

ORIGINAL ARTICLE

Diversity and dynamics of rare and of resident bacterial populations in coastal sands

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Coastal sands filter and accumulate organic and inorganic materials from the terrestrial and marine environment, and thus provide a high diversity of microbial niches. Sands of temperate climate zones represent a temporally and spatially highly dynamic marine environment characterized by strong physical mixing and seasonal variation. Yet little is known about the temporal fluctuations of resident and rare members of bacterial communities in this environment. By combining community fingerprinting via pyrosequencing of ribosomal genes with the characterization of multiple environmental parameters, we disentangled the effects of seasonality, environmental heterogeneity, sediment depth and biogeochemical gradients on the fluctuations of bacterial communities of marine sands. Surprisingly, only 3–5% of all bacterial types of a given depth zone were present at all times, but 50–80% of them belonged to the most abundant types in the data set. About 60–70% of the bacterial types consisted of tag sequences occurring only once over a period of 1 year. Most members of the rare biosphere did not become abundant at any time or at any sediment depth, but varied significantly with environmental parameters associated with nutritional stress. Despite the large proportion and turnover of rare organisms, the overall community patterns were driven by deterministic relationships associated with seasonal fluctuations in key biogeochemical parameters related to primary productivity. The maintenance of major biogeochemical functions throughout the observation period suggests that the small proportion of resident bacterial types in sands perform the key biogeochemical processes, with minimal effects from the rare fraction of the communities.

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Introduction

Marine coasts represent highly dynamic ecosystems where the atmosphere, continents and the ocean interact. Permeable sands constitute the dominant sediment type on continental shelves (Emery, 1968; Boudreau *et al.*, 2001) and have a central role for global carbon and nutrient cycles. They act as biocatalytic filters for various types of materials

advected by currents and winds, including dissolved and particulate organic matter derived from living and dead biomass of terrestrial or marine origin (de Beer *et al.*, 2005). Sandy sediments are also constantly subjected to biotic (e.g., bioturbation) and abiotic disturbances (e.g., mixing by currents, seasonal and tidal temperature fluctuation, anoxia; Boudreau *et al.*, 2001). They may also host human pathogens, depending on the human impact by, for example, recreational use of beaches, and temperature anomalies (Ruppert *et al.*, 2004; Dinsdale *et al.*, 2008).

The high diversity of niches provided by the sand habitat supports a rich community of bacteria fluctuating with environmental variations (e.g., Hunter *et al.*, 2006; Sørensen *et al.*, 2007; Mills *et al.*, 2008; Rusch *et al.*, 2009; Gaidos *et al.*, 2011). Yet, the dynamics of environmental parameters in coastal sands could still be a challenge to many microbial populations, selecting for few, but tolerant

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and well-adapted resident types (Gaidos *et al.*, 2011). Some of these produce extracellular polymeric substances to attach to sand grain surfaces. The resulting biofilm habitat can be considered as an active bioreactor accumulating diverse microbial populations, their extracellular enzymes, nutrients and organic matter from various origins (Flemming and Wingender, 2010). The pore volume between the sand grains represents a place of constant particle exchange due to turbulent flow of currents and pore-water advection, which may induce significant fluxes of particulate organic matter (Stoodley *et al.*, 2005). It remains yet unknown whether sand grain-associated biofilms trap microbes from terrestrial sources and from the water column flushing through the sand, and if they are a source of diversity to the sea around them. As they represent a transition zone between land and ocean characterized by high mixing rates, the presence of many rare bacterial types could be expected. In coarse-grained reef sediments, it has been shown that the typical patterns of rare microbial types found in DNA-based libraries (i.e., the characteristic long tail in rank-abundance curves; Pedrós-Alió, 2007) could not be observed in RNA-based libraries, suggesting that the rare biosphere may be inactive (Gaidos *et al.*, 2011). So far, few studies have examined the structure and ecology of microbial communities in coastal sediments, and these were mostly based on low-resolution community fingerprinting approaches (Urakawa *et al.*, 2000; Bertics and Ziebis, 2009; Böer *et al.*, 2009b). Although those molecular techniques have provided a first description of abundant populations, they generally did not describe rare populations that might represent considerable diversity (Acinas *et al.*, 2004). High-throughput sequencing strategies such as shotgun sequencing (Venter *et al.*, 2004) or pyrosequencing (Sogin *et al.*, 2006) allow a deeper exploration of microbial diversity. Pyrosequencing of ribosomal genes has already permitted the description of the rare microbial biosphere in several types of ecosystems, such as surface and deep-sea water (Sogin *et al.*, 2006), extreme environments (Huber *et al.*, 2007; Brazelton *et al.*, 2010), or the human hand surface (Fierer *et al.*, 2008). Patterns of the rare biosphere in the Arctic Ocean have been related to differences in water masses and attributed to dispersal limitation (Galand *et al.*, 2009). Here we focus on temporal fluctuations of resident and of rare microbial types in coastal sands, and on their link with fluctuations in environmental parameters.

Including the rare microbial biosphere in ecological analyses is challenging when considering the potential for technical noise in pyrosequencing data (Quince *et al.*, 2009; Kunin *et al.*, 2010). Here we apply a new analytical method to assess the effects of rarity on community structure and ecological interpretation (Gobet *et al.*, 2010). The main objective of this study was to explore the putative factors structuring bacterial diversity in temperate coastal

sands over six sampling dates within a year (2005–2006), and over different sediment depths (0–5 cm, 5–10 cm, 10–15 cm). For this purpose, we combined several data sets from the Long Term Ecological Research (LTER) Station, Sylt, Germany, including 454 massively parallel tag sequencing (MPTS) targeting the V6 region of the 16S rRNA gene (Gobet *et al.*, 2010) and concurrent environmental surveys (e.g., nutrients, enzymatic activities, bacterial production, weather and water parameters; Böer *et al.*, 2009a, b), to obtain as complete a description as possible of the changes in the bacterial community in their environmental context. We falsified the following hypotheses: (1) bacterial communities in coastal sands have the same composition across sediment depth and in comparison with the overlying water column, (2) as a biofilm community, their temporal fluctuation is low, and (3) the rare microbial biosphere in sands does not respond to environmental changes.

