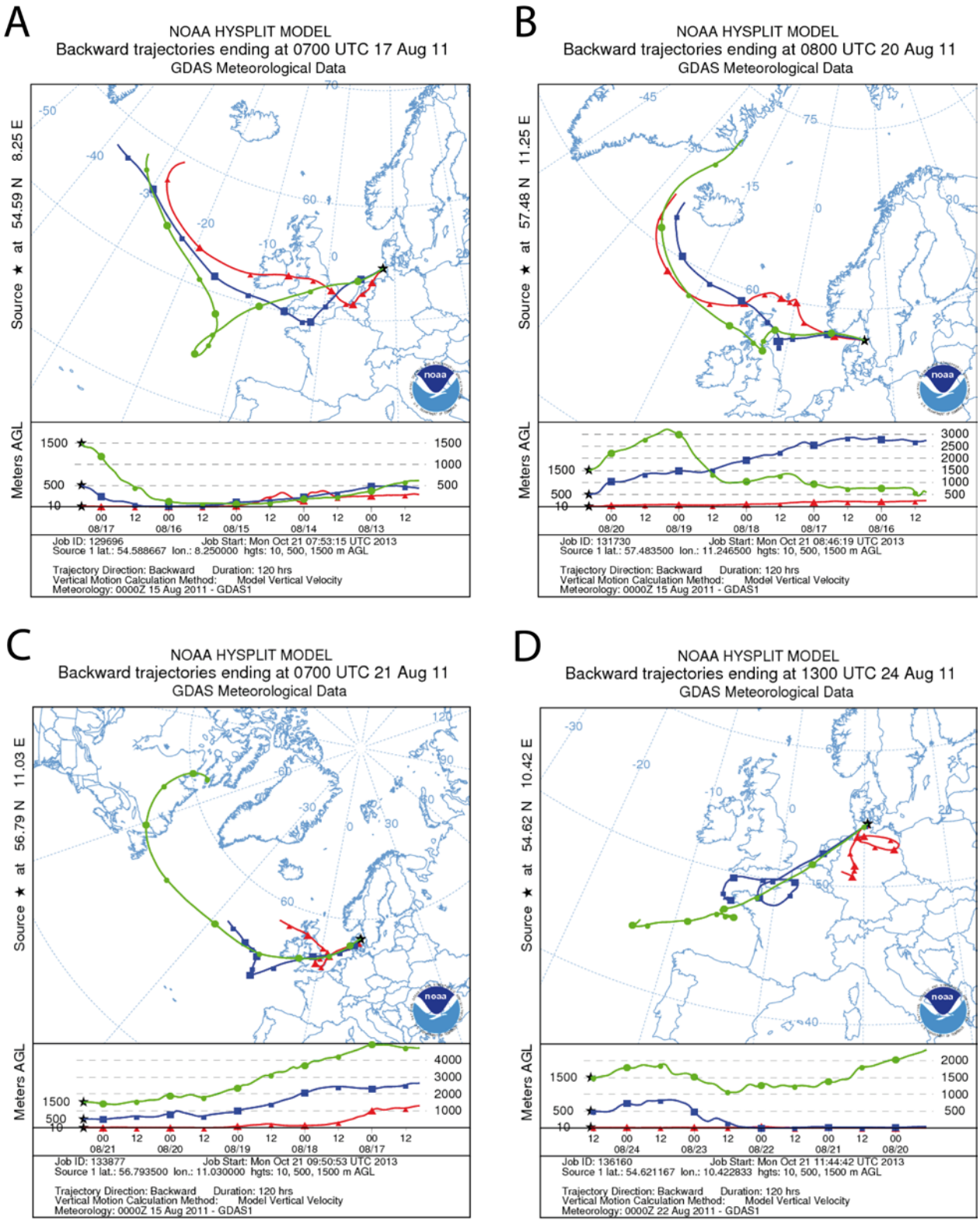

Figure 2 Backward trajectories calculated with the NOAA Hysplit Model (Draxler & Rolph, 2013) with three different transects with different arriving heights: red = 10m; blue = 500m; green = 1500m. Sampling time and place are indicated by the black asterisk (A = Sample 1; B = Sample 14; C = Sample 17; D = Sample 31).

Bacterial diversity

A total number of 293,011 reads was obtained after quality check via SILVAngs databank. Chloroplast sequences (125,993 = 43%) and mitochondrial sequences (49,607 = 17%) made up 60% of all sequences. The proportion of chloroplast and mitochondrial sequences varied between the samples (Table S8). Chloroplast sequence abundances ranged from 2.2 to 88.5% and mitochondrial sequences from 0.1 to 69.2%. Chloroplast and mitochondrial sequences as well as singletons (n = 1) were omitted from the analysis of the bacterial reads.

The remaining 117,232 bacterial sequences were highly variable among the samples and ranged from 8 to 11,537 sequences per sample (Table S8). Sample 27 (n = 8) was excluded from analysis due the small number of sequences. The bacterial sequences were classified in 753 OTUs. The rarefaction curves did not reach an asymptote for all samples, suggesting that the full extent of airborne bacterial diversity could not be recovered for all samples.

The calculated diversity values (Simpson's diversity index) showed no significant differences for most of the considered factors (BWT: $H_{K\text{-}W} = 7.663$, $p = 0.1048$; BWT influence: $H_{K\text{-}W} = 6.621$, $p = 0.0850$; cardinal direction: $H_{K\text{-}W} = 3.798$, $p = 0.2841$; wind direction: $H_{K\text{-}W} = 8.779$, $p = 0.1864$; height: $H_{K\text{-}W} = 2.650$, $p = 0.6180$; rain: $H_{K\text{-}W} = 4.062$, $p = 0.2548$). However, the factor 'sampling location' had a significant effect on the bacterial diversity ($H_{K\text{-}W} = 12.45$, $p = 0.0143$). This was particularly due to the differences between 'KC' and 'KT' ($p < 0.05$).

The PCO plot with 23.8% explained variation for the x-axis and 11.1% for the y-axis (34.9% in total), showed no clear patterns or grouping (Figure S1). However, the PERMANOVA of beta diversity revealed significant effects for the factors 'sampling location' ($p = 0.048$), 'cardinal direction' ($p = 0.040$) and 'wind direction' ($p = 0.001$; Table 2). Pairwise comparisons for 'sampling location' showed significant differences between the samples from the 'BS' and the 'NS' ($p = 0.049$) as well as the 'SK' ($p = 0.0437$; Table S3). In detail, the significant effect for 'cardinal direction' was caused by differences between 'E' and 'W' ($p = 0.043$) as well as between 'E' and 'N' ($p = 0.0437$; Table S4). Comparison of the wind directions revealed significant differences of 'SW' in direct comparison to 'W' ($p = 0.010$) and to 'NW' ($p = 0.002$) as well as between 'W' and 'E' ($p = 0.031$; Table S5). The factor 'rain' also had a significant effect on the beta diversity ($p = 0.035$; Table 2) although this could not be supported by pairwise comparisons (Table S6). The factors 'BWT', 'BWT influence' and 'height' had no significant effect on the bacterial diversity (BWT: $p = 0.507$; BWT Influence: $p = 0.123$; height: $p = 0.274$; Table 2).

Principal component analysis of environmental parameters displayed an even distribution of the individual sampling sites (Figure S2). However, there was a clear separation when the geographical group factors were taken into account. Within the data, 32.1% of the variation was accounted to PC1 and 25.6% was accounted to PC2. Therefore, 57.7% of the variation could be explained by these two components (Table S7).

DistLM analysis revealed significant effects of the factors 'longwave radiation' (p = 0.023), 'absolute wind direction' (p = 0.002), 'absolute wind speed' (p = 0.020), 'air temperature' (p = 0.020) and 'longitude' (p = 0.017; Table 3). The sequential test showed that the influence of 'temperature' (p = 0.001), 'longwave radiation' (p = 0.02), 'latitude' (p = 0.033) and 'wind direction' (p = 0.024) contributed significantly to the explained variation (Table 4). The first axis of the dbRDA explained 44% of fitted and 16.2% of total variance, whereas the second axis explained 14.8% of fitted and 5.6% of total variance (Figure 3). The separation along the x-axis was due to the factors 'wind direction' and 'temperature', which were negatively correlated, and due to latitude along the second axis.

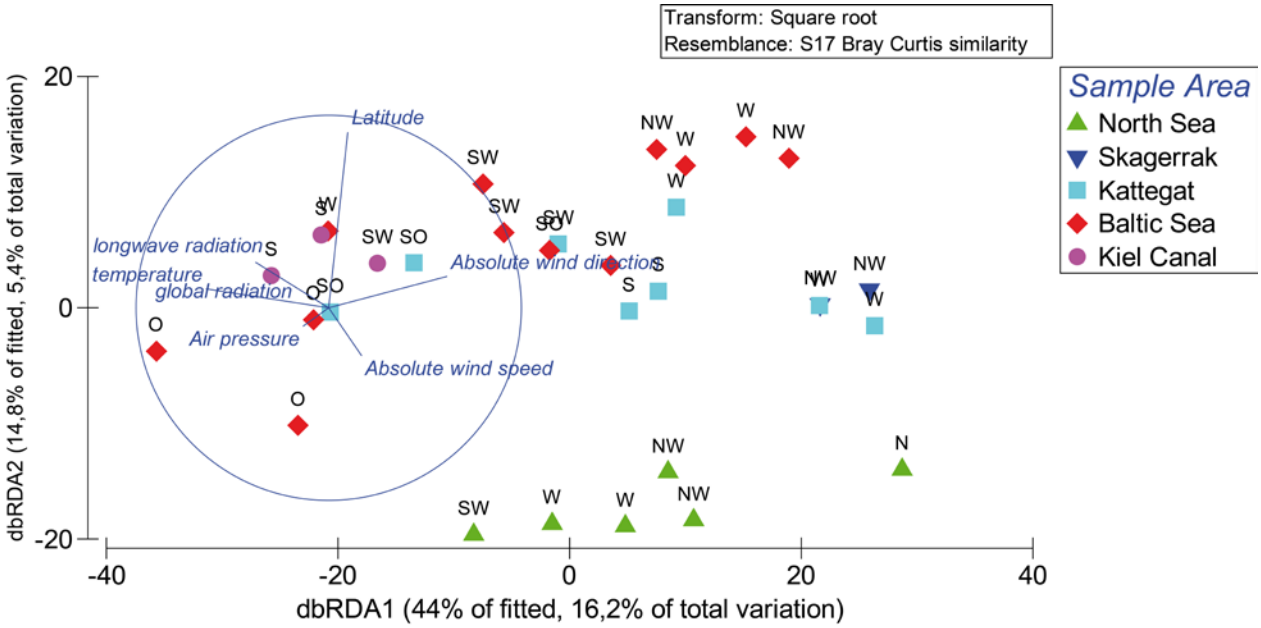

Figure 3 dbRDA ordination relating environmental variables with the OTU composition of samples taken. The '% of fitted' indicates the variability in the original data explained by the fitted model and '% of total variation' indicates the variation in the fitted matrix.

Table 2 PERMANOVA Main test of bacterial community composition based on Bray-Curtis dissimilarities of OTUs (16S rRNA gene amplicon sequencing).

Group	d.f.	SS	pseudo-F	p (perm)[1]	Sq. root
Sampling location	4	9998.4	1.3389	**0.048**	11.103
BWT	4	8921.6	1.2114	0.123	8.1096
BWT Influence	3	5432.3	0.95196	0.507	-4.8722
Cardinal direction	4	7948.0	1.4646	**0.040**	11.648
Wind direction	6	16674	1.6626	**0.001**	16.148
Height	4	826208	1.1068	0.274	7.4099
Rain	3	7928.1	1.4603	**0.035**	11.214

[1]Significant results (*p (perm)* < 0.05) are highlighted in bold
Displayed are tests for the factors 'BWT', 'BWT Influence', 'Cardinal direction', 'Wind direction', 'Height' and 'Rain' and the partitioning of multivariate variation. *p*-values were obtained using type III sums of squares and 999 permutations under the full model. d.f.: degrees of freedom, SS: sums of squares, Sq. root: square root of the component of variation attributable to that factor in the model, in units of Bray-Curtis dissimilarities.

Table 3 Distance-based linear models (DistLM) calculation for the influence of environmental variables on OTU composition.

Variable	Pseudo-F	p[1]	Prop.
global radiation	1.0319	0.359	3.44E-02
longwave radiation	2.0764	**0.023**	6.68E-02
Absolute wind direction	3.2902	**0.002**	0.1019
Absolute wind speed	1.9765	**0.020**	6.38E-02
Air pressure	1.4957	0.099	4.90E-02
Air temperature	4.0158	**0.001**	0.12163
Humidity	1.2687	0.168	4.19E-02
Longitude	2.1305	**0.017**	6.84E-02
Latitude	1.4607	0.093	4.80E-02

[1]Significant results (*p (perm)* < 0.05) are highlighted in bold

Table 4 Sequential test of distance-based linear models (DistLM) calculation. Tests for relationship between the OTU composition for different samples with environmental parameters. Amount explained by each variable added to model is conditional on variables already in the model.

Variable	Pseudo-F	p[1]	Proportion of variance	Cumulation
+Air temperature	4.0158	**0.001**	0.12163	0.12163
+longwave radiation	1.7393	**0.020**	5.14E-02	0.173
+Latitude	1.5829	**0.033**	4.58E-02	0.2188
+Absolute wind direction	1.7254	**0.024**	4.86E-02	0.26742
+Air pressure	1.3306	0.137	3.70E-02	0.30444
+Absolute wind speed	1.2629	0.167	3.48E-02	0.33921
+global radiation	1.0213	0.431	2.81E-02	0.3673

[1]Significant results (*p (perm)* < 0.05) are highlighted in bold

Pyrosequencing data analysis

The 37 most abundant OTUs, each present with more than 500 sequences, made up more than 76 % of all sequences (Table S9). The most abundant genus *Sphingomonas* was present with 17% of all bacterial reads.

According to the DistLM and PERMANOVA results, samples were combined based on wind direction and sample area (Table 4, Figure 3) and compared with each other. The number of samples comprised by wind direction groups ranged from 1 to 8 and the sequences represented by each wind direction group ranged from 756 to 27,955. The number of OTUs ranged from 96 to 573 (Table 5).

Samples per sampling location ranged from 2 to 12 samples, with 4,945 to 46,079 sequences and 221 to 578 OTUs per location (Table 6).

The phylogenetic analysis of bacterial communities was restricted to phyla, classes and families with an OTU occurrence of 1% or higher.

Four important phyla were detected in the aerosol samples: Actinobacteria (16.3%), Bacteroidetes (22.9%), Firmicutes (8.3%) and Proteobacteria (49.3%) (Figure 4). In combination with the Cyanobacteria (1.4%) the four main phyla represented 98.2% of all bacterial reads. The remaining bacterial sequences were affiliated with the classes Acidobacteria, Aquificae, Armatimonadetes, Chloroflexi, Deinococcus-Thermus, Fusobacteria, Gemmatimonadetes, Lentisphaerae, Nitrospirea, Planctomycetes, Tenericutes and Verrucomicrobia.

Proteobacteria was the dominant phylum in all groups of 'wind direction' and 'sampling location' with ca. 50% of all sequences (except for the wind directions: 'N' 45%, 'S' 43%, 'NW' 36%, and the sampling location 'SK': 45%).

Cyanobacteria were restricted to the wind directions 'N' (1.5%), 'W' (1.6%), and 'NW' (6.7%) and to 'SK' and 'BS' locations (2-3%).

Most abundant Proteobacteria were Alphaproteobacteria (see above) with *Sphingomonas* (17%), *Anderseniella* (3.9%) and *Rhizobium* (3.4%). Within the Betaproteobacteria, *Massilia* was most abundant with 2.8% of all sequences, whereas *Pseudoalteromonas* (2.8%), *Psychrobacter* (2.8%) and *Pseudomonas* (1.6%) were the most frequent Gammaproteobacteria.

Bacteroidetes were found in all groups ranging from 11% ('N') to 28-34% ('NW' and 'E'). For the other wind directions the numbers varied around 20%. Bacteroidetes were more evenly

distributed between the sampling locations (19-25%). Most abundant Bacteroidetes were from the class Bacteroidia *Prevotella* (2.4%), from the class Cytophagia *Hymenobacter* (4.7%), and from the class Flavobacteria *Brumimicrobium* (1.8%) and *Chryseobacterium* (1.8%).

Firmicutes were found at a low level with less than 1% in 'E' and fewer than 2% of all sequences in 'SE'. Higher abundances were observed in 'SW' (7%), 'S' and 'W' (11%), 'NW' (14.7%) and 'N' (25.5%). Firmicutes were found at all locations but with a wide range of abundances. Lowest abundances were detected in the 'NS', 'KC' and 'KT' samples (4-8%), slightly higher numbers were found in the 'BS' samples (11%) and 'SK' samples showed the highest abundances (19.5%).

The most frequent Firmicutes sequence was the Bacilli *Staphylococcus* (3.7%).

Actinobacteria were represented by 13-14% of all sequences in 'E', 'S', 'W' and 'NW' samples, with slightly higher numbers in 'N' (16.7%), 'S' and 'SW' direction (both 20.5%). For sampling locations we found slightly higher abundance in 'NS' and 'KT' samples (20-21.7%) than on the other locations (12-14.5%).

The most frequent Actinobacteria were unidentified Microbacteriaceae (2.4%), *Curtobacterium* (2.4%), *Arthrobacter* (2.2%) and *Propionibacterium* (2.4%).

Within the Proteobacteria (Figure 5), the three main classes Alpha-, Beta- and Gammaproteobacteria were found. Alphaproteobacteria were dominant in all wind and sampling location groups except for the 'S' group (30%), where Gammaproteobacteria clearly dominated Proteobacteria sequences (61%). Furthermore, in the samples from the 'SK' Betaproteobacteria clearly dominated with 45% over the Alphaproteobacteria (26.5%). In 'NW' and 'N' samples, Epsilonproteobacteria and Deltaproteobacteria were present in 1% of the total number of Proteobacteria, restricted to the 'SK' (1.8-3.2%).

Alphaproteobacteria were represented by 22 families (Figure 6). The highest diversity was observed in the 'NW' group with 14 detected families, followed by 'S', 'SK' and 'KC' with eleven families. The most dominant family was Sphingomonadaceae represented by the genus *Sphingomonas*, with the most abundant OTU in the entire dataset. Only in the 'N' and 'S' group and 'KC', the family Rhodobiaceae dominated with the genus *Anderseniella*. Although Rhodobiaceae were also present in other groups, they occurred in much lower numbers. Especially in 'NS' and 'SK' samples they made up only 2% and 4% of all sequences but gradually increased from the 'KT' (7.4%) to the 'BS' (21.3%) and the 'KC' (40.4%). Rhizobiaceae were found in all groups (9-23%), but less frequently in 'W', 'NW' and 'N' group

(>4%). The highest abundance was found within the 'E' group, gradually decreasing from 'E' to 'W' with the lowest percentages in 'NW' and 'N'. High numbers in the 'BS' (16%) were found, whereas abundances were lower at the 'KT' (13.4%), 'KC' (11.6%) and only 2-6.7% in the 'NS' and 'SK' samples.

Betaproteobacteria comprised nine families (Figure S3). The dominant Oxalobacteraceae (genera *Massilia* and *Oxalobacter*) represented more than 50% of all sequences in all groups, except for the 'NW' and 'N' group but were still the most abundant family. Neiserriaceae (uncultered Neisseriaceae) were found in all samples ranging from 2-7% ('E', 'SE', 'S' and 'SW') to 12-28% ('W', 'NW' and 'N'). Neisseriaceae were highly abundant in the 'BS' samples (21.5%), but occurred in lower numbers at other locations (2-6%). Comamonadaceae (genera *Variovorax* and *Acidovorax*) were evenly distributed to all wind directions and locations (13-29%) but were found in low numbers in 'SK' samples (6.5%). Alcaligenaceae occurred in all wind direction groupings in low numbers (2-5%) except for 'W' (20%). Alcaligenaceae were present with 1-3.4% in the samples collected in the 'NS', 'SK' and 'KC' and showed slightly higher numbers in the 'KT' and 'BS' groups (5-11%).

The class Gammaproteobacteria comprised 20 families with no dominant family/genus for all samples (Figure S4). Moraxellaceaea (*Psychrobacter*, *Acinetobacter*, *Enhydrobacter*) were present in all samples, dominating in 'W' (63%) and 'NW' (35%) and also in the 'NS' and 'BS' samples (42-48%). Pseudoalteromonadaceae (*Pseudoalteromonas*) were found in all samples in comparably low numbers (3-5%). However, clear dominances in 'S' (50%), 'KT' and 'KC' (42-45%) were detected. Enterobacteriaceae (*Pantoea*, Escherichia-Shigella, *Citrobacter*) were found in all groups with higher abundances in 'N' and 'E' (25-30%). In the 'NS', 'SK' and 'KT' (6-7%), however, abundances were slightly lower than in the 'BS' and the 'KC' (12.5-14.7%). Pseudomonadaceae (*Pseudomonas*) were present in all groups dominating in 'E' and 'SE' (42-44%) as well as in the 'SK' (36.9%). Abundances in 'S', 'W' and 'KT' (5.3-7.9%) were comparably low. Oceanospirillaceae (*Marinomonas*) occurred in low numbers 'NW', 'N', 'SK' and 'KC' (< 2%) and slightly higher numbers in 'S' and 'KT' (9%).

The phylum Actinobacteria was strongly dominated by the class Actinobacteria. Furthermore, in the groups 'W', 'NW', 'N', 'E', 'SW' the class Acidimicrobia was detected in low numbers (3-10%). In addition, Acidimicrobia were detected at all locations (1.5-2.5%) and in the two 'SK' samples Acidimicrobia made up 30.8 % of the Actinobacteria sequences.

The Actinobacteria comprised 18 families (Figure 7). Propionibacteriacea (*Propionibacterium*, *Friedmaniella*) were found in all groups, but were only dominant in 'NW' and 'N' (64-71%) and in 'SK' samples (48%). Micrococcaceae (*Arthrobacter*) were present in all groups (5-10%), clearly dominating in 'S' (43%). Microbacteriaceae (*Curtobacterium*, unident. Microbacteriaceae, *Agreia*, *Clavibacter*; *Microbacterium*, *Plantibacter*) were found in all groups and were dominant in 'W', 'E', 'SE', 'SW', 'NS' and 'BS' (55-77%). Lowest numbers were detected in 'N', 'NW' and the 'SK' group (8.9-9.7%). Acidimicrobiaceae were found in 'W', 'NW', and 'N' samples (2.5-9.1%). This family was present in low numbers at all other locations (except for the 'BS') with a higher numbers in 'SK' samples (29.6%). Coryneobacteraceae were not present in 'E' and 'SE' direction but at all locations.

Firmicutes were dominated by the class Bacilli in all samples except from E direction, where Clostridia were more abundant (Figure S5). Erysipelotrichi were restricted to 'NW' and 'SW' samples and 'KC'.

The 13 families of the class Clostridia were mostly evenly distributed (Figure S6). Clostridiaceae (*Clostridium*) was the dominant family in every group except for one sample from North, where the family XI Incertae Sedis was dominant (60%). For the other wind directions, abundances were lower (1.5-4.1%), which was similar in the location groups with slightly higher abundances in samples from the 'KC' and the 'NS' (8-15%). Lachnospiraceae and Rumicocaceae were constantly detected in every sample. Syntrophomonadaceae, however, only occurred in 'E', 'SE', 'S' samples in low abundances (2%), but were restricted to 'KC' samples with higher numbers (10.8%).

The detected 14 families of the class Bacilli were strongly dominated by Staphylococcaceae (genus *Staphylococcus*; 53-91%, except for 'E': 33%; Figure S7). The two families Bacillaceae (*Bacillus, Aeribacillus*) and Streptococcaceae (*Streptococcus*) were also present in all groups. Bacillaceae (5-11%, except for 'E': 30%) and Streptococcaceae (12-18%, except for 'N' and 'SW': 2% and 'NS', 'SK' and 'KC' 5.7-8.4%) showed an even distribution among the samples.

The phylum Bacteroidetes comprised four main classes with ten families which were present in in all groups (Figure S8, Figure S9). The class Bacteroidia dominated in 'N' (75%) and 'SK' (54.9%) but showed lower abundances in the other groups (2-23%). This class was represented by the three families Bacteriodaceae, Porphyromonadaceae and Prevotellaceae. Prevotellacea (genus *Prevotella*) was the most abundant Bacteroidia and was found in all groups, clearly dominating in the 'N' (67%) group. For the location groupings, Prevotellaceae

were present at all locations in abundances ranging from 3-14.6 %, representing 29.5% in the 'SK'. Cytophagia were dominant in 'SW' (45%) and 'W' (59%) and had high abundances in 'E' (39.3%), 'SE' (32.9%) but lower abundances in 'S' (10.8%), 'NW' (16.2%) and 'N' (4.65%). Cytophagia dominated samples from the 'NS' (55%) and the 'KC' (37.9%), increasing in the 'SK' samples (9.7%) and showing medium abundances in the 'KT' and the 'BS' (24.7-30.1%). Two families were found: the Cyclobacteriaceae and the Cytophagaceae. Cyclobacteriaceae (*Algoriphagus*) were only found in 'W' samples and the 'KT' in low numbers whereas Cytophagaceae (*Hymenobacter*, *Dyadobacter*¸ *Spirosoma*) were present in all groups with an emphasis in 'W', 'E', 'SE' and 'SW' directions. This was the dominant family in 'NS' samples (55.5%) and 'KC' samples. Flavobacteria dominated in the 'S' directions (61%) and the 'KT' location (45.9%) and were evenly distributed between the other locations and wind directions (9.9-24.3%). They comprised two families: Flavobacteriaceae were present in all wind direction and location groups in equal abundances whereas Cryomorphaceae were restricted to 'S' (41%) and 'NW' (< 2%) wind directions as well as the 'SK' (1.4%), 'KT' (26.3%) and the 'KC' (3.7%). Sphingobacteriia were dominant in 'NW' (50%) and 'SE' (39.8%) samples and in the 'BS' (45.5%). This was due to the most abundant Sphingobacteriaceae (*Pedobacter*), which also occurred in higher numbers in 'E', 'SE', 'SW', but lower numbers in 'W', 'N' and 'S' directions. For the location groupings, Sphingobacteriaceae dominated 'BS' samples (44.2%) with lower numbers in the 'NS', the 'SK' and in 'KT' samples (2.3-15%) whereas intermediate abundances were found in the 'KC' (27.9%). Chitinophagaceae were found in low abundances in all groups (location and Wind direction) except for 'NW' wind directions.

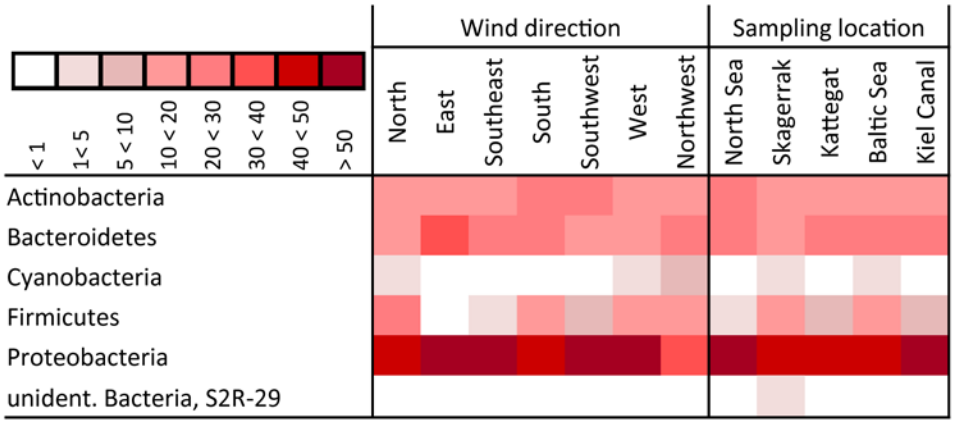

Figure 4 Taxonomic classification of bacterial reads grouped in wind direction and sampling location on phyla level using SILVA classifier based on 98% similarity omitting singletons (n = 1) and rare reads (< 1%). The amount of percentage proportion contribution of each phyla per group is indicated by colour of cell; darker colour represent higher contribution.

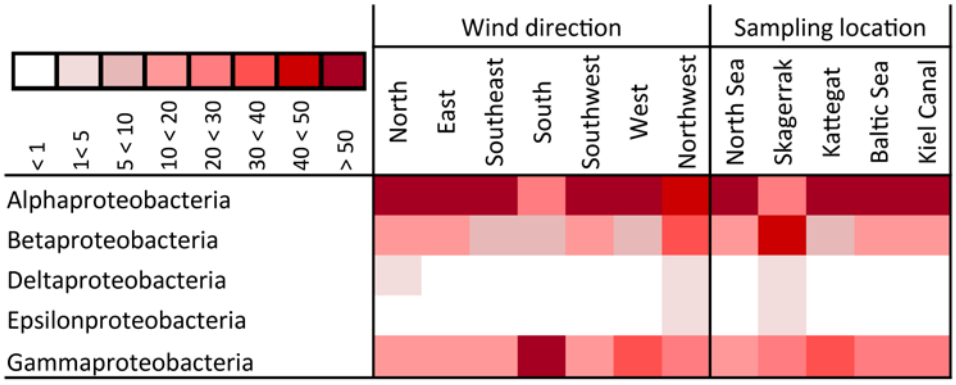

Figure 5 Taxonomic classification of Proteobacteria reads grouped in wind direction and sampling location on class level using SILVA classifier based on 98% similarity omitting singletons (n = 1) and rare reads (< 1%). The amount of percentage proportion contribution of each class per group is indicated by colour of cell; darker colour represents higher contribution.

