BACTERIAL HABITAT DIFFERENTIATION IN COLD- AND WARM-WATER CORAL REEF ECOSYSTEMS

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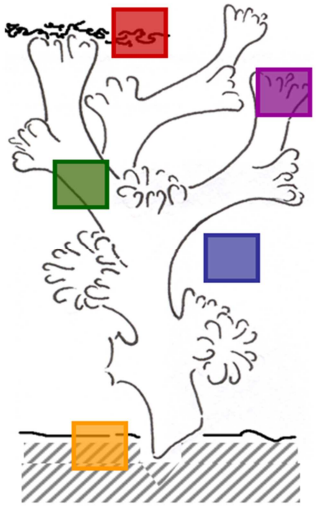

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Be infinitesimal under that sky.

Remember the way you are all possibilities
you can see and how you live best
as an appreciator of horizons,
whether you reach them or not.

DAVID WHYTE

SUMMARY

Coral reefs are highly diverse and complex ecosystems. Beyond harboring a unique plethora of different coral species as well as macro- and meiofauna, especially reef-associated microbial communities of coral reefs have recently come into focus. As only little is known about the diversity and ecological potential of reef-associated microbes, this thesis aimed at investigating some of the fundamental patterns of bacterial diversity in both cold-water and warm-water coral reef ecosystems. For this purpose, mainly the high-throughput fingerprinting technique ARISA (Automated Ribosomal Intergenic Spacer Analysis) and a suite of multivariate statistical tools were used to assess bacterial diversity patterns in context with different environmental gradients.

The first study (Schöttner *et al.* 2009; II.2) focused on principle patterns of bacterial diversity associated with and surrounding the main constructional cold-water coral *Lophelia pertusa.* The coral- and environment-specific structuring of bacterial communities was studied under both natural (reef) and controlled (aquaria) conditions, with an emphasis on four distinct microbial habitats: coral branch (i.e. skeleton surface), coral mucus, ambient seawater and proximal sediment. Overall, the observed community variations reflected a specific partitioning of bacterial assemblages between the different microbial habitats, potentially controlled by the prevailing environment.

Within the second study (II.3), intra- and inter-reef variations in bacterial diversity of four cold-water coral reef ecosystems were investigated in a multi-scale survey spanning five levels of spatial and ecological reef organization. The main focus was thereby on microbial habitats of the two constructional cold-water corals *Lophelia pertusa* and *Madrepora oculata.* Like in the first study, bacterial signatures of two coral-generated (branch, mucus) and two ambient (seawater, sediment) habitats were analyzed. The results confirmed the differences in community variation as related to coral-associated *versus* ambient microbial habitats. Further, it was revealed that bacterial community variation (i) was locally consistent with coral species and reef-internal zoning, and (ii) changed markedly from local to regional scale, exhibiting biogeographic patterns that resembled those of water- and sediment-dwelling bacteria.

Complementing the first two studies on cold-water coral reef ecosystems, the third study (II.4) focused on permeable carbonate and silicate reef sands of a warm-water coral reef ecosystem in the Red Sea. The two sand types were compared for their potential to

promote differences in bacterial diversity and biomass, thereby taking into account the influence of season and space. Confirming previous hypotheses, the results indicated pronounced sand type-related as well as spatio-temporal imprints, and emphasized the deterministic role of sediment mineralogy for the seasonal and vertical structuring of bacterial communities. Carbonate and silicate reef sands were characterized as distinct microbial habitats for specific, comparably diverse and highly dynamic bacterial assemblages.

In conclusion, this thesis provides the first analysis of the specificity and spatial scaling of bacterial associations with cold-water corals, and reveals that cold-water coral reef ecosystems are potential hotspots of microbial biodiversity in the deep sea due to the high community turnover on small spatial scales. The methods established in these studies also allowed the deciphering of environmental drivers of bacterial diversity in warm-water coral reef – and offer opportunities for further progress with investigating microbial community shifts in response to environmental changes and anthropogenic pressures to coral reef ecosystems.

Implemented within the framework of the graduate program International Max Planck Research School in Marine Microbiology (MarMic) at the Max Planck Institute for Marine Microbiology (MPI-MM), Bremen, and the Jacobs University Bremen (JUB), Bremen, this work was enabled by and carried out in the Microbial Habitat Group at the Max Planck Institute for Marine Microbiology, Bremen, and the Coral Reef Ecology Work Group (CORE) at the Ludwig Maximilians University (LMU), Munich.

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I. INTRODUCTION

Coral reef ecosystems have been recognized as unique and complex ecosystems hosting an exceptionally high diversity of different organisms (HOEGH-GULDBERG & BRUNO 2010). They are considered as "rainforests of the oceans" and comprise the largest structures of biological origin on earth due to the net accumulation of calcium carbonate produced by corals and other calcifying organisms (KLEYPAS *et al.* 1999). In the following, both warm-water coral (WWC) and cold-water coral (CWC) reef ecosystems will be described and compared in more detail, with an emphasis on their distribution, ecosystem structure and function, as well as their significance as biodiversity hotspots – and microbial habitats.

I.1 Distribution and Structure of Coral Reef Ecosystems

Rivaling old forests in longevity of their ecological communities, well-developed coral reefs reflect thousands of years of history (TURGEON & ASCH 2001). By forming enormous structures that are predominantly composed of their skeletal frameworks, corals create a highly complex array of habitats of varying sizes and endurance for a plethora of mobile and sessile organisms. Their intricate build-ups of calcium carbonate deposits provide three-dimensional living space and direct settlement substrate, but also alter current flow regimes and sedimentation rates, thereby changing the abiotic environment in time and space (ROBERTS *et al.* 2009 and references therein). As structural "ecosystem engineers" (JONES *et al.* 1994) with the capacity to directly and indirectly create, maintain and/or modify habitats, corals often enhance local diversity and richness (BERKE 2010 and references therein).

Global Distribution of Coral Reefs. The geographical distribution of WWC reefs is restricted to the circum-equatorial belt between the Tropic of Capricorn (30°S) and Tropic of Cancer (30°N), as well as to an average winter temperature of ≥18°C (SCHUHMACHER 1976, WILD 2003). Overall, WWC reefs cover a total area of ca. 6×10^5 km^2 (SMITH 1978, WILD 2003), including about 15% of the continental shelf areas and about 30% of the world's coastline (SMITH 1978, WILD 2003).By occupying 0.2% of the

world's ocean, WWC reefs provide habitation and breeding ground for over 25% of all known marine species (BRYANT *et al.* 1998). Due to their photosynthetic endosymbionts, the zooxanthella, WWC are restricted to water depths within the euphotic zone (<100 m in tropical oceans) and to environments with a low turbidity. Furthermore, they are limited to salinities of 32–34 PSU (VERON 1986).

CWC reefs have no photosynthetic symbionts, and are hence not restricted to surface waters of warm, tropical seas, but also thrive in the deeper, nutrient-rich high latitude waters where ocean currents prevent sedimentation. They can grow in deep waters (10–3000 m), and populate a diverse range of marine environments (ROBERTS *et al.* 2009 and references therein). What was considered to be enigmatic findings by fishermen just a few decades ago, has now been revealed as a giant deep-sea ecosystem, hosting a high density and biodiversity of marine life. Recent advances in deep-sea technology and exploration revealed the true extent and abundance of CWC reefs. Occurring throughout the world's oceans, CWC reefs are usually associated with topographic highs at continental margin features (i.e. slopes, ridges, canyons, and fjords) as well as with seamounts and mid-ocean ridges (FREIWALD *et al.* 2004, ROBERTS *et al*. 2009). The cosmopolitan scleractinian corals *Lophelia pertusa* (L.) and *Madrepora oculata* (L.) occur along much of the NW European continental margin and in some Scandinavian fjords. Both these CWC species have also recently been discovered in the Mediterranean Sea. Despite their intense study during the past few years, it is still not known, how widely distributed CWC are. CWC recruitment and proliferation in specific ecological niches devoid of sunlight are determined by a subtle interplay of factors, whereof the availability of elevated hard-bottom substrata, adequate food sources, and suitable temperatures, combined with high productivity of surface waters and vigorous hydrodynamical regimes constitute some of the most crucial prerequisites (MORTENSEN *et al*. 2001, DUINEVELD *et al.* 2004, FREIWALD *et al*. 2004, KIRIAKOULAKIS *et al.* 2005, WHITE *et al*. 2005, DODDS *et al.* 2007, DAVIES *et al.* 2008). CWC reefs develop over long periods of time, usually hundreds to thousands of years. With the deep sea being a low energy environment, CWC grow only very slowly, which is why the presence of a CWC reef usually indicates a stable, low-disturbance setting. The majority of CWC reefs have been found in the NE Atlantic Ocean, and are usually dominated by *L. pertusa*. The largest CWC reef complex in the world is the Røst Reef (35–50×3 km) off the Norwegian coast.

WWC Ecosystems. According to SCHUMACHER (1976), WWC reefs appear in different types, including fringing reefs, barrier reefs, atoll reefs and platform reefs. The most common type, which was also investigated within this thesis, is the fringing reef, "a reef belt orientated parallel and close to the coastline, usually with a width of less than 100 m" (WILD 2003). WWC reefs support a very high biodiversity, with approximately 350 species of scleractinian corals forming the basic reef structure and hosting 4000 species of molluscs, 1500 species of fish and 240 species of associated seabirds (LALLI & PARSONS 1997). Potentially, hundreds of thousands of species live in WWC reefs, many of which are not yet scientifically described (PORTER 1972). With more than 180 reported WWC genera, coral diversity itself is highest in the W Pacific (VERON 1993, WILD 2003).

As summarized by WILD (2003), the gross primary production in WWC reef ecosystems is estimated to 300–5000 g C m^{-2} y^{-1} (LEWIS 1977), exceeding primary production rates in the adjacent open sea by 1–3 orders of magnitude (ADEY 1987, HATCHER *et al.* 1987). Even WWC alone can produce 14.600 g C m^{-2} d^{-1} (HATCHER 1988), which is highly astonishing, because WWC reefs are usually found in oligotrophic regions where nutrient concentrations are very low (JOHANNES *et al.* 1983, D'ELIA & WIEBE 1990, RASHEED *et al.* 2002, WILD 2003). This apparent imbalance between nutritive input and output in WWC metabolism, known as "Darwin's paradox", is not yet fully elucidated. The prodigious biological productivity and limestone-secreting capacity of reef-building WWC may however be mainly based on the rapid recycling and mutual exchange of algal photosynthates and cnidarian metabolites (BARNES & LOUGH 1989, LEVINTON 1995, LALLI & PARSONS 1997, BARNES & HUGHES 1999, WILD *et al.* 2004).

CWC Ecosystems. Unlike their warm-water counterparts that usually generate extensive, continuous reefs and banks with clear energy-related zoning (e.g. Great Barrier Reef), CWC communities exhibit various forms of appearance, comprising isolated colonies and patchy accumulations as well as large reefs or giant carbonate mounds (FREIWALD *et al.* 2002, TAVIANI *et al.* 2005, DORSCHEL *et al.* 2007, ROSSI *et al.* 2008, LAVALEYE *et al.* 2009). These reef types can differ substantially with respect to their on-site spatial configuration (WILSON 1979, FREIWALD *et al.* 1997, ROGERS 1999, MORTENSEN *et al.* 2001, FREIWALD 2002). Not uncommonly, individual clusters of coral frameworks (rather than a single coalescent one) form entire reef complexes which, depending on local seabed geology as well as community history, exhibit distinctive geomorphologic and

taphonomic zoning, with marked transitions in sediment facies, faunal composition and proliferation stage (FREIWALD & WILSON 1998, MORTENSEN *et al*. 2001, FREIWALD *et al.* 2002, BUHL-MORTENSEN *et al*. 2010).

CWC ecosystems are increasingly recognized as key habitats and biodiversity hotspots in the deep sea. Fully developed CWC frameworks reveal an exceptional degree of species richness and turnover of associated mega-, macro- and meiofauna, including high proportions of suspension and filter feeders as well as commercial fish (JENSEN & FREDERIKSEN 1992, ROGERS 1999, HUSEBØ *et al.* 2002, HENRY & ROBERTS 2007). It is speculated that increased food supply due to elevation and currents (THIEM *et al.* 2006, KIRIAKOULAKIS *et al.* 2007) plays a pivotal role in sustaining CWC ecosystem diversity. Although many of the CWC-reef associated animals are not endemic to these ecosystems, their diverse and dense accumulation clearly distinguishes the reef environment from many off-reef habitats (BONGIORNI *et al.* 2010, BUHL-MORTENSEN *et al*. 2010, HENRY *et al.* 2010, ROBERTS *et al.* 2009 and references therein). In general, CWC reef ecosystems support fewer obligate associates than their warm-water counterparts, but exhibit high species packing, thereby enhancing regional diversity (LEVIN *et al.* 2010).

In conclusion, WWC and CWC reef ecosystems differ substantially with respect to their biogeography and oceanographic setting, as well as their reef-building coral species and associated communities (Table I.1; modified after FREIWALD *et al.* 2004, BEUCK 2008):

Table I.1 Differences between WWC and CWC reef ecosystems.

Attribute	**WWC**	**CWC**
Distribution	Sub-/Tropical seas, 30°N–30°S	Globally, 71°N–56°S
Temperature	20°–29°C	4°–13°
Depth	0–100 m	10–3000 m
Symbiotic algae	Yes	No
Coral nutrition	PS, zooplankton, suspended OM	Zooplankton, suspended OM
Growth rate	1–150 mm y^{-1}	1–25 mm y^{-1}
Number of reef-building species	~800	~6–14
Largest reef structure	Great Barrier Reef (30,000 km^2)	Currently known: Røst Reef (100 km^2)
Global coverage	> $2x10^5$ km^2	Unknown, probably >WWC
Reefs at risk	More than 30% destroyed by bleaching, tourism and pollution, 30% at risk of loss within 30 y	Unknown, damage by fisheries and littering observed in most areas

Abbrev: PS=photosynthesis, OM=organic matter

I.2 Factors Generating Biodiversity in Coral Reef Ecosystems

Biodiversity Hotspots and Underlying Factors. Biodiversity is often measured in terms of alpha and beta-diversity, with alpha-diversity consisting of the number of species in a given sample (i.e. species richness), and beta-diversity representing the variation in community composition between samples (i.e. species turnover; MAGURRAN 2004). Biodiversity hotspots can be defined as areas of exceptional species richness, which may also support numerous endemic species and exhibit demonstrable vulnerability to habitat loss (BELLWOOD & MEYER 1998, REID 1998). Despite the numerous evolutionary, ecological and conservation studies about biodiversity hotspots, our understanding of the factors shaping them is in its infancy, especially in the marine realm (CONNOLLY *et al.* 2003).

As to animal and plant life in marine systems, the greatest diversity is seen on WWC reefs, one of the world's greatest biodiversity hotspot, which lies within a single biogeographical region encompassing approximately two-thirds of the equatorial tropics (BELLWOOD & MEYER 1998). Within this region, coral reef biodiversity increases, both latitudinally and longitudinally, as one moves towards a hotspot in the Indo-Australian Archipelago (BELLWOOD & MEYER 1998). The strong longitudinal gradients preclude many of the tropical–temperate hypotheses to be invoked. Over the past 30 years, in order to explain the existence of such a marine biodiversity hotspot, discussions have revolved around four main alternate, but not mutually exclusive, models (reviewed in BELLWOOD & MEYER 1998, REAKA *et al.* 2008): The center-of-origin model has its roots in Darwinian pre-continental drift ideas, with species arising in a specific location or center, and dispersing out from it. In the center-of-overlap model, high biodiversity results from population division (vicariance) and subsequent range expansion, and this model has found many supporting examples associated with coral biodiversity hotspots. In the center-of-accumulation model, species arise outside the biodiversity hotspot, and thereafter accumulate in the region by immigration form nearby islands. This may be realized at the level of individual organisms or entire communities, which are transported by rafting, accretion or suturing of land masses following tectonic events. Numerous factors have been proposed that may account for this ability to accumulate species, including the geological history of the area, its geographic position, and the availability of large area of shallow-water habitats. Finally, the species diversity

hypothesis states that high species diversity itself may promote diversification, due probably to species interactions. For instance, speciation rates in fossil plankton groups over 2–20 my correlate with species diversity regardless of sampling intensity and area, and species interactions in diverse assemblages of stomatopods may cause shifts in their body size and changes in their life history and speciation/extinction patterns (REAKA *et al.* 2008). It is yet unknown, if microbial alpha- or beta-diversity is enriched in association with coral reefs and would follow any of the hypotheses described above.

Several CWC species, just like many WWC, create structural habitat that alters local hydrography (DAVIES *et al.* 2009), which thus creates and modifies the abiotic environment in ways that favor colonization of reefs by species-rich fish and invertebrate communities, with the richness of some taxa rivaling that found on shallow WWC reefs (HENRY *et al.* 2008, GHEERARDYN *et al.* 2009). Cyclical *L. pertusa* reef growth produces a dynamic mosaic of habitats that results in the vertical zonation of four major macro-habitats and therefore zones of significant invertebrate species turnover ranging from the reef summit to its flank (HENRY *et al.* 2008): (1) living coral framework, (2) sediment-clogged, mostly dead coral framework, (3) coral rubble, and (4) underlying sediments (ROBERTS *et al.* 2006). Beta-diversity on *L. pertusa* reefs is also influenced by gradients in live coral cover and depth. It may therefore vary with communities becoming more dissimilar as one moves from reef summit to flank macro-habitats, and as one compares communities from increasingly different or distant environments (HENRY *et al.* 2010 and references therein). Hence, it could be assumed that the same factors making WWC and CWC diversity hotspots for animals, also increase microbial community diversity. An alternate hypothesis could be that coral reefs host a subset of water column and sediment microbial communities and are less diverse as their surrounding environments.

Effects of Environmental Gradients and Spatial Location. Because reef species tend to occupy particular environmental niches, the spatial structure of communities may largely be controlled by environmentally deterministic processes. On shallow WWC reefs, environmental gradients thus explain significant amounts of beta-diversity in animals (CLEARY & DE VOOGD 2007, HENRY *et al.* 2010). Heterogeneous reef communities can also reflect the effects of spatial autocorrelation, in which processes governed by species traits (e.g. growth, predation, differential mortality), and/or random but spatially auto-correlated neutral processes (e.g. spatially limited dispersal) produce heterogeneous

communities that vary across purely spatial gradients (BORCARD *et al.* 1992, HUBBELL 2001, HENRY *et al.* 2010). Currently, there is only minor support for spatially auto-correlated coral reef communities (HENRY *et al.* 2010), but it is not known whether this conclusion indeed reflects spatial autocorrelation or the effects of unmeasured environmental variables that are often themselves spatially dependent (BECKING *et al.* 2006, CLEARY & DE VOOGD 2007), which impedes any pure spatially explicit effects on beta-diversity. Therefore, more studies are needed, especially across a broader range of species, communities and regions that simultaneously test the significance and relative importance of these processes in order to refine our understanding of beta-diversity on coral reefs worldwide.

Recently, the increasing environmental pressure on coral reef ecosystems induced by tourism, pollution and ocean warming and acidification has raised interest in factors influencing coral reef biodiversity. Over the past decades, coral reef ecosystems have been degrading at an alarming rate (HUGHES *et al.* 2003, BAKER *et al.* 2008). This degradation is to a large part a consequence of coral disease (HARVELL *et al.* 1999, HARVELL *et al.* 2004). To gain a better understanding of how variation in coral-associated microbial assemblages may lead to the onset of disease, numerous studies have compared bacterial communities between healthy and diseased corals. These studies have shown that both the composition and function of microbiota associated with healthy and diseased corals are different (MOUCHKA *et al*. 2010 and references therein).

I.3 Coral-Microbe Associations and Interactions

Microorganisms represent important community members that drive biogeochemical and ecological processes and thereby significantly influence ecosystem diversity, function and resilience (e.g., BALSER *et al.* 2006, SCHIMEL *et al.* 2007). A central question in microbial ecology is whether microorganisms fill defined niches within complex communities, or whether communities are comprised of functionally redundant, neutrally-selected taxa leading to random assemblages (FUHRMAN *et al.* 2006, MOUCHKA *et al.* 2010). In marine plankton, microbial assemblages are heterogeneously distributed between geochemical and productivity-defined habitats (MOESENEDER *et al.* 2001). Often, spatially distinct communities in the same habitat type are more similar to each other than to those in

adjacent habitats (HEWSON *et al.* 2006). A growing number of investigations suggest that coral reef environments, already renowned for the diversity and intricacy of their organismic associations, are no exception in this regard (e.g. KNOWLTON & ROHWER 2003, AINSWORTH & HOEGH-GULDBERG 2009). WWC have been found to harbor a wide variety of microbes, including heterotrophic eukaryotes, bacteria, archaea and viruses (ROSENBERG *et al.* 2007 and references therein, MOUCHKA *et al.* 2010). These microbes can be found in different microbial habitats and may be associated in different ways with the coral host.

Corals are known to form associations with both external and internal microbiota. The coral animal, its intracellular algal symbionts, and the diverse microorganisms found in association with coral tissues and exudates have been termed the ‘‘holobiont’’ (ROHWER *et al.* 2002, RESHEF *et al.* 2006). In WWC, the algal symbiont is an obligate partner supplying up to 95% of the host’s metabolic requirements for carbon and contributing to formation of the skeleton (MUSCATINE 1973), however, the roles of coral-associated bacteria have not been well elucidated.

Coral-Bacteria Associations. Several studies have evidenced the existence of specific associations between bacteria and their coral hosts, especially for WWC (ROHWER *et al.* 2002, BOURNE & MUNN 2005, PENN *et al.* 2006, YAKIMOV *et al*, 2006, WEBSTER & BOURNE 2007). The coral holobiont is a complex system containing diverse, abundant and active microbial representatives of all three domains of life (ROHWER *et al.* 2002, WEGLEY *et al*. 2004, WILD *et al.* 2004, BOURNE & MUNN 2005). Several microbial habitats and respective niches, such as those directly generated by the coral (skeleton, tissue, mucus), but also ambient seawater and reef sediments, are known to be available for reef-associated microorganisms (ROSENBERG *et al.* 2007 and references therein). The selection of specific bacterial associates could be induced by nutritional coral–microbe relationships, as coral exudates are known to attract specific, yet diverse populations of organo-heterotrophic bacteria (RITCHIE & SMITH 2004, ALLERS *et al*. 2008, NEULINGER *et al.* 2008), or by specific chemical mediation through the coral host (KELMAN *et al.* 2006, RITCHIE 2006). Yet, the drivers of microbial diversity and function within WWC reef ecosystems still lack a good understanding.

Recent studies on microbial communities in CWC reefs have also provided first indications of coral-bacteria associations (e.g. NEULINGER *et al.* 2008, HANSSON *et al.*

2009,KELLOGG *et al.* 2009). For example, while YAKIMOV *et al.* (2006) showed that living specimens of the stony CWC *L. pertusa* in the Mediterranean harbor specific bacterial communities that are different from those of dead coral material or sediments, another study evidenced spatial stability of CWC-microbe associations with an Antarctic soft coral across an environmental impact gradient (WEBSTER & BOURNE 2007). Characteristic bacterial assemblages were also found in an Alaskan octocoral, but exhibited minimal influence of transient water-column microbes (PENN *et al.* 2006).

Specificity of Associations. Apparently promoted by the availability of diverse microbial habitats and niches on, within and surrounding various reef dwellers, microbial colonization in WWC reefs is suspected to contribute significantly to their structural and functional complexity (AINSWORTH *et al.* 2010, ROSENBERG *et al.* 2007 and references therein). First studies of bacterial associations with WWC found similar bacterial ribotypes associated with the same coral species, distinct from those in surrounding seawater and sediments (FRIAZ-LOPEZ *et al.* 2002, ROHWER *et al.* 2002, BOURNE & MUNN 2005, PANTOS & BYTHELL 2006). This is supported by the observation that some bacterial ribotypes form host-species-specific associations with corals (ROHWER *et al.* 2001, FRIAS-LOPEZ *et al.* 2002, ROHWER *et al.* 2002, BOURNE & MUNN 2005, SEKAR *et al.* 2006, LAMPERT *et al.* 2008). It is hypothesized that this specificity is indicative of important interactions, structured in ways that maximize the health of the holobiont (ROHWER *et al.* 2002, RESHEF *et al.* 2006, MOUCHKA *et al.* 2010).

While the existence of such coral-bacterial specificity in WWC is widely accepted, the spatial and temporal stability of these interactions is debated. In seawater, for example, bacterial assemblages can be heterogeneous within the same habitat at spatial scales ranging from micrometers to kilometers (AZAM & LONG 2001, HEWSON & FUHRMAN 2006, HEWSON *et al.* 2006) In coral, some studies have shown that species-specific bacteria are geographically consistent (ROHWER *et al*. 2001, ROHWER *et al*. 2002, MOUCHKA *et al.* 2010). Variations over geographic scales and with host species may also reflect differential species responses (host and/or microbiota) to site-specific factors (LITTMAN *et al.* 2009). Taken together, the results from early studies of coral-bacteria associations highlight the multi-faceted and dynamic nature of microbial habitats in coral reefs.

Microbial Habitats in Coral Reefs. In general, delineating a (microbial) habitat is not necessarily straightforward and the definition is to some degree operational. Here, the definition given by MARTINY *et al.* (2006), who defined habitat type as "environment defined by the suite of its abiotic and biotic characteristics", is used**,** which can easily be applied to the micro-scale, with different surfaces and matrices offering different microbial habitats, each with their own specific set of niches. With respect to corals and their direct surrounding, those habitats include specifically coral skeleton (surface, interior), coral mucus (surface layer, mucus string released to water column), and coral tissues (coenosarc, polyp). In contrast, ambient seawater and proximal sediment comprise coral-ambient habitats. Environmental factors vary significantly over these complex structures and influence both host and microbial communities. Therefore, just as the reef provides multiple macro-habitats and niches for macro-organisms, each coral colony contains several micro-habitats for an array of microbial communities. (AINSWORTH *et al.* 2010). Each of thee microbial habitats on the reef is influenced by physical and biological environmental conditions that vary in time and space (AINSWORTH *et al.* 2010). Usually, the following (coral-associated and other) microbial habitats are differentiated:

Coral Mucus. Mucus from both WWC and CWC is a complex mixture of carbohydrates, peptides, and lipids (BROWN & BYTHELL 2005), the composition of which varies between coral species (Meikle *et al.* 1998), with depth and/or irradiance (CROSSLAND 1987), and with ageing and contamination upon release into the water column (DUCKLOW & MITCHELL 1979, DAUMAS *et al.* 1982, WILD *et al.* 2004). Directly upon secretion, it forms a viscous layer on the coral surface. After release into the water column, it partly dissolves, whereat the remaining strings and flocs trap sediment grains, marine snow particles, and microorganisms (NAUMANN *et al.* 2009). By sinking and transportation into the reef sediment, mucus ultimately contributes to an efficient recycling of energy and nutrients in the reef system (WILD *et al.* 2004). Due to its composition, coral mucus has shown to be an attractive food source for planktonic microbial communities in both WWC (ALLERS *et al.* 2008, WILD *et al.* 2004, WILD *et al.* 2005) and CWC reef ecosystems (WILD *et al.* 2008). It fulfills various functions such as protection against contamination with pollutants or pathogens, UVR or physical damage, desiccation, or sedimentation (BROWN & BYTHELL 2005). As coral surface mucus layer directly on the coral, it provides a vital interface between the coral epithelium and the seawater

environment. Previous studies revealed that the coral surface mucus layer harbors specific microbial communities (KELLOGG 2004, RITCHIE & SMITH 2004, RITCHIE 2006) which may be regulated through the coral host, and usually involves the production of antibiotic substances (e.g. RYPIEN *et al.* 2010).

Coral Tissues. Tissues of WWC and CWC generally comprise polyp bodies and the coenosarc covering (parts of) the coral skeleton. In WWC, microbial associates, other than endosymbiotic dinoflagellates, are rarely observed *in situ* (AINSWORTH & HOEGH-GULDBERG 2009). But several studies indicate diverse communities inhabiting healthy or diseased tissues of different WWC species (COONEY *et al.* 2002, PANTOS *et al.* 2003, LESSER *et al.* 2007, SUNAGAWA *et al.* 2010). In healthy CWC tissues, flask-like bacteria have shown to be associated with nematocyst batteries at the polyp tentacle surface, while filamentous cells were observed homogenously dispersed in the polyp body tissue (NEULINGER *et al.* 2009). In a diseased soft CWC species, an increase in bacterial concentration appears to coincide with tissue necrosis (HALL-SPENCER *et al.* 2007). The discrete analysis of tissues appears difficult as mucus is directly secreted by specific mucus-producing cells at the tissue surface, and often forms a viscous film on the coral (tissue) surface.

Coral Skeleton. From WWC skeletons, several endolithic microbial cells are known, which comprise photosysthetic algae, cynobacteria, fungi, and bacteria (AINSWORTH *et al.* 2010). Especially phototrophic microendoliths seem to constitute important members of the WWC holobiont (FINE *et al.* 2005, FINE *et al.* 2006). However, nothing is known yet on the occurrence of bacteria in CWC. Interestingly, microendolithic phototrophs were recorded on CWC in Chile (FÖRSTERRA & HÄUSSERMANN 2008). Furthermore, epibiotic communities can form biofilms on CWC skeleton surface (BEUCK *et al.* 2007). Often, early post-mortem biofilm formation on skeleton framework results in clearly visible gradients on the coral colony.

Reef Sponges. Sponges host extraordinarily dense and diverse microbial communities, which comprise up to 40% of sponge volume and contribute to many aspects of sponge biology, including carbon and nitrogen nutrition and chemical defense (HENTSCHEL *et al.* 2006). In many demosponges, bacterial population densities in the mesohyl matrix may

reach 10^8–10^{10} bacteria per gram of sponge wet weight, exceeding seawater concentrations by 2–4 orders of magnitude. Those sponges have been termed "bacteriosponges" or "high-microbial-abundance sponges" (VACELET & DONADEY 1977, HENTSCHEL *et al.* 2003), in contrast to other co-existing species that are essentially devoid of microorganisms ("low-microbial-abundance sponges"). In some sponges, bacteria are found within host bacteriocytes (VACELET & DONADEY 1977) and even within host nuclei (FRIEDRICH *et al.* 1999). Bacterial associates of sponges are phylogenetically complex, yet highly sponge-specific, and represent at least 16 recognized phyla and one candidate phylum (*Poribacteria*). Based on 16S rRNA gene surveys, the most common phyla recovered include the *Acidobacteria*, *Actinobacteria*, and *Chloroflexi* (HENTSCHEL *et al.* 2006). But also archaeal lineages have been identified (PAPE *et al.* 2006). It appears that sponge-associated microbial communities are stable within individuals and through time, and specific subsets of the overall community occur consistently within the same sponge species from different locations (WEBSTER & HILL 2001, HENTSCHEL *et al.* 2002, WEBSTER *et al.* 2004). Although most hitherto sponge studies are related WWC reef ecosystems, first insights into sponge communities in CWC reef ecosystems revealed also highly diverse and active microbial communities (HOFFMANN *et al.* 2006, JENSEN *et al.* 2008).

***Reef Algae*.** There are not many studies of algae-associated microbial communities in reef ecosystems, but from the research that has been carried out in other environments, a few basic trends emerge. Like corals and sponges, algae have conserved and potentially species-specific bacterial communities that are distinct from the surrounding water (LEWIS *et al.* 1985, LONGFORD 2007). Seasonal shifts, both in bacterial numbers and in species richness, have been documented (LAYCOCK 1974, MAZURE & FIELD 1980, SIEBURTH & TOOTLE 1981, BOLINCHES *et al.* 1988), and different bacterial communities have been found to be associated with different parts of the algae, like e.g. the thallus *versus* the frond (LAYCOCK 1974, MAZURE & FIELD 1980, CORRE & PRIEUR 1990). Most studies have been cultivation-based, and enumeration via plate counts and microscopy revealed 10^6–10^8 bacteria g^{-1} wet weight of algal biomass (LEWIS *et al.* 1985, JENSEN *et al.* 1996). Common genera from cultivation studies include *Flavobacterium* spp. (reviewed in BOLINCHES *et al.* 1988), *Bacillus*, *Vibrio*, *Pseudomona*s, and *Moraxella* (LEWIS *et al.* 1985). The application of fluorescently labeled probes showed *Bacteroidetes*, *Alpha*-,

Beta-, and *Gammaproteobacteria*, *Actinomyces*, *Planctomyces*, and 30% that could not be identified (HEMPEL *et al.* 2008).

Reef Water Column. Microbial communities are ubiquitous and active components of the microbial loop in coral reef waters (e.g. HEWSON & FUHRMANN 2007, WILD *et al.* 2008). In WWC reef ecosystems, high bacterial growth rates (Moriarty *et al.* 1985) and low inorganic nutrient concentrations (VAN DUYL *et al.* 2006) imply nutrient limitation of bacterioplankton communities. Cell densities and nutrient concentrations are, however, not uniformly distributed throughout the water column. Due to their high biotic complexity, reef cavities and crevices which occur throughout the coral reef framework, are an evident source of nutrients and sink of bacterioplankton (SCHEFFERS *et al* 2005, VAN DUYL *et al.* 2006). Although bacterial growth is enhanced in reef crevices, bacterial densities decrease from the overlying water through the bottom water into those crevices (range: $9–2\times10^5$ ml^{-1}), showing a transfer of bacterial biomass into the reef which depends mainly on water movement and bottom relief (GAST *et al.* 1998). During its passage over the coral reef, the water body thus obtains a reef signature that is often characterized by depleted phytoplankton and bacterioplankton concentrations and enhanced dissolved nutrient concentrations (AYUKAI 1993, YAHEL *et al.* 1998, VAN DUYL *et al.* 2002). Similar trends may exist in the water column of CWC reef ecosystems, as the composition of suspended particulate matter changes significantly during its passage over a proliferating reef environment, indicating preferential removal of nutrients by bacterioplankton communities (LAVALEYE *et al.* 2009). In general, CWC reef environments are characterized by nutrient- and carbon-rich waters, wherefore the diverse microbial assemblages (JENSEN *et al.* 2008, NEULINGER *et al.* 2008) are expected to be present in high numbers. First studies on activity indicate that microbial plankton in the water column above CWC reefs react with increased oxygen consumption to the addition of CWC mucus (WILD *et al.* 2008, WILD *et al*. 2009).