Materials and methods

Study site, sampling procedures and contextual parameters

Detailed sample processing and environmental measurements have been published elsewhere (Böer *et al.*, 2009a). Briefly, sediment samples were repeatedly collected every 2–3 months on a shallow (0.5–2.5 m), subtidal sand flat of the LTER site at the island of Sylt in the North Sea (55° 00′ 47.7″ N, 8° 25′ 59.3″ E) between February 2005 and March 2006. Sandy cores of 15 cm were directly sectioned into three 5 cm layers. Subsamples were stored at –20 °C for DNA extraction or used for the measurement of contextual parameters (extracellular enzymatic activities, nutrients, pigments, carbohydrates, bacterial cell counts; Böer *et al.*, 2009a). Water column data consisting of chlorophyll *a*, pH and water temperature, were obtained from the Sylt LTER time series (van Beusekom *et al.*, 2009), and data on wind speed was obtained from the German Weather Service (Deutsche Wetterdienst; weather station of List at Sylt).

To compare the communities of sediments, pore water and overlying water, at one time, additional samples were taken at Sylt Königshafen in April 2008 (sediments 55° 2′ 28″ N, 8° 24′ 26″ E; seawater 55° 1′ 41″ N, 8° 26′ 10″ E). Seawater of 1 l was successively filtered through 10 µm and 0.2 µm filters (type GTTP; diameter 47 mm) before DNA extraction. Pore water of the upper 5 cm of the sediment was separated from the sand grains (with bacterial biofilms) by low-speed centrifugation (10 min at 1801 g, at 4 °C) through GF-C filters (47 mm). The resulting ‘dry’ sediment (about 8 g) and 2-ml pore water (filtered through 0.2-µm GTTP-type filters; diameter 47 mm) were subsequently used for DNA extraction.

DNA extraction and 454 MPTS

For samples taken in 2008, DNA was extracted using an UltraClean Soil DNA Isolation Kit (MoBio

Laboratories Inc., Carlsbad, CA, USA) from (1) ca. 10 g of homogenized wet sediment separated in the dry sand and the pore water, and (2) 1 l of water filtered through 0.2- μ m filters (pore water and water column) cut into pieces with a sterilized cutter. DNA was further stored in a final volume of 50–100 μ l of Tris-EDTA buffer. To study temporal fluctuations and vertical community patterns in sediments, DNA from samples collected between 2005–2006 were similarly extracted from 1 g of sediment, as described previously (Böer *et al.*, 2009b).

DNA was deposited within the International Census of Marine Microbes project (<http://icomm.mbl.edu/>), where it was amplified using primers targeting the V6 region of the bacterial 16S rRNA gene, and which included 454 Life Science's A or B sequencing adapter according to Sogin *et al.* (2006). Pyrosequencing was performed on a Genome Sequencer 20 system (Roche, Basel, Switzerland) at 454 Life Sciences (Branford, CT, USA) by primer extension (Margulies *et al.*, 2005), yielding on average 60-bp long fragments after removing primer sequences.

Sequence analysis

Data from the 454 MPTS were retrieved from the publicly available 'Visualization and Analysis of Microbial Populations Structure (VAMPS)' website (<http://vamps.mbl.edu/>; projects AB_SAND_Bv6 and ICM_FIS_Bv6) in the framework of the International Census of Marine Microbes project. Sequences were taxonomically assigned by an automatic annotation pipeline (Sogin *et al.*, 2006), using several known databases (Entrez Genome, RDP, SILVA), allowing a taxonomic annotation of up to 94% of the data set. In our study, analyses were based on a definition of operational taxonomic units (OTU) as unique (i.e. two sequences belong to two different OTU_{unique}, when they differ by at least one base) to keep a consistent definition throughout. Other classifications used in the study are indicated as follows: OTU_{all} represents all, un-annotated sequences, PyroNoise_{0%} and PyroNoise_{3%} correspond to the PyroNoise-corrected data sets at 0% and 3% sequence identity, respectively (Quince *et al.*, 2009), OTU_{all}—x% rare, which represent the OTU_{all} data set truncated of its x% rarer OTU_{unique} according to Gobet *et al.* (2010).

Taxa-environment relationships

To investigate taxa-environment relationships, most of the parameters (except pH, water temperature, wind speed and salinity) were log₁₀-transformed, whereas the community matrices (OTU_{all} data set, and the resident single-sequence OTU relative (SSO_{rel}) data sets or the (potential) pathogen sequence abundance matrix (including the genera *Parachlamydia*, *Arcobacter*, *Francisella*,

Acinetobacter, *Rickettsiella*, *Pseudomonas* and *Ralstonia*) were standardized by Hellinger transformation (Legendre and Gallagher, 2001). A forward selection (based on a canonical redundancy analysis algorithm and 999 Monte Carlo permutation tests) of the environmental factors was done to find the set of parameters that could best explain the variation in the community table. The best-fitting models were chosen using the Akaike Information Criterion. Canonical variation partitioning (Borcard *et al.*, 1992; Ramette and Tiedje, 2007b) was then applied on the community data to disentangle the specific effects of environmental variables (pigments, nutrients, extracellular enzymatic activities, cell abundance) selected previously and of their covariation on microbial community structure. Statistical analyses were carried out using the R statistical environment (R version 2.10.0, R Development Core Team, 2009), using the vegan (Oksanen *et al.*, 2009), gplots (Bolker *et al.*, 2009) packages, and custom R scripts.