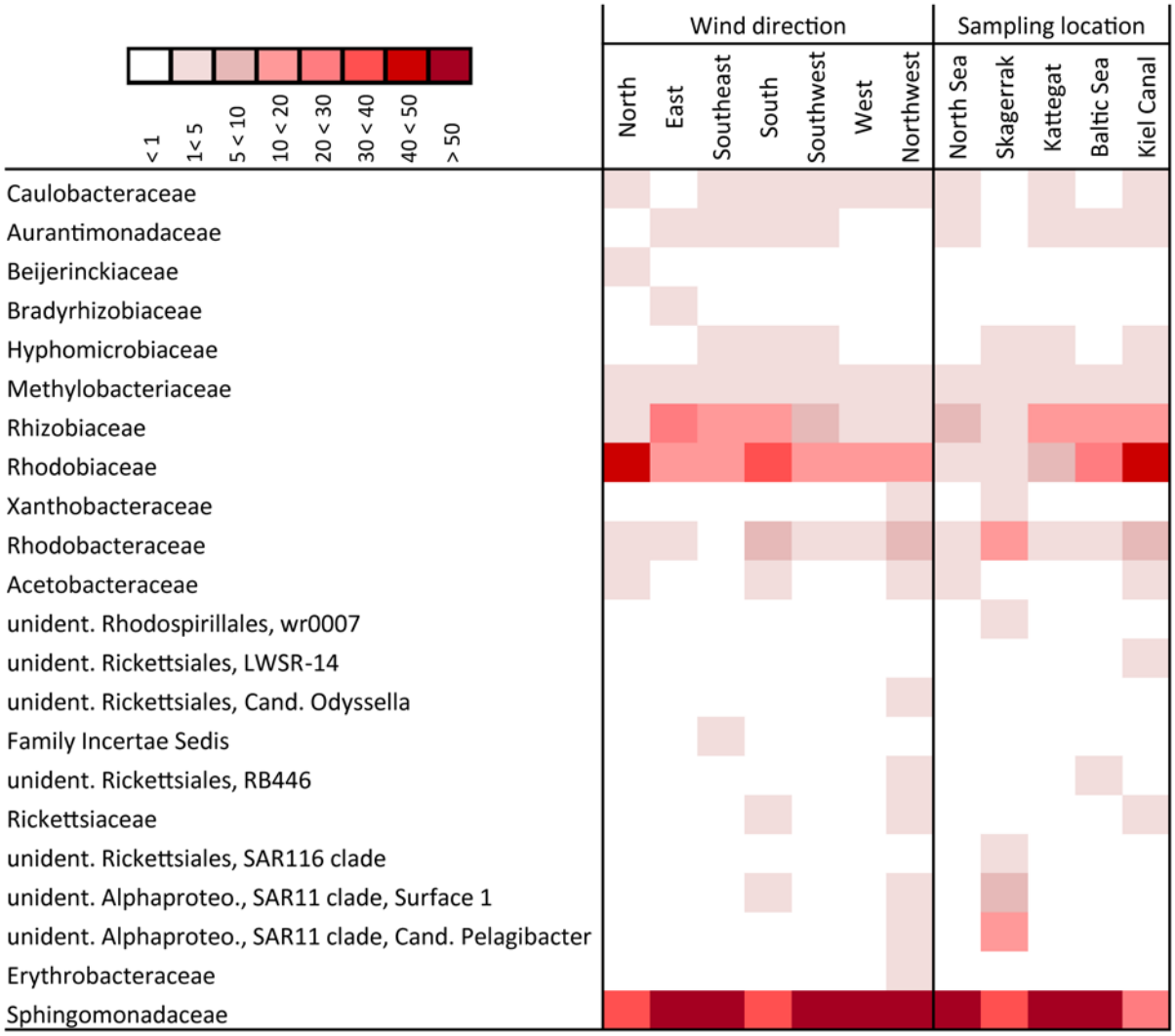

Figure 6 Taxonomic classification of Alphaproteobacteria family reads grouped in wind direction and sampling location on class level using SILVA classifier based on 98% similarity omitting singletons (n = 1) and rare reads (< 1%). The amount of percentage proportion contribution of each family per group is indicated by colour of cell; darker colour represents higher contribution.

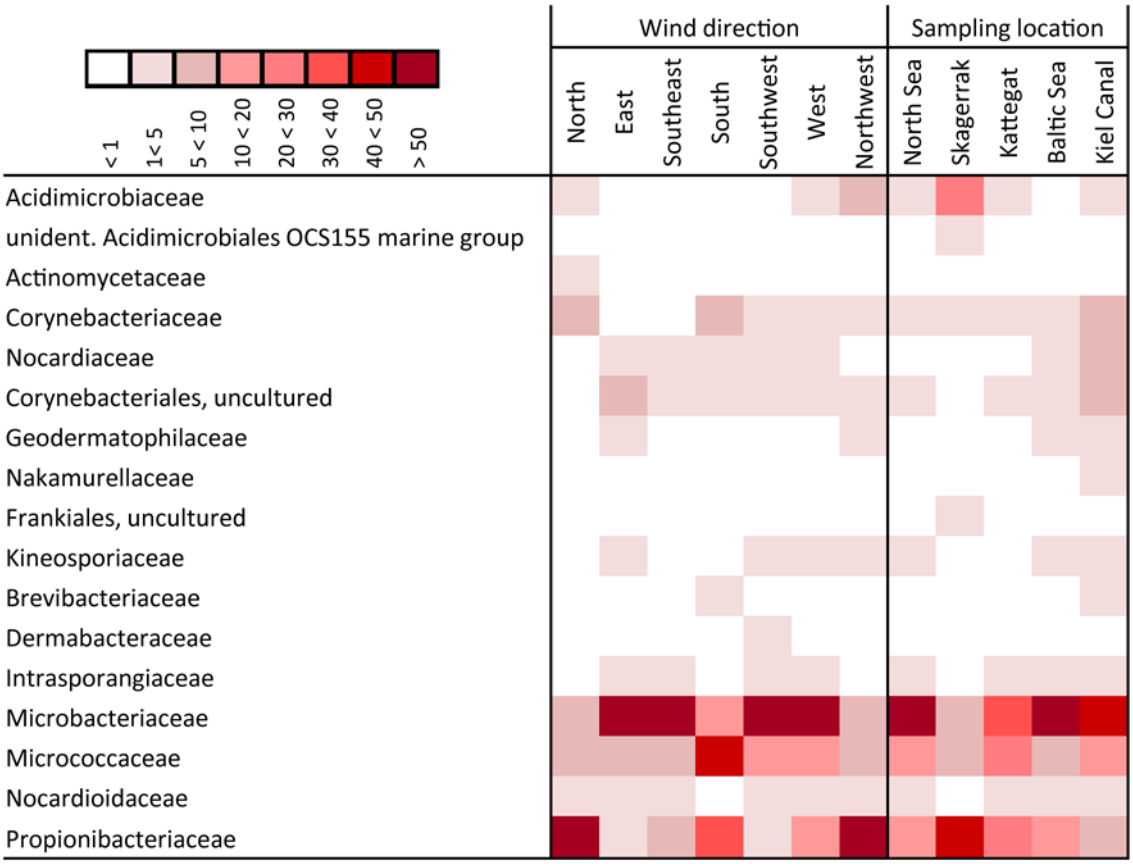

Figure 7 Taxonomic classification of Actinobacteria reads grouped in wind direction and sampling location on family level using SILVA classifier based on 98% similarity omitting singletons (n = 1) and rare reads (< 1%). The amount of percentage proportion contribution of each family per group is indicated by colour of cell; darker colour represents higher contribution.

Table 5 Number of samples, sequences and OTUs belonging to the different wind direction groups.

Group	Samples	Sequences	OTUs
North	1	756	96
East	3	16292	388
Southeast	3	13182	388
South	4	20364	444
Southwest	6	27955	573
West	8	21486	447
Northwest	6	17189	401

Table 6 Number of samples, sequences and OTUs belonging to the different wind direction groups.

Group	Samples	Sequences	OTUs
North Sea	6	25140	533
Skagerrak	2	4945	221
Kattegat	8	31786	511
Baltic Sea	12	46079	578
Kiel Canal	3	9274	452

Discussion

Bacterial diversity

The pyrosequencing reads contained a high amount of mitochondrial and chloroplast sequences. Chloroplast sequences are a common element of bioaerosol samples, particularly in the summer months, and have been reported from numerous studies (e.g. Maron et al. 2005; Brodie et al. 2007; Fahlgren et al. 2010; Fahlgren et al. 2011; Franzetti et al. 2011; Jeon et al. 2011; Zweifel et al. 2012; Bertolini et al. 2013), regardless of the applied molecular technique. The occurrence of mitochondrial sequences may be explained by aerolised microalgae and plant debris which most likely also caused the high amount of chloroplast sequences. There is a distinct lack of information about mitochondrial sequences in comparable studies, which could be due to different sampling techniques. Sampling methods in other investigations ranged from direct vacuum filtration on filters (Bowers et al. 2009; Fahlgren et al. 2010; Bowers et al. 2011a), low volume gravimetric samplers (Franzetti et al. 2011) and commercial vacuum cleaners (Zweifel et al. 2012), to high volume air samplers (Jeon et al. 2011), and wetted-wall cyclone samplers (Maron et al. 2005). Another explanation could be that mitochondrial sequences were simply disregarded in other studies.

The pyrosequencing revealed a high bacterial diversity in marine bioaerosols, which could not be accessed in total (deduction from the rarefaction curves). So even with our high throughput approach to gain as many reads as possible with 3,781 sequences on average per sample we could not survey the full extent of airborne bacterial community diversity with our data set. Overall, four important phyla were detected: Proteobacteria, Bacteroidetes, Firmicutes and Actinobacteria. The gram negative bacteria Proteobacteria and Bacteroidetes made up 72.2 % of all bacterial reads whereas the gram positive bacteria Firmicutes and Actinobacteria were less frequent with only 24.6%. This conforms with the findings of other studies, which used molecular approaches (see Després et al. 2012 and references therein).

Furthermore, 37 OTUs made up more than 75% of all sequences, implying the existence of a dominant core community of bacteria with a long tail of rare OTUs. A similar assemblage of bacteria was also reported by Fahlgren et al. (2010). According to Gandolfi et al. (2013), there may be a bacterial background in the atmosphere as several dominant taxa were found in independently conducted studies. Considering the results of Fahlgren et al. (2010) and Zweifel et al. (2012), our current results support this assumption. A similar hypothesis was postulated

by DeLeon-Rodriguez et al. (2013) for higher altitudes in the upper troposphere. The main OTU present in 17% of all bacterial sequences was affiliated with the genus *Sphingomonas*, which was the dominant OTU in all samples. This bacterial genus is known to be ubiquitous in the environment and has subsequently been reported from marine and aquatic environments as well as from terrestrial habitats and plant root systems (Harrison et al. 2005; Fahlgren et al. 2010; Li et al. 2010; Zweifel et al. 2012). Overall bacterial diversity was highly diverse ranging from typical marine bacteria such as *Pseudoalteromonas* and marine sediment bacteria like *Andersienella* to plant-related taxa (*Rhizobium, Massilia*), soil-related bacteria such as *Hymenobacter*, *Curtobacterium* and *Arthrobacter* and human-associated genera such as *Propionibacterium* and *Prevotella.* Furthermore, potential pathogenic genera including the Bacillus genera *Staphylococcus* and *Streptococcus* as well as potential ice nucleation bacteria such as *Pseudomonas* and *Pantoea* were found. Human-associated bacteria were also detected in other studies: Fahlgren et al. (2010) and Zweifel et al. (2012) found *Propionibacterium* in bioaerosols on coastal areas of Sweden and the boundary layer above the coastal areas of Sweden and Denmark. This would be a further indication for a wide atmospheric mixture as found by Li et al. (2010). However, members of the family Prevotellaceae were detected in aerosol samples at a high elevation site in Colorado, USA (Bowers et al. 2012). A broad occurrence of human associated bacteria may be due to the influence of waste water plants as point sources for aerolised bacteria to the atmosphere (Pascual et al. 2003; Karra & Katsivela 2007; Haas et al. 2010). Also potential ice condensation nuclei genera such as *Pantoea*, *Pseudomonas, Pedobacter* and *Psychrobacter* have been found widely (Fahlgren et al. 2010; Bowers et al. 2012). The detected soil associated genera were also reported from other studies which dealt with urban aerosols (Fierer et al. 2008). The genus *Anderseniella* consists of a single species, *Anderseniella baltica*. Although it was isolated from sediments of the Baltic Sea (Brettar et al. 2007), it was not found in the studies of Fahlgren et al. (2010) and Zweifel et al. (2012). This is probably due to their cloning approach which resulted in fewer sequences compared to the high-throughput approach of the current study. The occurrence of marine sediment associated bacteria may also underline the importance of beaches and/or coastal erosion processes for the formation of bioaerosols. Similar findings were made by Urbano et al. (2011) for the coastal area of San Diego (California, U.S.A.).

Many bacteria which occurred in high numbers in the present study were dominant in other bioaerosol studies which investigated marine/coastal and terrestrial ecosystems. This is a

further indication for a steady 'bacterial core community' in the atmosphere, probably originating from strong area sources and constantly getting distributed and mixed over long distances as stated by Gandolfi et al. (2013).

Influence of environmental factors

The bacterial community was strongly affected by temperature, wind direction and the sample location, whereas temperature and wind direction were negatively correlated. Only few studies identified environmental parameters which influence the structure of airborne microbial communities (Maron et al. 2006; Brodie et al. 2007; Bowers et al. 2012; Bertolini et al. 2013; Gandolfi et al. 2013). All these studies, however, conducted long-term sampling approaches at single (Maron et al. 2005; Maron et al. 2006; Brodie et al. 2007; Bowers et al. 2012; Bertolini et al. 2013) or two sampling sites (Brodie et al. 2007). This clearly contrasts with the sampling design of the current investigation where the sampling events were conducted at different locations. All studies found temperature to be one main factor influencing the bacterial community composition. Temporal variability may be influenced by combined effects of differences in several meteorological factors, chemical composition, particulate matter and the importance of the main source of PBAP rather than only temperature (Brodie et al. 2007; Bertolini et al. 2013). This is supported by our findings also for spatial variability. Temperature might be a function of wind direction, as samples were taken at different sites each featuring different temperatures. North winds were colder than southern winds which exhibit continental influence. The OTU sequencing results were therefore grouped into the wind directions, allowing for classification along a clearly defined system. The calculated backward trajectories revealed that several samples featured a stronger marine influence than the measured wind direction might anticipate. Although wind direction seems to be a good indicator it has to be regarded with caution as the days before sampling also need to be taken into account. Bacterial composition of marine bioaerosols seems to be influenced by several different environmental factors combined.

Additionally, differences in bacterial community composition among the sampling locations were affected by the sampling locality itself and their adjacent ecosystems. Similar studies for terrestrial environments revealed a strong influence of the adjacent land-use type (agricultural, rural, forest) on the airborne bacterial community and that the different sampling locations for

microbial communities were significantly distinct from each other (Bowers et al. 2011a). This also seems to apply to marine/coastal environments. For example, the detected Cyanobacteria showed a distinct distributional pattern in our samples grouped by sampling locations. While the few sequences in the samples from the Skagerrak were mostly accounted to *Prochlorococcus*, sequences in the Baltic Sea mostly belonged to *Synechococcus*. The latter genus is a common Cyanobacterium present in the low salinity environment of the Baltic Sea (Partensky et al. 1999), particularly thriving in shallow water depths with increasing light intensities in the summer months (Jochem 1988). Therefore, these findings corroborate the general assumption that the bubble bursting process of marine bioaerosol formation mostly transports bacteria on and near the sea surface whereas bacteria from deeper parts of the water column have a much lower chance to get aerolised. Similar conclusions were drawn by Cho & Hwang (2011) who compared airborne bacteria with bacteria from the sea surface at the same sampling location. Aller et al. (2005) also identified the sea surface microlayer as a source for marine bioaerosols.

The bacterial distribution was affected by different environmental factors (wind direction, sampling location) and this effect could already be seen on the phylum level. For example, Firmicutes bacteria were detected at all sampling locations but were nearly absent in eastern and southeastern winds. On class level, Gammaproteobacteria were present in every wind direction and location but showed a higher proportion of the sequences in southern wind directions where it was the dominant class. In the class Alphaproteobacteria the plant/root associated Rhizobiaceae (genus Rhizobium) were only present in 'E', 'SE', 'S' and 'SW' sampling direction but were found at all sampling locations except 'SK', where only northern and northwest winds were detected.

In contrast, Betaproteobacteria were evenly distributed among the different wind directions but were disproportionally more abundant in samples from 'SK'. A similar picture is present for the phylum Actinobacteria with the classes Actinobacteria and Acidimicrobia, where in 'SK' samples Acidimicrobia represented 31% (uncultured Acidimicrobiaceae) of all Actinobacteria sequences while it was below the 1% threshold at other sampling locations.

In summary, we found that a small number of bacterial OTUs made up more than 75% of all bacterial sequences. The most abundant OTU belonging to the genus *Sphingomona*s has also been reported in other bioaerosol studies from many different environments (urban, rural, forest, high alpine). This further supports the assumption of a bacterial core community in the

atmosphere, released from strong area aerolising sources and then getting mixed and dispersed over long distances. The most abundant bacterial genera are associated with different places of origin: marine (*Pseudoalteromonas*), marine sediment (*Anderseniella*), plant/root (*Rhizobium*), human (*Propionibacterium*), potential pathogens (*Staphylococcus*), soil (*Hymenobacter*). This highly diverse airborne bacterial community suggests the existence of several heterogeneous sources for bioaerosols of/near marine and coastal environments. The two most important environmental parameters were temperature as a function of wind direction and the sampling location. Some bacteria were found at all locations but were absent in some wind directions and *vice versa*. The wind direction can be a good indicator for possible influences (marine, continental) but additionally backward trajectories are needed for detailed information.

DNA yield after DNA extraction was comparably low. The combination of low DNA yield and high diversity contributed to rarefaction curves that did not reach a plateau. Thus, the chosen sampling duration limits the breadth of the conclusions that can be drawn, as not the whole bacterial community may have been accessed. Further clarification of sampling durations and sampling method evaluation for marine bioaerosols is needed to strengthen future investigations in that field.

Acknowledgements

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Supplementary material

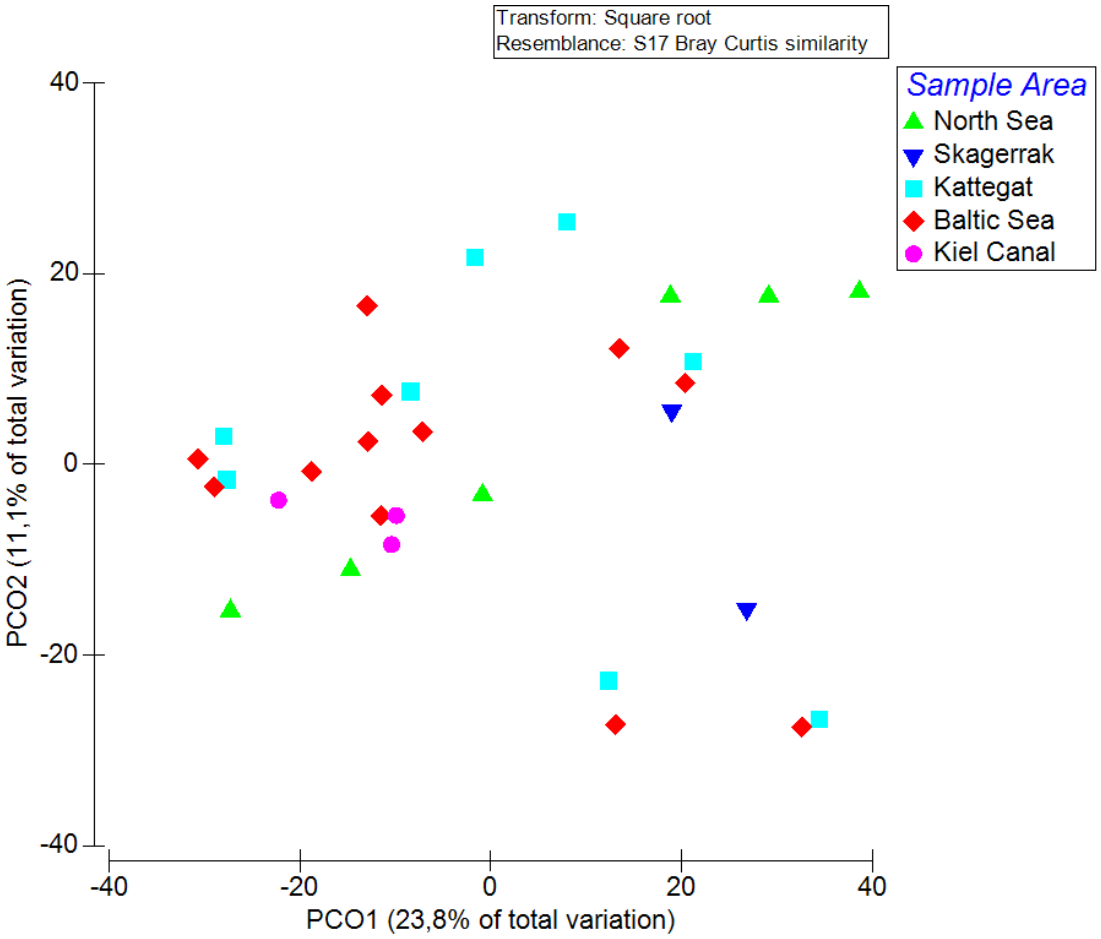

Figure S1 Principal coordinates analysis (PCO) of the pair-wise distances between bacterial communities between the five different sampling locations as calculated using Bray-Curtis dissimilarities.

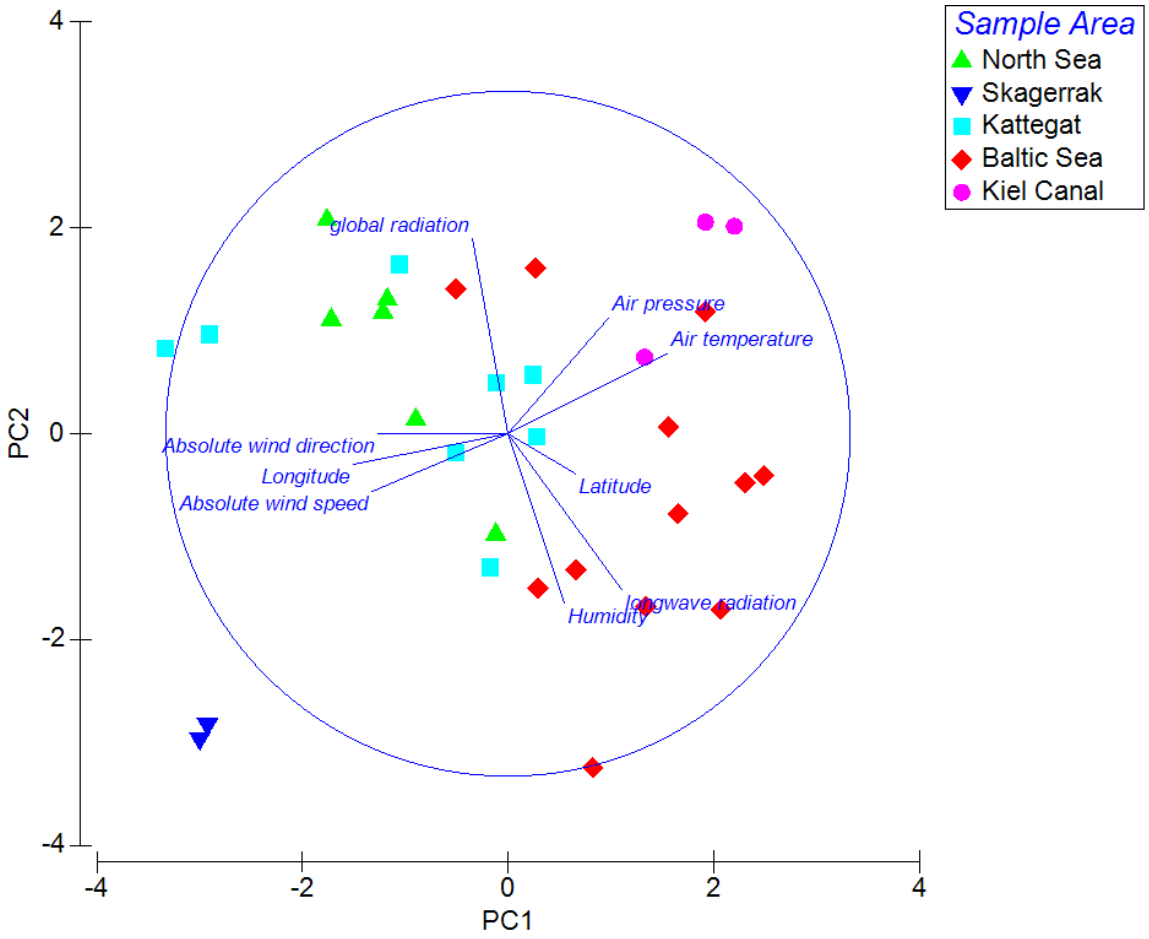

Figure S2 Principal components analysis (PCA) of the pair-wise distances of environmental variables measured for the samples calculated using the Euclidean distances.

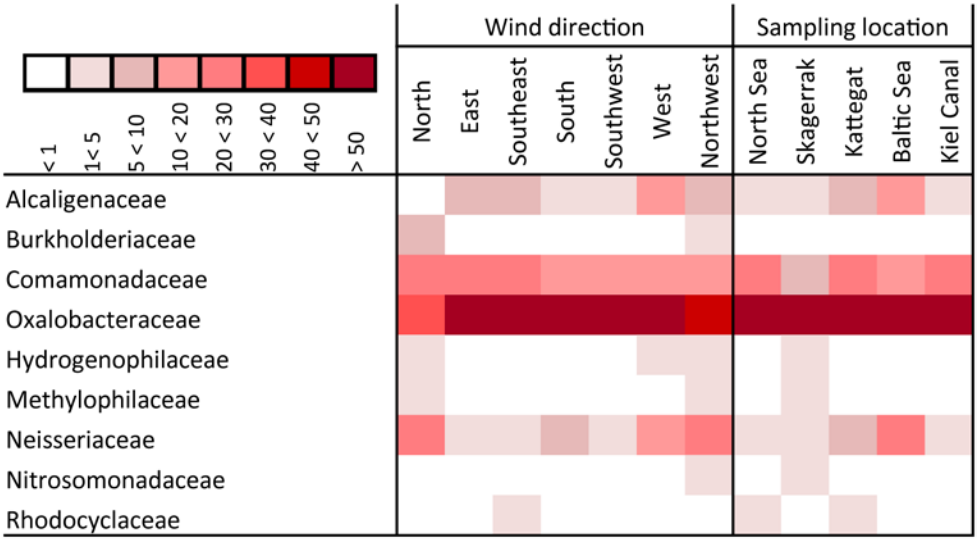

Figure S3 Taxonomic classification of Betaproteobacteria reads grouped in wind direction and sampling location on family level using SILVA classifier based on 98% similarity omitting singletons (n = 1) and rare reads (< 1%). The amount of percentage proportion contribution of each family per group is indicated by colour of cell; darker colour represents higher contribution.

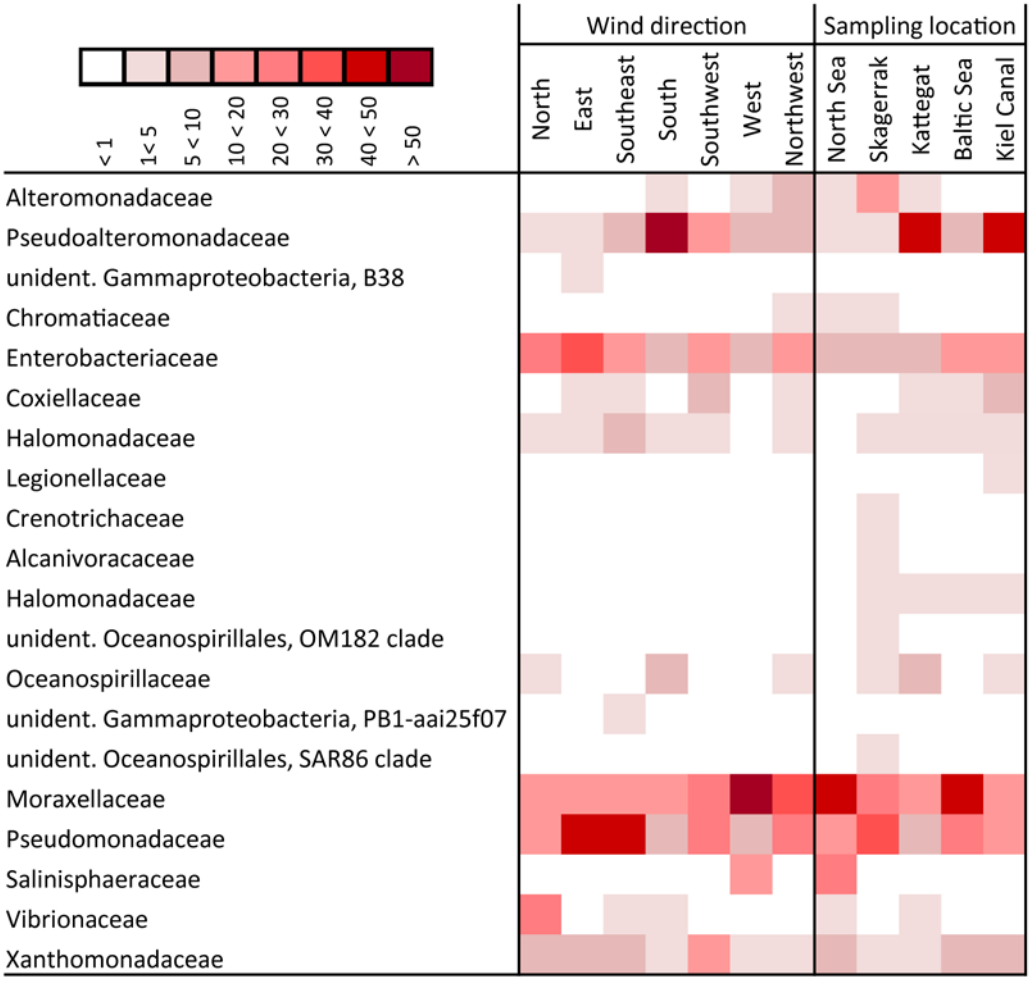

Figure S4 Taxonomic classification of Gammaproteobacteria reads grouped in wind direction and sampling direction on family level using SILVA classifier based on 98% similarity omitting singletons (n = 1) and rare reads (< 1%). The amount of percentage proportion contribution of each family per group is indicated by colour of cell; darker colour represents higher contribution.

