# Influence of Global Change on microbial communities in Arctic sediments

Dissertation zur Erlangung des Doktorgrades der Naturwissenschaften

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**Summary**

Understanding the impacts of global climate change on marine organisms is essential in a warming world in order to predict the future development and functioning of the benthic ecosystem. Only long-term observations allow for the discrimination between natural temporal ecosystem variations and climate change impacts, but few long-term observatories exist worldwide. The Arctic Ocean especially is changing fast, and, at the same time, remains understudied. The Arctic is impacted by warming surface waters and a shrinking sea-ice cover, both influencing primary productivity and subsequent organic matter export to the deep ocean. Furthermore, benthic bacteria that mainly depend on organic matter supply from the surface ocean and that play a major role in carbon cycling at the seafloor, will be affected by these changes. Benthic communities show variations along water depth gradients as organic matter availability changes. However, only little is known about spatial and temporal variations of microbial benthic communities in relation to climate change impacts on pelago-benthic coupling, due to the lack of benthic time-series studies in the Arctic. Therefore, the investigation of Arctic benthic microbial diversity patterns along spatial and water depth gradients and with interannual changes in surface ocean productivity were the major objectives of this thesis. The Long-Term Ecological Research (LTER) site HAUSGARTEN, established in 1999, provides a unique opportunity to study effects of variations in physical properties of the Arctic Ocean, and their impacts on organic matter export and deep-sea benthic communities.

In **Chapter I**, bacterial community composition and patterns along spatial gradients such as water depth and distance were explored in HAUSGARTEN sediments. This revealed a very diverse bacterial community comparable to other Arctic sediments and high numbers of unique bacterial types on spatial scales of few kilometers. Strong impacts of changes in the quantity of organic matter supply with water depth were encountered for the whole bacterial community and specific bacterial taxa changing with water depth differences were identified.

Results presented in **Chapter II** show that the bacterial community reacts rapidly (within the same year) to changes in interannual variations of organic matter supply from surface waters. A strong decrease of bacterial richness and shift in bacterial community structure was encountered with decreases in organic matter availability, yet individual bacterial taxa responded differently to such variations.

The influence of a decrease or even absence of organic matter deposition on sediments and its impacts on benthic bacterial community structure and functioning were studied over three years by an *in situ* experimental approach (**Chapter III**). It revealed that deep-sea benthic bacterial communities are stable over a short time period of one year when fresh organic matter is absent, but when starved for a longer time period, richness, structure and potential enzymatic activity for the degradation of organic matter are substantially altered.

Benthic eukaryotes were investigated along a water depth gradient and in relation to temporal changes in upper ocean processes in **Chapter IV**. A strong decrease in richness of eukaryotic taxa with increasing water depth, especially below 3000 m water depth, and a decrease in eukaryotic richness and change in community composition with a decrease in upper ocean productivity were observed.

The results of this thesis give unique insights into temporal variations of Arctic microbial benthic communities along a large gradient of water depth and in relation to upper ocean productivity and thus help to predict Arctic benthic ecosystem responses in a future Arctic impacted by climate change.

**Zusammenfassung**

Den Einfluss des Klimawandels auf marine Organismen zu verstehen ist essentiell in einer sich erwärmenden Welt, um die zukünftige Entwicklung und Funktionsweise des benthischen Ökosystems vorhersagen zu können. Es ist nur mit Hilfe von Langzeit-Beobachtungen möglich, zwischen natürlichen zeitlichen Schwankungen des Ökosystems und tatsächlichen Folgen des Klimawandels zu unterscheiden, dennoch existieren weltweit nur wenige Langzeit-Observatorien. Besonders schnelle Veränderungen sind im Arktischen Ozean zu beobachten, trotzdem blieb er bisher relativ unerforscht. Die Arktis wird sowohl von sich erwärmendem Oberflächenwasser, als auch von der zurückgehenden Meereisbedeckung beeinflusst und beides hat Auswirkungen auf die Primärproduktion und den damit verbundenen Export von organischem Material in die Tiefsee. Diese Veränderungen werden auch Folgen für benthische Bakterien haben, die größtenteils auf den Eintrag organischen Materials aus den oberen Wasserschichten angewiesen sind und eine wichtige Rolle im Kohlenstoffkreislauf am Meeresgrund spielen. Benthische Gemeinschaften verändern sich entlang von Wassertiefe-Gradienten, da sich auch die Verfügbarkeit des organischen Materials verändert. Es ist jedoch bislang nur wenig über die räumliche und zeitliche Variation von mikrobiellen benthischen Gemeinschaften im Zusammenhang mit den Auswirkungen des Klimawandels auf pelagisch-benthische Wechselwirkungen bekannt, vor allem aufgrund fehlender Zeitreihen-Untersuchungen in der Arktis. Die Hauptziele dieser Arbeit waren daher die Untersuchung von Diversitätsmustern benthischer mikrobieller Gemeinschaften in der Arktis, sowohl entlang räumlicher Gradienten, als auch entlang von Wassertiefe-Gradienten und zwischenjährlichen Schwankungen der Produktivität im Oberflächenwasser. Das Langzeit-Observatorium HAUSGARTEN, gegründet im Jahr 1999, gibt die einzigartige Möglichkeit die Folgen physikalischer Veränderungen im Arktischen Ozean zu untersuchen, sowie deren Auswirkungen auf den Export organischen Materials und auf die benthischen Tiefsee-Gemeinschaften.

In **Kapitel I** dieser Arbeit wurden Zusammensetzung und Muster bakterieller Gemeinschaften entlang räumlicher Gradienten, wie Wassertiefe und Entfernung, in HAUSGARTEN Sedimenten untersucht. Die Ergebnisse zeigen eine sehr diverse bakterielle Gemeinschaft, vergleichbar mit anderen arktischen Sedimenten, und eine hohe Anzahl einzigartiger bakterieller Typen innerhalb einer räumlichen Reichweite von wenigen Kilometern. Veränderungen der verfügbaren Menge an organischem Material mit der Wassertiefe hatten

starken Einfluss auf die gesamte bakterielle Gemeinschaft und es konnten spezifische bakterielle Taxa identifiziert werden, die sich mit Unterschieden in der Wassertiefe veränderten.

Die Ergebnisse in **Kapitel II** zeigten, dass die bakterielle Gemeinschaft schnell (innerhalb desselben Jahres) auf zwischenjährliche Veränderungen des Eintrags von organischem Material aus Oberflächenwasser reagiert. Wenn weniger organisches Material verfügbar war, konnte ein starker Rückgang der bakteriellen Vielfalt, sowie eine Veränderung der Struktur der bakteriellen Gemeinschaft beobachtet werden, individuelle bakterielle Taxa zeigten jedoch unterschiedliche Reaktionen auf die Veränderungen.

Der Einfluss von abnehmender oder sogar nicht vorhandener Ablagerung organischen Materials am Meeresboden und die damit verbundenen Auswirkungen auf die Struktur und Funktionsweise bakterieller Gemeinschaften wurden über einen Zeitraum von drei Jahren in einem *in situ* Experiment untersucht (**Kapitel III**). Es zeigte sich, dass benthische bakterielle Gemeinschaften in der Tiefsee unter Mangel von frischem organischem Material über den kurzen Zeitraum von einem Jahr stabil waren. Hungerten die Gemeinschaften jedoch für einen längeren Zeitraum, traten wesentliche Veränderungen der Vielfalt, Struktur und der potentiellen enzymatischen Aktivität für den Abbau von organischem Material auf.

In **Kapitel IV** wurden benthische Eukaryoten entlang eines Wassertiefe-Gradienten und in Zusammenhang mit zeitlicher Veränderung von Prozessen im oberen Ozean untersucht. Während mit zunehmender Wassertiefe, insbesondere unterhalb von 3000 m, ein starker Rückgang der Vielfalt eukaryotischer Taxa beobachtet wurde, zeigte sich mit abnehmender Produktivität im oberen Ozean neben einer Verringerung der Vielfalt auch eine Veränderung der Zusammensetzung der Gemeinschaft.

Die Ergebnisse dieser Arbeit geben erstmalige Einblicke in die zeitlichen Variationen von mikrobiellen benthischen Gemeinschaften in der Arktis entlang eines großen Wassertiefe-Gradienten und im Zusammenhang mit der Produktivität des oberen Ozeans. Sie helfen daher, Reaktionen des benthischen Ökosystems in der Arktis auf zukünftige Auswirkungen des Klimawandels vorherzusagen.

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# 1. Introduction

## 1.1. Global climate change

Global climate change has progressed rapidly in the last decades, manifesting in increasing air and water temperatures, sea-level rise and a decrease in snow and ice cover. All of these variables are interconnected and to a large extent driven by increasing atmospheric $CO_2$ levels (Myhre et al., 2013). Since the 1980s, steady increases of 0.254°C per decade have been recorded. Such increases between two consecutive decades have not been observed before, and furthermore the ten warmest years so far recorded have occurred since 1997 (Hartmann et al., 2013; Figure 1). At the same time ocean surface water temperatures have increased, resulting in rising ocean heat content (Rhein et al, 2013; Figure 2). This warming is most pronounced in the surface ocean, but also observable in the deep sea below 2000 m water depth (Rhein et al, 2013; Somavilla et al., 2013).

Current investigations aim to evaluate how increasing temperatures, and other climate change-related parameters (e.g. ocean acidification), affect marine ecosystems (e.g. reviewed by Hoegh-Guldberg and Bruno, 2010; Chavez et al., 2011; Doney et al., 2012). Impacts of climate change on the abundance, biomass and diversity of a variety of organisms have been observed with successional changes in species compositions over a decade and poleward shifts in spatial ranges of certain populations (see Doney et al., 2012 and references therein; Dornelas et al., 2014). Climate change in the Arctic is even more pronounced than the global average (e.g. Graversen et al., 2008), but only little is known about temporal natural Arctic ecosystem changes or impacts of climate change.

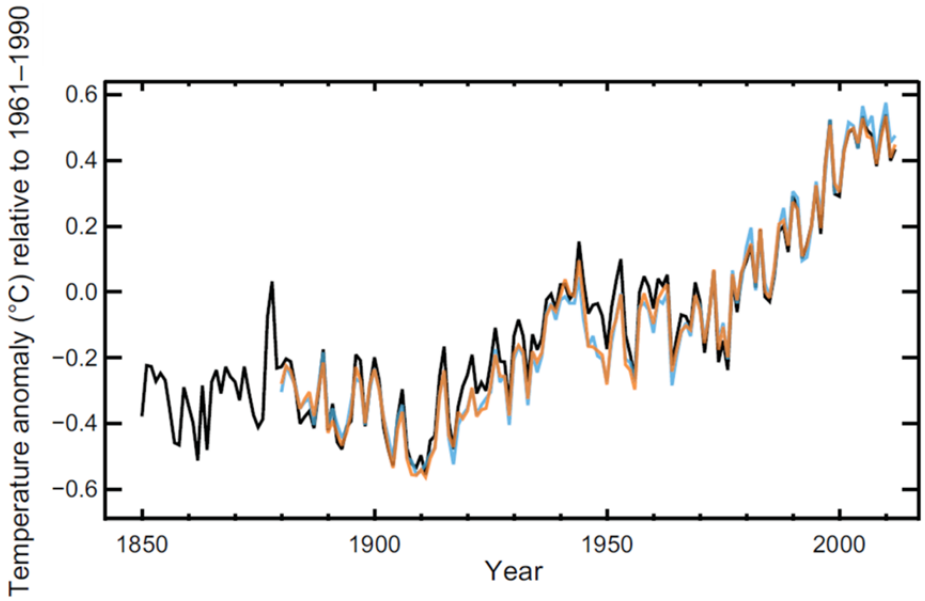


**Figure 1** Average global annual air temperature anomaly relative to 1961 – 1990. Data derived from different datasets as indicated by the different colors. Adapted from IPCC (2013).

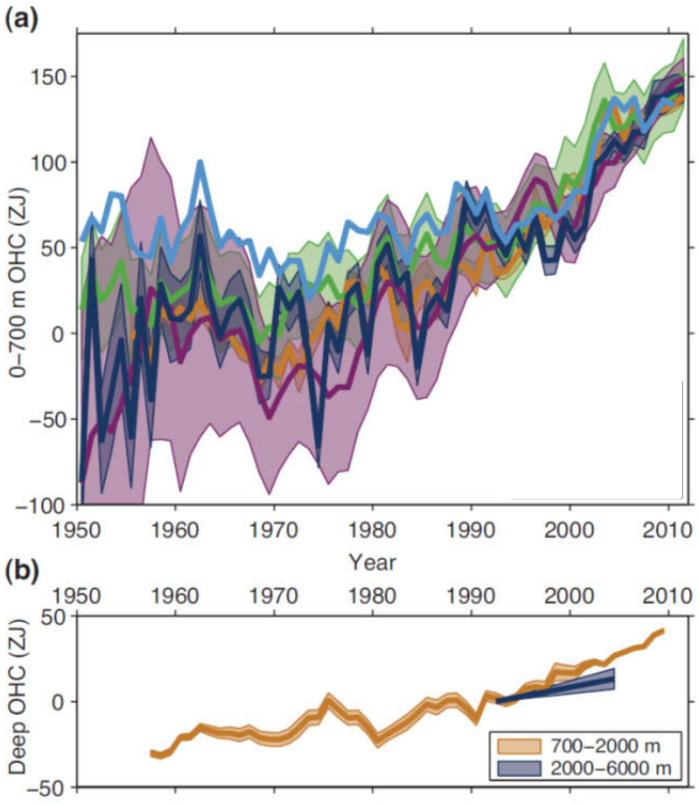


**Figure 2** (**a**) Observation-based estimates of average annual global heat content of the upper ocean (0 to 700 me depth) and uncertainties from different studies as indicated by color. (**b**) Observation-based estimates of average annual global heat content of the deeper ocean and standard deviations. Values are given in ZJ = $10^{21}$ Joules. For more detail see IPCC (2013).

## 1.2. The Arctic Ocean under changing conditions

The Arctic is one the most remote areas on Earth. Yet it is of high relevance, as it is rapidly changing due to climate change. Arctic air temperature is now roughly 2°C warmer than the average Arctic air temperature since 1900 (Polyakov et al., 2013; Figure 3). With the strongest increases measured since 1981 - at a rate of 0.63°C per decade (Comiso, 2010). Similar to air temperatures, Arctic surface water temperatures increased, most distinctly since the 1980s, by approximately 1.5 °C (Polyakov et al., 2013). Increased air and water temperatures have led to a decrease in summer sea-ice extent since satellite observations began in the 1970s, presently at a rate of more than 10% per decade (Comiso, 2010; Figure 6). It has been suggested that Arctic summer sea-ice extent will be reduced by 43% or more by the end of the 21st century (Collins et al., 2013). In combination with the general loss of sea-ice, changes towards younger and thus thinner sea-ice have been recorded in recent years (Maslanick et al., 2011). The rate at which multiyear ice decreases is higher than for perennial ice (Vaughan et al., 2013; Figure 4), which builds up every winter and melts in summer. Yet, multiyear sea-ice is important for cold- and ice-adapted species as it is more stable throughout the year and its decrease will most likely alter food web structure (Hop et al., 2006).

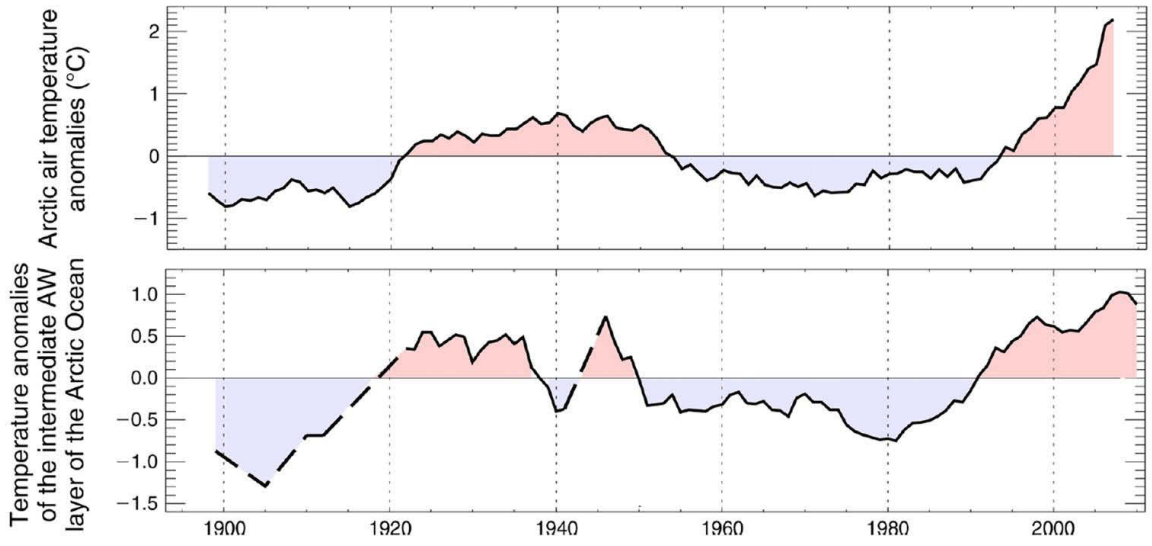


**Figure 3** Time-series of 7-year running mean temperature anomalies of surface air and water temperatures in the Arctic. Adapted from Polyakov et al. ( 2013).

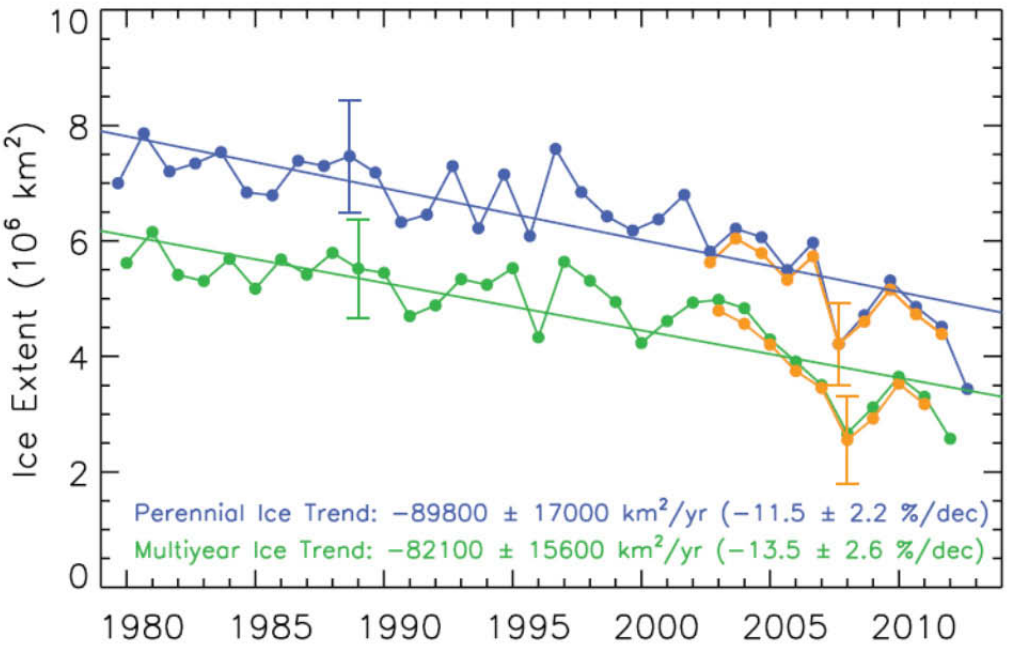


**Figure 4** Satellite derived annual Arctic sea-ice extent of perennial (blue) and multiyear (green). Values for perennial sea-ice derived from summer minimum extent and multiyear sea-ice are averages from winter extent. Gold line indicates data another dataset available since 2002. For more detail see Vaughan et al. (2013).

Changes in surface ocean conditions probably impact primary production and organic matter export. A typical Arctic food web is illustrated in Figure 6. Sea-ice is highly important for the Arctic ecosystem as its melting in spring directly impacts the onset of phytoplankton blooms (Hoegh-Guldberg and Bruno, 2010; Ji et al., 2013). Thus, strongest temporal anomalies in primary productivity were found along the ice edge (Wassmann et al., 2010). Some studies imply that primary productivity increases with the loss of sea-ice, since larger areas are exposed to sunlight (e.g. Arrigo et al., 2008; Slagstad et al, 2011), while other studies infer no effect or a decrease in primary productivity (Grebmeier et al., 2010). Overall, different scenarios are expected regarding different areas of the Arctic Ocean, depending on temperature, salinity and changes by freshwater input due to melting sea-ice and nutrient availability (Slagstad et al., 2011; Tremblay and Gagnon, 2009). The overall timing of phytoplankton blooms in the Arctic has shifted towards an earlier onset, but this varies for different regions (Kahru et al., 2011). Additionally, a shift towards smaller phytoplankton species was observed (Li et al., 2009) and sub-Arctic species appear to migrate into the Arctic Ocean (Drinkwater, 2011), potentially altering food web structures and ecosystem functioning (Weslawski et al., 2009). Changes in Arctic primary productivity and community composition

will impact the flux of organic matter to the deep ocean, where organisms depend on organic matter export from the surface (Grebmeier and Barry, 1991; Klages et al., 2004; Grebmeier, 2012). Since it is not yet clear how primary productivity will change in the future, it is also not clear whether there will be an increase or a decrease in organic matter export from surface waters (see Arrigo et al., 2008; Vancoppenolle et al., 2013). However, quantity and quality of organic matter due to changes in primary productivity and composition of primary producers respectively, is likely to change (e.g Bauerfeind et al., 2014; Lalande et al., 2013). Despite some uncertainties, climate change in the Arctic will consequently affect all parts of the marine community, from primary producers to detritus feeders (Wassmann, 2011).

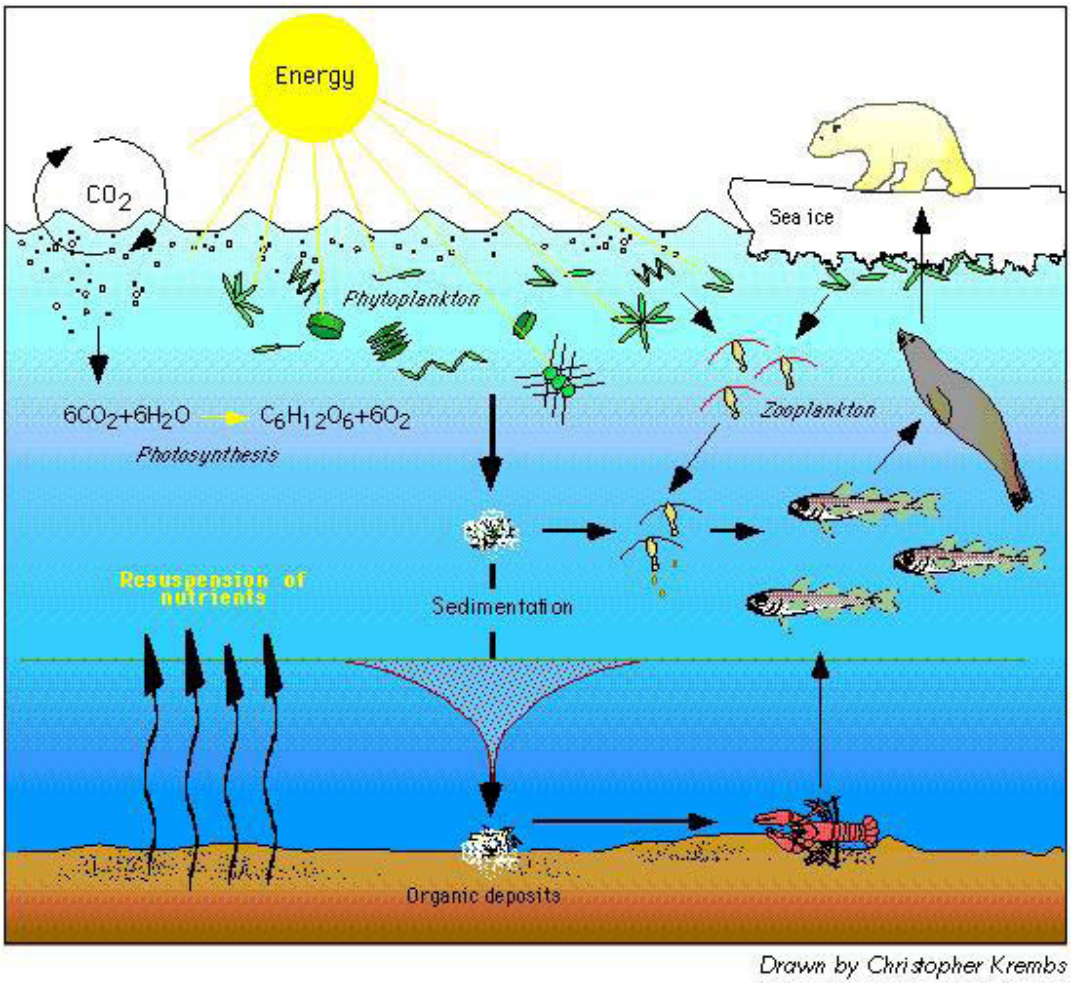


**Figure 6** Illustration of an Arctic food web. When sunlight is available during summer and sea-ice starts melting phytoplankton blooms form close to the ice edge, eventually sinking down to the sea floor where they serve as organic matter input to the benthos. Figure taken from www.sams.ac.uk.

Impacts of climate change on Arctic benthic organisms have been reported, despite a limited number of studies and the lack of a comprehensive baseline (see Wassmann et al., 2011). Elevated export of algal particles to the seafloor that are rapidly utilized by mobile megafauna, resulting in increased biomass, have been reported as a consequence of surface ocean warming and sea-ice retreat (Kortsch et al., 2012; Boetius et al., 2013). Over longer time periods however bottom water temperatures may continue to increase in the shallow and deep Arctic Ocean, therefore in combination with a decrease in organic matter input, the benthic macro- and megafaunal biomass and densities would eventually decrease (Soltwedel et al., 2005; Grebmeier et al., 2006; Bergmann et al., 2011). So far, most of the studies investigating Arctic benthic community response to climate change focus on larger organisms (Wassmann et al., 2011) and little is known on the response of e.g. microbial communities. Effects of climate change will affect the entire ecosystem, including e.g. competition and predation as well as food web structure. Therefore, studies on climate change impacts should include all faunal size classes (see Glover et al., 2010), especially microbial communities, as they are drivers of carbon cycling in the deep sea (van Oevelen et al., 2011).

Global climate change is predicted to continue over the coming years and decades (Collins et al., 2013). Changes will lead to a continued warming of land and ocean masses which will affect geochemical processes as well as biological communities. A major task will be to identify alterations within communities in response to these environmental changes and delineate them from natural variations. Long-term observations of ecosystems in strategically relevant areas are crucial to understand causes and effects of temporal variations in ecosystems. A better understanding of the natural systems will allow for improved future predictions under different climate scenarios.

## 1.3. Ecological open ocean long-term observations to investigate effects of climate change

Long-term observations are indispensable for studying the effects of global environmental changes on natural ecosystems, but are cost-, time and labor- intensive tasks. Before ecosystem changes can be attributed to climate change, natural temporal variations of marine communities need to be assessed, as they may enhance or weaken trends and thus complicate the interpretation of results (Magurran et al, 2010). Marine communities can exhibit seasonal, interannual and even decadal natural variations (e.g. Fuhrman et al., 2006; Ruhl et al., 2008; Gilbert et al., 2012). Also variations caused by variations in physical properties of water masses, e.g. El Niño-Southern Oscillation, North Atlantic Oscillation and Pacific Decadal Oscillation were recently observed in longer time-series studies (e.g. Ruhl and Smith, 2004; Smith et al., 2006; Chavez et al, 2011; Henson et al., 2012; Taylor et al., 2012) resulting in altered abundance, biomass and community composition. The ability to observe these trends is highly dependent on the time scale and temporal resolution of the datasets (Edwards et al., 2010). Therefore, multi-decadal time series are needed in order to evaluate whether variations in marine communities are related to climate change or reflect natural variations (e.g. Edwards et al., 2010; Glover et al., 2010; Wassmann, 2011; Doney et al., 2012). Such time-series should include measurements of physical, chemical and biological parameters in order to evaluate ecosystems in their environmental context.

Many physical and chemical oceanographic parameters can now be determined with automated systems, but changes in marine ecosystems can only be measured by time- and cost- intensive field campaigns. Therefore open ocean ecological time series are rare. The two longest ecological time-series are the Continuous Plankton Recorder (CPR; e.g. Richardson and Schoeman, 2004; for more information see www.sahfos.ac.uk) established in 1931 in the north Atlantic and the California Cooperative Oceanic Fisheries Investigations (CalCOFI; Roemmich and McGowan, 1995; for more information see www.calcofi.org) in the north Pacific established in 1949, both monitoring plankton communities over large spatial scales several times a year. While neither were established to investigate effects of climate change, they have become crucial for the study of long-term ecological responses of marine communities (Edwards et al., 2010). Other time-series study sites have been established, but

were not maintained over long time scales (e.g. over a decade or more) mostly due to the lack of funding (Edwards et al., 2010). Further, the few long-term ecological ocean sites that exist show spatial and temporal gaps. The deep-sea benthos is a large area where vast amounts of nutrient cycling and carbon turnover take place, deep-sea benthic ecological time series studies are however rare and even more constrained by spatial and temporal gaps (Glover et al., 2010). Nevertheless, continuous efforts were and are made in order to monitor variations in open oceans from surface to deep water in various areas including polar regions, e.g. by the OceanSITES network (see www.oceansites.org, Figure 7).

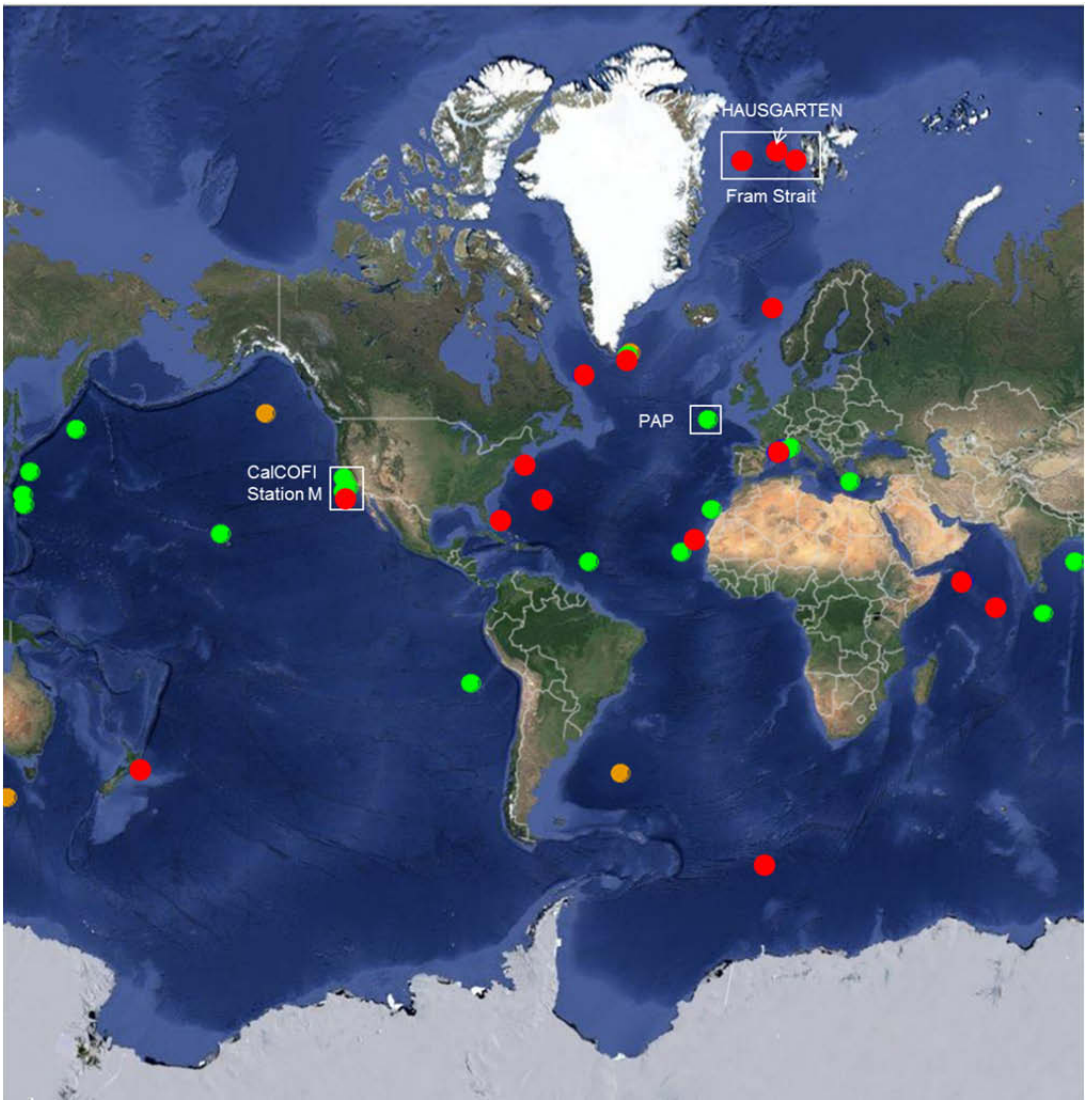


**Figure 7** Map of time-series sites investigating biological parameters and that are part of the OceanSITES network. Sites mentioned in this thesis are indicated by white rectangles. Green dots indicate the availability of real-time data on physical ocean properties, red dots indicate stations with delayed data availability. Orange dots mark stations that are currently planned. Map was created via www.oceansites.org.

### *1.3.1. Insights from deep-sea benthic ecological time-series studies*

The deep-sea is the largest ecosystem on earth, yet it is largely understudied. Despite some small energy rich hot spots, e.g. hydrothermal vents, the deep seafloor is mainly comprised of well oxygenated sediments down to several centimeters due to low organic matter availability (Jørgensen and Boetius, 2007). As no light penetrates to the deep sea, benthic organisms are ultimately dependent on organic matter supply from the euphotic zone. By far the largest fraction of phytoplankton biomass is however recycled within the surface ocean and only a small fraction (~ 1%) reaches the deep sea (Jahnke and Jackson, 1992). In terms of biomass, the deep-sea benthos is dominated by bacteria, followed by meio-, macro-, and megafauna (Wei et al., 2010, Figure 8). As a result of the remoteness of the deep-sea ecosystem, our knowledge on temporal processes that influence benthic deep-sea communities and on successional patterns is very limited (see Glover et al., 2010). In order to understand and predict variations in ecosystem structure and functioning on seasonal to decadal time scales and in relation to climate change, benthic time-series studies are crucial.

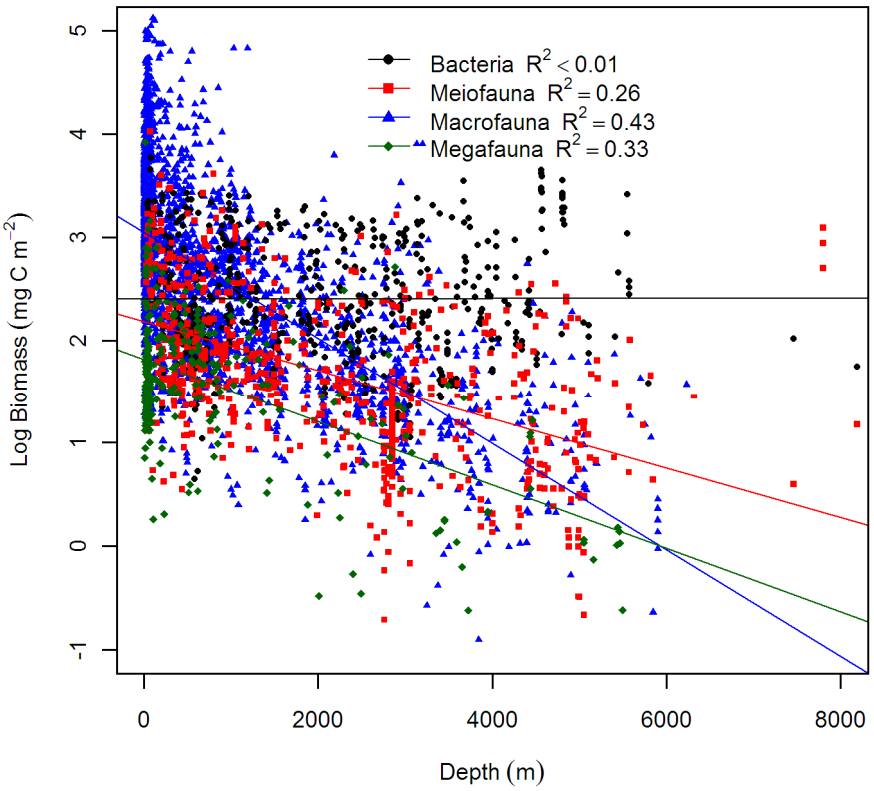


**Figure 8** Benthic biomass as a function of water depth. Bacterial biomass is constant while all faunal size classes decrease with increasing water depth. Adapted from Wei et al. (2011).

The first deep-sea benthic images were taken in the 1960s, while the first time-series studies began in the 1970s. In a recent review on temporal change in deep-sea benthic ecosystems by Glover at al. (2010), only 11 sites worldwide were identified where temporal variations had been investigated over several years. Only two of those sites were categorized as long-term studies, one is located in the Northeast Pacific Ocean (Station M, ~4100 m depth, since 1989, e.g. Ruhl et al., 2008; Smith et al., 2013) and the other one in the North Atlantic (PAP, ~4850 m depth, since 1989, e.g. Billett et al. 2001; Lampitt et al., 2001; Gooday et al., 2010). Benthic time-series studies at Station M revealed that altered organic matter export due to oscillation processes in the water column have an effect on phytodetritus supply to the benthos (Smith et al, 2006; Smith et al 2008). Higher organic matter supply generally resulted in an increase of total benthic remineralization rates (Ruhl et al., 2008; Smith et al., 2013). Moreover, abundance and biomass of meio-, macro- and megafaunal community increase and their composition is altered with a time lag of only weeks to several months at Station M and PAP (e.g. Ruhl et al., 2004; Ruhl et al., 2008) consistent with shorter studies from other oceanic regions (Billet et al., 2001; Danovaro et al., 2004; Bergmann et al., 2011; Grebmeier 2012; Meyer et al., 2013, Ramalho et al., 2014). Contrary to long-standing assumptions that the deep sea is a stable environment, we now know that it is instead highly dynamic and comprises a high biodiversity that is influenced by changes in surface ocean conditions (e.g. Glover et al., 2010; Danovaro et al., 2004). Most of the current studies however investigated only larger faunal size classes, leaving the smallest but most abundant benthic component, the microbial communities, understudied.

### *1.3.2. Temporal variations of bacterial communities*

Bacteria are the most abundant organisms in oligotrophic deep-sea sediments and make up the major fraction of benthic biomass (Wei et al., 2010). Benthic bacteria significantly contribute to the initial step of sinking organic matter degradation, making it available for larger benthic fauna (reviewed in Orcutt et al., 2011). Bacteria can react rapidly to pulses of organic matter supply by increased carbon uptake and changes in hydrolytic enzyme activity (Moodley et al., 2002; Witte et al., 2003) Thus, bacteria are important in the burial and remineralization of carbon reaching the deep sea (e.g. Rowe and Deming 1985). Nevertheless, investigations of total benthic bacterial community patters and function on seasonal or interannual time scales are limited.

Pelagic time series studies revealed that bacterial communities exhibit strong seasonal and annual patterns, depending on day length, water temperature and nutrient availability (Fuhrman et al., 2006; Gilbert et al., 2012). Due to the vast amounts of bacteria, these shifts probably occurred in relative abundances of certain bacterial taxa, rather than by extinction and recolonization of taxa (Caporaso et al., 2012). An overall decrease in community similarity in monthly obtained bacterioplankton samples over a time span of 10 years was observed with strong seasonal signals (Chow et al., 2013). Benthic microbial communities in coastal sediments were found to show temporal variations related to variations in primary productivity, yet without the reoccurring patterns observed in surface waters (Böer et al., 2009; Gobet et al., 2012). However, these studies were limited in length, covering periods of < 2 years, which may have been too short to detect typical seasonal or interannual patterns. Changes in organic matter supply over a four-year period were also shown to influence and alter bacterial community structure in abyssal surface sediments, despite maintenance of the major fraction of bacterial phylotypes (Moeseneder et al., 2012). This study however lacked in-depth analysis of the less abundant bacterial types and taxonomic information of shifting bacterial types.

Benthic bacteria are important drivers of carbon cycling in deep-sea sediment. They probably exhibit seasonal community variations as observed for pelagic bacterial communities and are impacted by changing organic matter supply as observed for larger organisms. Yet, benthic microbial community patterns remain largely understudied, especially in the rapidly changing Arctic Ocean. Therefore, this thesis aims to provide first insights into spatial and temporal patterns of bacterial communities in relation to changes in surface ocean conditions.

## 1.4. Long-term ecological research site HAUSGARTEN

Fram Strait is one of the key areas regarding investigations of changes in the Arctic Ocean (e.g. Wassmann, 2011). It is located between Svalbard and Greenland and is the gateway for most of the inflow and outflow of water masses to and from the Arctic Ocean (Manley, 1995; Hop et al., 2006; Figure 8). On the western side of Fram Strait, cold polar waters exit the Arctic Ocean at depth and sea-ice is transported out of the Arctic. In the eastern Fram Strait, warm Atlantic water masses are transported into the Arctic and supply the Arctic Ocean with the largest input of water and heat (Polyakov et al., 2011). Pronounced events of enhanced heat transported with Atlantic water masses were observed during the last decades (Piechura and Walczowski, 2009; Beszczynska-Möller et al., 2012) and with a delay of a few years, these events became evident in all other Arctic Ocean basins (Polyakov et al., 2011; Polyakov et al., 2013). The eastern Fram Strait is thus an early indicator for variations in surface ocean conditions and is a well suited area to study Arctic ecosystem variations due to global climate change at an early stage.

The long-term ecological research site HAUSGRARTEN was established in the Fram Strait in 1999 and is the only deep-sea time series site in the Arctic Ocean (Soltwedel et al., 2005, Figure 8). Initially, HAUSGARTEN included 15 permanent sampling stations. Due to the sea-ice retreat in recent years and thus accessibility of sampling sites further north, two additional stations further north were included. Sampling stations are located along two transects, one from East to West covering water depth of ~1000 m to 5500 m, the other one along a South-North transect at ~2500 m water depth including open water and ice-covered sites. The composition and density of all faunal size classes are investigated annually, in combination with biogeochemical measurements, e.g. organic carbon, phytopigment concentrations and carbon remineralization rates. In addition, physical oceanographic properties of surface waters, such as temperature, salinity and current velocities are recorded, in order to link pelagic and benthic processes. Export and composition of organic matter from the surface ocean to the deep sea are measured with sediment traps positioned in the upper and deeper water column.

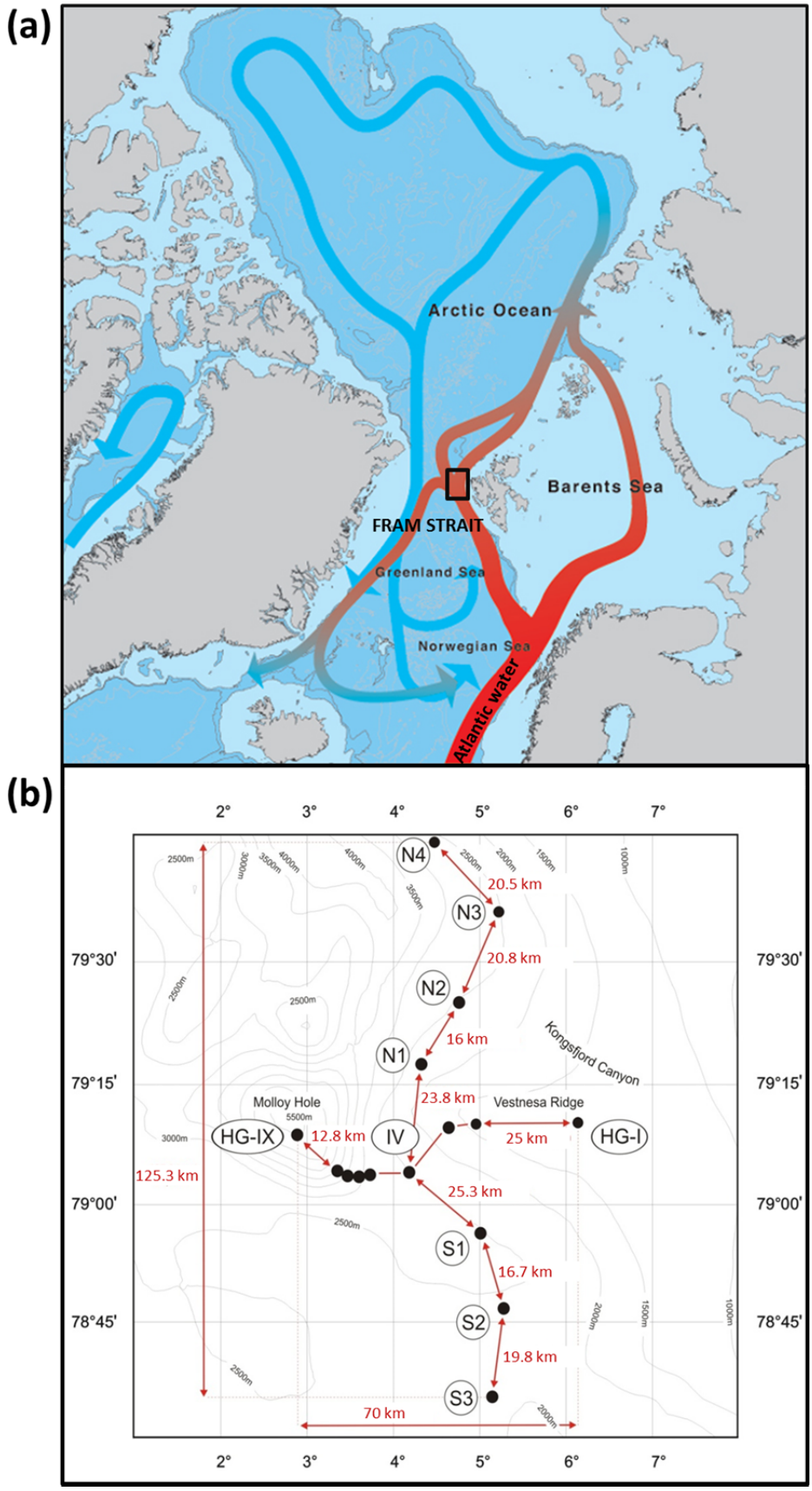


**Figure 9** Arctic warm surface Atlantic (red) and cold deep Polar (blue) water masses and location of LTER site HAUSGARTEN (a, black rectangle), and sampling network at HAUSGARTEN (b). Map with Arctic currents adapted from www.arcticsystem.no.

