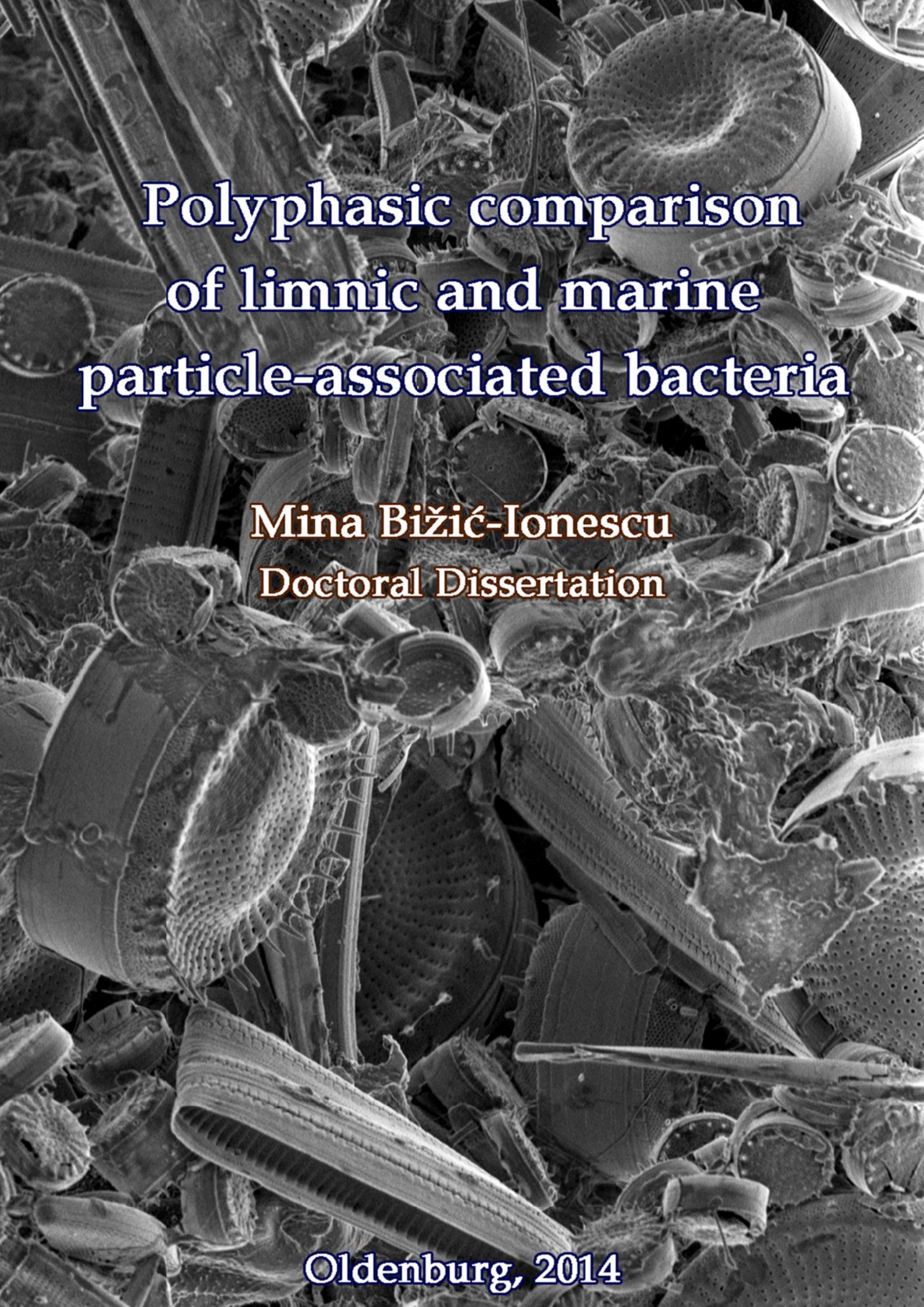

Polyphasic comparison of limnic and marine particle-associated bacteria

Mina Bižić-Ionescu

Doctoral Dissertation

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Polyphasic comparison of limnic and marine particle-associated bacteria

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Image on the front cover is a scanning electron micrograph (SEM) of a diatom aggregate.

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1. Gutachter: **Prof. Dr. Hans-Peter Grossart**

2. Gutachter: **Prof. Dr. Rudolf Amann**

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Eigenständigkeitserklärung

Hiermit versichere ich, Mina Bižić-Ionescu, dass ich die vorliegende Dissertation selbständig und ohne andere als die angegebenen Hilfsmittel verfasst habe. Stellen, die von anderen Werken dem Wortlaut oder Sinn nach übernommen sind, wurden als Zitate gekennzeichnet.

Oldenburg, March 2014

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Mina Bižić-Ionescu

DEDICATED TO THE MEMORY OF MY FATHER, DUŠAN,
WHO WOULD HAVE BEEN HAPPY TO SEE ME FOLLOW IN
HIS STEPS AS A FELLOW SCIENTIST

I feel you are always with me supporting and guiding...

"When I think of the floor of the deep sea, the single, overwhelming fact that possesses my imagination is the accumulation of sediments. I always see the steady, unremitting, downward drift of materials from above, flake upon flake, layer upon layer — a drift that has continued for hundreds of millions of years, that will go on as long as there are seas and continents….the most stupendous 'snowfall' the earth has ever seen"

Rachel Carson, ***The Sea Around Us*** (1951)

Summary

Lakes cover a little more than 3% of Earth surface as compared to 71% by oceans and seas, but they are estimated to have an equal contribution to CO_2 emissions and a larger contribution to carbon burial. In both limnic and marine aquatic systems particulate organic matter (POM) aggregates are responsible for the majority of carbon sequestration. These particles, however, are also hotspots of organic matter mineralization in the water column. Activities of particle-associated (PA) bacteria on OM aggregates affect both degradation and aggregation processes and thus control the flux of carbon to depth, as well as biogeochemical element cycling, and microbial food webs, in most aquatic systems.

In the first study of my thesis we provide an in depth comparison of PA bacterial communities between limnic and marine. To achieve this we compared multiple samples obtained from two distinct and well-studied limnic sites (the dimictic oligotrophic Lake Stechlin and the peat bog Lake Grosse Fuchskuhle) to two coastal marine sites (the mesotrophic North Sea and the eutrophic Northern Adriatic Sea) with a strong terrestrial influence to increase chance of detecting similarities. We combined Fluorescence *In Situ* Hybridization (FISH) with rRNA-targeted oligonucleotide probes and pyrosequencing of partial 16S rRNA to investigate the bacterial diversity and community composition on limnic and coastal marine particles. By using a novel semi-automated microscopy method we were able to analyze over 3000 individual particles. Limnic particles were more abundant, smaller in size, and more densely colonized than marine ones. Limnic PA bacteria consisted predominantly of *Alphaproteobacteria* and *Betaproteobacteria*, and unlike previously suggested sizeable populations of *Gammaproteobacteria*, *Actinobacteria* and *Bacteroidetes*. Marine particles were colonized by *Planctomycetes* and *Betaproteobacteria* additionally to *Alphaproteobacteria*, *Bacteroidetes* and *Gammaproteobacteria*. Large differences in individual particle colonization could be detected. High-throughput sequencing revealed a significant overlap of PA and free-living (FL) bacteria highlighting an underestimated connectivity between both fractions. Many bacterial families were shared between the limnic and marine PA fractions, but despite this similarity almost no OTUs were identical. This suggests infrequent bacterial transition across the obviously strong salinity barrier.

In the second study we followed a sudden under-ice bloom of heterotrophic bacteria in Lake Stechlin. Extraordinarily high bacterial abundance and biomass were fueled by the breakdown of a massive bloom of *Aphanizomenon flos-aquae* after ice formation. This led to a sudden proliferation of heterotrophic attached and subsequently of FL bacteria. Total bacterial protein production reached 201 μg C l^{-1} d^{-1}, ca. five times higher than spring-peak values that year. FISH and denaturing gradient gel electrophoresis at high temporal resolution showed pronounced changes in bacterial community structure. Pyrosequencing of 16S rRNA genes revealed that during cyanobacteria breakdown diversity of attached and FL bacterial communities were reduced to a few dominant families some of which were not detectable during the early stages of the cyanobacterial bloom. Our study suggests that in winter, unlike commonly postulated, carbon rather than temperature is the limiting factor for bacterial growth. Frequent phytoplankton blooms in ice-covered systems highlight the need for year-round studies of aquatic ecosystems including the winter season to correctly understand element and energy cycling through aquatic food webs, particularly the microbial loop.

In the third study of my thesis, we used a newly developed flow-through roller tanks system to observe temporal changes in PA bacteria and the genes expressed by these communities. We showed that the community profile obtained by DNA analysis differs significantly from the profile obtained by RNA analysis. While the former was dominated by *Caulobacteraceae* and *Flavobacteriaceae*, the latter was dominated by *Sporychtiaceae* and *Acidimicrobiaceae*. The discrepancy could be a result of extensive viral activity on the particles as one of the most abundant transcripts obtained was a complete *Microviridae* genome. The PA community obtained from single aggregates did not change throughout the experiment. Similarly, we did not observe any significant change in the expression of genes involved in carbon utilization. Hence we conclude that in our case more than 8 days is needed to obtain a community shift on particles. Beside extensive phage activity, antagonistic reactions on the particle include bacteria-eukaryote interactions, evident from the expression of protein secretion systems type III and VII as well as of genes involved in cyanide and phenazine production, both effective eukaryotic toxins. Intra-bacterial interactions include the above toxins as well as antibiotic production and degradation. The viral activity and the multitude of lytic processes which occur on a particle according to the transcriptome, make the particle a suitable location to be a

generator of genetic variability via lateral gene transfer. The latter is supported by the expression of genes involved in the uptake and integration of naked DNA into bacterial genomes.

Zusammenfassung

Während Ozeane und Meere 71% der Erdoberfläche bedecken, beträgt der Anteil der Seen nur knapp mehr als 3%. Trotzdem wird der Beitrag limnischer Wassermassen zur CO_2 Emission ähnlich hoch eingeschätzt wie die der marinen Wassermassen und der Anteil an der CO_2 Speicherung durch Seen ist vermutlich sogar noch größer. In limnischen wie auch in marinen aquatischen Systemen sind Aggregate aus partikulärem organischen Material (POM) für den Großteil der Kohlenstoffbindung verantwortlich. Zugleich sind diese Partikel jedoch auch Brennpunkte der Mineralisierung von organischem Material in der Wassersäule. Die Aktivität der mit den Partikeln assoziierten (PA) Bakterien beeinflusst sowohl die Abbau- als auch die Aggregationsprozesse auf makroskopisch großen Aggregaten und bestimmt dadurch den Kohlenstoffexport in die Tiefseee, die biogeochemischen Kreisläufe und die mikrobiellen Nahrungsnetze in den meisten aquatischen Systemen.

In der ersten Studie meiner Arbeit befasste ich mich mit einem detaillierten Vergleich zwischen bakteriellen PA Gemeinschaften aus dem limnischen und marinen Bereich. Für diese Studie wählten wir zwei deutlich unterschiedliche und gut untersuchte limnische Standorte aus (der dimikitische, oligotrophe Stechlinsee und der See Große Fuchskuhle) und verglichen diese mit zwei an der Küste gelegenen, marinen Standorten (die mesotrophe Nordsee und die eutrophe Nord-Adria). Für den marinen Bereich wurden Standorte mit hohem terrestrischen Einfluss ausgewählt, um die Vergleichbarkeit zu den limnischen Systemen zu verbessern. Wir haben Fluoreszenz-In-Situ-Hybridisierung (FISH) mit rRNA-ausgerichteten Oligonukeotid Sonden und Pyrosequenzierung partieller 16S rRNA kombiniert, um die bakterielle Diversität und Zusammensetzung der Gemeinschaft limnischer und küstennaher mariner Partikel zu untersuchen. Durch die Anwendung einer neuen halb-automatischen Mikroskopiermethode waren wir in der Lage, über 3000 individuelle Partikel zu analysieren. Limnische Partikel waren abundanter, kleiner und dichter besiedelt als die marinen. Limnische PA Bakterien waren überwiegend durch *Alphaproteobacteria* und *Betaproteobacteria* und, anders als zuvor vermutet, durch beträchtliche Populationen von *Gammaproteobacteria*, *Actinobacteria* und *Bacteroidetes* dominiert. Es wurden große Unterscheide in der individuellen Partikelbesiedlung

gefunden. Die Hochdurchsatz-Sequenzierung zeigte eine signifikante Überlappung von PA und frei lebenden (FL) Bakterien und hebt den unterschätzten Zusammenhang zwischen diesen beiden Fraktionen hervor. Viele bakterielle Familien waren sowohl in der limnischen als auch marinen PA Fraktion vorhanden, jedoch waren die OTUs trotz dieser Ähnlichkeit deutlich verschieden. Dies deutet darauf hin, dass Bakterien nur selten über die starke Salinitätsbarriere gelangen.

In der zweiten Studie untersuchte ich die Folgen einer plötzlichen Blüte heterotropher Bakterien unter dem Eis im Stechlinsee. Außergewöhnlich hohe bakterielle Abundanz und Biomasse wurden durch den Abbau einer massiven *Aphanizomenon flos-aquae* Blüte unter dem klaren Eis hervorgerufen. Die Cyanobakterien wurden durch die einsetzende Schneebedeckung der Eisschicht zunehmend Licht limitiert und physiologisch gestresst. Dies führte zu einer plötzlichen Proliferation heterotropher, PA Bakterien, und im Anschluss auch der FL Bakterien. Die Gesamtmenge bakteriell produzierten Proteins erreichte 201 $\mu g\ C\ l^{-1}\ d^{-1}$ und somit das ca. fünffache der Werte zu Zeiten der Frühlingsblüte desselben Jahres. FISH und denaturierende Gradienten-Gel-Elektrophorese in hoher zeitlicher Auflösung zeigten deutliche Veränderungen in der bakteriellen Gemeinschaftsstruktur. Pyrosequenzierung von 16S rRNA Genen zeigte, dass während des Abbaus von Cyanobakterien die Diversität von PA Bakterien und FL Bakterien auf wenige dominante Familien reduziert wurde, von denen einige in den frühen Stadien der cyanobakteriellen Blüte nicht nachweisbar waren. Unsere Studie deutet darauf hin, dass im Winter, anders als allgemein postuliert, Kohlenstoff, und nicht Temperatur, den limitierenden Faktor für das bakterielle Wachstum darstellt. Häufige Phytoplankton-Blüten in eisbedeckten Systemen unterstreichen die Notwendigkeit für ganzjährige Studien aquatischer Ökosysteme, einschließlich der Wintersaison, um Element- und Energie-Kreisläufe in aquatischen Nahrungsnetzen, insbesondere in der mikrobiellen Schleife besser zu verstehen.

In der dritten Studie meiner Arbeit haben wir neu entwickelte Durchfluss-Rolltank-Systeme benutzt, um zeitliche Veränderungen der PA Bakterien und die exprimierten Gene der entsprechenden Gemeinschaften zu untersuchen. Wir haben gezeigt, dass sich das Gemeinschaftsprofil, welches wir durch die DNA-Analyse erhielten, wurde, signifikant von dem Profil, welches die RNA-Analyse hervorbrachte, unterscheidet. Während die DNA-Analyse zeigte, dass *Caulobacteriaceae*

und *Flavobacteriaceae* dominierten, zeigte die RNA-Analyse, dass *Sporychtiaceae* und *Acidimicrobiaceae* dominierten. Diese Diskrepanz könnte das Ergebnis intensiver viraler Aktivität auf den Partikeln sein, eines der am häufigsten detektierten Transkripte war ein komplettes *Microviridae* Genom. Die PA Gemeinschaft einzelner Aggregate hat sich während des Experimentes nicht verändert. Ebenfalls haben wir keine signifikante Veränderung in der Exprimierung von Genen, welche in den Kohlenstoffgebrauch involviert sind, beobachten können. Demzufolge schlussfolgern wir, dass in unserem Fall mehr als 8 Tage benötigt wurden, um eine Veränderung der Bakteriengemeinschaft auf den Partikeln erkennen zu können. Abgesehen von intensiver Phagen-Aktivität, schließen antagonistische Reaktionen auf den Partikeln auch die Bakterien-Eukaryoten Interaktionen mit ein. Diese sind evident durch die Expression der Gene für die Typ III und VII Protein-sekretierenden Systeme sowie von Genen, welche in die Cyanid- und Phenazinproduktion (effiziente eukaryotische Toxine) involviert sind. Bakterielle Interaktionen umfassen sowohl die oben genannten Toxine, als auch Produktion und Abbau von Antibiotika. Die virale Aktivität und die Vielzahl lytischer Prozesse, welche entsprechend des Transkriptoms auf den Partikeln stattfinden, machen den Partikel zu einem Ort, der als Generator genetischer Variabilität via lateralem Gen-Transfer fungieren kann. Letzteres wird unterstützt durch die Exprimierung von Genen, welche in die Aufnahme und Integration von „naked DNA“ in das bakterielle Genom involviert sind.

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List of abbreviations

ANOSIM	Analysis of similarities
AT	Above thermocline
BCC	Bacterial community composition
BPP	Bacterial protein production
BT	Below thermocline
CARD FISH	Catalyzed reporter deposition fluorescence *in situ* hybridization
CRISPRs	clustered regularly interspaced short palindromic repeats
DAPI	4',6-diamidino-2-phenylindole
DGGE	Denaturing Gradient Gel Electrophoresis
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
EL	Epilimnion
EPS	Extracellular polymeric substance
FISH	Fluorescence *in situ* hybridization
FL	Free-living
FOV	Field of view
HL	Hypolimnion
LGT	Lateral gene transfer
MIA	Microscopic aggregates
NMDS	Non-metric multidimensional scaling
OM	Organic matter
ORF	Open reading frame
OTU	Operational taxonomic units
PA	Particle-associated
POM	Particulate organic matter

ROI	Region of interest
SMA	Single macroscopic aggregates
TEP	Transparent exopolymer particles

* Names of published software or algorithms that consist of acronyms and were used in this study are not given in this list

1. INTRODUCTION

The ubiquitous process of photosynthesis - the harvesting of light and nutrients to form organic matter (OM) unifies all surface water bodies regardless of their geographic location, temperature or salinity. In all cases, this OM gradually supports heterotrophic communities at different positions across the trophic scale, after which it is being mineralized to nutrients and CO_2 at various time scales. These timescale are the direct result of a "competition" between physics and biology, between the sinking and burial of particulate organic matter (POM) on the one hand and microbial decomposition on the other.

1.1 The POM-DOM continuum

The primary external source of dissolved organic matter (DOM) in aquatic environments is CO_2 fixation by photosynthetic organisms. Additionally, terrestrial input contributes as well to the aquatic DOM pool, with larger influences on coastal-marine and limnic environments. Once OM enters the aquatic system, it can alternate between the state of POM and DOM via a complex cycle involving physical, chemical and biological processes (Fig 1.1). Exudates from photosynthetic organisms consist of both labile and semi-labile substances. The former will be rapidly consumed by the microbial community. Semi-labile OM can be used by more specialized bacteria, but due to its slow rate of degradation, chemical properties and water movement, it will be converted into POM for example in the form of Transparent Exopolymer Particles (TEP) in the case of acidic polysaccharides

(Thornton et al. 2007). The sticky characteristics of TEP lead to the sequential formation of larger aggregates trapping also additional particles as described in section 1.4. Once formed, TEP is readily colonized by bacteria, which is the first stage in the transformation of POM back to DOM. Viral lysis of microorganisms, bacteria and phytoplankton excretions, as well as sloppy feeding by zooplankton are an additional significant internal source of DOM. This DOM will re-enter the DOM-POM aquatic cycle (Fig. 1.1).

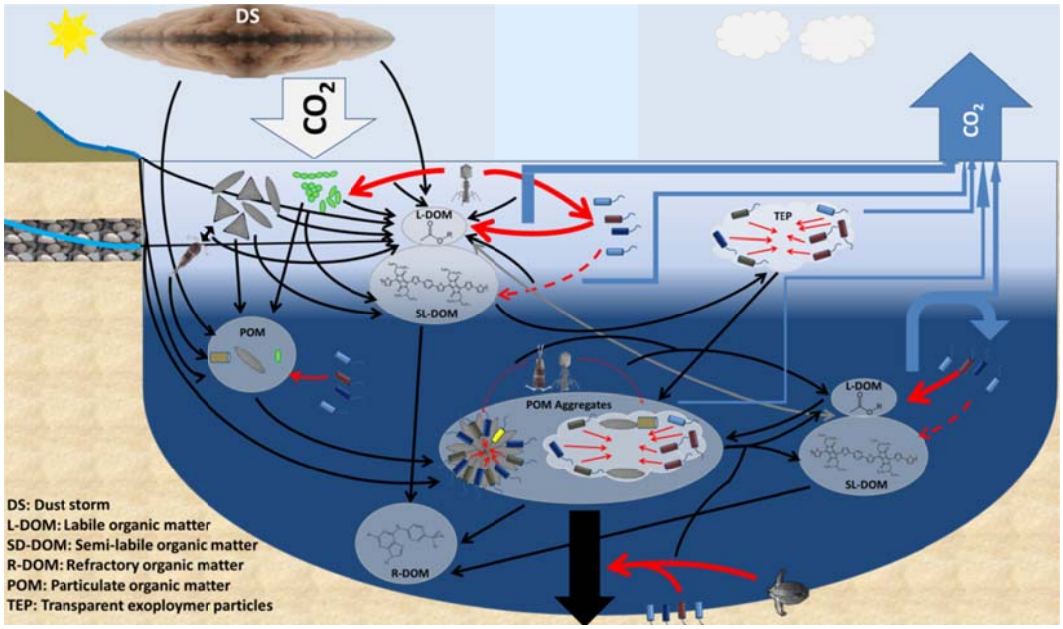

Figure 1.1 Schematic carbon transitions between the atmosphere and an aquatic system (limnic or marine) and within the system itself. Black and red lines represent contribution to or consumption from the organic matter pool at the end of the arrow, respectively. Blue lines represent export of carbon (as CO_2) from an aquatic system to the atmosphere. The grey line represents exchange between deep water and shallow water DOM pools via seasonal mixing, upwelling or down-welling. The figure is a synthesis of published concepts presented throughout the introduction.

1.2 Global significance of particles

Particles in aquatic systems hold a dual role. On the one hand, the flow of particles to the depths forms a critical link in the global carbon cycle called the 'biological carbon pump' (Alldredge & Silver 1988; Grossart & Simon 1998b; Revelle & Suess 1957). On the other hand, during sinking these organic particles are hotspots of microbial

activity and represent important loci for organic matter mineralization, nutrient redistribution and genetic material exchange in the water column.

Organic particles are primary vehicles for transporting surface derived organic material to deep water and the ocean floor (Alldredge & Silver 1988; Volkman & Tanoue 2002), where it remains buried for centuries and out of contact with the atmosphere. It is estimated that sinking particles transport up to a quarter of the carbon captured by phytoplankton in the surface ocean to depth (Falkowski et al. 1998). Nevertheless, this number varies and may be generally overestimated according to recent models (Yool et al. 2007). The physical frame of sinking is partly given by the stratification of the water body (Kindler et al. 2010) as often can be seen by an accumulation of POM aggregates along the pycnocline (Macintyre et al. 1995; Alldredge et al. 2002; McManus et al. 2003; Kindler et al. 2010). Thus, an increase in surface temperatures of water bodies, as predicted by recent climate models, may not only increase microbial activities but might also lead to increased stratification and the entrapment of POM in the upper water strata (Prairie et al. 2013). Since microbial mineralization would be increased two-fold: i) by temperature dependent microbial activities and ii) by prolonged residence times of the aggregates in the water column, overall carbon sequestration by stratified aquatic systems could be largely decreased.

Particles are important sites for biological processes like production, decomposition, and nutrient recycling in the water column (Simon et al. 2002; Alldredge & Silver 1988). The concentration of carbon and nutrients in organic particles can exceed those in sea water by >2 orders of magnitude (Blackburn et al. 1998; Shanks & Trent 1979; Grossart & Simon 1993). Cellular uptake of specific nutrients, like sugars and amino-acids, can differ significantly between free-living (FL) and particle-associated (PA) communities (Kirchman & Mitchell 1982; Lyons & Dobbs 2012; Grossart et al. 2003a; Kramer et al. 2013). Although, overall FL bacteria strongly outnumber those on particles, locally PA bacteria can reach higher concentrations than FL bacteria (Caron et al. 1982). Moreover, PA bacteria - due to a higher substrate availability in their microhabitats - are often more active than their FL counterparts (Grossart et al. 2003a; Caron et al. 1982; Fernández-Gómez et al. 2013), frequently contributing to >50% of total prokaryotic activity (Garneau et al. 2009; Crump et al. 1998, 1999; Ghiglione et al. 2007; Grossart et al. 2007; Fandino et al. 2001; Rösel & Grossart 2012; Stocker 2012). Consequently, PA bacteria may

significantly contribute to the overall microbial productivity in both limnic and marine systems, yet they are often excluded from studies for methodological reasons (Yooseph et al. 2007; Venter et al. 2004).

Sinking particles may be crucial for the survival of additional communities other than those that are already colonizing them (Smith et al. 1992, 1995). Thus, in deep water bodies such as ocean's bathypelagic zone, sinking particles provide a source of nutrition to organisms in the intermediate and deep water column (Smith et al. 2009). Additionally, once the particles have settled on the sediment, they often feed a community of deposit feeding organisms (Lauerman & Smoak 1997). In pelagic systems, particle density and composition varies in relation to factors including the source and proximity of terrestrial runoff, phytoplankton production, turbulence, and flocculation processes. Thus, many taxa respond to seasonal influx of POM to the ocean/lake floor as a result of which, for example, populations of opportunist species may increase.

An emerging concept is the importance of microscopic particles in the deep ocean as reservoir of microbial heterogeneity and metabolism (Eloe et al. 2011; Bochdansky et al. 2010; Nagata et al. 2010; Stewart 2013). Walsh et al. (2013) suggested that POM aggregates provide the necessary environmental and metabolic conditions for the frequent occurrence of lateral gene transfer (LGT). This is supporting earlier studies showing increased activity of known LGT mechanisms on particles (Lorenz 1988; Lorenz & Wackernagel 1990; Angles 1993).

1.3 Significance of marine vs. limnic systems to the global carbon cycle

Historically, many believed that inland waters play an insignificant role in regional and global carbon cycling, since they comprise only about 3% of the earth's continental surface (Downing et al. 2006). In spite of their relatively small areal coverage, lake sediments and wetland peat are globally-evident carbon pools because of their often very high carbon density and long residence times (Cole et al. 2007; Tranvik et al. 2009). Sediments of freshwater systems store 3 times more carbon (0.3 Pg y^{-1}) than marine ones (0.1 Pg y^{-1}) (~150 times more when normalized to area)

(Dean & Gorham 1998). Global annual burial of organic carbon in the sediments of lakes and reservoirs (~0.05 and 0.08 Pg yr^{-1}, respectively) exceeds that buried in ocean sediments (0.12 Pg yr^{-1}) (Dean & Gorham 1998; Sarmiento & Sundquist 1992; Tranvik et al. 2009). Global CO_2 emissions from freshwater have been estimated to 0.75-2.1 Pg y^{-1} (Cole et al. 2007; Raymond et al. 2013), equal, or higher than oceanic emissions (IPCC 2007). Hence, surface freshwater systems receive, sequester, and release carbon at high rates which makes them quantitatively important in regional and global budgets (Abril et al. 2005). In both environments, the biological uptake of CO_2 plays an important role in determining whether they are a carbon sink or a source. Under present climate conditions both marine and limnic systems serve as weak sinks for anthropogenic CO_2 (IPCC 2007; Gruber et al. 2009), nevertheless, this may change due to global warming (Le Quéré et al. 2013) as will be discussed in chapter 5.

POM is important for carbon sequestration in both marine and limnic systems; therefore, it is important to compare the biome associated with these particles in each environment. Despite the mineralization of POM occurring in all aquatic environments, the underlying microbial communities responsible for the degradation are substantially different based on the physico-chemical setup of the environment. While several studies have been carried out on describing and comparing the differences in bacterial community composition (BCC) of PA and FL microbial communities in either marine or freshwater ecosystems, it is one of the central aims of this work to compare limnic and marine PA in regard to their bacterial community composition and activity.

1.4 Formation and degradation of OM aggregates

POM aggregates consist of homogenous or heterogeneous assemblages of diverse live or senescent organisms such as diatoms, coccolithophorids, dinoflagellates, cyanobacteria, and macroalgae (e.g. Chlorophyta), alongside exudates or biological residues such as phytoplankton detritus (including diatom frustules), zooplankton molts and carcasses, abandoned larvacaean houses, pteropod feeding webs, fecal pellets and TEP. The composition of the particles is largely influenced by seasonal variations which lead to changes in phytoplankton species compositions and

abundance. Due to differences in the pelagic community in lakes and oceans, the composition of aggregates in both environments differs. Nevertheless, in both systems similar organisms such as diatoms are known to form aggregates due to their stickiness, which is caused by the excretion of polysaccharides (Kiørboe & Hansen 1993; Grossart et al. 1997).

Non biogenic components as clay and silt minerals, calcite, and additional particulate matter found in the water column may contribute to the aggregates' structure as well (Simon et al. 2002 and references therein). As lakes are intimately associated with their watersheds, they receive a large influx of organic and inorganic particulate matter from the surrounding terrestrial environments (Jansson et al. 2007; Tranvik et al. 2009). Microorganisms colonizing the particles have a major role in the continuous shaping of the POM aggregate and contribute to its degradation on the one hand and to its increase in size on the other hand (Grossart et al. 2006) as will be discussed.

To understand the ecological function of organic aggregates, it is important to study the processes leading to their primary formation. These include a combination of physical, chemical, and biological mechanisms (Eisma & Li 1993) which are often difficult to deconvolute one from the other. Turbulence and differential settling velocities are the most relevant physical mechanisms for the formation of aggregates in the marine pelagic zone (McCave 1984). In limnic systems aggregate formation is mostly triggered by wind-induced turbulence and shear (Grossart & Simon 1993) which seems to be contrary to observations in marine systems (Alldredge & Gotschalk 1989; Riebesell 1991). The ionic strength in the aquatic environment has a significant role in the formation of POM aggregates. The process of particle formation is likely to occur initially by coagulation followed by flocculation. In marine systems the strength of binding is expected to be greater than in fresh waters where divalent cations are found in lower concentrations. Nevertheless, aggregates formation via coagulation can be also a significant process in lakes, where it is mediated by divalent cations as well (Weilenmann et al. 1989). In an early study, Mulholland (1981) found that the addition of Ca^{2+} and Mg^{2+} to a soft freshwater lake caused an increase in the rate of POM formation as a result of a rapid physicochemical flocculation process. While generally divalent cations have a similar role in both marine and limnic systems, it was shown that due to higher ionic

strengths and turbulence, marine particles are usually denser than in freshwater systems (Logan & Kilps 1995; Chen & Eisma 1995). In contrast, aggregated particles in limnic systems are more voluminous and maintain to a certain extent the properties of the individual subunits (Logan et al. 1994).

Sticking of prime particles due to specific properties such as surface charges, coating by sticky compounds such as carbohydrates, and biological transformations play an important role in particle formation. Specifically to limnic systems, increased concentrations of humic substances in the water column may have a dual function with respect to particle formation. On the one hand they may slow down the process of aggregate formation as at high pH the humic substances become negatively charged and repulsive forces reduce aggregation (Weilenmann et al. 1989). On the other hand, von Wachenfeldt et al. (2008) showed that the spontaneous aggregation of humic substances by light plays a significant role in sequestration of allochthonous carbon in lakes.

Exudates, compounds secreted from cells, are ubiquitous in marine and fresh water systems and are important for attachment, locomotion, feeding and protection. They include Extracellular Polymeric Substances (EPS; a.k.a mucus, slimes, and biofilm matrices). EPS is often dominated by polysaccharides even though the composition varies with the organisms that produce them. In the process of aggregate formation, a key feature of EPS is their power to bind water after secretion (Sutherland 2001). In this process, which occurs in seconds, the small volume of the primary compounds expands to many times its original volume as water becomes incorporated into the matrix. One of the important differences, concerning types of exudates, between marine and fresh water is the absence of silk, a proteinaceous secretion, produced by some aquatic insects and aquatic spiders, in marine systems (Wotton 2011). Additionally, in lakes there is an obvious absence of Larvaceans, Doliolids and Pteropods; the mucus filter feeders that are often the major components of marine snow (Simon et al. 2002).

Free EPS from algae, bacteria, and other microorganisms is an important binding agent in aquatic systems (Lundkvist et al. 2007), due to the sticky quality of EPS supported by other biogeochemical processes, such as divalent cation binding (Wotton 2011 and the references therein). In surface water the fusion of aggregates leads to formation of TEP, a planktonic form of EPS. TEP forms sticky particles

that enable aggregation by providing the glue and the matrix of phytoplankton aggregates (Passow et al. 1994). Flocculation and stability of aggregates in fresh waters depend on free EPS, just as in the sea (Logan et al. 1994). Additionally cell spillover as a result of bacterial degradation increases the particle stickiness thus stabilizing it and increasing the chance of further matter adhering to the particle (Grossart et al. 2006).

The processes of coagulation and flocculation depend on the type of building material and will lead to formation of particles of different sizes accordingly. The size of these aggregates ranges from <1 μm to >10 cm, covering more than 6 orders of magnitude (Simon et al. 2002). Macroscopic organic particles >500 μm (marine) and >300 (limnic) μm in diameter are traditionally referred to as marine and lake snow, respectively (Suzuki & Kato 1953). Occasionally, these aggregates may reach gigantic sizes via mechanisms which are not fully understood yet. In the Northern Adriatic Sea and Tyrrhenian Sea such aggregates may be up to 5 m in length (Kaltenböck & Herndl 1992; Precali et al. 2005). Large (cm sized), mucous-rich marine snow aggregates were also observed in oil-contaminated surface waters of the Gulf of Mexico (Passow et al. 2012). Boetius et al. (2013) reported 50 cm large aggregates in the Arctic Sea. The abundance of aggregates ranges from <1 L^{-1} to >10^8 L^{-1} and is inversely related to aggregate size (Simon et al. 2002).

The aggregates formed in surface layers of aquatic systems are subject to degradation. They can be torn apart by physical disintegration and shear stress (Karl et al. 1988) as well as solubilized and remineralized by microorganisms (Ploug et al. 1999; Smith et al. 1992). The physical fragmentation of an aggregate is significant to the surrounding FL microbial community since it can result in an immediate release of interstitial DOC and macronutrients to surrounding water (Goldthwait & Carlson 2005). However, biological processes are more important for aggregate degradation. As aggregates are hotspots of heterotrophic activity in the water column they host a very rich and abundant flora and fauna (Alldredge & Silver 1988; Kiørboe 2000). Bacteria on aggregates occur in concentrations that are many orders of magnitude higher than in the surrounding water (Alldredge & Silver 1988) and their activity causes aggregates to solubilize and remineralize at high rates (Ploug et al. 1999). Numerous protozoans and metazoan species may also be abundant on particles and contribute to aggregate degradation by feeding on both bacteria and other

components of the aggregate (Kiørboe 2000; Kiørboe et al. 2003). Overall, leakage of DOM by physical fragmentation, feeding on aggregates by protozoans, mesozooplankton species and fish or the microbial heterotrophic degradation all contribute to aggregate degradation.

1.5 Colonization of particles

1.5.1 Bacterial colonization of particles

Within the water column bacteria can exist either as FL, or found in association with algae, zooplankton, detritus, or any other object that provides a surface for colonization. Eventually these units will form aggregates. These heterogeneous units that form particles, already harbour associated bacterial communities, as was previously shown for example for cyanobacteria (Behrens et al. 2008), diatoms (Amin et al. 2012; Gärdes et al. 2011) and Charophyceae (Fisher et al. 1998). The traditional point of view associates the phase of active growth of algae with a low number of attached bacteria while the increased number of bacteria coincides with late senescent stages of the bloom (Riemann et al. 2000; Grossart et al. 2005). On the contrary, Smith et al. (1995) found a high number of diatom-associated bacteria related to the early stages of the bloom, while Kaczmarska et al. (2005) report a low bacterial colonization in senescent stages of diatom bloom.

Since diatoms are the key component of phytoplankton assemblages (Armbrust 2009) and responsible for 20-25% of global C fixation (Smetacek 1999), the multitude of possible interactions between planktonic algae and bacteria were mainly studied with diatoms as model organisms (Grossart et al. 2005; Cole 1982; Grossart & Simon 2007).

In terms of general ecological concepts there are various modes of interactions between bacteria and algae (diatom) (Grossart 1999). Bratbak and & Thingstad (1985) described one of them as a competition where bacteria competes with the diatom for inorganic nutrients. In commensalistic interactions the bacteria benefit from the diatom without having any negative effect on it. However, commensalism

can easily turn to parasitism if the algae grow under environmental stress (Cole 1982). In the parasitism mode of interaction bacteria may either invade the host cell, or produce metabolites with algicidal activity which will eventually lead to cell lysis and death (Park et al. 2010), while algae can produce antibiotic compounds inhibiting bacterial growth (Leflaive & Ten-Hage 2009). In mutualism, bacteria can benefit from diatom exudates (Pete et al. 2010), while algae profit from bacterial products (e.g., vitamins) (Croft et al. 2005; Droop 2007).

The composition of aggregates (mainly the quality and quantity of OM) determines the community composition of the associated-microorganisms, often including microorganisms that otherwise may not be found in the water column (DeLong et al. 1993; Bidle & Fletcher 1995). To better understand the specific ecological role of aggregates it is necessary to first analyse their microbial diversity and composition. The phylogenetic affiliation may point to potential functional properties of PA microbes and their metabolic pathways such as sulfate reduction, ammonium oxidation, nitrate reduction, methanogenesis and methanotrophy (Stepanauskas & Sieracki 2007).

Factors that affect the dynamics of dominant colonizing phylotypes, such as viral infection and bacteria-bacteria antagonistic interactions, may play important roles in determining whether specific populations develop on decaying phytoplankton (Bidle & Azam 2001). Antagonistic relations were found between bacteria isolated from POM aggregates (Long & Azam 2001; Grossart et al. 2004). Among these *Actinobacteria* had the strongest activity (Grossart et al. 2004). Nevertheless, to the best of my knowledge, these interactions between bacteria were not yet demonstrated directly on particles.

PA bacteria harbour specific adaptations suited to the attached lifestyle. Bacteria are only rarely colliding with POM aggregates in the open ocean (Kiørboe et al. 2002). Therefore, many PA bacteria are motile (Grossart et al. 2001) and express chemotactic behaviour (Kiørboe et al. 2002). This allows them to detect and attach to sinking particles. Stocker (2012) concludes that copiotrophic bacteria, detecting microgradients such as formed by sinking aggregates, swim up to 5 times faster than oligotrophic bacteria. Once a bacterium is attached to the particle the external degradation of the substrate is done by extracellular enzymes. Accordingly, the activity of such enzymes was found to be 1-2 orders of magnitudes higher in PA

bacteria than in their FL counterparts (Karner & Herndl 1992; Smith et al. 1995). For example the protease activity of PA bacteria was up to 20 times higher than in FL bacteria. The study of a the genome of *Gramella forsetii* revealed a high abundance of adhesion related genes as well as a specialization for the degradation of high molecular weight organic carbon in the marine *Bacteroidetes* (Bauer et al. 2006). Single cell genomic studies of PA *Verrucomicrobia* showed that these organisms possessed an excessive number of glycoside hydrolase, esterases, sulfatases carbohydrate lyases and peptidases (Martinez-Garcia et al. 2012).

Ghiglione et al. (2009) concluded that PA and FL bacteria do not differ phylogenetically. This is in disagreement with previous studies (DeLong et al. 1993; Crump et al. 1999). Based on large numbers of similar OTUs between PA and the FL fraction, Riemann and Winding (2001) and Ghiglione et al. (2009) proposed that PA and FL fractions are not separate entities, but interacting assemblages. Low diversity of the PA was reported as a general feature in pelagic environments (Ghiglione et al. 2009; Acinas et al. 1997; Covert & Moran 2001; Moeseneder et al. 2001), however, recent studies, using high throughput sequencing techniques, show the opposite (Crespo et al. 2013; Ortega-Retuerta et al. 2013).

DeLong et al. (1993), using a clone library, showed that the structural BCC on marine snow is dominated by *Bacteroidetes* (55%), *Planctomycetes* (22%), and *Gammaproteobacteria* (11%). Nevertheless, most of the data available so far indicate that only *Bacteroidetes* and *Gammaproteobacteria* are the dominant colonizers of marine aggregates (Rath et al. 1998; Bidle & Azam 2001; Ploug et al. 1999; Simon et al. 1999; Rink et al. 2008). Bidle and Azam (2001), who detected *Bacteroidetes* and *Gammaproteobacteria* on diatoms, suggested their important role in transforming organic and inorganic matter. Riemann et al. (2000), reported *Alphaproteobacteria* dominating during *Thalassiosira* sp. (diatom) bloom. Eloe et al. (2011) systematically compared the diversity between PA and FL assemblages at 6000 m depth in the Puerto Rico Trench and found *Rhodobacterales*, unclassified *Myxococcales*, *Bacteroidetes* and *Planctomycetes* associated with particles whereas *Caulobacterales*, *Xanthomonadales* and *Burkholderiales* were dominating FL bacteria.

In contrast, the BCC of limnic particles is dominated by *Alphaproteobacteria*, *Betaproteobacteria* (Weiss et al. 1996; Grossart & Simon 1993) and *Bacteroidetes* (Brachvogel et al. 2001; Grossart & Simon 1998a; Schweitzer et al. 2001;

Lemarchand et al. 2006). During the *Dinobryon* sp. bloom, *Gammaproteobacteria* and to a lesser extent *Bacteroidetes* were the dominant PA bacteria (Brachvogel et al. 2001). Tang et al. (2012) showed that in limnic systems the *Planctomycetes* can represent a significant fraction of total bacteria as well.

Bacteroidetes in lakes are often found in high abundances during periods following cyanobacterial blooms (Newton et al. 2011; Eiler & Bertilsson 2007). Flavobacterium-like populations and some other Bacteroidetes are copiotrophs adapted to high-nutrient conditions (Newton et al. 2011; Zeder 2009).

1.5.2. Archaea on particles

Archaea were not often described on particles although their presence has been confirmed in several studies (Galand et al. 2013; Woebken et al. 2007; Eloe et al. 2011). The ecological niche(s) of aquatic Archaea are still a subject of active investigation (Pearson & Ingalls 2013). Analysing phylogenetic diversity of PA and FL archaeal assemblages from the Mackenzie River and Beaufort Sea in the western Canadian Arctic Galand et al. (2008) found *Euryarchaeota* dominating riverine and coastal waters whereas the marine waters contained most exclusively *Crenarchaeota*. They could not point at any specific archaeal group that was exclusively restricted to either PA or FL fraction. A large fraction of marine Archaea is associated with *Thaumarchaeota* (previously considered a class of *Crenarchaeota*) (Brochier-Armanet & Boussau 2008). These Archaea which are known to be ammonia oxidizers have been already shown on particles in oxygen minimum zones (Woebken et al. 2007). It would be interesting to further investigate their distribution on particles in comparison with bacterial nitrifying bacteria which are as well present on particles (Bianchi & Marty 1992; Phillips et al. 1999). Interestingly, a recent study has shown the presence of methanogenic Archaea on particles in the oxygenated water column of an oligotrophic lake (Grossart et al. 2011).

1.5.3. Particles can be islands for pathogens

It has been described before that important pathogens like *Vibrio cholerae* survive and spread on particles (Colwell et al. 2003). Danovaro et al. (2009), by using FISH, found on marine mucilage from the Mediterranean Sea a conspicuous number of pathogenic species (e.g., *Vibrio harveyi*) that were absent in surrounding water. Furthermore, particles have a significant role in the transfer of bacteria, including pathogens like *Vibrio parahaemolyticus* (Venkateswaran et al. 1990; Lyons et al. 2007) and *Vibrio cholera* (Colwell et al. 2003; Lyons et al. 2007), within and between similar ecosystems (Grossart et al. 2010). Particles are known to serve as a refuge to pathogenic bacteria (Tang et al. 2011; Grossart et al. 2010). Concentrations of pathogenic *Vibrio* species were higher on aggregates than in the surrounding water (Lyons et al. 2010).

1.5.4. Fungi on particles

Fungi make a large proportion of the microbial diversity on Earth (Hawksworth 2001; Hibbett et al. 2007). The number of fungal species was estimated to 1.5 million (Hawksworth 1991) based mainly on plant and soil associated species, not taking into consideration possible habitats such as freshwater or marine ecosystems. Following the introduction of modern molecular methods a revised estimate for fungal diversity stands between 3.5 and 5.1 million species (O'Brien et al. 2005). Nevertheless, given their large evolutionary complexity, coupled with unsuitable methodology as well as asymmetric sampling efforts of the different environments, worldwide abundance of fungi species is easy to overlook. Independent of the correctness of these estimates, it is likely that <5% of the fungal diversity is currently described and kept in culture (Richards et al. 2012).

Current knowledge brings us to believe that fungi are rare in marine environments (Richards et al. 2012), more abundant and diverse in freshwater systems (Wurzbacher et al. 2010) and without any doubt widespread and important in terrestrial ecosystems (James et al. 2006; Wang & Qiu 2006). Given the importance of these heterotrophic microorganisms, and their link to the microbial food web in

transferring nutrients directly and indirectly to other trophic levels, it is more likely that their abundance and diversity has not been fully unveiled in aquatic ecosystems (especially marine systems) and therefore represent the main unknown of the aquatic microbial world.

While in surface pelagic environments fungi are considered non-diverse and low-abundant (Richards et al. 2012), increased fungal diversity was recently discovered in deep or anoxic oceanic waters, hydrothermal vent environments, and deep-sea marine sediments (Bass & Howe 2007; Burgaud et al. 2009; Jebaraj & Raghukumar 2010; Le Calvez et al. 2009). This goes hand in hand with the fact that fungi require nutrient rich environments and surfaces to thrive. Fungi may be directly involved in the collapse of algal blooms and thus in POM aggregates formation (Wurzbacher et al. 2010, 2011). Algae-associated fungi were extensively studied in aquatic systems (e.g., Kohlmeyer & Kohlmeyer 1979; Canter-Lund et al. 1995; Alster & Zohary 2007). Alster & Zohary (2007) observed during a bloom of the dinoflagellate *Peridinium gatunense* a mass development of the chytrid fungus *Phlyctochytrium* sp. which led to a 1000-fold decline in *Peridinium* density within 9 days of the initial observation of the chytrid.

POM aggregates, readily colonized by bacteria, might be suitable, but yet understudied, fungal habitats. Many fungal sequences recovered from the deep-sea, branch close to terrestrial ones suggesting that they were transported from surface waters to the sea floor, possibly via particles. The transfer from shallow to deep water appears to be possible as some fungi are capable of altering their membrane composition to tolerate high hydrostatic pressure (Simonato et al. 2006). Only limited data is available on particle associate fungi. Wurzbacher et al. (2010) reported from a pelagic lake-sample, planktonic aggregates and zooplankton exuviae heavily colonized by chytridious fungi, suggesting their important role in degradation of particles and decomposition of allochthonous POM (e.g., leaf litter). This makes fungi an integral part of the microbial loop (Lefèvre et al. 2008). Though fungi are important in the marine system (Krause et al. 2013), there are currently no direct reports of fungi on POM aggregates in marine systems. Eloe et al. (2011) reported the occurrence of fungal sequences in the filter fraction >3 μm, nevertheless due to the larger size of fungi, this cannot be directly linked to their presence on POM aggregates. A more, interesting report comes from Damare and Raghukumar (2008)

who reported that fungi are found in macroaggregates in deep-sea sediment. Whether these aggregates are formed in the sediment or they sink as such and get buried is still unclear. It may be that the ecological niche of marine PA fungi is filled by fungal analogues - the *Thraustochytrids* (marine protists) (Raghukumar 2002). Thraustochytrids are known to produce a wide spectrum of enzymes involved in the hydrolysis of several classes of organic compounds and thus degrading a large variety of substrates (Bongiorni et al. 2005). Based on their high abundance in relation to chlorophyll a and POC (Raghukumar et al. 1990) it was suggested that *Thraustochytrids* might have an important role in degradation of autochthonous oceanic material and TEP (Damare & Raghukumar 2008).

1.5.5 Viral abundance on particles

Viruses of prokaryotic and eukaryotic microorganisms have a significant role in aquatic environments by regulating communities, releasing nutrients from lysed cells or exposing naked DNA for uptake (Suttle 2005; Fuhrman 1999; Proctor & Fuhrman 1992). As a result of their lytic activity, viruses contribute to POM aggregate formation and development. Weinbauer et al. (1993) suggested that viral termination of algal blooms leads to the formation of particles. Enhanced aggregate formation following a viral infection has been shown for *Phaeocystis globosa* (Sheik et al. 2013). Furthermore, the organic matter released during a lytic infection is often in the form of TEP which is known to trap particles from the water body and enhance aggregate formation (Brussaard et al. 2005).

Bacterial viruses (phages) are known to follow the presence of their hosts (Fuhrman 1999). Accordingly, as particles in aquatic environments are densely colonized by bacteria, phages are as well abundant on POM aggregates (Peduzzi & Weinbauer 1993; Müller-Niklas & Schuster 1994). Brussard et al. (2005) showed that viruses are rapidly attached to the TEP released from the decaying cells. Accordingly, TEP was suggested to be an infection site for bacteria where phages play a major role in prokaryotic diversity and productivity (Mari et al. 2007). Peduzzi and Weinbauer (1993), showed that phage concentration on particles is 3 orders of magnitude higher than in the surrounding water reaching values of ($8*10^7$ mL^{-1}). Such high abundances of particles-associated viruses led to the estimation that about 37% of PA bacteria are

killed by viruses (Proctor & Fuhrman 1992). The high viral abundance can be the result of a higher host density on particles (Müller-Niklas & Schuster 1994). Alternatively, this may be a result of physical adherence. Kernegger et al. (2009) found that the increase in abundance of FL bacteria is correlated with increase of PA viruses and suggested this is the result of the relevant viruses being scavenged by the particles. In contrast, Simon et al. (2002) suggested that increased activity of extracellular enzymes on particles makes this a harsh environment for viruses leading to a rapid decay in viral communities. Thus the high viral abundance on particles, as detected by microscopy, may not directly represent the truly active fraction.

Viruses may further play a role in the dynamics of the PA bacterial communities by lysis of specific phylotypes (Sheik et al. 2014). PA viruses may have conflicting roles in the ecology of the particle. On the one hand viruses can lead to an increase in particles size by locally decreasing DOM and affecting the particle's stickiness (Peduzzi & Weinbauer 1993; Weinbauer et al. 2009). On the other hand, presence of viral particles on POM aggregates may inhibit bacterial colonization of the particle and by this delay its degradation and/or structural evolution (Malits & Weinbauer 2009).

Several essential questions remain unanswered with respect to the ecology of PA viruses. First, there is hardly anything known regarding host-virus interaction on particles with Proctor and Fuhrman (1992) being, to the best of our knowledge, the sole study on this topic. Second, as a direct consequence of the latter understudied topic, we cannot correctly estimate the role of viral infections on particles in lateral gene transfer. It was experimentally shown that due to high bacterial density on particles, alongside a matching viral population, particles are hotspots for viral-induced transfer of genetic material (Riemann & Grossart 2008). It remains to be determined whether the bidirectional shifts between PA and FL lifestyle of bacteria is driven to some extent by viral activity on particles and in the water body.

1.5.6 Others on particles

POM aggregates readily colonized by bacteria are known to attract the respective predators of these communities. Thus aggregates often attract heterotrophic

flagellates, ciliates, and amoebas (Caron et al. 1982; Simon et al. 2002; Zimmermann-Timm 2002). The numbers of protozoa on particles can be enriched up to 4 orders of magnitudes as compared to the surrounding water (Caron et al. 1982; Grossart et al. 2003a). The presence of these predators was shown to largely affect the rate of particle colonization as well as bacterial density on the aggregate (Kiørboe et al. 2003). Protozoa not only affect the bacterial community by grazing on it. For example, FL *Sphingobium* cells are known to form POM aggregates or to adhere to existing ones in the presence of grazing protozoa (Blom et al. 2010). Though the topic of fungi is severely understudied with regards to POM aggregates (Simon et al. 2002; Zimmermann-Timm 2002; Wurzbacher et al. 2010), as protozoa are the natural grazers of fungi (Wurzbacher et al. 2010), they are expected to shape the PA fungal community as well. Similarly to the succession of bacterial communities on particles (Grossart et al. 2003b), protozoan communities change in time as well (Wörner et al. 2000). The nutrient trail of a degrading aggregate can be readily sensed by metazoa, thus attracting larger predators to the particle (Lombard et al. 2013). These predators do not only shape the bacteria-grazing community, but as they feed on the particle itself, they degrade it and alter its hydrodynamic proprieties (Iversen & Poulsen 2007).

1.6 Representative limnic and coastal-marine systems chosen for comparison

To more systematically evaluate differences in community composition of PA bacteria in limnic and coastal marine environments we selected marine sites with a strong terrestrial influence. The following section provides a short introduction into the environmental and chemo-physical characteristics of each of the study locations.

1.6.1 Limnic ecosystem - Lake Stechlin

During the last glacial period large parts of the northern hemisphere including Northern America, Asia and Europe were covered by ice. Approximately 12,000

years ago the scouring action of glaciers created thousands of lakes in northern Germany alone. Most of these temperate lakes are dimictic and completely overturn twice a year – in autumn before ice-on and in spring after ice-off. In summer the lakes are stratified with warmer water on top (epilimnion) and colder water on the bottom (hypolimnion). In winter ice-cover leads to an inverse temperature stratification with cooler water (0°C) staying near the surface and warmer, denser water (4°C) extending to the bottom (Bertilsson et al. 2013).