Reef Sediments. Due to their enormous structural and physico-chemical complexity (MEYER-REYL 1994 and references therein), sediments probably offer the most important array of interfaces for microbial colonization and biofilm formation. They contain high amounts ($10^8–10^9$ cells g^{-1}; RUSCH *et al.* 2009) of very diverse and active microbial cells, most of which are directly attached to grain and particle surfaces (MEADOWS & ANDERSON

1966). The resulting immobilization of cells within the sediment matrix leads to high micro-scale heterogeneity in microbial distribution and activity (MEYER-REIL 1994). In WWC reef ecosystems, permeable sediments with their high volume-specific surface area and associated microbial communities (YAMAMOTO & LOPEZ 1985, HANSEN *et al.* 1987, WILD *et al.* 2006) act as biocatalytic filter systems that enhance pelagic-benthic coupling via advectional porewater flow, and thereby ensure efficient energy and nutrient recycling in an oligotrophic environment (WILD *et al.* 2005). Reef sediment-associated phyla commonly identified by 16S rRNA gene-based techniques comprise the (*Gamma-*, *Delta-*, *Alpha-*)*Proteobacteria*, *Bacteroidetes*, *Cyanobacteria*, *Acidobacteria*, *Actinobacteria*, *Chloroflexi*, and *Planctomycetes*, as well as *Eury-* and *Crenarchaea* (SØRENSEN *et al.* 2007, UTHICKE & MCGUIRE 2007). As metabolic pathways and the structure of the catalyzing microbial communities are constrained by their redox environment, vertical variations are observed that reflect reef porewater chemistry (HEWSON & FUHRMAN 2006, SØRENSEN *et al.* 2007, RUSCH *et al.* 2009). Furthermore, communities may vary with location across gradients within and between different reefs (HEWSON & FUHRMAN 2006, UTHICKE & MCGUIRE 2007), and with season (RUSCH *et al.* 2009). Albeit not as extensively studied as WWC reef sands, also CWC reef sediments appear to contain diverse and active microbial assemblages, mainly including members of the (*Delta-*, *Gamma-*, *Alpha-*)*Proteobacteria*, *Bacteroidetes*, *Chloroflexi*, *Planctomycetes*, and *Actinobacteria* (YAKIMOV *et al.* 2006, JENSEN *et al.* 2008, NEULINGER *et al.* 2008). According to findings by WEHRMANN *et al.* (2009), sediment-associated anaerobic carbon mineralization is comparatively reduced due to de-coupling of sediment communities from processes occurring in the overlying reef framework. Sedimentary microbial activity may, however, also be responsible for inhibiting acid-driven dissolution of buried coral skeletons through buffering of the porewater carbonate system (WEHRMANN *et al.* 2009).

1.4 Coral Reefs under Global Change Pressures

Coral reefs are continuously deteriorating as a result of human influences (HUGHES *et al.* 2003). Anthropogenic pressures include overfishing and destructive fishing, especially dynamite and cyanide fishing. Pollution and sedimentation from land-based sources and costal development, tourism and anchoring of recreational and commercial ships are

further threats. Since 1980, coral bleaching (i.e. the whitening of corals due to stress-induced expulsion or death of their photosynthetic symbionts) increases globally. In the ocean, anthropogenically driven increases in atmospheric concentrations of carbon dioxide contribute to both ocean warming and acidification (HARVELL *et al.* 2007). Warming and acidification alone, and synergistically, have the potential to not only alter coral physiology directly (HOEGH-GULDBERG *et al.* 2007, KLEYPAS & YATES 2009), but also indirectly through impacts on coral-associated microorganisms, thereby potentially disrupting the normal function of the coral holobiont. This loss of function, in turn, may impact coral reef ecosystems as a whole (MOUCHKA *et al.* 2010). The concentration of carbon dioxide in the Earth's atmosphere exceeds already 380 ppm, which is more than 80 ppm above the maximum values of the past 740,000 years (PETIT *et al.* 1999). Under conditions expected for the 21st century, global warming and ocean acidification will compromise carbonate accretion, with negative consequences for coral growth. Climate change also exacerbates local stresses from declining water quality and overexploitation of key species, driving reefs increasingly toward the tipping point for functional collapse. (HUGHES *et al.* 2003, BRUNO & SELIG 2007, HOEGH-GULDBERG & BRUNO 2010). Also CWC are endangered by deep water fishing, littering and constructions.

As coral reef ecosystems are vulnerable to both direct (e.g. mining, waste dumping, bottom trawling, oil and gas exploration) and indirect (ocean acidification, temperature rise; e.g. GUINOTTE *et al.* 2006) impacts, reef-associated organisms, including bacteria, came into focus. This provided the basis for initiating several studies, including the ones presented in this thesis, to further explore microbial diversity and function associated with both WWC and CWC ecosystems. There is indeed evidence that coral-bacterial assemblages have the potential to be sensitive to the effects of climate change (MOUCHKA *et al*. 2010). The potential exists for very small modifications in temperature or pH associated with climatic change to increase the variability of coral-bacterial populations and in turn affect the health, life history, and species composition of coral reefs. It is possible that increasing temperatures and decreasing pH of the sea surface will alter the biogeochemical role that coral microbiota potentially play (MOUCHKA *et al.* 2010). Increases in seawater temperature can directly alter coral-associated bacterial structure and function, potentially leading to disease. THURBER *et al.* (2009) demonstrated that elevated temperatures shifted the microbiome of *Porites compressa* to a more disease-associated state.

Bacteria that colonize coral-associated microbial habitats must be able to withstand diurnal fluctuations in pH associated with algal photosynthesis. Despite being exposed to a large range of pH, there is some evidence that increasing acidity leads to variability in coral-associated microbiota. Similar to increasing temperatures, THURBER *et al.* (2009) found that decreasing the pH of seawater to 7.4 shifted the microbiome of *P. compressa* to a more disease-associated state. The mechanisms driving this shift are unknown, but like other environmental processes that drive changes in the structure of coral-associated bacterial assemblages, a complex interaction between direct and indirect effects on the coral holobiont is hypothesized (MOUCHKA *et al.* 2010). Furthermore, other than decreasing accretion rates, it is unknown how ocean acidification will alter the physiology and susceptibility to disease of the host. It is also possible that synergisms between increasing temperatures and decreasing pH could cause variation in coral–bacteria assemblages. Therefore, the synergisms between the two environmental factors may enable niche expansion of potentially pathogenic bacteria (MOUCHKA *et al.* 2010).

I.5 Objectives and Thesis Outline

Coral reef ecosystems, often called “rainforests of the sea”, represent intriguingly diverse, productive and dynamic environments in both shallow and deep realms of the ocean. During the past decade, a growing body of research has started to reveal the importance of these complex environments as structured landscapes for the “unseen majority” in the oceans, microbial communities. The emerging picture led to the recognition of microbial assemblages as highly diverse, abundant and active members of the coral holobiont and its whole reef ecosystem. Due to substantial limitations regarding the localization and accessibility of CWC reef ecosystems, however, respective investigations focused almost exclusively on the microbial ecology of WWC reefs. At the outset of this thesis, microbiology research in CWC reef ecosystems was thus still in its infancy, lacking fundamental insights into microbial diversity, distribution, abundance and function. Furthermore, it was unknown if similar principles of the distribution of microorganisms associated with WWC and CWC reef ecosystems exist, as these differ substantially concerning environmental settings and processes.

The general aim of this thesis was therefore to advance hitherto existing knowledge of microbial diversity in WWC and, particularly, CWC reef ecosystems by exploring the dynamics of bacterial community variation and potential drivers across a combination of different ecological (organizational, temporal, spatial) scales, with explicit focus on the importance of microbial habitat differentiation.

The studies presented in this thesis thereby contributed to the following overarching questions:

Question 1: *Are coral-associated microbial communities distinct from those in the surrounding reef water and sediment?*

Question 2: *Are coral reef habitats hotspots of microbial biodiversity?*

Question 3: *Which factors are responsible for structuring microbial diversity in coral reef ecosystems?*

CWC. With respect to CWC reef ecosystems, efforts were mainly targeted at "mapping" bacterial communities within and between different reef environments and at gaining an understanding of spatial scales relevant for significant community change. The respective work consisted of two studies, whereof the first (II.2) represented a pilot survey that provided the basic investigational framework applied in the second one (II.3).

II.2 The aim of the study presented in section II.2 was to obtain a basic characterization of bacterial diversity associated with constructional CWC under both in situ and ex situ conditions. This included, in particular, the identification of principle, coral- and environment-specific patterns in bacterial community structure as related to distinct microbial habitats associated with and surrounding a CWC, such as coral branch, coral mucus, ambient seawater and proximal sediment. Furthermore, it involved the assessment of potential shifts between communities sampled under natural (reef) versus controlled (aquarium) conditions. It was hypothesized that bacterial communities would exhibit a significant partitioning according to (i) microbial habitat type, and (ii) change in overall living condition.

II.3 The aim of the study presented in section II.3 was to determine the most important spatial and reef-organizational scales responsible for variations in bacterial diversity in CWC reef ecosystems. This was realized through the analysis of bacterial community differentiation from local (intra-reef) to regional (inter-reef) scale by applying a hierarchical sampling approach spanning five levels of spatial and ecological reef organization. Following prior results, special emphasis was thereby given to the discrimination of distinct microbial habitats associated with and surrounding CWC, so as to allow for a consistent cross-comparison of coral- and environment-specific patterns. It was hypothesized that the bacterial community structure would vary significantly from local to regional scale when accounting for (i) microbial habitat type, (ii) coral species and color, (iii) geomorphologic reef zoning, (iv) in-/out-reef location, and (v) reef site and type.

WWC. With respect to WWC reef ecosystems, efforts were mainly targeted at assessing the importance of permeable reef sands as specific microbial habitats that drive bacterial community variation. The respective work comprised a multi-phase study (II.4), which also presented the opportunity for a first comparative analysis of the overlap between CWC- and WWC-associated bacterial communities (preliminary insights).

II.4 The aim of the study presented in section II.4 was to investigate permeable WWC reef sands of differing mineralogy for their potential to promote variations in bacterial diversity and biomass, also over time and space. This was achieved by comparing locally co-occurring carbonate and silicate reef sands for deviances in bacterial diversity, community structure, and cell number and, further, by interrelating sand type-specific community patterns to seasonal and spatial variability. It was hypothesized that bacterial communities would differ significantly depending on (i) mineralogy per se, but also according to its synergy with changes in (ii) season, (iii) sediment depth, and (iv) in-/out-reef location.

The research objectives outlined above were mainly implemented by DNA-based molecular analyses targeting sequence and length heterogeneities of the 16S rRNA gene and corresponding internal transcribed spacer (ITS), respectively. This included the high-throughput fingerprinting technique ARISA (Automated Ribosomal Intergenic Spacer

Analysis; FISHER & TRIPLETT 1999), the construction of 16S(-ITS) rRNA gene clone libraries, the direct coupling of both these methods (BROWN *et al.* 2005), as well as a suite of multivariate statistical tools commonly applied in community ecology studies (RAMETTE 2007). Sampling was performed in four CWC reef ecosystems on the Norwegian continental margin and in a WWC fringing reef in the Northern Red Sea.

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II. RESULTS

II.1 Publication Outline and Author Contributions

This thesis comprises the following three manuscripts which have been published or prepared for publication in three international journals. Respective author contributions and financial support specify as follows:

II.2 SANDRA SCHÖTTNER, FRIEDERIKE HOFFMANN, CHRISTIAN WILD, HANS TORE RAPP, ANTJE BOETIUS, and ALBAN RAMETTE (2009) **Inter- and Intra-Habitat Bacterial Diversity Associated with Cold-Water Corals.** Published in: *ISME J* 3: 756–759.

SS: designed study; performed sampling, molecular work and statistical analyses; conceived, wrote, and edited manuscript. FH: designed study; assisted with sampling. CW: designed study; assisted with sampling. HTR: contributed samples; provided logistical support. AB: designed study; edited manuscript. AR: designed study; assisted with statistical analyses; edited manuscript. Funding was provided by the German Research Foundation to CW within the EuroDIVERSITY project MiCROSYSTEMS, by the German Research Foundation to FH, by the EU 6th FP HERMES, and by the Max Planck Society.

II.3 SANDRA SCHÖTTNER, CHRISTIAN WILD, FRIEDERIKE HOFFMANN, ANTJE BOETIUS, and ALBAN RAMETTE (In preparation) **Microbial Habitat and Spatial Variation in Bacterial Communities Associated with Cold-Water Corals.** In preparation for: *PLoS Biol.*

SS: designed study; performed sampling, molecular work and statistical analyses; conceived, wrote and edited manuscript. CW: assisted with sampling. FH: assisted with sampling. AB: helped with conceiving manuscript. AR: assisted with statistical analyses; edited manuscript. Funding was provided by the German Research Foundation to CW within the EuroDIVERSITY project MiCROSYSTEMS, by the German Research Foundation to FH, by the EU 6th FP HERMES and the EU 7th FP HERMIONE to AB, and by the Max Planck Society.

II.4 SANDRA SCHÖTTNER, BARBARA PFITZNER, STEFANIE GRÜNKE, MOHAMMED RASHEED, CHRISTIAN WILD, and ALBAN RAMETTE (In preparation) **Drivers of Bacterial Ddiversity Dynamics in Permeable Carbonate and Silicate Coral Reef Sands.** To be submitted to: *Environ Microbiol* in December, 2010.

SS: assisted with sampling, cell counts, molecular work, and statistical analyses; performed additional statistical analyses; conceived, wrote and edited manuscript. BP: performed sand characterization, cell counts, molecular work, and statistical analyses. SG: assisted with molecular work; edited manuscript. MR: assisted with sand characterization. CW: designed study; performed sampling; edited manuscript. AR: designed study; assisted with conceiving and writing manuscript; edited manuscript. Funding was provided by the German Research Foundation to CW, and by the Max Planck Society.

II.2 Inter- and Intra-Habitat Bacterial Diversity Associated with Cold-Water Corals

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Abstract

The discovery of large ecosystems of cold-water corals (CWC), stretching along continental margins in depths of hundreds to thousands of meters, has raised many questions regarding their ecology, biodiversity and relevance as deep-sea hard-ground habitat. This study represents the first investigation that explicitly targets bacterial diversity from distinct microbial habitats associated with the cosmopolitan reef-building coral *Lophelia pertusa*, and also compares natural (fjord) and controlled (aquarium) conditions. Coral skeleton surface, coral mucus, ambient seawater and reef sediments clearly showed habitat-specific differences in community structure and operational taxonomic unit (OTU) number. Especially in the natural environment, bacterial communities associated with coral-generated habitats were significantly more diverse than those present in the surrounding, non-coral habitats, or those in artificial coral living conditions (fjord *versus* aquarium). These findings strongly indicate characteristic coral–microbe associations and, furthermore, suggest that the variety of coral-generated habitats within reef systems promotes microbial diversity in the deep ocean.

Introduction

Cold-water coral (CWC) reefs have become increasingly recognized for their potential to locally enhance faunal biodiversity in the deep ocean (FREIWALD *et al*. 2004, ROBERTS *et al*. 2006). Microbial diversity and function within these ecosystems, have so far been only poorly understood. As evidenced by earlier investigations on warm-water coral (WWC) reefs, the coral holobiont (host animal plus all associated microorganisms) is a complex system containing diverse, abundant and active microbial representatives of all three domains of life (ROHWER *et al*. 2002, WEGLEY *et al*. 2004, WILD *et al*. 2004, BOURNE & MUNN 2005). Several microbial habitats and respective niches, such as those directly generated by the coral (skeleton, tissue and mucus), but also ambient seawater and reef sediments, are known to be available for WWC reef-associated microorganisms (see ROSENBERG *et al*. 2007, and references therein).

Recent studies on microbial communities in CWC reefs have also provided initial indications of coral– microbe associations. While YAKIMOV *et al*. (2006) showed that

living specimens of the stony CWC *L. pertusa* in the Mediterranean harbor, specific bacterial communities that are different from those of dead coral material or sediments, another investigation even showed the spatial stability of CWC–microbe associations with an Antarctic soft coral across an environmental impact gradient (WEBSTER & BOURNE 2007). Characteristic bacterial assemblages were also found in an Alaskan octocoral, but exhibited minimal influence of transient water-column microbes (PENN *et al*. 2006).

This work contributes the first high-resolution molecular fingerprinting analyses of bacterial communities associated with the cosmopolitan reef-building CWC, *L. pertusa* (L., 1758). Using the Automated Ribosomal Intergenic Spacer Analysis (ARISA, which targets the 16S–23S rRNA-gene spacer length polymorphism) and multivariate statistics, this study examines the relationships between bacterial community structure and distinct microbial habitats in a CWC, such as skeleton surface and mucus, associated with living coral specimens from both natural (fjord) and controlled (aquarium) environments. We tested the null hypothesis that the microbial diversity of CWC-generated habitats reflects that of the ambient seawater or of proximal reef sediments, and therefore contains no 'coral-specific' bacterial signatures.

Materials and Methods

A total of 12 coral fragments (5–12 cm in length) derived from three living colonies (white phenotype) from the Langenuen fjord (near Bergen, Norway) were collected in December 2006 at 59°56'5''N, 05°28'5''E, 167 m water depth, by the remotely operated vehicle *Aglantha* (Institute for Marine Research, Bergen, Norway). Seawater (2×2 l) was sampled nearby at 250 m depth with 5 l-Niskin bottles mounted on a conductivity-temperature depth rosette sampler and reef associated surface sediments (0–5 cm depth) were collected at 175 m depth using a Van–Veen grab (59°52'9''N, 05°31'5''E) in October 2006.

The aquarium samples used for comparative analyses comprised 12 living coral fragments (5–12 cm in length) and seawater (2×1 l), which were obtained from a flow-through aquarium with fjord bottom-water retrieved from about 100 m water depth off Bergen (University of Bergen, Norway; located about 30 km from the Langenuen fjord) in October 2006. The aquarium corals originated from three coral colonies (white

phenotype) collected earlier in the Langenuen fjord (59°52'9''N, 05°31'5''E) at 120 m depth in July 2006, and further maintained under conditions adjusted to their natural environment (WILD *et al*. 2008).

For sub-sampling, all selected coral fragments were maintained in *in-situ* water for not more than 30 min before thorough rinsing with sterile, 0.2-mm-filtered seawater. Freshly produced mucus (up to 0.3 ml per fragment) was collected directly from polyps using sterile syringes after induction of mucus excretion through air exposure. Skeleton surfaces (partially covered by coenenchyme; BEUCK *et al*. 2007) were then sampled using sterile scalpel blades by scraping two distinct, 1-cm^2 patches per coral fragment. Scraping was carried out both on the corallite directly surrounding a living polyp and on the skeletal part most distant to all polyps present, yielding a mixture of surface plaques and calcareous particles. Skeleton surface scrapings and sediments (1 g per DNA extraction) were directly used for DNA extraction, whereas mucus and seawater samples were first concentrated onto sterile 0.2 mm polycarbonate filters (Millipore, Eschborn, Germany). Immediate processing of samples was carried out at all steps to minimize biases that may be introduced during retrieval and maintenance of corals during sampling.

Total community DNA was extracted and purified with Ultra Clean Soil DNA kits (MoBio, Carlsbad, CA, USA). Bacteria-specific ARISA (in triplicate PCR) using normalized DNA quantities of 22 ng per reaction for all samples, subsequent data formatting and binning were carried out as described elsewhere (CARDINALE *et al*. 2004, HEWSON & FUHRMAN 2006). Non-metric multidimensional scaling (NMDS) and the following statistical tests (Bonferroni-corrected) were implemented in R (version 2.5.0). Between-group variation was tested by pairwise analysis of similarities (ANOSIM). Within-group variation (scatter in the NMDS ordination plot) was compared by dispersion analysis, that is, by evaluating whether the difference between sample location and the group centroid was significant, using pairwise Wilcoxon–Mann–Whitney tests after ensuring that an overall Kruskal–Wallis test was significant at $P<0.05$. Operational taxonomic unit (OTU) numbers were compared by pairwise Wilcoxon– Mann–Whitney tests. Redundancy analyses (RDA) and variation partitioning were carried out as described elsewhere (RAMETTE 2007). ARISA and statistical analyses were carried out twice independently. As consistent conclusions were obtained twice, only one set of results is presented here.

Results and Discussion

Molecular fingerprinting of bacterial communities from samples of coral skeleton surface, coral mucus, ambient seawater and reef sediments retrieved freshly from the field site (hereafter named "fjord samples") clearly showed significant differences in community structure and sample dispersion (Figure 1), as well as in OTU number (Figure 2). Contrary to our null hypothesis, CWC-associated microbial habitats (that is, skeleton surface and mucus, sampled from three coral colonies from one location) clearly exhibited specific bacterial signatures when compared with the surrounding habitats (that is, seawater and sediments; Figure 1; pairwise ANOSIM tests, all $P<0.001$). Bacterial communities from skeleton surface and mucus were even found to be significantly distinct from each other, regardless of the coral origin (fjord or aquarium; all $P<0.001$). It is interesting that all communities associated with coral-generated microbial habitats (that is, skeleton surface and mucus) showed significantly higher sample dispersion than did communities found in the surrounding environments (that is, seawater and sediments; dispersion analysis, all $P<0.001$).

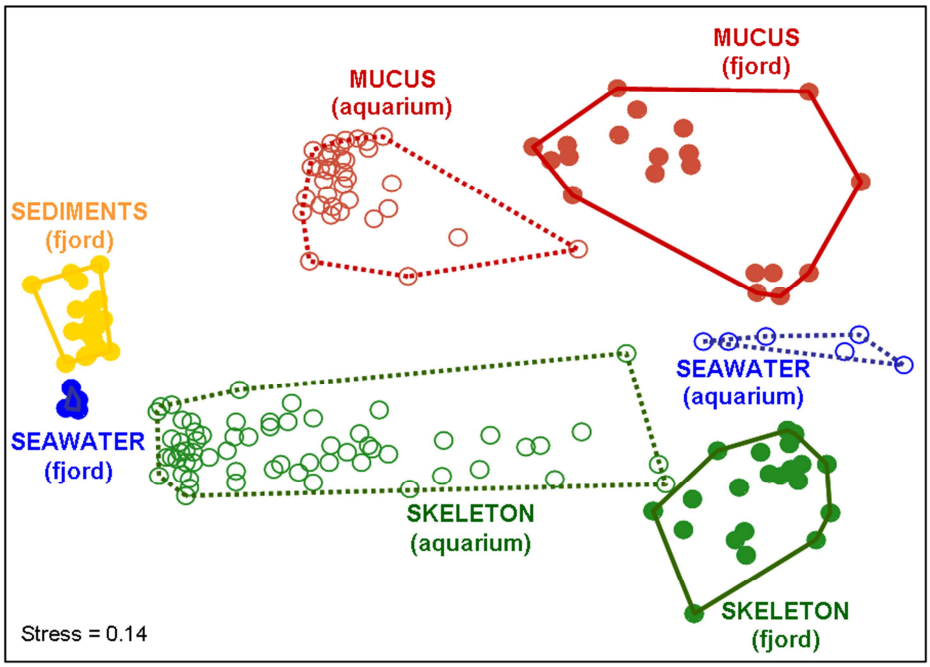

Figure 1. NMDS ordination plot (Bray–Curtis distance matrix) of ARISA profiles for coral-derived samples. ARISA was carried out on samples from coral skeleton surface, coral mucus, seawater, and sediments from natural (fjord) and controlled (aquarium) conditions. The proximity between samples in the plot corresponds to high-community similarity, and the quality of the ordination is indicated by a low-stress value.

The possibility of CWC-associated habitats offering different niches for bacterial communities could be explained by several factors, such as the availability of stable living conditions, distinct physico-chemical quality of the mucus or skeleton-derived surfaces, or the direct supply of nutritional sources such as CWC-derived organic matter. Furthermore, no community overlap between the respective habitats under different living conditions (fjord *versus* aquarium) was observed (Figure 1). Whether this indicates abiotic or biotic environmental effects on bacterial communities (that is, through the coral host) is not yet known, but similar findings have been obtained in a study comparing WWC mucus-associated bacteria from reef and aquarium environments (KOOPERMAN *et al.* 2007).

The OTU numbers differed substantially between the various habitats (Figure 2). In the aquarium, bacterial communities from skeleton surface and mucus yielded significantly higher OTU numbers (medians of 100 and 48, respectively) and greater sample variation than communities from ambient seawater (median of 9; pairwise Wilcoxon–Mann–Whitney tests, all $P<0.001$). The fjord skeleton surface and mucus, in contrast, showed fewer OTUs (medians of 7 and 14, respectively) and were associated with low variation, whereas samples retrieved from fjord seawater and sediments showed much higher OTU numbers (medians of 142 and 150, respectively) and higher variability (Figure 2).

For the fjord samples, the lower OTU numbers (Figure 2), but higher dispersion within coral-generated habitats (Figure 1), as compared with ambient habitats, seem to be rather counter-intuitive. It may be concluded that coral-generated habitats under natural conditions are associated with rather OTU-poor, but specific, bacterial communities. The selection of such bacterial associates could be induced by nutritional coral-microbe relationships, as coral exudates are known to attract specific, yet diverse populations of organo-heterotrophs (RITCHIE & SMITH 2004, ALLERS *et al.* 2008), or by specific chemical mediation by the coral host (KELMAN *et al.* 2006, RITCHIE 2006). Our findings are consistent with results from other studies that found specific associations between bacteria and their coral hosts (ROHWER *et al.* 2002, BOURNE & MUNN 2005, PENN *et al.* 2006, YAKIMOV *et al.* 2006, WEBSTER & BOURNE 2007). This indicates that a clear distinction in the bacterial community structure may exist between the different microbial habitats associated with scleractinian CWCs, such as *L. pertusa*.

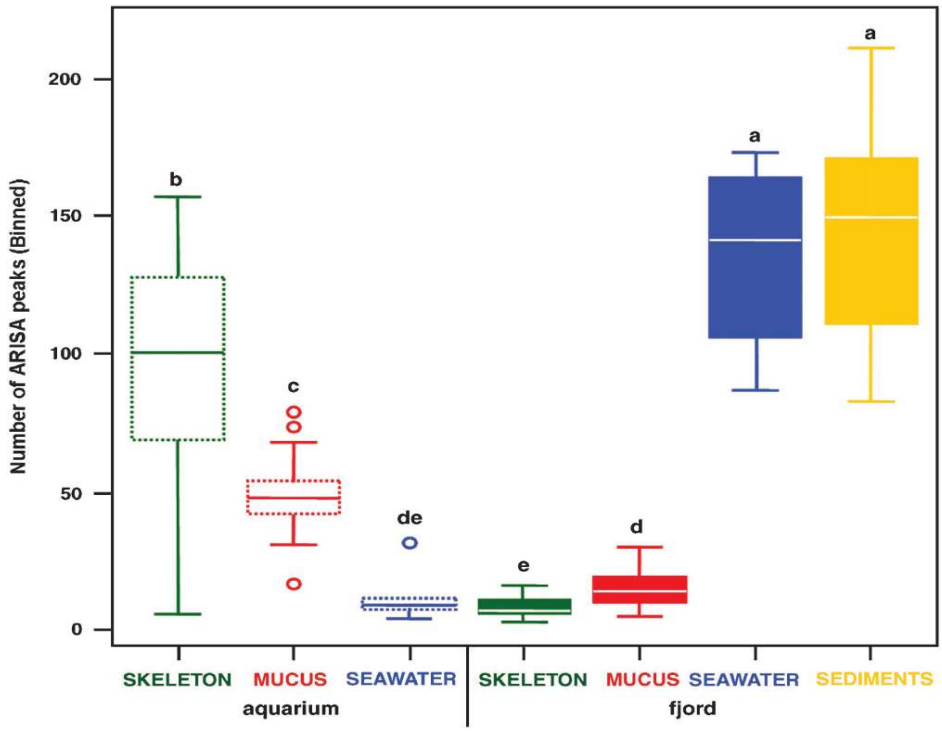

Figure 2. Bacterial OTU number as obtained by ARISA for coral-associated and environmental samples from both natural (fjord) and controlled (aquarium) conditions. The color coding for the different habitats corresponds to that used in Figure 1. The middle line in each box depicts the median of the respective data set. The box width represents 50% of the data, while both whiskers and outliers indicate the distribution of remaining data points, thus representing the overall variation. Different letters above each box denote a significant mean difference in OTU number between respective habitats (Wilcoxon–Mann–Whitney test, $P<0.05$).

The comparison between living conditions (fjord *versus* aquarium) and habitat type (coral-generated *versus* ambient) as diversity-generating factors showed that each factor contributed 9% to the total ARISA variation (variation partitioning; both $P<0.001$), but showed only 1% of co-variation (that is, the effects of the two factors were not confounded with each other). A large amount of community variation remained unexplained, suggesting that other factors may be likely to be at play. As the variation because of living condition was of the same amplitude as that caused by habitat type, keeping coral hosts even under the best-controlled conditions may seriously bias microbial diversity analyses. This has important practical implications for future work with coral-associated microbial communities. In conclusion, our data strongly suggest that the variety of coral-generated microbial habitats associated with CWCs promotes microbial diversity in the deep ocean.

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II.3 Microbial Habitat and Spatial Variation in Bacterial Communities Associated with Cold-Water Corals

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Abstract

Despite rising scientific recognition of cold-water coral ecosystems as biodiversity hotspots in the deep sea, insights into their associated bacterial communities are still limited. Assessing the magnitude of changes in bacterial communities is however critical for a better understanding of factors that contribute to the stability and functioning of these highly complex environments. Here we applied a hierarchical sampling approach spanning five levels of spatial and ecological reef organization to describe bacterial community differentiation in four Norwegian cold-water coral reef ecosystems by accounting for microhabitats present on corals, coral species and color, geomorphologic reef zoning, reef location, and reef type. Bacterial communities from coral-generated (branch, mucus) and ambient microbial habitats (water, sediment) were clearly distinct as indicated by Automated Ribosomal Intergenic Spacer Analysis (ARISA), and this partitioning was maintained over all spatial and ecological scales investigated. Coral-associated habitats were moreover characterized by highly site-specific patterns and community turnover. Furthermore, bacterial diversity patterns, albeit consistent with coral species and small-scale environmental complexity, changed markedly from local to regional scale, resulting in biogeographic patterns that parallel those of water and sediment bacteria. Supporting prior insights, our results demonstrate that the diversity of bacterial communities associated with cold-water corals may be structured by several factors acting at multiple spatial and organizational scales in cold-water coral reef ecosystems.

Introduction

Cold-water coral (CWC) reef ecosystems are increasingly portrayed as biodiversity hotspots in continental margin, seamount and mid-ocean ridge settings around the world (Roberts *et al.* 2009). As such, they appear as speciose, abundant and widespread as their warm-water counterparts (JENSEN & FREDERIKSEN 1992, FREIWALD *et al*. 2004, HENRY & ROBERTS 2007, HOVLAND 2008) and represent important species pools (ROBERTS *et al*. 2006, BUHL-MORTENSEN *et al*. 2010, HENRY *et al*. 2010) and speciation centers (LINDNER *et al*. 2008) in the deep sea. Their potential to foster a high degree of local diversity and

biomass is assumed to be mainly rooted in the ecosystem engineering capacity (JONES *et al*. 1994, BERKE 2010) of scleractinian constructional corals, such as the cosmopolitan key species *Lophelia pertusa* (L. 1758, Caryophylliidae) and *Madrepora oculata* (L. 1758, Oculinidae) (ROGERS 1999, FREIWALD 2002a, FREIWALD *et al*. 2004, ROBERTS *et al.* 2006). By forming enormous, dendritic skeletal frameworks, these corals provide three-dimensionally complex living space of varying size, structure and endurance for a plethora of mobile and sessile organisms (BONGIORNI *et al*. 2010, BUHL-MORTENSEN *et al.* 2010, HENRY *et al*. 2010). They also alter current flow regimes and sedimentation rates, thereby modifying the abiotic environment in time and space (ROBERTS *et al*. 2009 and references therein).

Structural habitat complexity is further promoted by the pronounced ecosystem discontiguity. Unlike warm-water coral (WWC) reef ecosystems that constitute contiguous reef environments with clear energy-related zonation (FREIWALD 2002a), cold-water coral ecosystems namely comprise several forms of appearance such as isolated colonies, small patch accumulations, large reefs or giant carbonate mounds, and can differ substantially with respect to their on-site spatial configuration (WILSON 1979, FREIWALD *et al*. 1997, ROGERS 1999, MORTENSEN *et al*. 2001, FREIWALD 2002a, FREIWALD *et al*. 2002b). Not uncommonly, individual clusters of coral frameworks (rather than a single coalescent one) form entire reef complexes which, depending on local seabed geology as well as community history, exhibit distinctive geomorphologic and taphonomic zoning, with marked transitions in sediment facies, faunal composition and proliferation stage (MORTENEN *et al*. 1995, FREIWALD & WILSON 1998, FREIWALD *et al.* 2002b, MORTENSEN *et al*. 2001, BUHL-MORTENSEN *et al*. 2010).

Despite mounting evidence of corals and reefs as structured landscapes of diverse and complex microbial communities (KNOWLTON & ROHWER 2003, ROSENBERG *et al*. 2007a, AINSWORTH *et al.* 2009), insights into the microbial diversity in CWC ecosystems are limited. Pioneering studies in this field mainly focused on microbes associated with colonial CWC scleractinians (YAKIMOV *et al*. 2006, GROßKURTH 2007, NEULINGER *et al*. 2008, HANSSON *et al*. 2009, KELLOGG *et al*. 2009, NEULINGER *et al*. 2009, SCHÖTTNER *et al*. 2009), but also with solitary scleractinians (FÖRSTERRA & HÄUSSERMANN 2008) and octocorals (PENN *et al*. 2006, BRÜCK *et al*. 2007, HALL-SPENCER *et al*. 2007, WEBSTER & BOURNE 2007). Respective findings based on community fingerprinting (Automated Ribosomal Intergenic Spacer Analysis, ARISA; Terminal Restriction Fragment Length

Polymorphism, T-RFLP; Denaturing Gradient Gel Electrophoresis, DGGE) or 16S rRNA gene sequencing show that bacterial communities colonizing living corals differ markedly from those present on dead corals or in the ambient environment like seawater or sediment (PENN *et al*. 2006, YAKIMOV *et al*. 2006, GROßKURTH 2007, NEULINGER *et al.* 2008, HANSSON *et al*. 2009, SCHÖTTNER *et al.* 2009). Even distinct coral-generated microbial habitats, such as skeleton surface, mucus, and tissue, further exhibit distinct bacterial signatures (GROßKURTH 2007, HANSSON *et al.* 2009, SCHÖTTNER *et al*. 2009).

Concerning the spatial patterning of bacterial communities associated with CWC habitats, diversity appeared consistent across an environmental impact gradient within and between different reef sites for some octocorals (WEBSTER & BOURNE 2007). On *L. pertusa*, several bacterial sequences were found in common from geographically separate regions, such as the Gulf of Mexico and the Trondheimsfjord in Norway, (NEULINGER *et al*. 2008, KELLOGG *et al*. 2009). Strict host specificity could not be evidenced though, as coral-associated communities show significant variations between sampling locations within the same geographic area or reef complex (GROßKURTH 2007, NEULINGER *et al*. 2008, HANSSON *et al*. 2009, KELLOGG *et al*. 2009), between colonies of the same coral species (HANSSON *et al*. 2009), between single polyps within the same coral colony (HANSSON *et al.* 2009), and between differently colored types within the same coral species (NEULINGER *et al.* 2008). In fact, evidence is mounting that coral-bacteria associations differ considerably with both host-associated microhabitats and prevailing environmental conditions. Due to the inconsistent choice of spatial scales and methodologies in past surveys, the relative importance of distinct factors that structure bacterial communities across various ecological scales may not be inferred without bias (CHASE 2003, WHEATLEY & JOHNSON 2009).