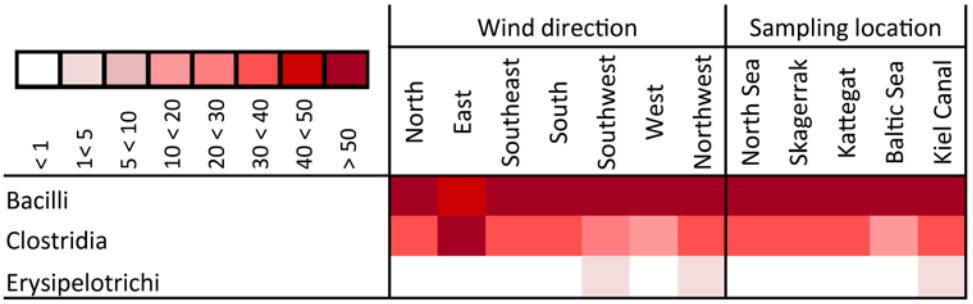

Figure S5 Taxonomic classification of Firmicutes reads grouped in wind direction and sampling location on class level using SILVA classifier based on 98% similarity omitting singletons (n = 1) and rare reads (< 1%). The amount of percentage proportion contribution of each class per group is indicated by colour of cell; darker colour represents higher contribution.

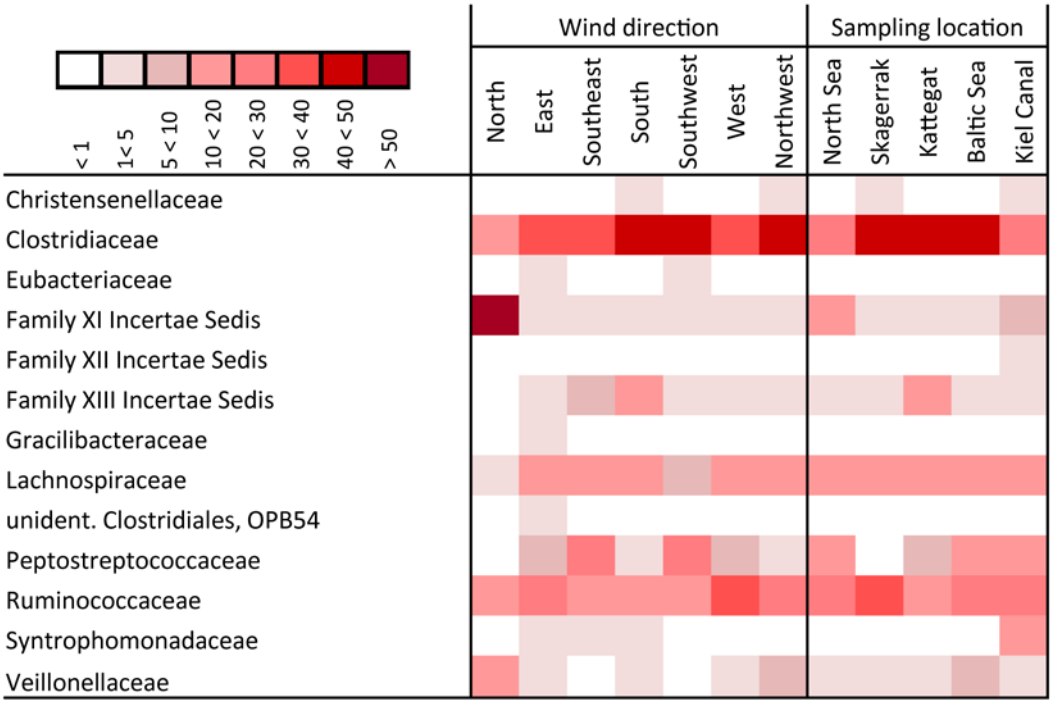

Figure S6 Taxonomic classification of Clostridia reads grouped in wind direction and sampling location on family level using SILVA classifier based on 98% similarity omitting singletons (n = 1) and rare reads (< 1%). The amount of percentage proportion contribution of each family per group is indicated by colour of cell; darker colour represents higher contribution.

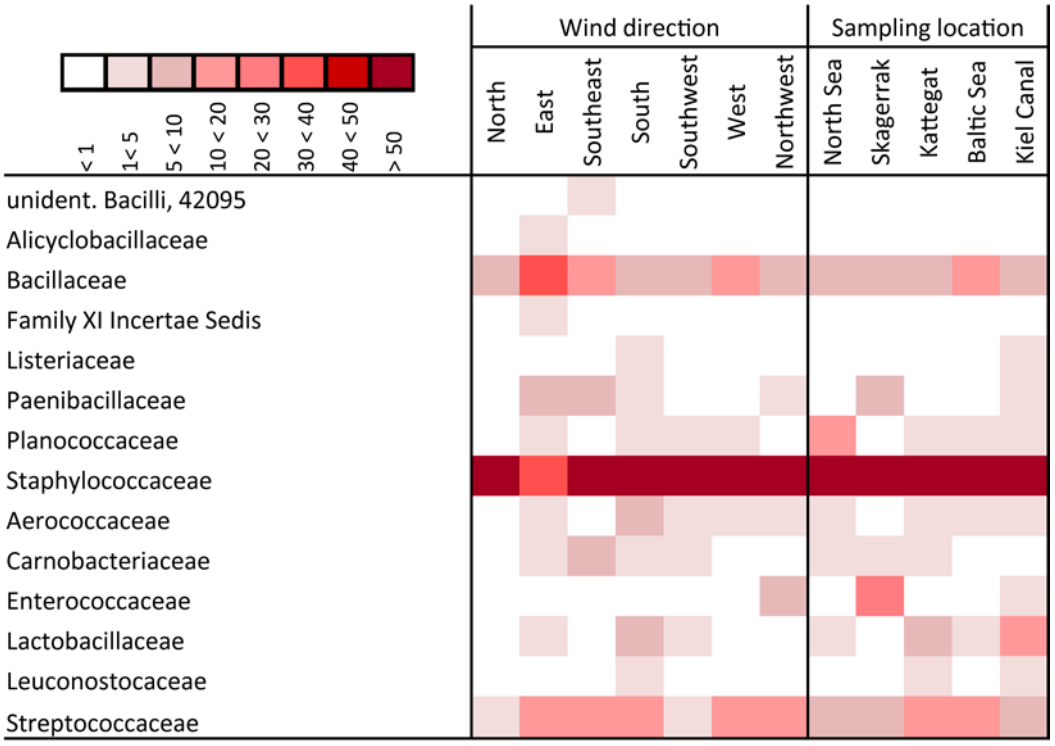

Figure S7 Taxonomic classification of Bacilli reads grouped in wind direction and sampling location on family level using SILVA classifier based on 98% similarity omitting singletons (n = 1) and rare reads (< 1%). The amount of percentage proportion contribution of each family per group is indicated by colour of cell; darker colour represents higher contribution.

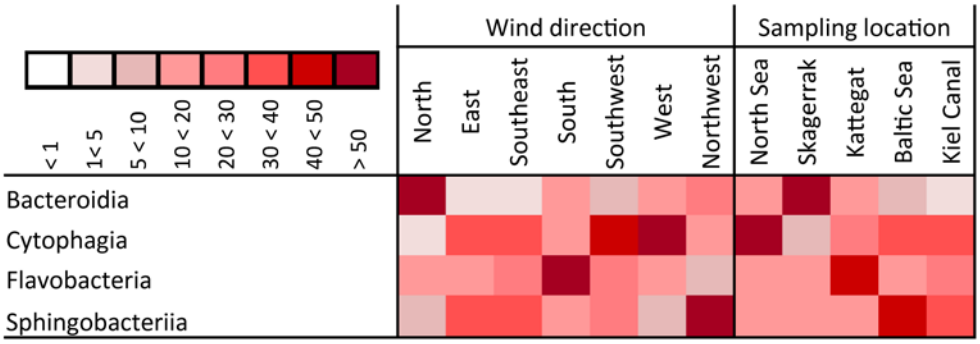

Figure S8 Taxonomic classification of Bacteroidetes reads grouped in wind direction and sampling location on class level using SILVA classifier based on 98% similarity omitting singletons (n = 1) and rare reads (< 1%). The amount of percentage proportion contribution of each class per group is indicated by colour of cell; darker colour represents higher contribution.

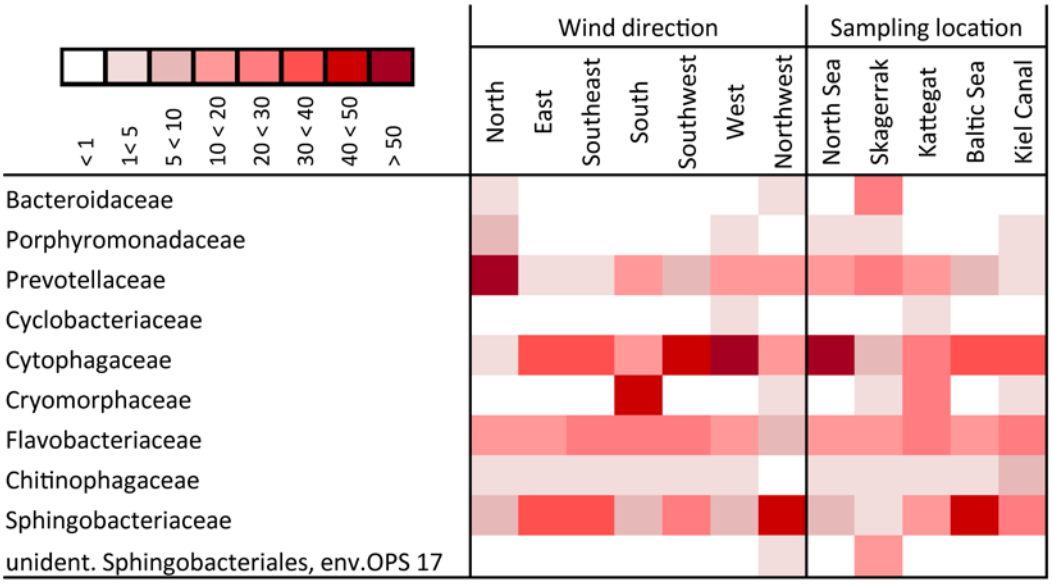

Figure S9 Taxonomic classification of Bacteroidetes reads grouped in wind direction and sampling location on family level using SILVA classifier based on 98% similarity omitting singletons (n = 1) and rare reads (< 1%). The amount of percentage proportion contribution of each family per group is indicated by colour of cell; darker colour represents higher contribution.

Table S1 Sample classifications for the different alpha and beta diversity testing groups.

Sample	Sample Area	BWT Influence	BWT	Cardinal direction	Wind direction	Height	Rain
H01	North Sea	mixed	crossing, no high altitude	W	SW	low	n
H02	North Sea	mixed	no crossing, no high altitude	W	W	low	n
H03	North Sea	mixed	no crossing, no high altitude	W	W	low	n
H04	North Sea	mixed	no crossing, no high altitude	W	NW	low	n
H06	North Sea	marine	no crossing, no high altitude	N	NW	1000	n
H08	North Sea	more marine	no crossing, no high altitude	N	N	low	n
H11	Skagerrak	marine	crossing, high altitude	W	NW	1500	0
H12	Skagerrak	marine	crossing, no high altitude	N	NW	500	0
H13	Kattegat	more marine	no crossing, no high altitude	W	W	500	n
H14	Kattegat	marine	no crossing, no high altitude	W	W	low	n
H15	Kattegat	more marine	no crossing, no high altitude	W	W	low	n
H16	Kattegat	more marine	no crossing, no high altitude	W	SW	low	n
H17	Kattegat	mixed	no crossing, no high altitude	S	S	1000	60
H18	Kattegat	mixed	no crossing, no high altitude	S	S	1000	60
H19	Kattegat	mixed	no crossing, high altitude	S	SE	2000	60
H20	Kattegat	mixed	no crossing, no high altitude	S	SE	500	n
H21	Baltic Sea	continental	no crossing, no high altitude	W	W	low	6
H22	Baltic Sea	more continental	no crossing, no high altitude	W	NW	low	6
H23	Baltic Sea	more continental	no crossing, no high altitude	W	NW	low	6
H24	Baltic Sea	more continental	no crossing, no high altitude	W	W	low	6
H25	Baltic Sea	mixed	no crossing, no high altitude	E	SE	low	n
H26	Baltic Sea	mixed	no crossing, no high altitude	E	E	low	n
H28	Baltic Sea	mixed	no crossing, no high altitude	E	E	low	n
H29	Baltic Sea	continental	no crossing, no high altitude	W	SW	low	60
H30	Baltic Sea	continental	no crossing, no high altitude	W	SW	low	60
H31	Baltic Sea	continental	no crossing, no high altitude	W	W	low	n
H32	Baltic Sea	continental	no crossing, no high altitude	E	E	low	6
H33	Baltic Sea	more continental	no crossing, no high altitude	W	SW	low	6
H34	Kiel Canal	more continental	crossing, no high altitude	W	SW	500	60
H35	Kiel Canal	more continental	crossing, no high altitude	S	S	low	n
H36	Kiel Canal	more continental	crossing, no high altitude	S	S	low	60

Table S2 Sampling Date, area, time, exact position and measured environmental parameters.

Sample	Date	Geography	UTC	Long	Lat	global radiation W/m²	longwave radiation W/m²	Absolute wind direction deg	Absolute wind speed m/s	Air pressure hpa	Air temperature °C	Humidity %
H01	August 17, 2011	North Sea	06:45-07:45	54.588667	08.250000	223.9	344.4	228.7	7.8	1014.2	16.9	89.7
H02	August 17, 2011	North Sea	10:40-11:45	54.579833	8.055667	697.1	311.5	274.4	8.5	1014.8	16.6	82.4
H03	August 17, 2011	North Sea	11:50-12:50	54.699	8.003167	746.1	299.6	267.4	8.4	1014.9	16.1	72.7
H04	August 18, 2011	North Sea	06:30-07:35	55.307167	7.787833	270.5	327.9	293.6	5.1	1016.6	15.7	82.9
H05	August 18, 2011	North Sea	07:40-08:45	55.461	7.562833	376.1	329.3	294.2	5.2	1016.8	15.8	82.9
H06	August 18, 2011	North Sea	11:00-12:05	55.7845	7.670333	647.4	319.7	328.2	3.8	1017.1	15.9	82.3
H07	August 18, 2011	North Sea	12:10-13:10	55.891167	7.68	644.3	304.2	335.9	3.8	1016.7	15.9	79.9
H08	August 18, 2011	North Sea	15:55-17:00	56.1275	7.702167	306.3	294.7	350.4	3.2	1015.3	15.9	79.3
H09	August 19, 2011	North Sea	06:55-08:00	56.993167	8.031167	52.3	348.8	58.3	4.5	1007.9	13.3	81.6
H10	August 19, 2011	North Sea	08:05-09:10	57.1445	8.329667	91.6	350.9	315.1	5.2	1007.9	13.2	84.2
H11	August 19, 2011	Skagerrak	12:50-13:55	57.522	9.3825	161.0	355.2	297.1	7.8	1006.3	13.9	84.3
H12	August 19, 2011	Skagerrak	14:00-15:05	57.672	9.639333	144.7	357.3	315.7	10.1	1005.8	14.6	83.3
H13	August 20, 2011	Kattegat	06:55-08:00	57.660833	11.051500	439.6	289.7	284.3	15.2	1012.8	15.8	72.9
H14	August 20, 2011	Kattegat	08:05-09:05	57.4835	11.2465	541.6	295.2	283.9	13.9	1013.9	15.9	74.9
H15	August 20, 2011	Kattegat	12:00-13:00	56.9265	11.438833	636.7	314.7	251.8	5.0	1016.0	17.2	72.5
H16	August 20, 2011	Kattegat	13:05-14:10	56.865333	11.111333	316.7	341.4	227.2	4.2	1015.9	17.9	74.7
H17	August 21, 2011	Kattegat	06:45-07:50	56.7935	11.03	191.7	347.6	185.3	5.8	1017.1	15.7	89.4
H18	August 21, 2011	Kattegat	07:55-08:55	56.655333	11.1595	317.7	321.0	176.5	7.2	1016.7	16.5	87.1
H19	August 21, 2011	Kattegat	11:15-12:20	56.4845	11.536833	568.1	322.3	136.9	5.6	1015.7	17.9	85.9
H20	August 21, 2011	Kattegat	14:10-15:15	56.461667	11.998	228.9	341.6	145.0	9.1	1014.1	19.4	73.9
H21	August 22, 2011	Baltic Sea	06:50-07:55	54.759833	13.067167	91.6	378.9	288.9	8.5	1013.3	17.2	96.9
H22	August 22, 2011	Baltic Sea	08:00-09:05	54.7145	12.975167	299.4	353.4	296.5	8.9	1014.2	17.8	95.0
H23	August 22, 2011	Baltic Sea	11:30-12:35	54.668	12.783333	730.3	303.4	297.4	7.8	1016.9	16.9	81.6
H24	August 22, 2011	Baltic Sea	12:40-13:40	54.644833	12.5565	606.4	311.4	291.9	4.5	1017.7	17.8	79.1
H25	August 23, 2011	Baltic Sea	06:45-07:50	54.321833	11.886333	182.2	344.0	130.0	3.3	1020.7	16.9	90.8
H26	August 23, 2011	Baltic Sea	07:55-09:00	54.2995	11.7695	180.1	359.2	96.3	4.9	1019.9	17.6	83.3
H27	August 23, 2011	Baltic Sea	11:35-12:40	54.234333	11.4525	581.3	338.8	103.9	5.9	1018.5	18.2	81.1
H28	August 23, 2011	Baltic Sea	12:45-13:45	54.1635	11.254	531.1	333.1	86.0	6.1	1017.5	18.8	79.6
H29	August 24, 2011	Baltic Sea	06:40-07:45	54.517667	10.845167	197.8	371.6	241.8	3.8	1013.4	17.9	93.2
H30	August 24, 2011	Baltic Sea	07:50-08:50	54.489833	10.808667	331.3	359.7	226.2	2.3	1013.8	18.3	93.7
H31	August 24, 2011	Baltic Sea	12:15-13:20	54.621167	10.422833	452.0	366.0	250.2	2.8	1013.8	19.8	85.0
H32	August 24, 2011	Baltic Sea	16:25-17:30	54.747667	10.112833	91.4	342.1	91.1	4.4	1013.4	19.0	92.1
H33	August 25, 2011	Baltic Sea	07:50-08:55	54.510667	10.1175	304.9	363.7	261.7	4.1	1015.2	16.1	96.1
H34	August 25, 2011	Kiel Canal	11:45-12:50	54.389	10.1945	483.4	357.4	232.5	2.9	1015.4	18.8	78.9
H35	August 25, 2011	Kiel Canal	12:55-13:55	54.361333	10.061667	582.8	340.3	196.9	1.3	1015.2	20.4	72.4
H36	August 25, 2011	Kiel Canal	14:00-15:00	54.362833	9.859	392.8	339.8	177.9	1.2	1014.8	21.4	69.2

Table S3 PERMANOVA pair wise test for the factor 'sampling area' based on Bray-Curtis dissimilarities of OTUs (16S amplicon sequencing).

Groups	t (perm)	p (perm)[1]
North Sea, Skagerrak	1.0615	0.197
North Sea, Kattegat	0.96675	0.462
North Sea, Baltic Sea	1.3509	**0.049**
North Sea, Kiel Canal	1.2275	0.121
Skagerrak, Kattegat	1.1613	0.175
Skagerrak, Baltic Sea	1.4988	**0.0437***
Skagerrak, Kiel Canal	1.8003	0.096
Kattegat, Baltic Sea	0.99075	0.404
Kattegat, Kiel Canal	1.0648	0.288
Baltic Sea, Kiel Canal	1.0376	0.267

[1]Significant results (*p (perm)* < 0.05) are highlighted in bold
Displayed are pair-wise *a posteriori* comparisons of the factor 'sampling area' with at least 100 unique permutations per comparison. Below 100 unique permutations the Monte Carlo permutation was used (*).

Table S4 PERMANOVA pair-wise test for the factor 'cardinal direction' group based on Bray-Curtis dissimilarities of OTUs (16S amplicon sequencing).

Groups	t (perm)	p (perm)[1]
W, N	1.915	0.106
W, S	0.99067	0.441
W, E	1.3059	**0.043**
N, S	1.2778	0.111
N, E	1.7473	**0.0411***
S, E	1.1389	0.223

[1]Significant results (*p (perm)* < 0.05) are highlighted in bold
Displayed are pair-wise *a posteriori* comparisons of the factor 'cardinal direction' with at least 100 unique permutations per comparison. Below 100 unique permutations the Monte Carlo permutation was used (*).

Table S5 PERMANOVA pair-wise test for 'wind direction' group based on Bray-Curtis dissimilarities of OTUs (16S amplicon sequencing).

Groups	t (perm)	p (perm)[1]
SW, W	1.5497	**0.01**
SW, NW	1.6776	**0.002**
SW, N	1.7433	0.149
SW, S	1.2842	0.057
SW, SE	1.0953	0.245
SW, E	1.0935	0.21
W, NW	1.0597	0.246
W, N	1.0127	0.568
W, S	1.1625	0.133
W, SE	1.2366	0.064
W, E	1.4072	**0.031**
NW, N	0.86427	1
NW, S	1.1162	0.14
NW, SE	1.3704	0.1096*
NW, E	1.4835	0.0666*
N, S	1.1725	0.181
N, SE	1.7674	0.234
N, E	1.9946	0.23
S, SE	1.2403	0.146
S, E	1.3476	0.118
SE, E	0.87567	0.731

[1]Significant results ($p\ (perm) < 0.05$) are highlighted in bold

Displayed are pair-wise *a posteriori* comparisons of the factor 'wind direction' with at least 100 unique permutations per comparison. Below 100 unique permutations the Monte Carlo permutation was used (*).

Table S6 PERMANOVA pair-wise test for 'rain' group based on Bray-Curtis dissimilarities of OTUs (16S amplicon sequencing).

Groups	T(perm)	P(perm)
n, 0	1.3498	0.051
n, 60	1.1376	0.164
n, 6	1.2004	0.113
0, 60	1.4160	0.061
0, 6	1.1806	0.144
60, 6	0.97765	0.421

Displayed are pair-wise *a posteriori* comparisons of the factor 'rain' with at least 100 unique permutations per comparison.

Table S7 Principal components analysis (PCA) calculated eigenvalues. Displayed are the eigenvalues for the first five axes including the percentage of variation explained by each axis and the accumulation of them.

PC	Eigenvalues	%Variation	Cum.%Variation
1	2.89	32.1	32.1
2	2.30	25.6	57.7
3	1.28	14.2	71.9
4	0.925	10.3	82.2
5	0.708	7.9	90

Table S8 Result of the 16S gene amplicon sequencing. Sequences obtained per sample, percentage of chloroplast and mitochondrial sequences and sequences and percentage of sequences which entered further analyses.

Sample	Date	Number of Sequences before trimming	Sequences after trimming	Mitochondrial sequences (%)	Chloroplast sequences (%)	Sequences left after trimming (%)
H01	August 17, 2011	12507	11537	0.4	7.1	92.2
H02	August 17, 2011	8518	7259	2.9	11.7	85.2
H03	August 17, 2011	1642	1435	0.1	12.4	87.4
H04	August 18, 2011	1111	483	0.5	55.9	43.5
H06	August 18, 2011	3968	3670	0.5	6.8	92.5
H08	August 18, 2011	6744	756	0.3	88.5	11.2
H11	August 19, 2011	2682	2116	1.4	19.7	78.9
H12	August 19, 2011	8139	2829	0.1	65.1	34.8
H13	August 20, 2011	6895	1765	0.9	73.5	25.6
H14	August 20, 2011	5270	1557	1.6	68.9	29.5
H15	August 20, 2011	5516	1533	26.1	46.1	27.8
H16	August 20, 2011	10250	5144	23.5	26.2	50.2
H17	August 21, 2011	8750	8537	0.1	2.2	97.6
H18	August 21, 2011	5041	4900	0.1	2.7	97.2
H19	August 21, 2011	19233	2627	69.2	17.2	13.7
H20	August 21, 2011	7451	5723	0.6	22.5	76.8
H21	August 22, 2011	3934	3812	0.2	2.9	96.9
H22	August 22, 2011	7414	6852	0.4	7.1	92.4
H23	August 22, 2011	22865	1239	58.0	36.6	5.4
H24	August 22, 2011	13687	2299	37.7	45.5	16.8
H25	August 23, 2011	11582	4832	0.1	58.2	41.7
H26	August 23, 2011	10864	2568	8.0	68.3	23.6
H28	August 23, 2011	15153	6953	32.9	21.2	45.9
H29	August 24, 2011	7663	2840	4.3	58.6	37.1
H30	August 24, 2011	11404	3570	0.2	68.5	31.3
H31	August 24, 2011	6405	1826	17.9	53.6	28.5
H32	August 24, 2011	12239	6771	0.9	43.7	55.3
H33	August 25, 2011	5424	2517	32.1	21.4	46.4
H34	August 25, 2011	15228	2347	8.6	76.0	15.4
H35	August 25, 2011	23327	3295	9.5	76.4	14.1
H36	August 25, 2011	12075	3632	5.4	64.5	30.1

Table S9 List of the 37 most abundant OTUs with classification, total number of sequences and sequences partitioned by wind direction and sampling location.