During the last 15 years of investigations at HAUSGARTEN, strong variations in physical and biological variables were observed. In 1999 - 2000 and 2005 – 2007 the Atlantic water masses reached further north than usual, resulting in warmer surface waters in the HAUSGARTEN region (Piechura and Walczowski, 2009; Walczowski et al., 2012; Beszcynska-Möller et al., 2012), causing so-called warm anomalies. Although primary productivity has steadily increased in the wider HAUSGARTEN area since 1998, with highest values in April-August (Cherkasheva et al., 2014), primary productivity and the export of organic matter decreased during the second warm anomaly (Lalande et al., 2013). Additionally, the community composition of surface waters changed from a diatom-dominated system to a coccolithophorid-dominated system (Bauerfeind et al., 2009). At the same time an increase in the proportion of Atlantic amphipod species relative to polar species was observed (Kraft et al., 2011), indicating a shift in species composition. The decrease in organic matter export from surface waters was reflected in the deep sea, where a lower input of phytodetritus was measured, and decreases in microbial biomass and megafaunal densities as well as changes in megafaunal composition were reported (Bergmann et al., 2011; Meyer et al., 2013). Despite the major role of microbial communities in organic matter remineralization in the deep sea benthos, nothing is known about variations of microbial community composition or patterns in response to variations in surface ocean characteristics at HAUSGARTEN.

## 1.5. Objectives

Benthic microbial communities depend on organic matter supply from the productive surface ocean and are able to rapidly react to the input of fresh organic matter. In the Arctic, where primary production only occurs when sunlight is available, the strongest pulse of organic matter usually reaches the seafloor in spring. With ongoing physical changes in the surface Arctic Ocean, i.e. warming and decreasing sea-ice extent, the location, quantity and quality of phytoplankton primary production will likely change and result in an altered organic matter flux to the deep sea. Little is known about how this will influence communities at the seafloor. Only few studies exist that investigate total bacterial or eukaryotic communities in Arctic sediments, and they are limited either in spatial or temporal resolution. It is however of high relevance to get a better insight into the factors shaping Arctic deep-sea benthic microbial communities, in order to establish well suited monitoring programs and help predict future benthic changes in relation to climate change.

Therefore the aim of this thesis was to improve our understanding of spatial and temporal variations of both bacterial and eukaryotic communities at the HAUSGARTEN site, and to determine how these are influenced by changing organic matter supply from the surface ocean. More specifically, the purpose was to investigate (i) whether spatial or temporal variations are more pronounced, (ii) how natural variations in organic matter supply affect the benthic bacterial community and (iii) if total benthic eukaryotic communities are shaped by similar environmental variations when compared to bacterial communities.

In order to answer the questions raised above, the following objectives led to the studies presented in the following thesis chapters:

1) Investigation of spatial variations in benthic bacterial diversity in relation to natural gradients in organic matter supply, along a water depth gradient and differences in the position of the ice edge. (Chapter I)

2) Examination of how and on which time scales benthic bacterial communities respond to natural inter-annual changes in organic matter supply. (Chapter II)

3) Identification of abundant and rare bacterial types that are specifically affected by spatial or temporal variations in organic matter supply. (Chapters I and II)

4) Determination of long-term bacterial community responses to the absence of fresh organic matter input. (Chapter III)

5) Exploration of spatial and temporal patterns in the total benthic eukaryotic community in relation to changes in organic matter supply. (Chapter IV)

## 1.6. Methods for microbial community structure determination

Microbial community structure is nowadays usually determined by sequencing parts of the genes encoding ribosomal RNA. Ribosomal genes are ubiquitously found in all organisms and are assumed to not be influenced by horizontal gene transfer, making them well-suited molecular markers (Woese, 1987). Ribosomes consist of a small and a larger subunit, which differ in bacteria and eukaryotes in terms of nucleotide combinations and sequence lengths. Especially for bacteria, sequencing of the 16S rRNA gene, encoding the small ribosomal subunit, became the method of choice for phylogenetic analyses resulting in the discovery of bacterial groups that could not be detected by traditional culturing approaches (Hugenholtz et al., 1998). While some microbial eukaryotes can be microscopically distinguished, sequencing also became a popular method for determining the small unicellular fraction (protists) of eukaryotic communities (see Bik et al., 2012) and recently even total community analyses (e.g. Pawlowski et al., 2011).

The two methods used to analyze microbial community composition and structure in this thesis are automated ribosomal intergenic spacer analysis (ARISA) and massively parallel tag sequencing (MPTS). ARISA is assumed to target more abundant bacteria in an environmental sample, while MPTS allows for the detection abundant and rare bacteria. Yet, both methods were shown to produce coherent diversity patterns over temporal or spatial scales (e.g. Gobet et al., 2013; Jacob et al., 2013). Thus, ARISA can be used as a starting point for the analysis of large datasets, from which a subset of samples is then selected for more thorough analysis using MPTS (Gobet et al., 2013).

### *1.6.1. Automated ribosomal intergenic spacer analysis (ARISA)*

Automated ribosomal intergenic spacer analysis (ARISA), was introduced by Triplett and Fisher in 1999 as a rapid and effective method to investigate natural bacterial communities and is a frequently used method to determine bacterial community variation in space and time (e.g. Bienhold et al., 2012; Chow et al., 2013).

After environmental DNA is extracted, a PCR is conducted with primers amplifying the variable region between the small and large subunit of the ribosomal rRNA gene. One of

these primers is labelled with a fluorescent dye for later detection of the PCR amplicon. After amplification, DNA sequences with up to 1200 base pairs are present, which can be discriminated by capillary electrophoresis. In an electropherogram, the different lengths of amplicons and fluorescent intensity of the dye can be visualized. A schematic of the ARISA workflow is presented in Figure 9. The electropherograms are the basis of the calculation of a so called "fingerprint" of the community in a given sample. A peak in an electropherogram represents one operational taxonomic unit (OTU) and the fluorescence intensity is used to calculate the relative abundance of each OTU. This can achieved with cleaning and binning procedures to obtain robust and reliable data (see Ramette, 2009 for detail).

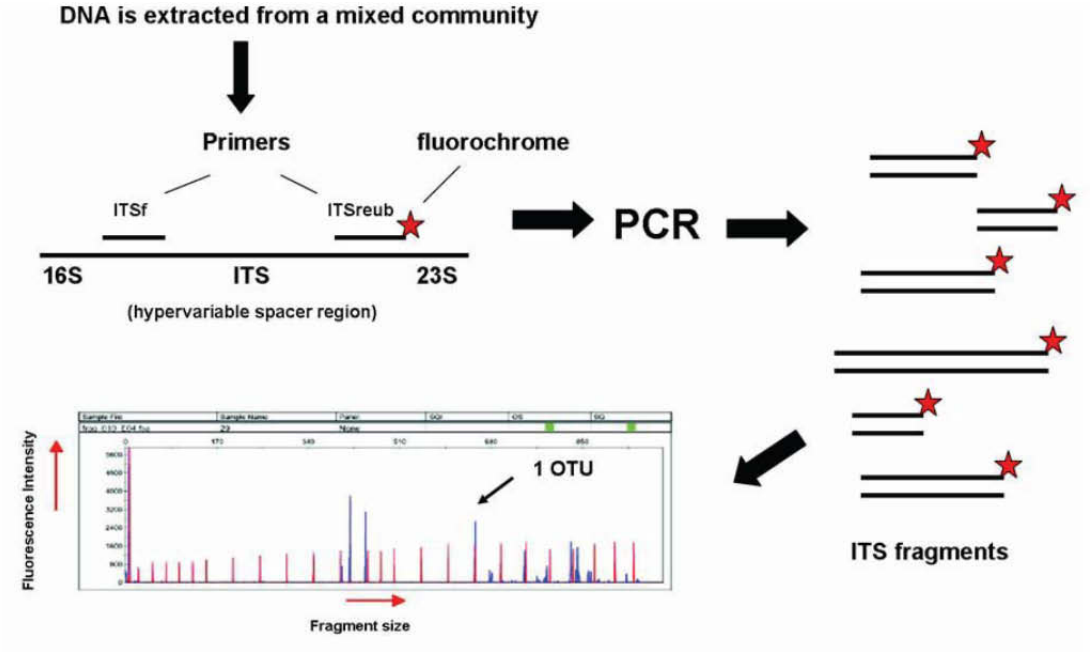


**Figure 9** ARISA workflow for bacterial community analysis. After extraction of total environmental DNA from samples, the intergenic spacer region (ITS) is amplified with a fluorescently labelled primer. The produced amplicons of different length are separated by capillary electrophoresis and can be visualized as different peaks in an electropherogram. Sketch adapted from Böer, 2008.

### *1.6.2. Massively parallel tag sequencing (MPTS)*

Massively parallel tag sequencing for the analysis of environmental microbial DNA was introduced by Sogin et al. in 2006. Although rather expensive in the beginning, this is a method which can quickly describe the large diversity in microbial communities.

After DNA extraction, a variable region in the small subunit of the rRNA genes is amplified and ligated with specific adapters that can immobilize the DNA fragment onto a bead. These beads are emulsified in a water-in-oil solution containing PCR reagents. Within each droplet, a PCR is carried out generating millions of copies of the original DNA template. Afterwards, DNA on the bead is denatured, resulting in single-stranded DNA captured around the bead, and each bead is deposited in a well of a fiber-optic PicoTiter plate. Smaller beads with immobilized enzymes needed for sequencing are added into the wells. PCR buffers and nucleotides are flowed sequentially across the plate and the incorporation of a certain nucleotide, which yields a light signal, is captured with a camera. A schematic of the massively parallel tag sequencing procedure is shown in Figure 10.

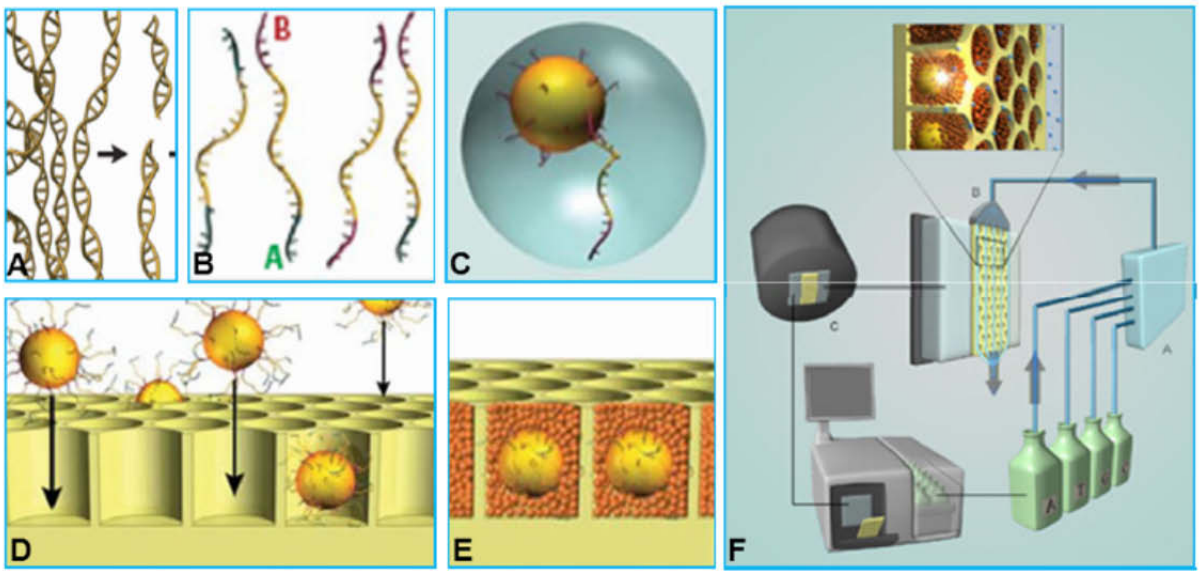


**Figure 10** Workflow of the procedure for massively parallel tag sequencing. (A) Fragmentation of DNA and ligation of adapters, resulting in (B). (C) Each fragment is immobilized onto a bead and placed in a well of a PicoTiter plate (D). Small beads with enzymes are added into the wells. (F) Nucleotides are flown one by one through the plate, releasing a light signal when incorporated to the sequence. Adapted from Margulies et al., 2005.

After the actual sequencing, further processing procedures are necessary in order to get reliable sequence data for community, taxonomic or phylogenetic analyses. MPTS read data (light signals) per PCR amplicon are stored in so called flowgrams, prior to translation into sequences. Due to the procedure of MPTS, it is assumed that homopolymers (repetition of the same nucleotide) are not accurately detected with the light signals. Also the production of chimera, sequences formed from two or more templates, can occur as a result of the PCR

amplification. Different algorithms were introduced to remove erroneous flowgrams, e.g. PyroNoise (Quince et al. 2009), DeNoiser (Reeder and Knight 2010) or AmpliconNoise (Quince et al., 2011). After the removal of noise, resulting sequence reads are clustered into operational taxonomic units, usually at a 3% identity level ($OTU_{3\%}$). By aligning the sequences or $OTU_{3\%}$ to sequences of known species, taxonomic assignments can be achieved.

The amounts of sequences that can be produced by MPTS allow for the detection and incorporation of rare (low abundant) community members into the investigation of microbial communities from environmental samples (e.g. Sogin et al., 2006; Pedros-Alio, 2012). Yet, the large amounts of data produced with MPTS make analyses computer intense. Another advantage of MPTS is the possibility to make taxonomic assignments and thus analyze not only the total community structure based on OTU, but also community patterns of specific groups of bacterial clades.

## 1.7. Publication outline

In the following four chapters, I will first give an insight into the typical bacterial richness and diversity in sediments from the long-term ecological research site HAUSGARTEN and determine spatial community patterns and their ecological drivers. Following, I will investigate natural temporal variations in bacterial community structure resulting from variations in organic matter supply. Finally, spatial and temporal community patterns of benthic eukaryotes are presented in comparison to the previously identified patterns for benthic bacteria.

**Chapter I: Biogeography of deep-sea benthic bacteria at regional scale (LTER HAUSGARTEN, Fram Strait, Arctic)**

Marianne Jacob, Thomas Soltwedel, Antje Boetius, Alban Ramette

(PLoS ONE (2013) 8(9): e72779)

This study shows that the bacterial community in Arctic sediments is highly diverse and is structured by the differences in organic matter availability at different water depth, yet with a high number of unique bacterial types on small spatial scales underlining the necessity of including several stations in sediment community analyses.

This study was designed by M. Jacob, A. Ramette, A. Boetius and T. Soltwedel. Molecular analyses and data assimilation were performed by M. Jacob. Environmental data were provided by T. Soltwedel. Statistical analyses were carried out by M. Jacob with help from A. Ramette. The manuscript was written by M. Jacob with support and input from all co-authors.

**Chapter II: Deep-sea microbial communities are fast indicators of particle flux variations in a warmer Arctic ocean**

Marianne Jacob, Thomas Soltwedel, Alban Ramette, Antje Boetius

(16.04.2014, in preparation for PNAS)

This study shows that the natural bacterial community reacts instantly to strong variations in the surface ocean and subsequent changes in organic matter supply, by a reduced overall diversity and shifted community structure with low organic matter availability, yet, individual bacterial taxa react distinctly.

The study was designed by M. Jacob, T. Soltwedel and A. Boetius. Molecular analyses and data assimilations were carried out by M. Jacob; additional environmental data were provided

by Thomas Soltwedel. Statistical analyses were performed by M. Jacob. Manuscript was written by M. Jacob and Antje Boetius. Surface ocean data of the LTER site HAUSGARTEN were kindly provided by Catherine Lalande, Eva Maria Nöthig, Eduard Bauerfeind and Alexandra Cherkasheva.

**Chapter III: Response of a benthic bacterial community to decreasing food availability: an *in situ* experimental approach at the Arctic deep-sea observatory HAUSGARTEN**

Marianne Jacob, Antje Boetius and Thomas Soltwedel

(19.04.2014 – in preparation for The ISME Journal as Short Communication)

This *in situ* experimental study shows that the bacterial community in Arctic sediments responds to starvation by a cut-off from particle flux with a reduction in diversity and a shift in enzymatic activity.

The study was designed by T. Soltwedel and M. Jacob. Molecular analyses and data assimilation was carried out by M. Jacob, and additional environmental data were provided by T. Soltwedel. Statistical analyses were performed by M. Jacob. Manuscript was written by M. Jacob with input from co-authors.

**Chapter IV: Temporal and spatial variations in eukaryotic diversity in Arctic deep-sea sediments**

Marianne Jacob and Antje Boetius

(16.04.2014 – in preparation for PLoS ONE)

This study shows that benthic eukaryotic community patterns as assessed by 454 tag sequencing resemble those of bacterial communities, with a distinct decrease in diversity along a depth gradient, and a varying community composition according to interannual variations in organic matter supply.

The study was designed by M. Jacob and A. Boetius. Statistical analyses were carried out by M. Jacob. The manuscript was written by M. Jacob with input from A. Boetius.

# 2. Thesis Chapters

# Chapter I

## Biogeography of deep-sea benthic bacteria at regional scale

## (LTER HAUSGARTEN, Fram Strait, Arctic)

Marianne Jacob, Thomas Soltwedel, Antje Boetius and Alban Ramette

HGF-MPG Research Group for Deep-Sea Ecology and Technology, Alfred-Wegener-Institut

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# Biogeography of Deep-Sea Benthic Bacteria at Regional Scale (LTER HAUSGARTEN, Fram Strait, Arctic)

**Marianne Jacob[1,2]*, Thomas Soltwedel[1,2], Antje Boetius[1,2], Alban Ramette[1,2]**

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## Abstract

Knowledge on spatial scales of the distribution of deep-sea life is still sparse, but highly relevant to the understanding of dispersal, habitat ranges and ecological processes. We examined regional spatial distribution patterns of the benthic bacterial community and covarying environmental parameters such as water depth, biomass and energy availability at the Arctic Long-Term Ecological Research (LTER) site HAUSGARTEN (Eastern Fram Strait). Samples from 13 stations were retrieved from a bathymetric (1,284–3,535 m water depth, 54 km in length) and a latitudinal transect (~ 2,500 m water depth; 123 km in length). 454 massively parallel tag sequencing (MPTS) and automated ribosomal intergenic spacer analysis (ARISA) were combined to describe both abundant and rare types shaping the bacterial community. This spatial sampling scheme allowed detection of up to 99% of the estimated richness on phylum and class levels. At the resolution of operational taxonomic units (97% sequence identity; $OTU_{3\%}$) only 36% of the Chao1 estimated richness was recovered, indicating a high diversity, mostly due to rare types (62% of all $OTU_{3\%}$). Accordingly, a high turnover of the bacterial community was also observed between any two sampling stations (average replacement of 79% of $OTU_{3\%}$), yet no direct correlation with spatial distance was observed within the region. Bacterial community composition and structure differed significantly with increasing water depth along the bathymetric transect. The relative sequence abundance of Verrucomicrobia and Planctomycetes decreased significantly with water depth, and that of Deferribacteres increased. Energy availability, estimated from phytodetrital pigment concentrations in the sediments, partly explained the variation in community structure. Overall, this study indicates a high proportion of unique bacterial types on relatively small spatial scales (tens of kilometers), and supports the sampling design of the LTER site HAUSGARTEN to study bacterial community shifts in this rapidly changing area of the world's oceans.

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## Introduction

Biogeographic patterns have been identified at global and regional scales for marine microbes, (e.g., [1,2]). In most studies, these patterns may be explained by a combination of spatial distance effects and contemporary environmental variations in physical, chemical and biological factors [3]. In an environmentally relatively uniform habitat such as the deep-sea floor, the influence of horizontal geographical distance on community patterns is likely related to dispersal limitation, resulting in a distance-decay relationship [2,4]. In a completely uniform habitat, this relationship could be entirely caused by drift [5]. In naturally patchy environments, selection pressures and historical processes will also play an important role [6]. However, so far it remains unclear at what spatial scales these different processes act on bacterial communities in deep-sea sediments. Information on such spatial patterns is not only important to understand the distribution range of bacterial species, it is also a prerequisite for monitoring and evaluating temporal variations in deep-sea ecosystems, for example by climate change and other anthropogenic disturbances [7], or for the implementation of marine protected areas [8].

A strong impact of spatial distance together with water depth and surface water productivity on variation in marine benthic bacterial community structure has already been detected on a global scale in coastal and deep-sea sediments [2]. In the South Atlantic, correlations between spatial distances and bacterial community structures at intermediate scale (up to 1,200 km distance), large scale (up to 3,500 km distance) and basin wide scale (up to 18,000 km distance) were observed [1]. Also in the Arctic sector, geographically related patterns of bacterial diversity were suggested based on surface sediment samples from two shallow (40 and 447 m water depth) and two deep stations (3,000 and 3,850 m water depth) in the Chukchi Sea and Canada Basin [9], while no such patterns were found in the western Greenland Sea (2,747–3,395 m water depth; 16 stations) [10]. Along the Siberian continental margin an energy-diversity relationship was found, which was tightly coupled to water depth differences, while accounting for spatial factors (37–3,427 m water depth; 17 stations) [11].

In this study of the Arctic Long-Term Ecological Research (LTER) site HAUSGARTEN in Fram Strait [12], we investigated the impact of spatial distance, water depth and environmental parameters related to food availability (phytodetrital pigments) and biomass on bacterial diversity and community structure, on a local to regional scale (~ 1–100 km distances). The part of the LTER site studied here covered 13 sampling sites arranged along two perpendicular transects. A bathymetric transect that spans water depths of 1,284 to 3,535 m (54 km length) and thereby incorporating a difference in phytodetritus input, and also a latitudinal transect covering a distance of 123 km along similar water depths (~ 2,500 m), lacking such a strong gradient in food availability [13] (Figure1). This allowed testing the hypotheses a) that spatial distances of 10–100 km can structure bacterial communities of the deep-sea floor; and b) that spatial patterns of bacterial communities can be linked to variations in food availability caused by different fluxes of particulate organic matter at different water depths. The objectives of this study were accordingly 1) to describe changes in bacterial diversity at the regional scale both in terms of local richness and community turnover, 2) to determine whether specific spatial and environmental factors explain changes in diversity patterns, and 3) to identify bacterial types that may be specifically affected by spatial or environmental factors.

## Materials and Methods

### Study Site

Fram Strait is the only deep-water connection to the Arctic Ocean. Here, warm Atlantic water masses enter the Arctic Ocean through the West Spitsbergen current, while cold Polar waters exit through the East Greenland Current [14,15]. Over the last decade, significant changes in sea ice distribution, temperature fluctuations of Atlantic water masses [16], changes in the biological composition of the water column [17,18] and the composition of export fluxes [19] have been observed. Due to a high efficiency of benthic-pelagic coupling [20,21,22], the ongoing changes of Arctic surface ocean conditions are predicted to directly affect the benthic environment [23,24], which depends on organic matter input from the more productive zone of the upper water column [25]. Main contributors to benthic carbon processing in Fram Strait are bacteria [26], which make up the major fraction of the small benthic infaunal biomass (up to 95%) [27]. Previous investigations on the bacterial community structure of this region include *in situ* experiments of bacterial colonization of artificial and deep-sea sediments [28], bacterial community response to chitin enriched sediments over different time scales [29] and around biogenic structures [30]. Natural spatial variation in benthic bacterial diversity was also investigated along a canyon at the Greenland continental rise over a distance of 200 km [10].

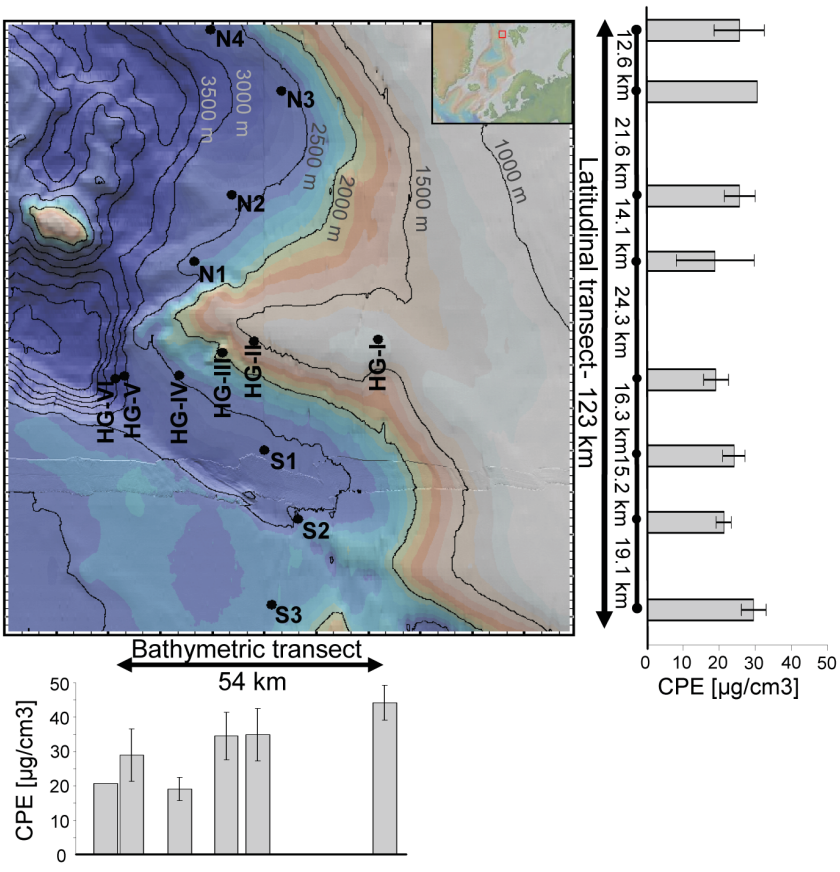


**Figure 1. Location of sampling stations of the LTER site HAUSGARTEN and corresponding pigment concentrations (CPE).** Distances in km between sampling stations were calculated from latitude or longitude only for the latitudinal and bathymetric transect, respectively. Map created with GeoMapApp [70].
doi:10.1371/journal.pone.0072779.g001

## Sampling Strategy

During the cruise ARK-XXIV/2 in July 2009 with the German research ice-breaker RV Polarstern to the LTER site HAUSGARTEN [12] west of Spitsbergen (Figure 1), samples of virtually undisturbed sediments where taken using a TV-guided multiple corer (TV-MUC) at 78.6–9.7°N and 3.5–6°E (Table S1). Six stations (HG-I to HG-VI) along a bathymetric transect from East to West from 1,284 m down to 3,535 m water depth as well as a latitudinal transect with eight stations (N1 to N4, HG-IV, and S1 to S3) at about 2,500 m water depth were sampled (Table S1). The most northern stations (N3 and N4) as well as the deepest station sampled in this study (HG-VI) were partly ice covered during sampling. TV-MUC cores were sub-sampled using modified 10-ml syringes (2 cm in diameter), sub-divided into 1-cm layers and only the uppermost centimeter representing the most active community was analyzed in this study [31]. Necessary permits for sampling were obtained from the Norwegian authorities (Fisheries directorate). The locations sampled are not privately-owned or protected areas, and the field studies did not involve endangered or protected species.

## Biotic and Abiotic Factors

Sample processing for all environmental parameters was done as described in [22]. In brief, concentrations of chlorophyll *a* and its degradation products phaeopigments, here summarized as chloroplastic pigment equivalents (CPE) [32], were determined using a Turner fluorometer. CPE concentrations serve as an indicator for food availability in form of phytodetritus originating from photosynthetic production in surface ocean layers. Porosity of sediments was assessed by the weight loss of wet sediment samples dried at 60°C. Phospholipids, indicating the total microbial biomass, were analysed by gas chromatography, and particulate proteins, indicating the biomass of detrital matter, were analysed photometrically [33]. Data is available at doi.pangaea.de/10.1594/PANGAEA.744673 -doi.pangaea.de/10.1594/PANGAEA.744685 (Table S1).

## DNA Extraction and Purification

Sediment from the uppermost centimeter originating from three different TV-MUC cores was pooled. Total DNA was extracted from 1 g of this homogenized slurry (comprising on average $4.22\times10^8$ bacterial cells as determined by acridine orange direct counting [34]) using the UltraClean Soil DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA) according to the manufacturer's instructions for maximum yields. Elution was carried out using 4×50 µl Tris-EDTA buffer (Promega, Madison, WI, USA). DNA extracts that showed a final DNA concentration lower than 4 ng $\mu l^{-1}$ (determined spectrophotometrically using a NanoDrop Spectrophotometer ND 1000, Thermo Fisher Scientific Inc., Wilmington, DE, USA) were purified via isopropanol precipitation. Final DNA concentrations ranged from 4–12 ng $\mu l^{-1}$.

## Automated Ribosomal Intergenic Spacer Analysis (ARISA)

ARISA PCR consisted of 1×Eppendorf PCR buffer (5′Prime Inc., Gaithersburg, MD, USA), 0.25 mM desoxynucleosidetriphosphate mix (Promega), 0.3 g $l^{-1}$ bovine serum albumin, 0.4 µM of each primer, 0.05 units Eppendorf Taq (5′Prime Inc.) and 20–25 ng DNA (determined spectrophotometrically using a Tecan Infinite 200, Tecan Group Ltd., Switzerland) in a total volume of 50 µl. Primers were used and PCR amplification (in triplicates per sample), separation of fragments by capillary electrophoresis, evaluation of signals and binning into operational taxonomic units (OTU) was done as described previously [35]. In order to get reliable data for statistical analyses, only those OTU that occurred in at least two of the PCR triplicates were kept for further analyses and their relative peak areas were averaged to produce one complete fingerprint per sample.

## 454 Massively Parallel Tag Sequencing (MPTS)

Extracted DNA was amplified at the Marine Biological Laboratory (Woods Hole, MA, USA) according to the protocol published on http://vamps.mbl.edu, using primers targeting the V4–V6 region of the bacterial 16 S rRNA gene. SFF files were deposited in the GenBank Sequence Read Archives (www.ncbi.nlm.nih.gov) under BioProject ID: PRJNA208712. Preparation of flowgrams and transformation into an OTU- by- Sample table were conducted with "mothur" [36] according to the standard operating procedure (SOP [37]) including the implemented denoising algorithm. Alignment of denoised sequences and taxonomic affiliation were carried out using the SILVA reference file for bacteria [38] (downloaded from http://www.mothur.org in March 2012) and chimeric sequences were identified using the mothur implemented *uchime* program. Cleaned sequences were clustered at a 97% identity level into operational taxonomic units ($OTU_{3\%}$) and the dataset was normalized by the total amount of sequences per sample to get relative abundances. To investigate the rare biosphere [39] we considered: a) $OTU_{3\%}$ that occurred with only one sequence in the whole denoised dataset (absolute singletons), called $SSO_{abs}$ and b) $OTU_{3\%}$ that consisted of only one sequence in at least one sample, and were not absolute singletons (relative singletons or $SSO_{rel}$), so the total number of sequences for any $SSO_{rel}$ was larger than one [40]. Taxonomic assignment up to the genus level was possible for 40% of all $OTU_{3\%,}$ but only 4% of all $OTU_{3\%}$ were assigned up to the species level. Therefore we only considered annotation up to genus level for subsequent analyses.

## Statistical Analyses

Chao1 richness estimates per sample were calculated on a normalized subset based on the sample with lowest number of $OTU_{3\%}$ (i.e. HG-II, 3,716 $OTU_{3\%}$). Turnover of OTU was calculated as percentage of pairwise shared, lost or gained OTU relative to the total number of OTU in the two samples. Shared OTU are those appearing in both samples, lost OTU are only present in the first sample and gained OTU are only present in the second sample. To compare bacterial classes found in this study to those found in other studies (i.e. [2,11]), we only considered the shared classes and then calculated their mean relative sequence abundances for each subset. To determine whether class proportions obtained in this study could be predicted from the previous studies, we used linear regression and determined whether the slope coefficients were significantly different from one by calculating the 95% confidence intervals of the respective slope coefficients (e.g. [35]).

Dissimilarity matrices based on community data and environmental tables were calculated using Bray-Curtis and Euclidean distances, respectively. Homogeneity of group dispersions were determined by calculating the average distance of a group member to the median of the group [41] and the central station HG-IV was included in both transects. Non-metric multidimensional scaling (NMDS) was performed together with a minimum-spanning tree between samples connecting nearest neighbours (i.e. the most similar stations) in terms of similarity of their community structure to visualize pairwise community similarities. Mantel tests with 999 Monte-Carlo permutations were used to test for the significance of

Spearman correlations between dissimilarity matrices or dissimilarity matrices and environmental parameters.

Except for longitude, latitude, spatial distance and water depth, all parameters were normalized by $\log_{10}$ transformation to meet the assumptions for regression analysis (see [42]). Distances between sampling stations were calculated in kilometer from only longitude or latitude for the bathymetric and latitudinal transect, respectively. Spatial distance between sampling stations of all stations were calculated with both, longitude and latitude. Redundancy analyses (RDA) were used to explore the degree of variation in community datasets that can be explained by environmental parameters. In order to look for pure effects of certain environmental parameters, canonical variation partitioning [42] was performed using the forward selected contextual parameters water depth and CPE concentrations. We used CPE concentrations as they explained more of the variability than chlorophyll *a* or phaeopigments alone. When referring to behaviour of certain taxa, the $OTU_{3\%}$ data was pooled using the "taxa.pooler.1.2" of the MultiCoLA software package [43] which groups all $OTU_{3\%}$ that were assigned to a taxonomic group at a predefined taxonomic level. $OTU_{3\%}$ that were not classified at a certain taxonomic level were combined into one group. All analyses were performed in R (v.2.14.1) [44] using *vegan* [45], *permute* [46] and *MASS* [47] packages.

## Results and Discussion

Biogeographic patterns of surface sediment bacterial communities were investigated at the Arctic LTER site HAUSGARTEN (~79°N, 4°E; Figure 1, Table S1). Shifts in bacterial community structure were investigated using automated ribosomal intergenic spacer analysis (ARISA) and 454 massively parallel tag sequencing (MPTS) of the V4–V6 variable regions. We found consistent community patterns derived from both data types at different taxonomic resolution levels (Table S2), thus we mostly focused on results based on MPTS data, including some comparisons to the patterns detected by ARISA.

### Richness of Bacterial Types

Using MPTS data, a total of 41 phyla, 78 classes, 136 orders, 215 families, and 410 genera were identified (Table S3). Most of the $OTU_{3\%}$ belonged to the phylum Proteobacteria (47% of all $OTU_{3\%}$) with the most abundant classes being Gammaproteobacteria (23%), Deltaproteobacteria (15%) and Alphaproteobacteria (7%). The second most $OTU_{3\%}$ abundant phylum was Bacteroidetes (9%) with, among others, the classes Flavobacteria (3%) and Sphingobacteria (5%). Other abundant phyla were Actinobacteria (3%), Acidobacteria (5%), and Verrucomicrobia (4%). Those proportions barely changed when excluding $SSO_{abs}$ from the dataset. These phyla and classes were also found as abundant members of Arctic sediments from the Pacific sector [9], in a fjord off Svalbard [48], the Siberian continental margin [11], as well as in other benthic environments [2].

The mean proportions of bacterial classes inhabiting HAUSGARTEN sediments were in very good agreement ($R^2 = 0.78$, $p<0.001$; determined by linear regression; Figure 2) with those predicted for globally distributed benthic deep-sea samples (262–5,347 m water depth), indicating a typical deep-sea microbiome [2]. Differences from the global average included for example lower Alphaproteobacteria and higher Gammaproteobacteria relative sequence abundances at HAUSGARTEN. When considering Siberian continental margin sediments (534–3,427 m water depth) [11], we found an even better relationship for mean class proportions ($R^2 = 0.85$, $p<0.001$; Figure 2). Those observations were corroborated by determining the slope coefficients of each comparison, and slope coefficients of $1.25\pm0.24$ (95% confidence interval assuming a Student's $t$ distribution with 30 degrees of freedom) and $1.1\pm0.19$ (24 degrees of freedom), were obtained for the comparison with the global dataset and the Siberian margin dataset, respectively. This shows that the best model (i.e. a slope coefficient of 1 and higher explained variance) is obtained in the latter case when only considering sediments from the Arctic.

Chao1 richness estimates were on average $3{,}010\pm642$ $OTU_{3\%}$ per sample at each station (Table S4), which is comparable to sediments from the Siberian continental margin [11] and higher than for samples from the deep Arctic Ocean water column [49]. Interestingly the variation in richness (coefficient of variation 0.21) was close to that observed for biomass (phospholipid concentration per sample, CV = 0.25 based on $12\pm3$ nmol $ml^{-1}$; Table S1). We found no correlation of the number of $OTU_{ARISA}$, nor of observed or estimated richness of $OTU_{3\%}$ per sample with pigment concentrations (CPE), water depth (Table S5) or with any other contextual parameter (latitude, porosity, particulate protein concentrations, phospholipid concentrations; data not shown). These observations did not change when removing singletons from the dataset (data not shown). Our findings differ from a previous investigation of the oligotrophic Siberian continental margin where both, numbers of $OTU_{ARISA}$ and estimated richness of $OTU_{3\%}$, correlated positively with phaeopigment concentrations below 4 μg $cm^{-3}$ [11]. However, in Fram Strait, phaeopigment concentrations were considerably higher (13–37 μg $cm^{-3}$) than at the Siberian continental margin (<8 μg $cm^{-3}$) [11]. This may indicate that, within the range of phytodetritus supply to the deep Fram Strait (Table S1), the observed local variations in bacterial richness might be driven by other factors than energy supply and water depth. For example, it is possible that the locally differing assemblages of benthic fauna [13,50,51] have an impact on local patterns in bacterial richness for example, by altering the sediment-water interface and particle deposition or grazing (see [30,52,53]), which remains to be further investigated.

### Sampling Effect on Diversity Discovery

The increase of newly detected $OTU_{3\%}$ with every sampled station was linear (Figure S1B). By sampling 12 of 13 stations, 95% of observed $OTU_{3\%}$ were detected and 36% of estimated richness was recovered, when considering all stations (Table S3). The $OTU_{3\%}$ accumulation curve could not reach a plateau because of the high numbers of singletons in the dataset (62% of all $OTU_{3\%}$). In contrast, the OTU accumulation curve for ARISA data did reach a plateau and only nine stations were needed to recover 95% of all observed $OTU_{ARISA}$ (Figure S1A). This reflects the technical limitations of ARISA such as the maximum number of detectable $OTU_{ARISA}$ (here 450) and 16–23 S length identity between different genera or species [54] (see Text S1).

To investigate the effects of taxonomic resolution, we used the taxonomic information associated to each $OTU_{3\%}$ from phylum to genus, according to [43] (see Table S3). Only 1.36% of all $OTU_{3\%}$ could not be assigned to a known phylum. Taking only seven stations into account, at least 95% of all observed phyla, classes or orders were recovered; in contrast, sampling of ten stations was needed to recover 95% of all occurring genera in the dataset (Figure 3). Considering all stations, 99% of the estimated richness of phyla and classes were described and 77% of the estimated richness of genera (Table S3). In order to determine which transect added most to the total diversity – the bathymetric transect covering water depth together with food availability differences and spatial distance, or the latitudinal transect representing mostly pure spatial distance - we analysed both

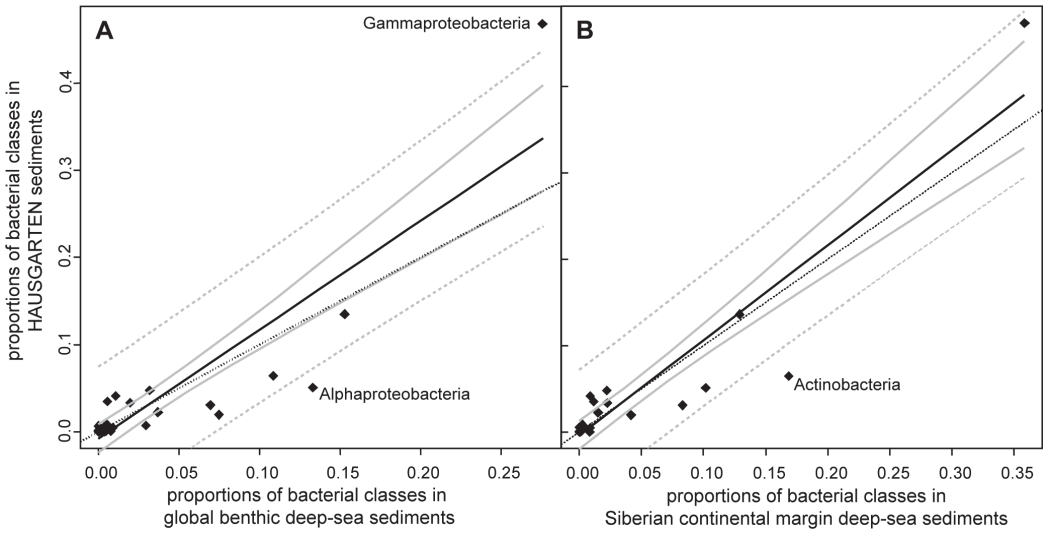


**Figure 2. Comparison of bacterial classes in sediments from HAUSGARTEN with other datasets.** A: Globally distributed sediments; B: sediments from the Siberian continental margin. The solid lines indicate the best fit using linear regression; solid grey lines indicate 95% confidence intervals; dotted grey lines indicate predicted intervals at a 95% confidence level; dotted black lines indicate the case where equal proportions were found in the datasets being compared (y = x).
doi:10.1371/journal.pone.0072779.g002

transects separately, but compared the recovered diversity with that of the whole dataset. From the latitudinal transect alone 5, 6, 5 and 8 stations were needed to cover 95% of all observed phyla, classes, orders and families, respectively, in the entire HAUSGARTEN dataset. With all stations from the latitudinal transect, 99% of the estimated total richness at the phylum, class and order level were recovered, 95 and 92% at the family and genus level, respectively. At the $OTU_{3\%}$ level, 78% of observed and 28% of estimated total richness was recovered. Along the bathymetric transect, 89%, 93%, 93%, 75% and 81% of the estimated total richness was recovered at the phylum, class, order, family and genus level, respectively. Only 50% of all observed $OTU_{3\%}$ were found at stations from the bathymetric transect, and only 18% of estimated richness could be recovered by sampling the six stations along this transect. Hence, a high amount of bacterial diversity came from the latitudinal transect. By sampling only this transect, most of the diversity discovery at coarse taxonomic levels was covered. The latitudinal transect hosted four unique candidate divisions WS1, OP9, SR1 and WCHB1–60, which did not occur in samples from the bathymetric transect. Overall, the near-complete coverage of diversity at coarse taxonomic resolution shows that our sampling scheme was suitable to examine bacterial diversity at the regional scale. Still, with every additional sample, new families, genera and, most of all, $OTU_{3\%}$ could be detected.

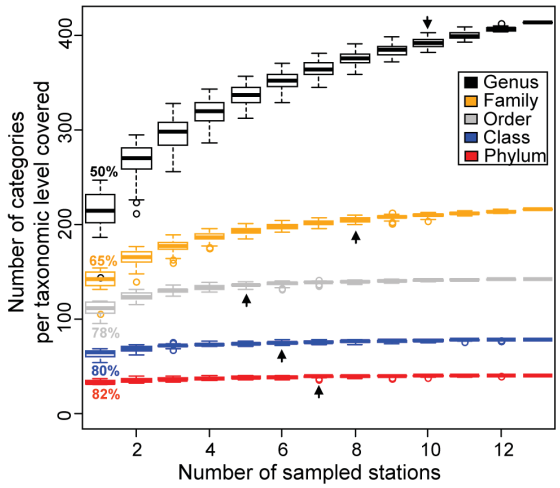


**Figure 3. Accumulation curves per taxonomic category based on MPTS data.** Arrows indicate how many stations are needed to recover 95% of categories per taxonomic level. The percentages indicated for n = 1 station correspond to how much diversity would be recovered on average by randomly sampling only one station.
doi:10.1371/journal.pone.0072779.g003

## Community Turnover and Structure along the Two Transects

On average 21±2% $OTU_{3\%}$ (32±3% when removing $SSO_{abs}$) were shared between any two samples at HAUSGARTEN (Table S6) which is higher than shared $OTU_{3\%}$ between coastal and deep-sea surface sediments (~ 14 $OTU_{3\%}$) around the whole globe [2]. Overall, no correlation of community composition (similarities in the presence and absence of $OTU_{3\%}$) with spatial distance between any two samples was observed ($p = 0.557$), neither for the whole data set, nor for samples of the latitudinal transect (13–123 km difference; $p = 0.246$) or of the bathymetric transect alone (2–52 km difference; $p = 0.107$) when based on MPTS data including singletons. Removing absolute singletons from the dataset led to the same conclusions (data not shown). In contrast, community composition of samples from the bathymetric transect based on ARISA – known to detect the more abundant types - significantly correlated with spatial distance ($r = 0.83$, $p = 0.013$).

Dissimilarities in community composition significantly correlated with water depth differences along the bathymetric transect ($r = 0.56$, $p = 0.032$; $r = 0.62$, $p = 0.034$ when removing $SSO_{abs}$; 263–2,251 m water depth differences). Pairwise shared $OTU_{3\%}$

gradually decreased from 25% to 19% (34%–27% when removing $SSO_{abs}$) from samples from the shallowest HAUSGARTEN station HG-I to station HG-V (1,821 m total depth difference; Table S6). The same trend was observed for bacterial community structure (similarities in the relative abundance of $OTU_{3\%}$) with a gradual increase in dissimilarities of community structure with increasing water depth differences (Figure 4C). For the latitudinal transect, no significant correlation of community composition or structure with spatial distance was found (Figure 4D, Table S6).