The aim of the present study was therefore to identify habitat-specific patterns of bacterial diversity in CWC reef ecosystems using a multi-scale, hierarchical sampling approach spanning five levels of spatial and ecological reef organization. Sources of community variation were assessed with respect to local (intra-reef) to regional (inter-reef) scale, and by further considering (i) microbial habitats on and around corals, (ii) coral species and color, (iii) geomorphologic reef zoning, (iv) in-/out-reef location and (v) reef type/site. Bacterial community DNA derived from two constructional corals (*L. pertusa*, *M. oculata*) as well as seawater and sediment as reference habitats were sampled within four cold-water coral reef ecosystems on the Norwegian continental margin, and

subjected to the high-throughput fingerprinting technique ARISA and a suite of multivariate statistical tools. According to our hypotheses, the bacterial community structure was expected to vary significantly among coral microbial habitats as well as between coral hosts, but also to show clear imprints of reef complexity and geographic separation.

Material and Methods

Study Sites. The Røst Reef (Figure 1A), discovered in 2002, is regarded as the largest proliferating CWC reef ecosystem in the world (THORSNES *et al.* 2004, NORDGULEN *et al.* 2006,). Situated on the northern mid-Norwegian continental slope at a water depth of 300–400m, it forms a 35–50 km long and 3 km wide belt (FOSSÅ *et al*. 2005) covering steep ridges in the headwall zone of the Trænadjupet submarine landslide (DAMUTH 1978, LABERG & VORREN 2000, LABERG *et al*. 2002a). The hydrodynamic regime around these ridges is controlled by the Norwegian Current, with strong northeast-oriented currents flowing approximately parallel to the reef (POULAIN *et al*. 1996, LABERG *et al*. 2002b). Owing to the specific ridge morphology, current dynamics and resulting strong variation in hemipelagic sediment deposition (LABERG *et al*. 2002b), the whole reef features a distinct geomorphologic habitat zoning. Ridge crests and upper slope parts, consisting of hard glacial clay, are covered by a dense frame work of living coral colonies which form giant apron-like terraces facing up-slope (FOSSÅ *et al*. 2005, WEHRMANN *et al.* 2009). Main spatial contributors comprise the constructional scleractinian species L. pertusa and *M. oculata* which occur in several color types, mostly white and red (Figure 1B).

The lower slopes, characterized by coral rubble-bearing facies (WEHRMANN *et al*. 2009), are highly sponge-dominated, and coral occurrence is reduced to isolated living colonies originating from an occasional framework rupture at the ridge top and subsequent debris transport down-slope. The depressions between ridges comprise a fine-grained, clay to silt-bearing matrix with embedded dead coral fractions (WEHRMANN *et al*. 2009), and are populated by various sponge communities, with only few living colonies in between. In general, the highest degree of coral proliferation and density (reef center) is found up-slope, in immediate vicinity of the headwall (high-relief area; FOSSÅ *et al*. 2005, NORDGULEN *et al.* 2006, JAGO team *personal comment*), while the down-slope reef periphery (low-relief area) exhibits only randomly occurring isolated colonies.

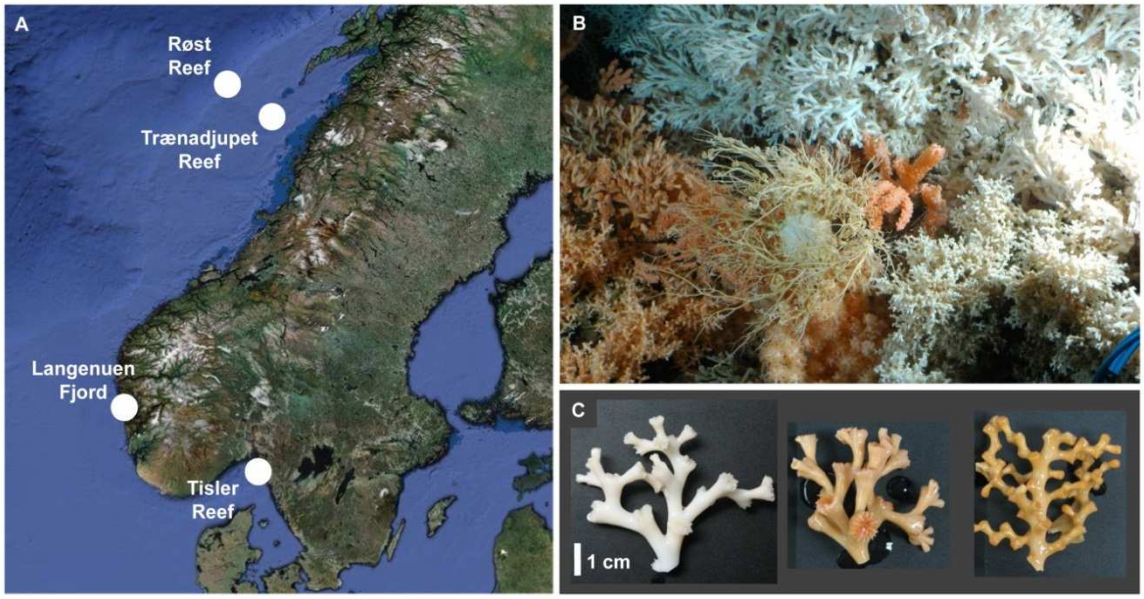

Figure 1. Study sites and coral species targeted in this study. (**A**) Offshore and inshore cold-water coral ecosystems along the Norwegian continental margin. (**B)** Living colonies of *L. pertusa* and *M. oculata* in their natural environment at Røst Reef, northern mid-Norwegian continental margin. (**C)** Fragments of freshly sampled white *L. pertusa* (*left*), red *L. pertusa* (*middle*), and red *M. oculata* (*right*).

The Trænadjupet Reef (Figure 1A) covers a circular embayment on the edge of Trænadjupet (HOVLAND & MORTENSEN 1999), an elongated transverse cross-shelf trough incising the mid-Norwegian shelf (OTTENSEN *et al*. 2005). At 300–330 m water depth, it covers deltaic sandy fan deposits forming distinct cigar-shaped structures (HOVLAND *et al.* 2005), and is exposed to the cyclonic circulation predominating in the Lofoten basin (POULAIN *et al.* 1996). In absence of a distinct habitat zoning, most of the cigar-shaped elevations are covered by a fine-grained matrix of silt to clay and biogenic debris, with coral rubble and dead framework atop (WEHRMANN *et al*. 2009). Living coral colonies of white *L. pertusa* are only found on some of the eastern tips of these structures, while sponges mark the dominant fraction of the overall reef community.

The Tisler Reef (Figure 1A), first discovered and documented in 2002, represents one of the largest and shallowest coastal reefs known worldwide (LUNDÄLV 2004, JONSSON 2006). Situated on a sill in the Hvaler/Kosterfjord region, northeast of the Tisler islands in the Norwegian Skagerrak, it encompasses an area of about 1200×200 m at a water depth of 70–160 m (LUNDÄLV 2004, JONSSON 2006). Due to the Kosterfjord deep-water connection to the open Skagerrak, the reef is exposed to strong currents being forced through a long, deep gully in northwest-southeast direction (LAVALEYE *et al.*

2009). The live coral cover is dominated by many large colonies of *L. pertusa* (JONSSON 2004), but also sponges constitute an integral part of the reef structure (PURSER *et al.* 2009). In the distal reef areas, large dead coral structures indicate severe trawl damage and suggest the original size of the living Tisler reef as about twice its present size (LUNDÄLV 2004, LAVALEYE *et al*. 2009).

The Langenuen Fjord in West-Norway near Bergen (Figure 1A), is a north-south water passage connecting the Hardangerfjord with the Korsfjord. One of the several patch reefs covering the rocky, steep slopes of the northern fjord section is located near Landrøyodden at a water depth of approximately 80–420 m. Atop of rock- and rubble-bearing facies, living colonies of *L. pertus*a and *M. oculata* occur among different types of sponges and other invertebrates.

Hierarchical Sampling Design. In order to study ecosystem-specific patterns of bacterial community variation from local to regional scale, sampling was performed hierarchically, spanning a total of 5 different levels of spatial and ecological reef organization (Figure 2). The first study level (factor: HABITAT, scale: µm–cm) comprised four potentially distinct types of bacterial habitat associated with and surrounding a scleractinian coral colony in its reef environment: Coral skeleton surface ("branch"), coral exudates ("mucus"), ambient seawater ("water") and proximal sediment ("sediment"). The second level (factors: COLOR, SPECIES, scale: 1 cm–2 m) featured a specific scleractinian species (*L. pertusa*, *M. oculata*) and coral color type (white, red). Geomorphologic reef zoning, as prevailing at Røst Reef, determined the third level (factor: ZONE, scale: 1 m–10 m). Respective zones included the terrace-covered ridge top ("crest"), the rubble- and sponge-dominated ridge slope ("slope"), and the clay-bearing, sparsely populated inter-ridge depression ("valley") in the reef center. At the fourth level (factor: IN-OUT, scale: 1 m–100 m), the up-slope reef center ("in-reef") was compared with the down-slope reef periphery ("out-reef"), in distances of 1 m, 10 m and 100 m away from the apparent reef margin. The fifth level (factor: REEF, scale: 100–1000 km) eventually contrasted the Røst Reef environment ("Røst") with the nearby Trænadjupet Reef ("Trænadjupet") as well as with the two inshore ecosystems, Tisler Reef ("Tisler") and Langenuen Fjord ("Langenuen").

Focusing on local, mainly reef-internal variations, levels 1–4 (Figure 2) were implemented in the Røst Reef area as main study site. Respective sampling of corals (*L.*

pertusa, *M. oculata*), seawater and sediment was performed within the scope of two mannend submersible dives describing a down-slope gradient across the reef (Table 1; see also Supplemental Information: Figure S1), with the first dive traversing two of the uppermost ridges near the headwall (reef center; in-reef), and the second dive extending former heading further down-slope beyond the apparent reef margin (reef periphery; out-reef), in a distance of approximately 2.5 km. At the other study sites (level 5; Figure 2), sampling involved the collection of *L. pertusa*, seawater and sediment at random locations within respective main reef area (Table 1). Selection of sampling locations was based on prior video reconnaissance.

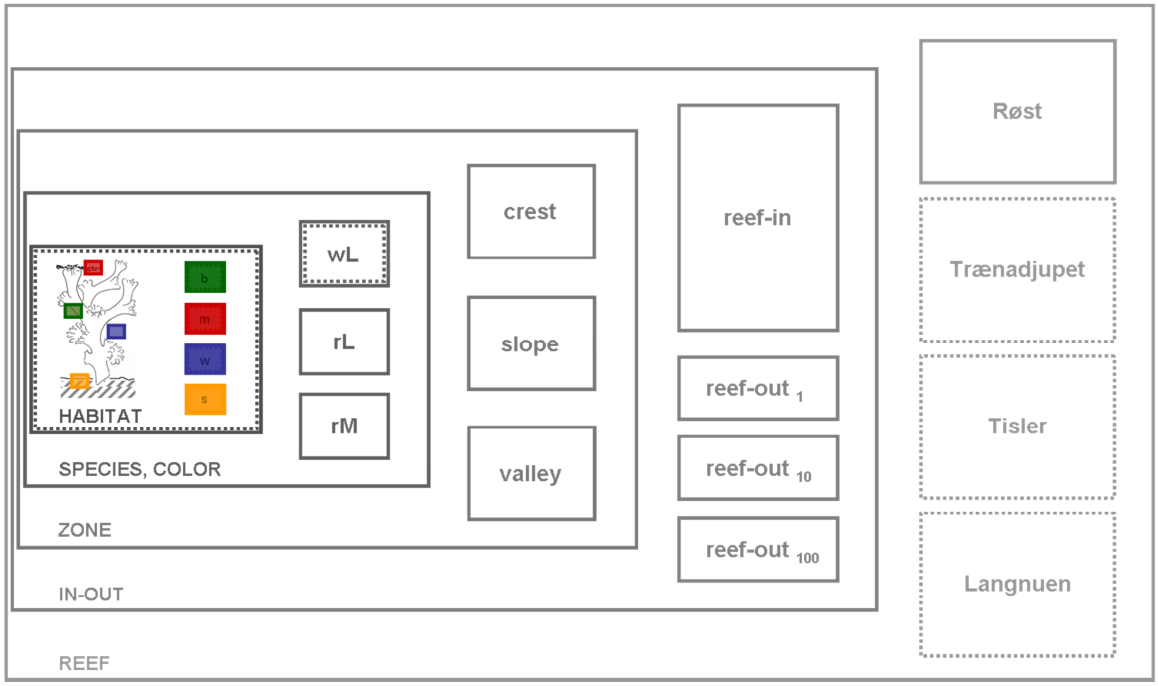

Figure 2. Multiscale hierarchical sampling design to investigate intra- and inter-reef specific patterns of bacterial habitat differentiation and interconnection from local to regional scale. Nested frames indicate the different study levels, with spatial scaling increasing from inside (*left*) to outside (*right*). Boxes within frames symbolize each lower level as integral part of respective higher level. At Røst Reef as main study site, sampling was implemented on all levels (continuous line); at all other sites, it was performed only on the lowest and highest level, respectively (dotted line). Study levels with respective scale and partitions: HABITAT (µm–cm): coral branch ("b"), coral mucus ("m") , ambient seawater ("w"), proximal sediment ("s"); SPECIES (cm–m): white *L. pertusa* ("wL"), red *L. pertusa* ("rL"), red *M. oculata* ("rM"); ZONE (1 m–10 m): ridge top with coral terraces ("crest"), ridge slope with single coral colonies on rubble ("slope"), ridge depression with single colonies on clay ("valley"); IN-OUT (1 m–100 m): reef center ("reef-in"), reef periphery in distances of 1, 10, 100 m away from the apparent reef margin ("reef-out"); REEF (km): Røst Reef ("Røst"), Trænadjupet Reef ("Trænadjupet"), Tisler Reef ("Tisler"), Langenuen Fjord ("Langenuen").

Table 1 Station list with sampling events of living corals (wL: white *L. pertusa*, rL: red *L. pertusa*, rM: red *M. oculata*), seawater, and sediment.

Study site	Research vessel	Expedition ID	Event ID and date	Sampling device	Sampling position	Water depth	Reef zone	Sample type	Sample amount	Coral type	Units used for ARISA
Røst-in	*RV Polarstern*	ARK XXXII/1a	PS 70/17-1 10 Jun, 2007	submersible *JAGO*	67°31'09'' N 09°28'47'' E	320 m	ridge crest	coral	3 colonies	wL, rL, rM	3x 1 fragment
					67°31'09'' N 09°28'47'' E	326 m	ridge valley	coral sediment	2 colonies 50 ml	wL, rL –	2x 1 fragment 3x 1 g
					67°31'10'' N 09°28'42'' E	318 m	ridge crest	coral water (*	2 colonies 2 l *	wL, rL –*	2x 1 fragment *
					67°31'10'' N 09°28'42'' E	328 m	ridge slope	coral water sediment	3 colonies 2 l 50 ml	wL, rL, rM – –	3x 1 fragment 2x 1l 3x 1 g
					67°31'10'' N 09°28'35'' E	340 m	ridge valley	coral water sediment	2 colonies 2 l 50 ml	wL, rM – –	2x 1 fragment 2x 1l 3x 1 g
Røst-out	*RV Polarstern*	ARK XXXII/1a	PS 70/31-1 15 Jun, 2007	submersible *JAGO*	67°31'18'' N 09°28'12'' E	365 m	1 m distance	coral water sediment	2 colonies 1 l 50 ml	wL, rM – –	2x 1 fragment 1x 1l 3x 1 g
					67°31'19'' N 09°28'11'' E	370 m	10 m distance	coral water sediment	2 colonies 2 l 50 ml	wL, rM – –	2x 1 fragment 2x 1 l 3x 1 g
					67°31'20'' N 09°28'01'' E	388 m	100 m distance	coral water sediment	2 colonies 2 l 50 ml	wL, rM – –	2x 1 fragment 2x 1l 3x 1 g
Trænadjupet	*RV Polarstern*	ARK XXXII/1a	PS 70/27-1 14 Jun, 2007	submersible *JAGO*	66°58'21'' N 11°06'37'' E	302 m	–	coral water sediment	1 colony 2 l 50 ml	wL – –	1x 1 fragment 2x 1 l 3x 1 g
					66°58'22'' N 11°06'33'' E	299 m	–	coral sediment	1 colony 50 ml	wL	1x 1 fragment 3x 1 g
Tisler	*RV Lophelia*	–	– 21 May, 2008	ROV *SubFighter*	58°59'51'' N 10°57'34'' E	107 m	–	coral	2 colonies	wL	2x 1 fragment
					58°59'53'' N 10°57'40'' E	91 m	–	coral	2 colonies	wL	2x 1 fragment
					58°59'50'' N 10°57'39'' E	100 m	–	coral	1 colony	wL	1x 1 fragment
					58°59'39'' N 10°57'50'' E	105 m	–	water sediment	2 l 50 ml	– –	2x 1l 3x 1g
					58°59'51'' N 10°57'39'' E	97 m	–	water sediment	2 l 50 ml	– –	2x 1l 3x 1g
Langenuen	*RV G.O. Sars*	2006118	– 07 Dec, 2006	ROV *Aglantha*	59°58'30'' N 05°22'30'' E	259 m	–	coral	1 colony	wL	1x 3 fragment
	RV G.O. Sars	2006118	– 07 Dec, 2006	CTD	–	250 m	–	water	4 l	–	2x 1l
	RV H. Brattstrøm	–	– 09 Oct, 2006	VanVeen grab	59°56'30'' N 05°28'30'' E	175 m	–	sediment	50 ml	–	6x 1g

* sample lost during further processing

In-situ Sample Collection. Living CWC were sampled by manned submersible (Røst, Trænadjupet) or video-assisted remotely operated vehicle (Tisler, Langenuen; Table 1). After visual assessment of each target colony *in situ*, a healthy looking fraction was picked from the colony's living outer rind using the manipulator arm, and placed into a separate compartment of the sampling reservoir. Directly back onboard, each specimen was inspected for epigrowth, impurities or degeneration, before selecting a fragment (5–15 cm in length) for sub-sampling of coral-associated microbial habitats. Fragments needed for skeleton surface and mucus sampling were maintained in flow-through tanks with *in-situ* water at a temperature of 10–11°C for ≤30 min until subsequent processing. Seawater was sampled with 2-l Niskin bottles attached to the submersible (Røst, Trænadjupet), or mounted on a conductivity-temperature-depth rosette sampler (Langenuen) or a video-assisted steel cable (Tisler). Immediately after retrieval, water samples were kept at 4°C, filtered in 500 ml aliquots onto sterile polycarbonate membranes (0.22 µm, Millipore, Billerica, MA), and stored at -20°C until further treatment. Surface sediments (approximately 0–5 cm sediment depth) were collected by custom sampling scoops operated via the submersible and vehicle manipulator arm (Røst, Trænadjupet, Tisler) or by Van-Veen grab (Langenuen). Right upon retrieval, sediment samples were transferred into sterile 50-ml vials and stored at -20°C until further processing/treatment. At Røst Reef, sediment sampling was not possible on ridge tops, owing to the high density of the prevailing coral framework cover.

Coral Sub-Sampling Procedures. After gentle rinsing with sterile-filtered (Whatman, Maidstone, UK) local seawater, skeleton surfaces of living CWC were sampled by scraping an area of up to 5 cm^2 per fragment with sterile scalpel blades, yielding a mixture of surface plaques, coenosarc tissue, and calcareous particles. Scraping was carried out on the primary, and partly secondary, branches of each fragment, avoiding fragile outer branches as well as polyp calices. All material accumulated per fragment was directly transferred into the provided DNA extraction tube (see below) by submerging the plaque-bearing scalpel blade in the contained solution. Freshly produced coral mucus was sampled by gently rinsing living coral fragments with sterile-filtered seawater and inducing mucus exudation through 2–5 min air exposure. After discarding exudates released during the first minute, subsequent production of up to 0.5 ml per fragment was collected directly from polyp surfaces by using sterile syringes. Resulting

mucus-seawater mixture was concentrated onto sterile polycarbonate filters (Whatman), and frozen at -20°C until DNA extraction. At all steps, immediate and careful processing of samples was of paramount importance to minimize biases introduced during retrieval, maintenance and sub-sampling of coral specimens.

DNA Extraction. Total community DNA was extracted and purified with the Ultra Clean Soil DNA Kit (MoBio, Carlsbad, CA, USA) following the manufacturer's instructions for maximum yield. Branch (scrapings from up to 5 cm^2 skeleton surface) and sediment (3×1 g) samples were directly transferred into extraction tubes, mucus (up to 0.5 ml) and water (2–4×500 ml) on respective filter membranes. Final elution of DNA was performed with 50–100 µl 1× TE buffer (Promega, Madison, WI). Concentration of yielded DNA was determined by NanoDrop spectrophotometry (NanoDrop, Wilmington, DE).

ARISA Fingerprinting. Universal bacterial ARISA (FISHER & TRIPLETT 1999) as well as subsequent data transformation and binning were carried out as described by RAMETTE (2009), with modifications. Universal primer ITSF (5'-GTCGTAACAAGGTAGCCGTA-3'; 5'-6-Fam-labeled, CARDINALE *et al.* 2004) and bacterial primer ITSReub (5'-GCCAAGGCATCCACC-3'; CARDINALE *et al.* 2004) were used for amplification of the rRNA intergenic spacer region ITS1 by polymerase chain reaction (PCR), with each 50 µl reaction (in triplicates per sample) containing 22 ng of template DNA. After a PCR product control via 1.5% agarose gel electrophoresis, all products were purified with Sephadex G-50 Superfine (Sigma-Aldrich, Buchs, Switzerland) and quantified with a NanoDrop spectrophotometer. For generating ARISA profiles, 100 ng/µl of cleaned PCR product were combined with a 15 µl-separation cocktail containing ROX 1000 internal size standard (MapMarker; BioVentures, Murfreesboro, TN) in deionized formamide. After denaturation at 95°C for 2 min and subsequent storage on ice, ARISA fragments were discriminated according to size by capillary electrophoresis using an ABI Prism 3130x Genetic Analyzer (Applied Biosystems, Carlsbad, CA). Resulting ARISA raw profiles were analyzed with GeneMapper software v3.7 (Applied Biosystems). The total peak area was normalized to 1, and only fragments with a relative fluorescence intensity (RFI) above 50 units (≥0.09% of total amplified DNA) and between 100–1000 bp length were chosen for further analyses. To account for size calling imprecision and compensate for slight peak shift

between single runs, GeneMapper-derived ARISA peaks were then subjected to a binning procedure with a fixed window size of 2 bp using custom scripts in R v2.9 (The R Project for Statistical Computing) as described by RAMETTE (2009). The binning frame yielding the highest pairwise similarities among samples was selected for a merging procedure where all RFI signals based on PCR replication were combined into a single consensus signal per sample, given the presence of a binned peak (henceforth: operational taxonomic unit, OTU) in at least two triplicates. The resulting sample-by-OTU consensus table, reflecting OTU presence-absence as well as relative abundance, was then subjected to numerical analyses.

Multivariate Statistics. All numerical analyses and graphical representations were implemented in PAST v2.0 (Palaeontological Statistics) as well as in R v.2.9 using standard and community ecology packages 'vegan', 'MASS', 'BiodiversityR', 'stats', 'labdsv', 'mgcv' and custom scripts. Total numbers of ARISA-derived OTU were compared in a box-and-whisker diagram and assessed for mean difference by applying an overall Kruskal-Wallis test (KW) as well as pairwise Wilcoxon-Mann-Whitney tests (WMW). Shifts in OTU presence-absence were analyzed by custom R functions for OTU partitioning and turnover. Multivariate patterns in bacterial community structure were analyzed based on Bray-Curtis distances among individual (Hellinger-transformed) abundance profiles. At first, visual inspection of variation was performed by Non-metric MultiDimensional Scaling (NMDS), cluster analysis, and heat-mapping. Significant differences between resulting *a posteriori* sample groupings were tested with Analysis of Similarity (ANOSIM), and corrected for multiple comparisons according to the Bonferroni criterion (e.g. RAMETTE 2007). Within-group diversification of bacterial communities was investigated by multivariate dispersion analysis (ANDERSON *et al.* 2006), including Analysis of Variance (ANOVA) and pairwise tests of Tukey's Honest Significant Differences (TukeyHSD) of mean distances to group centroids (i.e. mean group dispersions). Furthermore, bacterial community variation was assessed by Permutational Multivariate Analysis of Variance (PERMANOVA; Anderson 2001, MCARDLE & ANDERSON 2001), with three separate analyses performed: One examining local, intra-reef variation (factors: HABITAT, SPECIES, COLOR, ZONE; Figure 2) at the reef center of Røst, one examining extended local differences between the up-slope reef center (in-reef) and the down-slope reef periphery (out-reef; factor: IN-OUT; Figure

2), and one examining regional, inter-reef variation (factors: HABITAT, REEF; Figure 2) between the four different reef sites/types (Røst, Trænadjupet, Tisler, and Langenuen). All tests were performed using type I sums of squares and 999 permutations under the reduced model. In the specific case of intra-reef variation, testing followed a nested layout, whereat each factor (e.g. SPECIES) was hierarchically nested within the next higher factor (e.g. ZONE). With the higher factor being fixed, randomizations among all nested factor levels occurred only *within* each higher factor level, but not *across* all higher factor levels.

Results

Variations in Bacterial OTU Number. OTU numbers (that is, the total sum of binned ARISA peaks) per sample ranged between 9–223 OTU, from a total pool of 440 different OTU in the whole data set. The comparison of all samples revealed that variations in OTU number related highly significantly to habitat type (KW, $P<0.001$), despite marginal deviations between the different reef sites (KW, $P=0.05$) (Fig. 3). The most pronounced

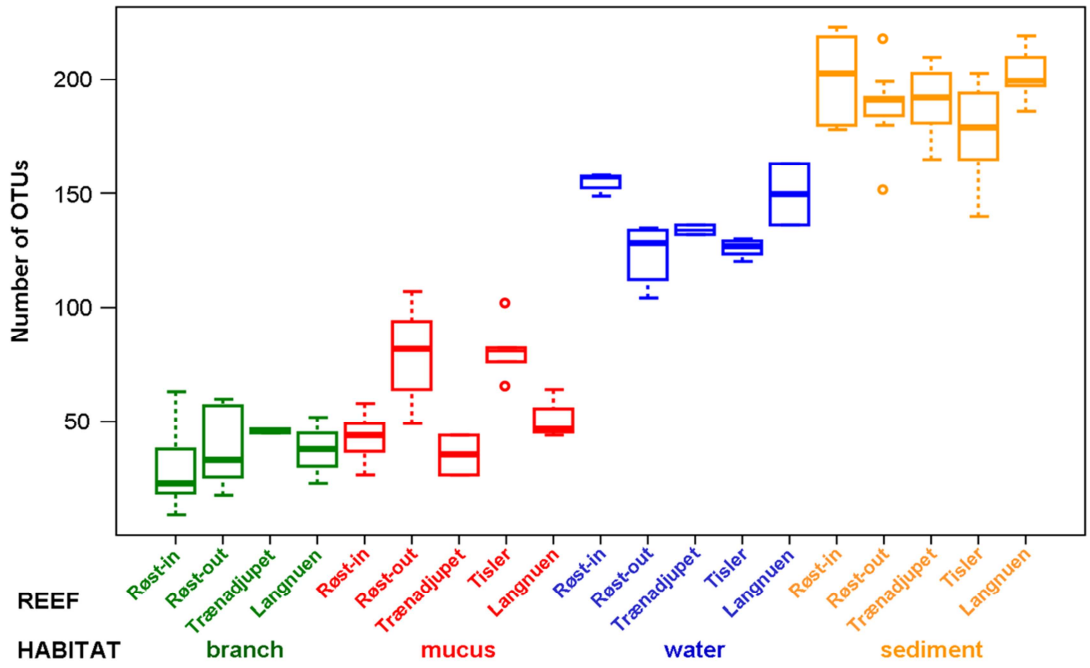

Figure 3 Number of ARISA-derived OTU in distinct microbial habitats at each reef site. Top, middle, and bottom lines of boxes represent the 25th (sample minimum), 50th (median), and 75th (sample maximum) percentiles; end of the whiskers represent the 5th and 95th percentiles; box size and line spacings indicate the degree of dispersion and skewness in the data; outliers above and below the whiskers denote extreme values.

habitat separation thereby occurred between coral-generated surfaces and the ambient environment, with branch (34 ± 15 OTU) and mucus (58 ± 22 OTU) featuring 30–80% lower mean OTU numbers than water (135 ± 16 OTU) and sediment (192 ± 19 OTU). At Røst, mean OTU numbers also showed distinctive changes between the in- and out-reef environment (KW, P=0.0398; Figure 3), which were mainly based on coral-associated habitats, especially mucus, causing a slight overall OTU increase from the up-slope reef center (Røst-in; 86 ± 11 OTU) to the down-slope reef periphery (Røst-out; 116 ± 62 OTU). When studying local trends at Røst-in and Røst-out separately, however, neither in-reef geomorphologic zoning (KW, P=0.098) nor out-reef gradual distances (of 1, 10, or 100 m) away from the apparent reef margin (KW, P=0.956) resulted in any significant OTU variation. No marked differences (mean totals of 36 ± 13 and 35 ± 17 OTU for *L. pertusa* and *M. oculata*, respectively) between both coral species were detected (KW, P=0.640). Likewise, OTU presence-absence was not related to variation in coral color (KW, P=0.538), as white (34 ± 14 OTU) and red (38 ± 14 OTU) corals harbored relatively similar OTU numbers.

OTU distribution among the four different microbial habitats was characterized by only 1 and 2 OTU specific to branch and mucus, respectively (Figure 4A) from a total of 390 OTU associated with *L. pertusa* at Røst, while 7 unique OTU (from 380 total) were identified on the respective same habitats on *M. oculata.* It may therefore be concluded that coral-derived habitats are not associated with extremely high bacterial specificity, especially when the surrounding water was characterized by 25–30 unique OTU and the sediment contained most of the habitat-specific OTU at a single reef site (up to 156 OTU at Røst; Figure 4A). Beyond Røst boundaries, these patterns were confirmed for all study sites, with only minor variations (data not shown). Yet, when all samples were combined (Figure 4B), the number of shared OTU increased, which remarkably reduced the number of unique OTU in water and sediment samples, while the number of branch- and mucus-specific OTU remained virtually unchanged.

OTU overlap between different microbial habitats and reef sites followed mainly habitat-specific trends and distinguished particularly coral-derived surfaces from the surrounding environment (Figure 5). In general, differences in OTU overlap clearly reflected variations in OTU number, with OTU-poor habitats (branch, mucus) sharing a much higher percentage of their OTU pool with OTU-rich habitats (water, sediment) than reciprocally. In this effect, branch and mucus shared at least half of their OTU with

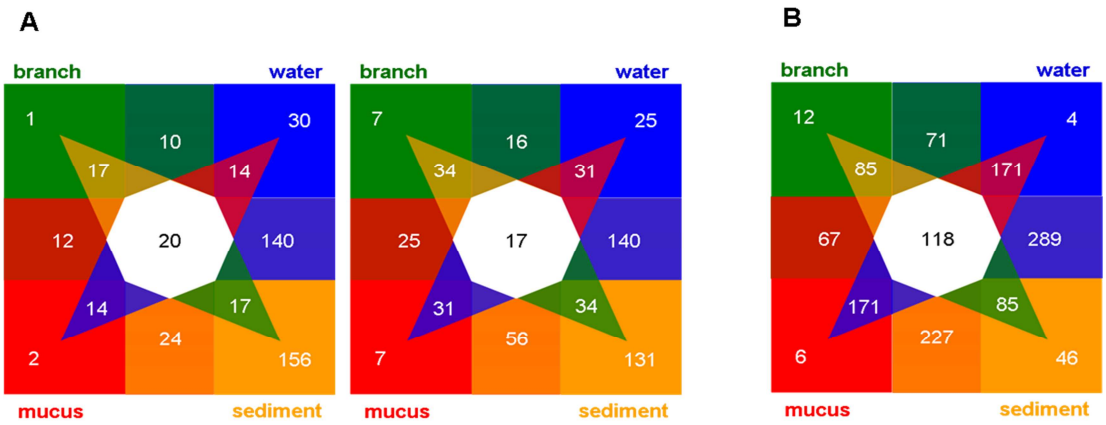

Figure 4. Partitioning of bacterial OTU between the distinct coral-associated and ambient microbial habitats targeted in this study. Numbers indicate the amount of OTU unique to each habitat (corner area), or common to any two (overlapping area) or all habitats (center area). (**A**) Bacterial OTU associated with samples of *L. pertusa (left)* and *M. oculata (right)* at Røst, (**B**) Bacterial OTU associated with all samples combined.

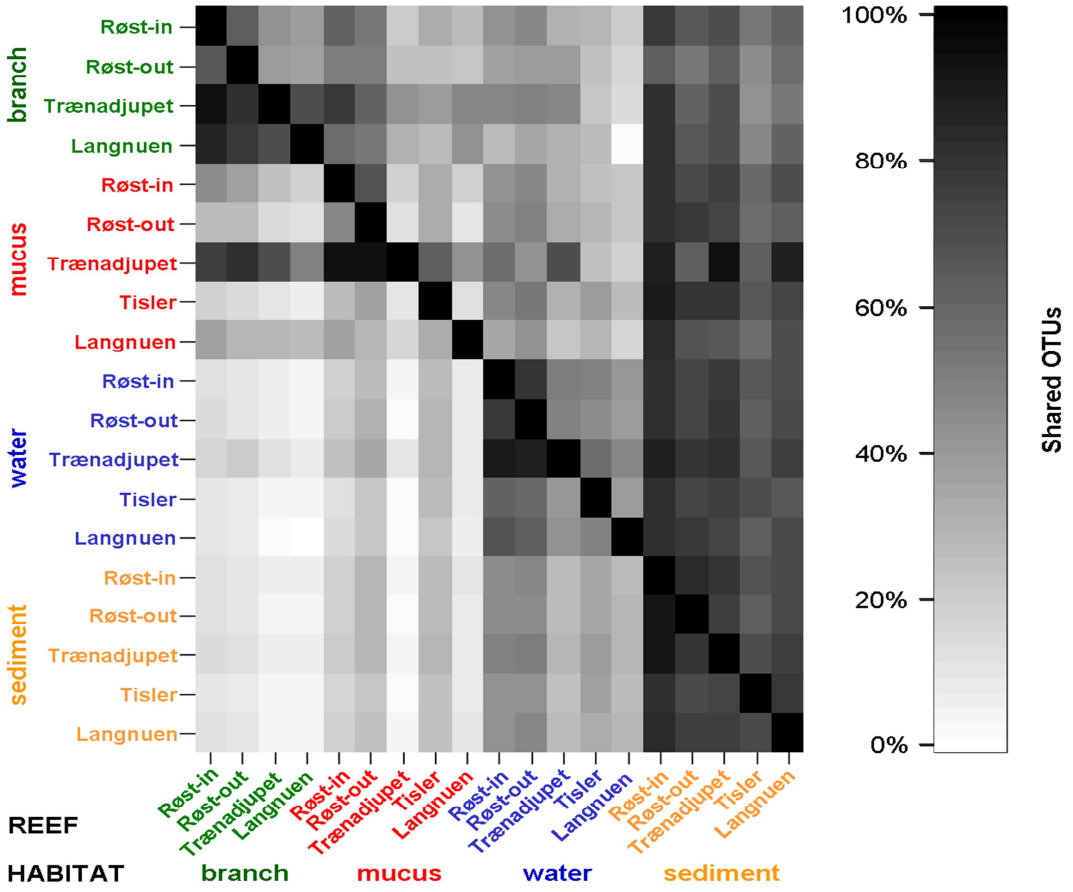

Figure 5. Heatmap representation of pairwise bacterial OTU overlap between the distinct microbial habitats at each reef site. Samples are grouped according to habitat type and reef. Cell position corresponds to the asymmetrical pairing of single sample groups, with rows specifying a group of reference and columns denoting respective group of comparison. Cell color indicates the respective OTU fraction (%) in the reference group being shared with OTU in the comparison group.

sediment (50% and 73%, respectively), and a comparatively lower fraction with water (25% and 36%, respectively). Conversely, only 9–10% and 17–18% of water and sediment OTU were also found among branch- and mucus-associated OTU, respectively. The number of OTU shared between the two coral-associated habitats amounted to a third of all OTUs (33–34%), and exhibited more variation for ambient habitats with water sharing higher fraction of its OTU pool with the sediment (74%) than vice versa (34%). Overall, OTU sharing between different reefs appeared remarkably more variable on coral-derived surfaces compared to the ambient environment (Figure 5).