Lake Stechlin, a dimictic oligotrophic lake situated in the Baltic Lake District (53° 10' N, 13° 02' E), is in many ways a true representative of the glacial lakes in the northern hemisphere. With a surface area of 4.25 km^2, a shoreline of 16.1 km and a maximal depth of 69.5 m (average 23.3 m), it represents the second deepest lake in northern Germany (Koschel & Adams 2003). Lake Stechlin stratifies during April-June and the stable thermocline is usually observed at the end of July. Inverse stratification occurs in winters during prolonged ice-cover. Lake Stechlin has a long history of ecological research and is also a member of the international Global Lake Ecological Observatory Network. The phytoplankton in the lake has been studied since 1959 and is dominated by centric diatoms, cyanobacteria of picoplanktonic size, and green algae (Padisák et al. 1998, 2003). Overall, given its physico-chemico-biological settings, Lake Stechlin is considered a good proxy for numerous lakes in North America.

In the period from 1966-1989, a nuclear power plant was built and operated on the shore of the lake. Cooling water was drawn from the Lake Nehmitz and the heated cooling water was pumped into the oligotrophic Lake Stechlin and from there led back to Lake Nehmitz (Casper 1985). This 20 years period turned Lake Stechlin into a ~97,000 m^3, long-term experiment. The biological chemical and physical properties of the lake during these 20 years were compared to those measured earlier and following the plant's operational period. This allowed for a thorough analysis of the effects the power plant had on the lake. The power plant led to a significant thermal pollution and reduced the mean retention time of water in the lake basin from 335 days to 202 days (Koschel et al. 2002). The increased nutrient load and 'thermal' pollution were the direct negative effects on the water quality of Lake Stechlin, but in spite of the observed tendencies towards eutrophication, the lake has

preserved its oligotrophic status (Koschel et al. 2002 and the references therein) with tendencies to meso-oligotrophy in the last decade.

The temperature of Lake Stechlin, averaged over the year and the entire lake body, was between 1960 and 2014 7.5°C, but calculations based on regional climate scenarios of the German Federal agency, predict that by the end of the century the temperature will increase to about 9°C. Overall this model indicates that climate change might alter the currently dimictic regime of Lake Stechlin to a warm monomictic one within the next 80 years.

The lake is slightly alkaline with an increase in pH from 8.2-8.9 during the last 3 decades. Oxygen saturation in the epilimnion is >90% while in the hypolimnion it drops to under 60% following the summer stagnation period (Koschel et al. 2002). Primary production between years 1970-2000 was estimated to 121 g C m^{-2} y^{-1}. The phosphorous load in the same period was 0.04-0.07 g TP m^{-2} y^{-1}. The annual averaged NO_3-N and NH_4-N decreased from 24.3±11.6 and 69±29.6 µg L^{-1}, respectively, during 1972-1977 to 21.2±9.7 and 39.9±34.2 µg L^{-1}, respectively between 1992-2000 (Koschel et al. 2002).

1.6.2 Limnic ecosystem - Lake Grosse Fuchskuhle

Lake Grosse Fuchskuhle (53° 10' N, 13° 02' E) is located as well in the Brandenburg Mecklenburg Lake District of Northeastern Germany and was also formed during the Weichsel glacial period (~12,000 years ago). It is a small lake with the surface area of ~0.02 km^2 and a maximum depth of 5.5 m (median 3.3 m). This dystrophic lake has no inlet or outlet, but is fed by rain and groundwater (Burkert et al. 2004). For the purpose of biomanipulation experiments, the naturally acidic lake was subdivided by plastic curtains in 1986 and 1991 into two and later four compartments: southwest (SW), northwest (NW), northeast (NE) and southeast (SE) (Kasprzak 1993). The basins differ in amounts of humic matter, pH (Allgaier & Grossart 2006a), and other physical and chemical parameters (Kasprzak 1993), microbial activity (Babenzien & Babenzien 1990), and microbial food web structure (Burkert et al. 2003; Grossart et al. 2008). Due to the division and a moderate wind-induced mixing of the water in the compartments, the lake is stratified from spring to autumn, with a metalimnion

between 2 and 3 m deep and with an anoxic zone near the sediment which can reach the metalimnion during summer. The pH value in the surface waters of Lake Grosse Fuchskuhle varies between the individual basins by 1-2 units (Koschel 1995). The non-divided lake had the pH value between 4.2 - 4.6 (Kasprzak 1993). After the division the western basins remained acidic (pH 4-5), while the eastern basins became slightly acidic to neutral (pH 5-7).

The eastern compartments have a lower proportion of humic substances and a significantly higher proportion of polysaccharides (Sachse et al. 2001). The western compartments, however, are characterized by a higher proportion of humic substances and a lower proportion of polysaccharides. These findings suggest that the DOC in the eastern basins is determined by the activity of phytoplankton and the emergence of the DOC is therefore attributable to autochthonous processes. This is confirmed by higher chlorophyll a concentrations and higher microbial activity (Šimek et al. 1998). In contrast, the humic-rich compartments by the neighboring peat bog area can therefore be characterized as allochthonous. Extracellular enzyme activities were significantly higher in the humic-rich SW compartment as compared to the low-humic matter NE compartment, suggesting high uptake of substances by bacteria from the environment (Burkert et al. 2004). Additionally, the individual compartments also differ on higher trophic levels as well as in the composition of the food web (Hehmann et al. 2001; Šimek et al. 1998).

1.6.3 Marine ecosystem - The German Bight of the North Sea (Helgoland)

The North Sea is a semi-enclosed, shallow and rather young ecosystem. It represents the northeastern arm of the Atlantic Ocean and was formed by the flooding of a landmass some 20,000 years ago (Holocene period). It is bounded by the coastlines of England, Scotland, Norway, Sweden, Denmark, Germany, the Netherlands, Belgium and France. Atlantic water characterized by salinity above 35 and elevated winter temperatures, enters the North Sea mainly from the north and circulates through it in anticlockwise direction. Additional, and similar, oceanic water enters the North Sea from the southwest through the English Channel. The North Sea receives a significant input of freshwater from riverine input. Radach and Patch (2007)

estimated the amount of freshwater entering the North Sea from 7 rivers on its German, Belgian and Dutch coasts as 133 km^3 y^{-1}. This freshwater input was found to fluctuate with a strong periodicity of 6-7 years between 100-200 km^3 y^{-1} (Radach & Pätsch 2007). Last, the North Sea also exchanges with the Baltic Sea via the Danish straights. Through this exchange, about 940 $km^3 y^{-1}$ of surface brackish-saline waters from the Baltic Sea enter the North Sea while 475 $km^3 y^{-1}$ saline sea water enter the Baltic Sea (Alhonen 1982). Temperature and salinity in the North Sea show annual, seasonal and decadal variability. Tidal currents vary from some of the strongest in the world to zero.

The German Bight is the southeastern bay of the North Sea and is bounded by the Netherlands and Germany to the south and Denmark and Germany to the east. The waters in this area can be characterized as a mixture between central North Sea water and a water body originating from the South West. The latter consists of coastal freshwater as well as ocean water coming through the English Channel (Becker et al. 1992).

The island of Helgoland is located in the German Bight about 60 km off shore. While it is fed by the North Atlantic current and the English Channel, the rivers Elbe and Weser discharge their terrestrial load into the German Bight directly across the island. This riverine input constantly inoculates the coastal marine environment with freshwater. Thus, due to its proximity to the shore it has a significant coastal freshwater influence. The salinity in the area varies seasonally between 28 and 33 and the temperature varies from above 20°C in the summer to below 0°C in the winter (Wiltshire & Manly 2004). The sampling station "Kabeltonne" (54°11.3'N, 07°54.0'E) located on the island has been regularly used as a long term ecological monitoring station since 1962 (Franke et al. 1998). The Kabeltonne area represents a mesotrophic coastal marine site with an overall reduction in nutrients since the 1980s. This change has been mainly attributed to a reduction in input (van Beusekom 2008) and to some extent to changes in the current regime in the area during the last decades (Wiltshire et al. 2009).

The continuous monitoring of environmental parameters alongside its strong connectivity to terrestrial freshwater sources makes this site ideal for a comparison of limnic and marine systems where one could follow the competitiveness of freshwater bacteria in a saline environment.

1.6.4 Marine ecosystem - The Northern Adriatic Sea

The Northern Adriatic Sea, extending from the Gulf of Trieste southwards to the Ancona-Pula line, is a shallow, semi-enclosed basin with a mean depth of about 30 m. It is characterized by high seasonal variability of oceanographic and biological conditions, mainly due to climatic fluctuations and external nutrient input principally from the River Po. The latter flows into the northern Adriatic along its northwestern coast and continuously supplies the basin with freshwater. Mixing of the freshwater brought by the river and the input of oligotrophic Mediterranean water, make the productivity of this basin one of the highest of the whole Mediterranean Sea. Intensive spring and autumn phytoptankton blooms have been found to appear regularly in the part of the basin under the direct influence of the Po River (Revelante & Gilmartin 1976). The northern Adriatic Sea is well known for the formation of large algal agglomerations (Najdek et al. 2002) which in combination with phosphorus depletion and a high N:P ratio have been frequently suggested to induce a characteristic accumulation of carbohydrate-rich algal mucilage.

Although it is a shallow basin the northern Adriatic Sea shows strong physical, chemical and biological gradients. It has a pronounced stratification and a sharp thermocline with difference in water temperature between the top and bottom water strata reaching up to 20 degrees during the summer months (Zavatarelli et al. 2000). The stratification is further increased by an eastward flow of freshwater (Russo et al. 2005). Gradients in nutrient availability are evident in the water column across the northern Adriatic as well as across from east to west. This for example dissolved inorganic nitrogen close to the Istrian coast increases from 0.97 μmol L^{-1} in the surface waters to 2.01 μmol L^{-1} at the bottom. However eastward, in front of the river Po, surface waters contain 3.93 μmol L^{-1} dissolved in organic nitrogen while bottom waters 2.91 μmol L^{-1}. Gradients are also observed with respect to phosphate, however, these can span over an order of magnitude (Radić et al. 2009). These gradients also affect the abundance and distribution of phytoplankton species (Radić et al. 2009).

The large amorphous aggregates of the northern Adriatic Sea are unique among the marine snow in the world's oceans. Thus, together with the gradual influence of freshwater on an east-west transect, the northern Adriatic Sea makes a

suitable second marine site to study the similarities and differences between freshwater and marine PA bacterial communities.

(All references are found at the end of the thesis starting on page 194.)

2. METHODOLOGICAL ASPECTS OF THE STUDY

Extended details about methodology used in my doctoral research are given in each manuscript (I-III). This section aims at highlighting novel methodology developed as part of my studies, in light of the current state-of-the-art.

2.1 Overview on current methods for studying POM aggregates

Particulate organic matter (POM) aggregates are fragile entities spanning a large range of sizes and composition. The methods used for studying the composition and development of particle-associated (PA) microbial communities on aggregates and related microbial processes are grouped into several general categories. Although there is no single perfect method that can be used to sample and study POM aggregates, each of them gives a good tool to look for specific processes and mechanisms.

The most direct methods rely on the direct sampling of natural POM aggregates by SCUBA divers. This includes the collection of macroscopic organic aggregates *in situ* in open-ended plastic syringes or widely opened bottles, followed by microscopic observations or incubation experiments in the laboratory (Alldredge et al. 1986; Alldredge & Gotschalk 1990; Ploug et al. 1999; Grossart et al. 2003a). Despite the advantages found in studying naturally occurring POM aggregates, this approach has several disadvantages; (i) it requires very careful sampling by SCUBA

divers to avoid disruption of fragile aggregates. Therefore, this method reduces significantly the yield and number of collected aggregates not allowing for large scale studies; (ii) it limits aggregate studies to depths shallower than 50 m; (iii) the composition and age of these aggregates can be very heterogeneous since they are randomly collected *in situ*; (iv) it limits our understanding of PA communities to the fraction of aggregates visible to the human eye and thus misses an entire smaller fraction or TEP.

A second group of methods that is commonly used to measure quantity and quality of particles sinking from the upper regions of the water column are sediment traps (Davis, 1967). Thereby, the processes on the particle are usually stopped because otherwise they cannot be associated to the water column processes, but rather to the effects of the trap itself. This is usually accomplished by poisoning with sodium azide or formalin (Knauer & Karl 1984; Taylor et al. 2009). This kind of study offers a glimpse of sinking particles without providing the ability to perform further incubation experiments. Thus, rates of bacterial respiration, bacterial production, as well as temporal changes in microbial community composition cannot be determined. Very recently LeCleir et al. (2014) designed and developed a nonlethal sediment traps that allowed for incubation of marine aggregates at *in situ* temperature and pressure following separate phytoplankton blooms in the southern Pacific Ocean.

A third group of methods for studying the dynamics of PA microbial communities depends on the use of model aggregates that mimic natural ones. For example, some studies rely on the use of millimeter sized model agar spheres to follow bacterial colonization (Grossart et al. 2007; Kiørboe et al. 2003). The idea behind using model particles is to understand specific mechanisms by simplifying complex processes on particles. Although the organic matter composition can be easily manipulated the processes occurring on artificial particles might deviate from natural aggregates. On the other hand the use of such model aggregates allows production of large numbers of aggregate-like particles of known and controlled size, age, and composition.

A fourth group of methods is based on the differential filtration of known volumes of water coupled with low-vacuum gravity filtration. This method allows collection of large numbers of aggregates from any time point at any depth of the

water column. The limitation of this method is that the aggregates may become physically disrupted. Additionally, even though it has a high potential for large-scale studies of aggregates and their associated communities, the methods for counting PA bacteria were so far limited, inaccurate and exhausting. As described below and in the first manuscript, we developed a method for high-throughput, reproducible, semi-automatic enumeration of PA microbial communities.

A fifth group of methods uses aggregates formed *in vitro* from natural material and follow the development and activity of the PA communities in time. This requires mimicking constant sinking of the aggregates through the water column. For example, aggregates can be produced in the laboratory from naturally occurring organic material using roller tanks (e.g., Shanks & Edmondson 1989) or Couette flocculators (e.g., Drapeau et al. 1994). The power of this method is that it allows for the production of large numbers of similar, robust, newly formed macro-aggregates from natural material that can be easily used for further investigations. The main drawback of this method is that so far only closed systems for their study were available. In such systems the continuous supply of bacteria as well as OM and nutrients from the habitat is disabled alongside with an accumulation of toxic metabolites. This leads to the formation of the bottle effect, a phenomena that has been known for nearly a century by now (Zobell & Anderson 1936) in which the natural microbial community is rapidly replaced by opportunistic bacteria. As described below (point 2.2.2) and in the 3rd Manuscript of this thesis, we developed a completely new flow-through roller tank. This device allows for the continuous flow of water from any natural source without the loss of the particulate matter inside. In addition to avoiding the bottle effect, this system allows for the constant supply of new OM, nutrients and bacteria from the habitat water, tightly following all the fine changes in natural systems and thus allowing for *in-situ*-like studies.

The strength of all of the above mentioned methodologies, in my opinion, is that they will never become obsolete. They may be further developed and improved but the ability to exploit the technology to its fullest extent, I believe, has not yet been attained. Keeping awareness of the whole plethora of methods in a given field can always be beneficial, and if not practical, then at least it provides a deep understanding of the field and a motivation for continuous improvement.

2.2 Methods developed in this study

2.2.1. Enumeration of particle-associated bacteria by automated and semi-automated microscopic analyses

The employment of automated microscopy in this study allowed for the analysis of PA microbial communities on over 3,500 individual POM aggregates, the results of which are part of manuscripts I and II. The employment of this method allowed for a time-efficient, reproducible enumeration of PA communities. Details of acquisition and analysis methods are given below as well as in manuscript I.

Automated microscopy analysis:

Free-living bacteria-image acquisition: Quantification of total microbial cell numbers and relative abundance of distinct phylogenetic clades was performed in a fully automated manner using a multi-purpose imaging system (MPISYS, modified after Zeder (2009)). It is based on a fully motorized epifluorescence microscope (AxioImager.Z2m, Zeiss, Germany). It features LED illumination devices for transmission and fluorescence illumination (Colibri, Zeiss), and a monochrome digital camera (AxioCam MRm). It uses a motorized x, y, z-stage capable of holding 8 microscope glass slides on each of which up to 8 FISH preparations can be mounted. The software SAMLOC was subsequently run to scan all slides with a 1 x objective (EC Plan-Neofluar, NA: 0.025, Zeiss), as described in Zeder et al. (2011a). The software is able to recognize the FISH preparations and an algorithm creates coordinate position lists for later high-resolution imaging. The optical filter set 62HE (Zeiss) was applied to image the fluorescent stains DAPI and Alexa-488. Autofluorescence was recorded upon excitation on 633 nm. Bacterial cells were imaged using a 63 × objective (Plan Apochromate, NA = 1.4). Imaging was usually performed autonomously overnight, using 3 channels with a fixed camera exposure time of 500 ms for the autofluorescence, and automatically determined exposure times for DAPI and FISH images. Eight filters are processed per run with an average speed of 4.7 positions per minute, including nested autofocusing, z-stack acquisition and extended depth of field imaging (Zeder & Pernthaler 2009, Zeder, et al. 2011) on each position.

Aggregates-image acquisition: Image acquisition of POM aggregates was performed on the same microscope, but running the MPISYS in a semi-automated mode – as the density of aggregates varied from sample to sample, fields of view had to be selected manually in the following manner: SAMLOC was used to define a list of 12 positions for each sample. The imaging routine was run in a semi-automated mode using the 63x objective and the 62HE filter set. On each position, the routine performed an autofocus and subsequently paused allowing the user to adjust the position in x, y, and to refocus. The user then searched in a random manner an aggregate close to the original position, centered the aggregate to the camera's field of view, and adjusted the focus to the center of the aggregate. In addition, the user had to set an optimal exposure time for imaging on the DAPI, Alexa-488, and autofluorescence channel. Upon confirmation, the system acquired a z-stack of 25 images around the focused position with an interval of 0.3 µm for each channel. The original stack was stored for deconvolution and 3D reconstruction. For 2D cell quantification, a maximum intensity projection was calculated on each channel. For each sample at least 10 aggregates were imaged per FISH probe.

Automated Cell Counting: Image quality control prior to cell counting is of crucial importance (Zeder et al. 2010). To ensure highest quality of this dataset, each image was manually checked and removed if it contained artifacts, was out of focus, or if it could otherwise not be evaluated. Cell counting of both, total cell numbers and hybridized cells, was done using the ACMEtool2 software (Zeder et al., unpublished). It includes automated dynamic thresholds for segmentation and uses the unbiased counting frame (Gundersen et al. 1988). This software analyses images in multiple fluorescence channels with a high level of automation and according to sophisticated, user-defined counting rules. The software and description is available at www.technobiology.ch.

Semi-automated Aggregate Analysis: Aggregate images were similarly evaluated with the ACMEtool2 software. As aggregates exhibit a high degree of morphological variation, their outlines had to be defined manually by drawing a simple polygon (region of interest [ROI]) along the aggregate's periphery. The software then automatically based the cell counting on the ROIs. Area and perimeter were also assessed for each ROI. A screenshot of the aCMEtool2 software during the analysis of a particle is given in Figure 2.1.

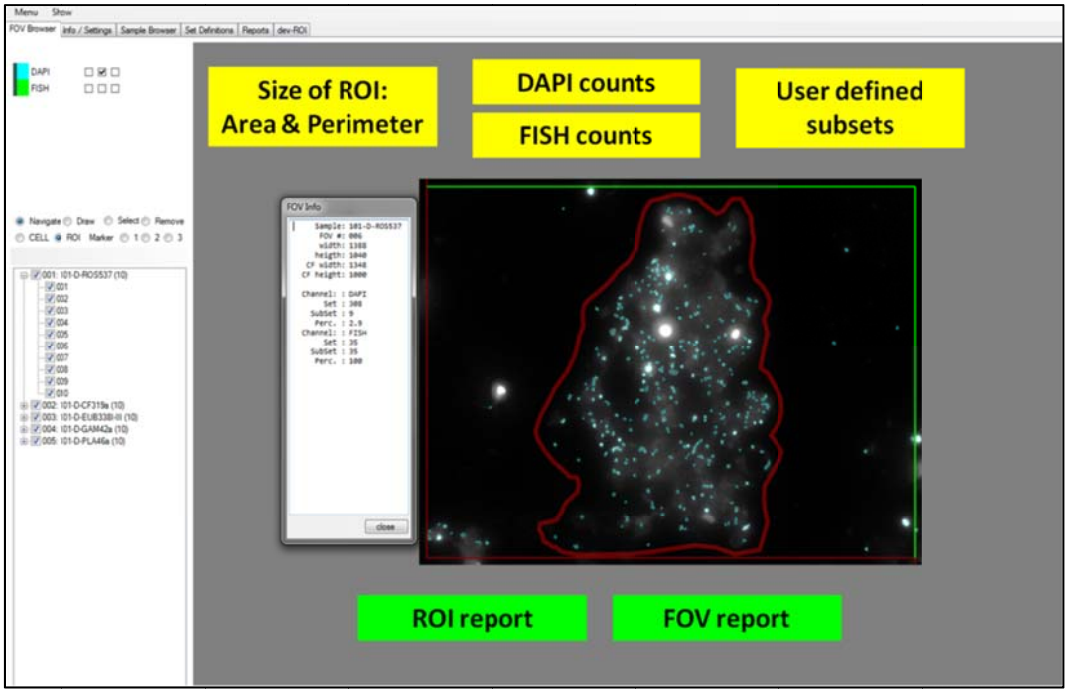

Figure 2.1 A screenshot of the analysis window of ACMEtool2 during the analysis of a FISH stained particle. The red line marks the region of interest (ROI; particle) for which cells should be counted. DAPI stained cells which were recognized by the software and match the predefined conditions are marked in blue. The software will provide as an output particles parameters such as area and perimeter. Bacteria data will include the number of DAPI and FISH stained cells per ROI and per entire Field of view (FOV) as well as the number of cells that are stained by both methods. Additionally the software will provide details on the distribution of cell sized and morphologies (length:width ratio).

2.2.2 Flow-through roller tank

To conduct long term experiments while avoiding the "bottle effect" we used a newly designed flow-through roller tank system (Ionescu et al., in prep). This device consists of an inner tube in which the particles roll and an external casing through which lake water flows in and out of the inner tube. The inlet and outlet of water occurs through 6 pores on each duct, respectively. The pores are ordered along the inlet and outlet tubes in such a manner that the inlet pores do not overlap with the outlet pores (Fig. 2.2). This creates a flow through the reactor while avoiding water crossing rapidly from the inlet to the outlet. By adjusting the rotation speed of the system and the flow velocity, aggregates can be maintained in the main reactor while

obtaining a constant steady flow of fresh water from the environment. For specific details regarding the use of the device in this study please see manuscript III.

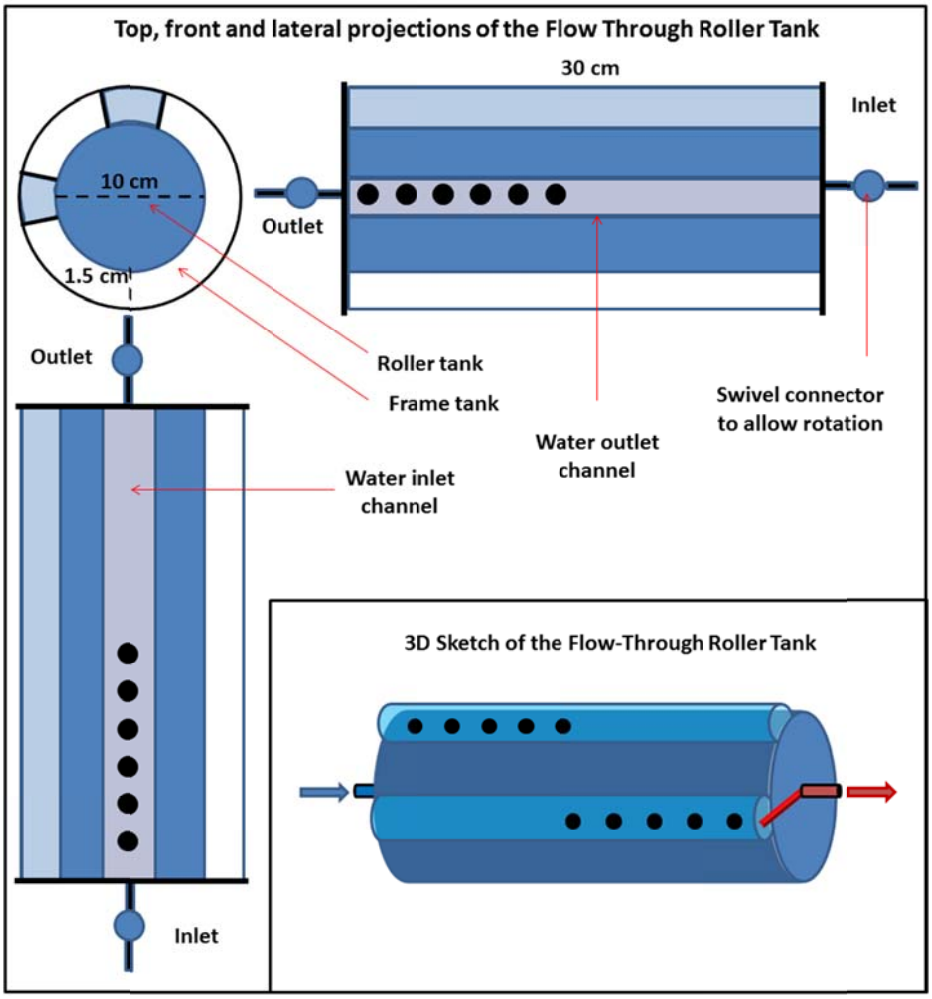

Figure 2.2 Schematics and 3D model of the flow-through roller tank system.

2.3 Overview of the methodology used in the different manuscripts

Manuscript I: ***Comparison of bacterial communities on limnic vs. coastal marine particles reveals profound differences in colonization***

Catalyzed Reporter Deposition Fluorescence *in situ* Hybridization (CARD-FISH)
Automated microscopy;
DNA Extraction;
Denaturing Gradient Gel Electrophoresis;
Tag-Pyrosequencing – data analysis

Manuscript II: ***Massive regime shifts and high activity of heterotrophic bacteria in an ice-covered lake***

Catalyzed Reporter Deposition Fluorescence *in situ* Hybridization (CARD-FISH)
Automated microscopy
DNA Extraction
Denaturing Gradient Gel Electrophoresis
Tag-Pyrosequencing – data analysis
Bacterial Protein Production-*employed by coauthors of the study*

Manuscript III: ***Macroscopic organic aggregates are hotspots for interactions between colonizing microorganisms as revealed by transcriptomics analysis***

Flow-Through Roller Tank
DNA extraction
RNA extraction
cDNA synthesis
Tag-Pyrosequencing – data analysis
Metagenome- data analysis
Metatranscriptome – data analysis
Scanning Electron Microscopy (SEM)

(All references are found at the end of the thesis starting on page 194.)

3. AIMS AND HYPOTHESES

Our knowledge and understanding of the ecological role of particulate organic matter (POM) aggregates and their associated microbial communities were accumulated for over 50 years and thus are accordingly extensive. Nevertheless, several key aspects of the microbial ecology of aggregates have not yet been resolved. This study therefore aimed at:

I) Elucidating similarities and differences between microbial communities associated with limnic and marine aggregates by applying high-throughput community analysis methods;

II) Revealing the driving force behind extensive heterotrophic activity and change in community composition of FL and PA bacteria in Lake Stechlin during ice-cover;

III) Generating a valid experimental system for conducting long-term laboratory studies on POM aggregates and using it to provide a deeper insight into temporal changes in gene expression on such particles.

These goals are summarized in Figure 3.1 and detailed below together with the specific working hypotheses of each study.

3.1 (I) Similarities and differences between limnic and marine particle-associated communities

POM aggregates in both limnic and marine systems consist of homogenous or heterogeneous assemblages of diverse live or senescent organisms, e.g. diatoms and cyanobacteria. Additionally, these particles may consist of or contain numerous source components such as phytoplankton and zooplankton detritus, diatom frustules, zooplankton molts and carcasses, abandoned larvacaean houses, pteropod feeding webs, fecal pellets, TEP etc. Therefore, it appears that the building stones of POM aggregates are generally relatively similar over the entire range of aquatic environments. This raises the question whether these seemingly identical niches harbor the same bacterial taxa.

Overall, marine and limnic microbial communities significantly differ from each other (Glöckner et al. 1999; Herlemann et al. 2011). However, hitherto no systematic comparison using identical methods has been performed between PA bacteria in lakes vs. marine systems, especially of coastal seas with a strong influence of freshwater input. The comparison between FL and PA microbial communities in different individual systems has led to controversial conclusions. Some studies suggest a strong separation between the two fractions (DeLong et al. 1993; Crump et al. 1999; Rösel & Grossart 2012) while others propose a large overlap (Ghiglione et al. 2009; Riemann & Winding 2001). Therefore, I hypothesized that (see Figure 3.1):

I.a) …despite the apparent similarity between the structural components of marine and limnic POM aggregates, they each will harbor a unique microbial community adapted to the general physico-chemical parameters of the system.

I.b) …high throughput sequencing will reveal connectivity between the PA and FL bacterial fractions, pointing out the common occurrence of mixed lifestyles among bacteria.

I.c) …using high throughput microscopic analysis of individual particles will unveil a high inter-particle heterogeneity in BCC pointing to the need of single-particles studies.

3.2 (II) Sudden under-ice bloom of heterotrophic bacteria in Lake Stechlin (northeastern Germany)

Although lakes cover a little more than 3% of Earth surface as compared to 71% by oceans and seas, they have an equal contribution to CO_2 emissions (IPCC 2007) and a larger contribution to carbon burial (Dean & Gorham 1998). On a global scale ice covers, either seasonally or permanently, up to 8.5% and 8.4% of saline and freshwater bodies (not including the Southern hemisphere), respectively (Benson & Magnuson 2007; Chapman & Walsh 1991). While under-ice phytoplankton blooms are common phenomena (e.g. (Dokulil & Herzig 2009; Boetius et al. 2013), little is known about the activity of heterotrophic bacteria during and following such blooms (Bertilsson et al. 2013). It is believed that as a result of low temperatures and reduced nutrient remineralization due to low protozoan grazing, microorganisms under-ice are killed, harmed or exist in a dormant state (Bertilsson et al. 2013; Greenbank 1945; Tulonen & Kankaala 1994). However, estimated biomass turnover for the overall bacterial communities from these studies usually ranges between 4-10 days indicating that winter microbial communities are actively growing and not just dormant. In winter 2009/2010, during a routine sampling, we noticed that the bacterial protein production was unusually high in the ice-covered Lake Stechlin. Since there was an ongoing bloom of *Aphanizomenon flos-aquae* at that time we hypothesized that:

II.a) …in light of nutrient availability which follows the winter mixing, heterotrophic bacteria communities will bloom if provided with sufficient suitable organic matter, despite near-zero water temperatures.

II.b) …the sudden availability of DOM and POM will be followed by a change in BCC.

3.3 (III) Temporal changes in active community and gene expression on POM aggregates – First insights from a new flow-through experimental system

Bulk microbial activity on particles has been studied using uptake of radiolabeled tracers and was found to be >50% of the overall bacterial productivity in water bodies during periods of bloom breakdown (Garneau et al. 2009; Crump et al. 1998, 1999; Ghiglione et al. 2007; Grossart et al. 2007; Fandino et al. 2001; Rösel & Grossart 2012; Stocker 2012) . Microbial activity was also studied on individual particles using microsensors (Grossart & Ploug 2001). Few studies have followed the temporal change in PA BCC mostly using artificial particles (Schweitzer et al. 2001; Simon et al. 2002; Grossart et al. 2003b; Kiørboe et al. 2003; LeCleir et al. 2014). Even fewer studies tried to link between the BCC and its activity on the particle (Schweitzer et al. 2001; Moran et al. 2013; Kong et al. 2013). The latter have recently focused on *in situ* environments (Moran et al. 2013; Kong et al. 2013), thus not providing any temporal resolution. Older studies conducted using closed roller tank systems (Schweitzer et al. 2001) suffer from two problems, a low taxonomic resolution due to available technology at the time they were conducted and a strong "bottle effect" due to a closed system setup. Therefore, by combining high-throughput methods and an open experimental system, I hypothesize that:

III.a) …the PA microbial community changes over time, probably due to changes in organic matter quality and structure.

III.b) …changes in carbon quality will lead to differential gene expression. This will occur within the same bacterial community on the short term and between different bacterial assemblages throughout time periods.

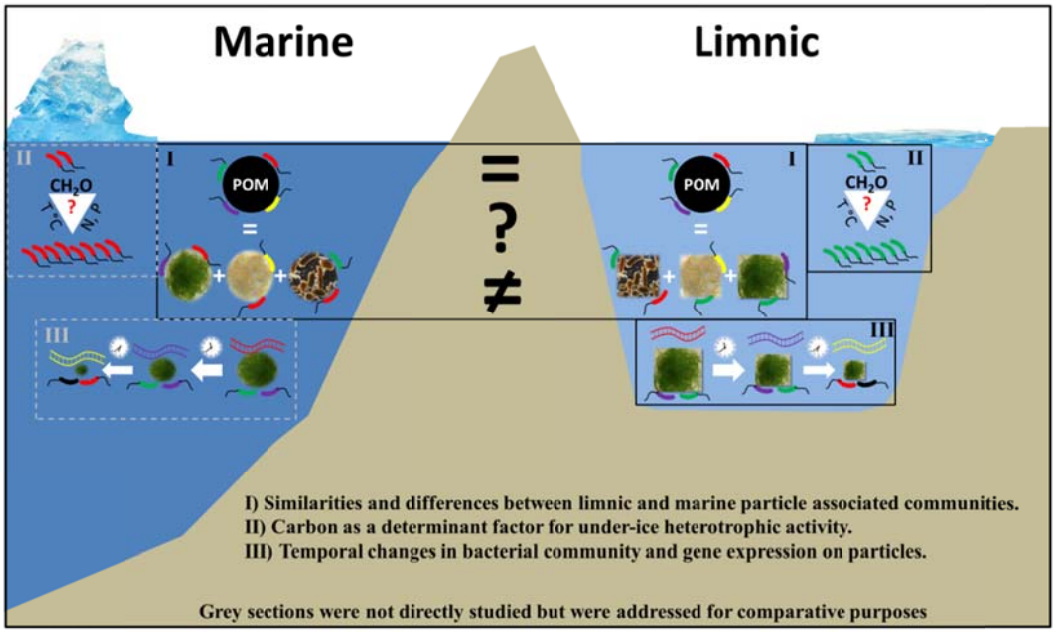

Figure 3.1 A graphic summary of the research goals of this study together with the main working hypotheses.

(All references are found at the end of the thesis starting on page 194.)

4. LIST OF MANUSCRIPTS PRESENTED IN THIS THESIS

I. Comparison of bacterial communities on limnic vs. coastal marine particles reveals profound differences in colonization

Mina Bižić-Ionescu, Michael Zeder, Danny Ionescu, Sandi Orlić, Bernhard M. Fuchs, Hans-Peter Grossart, Rudolf Amann

Published in ***Environmental Microbiology***

Author contributions given in the order of authors:
Conceived and designed the experiments: **MBI** HPG RA. Performed the experiments: **MBI**. Analyzed the data: **MBI** DI. Contributed reagents/materials/analysis tools: MZ SO HPG RA. Wrote the paper: **MBI** MZ DI SO BMF HPG RA.

II. Massive regime shifts and high activity of heterotrophic bacteria in an ice-covered lake

Mina Bižić-Ionescu, Rudolf Amann, Hans-Peter Grossart

Submitted to ***the Proceedings of the National Academy of Sciences USA (PNAS)***

Author contributions given in the order of authors:

Conceived and designed the experiments: **MBI** HPG. Performed the experiments: **MBI** HPG. Analyzed the data: **MBI** HPG. Contributed reagents/materials/analysis tools: RA HPG. Wrote the paper: **MBI** RA HPG.

III. Macroscopic organic aggregates are hotspots for interactions between colonizing microorganisms as revealed by transcriptomics analysis

Mina Bižić-Ionescu, Hans-Peter Grossart, Danny Ionescu

In preparation for ***ISME journal***

Author contributions given in the order of authors:

Conceived and designed the experiments: **MBI** HPG DI. Performed the experiments: **MBI** DI. Analyzed the data: **MBI** DI. Contributed reagents/materials/analysis tools: HPG. Wrote the paper: **MBI** HPG DI.

Manuscript I

Comparison of bacterial communities on limnic vs. coastal marine particles reveals profound differences in colonization

Mina Bižić-Ionescu, Michael Zeder, Danny Ionescu, Sandi Orlić, Bernhard M. Fuchs, Hans-Peter Grossart, Rudolf Amann

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Comparison of bacterial communities on limnic vs. coastal marine particles reveals profound differences in colonization

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Running title: Marine and limnic particle-associated bacteria

Summary:

Marine and limnic particles are hotspots of organic matter mineralization significantly affecting biogeochemical element cycling. Fluorescence-*in-situ*-hybridization and pyrosequencing of 16S rRNA genes were combined to investigate bacterial diversity and community composition on limnic and coastal marine particles >5 and >10 μm, respectively. Limnic particles were more abundant (average: 1×10^7 L^{-1}), smaller in size (average areas: 471 vs. 2,050 μm^2), and more densely colonized (average densities: 7.3 vs. 3.6 cells 100 μm^{-2}) than marine ones. Limnic particle-associated (PA) bacteria harbored *Alphaproteobacteria* and *Betaproteobacteria*, and unlike previously suggested sizeable populations of *Gammaproteobacteria*, *Actinobacteria* and *Bacteroidetes*. Marine particles were colonized by *Planctomycetes* and *Betaproteobacteria* additionally to *Alphaproteobacteria*, *Bacteroidetes* and *Gammaproteobacteria*. Large differences in individual particle colonization could be detected. High-throughput sequencing revealed a significant overlap of PA and free-living (FL) bacteria highlighting an underestimated connectivity between both fractions. PA bacteria were in 14/21 cases more diverse than FL bacteria, reflecting a high heterogeneity in the particle microenvironment. We propose that a ratio of Chao1 indices of PA/FL<1 indicates the presence of rather homogeneously colonized particles. The identification of different bacterial families enriched on either limnic or marine particles demonstrates that, despite the seemingly similar ecological niches, PA communities of both environments differ substantially.

Key words: Bacterial community composition/ Ecological niches/ FISH/ Free-living bacteria/ Limnic and marine particles/ Particle-associated bacteria/ Tag pyrosequencing

Introduction:

Organic particles are hotspots of microbial activity and represent important loci for organic matter (OM) mineralization in the water column of both limnic and marine systems. These particles with their associated microorganisms constitute a significant portion of the sinking particulate OM (Alldredge and Silver, 1988; Grossart and Simon, 1998b) and hence largely contribute to the "biological carbon pump" by transporting carbon to deep waters and sediments (Volkman and Tanoue, 2002). Although, overall free-living (FL) bacteria strongly outnumber those on particles, locally particle-associated (PA) bacteria can reach higher concentrations than FL ones (Caron et al., 1982). Moreover, PA bacteria - due to a higher substrate availability in their microhabitats - are often more active than their FL counterparts (Caron et al., 1982; Grossart et al., 2003; Fernández-Gómez et al., 2013), frequently contributing to >50% of total prokaryotic activity (Crump et al., 1998, 1999; Fandino et al., 2001; Ghiglione et al., 2007; Grossart et al., 2007; Garneau et al., 2009; Rösel and Grossart, 2012; Stocker, 2012). Consequently, PA bacteria may significantly contribute to the overall microbial productivity in both limnic and marine systems, yet they are often excluded from studies for methodological reasons (Venter et al., 2004; Yooseph et al., 2007).

Whereas differences in total microbial diversity and community composition have been found between limnic and marine habitats (Glöckner et al., 1999; Herlemann et al., 2011), no systematic comparison of PA bacteria in lakes vs. coastal seas with identical methods has been performed. There are several studies that compare PA and FL bacterial fractions at individual marine (DeLong et al., 1993; Rath et al., 1998; Acinas et al., 1999; Hollibaugh et al., 2000; Ghiglione et al., 2009; Crespo et al., 2013) and limnic sites (Allgaier and Grossart, 2006b; Rösel et al., 2012). They all indicate that the differences in bacterial diversity and community composition of both bacterial fractions are significant. Whereas DeLong et al. (1993) found by comparative 16S rRNA sequencing that marine particles are dominated by *Bacteroidetes*, *Planctomycetes* and *Gammaproteobacteria*, most of the other available studies (Rath et al., 1998; Ploug et al., 1999; Simon et al., 1999; Bidle and Azam, 2001; Rink et al., 2008) show that solely *Bacteroidetes* and *Gammaproteobacteria* are dominant on marine particles. Contrary, limnic particles seem to be dominated by *Alphaproteobacteria*, *Betaproteobacteria*, *Bacteroidetes* (Grossart and Simon, 1998a;

Brachvogel et al., 2001; Schweitzer et al., 2001; Lemarchand et al., 2006) and sometimes by *Planctomycetes* (Tang et al. 2012). Studies based on rather low numbers of particles and 16S rRNA sequences suggested a lower diversity of PA bacteria than FL bacteria (Ghiglione et al., 2007). However, when using next generation sequencing the opposite pattern was observed (Crespo et al. 2013; Ortega-Retuerta et al. 2013). The majority of earlier studies focused on marine and lake snow, rare macroaggregates with a diameter larger than 300 μm often collected by SCUBA diving (e.g. Alldredge & Silver 1988; Grossart & Simon 1993). More recently, filtration has been used to compare the diversity and genomic potential of various size classes of marine bacterioplankton (Zeigler Allen et al., 2012; Ganesh et al., 2013; Smith et al., 2013). Here, we used a similar standardized methodology for the comparative assessment of limnic and marine PA bacteria by next generation sequencing and microscopy.

For our comparison we have chosen two limnic and two coastal marine sites from which we collected multiple spatial and/or temporal samples. The limnic (Allgaier & Grossart 2006a) as well as the marine (Herndl 1988; Riebesell 1991) sites had been previously examined for particles and their associated bacteria. Lake Stechlin is a dimictic oligotrophic lake in northeastern Germany and serves as a microbial observatory site (Bertilsson et al., 2013). Its bacterial community is similar to those of many temperate lakes (Newton et al., 2011). The peat bog Lake Grosse Fuchskuhle (Germany; hereafter Lake Fuku) was artificially divided into four basins in 1990. This led to pronounced gradients in pH and humic matter concentration (Burkert et al., 2004) rendering it a good model system for a variety of temperate humic lakes of the Northern hemisphere (Newton et al., 2011). Whereas the South West (SW) basin is acidic and rich in humic substances (up to >80 mg L^{-1}), the North East (NE) basin receives a much lower humic matter input (<20 mg L^{-1}) and thus has a higher pH (Allgaier and Grossart, 2006b). The long-term ecological research station Kabeltonne represents a mesotrophic coastal marine site off the island Helgoland in the German Bight of the North Sea (Wiltshire et al., 2009). The Northern Adriatic Sea served as the second coastal marine site (Ivančić et al., 2010) where a transect was performed (<40 miles from shore). Both marine sites have a strong riverine/terrestrial influence.

Particulate and dissolved organic matter forms a size continuum (Verdugo et al., 2004). For the purpose of this study we defined particles as diverse objects larger than 5 and 10 μm (limnic and marine, respectively), which we obtained by filtration-based size fractionation. This included phytoplankton, zooplankton, dead fragments of any sort of aquatic organisms and mineral particles. We hypothesize (i) that our standardized methodology reveals that marine and limnic particles differ in their bacterial community composition; despite being seemingly similar; (ii) that high throughput tag sequencing uncovers a stronger connectivity between the PA and FL fractions than previously suggested; and (iii) that particles as heterogeneous microenvironments generally harbor a more diverse microbial community than the surrounding water. Additionally, by using high throughput microscopic analysis of individual particles we also expected to gain knowledge on the degree of inter-particle variability.

Results and Discussion:

The mean values of pH, temperature, O_2 saturation, conductivity/salinity (limnic/marine, respectively), chlorophyll a and dominant phytoplankton groups are given for the time of sampling at the different sites in Table 1. These parameters follow the trends previously published for these sites. Lake Stechlin due to photosynthesis had a higher average pH in the epilimnion (8.28) than in the hypolimnion (7.92). In the more acidic Lake Fuku, the SW basin had a lower average pH (4.66) as compared to an average pH of 5.92 in the NE basin. At the marine sites, differences between sample sites were not as pronounced as in the limnic systems. Lowest average O_2 saturation was observed in the SW basin of Lake Fuku, while it was the highest at Helgoland during 2011. Diatoms were the dominant phytoplankton group during 3 out of 4 sampling periods in the marine systems while in the limnic ones phytoplankton variability was higher.

More particles were found in limnic than in coastal marine samples, with an average of 1×10^7 (n = 9) and 7×10^5 (n = 15) particles L^{-1}, respectively (Fig 1A). This also stays true if the limnic counts are corrected to particles >10 μm (Fig. S1A). It should be noted that based on our particle definition and the methodology applied

those numbers are orders of magnitudes higher than previously estimated (1-6,000 L^{-1}; reviewed in Asper & Smith (2003)). Samples from both limnic and marine sites harbored a broad range of particle sizes (Fig. 1B), with the smaller size classes being much more abundant than the larger ones (Fig. S2). Since volumetric size measurements of the easily collapsing particles were problematic we used microscopy to determine the area covered by the particle after filtration onto polycarbonate membranes. With a mean of 491 μm^2 (median of 111 μm^2, range 18-53,636 μm^2, N=2,231), limnic particles were on average smaller than their marine counterparts which had a mean area of 2,050 μm^2 (median 529 μm^2, range 19-212,590 μm^2, N=5,057) (Fig. 1B). Again, this tendency did not change when we corrected size statistics to limnic particles >10 μm (Fig. S1B). Total bacterial abundance on both limnic and marine particles showed a positive relation with particle size (Fig. S2). Bacterial colonization density (Fig. 1C) as assessed by total cell counts (DAPI) on individual particles was significantly higher on limnic (7.3±3.3 cells 100 μm^{-2}) than on marine particles (3.6±2.1 cells 100 μm^{-2}). Whereas marine particles showed similar bacterial densities independent of particle size (Fig. 1D), areal densities increased with size on limnic particles. As some studies report particle colonization data with respect to particle volume we provide volumetric estimations of bacterial density in the supplementary information (Fig. S3).

Based on the particle abundance and colonization density, the number of PA bacteria was calculated. Limnic and marine PA bacteria were found to contribute an average of 15% and 4% of the total bacteria (Fig. 1E), respectively. The different colonization density patterns between limnic and coastal marine systems suggests that the structure of bigger particles in limnic environments is more complex than in marine environments and, therefore, offers more niches for colonization (Fig. S4). This notion is in line with previous studies showing that due to higher ionic strengths and turbulence, marine particles are usually denser than limnic particles (Logan & Kilps 1995, Chen & Eisma 1995).

Bacterial community composition: Fluorescence *in-situ* hybridization (FISH) with rRNA-targeted oligonucleotide probes revealed a high particle to particle variability. Already on the class level, large variations in colonization of particles in a single sample were observed (Fig. 2). PA communities in limnic systems (Fig. 2A) were dominated by *Alphaproteobacteria* and *Betaproteobacteria* which contributed on average to

26% (1-80%) and 17% (1-76%) of total bacteria, respectively. *Gammaproteobacteria*, *Bacteroidetes* and *Planctomycetes* accounted on average for 5%, 4% and 0.3% of the PA fraction, with maxima of 70%, 37% and 1%, respectively. *Actinobacteria* represented on average 12% of all limnic PA bacteria, but in acidic Fuku SW basin they occasionally reached up to 76% (Fig. S5). In marine systems, *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria* and *Bacteroidetes* constituted similar PA fractions with 23% (1-94%), 15% (1-72%), 18% (0-86%), and 25% (2-76%), respectively (Fig. 2B). In both, marine and limnic ecosystems some of the most abundant FL clades were not detected on particles. These included the marine SAR86, SAR92 and SAR324 clades (Table S1) as well as the marine and freshwater members of the SAR11 clade (SAR11 and LD12, respectively).

The FISH data confirm earlier findings, e.g., with respect to dominance of *Alphaproteobacteria* and *Betaproteobacteria* on limnic particles (Lemarchand et al., 2006), but we also detected significant numbers of *Actinobacteria*, *Gammaproteobacteria* and *Bacteroidetes*. In our limnic samples, we did not find elevated levels of PA *Planctomycetes* as previously reported, e.g., by Allgaier & Grossart (2006b) and Tang et al. (2012). For marine macroaggregates collected off the coast of Santa Barbara, DeLong et al. (1993) showed a dominance of *Bacteroidetes*, *Planctomycetes* or *Gammaproteobacteria*. This was subsequently corroborated for *Bacteroidetes* and *Gammaproteobacteria* by a study on bacterial colonization of coastal marine particles (Rink et al., 2008). Marine particles were colonized mainly by *Alphaproteobacteria*, *Bacteroidetes* and *Gammaproteobacteria*, but also *Planctomycetes* and *Betaproteobacteria* were detected. The latter, consisted mostly of the OM43 clade (Table S1). In summary, despite a high particle to particle variability our detailed FISH analyses revealed consistent differences in the colonization of marine and limnic particles.

Bacterial diversity: Tag pyrosequencing of the selected 16 limnic and 30 coastal marine samples yielded ~330,000 bacterial sequences (Table S2). For subsequent analysis, data from different samples were pooled together into sample-groups (Table S3). These are "all limnic", "all marine", "PA limnic", "PA marine", "FL limnic" and "FL marine". Overall, as well as in the individual samples, based on the Chao 1 index the diversity of marine bacteria was higher than that of limnic bacteria (57,383 vs. 11,728). In pooled PA fractions Chao 1 indices were higher than in FL in both limnic (6,433 vs. 4,225) and marine (32,180 vs. 21,175) environments (Table 2 and S2). In

14 of 21 PA/FL pairs the OTU richness was higher in PA. Shannon evenness, as calculated on bacterial families, was similar between PA and FL fractions in limnic (0.58 and 0.56, respectively) and marine systems (0.72 and 0.69, respectively; Table 2 and S2), yet significantly lower in all limnic than in marine systems ($P<0.0001$).

As hypothesized, the diversity detected on particles was in 14/21 cases higher than in the surrounding FL community. Earlier studies have suggested a lower diversity in PA as compared to FL (Ghiglione et al., 2007). These studies assume that particles upon their formation recruit a sub-fraction of the bacterial community present in the surrounding waters. However, our data matched recent findings by Crespo et al. (2013) and Ortega-Retuerta et al. (2013) who used comparable high-throughput sequencing methods. One explanation for this would be a high heterogeneity within and among particles which is partly due to the association of different bacteria with different phytoplankton groups (Fisher et al., 1998; Behrens et al., 2008; Gärdes et al., 2011; Amin et al., 2012). In 7/21 cases PA diversity was lower than FL diversity. In all of these cases this coincided with rather homogeneous phytoplankton blooms (for a detailed discussion see SI). Diatoms, known to select for a rather limited set of bacteria (Amin et al. 2012; Teeling et al. 2012), dominated in four of these cases. We would like to suggest that the PA/FL Chao 1 diversity index ratio of 16S rRNA genes could represent a robust and practical indicator for particle homogeneity. In our data set PA/FL ratios <1 are restricted to samples characterized by recent phytoplankton blooms. In contrast, a PA/FL ratio >1 indicates the presence of diversely colonized particles of different age, origin and composition as supported by our microscopy analyses. The PA Chao 1 diversity index alone, however, is not suitable for this purpose, since for methodological reasons carry-over of FL bacteria into the PA fraction cannot be excluded.

We further investigated whether the PA bacterial communities in samples with a Chao 1 PA/FL<1 differed from those on particles with a Chao 1 PA/FL>1 (Table S4A, S4B). Many genera were shared, yet in some cases unique genera could be identified (Table S4A). We also checked for frequency changes in shared genera (Table S4B). In the limnic system, 16S rRNA sequences of the genera *Flavobacterium*, *Caulobacter, Albidiferax* and *Stenotrophomonas* were more frequently found in the PA/FL <1 samples, while in the marine PA/FL<1 samples the genera *Formosa*, *Paracoccus*, *Roseovarius* and *Haliea* were overrepresented. Genera that were enriched on heterogeneous particles included *Pirellula* and *Fluviicola* in the limnic and marine

systems, respectively (Table S4B). Members of the genus *Formosa* have recently been shown to be early colonizers of a decaying diatom bloom (Teeling et al., 2012).

NMDS analysis of the 16S rRNA gene sequence frequency data clustered on the family level shows a clear separation between limnic vs. coastal marine samples (Fig. 3). Interestingly, contrary to our expectations, differences between the two marine environments are as large as between marine and limnic systems, while the latter are clustered close to each other. The high similarity of the sampled limnic sites could be a result of their relative geographical proximity as compared to the two marine sites. However, bacterial communities of the sampled limnic systems represent the majority of dominant bacterial phylotypes found in a global meta-analysis of limnic systems (Newton et al., 2011). Within each system, individual PA samples are separated from their FL counterpart (Fig. S6). ANOSIM on the same data shows that there is a significant difference between PA and FL communities in limnic, but not in the marine environments. Nevertheless, in each of the 21 PA/FL pairs the separation is significant (Table S5). Thus, at any given time point, the PA and FL communities differ from each other.