In a non-metric multidimensional scaling plot (NMDS), visualizing dissimilarities of bacterial communities between samples, those from the bathymetric transect were located further apart from one another and had significantly higher community dispersion as those from the latitudinal transect (Figure 5). The latter samples grouped together and were significantly less dispersed (mean distances to their centroid of 0.21, as compared to 0.27 for samples from the bathymetric transect; 0.18 and 0.24, respectively, when removing $SSO_{abs}$), as assessed by ANOVA of the distances to group centroids [41] ($p = 0.003$, $p = 0.002$ when removing $SSO_{abs}$). These findings indicate that samples taken within a water depth zone were more similar to each other than across the zones. Grouping of the communities indicated higher similarities within the depth zones ~1000–2000 m and>~2500 m, which was previously also found for meiofauna taxa densities [13]. Strong bathymetric gradients, but without this clear zonation, were found for macro- and megafauna in Arctic deep-sea sediments (e.g. [55]).

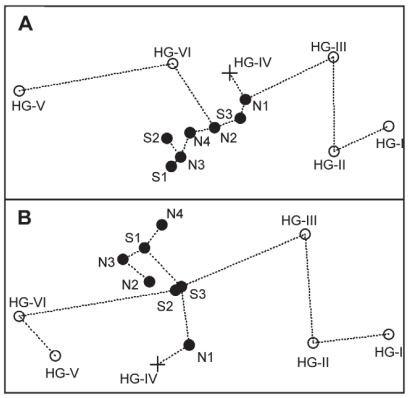


**Figure 5. Non-metric multidimensional scaling (NMDS) plot of community data.** MPTS (A) and ARISA (B) data based on Bray-Curtis dissimilarity matrices. Open circles indicate stations from the bathymetric transect, filled circles indicate stations from the latitudinal transect and the crosses indicate the central station. Dotted lines show a minimum spanning tree connecting nearest neighbours. Stress values: 0.05 for A and 0.06 for B.
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## Spatial and Environmental Effects on Community Structure

We determined which environmental variables could explain some of the variation in bacterial community structure. In these analyses, bacterial community structure refers to the relative abundance of $OTU_{3\%}$ including singletons (analyses without $SSO_{abs}$ led to the same conclusions; Table 1). Spatial variables consisted of longitude, latitude, spatial distance and water depth. Energy availability in the sediments in form of phytodetritus input from surface waters was estimated by measuring pigment concentrations (CPE). Porosity refers to the sediment water content. Protein and phospholipid concentrations were used to estimate total organic detritus and living microbial biomass, respectively. These environmental parameters have previously been shown to be related to differences in bacterial abundance, biomass and enzyme activities in Fram Strait (e.g. [22,27]), and

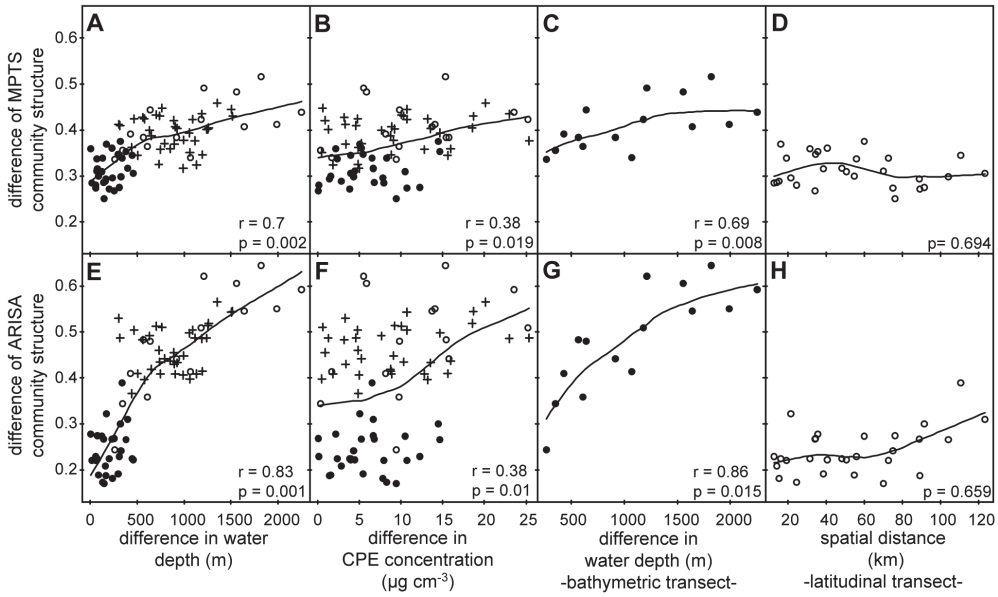


**Figure 4. Changes in bacterial community structure with water depth and CPE concentrations and along spatial distance for the two transects.** The plots A, B, C and D are based on MPTS data, plots in E, F, G and H are based on ARISA data. Filled circles indicate comparisons of samples from the latitudinal transect, open circles indicate comparisons of samples from the bathymetric transect, crosses indicate comparisons across transects. C, D, G and H are based on a subset of 6 and 8 samples for the bathymetric and latitudinal transects, respectively. Mantel tests were used to assess the significance of Spearman's correlation coefficients (r) based on 1000 permutations.
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**Table 1.** Community response to spatial and environmental factors.

| | $OTU_{3\%}$ | | | | | | $OTU_{ARISA}$ | |
|---|---|---|---|---|---|---|---|---|
| | All | | $SSO_{abs}$ removed | | $SSO_{rel}$ only | | | |
| | r[a] | $R^2$ adj[b]. | r | $R^2$ adj. | r | $R^2$ adj. | r | $R^2$ adj. |
| Spatial distance | ~ | ~ | ~ | ~ | ~ | ~ | ~ | ~ |
| Latitude | ~ | ~ | ~ | ~ | ~ | ~ | ~ | ~ |
| Longitude | 0.38* | 0.03* | 0.42* | 0.05* | ~ | 0.02* | 0.47** | 0.09* |
| Water depth | 0.70** | 0.07*** (0.05**) | 0.71*** | 0.09*** (0.07**) | 0.68** | 0.06*** (0.04**) | 0.83*** | 0.22*** (0.14**) |
| Phospholipids | 0.31* | ~ | 0.36* | ~ | 0.49** | ~ | 0.45** | ~ |
| CPE | 0.38* | 0.03* (~) | 0.36* | 0.04*(~) | 0.33* | 0.02* (~) | 0.38** | 0.12** (~) |
| covariation | | (0.02) | | (0.03) | | (0.02) | | (0.08) |

$OTU_{3\%}$: Clustered sequences from MPTS at 97% sequence identity; $OTU_{ARISA}$: OTU derived from ARISA fingerprinting; $SSO_{abs}$: $OTU_{3\%}$ with only one sequence in the whole dataset (absolute singletons); $SSO_{rel}$: $OTU_{3\%}$ with only one sequence in at least one sample but more than one sequence in the whole dataset (relative singletons). [a]The significance of Spearman's correlation coefficients (r) between relative OTU abundance tables and environmental parameter was determined by Mantel tests. [b]Redundancy analysis (RDA) and partial RDA (pRDA; in brackets; to evaluate factor effect while taking the effects of other parameters into account) were used to determine the amount of variation ($R^2$ adjusted) in the community data in a variation partitioning approach. For pRDA, the used parameters were water depth and CPE concentrations. Note that covariation effects cannot be tested for significance in the variation partitioning context (e.g. [71]). Significance levels are indicated as ***: p≤0.001, **: p≤0.01, *: p≤0.05, ~: not significant, p>0.05.

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were hence chosen as proxies to represent some of the complex factors that may impact the variation in community structure at the LTER site HAUSGARTEN.

Dissimilarities in bacterial community structure significantly increased with increasing differences in water depth ($r = 0.70$, $p = 0.002$) and longitude ($r = 0.38$, $p = 0.017$); Table 1), but not with latitude or spatial distance ($p = 0.971$ and $p = 0.342$, respectively). Water depth differences and bacterial community dissimilarity followed a continuous linear relationship within the investigated range of 1,284–3,535 m water depth (Figure 4). Redundancy analyses (RDA) revealed that water depth and longitude significantly explained 7% and 3% of variation in the $OTU_{3\%}$ dataset, respectively. Water depth was shown to correlate with bulk enzymatic activity, bacterial abundance and bacterial viability [13,31]. A number of environmental factors vary with water depth and may include additional controlling factors, e.g. food quality or presence of larger organisms (e.g. nematodes [56]). In addition, adaptation to pressure differences might influence the bacterial community structure (e.g. [57]).

Particle flux of organic matter to the deep sea generally decreases with increasing water depth (e.g. [58,59]). We observed that differences in CPE concentrations correlated positively with changes in bacterial community structure: stations with high differences in CPE concentrations showed more dissimilar community structures ($r = 0.38$, $p = 0.019$; Table 1, Figure 4) than those with similar CPE concentrations. A significant amount of 3% of the variation in bacterial community structure was explained by CPE concentrations (Table 1). Of course, CPE is just one proxy for phytodetritus input and does not necessarily reflect the complexity of food quantity and quality.

Although we did not find a significant correlation between water depth and CPE concentrations ($p = 0.112$; Table S5), they covaried and explained together with porosity 2% of the variation in community structure. Pure fractions of CPE concentrations (when the effect of covariation with water depth was removed) did not significantly explain variation in the community structure while pure fractions of water depth (when the effect of covariation with CPE was removed) still specifically explained 5% of the community variation. Porosity, proteins and phospholipids did not significantly explain variation in bacterial community structure ($p = 0.313$, $p = 0.845$ and $p = 0.149$, respectively) although differences in phospholipid concentrations significantly correlated with dissimilarities in community structure ($r = 0.31$, $p = 0.04$; Table 1). At the Siberian continental margin a relationship of bacterial community structure and phaeopigment concentration was found and a pure effect of phaeopigment concentrations (when the effect of water depth, spatial distance and protein concentrations was removed) could explain 5% of variation in community structure [11]. The reason why we did not find such a relationship could be explained by the smaller water depth range of this study (1284–3535 m water depth here, versus 37–3,427 m water depth at the Siberian continental margin), and the higher supply with phytodetritus at HAUSGARTEN.

Finally, we also tested the effect of grouping $OTU_{3\%}$ at coarser taxonomic resolution. In this case, community structure at every taxonomic level significantly correlated with differences in water depth and a high percentage (12% to 24%) of variation in community structure could be significantly explained (Table S7). This means that although most of the phyla and classes were common to all stations, their members significantly varied in relative abundances between different water depths. In contrast, no significant relationship between bacterial community structures at different taxonomic levels with CPE concentrations was found.

## Response of Individual Bacterial Taxa

Previous studies have shown that the abundance of Arctic deep-sea fauna either linearly decreased with decreasing water depth and food availability or peaked at intermediate water depth and thus phytodetritus input [60]. Therefore we used both linear and quadratic regression to test how individual bacterial taxa correspond to changes in water depth and CPE concentrations. Out of the 40 phyla identified in the dataset, 11 showed significant positive or negative relationships with increasing water depth (Table S8). Significant negative linear relationships with water depth were found for Verrucomicrobia and Planctomycetes, two related taxa which are ubiquitously found in soil and marine sediments, e.g. [61,62,63]. Their relevant contribution to benthic bacterial diversity was already reported from sediments in the Pacific sector of the Arctic Ocean [9], the Siberian margin [11] and coastal sites of Fram Strait [64,65], yet no relationship with

water depth had been detected. A positive quadratic relationship with water depth (minimum relative abundance at intermediate water depth) was found for Deferribacteres, which were previously found in coastal and deep-sea sediments [2,40] and were reported from sediments from the Laptev Sea [11]. The phylum Actinobacteria showed a negative linear relationship with CPE concentrations, while Planctomycetes and Verrucomicrobia showed a positive linear relationship (Table S8). Verrucomicrobia was previously found to be also positively correlated to pigment concentrations in samples from the Siberian continental margin [11].

### Rare Biosphere

The rare bacterial biosphere was shown to make up a high fraction of bacterial community diversity in deep-sea sediments (e.g. [2]). Members of the rare biosphere include types which may vary in space and time and may become abundant when favourable conditions are present [66]. Here we looked at a subset of the rare biosphere including only those $OTU_{3\%}$ occurring with exactly one sequence in at least one sample but with more than one sequence in the whole dataset ("relative singletons", $SSO_{rel}$, [40]). This group of rare bacterial types comprised 31% of all $OTU_{3\%}$ (25% of all sequences), and on average 38±8% per sample. Interestingly, it showed similar responses to water depth changes and CPE concentrations as the whole community: water depth differences were highly correlated with differences in community structure and explained 6% of the variation in the $SSO_{rel}$ community data, CPE concentrations correlated significantly with differences in community structure and explained 2% of the variation in the community (Table 1). When removing effects of covariation between water depth and CPE, the pure fraction of water depth still explained 4% of the variation in the $SSO_{rel}$ community data, but pure fractions of CPE concentrations did not significantly explain any variation in the $SSO_{rel}$ community data. This shows that the rare bacterial biosphere does vary with water depth, partly independent of phytodetritus concentrations. Likewise, differences in rare bacterial community structure with different water masses were found in the water column of the Arctic Ocean [67] and an effect of pigment concentrations on a part of low abundant bacterial types were reported from Arctic sediments [11].

Not only abundant types of bacteria but also rare members of the biosphere were found to be important for microbial processes (e.g. cellulose and chitin degradation [68]) and specific biogeochemical processes (e.g. sulphate reduction [69]). In Arctic sediments, high bacterial diversity was related to higher enzymatic activity and higher rates of organic matter degradation than in less diverse communities [65], and bacterial community patterns explained variations in enzyme activity [11]. Rare members of the biosphere might change in abundance with the varying availability of certain substrates (see [66] and references therein). Especially in variable environments such as the Arctic deep sea with a varying seasonal input of "fresh" phytodetritus, a high bacterial diversity and a complex community structure may be essential to react to environmental changes and for the functioning of the ecosystem [65,68].

## Conclusions

We found a spatially highly diverse bacterial community in surface sediments of the Long-Term Ecological Research site HAUSGARTEN (Eastern Fram Strait). With 13 sampling stations over an area of about 3,385 $km^2$ we assessed most of the estimated regional richness and found strong water depth related patterns of community structure along the bathymetric transect (54 km distance, 1,284–3,535 m water depth). Along the 120-km long latitudinal transect, no increasing bacterial community dissimilarity with increasing spatial distance could be observed. Nevertheless, a turnover of on average 79% $OTU_{3\%}$ (still 68% when absolute singletons were removed) was detected between any two samples taken within a distance of on average 13 km. Pigment concentrations as a proxy for energy supply in the form of phytodetritus sedimentation influenced bacterial community richness and structure, but no strong energy-diversity relationship was found within the investigated range. We identified indicator taxa that showed significant changes in relative sequence abundance with changes in water depth or pigment concentrations. This study demonstrates the complexity of bacterial community structure in deep-sea sediments and the necessity to investigate the regional biodiversity of deep-sea life not only at one single spot, but over scales of 1–100 km and different water depth zones, in order to better evaluate community responses related to environmental variations.

## Supporting Information

**Figure S1 OTU accumulation curves.**
(DOC)

**Table S1 List of samples taken during the Polarstern cruise ARK-XXIV/2 in 2009 and measured environmental parameters.**
(DOC)

**Table S2 Comparison of dataset structure based on ARISA and MPTS using Spearman correlation and Procrustes tests.**
(DOC)

**Table S3 Observed and estimated richness of OTU or taxa at different taxonomic levels and shared OTU or taxa between all stations.**
(DOC)

**Table S4 Observed and estimated richness of ARISA and MPTS data per station and in the total dataset.**
(DOC)

**Table S5 Spearman's correlation matrix of alpha diversity measures, water depth and pigment concentrations (CPE).**
(DOC)

**Table S6 Percentages of pairwise shared, lost and gained $OTU_{3\%}$(A), $OTU_{3\%}$ without $SSO_{abs}$ (B) and $OTU_{ARISA}$ (C).**
(DOC)

**Table S7 Community response to water depth at different taxonomic levels.**
(DOC)

**Table S8 Linear and quadratic regression of phyla and classes in the $OTU_{3\%}$ dataset.**
(DOC)

**Text S1 Comparison of ARISA and MPTS and Richness of OTU.**
(DOC)

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## Author Contributions

Conceived and designed the experiments: MJ AR TS AB. Performed the experiments: MJ. Analyzed the data: MJ AR. Wrote the paper: MJ TS AB AR. Provided environmental data: TS.

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## Supporting Information

### Comparison of ARISA and MPTS

Shifts in bacterial community structure were investigated using automated ribosomal intergenic spacer analysis (ARISA) and 454 massively parallel tag sequencing (MPTS) of the V4-V6 variable regions, which are both commonly used techniques to describe microbial communities over large spatial scales (e.g. [1, 2]). The two techniques differ to some extent: ARISA targets the length variability of the 16-23S intergenic region, but is limited in the number of detectable operational taxonomic units (OTU), thus representing abundant types of bacteria, which limits the use of richness estimates [3]. Moreover, it does not provide phylogenetic information [4]. In contrast, MPTS offers an in-depth view on community composition (based on presence or absence of OTU) and structure (based on the relative abundance of OTU), e.g. [5, 6]. At the resolution of family to genus, both community fingerprinting methods show highly congruent patterns (e.g. [7, 8]. Also in this study, we found consistent community patterns derived from both data types at different taxonomic resolution levels (Table S2). In this study, we mostly focused on results based on MPTS data, including some comparisons to the patterns detected by ARISA.

### Richness

On average 2,028 ± 463 $OTU_{3\%}$ occurred per sample at each station. After removing pyrosequencing and PCR-related technical errors, on average 572 ± 225 $OTU_{3\%}$ per sample were absolute singletons ($SSO_{abs}$; Table S4). This resulted in 7,430 $SSO_{abs}$ (62% of all $OTU_{3\%}$, 5% of all denoised sequences) in the whole dataset. In total, 3,705 $OTU_{3\%}$ (31% of all $OTU_{3\%}$, 25% of all denoised sequences) were relative singletons ($SSO_{rel}$) with on average 739 ± 116 $SSO_{rel}$ per sample. Overall this indicates that a large fraction of the recovered

bacterial diversity consisted of rare microbial types. Noticeably, total number of $OTU_{3\%}$, $SSO_{abs}$ and $SSO_{rel}$ were all correlated positively to each other (Table S5), indicating that more rare types (either absolute or with fluctuating sequence abundances) were discovered as observed richness increased.

**Table S1. List of samples taken during the Polarstern cruise ARK-XXIV/2 in 2009 and measured environmental parameters.**

| | Station | Water depth (m) | Latitude [N] | Longitude [E] | Porosity [% vol] | CPE* [µg $cm^{-3}$] | Phospholipids [nmol $ml^{-1}$] | Proteins [mg $cm^{-3}$] | Event label | Date (2009) | Pangaea Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Bathymetric transect | HG-I | 1284 | 79° 8' 2" | 6° 5' 46" | 72 ± 2 | 44 ± 5 | 13 ± 1 | 1.3 ± 0.2 | PS74/109-2 | 13 July | [9] |
| | HG-II | 1547 | 79° 7' 48" | 4° 54' 7" | 63 ± 1 | 35 ± 8 | 6 ± 2 | 1.0 ± 0.1 | PS74/108-2 | 12 July | [10] |
| | HG-III | 1895 | 79° 6' 29" | 4° 35' 56" | 55 ± 5 | 34 ± 7 | 13 ± 3 | 0.7 ± 0 | PS74/107-2 | 12 July | [11] |
| | HG-IV (central st.) | 2464 | 79° 3' 50" | 4° 10' 55" | 55 ± 3 | 19 ± 3 | 9 ± 1 | 0.7 ± 0.1 | PS74/121-1 | 16 July | [12] |
| | HG-V | 3105 | 79° 3' 47" | 3° 39' 32" | 58 ± 1 | 29 ± 8 | 17 ± 6 | 0.8 ± 0.1 | PS74/113-2 | 14 July | [13] |
| | HG-VI | 3535 | 79° 3' 25" | 3° 34' 16" | 54 ± 6 | 21’ | 16 ± 2 | 0.4 ± 0.1 | PS74/106-3 | 12 July | [14] |
| Latitudinal transect | N1 | 2401 | 79° 16' 59" | 4° 19' 44" | 53 ± 2 | 19 ± 11 | 11 ± 6 | 1.1 ± 0.2 | PS74/120-2 | 16 July | [15] |
| | N2 | 2545 | 79° 24' 36" | 4° 41' 24" | 66 ± 2 | 26 ± 4 | 15 ± 3 | 1.0 ± 0.6 | PS74/119-2 | 16 July | [16] |
| | N3 | 2786 | 79° 36' 14" | 5° 10' 1" | 54 ± 4 | 31’ | 9 ± 1 | 3.0 ± 2.3 | PS74/118-2 | 16 July | [17] |
| | N4 | 2802 | 79° 43' 1" | 4° 29' 10" | 57 ± 1 | 26 ± 7 | 11 ± 7 | 0.7 ± 0 | PS74/116-2 | 15 July | [18] |
| | S1 | 2637 | 78° 55' 1" | 5° 0' 4" | 60 ± 1 | 24 ± 3 | 9 ± 3 | 0.5 ± 0.1 | PS74/127-2 | 17 July | [19] |
| | S2 | 2473 | 78° 46' 48" | 5° 19' 37" | 66 ± 3 | 21 ± 2 | 11 ± 2 | 0.9 ± 0 | PS74/128-2 | 18 July | [20] |
| | S3 | 2339 | 78° 36' 29" | 5° 4' 23" | 60 ± 2 | 30 ± 3 | 11 ± 2 | 1.2 ± 0 | PS74/129-3 | 18 July | [21] |

*CPE: Chloroplastic pigment equivalents used as proxy for phytodetritus input. ‘: no replicates were available.

**Table S2. Comparison of dataset structure based on ARISA and MPTS using Spearman correlation and Procrustes tests.**

| Taxonomic level | Mantel test | Procrustes test |
|---|---|---|
| Phylum | 0.33 * | 0.63 ** |
| Class | 0.35 * | 0.60 * |
| Order | 0.54 ** | ~ |
| Family | 0.57 ** | ~ |
| Genus | 0.63 *** | ~ |
| $OTU_{3\%}$ | 0.87*** | 0.83*** |

$OTU_{3\%:}$ Clustered sequences from MPTS at 97% sequence identity. Significance: *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ~: not significant. Significance for Spearman correlation was determined by Mantel tests.

**Table S3. Observed and estimated richness of OTU or taxa at different taxonomic levels and shared OTU or taxa between all stations.**

| | No. of observed taxa /$OTU_{ARISA}$ | % $OTU_{3\%}$ annotated to taxonomic level | Chao1 richness estimator | % observed taxa of estimated taxa | No. of shared taxa/ $OTU_{ARISA}$ between all stations | % of shared taxa/ $OTU_{ARISA}$ between all stations |
|---|---|---|---|---|---|---|
| Phylum | 41 | 99 | 42 | 99 | 27 | 66 |
| Class | 78 | 97 | 80 | 98 | 46 | 58 |
| Order | 136 | 89 | 139 | 98 | 73 | 51 |
| Family | 215 | 68 | 260 | 83 | 80 | 25 |
| Genus | 410 | 30 | 529 | 77 | 85 | 21 |
| $OTU_{3\%}$ | 12011 | 5 | 33778 | 36 | 217 | 2 |
| $OTU_{ARISA}$ | 289 | | | | 46 | 16 |

Abbreviations: $OTU_{ARISA}$: Operational taxonomic unit as determined by binning ARISA peaks with a window size 2; $OTU_{3\%:}$ Clustered sequences from MPTS at 97% sequence identity.

**Table S4. Observed and estimated richness of ARISA and MPTS data per station and in the total dataset.**

| | | No. of $OTU_{ARISA}$ | No. of $OTU_{3\%}$ | No. of MPTS sequences | No. of $SSO_{abs}$ | No. of $SSO_{rel}$ | Chao1 richness estimates of MPTS |
|---|---|---|---|---|---|---|---|
| Bathymetric transect | HG-I | 133 | 1740 | 7382 | 423 | 657 | 2793 |
| | HG-II | 150 | 1063 | 3716 | 179 | 516 | 2619 |
| | HG-III | 153 | 2116 | 7408 | 703 | 785 | 4485 |
| | HG-IV (central st.) | 140 | 1444 | 5793 | 343 | 627 | 2972 |
| | HG-V | 137 | 1606 | 10993 | 384 | 570 | 2082 |
| | HG-VI | 154 | 2236 | 14174 | 533 | 735 | 2482 |
| Latitudinal transect | N4 | 156 | 2351 | 12166 | 739 | 863 | 3411 |
| | N3 | 158 | 1961 | 11020 | 572 | 718 | 3097 |
| | N2 | 164 | 2017 | 11534 | 475 | 800 | 2542 |
| | N1 | 128 | 2572 | 14943 | 643 | 880 | 2904 |
| | S1 | 157 | 2397 | 13192 | 831 | 790 | 3511 |
| | S2 | 163 | 2671 | 13264 | 1036 | 850 | 3729 |
| | S3 | 159 | 2196 | 11490 | 569 | 820 | 2502 |
| | Total* | 289 | 12011 | 137075 | 7430 | 3705 | 33778 |

Abbreviations: $OTU_{ARISA}$: Operational taxonomic unit as determined by binning ARISA peaks with a window size 2; $OTU_{3\%}$: Clustered sequences from MPTS at 97% sequence identity; $SSO_{abs}$ (absolute singletons): $OTU_{3\%}$ with only one sequence in the whole dataset. $SSO_{rel}$ (relative singletons): $OTU_{3\%}$ with only one sequence in a given sample but more than one sequence in the whole dataset; Chao1 richness estimates per station were calculated on normalized data based on the least abundant one (HG-II, 3,716 sequences). *: Total numbers in the whole dataset.

**Table S5. Spearman's correlation matrix of alpha diversity measures, water depth and pigment concentrations (CPE).**

| | **Water depth** | **CPE** | **$OTU_{3\%}$** | **$SSO_{abs}$** | **$SSO_{rel}$** | **Chao1 richness estimator** | **$OTU_{3\%}$ without $SSO_{abs}$** | **$OTU_{ARISA}$** | **reads** |
|---|---|---|---|---|---|---|---|---|---|
| **Water depth** | | 0.112 | 0.415 | 0.494 | 0.517 | 0.541 | 0.364 | 0.364 | 0.082 |
| **CPE** | -0.46 | | 0.058 | 0.344 | 0.078 | 0.845 | **0.044** | 1.000 | ***0.012*** |
| **$OTU_{3\%}$** | 0.25 | -0.54 | | ***0.000*** | ***0.000*** | 0.168 | ***0.000*** | 0.263 | ***0.000*** |
| **$SSO_{abs}$** | 0.21 | -0.29 | ***0.89*** | | ***0.000*** | ***0.008*** | ***0.010*** | 0.144 | ***0.011*** |
| **$SSO_{rel}$** | 0.20 | -0.51 | ***1.00*** | ***0.90*** | | 0.162 | ***0.000*** | 0.231 | ***0.000*** |
| **Chao1 richness estimator** | -0.20 | -0.06 | 0.41 | ***0.70*** | 0.41 | | 0.762 | 0.566 | 0.817 |
| **$OTU_{3\%}$ without $SSO_{abs}$** | 0.28 | **-0.57** | ***0.92*** | ***0.70*** | ***0.91*** | 0.09 | | 0.334 | ***0.000*** |
| **$OTU_{ARISA}$** | 0.28 | 0.00 | 0.34 | 0.43 | 0.36 | 0.18 | 0.29 | | 0.334 |
| **reads** | 0.50 | ***-0.67*** | ***0.90*** | ***0.68*** | ***0.87*** | 0.07 | ***0.93*** | 0.29 | |

Upper matrix triangle indicates p-values; lower matrix triangle indicates the Spearman's correlation value. Bold font indicates significant correlations; italic font indicates still significance after correction for multiple comparisons using the false discovery rate. Longitude, latitude, porosity, proteins and phospholipids did not show significant correlations and are therefore not shown.

**Table S6. Percentages of pairwise shared, lost and gained $OTU_{3\%}$ (A), $OTU_{3\%}$ without $SSO_{abs}$ (B) and $OTU_{ARISA}$ (C).**

| | **A** | HG-I | HG-II | HG-III | HG-IV | HG-V | HG-VI | N4 | N3 | N2 | N1 | S1 | S2 | S3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Bathymetric transect | HG-I | | 53 / 23 | 33 / 45 | 45 / 34 | 43 / 38 | 32 / 47 | 31 / 49 | 36 / 43 | 34 / 43 | 27 / 50 | 31 / 50 | 28 / 53 | 32 / 46 |
| | HG-II | 25 | | 20 / 60 | 30 / 49 | 29 / 53 | 20 / 62 | 19 / 63 | 22 / 58 | 20 / 58 | 15 / 65 | 19 / 64 | 17 / 67 | 19 / 61 |
| | HG-III | 22 | 21 | | 52 / 29 | 49 / 33 | 38 / 41 | 37 / 43 | 42 / 38 | 41 / 38 | 33 / 44 | 37 / 45 | 34 / 48 | 38 / 40 |
| | HG-IV | 21 | 21 | 19 | | 37 / 43 | 26 / 52 | 25 / 54 | 30 / 48 | 28 / 49 | 21 / 56 | 25 / 55 | 23 / 59 | 26 / 51 |
| | HG-V | 19 | 19 | 18 | 21 | | 28 / 49 | 29 / 51 | 34 / 46 | 32 / 46 | 26 / 54 | 29 / 52 | 26 / 55 | 31 / 49 |
| | HG-VI | 21 | 19 | 20 | 22 | 23 | | 37 / 40 | 43 / 35 | 41 / 35 | 33 / 42 | 37 / 41 | 35 / 45 | 39 / 38 |
| Latitudinal transect | N4 | 21 | 18 | 20 | 21 | 20 | 22 | | 44 / 33 | 43 / 34 | 35 / 41 | 39 / 40 | 36 / 44 | 41 / 37 |
| | N3 | 21 | 20 | 20 | 22 | 21 | 22 | 22 | | 38 / 39 | 30 / 46 | 33 / 45 | 31 / 49 | 35 / 42 |
| | N2 | 22 | 22 | 21 | 23 | 22 | 24 | 23 | 23 | | 29 / 44 | 34 / 44 | 31 / 48 | 35 / 40 |
| | N1 | 23 | 20 | 23 | 23 | 21 | 24 | 24 | 24 | 26 | | 41 / 37 | 38 / 40 | 42 / 32 |
| | S1 | 19 | 18 | 18 | 20 | 19 | 21 | 21 | 22 | 22 | 22 | | 37 / 44 | 42 / 36 |
| | S2 | 19 | 16 | 18 | 18 | 19 | 20 | 20 | 20 | 21 | 22 | 19 | | 45 / 34 |
| | S3 | 22 | 21 | 22 | 23 | 20 | 23 | 23 | 23 | 25 | 26 | 22 | 21 | |

| | **B** | HG-I | HG-II | HG-III | HG-IV | HG-V | HG-VI | N4 | N3 | N2 | N1 | S1 | S2 | S3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Bathymetric transect | HG-I | | 46 / 20 | 30 / 35 | 41 / 29 | 39 / 34 | 27 / 44 | 28 / 44 | 33 / 36 | 29 / 39 | 21 / 36 | 30 / 41 | 28 / 41 | 27 / 41 |
| | HG-II | 34 | | 20 / 50 | 29 / 43 | 28 / 48 | 18 / 57 | 18 / 55 | 21 / 50 | 18 / 53 | 13 / 60 | 19 / 54 | 18 / 56 | 17 / 55 |
| | HG-III | 35 | 31 | | 43 / 27 | 41 / 31 | 28 / 41 | 29 / 38 | 35 / 33 | 31 / 37 | 22 / 43 | 31 / 38 | 30 / 39 | 28 / 38 |
| | HG-IV | 30 | 28 | 30 | | 32 / 39 | 21 / 49 | 22 / 46 | 26 / 41 | 23 / 45 | 15 / 52 | 23 / 46 | 22 / 48 | 21 / 46 |
| | HG-V | 27 | 25 | 28 | 29 | | 23 / 45 | 26 / 44 | 30 / 39 | 27 / 42 | 21 / 50 | 27 / 43 | 25 / 44 | 27 / 45 |
| | HG-VI | 29 | 25 | 31 | 31 | 33 | | 35 / 31 | 40 / 27 | 36 / 30 | 28 / 37 | 36 / 31 | 35 / 32 | 35 / 32 |
| Latitudinal transect | N4 | 31 | 27 | 32 | 32 | 30 | 34 | | 37 / 27 | 34 / 31 | 26 / 38 | 33 / 32 | 32 / 33 | 32 / 33 |
| | N3 | 31 | 29 | 32 | 33 | 31 | 33 | 35 | | 29 / 36 | 21 / 43 | 28 / 36 | 28 / 39 | 27 / 38 |
| | N2 | 32 | 30 | 32 | 33 | 30 | 34 | 35 | 34 | | 23 / 39 | 33 / 34 | 31 / 35 | 30 / 34 |
| | N1 | 33 | 28 | 36 | 33 | 30 | 35 | 37 | 36 | 38 | | 40 / 26 | 38 / 27 | 37 / 25 |
| | S1 | 29 | 27 | 31 | 31 | 29 | 33 | 35 | 35 | 34 | 35 | | 32 / 35 | 31 / 34 |
| | S2 | 31 | 26 | 31 | 30 | 31 | 33 | 35 | 34 | 34 | 36 | 34 | | 33 / 32 |
| | S3 | 32 | 29 | 34 | 33 | 28 | 33 | 35 | 35 | 36 | 38 | 35 | 35 | |

Table 6 continued.

| | C | HG-I | HG-II | HG-III | HG-IV | HG-V | HG-VI | N4 | N3 | N2 | N1 | S1 | S2 | S3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Bathymetric transect | HG-I | | 12 / 22 | 17 / 28 | 28 / 31 | 29 / 31 | 25 / 35 | 23 / 34 | 24 / 36 | 21 / 36 | 29 / 27 | 25 / 36 | 20 / 35 | 21 / 34 |
| | HG-II | 66 | | 19 / 21 | 29 / 23 | 31 / 25 | 27 / 29 | 25 / 28 | 25 / 29 | 21 / 28 | 31 / 19 | 24 / 27 | 20 / 26 | 21 / 25 |
| | HG-III | 55 | 59 | | 30 / 24 | 33 / 25 | 28 / 28 | 24 / 25 | 27 / 29 | 20 / 26 | 31 / 17 | 25 / 26 | 22 / 27 | 20 / 23 |
| | HG-IV | 41 | 48 | 46 | | 23 / 21 | 22 / 29 | 17 / 26 | 14 / 23 | 11 / 24 | 21 / 14 | 14 / 23 | 13 / 26 | 10 / 20 |
| | HG-V | 39 | 44 | 42 | 56 | | 13 / 23 | 16 / 26 | 17 / 28 | 14 / 28 | 24 / 19 | 17 / 28 | 15 / 29 | 16 / 28 |
| | HG-VI | 40 | 45 | 44 | 49 | 64 | | 21 / 22 | 22 / 24 | 20 / 25 | 30 / 16 | 22 / 24 | 20 / 25 | 21 / 23 |
| Latitudinal transect | N4 | 43 | 47 | 51 | 57 | 58 | 57 | | 16 / 17 | 14 / 18 | 27 / 11 | 15 / 16 | 15 / 18 | 13 / 15 |
| | N3 | 39 | 47 | 45 | 63 | 55 | 54 | 66 | | 11 / 15 | 28 / 11 | 15 / 14 | 14 / 17 | 17 / 17 |
| | N2 | 43 | 52 | 54 | 65 | 58 | 55 | 68 | 74 | | 27 / 7 | 14 / 10 | 14 / 14 | 14 / 11 |
| | N1 | 44 | 49 | 52 | 65 | 57 | 54 | 61 | 61 | 66 | | 9 / 26 | 7 / 27 | 6 / 25 |
| | S1 | 39 | 49 | 49 | 63 | 56 | 54 | 69 | 71 | 76 | 65 | | 12 / 16 | 12 / 13 |
| | S2 | 45 | 54 | 50 | 61 | 56 | 55 | 67 | 69 | 72 | 65 | 72 | | 13 / 11 |
| | S3 | 46 | 54 | 57 | 70 | 56 | 56 | 72 | 66 | 75 | 69 | 75 | 76 | |

Lower-matrix triangle: Pairwise shared OTU; upper-matrix triangle: percentages of unique OTU in the station on the row and column respectively.
For example, 25 % of $OTU_{3\%}$ (a) are shared between stations HG-I and HG-II, 53 % are unique to station HG-I and 23 % are unique to station HG-II.

## Table S7. Community response to water depth at different taxonomic levels.

| | $OTU_{3\%}$ | | | | | |
|---|---|---|---|---|---|---|
| | All | | $SSO_{rel}$ only | | $SSO_{abs}$ removed | |
| | r[b] | $R^2$ adj.[b] | r | $R^2$ adj. | r | $R^2$ ad.j |
| Phylum | 0.29* | 24** | ~ | 14** | 0.26* | 23** |
| Class | 0.30* | 23** | 0.44** | 19** | 0.29* | 22** |
| Order | 0.34* | 13* | 0.48** | 14** | 0.33* | 13* |
| Family | 0.39* | 13* | 0.39** | 13** | 0.37* | 13* |
| Genus | 0.52** | 12* | 0.57** | 13** | 0.47* | 14* |
| $OTU_{3\%}$ | 0.70*** | 7** | 0.68*** | 6* | 0.71*** | 9*** |

$OTU_{3\%}$: Clustered sequences from MPTS at 97% sequence identity; $SSO_{rel}$: $OTU_{3\%}$ with only one sequence in at least one sample but more than one sequence in the whole dataset. $SSO_{abs}$: $OTU_{3\%}$ with only one sequence in the whole dataset (absolute singletons);[a]The significance of Spearman's correlation coefficients (r) between relative OTU abundance tables and water depth was determined by Mantel tests. [b]Redundancy analysis (RDA) was used to determine the amount of variation ($R^2$ adjusted) in the community data that can be explained by water depth. Significance levels are indicated as *** $p \leq 0.001$, ** $p \leq 0.01$, * $p \leq 0.05$, ~: not significant $p > 0.05$.

**Table S8. Linear and quadratic regression of phyla and classes in the $OTU_{3\%}$ dataset.**

| | | | water depth | | | | CPE | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | lm | | qm | | lm | | qm | | |
| | | No. of seq. | $R^2$ | sign | $R^2$ | sign | $R^2$ | sign | $R^2$ | sign | # |
| phyla | Actinobacteria | 7988 | | | | | 0.38 | - | | | 13 |
| | Acidobacteria | 6720 | 0.31 | - | | | | | | | 13 |
| | Verrucomicrobia | 6505 | 0.64* | - | | | 0.30 | + | | | 13 |
| | Planctomycetes | 5227 | 0.63* | - | | | 0.27 | + | 0.49 | + | 13 |
| | Deferribacteres | 978 | 0.43 | + | 0.62 | + | | | | | 13 |
| | Thermodesulfobacteria | 844 | | | 0.25 | - | | | 0.65 | + | 13 |
| | Lentisphaerae | 647 | 0.34 | - | | | | | | | 13 |
| | Candidate division OP3 | 354 | | | | | | | 0.35 | + | 13 |
| | BD1-5 | 320 | 0.31 | + | | | | | | | 13 |
| | Candidate division TM6 | 230 | | | 0.37 | - | | | 0.32 | + | 13 |
| | Chlorobi | 208 | | | | | | | 0.27 | + | 13 |
| | Candidate division TG-1 | 104 | | | 0.45 | + | | | | | 13 |
| | NPL-UPA2 | 72 | | | 0.30 | - | | | | | 11 |
| | Deinococcus-Thermus | 53 | 0.25 | - | | | | | | | 11 |
| | GOUTA4 | 19 | | | | | | | 0.23 | - | 10 |
| class | Actinobacteria | 7988 | | | | | 0.38 | - | | | 13 |
| | Acidobacteria | 3563 | 0.26 | - | | | | | | | 13 |
| | Alphaproteobacteria | 6154 | 0.48 | - | | | 0.36 | + | 0.62 | + | 13 |
| | Verrucomicrobiae | 4912 | 0.60* | - | | | 0.31 | + | | | 13 |
| | Planctomycetacia | 2108 | 0.70* | - | 0.86 | + | 0.39 | + | 0.65 | + | 13 |
| | RB25 | 2062 | 0.35 | - | | | | | | | 13 |
| | Unclassified Deferribacterales | 975 | 0.43 | + | 0.62 | + | | | | | 13 |
| | JTB23 | 870 | | | | | | | 0.31 | + | 13 |
| | Thermodesulfobacteria | 844 | | | 0.25 | - | | | | | 13 |
| | OM190 | 670 | | | | | | | | | 13 |
| | Opitutae | 657 | 0.48 | - | | | | | | | 13 |
| | Lentisphaeria | 647 | 0.34 | - | | | | | | | 13 |
| | TA18 | 264 | | | | | | | | | 13 |
| | Chlorobia | 208 | | | | | | | 0.27 | + | 13 |
| | OPB35 | 134 | | | 0.45 | + | | | | | 13 |
| | KD4-96 | 106 | 0.38 | - | | | | | | | 13 |
| | Candidatus Kuenenia | 42 | 0.53 | - | | | 0.35 | + | | | 13 |
| | Thermales | 39 | 0.29 | - | | | | | | | 11 |
| | Lineage I Endomicrobia | 31 | 0.30 | + | | | 0.28 | - | | | 11 |
| | Acidimethylosilex | 25 | | | | | 0.29 | - | | | 9 |
| | GIF3 | 10 | 0.36 | + | | | | | | | 7 |

Only those phyla and classes which showed a significant relation are shown here ($p < 0.05$); * indicates still significance after correction for multiple comparisons using the false discovery rate. Linear model: - and + indicate decrease or increase with increasing water depth or CPE concentrations, respectively; for quadratic model: - and + indicate maximum or minimum relative abundance at intermediate water depth or CPE concentrations, respectively. # indicates the number of samples where a taxon was present.

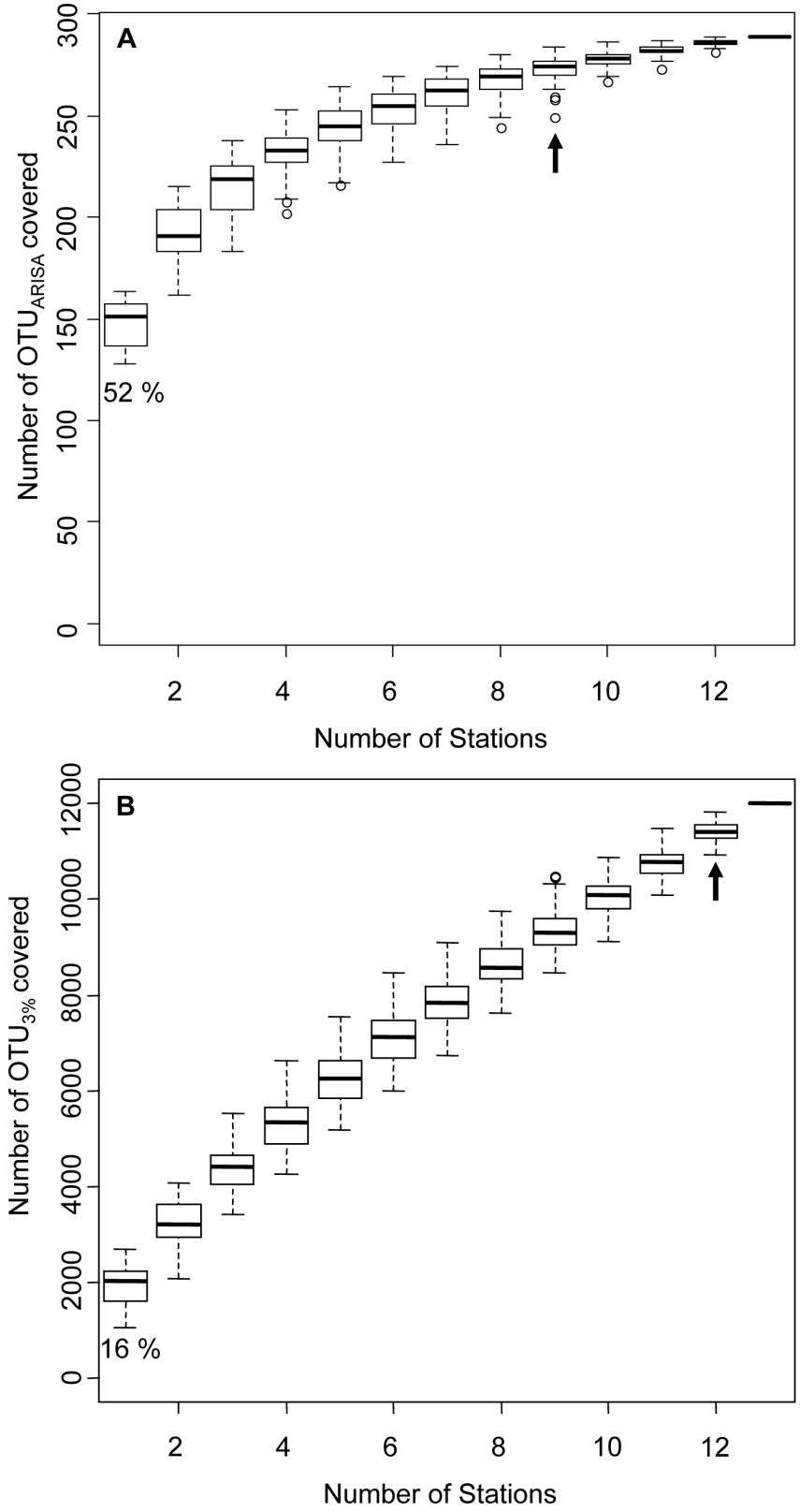


**Figure S1**. **OTU accumulation curves**. (A) based on ARISA data, (B) based on 454 MPTS data. The percentages indicated for n=1 station correspond to how much diversity would be recovered on average by randomly sampling only one station. The arrows indicate the number of stations needed to recover 95% of observed OTU.

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# Chapter II

## Deep-sea microbial communities are fast indicators of particle flux variations in a warmer Arctic ocean

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**Abstract**

The rapid warming of the Arctic has manifested in substantial sea ice decrease, but little is known on its ecological consequences for marine ecosystems. Model simulations suggest increasing primary productivity in the Arctic due to sea ice retreat, and field observations show that shifts in plankton composition may cause substantial interannual variations in particle flux. Here, we investigated the relationships between surface ocean processes and seafloor microbial community composition during an ocean warming anomaly. Annual samples were taken in the summers of 2003 to 2009 at the Long-Term Ecological Research (LTER) site HAUSGARTEN (79°N, 4°E), located in the eastern Fram Strait. During this time period, a substantial increase in water temperatures in combination with a low in ice-cover was recorded in Fram Strait in 2005-2007. Changes in seafloor bacterial diversity were tightly coupled to variations in surface ocean conditions and in phytodetritus export fluxes, but individual bacterial taxa of both rare and resident types responded differently to changes in food supply.