When studied within each microbial habitat separately, the mean number of OTU shared between any two reefs was the lowest in the mucus (37%), but also varying the most (11–94%). In contrast, reef-specific changes in the sediment were on average the highest (76%), but shared fractions among sediment samples were more homogeneous (63–90%). In branch and water, the different reefs exhibited a similarly high OTU overlap of 37–90% (mean: 63%) and 38–89% (mean: 57%), respectively. No conspicuous separation between off- and inshore reefs was detected, but patterns showed that several OTU occurring at Røst and Tisler were not found at Trænadjupet and Langenuen (Figure 5), while most of the mucus OTU from Trænadjupet matched those from other sites.

Variations in Bacterial Community Structure. As detected by NMDS (Figure 6) and confirmed by PERMANOVA (Table 2A), bacterial communities markedly differ according to habitat type. At Røst (PERMANOVA R^2=0.587, P<0.001), and for all study sites combined (R^2=0.467, P<0.001), samples exhibited a highly significant affiliation with either one of the four different habitats, irrespective of any other trends (see also Supplemental Information: Table S1). With PERMANOVA R^2 values ranging from 0.75–0.80 for the different reefs (Table 2A), this separation of branch-, mucus-, water- and sediment-specific communities was similarly pronounced at all study sites. In the NMDS ordination (Figure 6) and in the original Bray-Curtis dissimilarity matrix (Figure S2), a clear separation was obtained between coral-associated surfaces and ambient environment, which also proved consistent when analyzing samples from the different locations separately (data not shown).

Despite commonalities in habitat differentiation, however, the four reef sites differed markedly in their community structure (Table 2A), especially with regard to mucus (PERMANOVA R^2=0.754, P=0.001), water (R^2=0.835, P=0.001) and sediment

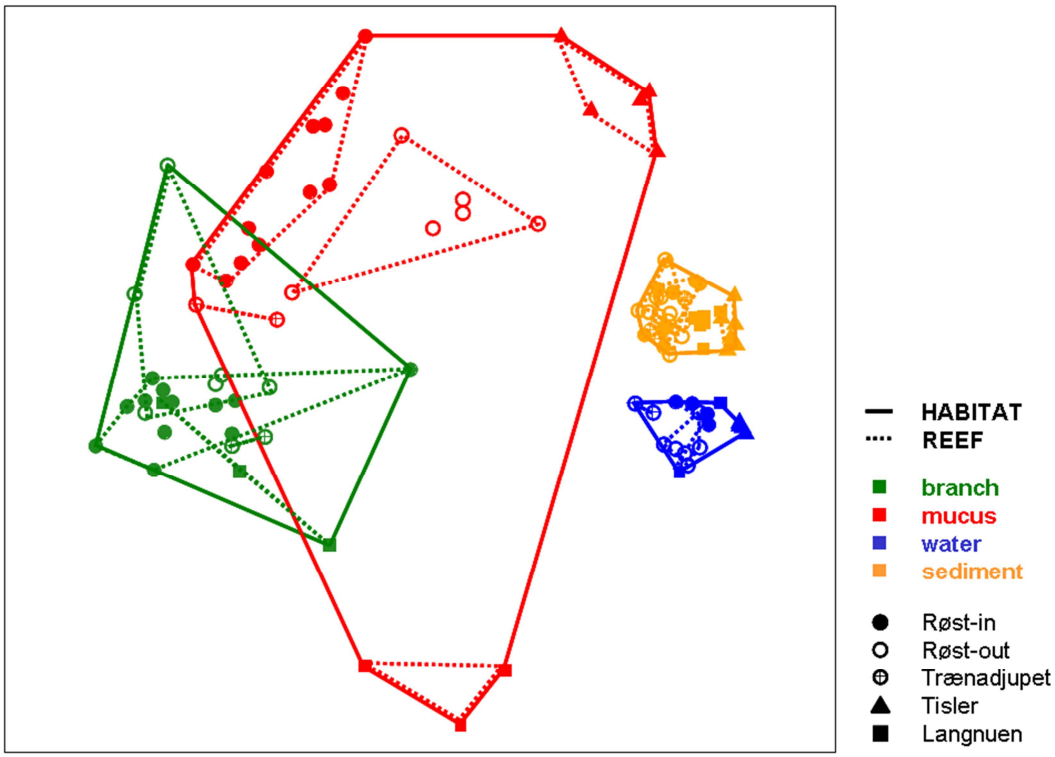

Figure 6. NMDS ordination generated from all ARISA-derived abundance profiles, describing variations in bacterial community structure as related to microbial habitat type and reef site/type. For each sample, consensus signals of PCR triplicates were used. Closer sample points indicate more similar community structure. *A posteriori* groupings specify microbial habitat and reef type/site. The low-stress value of 0.16 indicates the goodness-of-fit of the 2-dimensional representation compared to the original Bray-Curtis dissimilarity matrix.

(R^2=0.504, *P*=0.001), as also illustrated in the NMDS ordination (Figure 6) where the inter-reef segregation of communities was also strongly marked. Although additional community separation consisting of a split between offshore and inshore reef systems, and of specific reef type signatures in the water and sediment could be detected, the overall community patterning was mostly dominated by strong habitat effects. When analyzed for each habitat separately though, water showed mainly the regional separation between offshore and inshore sites, while sediment and mucus also revealed a further separation between the neighboring Røst and Trænadjupet (Figure S3). Significant intra-reef variations were already detected at Røst between the up-slope reef center (Røst-in) and the down-slope reef periphery (Røst-out) (Table 2B). Similar to above described regional patterns, this local effect was mainly evidenced in mucus (PERMANOVA R^2=0.319, *P*=0.001), water (R^2=0.300, *P*=0.01), marginally in sediment (R^2=0.165, *P*=0.003), and apparently not in branch (R^2=0.095, *P*=0.073; Fig. S3).

Interestingly, while the differences in the wider Røst area (i.e. between in- and out-reef environment/reef center and reef periphery) indicated some degree of local separation, the pronounced geomorphologic (including vertical) zoning at the reef center itself (Røst-in; Table 2C; Figure 4) revealed a weak trend among bacterial communities (R^2=0.102, *P*=0.049; Figure S3). Also, a purely horizontal segregation over distances of 1, 10, and 100 m in the reef periphery (Røst-out) had no significant effect (data not shown). Nevertheless, *L. pertusa* and *M. oculata* harbored bacterial assemblages with different structure (R^2=0.416, *P*<0.001; Table 2C), but overlapping to certain extent (Figure S3). In contrast, effects of bacterial variation related to coral color were, albeit overall significant, neither substantiated in branch (R^2=0.078, *P*=0.621) nor in mucus (R^2=0.096, *P*=0.434). With only minor deviances, all described results were supported by those of ANOSIM (Table S1) and cluster analysis (data not shown).

Discussion

Here we document the first multi-scale survey of bacterial diversity in CWC reef ecosystems across five levels of spatial and ecological reef organization. By assessing thedegree of bacterial community differentiation from local (intra-reef) to regional (inter-reef) scale and by considering (i) microbial habitat type, (ii) coral species and coral color type, (iii) geomorphologic reef zoning, (iv) in- and out-reef location, and (v) reef type, we aimed to identify the most important scales for changes in bacterial diversity associated with CWC reefs.

Microbial Habitats. Bacterial communities associated with the constructional CWC *L. pertusa* and *M. oculata* as well as their abiotic surrounding differed substantially according to the type of habitats colonized, with coral branch, coral mucus, ambient seawater and proximal sediment each consisting of a specific community structure that varied both in OTU composition and relative abundance. This habitat specificity appeared similarly pronounced for all samples at all study sites, and therefore clearly extend our earlier findings (SCHÖTTNER *et al*. 2009) beyond the local environment of a particular reef location or maintenance aquarium. This is in agreement with other studies that also partially evidenced such phenomenon for samples from the North-East Atlantic (GROßKURTH 2007, NEULINGER *et al.* 2008, HANSSON *et al.* 2009), the Central

Table 2A. PERMANOVA* of regional (inter-reef) bacterial variation as related to microbial habitat type and reef site.

Study site	Source of variation	R^2	F	*P*	
Røst-in Trænadjupet Tisler Langenuen	**HABITAT**	**0.467**	**17.222**	**0.001**	*******
	Røst	0.777	20.947	0.001	***
	Trænadjupet	0.794	10.273	0.001	***
	Tisler	0.801	24.135	0.001	***
	Langenuen	0.748	9.888	0.001	***
	REEF	**0.124**	**2.795**	**0.001**	*******
	branch	0.393	2.262	0.032	*
	mucus	0.754	11.210	0.001	***
	water	0.835	13.476	0.001	***
	sediment	0.504	7.452	0.001	***

Table 2B. PERMANOVA* of local (intra-reef) bacterial variation as related to in-/out-reef location, i.e. reef boundary.

Study site	Source of variation	R^2	F	*P*	
Røst-in Røst-out	**IN-OUT**	**0.048**	**3.100**	**0.006**	******
	branch	0.095	1.684	0.073	
	mucus	0.319	7.478	0.001	***
	water	0.300	3.433	0.01	*
	sediment	0.165	2.967	0.003	**

Table 2C. PERMANOVA* of local (intra-reef) bacterial variation as related to coral species, coral color, geomorphologic reef zoning, and microbial habitat type (nested tests).

Study site	Source of variation	R^2	F	*P*	
Røst-in Trænadjupet Tisler Langnuen	**ZONE**	**0.102**	**1.882**	**0.049**	
	branch	0.143	0.752	0.752	
	mucus	0.146	0.767	0.803	
	water	0.399	1.326	0.260	
	sediment	0.209	1.589	0.134	
	SPECIES (:: ZONE)	**0.416**	**11.742**	**0.001**	*******
	branch	0.236	3.086	0.001	***
	mucus	0.163	1.942	0.043	*
	COLOR (:: ZONE)	**0.024**	**0.398**	**0.868**	
	branch	0.011	0.878	0.607	
	mucus	0.082	0.631	0.794	
	HABITAT (:: SPECIES)	**0.587**	**23.411**	**0.001**	*******

* The significance of each model was assessed by 999 random permutations. Presented are the amount of explained variation (R^2), F-ratio, and P-value (*** = P<0.001). Shading indicates significance maintained after Bonferroni correction (P<0.002) .

Mediterranean (YAKIMOV *et al*. 2006), the Gulf of Alaska (PENN *et al.* 2006), as well as WWC reef ecosystems (FRIAS-LOPEZ *et al.* 2002, BOURNE & MUNN 2005, KOREN & ROSENBERG 2006). The most pronounced differences in bacterial community structure generally consist of a separation of coral-generated surfaces from the ambient environment, which was also accompanied by a strong OTU-number reduction in coral-derived samples. Those differences were also noted before (GROßKURTH 2007, NEULINGER *et al.* 2008, HANSSON *et al.* 2009, SCHÖTTNER *et al.* 2009), regardless of the molecular techniques being used.

Apparently, this marked contrast between OTU-poor coral-associated and OTU-rich ambient habitats also strongly determined the overall degree of bacterial distribution and overlap: Coral branch and mucus exhibited notably few unique bacterial signatures, as most of their respective OTU pool was shared with water and sediment. In turn, many of the signatures found in both ambient habitats were not contained in the coral-generated habitats, because water and, especially, sediment covered a much larger OTU pool. Irrespective of study site and local facies, the sediment generally featured the highest number of specific OTU and thereby highest potential diversity; a trend that is also known from other ARISA-based studies on WWC reefs (HEWSON & FUHRMAN 2006, SCHÖTTNER *et al.* unpublished data) and other marine ecosystems (Böer *et al.* 2009), and that may only be rivaled by sponge-contained bacterial signatures (HOFFMANN *et al*. unpublished data).

Albeit strongly reduced in overall bacterial OTU number and specificity, coral surfaces were characterized by a strikingly high inter-sample variability in community diversity, clearly exceeding the variability of OTU-rich water and sediment communities. Correspondingly, bacterial community changes across respective branch or mucus samples were relatively high and variable, suggesting marked intra-habitat heterogeneity. This may result from a combination of stochastic events during community assembly, such as the random attachment of environmental bacteria on coral surfaces (RITCHIE 2006), and of deterministic processes such as the selection of few opportunistic types through environmental filtering or antagonistic interactions (RYPIEN *et al.* 2010). Antagonistic interactions, usually involving inhibition through antibiotics as means of pathogen defense and resource competition (KOH 1997, HARDER *et al.* 2003, RITCHIE 2006), may represent an important structuring force of communities and would result in local community patterns. Furthermore, identified high variability in coral-associated

assemblages may reflect local, inter-colony differences in host status, such as genetic identity (e.g. LE GOFF-VITRY *et al.* 2004), physiological condition (e.g. HALL-SPENCER *et al*. 2007), or developmental state (BROWN & BYTHELL 2005 and references therein). In their study on *M. oculata*-associated microbes, HANSSON *et al.* (2009) also reported significant inter-colony variation, which was even further enhanced by intra-colony differences between single polyps. Within-colony variation has also been documented for branching WWC (ROHWER *et al.* 2002). In addition to these passive controls, bacterial colonization may also be actively regulated by the coral host (e.g., ROHWER & KELLEY 2004) in adaptation to changing environmental conditions (RESHEF *et al.* 2006, ROSENBERG *et al.* 2007b, ZILBER-ROSENBERG & ROSENBERG 2008).

Coral Species and Color. *L. pertusa* and *M. oculata* were characterized by significantly different bacterial communities, largely due to variations in relative OTU abundance. This corroborates preliminary evidence presented by HANSSON *et al.* (2009) who found DGGE signals from both species to group separately in NMDS and cluster analyses (yet, with >50% similarity). Like in our study, those corals originated from the same sampling location, where they occurred right next to each other. Hence, a mere spatial separation between bacterial assemblages may therefore not explain this pattern. Also, first (indirect) comparisons of 16S rRNA gene sequences from different studies (NEULINGER *et al*. 2008, HANSSON *et al.* 2009, KELLOGG *et al.* 2009) suggested divergence between *L. pertusa* and *M. oculata*-associated signatures. Interestingly, WILD *et al.* (2010) recently reported significant differences in carbohydrate composition between *L. pertusa* and *M. oculata* mucus from the Røst area. Furthermore, both coral species appear to differ with respect to tissue-contained acid concentrations (MANCINI *et al.* 1999), suggesting the involvement of host-related traits in structuring bacterial assemblages between both CWC. Species-specific variations in mucus composition and production are also known from WWC (MEIKLE *et al.* 1988, CROSSLAND 1987), and even held responsible for a close attuning of bacterial communities to host metabolism (DUCKLOW & MITCHELL 1979).

Concerning bacterial community changes related to *L. pertusa* color, no significant difference was evidenced: At first glance, this seemed contradictory with earlier findings by NEULINGER *et al.* (2008) who studied bacterial associates of white and red *L. pertusa* by 16S rRNA-based fingerprinting (T-RFLP) and sequence analysis, and

highlighted considerable phenotype-specific associations. These authors observed, however, that the fingerprinting method lacked resolution to resolve community differences among color types when compared to the phylogenetic approach. In principle, ARISA offers more resolution than T-RFLP to detect changes in marine bacterial communities for OTU accounting for less than 5% of the total amplified product (DANOVARO *et al.* 2006), as well as intra-genomic heterogeneities within closely related gene clusters (BROWN & FUHRMAN 2005). Observed general discrepancies between fingerprinting (ARISA, T-RFLP) and sequencing results may therefore be attributed to the fundamental difference between both analysis approaches (diversity screening for fingerprinting techniques *versus* diversity sampling for sequencing-based approaches; *sensu* BENT & FORNEY 2008) rather than to a mere lack of resolution. Apart from methodological concerns, the present study (Røst) yielded no phenotype-related differences while the prior one (Trondheimsfjord) did, which may also indicate that the segregation of bacterial communities according to coral color is, to some degree, environmentally controlled.

***Local to Regional Variability*.** Bacterial community variations recorded in the reef center of Røst (Røst-in) yielded unexpectedly homogeneous patterns, despite the pronounced geomorphologic reef zoning prevailing in this reef section. Related vertical gradients in topography and facies typically reflect local dynamics in e.g. current regime, sediment deposition and diagenesis, as well as organic matter quality, transport and remineralization within only few tens of meters (FREIWALD 2002a, FREIWALD *et al.* 2002b, WEHRMANN *et al.* 2009). Geomorphologic reef zoning was therefore assumed to contribute significantly to the structuring of bacterial assemblages, particularly so in water and sediment, but likely also in coral-derived habitats (branch, mucus). Instead, the finding of overall stable signatures may lead to the assumption that local, small-scale differences in the abiotic environment at Røst-in are not intense enough to generate significant community shifts in any of the microbial habitat types investigated.

The observed local consistency of bacterial signatures was not maintained beyond the reef center (Røst-in), because mucus, water and sediment exhibited significant changes in community patterns towards the reef periphery (Røst-out; Figure S1). Only out-reef branch communities were still similar to in-reef ones, and thereby marked an intriguing partition in the bacterial diversity in both coral-generated habitats. The finding

of a more spatially consistent community in branch *versus* mucus may be attributed to the circumstance that branch surface scrapings included traces of coenosarc tissue. As noted in a previous study based on fluorescence in-situ hybridization (FISH) of *L. pertusa* tissue-associated bacteria (NEULINGER *et al.* 2009), not only the peripheral ectoderm of polyp tentacles, but also the coenosarc covering parts of the coral skeleton harbor (extracellular) bacterial cells like those of the newly described "*Candidatus* Mycoplasma corallicola". Adapted to the rather stable conditions provided by the coral tissue, such tissue-associated bacteria may be slightly less susceptible to exogenous change than mucus-inhabiting assemblages which clearly appear to mirror local, meso-scale environmental shifts. Within Røst, such meso-scale changes in a/biotic conditions (and thereby bacterial community patterns) may occur as a function of the marked habitat transition from the reef center (Røst-in) to the reef periphery (Røst-out). The latter represents a quasi discrete interface ("ecotone"; *sensu* COSTELLO 2009) between the overall structurally complex reef ecosystem and the more uniform, sediment-covered level bottom down-sloping into the abyssal plain (BUHL-MORTENSEN *et al.* 2010 and references therein). As such, the reef periphery may already be partly disconnected from biotic and abiotic (e.g. LAVALEYE *et al.* 2009, WILD *et al.* 2008, WILD *et al.* 2009) processes taking place in the reef center, despite single occurrences of isolated coral colonies. Also some of the local variations observed in other studies may have resulted from ecosystem-internal habitat heterogeneity between different mounds (HANSSON *et al.* 2009), knoll sites (KELLOGG *et al.* 2009), or fjord features (NEULINGER *et al.* 2008). The underlying meso-scale environmental changes that bacteria experience between respective areas may thereby not necessarily follow linear distance relationships (as addressed by NEULINGER *et al.* 2008), but rather be subject to a whole interplay of locally different, ecosystem-specific factors.

The importance of environmental imprinting becomes even more evident through the finding of significant bacterial community variations between the four investigated reef types/sites. Remarkably, observed patterns not only reflected local, site-specific imprinting (– characteristic assemblages in all four reefs, even the two closely located ones, Røst and Trænadjupet), but also regional, province-specific imprinting (– marked separation between both offshore *versus* each of the inshore reefs; Figure S3). Certainly common to all those sites are geological and hydrological features that are pivotal for local cold-water coral recruitment and proliferation (for details refer to ROBERTS *et al.*

2009). Røst, Trænadjupet, Tisler and Langenuen differ, however, substantially with respect to their geographical setting and on-site configuration, which, depending on the interplay of local and regional environmental regimes, may result in clearly site-specific differences. These appear to be reflected in water- and, in particular, sediment-inhabiting bacterial communities which showed a very pronounced community separation according to local as well as regional characteristics. Also both coral-generated habitats exhibited site-specific bacterial variation; branch apparently less than mucus, which basically extends aforementioned branch-mucus partition from local (meso-) scale to the regional (large-) scale. The marked site-specificity of mucus communities may be attributed to a stronger direct coupling of those bacteria to exogenous change *per se* (see above) or an indirect coupling via the coral host. Mucus of *L. pertusa* namely revealed compositional differences between samples from Røst and Tisler (WILD *et al.* 2010), which may be governed by environmental controls, such as shown in studies on WWC, where the quantity (NAUMANN *et al.* 2010) and composition (DROLLET *et al.* 1997) of mucus changed when corals were exposed to different environmental parameters. Host-mediated reef-specificity in branch and mucus communities may also describe geographic fluctuations in coral reproduction strategy and genetic variability (LE GOFF-VITRY *et al.* 2004), as well as in local coral food supply and quality (KIRIAKOULAKIS *et al.* 2007, DODDS *et al.* 2009).

Overall, variations in bacterial community structure seemed to increase with spatial scale, however, not entirely independently of the respective type of microbial habitat colonized (mucus, water, and sediment reflected changes more intensely than branch). Interestingly, habitat differentiation thereby not only determined the degree of community differentiation but also that of community interconnection, with important contrasts between coral-derived surfaces and the ambient surrounding. Due to the comparatively higher OTU numbers contained in water and (particularly) sediment, for example, those habitats held a much higher potential for meso- and large-scale OTU sharing than the overall OTU-poor branch and mucus. Conversely, these coral-associated habitats had always a high fraction of their OTU pool replaced between and even within different reef sites, thereby giving each coral sample a highly individual character despite considerable OTU reduction. Altogether, the observed bacterial community changes from local (small- and meso-) to regional (large-) scale suggest a marked biogeographic imprinting on bacterial communities (HUGHES-MARTINY *et al.* 2006 and references therein, RAMETTE &

TIEDJE 2007 and references therein) in cold-water coral ecosystems, which manifests either directly, related to environmental characteristics, or indirectly, depending on coral-mediated effects.

Coral Host Specificity. In host-microbe research, the finding of low numbers of specific bacterial types, combined with significant community variations related to host taxonomy is often interpreted as a sign for host specificity, implying the selection of few beneficial associates as part of commensalistic, if not even mutualistic, relationships. Associations between host and microbes may, however, only be termed "specific" provided that respective community patterns are maintained over time and space. In the case of CWC, whether and to which degree bacteria form host-specific associations (as opposed to random epibiosis) is still unresolved, due to the relatively few available studies that have examined bacterial community variation over several spatial and ecological scales.

In this study, microbial habitat-specific bacterial patterns remained conserved over all sites, including differences between low and high numbers of (unique) bacterial types in coral-derived *versus* ambient habitats, respectively. Also, the coral species-related divergence between communities associated with *L. pertusa* and *M. oculata* appeared consistent, at least within a highly heterogeneous reef environment such as Røst. For the following reasons, however, strict bacterial host specificity can be ruled out: Most of the coral-associated bacterial signatures were also found in the ambient environment (water, sediment), which suggests that their specificity for coral-derived surfaces (branch, mucus) is mainly based on the relative abundance (and total mean occurrence) of community members rather than on the presence of unique ones. This overall resemblance with environment-contained signatures may also be one of the reasons why coral-associated bacterial communities furthermore changed considerably from local (small- and meso-) to regional (large-) scale, thereby exhibiting a biogeographic pattern that is almost comparable to that of bacterial communities present in the ambient surrounding. What specifically characterizes coral-associated communities, though, are the patterns they generate on coral surfaces, and implied local variability.

Overall, our findings revealed a fundamental separation of bacterial communities between distinct coral-generated and ambient microbial habitats, which appeared pivotal for

determining the differentiation or connectedness of bacterial communities over all spatial and organizational scales investigated. Especially the community variability associated with coral-derived surfaces led to highly site-specific diversity patterns and community changes. Furthermore, bacterial community patterns, albeit locally consistent with different coral species and reef-internal environmental complexity, changed markedly from local (small- and meso-) to regional (large-) scale, resulting in biogeographic patterns that resembled those of water- and sediment-dwelling bacteria. Combined with prior insights, this suggests that bacterial communities in cold-water coral ecosystems are highly diverse and heterogeneously distributed, and may be determined by both local community history (based on random and selective community assembly) as well as respective local and regional environment. Exploring bacterial diversity in CWC reef ecosystems is but one of the first steps in understanding coral-microbe relationships in such complex deep-sea environments. Further studies are now needed to broaden and substantiate our understanding on how changes in bacterial community diversity and structure affect the dynamics and functioning of CWC reef ecosystems.

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Supplemental Information

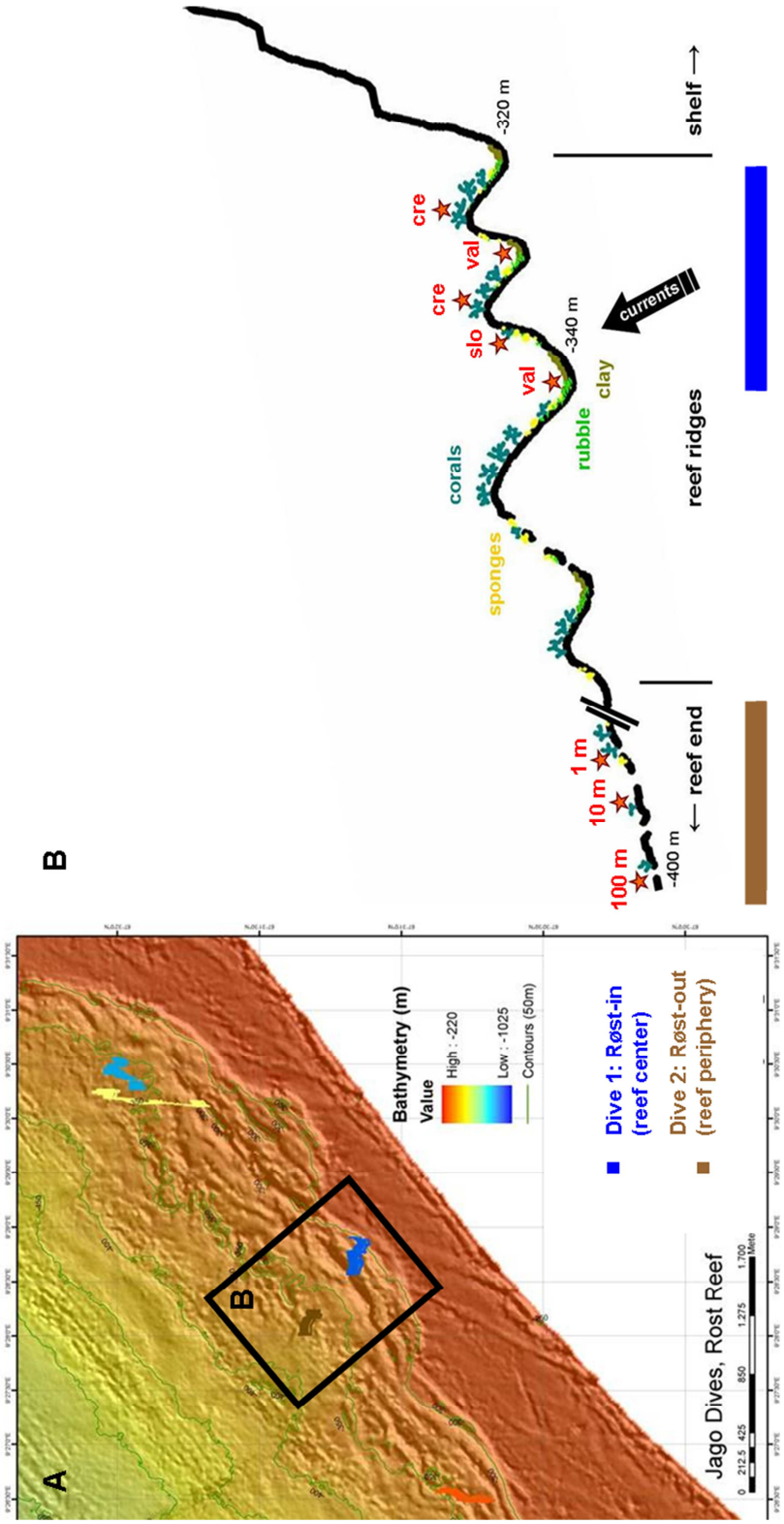

Figure S1 (A) Røst bathymetry, including dive transects at Røst-in and Røst-out, **(B)** Røst transversal scheme (not to scale) indicating topographical reef structure and sampling stations (marked by stars)

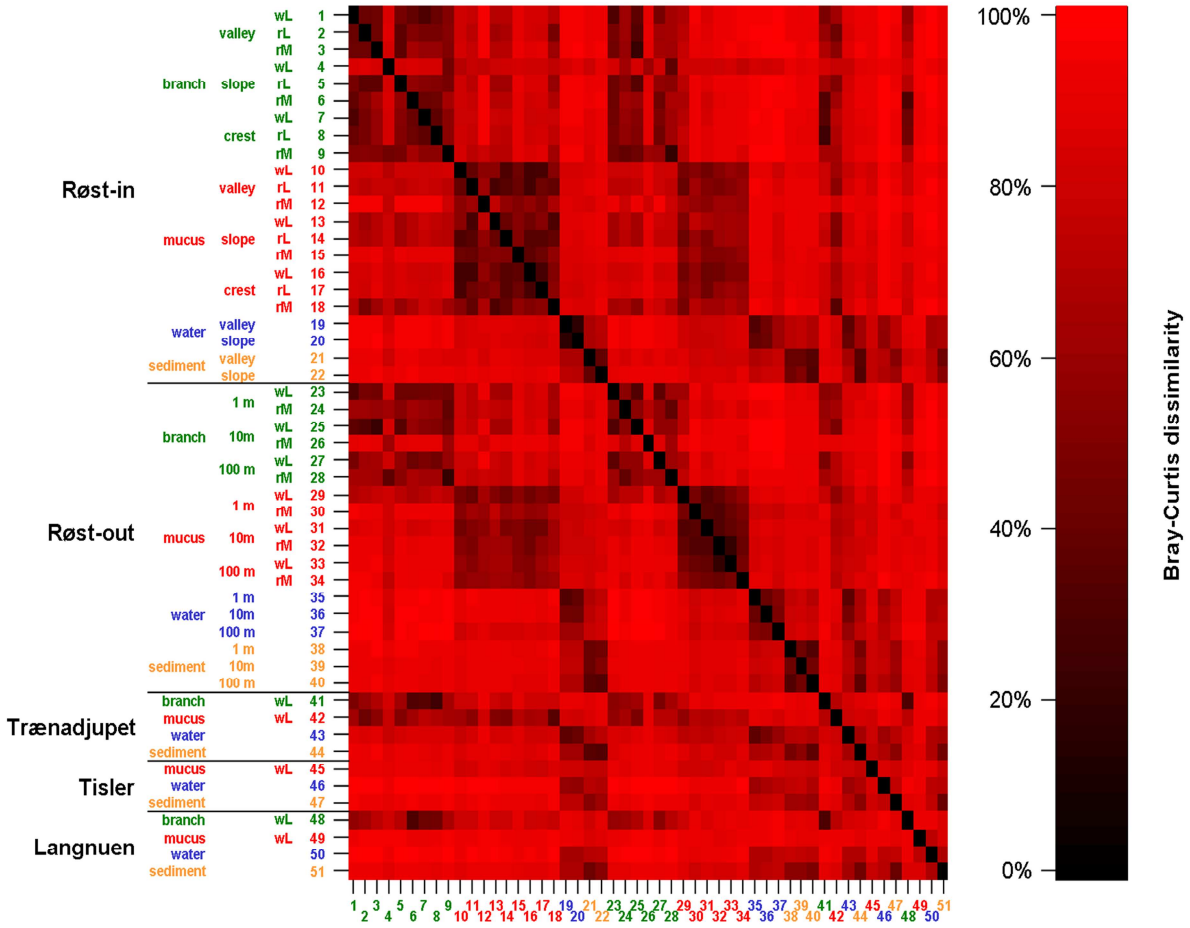

Figure S2 Bray-Curtis distance matrix depicting dissimilarity relationships between all ARISA-derived abundance profiles. Samples are grouped according to microbial habitat type, coral species, coral color phenotype, geomorphologic reef zoning, in-/out-reef location (incl. distances away from the apparent reef margin), and reef type/site.