A similarity percentage (SIMPER) analysis (Clarke, 1993) revealed that *Sporichthyaceae* are contributing the most of all taxa (13%) to the difference between limnic and marine system (Table S6). Overall species contributing over 1% each (n=23) to the difference between the two systems, add up to only <65% of the difference, while the rest is explained by the remaining >500 identified taxa (families). When comparing the limnic and marine PA fractions, *Candidatus* Alysiosphaera (*Rhodospirillales*) had the largest contribution to the difference between the two fractions, but that was only 4% (Table S6). The lack of a single species that differs between the limnic and marine PA fractions could be due to the overall low similarity between the two communities (Fig 3, Table S5).

Tag pyrosequencing revealed that the dominant groups analyzed by FISH accounted for 66-83% of all obtained bacterial 16S rRNA sequences when chloroplast sequences were excluded. The majority of the 16S rRNA gene sequences obtained in the PA limnic fraction was affiliated with *Alphaproteobacteria* (28%), *Betaproteobacteria* (18%) and *Cyanobacteria* (15%) (Fig. 4). *Gammaproteobacteria*, *Bacteroidetes*, *Planctomycetes*, *Actinobacteria* and *Verrucomicrobia* contributed 3%, 9%, 4%, 5% and 4%, respectively, to all amplicons. The PA marine fraction was dominated by

Alphaproteobacteria (32%), yet, in contrast to limnic particles, *Betaproteobacteria* accounted for <1% of amplicons obtained from marine particles. Amplicons affiliated with *Gammaproteobacteria*, *Bacteroidetes* and *Planctomycetes* were with 14%, 16% and 13%, respectively, more frequent on marine than on limnic particles. *Actinobacteria* and *Verrucomicrobia* accounted on average each for 3% of the marine PA amplicons. Cyanobacterial sequences were frequently retrieved from marine particles (11%). These results corresponded well with our FISH data for most of the clades (Fig. 2). There were, however, a few exceptions, e.g. FISH counts of on average about 10% *Betaproteobacteria* (mostly OM43) and 13% *Actinobacteria* on marine and limnic particles, respectively, that were not reflected by the <1% betaproteobacterial and actinobacterial sequences in the respective PA fraction. Factors contributing to these ambiguities could be differences in rRNA operon number per genome which is only 1 in OM43 (Giovannoni et al., 2008) and primer selectivity (Klindworth et al., 2013) in the case of *Actinobacteria.*

Based on tag sequences, the difference on the phylum/class level between PA and FL was more pronounced in limnic than in coastal marine systems. This further supports our previous suggestion that the exchange between the FL and PA fractions is higher in marine systems. In limnic systems, there was a clear enrichment of *Alphaproteobacteria* on particles and a strong depletion of *Actinobacteria* in most of the samples. The marine PA fraction was enriched in *Gammaproteobacteria*, *Bacteroidetes*, *Planctomycetes* and *Verrucomicrobia* and depleted in *Alphaproteobacteria* (tag sequences). Overall, this pattern was also maintained when comparing the different sub-systems with each other, however, not in some specific cases (Fig. S7).

Enrichment and depletion of bacterial groups in the PA fraction was further evaluated at a higher phylogenetic resolution by analyzing the dominant families. The latter were defined as bacterial families with a sequence frequency of >5% in at least one sample. Enrichment or depletion of the respective families on particles were considered only if a factor of 2 or higher was detected in one or more samples. Overall, we identified 29 dominant groups, 26 of them could be taxonomically resolved to the family level (Table 3). On limnic particles, 8 groups of heterotrophic bacteria (*Paenibacillaceae*, *Caulobacteraceae*, *Xanthobacteraceae*, alpha1 cluster (*Rhizobiales*), *Rhodobacteraceae*, *Sphinogomonadaceae*, *Alcaligenaceae* and *Oxalobacteraceae)* were enriched, but on marine particles there were only 3 groups (*Planctomycetaceae*, *Parvularculaceae* and

Crenotrichaceae). Additional groups were found enriched in the different environments within the studied limnic and marine systems (Table 3). Except for *Flavobacteraceae* and *Planctomycetaceae*, which were found to be enriched on particles in several limnic and marine environments, no overlap was observed between enriched groups of limnic and coastal marine samples.

Depletion in the PA fraction was evident for four limnic clades, *Sporichthyaceae* including AcI, the freshwater SAR11 clade LD12, *Burkholderiaceae* and *Opitutae* vadinHA64, and three marine SAR 11 clades (Table 3). The latter, nevertheless, made up a major part of marine PA sequences. Interestingly, 10-30% of the PA SAR11 OTUs (belonging to Surface clades I and IV) could not be found in the FL fractions. We consider this as an indication that particles are not only important as hotspots of OM mineralization, but also represent important refuges for FL bacteria. One possible scenario is that there was a rapid succession of SAR11 clades due to phage infection (Zhao et al., 2013) and that what we detected as "PA clades" indeed reflected SAR11 clades that had been removed by virus-induced mortality from the FL fraction.

To gain further insights in the classes and phyla of similar abundance in both marine and limnic systems (Fig. 4) an analysis at the family level was conducted. We focused on families with a frequency of >5% within their respective class or phylum (Fig. 5 and S8; the full phylogenetic data are given in Table S7). With increasing taxonomic resolution, limnic and marine PA clades (Fig. 5) were increasingly separated from FL clades (Fig. S8). Sequences affiliating with those of the families *Caulobacteraceae* (Class *Alphaproteobacteria*), *Alcaligenaceae* (Class *Betaproteobacteria*) and *Chitinophagaceae* (Phylum *Bacteroidetes*) were among the most frequent on lake particles, whereas sequences of *Rhodobacteraceae* (Class *Alphaproteobacteria*), *Alteromonadaceae* (Class *Gammaproteobacteria*) and *Flavobacteriaceae* (Phylum *Bacteroidetes*) were dominating on coastal marine particles. A detailed discussion regarding PA and FL families in each phyla/class is given in SI. With the exception of *Bacteroidetes* and *Planctomycetes*, we identified groups that are uniquely PA within each phyla or class (Fig. S8). Since we cannot exclude carry-over of particularly abundant FL clades to the PA fraction collected by fractionated filtration, we have additionally analyzed amplicon frequencies in the FL fraction. This allows us to consider enrichment and depletion in the PA fraction compared to the FL fraction. A common result of our

comparative 16S rRNA sequence analyses is a strong separation of limnic and marine PA clades. Despite a high inter-particle heterogeneity in both composition and size, we were able to detect consistent differences between freshwater and marine samples, although we specifically examined coastal sites with a high terrestrial input.

Limnic and marine systems shared in our dataset more than 50% of all validly described families identified in the whole data set (184/338 families; Fig. 6). The same applied to the PA fractions where 172 of 330 families were both found on limnic and marine particles. However, when the data analysis was refined to the OTU level (at 98% similarity), only 68 of 35,554 OTUs are shared between all limnic and marine sequences, and 41 of a total of 24,460 OTUs between the PA fractions of limnic and marine sequences (Fig. 6). The separation between PA and FL is generally much less pronounced. This supports previous findings by Crespo et al. (2013). On the OTU level within the same system 1,200 of 6,385 in limnic and 4,026 of 28,877 in marine are shared between the PA and FL fractions (Fig. 6). This suggests that a significant number of taxa might hop on and off particles (Ghiglione et al., 2007; Grossart, 2010). Given the added coverage by tag pyrosequencing it is expected to detect bacteria common to both fractions as well as those unique to each. Within both the limnic and marine systems, the PA fraction contains more unique genera and OTUs than the FL one (Fig. 6) as was previously shown by the Chao 1 index. This further supports the presence of bacteria already on the particle forming units prior to their aggregation and further bacterial recruitment from the FL fraction.

Out of the 41 OTUs shared between the limnic and marine PA fractions 19 are of known and potential pathogens such as *Staphylococcus aureus*, *S. epidermidis*, *S. pasteuri*, *Brevundimonas diminuta*, *Bosea massiliensis* and *Vibrio* sp. We cannot rule out that these sequences are anthropogenic contaminations which occurred during sampling or sample preparation. Yet, it has been described before that important pathogens like *Vibrio cholera* survive and spread on particles (Colwell et al., 2003; Danovaro et al., 2009).

In summary, we observed three different life styles: (i) Mostly FL clades like the marine SAR11, SAR86, and the limnic *Actinobacteria* of the clade Ac1. LD12, the limnic lineage of SAR11 is in parallel to its marine sister group also strongly depleted on particles. (ii) Clades that are preferentially found attached like the *Caulobacteraceae* which were enriched on all limnic particles examined in this study (Table 3) or

Planctomycetaceae for which we confirm earlier reports on their high abundance on marine particles (DeLong et al., 1993; Pizzetti et al., 2011). (iii) Clades found in both FL and PA fractions such as the *Comamonadaceae* and the *Flavobacteriaceae*. The latter had an inconsistent pattern (enriched in 3 of 8 subsystems investigated and depleted in a single subsystem). Such cases are not surprising considering that bacterial clades specialized for life on particles must also have a FL phase for dispersing between particles. Members of the genera *Caulobacter* and *Planctomyces* achieve this by formation of swarmer cells.

Conclusions:

Returning to our hypotheses we conclude that the separation of marine and limnic PA bacteria is very pronounced, although the investigated limnic and coastal marine sites were selected to provide a certain degree of connectivity, in the sense that the two coastal marine sites received constant input from rivers, and the lakes potentially obtained marine input by birds or air. Based on the low number of OTUs shared between limnic and marine systems both in FL and PA communities, we argue that similar ecological niches are filled by different bacterial species that do not easily cross the salinity barrier. From an evolutionary point of view, it is interesting that at the same time many genera and families are shared. These larger clades have likely evolved "genomic blue prints" that encode a preference for life on particles. *Flavobacteriaceae* represent a family in which the salinity barrier has obviously been crossed several times. Furthermore, our high-throughput data highlight a previously neglected connectivity between PA and FL habitats. This is evident from the high percentage of shared taxa between the FL and PA fractions in both the limnic and marine environments indicating that a significant portion of the microbial community alternates between a planktonic and a particle-associated lifestyle. The triggers for this remain to be investigated and could include refuge from phages, nutrients availability and competition.

Our single particle analyses provide evidence for a large heterogeneity in bacterial colonization within a single sample. This calls for targeted studies on defined particle classes which could be based on particles sorted on traits such as size, fluorescence as a means to differentiate pigmentation of phytoplankton clades or

even the biochemical composition of particles (e.g., by a-lectin-based staining of polysaccharides).

Experimental procedures:

Environmental parameters and site description: Main chemical and physical parameters, at the time of sampling (Table 1), were collected in the context of long-term monitoring programs at the Leibniz Institute for Freshwater Ecology and Inland Fisheries (Stechlin), the Helgoland Biological Station of AWI and the Rudjer Boskovic institute (Rovinj). Detailed physicochemical and biological data of these systems have been previously published (Allgaier and Grossart, 2006b; Wiltshire et al., 2009; Giani et al., 2012).

Sampling: Samples were collected monthly in 2009 from the epilimnion and hypolimnion of Lake Stechlin, the surface waters of the SW and NE basins of Lake Fuku monthly in 2009, the surface waters of Helgoland Kabeltonne in autumn 2009 and spring 2011, and from above and below the thermocline along the Po River Delta-Rovinj transect in July 2011 (Table S8). Samples were collected in parallel for DNA extraction and microscopy (for details see supplementary information (SI)). To achieve a reproducible operational differentiation between the particle-associated (PA) and free-living (FL) bacteria we used fractionated filtration with a cut-off of 5 and 10 μm as commonly used for limnic and marine particles, respectively (Pizzetti et al., 2011; Rösel et al., 2012). Thus, the limnic PA fractions were collected on a polycarbonate membrane (47 mm in diameter, Sartorius, Germany) and separated from FL fractions (5 μm filtrate collected on 0.2 μm polycarbonate membranes), whereas the marine PA fraction refers to particles >10 μm and the FL to the size fraction 0.2-3 μm. Overall, we have generated 126 different samples, 122 of which were used for microscopic analysis and 46 for tag sequencing (Table S8 and S3).

Catalyzed Reporter Deposition Fluorescence *in-Situ* Hybridization (CARD-FISH) and automated image analysis: CARD-FISH was done according to Pernthaler et al. (2004) with the following modifications: hybridization was performed at 46°C for 2.5 h and the washing step at 48°C for 5 min (SI). All probes used in this study are given in Table S9. Quantification of FL bacteria and relative abundance of distinct phylogenetic clades was performed in a fully automated

manner using a multi-purpose imaging system (MPISYS, modified after (Zeder, 2009) based on a motorized epifluorescence microscope (AxioImager.Z2m, Zeiss, Germany)). Eight samples (i.e. stained membrane filter pieces) were processed per run with an average speed of 4.7 positions per minute, including nested autofocusing, z-stack acquisition and extended depth of field imaging (Zeder and Pernthaler, 2009; Zeder et al., 2011) on each position. Image acquisition of 2,882 limnic and marine microbial particles (1,405 and 1,477, respectively) was performed on the same microscope, but running the MPISYS in a semi-automated mode – as fields of view had to be selected manually (as detailed in SI). Upon selection, the system acquired a z-stack of 25 images around the focused position with an interval of 0.3 μm for each channel. The original stack was stored for deconvolution and 3D reconstruction. For 2D cell quantification, a maximum intensity projection was calculated on each channel. For each sample, at least 10 particles were imaged. A total number of 2,882 particles were imaged with a rate of approximately 10 per hour. Image data are available on request. Particle abundance data was obtained from 24 representative samples (subsequently chosen for sequencing) using the MPISYS. Particles larger than 5 μm or 10 μm in diameter (limnic and marine, respectively) were counted from whole filter sections. Particle abundance per L was than extrapolated based on filter area and filtered sample volume. Control of image quality prior to cell counting is of crucial importance (Zeder et al., 2010). To ensure highest quality of this dataset, each image was manually checked and excluded from analysis if it contained artifacts or was out of focus.

Cell counting of both, total cell numbers and hybridized cells, was done using the ACMEtool2 software (Software and description are available at www.technobiology.ch). As particles exhibit a high degree of morphological variation, their outlines had to be defined manually by drawing a polygon (region of interest) along the particle's periphery as seen in the DAPI image. Subsequent calculations, including automated cell counting and particle area calculation, were confined to the defined region of interest.

DNA extraction and pyrosequencing: DNA was extracted from size-fractioned samples equivalent to those used for FISH analysis, as described previously (Ionescu et al., 2012) and detailed in SI. DNA extracts from a total of 46 limnic and marine (16 and 30, respectively; Table S3) samples were analyzed by tag pyrosequencing for

bacterial diversity using primer sets 28F and 519R (Lane, 1991). Pyrosequencing was done by MrDNA (Shallowater, Texas), using a Roche 454 FLX Genome Sequencer system as detailed in SI.

Sequence Analysis: Sequences were analyzed as previously described in (Ionescu et al., 2012), using the bioinformatics pipeline of the SILVA rRNA gene database project and the SILVA SSURef dataset (release 111) (Quast et al., 2013). Diversity and community structure analyses were performed on >330,000 bacterial sequences. Detailed sequence statistics including the total number of reads and length distribution, as well as the results of quality management, dereplication, and clustering, are given in Table S2. A detailed list of the final taxonomic affiliation of all analyzed sequences together with their relative abundances within the amplicon pool is provided in Table S7 (Bacteria). The sequences were submitted to the SRA. Accession numbers have not been obtained yet and will be added to the final version.

Statistical analyses: Non Metric Multidimensional Scaling (NMDS) analysis has been done for pyrosequencing data of all sampling sites. Data were clustered based on phylogeny at the family level. Distances were calculated using the Bray-Curtis algorithm. The clustering was tested by ANalysis of SIMilarity (ANOSIM) using the same clustering algorithm. Taxa that are significant to the difference between the different sample groups were detected by the SIMPER (Similarity Percentage; (Clarke, 1993)) algorithm. NMDS, ANOSIM, SIMPER and correlation analyses as well as T-tests and Mann-Whitney rank sum test were done using the respective algorithms as implemented in the PAST software (Harper and Ryan, 2001). Chao 1 diversity index was calculated based on a formula that accounts for sample size bias (Chao, 2005).

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Table 1. Environmental parameters of the sampling sites at the time of sampling. Sample name abbreviations: EL and HL - Epilimnion and Hypolimnion of Lake Stechlin, NE and SW - North-Eastern and South-Western basins of Lake Grosse Fuchskuhle (Fuku), '09 and '11 - Samples collected in 2009 and 2011 on Helgoland, AT and BT - samples collected above and below the thermocline along the Rovinj-Po transect in the Northern Adriatic Sea.

	Location	pH	Temperature [°C]	O_2 Saturation [%]	Conductivity / Salinity* [µS/cm] / [PSU]	Chlorophyll a [µg/L]	Dominant Phytoplankton
Limnic	**Stechlin EL**	8.28±0.23	8.9±3.8	95.6±10.9	297.1 ± 2.3	3.5±2.2	Jan-Jun: **Diatoms**; Jul-Dec: **Cyanobacteria**
	Stechlin HL	7.92±0.23	4.0±0.4	74.6±12.5	299.1±2.3	n/a	n/a
	Fuku NE	5.92±0.28	11.2±6.2	64.5±31.0	30.5±3.4	20.3±20.1	Jan-Jul & Nov-Dec: **Dinoflagellates**; Aug-Nov: **Raphidophytes**
	Fuku SW	4.66±0.11	9.7±5.2	31.9±25	46.0±1.6	12.4±10.1	Jan-Dec: **Chrysophytes**; Jun-Dec: **Cryptomonads**
Marine	**N. Adriatic AT**	8.28±0.04	20.5±3.4	91.7±8.4	37.1±0.6*	0.2±0.1	Jul: **Diatoms**
	N. Adriatic BT	8.15±0.04	11.6±0.4	53.6±2.8	37.6±0.1*	0.5±0.4	Jul: **Diatoms**
	Helgoland '09	n/a	13.9±2.5	n/a	32.7±0.5*	2.9±2.4	Sep-Dec: **Dinoflagellates**
	Helgoland '11	8.45±0.1	9.1±0.8	103.9±4.8	30.8±1.1*	5.3±4.4	April-Jun: **Diatoms**

Table 2. Chao 1, Shannon (H) and Shannon Evenness (E_H) diversity indices for sample-groups. The full list of samples, their respective sequence quality data and diversity indices are given in Table S2. Sample name abbreviations: EL and HL - Epilimnion and Hypolimnion of Lake Stechlin, NE and SW - North-Eastern and South-Western basins of Lake Grosse Fuchskuhle (Fuku), 09 and 11 - Samples collected in 2009 and 2011 on Helgoland (date given in ddmm format), AT and BT - samples collected above and below the thermocline along the Rovinj-Po transect in the Northern Adriatic Sea at the specified stations. PA and FL refer to particle-associated and free-living, respectively.

	Sample name	Chao 1	H	E_H
	Limnic PA	**6433**	**6.74**	**0.58**
	Limnic FL	4225	6.37	0.56
	Marine PA	**32 180**	**8.86**	**0.72**
	Marine FL	21 175	8.31	0.69
Limnic	**Stechlin EL-PA**	**1881**	**5.41**	**0.53**
	Stechlin EL-FL	1456	5.21	0.52
	Stechlin HL-PA	**2476**	**6.33**	**0.62**
	Stechlin HL-FL	1466	5.72	0.56
	FuKu NE-PA	**1538**	**5.07**	**0.48**
	FuKu NE-FL	1332	4.81	0.46
	FuKu SW-PA	**1296**	**5.32**	**0.53**
	FuKu SW-FL	1700	5.36	0.53
Marine	**Helgoland '09-PA**	**11 693**	**7.72**	**0.70**
	Helgoland '09-FL	2522	6.24	0.65
	Helgoland '11-PA	**6421**	**7.36**	**0.72**
	Helgoland '11-FL	5861	6.89	0.65
	Adriatic AT-PA	**10 123**	**7.83**	**0.69**
	Adriatic AT-FL	5819	7.24	0.68
	Adriatic BT-PA	**10 989**	**7.90**	**0.70**
	Adriatic BT-FL	9242	7.56	0.67

Table 3. Dominant families and respective PA/FL enrichment factors

Limnic					Marine							
Lim	EL	HL	NE	SW	Mar	09	11	AT	BT	Phylum	Class	Order	Family
						7		2		Actinobacteria	Acidimicrobiia	Acidimicrobiales	OCS155 marine group
13	38	12	20	9							Actinobacteria	Frankiales	Sporichthyaceae
	5	2	6						3	Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae
				3							Sphingobacteriia	Sphingobacteriales	Chitinophagaceae
2	3	3								Cyanobacteria	Cyanobacteria	SubsectionI	Family I
568	700	708										SubsectionIV	Family I
477				88						Firmicutes	Bacilli	Bacillales	Paenibacillaceae
								12	3	Planctomycetes	OM190		
	31										Phycisphaerae	Phycisphaerales	Phycisphaeraceae
		3			3	5		3	2		Planctomycetacia	Planctomycetales	Planctomycetaceae
11	41	3	49	7						Proteobacteria	Alpha-proteobacteria	Caulobacterales	Caulobacteraceae
					12			17	5			Parvularculales	Parvularculaceae
386				55								Rhizobiales	Xanthobacteraceae
11	180		15	2								Rhizobiales	alphaI cluster
6	21	4										Rhodobacterales	Rhodobacteraceae
					6	10		18	4			SAR11 clade	LD12 freshwater group
17	10	23	38										
					3	2	16	7	3				Surface 1
					3			13					Surface 2
5		8	4									Sphingomonadales	Sphingomonadaceae
17			37								Beta-proteobacteria	Burkholderiales	Alcaligenaceae
2		19		5									Burkholderiaceae
	2												Comamonadaceae
11			2	9									Oxalobacteraceae
							5				Gamma-proteobacteria	Alteromonadales	Alteromonadaceae
					4	103	1000	6				Methylococcales	Crenotrichaceae
						2	13	3				Oceanospirillales	SAR86 clade
						4	3	4		Verrucomicrobia	Opitutae	Puniceicoccales	Puniceicoccaceae
4	3	4										vadinHA64	

The sample-groups: Lim, EL, HL, NE, SW, Mar, 09, 11, AT and BT, include all samples in the respective environment as was defined in Table S3; EL and HL - Epilimnion and Hypolimnion of Lake Stechlin, NE and SW - North-Eastern and South-Western basins of Lake Grosse Fuchskuhle, 09 and 11 - Samples collected in 2009 and 2011 on Helgoland, AT and BT - samples collected above and below the thermocline along the Rovinj-Po transect in the Northern Adriatic Sea. Families were considered dominant if they appeared in at least one sample-group in over 5% of total sequences (excluding Chloroplast). Enrichment factors were calculated as ratios between sequence frequencies of the family in the PA and the FL fractions of a sample-group. Families that were found enriched or depleted on particles with a factor of 2 or more in at least one sample are marked with red or blue respectively. The *Flavobacteriaceae* (yellow) have shown both trends. Numbers represent enrichment or depletion factors.

Figure Legends:

Fig. 1 Abundance (A), size distribution (B) and bacterial colonization density (C) of particles over 5 μm and 10 μm in diameter in all limnic and all marine samples, respectively. Bacterial cell density was calculated for different size groups using area increments of 500 μm^2 (D) and was used to estimate the total number of particle-associated bacteria in limnic and marine samples (E). The size range plotted in panel (B) covers >95% and 100% of the total and FISH analyzed particles, respectively. Significance of the differences between limnic and marine samples was proven by the Mann-Whitney rank sum test. Boxes show 50% quartiles, whiskers 10 and 90 percentiles, and dots 5 and 95 percentiles. Full central line shows median and dashed line shows average.

Fig. 2 Distribution of relative abundances of major phylogenetic groups as assessed by CARD-FISH in (A) summed limnic and (B) marine systems (PA) particle-associated and (FL) free-living bacteria. Boxes show 50% quartiles, whiskers 10 and 90 percentiles, and dots 5 and 95 percentiles. Full central line shows median and dashed line shows average.

Fig. 3 Non Metric Multidimensional Scaling analysis of 454 data for Lakes Stechlin and Grosse Fuchskuhle, Helgoland and Northern Adriatic Sea. Data were clustered based on phylogeny at the family level. Distances were calculated with the PAST software using the Bray-Curtis algorithm (Stress level was 0.13). ANalysis Of SIMilarity (ANOSIM) results for the data are given in Table S5. For identification of individual samples see Fig. S6.

Fig. 4 Sequence frequencies of major phylogenetic groups in free-living (FL) and particle-associated (PA) bacterial communities from summed limnic and marine systems. Data for subsystems are provided in Fig. S7.

Fig. 5 Sequence frequencies of major particle-associated bacterial taxa at the family level within the different groups, normalized to the total number of sequences in each group. To simplify the image, only families making up over 5% of the sequences of the respective phyla/class in at least one sample are shown. Families listed in the legend. Family names marked with green or blue were found exclusively in either limnic or marine samples, respectively.

Fig. 6 Families and Operational Taxonomic Units (OTU) shared between limnic and marine samples (green); from top to bottom all particle-associated (PA) and free-living (FL) fractions, as well as between PA and FL fractions within each system. Clusters for which taxonomy at the family level could not be assigned are not accounted for; however, their identification would either not change or increase the number of shared families. OTUs were clustered at 98% sequence similarity.

Figure 1.

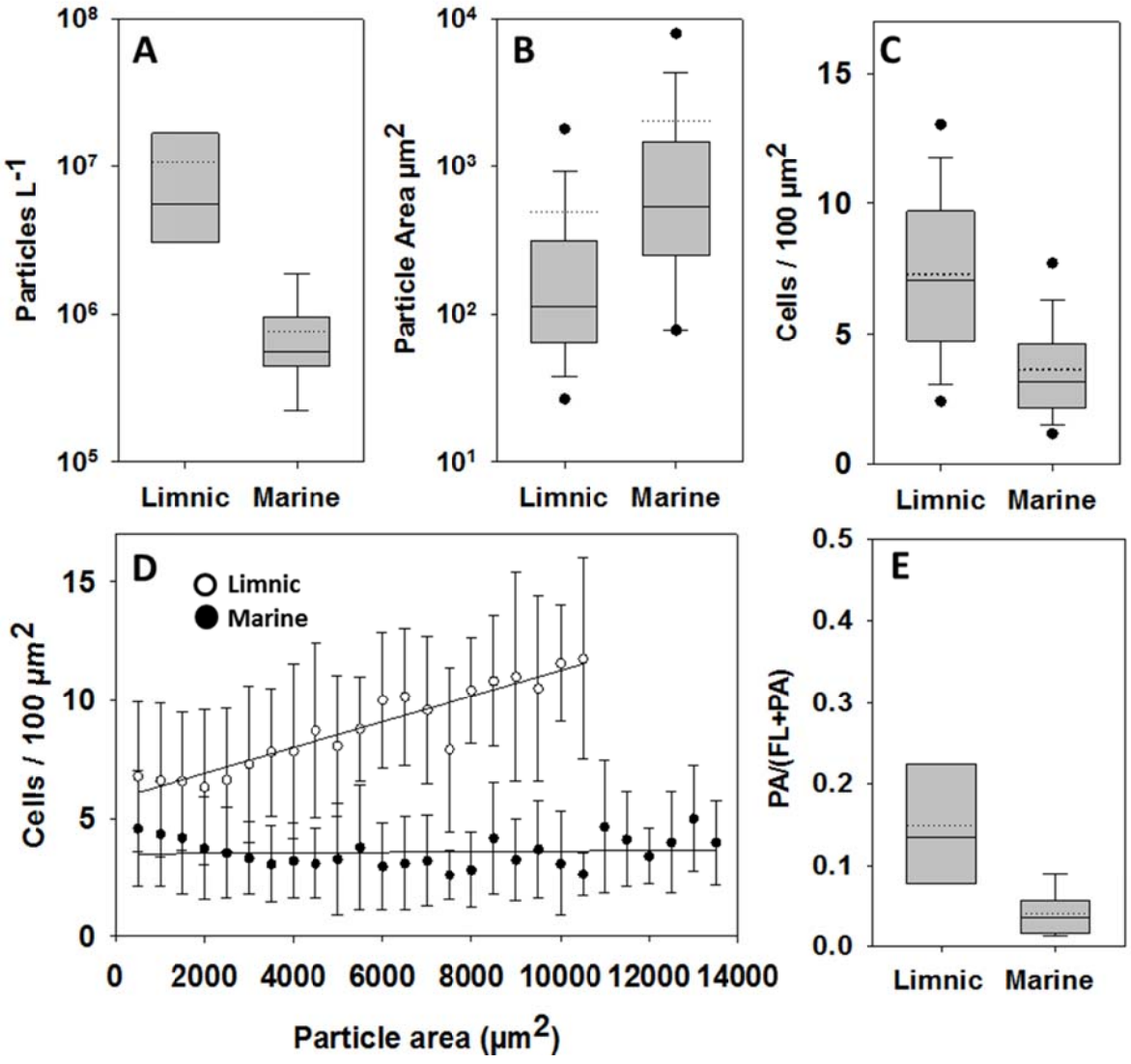


Figure 2.

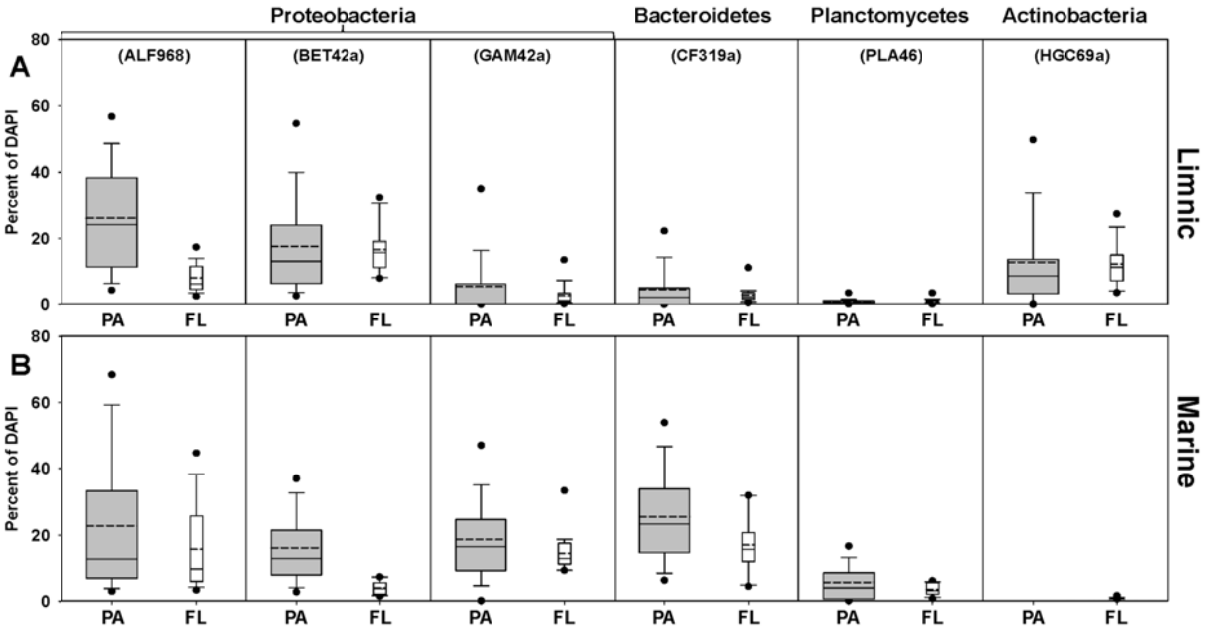


Figure 3.

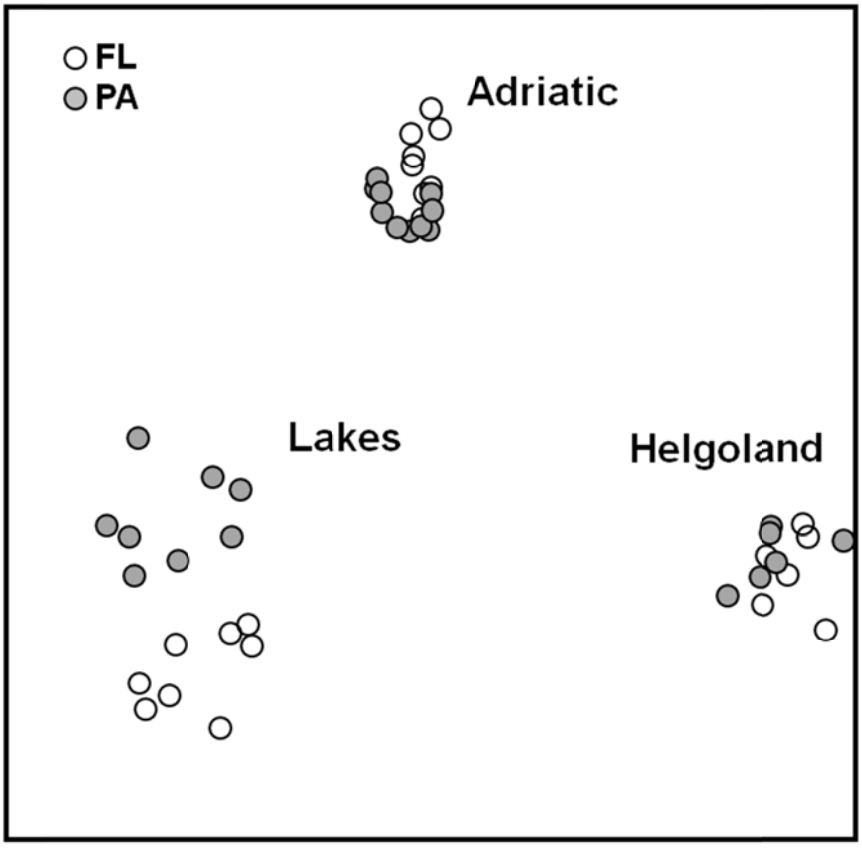


Figure 4.

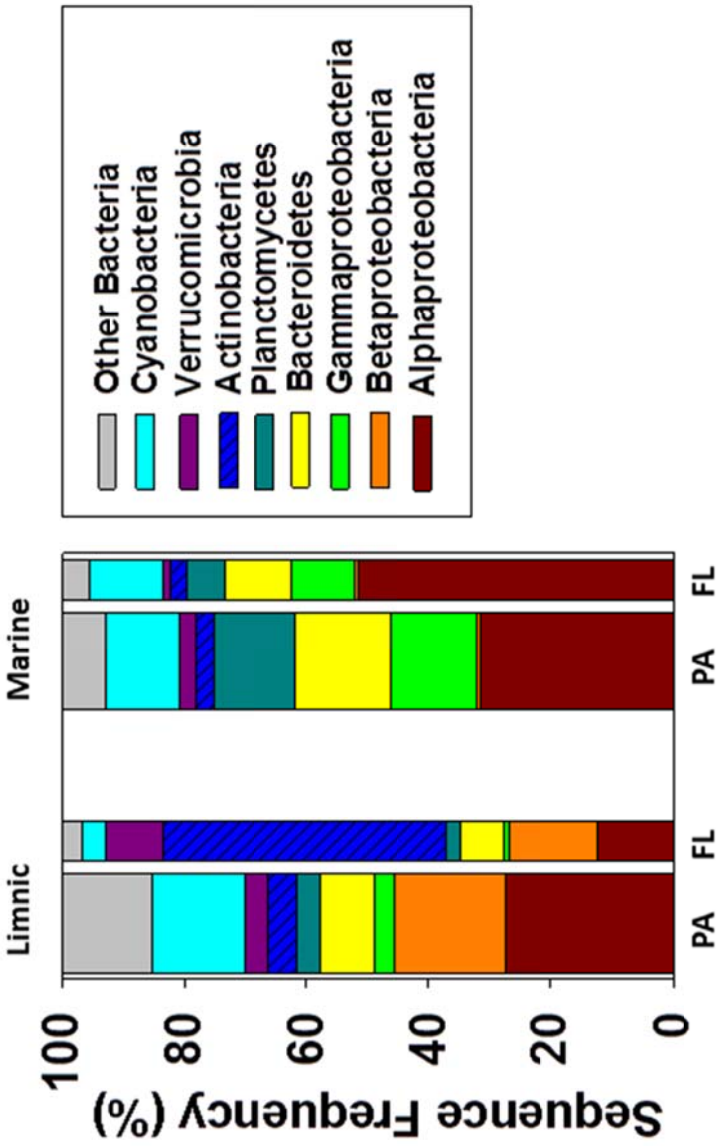

Other Bacteria
Cyanobacteria
Verrucomicrobia
Actinobacteria
Planctomycetes
Bacteroidetes
Gammaproteobacteria
Betaproteobacteria
Alphaproteobacteria
Limnic
Marine
PA
FL
PA
FL
Sequence Frequency (%)
0
20
40
60
80
100

Figure 5.

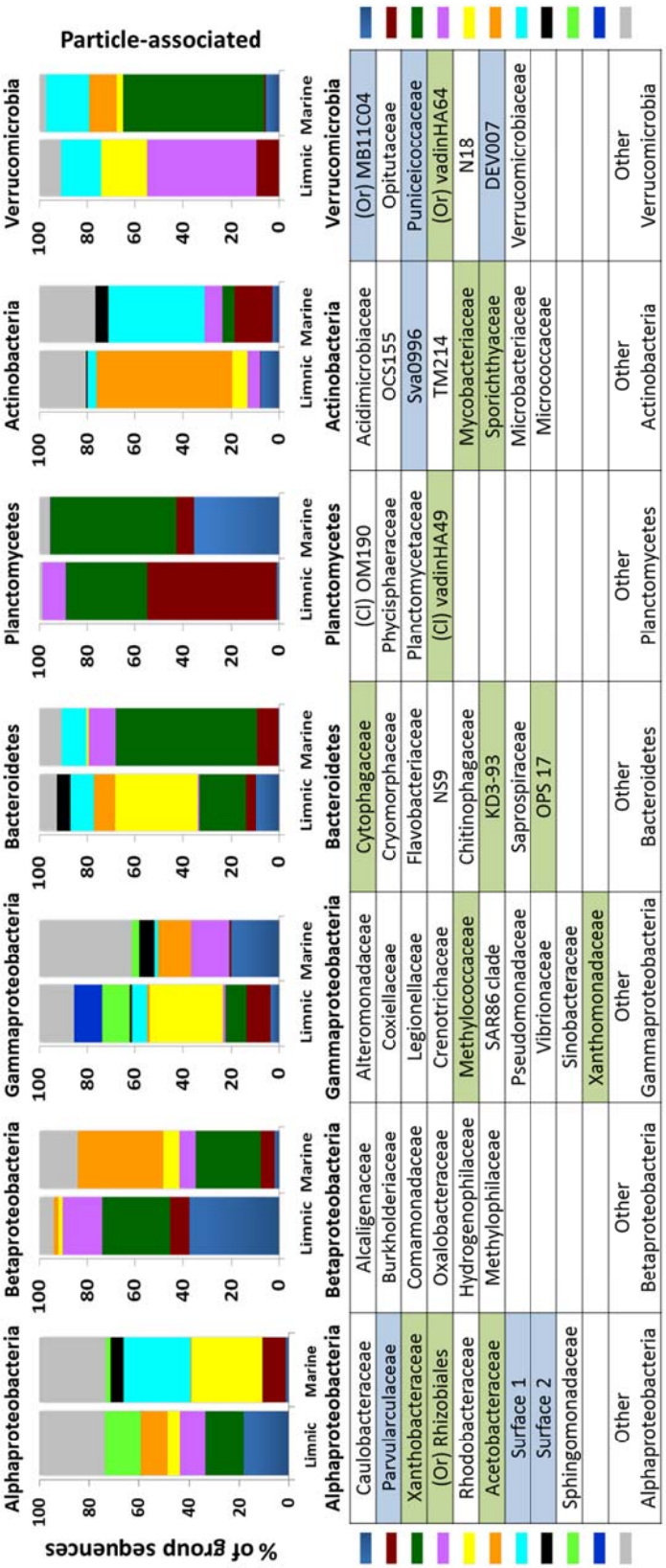
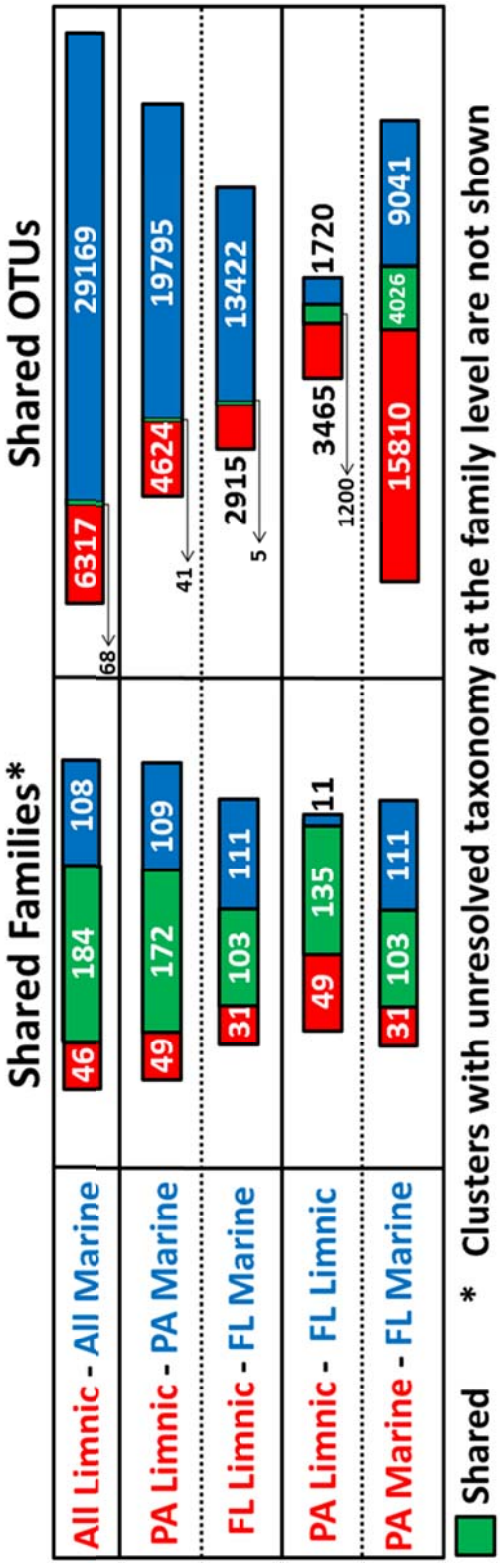

Particle-associated
% of group sequences
Limnic
Marine
Alphaproteobacteria
Betaproteobacteria
Gammaproteobacteria
Bacteroidetes
Planctomycetes
Actinobacteria
Verrucomicrobia
Caulobacteraceae
Parvularculaceae
Xanthobacteraceae
(Or) Rhizobiales
Rhodobacteraceae
Acetobacteraceae
Surface 1
Surface 2
Sphingomonadaceae
Other Alphaproteobacteria
Alcaligenaceae
Burkholderiaceae
Comamonadaceae
Oxalobacteraceae
Hydrogenophilaceae
Methylophilaceae
Other Betaproteobacteria
Alteromonadaceae
Coxiellaceae
Legionellaceae
Crenotrichaceae
Methylococcaceae
SAR86 clade
Pseudomonadaceae
Vibrionaceae
Sinobacteraceae
Xanthomonadaceae
Other Gammaproteobacteria
Cytophagaceae
Cryomorphaceae
Flavobacteriaceae
NS9
Chitinophagaceae
KD3-93
Saprospiraceae
OPS 17
Other Bacteroidetes
(Cl) OM190
Phycisphaeraceae
Planctomycetaceae
(Cl) vadinHA49
Other Planctomycetes
Acidimicrobiaceae
OCS155
Sva0996
TM214
Mycobacteriaceae
Sporichthyaceae
Microbacteriaceae
Micrococcaceae
Other Actinobacteria
(Or) MB11C04
Opitutaceae
Puniceicoccaceae
(Or) vadinHA64
N18
DEV007
Verrucomicrobiaceae
Other Verrucomicrobia

Figure 6.


Shared Families*
Shared OTUs
All Limnic - All Marine
PA Limnic - PA Marine
FL Limnic - FL Marine
PA Limnic - FL Limnic
PA Marine - FL Marine
46
184
108
6317
29169
68
49
172
109
4624
19795
41
31
103
111
2915
13422
5
49
135
11
3465
1720
1200
31
103
111
15810
4026
9041
Shared
* Clusters with unresolved taxonomy at the family level are not shown

Supporting Information (SI):

Sample treatment: Sixty-one limnic and marine (42 and 19, respectively) water samples were fixed for 1 h at room temperature (RT) (20-25°C) or 24 h at 4°C with formaldehyde (1% final concentration) for catalyzed reporter deposition fluorescence *in-situ* hybridization (CARD-FISH). Afterwards, to separate the particle-associated (PA) (>5 and >10 μm fractions for limnic and marine systems, respectively) and the free-living (FL) (0.2-5 and 0.2-3 μm fractions for limnic and marine systems, respectively) fraction, aliquots were size-fractioned, to a final number of 141 filter samples, under gentle vacuum (7 kPa). Polycarbonate filters (diameter, 47 mm; pore sizes 10, 5, 3 and 0.2 μm, [Millipore, Eschborn, Germany]) were used as detailed in Table S8. The intermediate marine fraction from 3-10 μm was excluded from this study as it did not contain a sizeable number of particles. Samples for DNA extraction were immediately size-fractionated following sampling and stored at -80°C for further processing.

Modifications of the standard CARD-FISH protocol: Prior to CARD-FISH, filters were embedded in 0.15% (w/v) agarose (gel strength >300 g cm^{-2}, Biozym, Oldendorf, Germany) (Pernthaler et al., 2002). Permeabilization treatments were done using lysozyme (10 mg ml^{-1}; Fluka, Steinheim, Germany; buffer contained 50 mM EDTA, 100 mM Tris-HCl, pH 7.4) for 1 h at 37°C. For optional detection of *Planctomycetes*, we followed the protocol by Pizzetti et al. (2011) in which the lysozyme treatment was followed by an achromopeptidase treatment (60 U ml^{-1}; Sigma, Steinheim, Germany; buffer contained 10 mM NaCl, 10 mM Tris-HCl, pH 8.0) for 30 min at 37°C. Endogenous peroxidases were inactivated by 0.15% H_2O_2 in absolute methanol for 30 min at RT. The same was done for the highest detection of *Actinobacteria* following the protocol by Sekar et al. (2003).

Automated microscopy analysis:

System specifications: The microscope system features the LED illumination devices for transmission and fluorescence illumination (Colibri, Zeiss), and a monochrome digital camera (AxioCam MRm). It uses a motorized x, y, z-stage capable of holding 8 microscope glass slides on each of which up to 8 FISH

preparations were mounted. The software SAMLOC was subsequently run to scan all slides with a 1 x objective (EC Plan-Neofluar, NA: 0.025, Zeiss), as described in Zeder et al. (Zeder et al., 2011). The software is able to recognize the FISH preparations and an algorithm creates coordinate position lists for later high-resolution imaging. The optical filter set 62HE (Zeiss) was applied to image the fluorescent stains DAPI and fluorescein isothiocyanate (FITC). Autofluorescence was recorded upon excitation on 633 nm. Bacterial cells were imaged using a 63 × objective (Plan Apochromate, NA = 1.4). Imaging was usually performed autonomously overnight, using 3 channels with a fixed camera exposure time of 500 ms for the autofluorescence, and automatically determined exposure times for DAPI and FITC image. Autofluorescence was used to exclude possible false positive FISH signals.

Procedure for particle field of view (FOV) selection: SAMLOC was used to define a list of 12 positions for each sample. The imaging routine was run in a semi-automated mode using the 63x objective and the 62HE filter set. On each position, the routine performed an autofocus and subsequently paused allowing the user to adjust the position in x, y, and to refocus. The user then searched in a random manner a particle close to the original position, centered the particle to the camera's field of view, and adjusted the focus to the center of the particle. In addition, the user had to set an optimal exposure time for imaging on the DAPI, FITC, and autofluorescence channel.

Particle volume approximation: In this study, particle volumes were not calculated. However, volumetric analysis of particles is possible by a sphere approximation from the 2D particle area as given by the ACMEtool analysis. We assume that particles in their original state are random arbitrarily-shaped 3D objects. Upon removal of water during the filtration process they collapse within themselves. Thus, their projected area in the original state is similar to that after filtration. The original z-dimension cannot be readily reconstructed from the 2D projection. Therefore, we suggest to estimate their volume (V) using a sphere approximation. The radius (r) of the sphere is equivalent to that of a circle with an equal area to that of our particle (A). Thus $r = \sqrt{\frac{A}{\pi}}$; and $V = \frac{4}{3} A \sqrt{\frac{A}{\pi}}$. Based on such an analysis, average-sized limnic and marine particles matching densities as shown in Fig. 1, would harbor 1.78 and 0.69

cells per 1000 μm^3, respectively. These values are similar to previously published cell densities (<1 cells 1000 μm^3) on particles (Kiorboe et al., 2003; Tang et al., 2012). Yet, this may be an underestimation of the actual cell density. Though sphere approximation of particle volumes are a common practice (e.g. (Alldredge and Gotschalk, 1988)) *in-situ* observations show that particles are often elongated spheroids. An adjustment of the above particles to an elongated spheroid would render denser colonized particles with 7.12 and 2.76 cells per 1000 μm^3, respectively. Interestingly, following an initial drop in density with volume increase, the volumetric density remains constant across the particle size range in both marine and limnic systems (Fig. S3). Based on these results we suggest that following an initial aggregation, limnic particles increase in volume by accumulation of equally colonized units. On the other hand, for marine particles the volume increase occurs without changes in bacterial numbers. This is in accordance with our initial hypothesis regarding the colonization mode of limnic and marine particles.

DNA extraction: Cells were lysed by incubation for 15 min at 95°C in a buffer containing 100 mM Tris-HCl, 50 mM EDTA, 100 mM NaCl and 1% SDS (pH 8.0). This was followed by 15 min incubation at 65°C with phenol (half sample volume) and a chloroform extraction. Following an additional phenol-chloroform (1:1) extraction the sample was extracted twice with chloroform and precipitated over night at -20°C using 2-propanol (1:1). The DNA was purified in 70% ethanol and dissolved in diethylpyrocarbonate treated water. Each step was followed by centrifugation at 14 000 g at 4°C.

PCR and pyrosequencing: Tag-encoded FLX amplicon pyrosequencing was carried out as previously described by Dowd et al. (Dowd et al., 2008). A 20 ng aliquot of each DNA sample was used for a 25 μl PCR reaction. A 30 cycle PCR using HotStarTaq Plus Master Mix Kit (Qiagen, USA) was used under the following conditions: 94°C for 3 minutes, followed by 30 cycles of 94°C for 30 seconds; 55°C for 40 seconds and 72°C for 1 minute; and a final elongation step at 72°C for 5 minutes. Following PCR, all amplicon products from different samples were mixed in equal volumes and purified using Agencourt-Ampure beads (Agencourt Bioscience Corporation, USA).

Sequencing data analysis: All reads were aligned using the SILVA Incremental Aligner against the SILVA SSU rRNA seed (Quast et al., 2013). Non-aligned reads

(putative contaminations/artifacts) were excluded from further downstream analysis. Additionally, all remaining reads shorter than 50 aligned nucleotides and reads with more than 2% of ambiguities or 2% of homopolymers were removed. Subsequently, reads of the filtered datasets were dereplicated, clustered and classified in parallel on a sample by sample basis. Dereplication (identification of identical reads ignoring overhangs) and clustering (OTU definition based on a non-redundant subset of reads) was done using cd-hit-est (http://www.bioinformatics.org/cd-hit) applying identity criteria of 1.00 and 0.98, respectively, both times with a word size of 8. For each cluster, the longest read was then used for taxonomic classification. The classification was performed by a local nucleotide BLAST search against the non-redundant version of the SILVA SSURef dataset (release 111; http://www.arb-silva.de) using blast-2.2.22+ http://blast.ncbi.nlm.nih.gov/Blast.cgi) with standard settings. To filter out low identity and artificial BLAST hits, hits for which the function '(%sequence identity +%alignment coverage)/2' did not exceed the value of 93.0 were discarded. For the analyzed reads with sufficiently good BLAST hits, the taxonomic classification of the best BLAST hit according to the SILVA taxonomy has been assigned to the read. Reads without any BLAST hits, or reads with weak BLAST hits only, were classified as 'No Relatives'. Finally, the taxonomic path of each cluster reference was mapped to all reads within the corresponding cluster as well as to their corresponding replicates. This last step allowed to obtain quantitative amplicon information (number of individual reads representing a taxonomic path), within the bounds of PCR and pyrosequencing biases.

Methodological considerations in the overlap of PA and FL fractions: The shared OTUs between PA and FL fractions might be also caused by methodological biases. In contrast to FISH where the physical contact to particles was verified, the comparative 16S rRNA gene sequence analysis of the PA and FL fractions relied on fractionated filtration. In particular, when particle concentrations are high, some of the 5 or 10 μm diameter pores will be partially blocked and thereby trap some of the highly abundant FL bacteria. Furthermore, despite we used low vacuum filtration (50 mm Hg) we cannot fully exclude fragmentation of particles during sampling and filtration which would result in a carry-over of PA bacteria to the FL fraction.