## Introduction

In the Arctic Ocean the season for phytoplankton growth and organic matter export to the deep sea is restricted to the months of May-August, due to light limitation during the rest of the year (Tremblay and Gagnon, 2009). Stratification of surface waters by warming, sea-ice melt and low wind-mixing lead to phytoplankton blooms in spring. Upon the depletion of nutrients in July (Vaquer-Sunyer et al., 2013), the phytoplankton biomass is exported to the deep sea and eventually nourishes benthic organisms (Klages et al., 2004). Climatic changes in the Arctic that lead to an increase of surface water temperatures and a decrease of sea ice extent and thickness (e.g. Reigstad et al., 2011; Wassmann, 2011; Beszczynska-Möller et al., 2012) will vary in effect between regions, but will generally alter the timing and location of pelagic primary production (Strass and Nöthig, 1996; Markus et al., 2009; Kahru et al., 2010; Cherkasheva et al., 2014).

Mathematical modelling of the effects of climatic changes in Fram Strait and the Barents Sea in the past decade mostly focused on productivity in surface waters, and found rather low interannual variations in productivity of < ±20% (70-90 g C $m^{-2}$ $yr^{-1}$) (Wassmann et al., 2010; Drinkwater, 2011 and references therein). Thus, surface productivity of the Barents Sea and Svalbard region did not appear to be affected by Climate Change until the extremely low sea-ice cover was observed in 2007 (Reigstad et al., 2011). This conclusion was recently confirmed by chlorophyll data from a remote sensing study on ocean color variations in the NW Svalbard region (Cherkasheva et al., 2014). However, sediment trap data revealed that the composition of the plankton communities and the quantities of export fluxes have changed considerably during the past decade (Bauerfeind et al., 2009; Kraft et al., 2011; Lalande et al., 2011). These variations could be related to the warming anomaly between 2005-2007 Beszczynska-Möller et al., 2012), that manifested in low organic matter export to the deep sea as well as a shift in the composition and quality of sinking organic matter (Lalande et al., 2013). The previously diatom-dominated community shifted to a coccolithophorid-dominated community since 2005 (Bauerfeind et al., 2009), and polar zooplankton was replaced by Atlantic water species (Kraft et al., 2011; Bauerfeind et al., 2014).

Arctic deep-sea benthic organisms are energy limited and fresh phytodetritus from settling blooms represents the major energy source for the benthic community. Up to 95% of benthic biomass in the deep sea is made up by bacteria (Soltwedel et al., 2000), which perform the

initial step in benthic organic matter degradation and provide degradation products to larger organisms (reviewed by Jørgensen and Boetius, 2007). Previous studies observed rapid responses of the deep-sea microbial communities to pulses of organic matter supply, such as changing respiration rates, biomass shifts and increased enzymatic activities (Boetius and Lochte, 1996; Pfannkuche et al., 1999; Moodley et al., 2002; Witte et al., 2003; Smith et al., 2013). Furthermore, a coupling between phytodetritus input and bacterial community richness was detected previously in Arctic sediments (Bienhold et al., 2011; Jacob et al., 2013). Here we investigate whether surface warming and sea ice retreat result in enhanced particle fluxes and increased seafloor bacterial community richness. In order to test responsiveness and resilience of deep-sea microbial communities to changes in surface ocean productivity and particle fluxes to the seafloor, comparative analyses of bacterial community composition at 2500 m water depth were carried out before, during and after the 2005-2007 warming anomaly.

## Results

*Interannual change in particle flux to the seafloor*

Average phytodetritus input to the seafloor, measured as the sum of chlorophyll *a* and its degradation products phaeopigments (chloroplastic pigment equivalents, CPE; Thiel, 1978) within sediments, generally decreased by >50% from 2003 to 2006 and was significantly elevated by 2-3-fold in subsequent years (Figure 1). CPE concentrations were significantly correlated to the total POC flux (data from Lalande et al., 2013) integrated over 60 days before sampling (Spearman's $\rho = 0.82$, $p = 0.023$). Likewise, average mixed layer depth (data from Cherkasheva et al., 2014) in spring was significantly negatively correlated with POC flux and CPE input to the seafloor ($\rho = -0.79$, $p = 0.036$ and $\rho = -0.93$, $p = 0.003$, respectively), suggesting that surface ocean dynamics substantially influence export fluxes to the seafloor.

*Composition of bacterial community at the HAUSGARTEN LTER*

Results of 454 massively parallel tag sequencing (MPTS) revealed that the bacterial community in 2003 at HAUSGARTEN was composed mostly of Proteobacteria (48% of detected operational taxonomic units clustered at 3% identity; $OTU_{3\%}$), followed by Verrucomicrobia (12%), Actinobacteria (10%), Bacteroidetes (9%), and Acidobacteria (7%). Most of the Proteobacterial OTU were classified as Gammaproteobacteria (63%), Deltaproteobacteria (19%), and Alphaproteobacteria (15%).

Only 42 $OTU_{3\%}$ accounted for 50% sequence abundance in the whole dataset (Table S1). These $OTU_{3\%}$ were present at all stations at every time and were classified to as 13 different classes. Most of these abundant $OTU_{3\%}$ showed little variation between years. The most abundant $OTU_{3\%}$ was affiliated with the family *Sinobacteraceae* (OTU ID 2) and had a total relative sequence abundance of 10%. The representative sequence of this $OTU_{3\%}$ was highly similar to sequences previously found in the Antarctic and Arctic (Fram Strait) as well as other more temperate oceanic regions, as determined by Geographic-BLAST (see Methods section).

*Interannual change in richness of bacterial taxa*

DNA fingerprinting with ARISA showed that the observed operational taxonomic units ($OTU_{ARISA}$) decreased substantially during the warm anomaly in 2005-2007 and again increased in 2008 to a similar level as in 2003 (Figure 1). Observed and estimated richness of $OTU_{3\%}$ showed a similar trend, although only a subset of stations was used (Figure 1, Table 1, Table S2). Chao1 richness estimates were lowest in 2006 and exhibited relatively elevated values in the years 2003 and 2007-2009. Chao1 richness estimates showed significant correlations with CPE concentrations ($\rho = 0.74$, $p = 0.001$) as well as the year of sampling ($\rho = 0.52$, $p = 0.046$) (Table 1, Table S3). The most abundant $OTU_{3\%}$ was affiliated with the family *Sinobacteraceae* (OTU 2) and showed a significant linear increase from 2003 to 2009 (Table S1). Other abundant $OTU_{3\%}$ that showed an increase with time were classified as Acidimicrobineae and Rubritalea, while abundant $OTU_{3\%}$ that decreased with time were classified as Nitrosospira and Deltaproteobacteria (Table S1). Only one abundant $OTU_{3\%}$, which was classified as Acidobacteria, showed a strong decrease with decreasing CPE concentrations (Table S1).

*Bacterial beta-diversity patterns*

Bacterial community structure (relative abundance of $OTU_{ARISA}$) showed strong interannual variations, with the year 2006 being most dissimilar to all other years (Figure S1). For the MPTS subset, patterns in community structure changed gradually from 2003 to 2009, except for the bacterial community structure in 2006, which differed from all other years (Canonical Redundancy Analysis: $p = 0.002$; Figure 1). This distinct bacterial community structure of 2006 compared to all other years of sampling was confirmed by sorting the order of $OTU_{3\%}$ according to their relative abundance per year and further subjecting them to pairwise

Spearman rank correlation tests (Table S4). The shift in community structure was detected in the rare biosphere as well as the resident bacterial types determined by MPTS and bacterial types ARISA (Figure S1).
Despite the decline in OTU richness in 2006, the bacterial community had up to 32% - 46% OTU in common with previous years (without considering singletons) (Table S5). Mostly $OTU_{3\%}$ with low sequence abundance were lost or gained in 2006, while those with higher abundances remained present.

*Indicator taxa for variations in food supply to the deep sea*
We aimed to identify deep-sea indicator taxa ($OTU_{3\%}$) for both low and high phytodetritus input by assessing shifts in sequence abundance between 2006 and other years. The strong interannual variation of CPE concentrations could explain variations in relative sequence abundances at the phylum (35%), class (36%) and genus (30%) levels (Table S6). Several of the $OTU_{3\%}$ showed significant linear relationships to CPE concentrations with high regression slope values (Table S7). $OTU_{3\%}$ with strong positive relationships were classified as *Roseospira* (Alphaproteobacteria), *Calditrhrix* (Deferribacteres), *Microscilla* (Bacteroidetes) or *Pelagibus* (Alphaproteobacteria). Other $OTU_{3\%}$ showed negative relationships and were classified as *Coxiella* (Gammaproteobacteria), *Fangia* (Gammaproteobacteria), or *Acidobacteriaceae* (Acidobacteria) (Table S7).

In total 26 $OTU_{3\%}$ exhibited higher relative sequence abundance in 2006 than in all other years (Table S8). Representative sequences of these indicator $OTU_{3\%}$ were highly similar to sequences previously found in other deep-sea regions, e.g. Pacific and Atlantic, as determined by Geographic-BLAST (see Methods section). All $OTU_{3\%}$ affiliated with the genus *Glaciecola*, a genus within the Gammaproteobacteria that was previously found in polar sea-ice and Arctic sediments (see Qin et al., 2013 and references therein), were absent in the years 2006 and 2007.

**Discussion**

High fluctuations of sea ice concentration, surface water temperature and primary productivity were observed at the LTER HAUSGARTEN between 2003 and 2009, as well as in the wider Svalbard area (Strass and Nöthig, 1996; Markus et al., 2009; Kahru et al., 2010; Reigstad et al., 2011; Wassmann, 2011; Beszczynska-Möller et al., 2012; Lalande et al., 2013;

Cherkasheva et al., 2014). Ice concentration in Fram Strait is mostly driven by transport of ice with the Transpolar Drift, and large variations are expected with the ongoing thinning of the Arctic ice cover (Krumpen et al., 2013). In spring/summer 2003 and 2008 ice concentrations were high compared to other years (Lalande et al., 2013) (Figure 1). In 2005-2007, warm Atlantic water masses reached further northward than in preceding years, leading to a record warm anomaly (Beszczynska-Möller et al., 2012; Walczowski et al., 2012). In this area increased ice melt can lead to higher stratification, which in turn leads to a stronger phytoplankton bloom earlier in the year (Cherkasheva et al., 2014). Furthermore, ice melt above the investigated site was shown to change magnitude and composition of POC exported to the deep sea, resulting in short-term POC pulses from the melting ice (Lalande et al., 2011; Lalande et al., 2013). Assuming a sinking speed of ~100 or 300 meter per day, as is it was observed for marine snow (see Alldredge and Silver, 1988) and feces (Pfannkuche and Lochte, 1993), respectively, it would take 8-25 days for organic matter to reach the seafloor at 2500 meter water depth. In the North Pacific, a sinking speed of ~ 100 m per day and time lag of 40-60 days was reported for depth of 4100 m (Baldwin et al., 1998), underlining the fast sinking of phytodetritus to the seafloor. This explains why the change in POC flux was directly reflected in sediment-bound pigment (CPE) concentrations first in a decline of CPE from 2003 to 2006 and then in an increase from 2007 to 2009. An important question investigated in this study was whether we could also observe changes in bacterial community structure.

Previous investigations on the Beaufort Shelf (Alaska) have shown that sediment bacteria can reflect differences in surface water characteristics (Hamdan et al., 2013). Deep-sea bacteria react to food pulses with increased respiration rates and hydrolytic enzyme activities (Lochte and Turley, 1988; Boetius and Lochte, 1996; Kanzog et al., 2009; Smith et al., 2013), that appear relatively unaffected by the cold temperatures of Arctic bottom waters of < 0°C (Boetius et al., 2013). The strength of benthic microbial response, e.g. increase in activity or biomass, is assumed to be dependent on the quantity of organic matter supply (Pfannkuche et al., 1999; Moeseneder et al., 2012). An influence of both quantity and quality of detritus input on bacterial community structure in HAUSGARTEN was previously observed in an *in situ* experiment where chitin was provided in different concentrations to living sediments at 2500 m water depth (Kanzog et al., 2009). Yet little is known on the time-scale and magnitude of community shifts induced by natural variations in POC flux. Here we investigate whether interannual variations in POC flux instantly impact the bacterial community. This hypothesis

was supported by several observations, such as the positive correlation of bacterial richness (observed and estimated) with pigment concentrations (Figure 1), and the substantial shift in bacterial community structure over time (Figure 2).

Similar to our observation at HAUSGARTEN, a positive relationship between bacterial richness estimates and sediment pigment concentrations was previously found on the Siberian margin and was explained by the strong food limitation in an area marked by POC fluxes < 1 g $m^{-2}$ $yr^{-1}$ (Bienhold et al., 2011). Such a relationship between food availability and deep-sea benthic community diversity is known for oligotrophic deep-sea regions, when energy supply is limiting population density and niche differentiation (Smith et al., 2008). We observed that the sequence abundance of several bacterial taxa declined between 2003 and 2006, while some taxa were no longer detected, but re-appeared after 2007. These observations indicate that their populations declined below detection limits due to the reduced organic matter availability. However, other bacterial types increased in relative sequence abundance in 2006, suggesting that some bacteria were adapted to the usually low energy supply in deep-sea ecosystem and to only episodic and short pulses of high energy supply. These substantial shifts in bacterial community structure, that coincided with a strong surface warming anomaly in the Svalbard area in the period 2005-2007 (Beszczynska-Möller et al., 2012; Figure 2), reveal the instant impact of surface water conditions on the benthic ecosystem.

During this warm anomaly, a relative decrease in diatom detritus exported to the deep sea was observed. Instead coccolithophores dominated the surface waters, altering the silicate to carbon ratio of the sinking matter (Bauerfeind et al., 2009; Lalande et al., 2013). Also fecal pellet volumes decreased during this phase, which might indicate a shift in zooplankton community composition (Lalande et al., 2013). Due to the high amounts of labile organic carbon in diatoms, bacteria can mineralize faster than carbon derived from fecal pellets (Mayor et al., 2012). The hydrographic change in Fram Strait had changed not only the the quantity but also the quality of organic matter deposited at the seafloor, which likely impacted the bacterial communities. This change in organic matter availability was also reflected in substantial interannual variations in megafaunal densities in the HAUSGARTEN area (Bergmann et al., 2011; Meyer et al., 2013). Overall megafaunal densities and diversity decreased from 2002 to 2007 and was dominated by only one feeding type in 2007 (Bergmann et al., 2011). Hence, compared to the shift in bacterial communities, the response in megafauna composition appeared to be delayed by a year. Previously, megafauna in the

deep northeast Pacific was observed to react with a time lag of 10 – 13 months to changes in the input of organic matter (Ruhl, 2008). Thus, megafaunal communities reflect changes in surface water conditions much slower than bacterial communities and did not impact the bacterial community structure. Similarly, abundances of nematodes, the most abundant metazoan taxon at HAUSGARTEN (Hoste et al., 2007), were shown to change with a time lag of 8-9 months to climate-related changes in food supply (Smith et al., 2009), and were probably also not a reason for the drastic changes in bacterial community structure in 2006.

Our observations suggest that benthic bacterial communities promptly respond to interannual variations in particle flux, which was in turn controlled by hydrographic variations such as warming or changes in ice concentrations. Warming and sea ice retreat is often assumed to result in enhanced primary productivity and organic matter export to the deep sea. In contrast, our observations indicate a substantial decline in food supply that is reflected by a strong shift of the benthic bacterial community. In view of the ongoing climate warming in the Arctic, our data suggest that major shifts both in surface and deep-sea life are to be expected. Monitoring the Arctic ecosystems at high spatial and temporal resolution is hence crucial to assess the impact of future climatic variations on benthic ecosystems.

## Methods

*Surface Ocean data*

Satellite based estimates of sea-ice concentration, primary production and mixed layer depth in the HAUSGARTEN area were previously published by Cherkasheva et al. (2014). Of these data, average values were calculated for a 60 day time period three to one month before benthic sampling. Data on the particulate organic matter (POC) export derived from sediment traps at 179 – 280 m water depth at the central HAUSGARTEN station HG-IV was published by Lalande et al. (2011), and the 60 day sum of the time period three to one month before benthic sampling was calculated. POC export data in 2005 was integrated from only 30 days of measurement. Data on primary productivity in 2008 and POC flux in 2004 was not available and was therefore calculated as the average of the previous and succeeding year.

*Sampling*

During six summer cruises to HAUSGARTEN observatory between 2003 and 2009, of which five were carried out using the German research ice-breaker RV Polarstern and one (in 2006) using the German research vessel RV Maria S. Merian, samples of virtually undisturbed sediments where taken using a TV-multiple corer (TV-MUC) (Tab. S1). Each year, samples were taken at up to eight distinct sampling stations across a latitudinal transect of the HAUSGARTEN area west of Svalbard (Soltwedel et al., 2005a) at 78.61 – 79.74°N and ~ 5°E (Figure S2) with water depths ranging from 2339 m to 2802 m (Table S1). TV-MUC cores were sub-sampled using modified 10-ml syringes (2 cm in diameter), sub-divided into 1-cm layers and only the uppermost centimeter representing the most active community (Quéric et al., 2004) was analyzed for bacterial community structure and environmental parameters in this study.

*Sample processing*

Sample processing for determining chloroplastic pigment equivalent (CPE) concentration and other sediment parameters was done as described in (Soltwedel et al., 2005b). Prior to DNA extraction, slurries from the uppermost centimeter of the sediments originating from three different TV-MUC cores were pooled. Total DNA was extracted from 1 g of homogenized sediment sample using the UltraClean Soil DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA) according to the manufacturer's instructions for maximum yields.

PCR for automated ribosomal intergenic spacer analysis (ARISA) was done as described in (Jacob et al., 2013) and separation of fragments by capillary electrophoresis, evaluation of signals and binning into operational taxonomic units (OTU) were done as described in Ramette (2009).

454 massively parallel tag sequencing (MPTS) of extracted DNA was performed at the Marine Biological Laboratory (Woods Hole, MA, USA) according the protocol published on http://vamps.mbl.edu using primers targeting the V4-V6 region of the bacterial 16S rRNA gene. Preparation of flowgrams and transformation into Sample by OTU tables were conducted with "mothur" software (Schloss et al., 2009) according to the standard operating procedure (SOP; Schloss et al., 2011) including the implemented denoising algorithm. Alignment of denoised sequences and taxonomic affiliation were carried out using the SILVA reference file for bacteria (Pruesse et al., 2007) (downloaded from http://www.mothur.org in March 2012) and chimeric sequences were identified using the mothur implemented uchime program. Cleaned sequences were clustered at a 97% identity level into operational taxonomic units ($OTU_{3\%}$) and the dataset was normalized by the total amount of sequences per sample to get relative abundances. The rare biosphere (Sogin et al., 2006) was considered as $OTU_{3\%}$ that consist of only one sequence in at least one sample, but with more than one sequence in the whole dataset (Gobet et al., 2012). $OTU_{3\%}$ that occurred with only one sequence in the whole denoised dataset, called absolute singletons, were subtracted from the whole dataset ($OTU_{3\%\text{ - abs singletons}}$) for some analysis (e.g. Figure S1, Table S5).

*Multivariate statistics*

For specific analyses, e.g. the comparison of shared OTU (Table S5), the $OTU_{3\%}$ table was merged according to year and the average relative abundance was calculated for each OTU per year. Spearman rank (rank-based) correlation analyses were used to find correlations between surface and benthic environmental parameters, between environmental parameters and bacterial richness, and to test whether the order of $OTU_{3\%}$ from high to low abundance correlates between any two years. Non-metric multidimensional scaling (NMDS) was carried out on Bray-Curtis distance matrices.

To determine which environmental factors significantly explained variations in bacterial community structure, redundancy analyses (RDA) were used. In order to find pure effects of certain environmental parameters, we first used stepwise selection (based on canonical

redundancy analysis) with the spatial variables longitude, latitude and water depth, the abiotic factor porosity, the biotic factors protein, CPE, Chl *a*: Phaeopigment ratio, and Sampling Year, and performed canonical variation partitioning using the *varpart* function in the *vegan* (Oksanen et al., 2012) package in the R software (R Development Core Team, 2008; Version 2.14.1). For the investigation of taxonomic groups, the $OTU_{3\%}$ table was grouped according to the taxonomic affiliation using the "taxa.pooler.1.2" of the MultiCoLA software package (Gobet et al., 2010). Indicator values per year were determined with the *indval* function in the *labdsv* (Roberts, 2013) package of the R software and linear regressions were performed to determine linear relationships of $OTU_{3\%}$ or taxa with CPE concentrations. For each $OTU_{3\%}$, one representative sequence with the smallest distance to all other sequences in the $OTU_{3\%}$ was chosen, and the Geographic-BLAST tool on the Megx.net webpage (Kottmann et al., 2010) was used with default options, which gives an overview on the global distribution of this particular sequence.

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## Tables and Figures

**Table 1.** Sequence reads, OTU abundance and richness for ARISA and MPTS datasets.

| Station | Year | $OTU_{ARISA}$ | MPTS sequence reads | $OTU_{3\%}$ | singleton $OTU_{3\%}$ | Estimated Chao1 richness |
|---|---|---|---|---|---|---|
| N1 | 2003 | 143 | 5082 | 1252 | 205 | 2501 |
| N2 | 2003 | 148 | 4866 | 1159 | 186 | 2605 |
| HGIV | 2004 | 132 | 9209 | 1873 | 330 | 2510 |
| N2 | 2004 | 171 | 3864 | 1096 | 176 | 2568 |
| N3 | 2004 | 103 | 13208 | 2670 | 911 | 3484 |
| N1 | 2006 | 75 | 9624 | 1282 | 241 | 1559 |
| N2 | 2006 | 116 | 7460 | 1274 | 280 | 2260 |
| N3 | 2006 | 73 | 12115 | 1656 | 426 | 1948 |
| N1 | 2007 | 151 | 6890 | 1828 | 527 | 3470 |
| HGIV | 2008 | 120 | 4698 | 1261 | 211 | 2539 |
| N2 | 2008 | 118 | 6232 | 1506 | 383 | 3718 |
| N3 | 2008 | 107 | 11817 | 2272 | 720 | 3532 |
| HGIV | 2009 | 140 | 5848 | 1432 | 352 | 3122 |
| N1 | 2009 | 128 | 15036 | 2566 | 600 | 2871 |
| N2 | 2009 | 164 | 11561 | 2014 | 466 | 2621 |
| N3 | 2009 | 158 | 11149 | 1963 | 533 | 3091 |
| N4 | 2009 | 156 | 12218 | 2329 | 738 | 3838 |
| total | | 285 | 150877 | 12262 | 7285 | |

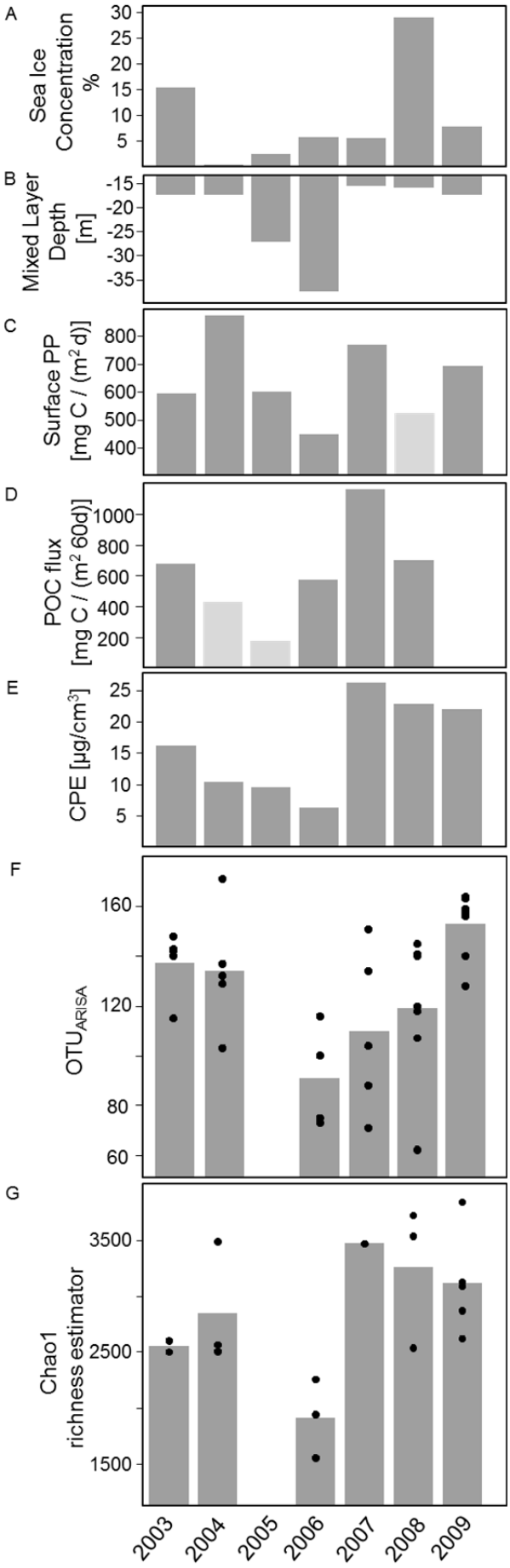


**Figure 1. Interannual variation of the environmental parameters and bacterial richness at HAUSGARTEN.**
(A) Satellite based estimated sea-ice concentration. (B) Mixed layer depth. (C) Modeled surface primary production. (A-C) shows averages for 2 and 3 month before sediment sampling. (D) POC flux at 179- 280 m water depth as sum of fluxes from 2 and 3 month before sampling. (E) Concentrations of chloroplastic pigment equivalents (CPE). (F) OTU richness determined by ARISA. (G) Estimated Chao1 richness of MPTS data. Data from (A), (B), and (C) is from Cherkasheva et al. (2014); data (D) is from Lalande et al. (2013). Light grey bars indicate integrated data (for details see Methods section).

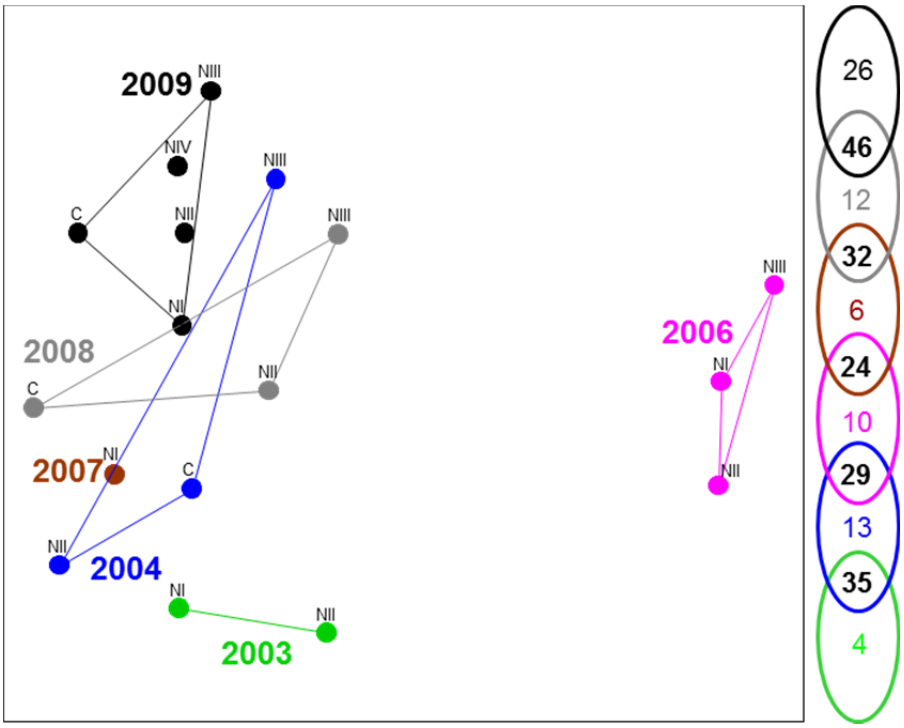


**Figure 2. Beta-diversity patterns of the bacterial community.** Non-metric multidimensional scaling of the dissimilarity in bacterial community structure for the $OTU_{3\%}$ dataset. Sampling station names are indicated above dots. On the right: Total pairwise shared $OTU_{3\%}$ in % between two consecutive years (bold) and unique $OTU_{3\%}$ to a certain year (color). For latter analysis singletons were removed from the dataset.

## Supplementary Tables and Figures

**Table S1** Taxonomic affiliations of most abundant $OTU_{3\%}$ comprising together 50% sequence abundance.

| Taxonomy | OTU ID | % seq. abundance | related to* |
|---|---|---|---|
| Acidobacteria, Acidobacteriales; Acidobacteriaceae; unclassified | 48 | 0.51 | |
| **Acidobacteria, Acidobacteriales; Acidobacteriaceae; unclassified** | 15 | 0.42 | *CPE* |
| **Actinobacteria, Acidimicrobidae; Acidimicrobiales; Acidimicrobineae; unclassified** | 1 | 2.20 | *Year* |
| Actinobacteria, Acidimicrobidae; Acidimicrobiales; Acidimicrobineae; unclassified | 20 | 1.18 | |
| Actinobacteria, Actinobacteridae; Actinomycetales; Corynebacterineae; Nocardiaceae; Williamsia; unclassified | 11 | 1.17 | |
| **Actinobacteria, Actinobacteridae; Actinomycetales; Micromonosporineae; Micromonosporaceae; Stackebrandtia; unclassified** | 5 | 2.05 | 2006 |
| Actinobacteria, unclassified | 50 | 0.71 | |
| Alphaproteobacteria, Rhizobiales; Rhodobiaceae; Parvibaculum; unclassified | 26 | 0.45 | |
| Alphaproteobacteria, Rhodospirillales; Rhodospirillaceae; Pelagibius; unclassified | 10 | 0.54 | |
| Alphaproteobacteria, Rhodospirillales; Rhodospirillaceae; unclassified | 54 | 0.59 | |
| Bacteroidetes; Flavobacteria, Flavobacteriales; Flavobacteriaceae; Ulvibacter; unclassified | 12 | 0.46 | |
| Bacteroidetes; Flavobacteria, Flavobacteriales; Flavobacteriaceae; unclassified | 17 | 0.84 | |
| **Betaproteobacteria, Nitrosomonadales; Nitrosomonadaceae; Nitrosospira; unclassified** | 14 | 1.48 | Year |
| Chloroflexi; Anaerolineae, Anaerolineales; Anaerolineaceae; unclassified | 59 | 0.42 | |
| Deltaproteobacteria, Myxococcales; JG37-AG-15; unclassified | 28 | 0.71 | |
| **Deltaproteobacteria, Sh765B-TzT-29; unclassified** | 19 | 1.95 | Year |
| **Deltaproteobacteria, Sh765B-TzT-29; unclassified** | 38 | 0.92 | 2009 |
| Gammaproteobacteria, Alteromonadales; Alteromonadaceae; OM60_NOR5_clade; Haliea; unclassified | 29 | 1.20 | |
| Gammaproteobacteria, Alteromonadales; Alteromonadaceae; OM60_NOR5_clade; Haliea; unclassified | 18 | 0.70 | |
| Gammaproteobacteria, endosymbionts; unclassified | 40 | 1.13 | |
| Gammaproteobacteria, endosymbionts; unclassified | 57 | 0.46 | |
| Gammaproteobacteria, JTB148; unclassified | 7 | 1.60 | |
| Gammaproteobacteria, KI89A_clade; unclassified | 36 | 0.54 | |
| Gammaproteobacteria, marine_group_E01-9C-26; unclassified | 65 | 0.59 | |
| Gammaproteobacteria, unclassified | 24 | 1.25 | |
| **Gammaproteobacteria, Xanthomonadales; Sinobacteraceae; unclassified** | 2 | 10.36 | Year |
| Gammaproteobacteria, Xanthomonadales; Sinobacteraceae; unclassified | 3 | 2.25 | |
| Gammaproteobacteria, Xanthomonadales; Sinobacteraceae ;unclassified | 13 | 2.23 | |
| **Gammaproteobacteria, Xanthomonadales; Sinobacteraceae; unclassified** | 4 | 1.57 | 2009 |
| Gammaproteobacteria, Xanthomonadales; Sinobacteraceae; unclassified | 23 | 1.39 | |
| Gammaproteobacteria, Xanthomonadales; Sinobacteraceae; unclassified | 27 | 0.61 | |
| Gammaproteobacteria, Xanthomonadales; Sinobacteraceae; unclassified | 32 | 0.60 | |
| Gammaproteobacteria, Xanthomonadales; Sinobacteraceae; unclassified | 16 | 0.56 | |
| Gammaproteobacteria, Xanthomonadales; Sinobacteraceae; unclassified | 79 | 0.40 | |
| Gemmatimonadetes, BD2-11; unclassified | 22 | 1.08 | |
| Gemmatimonadetes, Gemmatimonadales; Gemmatimonadaceae; unclassified | 42 | 0.47 | |
| **Gemmatimonadetes, PAUC43f_marine_benthic_group; unclassified** | 134 | 0.42 | 2006 |
| Thermodesulfobacteria, Thermodesulfobacteriales; Thermodesulfobacteriaceae; Thermodesulfatator; unclassified | 41 | 0.83 | |
| Verrucomicrobiae, Verrucomicrobiales; Rubritaleaceae; Rubritalea; unclassified | 8 | 0.94 | |
| **Verrucomicrobiae, Verrucomicrobiales; Rubritaleaceae; Rubritalea; unclassified** | 6 | 0.75 | *Year* |
| Verrucomicrobiae, Verrucomicrobiales; Verrucomicrobiaceae; Persicirhabdus; unclassified | 9 | 0.54 | |
| Verrucomicrobiae, Verrucomicrobiales; Verrucomicrobiaceae; unclassified | 43 | 0.90 | |

* indicates if OTU shows a significant negative linear relationship with year (*Year*) or CPE concentrations (*CPE*), a significant positive linear relationship with year (Year) or is an indicator value for a given year.

**Table S2** Metadata of sampling stations.

| Hausgarten Station | Event label | Date of event | °N | °E | Elevation of event |
|---|---|---|---|---|---|
| SI | PS64/445-1 | 2003-07-28 | 78.92 | 5.00 | -2636 |
| SII | PS64/484-1 | 2003-08-04 | 78.78 | 5.33 | -2474 |
| SIII | PS64/453-1 | 2003-07-30 | 78.61 | 5.07 | -2343 |
| NI* | PS64/477-1 | 2003-08-03 | 79.28 | 4.33 | -2401 |
| NII* | PS64/480-1 | 2003-08-04 | 79.41 | 4.70 | -2546 |
| C* | PS66/117-1 | 2004-07-09 | 79.08 | 4.08 | -2508 |
| NII* | PS66/126-2 | 2004-07-11 | 79.41 | 4.70 | -2544 |
| NIII* | PS66/127-2 | 2004-07-11 | 79.60 | 5.16 | -2791 |
| SI | PS66/113-2 | 2004-07-08 | 78.92 | 5.00 | -2635 |
| SII | PS66/112-2 | 2004-07-08 | 78.78 | 5.33 | -2460 |
| SIII | PS66/108-1 | 2004-07-08 | 78.63 | 5.05 | -2349 |
| NI* | MSM02/868-1 | 2006-09-05 | 79.28 | 4.33 | -2348 |
| NII* | MSM02/869-2 | 2006-09-05 | 79.41 | 4.71 | -2502 |
| NIII* | MSM02/864-1 | 2006-09-04 | 79.60 | 5.27 | -2650 |
| SII | MSM02/792-2 | 2006-08-26 | 78.78 | 5.33 | -2417 |
| NI* | PS70/193-1 | 2007-07-16 | 79.28 | 4.33 | -2406 |
| NIV | PS70/200-1 | 2007-07-17 | 79.74 | 4.43 | -2644 |
| SI | PS70/179-1 | 2007-07-15 | 78.92 | 5.00 | -2641 |
| SII | PS70/175-1 | 2007-07-14 | 78.78 | 5.33 | -2477 |
| SIII | PS70/174-1 | 2007-07-13 | 78.61 | 5.06 | -2354 |
| C* | PS72/122-2 | 2008-07-09 | 79.07 | 4.18 | -2462 |
| SI | PS72/125-2 | 2008-07-10 | 78.92 | 5.00 | -2637 |
| SII | PS72/126-2 | 2008-07-10 | 78.78 | 5.33 | -2465 |
| SIII | PS72/129-3 | 2008-07-10 | 78.61 | 5.06 | -2342 |
| NII* | PS72/147-3 | 2008-07-15 | 79.43 | 4.76 | -2587 |
| NIII* | PS72/146-1 | 2008-07-14 | 79.59 | 5.21 | -2768 |
| NIV | PS72/145-3 | 2008-07-14 | 79.74 | 4.49 | -2670 |
| C* | PS74/121-1 | 2009-07-16 | 79.06 | 4.18 | -2464 |
| NI* | PS74/120-2 | 2009-07-16 | 79.28 | 4.33 | -2401 |
| NII* | PS74/119-2 | 2009-07-16 | 79.41 | 4.69 | -2545 |
| NIII* | PS74/118-2 | 2009-07-16 | 79.60 | 5.17 | -2786 |
| NIV* | PS74/116-2 | 2009-07-15 | 79.72 | 4.49 | -2802 |
| SI | PS74/127-2 | 2009-07-17 | 78.92 | 5.00 | -2637 |
| SII | PS74/128-2 | 2009-07-18 | 78.78 | 5.33 | -2473 |
| SIII | PS74/129-3 | 2009-07-18 | 78.61 | 5.07 | -2339 |

* samples were used for 454 analyses

**Table S3** Spearman's correlation of environmental factors and bacterial richness or singleton abundance.

| | Year | Chl *a* | Phaeo-pigments | Chl *a* / phaeopigments | CPE | $OTU_{ARISA}$ | Chao1 estimate | absolute singletons | $OTU_{3\%}$ | absolute singletons / $OTU_{3\%}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| Year | | 0.074 | 0.008 | 0.181 | 0.040 | 0.450 | 0.046 | 0.020 | 0.033 | 0.051 |
| Chl *a* | | | 0.002 | 0.355 | 0.000 | 0.024 | 0.000 | 0.060 | 0.104 | 0.109 |
| Phaeopigments | 0.62 | 0.70 | | 0.103 | 0.000 | 0.073 | 0.001 | 0.064 | 0.147 | 0.086 |
| Chl *a*/ phaeopigments | | | | | | 0.606 | 0.701 | 0.903 | 0.844 | 0.801 |
| CPE | 0.50 | 0.81 | 0.82 | | | 0.141 | 0.001 | 0.174 | 0.275 | 0.229 |
| $OTU_{ARISA}$ | | 0.54 | | | | | 0.333 | 0.613 | 0.646 | 0.353 |
| Chao1 estimate | 0.49 | 0.81 | 0.75 | | 0.74 | | | 0.003 | 0.018 | 0.003 |
| absolute singletons | 0.56 | | | | | | 0.67 | | 0.000 | 0.000 |
| $OTU_{3\%}$ | 0.52 | | | | | | 0.56 | 0.96 | | 0.000 |
| absolute singletons / $OTU_{3\%}$ | | | | | | | 0.67 | 0.93 | 0.80 | |

Lower triangle shows Spearman's correlation statistic, upper triangle shows significance.

**Table S4** Pairwise spearman rank correlation tests of $OTU_{3\%}$ ranking for a) the whole dataset and b) $OTU_{3\% \text{ - abs singletons}}$.

a)

| | 2003 | 2004 | 2006 | 2007 | 2008 | 2009 |
|---|---|---|---|---|---|---|
| 2003 | | 2.20E-16 | 2.20E-16 | 2.20E-16 | 2.20E-16 | 2.20E-16 |
| 2004 | 0.29 | | 2.20E-16 | 2.20E-16 | 2.20E-16 | 2.20E-16 |
| 2006 | 0.20 | 0.10 | | 2.20E-16 | 2.20E-16 | 2.20E-16 |
| 2007 | 0.30 | 0.24 | 0.16 | | 2.20E-16 | 2.20E-16 |
| 2008 | 0.29 | 0.21 | 0.14 | 0.25 | | 2.20E-16 |
| 2009 | 0.25 | 0.17 | 0.07 | 0.21 | 0.16 | |

b)

| | 2003 | 2004 | 2006 | 2007 | 2008 | 2009 |
|---|---|---|---|---|---|---|
| 2003 | | 2.20E-16 | 2.20E-16 | 2.20E-16 | 2.20E-16 | 2.20E-16 |
| 2004 | 0.37 | | 4.20E-11 | 2.20E-16 | 2.20E-16 | 2.20E-16 |
| 2006 | 0.20 | 0.09 | | 2.20E-16 | 2.20E-16 | 6.33E-12 |
| 2007 | 0.38 | 0.36 | 0.20 | | 2.20E-16 | 2.20E-16 |
| 2008 | 0.37 | 0.35 | 0.17 | 0.39 | | 2.20E-16 |
| 2009 | 0.35 | 0.36 | 0.10 | 0.37 | 0.36 | |

Upper matrix triangle indicates significance of the correlation test, lower triangle indicates the r value.

**Table S5** Total shared a) $OTU_{3\%}$ and b) $OTU_{3\% \text{ - abs singletons}}$ between years in percent.

| **a)** | 2003 | 2004 | 2006 | 2007 | 2008 | 2009 |
|---|---|---|---|---|---|---|
| 2003 | | 22 | 18 | 23 | 22 | 18 |
| 2004 | | | 18 | 19 | 24 | 25 |
| 2006 | | | | 16 | 19 | 17 |
| 2007 | | | | | 20 | 16 |
| 2008 | | | | | | 23 |
| 2009 | | | | | | |

| **b)** | 2003 | 2004 | 2006 | 2007 | 2008 | 2009 |
|---|---|---|---|---|---|---|
| 2003 | | 35 | 27 | 32 | 35 | 32 |
| 2004 | | | 29 | 31 | 42 | 49 |
| 2006 | | | | 24 | 32 | 33 |
| 2007 | | | | | 32 | 29 |
| 2008 | | | | | | 46 |
| 2009 | | | | | | |

**Table S6** Explained variation in beta-diversity patterns.

| | $OTU_{3\%}$ | | | | | | $OTU_{ARISA}$ | |
|---|---|---|---|---|---|---|---|---|
| | full set | rel. singletons | resident OTU | phylum | class | genus | full set | reduced set |
| Year (-CPE) | 3%* | 2%* | 13%** | 11%* | 10%* | 8%* | 5%*** | 4%** |
| CPE (-Year) | 4%* | 4%** | 12%* | 17%* | 15%** | 12%* | 4%*** | n.s. |
| Year + CPE | - | - | 1% | 4% | 2% | - | - | 1% |
| Interannual (-CPE) | 13%*** | 10%*** | 35%*** | 35%*** | 36%*** | 30%*** | 22%*** | 19%** |
| CPE (-Interannual) | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |
| Interannual + CPE | 6% | 5% | 15% | 23% | 19% | 14% | 14% | 9% |

-: 0%, n.s.: not significant, *: $p < 0.05$, **: $p < 0.01$, *** $p < 0.001$.