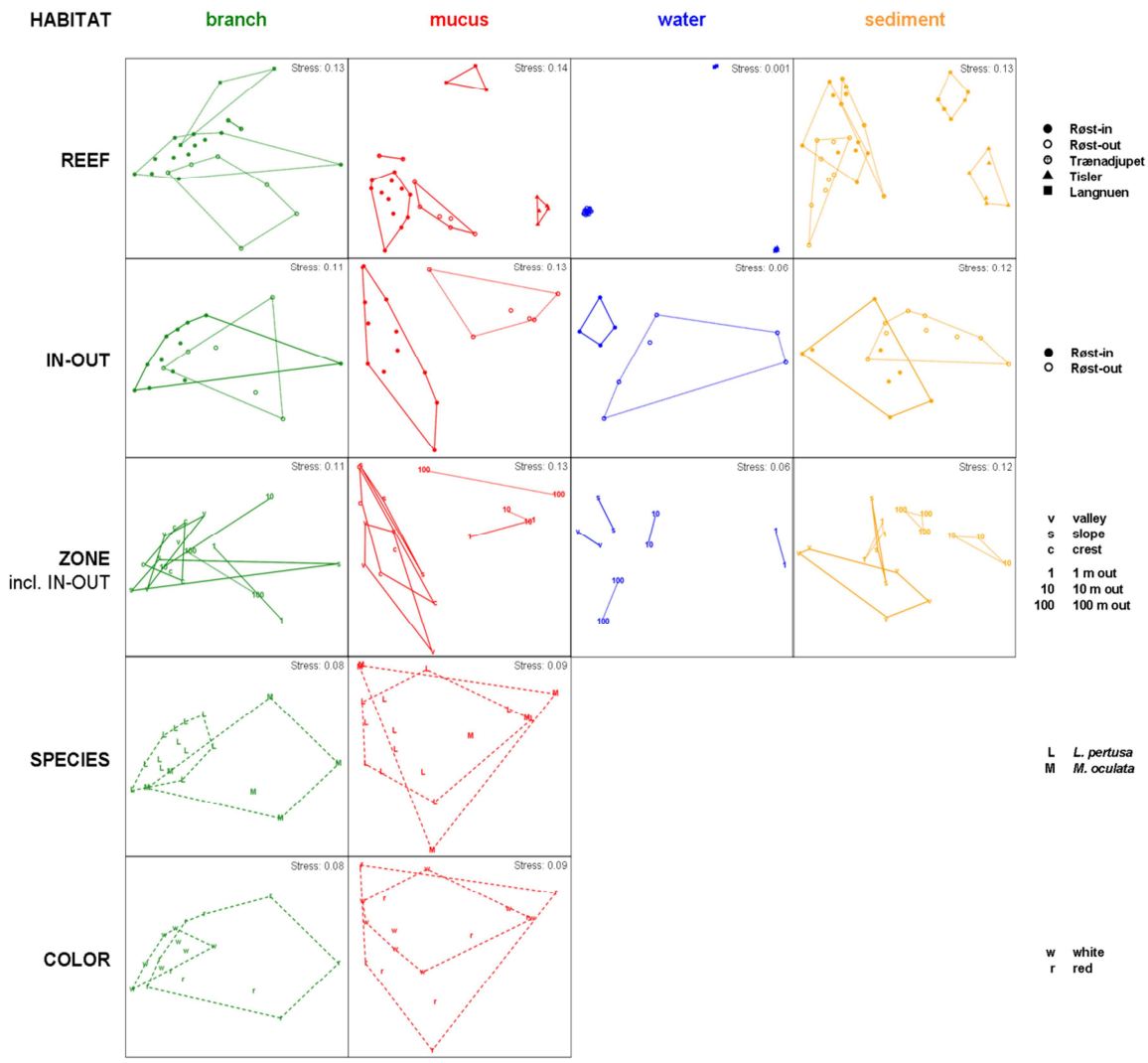

Figure S3 Single NMDS ordinations (Bray-Curtis distance) of all ARISA-derived abundance profiles describing variations in bacterial community structure for each microbial habitat type as related to reef type/site, in-/out-reef location, geomorphologic reef zoning, coral species, and coral color phenotype. Objects represent consensus signals for all PCR triplicates per sample and share a more similar community structure when plotting closer to each other. Stress values indicate the goodness-of-fit of the 2-dimensional representation compared to the original multi-dimensional matrix.

Table S1A. Global and pairwise ANOSIM of ARISA-derived bacterial community variation as related to microbial habitat type (factor: HABITAT) at all sites (samples: white *L. pertusa*). R values are presented in the upper matrix, Bonferroni-corrected *P*-values in the lower matrix.

Study site	Source of variation		Pairwise R / Pairwise *P*				Global
			branch	mucus	water	sediment	
all sites	**HABITAT**	branch	–	0.4695	0.9978	0.9996	R=0.874 *P*<0.0001
		mucus	0	–	0.7511	0.8855	
		water	0	0	–	0.9212	
		sediment	0	0	0	–	
Røst-in	**HABITAT**	branch	–	1	1	1	R=0.9998 *P*<0.0001
		mucus	0.0444	–	1	1	
		water	0.0456	0.0504	–	1	
		sediment	0.006	0.0042	0.0132	–	
Røst-out	**HABITAT**	branch	–	1	1	1	R=0.9998 *P*<0.0001
		mucus	0.5742	–	1	1	
		water	0.072	0.0702	–	1	
		sediment	0.027	0.0342	0	–	
Trænadjupet	**HABITAT**	branch	–	1	1	1	R=1 *P*<0.0001
		mucus	1	–	1	1	
		water	1	1	–	1	
		sediment	0.2094	0.2154	0.2154	–	
Tisler	**HABITAT**	branch	–	n.a.	n.a.	n.a.	R=1 *P*<0.0001
		mucus	n.a.	–	1	1	
		water	n.a.	0.0249	–	1	
		sediment	n.a.	0.0084	0.0102	–	
Langenuen	**HABITAT**	branch	–	1	1	1	R=0.996 *P*<0.0001
		mucus	0.6066	–	0.9167	1	
		water	0.5964	0.6174	–	1	
		sediment	0.0732	0.0708	0.2196	–	

n.a. = not applicable

Table S1B. Global and pairwise ANOSIM of ARISA-derived bacterial community variation as related to reef type/site (factor: REEF) at all sites (samples: white *L. pertusa*). R values are presented in the upper matrix, Bonferroni-corrected *P*-values in the lower matrix.

Habitat type	**Source of variation**		**Pairwise R** **Pairwise *P***					**Global**
			Røst-in	Røst-out	Trænadj.	Tisler	Langen.	
all habitats	**REEF**	Røst-in	–	0.03384	-0.04568	0.1488	0.1572	R=0.08949 *P*=0.0028
		Røst-out	1	–	-0.00825	0.08051	0.1603	
		Trænadj.	1	1	–	0.09753	0.0759	
		Tisler	0.125	0.578	0.571	–	0.1763	
		Langen.	0.109	0.127	0.958	0.033	–	
branch	**REEF**	Røst-in	–	0.08718	0.2364	n.a.	0.4769	R=0.3173 *P*=0.0362
		Røst-out	1	–	0.8333	n.a.	0.7037	
		Trænadj.	0.8514	0.618	–	n.a.	-0.3333	
		Tisler	n.a.	n.a.	n.a.	–	n.a.	
		Langen.	0.4296	0.612	1	n.a.	–	
mucus	**REEF**	Røst-in	–	0.8667	1	1	1	R=0.9277 *P*<0.0001
		Røst-out	0.156	–	1	1	1	
		Trænadj.	0.459	1	–	1	1	
		Tisler	0.075	0.186	0.437	–	1	
		Langen.	0.177	0.989	1	0.187	–	
water	**REEF**	Røst-in	–	0.4167	1	1	1	R=0.8103 *P*<0.0001
		Røst-out	0.298	–	0.2292	1	1	
		Trænadj.	0.693	1	–	1	1	
		Tisler	0.284	0.04	0.687	–	1	
		Langen.	0.709	0.356	1	0.659	–	
sediment	**REEF**	Røst-in	–	0.3216	0.1143	1	0.9651	R=0.7633 *P*<0.0001
		Røst-out	0.01	–	0.6747	1	0.9971	
		Trænadj.	1	0.002	–	1	0.9611	
		Tisler	0.003	0.002	0.027	–	0.9167	
		Langen.	0.003	0.001	0.018	0.019	–	

n.a. = not applicable

Table S1C. Global and pairwise ANOSIM of ARISA-derived bacterial community variation as related to in/out-reef location (factor: IN-OUT) at Røst (samples: all coral species and color phenotypes). R values are presented in the upper matrix, Bonferroni-corrected *P*-values in the lower matrix.

Habitat type	Source of variation		Pairwise R Pairwise *P*		Global
			Røst-in	Røst-out	
all habitats	**IN-OUT**	Røst-in	–	0.1029	R=0.1092 *P*=0.0052
		Røst-out	0.0063	–	
branch	**IN-OUT**	Røst-in	–	0.1612	R=0.1612 *P*=0.862
		Røst-out	0.0934	–	
mucus	**IN-OUT**	Røst-in	–	0.6519	R=0.6519 *P*<0.0001
		Røst-out	0.0003	–	
water	**IN-OUT**	Røst-in	–	0.4167	R=0.4167 *P*=0.0311
		Røst-out	0.0324	–	
sediment	**IN-OUT**	Røst-in	–	0. 3216	R=0.3216 *P*=0.001
		Røst-out	0.0003	–	

n.a. = not applicable

Table S1D. Global and pairwise ANOSIM of ARISA-derived bacterial community variation as related to geomorphologic reef zoning (factor: ZONE) at Røst-in (samples: all coral species and color phenotypes). R values are presented in the upper matrix, Bonferroni-corrected *P*-values in the lower matrix.

Habitat type	Source of variation			Pairwise R Pairwise *P*		Global
			valley	slope	crest	
all habitats	**ZONE**	valley	–	-0.07947	0.05467	R=0.01998 *P*=0.2818
		slope	1	–	0.1325	
		crest	0.5022	0.1446	–	
branch	**ZONE**	valley	–	0.09259	-0.05625	R=0-0.0414 *P*=0.3394
		slope	0.7884	–	0.0359	
		crest	1	1	–	
mucus	**ZONE**	valley	–	-0.01852	-0.0375	R=-0.03695 *P*=0.5798
		slope	1	–	-0.02564	
		crest	1	1	–	
water	**ZONE**	valley	–	0.25	n.a.	R=0.25 *P*=0.6687
		slope	0.6717	–		
		crest	n.a.	n.a.	n.a	
sediment	**ZONE**	valley	–	-0.04615	n.a	R=-0.04615 *P*=0.5486
		slope	0.5516	–	n.a	
		crest	n.a	n.a	n.a	

n.a. = not applicable

Table S1E. Global and pairwise ANOSIM of ARISA-derived bacterial community variation as related to coral species (factor: SPECIES) at Røst (samples: all coral species and color phenotypes). R values are presented in the upper matrix, Bonferroni-corrected *P*-values in the lower matrix.

Habitat type	Source of variation		Pairwise R / Pairwise *P*		Global
			L. pertusa	*M. oculata*	
both habitats	**SPECIES**	*L. pertusa*	–	0.1519	R=0.1519, *P*=0.0498
		M. oculata	0.0482	–	
branch	**SPECIES**	*L. pertusa*	–	0.5366	R=0.5366 *P*=0.0046
		M. oculata	0.0046	–	
mucus	**SPECIES**	*L. pertusa*	–	0.698	R=0.698, *P*=0.0047
		M. oculata	0.0045	–	

n.a. = not applicable

Table S1F. Global and pairwise ANOSIM of ARISA-derived bacterial community variation as related to coral color phenotype (factor: COLOR) at Røst (samples: all coral species and color phenotypes). R values are presented in the upper matrix, Bonferroni-corrected *P*-values in the lower matrix.

Habitat type	Source of variation		Pairwise R / Pairwise *P*		Global
			white	red	
both habitats	**COLOR**	white	–	-0.04937	R=-0.04937 *P*=0.7771
		red	0.7735	–	
branch	**COLOR**	white	–	-0.1263	R=-0.1263 *P*=0.8842
		red	0.8821	–	
mucus	**COLOR**	white	–	-0.047	R=-0.047 *P*=0.6063
		red	0.6031	–	

n.a. = not applicable

II.4 Drivers of Bacterial Diversity Dynamics in Permeable Carbonate and Silicate Coral Reef Sands

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Abstract

Permeable sediments and associated microbial communities play a fundamental role in nutrient recycling within coral reef ecosystems, hence, in ensuring high levels of primary production in these oligotrophic environments. A previous study on organic matter degradation within carbonate and silicate coral reef sands in the Red Sea suggested that the observed sand-specific differences in microbial activity could be caused by variations in microbial community structure. Here, we tested this hypothesis by comparing bacterial diversity and biomass in both sand types and by further exploring the structuring effects of time (season) and space (sediment depth, in/out-reef). Changes in bacterial community structure as determined by Automated Ribosomal Intergenic Spacer Analysis (ARISA) were primarily driven by mineralogy at specific seasons, sediment depths, and locations. By coupling ARISA with 16S rRNA gene sequencing, we observed that community shifts could already be detected at the bacterial class level, with (*Alpha*-, *Gamma*-, *Delta*-) *Proteobacteria* and *Actinobacteria* being prominent members of the highly diverse communities. Overall, these findings suggest that bacterial communities are structurally different between reef sediments biogenic and terrigenic of different mineralogy. Especially in synergy with environmental variation over time and space, mineralogical differences play a central role in maintaining high levels of community heterogeneity. The co-occurrence of carbonate and silicate sands within a single reef ecosystem thus significantly increases the availability of microbial niches and, potentially, of various microbial functions.

Introduction

Warm-water coral reefs are highly productive ecosystems characterized by complex trophic interactions and a/biotic environmental gradients. Reef-associated organisms occupy different habitats and niches that are ecologically structured in time and space (AINSWORTH *et al.*, 2009, BELLWOOD *et al.* 2004, CONNELL 1978). Insights into the diversity and functions of microbial communities have been gained from reef-associated microbial habitats such as tissues and exudates of sponges and corals (e.g. HENTSCHEL *et al.* 2006, WEGLEY *et al.* 2007, RYPIEN e*t al.* 2010), interstitial and ambient reef water

(e.g. GAST *et al.,* 1998, HEWSON *et al.,* 2007) and proximal reef sediments (e.g. HEWSON & FUHRMAN 2006, UTHICKE & MCGUIRE 2007, RUSCH *et al.* 2009). Due to their versatile metabolic capacities, reef microbes are involved in numerous pelagic and benthic processes (CAPONE *et al.* 1992, SAKKA *et al.* 2002, WERNER *et al.* 2008). Especially heterotrophic bacteria are important for the remineralization of organic matter and recycling of nutrients directly within the reef system (ALONGI *et al.* 2007, Wild *et al.* 2004). This is particularly critical, as coral reefs maintain high levels of primary production and biomass in extremely oligotrophic seawater (CROSSLAND & BARNES 1983, D'ELIA & WIEBE 1990, TRIBBLE *et al.* 1994).

Sandy reef sediments and frameworks are highly permeable structures, where current- wave-, and tide-induced pressure gradients promote advective transport between sediment porewater and overlying water column (HUETTEL *et al.* 2003, WHEATCRAFT & BUDDEMEIER 1981). By retaining dissolved and suspended matter that is hydraulically flushed into the (upper layers of the) sediment matrix and subsequently metabolized by grain-associated bacteria, these sands function as biocatalytic filter systems which ensure efficient pelagic-benthic coupling (FALTER & SANSONE 2000, RUSCH *et al.* 2003, WILD *et al.* 2004).

Biogenic carbonate sands, which are mainly composed of fragmentary remains from calcifying organisms, usually represent the dominant sediment type in coral reef environments. Depending on local atmospheric and geologic events, terrigenic silicate sands may also occur, especially in fringing reefs that receive terrestrial deposits from nearby river mouths (REIS & HOTTINGER 1984). Carbonate and silicate particles substantially differ in physico-chemical properties, such as surface structure, dissolution kinetics, light and heat attenuation, or buffering capacity (SCHROEDER & PURSER 1986). Also, grain size and concomitant sediment sorting can clearly differ between both sand types. Overall, carbonate sands are characterized by higher permeability, porosity, and specific surface area than silicate sands, because of the relatively large grain size and highly porous grain structure (including fissures, cavities and channels) of carbonates (AL-ROUSAN *et al.* 2006, RASHEED *et al.* 2003a, WILD *et al.* 2006).

In many fringing reefs in the northern Gulf of Aqaba, Red Sea, highly permeable carbonate and silicate sands co-occur within the same reef system. Although being exposed to identical environmental conditions, both kinds of sediments exhibit strong divergence in their spatio-temporal nutrient dynamics, as well as in their organic matter

filtration and degradation capacities (RASHEED *et al.* 2003b, WILD *et al.* 2005). In this context, a previous pilot study (WILD *et al,* 2005) revealed that the addition of energy-rich natural particulate organic matter (POM) stimulated sedimentary oxygen consumption (SOC) significantly higher in carbonate as compared to silicate sands. It was hypothesized that those differences could originate from variations in cell number and community structure of sand-associated heterotrophic microbial assemblages between the two sediment types.

Therefore, we specifically determined bacterial diversity and total abundance in co-occurring carbonate and silicate reef sands, so as to test the hypothesis that contrasting mineralogy would have marked effects on sediment bacterial communities. Given the strong spatio-temporal variations in nutrient and organic matter concentration prevailing in such reef sands, we also assessed seasonal and spatial effects as covariables. Finally, we taxonomically identified the main bacterial taxa colonizing both sands, and described respective changes in their temporal and spatial context by coupling ARISA profiles with 16S rRNA gene sequence information.

Materials and Methods

Study Site and Sample Collection. The study was conducted in a shallow fringing reef in the north-eastern Gulf of Aqaba, Red Sea, located within a marine reserve close to the Marine Science Station (29°27'N, 34°58'E; see also WILD *et al. 2*005). During three field expeditions (December 2006, August 2007, February 2008, with average water temperatures of 23°C, 27°C, and 21°C, respectively), permeable reef sediments were sampled by SCUBA at two neighboring reef sites, whereof the first site (2.5 m water depth) was covered by biogenic carbonate sands and the second (1.8 m water depth) by terrigenic silicate sands. Both sites were located in 1 m proximity to the reef crest, but with a distance of about 150 m to each other. At each site, samples were collected in three different spots (approx. 50 cm apart) within a total patch area of 2 m^2, using two scaled clipboards and sterile metal spoons. Each of the three spots was sampled in sediment depths of 0–2 cm ("surface layer"), 2–6 cm ("middle layer"), and 6–12 cm ("deep layer"), as corresponding to the locally prevailing oxic, suboxic and anoxic sediment horizon. Triplicates obtained for one depth horizon were directly transferred into a 15-ml tube, thereby producing a pooled sample for each layer. Between seasons, the exact sampling

spots varied only by a few centimeters in order to avoid horizontal heterogeneity in the data. In February 2008, additional carbonate and silicate surface sands (0–2 cm) were collected outside the reef ("out-reef") at water depths of 4.1 m and 3.5 m, respectively, and in a distance of 10 m (silicate sand) and several hundred meters (carbonate sand) to both initial sampling sites ("in-reef"). Within 1 h after collection, all samples were transported to the laboratory, homogenized with a sterile spatula, and transferred into 2 ml tubes. Aliquots for DNA-based analyses were immediately frozen at -20°C until further use. Aliquots for both microbial cell enumeration and sediment characterization were fixed with 4% paraformaldehyde (PFA), incubated on a shaker at 4°C overnight, and washed twice with a sterile seawater-ethanol solution (1:1) prior to storage at -20°C.

Sediment Characterization. Carbonate content was measured by complexometric titration according to MULLER (1967) and adapted by RASHEED et al. (2003b). Grain size and sorting coefficient were determined by fractional sieving based on Wentworth scaling (WENTWORTH 1922).

Microbial Cell Enumeration. Enumeration of sand-associated microbial cells was performed with triplicate carbonate and silicate surface (0–2 cm) samples from February 2008. Following an optimized protocol for sandy sediments introduced by WILD *et al.* (2006), microbial cells were first extracted from PFA-preserved samples by applying ultrasound in combination with acetic acid, and subsequently subjected to the acridine orange direct count (AODC) method. Microbial cells on filter wedges were counted in 25 randomly chosen fields. For the carbonate samples, obtained average and standard deviation of all counts were multiplied with the correction factor 1.87 (WILD *et al.* 2006) in order to account for the embedding of cells in the carbonate matrix.

DNA Extraction. From 0.5–1 g sediment sample, 3–5 replicates of total genomic DNA were extracted with the UltraClean Soil DNA Isolation Kit (MoBio, Carlsbad, CA) following the manufacturer's instructions for maximum yield. Final elution of DNA was performed with 50–100 µl 1× TE buffer (Promega, Madison, WI). Concentration of yielded DNA was determined using NanoDrop spectrophotometry (NanoDrop, Wilmington, DE).

ARISA Fingerprinting. Universal bacterial ARISA (FISHER & TRIPLETT 1999) as well as subsequent data transformation and binning (RAMETTE 2009) were carried out as described previously with slight modifications (see Supplemental Information). Resulting response table ("initial ARISA" data), containing relative abundance information for all binned operational taxonomic units (OTU_A; subscript "A" denotes ARISA), was used for multivariate analyses and, further, for coupling of single OTU_A with 16S rRNA gene sequence information.

16S-ITS rRNA Gene Clone Library Construction. For the construction of four 16S-ITS rRNA gene clone libraries, those carbonate and silicate surface samples with the highest OTU_A numbers were selected from the December 2006 and August 2007 sample sets. PCR amplification of the 16S-ITS rRNA gene region was performed with bacterial primers 27f (5´-AGA GTT TGA TCM TGG CTC AG-3'; LANE 1991) and ITSReub (5'-GCC AAG GCA TCC ACC-3'; CARDINALE *et al.* 2004), yielding fragments of potential lengths between 1500–3000 bp (see Supplemental Information).

Taxonomic Classification and Diversity Indices. For a total of 283 16S rRNA gene sequences (each approx. 1050 bp), taxonomic affiliations were determined using the RDP Classifier and SeqMatch functions (Ribosomal Database Project II, Release 10; COLE *et al*. 2009). Further, all sequences were imported into the ARB software package (LUDWIG 2004) and aligned by applying the Silva INcremental Aligner tool (SINA; PRUESSE *et al*. 2007), including manual alignment correction. Based on an OTU_S (subscript "S" denotes sequencing) definition of ≥98% sequence similarity for the whole data set, rarefaction curves, richness estimators and diversity indices were computed using DOTUR (SCHLOSS & HANDELSMAN 2005), after generating genetic distance matrices in ARB using the Jukes-Cantor correction. To determine whether differences in library composition were significant, the statistical tool ∫-LIBSHUFF was applied to genetic distance matrices, with significances assessed by Monte Carlo permutations and corrected for multiple comparisons (SCHLOSS *et al.* 2004). The statistical tool SONS (SCHLOSS & HANDELSMAN 2006) was used on 16S rRNA gene sequences to calculate the shared Chao1 (shared richness), J_{class} (community overlap), and $Theta_{yc}$ (community structure similarity) estimators. and aligned by applying the Silva INcremental Aligner tool (SINA; PRUESSE *et al*. 2007), including manual alignment correction. Based on an OTU_S (subscript "S"

denotes sequencing) definition of ≥98% sequence similarity for the whole data set, rarefaction curves, richness estimators and diversity indices were computed using DOTUR (SCHLOSS & HANDELSMAN 2005), after generating genetic distance matrices in ARB using the Jukes-Cantor correction. To determine whether differences in library composition were significant, the statistical tool ∫-LIBSHUFF was applied to genetic distance matrices, with significances assessed by Monte Carlo permutations and corrected for multiple comparisons (SCHLOSS *et al.* 2004). The statistical tool SONS (SCHLOSS & HANDELSMAN 2006) was used on 16S rRNA gene sequences to calculate the shared Chao1 (shared richness), J_{class} (community overlap), and $Theta_{yc}$ (community structure similarity) estimators.

Coupling of ARISA to 16S-ITS rRNA Gene Clone Libraries. Taxonomic information was linked to OTU_A as described by BROWN *et al.* (2005). For each sequenced clone, the length between (and including) the ITSF and ITSReub primer sites was calculated, and designated as the derived OTU_A length. Respective taxonomic information was inferred from the corresponding 16S rRNA gene portion, using 80% bootstrap confidence support at the bacterial class level (RDP Classifier). All taxonomically identified OTU_A* (superscript * denotes taxonomic linking) were subsequently processed as a new table ("linked ARISA" data) for overall and individual patterns of variation.

Statistical Analyses. All statistical tests and graphical representations were performed in R v.2.9 (The R Project for Statistical Computing) using packages 'stats', 'vegan', 'MASS', 'labdsv', and 'mgcv', as well as in PAST v.1.47 (Palaeontological Statistics) and CANOCO for Windows v4.5 (TERBRAAK & SMILAUER 2002). The initial ARISA data reflecting relative OTU_A abundance were used to calculate Bray-Curtis pairwise distances between samples. The resulting matrix was visually explored for mineralogical, seasonal and spatial patterns in community structure by applying non-metric multidimensional scaling (NMDS) and cluster analysis. To test for significant differences between the resulting *a posteriori* sample groupings, Analysis of Similarity (ANOSIM) was performed, and corrected for multiple comparisons according to the Bonferroni criterion (e.g. RAMETTE 2007). Within-group diversification of bacterial communities was assessed by multivariate dispersion analysis of Hellinger-transformed data, followed by Analysis of Variance (ANOVA) on the distances to group centroids and Tukey's Honest

Significant Differences (TukeyHSD) test for pairwise comparisons of group mean dispersions. The relative importance of the factors sand type, season and depth in explaining bacterial community variation alone or in combination was investigated by variation partitioning (LEGENDRE & LEGENDRE 1998) based on canonical redundancy analysis (RDA) of Hellinger-transformed data (RAMETTE & TIEDJE 2007). Single and combined fractions of variation were tested for significance by ANOVA, with respective global and reduced models being evaluated by 999 Monte Carlo permutations at $P<0.05$ each. Furthermore, the initial ARISA data reflecting OTU_A presence-absence were used to compare mean OTU_A numbers by an overall Kruskal-Wallis test (KW) and subsequent pairwise Wilcoxon-Mann-Whitney tests (WMW).

Prior to analyzing OTU_A*-specific variation in community structure, the initial and linked ARISA data were tested for concordance in bacterial community variation by applying the Mantel test (LEGENDRE & LEGENDRE 1998) based on the corresponding Bray-Curtis distance matrices. Response behaviors of all OTU_A* to the structuring effects of sand type, season and depth were studied by RDA focusing on inter-species correlations at the bacterial class level (≥80% similarity), while response of individual OTU_A* and respective bacterial classes were monitored by single regression analyses as well as the Dufrene-Legendre indicator species analysis (DUFRENE & LEGENDRE 1997).

Results and Discussion

Differences in Sediment Characteristics. The sedimentological properties of carbonate and silicate surface (0–2 cm) samples from February 2008 differed greatly between both sand types. While the biogenic carbonate sediment had an expectedly high $CaCO_3$ content of 86.7%, the terrigenic silicate sediment consisted of only 19.3% $CaCO_3$, with the remainder representing quartzous components (see also RASHEED *et al.* 2003b). Due to a grain size median of 553 µm and a sorting coefficient of 0.84, the carbonate sediment classified within the coarse sands (500–1000 µm; WENTWORTH 1922) and exhibited only moderate sorting, indicating a relatively high level of heterogeneity due to the presence of many different grain fractions. The silicate sediment, on the contrary, appeared to be of smaller grain size (326 µm) and represented typical medium sand (250–500 µm median range). Its very low sorting coefficient of 0.0016 is known to be characteristic for very well sorted, homogeneous sediments containing relatively few different grain fractions.

Consequences of those mineralogical differences for bacterial communities concern mode and degree of colonization, niche differentiation, as well as variations in cell abundance, distribution and overall community structure. The heterogeneous grain shape, highly porous surface structure, and thereby larger specific surface area of carbonate particles, as opposed to the comparatively round shape and smooth surface of silicate grains, are assumed to clearly promote microbial colonization in the carbonate sediment matrix, due to the increased supply of interfaces, micro-gradients, as well as shelter from mechanical damage or predation (NICKELS *et al.* 1981, MEYER-REIL 1994, RUSCH *et al.* 2006, WILD *et al.* 2006).

Changes in Bacterial Cell Number. No significant difference in cell numbers ($3.1 \pm 0.9 \times 10^9$ and $1.5 \pm 0.5 \times 10^9$ cm^{-3} for carbonate and silicate sands, respectively; Student's t-test, $P>0.05$) were observed between the sand types, and those estimates agreed with cell numbers in carbonate sediments of the Great Barrier Reef (HANSEN *et al*. 1987) and two Hawai'ian reefs (SØRENSEN *et al.* 2007, RUSCH *et al.* 2009). In contrast to carbonate and silicate sands previously collected in same area (RASHEED *et al.* 2003b), however, microbial counts in our study turned out to be considerably higher. This may be partly attributed to methodological differences, as we used a refined version of the AODC method (WILD *et al.* 2006), which considers the fact that cells may also be trapped within highly porous sediment grains.

The coarse-grained fraction of permeable sands can be expected to hold the major share of benthic bacteria (NICKELS *et al.* 1981, RUSCH *et al.* 2003), reaching levels of 10^9–10^{10} cells cm^{-3}, with up to one order of magnitude higher cell numbers in carbonate compared to silicate sands of the same grain size (WILD *et al.* 2005, RUSCH *et al.* 2006). In our study, similar levels of microbial biomass were obtained for both sand types, despite marked differences in grain complexity, which may result from the specific surface area of single carbonate particles being eventually down-balanced by their relatively high grain size. The total surface area effectively available for bacterial colonization in the whole carbonate sediment matrix would thus be reduced to a level similar to that offered by the smaller-grained silicate sediment. Accordingly, the significantly different sedimentary oxygen rates previously measured in carbonate and silicate reef sands of the same grain size (WILD *et al.* 2005) may not have resulted from strong differences in total microbial biomass.

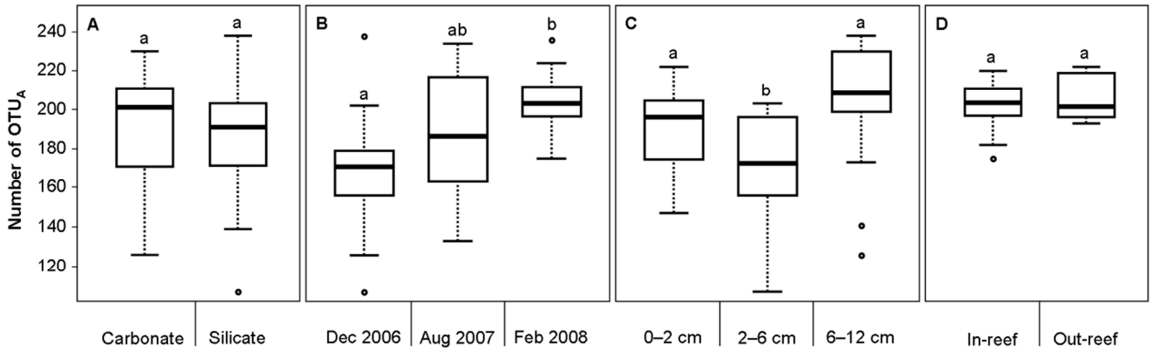

Figure 1. Number of ARISA-derived OTU_A per **(A)** sand type, **(B)** season, **(C)** sediment depth and **(D)** reef affiliation. Top, middle, and bottom lines of boxes represent the 25th (sample minimum), 50th (median), and 75th (sample maximum) percentiles; error bars represent the 5th and 95th percentiles; box size and line spacing indicate the degree of dispersion and skewness in the data; outliers above and below the error bars denote extreme values. Different letters above each box denote significant mean difference in OTU_A number based on pairwise Wilcoxon-Mann-Whitney testing.

Overall Bacterial Community Patterns. OTU_A numbers (i.e. the total sum of binned ARISA peaks) per sample ranged from 163 to 226 out of 438 OTU_A for the whole data set, and were similar to numbers reported by other ARISA-based studies in reef sands (HEWSON *et al.* 2006), different coastal marine sediments (DANOVARO & PUSCEDDU 2007, HEWSON *et al.* 2007, BÖER *et al.* 2009), or respective target reef water column (SCHÖTTNER *et al.* unpublished data). When averaged over all sampling times, depths, and in-/out-reef sites, carbonate and silicate sediments contained a similar mean total of 191 and 188 OTU_A, respectively (KW, P=0.56; Figure 1A). A seasonal trend could, however, be clearly evidenced (KW, P<0.001; Figure 1B), with lowest and highest OTU_A numbers in December 2006 (169 OTU_A) and February 2008 (204 OTU_A), respectively. When considering sand types individually, the temporal effect appeared to be mainly observed for carbonate sands (KW, P<0.01), but not in silicate sands (KW, P=0.09). In their ARISA-based study on intertidal sand communities of the North Atlantic, BÖER *et al.* (2009) also found lowest OTU_A levels in fall (November), however, highest in summer (August) instead of winter (February). This may be explained by general, ecosystem-specific differences in seasonal dynamics between virtually permanently submerged tropical reefs and strongly tide-affected temperate sand flats, which essentially include temporal shifts in peaks of allochthonous nutrient concentrations and ensuing primary production. In addition, OTU_A numbers exhibited sediment depth-related differences

(KW, P<0.001; Figure 1C), with a slight decrease from the sandy sediment surface (191 OTU_A) to the middle layer (176 OTU_A) and a subsequent increase to the deeper layer (205 OTU_A). Yet again, the two sand types revealed substantial difference, as this vertical effect proved significant only for the silicate (KW, P<0.001), but not for the carbonate communities (KW, P=0.07). Vertical variations in ARISA-derived OTU_A number were also identified in Australian reef sediments (HEWSON & FUHRMAN 2006), with a clear subsurface maximum and subsequent OTU decrease between 3–5 cm sediment depth. No marked horizontal differences in OTU_A number were yet detected in out-reef vs. in-reef surface sands (KW, P=0.91; Figure 1D).

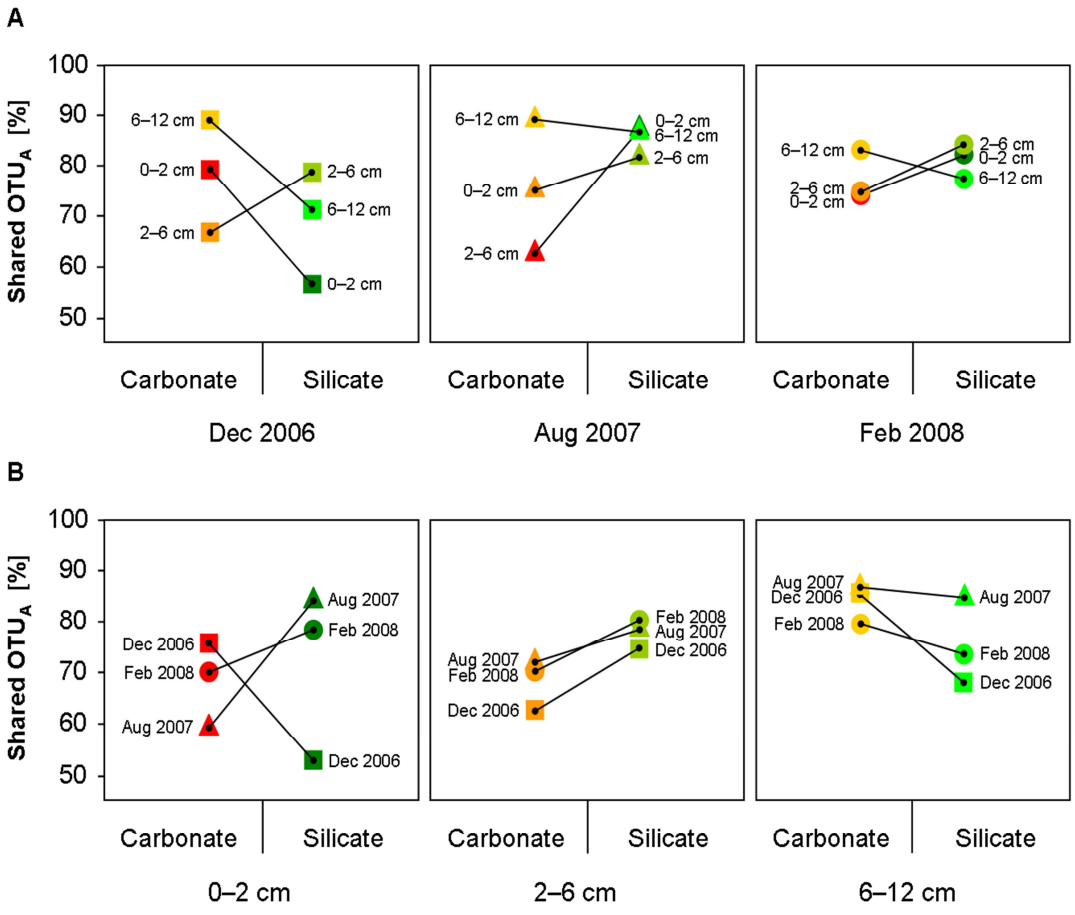

Figure 2. OTU_A turnover between carbonate and silicate in-reef samples over **(A)** season (at all depths), and **(B)** depth (in all seasons). Position along the vertical axis denotes the percentage of OTU_A shared between both sand types, with symbols/colors specifying sampling time/depth, and lines indicating respective paired comparison. Color and symbol coding are consistent with those of Figure 3.