Detailed discussion of PA and FL diversity: A similar frequency of alphaproteobacterial sequences of 26% and 23% was detected in the two PA

fractions, yet this class consisted of different families in the limnic and coastal marine samples (Fig. S8). The limnic PA *Alphaproteobacteria* were dominated by *Caulobacteraceae* (18%), *Xanthobacteraceae* (15%), and *Sphingomonadaceae* (15%). Sequences of these three families were at the same time enriched in the limnic PA fractions as compared to the FL fractions (Table 3). The *Caulobacteraceae* which occurred in all four limnic subsystems studied here are known for their attachment to surfaces by a cellular stalk, the prosteca, and a dimorphic life style that include swarmer cells. Sequences of *Xanthobacteraceae* were only present in the SW basin of Lake Fuku. This family is typically for soils, but was found in freshwater lakes before (Lee et al., 2005). The presence of this family in SW basin of Lake Fuku is probably a result of the runoff from a neighboring peat bog area. *Sphingomonadaceae* were previously described as a significant component of PA bacteria in freshwater systems (Selje and Simon, 2003). This family is known to degrade complex OM (Pinyakong et al., 2003), therefore, it is not surprising that we find it in higher numbers in the humic SW basin of Lake Fuku as compared to the other limnic sub-systems. The marine PA fraction was dominated by *Rhodobacteraceae* (28%) and *Parvularculaceae* (10%; yet in Northern Adriatic Sea only) which were also enriched on particles. The Surface 1 SAR11 clade made up a major part of the PA sequences; however, compared to the FL fraction it was depleted (Table 3). Interestingly, 10-30% of the SAR11 PA could not be found in the FL fractions of any of the samples most of which belonging to the Surface 1 clade. The *Rhodobacteraceae* sequences present in coastal marine and limnic PA fractions (28% and 5%, respectively) differed on the genus level (Table S7).

Within the class *Betaproteobacteria*, sequences affiliated with the families *Alcaligenaceae* (37%), and *Comamonadaceae* (28%) (Fig. S8) accounted for a significant fraction on limnic particles. The *Comamonadaceae* are diverse with 7 marine and 21 limnic species in our samples. The limnic *Comamonadaceae* were dominated by the genus *Limnohabitans* followed by genus *Albidiferax*. The latter is known to inhabit iron rich particles (Lu et al., 2013). *Limnohabitans* is known to have both PA and FL (Hunt et al., 2008) members as also seen by the OTU distribution in our study. The frequency of betaproteobacterial sequences retrieved from marine particles was low, reflecting the FISH data, except that betaproteobacterial clade OM43 was not found on this level.

Gammaproteobacteria represented the most diverse bacterial class (Fig. S8). The limnic PA fraction was dominated by the methanotrophic *Methylococcaceae* (30%). They were found almost exclusively in the SW basin of Lake Fuku, despite the fact that methane production was shown to occur also in Lake Stechlin (Grossart et al., 2011) and NE basin of Lake Fuku (Casper et al., 2003). Major gammaproteobacterial groups on marine particles comprised the families *Alteromonadaceae* (20%) and *Crenotrichaceae* (16%), and the SAR86 clade (14%). As members of the genus *Crenothrix* have been shown to be methane oxidizers (Stoecker et al., 2006), they may have a similar function on marine particles as the limnic *Methylococcaceae*. Similar to SAR11 clade of *Alphaproteobacteria* members of the clade SAR86 were found, but in relation to FL depleted in the PA fraction as reported earlier (Crespo et al., 2013). This is in line with the lack of detection of SAR86 on marine particles by FISH.

Within the phylum *Bacteroidetes*, the order *Sphingobacteriales* dominated the limnic PA fraction (Fig. S8). The most abundant family of this order was *Chitinophagaceae* (35%) which also contributed to >5% of all sequences (Fig. S8). Limnic and marine PA fractions contained sequences affiliating with *Flavobacteriaceae* (59% marine; 19% limnic), yet, again, no genera were shared between marine and limnic samples (Table S7). *Chitinophagaceae* as well as *Flavobacteriaceae* are well known for their ability to attach to surfaces on which they glide and degrade high molecular weight organic matter such as polysaccharides and proteins (Cottrell and Kirchman, 2000). The genus *Flavobacterium* is normally common in both marine and limnic systems (Adachi et al., 2002; Newton et al., 2011). In our case it was found mainly in limnic as those occurring in Lake Stechlin. Interestingly, though *Flavobacterium* sp. is known to contribute to samples from Lake Stechlin, which fits well to the observation by (Eiler and Bertilsson, 2007) that this genus is dominant after cyanobacterial blooms such the breakdown of *Gymnodinium* blooms (Adachi et al., 2002), such as the one during the Helgoland 2009 sampling, it was not found in any marine sample in this study. We found, however, *Formosa* related sequences in high frequency in our Helgoland samples from fall 2009 and spring 2011 on both PA and FL.

Although abundant in the actinobacterial PA fraction, the family *Sporichthyaceae* (Fig. S8) was depleted on limnic particles as compared to the surrounding water (Table 3). This family encompasses the globally distributed Ac1 freshwater cluster of *Actinobacteria* (Glöckner et al., 2000; Warnecke et al., 2004). Sequences of the family

Verrucomicrobiaceae were retrieved from both limnic and marine particles, yet otherwise the verrucomicrobial sequences differed strongly on the family level. Overall, the PA and FL bacterial distribution as shown by our study is in good agreement with previous studies on either of the systems. The OM190 (Rappe et al., 1997; Fuchsman et al., 2012) class was only abundant among marine planctomycetal PA sequences (36%) (Fig. S8) while *Phycisphaeraceae* were clearly more abundant in the limnic PA fraction (54%). Sequences affiliating with the family *Planctomycetaceae* were occurring in both limnic and marine samples. Again different genera dominated the limnic (*Schlesneria*, *Pirellula*) and marine (*Rhodopirellula*) PA fractions. Members of the order *Planctomycetales* are well known for their attachment to surfaces by a cellular holdfasts and for the formation of swarmer cells (Ward et al., 2006).

Detailed discussion of scenarios in which the index $Chao1_{PA}/Chao1_{FL}$ was <1:

In 3/8 (limnic) and 4/13 (marine) sequenced sample pairs the Chao 1 diversity was lower on particles. These samples consisted mostly of decaying diatom particles, matching the normally reoccurring phytoplankton distributions in the systems at the time of sampling (Table 1 and see also Wiltshire et al. 2008; IGB database). The SW basin of Lake Fuku was an exception as the particles consisted mostly of cryptomonads. Such uniform particles suppress the omnipresent background of heterogeneous particles. We hypothesize that these particles were likely colonized by rapidly growing specialists able to degrade the dominant substrates. This resulted in a lower diversity of the PA as compared to the FL community. It has recently been reported that the decay of diatom blooms results in substrate-driven successions of low-diversity bacterial communities (Amin et al., 2012; Teeling et al., 2012). Similarly, cryptomonads were also shown to be mainly colonized by a less diverse community (Pernthaler et al., 2001).

Table S1. CARD-FISH abundance data for specific groups

Particle-associated										
	Roseo-bacter	Sar11**	OM43	*Altero-monas*	*Pseudo-alteromonas*	Sar86	Nor5	SAR92	Sar324	*Eury-archaeota*
# particles	**54**	**20**		**17**	**39**	**20**	**29**	**20**	**20**	**47**
Average (% of DAPI)	8.3	<0,1		0.9	4.5	<0,1	8.1	<0,1	<0,1	12
Stdev	6.8	<0,1	**Not**	1.6	6.2	<0,1	11	<0,1	<0,1	13
Max	46	<0,1	**analyzed**	6.6	28	<0,1	46	<0,1	<0,1	51
Min	1.5	<0,1		0.0	0.0	<0,1	0.0	<0,1	<0,1	0.0
Median	7.4	<0,1		0.0	2.7	<0,1	2.8	<0,1	<0,1	8.7
Helgoland 2011 ('11)										
# particles	**32**	**20**								**25**
Average (% of DAPI)	9.8	<0,1								5.0
Stdev	6.3	<0,1								4.2
Max	33	<0,1	**Not analyzed**							14
Min	1.6	<0,1								0.0
Median	8.9	<0,1								4.5
Northern Adriatic - Above thermocline (AT)										
# particles	**38**	**20**	**30**	**30**	**7**	**20**			**20**	
Average (% of DAPI)	14	<0,1	3.4	1.8	0.6	<0,1			<0,1	
Stdev	14	<0,1	4.1	3.0	2.0	<0,1	**Not analyzed**		<0,1	**Not**
Max	63	<0,1	15.1	16	11	<0,1			<0,1	**analyzed**
Min	0.0	<0,1	0.0	0.0	0.0	<0,1			<0,1	
Median	11	<0,1	1.7	0.6	0.0	<0,1			<0,1	
Northern Adriatic - Below thermocline (BT)										
# particles	**78**	**20**	**30**	**41**	**33**	**20**			**20**	
Average (% of DAPI)	40	<0,1	7.6	4.4	3.4	<0,1			<0,1	
Stdev	23	<0,1	7.6	5.4	4.5	<0,1	**Not analyzed**		<0,1	**Not**
Max	94	<0,1	29.1	25	25	<0,1			<0,1	**analyzed**
Min	5.7	<0,1	1.1	0.0	0.0	<0,1			<0,1	
Median	37	<0,1	4.4	2.2	2.4	<0,1			<0,1	
Free-living*										
Helgoland 2009 ('09)										
# samples*	**6**	**6**		**6**	**6**	**6**	**6**	**6**	**6**	**6**
Average (% of DAPI)	9.7	30		1.7	0.7	4.2	1.9	1.4	0.8	7.6
Stdev	3.9	7.1	**Not**	1.3	0.4	0.8	0.9	0.3	0.2	1.8
Max	18	41	**analyzed**	4.7	1.5	5.6	3.4	1.9	1.3	12
Min	5.1	17		0.3	0.2	2.7	0.5	0.8	0.6	5.4
Median	9.1	30		1.4	0.7	4.2	1.6	1.4	0.7	7.3
Helgoland 2011 ('11)										
# samples*	**3**	**3**								**3**
Average (% of DAPI)	10	40								8.8
Stdev	2.1	7.9								4.8
Max	13	49	**Not analyzed**							13
Min	8.8	33								3.7
Median	9.6	38								9.6
Northern Adriatic - Above thermocline (AT)										
# samples*	**5**	**5**	**5**	**5**	**5**	**5**			**5**	**5**
Average (% of DAPI)	4.1	7.5	2.9	4.6	4.4	5.3			4.3	6.0
Stdev	1.1	2.1	1.7	1.8	2.1	2.2	**Not analyzed**		1.5	6.4
Max	5.7	11	5	7.4	7.9	9.2			6.8	6.0
Min	3.2	5.2	1.3	2.9	2.7	3.7			3.1	6.3
Median	3.8	7.8	2.5	4.5	3.7	4.5			3.8	6.0
Northern Adriatic - Below thermocline (BT)										
# samples*	**5**	**5**	**5**	**5**	**5**	**5**			**5**	**5**
Average (% of DAPI)	5.3	10	2.0	2.7	2.7	3.9			2.6	5.6
Stdev	2.1	3.1	0.4	0.6	1.3	1.1	**Not analyzed**		1.0	5.4
Max	8.2	14	2.7	3.6	4.9	5.7			4.3	5.2
Min	3.2	5.5	1.6	2.1	1.9	2.8			2.1	4.7
Median	4.4	10	1.9	2.6	2.2	3.5			2.2	5.6

*** In each sample between 32 and 56 Fields Of View (FOV)**

**** Limnic equivalent of SAR11, the LD12, was as well not found associated with particles**

Table S2. Sequence quality and diversity data for each sample.

sample name	#seq	min	avg	max	OTU	Clust‘	Rep‘	Rej‘	Goods	Chao1	H	E_H
EL-May-PA	7130	150	436	553	743	4513	1852	22	0.9655	978	5.23	0.55
EL-May-FL	7058	150	426	529	686	4142	2225	5	0.9602	1092	5.02	0.53
EL-Sep-PA	7740	151	429	542	615	4049	3065	11	0.9643	1061	4.46	0.46
EL-Sep-FL	5030	150	426	541	379	2750	1886	15	0.9734	512	4.19	0.45
HL-May-PA	3984	150	316	531	523	2267	1145	49	0.9571	664	5.22	0.58
HL-May-FL	5202	152	436	550	539	3409	1241	13	0.966	736	5.18	0.56
HL-Sep-PA	8988	150	436	554	1346	5556	2068	18	0.9362	2032	6.03	0.62
HL-Sep-FL	7974	150	423	558	682	4791	2486	15	0.9708	964	5.4	0.56
NE-May-PA	7149	150	423	764	650	3833	2623	43	0.9668	932	4.82	0.5
NE-May-FL	8755	150	418	543	643	4808	3291	13	0.9733	882	5	0.51
NE-Sep-PA	3582	151	439	545	465	2279	728	110	0.9397	748	4.61	0.52
NE-Sep-FL	3680	151	411	532	383	2002	1287	8	0.9598	556	4.63	0.52
SW-May-PA	5493	150	419	542	545	3158	1752	38	0.965	736	4.84	0.52
SW-May-FL	4434	150	407	531	226	2199	1996	13	0.9797	351	3.61	0.4
SW-Sep-PA	14602	150	443	551	565	8382	5340	315	0.9899	708	4.49	0.44
SW-Sep-FL	14476	150	412	540	946	6246	7252	32	0.9726	1453	4.74	0.46
'09-0610-PA	12109	200	417	532	2971	7796	1295	47	0.8676	5459	5.66	0.64
'09-0610-FL	3345	200	387	526	837	2068	439	1	0.8756	1380	5.9	0.67
'09-0109-PA	17571	200	444	530	4184	11262	2111	14	0.8821	7095	6.22	0.67
'09-0109-FL	3664	200	373	526	754	2284	626	0	0.8971	1330	7.31	0.7
'11-2804-PA	4462	200	357	516	1227	2578	618	39	0.8516	2300	6.32	0.7
'11-2804-FL	4741	200	395	513	852	3029	858	2	0.9129	1404	5.59	0.61
'11-0605-PA	3100	200	345	511	880	1688	496	36	0.8448	1645	5.94	0.68
'11-0605-FL	7321	200	404	520	1324	4656	1316	25	0.9088	2390	5.88	0.61
'11-1605-PA	4458	200	384	533	1252	2616	435	155	0.8499	2236	6.34	0.7
'11-1605-FL	3742	200	385	520	871	2343	491	37	0.8846	1488	5.92	0.66
'11-1905-PA	2888	200	396	525	899	1733	228	28	0.8314	1769	6.14	0.71
'11-1905-FL	5178	200	395	514	1190	3249	730	9	0.893	1859	6.96	0.69
BT-107-PA	10448	200	341	522	2359	6453	1604	32	0.8967	3817	6.93	0.7
BT-107-FL	9847	200	303	469	1747	6416	1620	64	0.9263	2593	6.47	0.65
AT-107-PA	8029	200	327	516	2069	4799	1110	51	0.884	3127	6.94	0.72
AT-107-FL	7831	200	302	472	1655	5183	932	61	0.9102	2494	6.54	0.68
AT-105-PA	9149	200	326	530	1967	5574	1513	95	0.901	3155	6.64	0.68
AT-105-FL	541	200	346	490	326	203	7	5	0.5527	1004	5.45	0.78
BT-105-PA	5728	200	314	515	1237	3708	729	54	0.908	1907	6.39	0.68
BT-105-FL	11644	200	306	465	1810	7944	1861	29	0.9347	2829	6.49	0.65
AT-103-PA	6061	200	330	528	1247	3909	838	67	0.9185	1765	6.35	0.68
BT-103-PA	1092	200	347	489	636	429	21	6	0.5668	2014	6.13	0.8
BT-103-FL	9738	200	298	621	1946	6105	1573	114	0.9052	3360	6.65	0.67
AT-101-PA	8574	200	400	558	1418	5502	1524	130	0.93	2154	6.27	0.64
AT-101-FL	1660	200	412	521	233	1110	314	3	0.9578	302	4.75	0.59
BT-101-PA	8554	200	292	471	1305	5695	1367	187	0.9455	1858	6.36	0.65
AT-108-FL	10163	200	377	510	1871	6201	2072	19	0.9172	2931	6.43	0.65
BT-108-PA	11959	200	291	487	2607	7505	1757	90	0.8965	4209	6.98	0.69
AT-108-PA	10467	200	306	463	1696	6571	2074	126	0.9309	2652	6.44	0.65
BT-108-FL	8532	200	304	487	1622	5553	1273	84	0.9211	2363	6.4	0.66

Sequence quality data for each sample. For each sample the number of sequences (#Seq), the minimal (min), average (avg) and maximal (max) sequence length found, number of OTUs are given. Additionally the number of cluster sequences (Clust‘), replicate sequences (Rep‘) and quality rejected sequences (Rej‘). The Goods coverage index and Chao 1, Shannon (H) and Shannon evenness (EH) diversity indices are porvided as well. Sample name abbreviations: EL and HL - Epilimnion and Hypolimnion of Lake Stechlin, NE and SW - North-Eastern and South-Western basins of Lake Grosse Fuchskuhle, 09 and 11 - Samples collected in 2009 and 2011 on Helgoland (date given in ddmm format), AT and BT - samples collected above and below the thermocline along the Rovinj-Po transect in the Northern Adriatic Sea at the specified stations. PA and FL refer to particle-associated and free-living, respectively.

Table S3. Sample composition of each Sample-group.

Environment	**Sample-group ID**	**# Of pooled results**	**Individual sample ID**
Lake Stechlin	EL-PA	2	May-2009 Sep-2009
	EL-FL	2	
	HL-PA	2	
	HL-FL	2	
Lake Grosse Fuchskuhle	NE-PA	2	May-2009 Sep-2009
	NE-FL	2	
	SW-PA	2	
	SW-FL	2	
Helgoland (Kabeltonne)	09-PA	2	01.09.2009 06.10.2009
	09-FL	2	
	11-PA	4	28.04.2011 06.05.2011 16.05.2011 19.05.2011
	11-FL	4	
Northern Adriatic Sea (Rovinj-Po River delta transect)	AT-PA	5	St.107 St.105 St.103 St.101 St.108
	AT-FL	5	
	BT-PA	5	
	BT-FL	5	

Name abbreviations: EL and HL-Epilimnion and Hypolimnion of Lake Stechlin, NE and SW-North-Eastern and South-Western basins of Lake Grosse Fuchskuhle, 09 and 11-Samples collected in 2009 and 2011 on Helgoland, AT and BT-samples collected above and below the thermocline along the Rovinj-Po transect in the Northern Adriatic Sea. PA and FL refer to particle-associated and free-living, respectively.

Table S4. Summary of OTU comparison between homogeneous and heterogeneous PA communities

A

	PA Sample compared	Chao1	$Chao1_{PA}/Chao1_{FL}$	Unique OTUs	Shared OTUs	Total OTUs	Unique Taxa	% of seqs	Shared tax paths	Total tax paths	Genera resolved#
Limnic	HL-Sep-PA	2032	2.11	626	98	968	25	2.5	172	208	145
	HL-May-PA	664	0.90	244			11	4.2			
	EL-Sep-PA	1061	2.07	250	62	695	27	15.07	87	149	115
	El-May-PA	978	0.90	383			35	7.5			
	SW-May-PA	736	2.10	166	104	380	19	1.3	58	90	71
	SW-Sep-PA	708	0.40	110			13	22.5			
Marine	'11-2804-PA	2300	1.64	293	29	643	14	25.3	102	120	88
	'11-0605-PA	1645	0.69	321			4	0			
	'11-1605-PA	2236	1.50	378	71	593	12	1.05	112	128	102
	'11-1605-PA	1769	0.95	144			4	0.25			
	AT-105-PA	3155	3.14	780	88	1081	8	1.45	194	213	164
	BT-105-PA	1907	0.67	213			11	6.098			
	BT-107-PA	3817	1.47	1087	90	1388	20	1.5	142	168	143
	BT-103-PA	2014	0.60	211			6	0.8			

B

Limnic	Marine
Taxa shared between homogenous* and heterogenous* PA communities	
Enriched in homogenous PA communities	
Acidimicrobiia;Acidimicrobiales;Acidimicrobiaceae;CL500-29 marine group	Flavobacteria;Flavobacteriales;Flavobacteriaceae;Formosa
Flavobacteria;Flavobacteriales;Flavobacteriaceae;Flavobacterium	Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Paracoccus
Alphaproteobacteria;Caulobacterales;Caulobacteraceae;Caulobacter	Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Roseovarius
Betaproteobacteria;Burkholderiales;Comamonadaceae;Albidiferax	Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Haliea
Gammaproteobacteria;Xanthomonadales;Xanthomonadaceae;Stenotrophomonas	
Enriched in heterogenous PA communities	
Planctomycetacia;Planctomycetales;Planctomycetaceae;Pirellula	Flavobacteria;Flavobacteriales;Cryomorphaceae;Fluviicola
Alphaproteobacteria;Rhizobiales;alphaI cluster;	
Betaproteobacteria;Nitrosomonadales;Nitrosomonadaceae;uncultured	
Taxa unique to either homogenous or heterogenous PA communities	
Unique to homogenous PA communities	
Cytophagia;Cytophagales;Cytophagaceae;Cytophaga	Actinobacteria;Frankiales;Geodermatophilaceae;Modestobacter
Cytophagia;Cytophagales;Cytophagaceae;Emticicia	Alphaproteobacteria;Rhizobiales;Phyllobacteriaceae;Aminobacter
Cytophagia;Cytophagales;Cytophagaceae;Flectobacillus	Alphaproteobacteria;Rhizobiales;Rhizobiaceae;Rhizobium
Firmicutes;Bacilli;Bacillales;Staphylococcaceae;Staphylococcus	Deltaproteobacteria;Myxococcales;0319-6G20;
Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Pseudorhodobacter	Gammaproteobacteria;Thiotrichales;Piscirickettsiaceae;uncultured
Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae;Sandarakinorhabdus	Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Roseibacillus
Betaproteobacteria;Burkholderiales;Comamonadaceae;	
Betaproteobacteria;Rhodocyclales;Rhodocyclaceae;Sulfuritalea	
Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Simiduia	
Gammaproteobacteria;Chromatiales;Chromatiaceae;Rheinheimera	
Gammaproteobacteria;Enterobacteriales;Enterobacteriaceae;Enterobacter	
Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;Pseudomonas	
Unique to heterogenous PA communities	
Armatimonadetes;	Acidimicrobiia;Acidimicrobiales;TM214;
Flavobacteria;Flavobacteriales;Flavobacteriaceae;Chryseobacterium	Actinobacteria;Micrococcales;Cellulomonadaceae;Demequina
Chloroflexi;SL56 marine group	Flavobacteria;Flavobacteriales;Flavobacteriaceae;Gramella
OM190	Flavobacteria;Flavobacteriales;Flavobacteriaceae;Lutibacter
Phycisphaerae;Phycisphaerales;Phycisphaeraceae;SM1A02	Flavobacteria;Flavobacteriales;Flavobacteriaceae;NS4 marine group
Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Hirschia	Flavobacteria;Flavobacteriales;Flavobacteriaceae;Polaribacter
Alphaproteobacteria;Rhodospirillales;Acetobacteraceae;Roseomonas	Sphingobacteriia;Sphingobacteriales;WCHB1-69;
Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;Elstera	Candidate division BRC1;
Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;Oceanibaculum	Gemmatimonadetes;Gemmatimonadetes;BD2-11 terrestrial group
Alphaproteobacteria;Rickettsiales;Family Incertae Sedis;Caedibacter	Lentisphaeria;Lentisphaerales;Lentisphaeraceae;Lentisphaera
Alphaproteobacteria;Sphingomonadales;GOBB3-C201;	Nitrospirae;Nitrospira;Nitrospirales;Nitrospiraceae;Nitrospira
Betaproteobacteria;Burkholderiales;Alcaligenaceae;Derxia	Planctomycetacia;Planctomycetales;Planctomycetaceae;Pirellula
Betaproteobacteria;Burkholderiales;Comamonadaceae;Simplicispira	Alphaproteobacteria;Rhizobiales;Rhodobiaceae;Rhodobium
Betaproteobacteria;oca12	Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Jannaschia
Deltaproteobacteria;Myxococcales;Sorangiineae;Phaselicystidaceae	Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Rhodobacter
Deltaproteobacteria;Myxococcales;Sorangiineae;Polyangiaceae	Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Ruegeria
Spartobacteria;Chthoniobacterales;DA101 soil group;	Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Sulfitobacter
Spartobacteria;Chthoniobacterales;FukuN18 freshwater group;	Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Tateyamaria
	Alphaproteobacteria;Rickettsiales;Ho(lab);
	Alphaproteobacteria;Rickettsiales;Rickettsiaceae;uncultured
	Alphaproteobacteria;Sphingomonadales;Erythrobacteraceae;Croceicoccus
	Alphaproteobacteria;Sphingomonadales;Erythrobacteraceae;Erythrobacter
	Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae;Stakelama
	Betaproteobacteria;Methylophilales;Methylophilaceae;OM43 clade
	Deltaproteobacteria;Desulfobacterales;Desulfobulbaceae;Desulfobulbus
	Deltaproteobacteria;Desulfobacterales;Nitrospinaceae;Nitrospina
	Gammaproteobacteria;Alteromonadales;Psychromonadaceae;Psychromonas
	Gammaproteobacteria;BD7-8 marine group
	Gammaproteobacteria;E01-9C-26 marine group
	Gammaproteobacteria;KI89A clade
	Gammaproteobacteria;Oceanospirillales;Alcanivoracaceae;Alcanivorax
	Gammaproteobacteria;Oceanospirillales;Halomonadaceae;Salinicola
	Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Reinekea
	Gammaproteobacteria;Oceanospirillales;Oleiphilaceae;Oleiphilus
	Gammaproteobacteria;Oceanospirillales;OM182 clade;
	Gammaproteobacteria;Oceanospirillales;ZD0405;
	Gammaproteobacteria;Order Incertae Sedis;Family Incertae Sedis;Marinicella
	Gammaproteobacteria;Pseudomonadales;Moraxellaceae;Psychrobacter
	Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;Pseudomonas
	Gammaproteobacteria;Thiotrichales;Thiotrichaceae;Leucothrix
	Gammaproteobacteria;Xanthomonadales;Sinobacteraceae;JTB255 marine benthic group
	Opitutae;Puniceicoccales;Puniceicoccaceae;Lentimonas
	Opitutae;Puniceicoccales;Puniceicoccaceae;marine group
	Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Haloferula

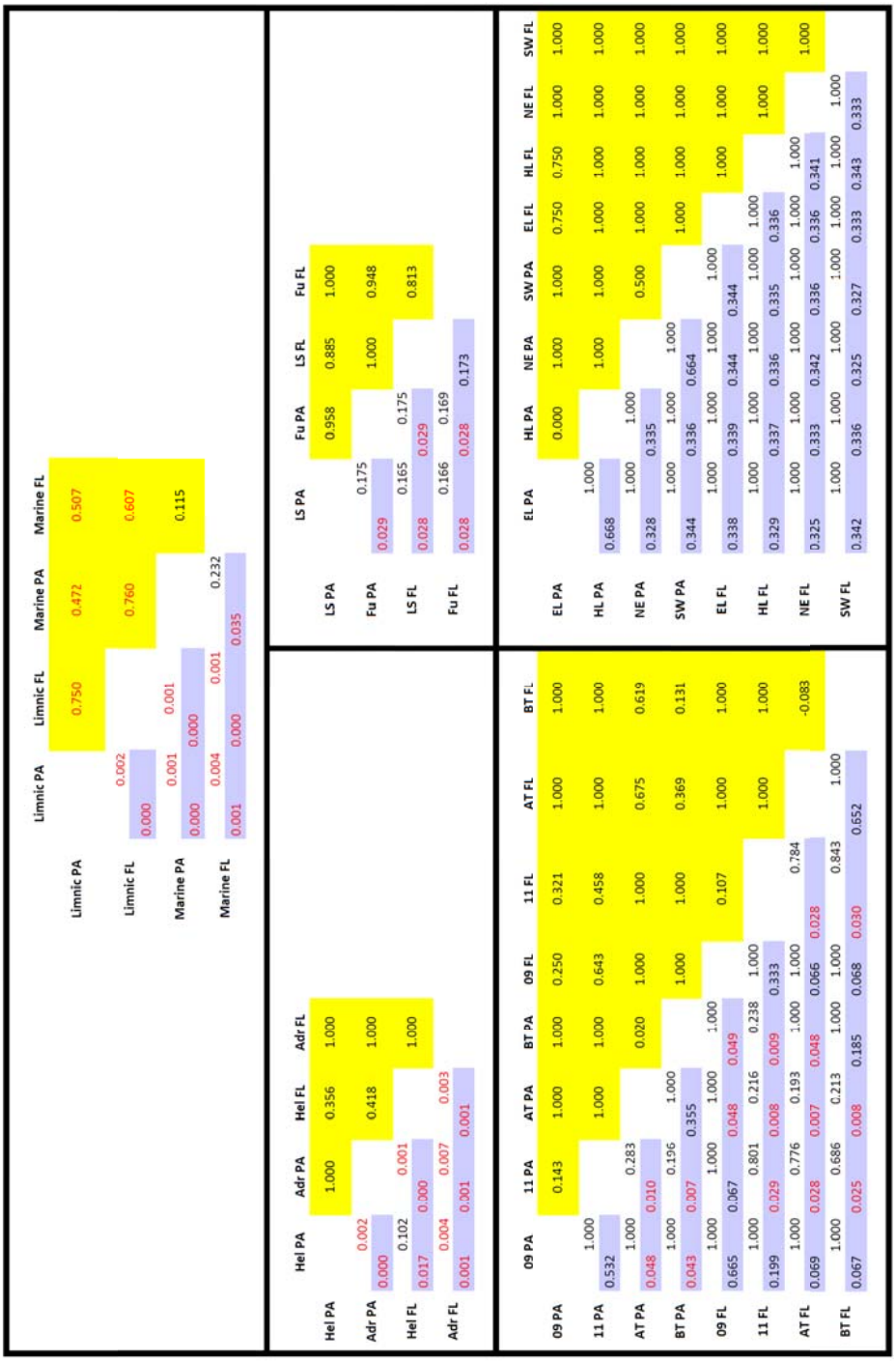
Taxonomy resolved to the genus level or an equivalent phylogenetic clade for which a valid nomenclature does not exist

* Homogenous and heterogenous PA communities are defined as having a Chao1 PA/FL<1 and PA/FL>1, respectively

All taxa specified are unique to their category in table B and occure in at least 2 of the samples

Among genera that were unique to either type of particles we identified as well such that reoccurred in more than one sample

Table S5. Analysis of similarity (ANOSIM)



	Limnic PA	Limnic FL	Marine PA	Marine FL
Limnic PA		0.750	0.472	0.507
Limnic FL	0.002		0.760	0.607
Marine PA	0.001	0.001		0.115
Marine FL	0.000	0.000	0.001	
	0.004	0.000	0.001	0.232
	0.001	0.000	0.000	0.035

	LS PA	Fu PA	LS FL	Fu FL
LS PA		0.175	0.165	0.813
Fu PA	0.029		0.175	
LS FL	0.028	0.029		
Fu FL	0.028	0.166	0.169	0.173
		0.028		

	Hel PA	Adr PA	Hel FL	Adr FL
Hel PA		1.000	0.356	1.000
Adr PA	0.002		0.418	1.000
Hel FL	0.000	0.102		1.000
Adr FL	0.017	0.300	0.001	
	0.001	0.004	0.007	0.003
		0.001	0.301	0.001

	EL PA	HL PA	NE PA	SW PA	EL FL	HL FL	NE FL	SW FL
EL PA		1.000	0.328	0.344	0.338	0.329	0.325	0.342
HL PA	0.668		0.328	0.344	0.338	0.329	0.325	0.342
NE PA	1.000	1.000		0.344	0.338	0.329	0.325	0.342
SW PA	0.500	0.500	0.500		0.338	0.329	0.325	0.342
EL FL	1.000	1.000	1.000	1.000		0.329	0.325	0.342
HL FL	1.000	1.000	1.000	1.000	1.000		0.325	0.342
NE FL	1.000	1.000	1.000	1.000	1.000	1.000		0.342
SW FL	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

	09 PA	11 PA	AT PA	BT PA	09 FL	11 FL	AT FL	BT FL
09 PA		0.143	1.000	1.000	0.250	0.321	1.000	1.000
11 PA	1.000		1.000	1.000	0.643	0.458	1.000	1.000
AT PA	0.532	1.000		0.020	1.000	1.000	0.675	0.619
BT PA	0.048	0.048	0.283		1.000	1.000	0.369	0.131
09 FL	1.000	1.000	0.196	1.000		0.107	1.000	1.000
11 FL	0.043	0.043	0.307	0.355	1.000		1.000	1.000
AT FL	1.000	1.000	1.000	1.000	1.000	1.000		1.000
BT FL	0.665	0.665	0.967	0.048	0.049	0.107	1.000	
	1.000	1.000	0.801	0.216	0.238	1.000	1.000	1.000
	0.199	0.199	0.029	0.008	0.009	0.333	1.000	1.000
	1.000	1.000	0.776	0.193	1.000	1.000	0.784	-0.083
	0.069	0.069	0.028	0.007	0.048	0.066	0.028	
	1.000	1.000	0.686	0.213	1.000	1.000	0.843	1.000
	0.067	0.067	0.025	0.008	0.185	0.068	0.030	0.652

Upper right triangle of each matrix (yellow shaded) shows the R values of the ANOSIM analsyis; The Lower left triangle of each matrix shows the p value (blue shaded) and the the Bonferoni corrected p above (non-shaded); Significant values are marked in red. Group names abbreviations: EL and HL- Epilimnion and Hypolimnion of Lake Stechlin (LS), NE and SW - North-Eastern and South-Western basins of Lake Grosse Fuchskuhle (Fu), 09 and 11 - Samples collected in 2009 and 2011 on Helgoland (Hel), AT and BT - samples collected above and below the thermocline along the Rovinj-Po transect in the Northern Adriatic Sea (Adr). PA and FL refer to particle associated and free-living, respectively.

Table S6. Contribution of individual taxa to difference between sample groups

	Contribution %	Cumulative %	Taxa
Limnic Vs Marine	13.37	13.37	Actinobacteria;Actinobacteria;Frankiales;Sporichthyaceae
	5.24	18.61	Proteobacteria;Alphaproteobacteria;SAR11 clade;Surface 4
	5.10	23.71	Proteobacteria;Alphaproteobacteria;Rhodospirillales;Candidatus Alysiosphaera
	4.07	27.78	Proteobacteria;Alphaproteobacteria;SAR11 clade;Surface 1
	4.03	31.81	Bacteroidetes;SB-1
	3.74	35.55	Proteobacteria;Betaproteobacteria;Burkholderiales;Comamonadaceae
	2.62	38.17	Verrucomicrobia;Opitutae;vadinHA64
	2.43	40.60	Proteobacteria;Alphaproteobacteria;SAR11 clade;Surface 2
	2.33	42.93	Proteobacteria;Alphaproteobacteria;SAR11 clade;LD12 freshwater group
	2.10	45.03	Proteobacteria;Alphaproteobacteria;Caulobacterales;Caulobacteraceae
	2.04	47.07	Proteobacteria;Betaproteobacteria;Burkholderiales;Alcaligenaceae
	1.83	48.90	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
	1.64	50.54	Bacteroidetes;Sphingobacteria;Sphingobacteriales;Chitinophagaceae
	1.63	52.17	Proteobacteria;Betaproteobacteria;Burkholderiales;Burkholderiaceae
	1.61	53.78	Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae
	1.60	55.38	Planctomycetes;OM190
	1.49	56.87	Bacteroidetes;Flavobacteria;Flavobacteriales;Flavobacteriaceae
	1.42	58.29	Planctomycetes;Phycisphaerae;Phycisphaerales;Phycisphaeraceae
	1.29	59.58	Proteobacteria;Alphaproteobacteria;Rhodospirillales;Acetobacteraceae
	1.27	60.85	Proteobacteria;Gammaproteobacteria;Oceanospirillales;SAR86 clade
	1.25	62.10	Proteobacteria;Alphaproteobacteria;SAR11 clade
	1.15	63.25	Proteobacteria;Betaproteobacteria;Burkholderiales;Oxalobacteraceae
	1.08	64.33	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae
Limnic PA Vs Marine PA	4.83	4.83	Proteobacteria;Alphaproteobacteria;Rhodospirillales;Candidatus Alysiosphaera
	4.26	9.09	Bacteroidetes;SB-1
	4.06	13.15	Proteobacteria;Alphaproteobacteria;Caulobacterales;Caulobacteraceae
	4.01	17.16	Proteobacteria;Betaproteobacteria;Burkholderiales;Alcaligenaceae
	3.98	21.14	Proteobacteria;Betaproteobacteria;Burkholderiales;Comamonadaceae
	2.46	23.60	Planctomycetes;OM190
	2.41	26.01	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
	2.35	28.36	Proteobacteria;Alphaproteobacteria;SAR11 clade;Surface 1
	2.34	30.70	Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae
	2.30	33.00	Proteobacteria;Alphaproteobacteria;Rhodospirillales;Acetobacteraceae
	2.24	35.24	Proteobacteria;Betaproteobacteria;Burkholderiales;Oxalobacteraceae
	2.09	37.33	Proteobacteria;Alphaproteobacteria;SAR11 clade;Surface 4
	2.02	39.35	Proteobacteria;Alphaproteobacteria;Parvularculales;Parvularculaceae
	1.97	41.32	Proteobacteria;Alphaproteobacteria;Rhizobiales;Xanthobacteraceae
	1.88	43.20	Verrucomicrobia;Opitutae;vadinHA64
	1.77	44.97	Bacteroidetes;Sphingobacteria;Sphingobacteriales;Chitinophagaceae
	1.74	46.71	Actinobacteria;Actinobacteria;Frankiales;Sporichthyaceae
	1.73	48.44	Bacteroidetes;Flavobacteria;Flavobacteriales;Flavobacteriaceae
	1.68	50.12	Proteobacteria;Gammaproteobacteria;Alteromonadales;Idiomarinaceae
	1.65	51.77	Planctomycetes;Phycisphaerae;Phycisphaerales;Phycisphaeraceae
	1.41	53.18	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae
	1.35	54.53	Proteobacteria;Alphaproteobacteria;Rhizobiales;alphal cluster
	1.29	55.82	Bacteroidetes;Flavobacteria;Flavobacteriales;NS9 marine group
	1.17	56.99	Firmicutes;Bacilli;Bacillales;Paenibacillaceae
	1.13	58.12	Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae
Limnic FL Vs Marine FI	23.98	23.98	Actinobacteria;Actinobacteria;Frankiales;Sporichthyaceae
	8.34	32.32	Proteobacteria;Alphaproteobacteria;SAR11 clade;Surface 4
	5.64	37.96	Proteobacteria;Alphaproteobacteria;SAR11 clade;Surface 1
	5.44	43.40	Proteobacteria;Alphaproteobacteria;Rhodospirillales;Candidatus Alysiosphaera
	4.07	47.47	Proteobacteria;Alphaproteobacteria;SAR11 clade;LD12 freshwater group
	3.86	51.33	Bacteroidetes;SB-1
	3.80	55.13	Proteobacteria;Alphaproteobacteria;SAR11 clade;Surface 2
	3.55	58.68	Proteobacteria;Betaproteobacteria;Burkholderiales;Comamonadaceae
	3.43	62.11	Verrucomicrobia;Opitutae;vadinHA64;
	2.46	64.57	Proteobacteria;Betaproteobacteria;Burkholderiales;Burkholderiaceae
	2.01	66.58	Proteobacteria;Alphaproteobacteria;SAR11 clade
	1.56	68.14	Proteobacteria;Gammaproteobacteria;Oceanospirillales;SAR86 clade
	1.54	69.68	Bacteroidetes;Sphingobacteria;Sphingobacteriales;Chitinophagaceae
	1.28	70.96	Proteobacteria;Alphaproteobacteria;Rickettsiales;SAR116 clade
	1.24	72.20	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
	1.16	73.36	Planctomycetes;Phycisphaerae;Phycisphaerales;Phycisphaeraceae
	1.15	74.51	Bacteroidetes;Flavobacteria;Flavobacteriales;Flavobacteriaceae
	1.10	75.61	Proteobacteria;Betaproteobacteria;Methylophilales;Methylophilaceae

	Contribution %	Cumulative %	Taxa
Limnic Pa Vs Limnic FL	29.22	29.22	Actinobacteria;Actinobacteria;Frankiales;Sporichthyaceae
	5.19	34.41	Proteobacteria;Alphaproteobacteria;SAR11 clade;LD12 freshwater group
	4.44	38.85	Verrucomicrobia;Opitutae;vadinHA64
	4.13	42.98	Proteobacteria;Alphaproteobacteria;Caulobacterales;Caulobacteraceae
	3.97	46.95	Proteobacteria;Betaproteobacteria;Burkholderiales;Alcaligenaceae
	3.00	49.95	Proteobacteria;Betaproteobacteria;Burkholderiales;Burkholderiaceae
	2.89	52.84	Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae
	2.48	55.32	Planctomycetes;Phycisphaerae;Phycisphaerales;Phycisphaeraceae
	2.32	57.64	Proteobacteria;Betaproteobacteria;Burkholderiales;Comamonadaceae
	2.27	59.91	Proteobacteria;Betaproteobacteria;Burkholderiales;Oxalobacteraceae
	2.08	61.99	Proteobacteria;Alphaproteobacteria;Rhizobiales;Xanthobacteraceae
	2.02	64.01	Proteobacteria;Alphaproteobacteria;Rhodospirillales;Acetobacteraceae
	1.40	65.41	Bacteroidetes;Sphingobacteria;Sphingobacteriales;Chitinophagaceae
	1.33	66.74	Proteobacteria;Alphaproteobacteria;Rhizobiales;alphaI cluster
Marine PA Vs Marine FL	12.00	12.00	Proteobacteria;Alphaproteobacteria;SAR11 clade;Surface 4
	9.81	21.81	Proteobacteria;Alphaproteobacteria;Rhodospirillales;Candidatus Alysiosphaera
	7.72	29.53	Proteobacteria;Alphaproteobacteria;SAR11 clade;Surface 1
	7.30	36.83	Bacteroidetes;SB-1
	5.19	42.02	Proteobacteria;Alphaproteobacteria;SAR11 clade;Surface 2
	3.21	45.23	Planctomycetes;OM190
	2.80	48.03	Proteobacteria;Alphaproteobacteria;SAR11 clade;
	2.59	50.62	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
	2.46	53.08	Bacteroidetes;Flavobacteria;Flavobacteriales;Flavobacteriaceae
	2.18	55.26	Proteobacteria;Alphaproteobacteria;Parvularculales;Parvularculaceae
	2.01	57.27	Proteobacteria;Gammaproteobacteria;Alteromonadales;Idiomarinaceae
	1.76	59.03	Proteobacteria;Alphaproteobacteria;Rickettsiales;SAR116 clade
	1.69	60.72	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae
	1.65	62.37	Proteobacteria;Gammaproteobacteria;Oceanospirillales;SAR86 clade
	1.55	63.92	Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae
	1.48	65.40	Verrucomicrobia;Opitutae;vadinHA64

Only groups with an individual contribution of >1% are shown

Table S7. Abundance of identifiable taxonomic paths across the different samples

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Table S8. List of the samples, sampling locations, sampling periods and the respective filtered fractions

<table>
<tr><th colspan="3">Limnic</th><th colspan="3">Marine</th></tr>
<tr><td colspan="3">Lake Stechlin
(53° 10’ N, 13° 02’ E)</td><td colspan="3">Helgoland (Kabeltonne)
(54° 11′ N, 7° 54′ E)</td></tr>
<tr><td>Sampling period</td><td>Layer</td><td>Fraction</td><td>Sampling period</td><td>Layer</td><td>Fraction</td></tr>
<tr><td rowspan="4">Monthly in 2009[a]</td><td rowspan="2">Epilimnion (EL)</td><td>Particle-associated (PA) >5 µm fraction</td><td rowspan="4">Biweekly Sep.-Dec. 2009[c] (’09) Apr.-May 2011[d] (’11)</td><td rowspan="4">Surface waters</td><td>Particle-associated (PA) >10 µm fraction</td></tr>
<tr><td>Free-living (FL) 0.2 - 5 µm fraction</td></tr>
<tr><td rowspan="2">Hypolimnion (HL)</td><td>Particle-associated (PA) >5 µm fraction</td><td rowspan="2">Free-living (FL) 0.2 - 3 µm fraction</td></tr>
<tr><td>Free-living (FL) 0.2 - 5 µm fraction</td></tr>
<tr><td colspan="3">Lake Grosse Fuchskuhle
(53° 10' N 13° 02' E)</td><td colspan="3">Northern Adriatic Sea
Rovinj-Po River delta transect (St.: 107, 105, 103, 101 & 108)
(45° 30′ N, 13° 20′ E - 44° 46′ N, 12° 45′ E)</td></tr>
<tr><td>Sampling period</td><td>Layer</td><td>Fraction</td><td>Sampling period</td><td>Layer</td><td>Fraction</td></tr>
<tr><td rowspan="4">Monthly in 2009[b]</td><td rowspan="2">Northeast basin (NE)</td><td>Particle-associated (PA) >5 µm fraction</td><td rowspan="4">Jul. 2011</td><td rowspan="2">Above thermocline (AT)</td><td>Particle-associated (PA) >10 µm fraction</td></tr>
<tr><td>Free-living (FL) 0.2 - 5 µm fraction</td><td>Free-living (FL) 0.2 - 3 µm fraction</td></tr>
<tr><td rowspan="2">Southwest basin (SW)</td><td>Particle-associated (PA) >5 µm fraction</td><td rowspan="2">Below thermocline (BT)</td><td>Particle-associated (PA) >10 µm fraction</td></tr>
<tr><td>Free-living (FL) 0.2 - 5 µm fraction</td><td>Free-living (FL) 0.2 - 3 µm fraction</td></tr>
</table>

[a]Except in Feb. and Jul.; [b] Except in Nov.; [c] Dates are: Sep.1st & 22nd, Oct.6th & 20th, Nov.3rd & 17th; [d]Dates are: Apr.24th & 28th, May.2nd.

Table S9. Oligonucleotide probes used in this study.

Probe	Target	Reference
ALF968	*Alphaproteobacteria*	(Neef, 1997)
ROS537	*Roseobacter* clade	(Eilers et al., 2001)
SAR11-441R	SAR11 clade (marine group)	(Morris et al., 2002)
LD12-121	SAR11 clade (freshwater group)	(Salcher et al., 2011)
BET42a	*Betaproteobacteria*	(Manz et al., 1992)
OM43-62R	OM43 clade	(Morris et al., 2006)
GAM42a	*Gammaproteobacteria*	(Manz et al., 1992)
ALT1413	*Alteromonas / Colwellia*	(Eilers and Pernthaler, 2000)
PSA184	*Pseudoalteromonas*	(Eilers and Pernthaler, 2000)
SAR86-1245 + helpers	SAR86 clade	(Eilers and Pernthaler, 2000)
NOR5-730 + helpers	NOR5/OM60 clade	(Eilers et al. 2001; Yan et al. 2009)
SAR92-627	SAR92 clade	(Stingl et al., 2007)
SAR324-1412	SAR324 clade	(Schattenhofer et al., 2009)
CF319a	*Bacteroidetes*	(Manz et al., 1992)
PLA42a	*Planctomycetes*	(Neef et al., 1998)
HGC69a	*Actinobacteria*	(Roller et al., 1994)
EUB338 I-III[a]	Bacteria	(Amann & Binder 1990; Daims et al. 1999)
EURY806	*Euryarchaeota* marine group II	(Teira et al., 2004)
NON338	Control	(Wallner et al., 1993)

[a]equimolar concentrations of probes EUB338 I, EUB338 II and EUB338 III

Titles and legends to supplementary figures:

Fig. S1 Abundance (A) and size distribution (B) of particles with a diameter larger than 10 μm in limnic and marine systems. Boxes show 50% quartiles, whiskers 10 and 90 percentiles, and dots 5 and 95 percentiles. Full central line shows median and dashed line shows average.

Fig. S2 Correlation between particle area (x axis) and DAPI counts (y axis); (A) overall and (B) separated into 8 subsystems. Sample name abbreviations: EL and HL - Epilimnion and Hypolimnion of Lake Stechlin, NE and SW - North-Eastern and South-Western basins of Lake Grosse Fuchskuhle, 09 and 11 - Samples collected in 2009 and 2011 on Helgoland, AT and BT - samples collected above and below the thermocline along the Rovinj-Po transect in the Northern Adriatic Sea.

Fig. S3 Bacterial cell density expressed as cells per 1000 μm^3 and plotted per particle volume.

Fig. S4 A model of available space for colonization in limnic and marine particles suggesting difference in available surfaces due to particle structure (A) and inner particle colonization in freshwater systems vs. surface colonization alone in marine systems (B) In our limnic samples both the inner core and the outer surface of the particles might have been colonized, whereas on marine particles colonization had occurred mainly on the surface of a particle. This model shows 4 adjunct spheres of volume V as a limnic particle and a single sphere of volume 4V as a marine particle. The first would offer a surface area 1.6 times greater than the second. As particles are often fractal structures (Richardson et al., 1990), each hypothetical sphere can in turn offer additional sub niches for colonization.

Fig. S5 Distribution of abundances of major phylogenetic groups (CARD – FISH) in 4 limnic (white field) and 4 marine (grey field) subsystems: (A) *Alphaproteobacteria*; (B) *Betaproteobacteria*; (C) *Gammaproteobacteria*; (D) *Bacteroidetes*; (E) *Planctomycetes*; (F) *Actinobacteria*. Boxes show 50% quartiles, whiskers 10 and 90 percentiles, and dots 5 and 95 percentiles. Full central line shows median and dashed line shows average. Sample name abbreviations: EL and HL - Epilimnion and Hypolimnion of Lake Stechlin, NE and SW - North-Eastern and South-Western basins of Lake Grosse Fuchskuhle, 09 and 11 - Samples collected in 2009 and 2011 on Helgoland, AT and BT - samples collected above and below the thermocline along the Rovinj-Po

transect in the Northern Adriatic Sea. PA and FL refer to particle-associated and free-living, respectively.

Fig. S6 Non Metric Multidimensional Scaling analysis of 454 data for Lake Stechlin, and Lake Grosse Fuchskuhle (A), Helgoland (B) and the Northern Adriatic Sea (C). Stress levels for each analysis were 0.14, 0.11 and 0.1, respectively. The data was clustered based on phylogeny at the family level. Distances were calculated with the PAST software using the Bray-Curtis algorithm. ANalysis Of SIMilarity (ANOSIM) results for the data are given in Table S5.

Fig. S7 Sequence frequencies of major phylogenetic groups of free-living (FL) and particle-associated (PA) bacterial communities from limnic and marine systems separated according to the different basins.

Fig. S8 Sequence frequencies of major bacterial taxa at the family level within the different groups, normalized to the total number of sequences in that group. To simplify the image, only families making up over 3% of the sequences of the respective phyla/class in at least one sample are shown. The list of families per groups is given in the legend table. Where no taxonomic information was available at the family level, taxonomy is provided at the level of class (cl) or order (or). The presence or absence of each family within the particle-associated (A) or free-living (F) fractions in Limnic (L) or Marine (M) systems is given by the 4 "+" or "—"symbols next to each family name. A symbol "E" means that a group was enriched in the respective fraction in at least one sample. A red symbol "E" appears in the cases when a group was simultaneously enriched on particles in at least one sample and depleted in other sample. Sample name abbreviations EL, HL – Lake Stechlin Epi- and Hypolimnion, respectively; NE, SW – North East and South West basins of Lake Grosse Fuchskuhle, respectively; 09, 11 – Helgoland 2009 and 2011, respectively; AT, BT – Above and Below the thermocline in the Northern Adriatic Sea, respectively. "PA" and FL" within the sample name refer to particle-associated and free-living fractions, respectively. Curly brackets represent clustering at the nearest lower taxonomy (order or class).

Figure S1.

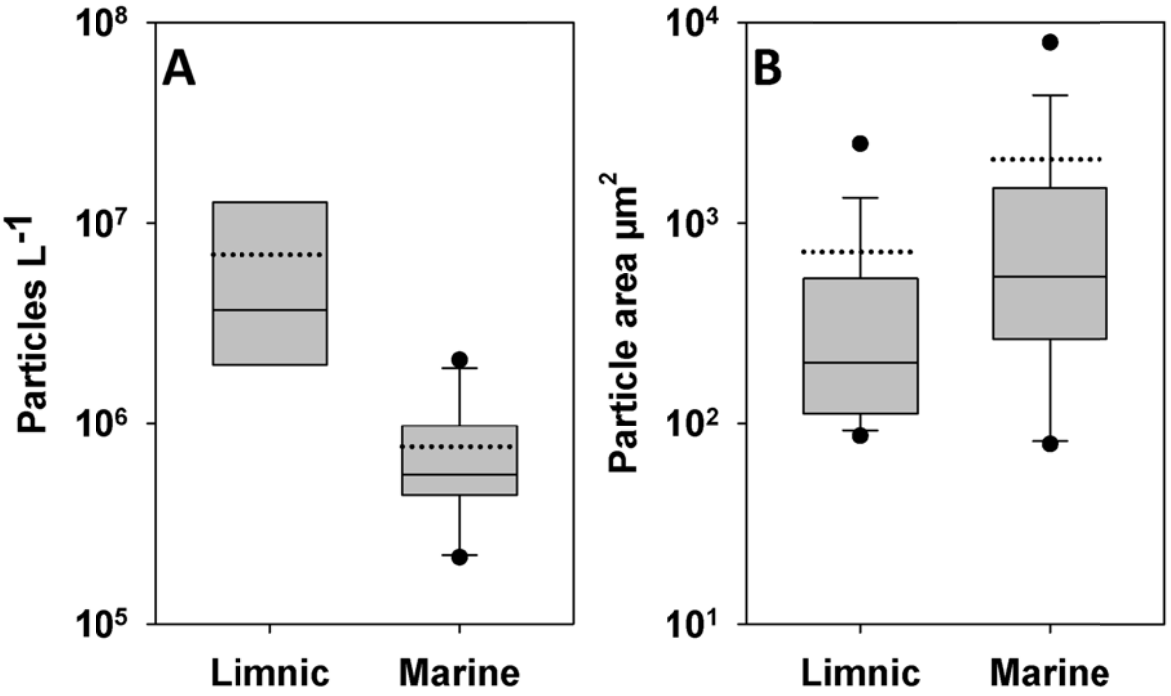


Figure S2.