**Table S7** Bacterial genera and $OTU_{3\%}$ that showed a linear relationship with CPE concentrations.

| | Adj. $R^2$ | p-value | Positive / negative correlation | # datapoints |
|---|---|---|---|---|
| **Genus - level** | | | | |
| Candidate_division_OP3;unclassified;unclassified;unclassified;unclassified | 0.56 | 0.000 | + | 17 |
| Bacteroidetes;Sphingobacteria;Sphingobacteriales;Saprospiraceae;Lewinella | 0.54 | 0.000 | + | 17 |
| Verrucomicrobia;Arctic97B-4;unclassified;unclassified;unclassified | 0.50 | 0.001 | + | 16 |
| Planctomycetes;Candidatus_Kuenenia;unclassified;unclassified;unclassified | 0.44 | 0.002 | + | 12 |
| Proteobacteria;Gammaproteobacteria;Alteromonadales;Moritellaceae;Moritella | 0.44 | 0.002 | + | 14 |
| Proteobacteria;Deltaproteobacteria;Sh765B-TzT-29;unclassified;unclassified | 0.43 | 0.003 | + | 17 |
| Actinobacteria;Actinobacteria;Actinobacteridae;Actinomycetales;Micromonosporineae | 0.38 | 0.005 | - | 17 |
| Bacteroidetes;Sphingobacteria;Sphingobacteriales;Flammeovirgaceae;Fulvivirga | 0.38 | 0.005 | + | 17 |
| Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhodobiaceae;Rhodobium | 0.37 | 0.006 | - | 17 |
| Chlamydiae;Chlamydiae;Chlamydiales;Parachlamydiaceae;Parachlamydia | 0.37 | 0.006 | - | 17 |
| Deferribacteres;Unclassified_Deferribacterales;LCP-89;unclassified;unclassified | 0.36 | 0.006 | + | 17 |
| Proteobacteria;Deltaproteobacteria;Desulfobacterales;Desulfobacteraceae;Desulfatiferula | 0.36 | 0.007 | + | 15 |
| Proteobacteria;Gammaproteobacteria;Legionellales;Coxiellaceae;unclassified | 0.36 | 0.007 | - | 17 |
| Chloroflexi;Anaerolineae;Anaerolineales;Anaerolineaceae;unclassified | 0.35 | 0.008 | - | 17 |
| Proteobacteria;Gammaproteobacteria;Chromatiales;Ectothiorhodospiraceae; Alkalilimnicola | 0.35 | 0.008 | - | 17 |
| Firmicutes;Clostridia;Clostridiales;Veillonellaceae;Anaerosinus | 0.34 | 0.008 | + | 12 |
| Proteobacteria;Deltaproteobacteria;Myxococcales;Nannocystineae;unclassified | 0.33 | 0.009 | + | 16 |
| Planctomycetes;Phycisphaerae;unclassified;unclassified;unclassified | 0.33 | 0.009 | + | 17 |
| Proteobacteria;Gammaproteobacteria;Thiotrichales;CHAB-XI-27;unclassified | 0.32 | 0.010 | - | 17 |
| Planctomycetes;vadinHA49;unclassified;unclassified;unclassified | 0.32 | 0.011 | + | 16 |
| Bacteroidetes;Flavobacteria;Flavobacteriales;Flavobacteriaceae;Flagellimonas | 0.31 | 0.012 | + | 7 |
| Chloroflexi;S085;unclassified;unclassified;unclassified | 0.31 | 0.012 | - | 17 |
| Proteobacteria;Gammaproteobacteria;Thiotrichales;Caedibacter;unclassified | 0.30 | 0.013 | - | 9 |
| Actinobacteria;Actinobacteria;Acidimicrobidae;Acidimicrobiales;Acidimicrobineae | 0.30 | 0.013 | - | 17 |
| Planctomycetes;Phycisphaerae;Phycisphaerales;Phycisphaeraceae;Phycisphaera | 0.30 | 0.014 | + | 17 |
| unclassified;unclassified;unclassified;unclassified;unclassified | 0.30 | 0.014 | - | 17 |
| Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;Pelagibius | 0.30 | 0.014 | + | 17 |
| Bacteroidetes;Sphingobacteria;Sphingobacteriales;unclassified;unclassified | 0.30 | 0.014 | + | 16 |
| Proteobacteria;Betaproteobacteria;Nitrosomonadales;Gallionellaceae;Gallionella | 0.29 | 0.015 | + | 10 |
| Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;Tistrella | 0.29 | 0.015 | + | 14 |
| Proteobacteria;Gammaproteobacteria;1013-28-CG33;unclassified;unclassified | 0.29 | 0.015 | - | 16 |
| Proteobacteria;Gammaproteobacteria;Thiobacillus;unclassified;unclassified | 0.29 | 0.016 | - | 17 |
| Acidobacteria;RB25;unclassified;unclassified;unclassified | 0.29 | 0.016 | + | 17 |
| Actinobacteria;Actinobacteria;unclassified;unclassified;unclassified | 0.28 | 0.016 | - | 17 |
| Proteobacteria;Deltaproteobacteria;Desulfobacterales;Desulfobacteraceae;Desulfotignum | 0.28 | 0.016 | + | 4 |
| Proteobacteria;Deltaproteobacteria;Syntrophobacterales;Syntrophobacteraceae | 0.28 | 0.017 | + | 9 |
| Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhodobiaceae;Parvibaculum | 0.28 | 0.017 | - | 17 |
| Proteobacteria;Deltaproteobacteria;Bdellovibrionales;Bdellovibrionaceae;OM27 | 0.27 | 0.018 | + | 16 |
| Proteobacteria;Gammaproteobacteria;marine_group_E01-9C-26;unclassified;unclassified | 0.27 | 0.018 | + | 17 |
| Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;unclassified | 0.27 | 0.019 | + | 17 |
| Bacteroidetes;Flavobacteria;Flavobacteriales;Flavobacteriaceae;unclassified | 0.27 | 0.019 | + | 17 |
| Bacteroidetes;Sphingobacteria;Sphingobacteriales;Cytophagaceae;Microscilla | 0.27 | 0.019 | + | 14 |
| Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae; Persicirhabdus | 0.27 | 0.020 | - | 17 |
| Firmicutes;Clostridia;Clostridiales;Veillonellaceae;Acidaminococcus | 0.26 | 0.020 | - | 17 |
| Acidobacteria;Holophagae;NKB17;unclassified;unclassified | 0.26 | 0.021 | + | 10 |

| | | | | |
|---|---|---|---|---|
| Bacteroidetes;Sphingobacteria;Sphingobacteriales;WCHB1-32;unclassified | 0.26 | 0.021 | + | 15 |
| Acidobacteria;Holophagae;Holophagales;Holophagaceae;unclassified | 0.26 | 0.022 | + | 17 |
| Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;Roseospira | 0.26 | 0.022 | + | 17 |
| Proteobacteria;Deltaproteobacteria;Desulfobacterales;Desulfobulbaceae;unclassified | 0.26 | 0.022 | - | 7 |
| Deferribacteres;Unclassified_Deferribacterales;Caldithrix;unclassified;unclassified | 0.26 | 0.022 | + | 16 |
| Actinobacteria;Actinobacteria;Actinobacteridae;Actinomycetales;Corynebacterineae | 0.25 | 0.023 | - | 17 |
| Candidate_division_TM6;unclassified;unclassified;unclassified;unclassified | 0.25 | 0.024 | - | 17 |
| Candidate_division_TG-1;unclassified;unclassified;unclassified;unclassified | 0.25 | 0.024 | + | 2 |
| Proteobacteria;Alphaproteobacteria;Rhizobiales;Candidatus_Liberibacter;unclassified | 0.25 | 0.024 | + | 2 |
| Proteobacteria;Deltaproteobacteria;Desulfobacterales;Desulfobulbaceae;Desulfopila | 0.25 | 0.024 | + | 2 |
| Firmicutes;Clostridia;Clostridiales;Family_XI_Incertae_Sedis;Helcococcus | 0.24 | 0.025 | + | 2 |
| Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Nereida | 0.24 | 0.025 | + | 2 |
| Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae; Robiginitomaculum | 0.24 | 0.026 | + | 3 |
| Nitrospirae;Nitrospira;Nitrospirales;Nitrospiraceae;Nitrospira | 0.24 | 0.026 | + | 17 |
| Lentisphaerae;Lentisphaeria;BS5;unclassified;unclassified | 0.24 | 0.027 | + | 17 |
| Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhodobiaceae;Afifella | 0.23 | 0.028 | + | 8 |
| Proteobacteria;Deltaproteobacteria;Desulfarculales;Desulfarculaceae;unclassified | 0.23 | 0.029 | + | 2 |
| Actinobacteria;Actinobacteria;Actinobacteridae;Actinomycetales;Frankineae | 0.23 | 0.030 | - | 14 |
| Proteobacteria;Gammaproteobacteria;Legionellales;Coxiellaceae;Coxiella | 0.23 | 0.030 | - | 17 |
| Bacteroidetes;Sphingobacteria;Sphingobacteriales;Sphingobacteriaceae; Sphingobacteriaceae | 0.23 | 0.030 | - | 17 |
| Bacteroidetes;Flavobacteria;Flavobacteriales;Flavobacteriaceae;Myroides | 0.23 | 0.031 | + | 9 |
| Proteobacteria;Deltaproteobacteria;Desulfuromonadales;Geobacteraceae; Geothermobacter | 0.22 | 0.032 | + | 17 |
| Proteobacteria;Gammaproteobacteria;Oceanospirillales;Hahellaceae;Hahella | 0.22 | 0.032 | - | 5 |
| Proteobacteria;Alphaproteobacteria;Rhizobiales;Hyphomicrobiaceae;Hyphomicrobium | 0.22 | 0.032 | - | 17 |
| Proteobacteria;Gammaproteobacteria;Thiotrichales;Fangia;unclassified | 0.22 | 0.032 | - | 17 |
| Verrucomicrobia;Spartobacteria;unclassified;unclassified;unclassified | 0.22 | 0.033 | - | 17 |
| Proteobacteria;Gammaproteobacteria;Oceanospirillales;Halomonadaceae; Modicisalibacter | 0.22 | 0.033 | - | 16 |
| Proteobacteria;Gammaproteobacteria;Oceanospirillales;Halomonadaceae;Carnimonas | 0.22 | 0.034 | + | 12 |
| Bacteroidetes;Sphingobacteria;Sphingobacteriales;Sphingobacteriaceae;Solitalea | 0.21 | 0.036 | + | 4 |
| Proteobacteria;Betaproteobacteria;Burkholderiales;Comamonadaceae;Delftia | 0.21 | 0.038 | - | 3 |
| Actinobacteria;Actinobacteria;Rubrobacteridae;AKIW543;unclassified | 0.20 | 0.040 | - | 16 |
| Proteobacteria;Gammaproteobacteria;Chromatiales;Ectothiorhodospiraceae; Alkalispirillum | 0.20 | 0.040 | - | 17 |
| Proteobacteria;Alphaproteobacteria;Rickettsiales;Holosporaceae;Holospora | 0.20 | 0.042 | + | 2 |
| Cyanobacteria;SubsectionIII;Halomicronema;unclassified;unclassified | 0.20 | 0.042 | - | 10 |
| Proteobacteria;Deltaproteobacteria;Myxococcales;unclassified;unclassified | 0.20 | 0.043 | + | 16 |
| Proteobacteria;Deltaproteobacteria;Desulfovibrionales;Desulfohalobiaceae; Desulfonauticus | 0.20 | 0.043 | - | 17 |
| Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;Novispirillum | 0.19 | 0.043 | + | 17 |
| Proteobacteria;Betaproteobacteria;Neisseriales;Neisseriaceae;Chromobacterium | 0.19 | 0.045 | - | 4 |
| Proteobacteria;Gammaproteobacteria;Chromatiales;unclassified;unclassified | 0.19 | 0.047 | - | 2 |
| Bacteroidetes;Flavobacteria;Flavobacteriales;Cryomorphaceae;Owenweeksia | 0.19 | 0.047 | + | 17 |
| Proteobacteria;Gammaproteobacteria;Methylococcales;Methylococcaceae;unclassified | 0.19 | 0.047 | - | 17 |
| Proteobacteria;Gammaproteobacteria;Legionellales;Legionellaceae;unclassified | 0.19 | 0.048 | - | 17 |
| Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Nitrincola | 0.19 | 0.048 | - | 8 |
| Bacteroidetes;Sphingobacteria;Sphingobacteriales;Flammeovirgaceae; Candidatus_Cardinium | 0.18 | 0.049 | + | 14 |
| Proteobacteria;unclassified;unclassified;unclassified;unclassified | 0.18 | 0.049 | + | 17 |
| **OTU - level** | | | | |
| 446 _ Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;Roseospira; | 0.59 | 0.000 | + | 13 |
| 1935 _ Deferribacteres;Unclassified_Deferribacterales;Caldithrix;unclassified;unclassified | 0.55 | 0.000 | + | 13 |

| | | | | |
|---|---|---|---|---|
| 3589 _ Bacteroidetes;Sphingobacteria;Sphingobacteriales;Cytophagaceae;Microscilla | 0.54 | 0.000 | + | 7 |
| 1598 _ Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;Pelagibius | 0.53 | 0.001 | + | 12 |
| 1415 _ unclassified;unclassified;unclassified;unclassified;unclassified;unclassified | 0.52 | 0.001 | - | 7 |
| 974 _ Proteobacteria;Deltaproteobacteria;Myxococcales;Nannocystineae;unclassified | 0.50 | 0.001 | + | 16 |
| 885 _ Bacteroidetes;Sphingobacteria;Sphingobacteriales;Saprospiraceae;Saprospira | 0.50 | 0.001 | + | 13 |
| 1574 _ Proteobacteria;Gammaproteobacteria;Legionellales;Coxiellaceae;Coxiella;unclassified | 0.47 | 0.001 | - | 7 |
| 3649 _ Proteobacteria;Gammaproteobacteria;Thiotrichales;Fangia;unclassified;unclassified | 0.47 | 0.001 | - | 7 |
| 116 _ Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;Pelagibius | 0.44 | 0.002 | + | 17 |
| 4765 _ Planctomycetes;Phycisphaerae;unclassified;unclassified;unclassified;unclassified | 0.43 | 0.003 | + | 5 |
| 15 _ Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;unclassified;unclassified | 0.42 | 0.003 | - | 17 |
| 53 _ Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;unclassified;unclassified | 0.42 | 0.003 | - | 16 |
| 2039 _ Proteobacteria;Deltaproteobacteria;unclassified;unclassified;unclassified;unclassified | 0.41 | 0.003 | + | 6 |
| 68 _ Nitrospirae;Nitrospira;Nitrospirales;Nitrospiraceae;Nitrospira;unclassified | 0.39 | 0.004 | + | 17 |
| 648 _ Candidate_division_OD1;unclassified;unclassified;unclassified;unclassified;unclassified | 0.39 | 0.004 | - | 9 |
| 849 _ Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;BD2-7 | 0.39 | 0.004 | + | 10 |
| 4044 _ Proteobacteria;Gammaproteobacteria;Thiotrichales;CHAB-XI-27; unclassified;unclassified | 0.38 | 0.005 | - | 3 |
| 2234 _ Bacteroidetes;Flavobacteria;Flavobacteriales;Flavobacteriaceae;Lutibacter;unclassified | 0.38 | 0.005 | + | 11 |
| 47 _ Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;unclassified;unclassified | 0.37 | 0.005 | - | 17 |
| 1480 _ Proteobacteria;Gammaproteobacteria;unclassified;unclassified;unclassified;unclassified | 0.37 | 0.005 | - | 4 |
| 1159 _ Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;Tistrella | 0.37 | 0.005 | + | 14 |
| 535 _ Proteobacteria;Gammaproteobacteria;Oceanospirillales;Halomonadaceae;Carnimonas | 0.37 | 0.005 | + | 12 |
| 574 _ unclassified;unclassified;unclassified;unclassified;unclassified;unclassified | 0.37 | 0.006 | - | 7 |
| 1500 _ Acidobacteria;RB25;unclassified;unclassified;unclassified;unclassified | 0.37 | 0.006 | + | 10 |
| 4072 _ Bacteroidetes;Flavobacteria;Flavobacteriales;Flavobacteriaceae;Robiginitalea;unclassified | 0.37 | 0.006 | - | 4 |
| 2067 _ unclassified;unclassified;unclassified;unclassified;unclassified;unclassified | 0.37 | 0.006 | + | 12 |
| 1420 _ Proteobacteria;Alphaproteobacteria;DB1-14;unclassified;unclassified;unclassified | 0.37 | 0.006 | - | 6 |
| 477 _ Proteobacteria;Gammaproteobacteria;unclassified;unclassified;unclassified;unclassified | 0.36 | 0.006 | - | 5 |
| 1381 _ Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Teredinibacter | 0.36 | 0.007 | - | 5 |
| 220 _ Bacteroidetes;Sphingobacteria;Sphingobacteriales;Saprospiraceae;unclassified | 0.36 | 0.007 | + | 17 |
| 2121 _ Verrucomicrobia;Arctic97B-4;unclassified;unclassified;unclassified;unclassified | 0.35 | 0.007 | + | 5 |
| 4544 _ Proteobacteria;Deltaproteobacteria;Myxococcales;Cystobacterineae;unclassified | 0.35 | 0.007 | + | 5 |
| 3883 _ Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;unclassified | 0.35 | 0.008 | - | 5 |
| 4633 _ Planctomycetes;Phycisphaerae;SHA-43;unclassified;unclassified;unclassified | 0.34 | 0.008 | + | 3 |
| 495 _ Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;unclassified | 0.34 | 0.008 | - | 3 |
| 369 _ Proteobacteria;TA18;unclassified;unclassified;unclassified;unclassified | 0.34 | 0.008 | - | 15 |
| 59 _ Chloroflexi;Anaerolineae;Anaerolineales;Anaerolineaceae;unclassified;unclassified | 0.34 | 0.008 | - | 17 |
| 3147 _ Proteobacteria;Gammaproteobacteria;Xanthomonadales;Sinobacteraceae;unclassified | 0.34 | 0.009 | + | 8 |
| 1019 _ Chloroflexi;Anaerolineae;Anaerolineales;Anaerolineaceae;unclassified;unclassified | 0.34 | 0.009 | - | 10 |
| 966 _ Candidate_division_OP3;unclassified;unclassified;unclassified;unclassified;unclassified | 0.33 | 0.009 | + | 9 |
| 3128 _ Planctomycetes;vadinHA49;unclassified;unclassified;unclassified;unclassified | 0.33 | 0.009 | + | 6 |
| 349 _ Bacteroidetes;Flavobacteria;Flavobacteriales;Flavobacteriaceae;unclassified;unclassified | 0.33 | 0.009 | + | 14 |
| 3103 _ Proteobacteria;Gammaproteobacteria;JTB148;unclassified;unclassified;unclassified | 0.33 | 0.010 | - | 6 |

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| 4545 _ Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;unclassified | 0.32 | 0.010 | + | 3 |
| 4844 _ Acidobacteria;Holophagae;Holophagales;Holophagaceae;unclassified;unclassified | 0.32 | 0.010 | + | 3 |
| 5217 _ Proteobacteria;Deltaproteobacteria;GR-WP33-30;unclassified | 0.32 | 0.010 | + | 3 |
| 2792 _ Bacteroidetes;Flavobacteria;Flavobacteriales;NS9;unclassified;unclassified | 0.32 | 0.010 | + | 8 |
| 196 _ Proteobacteria;Deltaproteobacteria;Desulfovibrionales;Desulfohalobiaceae; Desulfonauticus | 0.32 | 0.011 | - | 8 |
| 2101 _ Bacteroidetes;Flavobacteria;Flavobacteriales;Cryomorphaceae;Fluviicola;unclassified | 0.32 | 0.011 | + | 6 |
| 3709 _ Proteobacteria;Gammaproteobacteria;NKB5;unclassified;unclassified;unclassified | 0.32 | 0.011 | - | 8 |
| 312 _ Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;unclassified;unclassified | 0.31 | 0.012 | - | 17 |
| 2737 _ unclassified;unclassified;unclassified;unclassified;unclassified;unclassified | 0.31 | 0.012 | + | 7 |
| 751 _ Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Haloferula | 0.31 | 0.012 | - | 5 |
| 1898 _ Bacteroidetes;Flavobacteria;Flavobacteriales;Cryomorphaceae;Owenweeksia;unclassified | 0.31 | 0.012 | + | 10 |
| 2088 _ Acidobacteria;Holophagae;32-20;unclassified;unclassified;unclassified | 0.31 | 0.012 | + | 6 |
| 9 _ Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae; Persicirhabdus;unclassified | 0.31 | 0.012 | - | 17 |
| 55 _ Proteobacteria;Alphaproteobacteria;Rhizobiales;Hyphomicrobiaceae;Hyphomicrobium | 0.31 | 0.013 | - | 17 |
| 385 _ Proteobacteria;Deltaproteobacteria;Sh765B-TzT-29;unclassified | 0.30 | 0.013 | + | 15 |
| 2781 _ Proteobacteria;Deltaproteobacteria;Myxococcales;Sorangiineae;unclassified;unclassified | 0.30 | 0.014 | + | 12 |
| 26 _ Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhodobiaceae;Parvibaculum;unclassified | 0.30 | 0.014 | - | 17 |
| 1050 _ Proteobacteria;Gammaproteobacteria;unclassified;unclassified;unclassified;unclassified | 0.30 | 0.014 | - | 5 |
| 387 _ Proteobacteria;Deltaproteobacteria;SAR324;unclassified;unclassified;unclassified | 0.30 | 0.014 | + | 13 |
| 964 _ Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhodobiaceae;Rhodobium;unclassified | 0.29 | 0.015 | - | 17 |
| 692 _ Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;unclassified;unclassified | 0.29 | 0.015 | - | 3 |
| 3908 _ Cyanobacteria;ML635J-21;unclassified;unclassified;unclassified;unclassified | 0.29 | 0.015 | - | 4 |
| 860 _ Chloroflexi;S085;unclassified;unclassified;unclassified;unclassified | 0.29 | 0.015 | - | 15 |
| 1204 _ unclassified;unclassified;unclassified;unclassified;unclassified;unclassified | 0.29 | 0.015 | - | 9 |
| 100 _ Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhodobiaceae;Rhodobium;unclassified | 0.29 | 0.015 | - | 17 |
| 279 _ Proteobacteria;Deltaproteobacteria;Desulfuromonadales;Geobacteraceae; Geothermobacter;unclassified | 0.29 | 0.015 | + | 15 |
| 4457 _ Proteobacteria;Deltaproteobacteria;Sh765B-TzT-29;unclassified | 0.29 | 0.015 | + | 4 |
| 4970 _ Proteobacteria;TA18;unclassified;unclassified;unclassified;unclassified | 0.29 | 0.015 | + | 4 |
| 2183 _ Acidobacteria;Holophagae;Holophagales;Holophagaceae;unclassified;unclassified | 0.29 | 0.016 | + | 5 |
| 1747 _ Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;unclassified | 0.29 | 0.016 | + | 12 |
| 2062 _ Acidobacteria;RB25;unclassified;unclassified;unclassified;unclassified | 0.29 | 0.016 | + | 7 |
| 1971 _ unclassified;unclassified;unclassified;unclassified;unclassified;unclassified | 0.28 | 0.016 | + | 4 |
| 887 _ Proteobacteria;Gammaproteobacteria;unclassified;unclassified;unclassified;unclassified | 0.28 | 0.016 | - | 6 |
| 3818 _ Proteobacteria;Deltaproteobacteria;Myxococcales;Cystobacterineae;Myxococcaceae; Pyxidicoccus | 0.28 | 0.017 | - | 7 |
| 4057 _ Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;unclassified | 0.28 | 0.017 | - | 4 |
| 1883 _ Lentisphaerae;Lentisphaeria;Lentisphaerales;Lentisphaeraceae;Lentisphaera;unclassified | 0.28 | 0.017 | + | 10 |
| 4551 _ Bacteroidetes;Flavobacteria;Flavobacteriales;unclassified;unclassified;unclassified | 0.28 | 0.017 | + | 5 |
| 622 _ Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;unclassified;unclassified | 0.28 | 0.017 | - | 16 |
| 1919 _ Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;Pelagibius | 0.28 | 0.018 | + | 12 |
| 128 _ Proteobacteria;Gammaproteobacteria;Oceanospirillales;OM182;unclassified;unclassified | 0.28 | 0.018 | + | 15 |

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| 502 _ Actinobacteria;Actinobacteria;Actinobacteridae;Actinomycetales;Frankineae;Fodinicola | 0.28 | 0.018 | - | 10 |
| 120 _ Actinobacteria;Actinobacteria;Acidimicrobidae;Acidimicrobiales;Acidimicrobineae | 0.27 | 0.018 | - | 17 |
| 859 _ Bacteroidetes;Sphingobacteria;Sphingobacteriales;Saprospiraceae;unclassified | 0.27 | 0.018 | + | 11 |
| 114 _ Actinobacteria;Actinobacteria;Acidimicrobidae;Acidimicrobiales;Acidimicrobineae; Iamiaceae | 0.27 | 0.019 | - | 16 |
| 433 _ Candidate_division_TM6;unclassified;unclassified;unclassified;unclassified;unclassified | 0.27 | 0.019 | - | 9 |
| 182 _ Bacteroidetes;Sphingobacteria;Sphingobacteriales;B01R012;unclassified;unclassified | 0.27 | 0.019 | + | 17 |
| 7020 _ Cyanobacteria;SubsectionIII;Halomicronema;unclassified;unclassified;unclassified | 0.27 | 0.020 | - | 3 |
| 7205 _ Proteobacteria;Gammaproteobacteria;unclassified;unclassified;unclassified;unclassified | 0.27 | 0.020 | - | 3 |
| 7214 _ Proteobacteria;Gammaproteobacteria;Oceanospirillales;Halomonadaceae; Modicisalibacter | 0.27 | 0.020 | - | 3 |
| 1667 _ Lentisphaerae;Lentisphaeria;WCHB1-41;unclassified;unclassified;unclassified | 0.27 | 0.020 | + | 12 |
| 103 _ Proteobacteria;Gammaproteobacteria;Xanthomonadales;Sinobacteraceae;unclassified | 0.26 | 0.020 | + | 15 |
| 6948 _ Cyanobacteria;SubsectionV;Nostochopsis;unclassified;unclassified;unclassified | 0.26 | 0.020 | - | 3 |
| 500 _ Chloroflexi;S085;unclassified;unclassified;unclassified;unclassified | 0.26 | 0.021 | - | 12 |
| 2042 _ unclassified;unclassified;unclassified;unclassified;unclassified;unclassified | 0.26 | 0.021 | - | 12 |
| 901 _ Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;unclassified | 0.26 | 0.021 | - | 8 |
| 907 _ Bacteroidetes;Sphingobacteria;Sphingobacteriales;Flammeovirgaceae;Fulvivirga | 0.26 | 0.021 | + | 15 |
| 1879 _ Proteobacteria;Gammaproteobacteria;Alteromonadales;Moritellaceae;Moritella | 0.26 | 0.021 | + | 9 |
| 226 _ Deferribacteres;Unclassified_Deferribacterales;LCP-89;unclassified | 0.26 | 0.021 | + | 16 |
| 2106 _ Proteobacteria;Alphaproteobacteria;unclassified;unclassified;unclassified;unclassified | 0.26 | 0.022 | + | 12 |
| 1515 _ Proteobacteria;Gammaproteobacteria;Legionellales;Coxiellaceae;Coxiella;unclassified | 0.26 | 0.022 | - | 12 |
| 626 _ Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Rhodopirellula | 0.26 | 0.022 | - | 13 |
| 1463 _ Candidate_division_TM6;unclassified;unclassified;unclassified;unclassified;unclassified | 0.25 | 0.022 | - | 3 |
| 2745 _ Chloroflexi;Caldilineae;Caldilineales;Caldilineaceae;unclassified;unclassified | 0.25 | 0.023 | + | 9 |
| 745 _ Chlamydiae;Chlamydiae;Chlamydiales;Parachlamydiaceae;Parachlamydia;unclassified | 0.25 | 0.024 | - | 5 |
| 4797 _ Candidate_division_OP3;unclassified;unclassified;unclassified;unclassified;unclassified | 0.25 | 0.024 | + | 3 |
| 4875 _ Acidobacteria;RB25;unclassified;unclassified;unclassified;unclassified | 0.25 | 0.024 | + | 3 |
| 1913 _ Proteobacteria;Gammaproteobacteria;unclassified;unclassified;unclassified;unclassified | 0.25 | 0.024 | + | 10 |
| 88 _ Bacteroidetes;Sphingobacteria;Sphingobacteriales;Saprospiraceae;Lewinella;unclassified | 0.25 | 0.024 | + | 17 |
| 6893 _ Proteobacteria;Gammaproteobacteria;Thiotrichales;CHAB-XI-27;unclassified | 0.25 | 0.024 | - | 2 |
| 6910 _ Chlamydiae;Chlamydiae;Chlamydiales;Parachlamydiaceae;Neochlamydia;unclassified | 0.25 | 0.024 | - | 2 |
| 6919 _ Candidate_division_TM6;unclassified;unclassified;unclassified;unclassified;unclassified | 0.25 | 0.024 | - | 2 |
| 6954 _ Proteobacteria;Gammaproteobacteria;Thiobacillus;unclassified;unclassified;unclassified | 0.25 | 0.024 | - | 2 |
| 6959 _ Proteobacteria;unclassified;unclassified;unclassified;unclassified;unclassified | 0.25 | 0.024 | - | 2 |
| 6973 _ Proteobacteria;Gammaproteobacteria;Thiotrichales;Fangia;unclassified;unclassified | 0.25 | 0.024 | - | 2 |
| 6982 _ unclassified;unclassified;unclassified;unclassified;unclassified;unclassified | 0.25 | 0.024 | - | 2 |
| 6990 _ Proteobacteria;Gammaproteobacteria;unclassified;unclassified;unclassified;unclassified | 0.25 | 0.024 | - | 2 |
| 7009 _ Proteobacteria;Gammaproteobacteria;Chromatiales;Ectothiorhodospiraceae; Alkalilimnicola | 0.25 | 0.024 | - | 2 |
| 7032 _ Chlamydiae;Chlamydiae;Chlamydiales;Parachlamydiaceae;Parachlamydia;unclassified | 0.25 | 0.024 | - | 2 |
| 7038 _ Proteobacteria;Gammaproteobacteria;Thiotrichales;Fangia;unclassified;unclassified | 0.25 | 0.024 | - | 2 |
| 7039 _ Candidate_division_BRC1;unclassified;unclassified;unclassified;unclassified;unclassified | 0.25 | 0.024 | - | 2 |

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| 7045 _ Proteobacteria;Gammaproteobacteria;NKB5;unclassified;unclassified;unclassified | 0.25 | 0.024 | - | 2 |
| 7084 _ unclassified;unclassified;unclassified;unclassified;unclassified;unclassified | 0.25 | 0.024 | - | 2 |
| 7151 _ Cyanobacteria;ML635J-21;unclassified;unclassified;unclassified;unclassified | 0.25 | 0.024 | - | 2 |
| 7152 _ Proteobacteria;Gammaproteobacteria;Legionellales;Coxiellaceae;unclassified;unclassified | 0.25 | 0.024 | - | 2 |
| 7178 _ Proteobacteria;Gammaproteobacteria;Thiobacillus;unclassified;unclassified;unclassified | 0.25 | 0.024 | - | 2 |
| 7233 _ Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;unclassified | 0.25 | 0.024 | - | 2 |
| 7246 _ Proteobacteria;Gammaproteobacteria;unclassified;unclassified;unclassified;unclassified | 0.25 | 0.024 | - | 2 |
| 7277 _ Proteobacteria;Deltaproteobacteria;Myxococcales;Cystobacterineae;Myxococcaceae; Pyxidicoccus | 0.25 | 0.024 | - | 2 |
| 7306 _ Proteobacteria;Gammaproteobacteria;Legionellales;Coxiellaceae;unclassified;unclassified | 0.25 | 0.024 | - | 2 |
| 343 _ Bacteroidetes;Sphingobacteria;Sphingobacteriales;Saprospiraceae;unclassified | 0.25 | 0.024 | + | 16 |
| 2068 _ Planctomycetes;Pla4;unclassified;unclassified;unclassified;unclassified | 0.25 | 0.024 | + | 4 |
| 67 _ Deferribacteres;Unclassified_Deferribacterales;LCP-89;unclassified;unclassified | 0.25 | 0.025 | + | 14 |
| 318 _ Bacteroidetes;Sphingobacteria;Sphingobacteriales;Sphingobacteriaceae; Sphingobacteriaceae;Parapedobacter | 0.25 | 0.025 | - | 16 |
| 2015 _ Proteobacteria;Deltaproteobacteria;unclassified;unclassified;unclassified;unclassified | 0.25 | 0.025 | - | 9 |
| 4432 _ Proteobacteria;Gammaproteobacteria;unclassified;unclassified;unclassified;unclassified | 0.24 | 0.025 | + | 2 |
| 4878 _ Candidate_division_OP3;unclassified;unclassified;unclassified;unclassified;unclassified | 0.24 | 0.025 | + | 2 |
| 332 _ Verrucomicrobia;Spartobacteria;unclassified;unclassified;unclassified;unclassified | 0.24 | 0.026 | - | 15 |
| 981 _ Planctomycetes;OM190;unclassified;unclassified;unclassified;unclassified | 0.24 | 0.026 | + | 8 |
| 5 _ Actinobacteria;Actinobacteria;Actinobacteridae;Actinomycetales;Micromonosporineae; Micromonosporaceae | 0.24 | 0.026 | - | 17 |
| 4571 _ Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;unclassified | 0.24 | 0.026 | + | 2 |
| 4630 _ Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;unclassified;unclassified | 0.24 | 0.026 | + | 2 |
| 4732 _ Acidobacteria;RB25;unclassified;unclassified;unclassified;unclassified | 0.24 | 0.026 | + | 2 |
| 20 _ Actinobacteria;Actinobacteria;Acidimicrobidae;Acidimicrobiales;Acidimicrobineae | 0.24 | 0.026 | - | 17 |
| 1692 _ Proteobacteria;Gammaproteobacteria;Thiotrichales;Fangia;unclassified;unclassified | 0.24 | 0.026 | - | 3 |
| 2522 _ Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;unclassified | 0.24 | 0.027 | + | 5 |
| 721 _ Planctomycetes;Phycisphaerae;Phycisphaerales;Phycisphaeraceae;Phycisphaera | 0.24 | 0.027 | + | 4 |
| 821 _ Planctomycetes;Phycisphaerae;Phycisphaerales;Phycisphaeraceae; Urania-1B-19;unclassified | 0.24 | 0.027 | + | 9 |
| 4047 _ Chlamydiae;Chlamydiae;Chlamydiales;Parachlamydiaceae;Parachlamydia;unclassified | 0.24 | 0.028 | - | 7 |
| 2610 _ Proteobacteria;Gammaproteobacteria;Thiobacillus;unclassified;unclassified;unclassified | 0.23 | 0.028 | - | 8 |
| 1866 _ Proteobacteria;Alphaproteobacteria;Rhizobiales;Phyllobacteriaceae;Defluvibacter | 0.23 | 0.028 | - | 13 |
| 4485 _ Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;Novispirillum | 0.23 | 0.028 | + | 2 |
| 4525 _ Proteobacteria;Deltaproteobacteria;Myxococcales;Cystobacterineae;unclassified | 0.23 | 0.028 | + | 2 |
| 4546 _ Fusobacteria;Fusobacteria;Fusobacteriales;Fusobacteriaceae;Ilyobacter;unclassified | 0.23 | 0.028 | + | 2 |
| 4613 _ Candidate_division_TM6;unclassified;unclassified;unclassified;unclassified;unclassified | 0.23 | 0.028 | + | 2 |
| 4685 _ Proteobacteria;Alphaproteobacteria;Rhodospirillales;unclassified;unclassified;unclassified | 0.23 | 0.028 | + | 2 |
| 4737 _ Bacteroidetes;Flavobacteria;Flavobacteriales;Cryomorphaceae;Brumimimicrobium;unclas sified | 0.23 | 0.028 | + | 2 |
| 4749 _ Acidobacteria;Holophagae;Holophagales;Holophagaceae;unclassified;unclassified | 0.23 | 0.028 | + | 2 |
| 4764 _ Candidate_division_TG-1;unclassified;unclassified;unclassified;unclassified;unclassified | 0.23 | 0.028 | + | 2 |

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| 4774 _ unclassified;unclassified;unclassified;unclassified;unclassified;unclassified | 0.23 | 0.028 | + | 2 |
| 4793 _ Acidobacteria;unclassified;unclassified;unclassified;unclassified;unclassified | 0.23 | 0.028 | + | 2 |
| 4815 _ Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Marinobacterium;unclassified | 0.23 | 0.028 | + | 2 |
| 4851 _ Planctomycetes;Phycisphaerae;mle1-8;unclassified;unclassified;unclassified | 0.23 | 0.028 | + | 2 |
| 4966 _ Chlorobi;Chlorobia;Chlorobiales;OPB56;unclassified;unclassified | 0.23 | 0.028 | + | 2 |
| 5060 _ Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Haloferula;unclassified | 0.23 | 0.028 | + | 2 |
| 5099 _ Proteobacteria;Deltaproteobacteria;Myxococcales;Nannocystineae;Nannocystaceae;unclassified | 0.23 | 0.028 | + | 2 |
| 5121 _ Proteobacteria;Alphaproteobacteria;Kordiimonadales;Kordiimonadaceae;Kordiimonas;unclassified | 0.23 | 0.028 | + | 2 |
| 5160 _ Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula;unclassified | 0.23 | 0.028 | + | 2 |
| 5194 _ Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Pirellula;unclassified | 0.23 | 0.028 | + | 2 |
| 5200 _ Proteobacteria;TA18;unclassified;unclassified;unclassified;unclassified | 0.23 | 0.028 | + | 2 |
| 738 _ Proteobacteria;Alphaproteobacteria;OCS116;unclassified;unclassified;unclassified | 0.23 | 0.028 | - | 11 |
| 1593 _ Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Pirellula;unclassified | 0.23 | 0.028 | + | 9 |
| 543 _ Gemmatimonadetes;Gemmatimonadetes;BD2-11;unclassified;unclassified;unclassified | 0.23 | 0.028 | - | 11 |
| 3158 _ Proteobacteria;Deltaproteobacteria;Myxococcales;Nannocystineae;Nannocystaceae;Enhygromyxa | 0.23 | 0.028 | + | 3 |
| 50 _ Actinobacteria;Actinobacteria;unclassified;unclassified;unclassified;unclassified | 0.23 | 0.028 | - | 17 |
| 2011 _ Planctomycetes;OM190;unclassified;unclassified;unclassified;unclassified | 0.23 | 0.029 | + | 7 |
| 429 _ Actinobacteria;Actinobacteria;MB-A2-108;unclassified;unclassified;unclassified | 0.23 | 0.029 | - | 7 |
| 377 _ Proteobacteria;Deltaproteobacteria;Desulfovibrionales;Desulfohalobiaceae;Desulfonauticus;unclassified | 0.23 | 0.029 | - | 14 |
| 3401 _ Bacteroidetes;Sphingobacteria;Sphingobacteriales;Saprospiraceae;Lewinella;unclassified | 0.23 | 0.029 | + | 11 |
| 873 _ Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;unclassified;unclassified | 0.23 | 0.029 | + | 17 |
| 2003 _ Proteobacteria;Alphaproteobacteria;Rhodospirillales;MND8;unclassified;unclassified | 0.23 | 0.029 | + | 5 |
| 4442 _ Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Planctomyces;unclassified | 0.23 | 0.029 | + | 2 |
| 4472 _ Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;OM60_NOR5_clade;Haliea | 0.23 | 0.029 | + | 2 |
| 4553 _ Proteobacteria;Gammaproteobacteria;Legionellales;Coxiellaceae;unclassified;unclassified | 0.23 | 0.029 | + | 2 |
| 4603 _ Proteobacteria;Alphaproteobacteria;Rickettsiales;SM2D12;unclassified;unclassified | 0.23 | 0.029 | + | 2 |
| 4632 _ Proteobacteria;Gammaproteobacteria;Thiobacillus;unclassified;unclassified;unclassified | 0.23 | 0.029 | + | 2 |
| 4634 _ Candidate_division_OD1;unclassified;unclassified;unclassified;unclassified;unclassified | 0.23 | 0.029 | + | 2 |
| 4636 _ Nitrospirae;Nitrospira;Nitrospirales;0319-6A21;unclassified;unclassified | 0.23 | 0.029 | + | 2 |
| 4710 _ Candidate_division_OP3;unclassified;unclassified;unclassified;unclassified;unclassified | 0.23 | 0.029 | + | 2 |
| 4716 _ Proteobacteria;Alphaproteobacteria;unclassified;unclassified;unclassified;unclassified | 0.23 | 0.029 | + | 2 |
| 4718 _ Proteobacteria;Gammaproteobacteria;Xanthomonadales;Sinobacteraceae;Steroidobacter;unclassified | 0.23 | 0.029 | + | 2 |
| 4809 _ Proteobacteria;TA18;unclassified;unclassified;unclassified;unclassified | 0.23 | 0.029 | + | 2 |

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| 4811 _ Proteobacteria;Alphaproteobacteria;unclassified;unclassified;unclassified;unclassified | 0.23 | 0.029 | + | 2 |
| 4856 _ Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;Roseospira;unclassified | 0.23 | 0.029 | + | 2 |
| 4887 _ Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;Roseospira;unclassified | 0.23 | 0.029 | + | 2 |
| 4936 _ Bacteroidetes;Sphingobacteria;Sphingobacteriales;Flammeovirgaceae;Fulvivirga;unclassified | 0.23 | 0.029 | + | 2 |
| 5000 _ Cyanobacteria;Chloroplast;unclassified;unclassified;unclassified;unclassified | 0.23 | 0.029 | + | 2 |
| 5140 _ Planctomycetes;Phycisphaerae;vadinBA30;unclassified;unclassified;unclassified | 0.23 | 0.029 | + | 2 |
| 747 _ Proteobacteria;Deltaproteobacteria;Sh765B-TzT-29;unclassified;unclassified;unclassified | 0.23 | 0.029 | - | 4 |
| 1148 _ Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Planctomyces;unclassified | 0.23 | 0.029 | - | 10 |
| 4138 _ Acidobacteria;Holophagae;Holophagales;Holophagaceae;unclassified;unclassified | 0.23 | 0.030 | - | 4 |
| 33 _ Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;unclassified;unclassified | 0.23 | 0.030 | - | 17 |
| 4669 _ Proteobacteria;Gammaproteobacteria;NKB5;unclassified;unclassified;unclassified | 0.23 | 0.030 | + | 2 |
| 1217 _ Proteobacteria;Deltaproteobacteria;Sh765B-TzT-29;unclassified;unclassified;unclassified | 0.23 | 0.030 | + | 12 |
| 3496 _ Proteobacteria;Gammaproteobacteria;unclassified;unclassified;unclassified;unclassified | 0.23 | 0.030 | - | 9 |
| 924 _ Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;unclassified;unclassified | 0.23 | 0.030 | - | 8 |
| 209 _ Acidobacteria;Holophagae;Holophagales;Holophagaceae;unclassified;unclassified | 0.23 | 0.030 | + | 12 |
| 3363 _ Proteobacteria;Gammaproteobacteria;unclassified;unclassified;unclassified;unclassified | 0.23 | 0.031 | - | 9 |
| 99 _ Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;unclassified;unclassified | 0.23 | 0.031 | - | 17 |
| 109 _ Firmicutes;Clostridia;Clostridiales;Veillonellaceae;Acidaminococcus;unclassified | 0.23 | 0.031 | - | 16 |
| 4402 _ unclassified;unclassified;unclassified;unclassified;unclassified;unclassified | 0.23 | 0.031 | - | 3 |
| 2679 _ Proteobacteria;Alphaproteobacteria;DB1-14;unclassified;unclassified;unclassified | 0.23 | 0.031 | - | 10 |
| 3309 _ Proteobacteria;Gammaproteobacteria;unclassified;unclassified;unclassified;unclassified | 0.22 | 0.031 | + | 7 |
| 1394 _ Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Rubritaleaceae;Rubritalea;unclassified | 0.22 | 0.032 | + | 10 |
| 1251 _ Planctomycetes;Phycisphaerae;unclassified;unclassified;unclassified;unclassified | 0.22 | 0.032 | + | 15 |
| 4104 _ Proteobacteria;Gammaproteobacteria;unclassified;unclassified;unclassified;unclassified | 0.22 | 0.033 | - | 3 |
| 4205 _ Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;unclassified;unclassified | 0.22 | 0.033 | - | 3 |
| 837 _ Actinobacteria;Actinobacteria;Acidimicrobidae;Acidimicrobiales;Acidimicrobineae | 0.22 | 0.033 | - | 17 |
| 3808 _ Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;unclassified;unclassified | 0.22 | 0.034 | - | 6 |
| 1071 _ Proteobacteria;Deltaproteobacteria;Myxococcales;JG37-AG-15;unclassified | 0.22 | 0.034 | + | 5 |
| 971 _ Bacteroidetes;Sphingobacteria;Sphingobacteriales;Saprospiraceae;unclassified | 0.22 | 0.035 | + | 11 |
| 1863 _ Proteobacteria;Deltaproteobacteria;Desulfobacterales;Nitrospinaceae;Candidatus_Entotheonella;unclassified | 0.21 | 0.035 | + | 15 |
| 7040 _ Thermodesulfobacteria;Thermodesulfobacteria;Thermodesulfobacteriales;Thermodesulfobacteriaceae;Thermodesulfatator;unclassified | 0.21 | 0.036 | - | 2 |
| 7269 _ Candidate_division_TM6;unclassified;unclassified;unclassified;unclassified;unclassified | 0.21 | 0.036 | - | 2 |
| 155 _ Bacteroidetes;Sphingobacteria;Sphingobacteriales;Flammeovirgaceae;Fulvivirga;unclassified | 0.21 | 0.036 | + | 13 |
| 1461 _ Proteobacteria;Alphaproteobacteria;OCS116;unclassified;unclassified;unclassified | 0.21 | 0.036 | - | 10 |
| 6883 _ Proteobacteria;Gammaproteobacteria;NKB5;unclassified;unclassified;unclassified | 0.21 | 0.036 | - | 2 |

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|---|---|---|---|---|
| 6895 _ Proteobacteria;Gammaproteobacteria;Thiotrichales;CHAB-XI-27;unclassified | 0.21 | 0.036 | - | 2 |
| 6949 _ Proteobacteria;Gammaproteobacteria;Legionellales;Coxiellaceae;unclassified;unclassified | 0.21 | 0.036 | - | 2 |
| 7056 _ Thermodesulfobacteria;Thermodesulfobacteria;Thermodesulfobacteriales; Thermodesulfobacteriaceae;Thermodesulfatator;unclassified | 0.21 | 0.036 | - | 2 |
| 7078 _ Candidate_division_OD1;unclassified;unclassified;unclassified;unclassified;unclassified | 0.21 | 0.036 | - | 2 |
| 1881 _ Proteobacteria;Gammaproteobacteria;Xanthomonadales;Sinobacteraceae;unclassified | 0.21 | 0.036 | + | 12 |
| 144 _ Verrucomicrobia;Spartobacteria;unclassified;unclassified;unclassified;unclassified | 0.21 | 0.036 | - | 17 |
| 954 _ Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhodobiaceae;Parvibaculum;unclassified | 0.21 | 0.036 | - | 9 |
| 4490 _ Actinobacteria;Actinobacteria;Rubrobacteridae;Solirubrobacterales;Conexibacteraceae; Conexibacter | 0.21 | 0.036 | + | 2 |
| 4552 _ unclassified;unclassified;unclassified;unclassified;unclassified;unclassified | 0.21 | 0.036 | + | 2 |
| 4586 _ Proteobacteria;Gammaproteobacteria;Thiotrichales;CHAB-XI-27;unclassified | 0.21 | 0.036 | + | 2 |
| 4801 _ Proteobacteria;Gammaproteobacteria;endosymbionts;unclassified;unclassified | 0.21 | 0.036 | + | 2 |
| 4830 _ Proteobacteria;Gammaproteobacteria;Thiotrichales;CHAB-XI-27;unclassified | 0.21 | 0.036 | + | 2 |
| 4843 _ Proteobacteria;Deltaproteobacteria;unclassified;unclassified;unclassified;unclassified | 0.21 | 0.036 | + | 2 |
| 4874 _ Planctomycetes;Phycisphaerae;Phycisphaerales;Phycisphaeraceae; Urania-1B-19;unclassified | 0.21 | 0.036 | + | 2 |
| 4924 _ Verrucomicrobia;Arctic97B-4;unclassified;unclassified;unclassified;unclassified | 0.21 | 0.036 | + | 2 |
| 4968 _ Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae; Oceanicaulis;unclassified | 0.21 | 0.036 | + | 2 |
| 5081 _ Proteobacteria;Deltaproteobacteria;Myxococcales;Cystobacterineae;unclassified | 0.21 | 0.036 | + | 2 |
| 5196 _ Lentisphaerae;Lentisphaeria;WCHB1-41;unclassified;unclassified;unclassified | 0.21 | 0.036 | + | 2 |
| 4426 _ Bacteroidetes;Sphingobacteria;Sphingobacteriales;Saprospiraceae;unclassified | 0.21 | 0.037 | + | 2 |
| 3325 _ Actinobacteria;Actinobacteria;Acidimicrobidae;Acidimicrobiales;Acidimicrobineae; Acidimicrobiaceae | 0.21 | 0.037 | - | 5 |
| 218 _ Proteobacteria;Gammaproteobacteria;Enterobacteriales;Enterobacteriaceae; Enteric_Bacteria_cluster;Escherichia | 0.21 | 0.038 | - | 11 |
| 2596 _ Proteobacteria;Deltaproteobacteria;Myxococcales;Nannocystineae; Nannocystaceae;unclassified | 0.21 | 0.039 | - | 3 |
| 3181 _ Proteobacteria;Gammaproteobacteria;Chromatiales;Ectothiorhodospiraceae;unclassified | 0.20 | 0.039 | - | 3 |
| 34 _ Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhodobiaceae;Rhodobium;unclassified | 0.20 | 0.039 | - | 17 |
| 3626 _ Proteobacteria;Deltaproteobacteria;Myxococcales;Cystobacterineae;Myxococcaceae; Pyxidicoccus | 0.20 | 0.041 | - | 7 |
| 172 _ Acidobacteria;RB25;unclassified;unclassified;unclassified;unclassified | 0.20 | 0.041 | + | 13 |
| 4017 _ Proteobacteria;Deltaproteobacteria;Desulfuromonadales;Desulfuromonadaceae; Malonomas;unclassified | 0.20 | 0.041 | - | 4 |
| 3483 _ Proteobacteria;Gammaproteobacteria;NKB5;unclassified;unclassified;unclassified | 0.20 | 0.041 | + | 3 |
| 7872 _ Proteobacteria;Deltaproteobacteria;Syntrophobacterales; Syntrophobacteraceae;Desulfoglaeba;unclassified | 0.20 | 0.041 | + | 3 |
| 1086 _ Actinobacteria;Actinobacteria;Actinobacteridae;Actinomycetales;Corynebacterineae; Nocardiaceae | 0.20 | 0.042 | - | 11 |
| 1663 _ Chloroflexi;S085;unclassified;unclassified;unclassified;unclassified | 0.20 | 0.042 | - | 8 |
| 879 _ Planctomycetes;vadinHA49;unclassified;unclassified;unclassified;unclassified | 0.20 | 0.042 | + | 10 |
| 2142 _ Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae; Blastopirellula;unclassified | 0.20 | 0.042 | + | 9 |
| 4101 _ Actinobacteria;Actinobacteria;Actinobacteridae;Bifidobacteriales; Bifidobacteriaceae;Aeriscardovia | 0.20 | 0.042 | - | 2 |
| 4137 _ Proteobacteria;Gammaproteobacteria;Legionellales;Coxiellaceae;unclassified;unclassified | 0.20 | 0.042 | - | 2 |
| 4169 _ Proteobacteria;Gammaproteobacteria;Chromatiales;unclassified;unclassified;unclassified | 0.20 | 0.042 | - | 2 |
| 4173 _ Proteobacteria;Alphaproteobacteria;unclassified;unclassified;unclassified;unclassified | 0.20 | 0.042 | - | 2 |
| 4208 _ Proteobacteria;Gammaproteobacteria;unclassified;unclassified;unclassified;unclassified | 0.20 | 0.042 | - | 2 |
| 4217 _ Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae; | 0.20 | 0.042 | - | 2 |