OTU	Phylum	Class	Family	Genus	Sequences	Wind direction							Sampling location				
						N	E	SE	S	SW	W	NW	NS	SK	KT	BS	KC
37	Actinobacteria	Actinobacteria	Corynebacteriales, uncult.	Corynebacteriales, uncult.	580	0	188	49	61	198	59	25	137	5	137	235	66
82	Actinobacteria	Actinobacteria	Microbacteriaceae	unident. Microbacteriaceae	2809	1	295	263	94	1316	782	58	1217	18	827	642	105
90	Actinobacteria	Actinobacteria	Microbacteriaceae	Curtobacterium	2820	5	478	567	218	1093	424	34	698	8	586	1328	199
99	Actinobacteria	Actinobacteria	Microbacteriaceae	Microbacterium	572	1	96	92	49	233	71	30	162	5	173	194	38
113	Actinobacteria	Actinobacteria	Micrococcaceae	Arthrobacter	2608	5	105	69	1664	463	236	65	461	17	1678	259	192
132	Actinobacteria	Actinobacteria	Propionibacteriaceae	Friedmanniella	753	1	19	7	9	36	11	670	681	1	19	43	9
136	Actinobacteria	Actinobacteria	Propionibacteriaceae	Propionibacterium	2819	87	43	81	1242	140	495	731	262	272	1362	859	64
170	Bacteroidetes	Bacteroidia	Prevotellaceae	Prevotella	2791	58	241	61	734	311	560	826	733	260	853	893	52
180	Bacteroidetes	Cytophagia	Cytophagaceae	Dyadobacter	1145	0	299	301	137	248	100	60	60	0	308	626	151
183	Bacteroidetes	Cytophagia	Cytophagaceae	Hymenobacter	5486	4	1342	433	265	1721	1278	442	2471	72	703	1857	382
190	Bacteroidetes	Cytophagia	Cytophagaceae	Spirosoma	1085	0	361	130	94	298	85	117	146	1	127	682	129
192	Bacteroidetes	Cytophagia	Cytophagaceae	Cytophagaceae, uncult.	747	0	43	65	20	55	554	10	16	1	559	149	22
198	Bacteroidetes	Flavobacteria	Cryomorphaceae	Brumimicrobium	2072	0	9	3	2005	27	12	16	6	6	1968	26	66
206	Bacteroidetes	Flavobacteria	Flavobacteriaceae	Chryseobacterium	2085	7	533	475	173	570	111	215	410	24	488	965	197
210	Bacteroidetes	Flavobacteria	Flavobacteriaceae	Flavobacterium	568	0	169	100	52	90	123	34	132	2	139	235	60
222	Bacteroidetes	Flavobacteria	Flavobacteriaceae	Sufflavibacter	643	1	181	32	43	255	114	17	260	10	100	246	27
225	Bacteroidetes	Flavobacteria	Flavobacteriaceae	Winogradskyella	639	0	3	1	615	11	7	2	3	1	581	10	44
244	Bacteroidetes	Sphingobacteriia	Sphingobacteriaceae	Pedobacter	6452	5	1661	921	441	995	252	2177	371	20	1016	4578	467
278	Cyanobacteria	Cyanobacteria	FamilyI	Synechococcus	1347	5	12	23	22	39	266	980	26	39	88	1167	27
322	Firmicutes	Bacilli	Staphylococcaceae	Staphylococcus	4367	113	20	98	795	1050	1294	997	397	313	932	2573	152
344	Firmicutes	Bacilli	Streptococcaceae	Streptococcus	963	3	8	19	276	30	385	242	55	38	279	574	17
346	Firmicutes	Clostridia	Clostridiaceae	Clostridium	772	7	31	32	213	150	112	227	81	121	239	290	41
454	Proteobacteria	Alphaproteobacteria	Methylobacteriaceae	Methylobacterium	922	5	187	175	58	322	69	106	186	15	198	452	71
467	Proteobacteria	Alphaproteobacteria	Rhizobiaceae	Rhizobium	3937	4	1337	1075	329	881	244	67	584	7	1088	1932	326
469	Proteobacteria	Alphaproteobacteria	Rhodobiaceae	Anderseniella	4535	102	593	938	851	1130	677	243	160	24	595	2616	1139
545	Proteobacteria	Alphaproteobacteria	Sphingomonadaceae	Sphingomonas	20194	68	3192	3520	689	6571	5036	1116	7318	155	5527	6410	782
579	Proteobacteria	Betaproteobacteria	Comamonadaceae	Variovorax	547	1	117	52	55	204	58	60	157	11	80	235	64
586	Proteobacteria	Betaproteobacteria	Oxalobacteraceae	Massilia	3319	4	571	348	144	1034	358	860	911	787	357	1114	150
587	Proteobacteria	Betaproteobacteria	Oxalobacteraceae	Oxalobacter	929	16	58	30	315	366	98	46	30	2	165	348	384
598	Proteobacteria	Betaproteobacteria	Neisseriaceae	Neisseriaceae, uncult.	823	13	22	32	49	43	101	563	27	31	61	687	17
651	Proteobacteria	Gammaproteobacteria	Pseudoalteromonadaceae	Pseudoalteromonas	3280	2	33	45	2631	288	212	69	47	16	2352	307	558
667	Proteobacteria	Gammaproteobacteria	Enterobacteriaceae	Pantoea	556	0	220	63	79	144	38	12	74	2	52	350	78
690	Proteobacteria	Gammaproteobacteria	Oceanospirillaceae	Marinomonas	544	1	5	3	475	13	33	14	17	2	473	37	15
700	Proteobacteria	Gammaproteobacteria	Moraxellaceae	Acinetobacter	633	10	84	18	78	251	76	116	125	19	66	383	40
704	Proteobacteria	Gammaproteobacteria	Moraxellaceae	Psychrobacter	3277	0	28	41	739	91	2216	162	726	20	688	1716	127
707	Proteobacteria	Gammaproteobacteria	Pseudomonadaceae	Pseudomonas	1827	9	414	294	228	351	238	293	268	170	313	901	175
709	Proteobacteria	Gammaproteobacteria	Salinisphaeraceae	Salinisphaera	546	0	4	4	6	17	510	5	519	3	7	15	2

Chapter II

Temporal variability of airborne bacterial communities at a marine sampling site

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Abstract

The temporal variability of airborne bacterial communities in a marine environment was studied in an annual sampling campaign from August 2012 to June 2013 at the remote island of Helgoland (German Bight, North Sea) on a daily basis. Bacterial community structure was analysed with automated ribosomal intergenic spacer analysis (ARISA) and environmental data were recorded. The sampling month had a clear effect on both species richness and community structure, whereas the latter was also affected by the wind direction. Variability of the environmental data followed a clear hierarchical distribution (intra-week < intra-month < intra-season < sampling period). In contrast, the variability in the bacterial community structure proved to be at equally high levels over the whole sampling period (intra-week = intra-month = intra-season = sampling period). Furthermore, the results indicated that rather a combination of several different environmental parameters and not distinct single factors may be responsible for the observed patterns.

Introduction

Bacteria are ubiquitous in the atmosphere (Jaenicke et al. 2007) and represent an abundant component of the atmospheric aerosols, belonging to the primary biological aerosol particles (PBAP). PBAPs are defined as solid airborne particles derived from biological organisms such as microorganisms and fragments of biological materials (e.g. plant debris, animal dander), which can be released from all kind of surfaces and environments (IGAP 1992). The presence and importance of aerolised biological particles has been recognised and repeatedly been addressed in several investigations since the middle of the 19th century (e.g. Ehrenberg 1847; Pasteur 1860a; Carnelly et al. 1887; De Bary 1887). Investigations on airborne bacteria as PBAPs are important for several reasons: ecology, (i.e. dispersal of microorganisms), health (i.e. presence of pathogens; Husman 1996; Hirano & Upper 2000) and climate (i.e. nuclei for ice crystal and water droplets; Möhler et al. 2007). However, recent investigations on environmental aerobiology were only sporadic in nature, mainly demonstrating the presence of bacteria in the atmosphere in exemplary qualitative approaches (e.g. Meier 1935; Timmons et al. 1966; Imshenetsky et al. 1978). Most aerosol studies have been conducted in indoor environments, mostly for public health concerns such as hospitals and animal barns in order to detect potentially harmful microorganisms (Tang 2009; Eduard et al. 2012). Unfortunately, investigations in outdoor environments are comparably scarce (Gandolfi et al. 2013). The few outdoor investigations also mainly focused on the detection of potentially harmful microorganisms (Brodie et al. 2007; Polymenakou 2012), and assessed microbial dispersion from bio-hazardous industrial or agricultural plants (wastewater, composting, breeding farms; Dungan & Leytem 2009; Millner 2009; Korzeniewska 2011).

Although technological advances of recent years allow for studies with both quantitative and long-term sampling approaches, the field of aerobiology is still characterised by a large gap of knowledge (Burrows et al. 2009; Després et al. 2012; Gandolfi et al. 2013). Most studies still employed culture-dependent techniques in spite of their limitations and biases (preselection of growth media, incubation temperature and time) - only a small fraction of bacteria is known to be culturable

which is particularly pronounced in aerolised bacteria (Heidelberg et al. 1997; Colwell 2000). In recent years, the deployment of molecular techniques for investigations on aerolised bacteria allowed for comprehensive culture-independent studies on the bacterial community structure and their spatial and temporal variability. However, the main driving factors for shaping those community structures are still largely unknown.

The current study aimed to characterise the bacterial community structure of marine PBAP and its variability on a daily basis over the course of one year. Furthermore, our analyses tested for the most important environmental parameters which potentially define bacterial community structures of marine bioaerosols in the German Bight (North Sea). For this purpose, samples were consecutively collected in a period of ten months and analysed using Automated Ribosomal Intergenic Spacer Analyses (ARISA). This technique was chosen as it allows for rapid examination of structure of bacterial communities and its demonstrated ability to detect changes in complex bacterial communities (Ranjard et al. 2001; Maron et al. 2005; Maron et al. 2006; Banning et al. 2011; Abed et al. 2012).

Material and methods

Study site

All samples were continuously collected at Helgoland, on an exposed position near to a small tower of the German Bundeswehr 40 m above sea level (a.s.l.; Figure 1; 54°10'49.93 N and 7°53'2.83 E). The small island of Helgoland (only 1.7 km²) is situated in the centre of the German Bight, approximately 60 km off the German coastline (Figure 1). Therefore, its remote and exposed location is ideal for the study of marine bioaerosols. Samples were consecutively collected each working day for a time period of two hours in the morning from August 2012 to June 2013. However, sampling was only conducted under convenient weather conditions i.e. wind speeds lower than 15 m/s and/or absence of heavy rain- or snowfall, in order to prevent a damaging of the sampling gear.

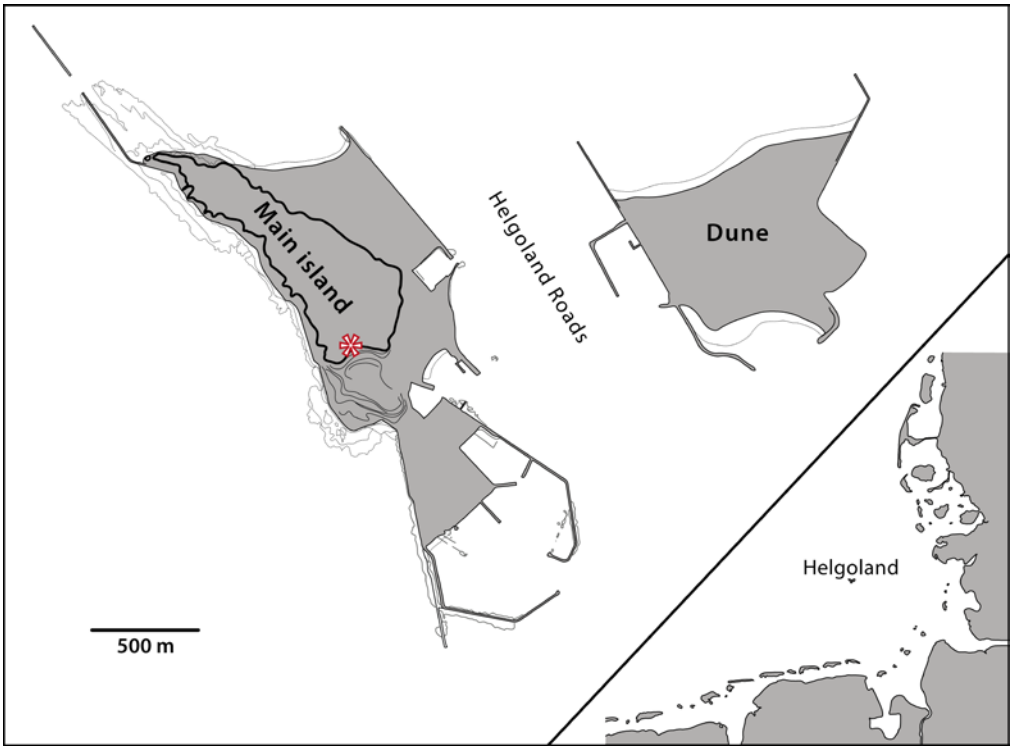

Figure 1 Map of Helgoland and its location in the German Bight (bottom right corner). Asterisk indicates the position of the sampling site Bundeswehr tower (54.1805 N, 7.8841 E), 40 m a. s. l. (map modified after Beermann 2014).

Sampling device

An impingement aerosol sampler XMX/2L MIL (Dycor, Edmonton, Canada) was employed for the collection of aerosols samples from August 2012 to June 2013. This device collects high volumes of air, stripes away large dust particles as well as very small micro debris and subsequently concentrates aerosols within a diameter of 1 to 10 µm. The air is absorbed with an intake flow rate of 690 l/min at the top of the air sampler and then passes three concentration stages operated by gravitational force. The secondary flow rate of 12 l/min describes the flow rate of air entering the buffer. The particles are then impinged into a sample collection vial (50 ml falcon tube) which contains 5 ml phosphate buffered saline (PBS) as sample liquid, washing out the bacteria and thereby preventing them from damage. Controlled by an integrated timer, each sample consisted of 12 runs à 10 minutes which were pooled thereafter. Samples from the first and the second hour of the sampling were each pooled separately on a 0.2 µm Isopore™ Membrane Filters (GTTP-type, diameter 13 mm; Millipore, Eschborn, Germany) and stored away at -20°C for later processing. In total, the volume of 1.44 m³ air was collected each day of the sampling period.

Meteorological conditions

Meteorological data (air temperature, relative humidity, atmospheric pressure, cloud coverage, sunshine duration, wind speed, perception depth and wind direction) were obtained from the German Meteorological Service's (DWD) monitoring station located in the direct vicinity of the sampling site (location: 54°10'36" N; 07°53'33" E, 4 m a.s.l.). Except for the wind directions, which were determined on an hourly basis, all parameters were averaged for the daily mean.

DNA extraction

Following the procedure of Sapp et al. (2006), a chemical cell lysis with subsequent phenol-chloroform-extraction and additional freeze-thaw-step (Maron et al. 2005) was performed. Concentration and pureness of the extracted DNA was determined photometrically, using the microplate reader Tecan Infinite© 200 from

NanoQuantphotometry (Tecan, Männedorf, Switzerland). DNA was stored at -20°C for later processing.

ARISA

ARISA was carried out following the protocol of Krause et al. (2012). The PCR forward primer L-D-Bact-132-a-A-18 (5'CCGGGTTTCCCCATTCGG-3') and the reverse primer S-D-Bact-1522-b-S-20 (5'-TGCGGCTGGATCCCCTCCTT-3') (Ranjard et al. 2000) were used. S-D-Bact-1522-b-S-20 was labeled with the infrared dye 682. The PCR reactions were carried out with a final volume of 25 µl. Each reaction contained 5 ng template DNA, 2.5 µl Taq Buffer (10x), 5 µl TaqMaster PCR Enhancer (5x), 0.7 µl of each primer (20 µM), 0.75 µl dNTPs (2.5 mM each), and 1.4 U Taq DNA polymerase (5Prime, Hamburg, Germany). Cycling conditions were set as follows: 95°C for 3 min, followed by 30 cycles of 95°C for 1 min, 50°C for 1 min and 68°C for 1 min, and a final step at 68°C for 5 min. The PCR products were verified with 1% agarose gels. Depending on band intensities, original or diluted PCR products were mixed with an equal volume of the stop mix. Samples and marker were heated to 95°C for 2 min to denature the DNA string and subsequently kept on ice for 10 min to ensure the single strand setting and 0.8 µl were separated in 5.5% polyacrylamide gels. Running conditions were 1500 V for 14 h on a LI-COR 4300 DNA Analyzer. A 15 min pre-run at 45°C was performed to precondition the gel prior to loading the samples. As size reference, a 50-1500 bp standard (all materials: LI-COR Biosciences, Lincoln, NE, USA) was used. ARISA-gels were analysed with the Bionumerics 7.1 software (Applied Maths, Sint-Martens-Latem, Belgium). Bands with intensities lower than 3% of the maximum value of the respective lane as well as bands smaller than 100 bp were neglected. Binning to band classes was performed conforming with Brown et al. (2005).

Air mass backward trajectories

In order to determine the origin of a given air parcels, backward trajectories were created for all sampled air masses using the HYSPLIT (HYbrid Single-Particle Lagrangian Integrated Trajectory) model (Draxler & Rolph 2013). Five-day backward trajectories were calculated for air parcels arriving at 10 m, 500 m (i.e. the upper part within the boundary layer) and 1500 m a.s.l. for each sampling period in order to detect possible mixing events between different air layers. The midway of each transect was chosen as starting point and time for the corresponding trajectory.

Statistical analyses

All statistical analyses were performed with the computer software packages PRIMER 6 (PERMANOVA+ ad-on; PRIMER-E Ltd.) and GraphPad Prism 5.04 (GraphPad Software, Inc.).

Species richness for each sample (day) was expressed by the number of bands on the ARISA gel profile, each gel band representing a single species (S). Similarity and diversity among the samples was calculated on presence/absence data of the ARISA-gel profiles with the Jaccard coefficient and visualised with Principle Coordinate analysis (PCO). Environmental parameter measurements (cloud coverage; absolute wind direction; absolute wind speed; atmospheric pressure; perception; air temperature; humidity) were log(x+1)-transformed and normalised in order to reduce the influence of extreme values, followed by a Principle Component analysis (PCA). The beta diversity for environmental variables between the samples was calculated with Euclidean distances.

Species richness

Due to the different amount of samples within each factor level, the data for species richness per sample did not fulfill the requirements for ANOVAs (Kolmogorov-Smirnov test, Bartlett test). Therefore, non-parametric Kruskal-Wallis tests were performed followed by Dunn's post hoc procedure.

Species richness and calculated Jaccard coefficients for the presence/absence data of the ARISA-gel profiles were tested for the following factors: 'backward trajectory

influence' (BWT Influence; factor levels: marine, more marine (75 %), mixed, more continental (75%), continental, 'cardinal direction' (factor levels: 'N', 'E', 'S', 'W'), 'wind direction' (factor levels: 'N', 'NE', 'E', 'SE', 'S', 'SW', 'W', 'NW'), 'season' (factor level: summer, autumn, winter, spring), 'month' (factor level: August, September, October, November, December, January, February, March. April, May, June) and 'height' (factor levels: low; 500 m a.s.l.; 1000 m a.s.l; 1500 m a.s.l; 2000 m a.s.l, 2500 m a.s.l, 3000 m a.s.l, 3500 m a.s.l, 4000 m a.s.l) (Table S1; for abbreviations see Table 1).

Whenever factors were positively tested to have a significant influence/effect on species richness, the environmental variables were separately tested for the respective factor. For this purpose, one-way ANOVAs (followed by Tukey's post hoc test) or Kruskal-Wallis tests were performed, in case the data did not meet the necessary requirements.

Table 1 Abbreviations

Group	Abbreviation
North	N
Northeast	NE
East	E
Southeast	SE
South	S
Southwest	SW
West	W
Northwest	NW

Beta diversity

Calculated Jaccard coefficients for the presence/absence data of the ARISA-gel profiles were analysed with one-factorial permutational ANOVAs (PERMANOVA) (Anderson 2001) with the same factors as for species richness (see above).

When the regarded factors where tested to have a significant influence/effect on overall similarity of communities, respective analyses were followed by a test of homogeneity of dispersions (PERMDISP) based on the sample distance to the group centroid. This allowed for the discrimination of significant beta diversity effects from

artefacts caused by different dispersions among the groups. Additionally, the environmental variables (Euclidean distances) were tested for the found significant factors with PERMANOVAs followed up by PERMDISP analysis.

Relationship between bacterial community structure and environmental variables

A distance-based linear model (DistLM) with distance based redundancy analysis (dbRDA) visualisation was performed on the calculated Jaccard coefficients of the ARISA profiles. This allowed for modeling the relationships of the ARISA profile data and environmental variables for the samples in order to test for possible influences of environmental parameters (predictor variables). Therefore, log(x+1)-transformed environmental variables were used, as a DistLM performs a normalisation of environmental variables.

Variability

The calculated Jaccard coefficients of the ARISA profiles were used for the comparison of variability within sampling weeks, sampling months, sampling seasons and over the total sampling period. The same procedure was applied to the calculated Euclidean distances of environmental variables.

Results

The results are delineated in the following way: (1) 'bacterial community' analysis, (2) 'environmental variables' analysis, (3) relationship between the bacterial community structure and environmental variables, and (4) variability within the environmental variables and the bacterial community. Accordingly, the bacterial community structure, including its species richness, is discussed first, as the analysis of the environmental variables built on the bacterial community analysis.

Bacterial community

Although the factor 'month' significantly affected bacterial species richness ($H_{K\text{-}W}$ = 23.44, p = 0.0092), no differences were found in the pair-wise comparisons of the respective species richness among the sampling months. No further significant effects of other regarded factors were detected (each p > 0.1; Table 2).

The PCO displayed strong aggregation of different samples despite their sampling date (Figure 2). However, the analysis of beta diversity (PERMANOVA) revealed significant differences among the months (p = 0.001), cardinal directions (p = 0.001), wind directions (p = 0.001) and heights (p = 0.048) (Table 3). The significant effect of the cardinal direction was due to the differences of 'S' against 'W' (p = 0.001) and 'N' (p = 0.021) as well as 'W' against 'E' (p = 0.03) and 'N' (p = 0.01). The significant effect of heights was due to the difference between 0 m and 2000 m (p = 0.028).

Pair wise comparisons of the factor 'months' displayed significant differences in beta diversity for November and May, which both clearly differed from the remaining months (each p < 0.05) (Figure 3, Table S2). However, when considering the PERMDISP analysis, this could be maintained only for May vs. January, April, August and October, November vs. August and January vs. February (Figure 2, Table S2).

In detail, pair wise comparisons for the factor 'wind direction' along with the PERMDISP showed 'NW' was distinctly different from all other wind directions (each p < 0.05) except the wind directions 'N' and 'W'. Additionally 'W' vs. 'S' and 'SW' (each p < 0.014) and 'N' vs. 'SE' (p = 0.032) were significantly different (Figure 4, Table S3).

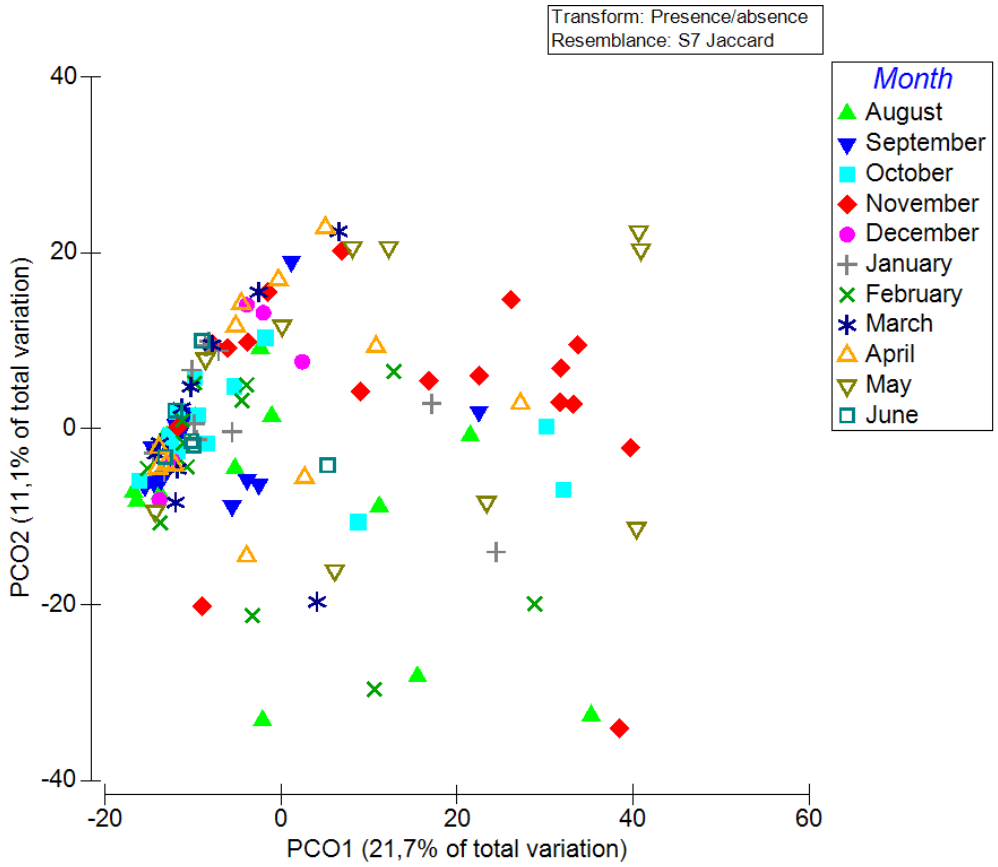

Figure 2 Principal coordinate analysis (PCO) based on the Jaccard dissimilarities of ARISA profiles of the impingement samples. Sampling period reached from August 2012 to June 2013.

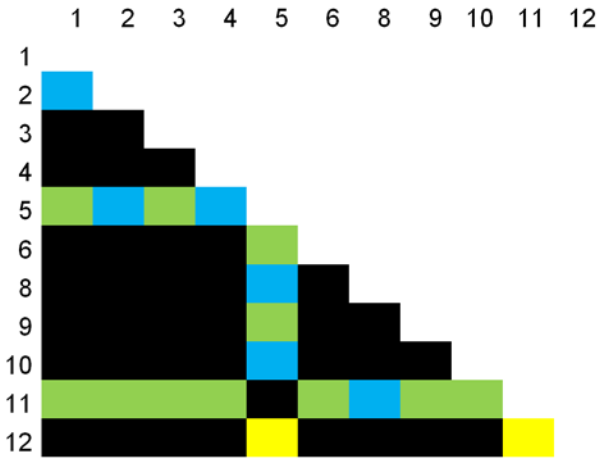

Figure 3 Response variable 'month' analysed with PERMANOVA and PERMDISP. Significant differences in the pairwise comparison of the PERMANOVA are highlighted in blue, significant result for PERMDISP comparison in yellow. If both are significant it is highlighted in green and no significance is represented by black

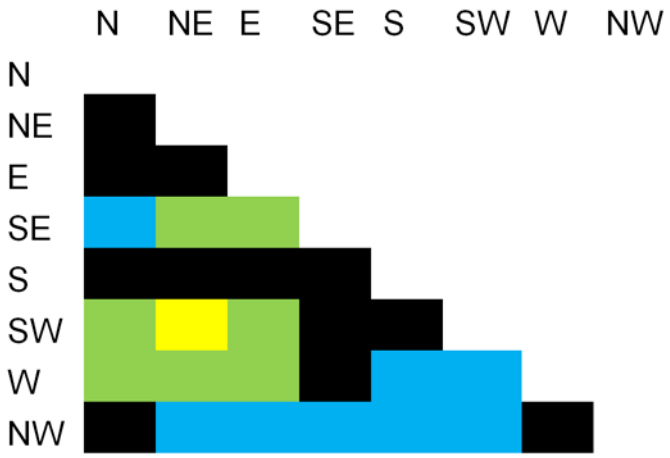

Figure 4 PERMANOVA and PERMDISP comparison of the response variable for factor 'wind direction'. Significant differences in the pairwise comparison of the PERMANOVA are highlighted in blue, significant result for PERMDISP comparison in yellow. If both are significant it is highlighted in green and no significance is represented by black.

Table 2 Species richness tested for different factors

Categories	H_{K-W}	P[1]
BWT Influence	6.604	0.1583
Height	7.226	0.3005
Season	1.828	0.6089
Month	23.44	**0.0092**
Cardinal direction	5.985	0.1123
Wind direction	10.56	0.159

[1]Significant differences ($p < 0.05$) are highlighted in bold

Table 3 PERMANOVA main tests comparing bacterial community compositions based on Jaccard dissimilarities of ARISA profiles of impingement samples.

Source	df	SS	Pseudo-F	p (perm)[1]	Sq.root
BWT Influence	5	8819.5	1.5395	0.057	5.6061
Height	7	11791	1.4777	**0.048**	6.1392
Cardinal direction	3	7479.9	2.1913	**0.001**	6.6605
Wind direction	7	13626	1.7317	**0.001**	7.3312
Season	3	4718	1.3548	0.055	3.6811
Month	10	18125	1.6268	**0.001**	7.9054

[1]Significant results (*p (perm)* < 0.05) are highlighted in bold.

Displayed are tests for the factors 'BWT influence', 'Height', 'Cardinal direction', 'wind direction', 'season' and 'month'. *p* (perm)-values were obtained using type III sums of squares and 999 permutations under the full model. d.f.: degrees of freedom, SS: sums of squares, Sq. root: square root of the component of variation attributable to that factor in the model, in units of Jaccard dissimilarities.

Environmental variables

Sampling was carried out from August 2012 to June 2013, thus, covering all four seasons. Throughout the year, a relatively high humidity was recorded ranging from 64% to 100% (Table S4). The climate was characterised by comparably mild summer and winter temperatures with a maximum of 18.6°C air temperature in August, and a minimum air temperature of -2.4°C in January. Average wind speed during the sampling ranged from 2.9 m/sec to 14.7 m/sec due to the necessity of cancelation at wind speeds exceeding 15 m/sec.

The PCA for the entire sampling period (August 2012 to June 2013) showed a good separation of summer months (August, September, June) and winter months (December, January, February, March), whereas autumn and spring months were

displayed intermediately, reflecting the transition from summer to winter (October, November, April, May; Figure 5).

As the factor 'month' had a significant effect on species richness, the different environmental variables were each tested accordingly. The analyses of environmental variables among the months showed significant differences for all measured parameters except for atmospheric pressure (Table 4 and 5; see Table S5 for post hoc comparisons).

The PERMANOVA analysis of the bacterial community structure showed significant effects of the factors 'month' and 'wind direction'. Therefore, the environmental variables were also analysed for the factor 'month' and 'wind direction'.

The follow-up PERMANOVA of environmental variables for the factor 'month' showed significant differences between nearly all months (Figure 6, Table S6). This finding was largely supported by the PERMDISP although two comparisons (August vs. February and April) could not be clearly assigned to differences in beta diversity (Figure 5).

For the follow up PERMANOVA of environmental variables for the factor 'wind direction' 'NW' was different from all wind directions ($p < 0.023$) except 'E' ($p = 0.205$), 'E' was different from 'SW', 'S', 'W' and 'NW' (each $p < 0.03$) and 'SW' from 'NW' and 'N' (each $p < 0.039$). The PERMDISP for the environmental variable 'wind direction' showed a significant dispersion difference only for 'W' vs. 'S'. Therefore, all differences detected by PERMANOVA could also be accounted to differences in the environmental data (Figure 7, Table S7).