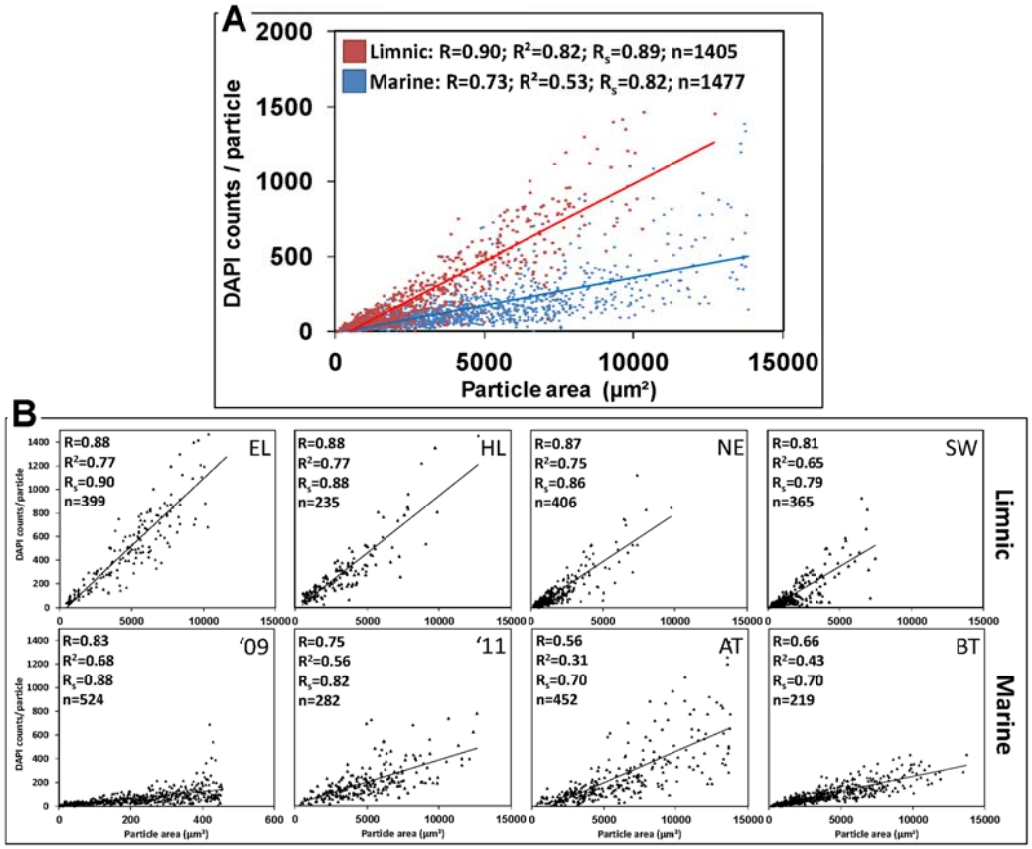
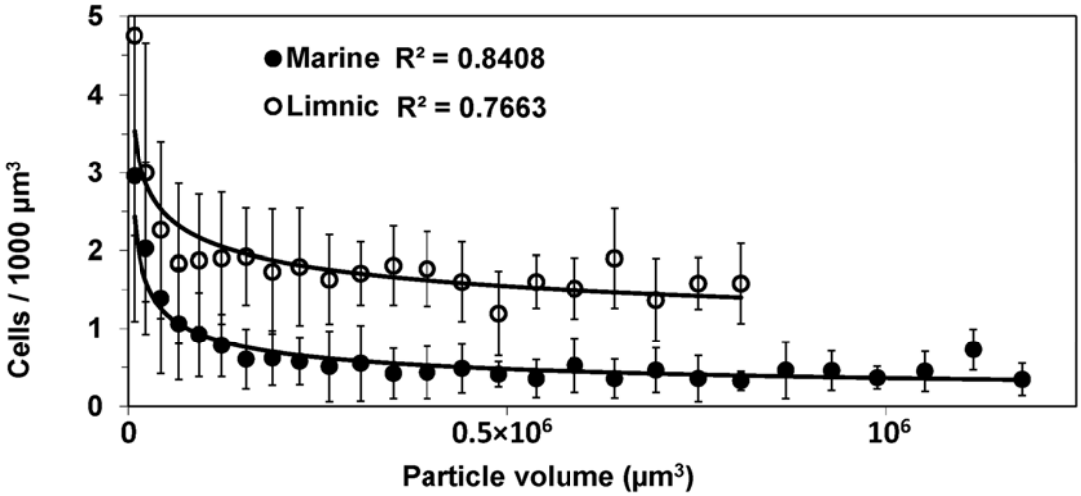


Figure S3.



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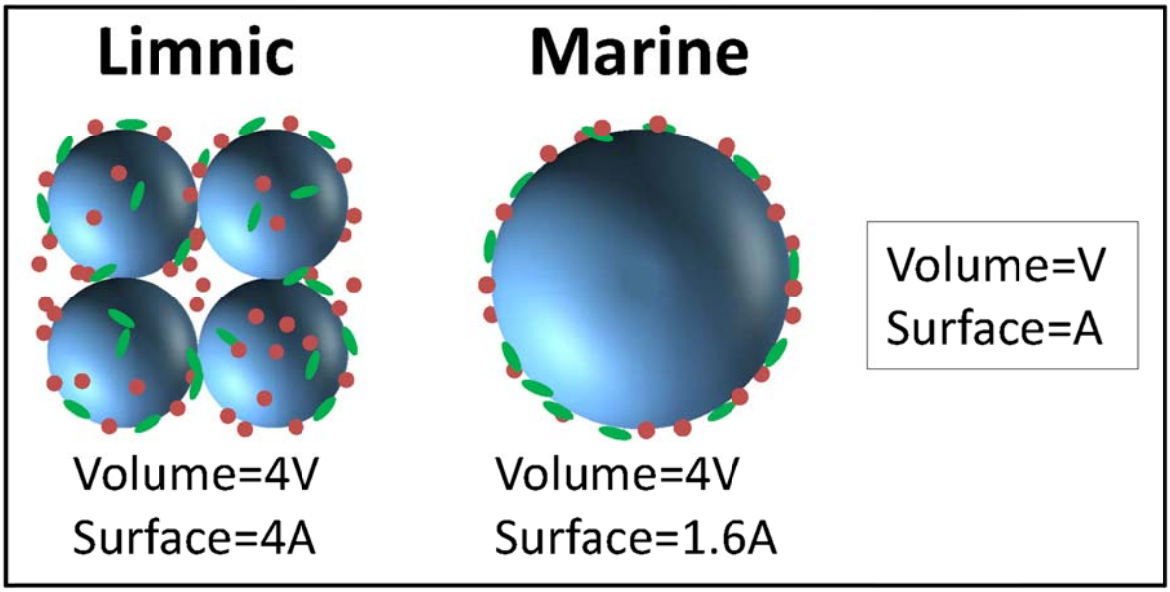


Figure S5.

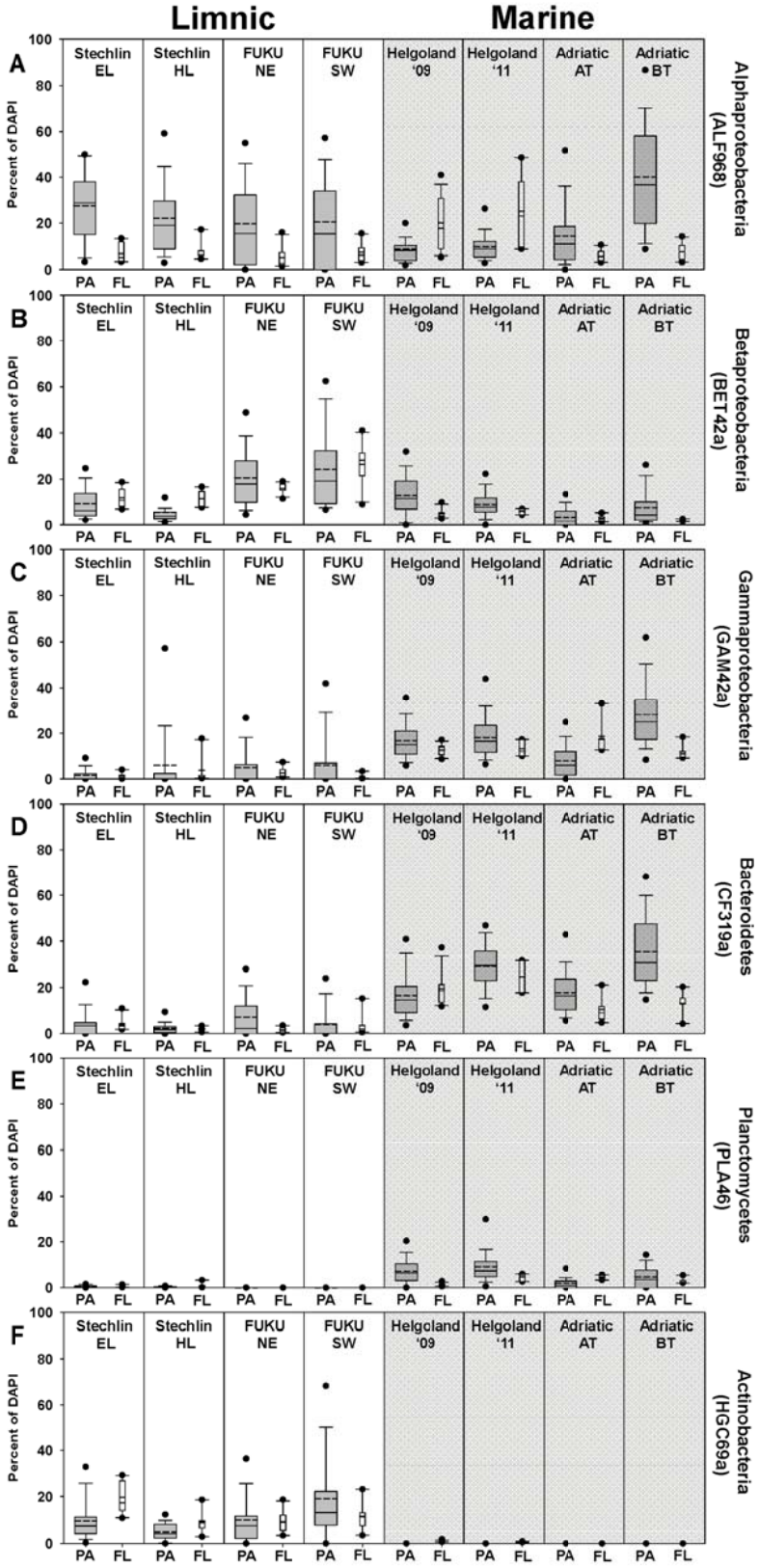

Limnic
Marine
A
B
C
D
E
F
Percent of DAPI
Stechlin EL
Stechlin HL
FUKU NE
FUKU SW
Helgoland '09
Helgoland '11
Adriatic AT
Adriatic BT
PA
FL
Alphaproteobacteria (ALF968)
Betaproteobacteria (BET42a)
Gammaproteobacteria (GAM42a)
Bacteroidetes (CF319a)
Planctomycetes (PLA46)
Actinobacteria (HGC69a)

Figure S6.

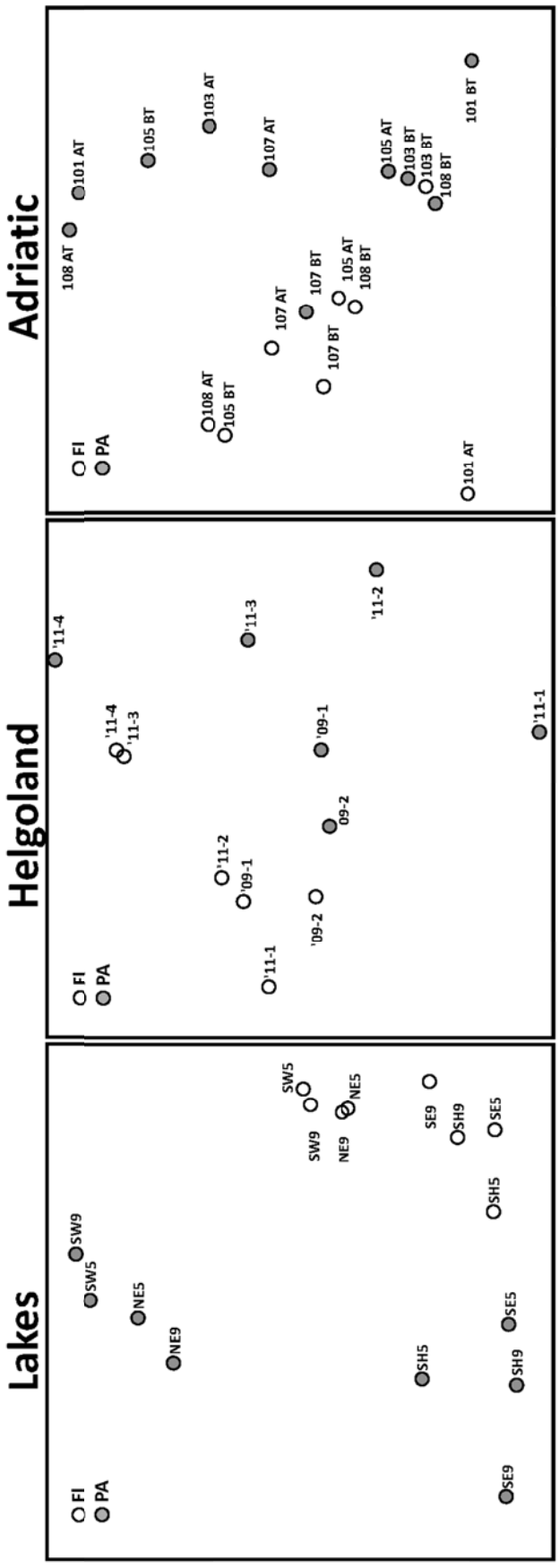

Lakes
Helgoland
Adriatic
FI
PA
SW9
SW5
NE5
NE9
SH5
SE5
SH9
SE9
'11-4
'11-3
'11-2
'09-1
'09-2
'11-1
101 AT
103 AT
105 AT
107 AT
108 AT
101 BT
103 BT
105 BT
107 BT
108 BT

Figure S7.

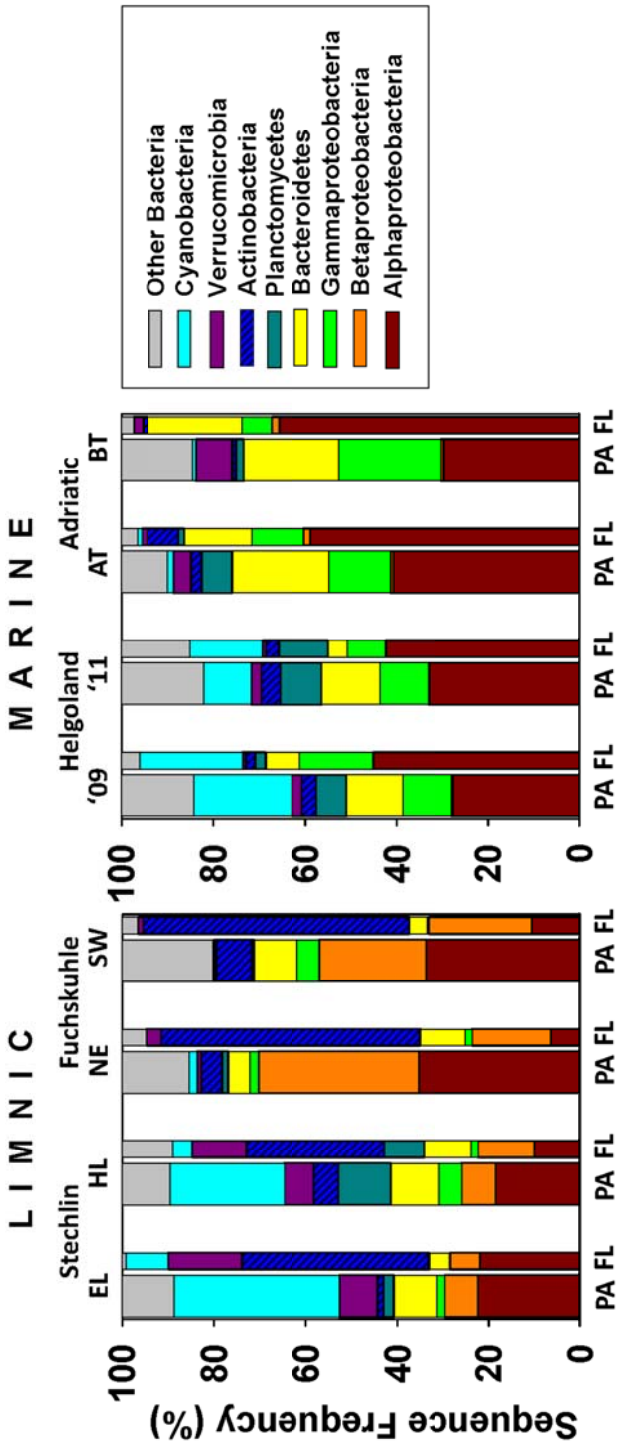
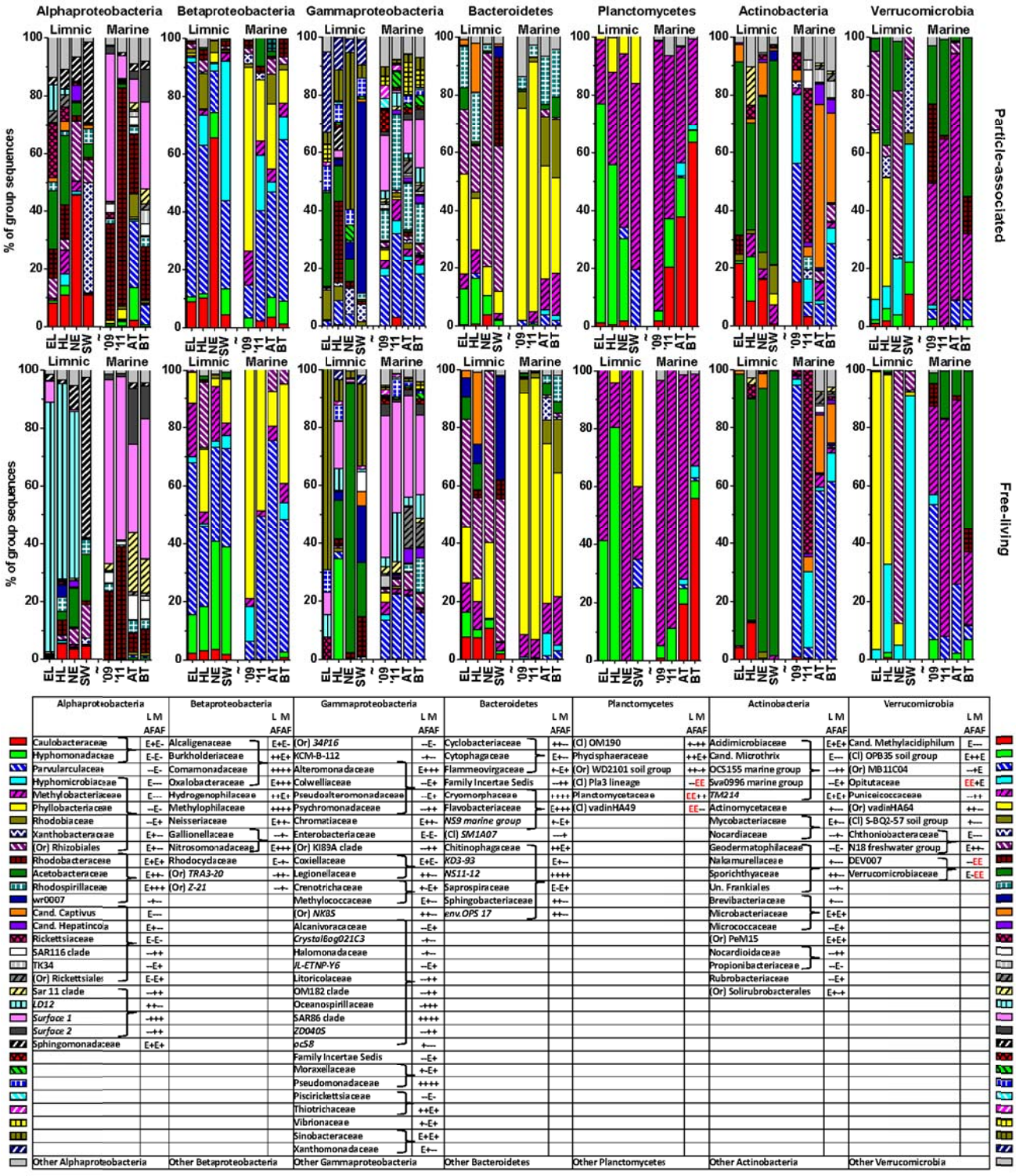

Other Bacteria
Cyanobacteria
Verrucomicrobia
Actinobacteria
Planctomycetes
Bacteroidetes
Gammaproteobacteria
Betaproteobacteria
Alphaproteobacteria
LIMNIC
MARINE
Stechlin
EL
HL
Fuchskuhle
NE
SW
Helgoland
'09
'11
Adriatic
AT
BT
PA FL
Sequence Frequency (%)
0
20
40
60
80
100

Figure S8.


Alphaproteobacteria
Betaproteobacteria
Gammaproteobacteria
Bacteroidetes
Planctomycetes
Actinobacteria
Verrucomicrobia
Limnic
Marine
% of group sequences
Particle-associated
Free-living
EL HL NE SW ~ '09 '11 AT BT
Caulobacteraceae
Hyphomonadaceae
Parvularculaceae
Hyphomicrobiaceae
Methylobacteriaceae
Phyllobacteriaceae
Rhodobiaceae
Xanthobacteraceae
(Or) Rhizobiales
Rhodobacteraceae
Acetobacteraceae
Rhodospirillaceae
wr0007
Cand. Captivus
Cand. Hepatincola
Rickettsiaceae
SAR116 clade
TK34
(Or) Rickettsiales
Sar 11 clade
LD12
Surface 1
Surface 2
Sphingomonadaceae
Alcaligenaceae
Burkholderiaceae
Comamonadaceae
Oxalobacteraceae
Hydrogenophilaceae
Methylophilaceae
Neisseriaceae
Gallionellaceae
Nitrosomonadaceae
Rhodocyclaceae
(Or) TRA3-20
(Or) Z-21
(Or) 34P16
KCM-B-112
Alteromonadaceae
Colwelliaceae
Pseudoalteromonadaceae
Psychromonadaceae
Chromatiaceae
Enterobacteriaceae
(Or) KI89A clade
Coxiellaceae
Legionellaceae
Crenotrichaceae
Methylococcaceae
(Or) NKB5
Alcanivoraceae
Crystal6og021C3
Halomonadaceae
IL-ETNP-Y6
Litoricolaceae
OM182 clade
Oceanospirillaceae
SAR86 clade
ZD0405
pc58
Family Incertae Sedis
Moraxellaceae
Pseudomonadaceae
Piscirickettsiaceae
Thiotrichaceae
Vibrionaceae
Sinobacteraceae
Xanthomonadaceae
Cyclobacteriaceae
Cytophagaceae
Flammeovirgaceae
Family Incertae Sedis
Cryomorphaceae
Flavobacteriaceae
(Cl) SM1A07
Chitinophagaceae
KD3-93
WS11-12
Saprospiraceae
Sphingobacteriaceae
env.OPS 17
(Cl) OM190
Phycisphaeraceae
(Or) WD2101 soil group
(Cl) Pla3 lineage
Planctomycetaceae
(Cl) vadinHA49
Acidimicrobiaceae
Cand. Microthrix
OCS155 marine group
Sva0996 marine group
TM214
Actinomycetaceae
Mycobacteriaceae
Nocardiaceae
Geodermatophilaceae
Nakamurellaceae
Sporichthyaceae
Un. Frankiales
Brevibacteriaceae
Microbacteriaceae
Micrococcaceae
(Or) PeM15
Nocardioidaceae
Propionibacteriaceae
Rubrobacteriaceae
(Or) Solirubrobacterales
Cand. Methylacidiphilum
(Cl) OPB35 soil group
(Or) MB11C04
Opitutaceae
Puniceicoccaceae
(Or) vadinHA64
(Cl) S-BQ2-57 soil group
Chthoniobacteraceae
N18 freshwater group
DEV007
Verrucomicrobiaceae
Other Alphaproteobacteria
Other Betaproteobacteria
Other Gammaproteobacteria
Other Bacteroidetes
Other Planctomycetes
Other Actinobacteria
Other Verrucomicrobia

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Manuscript II

Massive regime shifts and high activity of heterotrophic bacteria in an ice-covered lake

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Massive regime shifts and high activity of heterotrophic bacteria in an ice-covered lake

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Short title: Bacterial bloom in an ice-covered lake

Classification: Biological sciences (Environmental biology, microbiology, ecology)

Significance statement:

Under-ice heterotrophic bacterial activity is considered to be insignificant due to low temperatures and productivity, and therefore is often excluded from seasonal studies. Using a high temporal resolution limnic case study, we demonstrate that under-ice bacterial activity is carbon rather than temperature limited. During the decay of an under-ice cyanobacteria bloom (*Aphanizomenon flos-aquae*), bacterial activity was much higher than throughout the preceding year. This event was accompanied by a massive regime shift in bacterial community composition and the appearance of a few specialized bacterial phylotypes. Under-ice phytoplankton blooms are frequent in temperate and

polar aquatic ecosystems and thus their bacterial degradation has profound consequences for biogeochemical fluxes and biodiversity. To better understand and predict climate change induced alterations in biology and biogeochemistry on a global scale, year-round studies of aquatic ecosystems are needed.

Abstract:

In winter 2009/10, a sudden under-ice bloom of heterotrophic bacteria occurred in the seasonally ice-covered, temperate, deep, oligotrophic Lake Stechlin (Germany). Extraordinarily high bacterial abundance and biomass were fueled by the breakdown of a massive bloom of *Aphanizomenon flos-aquae* after ice formation. A reduction in light resulting from snow coverage exerted a pronounced physiological stress on the cyanobacteria. Consequently, these were rapidly colonized, leading to a sudden proliferation of heterotrophic attached and subsequently of free-living bacteria. Total bacterial protein production reached 201 μg C l^{-1} d^{-1}, ca. five times higher than spring-peak values that year. Fluorescence in situ hybridization and denaturing gradient gel electrophoresis at high temporal resolution showed pronounced changes in bacterial community structure coinciding with changes in cyanobacteria physiology. Pyrosequencing of 16S rRNA genes revealed that during cyanobacteria breakdown diversity of attached and free-living bacterial communities were reduced to a few dominant families. Some of these were not detectable during the early stages of the cyanobacterial bloom indicating that only specific, well adapted bacterial communities can colonize senescent cyanobacteria. Our study suggests that in winter, unlike commonly postulated, carbon rather than temperature is the limiting factor for bacterial growth. Frequent phytoplankton blooms in ice-covered systems highlight the need for year-round studies of aquatic ecosystems including the winter season to correctly understand element and energy cycling through aquatic food webs, particularly the microbial loop. On a global scale, such knowledge is required to determine climate change induced alterations in carbon budgets in polar and temperate aquatic systems.

Keywords: *Aphanizomenon flos-aquae* / Bacterial community composition (BCC) / Bacterial production / Climate change / *Flavobacteriaceae* / Tag pyrosequencing

Introduction:

On a global scale ice covers, either seasonally or permanently, up to 8.5% and 8.4% of saline and freshwater bodies (not including the Southern hemisphere), respectively (*1*, *2*). While under-ice phytoplankton blooms are common phenomena (e.g. (*3*, *4*)), little is known about the activity of heterotrophic bacteria during and following such blooms (*5*). Although lakes cover a little more than 3% of Earth surface as compared to 71% by oceans and seas, they have an equal contribution to CO_2 emissions (*6*) and a larger contribution to carbon burial (*7*). Therefore, it is surprising that annual studies of freshwater systems often neglect the entire period of ice coverage. Using a well-studied limnic system (Lake Stechlin, Germany), we bring forth evidence for the quantitative significance of heterotrophic bacterial activity due to rapid mineralization of phytoplankton biomass in ice-covered aquatic systems. Our findings thus relativize and extend recent findings on the rapid sinking of under-ice phytoplankton blooms in the Arctic Ocean (*4*).

It is believed that as a result of low temperatures and reduced nutrient remineralization due to low protozoan grazing, microorganisms under ice are killed, harmed or exist in a dormant state (*5*, *8*, *9*). However, estimated biomass turnover for the overall bacterial communities from these studies usually ranges between 4-10 days indicating that winter microbial communities are actively growing and not just dormant. Although seasonally ice-covered lakes are greatly understudied in the cold season, there is increasing evidence that winter dynamics of the microbiome is important for understanding bacterial community succession in the following ice-free period (*5*, *10*).

Powerful molecular tools have recently enabled systematic explorations of the diverse and largely uncultivable bacterioplankton communities (*11*) including the winter season (e.g. (*12*)). So far, only a few studies (*13–15*) have focused on the bacterial community composition of temporary ice-covered lakes during the winter season. These studies reveal a typical winter bacterioplankton community, which is distinct from that during the warm season indicating specific adaptation to environmental conditions in winter, e.g. little light, low temperatures and OM concentrations, and high inorganic nutrient availability during winter mixing and early ice cover stages.

In this study we followed a sudden and massive under-ice proliferation of heterotrophic bacteria in oligotrophic Lake Stechlin following the breakdown of an under-ice bloom of the low light-adapted cyanobacterium *Aphanizomenon flos-aquae* in March 2010. Based on this phenomenon during which OM became suddenly available at high concentrations in the cold season, we hypothesize that winter bacterial activity and community composition are mainly regulated by OM availability and subsequent protozoan grazing, and only to a lesser extent by low water temperatures as has been traditionally suggested.

Results:

Environmental conditions: In winter 2009/10, ice on the surface of Lake Stechlin first appeared at the end of December. Throughout January, the lake was covered by a thickening layer of clear ice. The ice (~30 cm thick) remained clear until mid-February, but thereafter the ice sheet was covered by snow. Thermal stratification under the ice was inverted with water <1 °C in the upper 5 m. Between 5 and 20 m the temperature increased to 3.1°C (Fig. 1A). Below 20 m the temperature increased gradually, but remained below 4°C. The ice-off occurred in the second half of March.

Oxygen was overall below 100% saturation (450 and 405 μmol L^{-1} in surface and bottom water, respectively) throughout the ice-coverage period with a background of 75% saturation (Fig. 1B). Until the end of February 2010, O_2 saturation level in the upper 4-5 m increased to maximal 95% following a surface bloom of the low light-adapted cyanobacterium *Aphanizomenon flos-aquae* (*16*). Thereafter, the O_2 saturation levels in the same water stratum decreased to 75% in early March (Fig. 1B) when the ice was covered by a thick layer of snow. The increase and decrease in O_2 saturation levels during February to March 2010 are accompanied by a respective increase and decrease in pH values (Fig. S1).

Cyanobacteria dynamics: Two cyanobacterial blooms were observed in Lake Stechlin in 2009, during September (*A. flos-aquae* and *Anabaena macrospora*) and December (*A. flos-aquae*), respectively (*16*). The second bloom, initiated by the mixing of the water column

in late fall, started in November and peaked during December. Although *A. flos-aquae* in Lake Stechlin forms heterocysts in summer, no heterocysts were visible in any of the analyzed filaments collected in winter (n >200 filaments), fitting data on available combined nitrogen (20 μmol L^{-1} NO_3; (*16*)). The second bloom persisted during the clear-ice period of the lake (December to mid-February). As the ice became covered by snow reducing the incident light to 40 and 5 μE at 1 and 5 m, respectively (*16*), the cyanobacteria were physiologically stressed (*16*) and a sudden bloom of heterotrophic particle-attached (PA) and subsequently free-living (FL) bacteria occurred.

Therefore, we divided the sampling period during lake ice coverage into two phases. Phase I (January 28th-February 22nd) comprises the late bloom of *A. flos-aquae*. Phase II (February 23rd-March 16th) depicts the breakdown of the cyanobacterial bloom and the sudden rise of heterotrophic bacteria. Differences between Phases I and II are evident from micrographs of DAPI and FISH stained cyanobacterial filaments collected from the epilimnion (Fig. 2). First, the bloom of *A. flos-aquae* consists of intact filaments that contain poly-P granules and are rarely colonized by heterotrophic bacteria (Fig. 2A). Subsequently, in Phase II damaged filaments with "ghost cells" are often aggregated and the poly-P granules are no longer associated with the filaments. These aggregates are heavily colonized by heterotrophic bacteria (Fig 2B).

In the epilimnion (0-10 m) Bacterial Protein Production (BPP) of PA and FL bacteria, increased abruptly (5 to 183 and 8 to 58 μg C l^{-1} d^{-1}, respectively) from Phase I to Phase II (Fig. 3A). This indicates that BPP can be very high at temperatures <1 °C as compared to the rest of the year in Lake Stechlin or to other aquatic ecosystems (*17*). The increase in BPP in Phase II was first seen in the FL fraction, but was followed by a larger increase in BPP of the PA fraction. The subsequent breakdown of the *A. flos-aquae* bloom led to a sudden rise in abundance of heterotrophic bacteria from 1 x 10^6 to 3.25 x 10^6 cells mL^{-1} (Fig. 3B). At the end of Phase II BPP rapidly decreased to 48 and 22 μg C l^{-1} d^{-1} in the PA and FL fractions, respectively, still several folds higher than at the end of January when the lake surface was covered by clear ice. BPP eventually decreased as the number of bacterial cells was reduced to 0.85 x 10^6 cells mL^{-1}, parallel to the increase in abundance of heterotrophic flagellates.

The sudden increase in bacterial abundance and activity were accompanied by a similarly rapid shift in Bacterial Community Composition (BCC) as evident from Denaturing Gradient Gel Electrophoresis (DGGE), Catalyzed Reporter Deposition Fluorescence In Situ Hybridization (CARD-FISH) and tag pyrosequencing results (Fig, S2; 3B; 4; 5, respectively). The swiftness of the change is best observed by high temporal resolution DGGE fingerprinting, showing the presence of two, stable, yet different communities in Phase I and II of the bloom (Fig. S2). In Phase I, members of *Alphaproteobacteria*, *Betaproteobacteria, Bacteroidetes* and *Planctomycetes* (11% (0-40%), 16% (0-33%), 7% (0-19%) and 3% (0-28%), respectively) were associated with *A. flos-aquae* cells (Fig. 4). In Phase II, we observed a simultaneously strong increase in relative abundances of *Betaproteobacteria* and *Bacteroidetes* (54% (4-88%) and 31% (4-63%), respectively). These were also the only two bacterial groups associated with *A. flos-aquae* at that time. *Alphaproteobacteria* and *Actinobacteria* (on average 24% and 44%, respectively) dominated the FL fraction in Phase I, but were replaced in Phase II by a dominance of *Bacteroidetes* and *Betaproteobacteria* (on average 49% and 23%, respectively) (Fig, 3B; 4). Comparative 16S rRNA gene sequencing of pooled PA and FL fractions of both the epi- and hypolimnion showed that the transition between Phase I and II resulted not only from a change in the bacterial groups, as already implied by CARD-FISH (Fig. 3B; 4), but also from a strong decrease in the number of dominant bacterial families, and the expansion of a few groups of specialist bacteria (Fig. 5). In Phase I, 50% of PA bacteria in the epilimnion belonged to 5 major families while the rest was distributed among 43 others. In contrast, in Phase II, 96% of the PA bacteria were dominated by 3 families alone: *Flavobacteriaceae* (21%), *Comamonadaceae* (27%) and *Oxalobacteraceae* (48%). While the first two families dominated the PA fraction in the epilimnion during Phase I, *Oxalobacteraceae* contributed to <0.5% of the BCC (Table S1). During the transition from Phase I to Phase II, FL bacteria in the epilimnion showed a similar succession as PA bacteria resulting in a decreased bacterial diversity. These changes in PA and FL BCC did not occur simultaneously. A succession between the PA and FL fractions is obvious from the DGGE fingerprinting, as several bands appeared first on particles and only later in the surrounding water (Fig. S2).

Our results indicate that the hypolimnion (20 m) was affected by the events in the epilimnion only towards the end of Phase II. Total bacterial numbers (FL) in the hypolimnion (20 m) were <0.5 x 10^6 cells mL^{-1} throughout the entire period. Accordingly, BPP was much lower than in the epilimnion (1-9 and 0.1-12 μg C l^{-1} d^{-1} in PA and FL, respectively) during the whole period and only slightly increased at the end of Phase II (Fig. 3A). This indicates that solely a small fraction of cyanobacterial cell debris reached this depth, and that the cyanobacterial biomass was efficiently remineralized in the epilimnion.

Discussion:

In temperate lakes, persistent cyanobacterial blooms frequently occur in winter under the ice (14, 15; Table S2). However, most ice-covered (meso-) oligotrophic lakes such as Lake Stechlin have been considered to be low carbon environments in winter (*5*). This is mainly because of two factors: 1) Primary productivity is usually low due to decreased light availability under the ice; 2) Input of atmospheric and terrestrial OM is blocked by the ice coverage and the available internal OM is rapidly consumed by the residual heterotrophic bacterial community. Accordingly, a low BPP was measured during Phase I of the cyanobacterial bloom. Coverage of Lake Stechlin with clear ice, however, allowed for penetration of sufficient light which together with a stable inverse stratification of the water column sustained growth of low temperature- and low light-adapted *A. flos-aquae* (*16*) in the cold epilimnion (<4°C).

Bacterial numbers and BPP remained low in Lake Stechlin in Phase I. At this time inorganic nutrients necessary for the blooming of *A. flos-aquae* were abundant after the mixing in early winter, including nitrogen as indicated by the lack of heterocysts and phosphorus by storage in form of poly-P granules. The high growth potential of the winter bacterioplankton - irrespective of temperature - became evident when *A. flos-aquae* became light limited (*16*) due to low irradiance under the snow-covered ice in Phase II. The sudden increase in bacterial abundance and BPP in the epilimnion during the transition from Phase I to Phase II in parallel to the relatively high availability of inorganic nutrients support the hypothesis that the winter bacterioplankton in Lake

Stechlin is controlled by carbon availability rather than temperature. The physiologically stressed cyanobacteria were rapidly colonized and degraded by heterotrophic bacteria leading to an extremely high bacterial abundance and BPP in Phase II. The degradation of cyanobacterial filaments resulted in the release of the protoplasmic content into the surrounding water leaving empty "ghost cells" and increasing OM availability also for FL bacteria. Although temperature remained <4 °C in the epilimnion in Phase II, high inorganic nutrient availability and the relief from carbon limitation led to an extreme increase in BPP, which was >5 times higher than measured during the peak of the subsequent spring bloom (*20*). From this notion it is evident that periodical events such as the under ice *A. flos-aquae* bloom in Lake Stechlin lead to high microbial OM turnover in winter when concentrations of herbivorous zooplankton are low (*5*). This suggests that availability of OM rather than temperature triggered BPP leading to a rapid mineralization of the phytoplankton biomass in ice-covered Lake Stechlin counter-acting its sinking and burial in the sediment. Thus, the rapid sinking of under-ice phytoplankton blooms in cold environments (*4*) should be seen in an environmental context and should not be considered as a general rule.

It can be assumed that the high heterotrophic activities measured in Phase II have the potential to strongly affect C cycling, particularly the release of CO_2 from the lake into the atmosphere after ice-off. CO_2 concentrations under the ice gas-barrier can exceed summer values (*21*) reaching concentrations of up to 650 μmol L^{-1} and often 2-3 folds higher than during the ice-free period (*22*). Hence, the resultant CO_2 buildup can be rapidly released to the atmosphere upon ice-off. So far, under ice blooms of heterotrophic bacteria have been neglected for modeling of metabolism and element cycling in lakes. However, Karlsson et al. (*23*) suggest that up 55% of the annual gas emissions from freshwater systems occur during ice-off. To better understand and predict environmental consequences of the biogeochemistry of lakes, year-round studies are required to also include massive under ice blooms of photoautotrophic and heterotrophic microorganisms. These may also set the frame for the subsequent food web development during the growing season (*5*).

High availability of OM during bacterial colonization and lysis of *A. flos-aquae* cells also dramatically affected biodiversity reflected by a temporal shift in BCC. Our DGGE

fingerprints suggest a sudden change of BCC between Phase I and II. The uniformity in quality of the available OM in Phase II of the bloom was indicated by the dominance of solely a small group of specialized bacteria different from those in Phase I. These bacteria first appeared in the epilimnic PA fraction and later in the FL fraction of the epilimnion and in the PA fraction of the hypolimnion. This suggests that a) bacterial colonization of senescent cyanobacteria resulted in detachment of bacteria and release of substrates into the surrounding water supporting the proliferation of these communities; b) a small fraction of colonized *A. flos-aquae* aggregates sank into the hypolimnion. The latter process was reflected by significant changes in BCC of hypolimnic PA bacteria greatly reducing microbial diversity in this fraction.

These shifts in microbial communities presented a striking limnic under-ice analogue to the substrate-driven succession of marine bacterioplankton populations induced by phytoplankton blooms recently described by Teeling et al. (*24*). Although temperatures and salt content greatly differed between the two studies, the *Flavobacteriaceae* family, known to degrade high-molecular-weight OM (*25*), were among the first to colonize the *A. flos-aquae* cells. Nevertheless, while the marine event harbored numerous genera from the *Flavobacteriaceae*, *Flavobacterium* was the sole genus detected under the ice (Fig. 5). In the marine analogue *Gammaproteobacteria* and to some extent *Alphaproteobacteria* succeeded the *Flavobacteriaceae*. In contrast, under-ice, *Betaproteobacteria*, specifically members of the genera *Undibacterium* and *Massilia* as well as an uncultured group of *Comamonadaceae* (Fig. 5) followed the initial colonization and rapidly exceeded the abundance of *Flavobacterium* (Fig. 4). We propose that the betaproteobacterial clades detected in this study fill in similar ecological niches in freshwater as the marine *Gammaproteobacteria* associated with a decaying algal bloom in Teeling et al. (*24*). Interestingly, both blooms independent of water temperature had a similar carrying capacity, leading to an increase in FL bacterial abundance from 0.5×10^6 to 3×10^6 cells mL^{-1}. This maximal carrying capacity could be a result of a protozoan top-down control similar to that observed in Lake Stechlin.

In our study, available OM affects the BCC and BPP. While the temperature in the epilimnion remains constant <1 °C throughout the period, and therefore can be excluded as an influencing factor, OM increases dramatically in Phase II due to

cyanobacterial decay. Based on our DGGE results, the BCC remained stable during Phase I (for at least 2 months) in parallel to a rather stable BPP. Nevertheless, during the rapid transition between Phase I and II of the bloom, while temperature remained constant, we observed a major change in BCC accompanied by large increase in activity. The initially low abundant *Flavobacteriaceae* in Phase I appeared in larger numbers in Phase II and the previously diverse *Comamonadaceae* were reduced to 3 genera solely. These phylotypes included cold adapted species such as *Flavobacterium psychrolimnae*, *F. fryxellicola*, *F. limicola*, *F. omnivorum*, *F. frigidarium* that are often reported from ice-covered aquatic systems (*26–29*). The bacterial phylotypes in Phase II grew rapidly and were able to degrade the cyanobacterial cells to a large extent. This suggests that availability of OM not only triggered BPP, but also succession in BCC.

Under-ice blooms of phytoplankton are not sporadic events since, during the last decades, they have been regularly described from limnic environments and more recently also from marine systems (Table S2). Using Lake Stechlin as an example, we show that temperate lakes bear the potential for high biological activity even during the cold winter season. Despite the low temperature (<4°C), carbon flow from primary production and, particularly, from cyanobacteria biomass during bloom breakdown has the potential to fuel massive blooms of heterotrophic microorganisms. These were soon top-down controlled in the epilimnion by heterotrophic nanoflagellates, demonstrating that also protozoa growth was less dependent on water temperatures than on prey availability. Consequently, under ice blooms of photoautotrophic and heterotrophic microorganisms greatly affect the lake's biogeochemistry and thus set the frame for further food web dynamics in the growing season.

Current climate change leads to a reduced intensity of ice coverage and hence to dramatic environmental changes with so far unknown ecological consequences, e.g., for biogeochemical processes such as greenhouse gas fluxes across the water-atmosphere boundary. Limnic systems are known to be supersaturated with greenhouse gases (*30*). While global CO_2 emissions from freshwater have been estimated to 0.75 Pg y^{-1} (*31*) (equal to oceanic emissions (*6*)), these estimates do not include emissions following ice-off. Sediments of freshwater systems store 3 times more carbon (0.3 Pg y^{-1}) than marine ones (0.1 Pg y^{-1}) (~150 times more when normalized to area) (*7*). Yet, this figure does

not include the unresolved fate of massive under-ice blooms in seasonally ice covered lakes. Since most of the OM in lakes is of autochthonous origin (*7*), the response of the heterotrophic bacterial community to massive under ice phytoplankton blooms potentially determines whether the lake acts as a C sink or a source. To better understand the role of lakes in global biogeochemical cycles and biodiversity throughout the whole season, it will be crucial to perform year-round studies with a greater focus on the winter season, which independent of the low temperature has the potential for high biological activities as long as phototrophic OM remains available for heterotrophic processing. Such studies should have a high temporal sampling resolution (as can be achieved by automatic sampling or monitoring) since as demonstrated in our study the entire bloom event can last less than two weeks. The short duration of such dynamic events points to the fact that monthly routine sampling is very likely to miss such events and that even in winter, boosts in biological activities and OM turnover occur. Extending earlier studies, e.g. those from Lake Baikal (*32*), usually covered by clear ice, we here suggest that energy and substrate availability rather than temperature are the key factors controlling dynamics of aquatic microorganisms. The dimensions of these carbon driven heterotrophic blooms are not only important for understanding their present ecological role in aquatic ecosystems, particularly when taking into account that in the future sea ice will rapidly reside (*33*) and perennially ice-covered aquatic ecosystems may fully disappear (*6*).

Materials and Methods:

Description of study site: Lake Stechlin (53'09 N, 13'02 E) is a seasonally ice-covered meso-oligotrophic freshwater lake formed after the last glacial period in northern Europe (Weichselian). With an area of 4.23 km^2 and a maximum depth of 69.5 m, this relatively small, lake is one of the deepest lakes of the Northeastern German Lowlands (*34*). Similar to other temperate deep lakes, Lake Stechlin overturns in spring and fall and thus is classified as a dimictic lake (*35*).

Sampling procedure: Our main focus was the ice-covered period and the microbial community residing beneath the ice. Samples were collected between 10th December

2009 and 30th March 2010 from the epilimnion (integrated from 0-10 m depth) and the hypolimnion (distinct depth of 20 m). They were processed for microscopy, bacterial protein production (BPP), catalyzed reporter deposition fluorescence in situ hybridization (CARD-FISH), denaturing gradient gel electrophoresis (DGGE) and tag pyrosequencing. Temperature, oxygen, pH and conductivity were measured with probes (WTW, Weilheim, Germany) to monitor thermal stratification and determine the potential cyanobacterial bloom period. BPP was determined by [^{14}C] - leucine incorporation in triplicates by in situ incubation for 1 h as described in Rösel & Grossart (*20*).

For CARD-FISH, water samples were fixed for 24 h at 4°C with formaldehyde (1% final concentration). Subsequently, aliquots were separated to determine particle-attached (PA) and free-living (FL) bacteria by sequential filtration through polycarbonate filters (5 and 0.2 µm pore size, respectively [Millipore, Eschborn, Germany]) as previously described in (*13*). CARD-FISH was done according to Pernthaler et al. (*36*) with minor modifications as described in Pizzetti et al. (*37*). All probes used in this study are given in Table S3. Automated image acquisition and analysis was done as previously described in Zeder et al. (*38*). Shortly, analysis of samples of FL bacteria was performed in a fully automated manner using a multi-purpose imaging system (MPISYS, modified after Zeder et al. (*38*) based on a fully motorized epifluorescence microscope (AxioImager.Z2m, Zeiss, Jena, Germany). To analyze PA bacteria the MPISYS was ran in a semi-automated mode as the density of particles varied from sample to sample. Upon selection, the system acquired a z-stack of 25 images around the focused position with an interval of 0.3 µm for each channel. For cell quantification, a maximum intensity projection was calculated on each channel. Following acquisition FL and PA sample images were analyzed using ACMEtool2 (www.technobiology.ch).

Samples for DNA extraction were size-fractionated immediately after sampling and stored at -80 °C for further processing. DNA from 20 epilimnic and 20 hypolimnic samples (10 PA and 10 FL per strata) (Table S4) was extracted as described by Ionescu et al. (*39*). To get the temporal resolution of the bacterial community composition (BCC) DNA extracts were analyzed by DGGE as detailed in Rösel et al. (*13*). Samples for tag pyrosequencing were then selected based on changes in BCC during the ice-coverage. A

total of 32 epilimnic and hypolimnic samples of PA and FL bacteria were pooled into 8 representative samples (Table S4). Tag pyrosequencing for bacterial diversity, using primer sets 28F and 519R (*40*), was done by MrDNA (Shallowater, Texas), using a Roche 454 FLX Genome Sequencer system equipped with titanium technology.

Sequence Analysis: Sequences were analyzed as previously described by Ionescu et al. (*39*), using the bioinformatics pipeline of the SILVA rRNA gene database project and the SILVA SSURef dataset (release 111; (*41*)). Diversity and community structure analyses were performed based on >132,000 bacterial sequences. A detailed summary of the analysis process of the pyrosequencing data of each sample, including the total number of reads and length distribution, as well as the results of quality management, dereplication and clustering, can be found in the supplement (Table S5). A detailed list of the final taxonomic affiliation of all analyzed sequences together with their relative abundances within the amplicon pool is given in Table (S1) (Bacteria).

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Figure 1. Thermal stratification (A), and oxygen saturation (B) profiles during the Phase I and Phase II of the bloom. Circles represent sampling points. Data is available at a higher spatial resolution (empty circles) only during Phase II of the bloom.

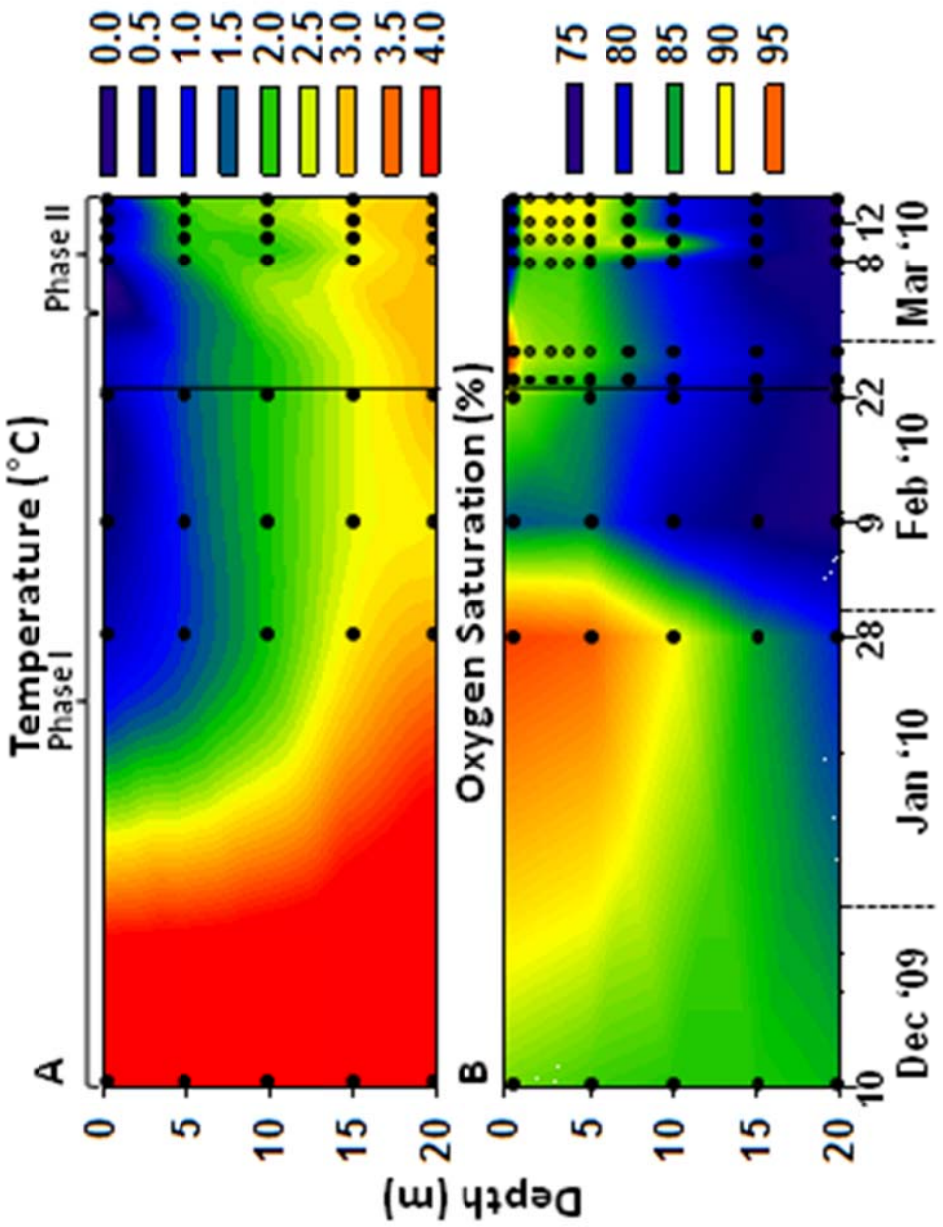


Figure 2. Micrographs of DAPI and FISH stained *Aphanizomenon flos-aquae* filaments and heterotrophic bacteria, respectively; collected from the epilimnion during Phase I (A) and Phase II (B). Cyanobacteria in Phase I are found in tufts (A1), the filaments contain poly-P granules (A2) and are hardly colonized by heterotrophic bacteria (A3). In Phase II single filaments with ghost cells are visible (B1). These are often aggregated with poly-P granules spilled onto the aggregate (B2) and heavily colonized by heterotrophic bacteria (B3). Significant increase in colonization between *Betaproteobacteria* and *Flavobacteria* in Phase I (A4-5) and Phase II (B4-5) is visible. End of Phase II is characterized by an increase in nanoflagellates (B6).