Results and discussion

The bacterial community composition of coastal sands

The three compartments, sand, pore water and the overlying water column, may be expected to share a large proportion of the same microbial assemblages, because the pore space of permeable sands is constantly flushed by the overlying water, trapping detritus and living cells from the water column (Boudreau *et al.*, 2001). We compared bacterial communities from one large, well-mixed sample of each compartment (sand, pore water and water column) at one time, to investigate the similarity in community composition between those coastal compartments, which are constantly mixed by wind and waves. However, microscopic observations (Figure 1a) confirm that sand grains are covered by a biofilm consisting of cells embedded in extracellular polymeric substances, and that there is potentially little exchange with cells flowing through the sediment (Rusch *et al.*, 2001). At any time, the pore water contains less than 0.2% of the cell abundance associated with the sand grains (this study and Rusch *et al.*, 2003). Accordingly, at that specific sampling time, we also found a rather low proportion of shared bacterial populations between the sand and the water column, at the level of both community composition and evenness (Figures 1b and c, Supplementary Figure S1). Only 2–3% of all unique OTU_{unique} (sequences from the original OTU_{all} data set that have at least one nucleotide difference) were shared between the three compartments, confirming previous findings (Epstein *et al.*, 1997; Llobet-Brossa *et al.*, 1998).

The Sylt (North Sea) water column was mainly dominated by *Bacteroidetes* and by the *Alpha*- and *Gamma*-subdivisions of the *Proteobacteria*, confirming previous results on the basis of fluorescence

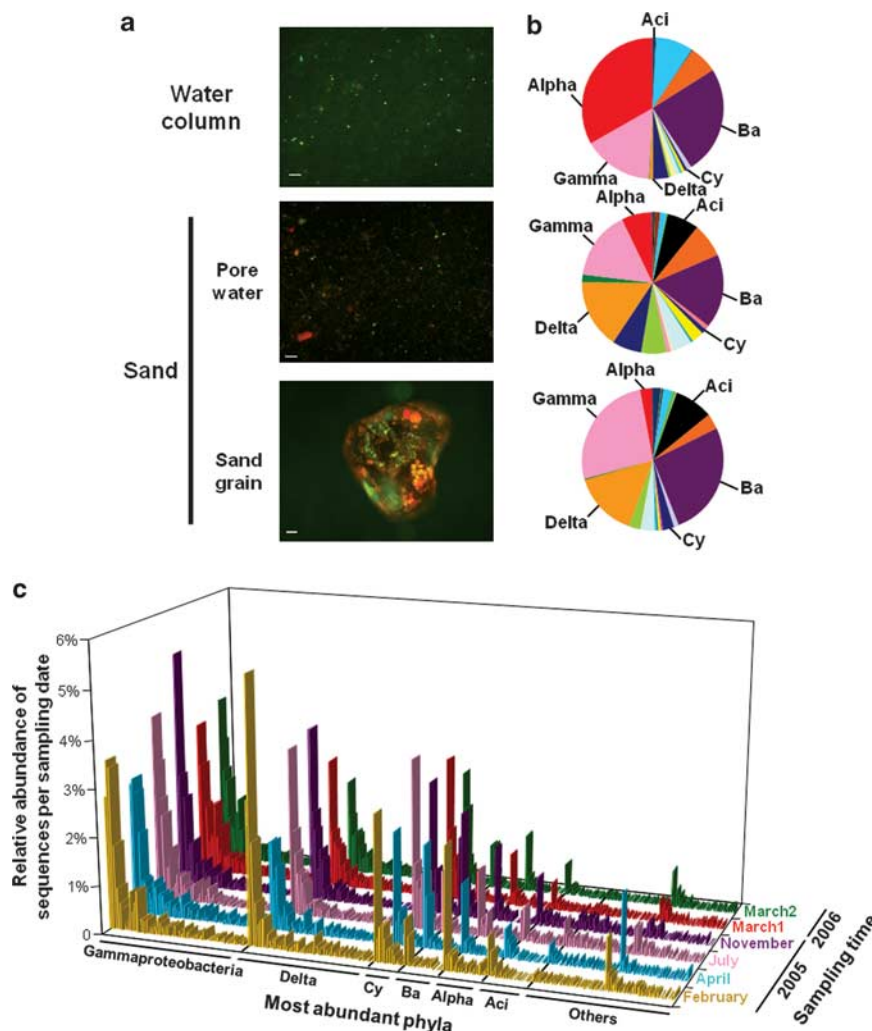


Figure 1 Microbial community distribution in the sand and the water column. (a) From top to bottom: acridine orange staining of bacteria in the water column, the pore water and on the surface of a sand grain (scale bar = 50 μ m). (b) Relative number of sequences in different compartments for the top 5-cm sand layer and in the overlying water column in April 2008. Here, the phylum level was chosen for illustrative purposes. (c) Sequence distribution in the sand over time, in which each bar represents an OTU_{unique} (only OTU_{unique} occurring more than 100 times in the whole data set are shown). The *Proteobacteria* phylum was further split into its corresponding classes, for example, *Alpha*, *Gamma*, *Delta*; *Cy*, *Cyanobacteria*; *Ba*, *Bacteroidetes*; *Aci*, *Acidobacteria*; *Others*: *Actinobacteria*, *NA*, not annotated *Proteobacteria*, *Planctomycetes*, *Chloroflexi*, *Verrucomicrobia*, *WS3*, *Firmicutes*, *Lentisphaerae*, *Deferribacteres* and *Gemmatimonadetes*.