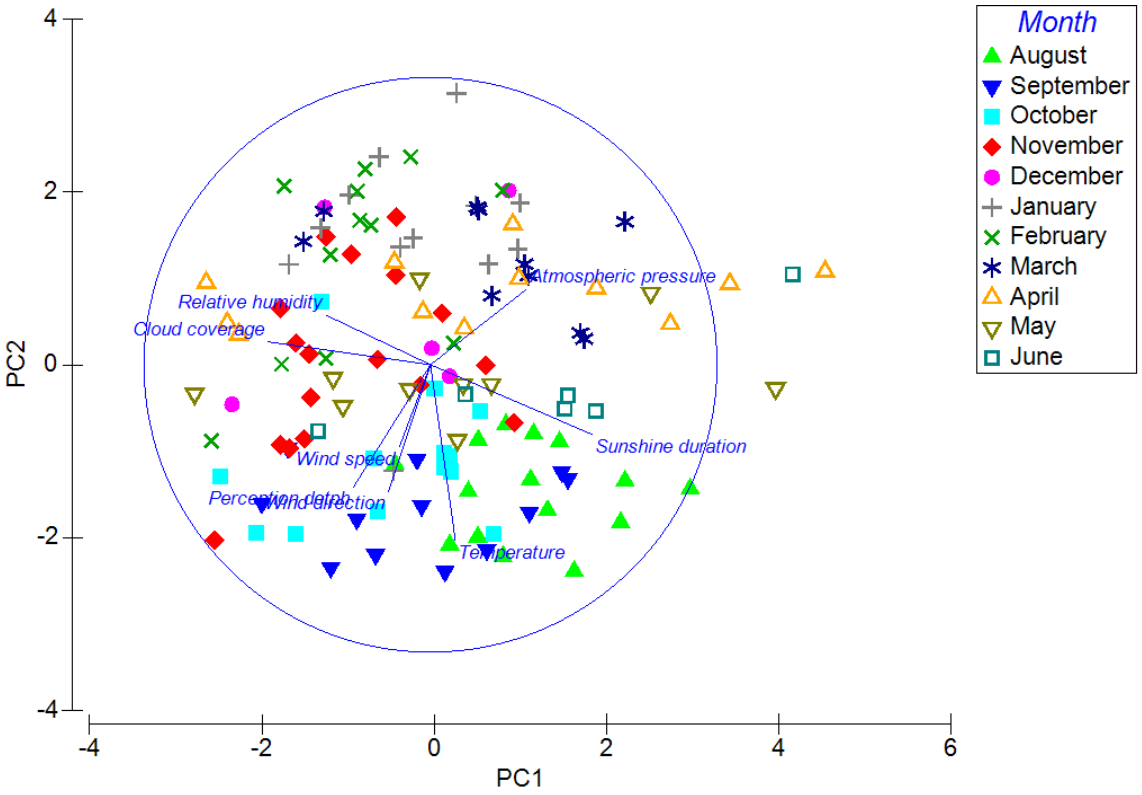

Figure 5 Principal component analysis (PCA) of environmental variables for the sampling period of the impingement sampler from August to June.

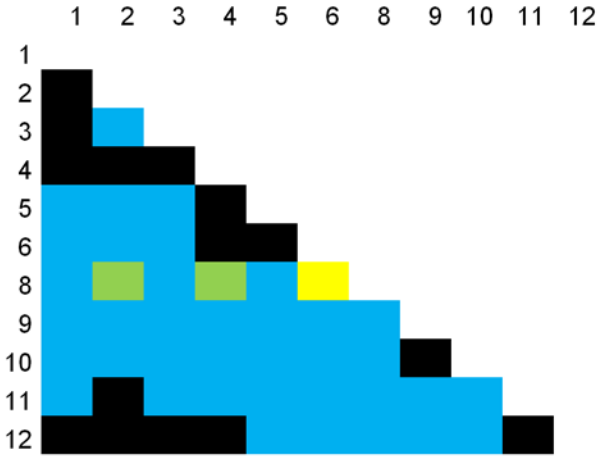

Figure 6 Environmental variable analysed for the factor 'month' with PERMANOVA and PERMDISP. Significant differences in the pairwise comparison of the PERMANOVA are highlighted in blue, significant result for PERMDISP comparison in yellow. If both are significant it is highlighted in green and no significance is represented by black.

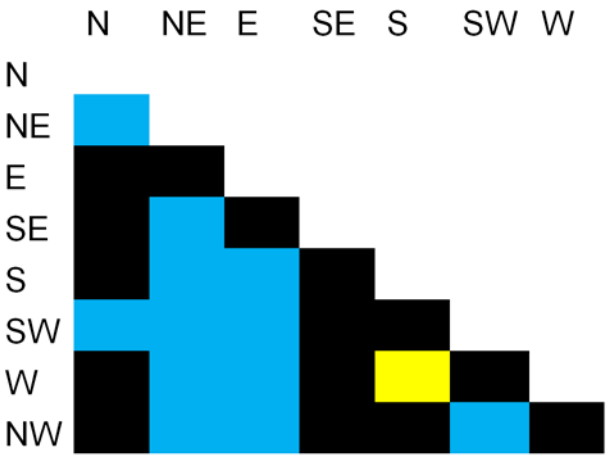

Figure 7 Environmental variable analysed for the factor 'wind direction' with PERMANOVA and PERMDISP. Significant differences in the pair wise comparison of the PERMANOVA are highlighted in blue, significant result for PERMDISP comparison in yellow. If both are significant it is highlighted in green and no significance is represented by black.

Table 4 Environmental parameters tested for factor 'month' with ANOVA

Environmental parameter	F	p[1]
Relative humidity	3.145	**0.0014**
Wind Speed	5.081	**0.0001**
Atmospheric pressure	1.65	0.1015

[1]Significant differences ($p < 0.05$) are highlighted in bold

Table 5 Environmental parameters tested for the factor 'month' with Kruskal-Wallis

Environmental parameter	H_{K-W}	p[1]
Cloud coverage	31.16	**0.0006**
Temperature	111	**0.0001**
Perception	26.41	**0.0032**
Sunshine duration	51.3	**0.0001**
Wind direction	23.26	**0.0098**

[1]Significant differences ($p < 0.05$) are highlighted in bold

Relationship between bacterial community structure and environmental variables

The DistLM analysis revealed significant effects of the factors 'relative humidity' (p = 0.001), 'sunshine duration' (p = 0.035) and 'wind direction' (p = 0.019) (Table 6). The sequential test showed that the influence of humidity (p = 0.004) and 'wind direction' (p = 0.039) contributed significantly to the explained variation of the bacterial community composition (Table 7). The x-axis of the dbRDA ordination explained 59.3% of fitted and 3.7% of total variance whereas the y-axis explained 20.5% of fitted and 1.3% of the total variance (Figure 8).

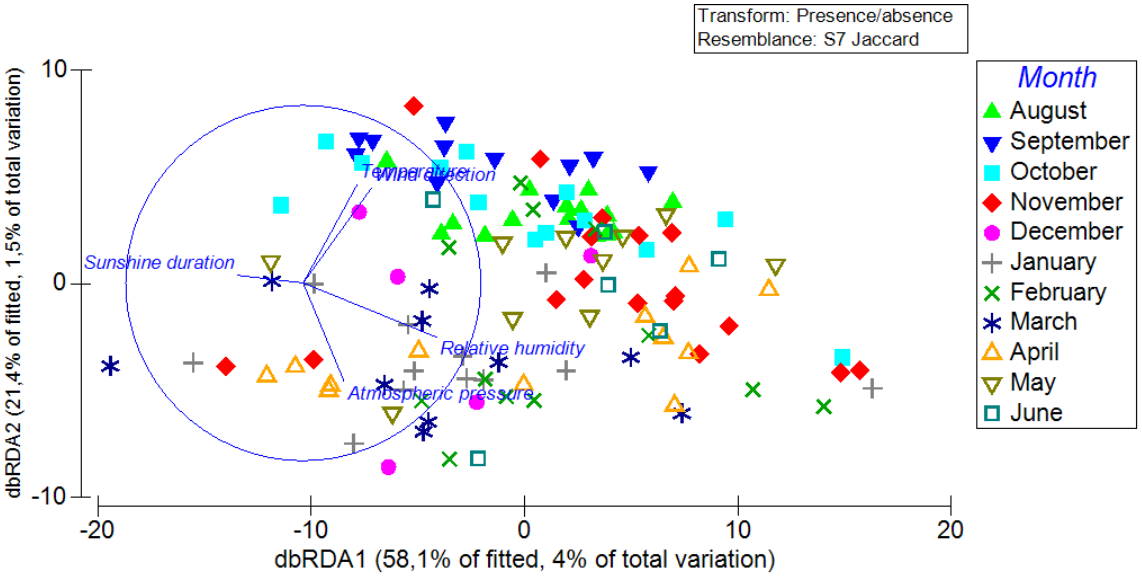

Figure 8 dbRDA ordination relating environmental variables with the bacterial community composition of taken samples based on Jaccard dissimilarities. The '% of fitted' indicates the variability in the original data explained by the fitted model and '% of total variation' indicates the variation in the fitted matrix.

Table 6 Distance-based linear models (DistLM) calculation for the influence of environmental variables on bacterial community composition.

Variable	SS(trace)	Pseudo-F	p[1]
Cloud coverage	1400.9	1.19840	0.256
Relative humidity	4022.4	3.50520	**0.001**
Air temperatur	1714.8	1.47020	0.111
Atmosphericpressure	1163.3	0.99343	0.400
Windspeed	997.34	0.85074	0.605
Precipitationdepth	1167.9	0.99741	0.435
Sunshine duration	2037.4	1.75070	**0.035**
Wind direction	2354.4	2.02750	**0.019**

[1]Significant results (*p (perm)* < 0.05) are highlighted in bold.

Table 7 Sequential test of distance-based linear models (DistLM) calculation. Tests for relationship between the bacterial community composition for different samples with environmental parameters. Amount explained by each variable added to model is conditional on variables already in the model.

Variable	SS(trace)	Pseudo-F	p[1]	Proportion of variance	Cumulation
Relative humidity	4022.4	3.5052	**0.004**	2.79E-02	2.79E-02
Wind direction	2158.4	1.8947	**0.039**	1.50E-02	4.29E-02
Atmospheric pressure	1415.7	1.2452	0.194	9.83E-03	5.27E-02
Sunshine duration	1472.0	1.2980	0.156	1.02E-02	6.30E-02

[1]Significant results (*p (perm)* < 0.05) are highlighted in bold.

Variability

The variability of the bacterial community composition within weeks proved to be as high as among months, seasons and over the whole sampling period (Figure 9), as no differences were detected. The variability of environmental variables showed a higher diversity with increasing intraspecific time period (intra-week < intra-month < intra-season < period) (Figure 10).

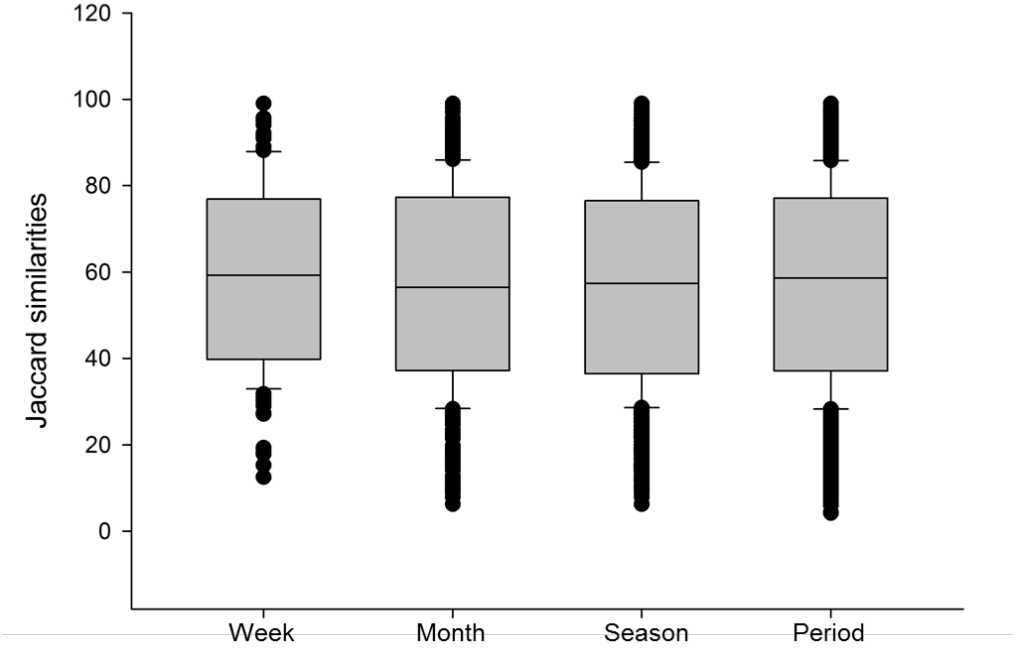

Figure 9 Variability of the response variable according to different time scales. Displayed are the intra group Jaccard similarities.

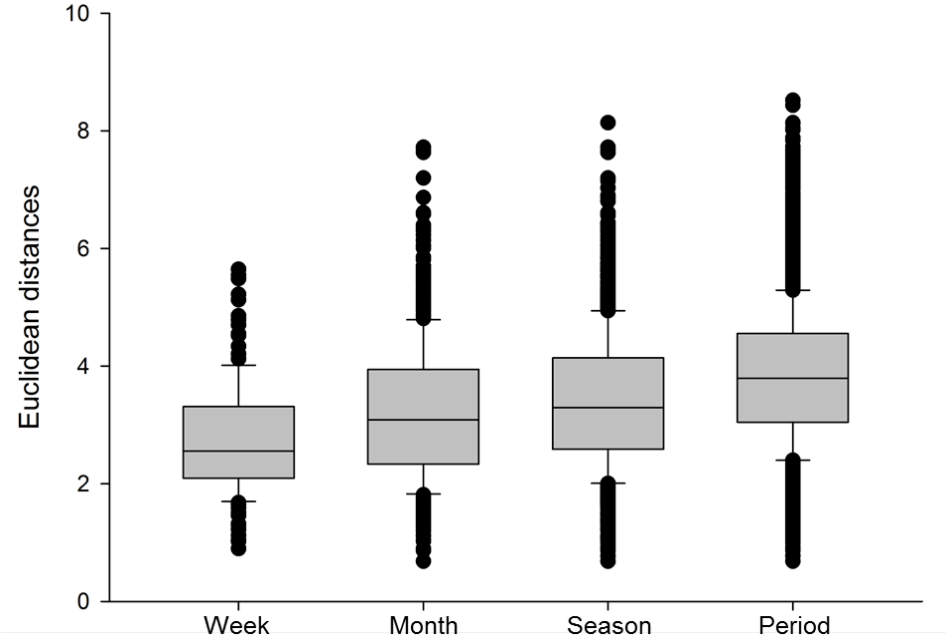

Figure 10 Variability of the environmental variables according to different time scales. Displayed are the intra group Euclidean distances.

Discussion

Temporal variability of airborne bacterial communities and the meteorological factors determining their composition are a highly complex topic which is currently still poorly understood. In the current study, a thorough analysis of a yearlong sampling campaign on airborne bacterial communities was conducted. For this purpose, a marine sampling site in the middle of the German Bight (Helgoland, Germany) was chosen and all gathered data were set in context with meteorological data for the analyses based on ARISA profiles.

The bacterial species richness was affected by the sampling month although no detailed differences among the months were detected. Not surprisingly, the considered environmental parameters were distinctly different between respective sampling months, as the whole sampling period ranged over 10 consecutive months. Therefore, the monthly comparisons of environmental parameters showed a similar pattern as the PCA visualisation.

The PCO displayed strong accumulation of the different samples despite their sampling date which indicates a high resemblance of the bacterial community structure in the collected samples. The beta diversity, however, was clearly influenced by the sampling month and the wind direction.

The relatedness with environmental variables (DistLM and dbRDA) revealed an effect of relative humidity and temperature on the bacterial community structure. Similar findings were made in previous studies where humidity and temperature affected bacterial community structure (Maron et al. 2006). The resolution/the amount of explained variance in our data set was low. Hence, several environmental parameters combined over the course of the seasons are most likely responsible for bacterial community dynamics. This conforms with other studies which found temperature to be a significant factor but also concluded that the findings were rather due to a combination of factors (Brodie et al. 2007; Bertolini et al. 2013) than temperature on its own.

As expected, the variability of environmental variables followed a hierarchical structure (intra-weekly < intra-monthly < intra-seasonal < sampling period). In contrast, the variability of the bacterial community composition was consistently

high for all considered timeframes (intra-week = intra-month = intra-season = sampling period). The current findings of this study are in clear contrast to the study of Maron et al. (2006) who investigated temporal airborne bacterial community structures at an urban site in France from December 2003 to April 2004. The authors found a hierarchy in the temporal variability of bacterial communities (daily < weekly < seasonally). The differential variabilities in bacterial community composition obtained in Maron et al. (2006) and the current study might be due to the different sampling locations. Urban study sites may have a more stable bacterial community originating from the surroundings like strong anthropogenic point sources, whereas marine sampling sites may be influenced by changes in environmental parameters (e.g. wind direction) to a much greater extent. Our selected dataset showed a considerable influence of the wind direction which is a strong indication for heterogeneous sources of bioaerosols (Burrows et al. 2009). Bacterial populations in aerosols around Helgoland may, thus, originate from heterogeneous sources which could be explained by its location in the German Bight where continental, marine and even polar influences may occur - depending on wind direction. This finding is consistent with the investigation of the spatial distribution of marine bioaerosols (see chapter I) were also a strong influence of wind direction on the bacterial community composition was detected.

As Maron et al. (2006) did not consider the wind direction for their analyses, it cannot be excluded that this may also be an explanation for their contrasting results. The microbial community in the atmosphere is permanently in a state of flux. Fierer et al. (2008), for example, found high variability of bacterial communities on consecutive days and even between consecutive hours which is in agreement to our results.

The sampling month and therefore sampling season had a significant effect on species richness and beta diversity/community structure in marine bioaerosols from the island of Helgoland. In continental areas, similar patterns were observed for the urban environments of Milan (Franzetti et al. 2011; Bertolini et al. 2013) and Colorado (Bowers et al. 2011b; Bowers et al. 2012). These seasonal differences were mainly related to plant-associated taxa such as Sphingomonadales in the summer

months and soil-related and/or spore-forming taxa in the winter months (Bowers et al. 2009; Franzetti et al. 2011). Furthermore, high seasonal variation of bacterial community composition may be shaped by both shifts in atmospheric conditions and the local terrestrial environment (Bowers et al. 2012). The authors showed significant differences between the four seasonal sampling sets due to those bacteria related to the ground coverage. In autumn, winter and spring bacteria, which were related to the snow coverage dominated the aerolised bacterial community, whereas summer samples were dominated by bacteria related to the vegetation coverage. It remains unclear which bacteria might be responsible for the observed shifts in the data set, as the utilised fingerprinting approach of this study did not allow for detailed identification of the bacterial content.

Molecular fingerprint techniques proved to be helpful tools for the investigation of bacterial populations in the atmosphere (Li et al. 2010; Jeon et al. 2011). The ARISA fingerprint method used in the current study also has been successfully applied in other studies (e.g. Maron et al. 2005; Maron et al. 2006; Banning et al. 2011; Abed et al. 2012) to analyse bacterial community structures in the atmosphere. In addition, fingerprinting allows for fast and cost-efficient analyses of bacterial community structures with high sample sizes (compared to sequencing approaches). However, the disadvantage of ARISA is its restriction to the evaluation and comparison of general patterns without an option for direct subsequent identification. Nevertheless, ARISA is perfectly suited to analyse general patterns in the bacterial community composition. Additionally, ARISA fingerprinting can be used for a pre-selection of sample types, which may later be used for follow-up analyses such as sequencing (e.g. Krause et al. 2012).

Altogether, our results showed sampling month had a clear effect on both species richness and community structure in marine airborne bacterial communities, whereas the latter was additionally affected by the wind direction. The comparison of the ARISA profiles revealed a general equal high amount of variability, whatever the time interval (intra-week = intra-month = intra-season = sampling period) even though the environmental variables showed a general order of magnitude (intra-week < intra-month < intra-season < sampling period). Furthermore, the results indicated

that rather a combination of several different environmental parameters and not distinct single factors may be responsible for the observed patterns.

Acknowledgements

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Supplementary material

Table S1 Categories assigned to the samples

Sample	Date MM/DD/YYYY	BWT Influence	Height a. s. l.	Season	Month	Cardinal direction	Wind direction
17	08/01/2012	mixed	1500	Summer	August	S	S
18	08/02/2012	more continental	1000	Summer	August	S	S
19	08/03/2012	marine	500	Summer	August	W	SW
20	08/06/2012	continental	0	Summer	August	S	SW
21	08/07/2012	mixed	0	Summer	August	W	W
22	08/08/2012	more marine	0	Summer	August	W	W
23	08/09/2012	marine	0	Summer	August	W	NW
24	08/10/2012	marine	1000	Summer	August	W	NW
25	08/13/2012	mixed	1500	Summer	August	E	O
26	08/14/2012	mixed	500	Summer	August	E	O
27	08/15/2012	mixed	2000	Summer	August	E	O
28	08/27/2012	marine	0	Summer	August	W	W
29	08/28/2012	continental	0	Summer	August	S	S
30	08/29/2012	mixed	1000	Summer	August	S	S
31	08/30/2012	mixed	3000	Summer	August	W	SW
32	09/05/2012	more marine	1000	Autumn	September	W	NW
33	09/11/2012	continental	0	Autumn	September	S	SW
34	09/12/2012	mixed	0	Autumn	September	W	W
35	09/13/2012	marine	1000	Autumn	September	N	N
36	09/17/2012	mixed	1000	Autumn	September	W	W
37	09/18/2012	mixed	0	Autumn	September	S	SW
38	09/19/2012	mixed	1000	Autumn	September	W	W
39	09/20/2012	mixed	1500	Autumn	September	W	SW
40	09/25/2012	continental	0	Autumn	September	S	S
41	09/26/2012	more continental	1000	Autumn	September	S	S
42	09/27/2012	continental	0	Autumn	September	S	SW
43	09/28/2012	mixed	2000	Autumn	September	S	SW
45	10/02/2012	more continental	0	Autumn	October	S	SW
46	10/04/2012	continental	0	Autumn	October	W	W
47	10/05/2012	mixed	1500	Autumn	October	S	S
49	10/09/2012	marine	1000	Autumn	October	W	NW
50	10/10/2012	more marine	2000	Autumn	October	W	NW
51	10/11/2012	more marine	2000	Autumn	October	S	S
52	10/12/2012	mixed	500	Autumn	October	E	SO
53	10/18/2012	more continental	1500	Autumn	October	S	S
54	10/19/2012	continental	3000	Autumn	October	S	S
55	10/22/2012	more continental	2000	Autumn	October	E	O
56	10/26/2012	more marine	2500	Autumn	October	S	S
57	10/30/2012	marine	0	Autumn	October	W	W
58	10/31/2012	more continental	0	Autumn	October	S	S
59	11/01/2012	continental	1500	Autumn	November	S	SO
60	11/05/2012	more continental	0	Autumn	November	N	N
62	11/07/2012	more marine	2000	Autumn	November	W	W
63	11/09/2012	more marine	1500	Autumn	November	S	SW
64	11/12/2012	more marine	1000	Autumn	November	W	SW
65	11/13/2012	mixed	2000	Autumn	November	S	SW
66	11/14/2012	mixed	1000	Autumn	November	S	SW
67	11/15/2012	continental	2500	Autumn	November	S	SW
68	11/16/2012	continental	3000	Autumn	November	S	S
69	11/19/2012	more marine	2500	Autumn	November	S	S
70	11/20/2012	continental	500	Autumn	November	S	S
71	11/21/2012	continental	1500	Autumn	November	S	SO
72	11/22/2012	more marine	3000	Autumn	November	S	SW
73	11/26/2012	mixed	2000	Autumn	November	S	SW
74	11/28/2012	more continental	500	Autumn	November	N	NW

Sample	Date MM/DD/YYYY	BWT Influence	Height a. s. l.	Season	Month	Cardinal direction	Wind direction
75	11/29/2012	more continental	500	Autumn	November	N	N
76	11/30/2012	more continental	500	Autumn	November	N	N
77	12/03/2012	more continental	2000	Winter	December	S	SW
79	12/06/2012	continental	1500	Winter	December	N	N
80	12/07/2012	more continental	1000	Winter	December	S	SO
81	12/10/2012	more continental	1500	Winter	December	N	N
82	12/11/2012	more continental	2500	Winter	December	N	N
83	01/07/2013	mixed	0	Winter	January	W	SW
84	01/10/2013	more marine	1000	Winter	January	N	N
85	01/11/2013	continental	1000	Winter	January	N	NO
86	01/14/2013	continental	500	Winter	January	S	S
87	01/15/2013	continental	0	Winter	January	S	S
88	01/16/2013	more continental	0	Winter	January	E	O
89	01/17/2013	continental	0	Winter	January	E	O
90	01/18/2013	more continental	0	Winter	January	E	O
91	01/23/2013	more continental	500	Winter	January	E	O
92	01/24/2013	more continental	1000	Winter	January	N	N
93	01/25/2013	more continental	500	Winter	January	S	S
94	01/28/2013	more marine	1000	Winter	January	W	SW
96	02/01/2013	more marine	0	Winter	February	W	SW
97	02/05/2013	more marine	1000	Winter	February	W	W
98	02/06/2013	more marine	1000	Winter	February	W	NW
99	02/07/2013	mixed	0	Winter	February	N	N
100	02/08/2013	continental	2000	Winter	February	N	NO
101	02/12/2013	continental	500	Winter	February	E	NO
102	02/13/2013	continental	1000	Winter	February	E	O
104	02/15/2013	continental	2000	Winter	February	S	S
105	02/18/2013	continental	4000	Winter	February	W	W
106	02/19/2013	mixed	0	Winter	February	E	NO
107	02/20/2013	more continental	500	Winter	February	N	NO
108	02/28/2013	mixed	1500	Winter	February	N	N
109	03/01/2013	marine	0	Spring	March	N	N
110	03/04/2013	marine	2500	Spring	March	S	SO
111	03/05/2013	mixed	2500	Spring	March	S	S
113	03/14/2013	more continental	0	Spring	March	N	NO
114	03/15/2013	more continental	2500	Spring	March	S	S
115	03/19/2013	mixed	500	Spring	March	E	NO
116	03/20/2013	more continental	1000	Spring	March	E	O
117	03/21/2013	more continental	1000	Spring	March	E	NO
118	03/22/2013	mixed	3000	Spring	March	E	O
119	03/26/2013	mixed	500	Spring	March	E	O
120	04/02/2013	more continental	1000	Spring	April	N	NO
121	04/03/2013	land	0	Spring	April	E	NO
122	04/04/2013	more continental	1500	Spring	April	E	NO
123	04/05/2013	mixed	1500	Spring	April	N	NO
124	04/08/2013	marine	500	Spring	April	E	O
125	04/11/2013	continental	500	Spring	April	E	O
126	04/12/2013	continental	0	Spring	April	W	SW
127	04/15/2013	mixed	1000	Spring	April	W	SW
128	04/16/2013	more continental	1000	Spring	April	S	S
130	04/25/2013	more marine	500	Spring	April	E	O
131	04/26/2013	more marine	500	Spring	April	N	N
132	04/30/2013	mixed	2500	Spring	April	E	O
133	05/02/2013	marine	3000	Spring	May	N	NO
134	05/03/2013	more marine	2500	Spring	May	E	O
135	05/07/2013	mixed	500	Spring	May	E	O
137	05/10/2013	continental	0	Spring	May	S	SW
138	05/14/2013	more marine	0	Spring	May	S	SW
139	05/15/2013	more continental	0	Spring	May	E	SO

Sample	Date MM/DD/YYYY	BWT Influence	Height a. s. l.	Season	Month	Cardinal direction	Wind direction
141	05/17/2013	more continental	500	Spring	May	W	NW
142	05/18/2013	marine	0	Spring	May	W	SW
143	05/28/2013	marine	2000	Spring	May	E	NO
144	05/31/2013	continental	2500	Spring	May	N	N
145	06/03/2013	marine	0	Summer	June	N	NW
146	06/04/2013	more marine	0	Summer	June	N	N
147	06/07/2013	marine	0	Summer	June	N	N
148	06/10/2013	marine	0	Summer	June	N	N
149	06/11/2013	marine	0	Summer	June	W	NW
150	06/12/2013	mixed	500	Summer	June	S	S

Table S2 PERMANOVA and PERMDISP pairwise comparison of the beta diversity for the factor 'month'.

Compared Months	PERMANOVA p (perm)[1]	PERMDISP p (perm)[1]
August, September	0.637	0.211
August, October	0.418	0.484
August, November	**0.002**	0.196
August, December	0.350	0.186
August, January	0.258	0.231
August, February	0.676	0.517
August, March	0.204	0.088
August, April	0.078	0.450
August, May	**0.007**	0.314
August, June	0.270	0.110
September, October	0.766	0.627
September, November	**0.007**	**0.004**
September, December	0.678	0.583
September, January	0.406	0.883
September, February	0.365	0.491
September, March	0.771	0.540
September, April	0.193	0.592
September, May	**0.004**	**0.025**
September, June	0.398	0.475
October, November	**0.017**	**0.031**
October, December	0.853	0.527
October, January	0.368	0.615
October, February	0.408	0.901
October, March	0.601	0.325
October, April	0.661	0.993
October, May	**0.022**	0.092
October, June	0.395	0.355
November, December	0.193	**0.001**
November, January	**0.010**	**0.006**
November, February	**0.004**	**0.032**
November, March	**0.015**	**0.001**
November, April	**0.018**	**0.033**
November, May	0.260	0.908
November, June	**0.026**	**0.001**
December, January	0.910	0.798
December, February	0.262	0.291
December, March	0.543	0.973
December, April	0.730	0.342
December, May	0.136	**0.005**

Compared Months	PERMANOVA p (perm)[1]	PERMDISP p (perm)[1]
December, June	0.480	0.968
January, February	**0.047**	0.498
January, March	0.816	0.699
January, April	0.764	0.570
January, May	**0.010**	**0.036**
January, June	0.375	0.608
February, March	0.142	0.219
February, April	0.101	0.908
February, May	**0.013**	0.073
February, June	0.155	0.231
March, April	0.489	0.256
March, May	**0.006**	**0.011**
March, June	0.249	0.776
April, May	**0.037**	0.063
April, June	0.356	0.294
May, June	**0.047**	**0.012**

[1]Significant results (*p (perm)* < 0.05) are highlighted in bold.