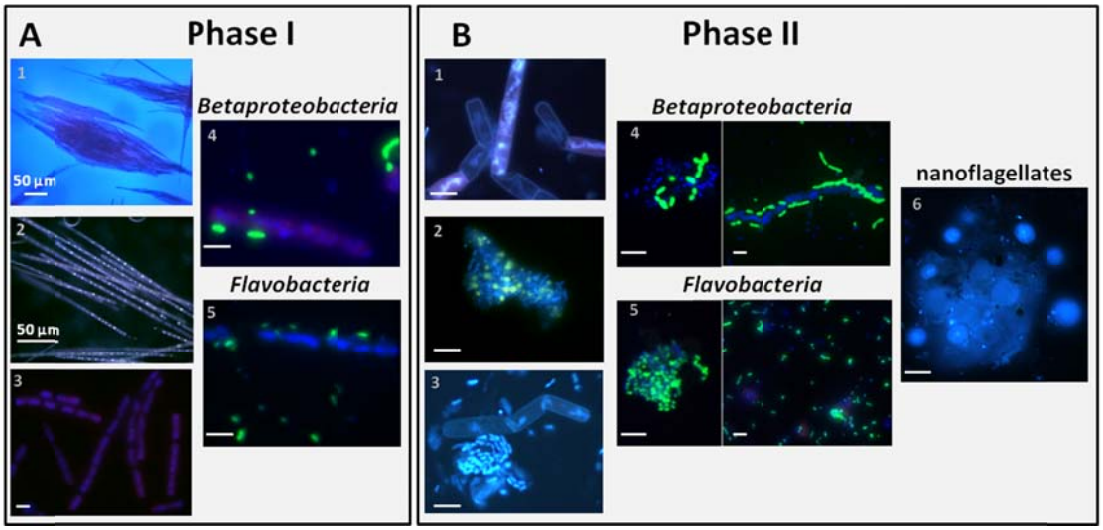


Figure 3. Bacterial protein production (BPP) of particle-attached (PA) and free-living (FL) communities in epilimnion and hypolimnion (A) and bacterial abundance of total community in epilimnion (B) and hypolimnion (C) during the two phases of the bloom.

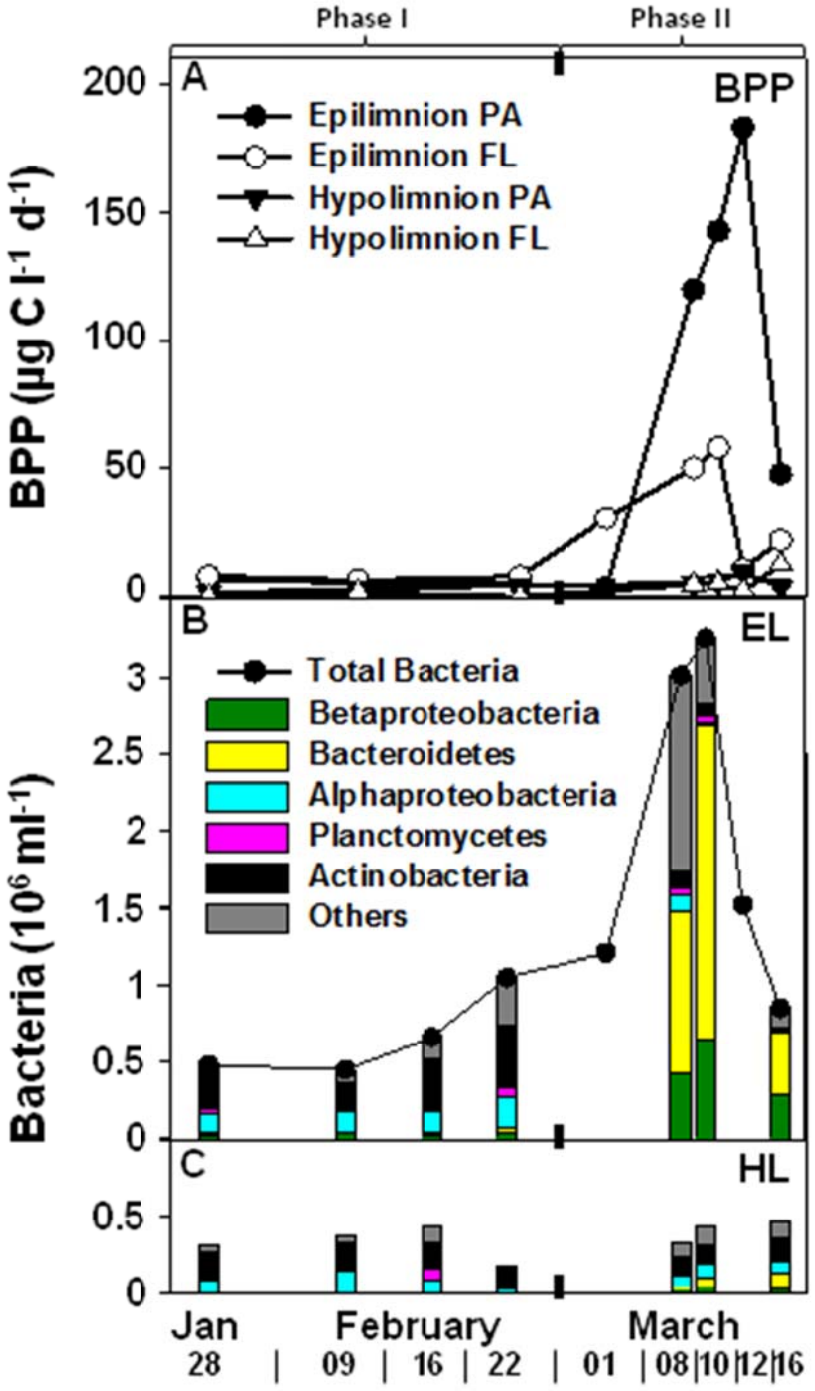


Figure 4. Distribution of relative abundances of major phylogenetic groups (% of DAPI - right axis): *Alphaproteobacteria*, *Betaproteobacteria*, *Bacteroidetes*, *Planctomycetes* and *Actinobacteria* as assessed by CARD-FISH in the particle-attached (PA) and free-living (FL) fractions of epilimnion and hypolimnion. Phase I and II of the bloom are separated by the dashed line. The abundance of *Aphanizomenon flos-aquae* (gray field) is presented during both phases of the bloom (left axis).

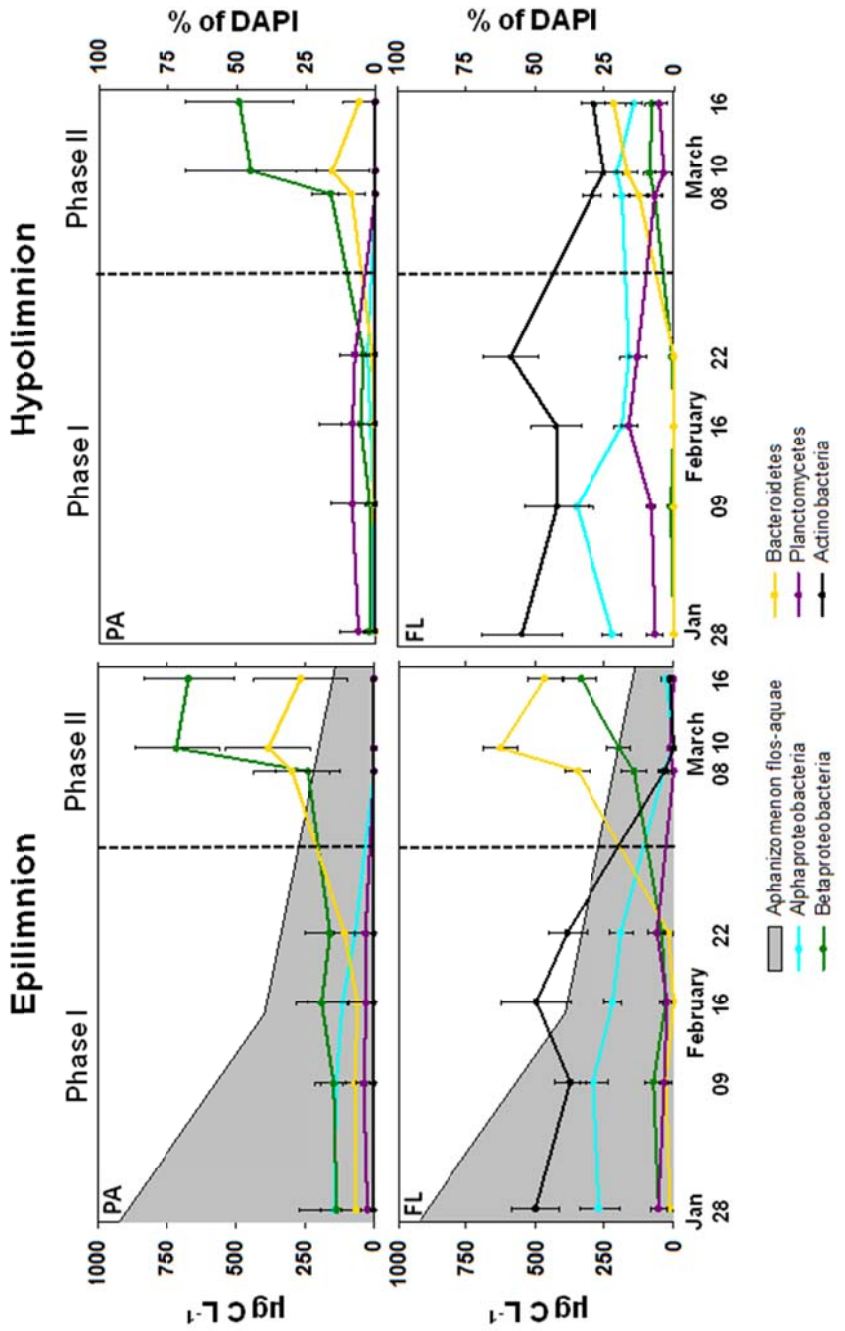
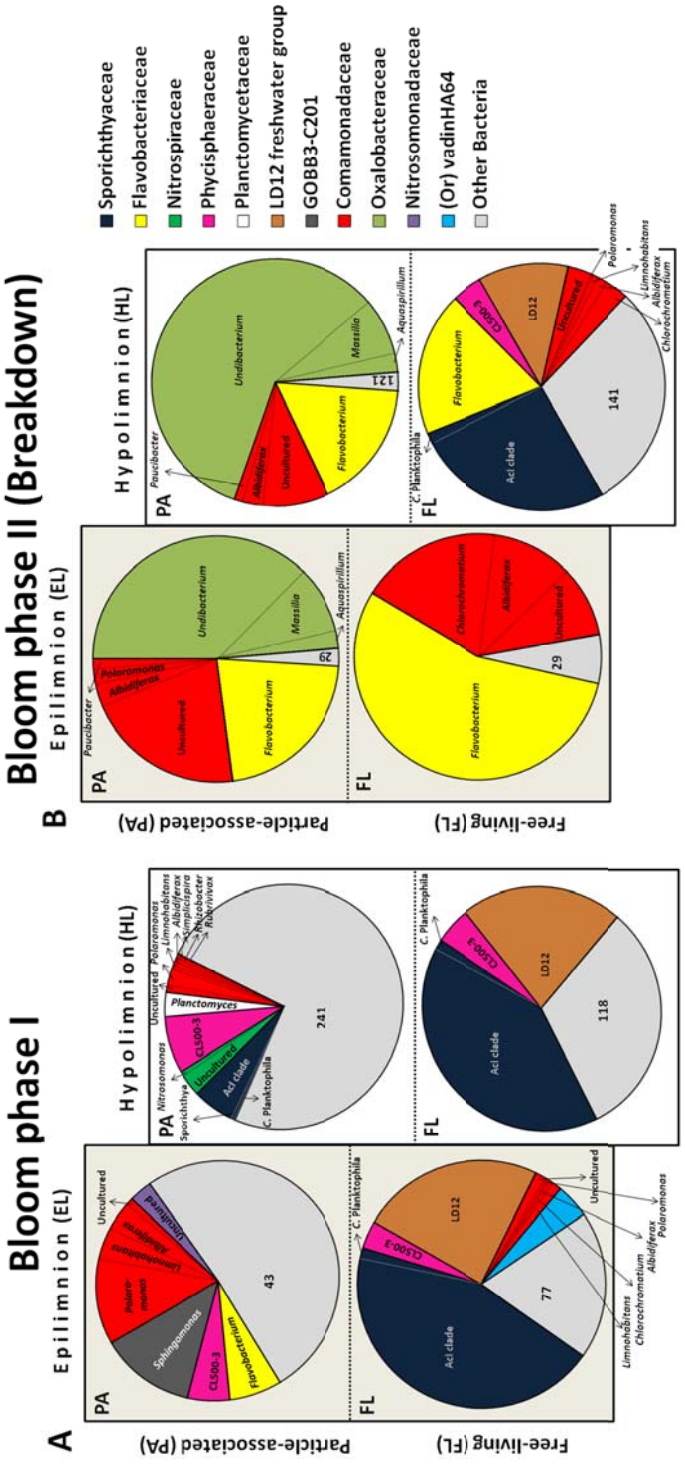


Figure 5. Sequence frequencies of major particle-attached (PA) and free-living (FL) bacterial genera in the epilimnion and hypolimnion of Lake Stechlin during Phase I (A) and Phase II (B) of the under-ice bloom. Genera are detailed only within families making up over 2% of the total sequences in at least one sample. Numbers in the gray fields refer to all the families that made less than 2% of the total sequences each.



Supplementary Materials:

Figures S1-S2

Tables S1-S5 (Table S1-Abundance of identifiable taxonomic paths across the different samples is not available in printed version)

Figure S1. pH profile

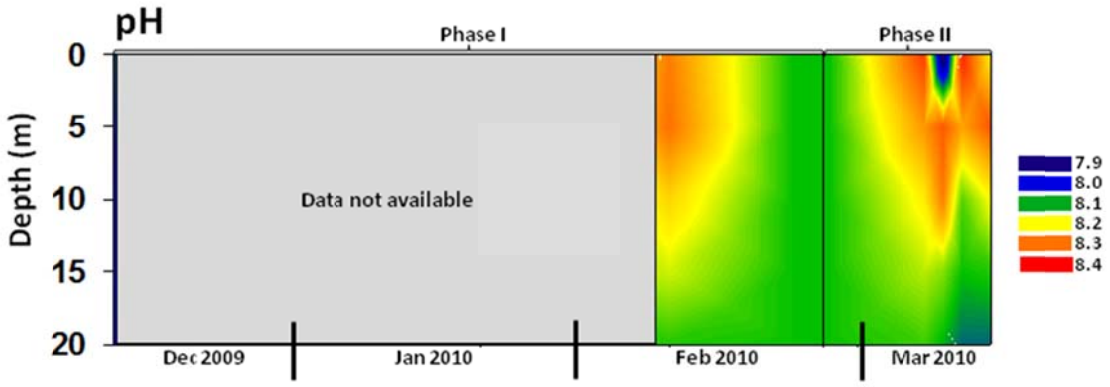


Figure S2. DGGE analysis of the bacterial diversity of the two phases of the bloom separated into particle-attached (PA) and free-living (FL) bacterial communities of epilimnion (EL) and hypolimnion (HL)

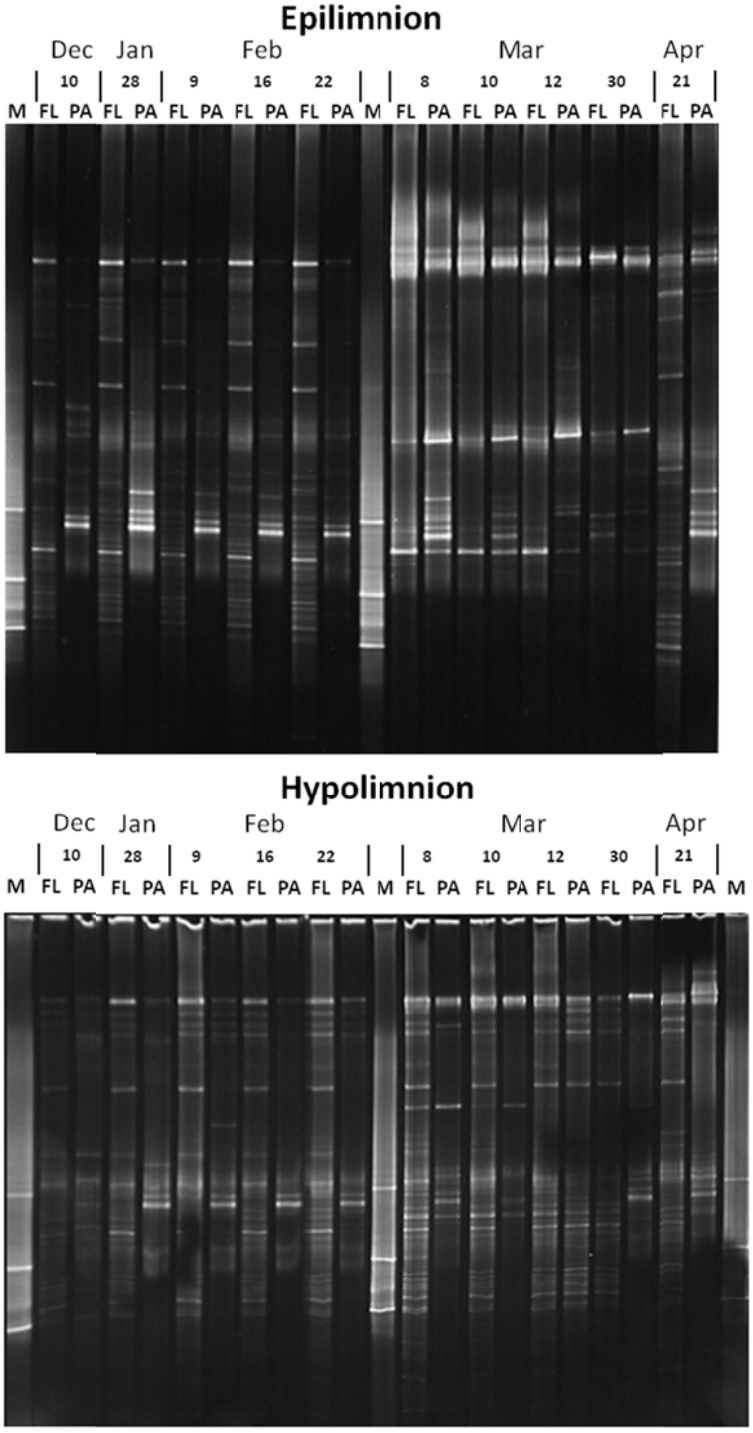


Table S2. Under-ice phytoplankton blooms in limnic and marine systems

Dominant phytoplankton	Sampling year	Place observed	Reference
Limnic Environments			
Diatoms Golden algae Dinoflagellates	1977-1978	White Heron Lake, Pennsylvania U.S.A.	(1)
Diatoms Cyanobacteria	2005	Lac Saint Pierre of St. Lawrence River Canada	(2)
Diatoms Golden algae Dinoflagellates Green algae	1962-1963	Black Lake Colorado, Pass Lake Ontario, Tea Lake Ontario	(3)
Diatoms Golden algae Green algae Cryptomonads	2005 1963	Lake Päijänne, Finland	(4, 5)
Golden algae Green algae	1969-1972	Heywood Lake Antarctica	(6)
Diatoms Golden algae Dinoflagellates Cryptomonads	1961-1962	Beaver Pond, Massachusetts, U.S.A	(7)
Green algae Cryptomonads Cyanobacteria	1990, 1991	Lake Opinicon, Canada Upper Rock Lake, Canada	(8)
Golden algae Green algae	Lake Rédo 1992, 1994; Schwarzsee ob Sölden 1994; Gossenköllesee 1994	Lake Redó, Spain, Schwarzsee ob Sölden, Austria Gossenköllesee, Austria	(9)
Diatoms, Green algae Cryptomonads Cyanobacteria	1968 - present	Neusiedler See, Austria	(10)
Diatoms Golden algae	2004	Lake Pääjärvi, Finland	(11)
Golden algae	1974	Lago Santo Parmense, Italy	(12)
Golden algae	1962-1963	Lake in the English Lake District, UK	(13)
Diatoms	1994	Lake Baikal, Russia	(14, 15)
Diatoms	1967-1971	Lake Haruna, Japap	(16)
Dinoflagellates		Lapland	(17)
Cyanobacteria Dinoflagellates	During the two austral summers	Antarctic lakes (Vanda and Bonney)	(18)
Cyanobacteria	2009-2010	Lake Stechlin	(19) and this study
Marine Environments			
Diatoms	2012	Arctic Sea	(20)
Diatoms		Arctic Sea	(21)
Diatoms	June 1998	Chukchi sea (Arctic)	(22)
Diatoms	June 1993	Northeast Greenland	(23)
Diatoms	1998	Ice camp drift in the Canadian Basin (Arctic)	(24)

Table S3. Oligonucleotide probes used in this study

Probe	Target	Reference
ALF968	*Alphaproteobacteria*	(25)
BET42a*	*Betaproteobacteria*	(26)
GAM42a*	*Gammaproteobacteria*	(26)
CF319a	*Bacteroidetes*	(26)
PLA42a	*Planctomycetes*	(27)
HGC69a	*Actinobacteria*	(28)
EUB338 I-III[a]	Bacteria	(29, 30)
NON338	Control	(31)

[a] equimolar concentrations of probes EUB338 I, EUB338 II and EUB338 III

* used in combination with a competitor

Table S4. List of samples analyzed for Bacterial Protein Production (BPP), Denaturing Gradient Gel Electrophoresis (DGGE), Tag Pyrosequencing (454) and Fluorescence in situ hybridization (FISH)

Date	BPP	DGGE	454	FISH	Stratum	Size fraction
10-Dec	-	+	-	-	**Epilimnion (EL)** & **Hypolimnion (HL)**	**Particle-attached (PA)** > 5 µm & **Free-living (FL)** 0.2 -5 µm
28-Jan	+	+	+ (Pooled DNA samples)	+		
9-Feb	+	+		+		
16-Feb	-	+		+		
22-Feb	+	+		+		
1-Mar	+	-	-	-		
8-Mar	+	+	+ (Pooled DNA samples)	+		
10-Mar	+	+		+		
12-Mar	+	+		-		
30-Mar	-	+		-		
21-Apr	-	+	-	-		
16-Mar	+	-	-	+		

Table S5. Sequence quality and diversity data for each sample

Sample name	# Sequences	Minimum length	Average length	maximum length	# OTU[1]	Clustered	Replicates	Rejected	Goods coverage	Shannon-diversity index (H)[2]	Shannon-Evenness (E_H)
Phase(I)-EL-PA	14249	150	452	540	300	5513	8420	16	0.99	3.10	0.79
Phase(I)-EL-FL	4195	150	418	531	516	2312	1364	3	0.95	2.06	0.47
Phase(II)-EL-PA	9095	150	420	531	530	4809	3735	21	0.98	1.15	0.33
Phase(II)-EL-FL	9230	150	416	530	520	4839	3848	23	0.98	0.99	0.29
Phase(I)-HL-PA	20728	150	448	563	1594	8703	10382	49	0.96	4.46	0.81
Phase(I)-HL-FL	13329	150	417	556	1125	7169	5001	34	0.97	2.30	0.48
Phase(II)-HL-PA	42603	150	417	544	1343	19311	21854	95	0.99	0.98	0.20
Phase(II)-HL-FL	19506	150	421	546	1700	11004	6753	49	0.97	2.66	0.53

[1] OTUs were clustered at 98% sequence similarity

[2] Based on differences in #seq, H was calculated for random subset of 4000 sequences

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Manuscript III

Macroscopic organic aggregates are hotspots for interactions between colonizing microorganisms as revealed by transcriptomics analysis

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Manuscript in preparation for the ISME journal

Macroscopic organic aggregates are hotspots for interactions between colonizing microorganisms as revealed by transcriptomics analysis

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Intended as an article in the ISME journal

Abstract:

We used a newly developed flow-through roller tank system to observe temporal changes in PA bacteria and the genes expressed by these communities. We show that the community profile obtained by DNA analysis differs significantly from the profile obtained by RNA analysis. While the former was dominated by *Caulobacteraceae* and *Flavobacteriaceae*, the latter was dominated by *Sporychthiaceae* and *Acidimicrobiaceae*. The discrepancy could be a result of extensive viral activity on the particle as one of the most abundant transcripts obtained was a complete *Microviridae* genome. The active particle-associated community obtained from single aggregates did not change throughout the experiment. Similarly, we did not observe any significant change in the expression of genes involved in carbon utilization. Hence we conclude that in our case more than 8 days is needed to obtain a community shift on particles. Beside extensive phage activity, antagonistic reactions on a particle include bacteria-eukaryote interactions, evident from the expression of protein secretion systems type III and VII as well as of genes involved in cyanide and phenazine production, both effective eukaryotic toxins. Intra-bacterial interactions include the above toxins as well as antibiotic production and degradation. The viral activity and the multitude of lytic processes which occur on the particle according to the transcriptome, make the particle a suitable location to be a generator of genetic variability via lateral gene transfer. The latter is supported by the expression of genes involved in the uptake and integration of naked DNA into bacterial genomes.

Key words: Antagonistic reactions/ Particle-associated bacteria/Phage/ Transcriptome/ *Microviridae*

Introduction:

Particulate organic matter (POM) aggregates and their surrounding plume of solutes are hot-spots of microbial activity (Kiørboe 2001). Macroscopic organic aggregates represent a collection of different particles, operationally defined to be larger than 300 μm and are derived from a number of sources. These include the coagulation of dead or senescent phytoplankton cells such as diatoms and cyanobacteria; zooplankton-carcasses; allochthonous organic matter (OM) such as leaf litter in limnic environments; fecal pellets of macroscopic organisms, or a combination of all of these (Alldredge & Silver 1988; Jackson 1980; Grossart & Simon 1993). The concentrations of organic and inorganic molecules, contained in POM aggregates, often exceed background levels by 2-4 orders of magnitude (Prézelin & Alldredge 1983; Grossart & Simon 1998). Hence, in a rather dilute aquatic environment POM aggregates provide the associated bacterial community with a "concentrated" energy and carbon source (Shanks & Trent 1979; McCarthy & Goldman 1979). As a result, these particles are heavily colonized by heterotrophic bacteria (Alldredge et al. 1986; Alldredge & Youngbluth 1985), whose activity plays an essential role in their degradation (Alldredge & Silver 1988; Simon et al. 2002). By involving a suite of surface-bound ectohydrolases, including lipases, proteases, chitinases, and phosphatases, bacteria hydrolyze POM into dissolved OM (Smith et al. 1992; Grossart & Simon 1998). This results also in the release of nutrients such as N, P, S and Fe (Azam & Malfatti 2007) which are necessary for cell growth. Nevertheless, to gain access to these "gold mines" for bacteria, solely encountering an aggregate (Kiørboe et al. 2002) is not sufficient, without the mechanisms to degrade it. Hence, it is expected that the composition of the aggregate (mainly quality and quantity of organic matter) will shape the nature of the associated bacterial community (Grossart et al. 2005; LeCleir et al. 2014; Eiler & Bertilsson 2004). For example, Fandino et al (2001) showed that during a dinoflagellate bloom, particle-associated (PA) bacteria belonged mainly to the *Bacteroidetes* phylum. These associated communities may differ even between seemingly very similar phytoplankton (Grossart 1999). Grossart et al. (2005) showed that two closely related diatom species harbour distinct PA communities which develop differently as a function of the physiological state of the algae.

PA bacteria have been shown to differ substantially from free-living (FL) ones (Grossart et al. 2005; Riemann & Winding 2001). Nevertheless, more recent data

(Bižić-Ionescu et al. 2014; Grossart 2010; Ghiglione et al. 2007) suggest there is a strong connectivity between the two fractions with a large fraction of the PA bacteria adopting a "hop-on hop-off" lifestyle. Several reasons can lead to the continuous attachment and detachment of bacteria to particles. First, while whole bacterial communities can often degrade a broad spectrum of organic substances, individual bacteria are usually specialized on utilization of only a few. For PA bacteria this means that following the consumption of a preferred organic matter pool, the respective bacteria will be outcompeted by more specialized ones leading to temporal shift in PA communities. Second, as PA communities are rarely homogenous, antagonistic reactions between different populations of microorganisms (e.g. bacteria-bacteria of fungi-bacteria) cannot be ruled out as these occur in various microenvironments (Grossart et al. 2004; Bidle & Azam 2001; Long & Azam 2001). Last, POM aggregates have been suggested to serve as a bacterial refuge from phages (Tang et al. 2011; Grossart & Tang 2010); however, they have also been shown to harbor large numbers of viral particles (Riemann & Grossart 2008; Peduzzi & Weinbauer 1993; Müller-Niklas & Schuster 1994; Proctor & Fuhrman 1992). Thus it is not unconceivable that while some FL bacteria seek refuge from phages on the particle, some PA bacteria will escape to the less densely populated off-particle environment.

Bulk bacterial activity on particles has been studied using uptake of radiolabeled tracers and was found to be >50% of overall bacterial productivity in the water body during periods of bloom breakdown (Crump et al. 1998, 1999; Fandino et al. 2001; Ghiglione et al. 2007; Grossart et al. 2007; Garneau et al. 2009; Rösel & Grossart 2012). Microbial activity was also directly studied on individual particles using microsensors (e.g. Grossart & Ploug 2001). Only few studies have followed the temporal change in PA bacterial community composition (BCC) (Grossart & Simon 1998; Schweitzer et al. 2001; Grossart et al. 2003b; Kiørboe et al. 2003; LeCleir et al. 2014). Solely, very few studies tried to link between the bacterial community composition and its activity on the particle (Schweitzer et al. 2001; Moran et al. 2013; Kong et al. 2013). The latter have recently focused on *in situ* environments (Moran et al. 2013; Kong et al. 2013), thus not providing any temporal resolution. Older studies conducted using closed roller tank systems (Grossart & Simon 1998; Schweitzer et al. 2001) suffer from two problems, a low taxonomic

resolution due to available technology at the time they were conducted and a strong "bottle effect" due to a closed system setup (Zobell & Anderson 1936).

To overcome the bottle effect and to allow for the constant supply of new OM, nutrients and bacteria from the habitat water, we connected a novel flow-through roller tank system (Ionescu et al. in prep) directly to Lake Stechlin (northeastern Germany). The flow-through roller tank was inoculated with aggregates formed from an axenic diatom culture and aggregate samples were collected, on several time points, for respiration and photosynthesis measurements (Ionescu et al. in prep), BCC, metagenomics and metatranscriptomics. This system allowed us to directly follow temporal shifts in the diversity and activity of microbial communities on a fine scale. Our study aims at identifying the first colonizers on particles and to provide information on their arsenal used against the "host" and other competing colonizers. We further aim at linking temporal changes in BCC with their functional role as the degradation of the particles may change the type of available carbon.

Materials and Methods:

Algal culture: An axenic culture of freshwater diatom *Navicula* sp., isolated from Lake Stechlin, was grown in 900 mL of sterile Z-medium (Staub 1961) in 10 - 1 L Shott Duran bottles. The culture was grown under a 12 h dark-light cycle at 15 °C for 2 weeks. Afterwards, the diatoms in the bottles were placed on a roller table to promote aggregation and create diatom flocks prior to *in situ* inoculation with freshwater bacteria from Lake Stechlin.

Experimental setup: To conduct long term experiments with a constant supply of fresh OM, nutrients and bacteria from the habitat water while avoiding the "bottle effect" we used a newly designed flow-through roller tank system (Ionescu et al. in prep). Shortly, this device consists of an inner tube in which the continuous sinking of particles is simulated and external casing through which lake water flows in and out of the inner tube without disturbing the aggregates (Fig 1).

Freshly formed diatom flocks were carefully transferred to the prefilled inner core of the rolling device carefully avoiding air bubbles. The device was then sealed and allowed to roll for up to an hour prior to onset of lake water flow. Lake water was pre-filtered through a 100 μm pore size sieve to prevent large objects from

entering the experimental setup. Flow was maintained at 300 ml h^{-1} and rotation speed was about 2 RPM. Four such reactors were set initially, two of which were sampled after 24 h and two after 8 days. Two additional reactors were inoculated once the first set was sampled. These reactors were sampled after 7 days of uninterrupted rolling with continuous flow. To obtain one more set of short term incubation two more reactors were inoculated and sampled after 1 day at the end of the experiment. See Fig 2 for the sampling scheme.

Sampling procedure: For sampling each roller tank was opened and single macroscopic aggregates (SMA) were carefully collected with a cut-end syringe while trying to avoid collection of surrounding water as much as possible. Collected SA were rapidly gathered and filtered through a 5 μm pore size polycarbonate filter (47 mm in diameter, Sartorius, Germany) to make sure no free-living (FL) bacteria are interfering with our study. Upon filtration the filters were immediately placed in Z6-buffer (8 M guanidinium-HCl, 20 mM MES, 20 mM EDTA [pH 7.0] and 0.7% [v/v] 2-mercaptoethanol) to deactivate RNAses and denature all proteins. To collect the fraction of the remaining microscopic aggregates (MIA), the total water volume of each roller tank (1.8 L) was filtered through several 5 μm pore size filters. Filters were changed each time the filtration speed slowed down to avoid clogging and collection of free-living bacteria on the MIA fraction. The filters were immediately placed in 700 μL of Z6 buffer and placed on ice for further analyses.

DNA and RNA extraction: Nucleic acids were extracted as described for DNA in Ionescu et al. (2012) except for the last desalting step. Shortly, cells were lysed by incubation for 15 min at 95 °C in a Z6-buffer followed by 15 min incubation at 65 °C with phenol (0.5:1 phenol:sample, v:v; Phenol pH 8 and 4.3 for DNA and RNA, respectively) after which the aqueous phase was extracted with chloroform. After an additional phenol-chloroform (1:0.5:0.5, sample:phenol:chloroform, v:v:v) extraction the sample was extracted twice with chloroform and precipitated over night at -20 °C using 2-propanol (1:1). The DNA was cleaned in 70% ethanol and dissolved in Diethylpyrocarbonate (DEPC) treated water. Following the extraction the RNA, samples were treated with TurboDNAFree (Ambion) according to the manufacturer's instructions.

mRNA enrichment and cDNA synthesis: Since ribosomal RNA is the most common transcript in RNA extractions we have enriched the mRNA proportional

concentration by removing rRNA transcripts following the procedure described in (Stewart et al. 2010). Shortly, we generated long 16S rRNA and 23S rRNA probes by obtaining sample specific rRNA genes PCR amplicons which were later transcribed to biotin labelled sample specific RNA probes using the MegaScript T7 kit (Ambion) and biotin labelled C and U (Roche). To achieve maximum rRNA removal all probes from all samples were pooled and equal aliquots were added to the RNA samples. Following an incubation of 5 min at 70 °C, the hybridized rRNA molecules were extracted with streptavidin coated magnetic beads. The mRNA enriched RNA was concentrated using the Easy RNA kit (Qiagen) into a working volume. First strand cDNA was created using the SuperscriptIII kit (Invitrogen) according to the manufacturer's instructions.

Sequencing:

Shotgun sequencing from DNA: Metagenome sequencing steps include fragmentation of genomic DNA, ligation to sequencing adapters and purification. Following the amplification and denaturation steps, libraries were pooled and sequenced. We used 50 ng of DNA from each sample to prepare the libraries using Nextera DNA Sample Preparation Kit (Illumina). Library insert size was determined by Experion Automated Electrophoresis Station (Bio-Rad). The insert size of the libraries ranged from 300 to 1400 bp. Pooled library (12pM) was loaded to a 600 Cycles v3 Reagent cartridge (Illumina) and the sequencing was performed on Miseq (Illumina).

cDNA sequencing: cDNA sequencing steps include purification and generation of blunt end cDNA followed by ligation to sequencing adapters, amplification, denaturation and sequencing. We used 250 ng of double strand cDNA from each sample to prepare the libraries using TruSeq RNA sample preparation kits (Illumina). Pooled library (10pM) was loaded to a 600 Cycles v3 Reagent cartridge (Illumina) and the sequencing was performed on Miseq (illumina).

Sequence quality control: Sequence quality was assessed using the FastQC program (Babraham Institute) after which the each sequence file was cleaned from low quality sequences and traces of the sequencing method with the Clip tool from the Nesoni package (Victoria bioinformatics) using the following parameters: the first 12 and last 3 bases were trimmed and sequences shorter than 50 nucleotides were removed; from the remaining data, sequences with an overall quality score below 25

were removed as well. The output files were re-evaluated using FastQC and specific samples were treated de-novo until the output file contained only high quality data (i.e. quality >25, no ambiguous nucleotides, no adapter sequences).

Sequence assembly and analysis:

Metagenome: For best assembly the two paired end libraries (SMA and MIA) for metagenome analysis were concatenated into single right and left read files. Similarly, the non-paired files obtained containing sequences whose pair did not pass the quality control, were concatenated too. Contigs for metagenome analysis were created using the Velvet software (Zerbino & Birney 2008) with a kmer of 49 and a coverage cutoff of 10. The assembled contigs were binned in MetaWatt (Strous et al. 2012) based on the tetranucleotide signature of the sequence. The main, associated with *Brevundimonas* and *Pseudomonas* bins were exported and analyzed on the RAST server (Aziz et al. 2008). The metagenome was analyzed on the MG-RAST server (Meyer et al. 2008) and the results will be discussed elsewhere.

Transcriptome: All paired end and single end libraries from the transcriptomics analysis were concatenated into single left, right and non-paired files. Ribosomal RNA transcripts were removed prior to cDNA formation, yet we cannot assure that the efficiency of the process was equal in all samples. Hence, for better differential expression analysis of coding sequences rRNA transcripts were removed from the data. For this, an rRNA database was created by concatenating the SSU (16S rRNA) and LSU (23S rRNA) Silva databases (Quast et al. 2013; V 115) and the 5S rRNA database (Szymanski et al. 2002; last updated in 2005). The transcriptome data were aligned against the complete rRNA databse using Bowtie2 (Langmead & Salzberg 2012) and rRNA and non-rRNA sequences were separated into separate libraries. The latter was then used for further processing. A second analysis of rRNA sequences was performed following the transcriptome assembly and matching sequences were removed from differential expression analysis.

Transcripts were assembled and analyzed using the Trinotate pipeline (http://trinotate.sourceforge.net). This includes transcripts assembly with the Trinity software (Grabherr et al. 2011), open reading frame prediction using TransDecoder (part of the Trinity software package), protein identification using BlastX and BlastP (Altschul et al. 1990) against the Uniprot protein database (The UniProt Consortium 2014), protein domain prediction using the HMMER (Finn et al. 2011) against the

Pfam database (Finn et al. 2014). To obtain additional annotation data all transcripts were run through the KEGG KAAS system (Moriya et al. 2007). To try and annotate potential partial transcripts that could not be identified due to incomplete sequence, we compared the assembled transcripts to the MG-RAST annotated metagenome. This provided as well data on the taxonomy of the transcripts. For specific cases (see results), assembly quality was validated using a second assembler (Velvet) as well as the REAPR program (Hunt et al. 2013) which identifies faulty assemblies based on read coverage.

Differential expression analysis was conducted with NOISeq (v 2.4; (Tarazona et al. 2011)) using the TMM normalization mode. The four samples in each group (SMA or MIA) were compared as individuals using the NOISeq-sim mode to simulate technical replicates. Additionally, 24 h incubations were compared to long incubations using NOISeq-bio with each pair of samples being considered as a biological replicate.

The presence of CRISPRs (Clustered Regularly Interspaced Short Palindromic Repeats) was tested using CRISPRfinder (Grissa et al. 2007). Spacer-sequences from candidate CRISPRs were than compared to the entire transcriptome data as well as to the assembled transcripts using Bowtie2 and BLAST, respectively.

Reconstruction of bacterial community:

Microbial community composition was reconstructed using EMIRGE (Miller et al. 2011) using the default parameters and the SILVA SSU (V. 111 (Quast et al. 2013) database. The obtained OTU abundance was used to create artificial fasta files consisting of replicate sequences of the respective distributions. Each such file containing 10,000 sequences was then run through the SILVA NGS (Ionescu et al. 2012) system. The 8 metatranscripome and 2 metagenome data sets generated in this study are available in MG-RAST as public databases under numbers: 4551019-4551027 (4551023 excluded) and 4551388-4551389, respectively.

Results:

Community composition:

To examine the bacterial community composition (BCC) in the flow-through roller tank experiment we compared the community profile obtained from the 16S rRNA (RNA) to that obtained from the 16S rRNA gene (DNA), representing the active and total community, respectively (Fig. 3A). The comparison was done for single macroscopic aggregates (pooled together; hereafter SMA; Fig. 3A) as well as for microscopic aggregates in the roller tank which were larger than 5 μm (hereafter MIA; Fig. 3A). The DNA-based community analysis showed a dominance (>5% of sequences) of *Flavobacteriaceae* (46% SMA; 29% MIA), *Caulobacteriaceae* (26% SMA; 18% MIA), *Comamonadaceae* (10% SMA; 12% MIA) and *Pseudomonadaceae* (7% SMA; 14% MIA). In contrast the SMA RNA based (active) community was dominated by *Sporichtyaceae* (36-42%), *Acidimicrobiaceae* (11-22%) and *Flavobacteriaceae* (7-11%). The MIA RNA based community was less homogenous throughout the experiment with *Sporichthyaceae* (37%) being the dominant family on the first day and *Caulobacteraceae* (37%) on the last day.

To check whether the community profile reconstructed from the 16S rRNA data is not biased by the rRNA removal protocol, we analyzed each transcriptome on the MG-RAST (Meyer et al. 2008) server. This produced a community profile based on all sequences that could be annotated. The obtained communities (Fig. 3B) where more diverse than those reconstructed from the 16S rRNA alone (Fig. 3), yet they clearly differed from the community profile obtained from the metagenomic data analyzed in a similar manner (Fig. 3B). A much higher similarity was obtained when the two RNA analysis methods were compared at the class level (Fig. S1).

Archaeal sequences were detected as well, mostly dominated by *Euryarchaeota* from the *Halobacteria* family. Additionally several families of methanogenic Archaea were identified (Fig. S2). Due to their relative low abundance (2-100 reads with recognizable taxonomic afiliation), *Archaea* will not be further discussed in this manuscript.

Functional annotation:

The Trinity assembly pipeline (Table 1) resulted in 4611 transcripts located on 4352 contigs larger than 90 nt (30 amino acids). Of these, 2294 could not be identified by any of the analysis tools used (Table 1). The remaining transcripts contained 2003

identifiable or known-hypothetical proteins and 314 rRNA molecules which were not efficiently removed in the preliminary sequence treatment (see methods section). These rRNA sequences were removed from further analyses of differential expression.

The longest and one of the most expressed transcripts obtained was a complete phage genome (5386 nt) belonging to the *Microviridae* family and with high similarity to various strains of *Microvirus*. The same genome was found as well in the metagenome. Nevertheless, assemblies of the two comparable DNA and RNA sequences were not identical with the DNA sequence being 59 nt longer (5442 nt; Fig. 4) than the RNA one. In the latter assembly, the phage replication protein was split between the beginning and end of the genome (Fig. 4). Read coverage analysis conducted by using the entire transcriptome on both the DNA and RNA assemblies shows that both sequences are present in the transcriptome (Fig. 4). The phage genome was obtained individually from both metagenomes as well as from the 8 transcriptomes. In contrast to the DNA, where all phage proteins were intact, a truncated protein (mostly capsid or replication) was found in all 8 individual transcripts (Fig. S3).

To obtain an overview of the functions represented in the Trinity assembled transcriptome the KEGG pathways were summed up (Table 3). Nucleotide and carbohydrate metabolism contained the most transcripts (349 and 270, respectively). Within each of these categories the individual transcripts were rather equally represented with few exceptions. Two component systems were the most common transcripts in the signal transduction group.

The Trinity pipeline does not provide information based on transcripts not assigned to contigs. To obtain most information from the RNA data, the individual samples were submitted to the MG-RAST server for analysis. An overview comparing the transcript abundance of each functional group between pairs of samples is given in Fig. S4A-D. The transcript abundance was Log2 normalized to account for differences in read coverage and was cantered per sample by the MG-RAST system. A similar expression pattern is obtained when comparing the 1 day incubation to 8 days incubation as to 7 days of incubations (Fig S4A-B). Twenty three functional groups show a clear over-expression in either the short (1 day; red) or the long incubations (8, 7 days; green). Functional groups that show preferential

expression in the first 24 h include genes involved in adhesion and invasion of host cells, protein secretion systems type III and VII and toxin synthesis. Genes responsible for the uptake, synthesis and use of amino sugars and polysaccharides are as well more expressed in the short term incubation experiment. Among the groups overexpressed in the longer incubations are the Gene Transfer Agent which is involved in lateral gene transfer, plasmid related functions, protein secretion system type VIII and genes responsible for proteolysis.

Differential expression of the Trinity assembled transcripts was calculated with NOISeq-sim to simulate technical replicates. When the 8 days and 1 day incubations were compared, 26 transcripts were overexpressed in the former and 10 in the latter. Among the 26 transcripts differentially expressed in the longer incubation, 19 could be annotated, 12 of which were related to phage activity, mainly reverse transcriptases. The rest were annotated as conserved hypothetical proteins or as eukaryotic domains. A similar comparison between the 1 day and 7 day incubation revealed 90 transcripts overexpressed in the long incubation and 35 in the short one. The latter included 6 transcripts that were overexpressed in the short experiment also when compare to the 8 days incubation however those that could be assigned to a function were proteins of general function (Pyridoxine 5'-phosphate synthase, Fumarate hydratase class I, ATP-dependent Clp protease proteolytic subunit, Putative O-methyltransferase).

Possible mechanisms for viral defense:

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) sequences consist of several similar stem-and-loop palindromic sequences separated by spacers of different sequence. The palindromic repeats cannot be found in transcript sequences and the spacers are often unique to the bacteria analysed. Therefore to identify potential sequences we have analysed the metagenomes of the SMA and MIA and have found 83 putative CRISPRs which include 175 different spacers (Table 3). Among these, 145 could be identified in the overall transcriptome of the samples and 134 had good matches in the Trinity assembled transcripts. Among the latter only 62 matched annotated sequences of which 11 were of viral origin (Table 3). Additionally, we identified in the metatranscriptome the expression of 6 different CAS (CRISPR Associated) genes including CAS1, CAS3 and several smaller CAS families.

A second defence mechanism employed by bacteria against foreign viral DNA is the use of restriction endonucleases (Restriction enzymes). Overall 12 and 9 different endonucleases were identified in the Trinity assembled transcripts and the MG-RAST analysis, respectively. Nevertheless, the specific target sequence for these enzymes and whether they digest RNA or DNA molecules could not be determined.

Discussion:

Our results portray the important role of the POM aggregate as hotspots for microbial interactions. First we show a discrepancy between the present bacteria and those that are metabolically active on the particle. Second, we suggest that the aggregate is an active "battle zone" between microbes and eukaryotes, microbes among themselves and, particularly, between microbes and phages.

Active vs. Present Bacteria:

Community analysis based on the gene encoding for the 16S rRNA is a common tool in microbial ecology (Glöckner et al. 2000; Crespo et al. 2013) and has been recently used for the analysis of particle-associated (PA) bacteria as well. In light of continuous changes in the dominant members of the microbial community (Teeling et al. 2012), DNA based methods describe the full extent of the present microbial community. To obtain an accurate idea regarding the active community at the time of sampling it is necessary to focus on the RNA and particularly at the 16S rRNA molecule itself (Nikolausz et al. 2004). Nevertheless, it is important to mention that the active microbial community (RNA) will not reflect the abundance of the microbial community *per se*, but rather the distribution of their "workload". Thereby, equally abundant bacteria with different activity levels or physiological conditions will have different quantities of mRNA and rRNA molecules (Kemp et al. 1993).

We, therefore, compared the community profile obtained from both the DNA and RNA using 16S rRNA gene and transcript analyses as well as metagenomic and metatranscriptomic analyses (Fig. 3A-B, respectively). While differences are to be expected between the two methods (Moeseneder et al. 2005; Brettar et al. 2012), to our surprise there was hardly any overlap between the obtained DNA and RNA results. While the DNA showed that the SMA community is dominated by *Caulobaceraceae* and *Flavobacteriaceae*, the RNA suggested that *Actinobacteria* are responsible for over 60% of the activity (Fig. 3A). Since we have used a mRNA

enrichment method which includes the targeted removal of rRNA molecules of the specific community, it was essential to verify that the obtained result is not purely a methodological artefact. Thus, we have obtained community profiles from the entire metatranscriptome of each sample as well as from the two metagenomes (Fig. 3B). While the two methods for RNA analysis resulted in different community profiles, in both cases the results were drastically different from the respective DNA based results. The difference between the 16S rRNA and the whole transcriptome analysis is to be expected due to a substantial difference in available information between the rRNA databases and the number of available genomes for comparison (Fig. S5). Furthermore, while 16S rRNA databases contain a large and uniform number of sequences across multiple taxa, the available genomes are poorly distributed with a large inclination towards medically relevant species. For example, there are 795 genomes of *Mycobacteria tuberculosis* strains and only one genome of a species belonging to the *Sporichthyaceae* family (NCBI database 14th of Feb 2014). Therefore, we redeem the rRNA obtained community profile as reliable and conclude that the active community at the time of sampling was dominated by *Actinobacteria*, specifically *Sporychthiaceae* and *Acidimicrobiaceae*. This is interesting as among the *Actinobacteria* mainly *Micrococcaceae* and *Mycobacteriaceae* are known as PA (Allgaier et al. 2007; Grossart et al. 2004).

While the SMA microbial community describes the active members on the experimental *Navicula* sp. particles we used as an inoculum, the MIA community describes to some extent the community on fragmented experimental aggregates but mainly of the PA community of the lake itself at the time of sampling. The two communities remained highly similar during the first 24 h of incubation. As our experiment was started immediately after the ice-off of Lake Stechlin, these results show that *Sporichthyaceae*, *Acidimicrobiaceae* and *Flavobacteriaceae* are the first main colonizer of particles once these become available. It is very likely that due to the lack of organic substrates at this time, the first MIA sample consisted mainly of fragmented experimental material. The latter would also come apart easier as it has only gone through minimal bacterial degradation which is required to increase the particle's stability (Grossart et al. 2006; Gärdes et al. 2011). The onset of the spring diatom bloom in the lake (immediately after ice-off) resulted in a new algal composition (Fig. S6A-B) and the release of new substrates leading to a shift in the lake's PA community. Interestingly, even though the *Navicula* particles did not remain

immune to the adhesion of additional diatom species (Fig. S6B), the microbial community on the SMA remained stable throughout the experiment while at the same time the PA community on MIA in the lake changed. This suggests that initial aggregate colonization, e.g. niche occupation, is of major importance for the subsequent temporal succession of the PA community. Interestingly, PA *Actinobacteria* in the lake decreased in abundance on the expense of a larger bacterial diversity rather than selecting for another dominant member.

Comparing the two 24 h incubations which differ in their starting time by 10 days resulted in a very different microbial community in both the SMA and MIA fractions supporting the above given suggestion. In particular, the MIA fraction seems to be controlled by the newly produced particulate matter in the lake. The observed diversity on the SMA fraction is most likely a combination of the changes in particulate organic matter and in the FL BCC in the lake. In addition, adhesion of the diatoms from the bloom in the lake to the *Navicula* aggregates led to the import of their associated microbial communities to the SMA fraction. Second, as the microbial community in the lake develops over time (following the ice off and the co-occurring bloom), additional bacterial species that are primary colonizers of aggregates may occur. Nevertheless, the significant abundance of *Sporichthyaceae* and *Acidimicrobaiceae* on both fractions, 10 days apart indicates them as important primary colonizers after ice-off and while the lake is still at low temperatures.

Particles are hotspots for microbial interactions:

As particles have the ability to concentrate bacteria on their surfaces as compared to the surrounding water (Caron et al. 1982), it can be expected that phages will follow their hosts. The occurrence of phages on particles has been shown before (Riemann & Grossart 2008; Müller-Niklas & Schuster 1994; Peduzzi & Weinbauer 1993) and is supported by our results, where one of the most abundant transcripts in all 8 samples was a complete phage genome. This phage best matches in sequences *Microvirus* sp. of the *Microviridae* family. These small sizes ssDNA viruses are known to have a small and conserved genome (4.5-6 kb) thus matching our results of 5.4 kb. They were originally shown to infect enterobacteria (Ackermann 2003) and later also *Caulobacter* and *Pseudomonas* (Ackermann 2006). Our genome matches well with both an *Enterobacteria* (*Gammaproteobacteria*) phage as well as a *Helicobacter* (*Epsilonproteobacteria*) phage. Given their conserved genome, one cannot rule out that the main target of this phage is in fact *Caulobacter*. If this is the case, the DNA of the lysed *Caulobacer*

cells would be detected in the DNA but they will have minimal presence in the RNA. Therefore, it may be that members of the *Caulobacteraceae* are as well among the first particle colonizers yet were susceptive to a broad viral attack which deemed them inactive. Using scanning electron microscopy we were able to detect a *Caulobacter*-like cell with lysis marks as well as symmetric viral-like structures still attached to the cell (Fig. S6C).