in situ hybridization and 16S rRNA gene-based clone libraries (Glöckner *et al.*, 1999; Eilers *et al.*, 2000; Zubkov *et al.*, 2002). Sequences of the phyla *Verrucomicrobia* and *Actinobacteria* were also abundant (Figure 1b). The top 5 cm of Sylt sands were dominated by *Bacteroidetes*, *Gammaproteobacteria*, *Deltaproteobacteria* and *Planctomycetes*, also in accord with previous clone libraries obtained from coastal sediments of the Wadden Sea (Llobet-Brossa *et al.*, 1998; Musat *et al.*, 2006), or other permeable sediments from shelf sands from South Atlantic Bight and coastal sediment in Hawaii (Hunter *et al.*, 2006; Sørensen *et al.*, 2007). In contrast, tropical coral reef sands were less consistent, with a dominance of *Proteobacteria*, *Actinobacteria* (Gaidos *et al.*, 2011), or *Alphaproteobacteria*

and *Spirochaetes* (Schöttner *et al.*, 2011) in the community. *Acidobacteria* sequences were abundant in temperate (Figure 1b, Supplementary Figure S2) and tropical sands (Gaidos *et al.*, 2011; Schöttner *et al.*, 2011). At the phylum level, the pore water microbial composition resembled the sand community with a dominance of *Bacteroidetes*, *Gammaproteobacteria*, *Deltaproteobacteria* and *Acidobacteria* (Figure 1b). At the OTU_{unique} level, substantial differences were detected between the pore water and the sand-associated bacterial community or the water column (Supplementary Figure S1C). The further analysis of temporal fluctuations was restricted to wet sands containing both the biofilm community, as well as the pore water types. Certainly, temporal variation and the role of the

environment in the exchange of microbial communities between land and sea, intertidal and subtidal flats with their different physical compartments deserve further studies.

The characterization of coastal sand communities over six sampling dates (Figure 1) provided a total of 197 684 rRNA gene sequences, corresponding to 27 630 OTU_{unique}. Per sample, sequences ranged from about 5000–19 000, corresponding to 496–2993 OTU_{3%} (i.e., OTU defined at 3% sequence difference after denoising the V6 sequences by PyroNoise; Supplementary Table S1). Noticeably, the high bacterial richness estimates for coastal sands were comparable to 454 MPTS-based estimates reported for coarse carbonate sands from coral reefs (Gaidos *et al.*, 2011), soils (Roesch *et al.*, 2007), marine sediments and crusts (Sogin *et al.*, 2006; Huber *et al.*, 2007; Brazelton *et al.*, 2010). A high, yet unexplored diversity in these habitats was already previously proposed based on clone libraries (Hunter *et al.*, 2006; Mills *et al.*, 2008; Rusch *et al.*, 2009). In addition, here we found that total sequence and OTU numbers systematically increased with depth from 0–15 cm at most of the sampling dates (Supplementary Table S1). A positive relationship between sediment depth and bacterial diversity was previously observed using different fingerprinting techniques (Urakawa *et al.*, 2000; Böer *et al.*, 2009b), and explained by increasing physico-chemical stability of the habitat. The *Delta*- and *Beta*-subdivisions of the *Proteobacteria*, *Deferribacteres*, *Spirochaetes* and *Nitrospira*, all showed an increasing OTU richness with depth. In contrast, *Cyanobacteria* and *Bacteroidetes* decreased with depth (Supplementary Figure S2).

Turnover of bacterial diversity with sediment depth and time

The phylum to class levels are commonly used to describe the diversity of bacterial communities on the basis of whole-cell fluorescence *in situ* hybridization or 16S-based clone libraries (Llobet-Brossa *et al.*, 1998; Musat *et al.*, 2006). At those levels, microbial community patterns in sands mostly remained unchanged over time and sediment depth (Supplementary Figure S2). However, drastic changes in bacterial community composition were evidenced when finer taxonomic resolutions were used; overall, only 152 OTU_{unique} (0.55% of the total number of 27 630 OTU_{unique}) were present in all samples at all times, and their sequence abundance ranged from 54–6550. Also, only 3–5% of all OTU_{unique} within a sampling depth layer were present at all times (Table 1). The majority (77%) of these OTU_{unique}, which we define as resident OTU, consisted of the phyla *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Cyanobacteria*, *Alpha*-, *Delta*- and *Gammaproteobacteria* and *Verrucomicrobia*. For the OTU_{all} data set (i.e., data set with all OTU_{unique}), a low percentage of OTU_{unique} were

shared between sediment layers or any two sampling dates. Only about 20% of sequences were shared between the deeper layers and the upper layer (Supplementary Figure S3A), the communities of the two deeper layers were slightly more similar (Figure 2a). Moreover, resident OTUs seemed to be characterized by a high and relatively stable number of sequences (Figure 3b). About 70% of the resident OTUs had abundances of more than 10 sequences per sample (Table 1, Figure 3b), suggesting that they belonged to archetypal sand microbial communities.

When time was considered alone, only 18–37% of OTU_{unique} were found to be shared between any two sampling times (Figure 2b, Supplementary Figure S3A). This indicates that a very large fraction of the community may be constantly replaced. Yet, the fact that the turnover rate did not increase with sampling time suggests that some populations vanished and reappeared during the investigated time period (Supplementary Figure S3). Some of the most sequence-abundant groups were found to be positively correlated with time (Figure 4); for example, *Gammaproteobacteria* and *Planctomycetes*, which followed the seasonal fluctuations in cell abundances observed in Sylt sediment (Musat *et al.*, 2006). Remarkably, the fluctuation within a month (beginning and end of March 2006) was almost as high as within a year. This could be explained by the observed large environmental variations that occurred in March 2006, starting with cold temperatures and high nutrient levels at the end of the winter period, and ending with spring blooms and stormy conditions (Böer *et al.*, 2009b).

An interesting aspect is the abundance and role of the resident, potentially sand-biofilm associated populations versus rare and transient populations getting advected through the sands. Indeed, in our data set, more than 50% of the OTU_{unique} present at all times in the three depth layers were members of the *Gammaproteobacteria* and *Deltaproteobacteria*, two classes representing up to 23% and up to 10%, respectively, of the total cell counts in such sands (Musat *et al.*, 2006). But also, 40% of all OTU_{unique} appearing only once in a given sample or in the whole OTU_{all} data set belonged to these classes. Noticeably, the rare members of dominant classes never became abundant at any sampling time, similarly to observations made in Arctic waters (Galand *et al.*, 2009).