Table S3 PERMANOVA and PERMDISP pair-wise comparisons of bacterial community composition based on Jaccard dissimilarities of ARISA profiles for the factor 'wind direction' of impingement samples.

Compared Groups	PERMANOVA p (perm)[1]	PERMDISP p (perm)[1]
S, SW	0.107	0.258
S, W	**0.014**	0.21
S, NW	**0.009**	0.746
S, E	0.626	0.196
S, N	0.591	0.424
S, SE	0.137	0.336
S, NE	0.578	0.382
SW, W	**0.006**	0.651
SW, NW	**0.002**	0.084
SW, E	**0.008**	**0.007**
SW, N	**0.016**	**0.018**
SW, SE	0.458	0.695
SW, NE	0.051	**0.015**
W, NW	0.223	0.129
W, E	**0.008**	**0.011**
W, N	**0.048**	**0.041**
W, SE	0.133	0.988
W, NE	**0.006**	**0.039**
NW, E	**0.011**	0.303
NW, N	0.071	0.651
NW, SE	**0.017**	0.141
NW, NE	**0.007**	0.633
E, N	0.867	0.641
E, SE	**0.043**	**0.017**
E, NE	0.628	0.646
N, SE	**0.032**	0.054
N, NE	0.349	0.971
SE, NE	**0.041**	**0.041**

[1]Significant results (*p (perm)* < 0.05) are highlighted in bold.

Table S4 Environmental parameter measured for sample taken with the XMX/2L MIL impingement sampler.

Sample	Cloud coverage eighth	Relative humidity %	Air temperatur °C	Atmospheric pressure hpa	Absolut windspeed m/sec	Perception depht mm	Sunshine duration h	Wind direction deg
17	5.8	81.63	18.2	1013.2	6.2	7.7	8.767	170
18	5.9	85.88	18.3	1009.74	6.3	1.2	2.15	190
19	3.1	81.46	17.7	1013.57	4.2	3.6	8.8	230
20	4.9	85.63	17.6	1006.53	6.2	0.9	7.967	205
21	4.2	80.88	16.9	1013.43	9.3	3	10.167	265
22	4.5	79.54	16.6	1020.02	5.9	1.4	7.3	285
23	6	70.29	16.1	1023.95	6.3	0	6.183	305
24	6.4	73.38	16.2	1025.03	7.1	0	2.9	295
25	1.9	74	18.1	1016.64	10.2	0	13.017	105
26	3	77.08	18.4	1015.63	10	0	12.233	110
27	3	75.96	18.6	1013.7	10.4	5.1	12.8	100
28	4	65.88	17.4	1017.42	6.5	0.7	11.1	290
29	5.3	81.54	18.1	1013.11	7.3	0	3.7	165
30	4.8	84.21	18.5	1014.66	5.2	0	8.6	175
31	5.5	76.54	17.9	1013.84	4.8	6.1	7.667	235
32	5.4	66.08	16.2	1020.72	8.4	0	5.6	305
33	6.6	78.25	16.7	1007.19	9.7	2.7	1.433	205
34	6.1	67.88	14.3	1008.6	7.6	7.1	5.467	270
35	5.3	64.75	14.6	1012.92	5.7	0	6.333	345
36	4.8	72.58	16.6	1008.83	9.2	0	7.85	280
37	5.7	74.83	14.8	1006.34	11.4	5	1.983	215
38	5.2	64.79	13	1012.84	9.6	3.9	4.233	290
39	6	75.38	13	1016.88	9.6	32.6	0.917	230
40	4.8	79.29	14.6	994.98	11.8	0	4.067	185
41	6.5	84	14	997.8	10.1	0.6	0.167	160
42	5.6	85.29	13.5	1001.16	9.6	5.4	0	210
43	6.3	79.21	14	1009.68	11.5	0.1	2.733	220
45	6.3	84.42	15	1010.19	10.5	0.1	6.783	215
46	6.3	74.96	12.7	1003.72	9.5	2.2	2.333	255
47	7.1	82.04	12.3	1002.98	11.3	11.7	0.667	165
49	4.3	67.04	10.9	1010.61	10.1	3	4.333	310
50	5.8	73.58	11.1	1013.14	7.1	2	3.85	310
51	4.3	79.58	11.6	1012.15	6	0.8	3.25	185
52	5.6	84	10.7	1004.13	14	13.9	1.383	120
53	7.8	89.5	14.3	1006.45	6.6	10.8	0	175
54	6	89.92	14.5	1009.88	4.2	0.4	4.883	170
55	8	94.96	12.9	1022.57	9.1	0	0	110
56	5.2	63.63	6	1011.34	5	10.1	1.783	180
57	5.4	71.08	8.6	999.68	6.5	0.7	4.583	260
58	6.1	82.08	9.4	995.18	12.7	0	4.05	190
59	6.9	82.54	9.3	983.17	13.5	5	1.017	150
60	6.4	82.29	7.3	997.31	7.1	2.1	0.567	355
62	7.1	82.04	10.9	1013.46	11.9	1.5	0.1	290
63	6.8	82.17	10.1	1010.56	9.7	0.9	0	215
64	6.1	84	9.1	1019.63	8.9	0.7	2.433	230
65	7.8	92.83	8.5	1026.56	10	2	0	210
66	7.9	92.63	10	1028.08	4.9	0	0	210
67	7.3	79.38	9.8	1023.05	3.8	0	0	220
68	7.4	89.33	3.6	1017.83	6	0	0	175
69	4.4	83.33	8.1	1015.17	9	0	3.9	165
70	6.8	89.38	9.6	1014.68	9.9	0	1.833	180
71	7.3	94	6.5	1011.85	11.2	0.1	0	140
72	3.3	84.71	9.5	1014.59	12.2	0	5.25	225
73	7.3	88.67	8.3	1003.18	8.7	3.8	1.983	205
74	7.2	84.17	6.5	1005.11	4.6	0.8	0.483	335
75	6.3	78.38	4.5	1003.91	9.4	0	0.6	10
76	5.2	75.21	4.5	1006.22	4.8	11.3	3.45	10
77	7	86.21	3.8	1003.78	10.4	4.4	0.017	205
79	4.9	70.13	2.3	1007.76	9.2	0	1.783	325
80	5.2	79	1.8	1005.93	11	0	3.383	155
81	7.5	84.96	2.3	1010.26	12.9	0	0	20

Sample	Cloud coverage eighth	Relative humidity %	Air temperatur °C	Atmospheric pressure hpa	Absolut windspeed m/sec	Perception depht mm	Sunshine duration h	Wind direction deg
82	4.9	78.79	0.8	1022.71	7.1	0.7	2.4	20
83	8	98.17	6.2	1024.51	4.3	1.7	0	235
84	6.9	81.75	3.6	1010.44	7.5	0.5	1.667	30
85	6.2	65.92	1.3	1015.99	5.8	0	1.95	25
86	7	75.92	-1.5	1013.36	5.4	0	1.433	160
87	4.1	72.96	-1.3	1008.6	3	0	3.267	175
88	5.9	82.79	-2.4	1012.14	4.3	0	0.15	75
89	5.8	77.08	-0.5	1018.56	5.7	0	2.083	70
90	7.2	85.63	0.2	1013.53	9.8	0	0	90
91	7.2	79.75	-1	1011.78	6.4	0	0.017	75
92	6	77.58	-2.1	1016.53	3.5	0	0.65	20
93	5.3	82.17	-1.3	1018.53	7.1	0	5.45	170
94	3.3	91	4	1003.31	9.1	7.3	5.15	230
96	6.7	93.58	4.7	996.28	7.3	0.4	1.417	240
97	7	87.92	3.2	991.41	7.8	4.8	0.7	285
98	5.9	88.42	3.4	996.21	6.4	0.1	1.75	310
99	5	80.13	1.9	1006.67	5.5	0	4.6	360
100	7.4	79.38	0.7	1013.31	6.8	0.2	0.033	25
101	7.8	81.92	0	1016.84	7.7	0	0.2	60
102	7.8	74.04	-0.3	1023.8	3.5	0	0	110
104	8	98.04	1	1015.4	4.7	0	0	160
105	8	82.58	2.8	1020.73	2.9	2	0.033	255
106	7.6	83.79	2.3	1017.52	8	0.4	0.4	50
107	6	79.08	0.3	1026.81	7.6	0	2.717	45
108	7.5	97.33	2.9	1026.82	4.3	0	1.267	355
109	4.6	85.54	2.8	1023.73	5.9	0	3.633	345
110	3.8	96.38	1.9	1019.45	3.6	0	2.917	145
111	2.6	81.96	4.5	1009.2	4.8	0	9.833	170
113	3.6	68	-0.8	1011.18	5.1	0	8.533	25
114	3.5	69.71	0.5	1012.5	6.8	0	7.117	185
115	7.5	83.58	-0.3	1001.2	9.5	0	0.317	55
116	7.5	86.17	-0.8	1008.14	7.6	0	0.417	70
117	6	81.92	-0.9	1018.82	6.7	0	3.883	60
118	4.5	79.75	-1.2	1023.61	14.7	0	5.683	80
119	4.1	75.5	0.4	1019.58	10.2	0	2.783	70
120	0.1	78.29	2.7	1016.72	4.4	0	11.967	45
121	1.6	70.96	2.6	1020.85	6	0	11.2	55
122	4	73.63	2.2	1017.6	7.8	0	9.5	60
123	6.2	77.25	2.6	1015.05	5.4	0	5.6	35
124	5	84.79	2.7	1011.1	5	0	11.033	90
125	8	98.92	3.9	998.93	7	1.4	0	70
126	7.9	100	3.7	998.55	5.9	0.7	0.683	240
127	5.2	92.88	6.9	1017.03	6	0	6.1	230
128	5.2	95	6.6	1015.87	7.6	0	3.15	160
130	7.4	94.25	7.5	1020.15	4.4	1.2	2.7	70
131	7.7	95.42	5.1	1010.1	5	2.3	0.033	340
132	1.8	85.67	6.5	1022.2	6.4	0	1.3483	105
133	3.1	78.79	7.6	1023.14	8.3	0	1.195	30
134	1.2	66.04	10	1018.2	5	0	1.2467	105
135	6.3	82.25	12.6	1017.52	5.1	2	8.467	75
137	5.4	87.33	9.5	1009.24	6.4	0.7	3.117	205
138	5.6	85.17	8.7	1005.89	5	0.2	8.683	210
139	6.8	90.92	10.2	1000.2	6.9	1.1	3.533	130
141	7	96.79	9.7	999.32	3.9	0.3	3.717	315
142	7.8	97.58	8.5	1005.6	3.9	10	0	235
143	6.2	95.5	10.3	1005.26	2.9	0.2	4.817	60
144	5.7	90.63	11.9	1008.87	7.4	0	11.217	340
145	4.1	86.88	10.4	1025.89	9.2	0	12.267	330
146	4.1	83.04	12.5	1023.06	5.2	0	15.167	360
147	0.6	88.63	12.6	1022.25	5.8	0	15.25	10
148	6.3	84.88	12.1	1012.35	4.3	0	6.833	345
149	4.8	69.88	12.4	1016.08	3.5	0	5.667	295
150	6.9	87.67	14.8	1014.01	4.8	6.4	0.633	160

Table S5 Post hoc test for the significant environmental parameters for the factor 'month'. Significant result are represented by asterisk (* = p < 0.05 ; ** = p < 0.01; *** = p < 0.001)and not significant results are represented by n.s.. Environmental parameters marked with '#' were tested with Tukey's multiple comparison test and '+' indicates Dunn's multiple comparison test.

Compared Months	Relative humidity #	Temperature +	Wind speed #	Perception +	Sunshine duration +	Wind direction +
August vs September	ns	ns	ns	ns	ns	ns
August vs October	ns	ns	ns	ns	ns	ns
August vs November	ns	**	ns	ns	***	ns
August vs December	ns	***	ns	ns	ns	ns
August vs January	ns	***	ns	ns	**	ns
August vs February	ns	***	ns	ns	***	ns
August vs March	ns	***	ns	ns	ns	ns
August vs April	ns	***	ns	ns	ns	ns
August vs May	ns	ns	ns	ns	ns	ns
August vs June	ns	ns	ns	ns	ns	ns
September vs October	ns	ns	ns	ns	ns	ns
September vs November	*	ns	ns	ns	ns	ns
September vs December	ns	**	ns	ns	ns	ns
September vs January	ns	***	*	ns	ns	ns
September vs February	*	***	*	ns	ns	ns
September vs March	ns	***	ns	ns	ns	ns
September vs April	**	**	**	ns	ns	ns
September vs May	*	ns	**	ns	ns	ns
September vs June	ns	ns	*	ns	ns	ns
October vs November	ns	ns	ns	ns	ns	ns
October vs December	ns	ns	ns	ns	ns	ns
October vs January	ns	***	ns	ns	ns	ns
October vsFebruary	ns	**	ns	ns	ns	ns
Octoberv s March	ns	***	ns	**	ns	ns
October vs April	ns	ns	ns	ns	ns	ns
October vs May	ns	ns	*	ns	ns	ns
October vs June	ns	ns	ns	ns	ns	ns
November vs December	ns	ns	ns	ns	ns	ns
November vs January	ns	*	ns	ns	ns	ns
November vs February	ns	ns	ns	ns	ns	ns
November vs March	ns	ns	ns	ns	ns	ns
November vs April	ns	ns	ns	ns	ns	ns
November vs May	ns	ns	*	ns	*	ns
November vs June	ns	ns	ns	ns	*	ns
December vs January	ns	ns	*	ns	ns	ns
December vs February	ns	ns	*	ns	ns	ns
December vs March	ns	ns	ns	ns	ns	ns
December vs April	ns	ns	*	ns	ns	ns
December vs May	ns	ns	*	ns	ns	ns
December vs June	ns	ns	*	ns	ns	ns
January vs February	ns	ns	ns	ns	ns	ns
January vs March	ns	ns	ns	ns	ns	ns
January vs April	ns	ns	ns	ns	ns	ns
January vs May	ns	*	ns	ns	ns	ns
January vs June	ns	**	ns	ns	ns	ns
February vs March	ns	ns	ns	ns	ns	ns
February vs April	ns	ns	ns	ns	ns	ns
February vs May	ns	ns	ns	ns	ns	ns
February vs June	ns	*	ns	ns	*	ns
March vs April	ns	ns	ns	ns	ns	ns
March vs May	ns	*	ns	ns	ns	ns
March vs June	ns	**	ns	ns	ns	ns
April vs May	ns	ns	ns	ns	ns	ns
April vs June	ns	ns	ns	ns	ns	ns
May vs June	ns	ns	ns	ns	ns	ns

Table S6 PERMANOVA and PERMDISP pairwise comparison of the environmental parameter for the factor 'month'.

Compared Month	PERMANOVA p (perm)[1]	PERMDISP p (perm)[1]
August, September	**0.001**	0.250
August, October	**0.001**	0.053
August, November	**0.001**	0.078
August, December	**0.001**	0.151
August, January	**0.001**	0.399
August, February	**0.001**	**0.007**
August, March	**0.001**	0.109
August, April	**0.001**	**0.005**
August, May	**0.002**	0.165
August, June	0.074	**0.048**
September, October	0.385	0.499
September, November	**0.001**	0.382
September, December	**0.004**	0.678
September, January	**0.001**	0.982
September, February	**0.001**	0.249
September, March	**0.001**	0.669
September, April	**0.001**	0.139
September, May	**0.001**	0.535
September, June	**0.001**	0.295
October, November	**0.037**	0.768
October, December	**0.012**	0.901
October, January	**0.001**	0.607
October, February	**0.001**	0.721
October, March	**0.001**	0.777
October, April	**0.001**	0.318
October, May	**0.008**	0.878
October, June	**0.004**	0.467
November, December	0.251	0.818
November, January	**0.004**	0.471
November, February	0.066	0.978
November, March	**0.001**	0.621
November, April	**0.010**	0.509
November, May	**0.005**	0.926
November, June	**0.006**	0.664
December, January	0.126	0.776
December, February	0.115	0.679
December, March	0.267	0.903
December, April	0.122	0.501
December, May	**0.008**	0.857
December, June	**0.006**	0.726
January, February	0.426	0.401
January, March	0.309	0.775
January, April	0.133	0.215
January, May	**0.001**	0.593
January, June	**0.002**	0.355
February, March	**0.021**	0.440
February, April	0.074	0.408
February, May	**0.008**	0.947
February, June	**0.001**	0.613
March, April	0.354	0.266
March, May	**0.007**	0.721
March, June	**0.005**	0.473
April, May	0.252	0.534
April, June	0.238	0.991
May, June	0.367	0.681

[1]Significant results ($p\ (perm) < 0.05$) are highlighted in bold.

Table S7 PERMANOVA and PERMDISP pairwise comparison of the environmental parameter for the factor 'wind direction'.

Compared Groups	PERMANOVA p (perm)[1]	PERMDISP p (perm)[1]
S, SW	0.110	0.394
S, W	0.288	**0.030**
S, NW	0.144	0.575
S, E	**0.009**	0.130
S, N	0.185	0.116
S, SE	0.479	0.096
S, NE	**0.001**	0.149
SW, W	0.573	0.094
SW, NW	**0.037**	0.864
SW, E	**0.001**	0.358
SW, N	**0.002**	0.311
SW, SE	0.456	0.171
SW, NE	**0.001**	0.279
W, NW	0.626	0.113
W, E	**0.003**	0.438
W, N	0.057	0.372
W, SE	0.299	0.705
W, NE	**0.001**	0.991
NW, E	**0.030**	0.336
NW, N	0.257	0.256
NW, SE	0.075	0.227
NW, NE	**0.001**	0.404
E, N	0.499	0.968
E, SE	0.062	0.423
E, NE	0.205	0.713
N, SE	0.111	0.429
N, NE	**0.023**	0.675
SE, NE	**0.019**	0.799

[1]Significant results (*p (perm)* < 0.05) are highlighted in bold.

Chapter III

Quantification of marine airborne bacteria: comparative study on C-FLAPS vs. q-PCR

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Abstract

The capability of a Fluorescent Aerosol Particle Sizer (FLAPS) for the quantification of bacterial bioaerosol particles was tested in an outdoor setting and compared with quantitative real-time PCR (q-PCR). For this purpose, samples for the q-PCR analysis were collected with an impingement sampler (Dycor XMX/2L MIL). Additionally, the direct counting of the FLAPS (total and fluorescent particles) and q-PCR quantification were compared and tested for the influence of environmental parameters on their performance. No correlation was found between the quantification results of the FLAPS and the q-PCR, whereas the two methods were differently affected by environmental parameters. It is thus concluded that the FLAPS is probably not suited for the quantification of particular bioaerosol particles (e.g. bacteria, pollen or fungi) in outdoor environments, but represents a good tool to count the total amount of bioaerosol particles. Future studies need to address further comparisons of quantification methods (e.g. q-PCR vs. counting of fluorescent dyed filters) in order to identify and establish suitable standardised quantification methods for airborne bacterial communities.

Introduction

The presence of particles in the atmosphere has been known for centuries (Gregory 1971). A wide range of biogenic aerosol particles such as bacteria are ubiquitous in the atmosphere (Jaenicke 2005; Elbert et al. 2007; Jaenicke et al. 2007). Although their investigation started already with Louis Pasteur (Pasteur 1860a, 1860b), until recently most studies on airborne bacteria were sporadic, qualitative and exploratory in nature, whereas only few attempts for systematic and quantitative studies were made. As recognised in several reviews (Burrows et al. 2009; Després et al. 2012; Gandolfi et al. 2013), existing literature on bioaerosols is also characterised by a lack of standardised state of the art methods. The heterogeneity of quantification techniques therefore complicates comparability of results as they can differ by several orders of magnitudes (Burrows et al. 2009).

There is an urgent need for sound knowledge on the concentration and distributional patterns of airborne bacteria on a global scale. This would allow for assessing their importance for climate and health effects including cloud formation/ development, microbial biodiversity, and atmospheric chemistry (Pöschl 2005). The assessment of the bacterial concentration in the atmosphere is thus one of the central tasks for the description of airborne microbial communities.

Although a large variety of methods for determination of bacterial concentration in the atmosphere were applied in the past, results are barely comparable with each other in the majority of cases. Until now most quantification approaches were carried out employing culture-dependent methods. These culture-dependent methods bare a couple of problems, such as potential pre-selection by the choice of culture medium, incubation time and temperature. Another main problem is that only a small fraction of environmental bacteria is culturable (Colwell 2000), and culturability of bacteria rapidly declines after aerolisation of the microbes (Heidelberg et al. 1997). Concerning culture-independent methods, total bacterial counts is an often used method and is considered as an important variable for meteorology as dead cells and fragments can function as condensation nuclei for water and ice (Möhler et al. 2008). Hence the present study aims to overcome these problems using also culture-independent methods. This can be achieved, for example, by direct counting of

bacteria on a filter with fluorescence dye where all DNA-yielding particles are stained. However, this method is laborious and time-consuming as counting and discrimination of particles has to be done manually to avoid misinterpretation of non-biological debris. Additionally, a considerably high amount of airborne bacteria must be present in the samples (over 10^4 cells l^{-1}) to obtain reliable result (Gandolfi et al. 2013). In contrast, quantitative PCR (q-PCR) allows for automated quantification of bacteria by means of eubacterial primers. Fluorescent Aerosol Particle Sizers such as the FLAPS II (Dycor Technologies, Edmonton, Canada) represent an additional method for direct quantification of bioaerosol particles in the field. A prototype of this system was developed in 1997 and subsequently tested and improved ever since (Hairston et al. 1997; Brosseau et al. 2000; Agranovski et al. 2003; Agranovski et al. 2004). The FLAPS II is able to count bioaerosol particles due to the content of NAD(P)H and Riboflavin, two components linked to the metabolism of living organism, which exhibit fluorescence under stimulation at a certain wavelength and can be identified and quantified. As stated by Agranovski et al. (2004) conventional methods monitoring airborne bacteria (culture-dependent and -independent) are laboratory based and need 24 h or more to provide any results. Especially in the context of bioterrorism the exposure to a risk organism has to be detected contemporary to react. For this kind of threat the FLAPS was designed as a real-time monitoring for the detection of aerosol particles which may contain microorganism (Ho et al. 1995). The FLAPS II system is designed to alert in case of a sudden increase of fluorescent aerosol particles exceeding typical background level despite the air-masses throughput. The current study compares and correlates the applicability of the FLAPS II to quantify/detect fluorescent bioaerosol particle with the quantification of atmospheric bacteria via q-PCR. The two quantification methods are compared and evaluated for the first time in an outdoor marine environment analysing natural samples.

Material and Methods

Study area

All samples were collected on Helgoland (German Bight, North Sea) from an exposed position at a small tower of the German Bundeswehr (54°10'49.93 N, 7°53'2.83 E), 40 m above sea level (a.s.l.). The small island of Helgoland (1.7 km²) is situated in the centre of the German Bight, approximately 60 km offshore (Figure 1). Its remote and exposed location is, thus, ideal for the study of marine bioaerosols.

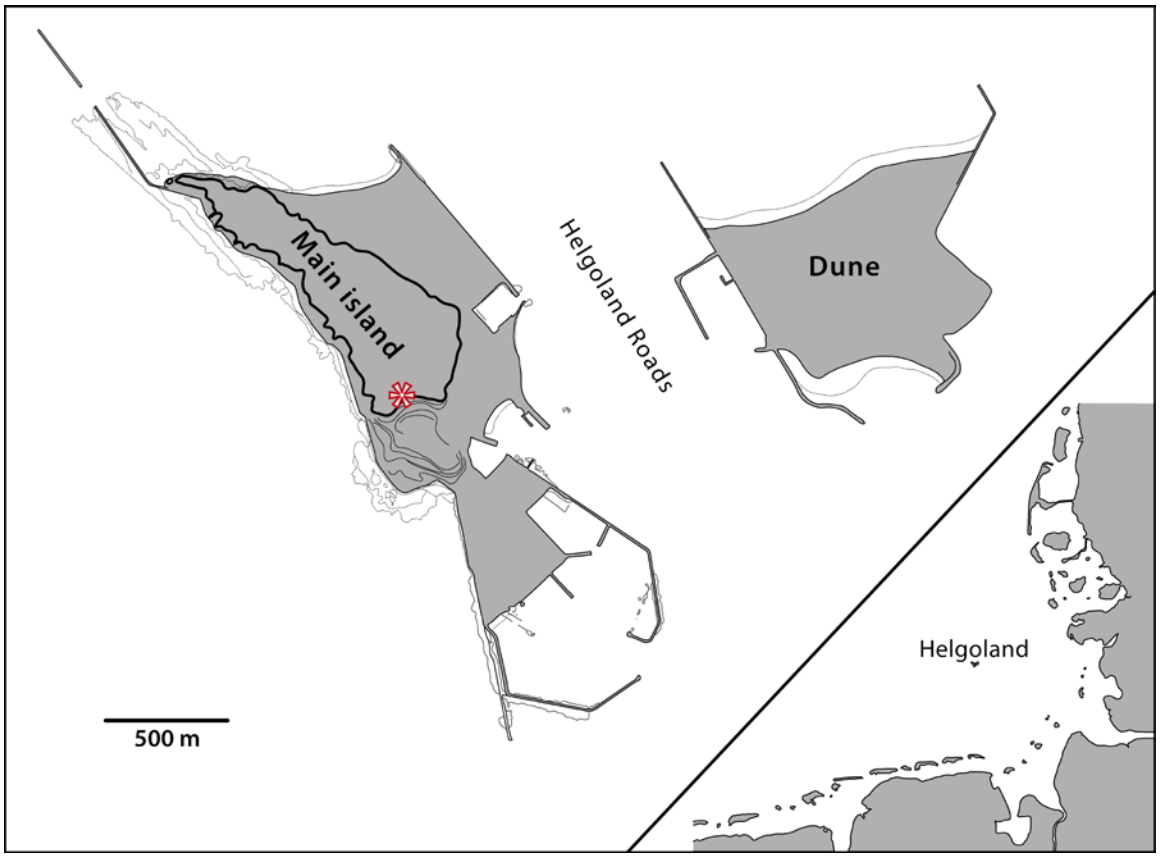

Figure 1 Map of Helgoland and its location in the German Bight (bottom right corner). Asterisk indicates the position of the sampling site Bundeswehr tower (54.1805 N, 7.8841 E), 40 m a. s. l. (map modified after Beermann 2014).

Sampling devices

Impingement sampler

An impingement aerosol sampler XMX/2L MIL (Dycor, Edmonton, Canada) was used to sample aerosols from August 2012 to June 2013. This sampling device collects high volumes of air, strips away larger dust particles and very small micro debris, and concentrates aerosols within a diameter of 1 to 10 µm. Particles were then impinged into a sample collection vial (50 ml falcon tube) containing 5 ml phosphate buffered saline (PBS) as a sample liquid, washing out the bacteria and preventing them from any damage. In total 12 l/min were collected in the sampling liquid. Each sample consisted of 12 runs à 10 minutes (controlled by an integrated timer) which were pooled afterwards. The samples of the first and the second hour were pooled separately on a 0.2 µm Isopore™ membrane filters (GTTP-type, diameter 13 mm; Millipore, Eschborn, Germany) each and stored away at -20°C for later processing.

FLAPS II direct online measurement of aerosol particle

Ambient air was sampled through an upstream tube (15 cm in diameter). Air flow through the tube was considered laminar at all times, and diffusion losses were, hence, considered negligible for all sampled particle sizes. A linked Fluorescent Aerodynamic Particle Sensor (FLAPS; TSI Inc. Model 3313, ST. Paul, MN) was then employed, following standard manual advised procedures. As detailed descriptions of the instrument have been provided by Hairston et al. (1997) and Brosseau et al (2000), only a brief introduction will be given below. An integrated optical biodetection system provides nearly real-time point detection of particles of biological origin, utilising laser to induce auto fluorescent intrinsic conductivity of biological particles (Huffman et al. 2010). The system was comprised by two major components: the FLAPS II (TSI Model 3313) and a particle concentrator (XMX/2A, Dycor technologies). The software CBNET3 from Dycor was employed for controlling and monitoring the sampling, being able to measure the particle size as well as the total and fluorescent particle count simultaneously. The whole setup is primarily designed for the detection of bio-hazardous events and has limited capability for accurate quantification. Additionally, detected bio-particles cannot be

identified automatically. The FLAPS II uses a pulsed ultraviolet laser beam at a wavelength of 355 nm for the stimulation of fluorescence in bioaerosol particles. Light at the range from 420 to 575 nm is than detected, corresponding to the auto fluorescence of reduced pyridine nucleotides (NAD(P)H) and riboflavin. This way, the setup detects specific bio-markers for living cells (Harrison & Chance 1970; Eng et al. 1989; Kell et al. 1991; Li et al. 1991; Iwami et al. 2001; Hill et al. 2009; Huffman et al. 2010). The FLAPS II was provided and calibrated by the Department of B-Detection of Bundeswehr Research Institute for Protective Technologies (Munster, Germany).