All RNA-originating viral genomes consist of a truncated protein as compared to the intact sequences which appear in the metagenome (Fig S3). To check whether this phenomenon is a result of computational error we examined the read coverage in a 3 nt windows along the assembled genomes. A misassembled area would be poorly covered in the sequence library. Our results show that the genome containing the truncated protein is correctly assembled. Therefore, we propose that the obtained truncated sequence is a result of a bacterial defense mechanism consisting of a ribonuclease (RNAse) as commonly used by bacteria against viruses (Beloglazova et al. 2008; Durand et al. 2012). It may be, however, that the truncation occurred already at the DNA stage of the phage, resulting later in the transcription and translation of a partial, non-functioning, protein. Interestingly, all truncation sites obtained in the 8 samples match recognition sites of the FspJI and MspJI restriction sites. The activity of these DNA restriction modification enzymes depends on the methylation of the phage DNA which is a known mechanism by which bacteria target phage DNA for digestion (Adams & Burdon 1985). Recognition sites for these enzymes and other of similar nature are spread throughout the DNA of the phage, thus it is possible that the phage is further digested by the invaded bacteria than actually seen in the assembled transcript. While we bring forth data suggestive of an on-going bacteria-phage interaction on the particles, further experimental work is needed to fully elucidate the specific mechanisms involved.

We can probably rule out CRISPRs were used as a defense mechanism against the phage dominating the transcriptome. With the exception of the last 59 nucleotides of the phage genome, no sequences are missing from the assemblies with truncated proteins as compared to the intact genome of the bacteria. As in CRISPR systems the spacer sequences are used to target phage DNA for digestion (Sorek et al. 2008), one would expect specific sequences to be missing from the transcriptome assembly, which is not the case. Interestingly, among the transcripts we can detect several toxin-antitoxin systems such as the *relBE* and *mazEF* systems. The *mazEF*

system, which is present in numerous bacteria, has been shown to promote programmed cell death in *Escherichia coli* as a mechanism to prevent the spreading of phage infections (Engelberg-Kulka et al. 2005). Thus, by lowering the abundance of host cells, the infected community raises the chance of individual survivors as the probability of the phage to encounter a host is drastically reduced.

The same read-coverage analysis as given above was conducted by using the intact genome (obtained from DNA) and the transcriptome library. The results suggest that this version of the genome as well does not contain erroneous assemblies. This suggests that both intact and truncated sequences are present in the different transcriptomes. Although the bacteria seem to be able to fight a part of the viral attack, it appears that a fraction of the viral community remains active. If indeed the abundance of the host is self-regulated via PCD, the latter will probably disappear with the decay of the target community. While co-evolution was shown to occur in phage-host systems (Riemann & Grossart 2008; Pal et al. 2007), no change in sequence has been seen between the two genome versions other than the truncation itself and the missing 59 nt in the genome assembled from the RNA.

Bacterial colonization of POM aggregates is unlikely to occur without a competitive effort from numerous microbial groups (Grossart et al. 2003a+b). This also includes the phytoplankton species from which the particle is made of, if it is still alive (Amin et al. 2012). Studies have shown *in vitro* antagonistic behaviour between isolates obtained from POM aggregates (Long & Azam 2001) and competitive colonization of particles (Grossart et al. 2003b). Our data fully supports the competitive nature of PA bacteria and their attempt to inhibit the particle-forming diatom. Cytostatic compounds are over expressed after 24h as compared to 8 days incubation (Fig. S4D) which fits with the initial colonization step of the particle. We have found evidence for toxin production in our transcriptome alongside mechanisms for resistance. Among these toxins, we found multiple genes involved in the synthesis of cyanide, a substance which has been shown to work as bacterial produced bactericide (Blumer & Haas 2000), or plant inhibitor (Kremer & Souissi 2001). Cyanide has also been proved to be toxic for diatoms (Pablo et al. 1997) which would suggest it may be used by the colonizing bacteria to penetrate the host. In addition, genes involved in the synthesis of phenazine, a toxic *Pseudomonas* sp., pigment (Gibson et al. 2009) were also found among the transcripts. This pigment, both in natural and modified forms was shown to be a toxin for several

eukaryotic organisms (Cezairliyan et al. 2013) though no information is available regarding diatoms.

A more direct indication of bacterial – host virulence is seen in the expression of type III and VII protein secretion systems, which seem to be overexpressed in the short term incubations. Type III secretion systems have been by now identified in numerous gram negative bacteria and have been shown to be an ancient mechanism which was transferred by lateral gene transfer (Gophna et al. 2003). These systems are involved in bacteria-eukaryote pathogeny as well as other symbiotic interactions (Galán & Wolf-Watz 2006). No data are yet available on the role of this secretion system in bacteria-diatom interactions, but a role in bacteria-fungi relations has been suggested (Warmink & van Elsas 2008). Type VII secretion systems have been identified already in the 1920s from *Mycobacterium bovis* and were associated with *Mycobacteria* sp. ever since (Abdallah et al. 2007). Nevertheless, recent studies have shown this system to be present in other gram positive bacteria (Abdallah et al. 2007) first of which the entire group of *Actinobacteria* (Pallen 2002) as well as *Staphylococcus* sp., (Burts et al. 2005) and *Bacillus* sp. (Pallen 2002). Type IV secretion system which is involved in the virulent injection of protein effectors or DNA into eukaryotic cells has also been detected with similar levels of expression throughout the experiment (Christie 2004). The expression of these protein translocation systems from a broad spectra of bacteria suggest they have a significant role in allowing bacteria to colonize a live POM aggregate. Nevertheless, further studies are required to prove and elucidate this mechanism.

Interestingly, we could not detect the expression of genes directly involved in the synthesis of known antibiotic compounds. Nevertheless, we witnessed a plethora of genes associated with resistance to antibiotics such as polymixin, erythromycin, spectinomycin and streptomycin. We suggest that this supports our hypothesis of an antagonistic and hence partly competitive behaviour between different bacteria (and perhaps fungi), yet this is not direct evidence and further studies are needed.

Hotspots of antagonism as source of genetic variability:

The multitude of interactions between the different bacteria alongside the abundant viral activity support previous suggestions of particles as a hotspot for lateral gene transfer (Walsh et al. 2013; Riemann & Grossart 2008). In our transcriptome we were able to assemble the complete (yet truncated) genome of a *Microvirus*, suggesting this

was the most abundant viral entity on the particles. Nevertheless, the entire data set suggests the presence of up to 100 different viruses or phages (e.g. Fig S4D). The transfer of genetic material between different bacteria is thought to be limited by host abundance in the water column (Weinbauer 2004). This would probably not be the case on the small surface of the highly populated POM aggregate, where accordingly numerous transduction events can occur.

Bacteria are also capable of taking up naked DNA from the environment and incorporating it into their genomes (Chen & Dubnau 2004). In the particle environment DNA is expected to be found in the extracellular matrix due to natural cell lysis, phage infection and bactericide activity (Böckelmann et al. 2006). The uptake of extracellular DNA is a complex process which depends on the function of a Type II protein secretion system, and Type IV pili while its integration often occurs via homologous recombination (Chen & Dubnau 2004). Our transcripts include both sequences of Type II secretion systems as well as the recA and recR recombination proteins. Additionally we found the expression of the *comEA* genes which encodes for a protein essential for DNA uptake (Provvedi & Dubnau 1999).

Temporal shifts in carbon utilization:

One of the main aims of this study was to evaluate the change in POM quality over time during the degradation of the particles. We hypothesized that, after the initial colonization, the change in available substrates due to degradation will first determine gene expression and later community composition. Our result do not contradict this hypothesis but suggests that the timeframe of the experiment was insufficient to cause major shifts in either the expression of genes related to carbon utilization (Fig. S7) nor to shift the active community (Fig. 3). The latter is clearly evident from the stable community profile on single aggregates throughout the experiment. Minor changes in the abundance involved in the degradation of mono and polysaccharides are evident between the different incubation times; however, due to the unexpected high abundance of viral transcripts it is probable that the sequencing depth was not sufficient to reliably detect and quantify such subtle change in carbon utilization. Last, microsensor measurements on parallel particles have shown that the algal photosynthesis has decreased significantly only after 9 days (Ionescu et al. in prep). This suggests a slow degradation process of the particles, dictated by bacteria inhibiting the algal activity. For as long as the algae are active there will be a decreasing yet constant supply of algal DOM. Under these conditions,

major changes in carbon utilization or community are not expected. Nevertheless, studies which compare transcriptomic and proteomic data show that changes in protein activity is not always reflected in the gene expression (Foss et al. 2007; Fu et al. 2009; Waldbauer et al. 2012) due to a significant role of posttranscriptional regulation. Thus we cannot rule out that PA bacteria express a plethora of carbon utilization mechanisms, yet activate and deactivate them directly at the protein level.

To conclude, our study demonstrates that particles are complex micro-niches in which communities are determined by a plethora of competitive, antagonistic activities, between all entities (Fig. 5B). These activities turn the particle into a generator of genetic variability (Fig. 5C). We further show that to directly link between phylogeny and activity on particles, studies should be conducted at the RNA level since the DNA may represent an inactive, perhaps lysed, bacterial community. Last, we suggest that long term transcriptional studies are required to understand the carbon metabolism from individual POM aggregates and its effect on the dynamics of the colonizing communities. Such controlled experiments are now possible at nearly *in situ* conditions using the newly developed flow-through roller tank system presented in Ionescu et al. (in prep) and here.

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Table 1: Statistical overview of the transcriptome annotation using the Trinotate pipeline

Assemebley		**Annotation Summary**			
Contigs	**Transcripts**	**Protein**	**rRNA**	**Not Annotated**	**ncRNA**
4352	4611	2003	314	2294	2
Annotation success of individual tools					
BlastX	**BlastP**	**Pfam**	**eggnog**	**Kegg**	**MG RAST**	**rRNA**
1349	1159	1334	928	878	1687	314

Table 2: Kegg pathways summary

Pathway Group	Pathway	#	total/ group
Nucleotide metabolism	Purine metabolism	69	
	Pyrimidine metabolism	51	
	Alanine, aspartate and glutamate metabolism	23	
	Glycine, serine and threonine metabolism	32	
	Cysteine and methionine metabolism	25	
	Valine, leucine and isoleucine degradation	29	
	Valine, leucine and isoleucine biosynthesis	10	
	Lysine biosynthesis	11	
	Lysine degradation	14	
	Arginine and proline metabolism	26	
	Histidine metabolism	14	
	Tyrosine metabolism	16	
	Phenylalanine metabolism	10	
	Tryptophan metabolism	10	
	Phenylalanine, tyrosine and tryptophan biosynthesis	9	349
Carbohydrate metabolism	Glycolysis / Gluconeogenesis	31	
	Citrate cycle (TCA cycle)	27	
	Pentose phosphate pathway	16	
	Pentose and glucuronate interconversions	12	
	Fructose and mannose metabolism	9	
	Galactose metabolism	8	
	Ascorbate and aldarate metabolism	7	
	Starch and sucrose metabolism	26	
	Amino sugar and nucleotide sugar metabolism	27	
	Pyruvate metabolism	31	
	Glyoxylate and dicarboxylate metabolism	19	
	Propanoate metabolism	21	
	Butanoate metabolism	18	
	C5-Branched dibasic acid metabolism	7	
	Inositol phosphate metabolism	11	270
Signal transduction			
	Two-component system	77	
	Ras signaling pathway	2	
	Rap1 signaling pathway	4	
	MAPK signaling pathway	4	
	MAPK signaling pathway - fly	1	
	ErbB signaling pathway	5	
	Wnt signaling pathway	2	
	Notch signaling pathway	2	
	Hedgehog signaling pathway	1	
	TGF-beta signaling pathway	1	
	Hippo signaling pathway	3	
	Hippo signaling pathway -fly	2	
	VEGF signaling pathway	1	
	Jak-STAT signaling pathway	1	
	NF-kappa B signaling pathway	3	
	TNF signaling pathway	4	
	HIF-1 signaling pathway	12	
	FoxO signaling pathway	7	
	Calcium signaling pathway	3	
	Phosphatidylinositol signaling system	7	
	PI3K-Akt signaling pathway	8	
	mTOR signaling pathway	4	154

Pathway Group	Pathway	#	total/ group
Energy metabolism	Oxidative phosphorylation	46	
	Photosynthesis	10	
	Carbon fixation in photosynthetic organisms	15	
	Carbon fixation pathways in prokaryotes	23	
	Methane metabolism	21	
	Nitrogen metabolism	15	
	Sulfur metabolism	19	149
Metabolism of cofactors and vitamins	Thiamine metabolism	4	
	Riboflavin metabolism	7	
	Vitamin B6 metabolism	5	
	Nicotinate and nicotinamide metabolism	12	
	Pantothenate and CoA biosynthesis	6	
	Biotin metabolism	6	
	Lipoic acid metabolism	2	
	Folate biosynthesis	9	
	One carbon pool by folate	9	
	Retinol metabolism	6	
	Porphyrin and chlorophyll metabolism	13	
	Ubiquinone and other terpenoid-quinone biosynthesis	6	85
Replication and repair	DNA replication	16	
	Base excision repair	13	
	Nucleotide excision repair	16	
	Mismatch repair	14	
	Homologous recombination	18	
	Non-homologous end-joining	1	
	Fanconi anemia pathway	2	80
Xenobiotics biodegradation and metabolism	Benzoate degradation	4	
	Aminobenzoate degradation	9	
	Chloroalkane and chloroalkene degradation	9	
	Chlorocyclohexane and chlorobenzene degradation	3	
	Toluene degradation	3	
	Ethylbenzene degradation	2	
	Styrene degradation	2	
	Atrazine degradation	1	
	Caprolactam degradation	3	
	Bisphenol degradation	1	
	Naphthalene degradation	4	
	Polycyclic aromatic hydrocarbon degradation	4	
	Metabolism of xenobiotics by cytochrome P45	7	
	Drug metabolism - cytochrome P45	8	
	Drug metabolism - other enzymes	8	68
Lipid metabolism	Fatty acid biosynthesis	8	
	Fatty acid elongation	1	
	Fatty acid degradation	17	
	Synthesis and degradation of ketone bodies	2	
	Steroid biosynthesis	2	
	Primary bile acid biosynthesis	2	
	Steroid hormone biosynthesis	3	
	Glycerolipid metabolism	5	
	Glycerophospholipid metabolism	8	
	Ether lipid metabolism	2	
	Sphingolipid metabolism	3	
	Arachidonic acid metabolism	2	
	Biosynthesis of unsaturated fatty acids	6	61

Table 2 continued: Kegg pathways summary

Pathway Group	Pathway	#	total/ group	Pathway Group	Pathway	#	total/ group
Metabolism of other amino acids	beta-Alanine metabolism	15		Translation			
	Taurine and hypotaurine metabolism	3			Aminoacyl-tRNA biosynthesis	19	
	Selenocompound metabolism	12			RNA transport	6	
	Cyanoamino acid metabolism	7			mRNA surveillance pathway	2	
	D-Glutamine and D-glutamate metabolism	2			Ribosome biogenesis in eukaryotes	3	30
	D-Arginine and D-ornithine metabolism	1		Cell motility	Bacterial chemotaxis	18	
	D-Alanine metabolism	2			Flagellar assembly	8	
	Glutathione metabolism	16	58		Regulation of actin cytoskeleton	4	30
Transcription				Biosynthesis of other secondary metabolites	Phenylpropanoid biosynthesis	7	
					Isoquinoline alkaloid biosynthesis	3	
	RNA polymerase	14			Tropane, piperidine and pyridine alkaloid biosynthesis	3	
	Basal transcription factors	2			Caffeine metabolism	1	
	Spliceosome	3			Betalain biosynthesis	1	
	Ribosome	39	58		Penicillin and cephalosporin biosynthesis	1	
Membrane transport					beta-Lactam resistance	2	
					Streptomycin biosynthesis	4	
	ABC transporters	35			Butirosin and neomycin biosynthesis	1	
	Phosphotransferase system (PTS)	1			Novobiocin biosynthesis	2	25
	Bacterial secretion system	11	47	Transport and catabolism	Endocytosis	4	
Folding, sorting and degradation	Protein export	6			Phagosome	1	
	Protein processing in endoplasmic reticulum	4			Lysosome	4	
	Ubiquitin mediated proteolysis	6			Peroxisome	12	
	Sulfur relay system	6			Regulation of autophagy	1	22
	Proteasome	1		Excretory system	Aldosterone-regulated sodium reabsorption	1	
	RNA degradation	20	43		Proximal tubule bicarbonate reclamation	2	
Cell growth and death	Cell cycle	7			Phototransduction	2	
	Cell cycle - yeast	7			Phototransduction - fly	2	
	Cell cycle - Caulobacter	18			Olfactory transduction	3	
	Meiosis - yeast	4			Dorso-ventral axis formation	1	
	Oocyte meiosis	1			Axon guidance	5	
	Apoptosis	4			Osteoclast differentiation	3	19
	p53 signaling pathway	2	43	Environmental adaptation			
Glycan biosynthesis and metabolism	N-Glycan biosynthesis	2			Circadian rhythm	1	
	Other types of O-glycan biosynthesis	3			Circadian entrainment	6	
	Glycosaminoglycan biosynthesis - chondroitin sulfate /	1			Circadian rhythm - fly	1	
	Glycosaminoglycan biosynthesis - heparan sulfate / hep	1			Plant-pathogen interaction	7	15
	Glycosaminoglycan biosynthesis - keratan sulfate	1		Signaling molecules and interaction			
	Glycosaminoglycan degradation	2					
	Glycosylphosphatidylinositol(GPI)-anchor biosynthesis	3			Neuroactive ligand-receptor interaction	7	
	Glycosphingolipid biosynthesis - lacto and neolacto seri	1			Cytokine-cytokine receptor interaction	1	
	Lipopolysaccharide biosynthesis	5			ECM-receptor interaction	1	
	Peptidoglycan biosynthesis	15			Cell adhesion molecules (CAMs)	1	10
	Other glycan degradation	4	38	Cell communication			
Metabolism of terpenoids and polyketides							
					Focal adhesion	4	
	Terpenoid backbone biosynthesis	8			Adherens junction	1	
	Carotenoid biosynthesis	1			Tight junction	2	
	Zeatin biosynthesis	1			Gap junction	3	10
	Limonene and pinene degradation	6					
	Geraniol degradation	7					
	Biosynthesis of ansamycins	3					
	Tetracycline biosynthesis	2					
	Polyketide sugar unit biosynthesis	1					
	Biosynthesis of vancomycin group antibiotics	1	30		Other pathways		598

Table 3: Matching distribution of predicted C**lustered Regularly Interspaced Short Palindromic Repeats** (CRISPRs) from the metagenome in the transcriptome.

			Match to transcripts		Expressed transcripts Annotation		Matched viral Transcripts	
Total CRISPR candidates	Total Spacers	Match sampleRNA	Expressed	Not expressed	Annotated	Not Annotated	Good Match (long)	Patial match (short)
83	175	145	134	11	62	62	1	10

Figure legends:

Figure 1. The flow-through roller tank system schematics (A) inoculation (B) and operation (C).

Figure 2. Sampling time scheme of single macroscopic aggregates (SMA) and the fraction larger than 5 μm (MIA). Each sample consists of 2 roller-tanks whose RNA was pooled for sequencing purposes.

Figure 3. Community composition of the Single Macroscopic Aggregates (SMA) and Microscopic Aggregates (MIA) according to 16S rRNA analysis (A) and whole transcript/metagenome annotation (B). Samples for DNA were collected after 2 days of incubation (D2) and were used for 16S rRNA gene sequencing (A) or for metagenomic analysis (B). RNA samples were collected after 1, 7, 8 days of incubation (D1, D1A, D7, D8). Samples were inoculated and sampled as detailed in Fig. 2.

Figure 4. A complete Microvirus genome as assembled either from the metagenome or from all transcriptome data sets combined (for separate assemblies see Figure S5). The read coverage plot was calculated using the transcriptome data on both genomes.

Figure 5. Conceptual summary of event leading from a potential (A) to an actual (C) community of particle colonizing bacteria as can be deduced from the transcriptome data. The sum of antagonistic reactions includes bidirectional repelling (red) and bacteriocidic (black) reactions between the aggregate forming organisms and the colonizing bacteria (B). Phage activity has also an influence on colonizing bacteria. The lytic activities, phage infection and bacterial density will allow for genetic material transfer by several mechanisms (C).

Figure 1.

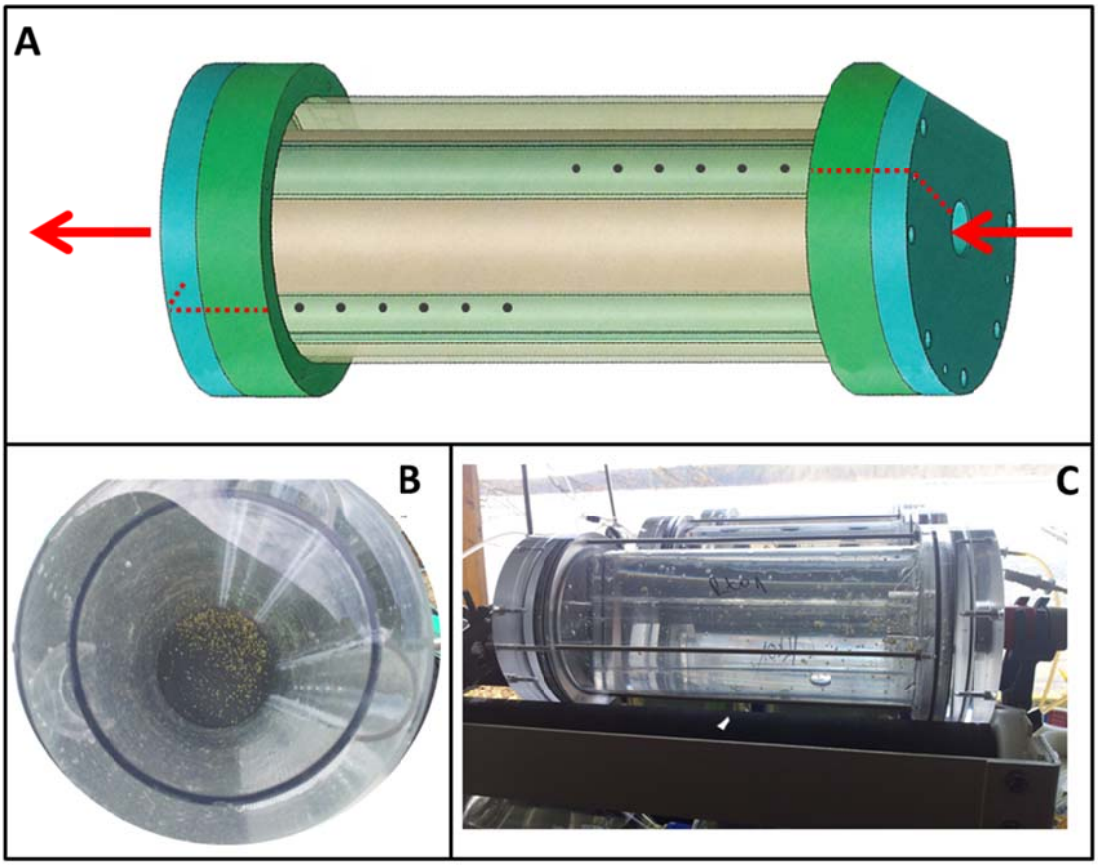


Figure 2.

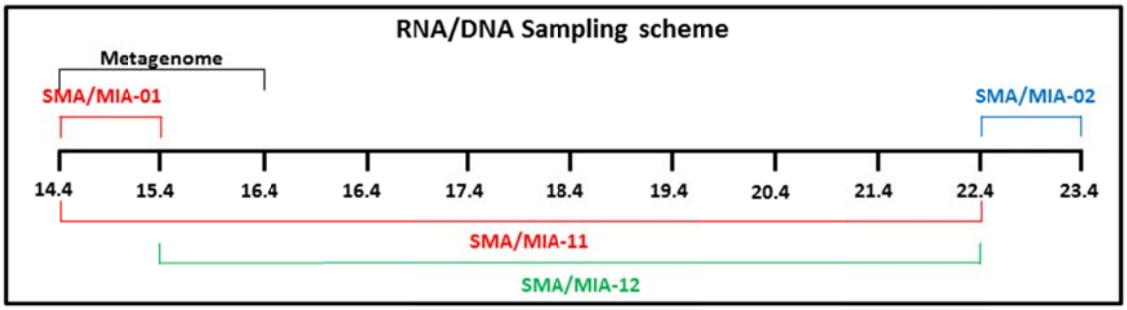


Figure 3.

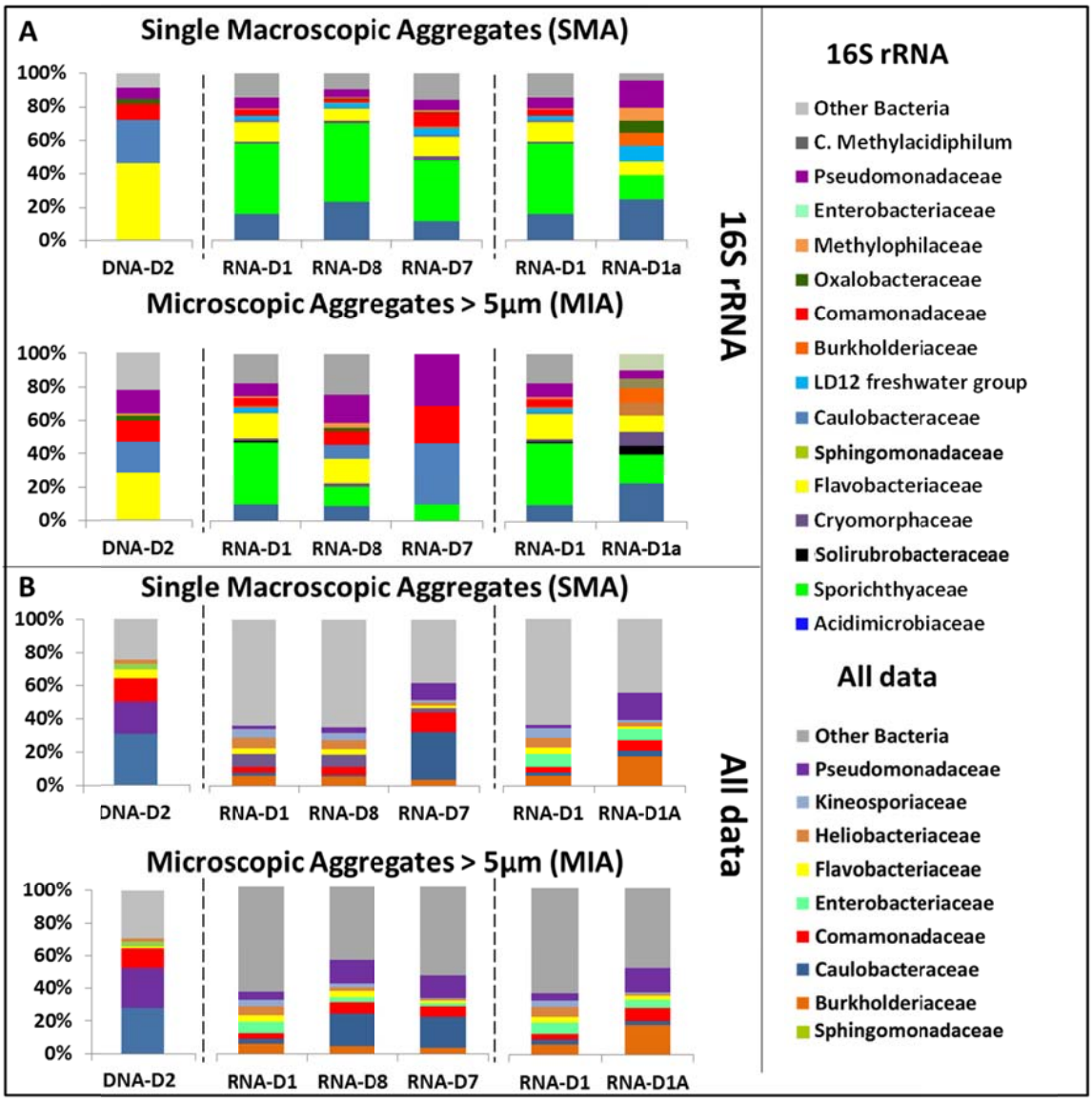
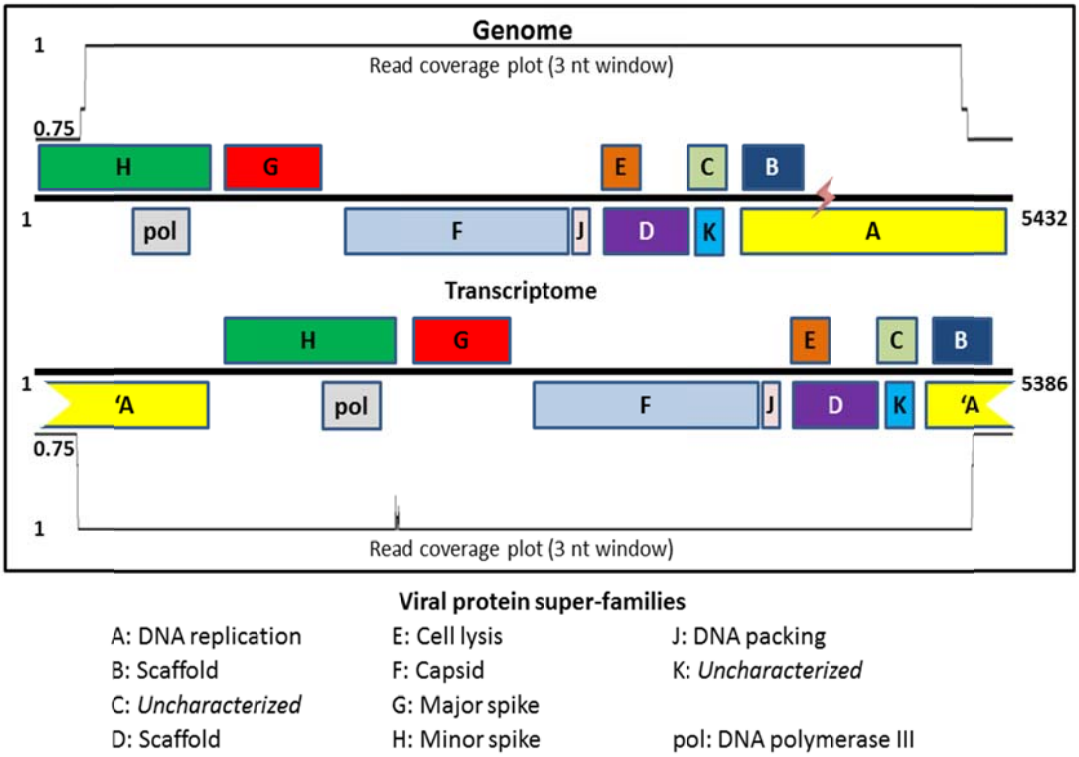

A
Single Macroscopic Aggregates (SMA)
100%
80%
60%
40%
20%
0%
DNA-D2
RNA-D1
RNA-D8
RNA-D7
RNA-D1
RNA-D1a
Microscopic Aggregates > 5µm (MIA)
16S rRNA
B
Single Macroscopic Aggregates (SMA)
RNA-D1A
Microscopic Aggregates > 5µm (MIA)
All data
16S rRNA
Other Bacteria
C. Methylacidiphilum
Pseudomonadaceae
Enterobacteriaceae
Methylophilaceae
Oxalobacteraceae
Comamonadaceae
Burkholderiaceae
LD12 freshwater group
Caulobacteraceae
Sphingomonadaceae
Flavobacteriaceae
Cryomorphaceae
Solirubrobacteraceae
Sporichthyaceae
Acidimicrobiaceae
All data
Other Bacteria
Pseudomonadaceae
Kineosporiaceae
Heliobacteriaceae
Flavobacteriaceae
Enterobacteriaceae
Comamonadaceae
Caulobacteraceae
Burkholderiaceae
Sphingomonadaceae

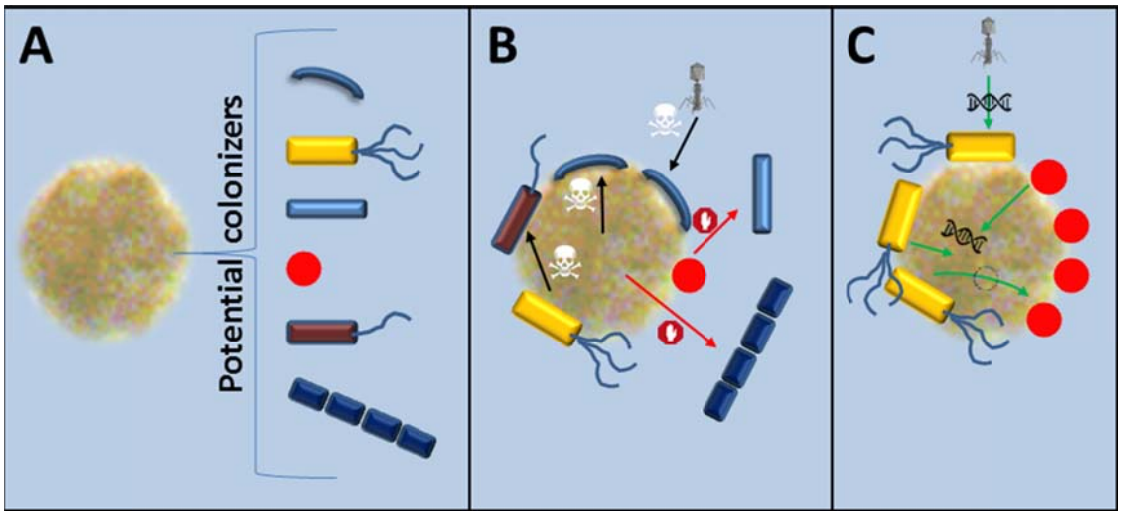
Figure 4.



Viral protein super-families

A: DNA replication	E: Cell lysis	J: DNA packing
B: Scaffold	F: Capsid	K: *Uncharacterized*
C: *Uncharacterized*	G: Major spike	
D: Scaffold	H: Minor spike	pol: DNA polymerase III

Figure 5.



Supplementary figures legends:

Figure S1. 16S rRNA and whole transcript community composition at the class phylogenetic depth.

Figure S2. Phylogenetic distribution of Archaea derived from all taxonomically annotatable transcripts. In sample D1a only 2 sequences could be associated with known Archaeal taxa.

Figure S3. Assembly of the *Microvirus* genome from each individual sample.

Figure S4. A tree representation of clusters of functions of annotated transcripts. The stacked bars represent the read abundance per compared dataset per function. Annotated transcripts may be associated with more than one function. The functions are defined according to the SEED database as incorporated into MG-RAST. Comparisons are shown only for the SMA fraction and as follows: Day 1 and Day 8 (A), Day 1 and Day 7 (B), Day 7 and Day 8 (C) and Day 1 and Day 1a (D). The figures were generated via MG-RAST analysis interface.

Figure S5. Comparison of sequence distribution across the Bacterial phylogenetic tree between the SILVA nrSSU database and all available sequenced genomes.

Figure S6. Scanning electron microscopy of a particle from Day 1 (a) and Day 5 (B). Phage-like particles were observed on cells resembling *Caulobacter* (c) as well as on other bacteria (d). Red and white arrows represent potential exit sites of phage particles and attached pahges, respectively.

Figure S7. The distribution of carbohydrate metabolism annotated transcripts in each dataset among the different functional groups. Annotated transcripts may be associated with more than one function. The functions are defined according to the SEED database as incorporated into MG-RAST. The figures were generated via MG-RAST analysis interface and the Krona application.

Figure S1.

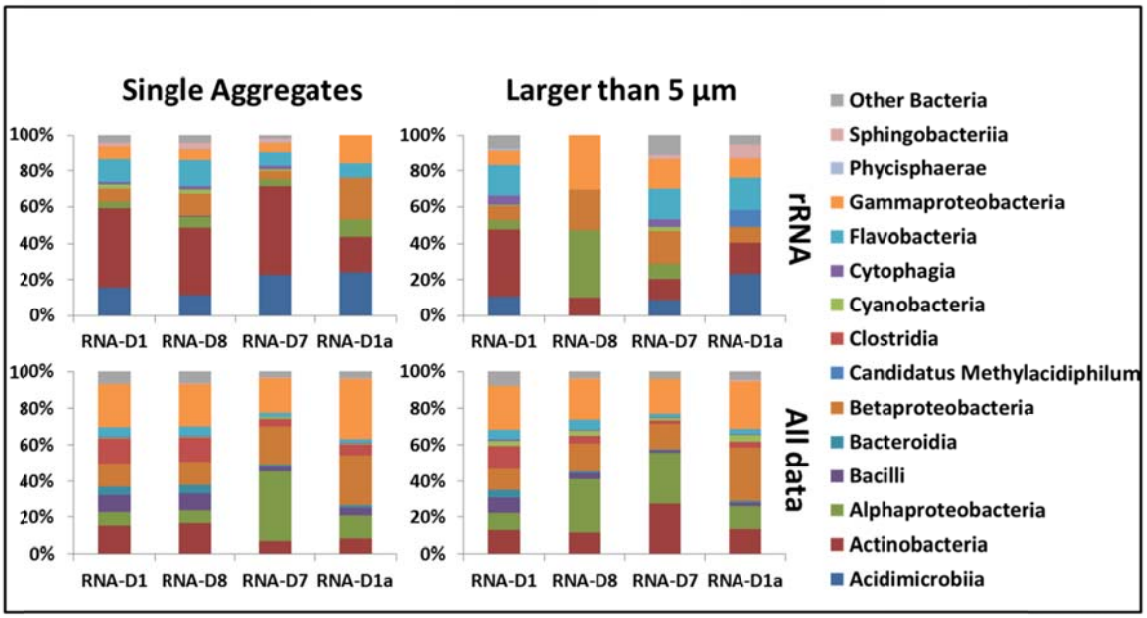


Figure S2.

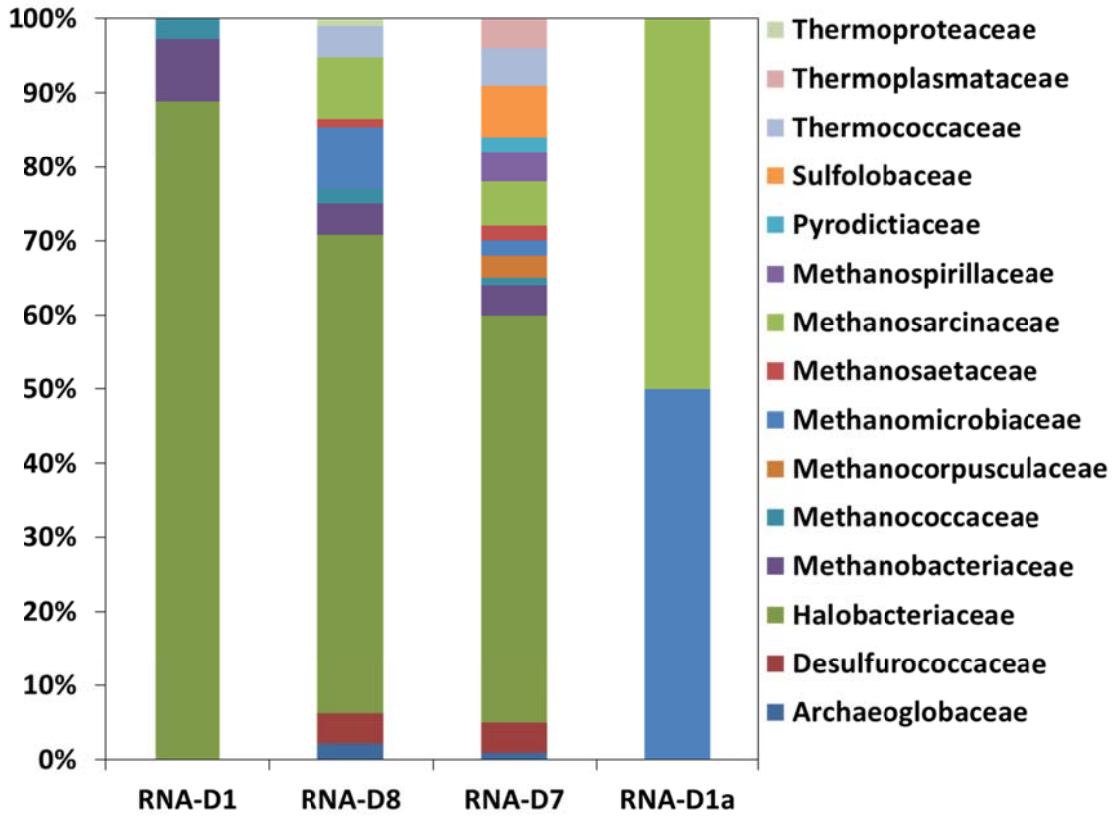


Figure S3.

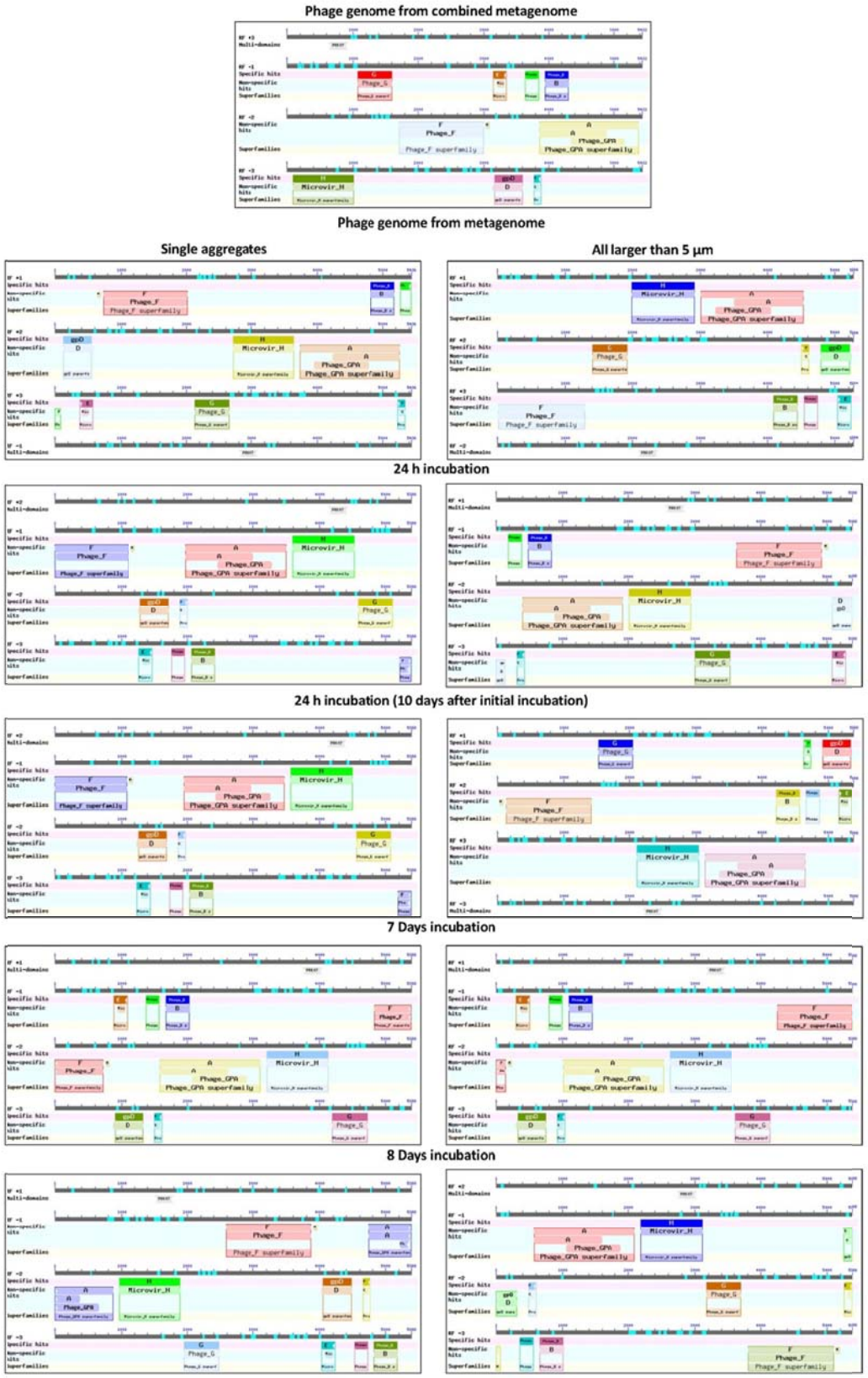

Phage genome from combined metagenome
Phage genome from metagenome
Single aggregates
All larger than 5 µm
24 h incubation
24 h incubation (10 days after initial incubation)
7 Days incubation
8 Days incubation

Figure S4-A.

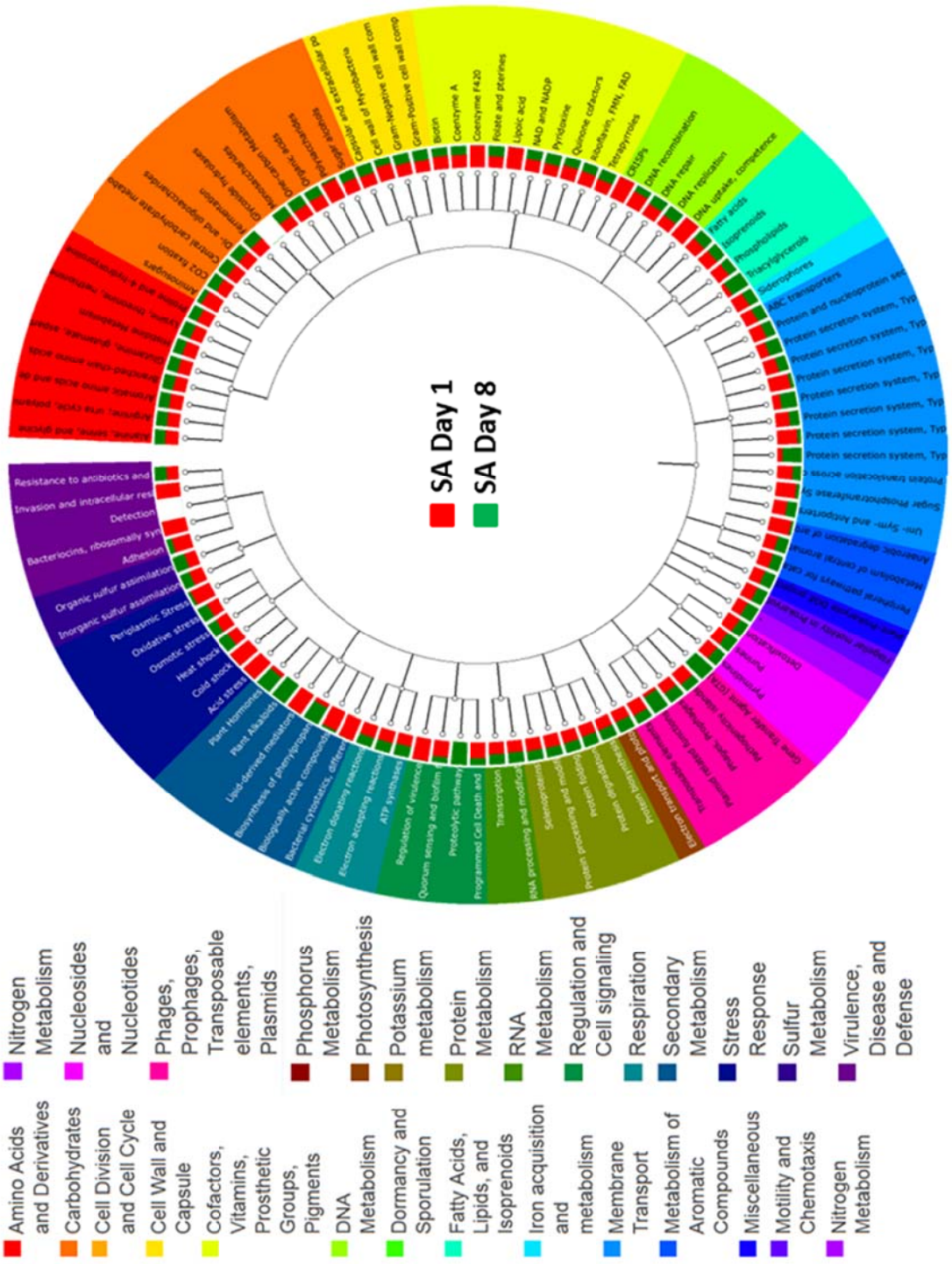

SA Day 1
SA Day 8
Nitrogen Metabolism
Nucleosides and Nucleotides
Phages, Prophages, Transposable elements, Plasmids
Phosphorus Metabolism
Photosynthesis
Potassium metabolism
Protein Metabolism
RNA Metabolism
Regulation and Cell signaling
Respiration
Secondary Metabolism
Stress Response
Sulfur Metabolism
Virulence, Disease and Defense
Amino Acids and Derivatives
Carbohydrates
Cell Division and Cell Cycle
Cell Wall and Capsule
Cofactors, Vitamins, Prosthetic Groups, Pigments
DNA Metabolism
Dormancy and Sporulation
Fatty Acids, Lipids, and Isoprenoids
Iron acquisition and metabolism
Membrane Transport
Metabolism of Aromatic Compounds
Miscellaneous
Motility and Chemotaxis
Nitrogen Metabolism

Figure S4-B.

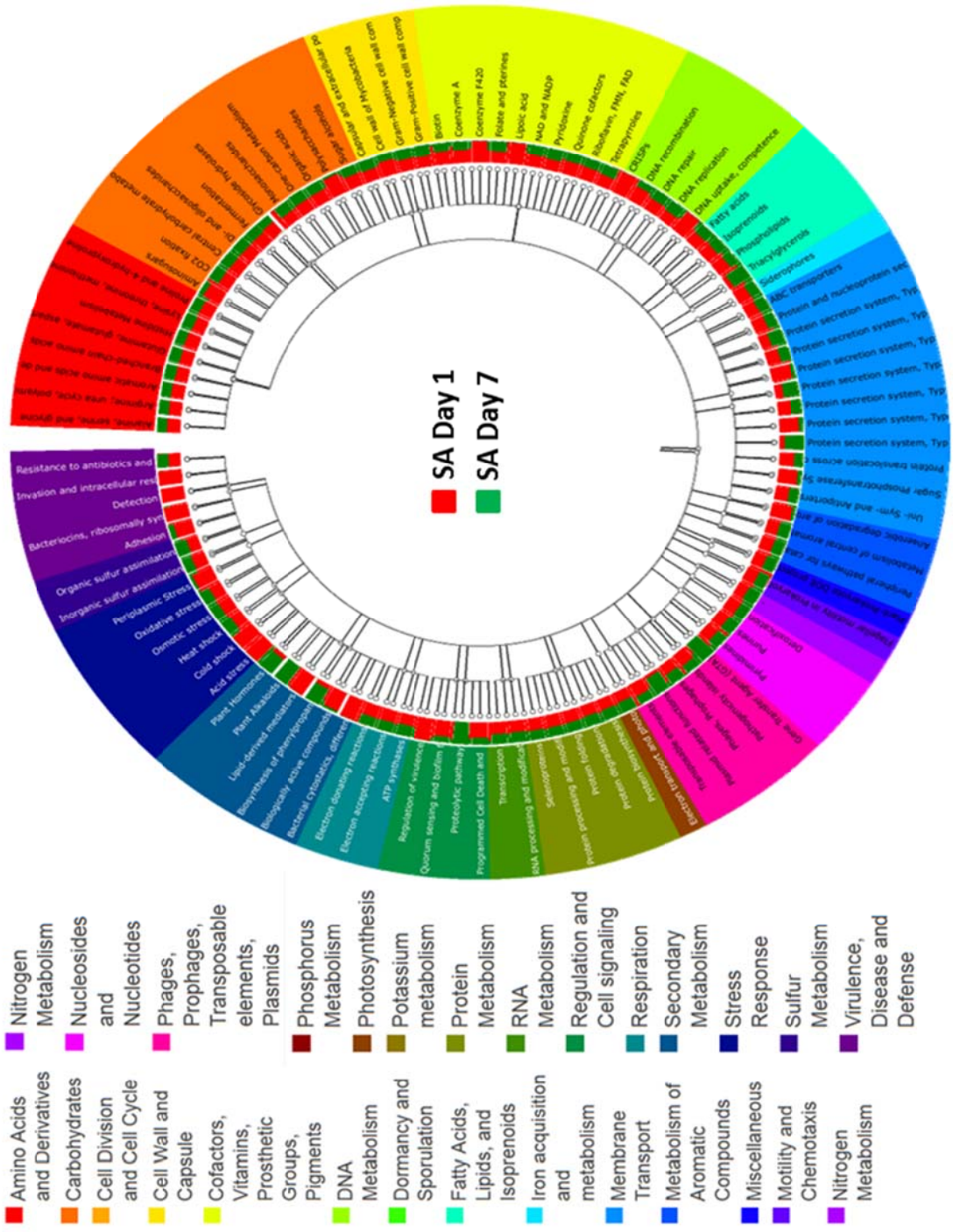

SA Day 1
SA Day 7
Amino Acids and Derivatives
Carbohydrates
Cell Division and Cell Cycle
Cell Wall and Capsule
Cofactors, Vitamins, Prosthetic Groups, Pigments
DNA Metabolism
Dormancy and Sporulation
Fatty Acids, Lipids, and Isoprenoids
Iron acquisition and metabolism
Membrane Transport
Metabolism of Aromatic Compounds
Miscellaneous
Motility and Chemotaxis
Nitrogen Metabolism
Nitrogen Metabolism
Nucleosides and Nucleotides
Phages, Prophages, Transposable elements, Plasmids
Phosphorus Metabolism
Photosynthesis
Potassium metabolism
Protein Metabolism
RNA Metabolism
Regulation and Cell signaling
Respiration
Secondary Metabolism
Stress Response
Sulfur Metabolism
Virulence, Disease and Defense

Figure S4-C.