Observing a high turnover for a substantial fraction of the bacterial community in marine sands raises several questions about the ecological significance of these dynamics. Large turnover in community composition could be due to various phenomena, which are not mutually exclusive, such as: (1) migration of non-resident bacterial populations into and out of an ecosystem (Sloan *et al.*, 2006). This could be due to the rapid physical transport processes prevailing in marine coastal sand ecosystems. (2) High adaptability of microbial communities to ever-changing and complex

Table 1 Temporal patterns of OTU variation^a

	<i>OTU_{all}</i>	<i>Sequence similarity thresholds for PyroNoise-corrected data</i>		
		<i>PyroN_{0%}</i>	<i>PyroN_{3%}</i>	<i>PyroN_{5%}</i>
<i>Number of OTU (%)^b</i>				
Total	19 785 (100)	9036 (100)	7668 (100)	5927 (100)
SSO _{abs}	13 676 (69)	5272 (58)	3970 (52)	2624 (44)
SSO _{rel}	3735 (19)	2136 (24)	2268 (30)	2087 (35)
DSO _{abs}	1108 (6)	697 (8)	536 (7)	386 (6)
TSO _{abs}	236 (1)	187 (2)	170 (2)	133 (2)
With ≥5 sequences minimum in any sample	203 (1)	124 (1)	125 (1)	135 (2)
With ≥10 sequences minimum in any sample	106 (0.5)	67 (0.7)	67 (0.9)	69 (1)
<i>Temporal patterns^c</i>				
Feb Apr Jul Nov Mar 1 Mar 2				
■ ■ ■ ■ ■ ■	654 (3)	394 (4)	412 (5)	443 (7)
■ ■ ■ ■ ■ ■	29 (0.2)	11 (0.1)	12 (0.2)	10 (0.2)
■ ■ ■ ■ ■ ■	157 (0.8)	110 (1)	116 (1)	106 (2)
■ ■ ■ ■ ■ ■	34 (0.2)	21 (0.2)	23 (0.3)	23 (0.4)
■ ■ ■ ■ ■ ■	32 (0.2)	18 (0.2)	18 (0.2)	19 (0.3)
■ ■ ■ ■ ■ ■	1998 (10/9) ^d	685 (8/6)	515 (7/5)	304 (5/4)
■ ■ ■ ■ ■ ■	1371 (7/6)	622 (7/6)	492 (6/5)	345 (6/5)
■ ■ ■ ■ ■ ■	1807 (9/8)	704 (8/7)	554 (7/6)	349 (6/5)
■ ■ ■ ■ ■ ■	3657 (18/17)	1571 (17/15)	1171 (15/13)	821 (14/11)
■ ■ ■ ■ ■ ■	3539 (18/16)	1706 (19/15)	1301 (17/13)	915 (15/12)
■ ■ ■ ■ ■ ■	2833 (14/13)	1018 (11/9)	767 (10/8)	501 (8/7)
<i>Type of temporal relationships^e</i>				
Linear (total)	490 (2)	287 (3)	284 (4)	261 (4)
Linear increase	375 (2)	231 (2)	230 (3)	215 (4)
Linear decrease	115 (0.6)	56 (0.6)	54 (0.7)	46 (0.8)
Quadratic (total)	227 (1)	113 (1)	114 (1)	103 (2)
Quadratic positive	208 (1)	100 (1)	97 (1)	90 (1)
Quadratic negative	19 (0.1)	13 (0.1)	17 (0.2)	13 (0.2)

Abbreviations: DSO_{abs}, double sequence OTU absolute; OTU, Operational Taxonomic Units; PyroN, PyroNoise; SSO_{abs}, single sequence OTU absolute, SSO_{rel}, single sequence OTU relative; TSO_{abs}, triple sequence OTU absolute; VAMPS, Visualization and Analysis of Microbial Populations Structure.

^aSequences from the first top 10-cm sediment layers pooled were processed with the VAMPS pipeline and with the PyroNoise algorithm to remove pyrosequencing and PCR amplification artifacts. Thresholds from 0–5% sequence similarity were used to define OTU.

^bNumbers of sequences are given for single-sequence OTU occurring in the whole data set (SSO_{abs}) or at least in one sample (SSO_{rel}), double sequence OTU (DSO_{abs}), or triple sequence OTU (TSO_{abs}) in the whole data set. Figures in parentheses correspond to the percentage of the total number of sequences in the first top 10-cm sediment layers.

^cOccurrence patterns were defined as the presence (black square) or absence of OTU at specific sampling dates from February 05 (Feb), April 05 (Apr), July 05 (Jul), November 05 (Nov), beginning of March 06 (1 Mar), to end of March 06 (2 Mar).

^dThe second value in parentheses indicates the respective percentage of SSO_{abs}.

^eDifferent patterns of OTU abundance were examined by specifying linear or quadratic models of change with time. A positive quadratic relationship with time implies a U-shape relationship, which is associated with high abundance at the beginning and at the end of the study, and low abundance observed at the third and fourth sampling times. A negative quadratic relationship would be conversely described by an inverted U-shape abundance function over time.

environments, as previously observed in the laboratory (Rosenzweig *et al.*, 1994). (3) Emergence of latent ‘rare’ prokaryotic stages that may become dominant when appropriate conditions are met (Pedrós-Alió, 2006). This fluctuation from rare to dominant types may be supported by the ‘seed bank’ hypothesis (Finlay, 2002). (4) Finally, the presence of extracellular DNA could also have strongly impacted our estimates of community turnover. This could be possible because, first, sands are known to act as natural filters that concentrate particles and DNA in suspension (Naviaux *et al.*, 2005). Second, our molecular approaches did not differentiate between DNA from living cells and extracellular DNA settling from seawater (Corinaldesi *et al.*, 2005). However, the OTU_{unique} dissimilarity between water

column and sand described above may indicate that the described bacterial community is typical of the sand ecosystem, and that a large effect of extracellular DNA on our results is not likely. To further test hypotheses 1–3, we investigated the contribution of the rare biosphere to the overall community turnover and determined how the rare biosphere depends on environmental conditions.