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|---|---|---|---|---|
| Planctomyces;unclassified | | | | |
| 4246 _ Planctomycetes;Planctomycetacia;Planctomycetales; Planctomycetaceae;unclassified;unclassified | 0.20 | 0.042 | - | 2 |
| 4350 _ Planctomycetes;Planctomycetacia;Planctomycetales; Planctomycetaceae;Pirellula;unclassified | 0.20 | 0.042 | - | 2 |
| 4358 _ Proteobacteria;Gammaproteobacteria;unclassified;unclassified;unclassified;unclassified | 0.20 | 0.042 | - | 2 |
| 4404 _ Proteobacteria;Gammaproteobacteria;Chromatiales;Ectothiorhodospiraceae; Alkalilimnicola;unclassified | 0.20 | 0.042 | - | 2 |
| 4088 _ Proteobacteria;Gammaproteobacteria;Thiotrichales;Fangia;unclassified;unclassified | 0.20 | 0.043 | - | 3 |
| 411 _ Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;unclassified;unclassified | 0.20 | 0.043 | + | 14 |
| 4112 _ Proteobacteria;Gammaproteobacteria;Chromatiales;Ectothiorhodospiraceae; Alkalilimnicola;unclassified | 0.20 | 0.043 | - | 2 |
| 3839 _ Proteobacteria;Gammaproteobacteria;Thiobacillus;unclassified;unclassified;unclassified | 0.20 | 0.043 | - | 2 |
| 3914 _ Proteobacteria;Deltaproteobacteria;Desulfuromonadales; M20-Pitesti;unclassified;unclassified | 0.20 | 0.043 | - | 2 |
| 4032 _ Proteobacteria;Gammaproteobacteria;Legionellales;Coxiellaceae;unclassified;unclassified | 0.20 | 0.043 | - | 2 |
| 4076 _ Proteobacteria;Gammaproteobacteria;Thiotrichales;CHAB-XI-27;unclassified | 0.20 | 0.043 | - | 2 |
| 4133 _ Proteobacteria;Gammaproteobacteria;Thiotrichales;Fangia;unclassified;unclassified | 0.20 | 0.043 | - | 2 |
| 4295 _ Proteobacteria;Gammaproteobacteria;Thiotrichales;Fangia;unclassified;unclassified | 0.20 | 0.043 | - | 2 |
| 194 _ Proteobacteria;Gammaproteobacteria;1013-28-CG33;unclassified | 0.19 | 0.044 | - | 16 |
| 691 _ Actinobacteria;Actinobacteria;Rubrobacteridae;AKIW543;unclassified;unclassified | 0.19 | 0.044 | - | 11 |
| 1090 _ Chloroflexi;SAR202;unclassified;unclassified;unclassified;unclassified | 0.19 | 0.044 | + | 14 |
| 317 _ Proteobacteria;Deltaproteobacteria;Myxococcales;Nannocystineae;Haliangiaceae; Haliangium | 0.19 | 0.044 | + | 15 |
| 7552 _ Chlamydiae;Chlamydiae;Chlamydiales;Simkaniaceae;Simkania;unclassified | 0.19 | 0.044 | + | 3 |
| 7294 _ Proteobacteria;Gammaproteobacteria;Thiotrichales;Caedibacter;unclassified;unclassified | 0.19 | 0.045 | - | 3 |
| 1273 _ Proteobacteria;Deltaproteobacteria;Myxococcales;VHS-B3-70;unclassified | 0.19 | 0.045 | + | 7 |
| 504 _ Proteobacteria;Gammaproteobacteria;Chromatiales;Ectothiorhodospiraceae; Alkalilimnicola;unclassified | 0.19 | 0.045 | - | 7 |
| 3447 _ Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae; Defluviicoccus;unclassified | 0.19 | 0.046 | + | 5 |
| 2058 _ Acidobacteria;Holophagae;Holophagales;Holophagaceae;unclassified;unclassified | 0.19 | 0.047 | + | 9 |
| 2103 _ Bacteroidetes;Flavobacteria;Flavobacteriales;Cryomorphaceae;unclassified;unclassified | 0.19 | 0.047 | + | 12 |
| 1221 _ Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae; OM60_NOR5_clade;Haliea | 0.19 | 0.047 | - | 3 |
| 3326 _ Proteobacteria;Gammaproteobacteria;Thiotrichales;CHAB-XI-27;unclassified | 0.19 | 0.047 | - | 3 |
| 382 _ Acidobacteria;Holophagae;Holophagales;Holophagaceae;unclassified;unclassified | 0.19 | 0.047 | + | 15 |
| 3938 _ Firmicutes;Bacilli;unclassified;unclassified;unclassified;unclassified | 0.19 | 0.047 | - | 3 |
| 97 _ Proteobacteria;Deltaproteobacteria;Sh765B-TzT-29;unclassified | 0.19 | 0.048 | + | 17 |
| 64 _ Chloroflexi;Anaerolineae;Anaerolineales;Anaerolineaceae;unclassified;unclassified | 0.19 | 0.048 | - | 17 |
| 4488 _ Chloroflexi;Caldilineae;Caldilineales;Caldilineaceae;unclassified;unclassified | 0.19 | 0.048 | + | 3 |
| 5168 _ Proteobacteria;Deltaproteobacteria;Desulfobacterales;Nitrospinaceae;unclassified | 0.19 | 0.048 | + | 3 |
| 143 _ Planctomycetes;Phycisphaerae;SHA-43;unclassified;unclassified;unclassified | 0.19 | 0.048 | + | 14 |
| 746 _ Proteobacteria;Betaproteobacteria;Burkholderiales;Comamonadaceae;Delftia;unclassified | 0.18 | 0.048 | - | 3 |
| 2848 _ Bacteroidetes;Sphingobacteria;Sphingobacteriales;Cytophagaceae; Microscilla;unclassified | 0.18 | 0.048 | + | 5 |
| 4822 _ Proteobacteria;Deltaproteobacteria;unclassified;unclassified;unclassified;unclassified | 0.18 | 0.049 | + | 4 |
| 6225 _ Bacteroidetes;Flavobacteria;Flavobacteriales;Cryomorphaceae;Crocinitomix;unclassified | 0.18 | 0.049 | + | 4 |
| 4494 _ Proteobacteria;Deltaproteobacteria;Desulfobacterales;Desulfobacteraceae;Desulfatiferula; unclassified | 0.18 | 0.049 | + | 3 |
| 5180 _ | 0.18 | 0.049 | + | 3 |

| | | | | |
|---|---|---|---|---|
| Candidate_division_OP3;unclassified;unclassified;unclassified;unclassified;unclassified | | | | |
| 4339 _ Proteobacteria;Deltaproteobacteria;Desulfuromonadales;Desulfuromonadaceae; Malonomas;unclassified | 0.18 | 0.049 | - | 2 |
| 1065 _ Actinobacteria;Actinobacteria;Actinobacteridae;Actinomycetales;Corynebacterineae; Nocardiaceae | 0.18 | 0.049 | - | 6 |
| 871 _ Proteobacteria;Deltaproteobacteria;Myxococcales;Nannocystineae;unclassified | 0.18 | 0.050 | + | 12 |

**Table S8** Bacterial taxa at different taxonomic levels and $OTU_{3\%}$ that occurred with a significantly higher relative abundance in a certain year compared to all others.

| | Year | indicator value |
|---|---|---|
| **Phylum - level** | | |
| Candidate_division_OP3 | 2007 | 0.33 |
| **Class - level** | | |
| Candidate_division_OP3.unclassified | 2007 | 0.33 |
| Cyanobacteria.SubsectionIII | 2006 | 0.40 |
| Cyanobacteria.SHA.109 | 2007 | 0.39 |
| Candidate_division_TG.1.unclassified | 2007 | 0.89 |
| Firmicutes.Clostridia | 2007 | 0.29 |
| Bacteroidetes.Bacteroidia | 2007 | 0.90 |
| Verrucomicrobia.Arctic97B.4 | 2007 | 0.39 |
| **Genus - level** | | |
| Acidobacteria.Holophagae.iii1.8.unclassified.unclassified | 2003 | 0.43 |
| Proteobacteria.Gammaproteobacteria.Alteromonadales.Alteromonadaceae.Glaciecola | 2003 | 0.59 |
| Proteobacteria.Deltaproteobacteria.Desulfobacterales.Desulfobulbaceae.Desulfotalea | 2003 | 0.86 |
| Bacteroidetes.Flavobacteria.Flavobacteriales.Flavobacteriaceae.Gillisia | 2003 | 0.72 |
| Bacteroidetes.Flavobacteria.Flavobacteriales.Flavobacteriaceae.Arenibacter | 2003 | 0.88 |
| Verrucomicrobia.Opitutae.Puniceicoccales.Puniceicoccaceae.unclassified | 2003 | 0.33 |
| Acidobacteria.Holophagae.Holophagales.Holophagaceae.Geothrix | 2003 | 0.51 |
| Firmicutes.Bacilli.Bacillales.Paenibacillaceae.Cohnella | 2004 | 0.80 |
| Proteobacteria.Gammaproteobacteria.Enterobacteriales.Enterobacteriaceae.endosymbionts | 2004 | 0.81 |
| Chloroflexi.Anaerolineae.Anaerolineales.Anaerolineaceae.Bellilinea | 2004 | 0.57 |
| Proteobacteria.Gammaproteobacteria.Oceanospirillales.unclassified.unclassified | 2004 | 0.62 |
| Proteobacteria.Gammaproteobacteria.Oceanospirillales.Oceanospirillaceae.Pseudospirillum | 2004 | 0.45 |
| Cyanobacteria.SubsectionIII.Halomicronema.unclassified.unclassified | 2006 | 0.64 |
| Firmicutes.Bacilli.Bacillales.Planococcaceae.Marinibacillus | 2006 | 1.00 |
| Firmicutes.Bacilli.Bacillales.Planococcaceae.Sporosarcina | 2006 | 1.00 |
| Proteobacteria.Gammaproteobacteria.1013.28.CG33.unclassified.unclassified | 2006 | 0.41 |
| Firmicutes.Bacilli.Bacillales.Bacillaceae.Bacillus | 2006 | 1.00 |
| Proteobacteria.Gammaproteobacteria.Legionellales.Coxiellaceae.Coxiella | 2006 | 0.42 |
| Actinobacteria.Actinobacteria.Actinobacteridae.Actinomycetales.Micromonosporineae | 2006 | 0.34 |
| Chlamydiae.Chlamydiae.Chlamydiales.Parachlamydiaceae.Parachlamydia | 2006 | 0.34 |
| Proteobacteria.Gammaproteobacteria.Chromatiales.Ectothiorhodospiraceae.Alkalilimnicola | 2006 | 0.27 |
| Proteobacteria.Alphaproteobacteria.Rhodospirillales.Rhodospirillaceae.Novispirillum | 2007 | 0.43 |
| Proteobacteria.Deltaproteobacteria.Desulfuromonadales.Desulfuromonadaceae.unclassified | 2007 | 0.57 |
| Candidate_division_OP3.unclassified.unclassified.unclassified.unclassified | 2007 | 0.33 |
| Cyanobacteria.SHA.109.unclassified.unclassified.unclassified | 2007 | 0.39 |
| Proteobacteria.Gammaproteobacteria.Thiohalophilus.unclassified.unclassified | 2007 | 0.29 |
| Proteobacteria.Alphaproteobacteria.Caulobacterales.Hyphomonadaceae.Oceanicaulis | 2007 | 0.35 |
| Candidate_division_TG.1.unclassified.unclassified.unclassified.unclassified | 2007 | 0.89 |
| Planctomycetes.Phycisphaerae.unclassified.unclassified.unclassified | 2007 | 0.47 |
| Proteobacteria.Alphaproteobacteria.Rhizobiales.Candidatus_Liberibacter.unclassified | 2007 | 0.89 |
| Proteobacteria.Alphaproteobacteria.Rhizobiales.Rhodobiaceae.Afifella | 2007 | 0.60 |
| Proteobacteria.Deltaproteobacteria.Desulfobacterales.Desulfobacteraceae.Desulfatiferula | 2007 | 0.38 |
| Proteobacteria.Epsilonproteobacteria.Campylobacterales.Helicobacteraceae.Sulfurimonas | 2007 | 0.77 |
| Bacteroidetes.Flavobacteria.Flavobacteriales.Flavobacteriaceae.Dokdonia | 2007 | 0.64 |
| Firmicutes.Clostridia.Clostridiales.Family_XI_Incertae_Sedis.Helcococcus | 2007 | 0.90 |
| Proteobacteria.Gammaproteobacteria.Legionellales.Coxiellaceae.Rickettsiella | 2007 | 0.58 |
| Proteobacteria.Deltaproteobacteria.Desulfobacterales.Desulfobulbaceae.Desulfobacterium | 2007 | 0.73 |
| Proteobacteria.Alphaproteobacteria.Rhizobiales.Aurantimonadaceae.Aurantimonas | 2007 | 0.63 |
| Proteobacteria.Deltaproteobacteria.Desulfobacterales.Desulfobacteraceae.Desulfotignum | 2007 | 0.71 |

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|---|---|---|
| Proteobacteria.Alphaproteobacteria.Rhodobacterales.Rhodobacteraceae.Nereida | 2007 | 0.90 |
| Proteobacteria.Alphaproteobacteria.Rhizobiales.Hyphomicrobiaceae.Ancalomicrobium | 2007 | 0.49 |
| Proteobacteria.Deltaproteobacteria.Desulfobacterales.Desulfobulbaceae.Desulfopila | 2007 | 0.89 |
| Planctomycetes.Phycisphaerae.Phycisphaerales.Phycisphaeraceae.Phycisphaera | 2007 | 0.32 |
| Firmicutes.Clostridia.Clostridiales.Ruminococcaceae.Hydrogenoanaerobacterium | 2007 | 0.81 |
| Proteobacteria.Gammaproteobacteria.Methylococcales.Methylococcaceae.Methylococcus | 2007 | 0.66 |
| Proteobacteria.Gammaproteobacteria.Sva0071.unclassified.unclassified | 2007 | 0.38 |
| Proteobacteria.Gammaproteobacteria.Alteromonadales.Pseudoalteromonadaceae.Pseudoalteromonas | 2007 | 0.81 |
| Proteobacteria.Deltaproteobacteria.Desulfuromonadales.Sva1033.unclassified | 2007 | 0.48 |
| Deferribacteres.Unclassified_Deferribacterales.LCP.89.unclassified.unclassified | 2007 | 0.27 |
| Firmicutes.Clostridia.Clostridiales.Peptococcaceae.Desulfurispora | 2007 | 0.73 |
| Proteobacteria.Alphaproteobacteria.Rhodospirillales.Rhodospirillaceae.Telmatospirillum | 2007 | 1.00 |
| Verrucomicrobia.Arctic97B.4.unclassified.unclassified.unclassified | 2007 | 0.39 |
| Firmicutes.Clostridia.Clostridiales.Lachnospiraceae.Incertae_Sedis | 2007 | 0.91 |
| Proteobacteria.Deltaproteobacteria.Desulfuromonadales.Desulfuromonadaceae.Desulfuromonas | 2007 | 0.75 |
| Bacteroidetes.Flavobacteria.Flavobacteriales.Flavobacteriaceae.Croceibacter | 2008 | 0.63 |
| Proteobacteria.Deltaproteobacteria.Syntrophobacterales.Syntrophobacteraceae.Desulfoglaeba | 2009 | 0.59 |
| **OTU - level** | | |
| 2913 _ Proteobacteria;Gammaproteobacteria;unclassified;unclassified;unclassified;unclassified | 2003 | 0.74 |
| 431 _ Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Glaciecola;unclassified | 2003 | 0.60 |
| 3278 _ Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Planctomyces;unclassified | 2003 | 0.66 |
| 2803 _ Proteobacteria;Deltaproteobacteria;Myxococcales;Nannocystineae;Nannocystaceae;Plesiocystis | 2003 | 0.63 |
| 994 _ Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Planctomyces;unclassified | 2003 | 0.48 |
| 62 _ Bacteroidetes;Flavobacteria;Flavobacteriales;Flavobacteriaceae;Gillisia;unclassified | 2003 | 0.72 |
| 1348 _ Bacteroidetes;Flavobacteria;Flavobacteriales;Flavobacteriaceae;Arenibacter;unclassified | 2003 | 0.88 |
| 386 _ Proteobacteria;Gammaproteobacteria;Thiobacillus;unclassified;unclassified;unclassified | 2003 | 0.52 |
| 3521 _ Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;unclassified;unclassified | 2003 | 0.67 |
| 512 _ Bacteroidetes;Sphingobacteria;Sphingobacteriales;Saprospiraceae;unclassified;unclassified | 2003 | 0.44 |
| 90 _ Verrucomicrobia;Opitutae;Puniceicoccales;Puniceicoccaceae;unclassified;unclassified | 2003 | 0.34 |
| 1024 _ Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;unclassified;unclassified | 2003 | 0.51 |
| 104 _ Bacteroidetes;Sphingobacteria;Sphingobacteriales;Flammeovirgaceae;unclassified;unclassified | 2003 | 0.32 |
| 76 _ Proteobacteria;Gammaproteobacteria;Chromatiales;Ectothiorhodospiraceae;Thioalkalivibrio;unclassified | 2003 | 0.35 |
| 1391 _ Proteobacteria;Gammaproteobacteria;JTB148;unclassified;unclassified;unclassified | 2003 | 0.45 |
| 1253 _ Acidobacteria;Holophagae;Holophagales;Holophagaceae;unclassified;unclassified | 2003 | 0.64 |
| 3310 _ Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula;unclassified | 2003 | 0.66 |
| 131 _ Proteobacteria;Alphaproteobacteria;OCS116;unclassified;unclassified;unclassified | 2003 | 0.53 |
| 3709 _ Proteobacteria;Gammaproteobacteria;NKB5;unclassified;unclassified;unclassified | 2003 | 0.48 |
| 252 _ Proteobacteria;Deltaproteobacteria;Myxococcales;Nannocystineae;Nannocystaceae;unclassified | 2003 | 0.46 |
| 124 _ Bacteroidetes;Flavobacteria;Flavobacteriales;Cryomorphaceae;Crocinitomix;unclassified | 2004 | 0.55 |
| 398 _ Chloroflexi;SAR202;unclassified;unclassified;unclassified;unclassified | 2004 | 0.40 |
| 430 _ Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;OM60_NOR5_clade;Haliea | 2004 | 0.49 |
| 462 _ Proteobacteria;Alphaproteobacteria;MNG3;unclassified;unclassified;unclassified | 2004 | 0.43 |
| 1019 _ Chloroflexi;Anaerolineae;Anaerolineales;Anaerolineaceae;unclassified;unclassified | 2006 | 0.71 |
| 3821 _ Chlamydiae;Chlamydiae;Chlamydiales;Parachlamydiaceae;Parachlamydia;unclassified | 2006 | 0.70 |
| 2182 _ unclassified;unclassified;unclassified;unclassified;unclassified;unclassified | 2006 | 0.66 |
| 3649 _ Proteobacteria;Gammaproteobacteria;Thiotrichales;Fangia;unclassified;unclassified | 2006 | 0.64 |
| 3808 _ Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;unclassified;unclassified | 2006 | 0.64 |
| 3818 _ Proteobacteria;Deltaproteobacteria;Myxococcales;Cystobacterineae;Myxococcaceae;Pyxidicoccus | 2006 | 0.59 |
| 50 _ Actinobacteria;Actinobacteria;unclassified;unclassified;unclassified;unclassified | 2006 | 0.56 |
| 946 _ Chloroflexi;S085;unclassified;unclassified;unclassified;unclassified | 2006 | 0.53 |
| 196 _ Proteobacteria;Deltaproteobacteria;Desulfovibrionales;Desulfohalobiaceae;Desulfonauticus;unclassified | 2006 | 0.52 |
| 3430 _ Proteobacteria;Gammaproteobacteria;Thiobacillus;unclassified;unclassified;unclassified | 2006 | 0.51 |
| 3496 _ Proteobacteria;Gammaproteobacteria;unclassified;unclassified;unclassified;unclassified | 2006 | 0.50 |
| 369 _ Proteobacteria;TA18;unclassified;unclassified;unclassified;unclassified | 2006 | 0.49 |
| 964 _ Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhodobiaceae;Rhodobium;unclassified | 2006 | 0.46 |
| 782 _ Proteobacteria;Gammaproteobacteria;unclassified;unclassified;unclassified;unclassified | 2006 | 0.46 |

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|---|---|---|
| 2546 _ Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;unclassified;unclassified | 2006 | 0.45 |
| 412 _ Proteobacteria;Deltaproteobacteria;Desulfuromonadales;unclassified;unclassified;unclassified | 2006 | 0.44 |
| 582 _ Chloroflexi;S085;unclassified;unclassified;unclassified;unclassified | 2006 | 0.44 |
| 437 _ Candidate_division_BRC1;unclassified;unclassified;unclassified;unclassified;unclassified | 2006 | 0.43 |
| 194 _ Proteobacteria;Gammaproteobacteria;1013-28-CG33;unclassified;unclassified;unclassified | 2006 | 0.42 |
| 543 _ Gemmatimonadetes;Gemmatimonadetes;BD2-11;unclassified;unclassified;unclassified | 2006 | 0.42 |
| 1874 _ Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Pirellula;unclassified | 2006 | 0.40 |
| 622 _ Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;unclassified;unclassified | 2006 | 0.39 |
| 144 _ Verrucomicrobia;Spartobacteria;unclassified;unclassified;unclassified;unclassified | 2006 | 0.38 |
| 837 _ Actinobacteria;Actinobacteria;Acidimicrobidae;Acidimicrobiales;Acidimicrobineae;unclassified | 2006 | 0.35 |
| 5 _ Actinobacteria;Actinobacteria;Actinobacteridae;Actinomycetales;Micromonosporineae;Micromonosporaceae | 2006 | 0.34 |
| 42 _ Gemmatimonadetes;Gemmatimonadetes;Gemmatimonadales;Gemmatimonadaceae;unclassified;unclassified | 2006 | 0.28 |
| 644 _ Planctomycetes;Phycisphaerae;SHA-43;unclassified;unclassified;unclassified | 2007 | 0.59 |
| 1045 _ Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;unclassified;unclassified | 2007 | 0.59 |
| 3014 _ Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;Candidatus_Chloroacidobacterium;unclassified | 2007 | 0.70 |
| 158 _ Proteobacteria;Deltaproteobacteria;Desulfuromonadales;Geobacteraceae;Geothermobacter;unclassified | 2007 | 0.44 |
| 821 _ Planctomycetes;Phycisphaerae;Phycisphaerales;Phycisphaeraceae;Urania-1B-19;unclassified | 2007 | 0.50 |
| 840 _ Acidobacteria;RB25;unclassified;unclassified;unclassified;unclassified | 2007 | 0.44 |
| 1251 _ Planctomycetes;Phycisphaerae;unclassified;unclassified;unclassified;unclassified | 2007 | 0.53 |
| 2064 _ Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;OM60_NOR5_clade;Haliea | 2007 | 0.73 |
| 2088 _ Acidobacteria;Holophagae;32-20;unclassified;unclassified;unclassified | 2007 | 0.75 |
| 296 _ Acidobacteria;RB25;unclassified;unclassified;unclassified;unclassified | 2007 | 0.35 |
| 1111 _ Candidate_division_OP3;unclassified;unclassified;unclassified;unclassified;unclassified | 2007 | 0.40 |
| 2788 _ unclassified;unclassified;unclassified;unclassified;unclassified;unclassified | 2007 | 0.53 |
| 2734 _ Proteobacteria;Deltaproteobacteria;SAR324;unclassified;unclassified;unclassified | 2007 | 0.67 |
| 2731 _ Planctomycetes;Phycisphaerae;Phycisphaerales;Phycisphaeraceae;Urania-1B-19;unclassified | 2007 | 0.69 |
| 174 _ Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;unclassified;unclassified | 2007 | 0.37 |
| 714 _ Proteobacteria;Alphaproteobacteria;Rhizobiales;Hyphomicrobiaceae;Ancalomicrobium;unclassified | 2007 | 0.51 |
| 666 _ Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Roseibacillus;unclassified | 2007 | 0.61 |
| 4439 _ Verrucomicrobia;Arctic97B-4;unclassified;unclassified;unclassified;unclassified | 2007 | 0.70 |
| 4751 _ Planctomycetes;Phycisphaerae;Phycisphaerales;Phycisphaeraceae;CL500-3;unclassified | 2007 | 0.69 |
| 966 _ Candidate_division_OP3;unclassified;unclassified;unclassified;unclassified;unclassified | 2007 | 0.60 |
| 1747 _ Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;unclassified;unclassified | 2007 | 0.47 |
| 116 _ Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;Pelagibius;unclassified | 2007 | 0.49 |
| 2146 _ Candidate_division_BRC1;unclassified;unclassified;unclassified;unclassified;unclassified | 2007 | 0.51 |
| 4820 _ BD1-5;unclassified;unclassified;unclassified;unclassified;unclassified | 2007 | 0.54 |
| 1338 _ Actinobacteria;Actinobacteria;Acidimicrobidae;Acidimicrobiales;Acidimicrobineae;Iamiaceae | 2007 | 0.48 |
| 387 _ Proteobacteria;Deltaproteobacteria;SAR324;unclassified;unclassified;unclassified | 2007 | 0.38 |
| 2097 _ Verrucomicrobia;Opitutae;Puniceicoccales;Puniceicoccaceae;Cerasicoccus;unclassified | 2007 | 0.57 |
| 2058 _ Acidobacteria;Holophagae;Holophagales;Holophagaceae;unclassified;unclassified | 2007 | 0.66 |
| 1273 _ Proteobacteria;Deltaproteobacteria;Myxococcales;VHS-B3-70;unclassified;unclassified | 2007 | 0.66 |
| 2781 _ Proteobacteria;Deltaproteobacteria;Myxococcales;Sorangiineae;unclassified;unclassified | 2007 | 0.46 |
| 189 _ Proteobacteria;Deltaproteobacteria;Desulfobacterales;Nitrospinaceae;Nitrospina;unclassified | 2007 | 0.52 |
| 1414 _ Proteobacteria;Deltaproteobacteria;Sh765B-TzT-29;unclassified;unclassified;unclassified | 2007 | 0.63 |
| 2688 _ Chloroflexi;Anaerolineae;Anaerolineales;Anaerolineaceae;unclassified;unclassified | 2007 | 0.59 |
| 5203 _ Proteobacteria;Gammaproteobacteria;Chromatiales;Ectothiorhodospiraceae;Alkalilimnicola;unclassified | 2007 | 0.63 |
| 826 _ Proteobacteria;Deltaproteobacteria;Sh765B-TzT-29;unclassified;unclassified;unclassified | 2007 | 0.40 |
| 2745 _ Chloroflexi;Caldilineae;Caldilineales;Caldilineaceae;unclassified;unclassified | 2007 | 0.45 |
| 1397 _ Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;unclassified;unclassified | 2007 | 0.60 |
| 456 _ Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;unclassified;unclassified | 2008 | 0.48 |
| 1357 _ Planctomycetes;Phycisphaerae;SHA-43;unclassified;unclassified;unclassified | 2008 | 0.54 |
| 1976 _ Proteobacteria;TA18;unclassified;unclassified;unclassified;unclassified | 2008 | 0.42 |
| 458 _ Proteobacteria;Deltaproteobacteria;Sh765B-TzT-29;unclassified;unclassified;unclassified | 2008 | 0.39 |
| 899 _ Bacteroidetes;Sphingobacteria;Sphingobacteriales;Saprospiraceae;unclassified;unclassified | 2009 | 0.57 |
| 315 _ Proteobacteria;Gammaproteobacteria;Xanthomonadales;Sinobacteraceae;unclassified;unclassified | 2009 | 0.52 |
| 164 _ Proteobacteria;Deltaproteobacteria;Sh765B-TzT-29;unclassified;unclassified;unclassified | 2009 | 0.29 |

| | | | | |
|---|---|---|---|---|
| 80 | _ | Deferribacteres;Unclassified_Deferribacterales;PAUC34f;unclassified;unclassified;unclassified | 2009 | 0.36 |
| 38 | _ | Proteobacteria;Deltaproteobacteria;Sh765B-TzT-29;unclassified;unclassified;unclassified | 2009 | 0.29 |

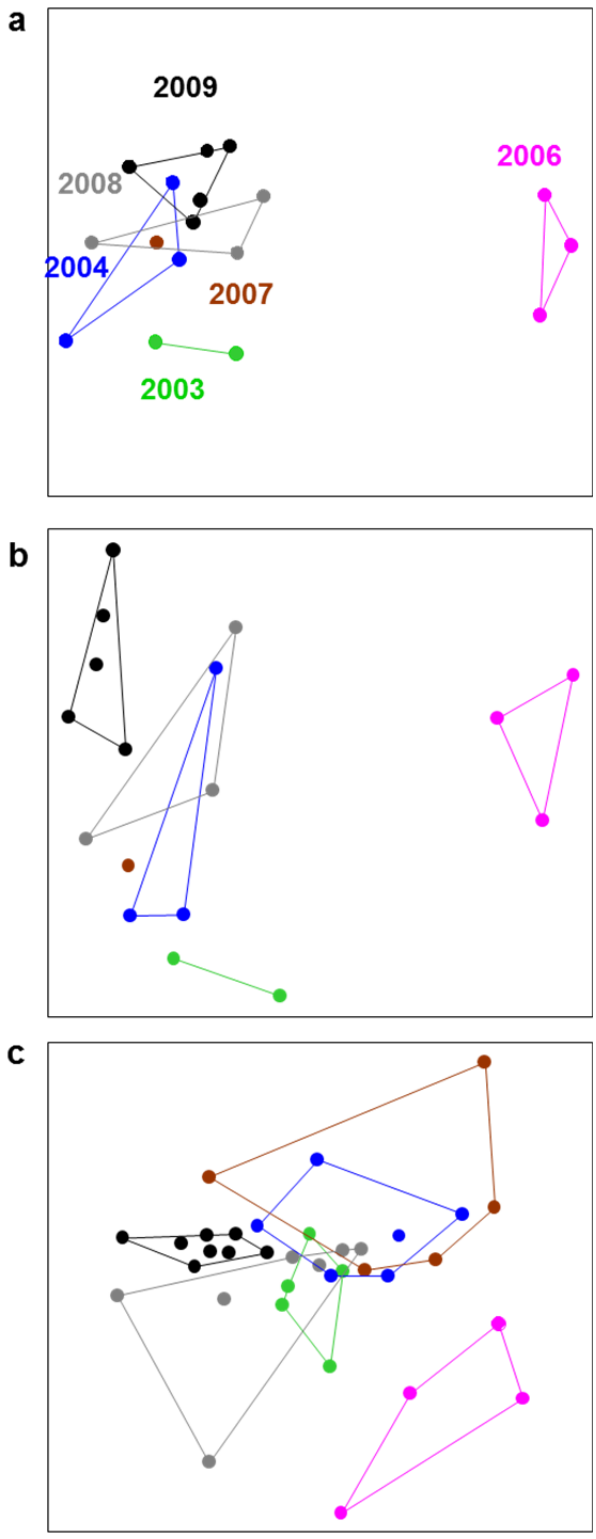


**Figure S1. Non-metric multidimensional** scaling of the dissimilarity in bacterial community structure for (**a**) rare $OTU_{3\%}$ , (b) resident $OTU_{3\%}$ and (c) $OTU_{ARISA}$. Colors per year are according to (**a**) for (**b**) and (**c**).

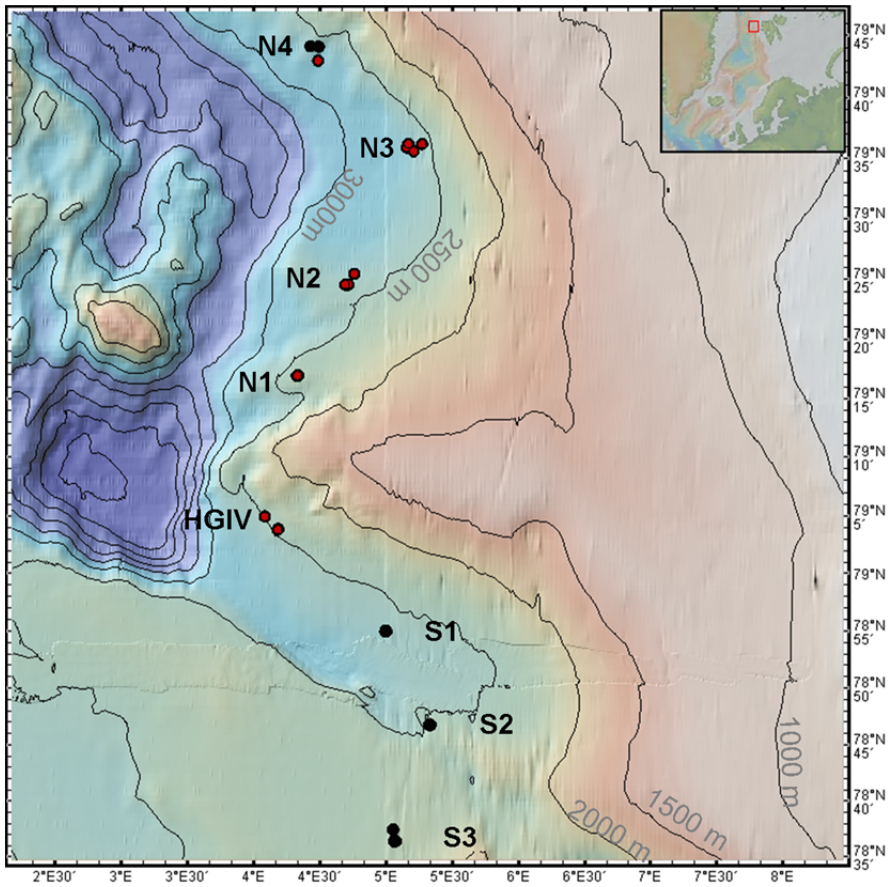


**Figure S2. Map of LTER HAUSGARTEN sampling stations**. Red dots indicate those stations that were used for 454 MPTS

# Chapter III

## Response of a benthic bacterial community to decreasing food availability: an *in situ* experimental approach at the Arctic deep-sea observatory HAUSGARTEN

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**Abstract**

Changing conditions in the Arctic Ocean such as warming of surface waters and sea-ice retreat may lead to changes in primary production and particle export to the deep-sea. Settling organic matter (OM) is the main food source to benthic deep-sea communities, with bacteria as the dominant component in terms of biomass and diversity. We investigated the in situ response of a deep-sea benthic bacterial community to a cut-off from particle flux over a three-year time period at 2500 m water depth. During this time, bacterial taxa richness was reduced by ~50% and bacterial community structure was altered considerably. Potential hydrolytic enzymatic activity per cell increased, indicating adaptations to the utilization of increasingly degraded polymeric matter. Our observations exhibited strong alterations of bacterial community structure to decreased organic matter supply and emphasize the necessity for long-term monitoring of Arctic benthic ecosystem changes.

Climate change in the Arctic Ocean has resulted in a decreased sea-ice cover and thickness as well as in increased water temperatures and stratification of surface waters (Maslanik et al., 2011; Rabe et al., 2014). Whether and where these trends lead to an increase in primary productivity is under debate (Arrigo et al., 2008; Tremblay and Gagnon, 2009; Wassmann et al., 2010) but biogeochemical models predict no or even negative changes in productivity and export flux in the Barents Sea and Fram Strait (Forest et al., 2010; Slagstad et al., 2011). Accordingly, recent investigations from the Long-Term Ecological Research (LTER) site HAUSGARTEN in Fram Strait (~79°N/04°E) revealed reduced primary production and export flux as well as a distinct shift in phytoplankton species due to sea ice retreat and warming between 2005 and 2008 (Kraft et al., 2011; Lalande et al., 2013; Cherkasheva et al., 2014).

Annual phytodetritus supply in early summer is the main food source for Arctic benthic organisms. Such pulsed sedimentation can usually be detected by elevated concentrations of chloroplastic pigment equivalents in surface sediments (e.g. Pfannkuche et al., 1999; Bianchi et al., 2002). It is known that deep-sea benthic bacterial communities react within days to the increased organic matter (OM) availability by enhanced carbon uptake and oxygen consumption, as well as by changes in the extracellular hydrolytic enzyme activity (Moodley et al., 2002; Witte et al., 2003). Moreover, Franco et al. (2007), Wei et al. (2010), as well as Bienhold et al. (2012) showed a positive relationship between food availability, bacterial biomass and bacterial diversity (richness). Previous *in situ* enrichment experiments carried out at HAUSGARTEN, showed that the effect of enhanced OM availability on bacterial biomass, activity and community structure lasted over a time span of one year (Kanzog and Ramette, 2009; Kanzog et al., 2009). Periods of deficits in carbon and energy supply by particle flux to the deep sea have also been observed as a consequence of Climate Change, and lead to transitions in respiration rates and body size of metazoan fauna (Ruhl et al., 2008). Yet, effects of reduced organic matter availability leading to food deficits in natural benthic bacterial communities are still unknown.

This study tested effects of the absence of the natural annual sedimentation of organic matter to the deep-sea floor on a benthic bacterial community by an *in situ* experimental approach. In summer 2008, four metal cages (2x2 m in length, 50 cm height) with a mesh at the sides (mesh size 1 cm) and solid lids preventing vertical particle sedimentation were deployed in an area of ~3.5 $km^2$ (2462 to 2472 m water depth) at the deep-sea observatory HAUSGARTEN

in Fram Strait (Figure S1). Surface sediments from 0-1 and 1-2 cm depth, which are directly influenced by deposition of organic matter, as well as a deeper layer at 4-5 cm depth, were sampled after one year (three sediment samples from inside one cage), and after three years (three sediment samples from inside each cage) using push-corers operated by a Remotely Operated Vehicle (ROV). Reference samples were taken ~2 km away at 2462 m water depth. Chloroplastic pigment concentrations, the potential activity of ester-cleaving hydrolytic enzymes and bacterial cell counts were determined as described in Shuman and Lorenzen (1975), Meyer-Reil (1983) and Köster et al. (1991), respectively. Bacterial diversity was investigated by the ARISA DNA-fingerprinting method (Fisher and Triplett, 1999; see Supplementary Information for details).

In accordance with previous studies (e.g. Pfannkuche et al., 1999; Soltwedel and Vopel, 2001), pigment concentrations, potential esterase activity, and cell numbers significantly decreased with increasing sediment depth (Spearman's $\rho$=-0.85, $p<0.001$, $\rho$=-0.56, $p<0.001$ and $\rho$=-0.85, $p<0.001$, respectively). Total pigment concentrations also significantly decreased with time in all sediment layers ($\rho$=-0.49, $p=0.001$) and were reduced by ~40% after three years of cutting of vertical particle flux (Figure 1a). The half-live of chlorophyll degradation products such as phaeophytin in oxic sediments is assumed to be on the order of weeks (Furlong and Carpenter, 1988; Sun et al., 1993; Graf et al., 1995).

To assess changes in hydrolytic enzymatic activity as a result of food limitation, we measured potential unspecific esterase activity. Esterases are relevant in the degradation of polymeric substances. Previous investigations suggested that their production is not directly induced by labile OM supply to benthic bacterial communities (Boetius and Damm, 1998; Pfannkuche et al., 1999), in contrast to other hydrolytic enzymes, which are substrate inducible (e.g. Boetius and Lochte, 1996; Kanzog et al., 2009). Here, probably as a result of cut-off from particle sedimentation, potential esterase activity was significantly elevated after three years in all sediment layers of the experiment ("Mann-Whitney"-test: $p<0.05$; Figure 1b). Similar responses have previously been observed in investigations of bacterial communities under starvation (Morita, 1982; Albertson et al., 1990).

Bacterial cell numbers exhibited minor variation throughout the experiment (Figure 1c). However, the average richness of bacterial operational taxonomic units (OTU) decreased by ~50% after three years ($\rho$=-0.7, $p<0.001$; Figure 1d). While still 78-85% of OTU found at the

beginning of the experiment remained after one year in the different sediment layers, only 30-37% were found after three years. Additionally, samples taken after three years of starvation only had on average 49-55% OTU per sediment layer in common and OTU evenness was lowest (average Pielou's evenness: 0.79±0.03) compared to 78-86% of shared OTU in one year samples and an evenness of 0.92±0.01 and an evenness of 0.89±0.03 in reference samples.

Bacterial community structure (based on relative abundances of OTU) changed only slightly after one year compared to reference samples, while community structure in upper and deeper sediment layers were maintained (Figure 2), indicating a good adjustment of the community to the naturally low, seasonally pulsed supply of OM. However, after three years, the bacterial community structure was significantly altered in all sediment layers, yet strongest in the surface layer (Figure 2a), probably due to the high loss of abundant OTU or due to adaptation of certain bacterial species to lower OM concentrations, or both. Dissimilarities of community structure in third-year samples were higher than between reference and one year samples, and a clear distinction in community structures of upper and lower sediment layers was missing (Figure 2b), indicating adaptation to the use of old degraded matter.

Our results indicate that the predicted decrease in OM/energy flux to the deep arctic sea due to climate change will substantially affect the bacterial community. Both, a decrease in richness of taxa and an elevated potential esterase activity could lead to general alterations in deep-sea ecosystem functioning. Further long-term monitoring in combination with experimental work under *in situ* conditions is needed to assess how changes in climate-driven surface ocean conditions may affect benthic ecosystem status and whether these changes are reversible.

Acknowledgements:
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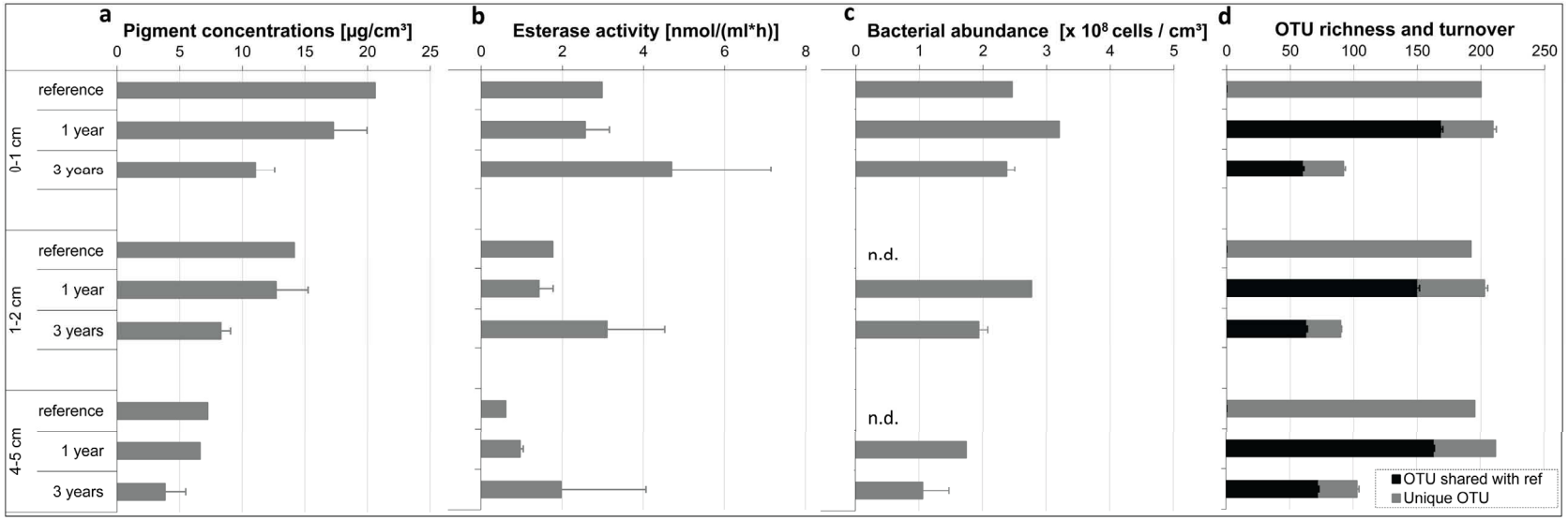


**Figure 1** Spatial and temporal variations in (**a**) pigment concentrations and (**b**) potential activity of ester-cleaving hydrolytic enzymes. (**c**) Bacterial cell numbers. (**d**) ARISA OTU richness as sum of shared OTU with the reference site and unique OTU. (**a**, **b**, **d**): except for the reference samples, average values per sample with standard deviations are displayed (one year: n=3 per depth layer, three years: n=12 per depth layer). (**c**): Standard deviations indicate differences in bacterial numbers between cages (n=4 per depth layer). n.d.: not determined.