Aerosol sampling was performed with an airflow of 5 l/min, being internally split in 1.0 ± 0.1 l/min for optical measurement, and a sheath flow of 4.0 ± 0.1 l/min for pressure difference feedback control. An external computer was connected for total and inner flow rate control via CBNET reading of the instrument settings, and for measurement data recording with CBNET3. Furthermore, aerodynamic diameter, side scatter intensity and fluorescent intensity measurements were recorded every 3 seconds each over a total sampling time of 2 hours.

DNA extraction

Following the procedure of Sapp et al. (2006), a chemical cell lysis with subsequent phenol-chloroform-extraction and additional freeze-thaw-step (Maron et al. 2005) was performed. Concentration and pureness of the extracted DNA was determined photometrically using the microplate reader Tecan Infinite© 200 from NanoQuantphotometry (Tecan, Männedorf, Switzerland). DNA was stored at -20°C for later processing.

q-PCR

Quantitative PCR (q-PCR) assays were performed with extracted DNA from all samples with the LightCycler® 480 SYBR Green I Master kit on a Lightcycler 480 II (Roche, Mannheim, Germany) following the procedure of Einen et al. (2008). For calibration, a marine *Pseudoalteromonas* isolate (H71, AccNo: AF069653, Wichels et al. 1998) was deployed. Reference curves and calculation of starting concentration

was implemented after An et al. (2006). The bacterial cell concentration of the reference curves was verified by CFU counts following Mao et al. (2007); and the primers 534r (5'-ATT ACC GCG GCT GCT GC-3') as well as 341f (5'-TAG GGG AGG CGA CAG-3') were used (Muyzer et al. 1993). DNA from all samples were run in duplicate. Triplicate 10-fold dilution series of genomic *Pseudoalteromonas* sp. DNA served for generation of a standard curve with an r^2 value of 0.996, and amplification efficiency was estimated to be 96%.

Meteorological conditions

Meteorological data (air temperature, relative humidity, atmospheric pressure, cloud coverage, sunshine duration, wind speed, perception depth and wind direction) was obtained from a measuring station of the German Meteorological Service (DWD), situated on Helgoland (location: 54°10'36" N; 07°53'33" E, 4 m a.s.l.). Except for wind direction, which was determined on an hourly basis, all parameters were averaged for the daily mean.

Statistical analyses

All statistical analyses were performed with the computer software package GraphPad Prism 5.04 (GraphPad Software, Inc.).

As particles measured with the FLAPS II system could not be quantified in relation to the sampled air volume, Spearman-rank correlation analyses were performed for recorded total particle number/fluorescent particle numbers against q-PCR quantification results. Furthermore, environmental parameters were correlated to the quantification results in order to check for possible influences on respective sampler/quantification methods.

Results

Total particle size distribution showed a clear emphasis on particles ranging from 0.5 to 4.0 µm, which was rarely exceeded (Figure 2). For the whole sampling period, total particle (TP) count ranged from 267 to 43,566 units, whereas the fluorescent particle (FP) count ranged from 53 to only 2,288 units. Although the statistical analysis indicated a significantly positive correlation of TP and FP count ($p < 0.0001$), this correlation is not absolute ($r_s = 0.693$). The aerolisation of TP and FP are positively correlated although numerical proportions may strongly differ. This is evident in the comparison of the course of the curves for TP and FP (Figure 3), which did not the same progression after inspection, as well as from the relative amount of fluorescent particles, which fluctuated between 1% to 46% of the total particle count (Figure 4).

The quantification by q-PCR showed a wide range of calculated abundances from 1.28×10^2 cells per m³ air to 1.4×10^4 cells per m³ air during the sampling period from August to June (Figure 5). The bacterial cell count by q-PCR and TP/FP count by FLAPS II as well as FP frequencies were not correlated with each other (TP: $r_s = -0.0582$, $p = 0.6618$; FP: $r_s = 0.0995$, $p = 0.4532$; FP frequency: $r_s = 0.1728$, $p = 0.1907$) (Figure 6-8, Table 2). The correlation analysis of bacterial cell count by q-PCR with environmental parameters revealed a significant, negative correlation with relative humidity ($r_s = -0.35$; $p= 0.003$) and perception depth ($r_s = -0.24$; $p = 0.0485$), whereas it was positively correlated with sunshine duration ($r_s = 0.25$; $p = 0.039$). The FLAPS TP count, however, was positively correlated with relative humidity ($r_s = 0.4442$; $p = 0.0001$), air temperature ($r_s = 0.4525$; $p = 0.0001$), perception depth ($r_s = 0.2723$; $p = 0.0207$) and wind direction ($r_s = 0.3035$; $p = 0.0096$). In contrast, the fluorescent particle count showed no direct correlation with any of the measured environmental factors, but the relative amount of FP to TP was negatively correlated with relative humidity ($r_s = -0.407$; $p = 0.0004$), air temperature ($r_s = -0.4082$; $p = 0.0004$), perception depth ($r_s = -0.3770$; $p = 0.0011$) and wind direction ($r_s = -0.2523$; $p = 0.0325$).

Table 1 Spearman correlation of environmental parameters with bacterial cells per m³ air, the total and fluorescent particle count and the frequency of fluorescent particles detected with FLAPS II.

	q-PCR		Total particles		Fluorescent particles		%	
	r_s	p[1]	r_s	p[1]	rs	p[1]	r_s	p1
Cloud coverage	-0.2090	0.0803	0.1530	0.1994	0.0964	0.4205	-0.1491	0.2113
Relative humidity	**-0.3476**	**0.0030**	**0.4442**	**0.0001**	0.1949	0.1009	**-0.4070**	**0.0004**
Air temperature	0.0043	0.9715	**0.4525**	**0.0001**	0.1582	0.1845	**-0.4082**	**0.0004**
Atmospheric pressure	0.1502	0.2113	-0.0696	0.5611	-0.0420	0.7261	0.0356	0.7667
Wind speed	0.0166	0.8907	0.0747	0.5326	-0.0791	0.5089	-0.1151	0.3357
Perception depth	**-0.2351**	**0.0485**	**0.2723**	**0.0207**	0.0889	0.4578	**-0.3770**	**0.0011**
Sunshine duration	**0.2452**	**0.0393**	0.0087	0.9424	0.0845	0.4802	0.1254	0.2938
Wind direction	-0.0881	0.4649	**0.3035**	**0.0096**	0.1509	0.2059	**-0.2523**	**0.0325**

[1]Significant results ($p < 0.05$) and corresponding correlations are highlighted in bold.

Table 2 Spearman correlations of bacterial cells per m³ air measured with q-PCR with the total and fluorescent particle count and frequency of fluorescent particles detected with FLAPS II.

	Total particles		Fluorescent particles		Fluorescent particle frequency	
	r_s	p	r_s	p	r_s	p
q-PCR	-0.0582	0.6618	0.0995	0.4532	0.1728	0.1907

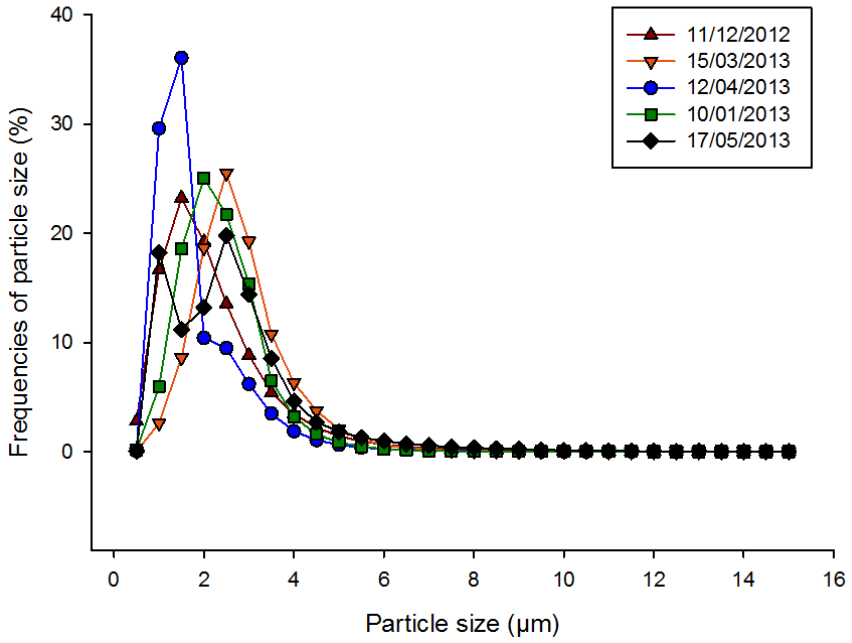

Figure 2 Total count particle size distribution measured with FLAPS II. Represented are particle distributions of five sampling events distributions: 11.12.2012 (brown), 15.03.2013 (orange), 12.04.2013 (blue), 10.01.2013 (green) and 17.05.2013 (black).

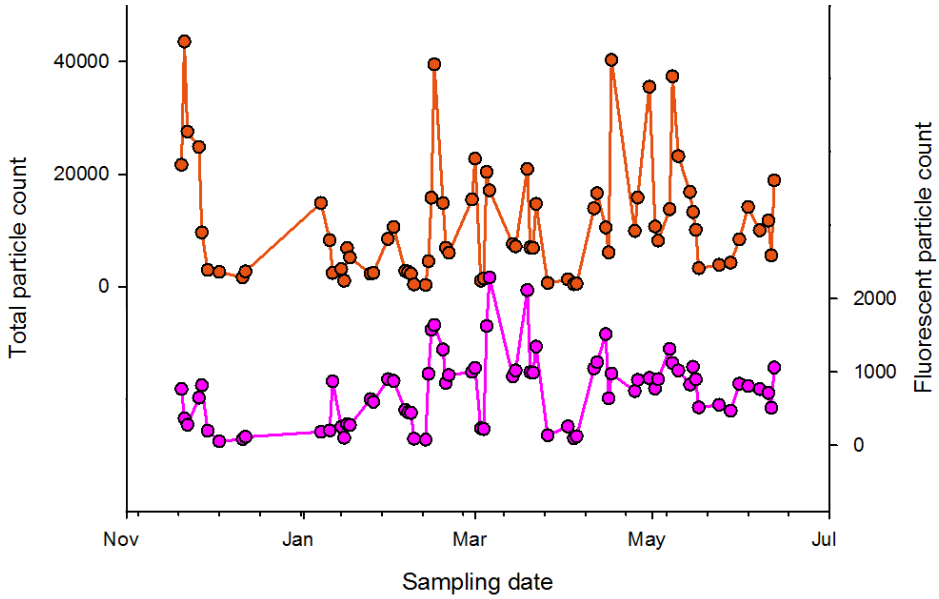

Figure 3 Mean total and fluorescent particle count measured with FLAPS II. Total particle counts (orange) are presented by the left y-axis and fluorescent particle (pink) counts by the right y-axis.

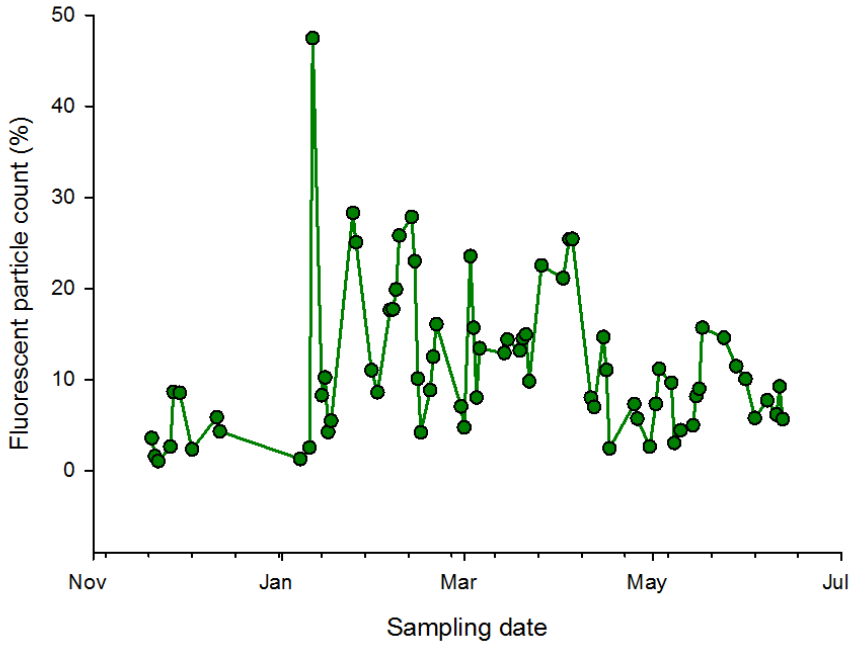

Figure 4 The frequency of fluorescent particle of the total particle count shown in percentage.

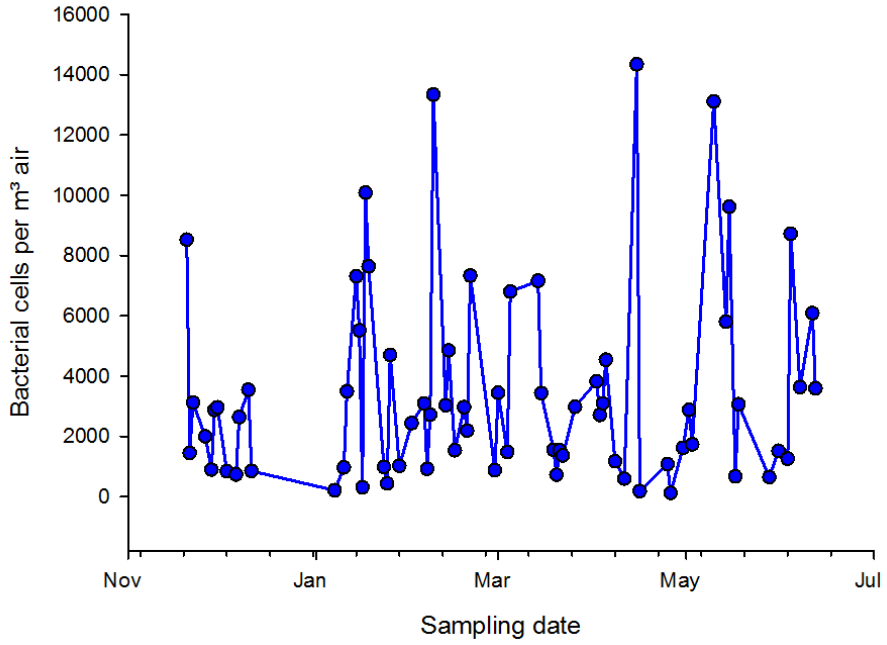

Figure 5 Quantification of marine bioaerosols sampled by impingement sampler via q-PCR.

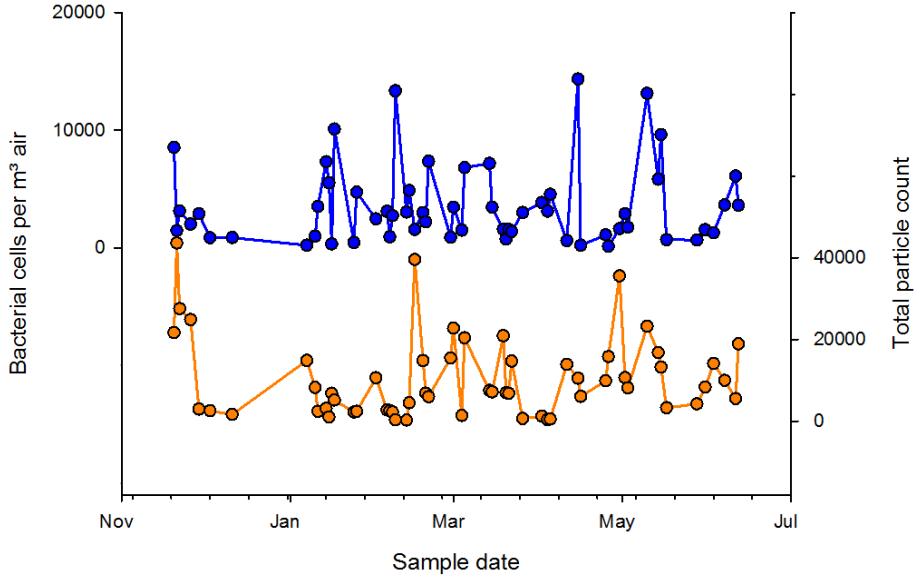

Figure 6 Comparison of bacterial count per m³ air (blue) and the mean total particle count via FLAPS II (orange). The upper graph refers to the left y-axis and the lower graph to the right y-axis.

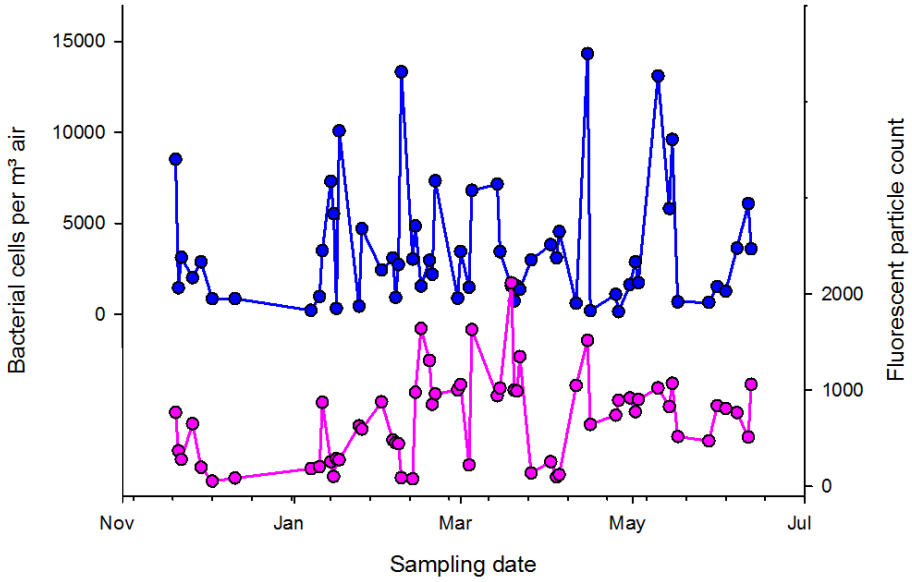

Figure 7 Comparison of bacterial count per m³ air (blue) and the mean fluorescent particle count via FLAPS II (pink). The upper graph refers to the left y-axis and the lower graph to the right y-axis.

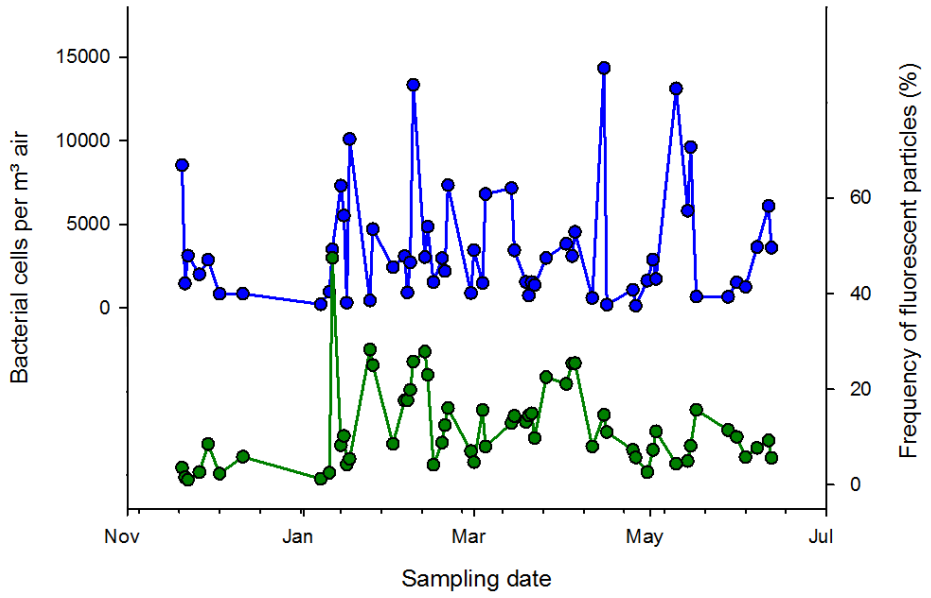

Figure 8 Comparison of bacterial count per m³ air (blue) and the percentage of fluorescent particles from the total particle count measured with FLAPS II (green). The upper graph refers to the left y-axis and the lower graph to the right y-axis.

Discussion

Assessing the bacterial concentration in the atmosphere is one of the central tasks in the field of aerobiology due to its importance for the climate (cloud formation, atmospheric chemistry) and health concerns. However, this research area is still characterised by a lack of standardised quantification methods. This results in an overall heterogeneity of quantification techniques and, accordingly barely comparable results among the few published studies. The current investigation, therefore, aimed to compare two available quantification techniques (direct counting via FLAPS II system and lab-based method q-PCR) analysing the comparability and suitability of this techniques in an outdoor environment.

The particle size distribution showed maximum peaks in the frequency at/between 1 µm to 4 µm, whereas larger particles were rare. In other studies, a medium particle size of 4 µm has been observed at continental sites and a medium particle size of 2 µm at coastal size (Shaffer & Lighthart 1997; Tong & Lighthart 2000; Wang et al. 2007). Hence, a higher proportion of 4 µm particles might indicate the influence of continental air, and a higher proportion of smaller particles might indicate marine influence. However, we were not able to observe direct correlation of wind direction and particle size. Accordingly, this assumption could not be supported by our current findings.

A comparison of the quantification via q-PCR and the direct measurement of fluorescent particle count with the FLAPS II showed no correlation. Similar results were obtained for the correlation of total particle flow and fluorescent particle frequencies. Thus, there was no linear relationship between the bacterial quantification via q-PCR and the direct counting of particles (total and fluorescent) of the FLAPS; the two quantification methods seemed to be incomparable to each other.

The correlation of the environmental parameters with the different bacterial cell count, FP and TP count and the frequency of FP particles showed that different environmental parameters correlated with quantification techniques and units. The q-PCR results show a slightly positive correlation with the sunshine duration and a slightly negative correlation with perception and relative humidity. The TP count in

contrast was positively influenced by relative humidity, air temperature, perception and wind direction while no correlation was detected for the FP count. The two quantification methods, therefore, seem to be influenced by different meteorological parameters, which is a further indication for their incomparability.

The frequency of FP particles was negatively correlated with the environmental parameters which were, in turn, positively correlated with the TP count being a further indication for a non-proportional increase in FP particles to the TP count. Therefore, the aerolisation of organic and anorganic particles does not seem to be linked proportionally to each other.

The different results for quantification may have also resulted from the sampling devices itself which could have been affected by environmental parameters differently. This would further complicate the interpretation of the data for the TP count and q-PCR results. The effects of relative humidity, temperature and air currents on the TP count are probably also the most important for the overall bioaerosol setting (Stetzenbach et al. 2004).

To date, there is only a single study on quantification method evaluation with a FLAPS device by Kanaani et al. (2008). The authors compared a FLAPS against an impingement sampler with subsequent CFU counting of two airborne fungi. The authors report that background fluorescent particle count was below detection before the start of the experiment and found a linear correlation between the direct counting of fluorescent particle with the FLAPS and the CFU count of the impingement samples. However, as the experiment was conducted in a Biological Safety Cabinet (exclusion of outdoor air), their findings are presumably based upon the specific conditions in a stable but artificial environment, which of course does not correspond to natural environments. In contrast, bioaerosol sampling of the current study was performed in situ where environmental conditions can neither be manipulated nor be controlled. Therefore, we were not able to exclude a fluorescent background (other bioaerosol particle classes) so the FLAPS II does not discriminate different types of bioaerosols such as bacteria, fungi, plant debris, lichens, spores. In contrast the quantification of bioaerosol particles using q-PCR selected for specific groups of organisms e.g. bacterial DNA using a eubacterial primer set. This is also possible

with other groups like fungi with universal fungi primer sets. So it is actually possible to quantify different bioaerosol groups from the same dataset and also compare the amount of different bioaerosol types. The non-correlation of q-PCR and FLAPS II is most likely due to a large number of non-bacterial fluorescent particles in the sampled bioaerosols. The FLAPS II, thus, is in general a detection method for the quantification of bioaerosols with predefined content or for determination of total biological content in the atmosphere (Huffman et al. 2010; Jamriska et al. 2012), but may be unsuitable for the quantification of selected biological particles in an outdoor environment. For this purpose other quantification methods need to be deployed like e.g. direct counting of fluorescent dyed filters (Rinsoz et al. 2008) or q-PCR (Cho & Hwang 2011).

Acknowledgements

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General discussion

There is a great lack of knowledge about aerolised bacterial communities (Burrows et al. 2009; Després et al. 2012; Gandolfi et al. 2013) which is further complicated by a lack of standardised methods for sample collection as well as for qualitative and quantitative analyses. To date, most studies were conducted in indoor environments and the few outdoor environmental studies were mainly focused on urban and rural areas. Respective investigations in both environments were mainly conducted with a clear emphasis on public health concerns (i.e. the presence of pathogens and allergens). Despite the fact that the oceans cover more than 70% of the earth's surface, emitting large amounts of PBAPs, in comparison to terrestrial environments aerosols in marine and coastal environments are still understudied. The current thesis addressed two important aspects of marine PBAPs: the spatial and the seasonal variability of marine bioaerosols. The spatial distribution of marine bioaerosols was investigated with samples ranging from the North to Baltic Sea via Skagerrak and Kattegat. The temporal variability was studied in a yearlong survey at the remote island of Helgoland (German Bight). Both investigations were accompanied by simultaneous measuring of environmental parameters and the calculation of backward trajectories. Furthermore, the investigation deployed culture-independent methods (next generation sequencing (NGS), ARISA, q-PCR) in order to eliminate the known biases of culture-dependent methods.

Spatial distribution of marine airborne bacterial communities

The spatial distribution of bacterial populations in marine bioaerosol samples was studied on a research cruise from the North Sea to the Baltic Sea via Skagerrak and Kattegat. In total, 36 bioaerosol samples were collected with an impingement sampler, subsequently analysed by a microbial 16S rRNA gene tagged high-throughput sequencing approach and finally related to meteorological parameters. The majority of former published studies either used cloning (Fahlgren et al. 2010) or DGGE approaches (Cho & Hwang 2011) for species identification, thereby detecting only the most abundant OTUs and disregarding the respective total

bacterial communities. In order to overcome this insufficiency a high-throughput NGS approach was deployed. The total number of 117,232 bacterial sequences with an average of 3,500 sequences per sample was obtained. Even though the high-throughput sequencing approach was used, the rarefaction curves displayed high bacterial diversity which was insufficiently captured. To date, only few studies applied a NGS approach on airborne bacteria of which all were conducted in terrestrial environments (Bowers et al. 2009; Bowers et al. 2011a). In a study from 2009, Bowers et al. obtained around 5,000 sequences with an average of 407 sequences per sample, whereas Bowers and co-authors reached around 11,000 sequences with an average of 730 sequences per sample in 2011. The authors concluded, that in both studies the bacterial community had been assessed insufficiently (Bowers et al. 2009; Bowers et al. 2011a). In the light of the obviously vast diversity of airborne bacterial communities, this also underlines the insufficiency of culture-dependent methods, as they only allow for detecting few culturable bacterial groups, which was coined as 'the great plate count anomaly' by Staley & Konopka in 1985. Accordingly, concerned studies will always access only small parts of respective bacterial communities, thus underestimating the total bacterial community diversity.

In the current study *Sphingomonas* sp. represented 17% of the total bacterial reads. This genus has also been found to be the dominant taxon in a study from the Baltic Sea (Fahlgren et al. 2010). Furthermore, a bacterial core community comprising 37 taxa (see Table S9, chapter I) which represented more than 75% of all bacterial reads was identified. The core community taxa were also detected in previous studies on bioaerosols (e.g. Fierer et al. 2008; Fahlgren et al. 2010; Bowers et al. 2012; Zweifel et al. 2012). This supports the assumption that strong areal sources (e.g. tropic rainforest, agricultural fields, soils) may emit bioaerosols which are then globally distributed by air currents, forming a bacterial core community (see Gandolfi et al. 2013).

The core community in the current study comprised bacteria being typically associated with different source environments (e.g. plants, soil, human, marine) which is a strong indication for a mixed origin of the bacterial communities sampled

during the research cruise to the North and Baltic Sea. Unfortunately, the frequent taxon *Sphingomonas* sp. ubiquitously occurs in the environment and can originate from both aquatic and terrestrial areas. Therefore, a direct association of this genus with a distinct source of origin is not possible. In contrast to terrestrial systems (Bowers et al. 2009; Bowers et al. 2011a; Bowers et al. 2011b; Bowers et al. 2012), marine airborne bacterial communities in the North and the Baltic Sea were not mainly characterised by bacteria from the subjacent ecosystem/environment. However, it remains unclear if this also applies to offshore areas in the middle of the Pacific, the Atlantic or the Indian Ocean, as the influence of terrestrial environments might be much lower in areas being 2,000 km off the coasts.

Additionally, human associated bacteria (e.g. *Propionibacterium*, *Prevotella*), potential pathogenic bacteria (e.g. *Staphylococcus*, *Streptococcus*) and potential ice nucleation bacteria (e.g. *Pseudomonas*, *Pantoea*, *Pedobacter* and *Psychrobacter*) were found in the current study. Human associated bacteria were also detected in other bioaerosol studies (Fahlgren et al. 2010; Bowers et al. 2012; Zweifel et al. 2012) but their occurrence has barely been discussed (Bowers et al. 2011b; DeLeon-Rodriguez et al. 2013). The repetitive findings of human associated bacteria may underline the importance of wastewater/sewage plants as point sources for aerolised bacteria in the atmosphere (Pascual et al. 2003; Karra & Katsivela 2007; Haas et al. 2010).