Shifts in OTU_A presence-absence between sand types revealed that most OTU_A were present in all samples, with generally high numbers of OTU_A shared between the different sand types, seasons, and sediment depths, as well as between in- and out-reef site. A total of 381 OTU_A were detected in both sand types together, irrespective of season or sediment depth. With 25 and 31 OTU_A being unique to the carbonate and silicate samples, respectively, the two sands thereby exhibited a general OTU_A overlap of 92–94%. While only 8 and 5 OTU_A turned out specific to December 2006 and August 2007, respectively, 44 OTU_A were associated with February 2008, amounting to an overall seasonal OTU_A overlap of 81–98%. When studying OTU_A presence per depth, 25 OTU_A were found only in the surface layer, 6 OTU_A in the middle layer, and 11 OTU_A in the deep layer, corresponding to an overall vertical OTU_A overlap of 85–97%. Finally, at the sediment surface (total pool of 399 OTU_A), in- and out-reef sediment samples contained 57 and 31 unique OTU_A, respectively, resulting in an OTU_A overlap with location of about 82–89%.

The overall high OTU_A overlap identified for each study factor decreased partly considerably when factors were combined: At defined seasons and sediment depths, carbonate-silicate OTU_A overlap then varied between 57–90% (Figure 2A–B). When studied specifically over season (at all sediment depths), it was lowest in December 2006 (especially in the silicate sand) and highest in August 2007 (Figure 2A). When examined specifically over sediment depth (in all seasons), the carbonate deep layer showed the highest fractions of vertically shared OTU_A (44–90%), whereas the silicate sand revealed no clear spatial trend (Figure 2B). For defined sand types and sediment depths, seasonal OTU_A overlap (data not shown) generally varied between 42–95%, with the carbonate surface (52%) and silicate middle layer (42%) exhibiting the overall lowest fractions of seasonally shared OTU_A, and the deep layers of both sand types (95%) the overall highest. For defined sand types and seasons, vertical OTU_A overlap (data not shown) varied between 55–95%, with the overall lowest OTU_A sharing for the carbonate samples from December 2006 (56%) and for the silicate samples from August 2007 (60%) and December 2006 (65%).

When bacterial community patterns in carbonate and silicate sands were altogether visualized by NMDS (Figure 3), sand type had the largest structuring effect (Fig. 3A), and was followed by the effects of sampling time, sediment depth horizon and in-/out-reef sampling location (Figure 3B–C). Those *a posteriori* groupings were also supported by

significant ANOSIM results, which attributed the highest community separation to sand type (ANOSIM R=0.56, $P<0.001$), despite the similarly high degree of community diversification in both sands (Tukey HSD, $P=0.29$). Also seasonal and vertical separations of communities were significant (ANOSIM R=0.44 and R=0.27, respectively; both $P<0.001$), but greatly depended on the sand type. Within the carbonate sand, for example, samples were mainly separated by season (ANOSIM R=0.80, $P<0.001$), with a noticeable segregation of winter (February 2008) from fall (December 2006) and summer (August 2007) communities (Figure 3B). Sediment depth-related patterns did not appear

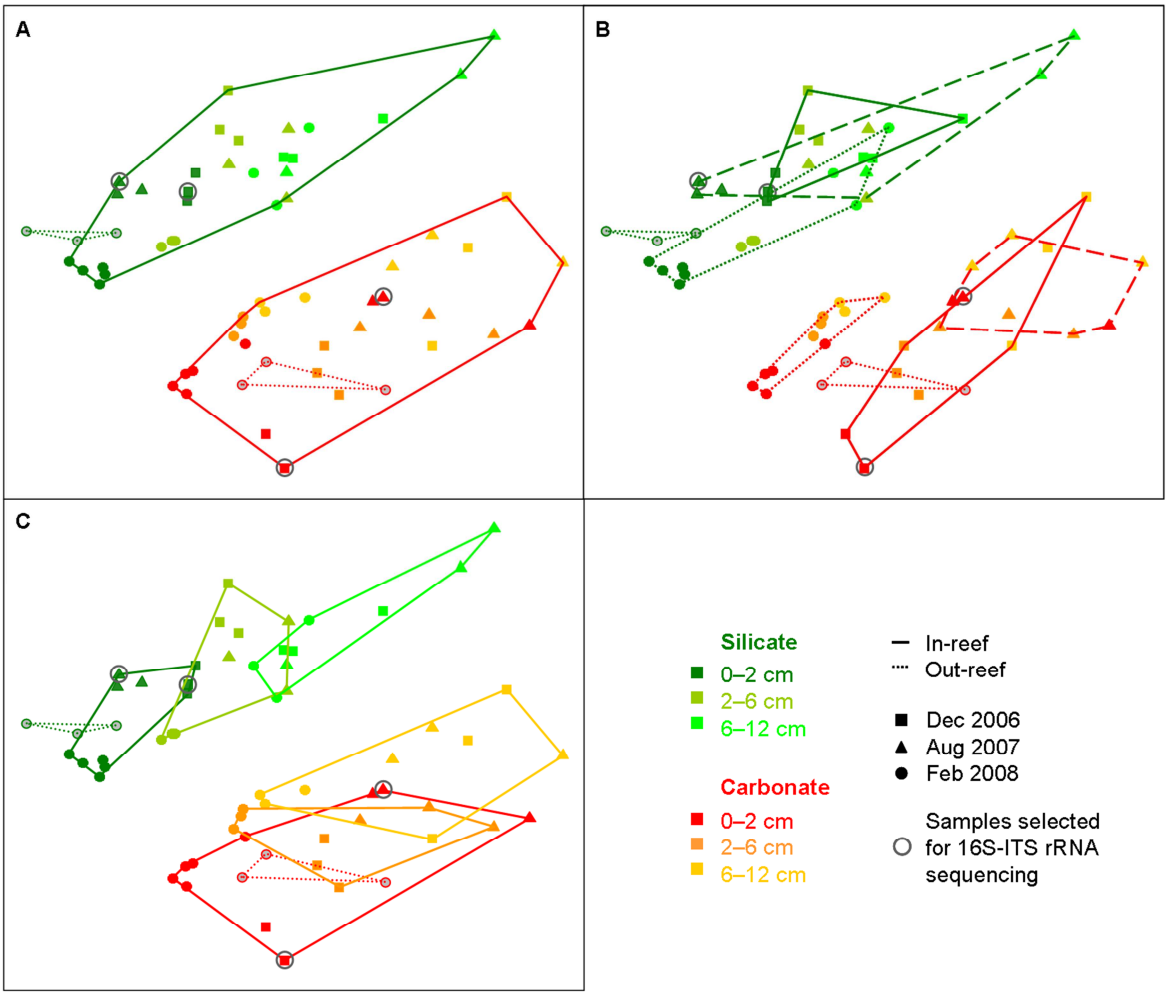

Figure 3. NMDS ordination (Bray-Curtis distance) of ARISA-derived bacterial community profiles, with at least three sediment replicates per sample. *A posteriori* groupings are specified according to **(A)** sand type, **(B)** season, and **(C)** depth. Samples chosen for 16S-ITS rRNA gene clone libraries are encircled. Objects plotting closer to each other share a more similar community structure relative to other, more distant objects. The stress value indicates the goodness-of-fit of the 2-dimensional representation compared to the original matrix.

very pronounced (ANOSIM R=0.12, $P<0.05$), but were consistent with a gradual change in community structure from the sediment surface down to the deep layer (Figure 3C). Variation in the silicate sand, by contrast, largely followed a vertical pattern (ANOSIM R=0.59, $P<0.001$; Figure 3C), while a seasonal effect, albeit significant (ANOSIM R=0.49, $P<0.001$), was not clearly observed in the NMDS ordination (Figure 3B). Cluster analysis results largely confirmed all major community groupings revealed by NMDS (data not shown).

This strong partitioning between sand types may be clearly attributed to the fact that biogenous carbonates and terrigenous silicates substantially differ in their mineralogical and morphological properties (SCHROEDER & PURSER 1986, RASHEED *et al.* 2003b). By imposing specific abiotic and biotic conditions within each sediment matrix, sand type may thereby represent a factor promoting niche creation and diversification of associated bacterial communities. Among the many parameters controlling bacterial distribution and diversity in sediments, grain texture and micro-topography have already proven as fundamentally important (MEADOWS & ANDERSON 1966, WEISE & RHEINHEIMER 1978, NICKELS *et al.* 1981, MEYER-REIL 1994). While the comparatively round-shaped silicates exhibit a rather regular, smooth surface, carbonates contain many micro-discontinuities, such as crevices, pores and depressions (WILD *et al.* 2006), which give each grain a highly porous and heterogeneous character. For cells residing within the carbonate sediment matrix, depression areas offer shelter from mechanical abrasion as well as predation (FRANKEL 1977, DEFLAUN & MAYER 1983), and promote the formation of extracellular polysaccharides that serve as multi-purpose binding agents in cell attachment and biofilm formation (BOYLE & READE 1983, MEYER-REIL 1994 and references therein).

As factors sand type, season, and sediment depth significantly explained changes in community variation, canonical RDA variation partitioning was used to disentangle the respective effects of each factor while taking the other ones into account. Each factor specifically contributed with 14% (sand type), 8% (season) and 8% (sediment depth) of the total community variation, with only 1% of co-variation between season and sediment depth (Table 1). While sand type obviously represented the dominant factor in the study, the relative effects of season and sediment depth differed considerably when carbonate and silicate samples were examined separately (Table 1). In carbonate sand alone, season (14%) exerted an almost 3-fold higher influence on the bacterial community structure

Table 1. Canonical partitioning of the bacterial variation into the relative effects of sand type, season, and sediment depth, individually or in combination.

Multivariate model	D_f	R^2_{adj}	F-ratio
Carbonate + Silicate			
Type + Season + Depth	3	0.30	08.812 ***
Type | (Season, Depth)	1	0.14	11.342 ***
Season | (Type, Depth)	1	0.08	02.729 ***
Depth | (Type, Season)	1	0.08	06.979 ***
Type * Season	3	0.25	07.187 ***
Season * Depth	3	0.17	04.929 ***
Depth * Type	3	0.24	06.775 ***
Carbonate			
Season + Depth	2	0.21	04.590 ***
Season | Depth	1	0.14	05.705 ***
Depth | Season	1	0.05	02.658 ***
Season * Depth	3	0.24	03.888 ***
Silicate			
Season + Depth	2	0.31	07.227 ***
Season | Depth	1	0.12	05.780 ***
Depth | Season	1	0.19	08.339 ***
Season * Depth	3	0.33	05.677 ***

The significance of contribution of each model to ARISA variation was determined by 999 permutations under RDA reduced models. For each global or partial model, the number of degrees of freedom (D_f), amount of explained variation (R^2_{adj}), and F-ratio (*** = P<0.001) are presented. Characters plus (+) and star (*) denote the addition and interaction, respectively, of two factors, while the vertical bar (|) indicates respective partial regression model, wherein the effects of the factors in paratheses were partialled out.

than sediment depth (5%). Conversely, changes characterizing only the silicate sand communities were more due to sediment depth (19%) than to season (12%). Those observations were also supported by an analysis of factor interactions (Table 1), which indicated that significant structuring effects were also due to the combinations of sand type with season and sediment depth, respectively. For instance, the sum of the pure

effects of sand type and season in the model accounted for 22% of the explained variation, while the additional inclusion of their crossed effect increased the explained community changes to 25% (Table 1). A similar relation was found for the combined effect of sand type and sediment depth, where the explained variation increased from 22% to 24%. Hence, season and sediment depth not only provided additional structuring for an already mineralogy-controlled community variation, but their respective effect depended partly directly on the prevailing sand type.

The different seasons clearly featured temporally distinct bacterial assemblages, whereat the winter (February 2008) samples marked the strongest segregation in community structure and overall lowest diversification. It is assumed that this relates to the overall enhanced primary productivity during winter (November-March; LEVANON-SPANIER *et al*, 1979), which is triggered by the wind-driven inflow of Red-Sea offshore water into the Gulf of Aqaba during October, and concomitant upwelling of deep, nutrient-rich water to the surface and into the reef (AL-NAJJAR 2000, MANASRAH *et al.* 2004, MANASRAH *et al*. 2006). This period ends in May, when the intrusion of offshore water decreases and nutrient concentrations in the reef drop and stabilize again. According to the organic carbon and nutrient inventories measured by RASHEED *et al.* (2003b) in the coastal water, those transitions (which are clearly marked by changes in e.g. wind speed/direction, temperature, or salinity) generate two main seasonal patterns: a winter period (with increased substrate concentrations) that includes October to April, and a summer period (with decreased substrate concentrations) that lasts from July until September. Due to effective pelagic-benthic coupling, whereby advective fluid exchange transmits seasonal changes from the water column into the porewater, carbon and nutrient inventories of the surface sediment layer clearly mirror the changes in the water column (RASHEED *et al.* 2003b). This not only supports the existence of a nutritional link between seasonal dynamics and sediment-associated communities, but also validates our finding of a stronger seasonal imprint on bacterial assemblages inhabiting the highly advection-driven carbonate sands as compared to the mostly diffusion-limited silicate sands.

Vertical differences in community structure, albeit unequally pronounced in carbonate and silicate sands, are likely related to gradients in redox potential, as well as organic matter and nutrient concentration. Stratification of the sediment column generally features steep transitions between the well-oxygenated, substrate-rich surface layer and the underlying sub-oxic and anoxic deeper layers, which is why both sands exhibited

community variations from 0 cm down to 12 cm. However, while enhanced advectional flushing of the carbonate matrix results in rapidly changing porewater geochemistry (RUSCH *et al.* 2009) with intensified and deeper-reaching supply of oxygen, organic matter and nutrients (RASHEED *et al.* 2003b), the comparatively reduced transport in the silicate sand likely causes a much tighter and more differentiated graduation of substrates. In addition to these physico-chemical constraints, macrofaunal activity such as bioturbation (RIDDLE 1988), grazing (MORIARTY *et al.* 1985, EPSTEIN 1997) or nutrient regeneration (UTHICKE 2001) can also play a substantial role in structuring bacterial communities associated with the sediment surface layer.

Overall, not only sand type-specific changes *per se*, but also seasonal and vertical shifts in bacterial diversity clearly reflected the fundamental difference in mineralogy and, ultimately, filtration efficiency of carbonate and silicate reef sands. Apart from the general temporal and spatial imprints detected in all samples, however, carbonate communities shifted mainly with season while silicate communities rather shifted vertically with sediment depth.

16S-ITS rRNA Gene Clone Library Analysis. The four libraries yielded a total of 283 non-chimeric sequences (average length: 1070 bp), which consisted of 168 OTU_S (≥98% similarity), with the highest and lowest OTU_S richness found in library Ca06 (66 OTU_S) and library Ca07 (38 OTU_S), respectively (Table 2). Total richness estimates (Chao1) showed that both fall libraries (Ca06 and Si06) contained the highest degree of richness at a confidence level of 0.05 (Table 2), with the carbonate sample being even more diverse than the silicate sample. Rarefaction curves (see Supplemental Information: Figure. S1) displayed a steeper slope, hence, higher diversity for library Ca06, compared to the very similar curve progressions of libraries Ca07, Si06 and Si07. Rarefaction analysis also showed that no saturation was achieved for any of the four libraries, suggesting that more sequences would be needed in order to obtain a more reliable estimate of diversity. The high reciprocal Simpson index of 125 for library Ca06 indicated a diversity profile with relatively even distribution of the different OTU_S, but also represented a marked contrast to the low index of <50 for library Si06, which usually denotes a typical dominance profile (STACH *et al.* 2003). The Shannon-Weaver index suggested library Ca06 and Si06 as the most and least diverse libraries, respectively, although values did not greatly differ

(Table 2). Noticeably, diversity patterns inferred from clone library analysis were very concordant with those inferred from ARISA (see Supplemental Information: Table S2). Libraries Ca06, Ca07, Si06, and Si07 contained 11, 6, 6, and 9 different phyla, respectively. Bacterial communities were dominated by the phylum *Proteobacteria* (41%), which accounted for 58%, 39%, 29%, and 36% of the respective libraries (Fig. 4). The majority of those OTU_S (67 sequences) in all samples belonged to the class *Gammaproteobacteria* (23.8%), followed by *Deltaproteobacteria* (9.2%) and *Alphaproteobacteria* (7.4%). In addition to the *Proteobacteria*, *Actinobacteria* appeared to be the most common phylum in all 16S rRNA gene libraries, with a total of 48 sequences (17%). Further phyla comprised the *Acidobacteria, Chloroflexi, Spirochaetes and Firmicutes* (Figure 4).

Overall, many of the detected phyla were also reported by other studies on coral reef sediments, coral and sponge tissue, or permeable shelf sediments (see Supplemental Information). According to RDP SeqMatch analyses, almost all of the identified sequences were closely related to uncultured bacterial lineages from marine habitats, including coral reefs and heavily impacted coastal environments (see Supplemental Information: Table S3).

Table 2. Richness and diversity indices for the 16S-ITS rRNA gene clone libraries constructed from carbonate and silicate surface samples from December 2006 and August 2007.

Sand type	**Sampling date**	**Clones**	**Total OTU_S ***	**Unique OTU_S***	**Chao1 richness**	**Simpson evenness (1/D)**	**Shannon-W.diversity (H)**
Carbonate	Dec 2006	85	66	54	245 (145, 417)	125	4.1 (3.9, 4.3)
Carbonate	Aug 2007	72	48	34	110 (73, 202)	63	3.7 (3.5, 3.9)
Silicate	Dec 2006	53	38	32	203 (94, 596)	44	3.4 (3.2, 3.7)
Silicate	Aug 2007	73	51	39	133 (86, 247)	59	3.8 (3.5, 4.0)

* 98% similarity level

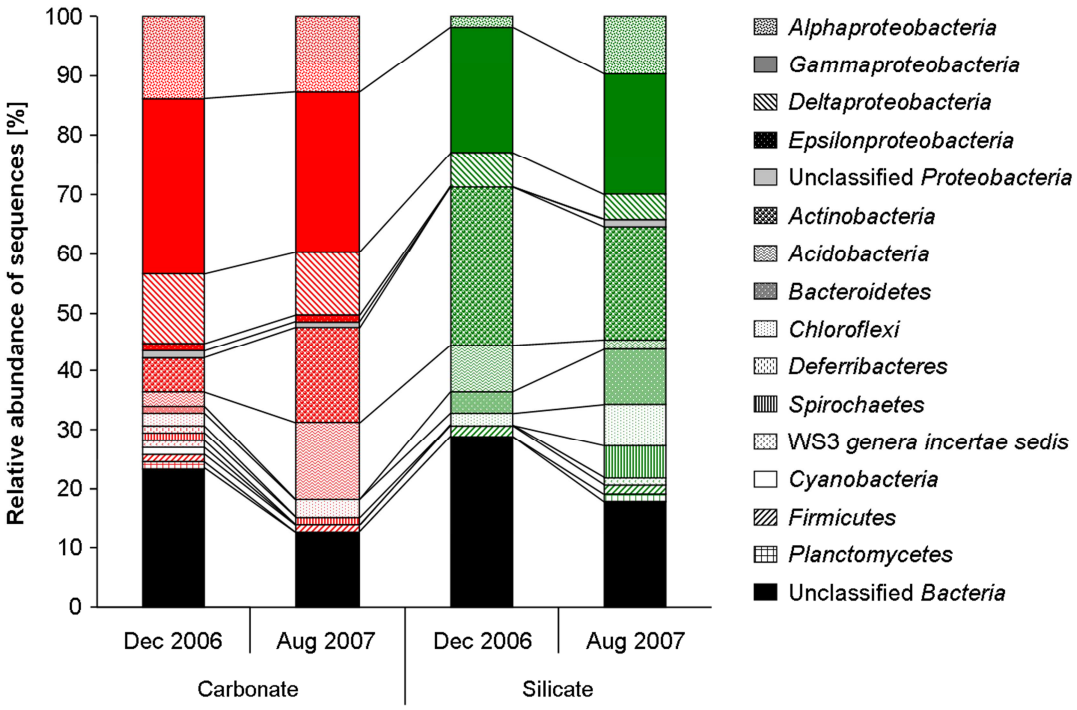

Figure 4. Frequencies of bacterial lineages detected in the 16S-ITS rRNA gene clone libraries constructed from carbonate and silicate surface samples from December 2006 and August 2007.

Taxon-Specific Patterns Inferred from Linking ARISA to Taxonomy. A total of 76 (out of 438) OTU_A could be matched with 16S rRNA gene sequences and their respective taxonomic assignments at the bacterial class level (≥80% similarity), resulting in an overall assignment success of 17.4% (see Supplemental Information: Table S3). Of the 76 identified OTU, 68 OTU_A* were affiliated with a discrete taxon each, whereas 5 OTU_A* corresponded to two distinct bacterial classes and 2 OTU_A* corresponded to three phylogenetically unrelated clones.

The diversity patterns obtained with the subset of OTU_A* were very consistent with those inferred from the whole ARISA dataset (Mantel test R=0.8449, P<0.001). It was therefore not surprising to observe that the main patterns of variation of OTU_A*, when analyzed by canonical RDA, were significantly related to sand type along the first ordination axis (representing 47.6% of the variance) and by season and sediment depth along the second axis (25.6% of the variance; see Supplemental Information: Figure S4, Table S5A). While the carbonate sand was positively correlated with two of the three seasons (i.e. August 2007 and February 2008) and negatively correlated with sediment depth, silicate sands exhibited the exact opposite relationships, which once more illustrated the aforementioned sand type-specific variations in seasonal and vertical

community response (see Supplemental Information: Table S5B). Furthermore, the relatively strong divergence among the different seasonal factor levels re-emphasized the peculiarity of the winter (February 2008) samples in comparison to those collected during fall (December 2006) and summer (August 2007).

Individual RDA ordination plots depicting OTU_A*-factor relationships for each of the most prominent different bacterial classes (Figure 5) indicated various distribution patterns for members of the *Gammaproteobacteria*, *Deltaproteobacteria*, *Actinobacteria*, and *Sphingobacteria*, which may reflect class-specific versatility in niche differentiation. In contrast, *Alphaproteobacteria* and *Spirochaetes* were mostly associated with the surface and middle layer of both sand types in February 2008. *Rhodobacterales* (accounting for most of the *Alphaproteobacteria*-related OTU_A*) usually alternate between chemoorganotrophic and phototrophic growth, which could explain this surface-specific occurrence. *Spirochaetes* are known as mainly anaerobic organisms, but OTU_A* included in the analysis may as well comprise aerotolerant types. Furthermore, all *Anaerolineae* specifically grouped with the upper layer(s) of the carbonate samples only. How these strictly anaerobic organisms are able to thrive at the usually oxygen-rich sediment surface is unknown, but they may benefit from the presence of anoxic depressions on the carbonate grains as well as other metabolic strategies known from anaerobic lineages. *Acidobacteria* were mostly found in the middle and deep layer of December 2006 and August 2007, wherefore sampled members of this group (all *Acidobacteriales*) are assumed to favor sub- or anoxic conditions.

As several ribosomal operons may exist within a bacterial cell (KLAPPENBACH *et al.* 2000) and may greatly vary in length (BROWN & FUHRMAN 2005), obtaining multiple OTU_A* within a given bacterial lineage cannot be directly equated with a high level of diversity (BROWN *et al.* 2005). It must be noted that, in our study, patterns of OTU_A* for a given lineage were found to be very different from each other (Figure 5), therefore suggesting that most of them may not originate from the same organisms, but may rather reflect the high diversity also identified from clone library analyses (Table 2; see Supplemental Information). Such high diversity could be explained by the existence of various ecological responses within a given lineage to environmental conditions (as reconstructed by RDA; Figure 5). Yet, the relationships between the presence of multiple rRNA operons from single organisms in response to resource availability was found to be insignificant in oligotrophic marine seawater (BROWN & FUHRMAN 2005 and references

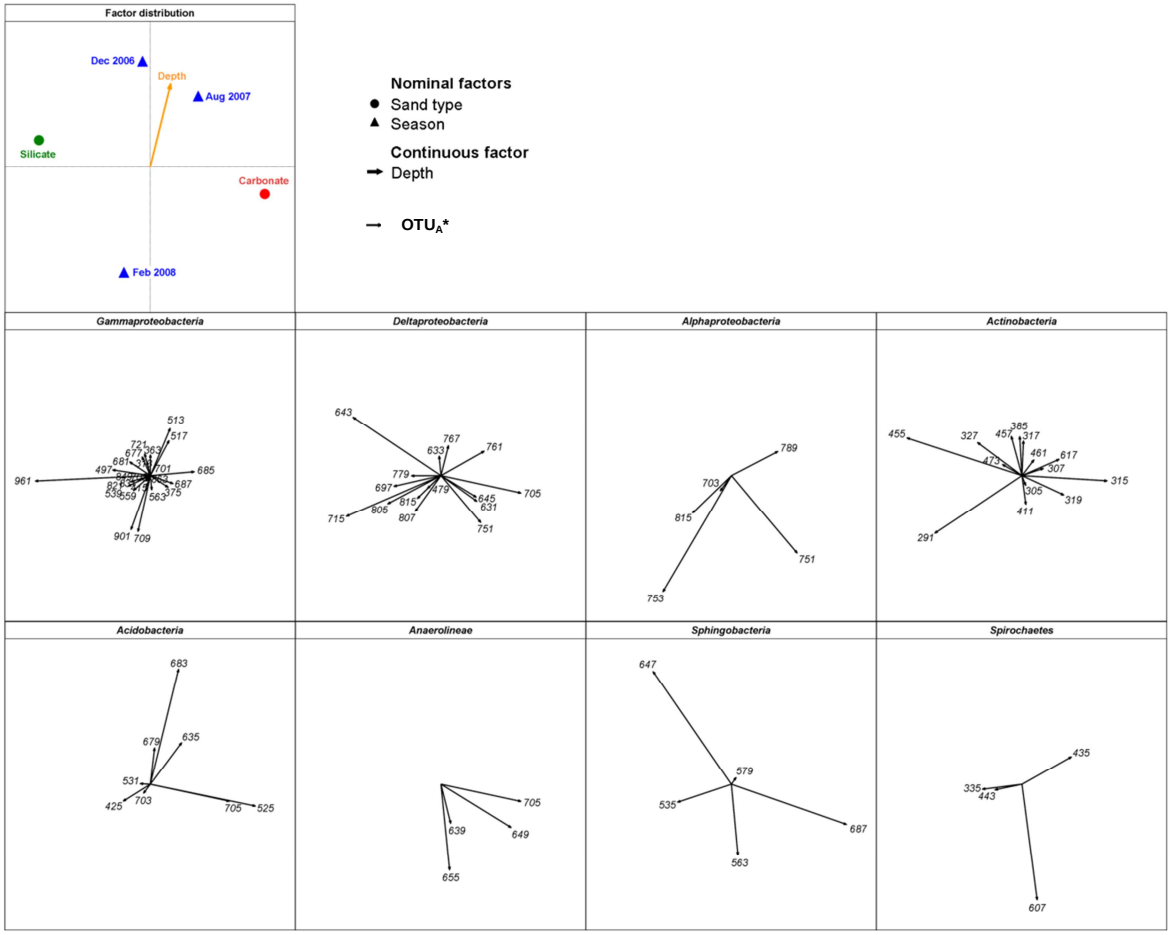

Figure 5. Relationships between sand type, season, and sediment depth, as well as changes in bacterial community structure, with focus on specific OTU_A* shifts at the bacterial class level (≥80% sequence similarity). All biplots represent the same RDA ordination of linked ARISA data (Bray-Curtis distance) under direct constraint of explanatory factors, whereby factor levels of sand type and season were set as nominal variables, and those of depth as continuous variables. Vectors represent variation patterns of OTU_A* that were linked to 16S-ITS rRNA gene sequences of a given bacterial class, with numbers denoting the respective OTU_A (i.e. ARISA fragment) length in base pairs. Angles between vectors indicate the correlation between individual OTU_A* distribution patterns, with collinear, opposite, and orthogonal vectors indicating positive, negative and independent OTU_A*covariation patterns, respectively.

therein). Permeable reef sands, with their high input of different substrates and fast-changing biogeochemical conditions, may actually foster the concomitant development of several ecological strategies. Such possible diversification into "ecotypes" with ecologically relevant physiological differences (ROCAP *et al.* 2003) is assumed to support the relative fitness and overall resilience of a lineage in response to key environmental variables (KITANO 2004).

In addition to RDA, regression analyses of individual OTU_A* variation against each factor and respective factor levels were used to identify putative indicator OTU_A (see Supplemental Information, Table S6A–C). The selection of the 10 strongest OTU_A*-factor relationships (highest and most significant R^2) confirmed that *Actinobacteria* and *Gammaproteobacteria* were mostly associated with overall type-, season-, and depth-related variations (see Supplemental Information).

In conclusion, our results suggest that permeable biogenic carbonate and terrigenic silicate reef sands represent distinct and dynamic microbial habitats that harbor specific, comparably diverse bacterial communities over time and space. By offering locally contrasting environmental conditions, both sands thereby contribute to an enhanced ecological structuring of bacterial diversity and potential functions within a single reef ecosystem. Many of the organisms identified in this study were also found in other reef or sediment habitats. For the future, it would therefore be relevant to determine whether molecular techniques offering higher taxonomic resolution levels could further advance our understanding of the ecological patterns of reef-associated microbial communities.

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Supplemental Information

16S-ITS rRNA Gene Clone Library Construction. Reactions containing 22–40 ng DNA extract in a final volume of 20 µl were set up as follows: 0.4 µM of primers 27f and ITSReub (Biomers, Ulm, Germany), 250 µM of each dNTP (Roche, Mannheim, Germany), 0.3 mg ml^{-1} bovine serum albumin (Sigma-Aldrich, Munich, Germany), 1 × *Taq*Master PCR Enhancer (Eppendorf, Hamburg, Germany), 1 × Master*Taq* buffer with 1.5 mM Mg^{2+} (Eppendorf), and 0.05 U $µl^{-1}$ *Taq* Polymerase (Eppendorf). Between an initial denaturation step at 94°C for 2 min and the final elongation at 72°C for 7 min, annealing was performed under two different conditions in order to particularly facilitate amplification of longer DNA fragments (>1500 bp): 10 cycles at 94°C for 30 s, 55°C for 1 min, 72°C for 3 min, then 18 cycles at 94°C for 30 s, 55°C for 30 s, 72°C for 3 min. Each sample was amplified in four replicates that were combined directly afterwards in order to maximize diversity coverage. PCR products were purified using the QIAquick PCR Purification Kit (Qiagen, Valencia, CA), ligated into the pGEM-T Easy vector (Promega, Madison, WI) and transformed into One Shot TOP10 Chemically Competent *E. coli* cells (Invitrogen, Carlsbad, CA) according to specified instructions. Representative clones, as determined after partial sequencing with the vector primer pair M13F/M13R, were selected for plasmid preparation with the Montage Plasmid Miniprep Kits (Millipore, Billerica, MA) and subjected to *Taq* cycle sequencing with an ABI Prism 3130x Genetic Analyzer (Applied Biosystems, Foster City, CA). Assembly of almost full-length sequences (approximately 1050 bp in average length) was carried out by using Sequencher v4.6 (Gene Codes, Ann Arbor, MI). Screening for chimeric signals by using Bellerophon (HUBER *et al*. 2004), Mallard (ASHELFORD *et al*. 2006), and RDP Chimera Check, which is part of the SimRank 2.7 package available through the Ribosomal Database Project II (COLE *et al*. 2009), resulted in the exclusion of 14 sequences from further analyses.

16S-ITS rRNA Gene Clone Library Composition. Bacterial communities were dominated by the phylum *Proteobacteria* (41%), which accounted for 58%, 39%, 29%, and 36% of the respective libraries. The majority of those OTU_S (67 sequences) in all samples belonged to the class *Gammaproteobacteria* (23.8%), mainly including members of the order *Chromatiales*, but also *Legionellales*, *Oceanospirillales*, *Alteromonadales*,

Pseudomonadales, *Thiotrichales*, and *Vibrionales*. A total of 26 sequences were affiliated with the *Deltaproteobacteria* (9.2%), with nearest known representatives grouping within the orders *Desulfobacterales*, *Desulfovibrionales*, *Desulfuromonadales*, *Myxococcales* and *Synthrophobacterales*. With a fraction of 7.4% of all clones, *Alphaproteobacteria* were mostly represented by members of the *Rhodobacterales*, but also *Sphingomonadales*, *Rhizobiales*, and *Rhodospirillales*. *Betaproteobacteria* and *Epsilonproteobacteria* were represented only by a single sequence in each carbonate sample.

In addition to the *Proteobacteria*, *Actinobacteria* appeared to be the most common phylum in all 16S rRNA gene libraries. A total of 48 sequences (17%) grouped within the orders *Actinomycetales*, as well as *Acidimicrobiales*, *Coriobacteriales* and *Rubrobacterales*. The phylum *Acidobacteria* (6.7%) was present with 19 sequences, which all grouped within the order *Acidobacteriales*, comprising 3.9% of the total 16S rRNA gene sequences, the phylum *Chloroflexi* was represented by sequences mostly belonging to the newly designated classes *Anaerolineae* and *Caldilineae*. Members of the *Bacteroidetes* (3.4%; formerly: *Cytophaga-Flavobacteria-Bacteroidetes* super-phylum), which were almost exclusively recovered from library Si07, were represented by the orders *Sphingobacteriales*, *Bacteroidales*, and *Flavobacteriales*, *Spirochaetes* and *Firmicutes* accounted for 2.1% and 1.4%, respectively, of all sequences found in all libraries. Two sequences, each from Ca06 and Ca07, respectively, grouped with members of the candidate division WS3 genera *incertae sedis*. Other lineages, represented by 60 sequences with ≥80% similarity at the bacterial phylum level, comprised the novel phylum *Gemmatimonadetes*, as well as *Lentisphaerae*, *Deferribacteriales*, *Planctomycetales*, and *Cyanobacteria*.