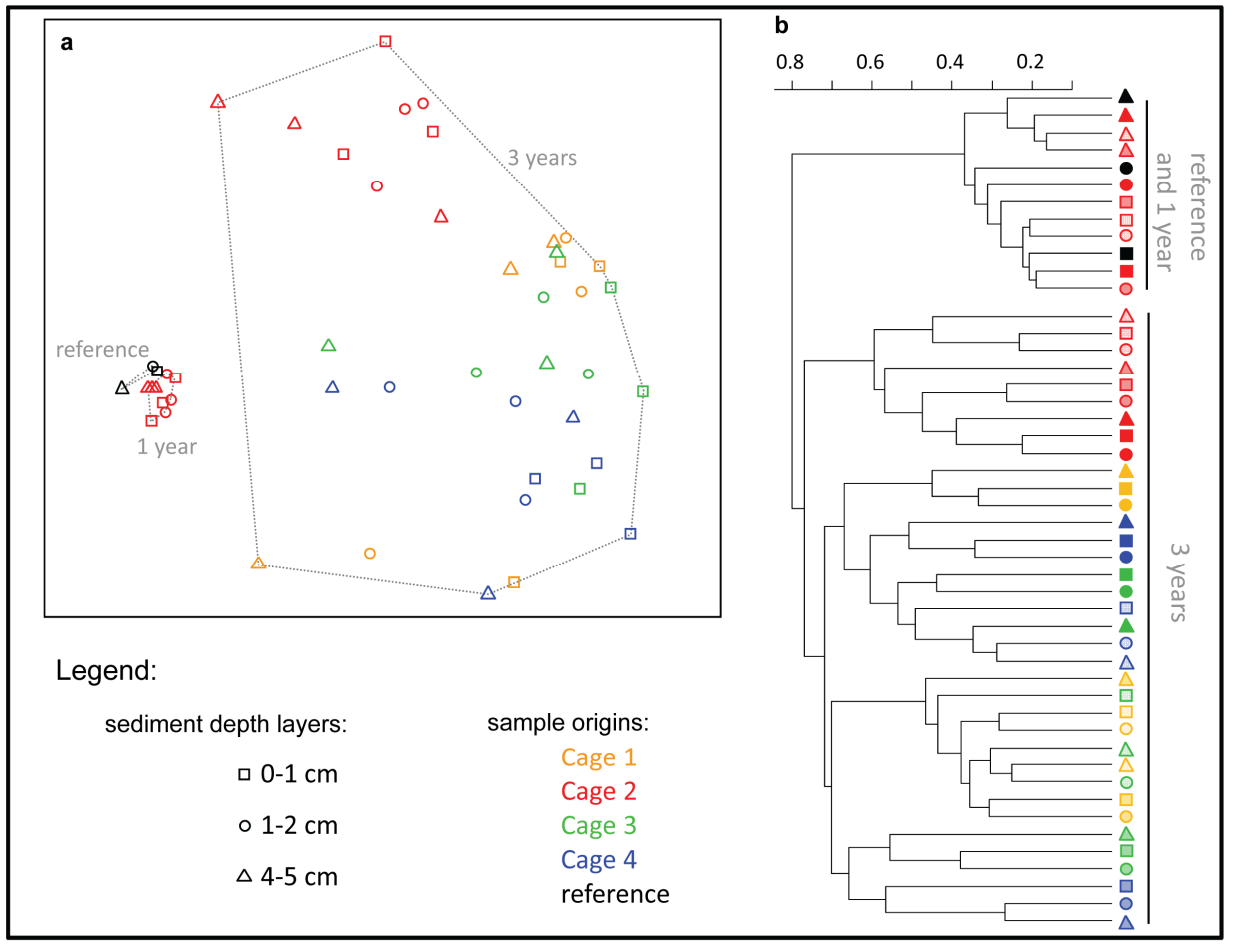


**Figure 2** (**a**) Non-metric multidimensional scaling plot (samples are encircled according to sampling time), and (**b**) cluster dendrogram of bacterial community structure based on Bray-Curtis dissimilarities; Symbol shadings in (**b**) indicate samples from the same push-corer per cage.

## Supplementary Information

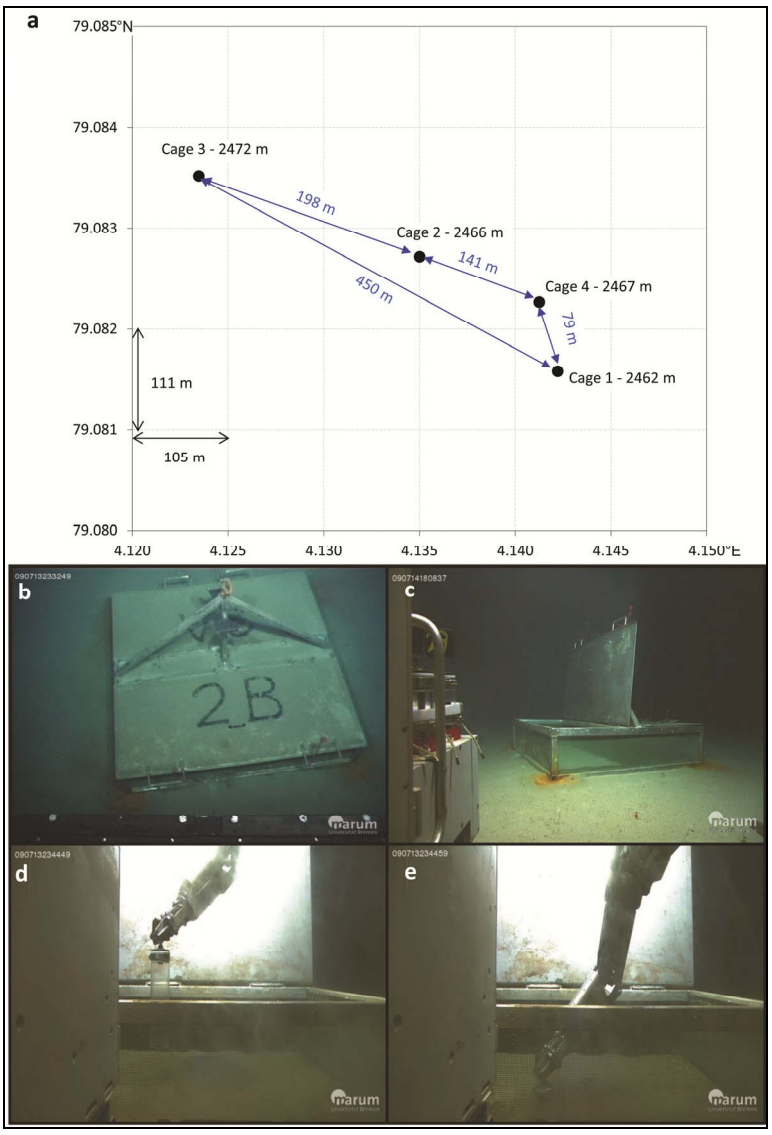


**Figure S1** (**a**) Cage positions at HAUSGARTEN observatory, (**b**, **c**) images of the cage 2 at the seafloor, and (**d**, **e**) pushcoring of sediment samples in 2009; Images were taken by the ROV QUEST 4000 (Marum, Bremen, Germany).

# Chapter IV

## Temporal and spatial variations in eukaryotic diversity in Arctic deep-sea sediments

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In preparation for PLoS ONE

**Abstract**

Interannual variations in plankton community composition related to interannual changes in hydrographic conditions were reported from a decade of observations in the Fram Strait region. Deep-sea benthic communities rely on the supply of energy and carbon from surface waters, yet it is not well understood how bacteria, protists and metazoan communities respond to natural variations in organic matter supply. We studied the benthic eukaryotic community composition by 454 massively parallel tag sequencing of the V9 region of the 18S rRNA gene as recovered from sediments of the long-term ecological research site HAUSGARTEN. We compared community composition in annual samples from 2003-2009 (~2500 m water depth), and along a bathymetric transect (~1000 – 3500 m water depth). Eukaryote community composition was highly diverse, comparable to that found in temperate deep-sea regions. DNA sequences of freshly sedimented plankton from surface waters, especially of diatoms, indicated a decrease in the input with water depth, and during a warm anomaly of surface waters in 2005-2007. According to the decrease in organic matter availability, benthic protist and metazoan taxonomic groups exhibited strong decreases in richness along the bathymetric transect. Moreover, interannual variations across all eukaryotic size classes were observed as a response to a decrease in organic matter supply. Similar to benthic bacterial communities, the eukaryotic community reacts rapidly to variations in surface ocean conditions, supporting the hypothesis of a tight pelago-benthic coupling in the Arctic Ocean and a rapid response of the deep-sea ecosystem to climate change.

## Introduction

Fram Strait is the only deep-water connection between the Atlantic and Arctic Ocean. On the eastern side of Fram Strait, west off Spitzbergen (Svalbard), warm Atlantic water flows into the Arctic Ocean. In the west, the Greenland current transports ice and polar water from the Arctic through Fram Strait. In this area, strong regional and interannual variations in surface Ocean conditions were recorded (Rudels et al., 2012). Substantial warming of the West Spitzbergen current occurred between 2004 and 2008, together with a retreat of sea ice (Beszczynska-Möller et al., 2012). As a result, the mixed layer deepened, primary production decreased and less organic matter was exported to the deep-seafloor (Lalande et al., 2013; Cherkasheva et al., 2014). Furthermore, the plankton composition in surface waters changed, with a shift from diatoms to coccolithophores (Bauerfeind et al., 2009), an increased proportion of Atlantic amphipod species (Kraft et al., 2011) as well as changing fecal matter composition (Lalande et al., 2013). Recently, regional variations in plankton composition in surface waters along Fram Strait were investigated via 454 tag sequencing of plankton by (Kilias et al., 2013), confirming a difference in the plankton composition in polar (diatom-dominated) and Atlantic-influenced (dinoflagellate- and Micromonas-dominated) surface waters along a West-East transect across Fram Strait (Wassmann et al., 2006 and literature therein).

Deep-sea benthic ecosystems rely on organic matter input from surface waters in their energy and carbon demand. Changes in surface ocean conditions and particle fluxes consequently impact the organic matter supply for the typically energy-limited deep-sea benthic communities (Smith et al., 2013). In the Arctic Ocean, pelago-benthic coupling is particularly tight, given the strong seasonality of primary production and particle export (Wassmann et al., 2006). The interannual surface ocean variations in the eastern Fram Strait between 2003-2009, especially the strong warming anomaly in 2005-2007, were shown to directly impact particle flux (Lalande et al., 2013) and the benthic bacterial community composition at 2500 m water depth (Jacob et.al, unpublished; Chapter II). Furthermore, megafaunal densities and trophic diversity shifted during the warming of surface waters with a dominance of suspension feeders in 2007 (Bergmann et al., 2011; Meyer et al., 2013). With increasing water depth and thus lower organic matter supply, both bacterial and eukaryotic communities decrease in richness and their community composition changes (Wei et al., 2010; Bienhold et al., 2011). In Fram Strait, bacterial richness (Jacob et al., 2013) as well as benthic nematode

and copepod densities (Hoste et al., 2007) were shown to decrease with increasing water depth.

Recent advances in DNA massively parallel tag sequencing allow for the investigation of the total benthic eukaryotic diversity in great detail (e.g. Amaral-Zettler et al., 2009; Pawlowski et al., 2011; Bik et al., 2012), using a similar approach as for bacterial diversity analyses (e.g. Sogin et al., 2006; Zinger et al., 2011). Such diversity fingerprinting studies recently revealed a higher eukaryotic richness than previously assumed and detected novel types of single cell eukaryotes (Stock et al., 2009; Scheckenbach et al., 2010; Lecroq et al., 2011; Pawlowski, 2013). Similarly to bacterial community patterns, large-scale biogeographic patterns were reported (e.g. Scheckenbach et al., 2010; Pawlowski et al., 2011; Bik et al., 2012). Yet, systematic analyses of interannual variations in deep-sea benthic eukaryote diversity and variations along spatial and bathymetric gradients are missing (Lecroq et al., 2011).

In this study, we investigated the composition of the eukaryotic community of bathyal sediments from the long-term ecological research site HAUSGARTEN by 454 massively parallel tag sequencing along a bathymetric gradient (~1000 to 3500 m) and with annual resampling from 2003 to 2009. Results were compared to previous investigations of the distribution and densities of bacteria, protozoa and metazoa at HAUSGARTEN and other polar deep-sea regions. Furthermore, we assessed the composition of potentially deposited eukaryotic DNA from surface waters to evaluate its relationship with the eukaryotic community composition of surface waters and sedimenting plankton.

## Material and methods

*Sampling strategy*

Samples were taken at the long-term ecological research site HAUSGARTEN, west of Svalbard (Soltwedel et al., 2005) between 78.6 – 79.7°N and 3.6 to 6.1° E. We sampled six stations (HG-I to HG-VI) along an East to West bathymetric transect from 1,284 m to 3,535 m water depth, and eight stations along a latitudinal transect (N1 to N4, HG-IV, and S1 to S3) at about 2,500 m water depth (Figure 1), during 6 cruises in summer 2003 to 2009, of which 5 were carried out using the German research ice-breaker Polarstern and one in 2006 using the German research vessel Maria S. Merian, (Table 1). Samples of virtually

undisturbed sediments where taken using a TV-multiple corer (TV-MUC) and the uppermost sediment layer of each core (1 cm) was analyzed for this study.

*DNA extraction and purification*

Sediment from the uppermost centimeter originating from three different TV-MUC cores was pooled to account for small scale horizontal variation. Total DNA was extracted from 1 g of the homogenized slurry using the UltraClean Soil DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA) according to the manufacturer's instructions for maximum yields. Elution was carried out using 4 x 50 µl Tris-EDTA buffer (Promega, Madison, WI, USA). DNA extracts that showed a final DNA concentration lower than 4 ng $\mu l^{-1}$ (determined spectrophotometrically using a NanoDrop Spectrophotometer ND 1000, Thermo Fisher Scientific Inc., Wilmington, DE, USA) were purified via isopropanol precipitation.

*454 massively parallel tag sequencing (MPTS)*

PCR amplification and tag generation of DNA extracts, including quality filtering and trimming of raw sequence reads, were carried out at the Marine Biological Laboratory (Woods Hole, MA, USA) as described in (Amaral-Zettler et al., 2009), using primers targeting the v9 region of the 18S rRNA gene. Cluster of operational taxonomic units (OTU) based on 97% sequence identity were produced first using a single-linkage clustering approach to reduce sequencing errors, followed by an average-linkage clustering (Huse et al., 2010). Representative sequences for each OTU (i.e. the most abundant sequence per OTU) was used for taxonomic classification as described in the SILVAngs user guide (Quast et al., 2013) using the eukaryotic taxonomy of the SILVA 111 release (Adl et al., 2005; Pruesse et al., 2007).

A total of 140,363 reads were obtained (Table 2), sequence read abundances ranged from 14,263 in sample NI_03 to 269 in HG-IV_04. After de-replication of sequences and clustering of unique sequences into operational taxonomic units (OTU) at 3% identity level, a total of 4,198 OTU were present in the dataset. OTU per sample ranged from 1,150 in HG-I (2009) to 123 in HG-IV_04. Due to strong variations of rRNA copy numbers between and within eukaryotic taxa (see Bik et al., 2012 and references therein), we focused on OTU abundance (= OTU richness), not read abundance, for taxonomic groups.

OTU that were classified as Bacteria or Archaea or non-marine Eukaryotes (about 7% of reads) were excluded from further analyses (Table 2). The latter included Charophytes (fresh water green algae), Embryophta (land plants) and Glaucophytes (fresh water algae). In order to investigate temporal variation in the dataset, OTU detected in station NI, NII and HG-IV were combined per year by keeping all OTU present in at least one of the samples.

## Results and Discussion

*Richness of major eukaryotic taxonomic groups*

Distribution of relative OTU richness of major taxonomic groups at the "supergroup" level (Keeling et al., 2005) only slightly varied between samples, despite the high differences in sequence read and OTU abundances per sample (Table 3). Supergroups with highest relative total OTU richness were Cercozoa (18% of all OTU), Metazoa (11%), Euglenozoa (8%) and Protalveolata (8%) (Figure 2). Cercozoa dominated in every sample, while the distribution of other relatively abundant taxonomic groups varied. Foraminifera were among the five richest taxonomic groups in 2003, 2004, 2008 and 2009, in 2006 Labyrintholumycetes and the group of marine Stramenopiles (MAST) belonged to the five richest taxonomic groups. These results are not in accordance with a previous study from the Kara Sea (Arctic Ocean), where Dinoflagellates constitute the richest taxonomic group, followed by Cercozoa, Metazoa and Ciliophora (Pawlowski et al., 2011), indicating differences of eukaryotic communities in different Arctic sediments.

*Taxonomic richness of Metazoa*

A total of 458 OTU were assigned to Metazoa, whose abundance ranged widely between samples, from 13 OTU in NII (2006) to 113 in HG-I (2009) and were classified as 19 distinct phyla (Table 4). Highest diversity was found amongst Nematodes (179 OTU) and Arthropoda (87 OTU), together accounting for 58% of metazoan OTU richness (Figure 3). Previous investigations on the metazoan meiofauna in sediments from HAUSGARTEN revealed that nematodes were the most abundant taxon, making up 80 – 99% of the total meiofauna, followed by harpacticoid copepods and nauplii (Hoste et al., 2007; Gallucci et al., 2009). Interestingly, most of the arthropod OTU (59%) found in this study were classified as Maxillopoda, a class including copepods, the second most abundant group. Other OTU rich phyla included Platyhelminthes, Annelida and Cnidaria. The overall distribution of metazoan groups were in accordance with previous studies from HAUSGARTEN (Bergmann et al.,

2009) and other Arctic and Antarctic regions (Pawlowski et al., 2011), as well as Atlantic and Pacific sites (Bik et al., 2012). Kinorhyncha, Tardigrada and Gastrotricha for example were reported from HAUSGARTEN sediments in very low numbers (Hoste et al., 2007), which we detected with only 1-4 OTU (Table 4).

In total, 178 OTU were classified as Nematoda of which the families Monohysteridae, Siphonolaimidae, Xyalidae and Plectidae, all belonging to Chromadorea were the richest (Figure 3, Table 5). Monohysteridae and Xyalidae are common, abundant and diverse in deep-sea sediments (C. Hasemann, pers. communication) and were previously reported from HAUSGARTEN and Fram Strait sediments at high abundances (Hoste et al., 2007). Previous studies on HAUSGARTEN sediments detected high small-scale diversity of nematodes (Gallucci et al., 2009) with Microlaimiae and Desmoscolecidae as the most abundant families (Hoste et al., 2007; Hasemann and Soltwedel, 2011), which were not detected by tag sequencing.

*Taxonomic richness of Foraminifera*

We detected 251 foraminiferal OTU (6% of all OTU, 2% of all sequence tags), which were classified as the multi-chambered classes Globothalamea and Tubothalamea. Among Globothalamea only the order Rotaliida (243 OTU) with seven genera and among Tubothalamea only the order Miliolida with three genera were detected (Table 6). We did not detect any monothalamous genera, which were previously found to be diverse in polar sediments (Pawlowski et al., 2011). Operculina, Pararotalia and Epistominella accounted for 60% of the total foraminiferal OTU richness. A previous study on deep-sea fauna in Northern Fram Strait by Schewe and Soltwedel (2003) reported foraminifera as the most abundant meiofaunal group with Epistominella as the most abundant genus. Tag sequencing of the v9 region from other Arctic and Antarctic sediments showed a rather low foraminiferal OTU richness (Pawlowski et al., 2011) similar to our observations, indicating the v9 region might be too short to detect the vast foraminiferal diversity.

*Water depth zonation of eukaryote diversity*

The bacterial community in HAUSGARTEN sediments was shown to change gradually with increasing water depth (Jacob et al., 2013), which could be related to the decrease in phytodetritus input measured as chloroplastic pigment equivalents (CPE). In accordance, total observed eukaryotic OTU richness decreased with increasing water depth, with a reduction by

~60% from 2500 m to 3000 m depth (Table 3, Figure 6). Overall metazoan OTU richness and OTU richness per phylum decreased with increasing water depth (Table 4). Nematode diversity for example decreased in a stepwise fashion between samples from water depths up to 2500 m and samples from greater depths, as previously described (Hoste et al., 2007), with only three OTU in the deepest sample (3500 m, Table 5). The decrease of overall meiofaunal densities and diversity with increasing water depth at HAUSGARTEN was likely linked to the general reduction of organic matter quality and quantity with increasing water depth (Hoste et al., 2007).

*Interannual variations of eukaryote diversity*

Strong interannual variations in surface water conditions of the HAUSGARTEN area were observed between 2003-2009 with a distinct warming between 2005-2007, causing a reduction of sea-ice coverage and a decrease in organic matter export (Beszczynska-Möller et al., 2012; Lalande et al., 2013). These variations were reflected in changing benthic pigment concentrations with a strong decrease recorded in 2006 (Figure 6). Moreover, the benthic bacterial community structure was significantly altered in 2006 compared to previous and following years (Chapter II). Similarly, eukaryotic OTU richness decreased in 2006 and increased in the following years (Figure 6). The proportion of relative shared OTU per year compared to the baseline in 2003 showed a general decreasing trend with time for all taxonomic groups (Table 7). The highest similarity was detected in 2006 (61.5% - 100%), but as OTU richness was low in this year, this indicates that the 2006 eukaryote community represented a subset of the community found in 2003, rather than a replacement by other taxa. Metazoan OTU distribution also reflected the increase in temperature in 2006, where no arthropod OTU were detected in contrast to other years. In 2007 more OTU classified as Annelida and Cnidaria were observed compared to other years (Table 4). In accordance, most Nematode families were present in each year except 2006 (Table 5). Foraminiferan OTU richness per genus hardly varied temporally (Table 6), yet none of the genera were found in every sample. Interestingly only 2 OTU classified as Epistominella and Heterostegina were detected in 2006 (Table 6, Table 7).

*Contribution of taxonomic groups with pelagic origin*

Environmental DNA from surface waters can be exported via particle sedimentation and deposition on deep-sea sediments (e.g. Lochte and Turley, 1988). Assuming a sinking speed of phytodetritus of ~100 m $d^{-1}$ (see Alldredge and Silver, 1988; Baldwin et al., 1998),

planktonic DNA sequences relate to surface conditions several weeks before benthic sampling. We screened for OTU that have previously been classified as taxa of planktonic groups (Pawlowski et al. 2011) and thus likely originated from surface waters. Only 10% (418) of all OTU were classified as planktonic OTU, which is much lower than previous estimates of >30% in Antarctic and Arctic sediments from water depths of ~700 – 4000 m (Pawlowski et al., 2011). Highest OTU richness throughout the dataset was found among the dinoflagellates (152) and diatoms (85) comprising up to 53% of planktonic OTU richness (76% of planktonic tag sequences; Figure 4). Different phylotypes of Bacillariophyta (Diatoms) and Dinophyceae (Dinoflagellates) were present but rare in the Eastern stations, yet highly abundant in samples from the western Fram Strait, which is influenced by polar water and was ice covered during sampling. This distribution however is not in accordance with the eukaryotic diversity observed in surface waters in Fram Strait in 2010 (Kilias et al. 2013), where Dinophyceae and Micromonas (Mamiellophyceae) were most abundant in Eastern Fram Strait, while diatoms dominated in polar waters of the Greenland current (West Fram Strait).

Planktonic OTU richness decreased substantially with increasing water depth, and several planktonic groups that occurred in relatively high richness in shallow stations, i.e. Prasinophytae and Mamiellophyceae, were absent in deeper stations. Dinophycea (dinoflagellates) and Bacillariophytina (diatoms) instead were found as richest planktonic groups in sediments from all water depths (Figure 4). Most of the diatoms observed were only found in the shallower stations (Figure 5), and only Mediophyceae, Fragilariales and Rhizosolenids could be detected in sediments below 3000 m water depth. Bauerfeind et al. (2009) reported Thalassosira, Chaetoceros and Fragilariopsis as the most abundant diatoms in sediment traps (~300 m) from HAUSGARTEN, which we also found with the highest sequence reads (Table 6, Figure 5).

Strong interannual variations in planktonic OTU richness only became evident in 2006, when strongest variations in surface ocean conditions and organic matter flux were observed (Lalande et al., 2013). Only 13 OTU could be detected, belonging to Dinophyceae, Chloroplastida, and Mediophyceae (Figure 4 and Figure 5). As indicated by the silicate flux, in 2005 and 2006 very low flux rates of diatoms from surface waters were measured compared to 2003 and later years (Lalande et al., 2013), which is in accordance with the low diatom OTU detected in 2006 (Figure 5). A shift from a diatom-dominated system towards a

dominance of Coccolithophores between 2003 and 2005 in surface waters has been reported previously (Bauerfeind et al., 2009), yet, no OTU belonging to Haptophyta were detected in 2006 in benthic samples .

*Conclusion*

In summary, the Fram Strait benthic eukaryote community shows a similar composition as previously described from Kara Sea sediments. Abundant taxonomic groups reported previously from HAUSGARTEN sediments by classical meiofauna enumeration methods could be well retrieved by 454 tag sequencing of the V9 region of the 18S rRNA gene, with the exception of some groups of nematodes and foraminifera. With increasing water depth and accordingly a decrease in food supply by particle flux, total eukaryotic richness was very low, such as in sediments deeper than 3000 m. This led to the absence of sequences of various eukaryotic taxa, which can be detected by microscopy in the typically larger sediment samples used for meiofauna studies compared to DNA extracts.

Interestingly, we observed a strong reduction in benthic eukaryote richness in 2006, when particle flux strongly declined due to a warming anomaly in surface waters of Fram Strait. Only 10% of all OTU were assigned to typical planktonic OTU, which however reflected differences in regional and temporal variations at phylum to supergroup level. Thus, our observations confirm that the 454 massively parallel tag sequencing is a good approach for rapid biodiversity assessment and the detection of spatial and temporal shifts in benthic eukaryote diversity. However, the method is limited by the available taxonomic data bases for abundant meio- and macrofauna types, as well as typical phytoplankton taxa in surface waters that may contribute to export flux. Furthermore, this study confirms that surface warming has a substantial impact on deep sea eukaryotic community composition. In light of ongoing global warming, further investigations are strongly required to assess the impact of such community composition changes on total ecosystem functioning.

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Table 1. Sampling name, year and geographic location.

| | Hausgarten | Year | Water depth | Event label | Latitude (°N) | Longitude (°E) | Date and Time |
|---|---|---|---|---|---|---|---|
| **N1_2003** | NI | 2003 | 2401 | PS64/477-1 | 79.283 | 4.334 | 2003-08-03T00:00:00 |
| **N2_2003** | NII | 2003 | 2546 | PS64/480-1 | 79.41 | 4.695 | 2003-08-04T00:00:00 |
| **HG-IV_2004** | HG-IV | 2004 | 2508 | PS66/117-1 | 79.083 | 4.083 | 2004-07-09T10:14:00 |
| **N2_2004** | NII | 2004 | 2544 | PS66/126-2 | 79.41 | 4.698 | 2004-07-11T13:13:00 |
| **N1_2006** | NI | 2006 | 2348 | MSM02/868-1 | 79.2827 | 4.3263 | 2006-09-05T08:48 |
| **N1_2007** | NI | 2007 | 2406 | PS70/193-1 | 79.2829 | 4.3283 | 2007-07-16T15:30 |
| **HG-IV_2008** | HG-IV | 2008 | 2462 | PS72/122-2 | 79.065 | 4.184 | 2008-07-09T01:23 |
| **N2_2008** | NII | 2008 | 2587 | PS72/147-3 | 79.425 | 4.759 | 2008-07-15T05:12 |
| **N1_2009** | NI | 2009 | 2401 | PS74/120-2 | 79.283 | 4.329 | 2009-07-16T11:14 |
| **N2_2009** | NII | 2009 | 2545 | PS74/119-2 | 79.41 | 4.69 | 2009-07-16T07:09 |
| **N4_2009** | NIV | 2009 | 2802 | PS74/116-2 | 79.717 | 4.486 | 2009-07-15T06:46 |
| **S3_2009** | SIII | 2009 | 2339 | PS74/129-3 | 78.608 | 5.073 | 2009-07-18T07:42 |
| **HG-I** | HG-I | 2009 | 1284 | PS74/109-2 | 79.134 | 6.096 | 2009-07-13T03:10 |
| **HG-II** | HG-II | 2009 | 1547 | PS74/108-2 | 79.13 | 4.902 | 2009-07-12T23:38 |
| **HG-III** | HG-III | 2009 | 1895 | PS74/107-2 | 79.108 | 4.599 | 2009-07-12T20:36 |
| **HG-IV** | HG-IV | 2009 | 2464 | PS74/121-1 | 79.064 | 4.182 | 2009-07-16T14:10 |
| **HG-V** | HG-V | 2009 | 3105 | PS74/113-2 | 79.063 | 3.659 | 2009-07-14T10:40 |
| **HG-VI** | HG-VI | 2009 | 3535 | PS74/106-3 | 79.057 | 3.571 | 2009-07-12T15:23 |

Table 2. Abundance of sequence reads and OTU.

| | total | NI_03 | NII_03 | HG-IV_04 | NI_04 | NI_06 | NI_07 | HG-IV_08 | NII_08 | NI_09 | NII_09 | NIV_09 | S3_09 | HG-I | HG-II | HG-III | HG-IV | HG-V | HG-VI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **454 reads** | 140363 | 14519 | 8756 | 289 | 5672 | 819 | 9157 | 9539 | 12811 | 7213 | 2087 | 9096 | 10255 | 13223 | 12754 | 8484 | 11678 | 3574 | 437 |
| **archaeal reads** | 405 | 17 | 21 | 1 | 17 | | 83 | 41 | 4 | 13 | 21 | 22 | 32 | 12 | 19 | 39 | 45 | 16 | 2 |
| **bacterial reads** | 7474 | 182 | 526 | 14 | 292 | 21 | 510 | 627 | 275 | 546 | 428 | 652 | 763 | 516 | 572 | 461 | 792 | 241 | 56 |
| **eukaryotic reads** | 132484 | 14320 | 8209 | 274 | 5363 | 798 | 8564 | 8871 | 12532 | 6654 | 1638 | 8422 | 9460 | 12695 | 12163 | 7984 | 10841 | 3317 | 379 |
| **eukaryotic OTU** | 5619 | 475 | 743 | 126 | 683 | 130 | 945 | 823 | 894 | 750 | 228 | 734 | 1018 | 1174 | 1145 | 935 | 937 | 367 | 133 |
| **unassigned OTU** | 330 | 27 | 43 | 5 | 28 | 5 | 42 | 58 | 49 | 35 | 13 | 34 | 66 | 48 | 68 | 51 | 53 | 20 | 6 |
| **excluded OTU*** | 65 | 13 | 8 | 3 | 10 | 2 | 12 | 14 | 14 | 17 | 2 | 16 | 14 | 23 | 15 | 13 | 12 | 7 | 2 |
| **final reads** | 130131 | 14263 | 8117 | 269 | 5304 | 786 | 8387 | 8658 | 12138 | 6355 | 1634 | 8305 | 9223 | 12585 | 11980 | 7909 | 10612 | 3278 | 328 |
| **final OTU** | 4198 | 461 | 734 | 123 | 672 | 128 | 932 | 808 | 879 | 732 | 225 | 717 | 1003 | 1150 | 1129 | 921 | 924 | 360 | 130 |

Table 3. Total and relative OTU richness for major taxonomic groups for the bathymetric transect and combined per year.

| | Total OTU richness | Average relative richness (%) | Relative OTU richness (%) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | HG-I | HG-II | HG-III | HG-IV | HG-V | HG-VI | 2003 | 2004 | 2006 | 2007 | 2008 | 2009 |
| **Chlorophyta** | 116 | 3 ± 1 | 3 | 3 | 4 | 3 | 3 | 1 | 3 | 4 | 5 | 3 | 3 | 3 |
| **Rhodophyceae (mostly Stylonematales)** | 38 | 1 ± 0 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| **Euglenozoa** | 345 | 5 ± 2 | 7 | 7 | 7 | 6 | 6 | 2 | 7 | 6 | 2 | 6 | 7 | 6 |
| **other Excatvata** | 16 | 0 ± 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | | 0 | 0 | 0 |
| **Amoebozoa (mostly Discosea)** | 218 | 4 ± 1 | 4 | 5 | 4 | 4 | 2 | 4 | 5 | 5 | 1 | 4 | 5 | 4 |
| **CCTH (without T)** | 259 | 7 ± 1 | 6 | 7 | 5 | 6 | 8 | 5 | 7 | 5 | 5 | 6 | 8 | 6 |
| **Fungi** | 131 | 3 ± 1 | 3 | 2 | 2 | 3 | 3 | 4 | 3 | 3 | 6 | 3 | 3 | 3 |
| **Metazoa** | 458 | 10 ± 1 | 10 | 8 | 9 | 10 | 13 | 14 | 8 | 10 | 10 | 11 | 9 | 11 |
| **other Opistokonta** | 91 | 2 ± 0 | 3 | 2 | 2 | 1 | 1 | 2 | 2 | 3 | 1 | 2 | 2 | 2 |
| **Ciliophora** | 236 | 3 ± 1 | 3 | 3 | 4 | 4 | 4 | 3 | 5 | 3 | | 4 | 4 | 4 |
| **Dinoflagellata** | 176 | 5 ± 1 | 4 | 4 | 4 | 5 | 6 | 8 | 4 | 4 | 4 | 5 | 4 | 5 |
| **Protalveolata** | 322 | 8 ± 1 | 8 | 7 | 7 | 8 | 8 | 8 | 7 | 7 | 6 | 9 | 7 | 8 |
| **Apicomplexa** | 91 | 1 ± 0 | 2 | 2 | 2 | 2 | 0 | 2 | 1 | 1 | 2 | 2 | 2 | 2 |
| **other Alveolata** | 23 | 1 ± 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 |
| **Cercozoa** | 752 | 24 ± 2 | 24 | 25 | 26 | 23 | 22 | 25 | 22 | 23 | 33 | 21 | 21 | 20 |
| **Endomyxa** | 4 | 0 ± 0 | | | 0 | 0 | | 1 | 0 | | | | | 0 |
| **Foraminifera** | 251 | 5 ± 1 | 5 | 5 | 5 | 6 | 6 | 5 | 7 | 6 | 2 | 5 | 6 | 7 |
| **Radiolaria** | 91 | 2 ± 1 | 2 | 3 | 2 | 2 | 4 | 1 | 2 | 2 | 1 | 3 | 2 | 2 |
| **Chrysophyceae** | 40 | 2 ± 0 | 1 | 1 | 2 | 1 | 2 | 1 | 1 | 1 | 1 | 2 | 1 | 1 |
| **Diatomea** | 85 | 3 ± 1 | 2 | 2 | 3 | 2 | 2 | 5 | 2 | 3 | 2 | 2 | 2 | 3 |
| **Labyrinthulomycetes** | 97 | 3 ± 1 | 2 | 2 | 2 | 3 | 2 | 2 | 4 | 3 | 5 | 2 | 2 | 2 |
| **MAST-1-12 excl. 5,10,11** | 71 | 2 ± 1 | 2 | 2 | 2 | 2 | 1 | 3 | 2 | 2 | 7 | 2 | 2 | 2 |
| **other Stramenopiles** | 139 | 4 ± 0 | 4 | 4 | 4 | 4 | 3 | 3 | 4 | 4 | 3 | 4 | 4 | 4 |
| **other eukarya** | 149 | 3 ± 1 | 3 | 2 | 4 | 3 | 3 | 2 | 4 | 4 | 4 | 4 | 4 | 4 |
| **Total OTU richness** | 4199 | | 1151 | 1130 | 922 | 925 | 360 | 131 | 927 | 712 | 128 | 933 | 1255 | 1398 |

*CCTH: Cryptophyta, Centroheliozoa, Haptophyta, Telonemia; missing values indicate no observed OTU.

Table 4. Total and relative OTU richness of metazoan phyla for the bathymetric transect and combined per year.

| | Total OTU richness | Relative OTU richness (%) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | HG-I | HG-II | HG-III | HG-IV | HG-V | HG-VI | 2003 | 2004 | 2006 | 2007 | 2008 | 2009 |
| **Annelida** | 40 | 6 | 8 | 6 | 7 | 9 | 28 | 10 | 8 | 8 | 11 | 6 | 7 |
| **Arthropoda** | 87 | 20 | 11 | 14 | 16 | 13 | 28 | 18 | 18 | 0 | 25 | 18 | 19 |
| **Brachiopoda** | 1 | | | | | | | | | | | | |
| **Bryozoa** | 1 | | | | | | | 1 | | | | | |
| **Cnidaria** | 18 | 2 | 4 | 4 | 3 | 4 | | 4 | 3 | | 11 | 4 | 3 |
| **Echinodermata** | 4 | | | | 1 | | 6 | 1 | | | | 1 | 2 |
| **Entoprocta** | 1 | | | | 1 | | | | | | | | 1 |
| **Gastrotricha** | 4 | 2 | 1 | | | | 6 | | 3 | | 2 | | |
| **Hemichordata** | 1 | | | | | | | | | | 1 | | 1 |
| **Kinorhyncha** | 1 | | | | | | | 1 | | | | | |
| **Loricifera** | 9 | | | 6 | 2 | 2 | | | 1 | 8 | | 1 | 1 |
| **Mollusca** | 18 | 3 | 4 | 3 | 3 | | | 1 | | | 1 | 5 | 3 |
| **Myxozoa** | 4 | 1 | 1 | | | 2 | | | 3 | | | | 1 |
| **Nematoda** | 179 | 50 | 49 | 50 | 48 | 51 | 17 | 45 | 48 | 62 | 38 | 48 | 41 |
| **Nemertea** | 4 | | 2 | | 1 | 2 | | | | | | 1 | 1 |
| **Platyhelminthes** | 49 | 9 | 8 | 9 | 9 | 4 | | 9 | 7 | 8 | 4 | 9 | 10 |
| **Porifera** | 7 | 1 | 3 | | 1 | 7 | 11 | | | | | 1 | 1 |
| **Rotifera** | 8 | 3 | 1 | 1 | 1 | | | | | | 1 | 2 | 3 |
| **Tardigrada** | 1 | | 1 | | | | | 1 | | | | | 1 |
| **other Metazoa** | 21 | 4 | 5 | 8 | 7 | 4 | 6 | 6 | 10 | 15 | 7 | 5 | 6 |
| **Total OTU richness** | 458 | 113 | 96 | 80 | 90 | 45 | 18 | 77 | 73 | 13 | 101 | 110 | 150 |

Missing values indicate no observed OTU.

Table 5. Total and relative OTU richness of nematodes for the bathymetric transect and combined per year.

| | | | Relative OTU richness (%) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **Total OTU richness** | **HG-I** | **HG-II** | **HG-III** | **HG-IV** | **HG-V** | **HG-VI** | **2003** | **2004** | **2006** | **2007** | **2008** | **2009** |
| **Chromadorea** | **Axonolaimidae** | 13 | 7 | 4 | 8 | 10 | 4 | | 9 | 6 | | 3 | 6 | 7 |
| | **Chromadoridae** | 11 | 11 | 6 | 5 | 12 | | | | | | | | 2 |
| | **Cucullanidae** | 8 | 5 | 2 | 10 | | | | 3 | 6 | | | | 2 |
| | **Cephalobidae** | 7 | 4 | | 3 | | | | 3 | 6 | | 3 | 4 | 3 |
| | **Leptolaimidae** | 10 | | 4 | 5 | 7 | 9 | | 12 | 9 | 14 | 11 | 6 | 7 |
| | **Monhysteridae** | 30 | 20 | 26 | 30 | 31 | 26 | | 26 | 31 | 57 | 29 | 32 | 21 |
| | **Plectidae** | 17 | 2 | 11 | 8 | 7 | 17 | | 9 | 3 | 14 | 11 | 8 | 8 |
| | **Siphonolaimidae** | 22 | 13 | 9 | 10 | 7 | 13 | | | 3 | | 8 | 11 | 11 |
| | **Xyalidae** | 20 | 18 | 17 | 13 | 10 | 9 | | 15 | 9 | | 13 | 9 | 13 |
| | **Rhabditidae** | 7 | 5 | 4 | 3 | 7 | 9 | 33 | 6 | 3 | | 3 | 8 | 5 |
| | **other Chromadorea** | 9 | 5 | 6 | 5 | 5 | 4 | 33 | 12 | 17 | 14 | 11 | 9 | 11 |
| **Enoplea** | **Oxystominidae** | 15 | 7 | 2 | 3 | 2 | 9 | | 6 | 3 | | 5 | 6 | 7 |
| | **other Enoplea** | 9 | 4 | 9 | | 2 | | 33 | | 6 | | 5 | 2 | 3 |
| **Total OTU richness** | | 178 | 56 | 47 | 40 | 42 | 23 | 3 | 34 | 35 | 7 | 38 | 53 | 61 |

Missing values indicate no observed OTU.

Table 6. Total and relative OTU richness of foraminifera for the bathymetric transect and combined per year.

| | | | | Relative OTU richness (%) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | **Total OTU richness** | **HG-I** | **HG-II** | **HG-III** | **HG-IV** | **HG-V** | **HG-VI** | **2003** | **2004** | **2006** | **2007** | **2008** | **2009** |
| **Globothalamea** | **Rotaliida** | **Ammonia** | 1 | | | | 2 | | | | | | | | 1 |
| | | **Bulimina** | 19 | | 6 | 13 | 5 | 14 | | 5 | 7 | | 7 | 8 | 9 |
| | | **Calcarina** | 7 | 6 | 2 | 4 | 5 | 5 | 17 | 3 | 2 | | 4 | 3 | 4 |
| | | **Epistominella** | 29 | 13 | 11 | 19 | 17 | 14 | 17 | 13 | 13 | 50 | 16 | 14 | 12 |
| | | **Heterostegina** | 8 | 2 | | 2 | 3 | | 17 | 3 | 4 | 50 | 2 | 5 | 3 |
| | | **Operculina** | 64 | 23 | 24 | 21 | 27 | 18 | | 30 | 20 | | 29 | 24 | 26 |
| | | **Pararotalia** | 58 | 26 | 26 | 21 | 20 | 23 | | 25 | 24 | | 18 | 20 | 23 |
| | | **other Rotalida** | 57 | 28 | 27 | 21 | 22 | 27 | 50 | 20 | 22 | | 24 | 22 | 20 |
| **Tubothalamea** | **Miliolida** | **Amphisorus** | 3 | | | | | | | 2 | 4 | | | 3 | 1 |
| | | **Archaias** | 2 | | 2 | | | | | | 2 | | | | |
| | | **Laevipeneroplis** | 2 | 2 | 2 | | | | | | | | | | |
| | | **other Miliolida** | 1 | | | | | | | | | | | 1 | |
| **total** | | | **251** | **53** | **62** | **48** | **60** | **22** | **6** | **61** | **45** | **2** | **45** | **74** | **91** |

Missing values indicate no observed OTU.

Table 6. Total OTU richness of Diatomea.

| | | | OTU | reads |
|---|---|---|---|---|
| **Bacillariophytina** | **Bacillariophyceae** | Bacillaria | 1 | 32 |
| | | CCMP2297 | 2 | 26 |
| | | Cylindrotheca | 1 | 2 |
| | | Cymbella | 2 | 9 |
| | | Cymbopleura | 1 | 10 |
| | | Fistulifera | 1 | 1 |
| | | Fragilariopsis | 2 | 164 |
| | | NA | 1 | 1 |
| | | Navicula | 3 | 71 |
| | | Neidium | 1 | 1 |
| | | Nitzschia | 1 | 62 |
| | | Placoneis | 1 | 1 |
| | | Pleurosigma | 1 | 14 |
| | | Prestauroneis | 1 | 1 |
| | | Pseudo-nitzschia | 3 | 4 |
| | | Sellaphora | 1 | 15 |
| | | Stauroneis | 2 | 2 |
| | | Zeuk10 | 1 | 1 |
| | **Mediophyceae** | Attheya | 2 | 46 |
| | | Chaetoceros | 24 | 8382 |
| | | Cymatosira | 1 | 1 |
| | | Ditylum | 1 | 1 |
| | | Minutocellus | 1 | 56 |
| | | NPK2-133 | 1 | 2 |
| | | Porosira | 1 | 5 |
| | | Skeletonema | 6 | 16 |
| | | Thalassiosira | 4 | 399 |
| | | Triceratium | 1 | 1 |
| **Coscinodiscophytina** | **Coscinodiscids** | Actinocyclus | 1 | 2 |
| | **Fragilariales** | Grammonema | 1 | 1 |
| | | Hyalosira | 2 | 10 |
| | **Melosirids** | Aulacoseira | 1 | 4 |
| | | Melosira | 1 | 1 |
| | | Stephanopyxis | 2 | 5 |
| | **Rhizosolenids** | Guinardia | 3 | 53 |
| | | Leptocylindrus | 3 | 336 |
| | | Rhizosolenia | 1 | 1 |
| **ME-Euk-FW10** | | | 2 | 83 |

Table 7. Turnover and OTU richness for depicted taxonomic groups.

| | | 2003 | 2004 | 2006 | 2007 | 2008 | 2009 |
|---|---|---|---|---|---|---|---|
| **% of total OTU* shared with 2003** | Metazoa | | 47.9 | 61.5 | 33.7 | 35.5 | 30 |
| | Nematoda | | 54.3 | 62.5 | 52.6 | 41.5 | 40.3 |
| | Foraminifera | | 46.7 | 100 | 51.1 | 41.9 | 39.6 |
| | OTU with planktonic origin | | 67.1 | 61.5 | 50 | 46.8 | 34.2 |
| | Diatoms | | 68.4 | 100 | 75 | 62.5 | 31.4 |
| | Total | | 56.7 | 69.5 | 46.6 | 42.9 | 38.2 |
| **total observed OTU** | Metazoa | 77 | 73 | 13 | 101 | 110 | 150 |
| | Nematoda | 35 | 35 | 8 | 38 | 53 | 62 |
| | Foraminifera | 61 | 45 | 2 | 45 | 74 | 91 |
| | OTU with planktonic origin | 96 | 76 | 13 | 96 | 124 | 161 |
| | Diatoms | 23 | 19 | 2 | 16 | 24 | 35 |
| | Total | 927 | 712 | 128 | 933 | 1255 | 1398 |

*total OTU that are present in the two years compared.