The bacterial communities were mainly affected by air temperature and wind direction, as well as the geographical sampling locations of the sampled air parcel. Not surprisingly, the air temperature was correlated with the wind direction, as winds from northern direction were colder than southern (continental) winds. Accordingly, the influence of air temperature and wind direction could not be differentiated and, as a consequence, was treated as a single factor. The wind direction was given preference here as it facilitated an easy classification of samples in clearly defined groups. Some bacteria were found at all locations but were absent in some wind directions and *vice versa*.

Temperature appeared to be a major factor influencing the airborne bacterial community composition according to previous studies (Maron et al. 2005; Brodie et

al. 2007; Bertolini et al. 2013). However, also these studies concluded, that not a single factor (e.g. temperature) but a combination of differences in meteorological parameters and chemical composition may shape airborne bacterial communities besides the relative importance of the origin of particles (Brodie et al. 2007; Bertolini et al. 2013).

In summary, we observed that a small number of bacterial taxa made up more than 75% of all bacterial sequences and these abundant taxa have also been reported in other bioaerosol studies from many different environments (urban, rural, forest, high alpine). This further supports the assumption of a bacterial core community in the atmosphere. In contrast to terrestrial samples, the impact of the underlying ecosystem seemed to be comparably low. The two most important environmental parameters that affected the spatial distribution of bacterial communities were temperature as a function of wind direction and the sampling location. Therefore, wind direction could be a good predictor for possible influences (marine, continental) but additional backward trajectories are needed for detailed information.

Temporal aspect

In a yearlong survey, marine airborne bacteria were sampled at Helgoland, analysed with the fingerprint method ARISA, and set in context with meteorological data. The sampling season had a clear influence on species richness and community structure, the latter additionally being influenced by wind directions. Similar patterns were observed for urban environments of Milan (Franzetti et al. 2011; Bertolini et al. 2013) and Colorado (Bowers et al. 2011b; Bowers et al. 2012). In these studies, seasonal differences were mainly attributed to plant-associated taxa such as Sphingomonadales in the summer months and soil-related and/or spore-forming taxa in the winter months (Bowers et al. 2009; Franzetti et al. 2011). Furthermore, high seasonal variation in bacterial community composition may be shaped by both shifts in atmospheric conditions and the local terrestrial environment (Bowers et al. 2012). The authors showed significant differences between the four seasonal sampling sets due to bacteria which are associated with the ground coverage. In autumn, winter and spring they detected bacteria, which were attributed to the snow coverage, whereas

summer samples were dominated by bacteria associated with the vegetation coverage. This conforms to the observed patterns in spatial distribution analysis of the current study. Although the DistLM calculation indicated that relative humidity and temperature were most important for the bacterial community structure, the overall explanatory power was very low. A combination of differential factors over the course of a year may thus be responsible for the observed variation. Similar conclusions were also drawn from other studies (Brodie et al. 2007; Bertolini et al. 2013).

High variability of bacterial communities is a common phenomenon and may change in timeframes ranging from days to even minutes (Fierer et al. 2008) and seasonal variability might even be higher (Maron et al. 2005; Maron et al. 2006; Brodie et al. 2007; Lee et al. 2010). The comparison of environmental factors in this study revealed a clear hierarchy in variability (intra-week < intra-month < intra-season < sampling period). In contrast, variability of bacterial community structures was always equally diverse (intra-week = intra-month = intra-season = sampling period). This clearly contrasts to results of Maron et al. (2006) for an urban site in France, who found a hierarchical order of variability in bacterial community structures (daily < weekly < seasonal). The authors concluded that anthropogenic influences may be responsible for the variability on a daily and weekly scale, whereas high seasonal variability was probably due to environmental factors. As the island of Helgoland is characterised by its remote location in the middle of the German Bight, anthropogenic influences should be comparably low while the influence of environmental parameters probably prevails. This might also explain the high variability on all timeframes considered.

Molecular fingerprint techniques proved to be helpful tools for the investigation of variability, dynamics, richness of bacteria and bacterial community structure the atmosphere (Li et al. 2010; Jeon et al. 2011). In addition, fingerprinting allows for fast and cost-efficient analyses of bacterial community structures, particularly in investigations with high sample sizes. However, it is restricted to the evaluation and comparison of general patterns, not allowing for direct identification of bacteria in the absence of subsequent analysis steps. Nonetheless, fingerprint methods can be

used for a pre-selection of samples, later being used for following analyses such as next generation sequencing (e.g. Krause et al. 2012).

Longterm data comparison: aerolised bacterial community structures vs. marine bacterial community structures

Recent studies from terrestrial environments indicated that airborne bacterial communities are largely related to the sampling location and the land use types (Bowers et al. 2011a; Bowers et al. 2011b; Bowers et al. 2012). For marine environments, Cho & Hwang (2011) compared water and bioaerosol samples and found an overlap in the detected taxa. This suggests that community composition of marine bioaerosols may also depend on their underlying water masses. However, their investigation used a DGGE approach which resulted in nearly 100 sequences for the analysis. Its validity for conclusions on the marine airborne bacterial community may thus be insufficient. Additionally, the results of the current investigation on the spatial distribution of marine airborne bacterial communities (see above) implicated only a low influence from the underlying ecosystem.

In order to test if underlying water masses also influenced the sampled bioaerosols in our yearlong survey (see *Chapter* 2), the gathered ARISA-profiles were compared to data from Helgoland Roads (Judith Lucas, unpublished data) for the period from August 2012 to February 2013. Water samples were filtered on 0.2 µm polycarbonate filter (GTTP, Millipore) corresponding to free-living bacteria.

All statistical analyses were performed with the computer software packages PRIMER 6 (PERMANOVA+ ad-on; PRIMER-E Ltd.) and GraphPad Prism 5.04 (GraphPad Software, Inc.). Based on their ARISA-profiles, the impingement sample data, membrane-filter sample data and water sample data (weekly sampling at Helgoland Roads) were compared. Similarity and diversity among the samples was calculated on presence/absence data of the ARISA-gel profiles with the Jaccard coefficient and visualised in a principle coordinate analysis (PCO). Calculated Jaccard coefficients were analysed with one-factorial permutational ANOVAs (PERMANOVA) (Anderson 2001) using the factor 'medium' (factors levels: water; air/filter; air/impingement). Additionally, calculated Jaccard coefficients of the

ARISA profiles were used for the comparison of variability within sampling weeks, sampling months, sampling seasons and over the total sampling period. Due to the weekly sampling, marine samples were only tested among months as well as seasons and over the whole sampling period. As sampling with the membrane-filter sampler started at the end of November, comparison between different seasons was impossible.

The results showed significant differences between the two aerosol sampling devices and the water samples ($p = 0.001$, Table 1) which was also clearly visible in the PCO plot (Figure 1). This indicates that bioaerosol communities at Helgoland may not mainly be affected by the subjacent marine bacterial communities. Therefore, the findings of studies conducted in terrestrial environments can probably not be generalised.

As shown in chapter 2, variability of the bacterial community composition within the impingement samples was equally high at all timeframes (Figure 2A). A similar pattern was found for the membrane-filter samples (Figure 2B). The variability of the water samples, in contrast, showed a clear hierarchical order (intra-month < intra-season < sampling period) (Figure 2C). Variability of bacterial communities in marine bioaerosol and marine water samples thus most likely comply with differential processes.

Similar to the findings of Cho & Hwang (2011), there may be an overlap in some OTUs between water and bioaerosol samples, although this cannot be confirmed without clear species identification. However, the current results strongly suggest that the captured aerosol bacteria/bacterial community mainly evict from other sources than the surrounding waters of Helgoland.

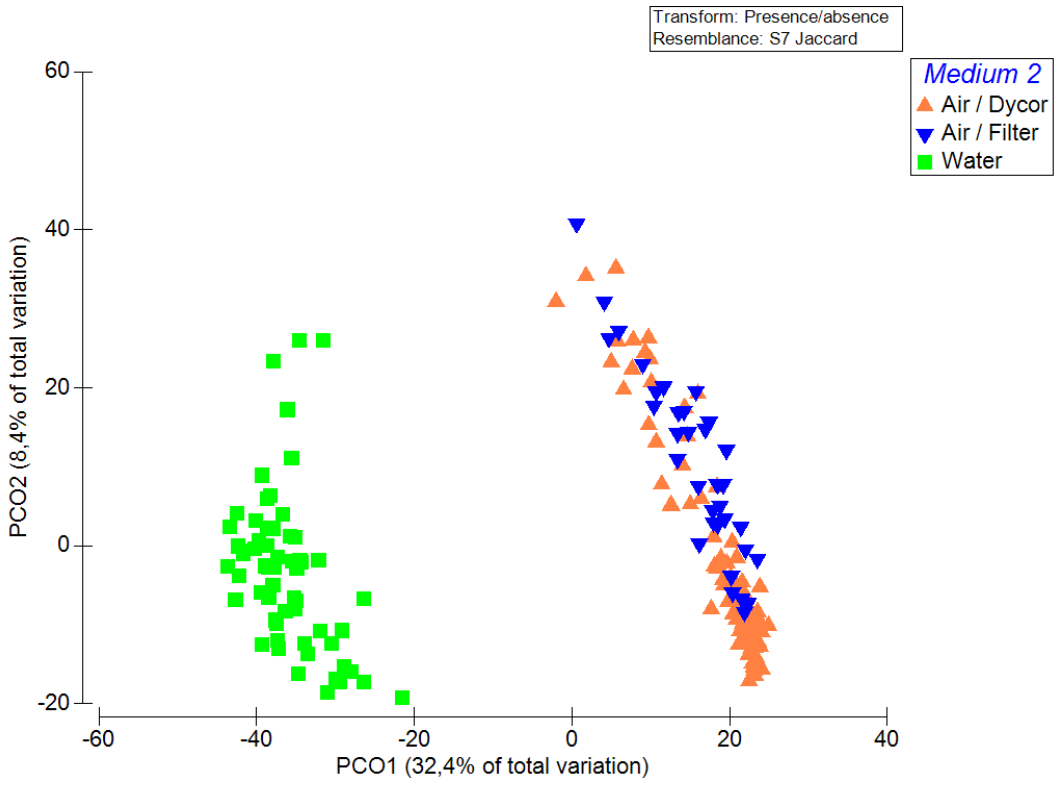

Figure 1 Principal coordinate analysis (PCO) based on the Jaccard dissimilarities of ARISA profiles of the impingement (orange) and membrane-filter samples (blue) with water samples (green). Sampling period reached from August 2012 to February 2013.

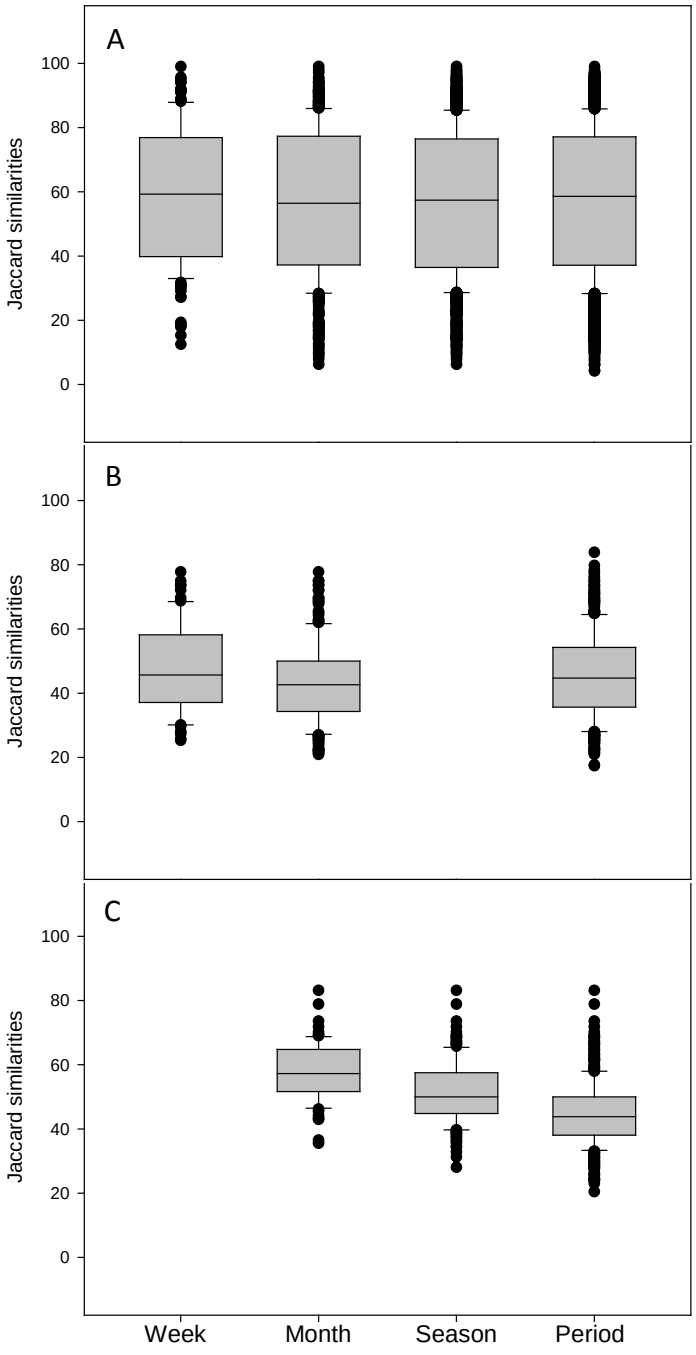

Figure 2 Variability of the response variable according to different time scales. Displayed are the intra-group Jaccard similarities for impingement (A) and membrane-filter bioaerosol samples (B) as well as water samples (C).

Table 1 PERMANOVA pair-wise comparison of the bacterial community structure of bioaerosol (impingement and membrane-filter) and water samples taken at Helgoland from August 2012 to February 2013.

Groups	t	p (perm)[1]
Air / Dycor vs. Air / Filter	2.3453	**0.001**
Air / Dycor vs.Water	7.6693	**0.001**
Air / Filter vs. Water	5.7198	**0.001**

[1]Significant results (*p (perm)* < 0.05) are highlighted in bold.

Comparison of quantification methods

The quantification of airborne bacteria represents an essential prerequisite for ecological studies, public health concerns (potential spreading of pathogens) and meteorological observation (potential to function as ice and water droplet condensation nuclei). The current evaluation compared the performance of two culture-independent methods - FLAPS and q-PCR. Additionally, in contrast to artificial environments of an aerosol test chambers, this is the first comparison of the two methods in an outdoor setting. The results of the q-PCR did not correlate with the direct counting of the FLAPS, neither for total particle count nor fluorescent particle count. Furthermore, both methods were each influenced by differential meteorological parameters. The two quantification methods seemed to be incomparable to each other.

When carried out in a biological safety cabinet, the comparison of a FLAPS against CFU count for the quantification of fungal spores showed a good correlation (Kanaani et al. 2008). Therefore, the FLAPS seems to be able to accurately count bioaerosol particles. However, as that comparison was run under controlled conditions, the findings may not apply for aerosol studies which select for particular groups of bioaerosol particles (e.g. bacteria and/or fungi) in the absence of a biological safety cabinet. The FLAPS counts all particles which exhibit fluorescent emission (all particles containing riboflavin and/or NAD(P)H). Therefore, it does not distinguish between pro- and eukaryotes. Accordingly, plant debris, microalgae and other particles were also counted, thus, distorting the general results. Quantitative

real-time PCR on the other hand specifically selected for bacterial DNA using a eubacterial primer set. Thus q-PCR allows for the quantification of different bioaerosol groups from the same dataset, also comparing the amount of different bioaerosol types. The observed non-correlation of q-PCR and FLAPS II is most likely due to a large number of non-bacterial fluorescent particles in the sampled bioaerosols. Quantitative real-time PCR, seems to be a good and reliable quantification method for specific bioaerosol types (e.g. bacteria, fungi). This is due to the possibility to employ different primer sets. At the same time, it is time efficient as many samples can be quantified within a single reaction, while the results are reproducible and comparable with each other. Accordingly, q-PCR was successfully applied in other investigations on airborne bacteria (e.g. Cho & Hwang 2011). Further comparison of quantification techniques is needed in order to establish standardised methods for future investigations.

Comparison of sampling devices

In addition to the variety of quantification methods (*see Chapter 3*), there is still a lack of standardised sampling methods for investigations on bioaerosol particles. Currently, many differential sampling techniques/devices are available and were applied in respective studies. However, it still remains unclear how and to which degree the obtained results of different studies are comparable. Inconsistencies among available studies may therefore either result from artefacts of different sampling methods or from true differences among the collected environmental samples. During the yearlong sampling campaign at the German Bundeswehr Tower on Helgoland (*Chapter 2*), a membrane-filter aerosol sampling device was added to the sampling routine from November to June. This commonly used type of sampling device consisted of a vacuum laboratory pump (N 820 FT.18 KFN Lab, Freiburg, Germany) with upstream build in airflow meter (massflow meter 4143, TSI, Shoreview, USA) and a triplicate filter holder. Despite its advantages (robust, easy-to-transport, cost-efficient), membrane-filter aerosol samplers have a lower air sampling rate compared to a high volume sampler (membrane-filter aerosol sampler: 4-10 l/min, High Volume Sampler: 12.5-800 l/min). Additionally, the sampled

bacteria might experience more stressful conditions: Griffin et al. (2011) compared an impingement sampler with a membrane-filter aerosol sampler based on colony forming unit (CFU) counts. The comparison of agar media showed that more colonies grew from impingement samples than from samples which were collected with membrane filters. However, as the authors did not identify the bacterial colonies, it remains unclear if the results were caused by mechanical damage of bacterial cells or if they were related to the lower sample volumes.

Despite the fact that the two bioaerosol samplers processed different amounts of air (higher turnover of the impingement sampler by the factor of 3), all obtained filters were treated in the same way followed by ARISA fingerprint analyses (for laboratory procedures see Chapter 2). In both cases 5 ng DNA was used for ARISA-PCR amplification, thus even lower DNA yields caused by different sampling methods should have been compensated. However, due to the potentially higher accumulation of DNA in the impingement sampler, rare bacteria were more likely to be sampled than in the membrane filter aerosol sampler.

Both data sets were then compared with regard to species richness (number of bands) and beta diversity (Jaccard coefficient). The membrane-filter aerosol sampler generally displayed significantly lower species richness (Mann-Whitney-U test: $p < 0.00001$). In addition, the direct comparison of the frequency of different amplicons revealed always lower abundances in the membrane-filter aerosol samples (Figure 3). Also the comparison of beta diversity between the two sampling devices showed that they are not correlated/related with each other (Relate: $p = 0.035$) which is also visible in the PCO (Figure 4).

Even though the samples were collected at the same location and time, the comparison of the membrane-filter aerosol sampler and the impingement sampler with their respective ARISA profiles revealed distinctly different bacterial community structures. Both devices, however, were not differently affected by environmental parameters.

Fahlgren et al. (2011) reported an overlapping of sequences of 16S rRNA clone libraries, suggesting that samples from different sampling devices (impingement sampler vs. biocapturer vs. membrane-filter aerosol sampler) can be compared with

each other to a certain degree. Hoisington et al. (2014), in turn, compared six different aerosol sampling devices (two passive filter samplers placed in the ventilation system and 4 different active samplers with different sampling volumes, reaching from 4 to 100 l/min) in respect to the comparability of the captured airborne microbial community with a high throughput NGS approach. Although the authors obtained similar results to Fahlgren et al. (2011) with an overlap between the bacterial communities of 25%, the statistical analysis of OTU composition and overall beta diversity revealed a selectivity of the sampling devices for different bacterial taxa. Samples, which were collected on different days but with the same sampling device, were much closer related than samples which were taken on the same day but with different sampling devices. This conforms to the findings of the current study using ARISA profiles. Different sampling devices probably capture the most abundant OTUs at the same sampling location in a similar way but emphasise certain bacterial groups. Previous studies have shown that even when sampling devices have nearly the same intake flow rate, they select for distinctly different bacterial communities (Hoisington et al. 2014: 10 to 12.5 l/min). Thus, the selectivity cannot exclusively be attributed to differences in the volumes of sampled air.

The processing of comparably lower amounts of air with a filter sampler inevitably requires much longer time periods for the sampling of high volumes. As long sampling periods can severely damage the gathered bacteria samples and might result in a subsequent degradation of DNA (Griffin et al. 2011), differences between sampling devices could also reflect differences in overall sampling duration. Therefore, high volume turnover samplers should be employed for future investigations in order to maximise integrity of detection.

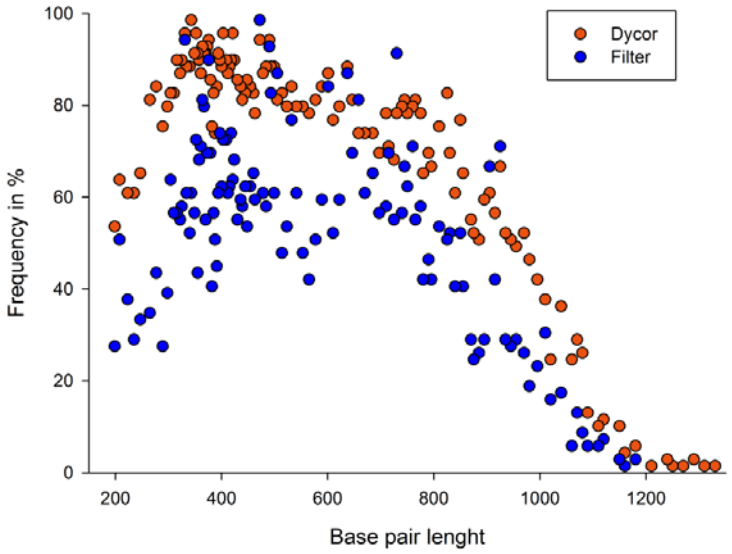

Figure 3 Comparison of base pair length frequencies between impingement sampler (orange) and membrane-filter aerosol sampler (blue).

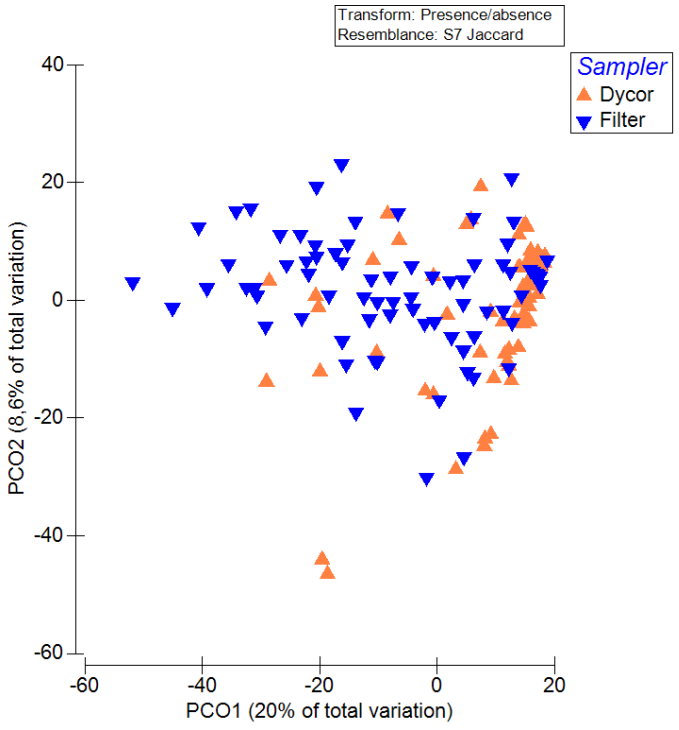

Figure 4 Principle coordinate analysis (PCO) based on the Jaccard dissimilarities of ARISA profiles of sampling two sampling devices membrane aerosol sampler and impingement sampler. Symbols represent the different sampling devices (blue: membrane-filter sampler; orange: Impingement sampler).

Conclusions

In the current thesis, the spatial and temporal dynamics of bacterial communities in marine bioaerosols were investigated. Statistical analyses were applied to identify the most influential environmental factors, gathering valuable basic knowledge for this understudied area which suffers from a lack of standardised methods. Culture-independent methods were deployed in order to minimise any possible biases.

The analysis of the spatial distribution of marine airborne bacterial communities showed that northern European waters feature a highly diverse bacterial community. At the current state, this high diversity is barely assessable even by means of modern techniques such as high-throughput sequencing approaches. The importance to implement modern approaches (e.g. high-throughput sequencing) for coverage of the complete bacterial diversity is therefore clearly underlined.

The analyses of marine bioaerosols both on the spatial and temporal scale identified the wind direction to be one of the main influencing factors for bacterial community structure, which is an indication for strong heterogeneous sources of airborne bacteria. This is corroborated by the detection of many bacterial groups which are typically associated with diverse environments (e.g. plant, soil, human, marine). When looking at the overall spatial distribution, the sampling location had a clear effect on the bacterial community composition. Additionally, the sampling month/season affected the temporal variation, which was probably due to a shift in the species composition, although it currently remains unclear which bacteria taxa were responsible for this pattern.

In contrast to terrestrial environments, marine airborne bacterial communities featured a distinct bacterial community which does not seem to be related to the underlying ecosystem. Marine bioaerosols, instead, seem to feature a bacterial core community which may evict from strong areal sources. Members of this core community such as plant and soil associated bacteria as well as potential pathogens, may then be transported via air currents over long distances, potentially functioning as ice and water droplet condensation nuclei.

This work further emphasises the given problem of a lack of standardised methods (sampling and analysis methods) for investigations on bioaerosols. The choice of the

sampling device was shown to have a strong influence on the outcome of a sampled bacterial community which can have severe impact on subsequent analyses. This also applies to the choice of the quantification method as only few of the available quantification methods are suitable for bioaerosol studies in outdoor environments. Thus, there is an urgent need for standardisation of sampling and quantification to allow for comparability among different studies. This would be an essential step towards proper understanding of bioaerosols on a global scale.

All things considered, the current findings elucidated the highly diverse bacterial communities in marine bioaerosols which were subject to strong temporal and spatial fluctuations. The evaluation of modern methodologies to assess this diversity clearly underlined the urgent need for further investigations. Therefore, the current thesis represents an important step towards a better understanding of patterns and processes within airborne bacterial communities, thereby advancing our knowledge on bioaerosols and their driving factors.

Summary

Airborne bacteria are omnipresent in the atmosphere, having a substantial impact on ecological patterns (distribution of organisms), medical concerns (spreading of pathogens) and climate functioning (condensation nuclei). Despite its unquestionable importance for ecosystem functioning, airborne bacteria, and particularly those in marine bioaerosols, are still clearly understudied. The absence of standardised methods further complicates proper comparison of the few studies on bioaerosols that exist to date.

The current thesis aimed to gather basic knowledge about abundances and composition of bacterial communities in marine bioaerosols and how their spatial and seasonal dynamics may be affected by environmental factors. Furthermore, a complementing evaluation of quantification methods was performed to identify suitable procedures with the potential to be used in standardised investigations. All analyses were carried out using culture-independent methods (pyrosequencing, ARISA, q-PCR) in order to avoid any possible biases which would have been introduced by culture-dependent methods.

The spatial aspect was studied in samples which were taken during a cruise with the research vessel Heincke from the North to Baltic Sea. A high-throughput sequencing approach revealed a highly diverse bacterial community in the samples with taxa that were typically associated with different areas of origin (plant, soil, marine, human). Wind direction and the sampling location were the most influential factors for the bacterial community composition. The current findings further support the existence of a bacterial core community in the atmosphere which may have a stronger influence on the bacterial community composition than the respective underlying ecosystem.

A continuous yearlong sampling was carried out at the remote island of Helgoland to investigate the temporal aspects of marine bioaerosols. Changes in the bacterial community composition were displayed using the culture-independent fingerprint method ARISA and analysed in context of the environmental data. In addition to a seasonal effect which was probably caused by a species shift, the wind direction again had a clear effect on the airborne bacterial communities. Further species

identification is needed to elucidate which bacteria might have been responsible for the shift. Furthermore, there was a continuously high variability in bacterial community composition, regardless of the regarded timespan (intra-week = intra-month = intra-season = whole sampling period). This finding is a clear indication for an unstable airborne bacterial community at Helgoland.

Associated with the yearlong sampling, two quantification methods were compared: a q-PCR and the direct counting of fluorescence particles using a FLAPS. The two quantification methods generated differential results. This was most likely caused by the FLAPS counting all biological particles (containing riboflavin and/or NAD(P)H), whereas the q-PCR specifically detected for bacteria. Thus, FLAPS does not seem to be well suited for outdoor investigations, underlining the urgent need for the standardisation of bioaerosol quantification. This important task needs to be addressed by future studies in order to facilitate comparable investigations, promoting our understanding of airborne bacteria in bioaerosols.

All things considered, the current findings elucidated the highly diverse bacterial communities in marine bioaerosols which were subject to strong temporal and spatial fluctuations. The evaluation of modern methodologies to assess this diversity clearly underlined the urgent need for further investigations. Therefore, the current thesis represents an important step towards a better understanding of patterns and processes within airborne bacterial communities, thereby advancing our knowledge on bioaerosols and their driving factors.

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Statutory Declaration

I, Jasmin Sabrina Seifried, hereby declare that I have written this PhD thesis independently, unless where clearly stated otherwise. I have used only the sources, the data and the support that I have clearly mentioned. This PhD thesis has not been submitted for conferral of degree elsewhere.

Signature