According to RDP SeqMatch analyses, almost all of the identified sequences were closely related to uncultured bacterial lineages from marine habitats, including coral reefs and heavily impacted coastal environments (see Supplemental Information: Table S3). Numerous OTU_S identified in our study were affiliated with the phyla *Proteobacteria*, *Actinobacteria*, *Acidobacteria*, *Bacteroidetes*, *Chloroflexi*, and *Spirochaetes*. Although care must be taken when cross-referencing clone libraries based on different primers sets and different numbers of clones, observed predominance of *Proteobacteria*-related sequences was well in concordance with previous studies on bacterial communities in permeable sands of two Hawai'ian reefs (SØRENSEN *et al.* 2007, RUSCH *et al.* 2009), the

Great Barrier Reef (GBR; UTHICKE & MCGUIRE 2007), as well as cold-water coral reefs (JENSEN *et al.* 2008, YAKIMOV *et al.* 2006). However, such pattern has also been reported from environments as contrasting as deltaic muds of Southeastern Papua New Guinea and French Guiana (TODOROV *et al*. 2000, MADRID *et al*. 2001), polar regions (RAVENSCHLAG *et al*. 1999, BOWMAN & MCCUAIG 2003), or the deep sea (LI *et al.* 1999, SCHAUER *et al*. 2009).

The *Gammaproteobacteria*, a group with many copiotropic members (GLÖCKNER *et al.* 1999) showed highest relative frequencies in all four libraries, with the majority of sequences grouping with the *Chromatiales*. Cultured members of this order are mainly known as phototrophic sulfur oxidizers, while a few have recently been proposed as chemolithoautotrophs (SOROKIN *et al.* 2001). The *Legionellales*, *Oceanospirillales*, *Alteromonadales*, and *Pseudomonadales* represent aerobic or facultatively anaerobic chemoorganoheterotrophs which are notoriously versatile in their ability to adapt to a variety of environmental conditions. Many of the identified sequences showed highest similarities to those sampled from contaminated habitats. In agreement with our results, *Deltaproteobacteria* were isolated from the surface layers of the GBR (UTHICKE & MCGUIRE 2007) and the Hawai'ian Checker Reef (RUSCH *et al.* 2009). In the nearby Moku O' Loe Reef, however, they were only detected in suboxic and anoxic depths (SØRENSEN *et al.* 2007). As *Deltaproteobacteria* include mostly anaerobic sulfate-reducing and syntrophic organisms (e.g. *Desulfobacterales*, *Desulfovibrionales*, *Syntrophobacterales*), they usually occupy suboxic and anoxic niches in sediments, such as the sulfate-rich zone several centimeters deep. But also oxygen-depleted micro-patches in surface sediments that are temporarily less impacted by disturbances (hence, oxygen entry) or totally oxygen-depleted due to organic matter degradation, represent suitable habitats. The relatively high, depth-independent sulfate-reduction rates measured previously in permeable reef sediments (WERNER *et al.* 2006) support this assumption. Identified sequences grouped mainly with sediments from habitats as diverse as seagrass meadows, salt marshes, intertidal flats, and coral reefs. *Alphaproteobacteria* have been described as dominant members of the bacterial communities in 16S rRNA gene libraries from permeable shelf sediments (MILLS *et al.* 2008, HUNTER *et al*. 2006), marine bacterioplankton communities (SUZUKI *et al*. 2001, STOICA & HERNDL 2007) and in tropical as well as temperate reef sediments (RUSCH *et al*. 2009, SØRENSEN *et al*. 2007, UTHICKE & MCGUIRE 2007). Although such predominance was not evidenced here,

Alphaproteobacteria-affiliated sequences were present in all four libraries. The majority of sequences grouped within the *Rhodobacterales*, whose related genera are known to alternate between chemoorganotrophic and phototrophic growth (IMHOFF 2001, GUYONEAUD *et al.* 2002). Sequences related to this class, were associated with bacteria recovered from coral tissue (KOREN & ROSENBERG 2006, KLAUS *et al.* 2007, SEKAR *et al.* 2008) and, again, sandy carbonate sediment in a Hawai'ian reef (SØRENSEN *et al.* 2007).

Next to the *Proteobacteria*, *Actinobacteria* represented the most common phylum in all 16S rRNA gene libraries. Interestingly, no *Actinobacteria*-related sequences could be identified in sands of the Checker Reef or GBR (UTHICKE & MCGUIRE 2007, RUSCH *et al.* 2009), and only few were detected in subsurface depths of the Hawai'ian Moku O' Loe Reef (SØRENSEN *et al.* 2007). Members of the *Actinomycetales* are mostly aerobic decomposers of complex organic materials (e.g. cellulose and chitin) and produce a variety of secondary metabolites frequently found in marine sediments (BULL *et al.* 2005). As *Actinobacteria* are often associated with reef-inhabiting invertebrates such as sponges (MONTALVO *et al.* 2004, WEBSTER *et al.* 2009) and corals (WEGELY *et al.* 2007, LAMPERT *et al.* 2008, NEULINGER *et al*, 2008), their true source and role in permeable reef sediments are still to be elucidated.

Similar to previous studies on permeable sands of reefs (SØRENSEN *et al*, 2007, UTHICKE & MCGUIRE 2007, JENSEN *et al.* 2008) and continental shelfs (BOWMAN & MCCUAIG 2003, HUNTER *et al*. 2006, MILLS *et al*. 2008), *Acidobacteria* also represented a relevant fraction of all bacterial phyla detected. In the Hawai'ian Checker Reef sediments, these mostly oligotrophic organisms even comprised the largest fraction in the oxic and suboxic zone (RUSCH *et al*. 2009). The phylum *Chloroflexi* was represented by sequences mostly belonging to the newly designated classes *Anaerolineae* and *Caldilineae* (YAMADA *et al.* 2006). Such organisms were also detected in oxic to suboxic depths of the two Hawai'ian reefs in Kane'ohe Bay, but not from GBR sediments. How these strictly anaerobic organisms are able to thrive in the upper, usually oxygen-rich sediment layers is unknown, but could be analog to strategies from other anaerobic lineages. Sequences attributed to the *Bacteroidetes* (formerly: Cytophaga-Flavobacteria-Bacteroidetes super-phylum) were mainly recovered from the silicate sand (Si07), comprising members of the *Sphingobacteriales*, *Flavobacteriales* and *Bacteroidales*. As cultured bacteria of the *Bacteroidales* are obligate fermenters (KIRCHMAN 2002), their growth in the surface layer may be favored by anoxic patches due to decreased advection in well-sorted sediments,

especially during summer. *Sphingobacteriales* and *Flavobacteriales*, on the contrary, are known as mostly aerobic chemoorganotrophs which degrade a variety of organic compounds and (high molecular mass) biopolymers. Consequently, they would have a multitude of niches available in well-oxygenated surface sediments that experience organic matter input from the reef via the water column. While *Bacteroidetes*-associated sequences were only sparsely detected in the Checker Reef off Hawai'i (RUSCH *et al.* 2009), they were well established in all sampled depths of the nearby Moku o Loe Reef and surface sediments of the GBR (SØRENSEN *et al.* 2007, UTHICKE & MCGUIRE 2007).

Recovery of *Spirochaetes*-affiliated phylotypes was neither reported in any of the three studies on tropical reef sands, nor in those targeting permeable shelf sediments (HUNTER *et al.* 2006, MILLS *et al.* 2008) or deep- sea deposits (LI *et al.* 1999, SCHAUER *et al.* 2008). Their detection in coral reef sediments, however, was supported by the study from JENSEN *et al.* (2008), who found *Spirochaetes* associated with sediments of a cold-water coral reef. Most *Spirochaetes* are free-living and anaerobic, but there are numerous exceptions. In our study, all sequences grouping with this phylum belonged to the order *Spirochaetales*. Only two of the sequences belonging to the *Firmicutes* showed sequence similarity with previously published ones from polluted harbor and subsurface salt marsh sediments.

Diversity Patterns Based on Sequence Data. According to ∫-LIBSHUFF, all four libraries revealed significant separation in bacterial community composition ($P<0.001$; see Supplemental Information: Table S2A), indicating that each library was dominated by its own suite of characteristic OTU_S. Estimation of shared Chao1 richness among the four libraries using SONS (see Supplemental Information: Table S2B) showed that carbonate and silicate sands shared more OTU_S in August 2007 (40 OTU_S) than in December 2006 (30 OTU_S), indicating season-specific differences. Compared over both seasons, however, similar numbers of OTU_S were shared within the carbonate and silicate samples 44 OTU_S and 46 OTU_S), respectively, implying a similar degree of richness in both sands. Overlap in bacterial community, as predicted by the J_{class} estimator (Supplemental Information: Table S2B), was relatively low, ranging between 7.5 and 11.3%. The December 2006 libraries (Ca06, Si06) exhibited an overlap of 8.3% between carbonate- and silicate associated communities, which was in no real contrast to the 9.8% overlap found in the August 2007 libraries (Ca07, Si07), indicating a relatively high sand type

specificity in both seasons. With only 7.4% overlap between communities from December 2006 and August 2007, the carbonate libraries, however, showed a higher level of seasonal dependence and OTU_S specificity than the silicate libraries (11.3% overlap). Estimation of differences in community structure by means of the $Theta_{YC}$ index (see Supplemental Information: Table S2B) revealed clearly that the carbonate libraries (Ca06, Ca07) from December 2006 and August 2007 shared a much lower similarity (11.0%) than the silicate libraries (Si06, Si07) from both seasons (27.6%). This seemed to be yet another indication that the seasonal effect on bacterial communities was mainly reflected within the carbonate sediment.

Concerning the dynamics of sand type-specific indicator OTU_A* (see Supplemental Information: Figure S6A), Carbonate sand was first and foremost characterized by Actino314 (order: *Actinomycetales*), Acido525 (*Acidobacteriales*), and Alpha751/Delta751 (*Rhodobacterales/Desulfobacterales*), while typical signatures in the silicate sand comprised Actino454 (*Actinomycteales*), Gamma961 (*Chromatiales*), and Gamma497 (*Legionellales*). The most important season-driven OTU_A* (see Supplemental Information: Figure S6B) included Anaero654 (*Caldilineae*), Sphingo687 (*Sphingobacteriales*) and Anaero649 (*Caldilineae*) in December 2006, Gamma839 (*Legionellales*), Delta767 (*Desulfobacterales*), and Delta715 (*Myxococcales*) in August 2007, as well as Acido635 (*Acidobacteriales*), Delta715 (*Myxococcales*) and Actino305 (*Acidimicrobiales*) in February 2008. OTU_A* significantly attributed to the different depth horizons (see Supplemental Information: Figure S6C) were Actino291 (*Actinomycetales*), Gamma900 (*Rhodobacterales*), and Spiro443 (*Spirochaetales*) in the surface layer, Delta715 (*Myxococcales*), Acido530 (*Acidobacteriales*) and Gamma961 (*Chromatiales*) in the middle layer, and Gamma677 (*Legionellales*), Acido679 (*Acidobacteriales*), Actino291 (*Actinomycetales*) in the deep layer.

Individual OTU_A* variations were also studied by the Dufrene-Legendre indicator species analysis (data not shown) which, in contrast to the single regression procedure, identified all those OTU_A* that significantly ($P<0.05$) contributed to factor level-specific variations in bacterial community structure by still taking respective other levels into account. Results therefore differed slightly from those obtained by regression analysis on both single OTU_A* and global RDA, but the general trends were largely confirmed.

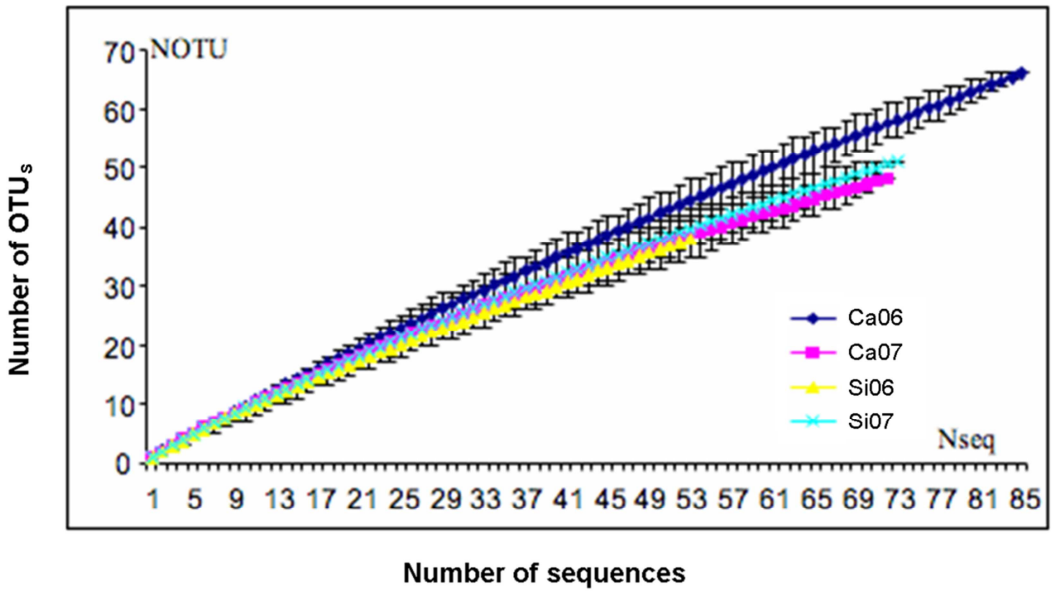

Figure S1. Rarefaction curves for the 16S-ITS rRNA gene clone libraries constructed from carbonate and silicate surface (0–2 cm) samples from December 2006 and August 2007, as calculated with DOTUR using the furthest neighbor assignment algorithm. Error bars represent the upper and lower bound of the 95% confidence interval.

Table S2A. Characterization of bacterial diversity and community structure for the 16S-ITS rRNA gene clone libraries constructed from carbonate and silicate surface (0–2 cm) samples from December 2006 and August 2007, based on the statistical tool ∫-LIBSHUFF.

	Ca06	**Ca07**	**Si06**	**Si07**
Ca06	0	0.0145	0.4896	0.9003
Ca07	0.0184	0	0.2524	0.001
Si06	0.7889	0.2678	0	0.2678
Si07	0.6539	0.0009	0.3166	0

Table 2B. Characterization of bacterial diversity and community structure for the 16S-ITS rRNA gene clone libraries constructed from carbonate and silicate surface (0–2 cm) samples from December 2006 and August 2007, based on the statistical tool SONS.

Shared Chao1	**Ca06**	**Ca07**	**Si06**	**Si07**
Ca06	–			
Ca07	44	–		
Si06	30	14	–	
Si07	54	40	46	–
J_{class}	**Ca06**	**Ca07**	**Si06**	**Si07**
Ca06	–			
Ca07	7.4	–		
Si06	8.3	7.5	–	
Si07	11.2	9.8	11.3	–
Theta $_{YC}$	**Ca06**	**Ca07**	**Si06**	**Si07**
Ca06	–			
Ca07	11.1	–		
Si06	17.2	18.5	–	
Si07	18.8	17.8	27.6	–

Table S3. Compilation of all ARISA-derived OTU_A that were successfully linked to 16S rRNA gene taxonomy, based on RDP Classifier and SeqMatch analyses. Single matches for each OTU_A are indicated by clone number, calculated fragment length and library name, as well as respective bacterial class (≥80% similarity), nearest neighbor, "S_ab" score, accession number, and environmental source.

ARISA		Sequencing			Phylogenetic assignment					
#	OTU_A	Clone	Frag.	Library	Bacterial class	%	Nearest neighbor	Score	Accession	Environmental source
1	**291**	65	292	Ca07	*Actinobacteria*	98%	uncultured bacterium SHFG434	0.931	FJ203051	coral with white band disease, Caribbean
	291	80	292	Ca07	*Actinobacteria*	95%	uncultured bacterium SHFG434	0.932	FJ203051	coral with white band disease, Caribbean
	291	87	292	Ca07	*Actinobacteria*	91%	uncultured bacterium SHFG434	0.938	FJ203051	coral with white band disease, Caribbean
	291	11	292	Si06	*Actinobacteria*	97%	uncultured bacterium SHFG434	0.925	FJ203051	coral with white band disease, Caribbean
	291	23	292	Si07	*Actinobacteria*	95%	uncultured bacterium SHFG434	0.875	FJ203051	coral with white band disease, Caribbean
2	**305**	2	306	Ca07	*Actinobacteria*	100%	uncultured bacterium KZNMV-5-B68	0.841	FJ712481	submarine mud-volcano sediment, Med
3	**307**	19	308	Si07	*Actinobacteria*	100%	uncultured bacterium KZNMV-5-B68	0.862	FJ712481	submarine mud-volcano sediment, Med
4	**313**	16	313	Ca07	*Gammaproteobacteria*	100%	uncultured gamma proteobacterium ARTE12_229	0.842	GU230347	water column, S-Atlantic
5	**315**	66	315	Ca07	*Actinobacteria*	94%	uncultured actinobacterium 3G1820-55	0.868	DQ431888	permeable marine sediment, N-GOM
6	**317**	41	317	Si07	*Actinobacteria*	94%	uncultured actinobacterium V1SC07b82	0.849	GU369874	volcano-assoc. hydrothermal vent, Tonga
	317	67	317	Si07	*Actinobacteria*	96%	uncultured actinobacterium V1SC07b82	0.868	GU369874	volcano-assoc. hydrothermal vent, Tonga
	317	14	318	Si07	*Actinobacteria*	97%	uncultured actinobacterium V1B07b85	0.906	GU369909	volcano-assoc. hydrothermal vent, Tonga
7	**319**	95	319	Ca07	*Actinobacteria*	91%	uncultured actinobacterium 3G1820-55	0.884	DQ431888	permeable marine sediment, N-GOM
	319	52	319	Si07	*Actinobacteria*	91%	uncultured actinobacterium V1B07b85	0.9	GU369909	volcano-assoc. hydrothermal vent, Tonga
8	**327**	33	327	Ca06	*Cyanobacteria*	96%	uncultured bacterium CI5cm.G09	0.982	EF208693	sandy carbonate reef sediment, Hawai'i
	327	26	327	Si07	*Actinobacteria*	92%	uncultured actinobacterium 3G1820-55	0.841	DQ431888	permeable marine sediment, N-GOM
9	**335**	36	335	Si07	*Spirochaetes*	98%	*Spirochaeta cellobiosiphila* (T) SIP1	0.585	EU448140	saline spring, Russia
10	**363**	24	364	Ca07	*Gammaproteobacteria*	100%	*Pseudomonas fulva* (T) AJ 2129	0.993	AB046996	
	363	74	364	Ca07	*Gammaproteobacteria*	100%	*Pseudomonas fulva* Z58zhy	0.997	AM410620	deep-sea
11	**375**	41	375	Ca06	*Gammaproteobacteria*	100%	uncultured bacterium CI5cm.D04	0.577	EF208686	sandy carbonate reef sediment, Hawai'i
12	**385**	68	386	Si07	*Actinobacteria*	100%	uncultured actinobacterium b6	0.939	GQ472798	water column, N-Bering Sea
13	**411**	64	412	Ca07	*Actinobacteria*	96%	uncultured bacterium Fe_B_116	0.816	GQ356933	methane seep sediment, California
	411	35	412	Si07	*Actinobacteria*	99%	uncultured bacterium Fe_B_116	0.867	GQ356933	methane seep sediment, California
14	**415**	25	415	Ca07	*Gammaproteobacteria*	100%	uncultured Legionellales bacterium TDNP_USbc97	0.651	FJ516889	carbonate wetland, Spain
	415	41	415	Ca07	*Gammaproteobacteria*	100%	uncultured Legionellales bacterium TDNP_USbc97	0.651	FJ516889	carbonate wetland, Spain
15	**425**	33	426	Ca07	*Acidobacteria_Gp10*	100%	uncultured bacterium D8S-76	0.862	EU652606	yellow Sea marine sediment
16	**435**	55	436	Si07	*Spirochaetes*	90%	uncultured bacterium 075B7	0.874	EU734981	pelagic Bacteria N-Bering Sea
	435	82	436	Si07	*Spirochaetes*	97%	uncultured bacterium 075B7	0.861	EU734981	pelagic Bacteria N-Bering Sea
17	**443**	91	444	Ca07	*Spirochaetes*	100%	uncultured organism MAT-CR-P2-H07	0.613	EU246077	courtyard soil, Boston
18	**449**	12	450	Ca06	WS3 *gen. incertae sedis*	100%	uncultured bacterium Out12bac85	0.898	GU302492	sediment under Beggiatoa mat, GOM
19	**455**	57	455	Si06	*Actinobacteria*	99%	uncultured bacterium 47SZ4	0.798	GU270862	pristine coastal sediment, Black Sea

20	**457**	10	457	Si06	*Actinobacteria*	100%	uncultured bacterium 47SZ4	0.81	GU270862	pristine coastal sediment, Black Sea
	457	60	457	Si06	*Actinobacteria*	100%	uncultured bacterium 47SZ4	0.81	GU270862	pristine coastal sediment, Black Sea
21	**461**	39	462	Si07	*Actinobacteria*	99%	uncultured bacterium Sd1-56	0.905	GQ246337	marine sediment, N-Yellow Sea
22	**473**	36	473	Ca07	*Actinobacteria*	100%	uncultured bacterium 1C226763	0.89	EU799192	estuarine water column, Chesapeake Bay
	473	91	473	Si07	*Acidobacteria_Gp23*	100%	uncultured bacterium 4.1E+70	0.814	EU925849	water column, N-Bering Sea
23	**479**	38	480	Ca06	*Deltaproteobacteria*	100%	uncultured bacterium PropaneSIP20-4-01	0.903	GU584771	hydrocarbon seeps
24	**485**	8	486	Ca06	*Caldilineae*	85%	uncultured *Chloroflexus sp*. XA1E08F	0.602	FJ481225	marine sponges
25	**497**	50	498	Ca07	*Gammaproteobacteria*	100%	uncultured gamma proteobacterium ARTE12_229	0.828	GU230347	water column, S-Atlantic
26	**513**	23	513	Ca07	*Gammaproteobacteria*	100%	uncultured gamma proteobacterium pItb-vmat-47	0.8	AB294952	reef-assoc. hydrothermal vent, Japan
27	**517**	5	517	Ca07	*Gammaproteobacteria*	100%	uncultured Legionellales bacterium TDNP_Bbc97	0.711	FJ516765	carbonate wetland, Spain
	517	35	518	Ca06	*Gammaproteobacteria*	100%	uncultured gamma proteobacterium A07-02F	0.662	FJ542878	earthworm intestines
28	**525**	73	526	Ca07	*Acidobacteria_Gp10*	100%	uncultured bacterium D8S-76	0.857	EU652606	marine sediment, Yellow Sea
29	**531**	7	531	Ca07	*Acidobacteria_Gp10*	100%	uncultured bacterium D8S-76	0.883	EU652606	marine sediment, Yellow Sea
	531	12	531	Ca07	*Acidobacteria_Gp10*	100%	uncultured bacterium D8S-76	0.882	EU652606	marine sediment, Yellow Sea
	531	56	531	Ca07	*Acidobacteria_Gp10*	100%	uncultured bacterium D8S-76	0.869	EU652606	marine sediment, Yellow Sea
30	**535**	49	535	Si07	*Sphingobacteria*	99%	uncultured bacterium Sd1-55	0.76	GQ246336	marine sediment, N-Yellow Sea
31	**539**	63	539	Si06	*Gammaproteobacteria*	100%	uncultured gamma proteobacterium 43 EDB3	0.889	AM882561	oil-contamin. coastal sediment, France
32	**559**	37	559	Si07	*Gammaproteobacteria*	100%	uncultured *Chromatiales* bacterium TDNP_Wbc97	0.828	FJ517068	carbonate wetland water & sediment, Spain
33	**563**	71	563	Ca07	*Gammaproteobacteria*	100%	uncultured gamma proteobacterium 2C75	0.962	FN424387	impacted coastal lagoon, Tunisia
	563	12	563	Si07	*Sphingobacteria*	100%	uncultured *Cytophaga sp*. VHS-B5-77	0.707	DQ395043	polluted harbor sediment
	563	51	563	Si07	*Sphingobacteria*	99%	uncultured *Cytophaga sp*. VHS-B5-77	0.697	DQ395043	polluted harbor sediment
34	**581**	3	581	Si07	*Sphingobacteria*	100%	uncultured *Bacteroidetes* bacterium MBAE20	0.721	AJ567581	deep-sea nodule province, Pacific
35	**599**	76	600	Ca07	*Betaproteobacteria*	100%	*Ralstonia sp*. MCT1	0.983	DQ232889	cultures
36	**607**	79	607	Si07	*Spirochaetes*	94%	uncultured bacterium SGUS1039	0.684	FJ202296	coral with white band disease, Caribbean
37	**611**	43	612	Ca06	*Planctomycetacia*	91%	unidentified bacterium wb1_E15	0.492	AF317763	Nullarbor cave, Australia
38	**617**	51	617	Ca07	*Actinobacteria*	99%	uncultured bacterium Fe_B_116	0.868	GQ356933	methane seep sediment, California
39	**631**	61	631	Ca07	*Deltaproteobacteria*	100%	uncultured bacterium SSS16A	0.886	EU592471	eutrophic lake, California
40	**633**	10	634	Ca07	*Deltaproteobacteria*	98%	uncultured bacterium 084L59	0.881	FJ416109	marine sediment, N-Bering Sea
41	**635**	40	636	Ca07	*Acidobacteria_Gp10*	100%	uncultured bacterium MSB-2Y4	0.929	EF125465	mangrove soil
42	**639**	71	640	Si07	*Anaerolineae*	99%	uncultured bacterium Ld1-28	0.886	GQ246423	marine sediment, N-Yellow Sea
43	**643**	17	644	Ca07	*Deltaproteobacteria*	100%	uncultured delta proteobacterium SL13	0.87	AY771942	intertidal mud flat sediment, N-Atlantic
44	**645**	67	646	Ca07	*Deltaproteobacteria*	100%	uncultured bacterium LARIS_58-01B08	0.789	FN550053	methane seep sediment
45	**647**	20	647	Si07	*Sphingobacteria*	100%	uncultured bacterium ELB16-214	0.637	DQ015814	ice-covered lake water, Antarctica
46	**649**	79	650	Ca07	*Anaerolineae*	100%	uncultured bacterium Ld1-28	0.858	GQ246423	marine sediment, N-Yellow Sea
47	**655**	40	655	Si07	*Anaerolineae*	100%	uncultured bacterium LARHR_38-01F05	0.83	FN549949	methane seep sediment, California
	655	69	655	Si07	*Anaerolineae*	99%	uncultured actinobacterium V1SC07b82	0.849	GU369874	methane seep sediment, California
	655	80	655	Si07	*Anaerolineae*	100%	uncultured bacterium livecontrolB12	0.833	FJ264746	methane seep sediment, California
48	**677**	89	678	Ca07	*Gammaproteobacteria*	100%	uncultured gamma proteobacterium MSB-4B8	0.696	DQ811840	mangrove soil
49	**679**	60	680	Ca07	*Acidobacteria_Gp26*	100%	uncultured bacterium MidBa64	0.919	EF999388	estuarine sediment, China

50	**681**	14	682	Ca06	*Gammaproteobacteria*	100%	uncultured bacterium S1-27	0.869	FJ545457	marine sediment & water, N-Yellow Sea
51	**683**	63	684	Ca07	*Acidobacteria_Gp26*	100%	uncultured bacterium MidBa64	0.905	EF999388	estuarine sediment, China
52	**685**	68	685	Ca07	*Gammaproteobacteria*	100%	*Alkalilimnicola ehrlichii* MLHE-1	0.599	CP000453	
53	**687**	17	688	Ca06	*Gammaproteobacteria*	100%	uncultured gamma proteobacterium SGST691	0.75	GQ348115	marine oxygen minimum zone, Canada
	687	1	688	Si07	*Sphingobacteria*	99%	uncultured *Saprospiraceae* bacterium TDNP_Wbc97	0.829	FJ517112	carbonate wetland, Spain
53	**689**	44	698	Ca06	*Deltaproteobacteria*	97%	uncultured bacterium CK_2C5_16	0.947	EU488447	bacterial lucinid bivalve symbionts
	701	30	701	Ca06	*Gammaproteobacteria*	100%	uncultured gamma proteobacterium MSB-4D7	0.93	DQ811842	mangrove soil
54	**703**	62	703	Si07	*Alphaproteobacteria*	100%	uncultured alpha proteobacterium Bac24_Flocs	0.922	AB491826	microbial flocs, shrimp hatchery, Java
55	**703**	28	704	Ca06	*Gammaproteobacteria*	100%	uncultured bacterium Tokyo.16S.Bac.11	0.976	AB530211	marine bays, Suez and Tokyo
56	**703**	42	704	Ca06	*Gammaproteobacteria*	100%	uncultured bacterium S1-58	0.983	FJ545485	marine sediment & water, N-Yellow Sea
	703	54	704	Ca07	*Acidobacteria_Gp26*	100%	uncultured *Acidobacterium sp*. HCM3MC90_12F_FL	0.819	EU373917	deep-sea surface sediment, S-Atlantic
	705	3	705	Ca07	*Anaerolineae*	100%	uncultured bacterium A13S-56	0.916	EU617894	marine sediment, Yellow Sea
57	**705**	57	705	Ca07	*Acidobacteria_Gp26*	100%	uncultured deep-sea bacterium Ucb15709	0.826	AM997868	deep-sea surface sediment, S-Atlantic
	705	2	706	Si06	*Deltaproteobacteria*	99%	uncultured bacterium Mn3b-B8	0.914	FJ264596	methane seep sediment, California
	709	2	710	Ca06	*Gammaproteobacteria*	99%	uncultured gamma proteobacterium 42 EDB3	0.913	AM882567	oil-contamin. coastal sediment, France
58	**715**	46	716	Ca06	*Deltaproteobacteria*	100%	uncultured bacterium 40_st3_4-6cm	0.885	EU290707	marine sediment, upwelling system, Namibia
59	**721**	58	722	Si07	*Gammaproteobacteria*	100%	uncultured gamma proteobacterium pItb-vmat-20	0.982	AB294936	reef-assoc. hydrothermal vent, Japan
60	**751**	26	752	Ca06	*Deltaproteobacteria*	100%	uncultured bacterium CI75cm.2.07	0.917	EF208703	sandy carbonate reef sediment, Hawai'i
61	**751**	45	752	Ca06	*Alphaproteobacteria*	100%	uncultured alpha proteobact. Belgica2005/10-130-28	0.89	DQ351770	metal contamin. marine sediment
	753	7	753	Ca06	*Alphaproteobacteria*	100%	uncultured alpha proteobact. Belgica2005/10-130-28	0.895	DQ351770	metal contamin. marine sediment
62	**753**	18	753	Ca06	*Alphaproteobacteria*	100%	uncultured alpha proteobact. Belgica2005/10-130-28	0.9	DQ351770	metal contamin. marine sediment
63	**761**	15	761	Ca06	*Deltaproteobacteria*	100%	uncultured delta proteobacterium 4aFS	0.936	AM039962	marine sediment, Elba
64	**675**	9	765	Ca06	*Epsilonproteobacteria*	100%	uncultured *Campylobacterales* bacterium DS057	0.858	DQ234141	river estuary, N-Taiwan
65	**767**	21	768	Ca07	*Deltaproteobacteria*	100%	uncultured bacterium livecontrolB19	0.839	FJ264753	methane seep sediment, California
66	**781**	92	781	Ca07	*Deltaproteobacteria*	100%	uncultured delta proteobacterium 4aFS	0.932	AM039962	marine sediment, Elba
67	**791**	4	791	Ca06	*Alphaproteobacteria*	100%	uncultured bacterium Dstr_M20	0.915	GU118190	corals, Caribbean
68	**805**	1	805	Si06	*Deltaproteobacteria*	100%	uncultured delta proteobacterium SI29	0.895	AY771939	intertidal mud flat sediment, N-Atlantic
69	**807**	88	808	Ca07	*Deltaproteobacteria*	100%	uncultured delta proteobacterium 3B1820-38	0.9	DQ431902	permeable marine sediment, N-GOM
70	**815**	70	815	Ca07	*Deltaproteobacteria*	97%	uncultured deep-sea bacterium Ucb15723	0.685	AM997862	deep-sea surface sediment, S-Atlantic
	815	23	816	Ca06	*Alphaproteobacteria*	100%	uncultured alpha proteobacterium STX_15f	0.925	EF123331	coral with black band disease, Caribbean
71	**821**	11	822	Ca07	*Gammaproteobacteria*	100%	uncultured bacterium G8_10.3_1	0.932	FJ717220	bioturbated mesocosm
72	**831**	22	832	Ca06	*Gammaproteobacteria*	100%	uncultured bacterium CK_1C4_36	0.951	EU488063	lucinid bivalve symbionts
73	**849**	20	850	Ca06	*Gammaproteobacteria*	100%	uncultured gamma proteobacterium Cobs2TisB5	0.837	EU246800	coral, S-Pacific
74	**863**	33	863	Si07	*Gammaproteobacteria*	100%	uncultured gamma proteobact. Belgica2005/10-140-23	0.933	DQ351795	metal-contamin. marine sediment
75	**901**	58	901	Ca07	*Gammaproteobacteria*	100%	uncultured bacterium SHFH407	0.904	FJ203377	coral with white band disease, Caribbean
76	**961**	2	962	Si07	*Gammaproteobacteria*	100%	uncultured bacterium CK_1C4_36	0.947	EU488063	bacterial lucinid bivalve symbionts

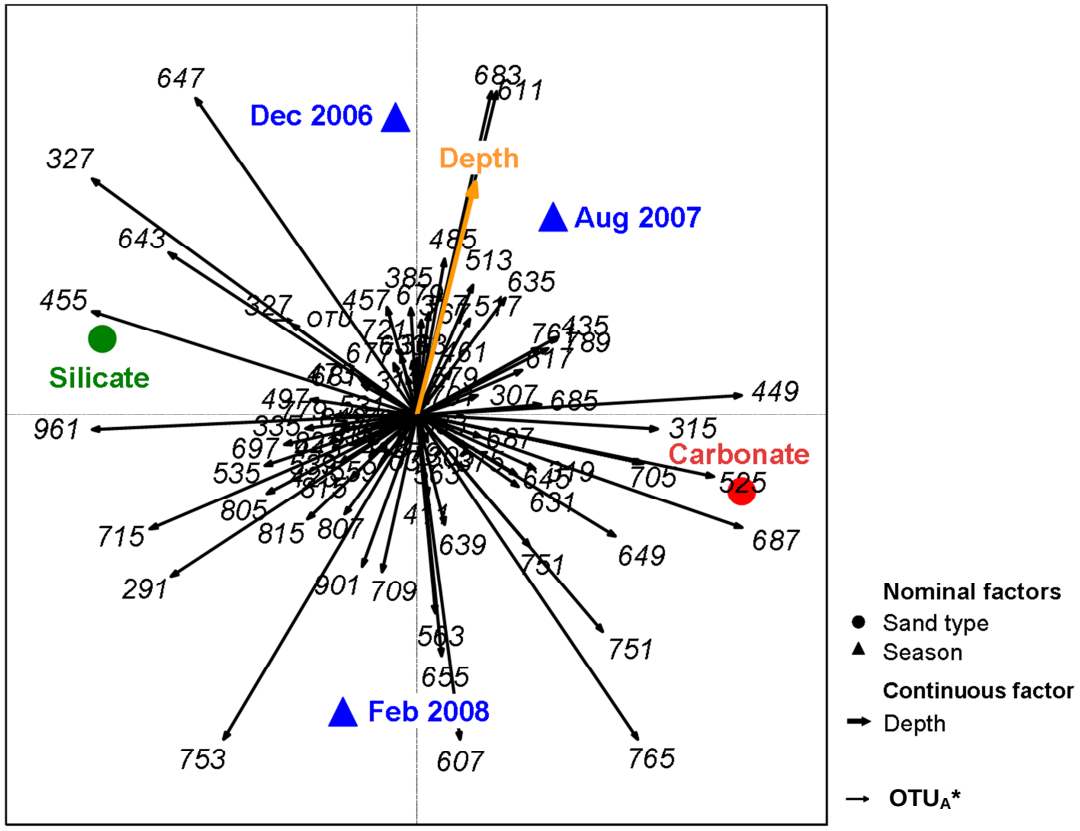

Figure S4. Relationship between factors sand type, season, and depth, and changes in bacterial community structure, with focus on specific OTU_A* shifts at the bacterial class level (≥80% sequence similarity). The biplot represents a RDA ordination of linked ARISA data (Bray-Curtis distance) under direct constraint of explanatory factors, whereby factor levels of sand type and season were set as nominal variables, and those of depth as continuous variables. Vectors represent all 76 OTU_A that were linked to a16S-ITS rRNA gene sequence, with numbers indicating the respective OTU_A* (i.e. ARISA fragment) length in base pairs. For respective phylogenetic assignments, see Table S3.