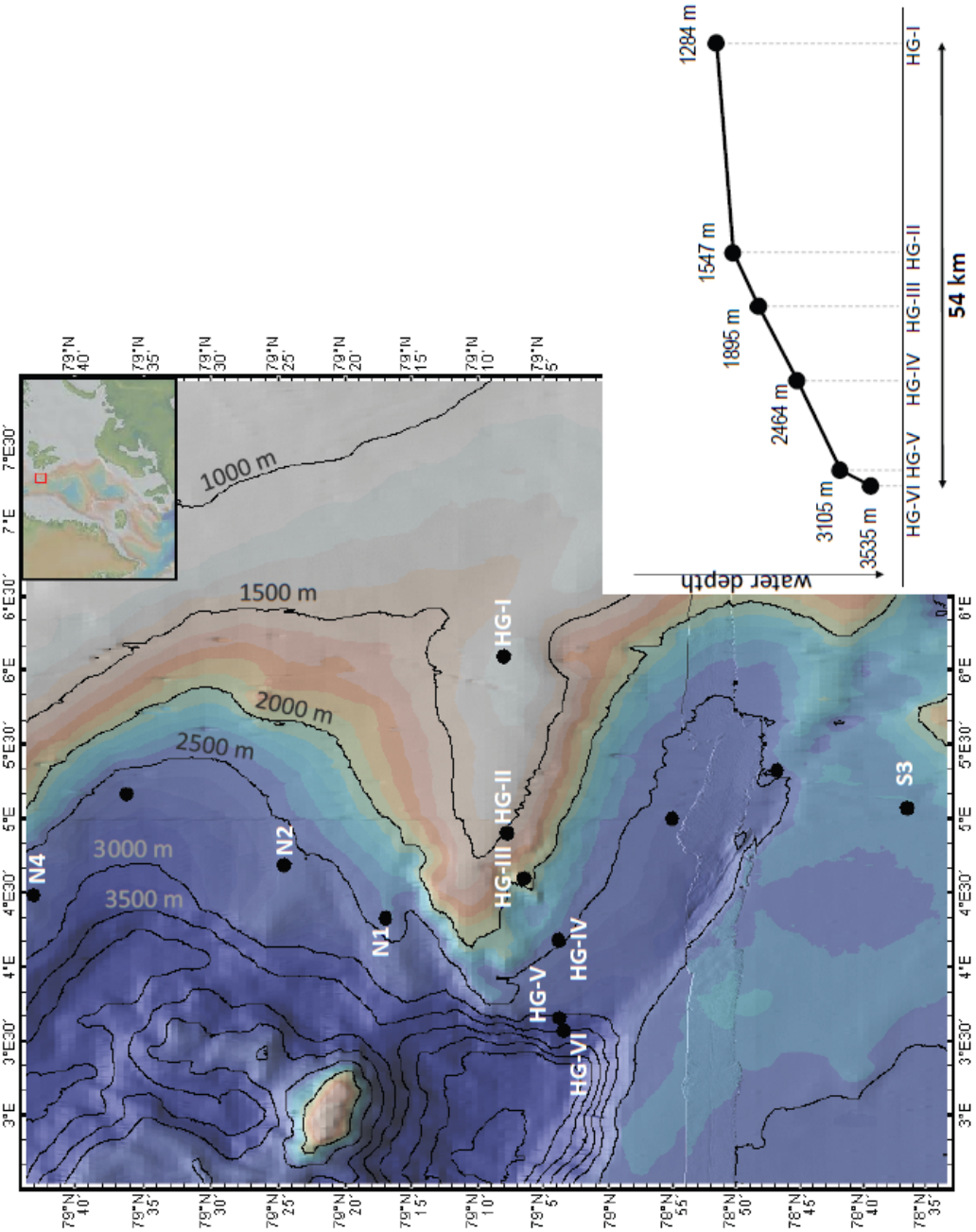


**Figure 1** Map of the location and stations of the long-term ecological research station HAUSGAERTEN.

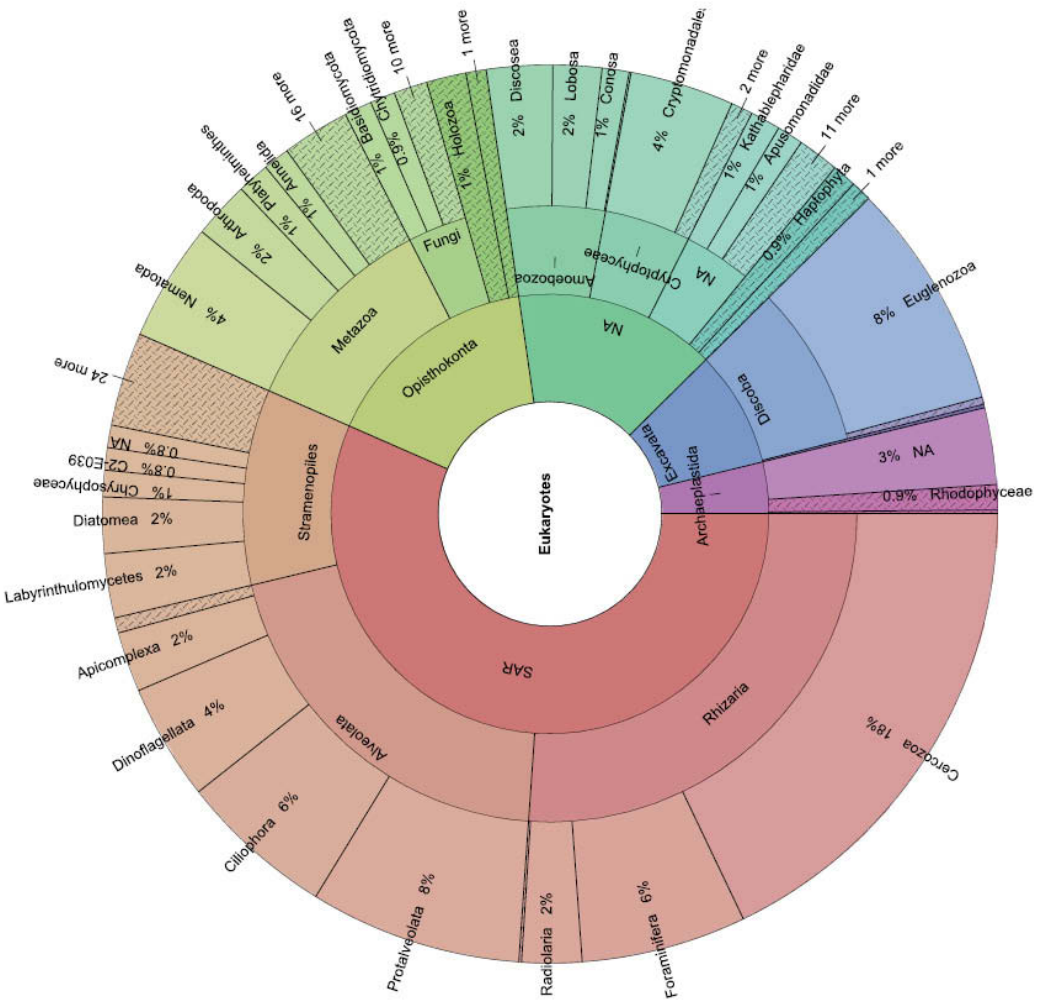


**Figure 2** Distribution of total OTU richness of major eukaryotic lineages found in HAUSGAERTEN surface sediments.

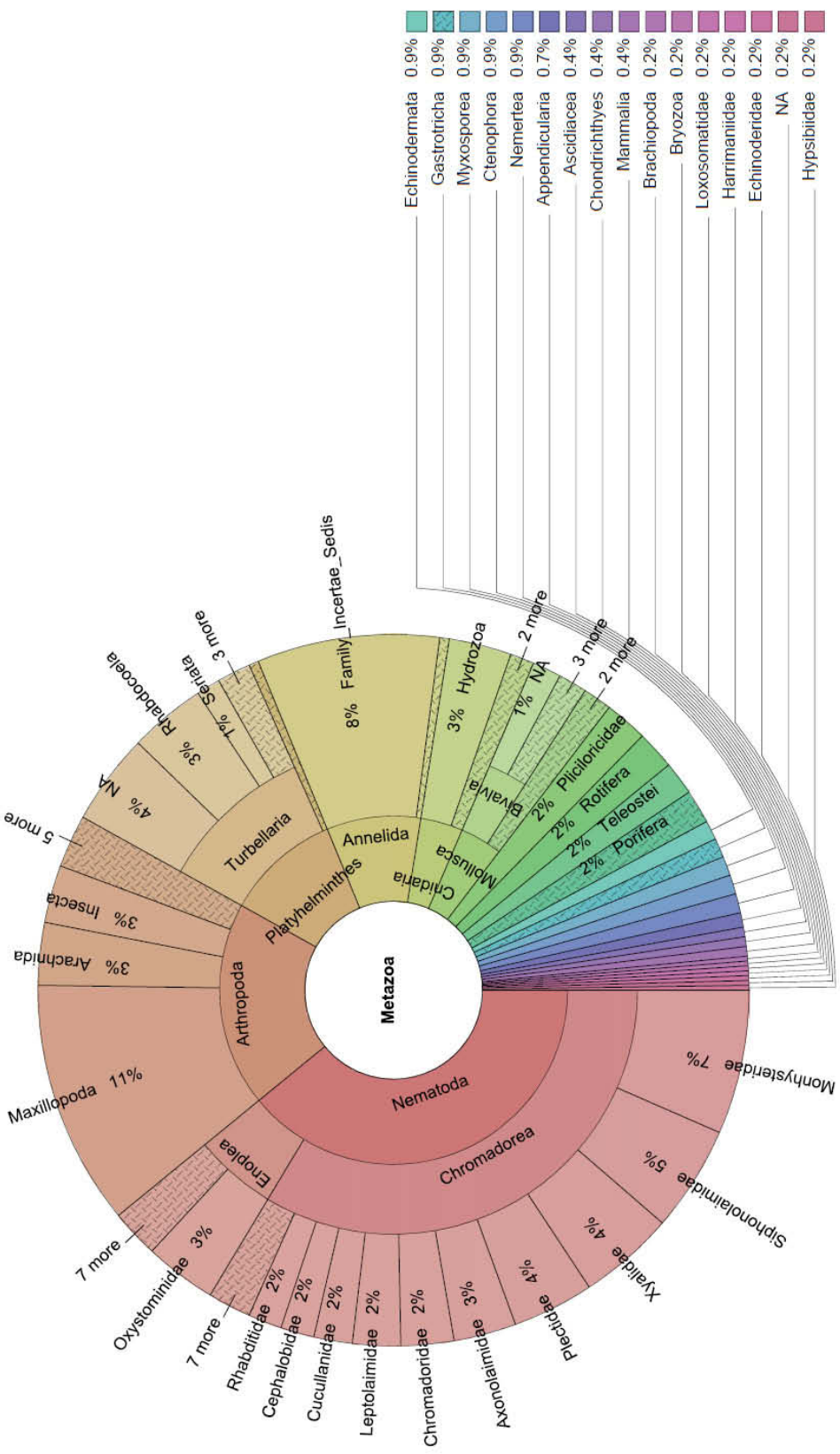


**Figure 3** Distribution of total OTU richness of Metazoa found in HAUSGAERTEN surface sediments.

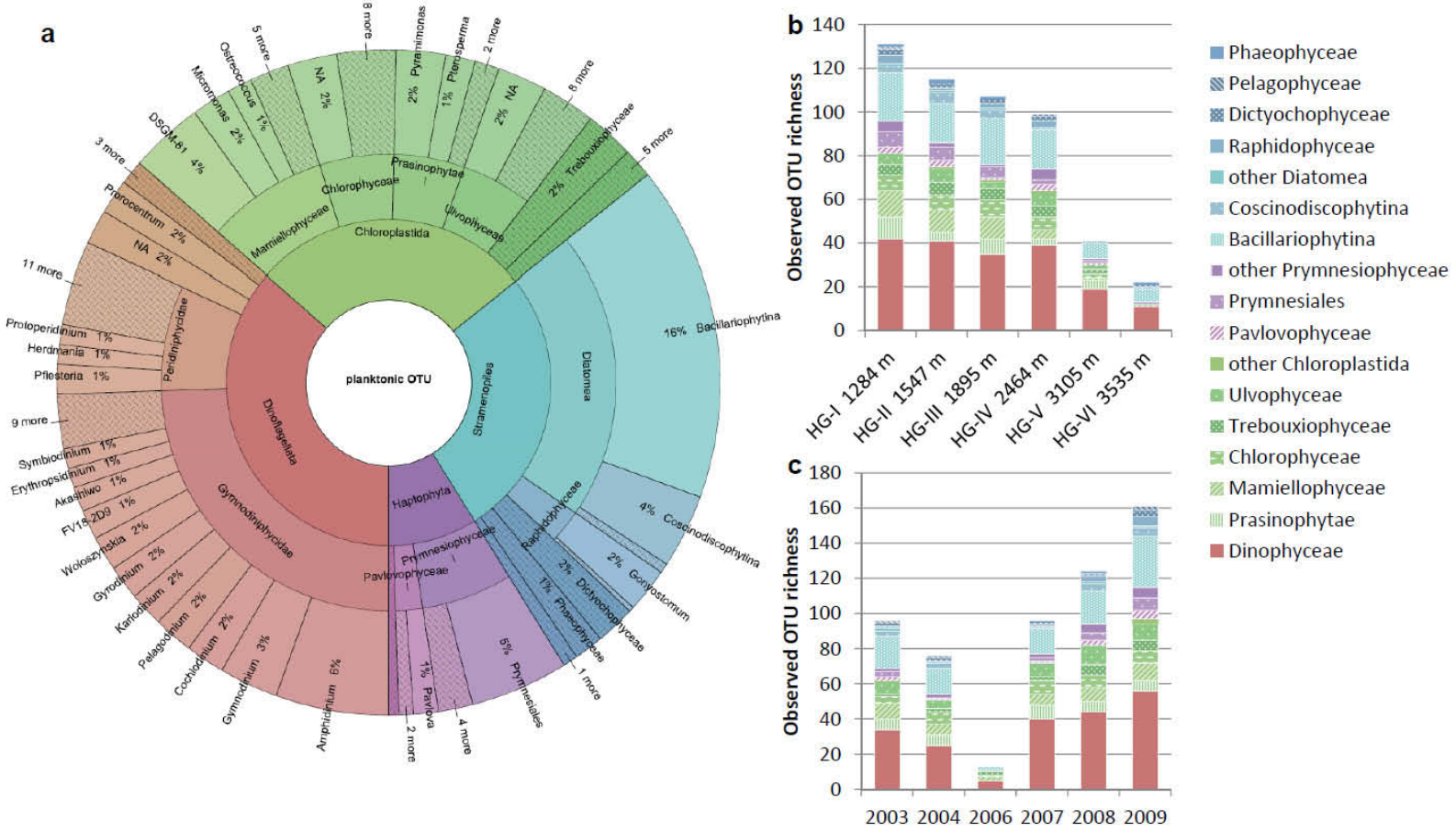


**Figure 4** Distribution of total richness (**a**), richness along the bathymetric transect (**b**) and richness per year (**c**) of OTU with a planktonic origin. Color in (**b**) and (**c**) are accordingly to (**a**), patterns were chosen to better discriminate taxa with similar coloring.

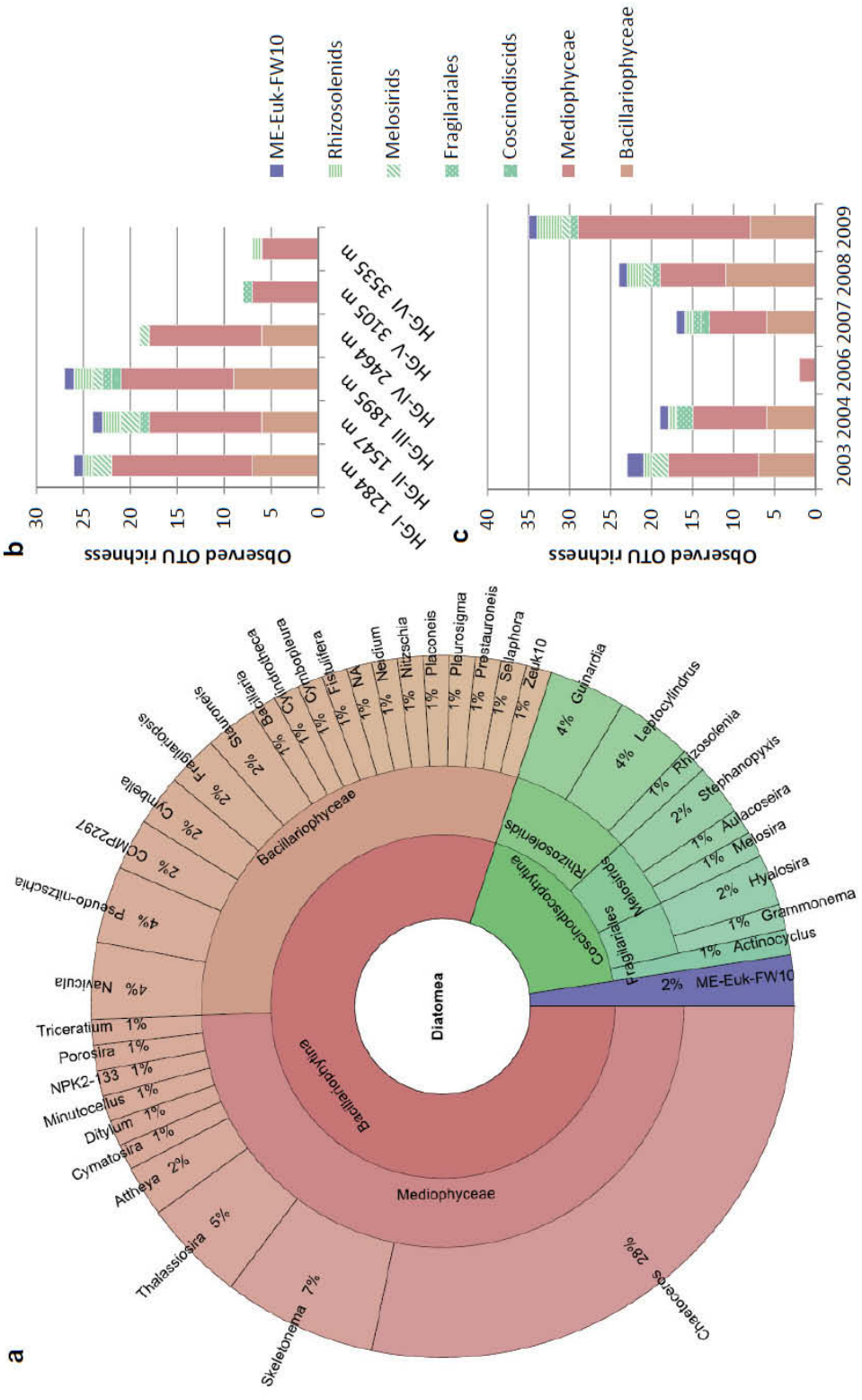


**Figure 5** Distribution of total OTU richness (**a**), OTU richness along the bathymetric transect (**b**) and OTU richness per year (**c**) of Diatoms. Color in (**b**) and (**c**) are accordingly to (**a**), patterns were chosen to better discriminate taxa with similar coloring.

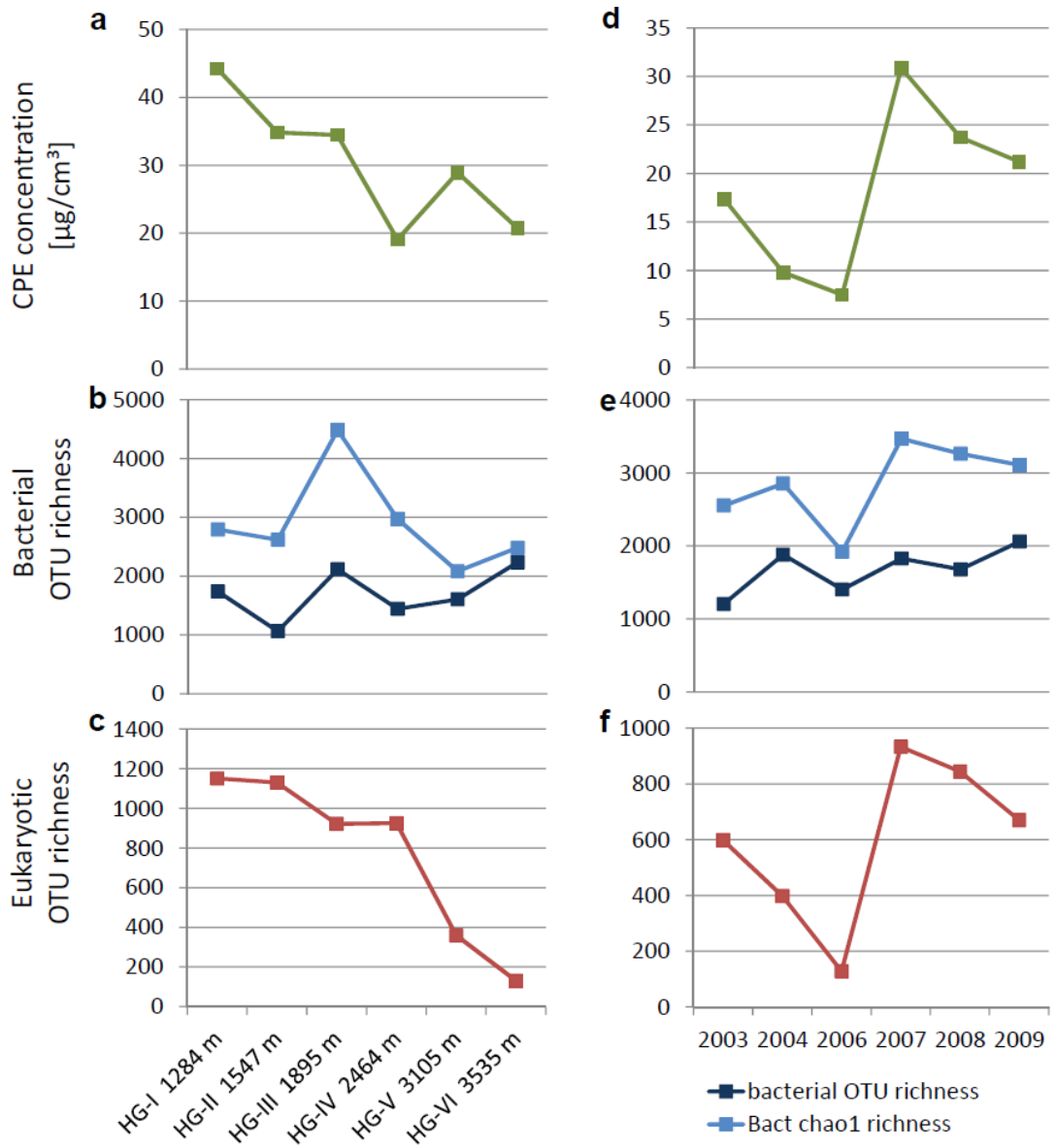


**Figure 6** Concentrations of chloroplastic pigment equivalents (CPE) (**a**), bacterial OTU richness (**b**) and eukaryotic richness (**c**) in surface sediments along the bathymetric transect and in the different years.

# 3. Discussion

Global change is having a rapid effect in the Arctic. The decrease in sea-ice cover and increased temperatures observed over the last decades are affecting physical properties of the Arctic Ocean, and influencing biological processes. Melting of sea-ice in spring is the starting point of phytoplankton blooms that eventually sink down and serve as organic matter supply to the oligotrophic deep sea. As a result of the physical environmental changes, the location as well as the composition of phytoplankton blooms has changed, consequently changing the quality and quantity of organic matter export. Due to the remoteness of the Arctic deep sea, spatial and temporal variations of benthic deep-sea communities in relation to changes in surface ocean productivity were not well studied when I started this thesis. Owing to the research at the long-term ecological research (LTER) site HAUSGARTEN, I was able to obtain sediment samples from natural gradients of organic matter supply that covered a time frame of seven years, including years where strong variations in surface ocean conditions were observed. The application of DNA fingerprinting and high throughput sequencing techniques enabled the investigation of total bacterial and eukaryotic community patterns as well as in-depth analyses of variations in specific taxonomic groups. Interpretation of microbial community patterns in conjunction with sediment environmental variables was facilitated by the application of multivariate statistics. This allowed for the investigation and comparison of spatial and temporal patterns in Arctic benthic bacterial and eukaryotic communities and their environmental drivers for the first time. Additionally, surface ocean characteristics are monitored at LTER site HAUSGARTEN, which enabled the direct investigation of how changes in surface ocean characteristics affect the deep-sea ecosystem, further advancing our understanding of the tight coupling between these compartments. The results presented in the chapters of this thesis show that benthic microbial communities exhibit strong spatial patterns, partly in accordance with differences in organic matter availability which is in turn directly influenced by changes in the availability of annual phytodetritus input from the surface ocean. These are the first insights into interannual variations of Arctic deep-sea benthic microbial communities and improve our understanding of the coupling of variations in surface Arctic Ocean conditions and Arctic benthos under climate change.

## 3.1. Spatial versus temporal variations in benthic bacterial communities

Total richness of bacterial types at all taxonomic levels increased with increasing amounts of sampling stations considered (Chapter I), as previously observed for microbial communities from other areas (Horner-Devine et al., 2004; Green and Bohannan 2006). This emphasizes the importance of spatial coverage in order to determine and predict general benthic community dynamics. Bacterial community structure gradually changed with increasing water depth, while richness of bacterial operational taxonomic units (OTU) stayed rather stable. Along the North-South transect of HAUSGARTEN at 2500 m water depth, bacterial communities from samples taken 20 km to 120 km apart showed a similar community structure, more similar than communities in sediments taken at 500 m water depth difference (Chapter I). Therefore samples from the North-South transect were used to investigate interannual changes in community structure (Chapter II). Strong interannual variations in bacterial community patterns driven by a decrease in organic matter availability due to changes in surface ocean characteristics have been encountered and were not delayed in comparison to surface ocean dynamics, as observed for larger faunal organisms (e.g. Ruhl and Smith, 2008; Bergmann et al., 2011). Changes in community structure with water depth and with time could be partly explained by changes in organic matter availability. Although organic matter availability is a major factor influencing benthic bacterial communities along water depth gradients (e.g. Bienhold et al., 2012), it became obvious that other changes with water depth and throughout the years have a significant impact on community structure. These factors could be of physical, e.g. pressure (e.g. Bartlett et al., 1995) or biological nature, e.g. species-species interactions or impact of larger faunal organisms (e.g., De Mesel et al., 2004; Fuhrman et al., 2006). Moreover, the quality of organic matter varies with water depth, e.g. more degraded material at the deeper stations, or with distance to the ice-edge and different years due to changes in phytoplankton composition in overlying waters. As an indicator of the freshness or organic matter, the ratio of chlorophyll *a* to phaeopigments (degradation product of chlorophyll *a*) was determined but did not correlate with changes in bacterial community structure maybe because chlorophyll *a* in sediment from HAUSGARTEN was always low (< 30%). Additional knowledge on the state and changes in composition of organic matter reaching the sea floor may help to better understand spatial and temporal variations in bacterial community patterns.

The two studies presented in Chapters I and II were separated in order to identify drivers of community structure over spatial and temporal scales independently. This allowed the detection of a strong influence of water depth differences and accompanying differences in organic matter availability and the detection of an immediate response of the bacterial community to a decreased availability of organic matter. Yet, in order to predict future changes in Arctic Ocean sediments due to changes in surface ocean dynamics and organic matter export, we need to better understand temporal dynamics of bacterial communities over larger spatial scales. In Figure 11, a non-metric multidimensional scaling plot indicating community similarity between surface sediment samples from all stations and years that were available for my study is shown. In addition, spatial, environmental, and temporal contextual parameters and their respective effects on variations in community structure are displayed. Variations in bacterial community structure could be best explained by differences in water depth (16% of community variation explained) which partly covaried with changes in pigment concentrations. A gradual change in bacterial community structure along the bathymetric transect becomes apparent in the NMDS plot (Figure 7a), similar to the one that is reported in Chapter I. Samples from the North-South transect (N and S) grouped together with other samples from 2500 m water depth. Yet, nine percent of the variation in community structure was explained by interannual variations, and there were strong differences in community structure in samples from the same water depth but sampled in different years. These patterns are similar to spatio-temporal changes in meiofaunal densities at HAUSGARTEN (Hoste et al., 2007). Thus, although spatial effects (i.e. water depth) seem to have a stronger impact on bacterial community structure than interannual effects for a time period of seven years, a significant temporal effect on bacterial communities in sediments from all water depth could still be detected. Additionally, some bacteria may vary with both water depth and interannual changes in organic matter availability, as for example shown for Verrucomicrobia (Chapters I and II), which may result in an amplification of natural variations in community structure over spatial scales by interannual variations due to climate change.

Forecasting of interannual changes of surface ocean characteristics is difficult. Therefore our investigations and observations were only possible by the continuous annual sampling at the LTER site HAUSGARTEN that allowed study of the benthic bacterial community before, during and after strong variations in the surface ocean. Thus, only long-term observation along spatial gradients will allow for a comprehensive determination of climate change

impacts on benthic communities that may be eventually serve for predictions of changes in other oceanic regions.

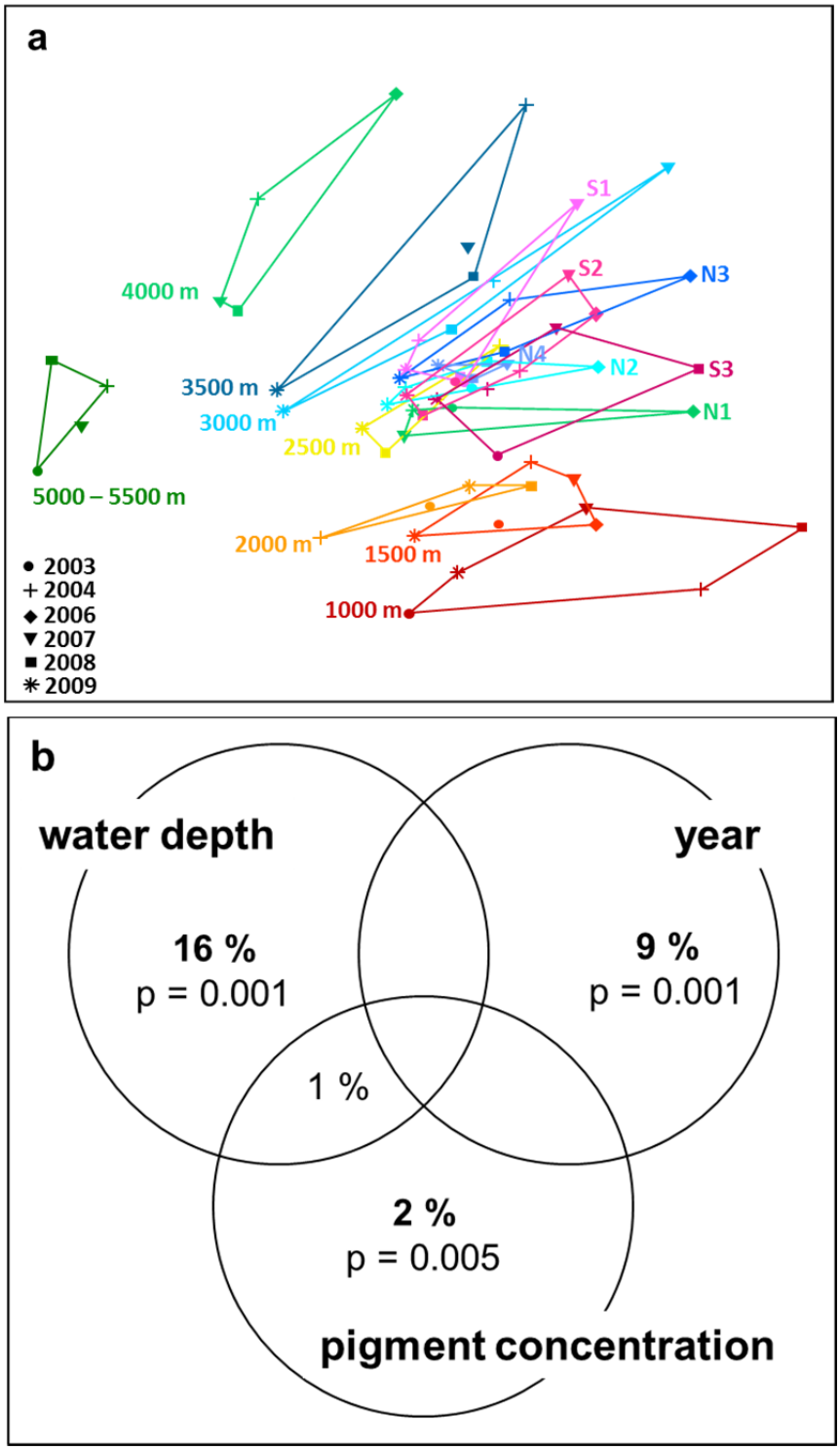


**Figure 11 (a)** Non-metric multidimensional scaling of ARISA data from surface sediment samples covering a time frame from 2003 to 2009. Community structure in samples from different water depths are indicated by color, from different years by symbols. **(b)** Partitioning of the biological variation in bacterial community structure derived with ARISA for the years 2003-2008 between the parameters water depth, sampling year and pigment concentrations as indicator for organic matter availability.

## 3.2. Influence of decreased organic matter export

The samples analyzed during my thesis covered the period from 2005-2007, during which time warm Atlantic water masses reached further north than usual, resulting in increased surface water temperatures at HAUSGARTEN (Beszcysnak-Möller et al., 2012). Additionally, the ice-edge retreated further north, resulting in low or absent sea-ice cover in the HAUSGARTEN area during 2005 and 2006. Warmer waters and the absence of ice led to low primary productivity and organic matter export to the deep sea during that time (Lalande et al., 2012; Cherkasheva et al., 2014). The observed interannual variations in benthic bacterial community structure (Chapter II) were in accordance with these changes in organic matter supply, with a strongly reduced bacterial richness and shifts in bacterial community in 2006. Unfortunately, no sediment samples were available for 2005, which was already a year with decreased organic matter supply to the benthos. The bacterial community might have already changed in this year, which then resulted in an even stronger shift in 2006. Yet it is surprising that already in 2007, when again more organic matter reached the seafloor, the bacterial community had shifted back to a community similarly rich and with a similar community structure to the years before the warming. It was shown that benthic bacterial communities are able to react rapidly to inputs of organic matter (e.g. Witte et al., 2003), which might explain this rapid recovery of the bacterial community. With further warming of the Arctic Ocean and a continued loss of sea-ice, ice-edge blooms may progress further north. Consequently, primary production at HAUSGARTEN may decrease leading to lower organic matter supply to the ocean floor. This was mimicked by an *in situ* experiment at HAUSGARTEN, where we studied the response of the benthic bacterial community to an absence of fresh organic matter input over several years (Chapter III). The bacterial community was stable after one year of starvation in terms of community composition, structure and function. However, after a three year period of starvation, bacterial diversity had decreased, community structure shifted and a starvation signal in the form of increased enzymatic activity could be measured, indicating a change in the functioning of benthic bacterial community.

These two studies indicate that benthic bacterial communities can survive short periods of up to two years without fresh organic matter supply and are able to recover when fresh organic matter is available again. Yet, the bacterial community was altered during the time of starvation and was less diverse. Some bacteria were able to thrive during the time of low organic matter supply (e.g. Verrucomicrobia), while other bacterial groups diminished (e.g.

Actinobacteria). Additionally, the increased enzymatic activity over longer periods of starvation might indicate a stress response of the bacterial community. Further starvation may lead to changes in community composition to an extent that no recovery to the initial state is possible anymore. Bacterial community composition and functioning is, to a certain extent, linked (Reed and Martiny, 2013), and more diversified communities are accompanied by broader enzymatic capabilities for organic matter degradation (Teske et al., 2011). Thus, the decreased richness and shift in community composition may change the efficiency of remineralization and burial of organic carbon. Yet, this needs to be confirmed by further monitoring of the benthic community and changes in quantity and quality of organic matter reaching the seafloor *in situ*.

As well as continued monitoring of the Arctic benthos, further *in situ* or *ex situ* experiments may improve understanding of possible developments of benthic communities under changing conditions. Results from the *in situ* experiment shown in Chapter III were preliminary, and the continuation of the experiment will give valuable insight into to the long-term effect of starvation on natural benthic bacterial community structure and function. Further, experiments investigating the effects of variations in organic matter composition resulting from changes in plankton composition, will help to predict benthic ecosystem responses to possible future changes in the surface Arctic Ocean.

## 3.3. Comparison of eukaryotic and bacterial diversity patterns

Investigation of the eukaryotic community by sequencing harbors many difficulties, as reviewed by Bik et al. (2012). Firstly, the eukaryotic community is comprised of organisms of various size classes, from single-cell to multi-cellular organisms. Thus, targeting the bulk ribosomal DNA in an environmental sample probably results in an overrepresentation of multi-cellular organisms. Secondly, the gene copy numbers for ribosomes vary strongly between eukaryotic organisms, even within species. This might add to an overrepresentation of certain eukaryotic species. Therefore, we only investigated eukaryotic community composition based on the presence or absence of OTU (Chapter IV), unlike the investigation of bacterial community patterns which are described by relative abundances of OTU.

We identified a strong decrease in total eukaryotic richness and richness of different eukaryotic taxonomic groups with a decrease in organic matter availability, both with water depth, and resulting from changes in the surface ocean. For the bacterial community, a similar decrease in richness was found with the decrease of organic matter availability due to surface ocean changes, but not with water depth. The eukaryotic community composition seems to be structured more by water depth differences and accompanying environmental parameters than bacterial community composition, which may be due to differences in cellular structure.

Bacteria and eukaryotes seem to be similarly structured by the availability of organic matter on spatial and temporal scales. Yet, interconnections between bacteria and eukaryotes also exist. Parts of the nematode community, which dominate metazoan meiofauna (Hoste et al., 2007), and deposit-feeding macrofauna were shown to feed on bacteria in HAUSGARTEN sediments (van Oevelen et al., 2011), thus probably impacting bacterial abundance. Additionally, due to selective feeding of nematodes, different nematode species impact bacterial community composition and structure differently (De Mesel et al., 2004). Microbial network analysis was used to identify interactions between bacteria, archaea, viruses and marine protists in surface ocean waters (Steele et al., 2011, Chow et al., 2014). With the information on total benthic eukaryotic and bacterial community composition obtained by sequencing it may be possible to expand such network analysis to investigate interactions of the whole benthic community in the future. This may help to get a better insight into the benthic food web and help and thus infer whole ecosystem response to climate change.

*Concluding remarks*

Arctic benthic bacterial and eukaryotic communities in surface sediments from the LTER site HAUSGARTEN are spatially structured and impacted by interannual changes in the water column that result in altered organic matter export. The composition and relative abundance of bacterial classes is highly similar to bacterial community composition reported from other Arctic sediments, thus HAUSGARTEN is a suitable site to represent dynamics in the Arctic benthos. This thesis presents unique insights into interannual variations of Arctic deep-sea benthic microbial communities and advances our understanding of the tight coupling between surface ocean productivity and benthic microbial communities, which was only possible by the long-term observation and sampling at HAUSGARTEN. With the predicted changes in the Arctic Ocean due to global climate change, such as sea-ice retreat and warming of water masses, composition of primary producers and efficiency of primary production will probably be altered and thus also organic matter export to the deep sea. Changes in organic matter availability affects all size classes of the community in deep-sea sediments and may irreversibly change community composition and ecosystem functioning, when persisting over several years. The results obtained during this thesis stress the need for long-term observations, in order to observe variations and predict changes in benthic ecosystems under future climate scenarios.

# 4. Perspectives

*Monitoring of Arctic benthic microbial communities in the future*

Global change is rapidly progressing around the world including the Arctic, but baseline studies of variations in benthic microbial communities are missing. This thesis provided first insights into temporal variations of Arctic microbial communities, but at a time when the area was already affected by global change. These times of rapid changes call for a strategic and long-term oriented monitoring of marine communities.

The annual samples of HAUSGARTEN sediments analyzed during this thesis provided evidence for interannual variability in benthic microbial communities. Yet, the time of sampling varied between June and August and was not carried out in a consistent temporal proximity to the deposition of organic matter. In order to better evaluate impacts of variations in the surface ocean on benthic communities, a higher temporal resolution of benthic samples would be needed. As observed for pelagic bacterial and benthic macrofaunal communities, benthic bacterial communities probably exhibit strong seasonal patterns in relation to the deposition of organic matter in spring. It is yet unknown how Arctic deep-sea benthic communities vary over seasonal scales, especially in winter when the Arctic is ice-covered and thus difficult to reach for ship-based expeditions. The magnitude of variations in community structure and functions before and after the deposition of organic matter also remains unknown. As it is difficult to estimate the exact timing of the deposition of organic matter, this would best be done by automated sampling systems installed in the deep sea. Such an automated sampling infrastructure was proposed for the HAUSGARTEN area, and would combine year-round monitoring of oceanographic and biological parameters in the surface ocean, as well as benthic monitoring using sediment sampling and photography (Soltwedel et al., 2013). This would enable sediment sampling during winter and would allow for a better temporal resolution and thus ability to track variations in the microbial community during the time of organic matter deposition and its degradation. Thus, year-round sediment sampling would lead to a better understanding of Arctic microbial community dynamics in relation to upper ocean processes, and would improve the evaluation of community changes related to global change.

Additional to the temporal monitoring of the benthic ecosystem, further *in situ* and *ex situ* experiments should be carried out, as proposed in section 3.2. Such experiments should not only target changes in benthic community structure, but also functioning. Bacterial community structure and functioning are linked, yet this linkage is not fully understood. The investigation of functional genes, that encode enzymes involved in the degradation of organic matter, may give insight into the potential to remineralize various sources of organic carbon. Actual expression patterns of functional genes can be determined by sequencing the metatranscriptome (Gilbert et al., 2008) or metaproteome (Wilmes and Bond, 2006). This would allow a comprehensive view of the functional and structural changes of microbial communities to variations in organic matter availability.

*Methodological considerations for long-term studies of microbes*

Sequencing technology for microbial studies is rapidly advancing since the first massively parallel tag sequencing approach was published in 2006 (Sogin et al., 2006). Back then thousands of sequence reads were produced for each sample with sequence lengths of approximately 60 base pairs. Sequencing used in this PhD study was carried out in 2012 when stretches of roughly 250 base pairs could be sequenced. Nowadays, sequencing technologies enable sequencing millions of reads of a few hundred base pairs (Caporaso et al., 2012). This allows in-depth investigation of not only resident and abundant bacterial species, but also of rare bacteria and their fluctuations. Additional to the advances in sequencing length, different variable regions on the ribsosomal rRNA gene are used in different studies. Yet, data compiled from different sequence lengths, loci or sequencing platforms are not directly comparable (e.g. Dunthorn et al., 2012) and result in different amounts of observable bacterial taxa and taxonomic composition (Yu and Morrison, 2004; Stoeck et al., 2010). Even though overall community patterns derived with different sequencing approaches seem to be robust (Gobet at al., 2013), rapid advances in sequencing technology can make comparisons of microbial communities difficult for long-term monitoring. Environmental samples for temporal investigations of microbial communities are often first collected over several years and then analyzed together with the same method to maintain comparability. Yet, for long-term observations over several decades this is not practicable, because a detection of changes would only be possible long after they occurred. There are different possibilities to circumvent this problem. One possibility would be to decide for one technique in advance and only use this one technique throughout the whole long-term investigation. This may hinder

the resolution of detectable community variations since newer techniques mostly yield more in-depth analyses of the whole bacterial community; in addition it would hinder comparisons to newer studies. Another possibility would be to re-sequence samples every time new methods are available or after a few years of sample collection. Thus, for long-term monitoring of microbial communities and comparisons of different studies, more knowledge is needed on how data between older and more advanced techniques and methods with different sequencing power can be compared. At best, this could lead to the development of algorithms capable of combining sequencing data from different genomic regions. This would allow re-using sequencing data, despite technical differences between studies, making long-term monitoring and comparison of new and old sequencing data of microbial communities possible.

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## Poster and Oral presentations

**M. Jacob**, A. Ramette, T. Soltwedel, M. Klages and A. Boetius (**2010**) Spatial and Temporal Variations of Deep-sea Microbial Diversity at the Arctic Long-Term Observatory HAUSGARTEN. IPY Oslo Science Conference, Oslo, June 8-12 2010. Poster presentation.

**M. Jacob**, A. Ramette, T. Soltwedel, M. Klages and A. Boetius (**2010**) Spatial and Temporal Variations of Deep-sea Bacterial Diversity at the Arctic Long-Term Observatory HAUSGARTEN. GfÖ 40th Anniversary Meeting, Giessen, Aug 30 - Sept 3 2010. Oral presentation.

**M. Jacob**, C. Bienhold, M. Bergmann, A.Bracher, E-M. Nöthig, A. Ramette, I. Schewe, T. Soltwedel, M. Klages and A. Boetius (**2011**) Observing microbial biodiversity shifts related to climate change in the Arctic Ocean. ASLO, San Juan, Feb 13-18, 2011. Oral presentation

**M. Jacob**, T. Soltwedel, A. Ramette and A. Boetius (**2012**) Spatial scaling of bacterial diversity in Arctic deep-sea sediments. Gordon Research Conference on Marine Microbes, Italy, June, 24-29 2012. Poster presentation.

## Cruise participations

RV Polarstern, ARK XXIV-2, Fram Strait, 10.07.2009 – 03.08.2009

RV Polarstern, ARK XXV-2, Fram Strait, 30.06.2010 – 29.07.2010

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