Impact of the rare biosphere on community turnover

The rare biosphere has been postulated to consist of low-abundance microbial organisms that would not be subjected to predation or viral lysis, and would likely represent a huge proportion of microbial communities, as generally indicated by long

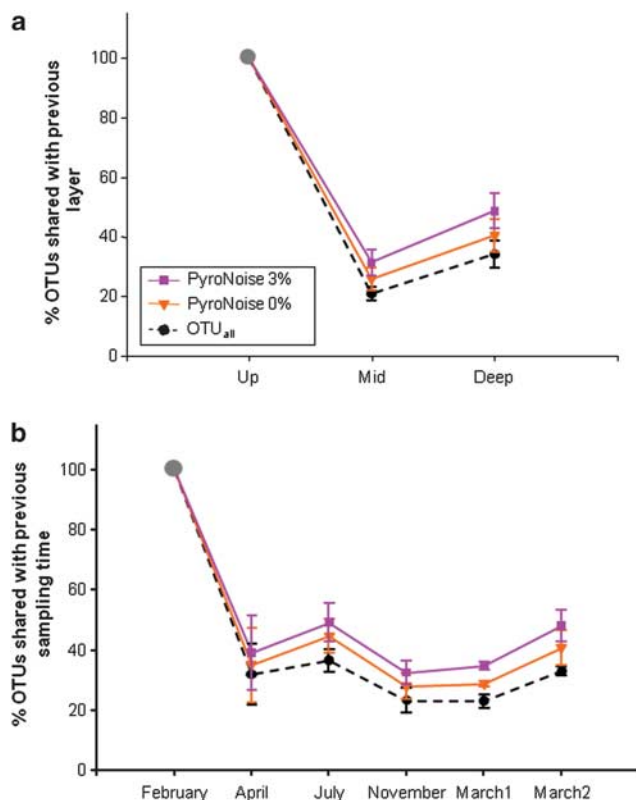


Figure 2 Bacterial community turnover between two consecutive depth layers (a) or sampling times (b). The percentage of OTU shared between a sampling depth (or date) and the previous one was calculated after PyroNoise correction and OTU clustering of the 454 MPTS data set at several levels of sequence dissimilarity. Bars correspond to s.d. calculated over 4–6 sampling dates (a) or three depth layers (b), except for July and November 2005 in which two depth layers were considered. The first depth layer and sampling date (February 2005) are indicated by the grey point as 100% of OTU. OTU_{all} represents the original data set with all OTU_{unique}. It is used here as a reference data set to study the effects of various correction levels on the observed dynamics of the bacterial community composition.

distribution tails in rank-abundance curves (Pedrós-Alió, 2007). For example, among the rare bacteria in coastal sands were the potential human pathogens (including *Parachlamydia*, *Arcobacter*, *Francisella*, *Acinetobacter*, *Rickettsiella*, *Pseudomonas* and *Ralstonia*) with a total of 16–88 sequences corresponding to 2–54 OTU_{unique} in the OTU_{all} data set (0.22% of all sequences in OTU_{all}). To understand what fraction of the community may be associated with the large diversity turnover observed in the sands, we gradually removed increasing fractions of the rare sequences in the data set starting from the rarest ones (Gobet *et al.*, 2010); the large turnover previously described for the complete data set over both sediment depth and time was no longer observed when up to 50% of the rare sequences were removed (Supplementary Figure S3C), indicating that most of the community turnover was due to changes in the rare tail of the data set.

The rare biosphere could be identified as OTU appearing only once in a given sample (i.e., SSO_{rel},

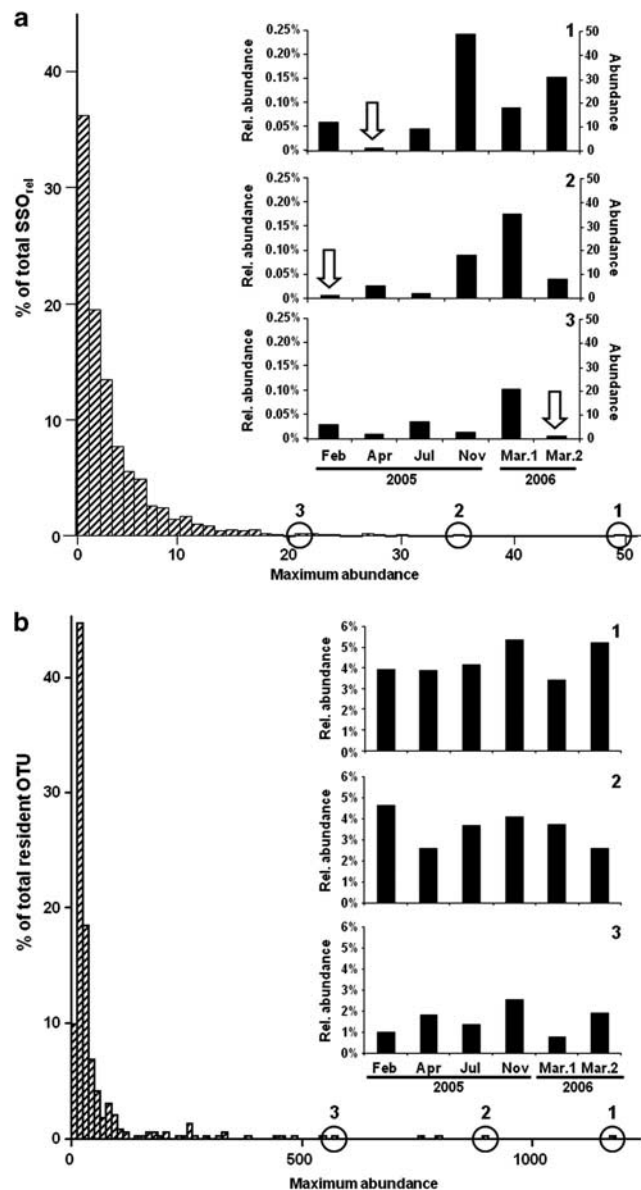


Figure 3 Distribution of the maximum abundance of (a) SSO_{rel} (i.e., those OTU_{0%} that, at least in one sample, consisted of only one sequence) and (b) resident OTU_{0%} (i.e., OTU_{0%} present at all times) in the top 10-cm layer. In (a), panels 1, 2 and 3 are examples of cases, in which particular high fluctuations from the single-sequence case (white arrow) to higher-sequence abundances were observed. In (b), panels 1, 2, 3 illustrate cases of particularly high fluctuations in relative abundances (no absolute abundances were calculated in this case). All data were initially processed to remove pyrosequencing noise. Rel. abundance, relative abundance to the total number of sequences per sampling time: February 2005 (13 285), April 2005 (7902), July 2005 (10 910), November 2005 (21 931), 1 March 2006 (17 302), 2 March 2006 (17 897).