Table S5A. RDA correlation matrix for single explanatory factors and the two main axes.

	RDA axis 1	**RDA axis 2**
Carbonate	0.9154	-0.1994
Silicate	-0.9154	0.1994
Dec 2006	-0.0373	0.4686
Aug 2007	0.2448	0.3210
Feb 2008	-0.1893	-0.7104
Depth	0.1640	0.5969

Table S5A. RDA correlation matrix for explanatory factors.

	Carbonate	**Silicate**	**Dec 2006**	**Aug 2007**	**Feb 2008**	**Depth**
Carbonate	1					
Silicate	-1	1				
Dec 2006	-0.0261	0.0261	1			
Aug 2007	0.01	-0.01	-0.3845	1		
Feb 2008	0.0142	-0.0142	-0.5437	-0.5657	1	
Depth	0.0146	-0.0146	0.1313	0.0967	-0.2052	1

III. DISCUSSION

III.1 Synopsis

Coral reef ecosystems, often called “rainforests of the sea”, represent intriguingly diverse and dynamic environments in both shallow and deep ocean realms. They are structurally highly complex, dynamic and species-rich environments, but still little is known about the role they play for the “unseen majority” in the oceans: microbial communities. This work includes the first multi-scale study on cold-water coral bacterial diversity. In addition, some of the fundamental patterns of bacterial diversity in both warm-water (WWC) and cold-water coral (CWC) reefs were investigated on different ecological scales, with particular emphasis on the macro- and micro-scale heterogeneity of microbial habitats. By applying mainly the high-throughput, high-resolution fingerprinting technique ARISA (Automated Ribosomal Intergenic Spacer Analysis) and a suite of multivariate statistical tools, it was possible to screen many samples and study patterns without being constrained by gaps originating in methodological differences. In case of WWC sands, fingerprinting was further combined with a standard molecular approach, including 16S rRNA gene sequencing, to taxonomically identify bacterial sand associates and to study their dynamics in relation to ecological factors. In the following, the main findings emerging of this work are summarized and evaluated. Challenges and limitations are addressed, and perspectives for future research are given.

Question 1: *Are coral-associated microbial communities distinct from those in the surrounding reef water and sediment?*

Within the first study (II.2, SCHÖTTNER *et al.* 2009), principle patterns of bacterial diversity associated with and surrounding the main constructional CWC, *L. pertusa*, under both natural (reef) and controlled (aquaria) conditions were characterized. Of particular interest was the coral- and environment-specific structuring of bacterial communities as related to distinct microbial habitats such as coral branch, coral mucus, ambient seawater and proximal sediment. Overall, the observed, non-random community

variations suggested a specific partitioning of bacterial assemblages between different microbial habitats on and surrounding scleractinian CWC and, furthermore, a potential control of community dynamics through the prevailing environment. In particular, this study revealed that coral-generated surfaces, such as skeleton surface (branch) and mucus, each are colonized by very specific bacterial assemblages. Bacteria hosted directly by the coral differed significantly in community structure and OTU (operational taxonomic unit) number from those inhabiting the surrounding seawater or proximal sediment, and also exhibited a much higher inter-sample variability and therefore a higher overall diversity. In addition, the comparison of fjord and aquarium samples showed a clear bacterial community shift related to coral living conditions. These findings suggest a marked habitat specificity of bacterial assemblages, which may also be subject to environmental conditioning.

The second study (II.3) supported the hypothesis on such specificity of bacterial assemblages for each of the different microbial habitats, with most conspicuous contrasts for coral-derived surfaces versus ambient environment. This habitat-specific partitioning of communities at the intra-ecosystem level was present in all reefs and dominated over any other spatial effect. In addition, bacterial community structure clearly reflected coral host identity, but also respective reef affiliation (i.e. biogeography), which characterized coral-bacteria associations as host-specific *sensu lato* only. Imprints of within-reef geomorphologic zoning, on the contrary, were not detected in coral-associated profiles, although they were unambiguously marked in seawater and sediment communities. At the inter- and cross-ecosystem level, sharing of bacterial signatures manifested mainly in the ambient environment seawater and sediment, while community turnover and reef-specificity were rather promoted by coral-generated surfaces.

When comparing WWC and CWC reef ecosystems, microbial habitat differentiation shows similar patterns in bacterial diversity with regard to the distinction between mucus, skeleton surface (branch), tissue, water, and sediment (e.g. ROHWER *et al.* 2001, BOURNE & MUNN 2005, RITCHIE 2006). Also, many bacterial signatures (OTU) are similar in CWC and WWC ecosystems, resulting in at least 75% overlap in sediments (SCHÖTTNER *et al*. unpublished data not included in this thesis). Further, the role of coral mucus as microbial habitat and food source seems common to CWC and WWC (WILD *et al*. 2005, NAUMANN *et al.* 2009, WILD *et al*. 2009, WILD *et al.* 2010). It has already been shown for animals how coral reef ecosystems represent a continuum from shallow waters to the

deep sea, with WWC and deep-sea CWC as the two endmembers and mesophotic reefs inbetween. The latter may play a critical role in connectivity of coral reef environments (OLSON & KELLOGG 2010), and may also be important for interconnection of their associated microbial communities.

Overall, these findings support the assumption that CWC surfaces represent very specific microbial habitats that select for a certain variety of bacterial colonizers, thereby increasing microbial diversity in the deep sea. Moreover, they suggest that bacterial niche differentiation in CWC reef ecosystems is strongly influenced by the type of microbial habitat available for colonization, as well as by the taxonomic affiliation of the coral host, but also indicated that large-scale environmental conditioning and/or distance effects, rather than within-reef geomorphology and respective medium-scale changes in (a)biotic parameters, may play an important role.

Question 2: *Are coral reef habitats hotspots of microbial biodiversity?*

This thesis includes one of the few pioneering efforts to characterize the microbial diversity of CWC reef ecosystems and provides the investigational framework for advanced work targeting bacterial diversity within and beyond a whole CWC reef ecosystem. Local (intra-reef) to regional (inter-reef) variations in bacterial diversity associated with two constructional CWC, *L. pertusa* and *M. oculata,* were investigated in a multi-scale survey spanning five levels of spatial and ecological reef organization (II.3). The main goal was the identification of scales most important for bacterial community variation in CWC reef ecosystems, in consideration of the distinct microbial habitats offered by those corals and their surrounding. This was achieved by investigating variations in bacterial community structure of four different CWC reef ecosystems. A fundamental separation of bacteria between distinct coral-generated and ambient microbial habitats was found, which appeared pivotal for determining the differentiation and interconnection of bacterial communities over all spatial and organizational scales investigated. It was also revealed that bacterial community variation, albeit locally consistent with coral species and reef-internal environmental complexity (i.e. geomorphologic reef zoning), changed markedly from local to regional scaleComparative investigations of *L. pertusa*-associated bacterial signals at all four CWC reef study sites

clearly showed that bacterial communities associate very specifically with distinct microbial habitats available in the reef system. Coral skeleton surface (branch) and mucus as well as ambient seawater and reef sediment exhibited clear differences in bacterial community structure and OTU number. On average, coral mucus and skeleton surface each contained about 25% unique OTUs relating those significant differences in bacterial diversity between coral-associated *versus* ambient microbial habitats to a quarter of the respective community structure. Although mean OTU numbers, giving a first indication for potential bacterial richness, were generally much lower in coral-derived surfaces than in ambient habitats, community structure, again, turned out as much more variable in bacterial assemblages directly associated with the coral compared to those present in the coral environment. Hence, compared to water- and sediment-associated bacterial communities, bacteria in CWC reef ecosystems showed a very high turnover on small scales within the reef, resulting in a high beta-diversity, likely structured by several factors operating at multiple spatial and reef organizational scales.

Overall, this suggests that coral reefs are hotspots of microbial biodiversity, as they provide various small-scale habitats for microbial colonization within each colony, each of them influenced by specific abiotic and biotic environmental conditions that vary in space and time (AINSWORTH *et al*. 2009). The presence of different microbial habitats within a given coral colony enhances microbial diversity and function in coral reef ecosystems. This might be based on spatial separation through habitat-specific separation (e.g. certain microbes are specific for mucus, while others are found in tissue), or on the contrary, by habitat-specific interaction (e.g. when locally co-occurring bacteria compete for resources through antagonistic interaction, as previously observed in WWC; RYPIEN *et al*. 2010). Discriminative colonization of microbial habitats, especially between coral-derived surfaces and ambient environment, increases local but also regional diversity.

Question 3: *What factors are responsible for structuring microbial diversity in coral reef ecosystems?*

The ecosystem engineering capacity of constructional corals (with their complex framework architecture) has been shown to enhance animal diversity at both local andregional scales (ARIAS-GONZALES *et al.* 2008, BUHL-MORTENSEN *et al.* 2010). As

benthic biological structures, both WWC and CWC represent a source of habitat complexity and heterogeneity, especially in low-structure surroundings. They offer settlement substrate, shelter, and feeding opportunities for various sessile and free-living organisms of different sizes.

Within the multi-level reef study (II.3), analyses on the relative importance of some of the most prominent ecological organization levels (i.e. microbial habitat, coral species, coral color type, reef zone, reef type and location) as drivers of bacterial diversity, both within a highly proliferating reef system and between several different CWC reefs, revealed the following: Variations in bacterial community structure were mainly determined by microbial habitat (mucus, skeleton, seawater, sediment) and reef site/type (offshore *versus* inshore; distance), as well as by coral species (*L. pertusa*, *M. oculata*). This clearly confirmed that bacterial communities associate specifically with coral-derived *versus* ambient habitats, and was even further supported by the observation that patterns of reef zoning could not be detected in coral-associated assemblages. This implies strong differences in community assembly as well as local environmental conditioning, both dependently and independently of mediation by the coral host. The finding that bacterial signatures differed significantly between the four investigated reef sites (Røst, Trænadjupet, Langenuen, Tisler), however, indicated that specificity of coral-associated communities may also be partly controlled by the environmental conditions prevailing in each reef system.

An additional investigation (SCHÖTTNER *et al.* unpublished data not included in this thesis) was dedicated to directly contrasting bacterial diversity patterns in (eutrophic) CWC *versus* (oligotrophic) WWC reef ecosystems. This work aimed, in particular, at identifying global patterns and relevant scales of coral-microbe associations in coral reef ecosystems. Findings revealed overall highly significant differences in bacterial community structure, reflecting the fundamental difference between both ecosystems and thereby the importance of large-scale environmental conditioning. Bacterial habitat specificity, however, turned out highly similar in CWC and WWC reef ecosystems. In both the CWC and WWC samples, bacterial assemblages associated very specifically with coral-derived surfaces (mucus, branch) *versus* ambient environment (water, sediment) and exhibited the same patterns in community structure and OTU number. Despite described ecosystem-specific differences, analyses also clearly showed that aconsiderable proportion of bacterial signatures (coral-associated: 25–70%, ambient:

50–85%) was shared between cold- and warm-water reefs, which highlights not only the high adaptation potential of prokaryotes, but also possible similarities regarding bacterial niche formation in these distinct ecosystems. This strongly suggests the existence of analogous patterns in bacterial colonization of CWC and WWC-derived surfaces, which can be mainly attributed to the discriminative colonization of the different microbial habitats in each ecosystem.

Structuring of microbial communities within the reef ecosystem may also be enhanced due to the substantial structural and physical-chemical heterogeneity inherent in reef sediments. This was shown in the third study (II.4), where permeable carbonate and silicate WWC reef sands were compared for their potential to promote differences in bacterial diversity and biomass, including those over season and space. The results revealed pronounced sand type-related as well as spatio-temporal imprints, and emphasized the deterministic role of sediment mineralogy for the seasonal and vertical structuring of bacterial communities. Furthermore, (*Gamma-*, *Delta-*, *Alpha-*) *Proteobacteria* and *Actinobacteria* were identified as prominent members of the overall highly diverse bacterial communities in both sands, with specific shifts already detectable at the bacterial class level. These findings characterized carbonate and silicate reef sands as distinct microbial habitats for specific, comparably diverse and highly dynamic bacterial assemblages. The local co-occurrence of both sand types enhanced the ecological structuring of bacterial communities within a single WWC reef ecosystem.

Overall, these results suggest that intra-reef variation of bacterial diversity in CWC reef ecosystems is mainly driven by a combination of local community history and (a)biotic conditioning, while inter-reef and cross-ecosystem divergence depends on rather large-scale, regional environmental factors and a separation of communities over distance. In this regard, multi-scale differentiation and interconnection of bacterial assemblages in distinct reef ecosystems seem to be largely determined by the discriminative colonization of coral-associated versus ambient habitats, with the former promoting primarily local and the latter mostly regional to global selection of bacterial assemblages.

III.2 Perspectives

The studies presented within this thesis portrayed bacterial communities as intriguingly diverse, complex and dynamic inhabitants of CWC and WWC reef ecosystems. Thereby, this work provided a baseline for future comprehensive investigations on microbial ecology (diversity and function) in complex ecosystems like coral reefs. Only a holistic approach can help understanding and mitigating problems arising from increasing environmental pressures, both human-induced and natural, as both WWC and CWC already show dramatic impacts. In the following, ideas for further studies on the microbial ecology of coral reef ecosystems are summarized.

Evidence for Symbiosis between Microbes and CWC – Polyp Tissue as Microbial Habitat. As indicated by previous studies on CWC and WWC, microbial communities also colonize healthy coral tissue (e.g. FRIAS-LOPEZ *et al.* 2002, BOURNE & MUNN 2005, KLAUS *et al.* 2005, NEULINGER *et al*. 2009). In this thesis, signatures of the "fifth" coral-associated microbial habitat (polyp tissue) could not be studied in parallel to other habitats, due to methodological constraints (PCR amplification), which may be partly caused by a low concentration of bacterial cells in these tissues. Protocol optimization was hindered by the overall low amount of sample material. However, preliminary results show that polyp tissue is characterized by specific bacterial signatures that are distinct to those associated with skeleton surface scrapings, mucus or the ambient environment (water, sediment). Although branch samples likely also contain tissue (coenosarc), which may explain observed similarities in community structure and OTU number, the overall polyp tissue signature is significantly different from the branch signature, suggesting differences between bacteria growing outside on skeleton (incl. coenosarc) and within the polyp tissue. This could be due to the circumstance that both represent different types of interfaces, but also be related to host physiology. For future analyses it is recommended that polyp tissue is further investigated, as it may contribute to habitat heterogeneity and, generally, to the within-colony structuring of communities on corals.

Additional Microbial Habitats in Coral Reef Ecosystems. Previous tests on sampling at different coral branch locations (stem *versus* polyp calice) revealed no significant effect on OTU diversity and structure, building the basis for the chosen approach: Samples from

different polyps on one coral colony were combined. However, this is also why direct inferences about the specific extent of within-colony variation were not possible. Nevertheless, within-colony variability may be important according to the findings by HANSSON *et al.* (2009) on CWC. It seems very likely, that community patterns in CWC may greatly differ between polyps, as their tissues are not connected inside the skeleton (BEUCK 2008, ROBERTS *et al*. 2009 and references therein). CWC polyps have already shown to differ greatly in age and thereby in growth capacity and metabolic activity (BROOKE *et al.* 2009, MAIER *et al.* 2009).

Furthermore, samples of dead coral framework should be included in future studies as it constitutes an important, most often dominant fraction of the overall reef structure. For CWC-associated fauna, the diverse habitats provided by dead coral skeletons are the ones facilitating faunal diversity (MORTENSEN & FOSSÅ 2006 and references therein). Further, significant differences have also been found when comparing bacterial communities of dead *L. pertusa* fragments with living specimens (GROßKURTH 2007, YAKIMOV *et al*. 2006). However, sampling was not conducted along the taphonomic gradient within the whole framework of a colony. There are already indications that microbes, together with boring organisms, represent the main colonizers of dead coral skeleton and are responsible for early post-mortem processes (FREIWALD *et al*. 1997, BEUCK 2008).

At another level of spatial analysis, a global investigation of coral reef-associated microbial communities is still needed. In this thesis, all CWC samples were obtained from reefs in the NE Atlantic. In the future, expanding study sites to reefs from the other side of the Atlantic Ocean, as well as other oceanic provinces and regions, might yield new insights into local to regional patterns, up to a global scale. In addition, investigation of further coral species, including soft and lace corals, as well as other microbial components such as archaea, fungi, protists, and viruses seems important (FREIWALD *et al.* 1997, KELLOGG 2004, ROSENBERG *et al.* 2007, WEGLEY *et al.* 2007, OLSON & KELLOGG 2008, AINWORTH *et al.* 2009).

High-Resolution Studies of Microbial Diversity Associated with Coral Reefs. The fingerprinting method ARISA provides a very fast, cost-effective and high-throughput solution for bacterial community screening of many samples. In comparison to other fingerprinting techniques (e.g. Terminal Restriction Fragment Length Polymorphism;

DANOVARO *et al*. 2006), ARISA offers more resolution to detect changes in marine bacterial communities, as well as intra-genomic heterogeneities within closely related gene clusters (BROWN & FUHRMAN 2005). Fingerprinting techniques may, however, only account for variations of the most abundant bacterial signatures, while many rare species are overlooked. Although probably not responsible for the main ecosystem functions at the point of sampling, rare types also represent important community members that should not be missed out in a "microbial mapping" effort. It would therefore be recommendable to combine molecular fingerprinting efforts with further methods, such as massive parallel tag sequencing, metagenomics, and transcriptomics, as well as single-cell techniques like secondary ion mass spectrometry at nano-scale ('NanoSIMS') and high-resolution microscopy, in order to achieve a multi-phase approach that allows for an effective, holistic characterization of microbial key players in coral reef ecosystems.

In coherence with a future multi-phase approach, it would also be desirable to relate fingerprinting diversity data (e.g. OTU numbers) to microbial cell numbers and biomass. It is expected that coral surfaces harbor less than the ambient environment, thereby (indirectly) reflecting obtained OTU number results. For example, preliminary cell counts of mucus-associated microbes revealed that many bacteria were associated with this seemingly attractive food source, even though they were not as concentrated as expected (less than in water). In addition, bacterial biomass could be used for including reef-associated microbial communities in ecosystem modeling (ALLISON & MARTINY 2008).

Functional Potential of Ccoral-Associated Microbes. As bacterial communities are, to some extent, directly driven by environmental factors, it would be very useful to link community data directly to measured environmental parameters (such as nutrient and oxygen concentration, temperature, or pH). If not contemporary environmental factors, then historical factors such as dispersal limitation may be responsible for variation of communities over larger spatial scales. It is yet to be explored how the extent of microbial habitat variability (and resulting coral microbial ecology) is ultimately manifested in the physiology and performance of the coral reef (AINSWORTH *et al*. 2009). In other ecosystems, small-scale variability significantly impacts both the host organism's physiology and ecosystem function. For example, biofilms and microbial associations of plant surfaces are strongly adapted to and influenced by small-scale environmental variability (RAMEY *et al*. 2004, HODGE *et al*. 2006). To assess the functional role of the

association between microbes and corals, it would be important to study the impacts of environmental variations on ecosystem function via direct and indirect effects on the microbial communities. Gaining insight into the functional potential of coral-associated bacteria may reveal the prevailing trophic interrelatedness in the reef ecosystem, both among the microbes themselves and between microbes and corals.

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IV. FURTHER CONTRIBUTIONS

IV.1 Sponge-Associated Bacterial Communities Reflect the Evolutionary History of their Hosts

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Abstract

Cold-water coral reefs are known to locally enhance faunal and possibly also microbial biodiversity on continental margins and in the deep ocean. Sponges are the most diverse faunal group on cold-water coral reefs and many sponges host huge amounts of associated microbes. Bacterial communities of 12 sponge species from 3 cold-water coral reefs off Norway, covering a broad range of sponge taxonomy, were investigated using the high-resolution molecular technique ARISA. The aim of this study was to investigate the contribution of sponges to microbial diversity on the reefs; to identify factors that structure microbial diversity in sponges; to explore the correlation between sponge phylogenetic distance and microbial community dissimilarity and, finally, to conclude whether these findings give arguments for sponge-microbe co-evolution. ARISA analyses showed that sponges on cold-water coral reefs host species-specific yet highly diverse bacterial communities which are different from those of the surrounding sediments. Family, species and ecological concept (high microbial abundance vs low microbial abundance sponges) are the main factors structuring bacterial communities in sponges, while spatial/depth effects are less important. Sponge phylogeny and bacterial community are directly correlated within the sponge family level, but not on a higher taxonomic level. It can be concluded that the different sponge species on cold-water coral reefs represent many specialized microbial habitats in this ecosystem, and therefore increase the microbial diversity on the reef system. While sponge phylogeny is the main factor structuring bacterial communities on lower sponge taxonomic levels, ecological and morphological features, namely growth form, tissue ventilation and amount of associated microbes, become more important on higher taxonomic levels. Linear correlation between bacterial community dissimilarity and sponge phylogenetic distance within the family of the *Geodiidae* strongly indicates sponge-microbe co-evolution within this ancient sponge family. The weak spatial (i.e. depth) effect on bacterial community structure further supports the idea of sponge-microbe co-evolution. These results indicate that sponges on cold-water coral reefs shape niches for specific microbial communities, which are stable over geographic distances and even during evolutionary times. By this, sponges may interconnect microbial ecosystems of the deep sea in time and space.

IV.2 Evidence of Nitrification and Denitrification in High and Low Microbial Abundance Sponges

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Abstract

Aerobic and anaerobic microbial key processes were quantified and compared to microbial numbers and morphological structure in Mediterranean sponges. Direct counts on histological sections stained with DAPI showed that sponges with high microbial abundances (HMA sponges) have a denser morphological structure with a reduced aquiferous system compared to low microbial abundance (LMA) sponges. In *Dysidea avara*, the LMA sponge, rates of nitrification and denitrification were higher than in the HMA sponge *Chondrosia reniformis*, while anaerobic ammonium oxidation and sulfate reduction were below detection in both species. This study shows that LMA sponges may host physiologically similar microbes with comparable or even higher metabolic rates than HMA sponges, and that anaerobic processes such as denitrification can be found both in HMA and LMA sponges. A higher concentration of microorganisms in the mesohyl of HMA compared to LMA sponges may indicate a stronger retention of and, hence, a possible benefit from associated microbes.

IV.3 A Comparative Study on Organic Matter Degradation Characteristics in Sediments of Cold-Water Coral Reef Ecosystems

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Abstract

The degradation state of sedimentary organic matter was investigated at two cold-water coral reefs, Røst Reef and Trænadjupet Reef, on the Norwegian margin and at two cold-water coral mounds, Beta and Gamma Mound, from Pen Duick Mound Province in the Gulf of Cadiz. A set of indicators based on different components of the bulk organic matter pool suggested highly degraded material throughout depth at the mound sites. These indicators included the chlorin index based on chlorophyll degradation products and the amino acid-based Degradation Index developed by DAUWE & MIDDELBURG (1998, Amino acids and hexosamines as indicators of organic matter degradation state in North Sea sediments. *Limnol Oceanogr* 43: 782–798). Concentrations of total hydrolysable amino acids (THAA) at the reef sites were twice as high as at the mound sites but low compared to other ocean margin sites. The relative contribution of amino acids to total organic carbon (%T_{aa}C) in surface sediments ranged from 5–7% at the Norwegian reefs to 3–5% at the mound sites, and the amino acid contribution to total nitrogen (%T_{aa}N) was 17–25% at the reef and ~11% at the mound sediments. We attributed the low percentages of %T_{aa}C and %T_{aa}N to extensive pre-depositional degradation of the organic matter. In conjunction, the coral-bearing sediments were characterized by extremely low mole-% contributions of aspartic acid (<4%) and glutamic acid (<8%) and elevated fractions of the non-protein degradation products β-alanine and γ-amino butyric acid. At the Norwegian cold-water coral reefs, the relative composition of the remaining THAA pool remained largely unchanged with sediment depth, while the cold-water coral mounds exhibited distinct compositional changes with depth indicating selective amino acid degradation and/or uptake. The contribution of amino acid nitrogen from living bacteria to the sedimentary THAA nitrogen yield for Beta Mound revealed that <3.5% of the total amino acid nitrogen pool can be explained by the living bacterial community.

IV.4 Organic Matter Release by Cold-Water Corals and its Implication for Fauna–Microbe Interaction

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Abstract

Particulate (POM) and dissolved organic matter (DOM) released by the cold water corals *Lophelia pertusa* (L.) and *Madrepora oculata* (L.) was collected, analysed and quantitatively compared to that released by warm water reef-building corals. Particulate nitrogen (PN) and particulate organic carbon (POC) release rates of *L. pertusa* were 0.14 ± 0.07 mg N m–2 h–1 and 1.43 ± 1.22 mg C m–2 h–1, respectively, which is in the lower range of POM release rates measured for warm water corals, while dissolved organic carbon (DOC) release was 47 ± 19 mg C m^{-2} h^{-1}. The resulting high DOC:POC ratio indicates that most cold water coral-derived organic matter immediately dissolved in the water column. Cold water corals, similar to their warm water counterparts, produced large amounts of nitrogen-rich coral mucus with C:N ratios of 5 to 7 for *Lophelia*- and 7 to 9 for *Madrepora*-derived mucus. A 7-fold increase in the oxygen consumption rates in cold water coral mucus-amended seawater containing the natural microbial assemblage indicates that this organic matter provided an attractive food source for pelagic microbes. *In situ* investigations at Røst Reef, Norway, showed that microbial activity in the seawater closest to the reef was 10 times higher than in the overlying water column. This suggests that cold water corals can stimulate microbial activity in the direct reef vicinity by the release of easily degradable and nutrient-rich organic matter, which may thereby function as a vector for carbon and nutrient cycling via the microbial loop in cold water coral reef systems.

IV.5 Microbial Degradation of Cold-water Coral-derived Organic Matter: Potential Implication for Organic C Cycling in the Water Column above Tisler Reef

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Abstract

Cold-water corals release organic matter, in particular mucus, but its role in the ecological functioning of reef ecosystems is still poorly understood. The present study investigates the planktonic microbial degradation of mucus released by *Lophelia pertusa* colonies from Tisler Reef, Skagerrak. Results are compared to the degradation of dissolved and particulate organic substrates, including the carbohydrates glucose and starch, as well as gum xanthan and the cyanobacterium *Spirulina* spp. as the model organism for phytoplankton. Resulting microbial organic C degradation rates for the dissolved fraction of *L. pertusa*-derived mucus showed nearly linear progression over time and revealed similar degradation rates compared to glucose and starch. Degradation of the particulate mucus fraction, in contrast, displayed exponential progression and was much faster than degradation of the dissolved fraction. In addition, particulate mucus degradation showed a 4-fold increase compared to that of the added *Spirulina* spp. suspension. Mucus-associated microbial communities apparently play a key role in organic matter recycling, as degradation rates more than doubled in untreated compared to sterile coral-derived mucus over 3 d of incubation. Quantification of O_2 consumption in the water column above Tisler Reef showed significantly increased values in the direct vicinity of the reef. C-stable isotope signatures of suspended particulate organic matter close to Tisler were close to those of *L. pertusa*-derived mucus, and high dissolved organic carbon (DOC) concentrations were detected above Tisler Reef. These findings demonstrate the stimulating effect of cold-water coral reefs on microbial activity in the adjacent water column and may indicate some control over organic C cycling.

IV.6 Fingerabdrücke Mikrobieller Gemeinschaften im Meer

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Abstract

Die weitgehend unbekannten mikrobiellen Gemeinschaften im Meeresboden können durch den Vergleich von molekularen Fingerabdrücken in ihrer Wechselwirkung mit der Umwelt und dem globalen Wandel untersucht werden.

Molecular fingerprinting tools allow an assessment of environmental controls of marine microbial community structure, including those related to global change.

V. APPENDIX

V.1 Expeditions and Fieldwork

Tisler cold-water coral reef, Skagerrak (2008) *MiCROSYSTEMS research day cruise ("RV Lophelia") and fieldwork, May 18–28, Sven Lovén Centre for Marine Sciences, Tjärnö, Sweden*

Aqaba warm-water coral reef, Gulf of Aqaba, Red Sea (2008) *CORE fieldwork, Feb 7–26, Marine Science Station, Aqaba, Jordan*

Røst and Trænadjupet cold-water coral reefs, Norwegian continental margin (2007) *HERMES research cruise ARKXXII/1a ("RV Polarstern"), May 29–June 22, Alfred Wegener Institute for Polar and Marine Research, Bremerhaven, Germany*

Langenuen cold-water coral reefs, West-Norway (2006) *Research cruise ("RV G.O. Sars"), Dec 3–12, University of Bergen, Bergen, Norway*

Langenuen cold-water coral reefs, West-Norway (2006) *Research day cruise ("RV Hans Brattstrøm") and fieldwork, Oct 2–23, University of Bergen, Bergen, Norway*

V.2 Poster and Oral Presentations

SCHÖTTNER S, WILD C, HOFFMANN F, BOETIUS A, RAMETTE A (2010) Bacterial habitat differentiation and interconnectivity – Fundamental insights from cold-water coral reef ecosystems. Oral presentation. *13th International Symposium on Microbial Ecology, August 22–27, 2010, Seattle, USA*

SCHÖTTNER S, PFITZNER B, WILD C, RAMETTE A (2010) Drivers of bacterial diversity in permeable carbonate and silicate reef sands. Poster presentation. *13th International Symposium on Microbial Ecology, August 22–27, 2010, Seattle, USA*

HOFFMANN F, SCHÖTTNER S (2010) Cold-water coral reefs – Hotspots for microbial diversity in the deep sea. Oral presentation. *The Arctic Conference Days, May 31–June 4, Tromsø, Norway*

SCHÖTTNER S, WILD C (2010) Microbial activity and diversity in carbonate and silicate reef sands from the Northern Red Sea. Oral presentation. *BIOSAND Workshop, April 24–May 8, Pianosa/Elba, Italy*

SCHÖTTNER S, WILD C (2009) The interdisciplinary "Tjärnö mucus venture" – exploring the ecological role of cold-water coral-derived organic matter. Oral presentation. *CORE Mini-Symposium, May 4, Munich, Germany*

PFITZNER B, SCHÖTTNER S, RAMETTE A, BOETIUS A, WILD C (2008) Carbonate and silicate reef sands from the northern Red Sea provide different micro-habitats for specific microbial communities. Poster presentation. *11th International Coral Reef Symposium, July –11, Fort Lauderdale, USA*

SCHÖTTNER S, WILD C, RAMETTE A, HOFFMANN F, BOETIUS A (2008) Microbial diversity and activity associated with cold-water coral ecosystems. Oral presemtation. *EGU General Assembly, April 13–18, Vienna, Austria*

SCHÖTTNER S, WILD C, RAMETTE A, HOFFMANN F, BOETIUS A (2008) Habitat differentiation by the cold-water coral *Lophelia pertusa* (Scleractinia) governs bacterial diversity. Poster presentation. *EGU General Assembly, April 13–18, Vienna, Austria*

SCHÖTTNER S, RAMETTE A, HOFFMANN F, WILD C, BOETIUS A (2008) Microbial habitat selection in cold-water coral reef ecosystems. Poster presentation (awarded). *HERMES Annual Meeting, March 31–April 04, Carvoeiro, Portugal*

SCHÖTTNER S, HOFFMANN F, WILD C, BOETIUS A, RAMETTE A (2008) Habitat differentitation by the cold-water coral *Lophelia pertusa* governs bacterial diversity. Oral presentation. *CORE Mini-Symposium, May 2, Munich, Germany*

SCHÖTTNER S, RAMETTE A, HOFFMANN F, WILD C, BOETIUS A (2007) Diversity and Specificity of bacterial communities associated with the cold-water scleractinian *Lophelia pertusa*. Poster presentation. *1st ESF EuroDIVERSITY Conference, October 3–5, Paris, France*

SCHÖTTNER S, WILD C, HOFFMANN F, BOETIUS A, RAMETTE A (2007) Microbial diversity and dynamics in cold-water coral reef habitats. Oral presentation. *CORE Mini-Symposium, May 2, Munich, Germany*

V.3 Courses and Workshops

Summer Course on Microbial Oceanography: Genomes to Biomes (2008) *Agouron Institute & University of Hawai'i, June 2–July 11, Honolulu, Hawai'i, USA*

European-American TRACES Workshop (2008) *HERMES Annual Meeting, March 29–30, Carvoeiro, Portugal*

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MAMA & PAPA

…for all your wonderful **guidance**, **support** and **inspiration**!

STATEMENT OF SOURCES

Declaration

I, SANDRA IRIS SCHÖTTNER, certify that this thesis is my own work and has not been submitted at another university or other institution of tertiary education for the conferral of a degree or diploma. Information derived from published or unpublished scientific work has been cited in the text and listed in the references.