representing about 20% of all PyroNoise-corrected OTU_{0%}, or as OTU appearing only once in the whole data set (i.e., SSO absolute (SSO_{abs}), representing about 58% of the OTU_{0%}), as compared with the 3–5% of the OTU_{0%} that were resident (Table 1). Moreover, when only the SSO_{rel} fraction of the data

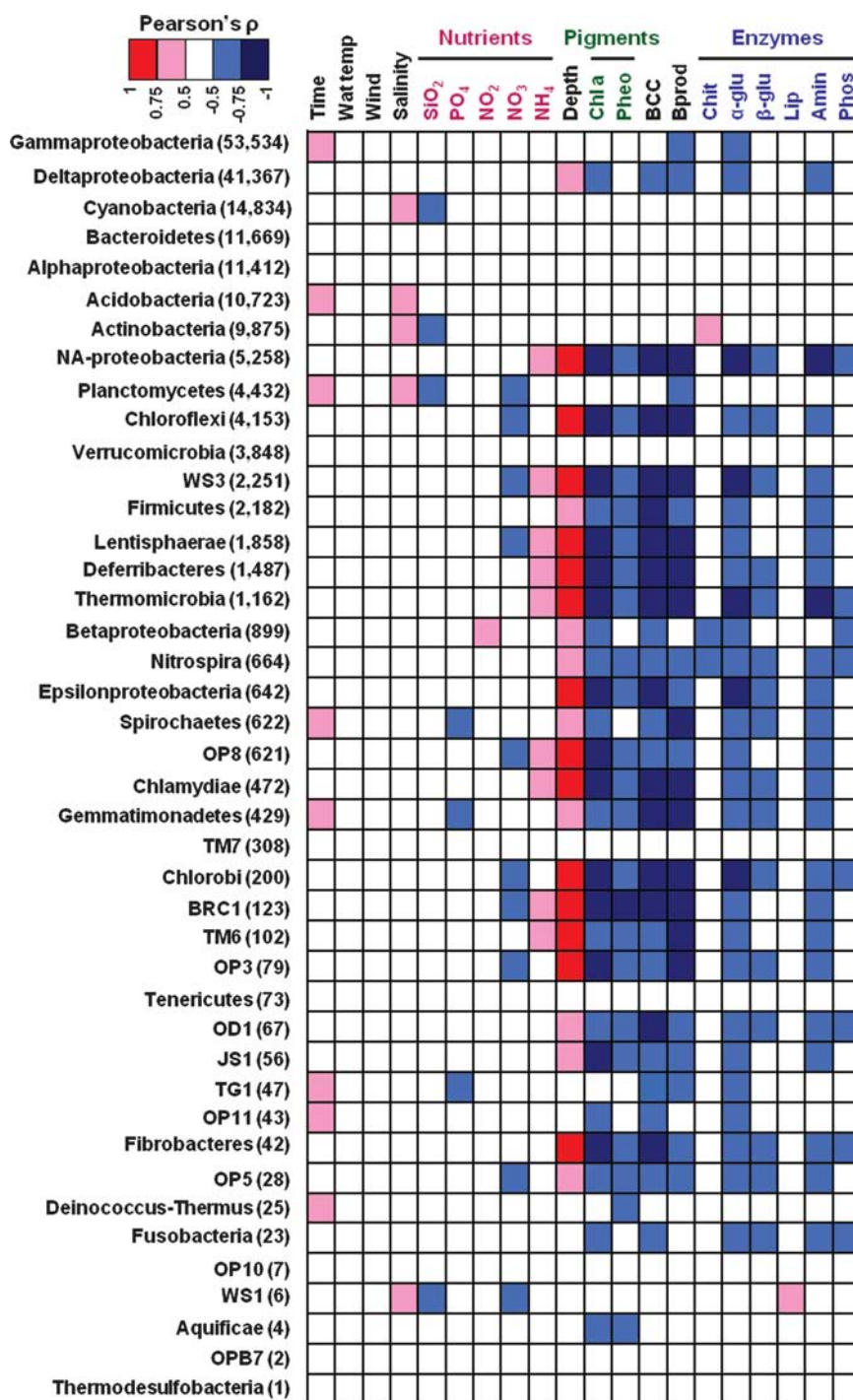


Figure 4 Environmental factors associated with variations of the bacterial community structure at the phylum level. Pearson's ρ indicates correlations between phylum sequence abundance and several environmental parameters. For example, a red square between sediment depth and *Chloroflexi* indicates a higher number of sequences with increasing depth; nonsignificant relationships between water temperature and sequence variation in any of the bacterial groups are indicated by white squares; a blue square between chlorophyll *a* and *Chloroflexi* denotes a decrease in sequences as chlorophyll *a* concentration gets higher (the latter being concordant with the relationship between increasing depth and *Chloroflexi* sequences number). The *Proteobacteria* phylum level was separated into its corresponding classes to obtain a higher resolution. NA-*Proteobacteria* are the *Proteobacteria* with missing class annotation. The total number of sequences in each phylum is indicated in parentheses. SiO_2 , silicate; PO_4 , phosphate; NO_2 , nitrite; NO_3 , nitrate; NH_4 , ammonium; Chl *a*, chlorophyll *a*; Pheo, pheophytin; BCC, bacterial abundance; Bprod, bacterial carbon production; Chit, chitinase; α -glu, α -glucosidase; β -glu, β -glucosidase; Lip, lipase; Amin, aminopeptidase; Phos, phosphatase.

set was retained, very similar fractions of the total explained-biological variation were identified as for the total data set (Supplementary Figure S4). This study therefore reports that not only a large fraction of bacterial communities consists of rare types, as previously found in many 454 MPTS-based studies in other environmental samples (e.g., Gilbert *et al.*, 2009; Bolhuis and Stal, 2011), which may undergo substantial replacement over few centimeters of sediment depth, as previously observed using lower resolution molecular techniques (Hewson *et al.*, 2007), but may also dramatically change over few months of time. In addition, rare bacterial types seemed to remain rare in the bacterial community over time and sediment depth; 94% of the SSO_{rel} had a maximum abundance of 10 sequences only (Figure 3a). In conclusion, both the presence of this large proportion of singleton OTU_{0%} in sandy sediments and the large turnover in community composition could be explained by the dispersal of OTU from other sand locations by advective transport and physical mixing (Boudreau *et al.*, 2001). Further research would be needed to examine more in detail the biogeography of rare and of resident bacterial types in coastal sands.

Ecological interpretation of overall microbial diversity patterns

To determine whether observed temporal patterns may be attributed to either deterministic (niche-based) or stochastic processes, or both (Ramette and Tiedje, 2007a), bacterial community structure was interpreted in concert with previously published contextual data (Böer *et al.*, 2009a) for the investigated samples, to test the effects of time, sediment depth, cell abundance and biogeochemical gradients (i.e., pigments, nutrients and extracellular enzymes, Figure 4). A multivariate variation partitioning approach (Borcard *et al.*, 1992; Ramette and Tiedje, 2007b) showed that biogeochemical gradients (pigments, nutrients and extracellular enzymes), cell abundance and their covariation were directly related to the major changes in community structure (Supplementary Figure S4), as previously assessed on the basis of a lower-resolution fingerprinting technique for the most abundant OTUs (Böer *et al.*, 2009b). This study, including also the rare types, showed that significant biogeochemical variables included in the most parsimonious multivariate models were qualitatively almost the same for the resident OTU, the phylum, OTU_{all} and SSO_{rel} levels (Supplementary Figure S4 and Supplementary Table S2). Also, a greater amount of biological variation could be explained when a lower taxonomic resolution was used, leading to a less complex data set (Gobet *et al.*, 2010).

The main parameters significantly influencing the variation for several OTU definition levels were chlorophyll *a*, activity of the extracellular enzyme phosphatase and cell abundance (Supplementary

Table S2), with all three parameters being highly correlated with sediment depth. Cell abundance was also identified as an important factor influencing the variation in the different OTU data sets (Supplementary Table S2). Noticeably, community diversity and composition varied much more than microbial functions (e.g., biomass, benthic oxygen consumption and extracellular enzymatic activities). This may suggest that the few continuously abundant, resident microbes perform these main microbial functions in sandy ecosystems, and that the rare types being replaced at high rates have little effects on bulk functions. Furthermore, with regard to heterotrophic degradation of organic matter, a substantial level of functional redundancy may exist in the dominant taxa of the Sylt sands, comprising both resident and transient organisms.

Our statistical analyses showed that the temporal dynamics of bacterial communities in temperate sandy sediments were clearly distinct from the seasonally reoccurring, cyclic bacterial patterns that were observed in water column samples offshore Southern California (Fuhrman *et al.*, 2006), in the English channel (Gilbert *et al.*, 2009), or in the Baltic Sea (Andersson *et al.*, 2010). Those patterns also differed from the observed stability of the Arctic bacterioplankton community through seasons (Kirchman *et al.*, 2010). This indicates that contrasting patterns of temporal variation may be associated with different microbial habitats and ocean regions, thus inviting further studies on the main drivers of temporal fluctuation or stability in marine ecosystems.

The rare biosphere responds to environmental drivers

The biogeography (Galand *et al.*, 2009) and the temporal dynamics over thousands of years (Brazelton *et al.*, 2010) of the rare microbial biosphere have previously been described in different marine habitats, but not much is known about factors influencing the composition of the rare microbial biosphere. To better understand whether patterns of the rare fraction were random or environmentally driven, the proportion of SSO_{rel} (Supplementary Table S3) in each sample was correlated with environmental parameters. Notably, fluctuations in SSO_{rel} were not random (as determined by model selection using Monte Carlo permutation test and by selecting the lowest Akaike Information Criterion model values) and seemed to be negatively correlated with pigments (chlorophyll *a*, pheopigments) and bacterial carbon production in the sediments (Supplementary Figure S5). For OTU_{0%}, the corresponding Pearson correlation coefficients between the proportions of SSO_{rel} and chlorophyll *a*, pheopigments and bacterial carbon production were -0.730 , -0.695 and -0.626 , respectively, and were all highly significant. However, neither sediment depth nor sampling time could significantly explain the variation in the proportion of rare types. Because

pigment concentration and bacterial carbon production may indicate food availability in sand ecosystems (Rusch *et al.*, 2003), an increased proportion of rare types that is concomitant with a decrease of those parameters would indicate that rarity becomes more prevalent when the environmental conditions become harsher for microbial life. Those hypotheses are concordant with the fact that primary and secondary productions in Sylt sands reach a minimum in late winter, when temperatures are close to the freezing point and strong winds dominate (van Beusekom, personal communication). Therefore, our previous hypotheses 2 (microbial adaptability) and 3 (seed bank hypothesis) to explain the observed high turnover in sandy sediments may be both supported by this finding of significant environmental control on the fluctuations of rare microbial types. Future structural and functional community analyses are needed to examine whether the large proportion of single OTU can be linked to specific environmental conditions in other marine coastal sandy habitats and hence typical for this microbial realm dominated by physical transport.

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