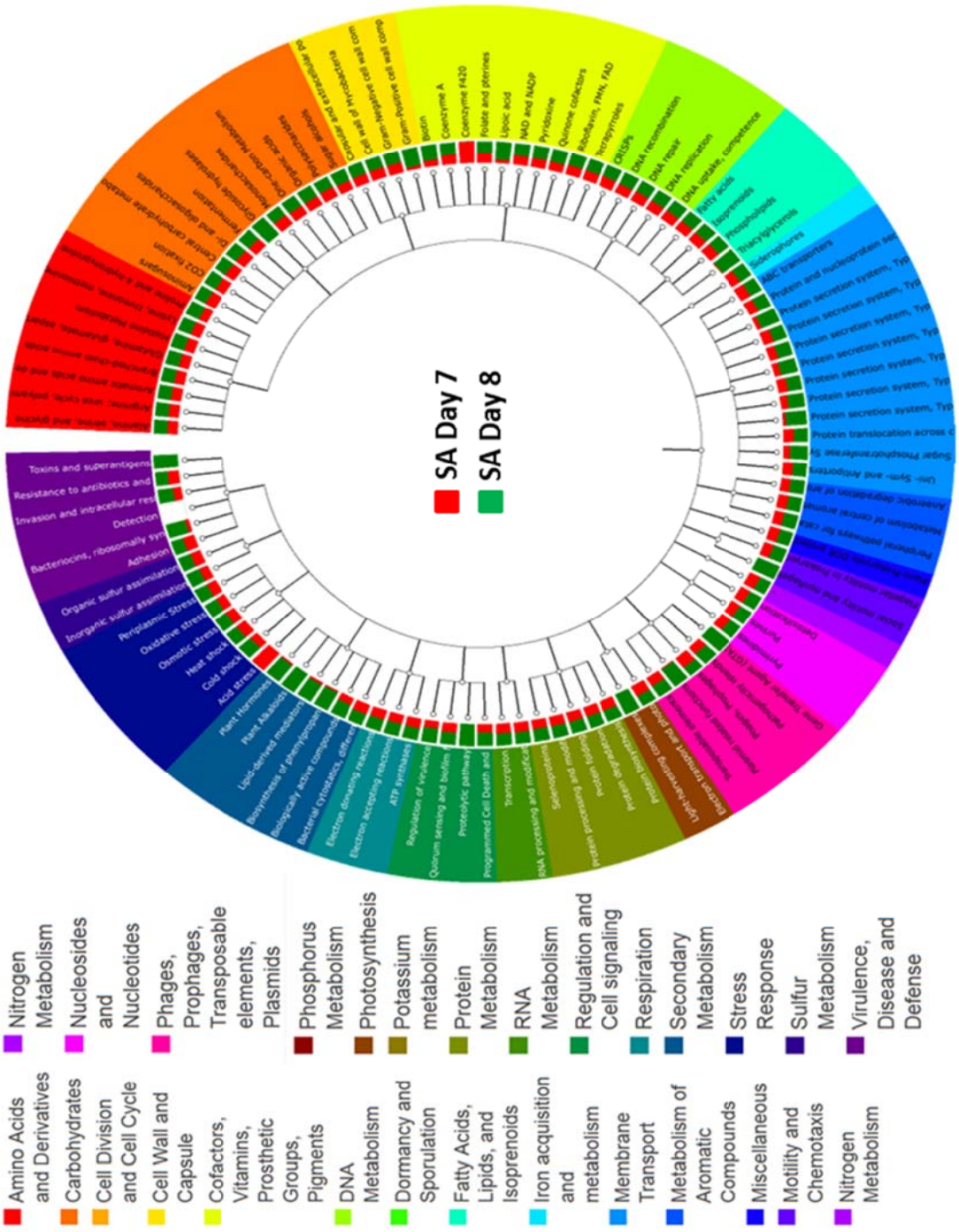

SA Day 7
SA Day 8
Nitrogen Metabolism
Nucleosides and Nucleotides
Phages, Prophages, Transposable elements, Plasmids
Phosphorus Metabolism
Photosynthesis
Potassium metabolism
Protein Metabolism
RNA Metabolism
Regulation and Cell signaling
Respiration
Secondary Metabolism
Stress Response
Sulfur Metabolism
Virulence, Disease and Defense
Amino Acids and Derivatives
Carbohydrates
Cell Division and Cell Cycle
Cell Wall and Capsule
Cofactors, Vitamins, Prosthetic Groups, Pigments
DNA Metabolism
Dormancy and Sporulation
Fatty Acids, Lipids, and Isoprenoids
Iron acquisition and metabolism
Membrane Transport
Metabolism of Aromatic Compounds
Miscellaneous
Motility and Chemotaxis
Nitrogen Metabolism

Figure S4-D.

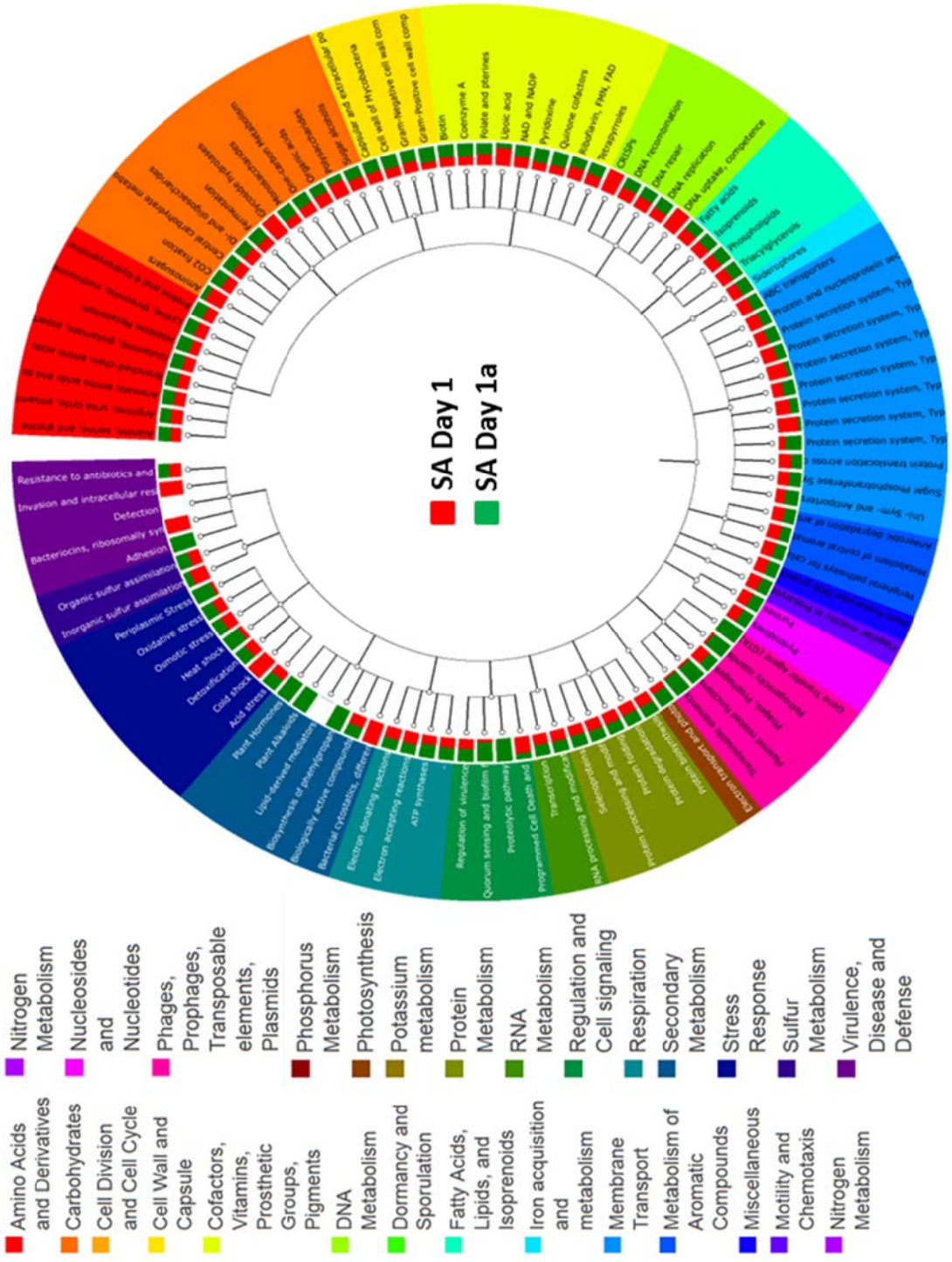

SA Day 1
SA Day 1a
Nitrogen Metabolism
Nucleosides and Nucleotides
Phages, Prophages, Transposable elements, Plasmids
Phosphorus Metabolism
Photosynthesis
Potassium metabolism
Protein Metabolism
RNA Metabolism
Regulation and Cell signaling
Respiration
Secondary Metabolism
Stress Response
Sulfur Metabolism
Virulence, Disease and Defense
Amino Acids and Derivatives
Carbohydrates
Cell Division and Cell Cycle
Cell Wall and Capsule
Cofactors, Vitamins, Prosthetic Groups, Pigments
DNA Metabolism
Dormancy and Sporulation
Fatty Acids, Lipids, and Isoprenoids
Iron acquisition and metabolism
Membrane Transport
Metabolism of Aromatic Compounds
Miscellaneous
Motility and Chemotaxis
Nitrogen Metabolism

Figure S5.

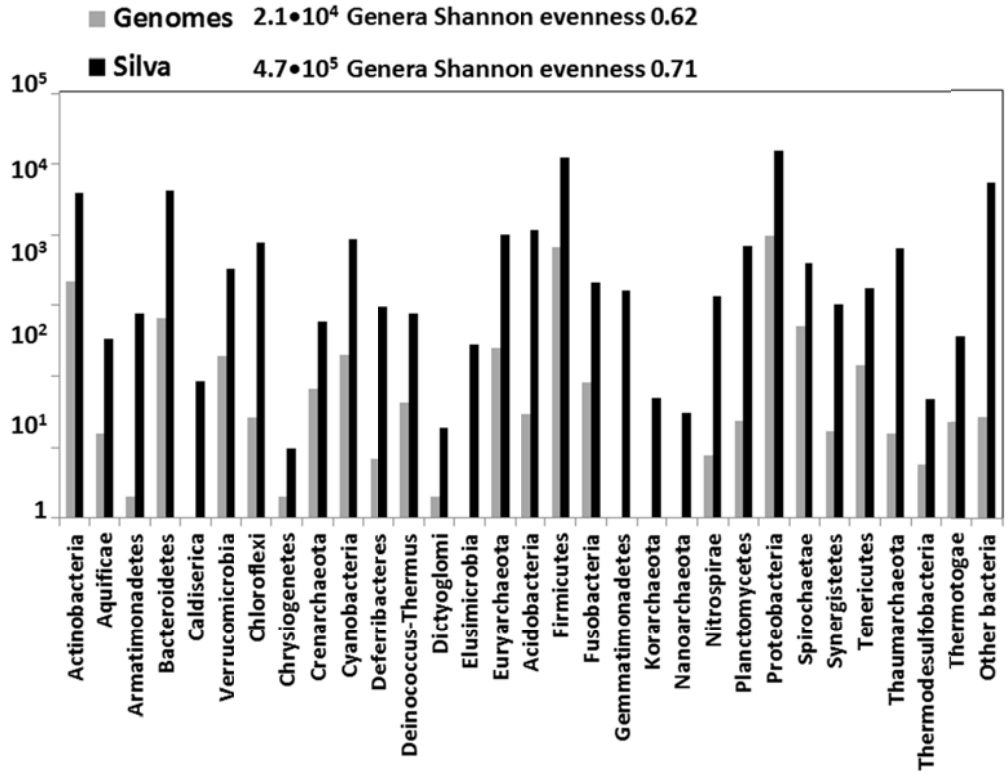


Figure S6.

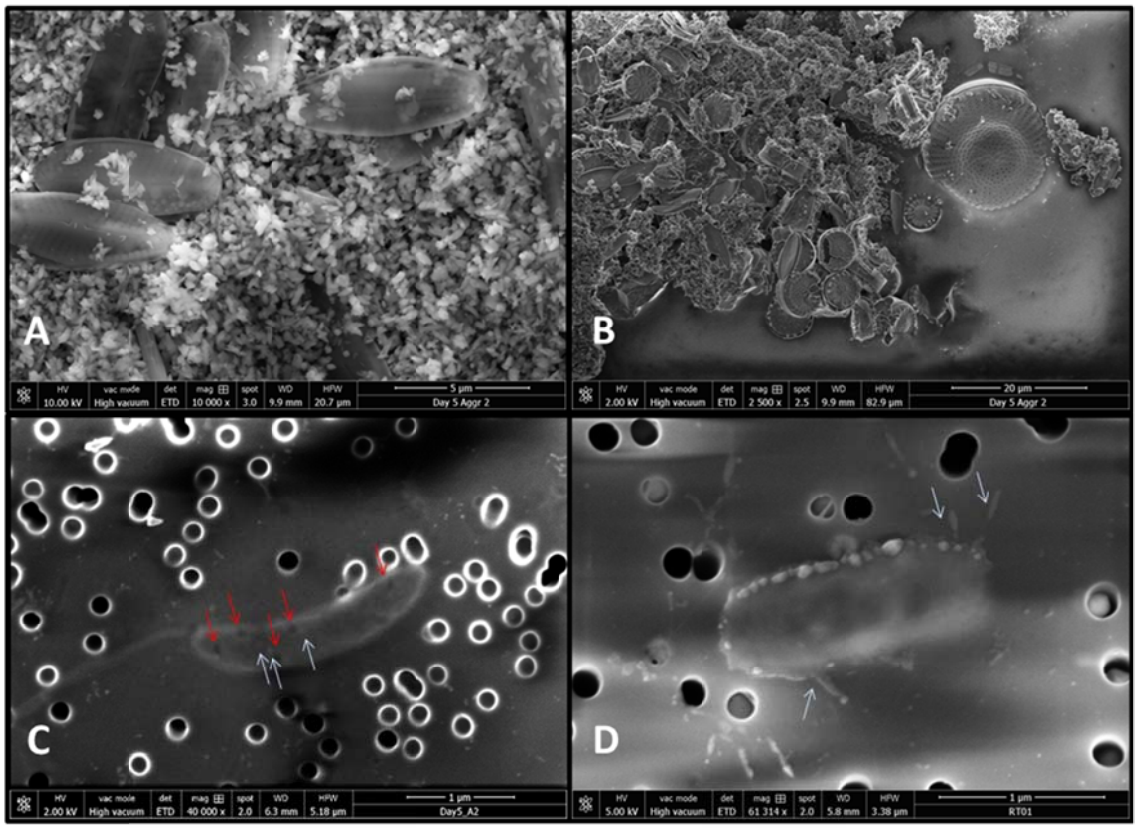
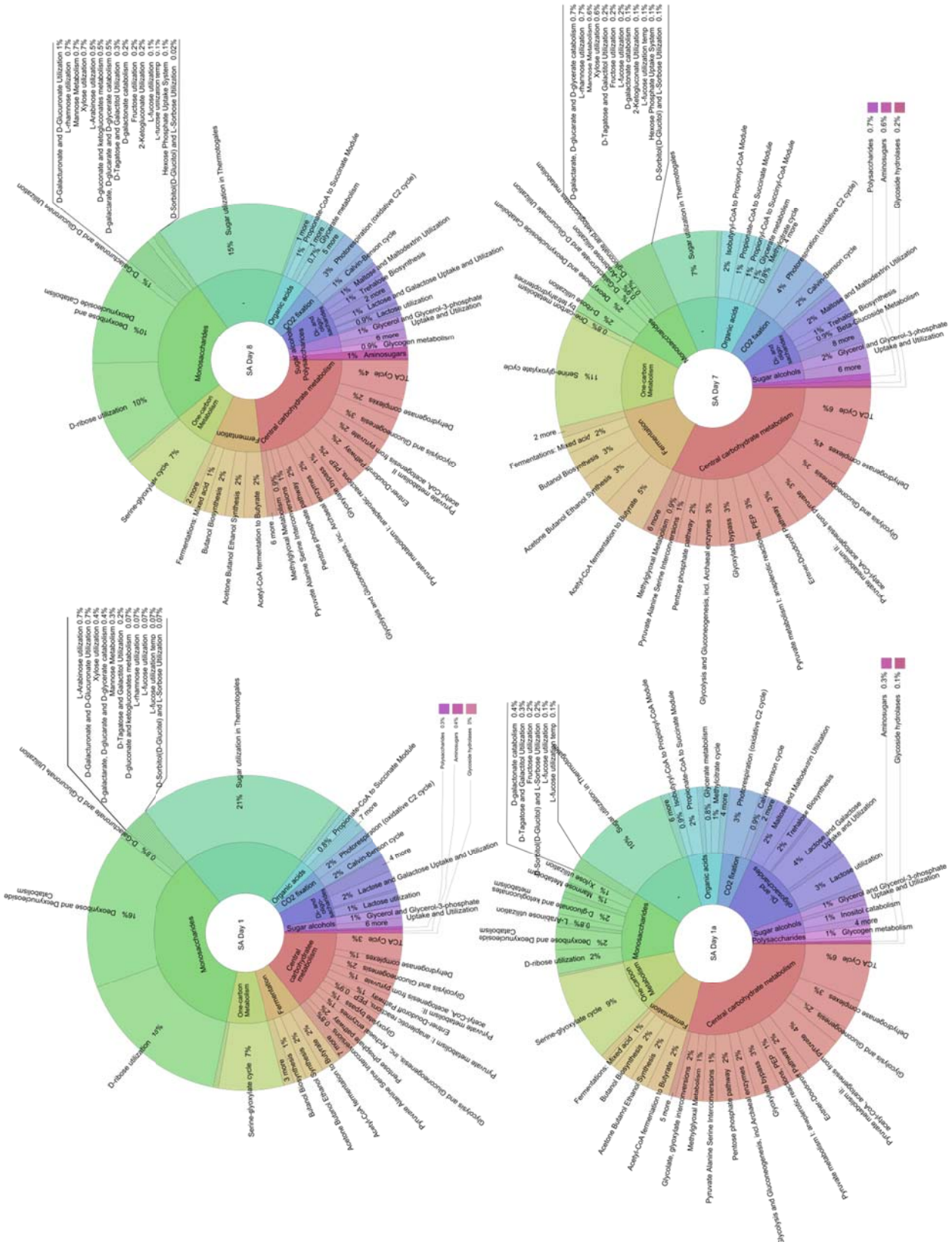


Figure S7.


SA Day 8
SA Day 7
SA Day 1
SA Day 1a
Sugar utilization in Thermotogales
Central carbohydrate metabolism
Monosaccharides
Organic acids
CO2 fixation
Sugar alcohols
Polysaccharides
One-carbon Metabolism
Fermentation
TCA Cycle
Glycosidases
Aminosugars
Glycoside hydrolases

5. DISCUSSION AND OUTLOOK

5.1 A holistic view of aquatic science – Lakes and Oceans as a single functional unit

Limnic and marine ecosystems are linked by the medium that defines both of them—water. It facilitates connections among aquatic systems by virtue of its physico-chemical characteristics, such as the nonlinear relationship between its density and temperature, high heat capacity, high viscosity, and its capacity to dissolve more substances than any other liquid (Dobson & Frid 2008). Throughout the history of aquatic sciences, limnic and marine subdisciplines have developed independently. The obvious reasons for these separations were differences in chemical composition, organisms, and the size of the aquatic ecosystem studied (Dobson & Frid 2008). Despite the difference of their communities and complexity of physical, chemical, and biological processes, both systems operate in a very similar way. Lakes vary by orders of magnitude in size, age, salinity, depth and allochthonous input. There are millions of limnic systems, present at almost all latitudes and altitudes, and in a variety of geological and hydrological settings. Studying and comparing the different properties of lakes along gradients in size, chemical composition and other features may help us understand the factors controlling aquatic communities and biogeochemical cycles. In the words of Schindler (1991): *"In making such comparisons it is in many cases convenient to treat oceans simply as end members; that is as our largest, deepest and oldest lakes."* Hence I provide here a comparative discussion and a call for such studies in cases which have not been tested so far.

5.2 The freshwater/salt barrier - how strong is it really?

Saline and freshwater body systems are usually separate entities, but transition of microorganisms from one to another constantly occurs through various mechanisms. Rivers unidirectionally transport biomass from freshwater to the marine system. Bidirectional transport mechanisms include aerosols, plants, euryhaline (anadromous) fish, birds, and on an evolutionary more recent timescale there is also an increasingly anthropogenic contribution.

The separation between limnic and marine bacteria can be driven by several factors. Salinity of natural waters is one of the main environmental factors with a major contribution to structure and functional characteristics of aquatic microbiota. Hence, it can be an important factor for the separation of bacterial communities (Bouvier & Giorgio 2002; Selje & Simon 2003; Telesh et al. 2013). To cope with external salinity, bacteria use two different mechanisms for osmoregulation. The "salt in" mechanism, utilized mostly but not exclusively by extreme halophiles and "compatible solutes" used by moderate halophiles and halotolerant bacteria (Wood 2011). Freshwater bacteria have been shown to survive in marine systems since the 1950s when the fate of sewage outflow into the ocean was investigated (Carlucci & Pramer 1959; Székely et al. 2013). Recent metagenomic studies have shown that while freshwater bacteria probably use the "salt in" osmoregulatory pathways, they have a significantly lower number of Na pumps in comparison to marine bacteria (Oh et al. 2011). This would impair their ability to deal with the Na influx in saline waters or increase its energetic cost. Results showing that competition with resident communities have the strongest influence (Székely et al. 2013) would favor for the latter option. An additional factor that may play a role in the transition of bacteria from freshwater to a marine environment is pH. While the pH in freshwater may vary dramatically between systems, ranging between <2 to >12 (Joint et al. 2011), the oceanic pH is rather stable at slightly alkaline values of about 8.1-8.3.

In one of my projects, by comparing samples collected over a prolonged period of time from two different limnic and two different marine systems, we tried to evaluate the frequency of taxa common to both types of environments. Interestingly, only 68 OTUs (clustered at 99% similarity) out of 35,554 were shared

between the limnic and marine systems. Out of these, 41 OTUs belonged to PA bacteria, 19 of which were known as potential pathogens. These include bacteria such as *Staphylococcus aureus*, *S. epidermidis*, *S. pasteuri*, *Brevundimonas diminuta*, *Bosea massiliensis,* and *Vibrio* sp. Although we cannot rule out that these sequences are anthropogenic contaminations which occurred during sampling or sample preparation, particles are known to serve as a refuge of pathogenic bacteria (Tang et al. 2011; Grossart et al. 2010). It has been described before that important pathogens like *Vibrio cholera* survive and spread on particles (Colwell et al. 2003; Danovaro et al. 2009). Nevertheless, this is the first time that 16S rRNA gene sequences identical to that of pathogenic bacteria are described simultaneously, on particles from both limnic and marine systems. Our findings suggest that particles could serve as transfer vehicle between different ecosystems. It has been observed that in estuaries, environments with transitional properties between limnic and marine systems, >50% of the bacteria are found associated to particles (Crump et al. 1998; Simon et al. 2002). Therefore, it is possible that the microniche of the particle protects the bacteria against sudden changes in the environmental conditions. A similar observation was made for bacteria "hiding" from UV irradiation in wastewater treatment plants (Tang et al. 2011). While a particle can shade the UV light thus offering the bacteria a protective environment, it remains to be determined if it can also provide a safe passage through a strong salinity gradient.

To establish the validity of these results on a global scale, I have done a similar, yet small scale, comparison using the SILVA PARC small rRNA subunit database. I extracted 367,000 marine and freshwater sequences (out of 3,800,000), using text identifiers applied to the sequence isolation location. A similar comparison was done by Barberán and Casamayor (2010) using >7000 surface water sequences which clustered at 97% similarity. When clustering the sequences obtained from the SILVA database (Quast et al. 2013) at different similarity levels, I observed the omnipresence of a certain set of sequences between marine and freshwater systems (Fig. 5.1). Interestingly, the number of obtained OTUs (with 90-99% sequence similarity) shared between marine and freshwater systems appear to be rather conserved, ranging between 4850 and 5000 OTUs, and only dropping to 2279 at 100% similarity. Nevertheless, these OTUs show a decrease in their proportional significance from the entire dataset decreasing between from 25% to 0.81% out of the total OTUs when the similarity criteria was increased from 90-100%, respectively.

In our study we were able to provide a PA/FL distribution of the taxa shared between limnic and marine habitats, however, this was not possible for the SILVA database as the information is not available.

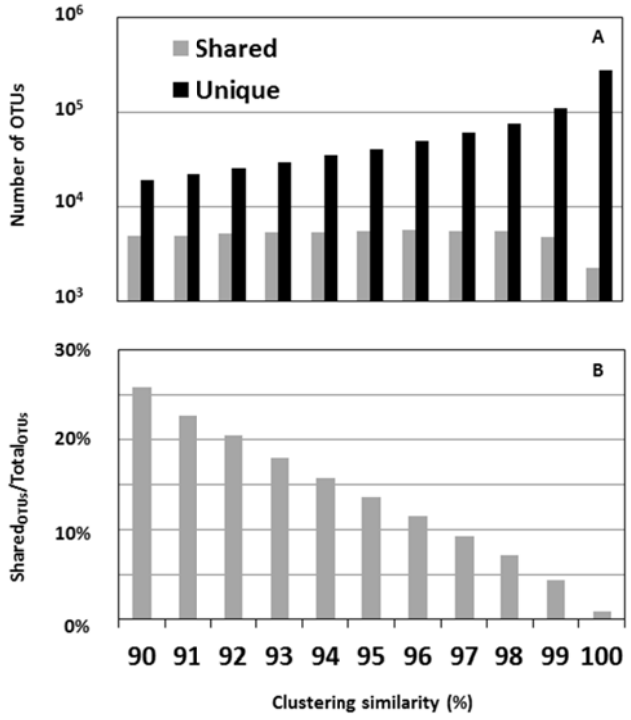


Figure 5.1 Total number of operational taxonomic units (OTUs) obtained by clustering sequences from marine and freshwater environments available in the SILVA SSU PARC 115 database alongside the number of shared OTUs between the two environments (A). Percentage of shared OTUs out of the total for each sequence similarity criteria (B). The shared OTUs span over the three domains of life: *Archaea*, *Bacteria* and *Eukaryota*.

The number of globally shared OTUs at 99% similarity clustering is much higher than obtained in our localized study (4850 vs. 68 OTUs making up 4.3% and 0.2% of all OTUs, respectively). Part of the large number of shared taxa could be the result of wrong data mining coupled with incomplete data submission for the deposited sequence, but this could not account for the entire phenomenon. Interestingly, the clustering of these taxonomic entities is conserved up to 99% sequence similarity, suggesting that these sequences belong to conserved clusters. The shared OTUs are spread across numerous families with those making over 1% of the prokaryotic sequences shown in Fig. 5.2. Families with the largest number of shared sequences include *Acidimicrobiaceae* (*Actinobacteria*) *Acidithiobacillaceae* (*Gammaproteobacteria)*,

Burkholderiaceae and *Comamonadaceae* (*Betaproteobacteria*), and the gram positive *Bacillaceae*. The *Betaproteobacteria* have been suggested before to have made several transitions between freshwater and marine environments (Rappé et al. 2000), therefore it is expected that they make the largest fraction of the shared prokaryotic sequences. Nevertheless, Walsh et al. (2013) point out only the *Methylophilalles* (OM43 clade) as a shared taxa. Interestingly, other groups that were suggested to be shared between freshwater and marine environments such as the *Planctomycetes* (Glöckner et al. 1999; Pizzetti et al. 2011), *Verrucomicrobia* (Arnds et al. 2010; Lee et al. 2009) make up only a very small fraction (<1%) of the shared taxa.

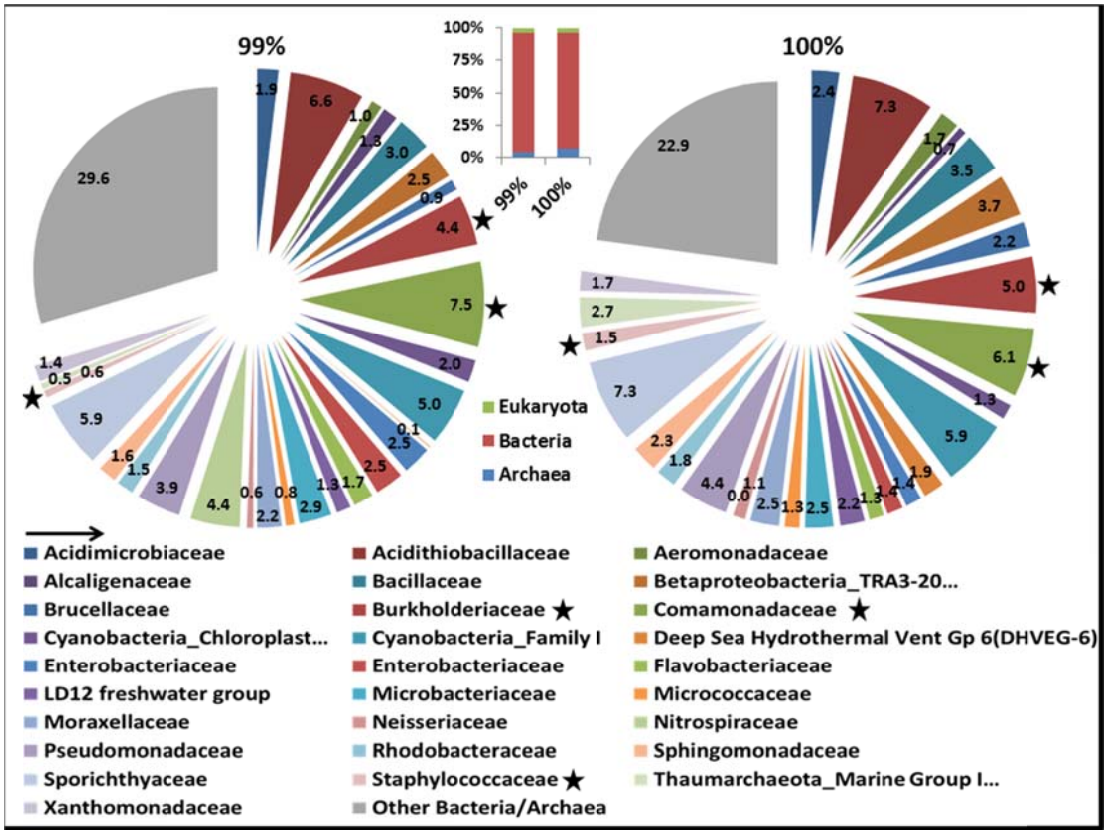


Figure 5.2 Taxonomic distributions at the family level of prokaryotic sequences shared between freshwater and marine environments at 99% and 100% sequence similarity, respectively. Only families which make up over 1% of the total prokaryotic sequences in either of the clustering criteria are shown (the abundance values are given in the figure). Families marked with a star were also found to be common in our specific comparative study (Manuscript I). The distribution among the three domains of life of the sequences shared between marine and freshwater environments is shown in the central panel for each clustering criteria. Groups for which taxonomic data is not available at the family level are represented by the last two defined taxonomic levels followed by three dots.

An interesting result comes from the SAR11 clade. This clade harbors the ubiquitous marine SAR11 (Giovannoni et al. 2005) and its freshwater counterpart LD12 (Newton et al. 2011). At 97% similarity this bacterial group spans over all aquatic environments (Logares et al. 2010) yet at higher resolution the marine and freshwater species are believed to be separated (Logares et al. 2010). Interestingly, while we do not find marine designated SAR11 sequences to be shared with freshwater environments, OTUs recognized as LD12 make up 1.3 and 2.2 % of the shared sequences at 99% and 100% similarity. If this result holds true, when sequences are chosen from better defined locations, it suggests that some members of the freshwater adapted SAR11 bacteria maintained their ability to survive in saline waters. Interestingly, Zaremba-Niedzwiedzka et al. (2013) suggest following the analysis of 57 LD12 clade genomes that these organisms are highly adapted to their freshwater environment, unlike their marine counterparts which show a large genetic variability. This is in contrast to our hypothesis where some LD12 bacteria maintained their ability to return to the sea. This contradiction can perhaps be resolved by direct investigation of marine LD12 isolates.

5.2.1 Outlook

When expanding the borders of the study to the global scale two things become evident: 1) Indeed, transitions between freshwater and marine environments are either not frequent or have not left a strong evolutionary footprint on the microbial community; 2) Despite their low contribution to the overall aquatic communities, omnipresent bacterial groups do exist, and they span across a large part of the phylogenetic tree.

Walsh et al., (2013) suggest that a border-crossing bacteria may on the one hand benefit from the lack of competition from his source community, as known from invasion events of higher organisms (e.g. fish, mussels or copepods (Lee 1999; Lee & Bell 1999), while on the other hand it may perish, as it is not well adapted to the new environment. The latter however, may place the invader in the "rare biosphere" (Sogin et al. 2006; Galand et al. 2009) accounting for the relatively low abundance of shared freshwater-marine taxa.

To understand the full extent of the phenomenon additional work is needed. First, as large datasets obtained from well-defined marine and freshwater locations are more frequently available, an extensive comparison should be made to define the overlapping taxa. The sequencing depth obtained with the use of new high-throughput methods will penetrate the rare biosphere (Sogin et al. 2006) providing information about less successful crossing events. With the increase in the awareness for the separation between FL and PA communities (Allgaier & Grossart 2006b; Rösel et al. 2012; Crespo et al. 2013; Bižić-Ionescu et al. 2014) further tuning of the *in silico* comparison using these two fractions may help understand whether particles serve as a transition vehicle between the two different ecosystems.

Isolation of organisms that have successfully crossed over between the two environments would provide useful information on the mechanisms that allowed for a competitive behaviour in the new environment. Nevertheless, as isolation of aquatic organisms is not always successful, the answer may lie in comparative metagenomic studies. Deep sequencing coupled with powerful binning algorithms allow for the assembly of full genomes of rare organisms from the environment (Albertsen et al. 2013). Therefore, comparing the genomes of multiple successful and less-successful invading bacteria can hold the key to understanding the phenomenon. Zaremba-Niedzwiedzka et al. (2013) provided an exemplary study showing the low genetic variability, hence high adaptation of freshwater SAR11 (LD12) in comparison to their marine counterparts. Similar studies if conducted on identical taxa found in freshwater and marine systems (e.g. LD12 in both systems rather than LD12 and SAR11) could show the adaptive genetic traits of the organisms that crossed the salinity barrier. Hawkins & Purdy (2007) showed by fingerprinting techniques a change in *Desulfobulbus* genotypes along an estuarine salinity gradient. A follow-up of this study would investigate the genomes of these organisms.

5.3 Particle-associated bacteria - at home or in transit?

The classification of bacteria as either oligotrophs or copiotrophs (Semenov 1991) leads to the expectation that particle association or FL are obligatory lifestyle for the

respective bacteria. Early studies of aquatic microbial communities which separated the FL and PA fraction supported this claim (Crump et al. 1999; DeLong et al. 1993; Allgaier & Grossart 2006b; Grossart et al. 2005). Newer studies using a deeper molecular characterization of the same type of communities start showing taxonomic overlaps at high sequence similarities between the two fractions (Bižić-Ionescu et al. 2014; Grossart 2010; Ghiglione et al. 2007; Crespo et al. 2013). The discovery of species common to both FL and PA suggests that these organisms are able to switch lifestyle according to environmental setting and metabolic needs or restrains (Polz et al. 2006; Bižić-Ionescu et al. 2014). FL bacteria, often adapted to oligotrophy (e.g. SAR11; (Giovannoni et al. 2005)) may resort to an attached lifestyle in the absence of nutrients or as a refuge from viral infection (Zhao et al. 2013). On the other hand PA bacteria may abandon their "garden of Eden" due to change in carbon source quality (i.e. the particle being finally degraded to their metabolic limit), antagonistic interactions between the multitude of organisms trying to benefit from the particle and similarly to the FL bacteria, to escape from a viral infection (Riemann & Grossart 2008). In addition to all these there are physico-chemical parameters involved as well. For example, the high abundance of sticky POM particles may act as a trap for FL bacteria; while bacteria from an over-colonized aggregate may detach off into the surrounding medium as usable resources become scarce (see Manuscript II).

The finding of unique clades of PA SAR11 during this study is an antidogmatic example for PA-FL connectivity. SAR11 have always been considered oligotrophic FL bacteria (Morris et al. 2002). Their occurrence on the PA fraction is therefore often attributed to methodological errors such as filter clogging (Mohit et al. 2014). Nevertheless, the finding that up to 40% of the PA SAR11 OTUs could not be accounted for in the FL fraction cannot be simply explained by sampling error. Therefore, we conclude based on these findings that a specific group of SAR11 bacteria was PA at the time of sampling. In the limnic system, the SAR11 parallel group, LD12, showed as well a high abundance on particles in some of the samples, however, all PA OTUs were found in the FL fraction as well. While this could easily be attributed to a methodological error, one may also consider a transient state where members of all clades are found in both fractions. This is in light of the fact that not all FL LD12 OTUs are found on particles.

5.3.1 Outlook

Overall in our comparative study (Manuscript I) we saw in both the freshwater and marine systems a large overlap between the FL and PA fractions suggesting a strong connectivity between the two. This was further confirmed when following the breakdown of an under-ice cyanobacterial bloom (Manuscript II). There, we observed a succession process in which PA bacteria appear as FL once the particles cannot longer metabolically support the attached community. Though we were able to record community snapshots showing these PA-FL transitions, we are still unable to reveal the forces driving this process at the level of individual taxa, beyond the state of hypotheses.

In this thesis two methodologies were developed that, if combined, could provide some answers to these above raised questions. In our 1st manuscript, we highlighted the large inter-particle heterogeneity in bacterial community composition. This heterogeneity is probably a result of particle age and source, both of which translate to organic matter quality. Using the high-throughput semi-automated microscopy we are now at a technological level in which we can quantitatively link between carbon source (e.g. cyanobacteria, diatom, dinoflagellate, fecal pellet) and colonizing community. This information could be then used as the basis for the experimental design. Using the flow through roller tank system which was presented for the first time in Manuscript III, we could setup long term experiments using defined substrates and *in situ* bacterial communities and water, following the change in colonization over time. Experiments in this direction have been done in the past (Schweitzer et al. 2001; Grossart et al. 2003b; Kiørboe et al. 2003; LeCleir et al. 2014; Grossart & Simon 1998a). They have set the foundation to our understanding of these processes. Nevertheless, we have shown that closed systems such as the classical "roller tank" devices commonly used for long term experiment, suffer from a strong bottle effect which biases the results (Ionescu et al., in prep; Manuscript III). Furthermore, we have demonstrated in the 3rd manuscript that we are able to use live, natural particles, which may be more similar to the natural environment than artificial substrates. Artificial substrates offer simplified, easy to model, to observe, to quantify and to measure particles of uniform and comparable size. Nevertheless, particles formed from living phytoplankton, will continuously interact with the colonizing bacteria by providing it with freshly synthesized OM and at the same time

apply antagonistic maneuvers to remove the colonizer. The latter will also interact with the host and spend energy in penetrating its defense lines. Additionally, the fractal nature of a natural particle may offer more niches for bacterial colonization, thus it may minimize the interaction between different bacteria as compared to a relatively uniform artificial particle.

5.4 Particles in light of a changing climate

Scenarios of climate changes that have fed the creative minds of movie makers for the last two decades are in fact hard to accurately generate based on the current knowledge and understanding of aquatic systems. It is necessary to predict, on the one hand, the anthropogenic impact in the long run, and, on the other hand, the respective global response. This leads to the formation of models varying significantly in their predictions. For example, the predicted increase in air temperature until 2100 varies between 0.1 and 4.6°C according to different models (IPCC 2013). In contrast, temperature measurements have shown that no increase in temperature occurred in the last 15 years (Foster & Rahmstorf 2011; Easterling & Wehner 2009; Kosaka & Xie 2013). The latter, however, is attributed to heat absorption by the global oceans (Balmaseda et al. 2013) which has numerous negative aspects (Solomon et al. 2007; Zeebe et al. 2008; Cao & Caldeira 2008; Le Quéré et al. 2013; Cocco et al. 2012; Doney et al. 2012; Steinacher et al. 2010). Our work brings forward two issues which have not been given full consideration in previous models.

First, we provide information on the numbers of small sized organic matter particles in limnic and marine environments (Bižić-Ionescu et al. 2014). Particulate organic matter has been accounted for in global carbon budgets and as of recently size distribution has been given some consideration as well (Guidi et al. 2008) yet it is unclear from present literature if this has been incorporated into global models. While POM is often considered a CO_2 sink via the oceanic / limnic carbon pump (De La Rocha & Passow 2007), the sinking rate of small sized particles differs significantly from that of the large marine / lake snow (Grossart 1995; Asper &

Smith 2003; Guidi et al. 2008; Ploug et al. 2008). This difference in sinking velocity results in larger particles being removed from the upper water column while smaller and more abundant particles remineralized still in the euphotic zone, releasing the CO_2 back to the environment for primary productivity, or to the atmosphere (Riley et al. 2012). Therefore, it appears that sediment traps are still the main reliable method for the estimation of POM flux to the depth.

Second, we show that heterotrophic activity in ice-covered lakes can exceed that of the rest of the year. Figure 5.3 is a revised version of Figure 1.1 with the addition of a nearly unknown compartment of microbial activity in ice-covered systems.

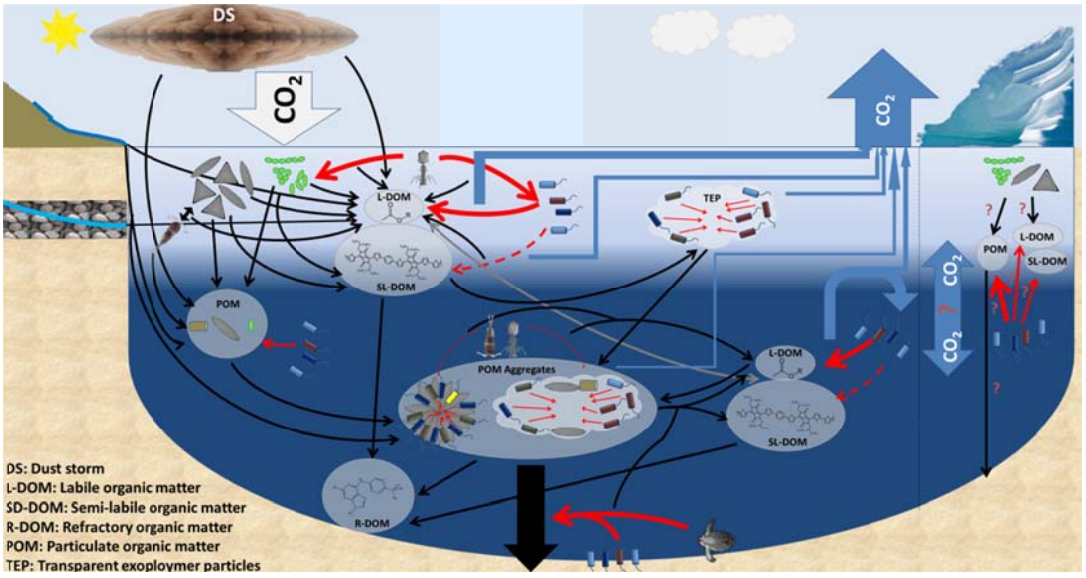


Figure 5.3 A revised version of the carbon cycle as depicted in Fig. 1.1 with the addition of the unknown compartment of autotrophic and heterotrophic activity in ice-covered systems (right side of figure)

On a global scale ice covers, either seasonally or permanently, up to 8.5% and 8.4% of saline and freshwater bodies (not including the Southern hemisphere), respectively (Benson & Magnuson 2007; Chapman & Walsh 1991). Under-ice phytoplankton blooms are common (e.g. (Dokulil & Herzig 2009; Boetius et al. 2013)), but little is

known about the activity of heterotrophic bacteria during and following such blooms (Bertilsson et al. 2013).

It can be assumed that the high heterotrophic activities measured in our study (Manuscript II) have the potential to strongly affect C cycling, particularly the release of CO_2 from the lake into the atmosphere after ice-off. CO_2 concentrations under the ice gas-barrier can exceed summer values (Kling et al. 1992) reaching concentrations of up to 650 µmol L^{-1} and are often 2-3 folds higher than during the ice-free period (Kankaala et al. 2013). Hence, the resultant CO_2 build-up can be rapidly released to the atmosphere upon ice-off. So far, under ice blooms of heterotrophic bacteria have been neglected for modeling of metabolism and element cycling in lakes. However, Karlsson et al. (2013) suggest that up 55% of the annual gas emissions from freshwater systems occur during ice-off. To better understand and predict environmental consequences of the biogeochemistry of lakes, year-round studies should also include under ice blooms of photoautotrophs such as *Aphanizomenon flos-aquae* and the response of heterotrophic microorganisms. These may also set the frame for the subsequent food web development during the growing season (Bertilsson et al. 2013).

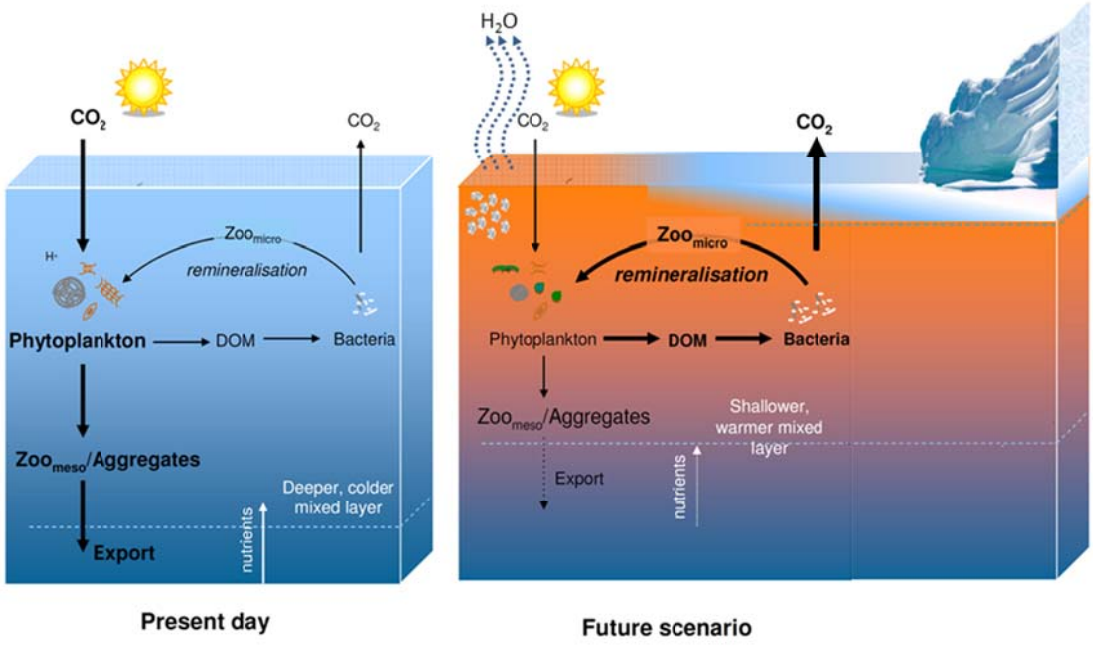


Figure 5.4 A simplified view on the marine carbon cycle under current conditions and following an increase in ocean and atmosphere temperatures. The ice-coverage was excluded from the current climate regime due to space limitations. Figure modified after Engel et al. (unpublished: *www.darwincenter.nl/Content/Downloads/Talk_Engel.pdf*).

Under current climate conditions particles serve as a carbon sequestering machinery, yet with increasing ocean (and atmosphere) temperatures their role may change (Laws et al. 2000), leading to a continuous acceleration of CO_2 release from the oceans. Figure 5.4 shows a summary of the mechanisms which may affect the role of POM aggregates. These include an increase in stratification and a reduction of the ice coverage across aquatic systems. The effect of the former have been previously discussed (Hughes et al. 2013), yet the effects of ice removal are not fully understood (Hughes et al. 2013) and would probably be incomplete without the recent information regarding under-ice autotrophic and heterotrophic blooms.

Two separate mechanisms will lead to a more pronounced stratification of the ocean (Fig. 5.4); the first, increasing temperature of the upper water layer which will raise the thermocline; and the second, stratification due to increased flow of ice melt-water, specifically at high latitudes. The shallower thermo/picno cline leads to the confinement of phytoplankton and particles to the upper water layer. This has several possibly cumulative effects on carbon remineralization and CO_2 release: a) Increase in ocean temperatures leads to decrease in CO_2 solubility, thus less CO_2 is absorbed by the ocean; b) particles will be remineralized in the upper water layer releasing CO_2; this coupled with the decreased solubility of CO_2 may lead to increased outgassing (or contribute to primary productivity); c) stratification means less mixing of the water column – thus no nutrients coming from deep water for the phytoplankton – this in turn can lead to two scenarios. On the one hand, we may witness a significant decrease in phytoplankton abundance (Boyce et al. 2010) hence decreased CO_2 fixation; on the other hand, in the presence of high light and absence of nutrients for biomass building, phytoplankton may release excessive amount of exudates due to the need to channel electrons. The latter will lead to excessive amounts of DOM (TEP) which will be trapped in the upper water layer.

It is hard to predicting the effect ice removal will have on the aquatic systems because we do not fully understand the current carbon cycling in such ice-covered systems. Hence dramatic environmental changes with so far unknown ecological consequences might occur. In many lakes ice-coverage duration has already been reduced as air temperatures have increased (Weyhenmeyer et al. 2011). Consequently, the number of seasonally frozen lakes will likely increase, on the expense of permanently frozen systems. These will experience warmer temperatures and exhibit

open-water conditions for at least some periods of the year (Solomon et al. 2007; IPCC 2007). The decrease in ice-coverage period or the removal of a permanent ice sheet will have major impact on the physico-chemical characteristics of the water body. Lakes that were not stratified nor had a permanent inverse stratification may now experience the formation of an upper layer of warmer waters. Furthermore, removal of the ice exposes the upper water layer to wind induced mixing. The latter, coupled with the prolonged exposure to the atmosphere will change the concentration of dissolved gases in the water. Specifically for limnic systems, decrease of ice-coverage duration can be directly translated to increase in the input of allochthonous OM, thus changing the eutrophication state of the lake. This cannot be excluded from seawater, though to a lower extent and not via terrestrial runoff but rather through atmospheric transport mechanisms such as dust. Changes in stratification patterns by the formation of thermoclines or the removal of permanent stratifications will alter the nutrient flow within the system. This, when coupled with the availability of allochthonous OM may lead to prolonged blooms of heterotrophs. Last, the removal of ice will alter the current light conditions in the respective systems. Phytoplankton as well as other microorganisms will be affected also by the removal of the ice as an attachment surface.

While numerous potential changes may and will occur in aquatic system upon shifts in ice-coverage regimes we lack the understanding of current events in these systems to make accurate predictions. More research on the aquatic food webs is therefore needed.

5.5 Concluding remarks

In summary, I have demonstrated that the limnic and marine PA communities differ one from the other. Nevertheless, further work is needed to understand which organisms thrive in parallel ecological niches in both systems. The newly developed flow-through roller tank system, designed in this study provides an experimental system that will allow answering such questions in the future. It also enables us to better understand the molecular basis and the biogeochemical consequences of

bacterial interactions on macroscopic organic aggregates which are important hotspots of microbial organic matter remineralization in aquatic systems. I have implemented a method for high-throughput microscopy of particles that for the first time allows the analysis of thousands of individual aggregates in a manageable period of time. This allows us to target environmentally-induced temporal and spatial changes in bacterial activities and BCC in a more defined manner. Further, I have shown that our understanding of heterotrophic activity in ice-covered aquatic systems is incomplete, yet essential for the formation of accurate models to evaluate future climate changes.

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6. APPENDICES

Appendix A: Microbial and chemical characterization of underwater fresh water springs in the Dead Sea

Appendix B: Diversity of iron oxidizing and reducing bacteria in bioreactors set in the Äspö Hard Rock Laboratory

Appendix C: *Curriculum Vitae*

Appendix A

Microbial and chemical characterization of underwater fresh water springs in the Dead Sea

Danny Ionescu, Christian Siebert, Lubos Polerecky, Yaniv Y. Munwes, Christian Lott, Stefan Häusler, **Mina Bižić-Ionescu**, Christian Quast, Jörg Peplies, Frank Oliver Glöckner, Alban Ramette, Tino Rödiger, Thorsten Dittmar, Aharon Oren, Stefan Geyer, Hans-Joachim Stärk, Martin Sauter, Tobias Licha, Jonathan B. Laronne, Dirk de Beer

Published (2012) in PloS One, 7(6):e38319

Author contributions given in the order of authors:

Conceived and designed the experiments: DI CS. Performed the experiments: DI CS YYM CL SH **MBI** TR TD. Analyzed the data: DI CS LP **MBI** CQ. Contributed reagents/materials/analysis tools: CQ JP FOG AR AO SG HJS MS TL JBL DdB. Wrote the paper: DI CS LP CQ JP AR DdB.

Abstract

Due to its extreme salinity and high Mg concentration the Dead Sea is characterized by a very low density of cells most of which are Archaea. We discovered several underwater fresh to brackish water springs in the Dead Sea harboring dense microbial communities. We provide the first characterization of these communities; discuss their possible origin, hydrochemical environment, energetic resources and the putative biogeochemical pathways they are mediating. Pyrosequencing of the 16S rRNA gene and community fingerprinting methods showed that the spring community originates from the Dead Sea sediments and not from the aquifer. Furthermore, it suggested that there is a dense Archaeal community in the shoreline pore water of the lake. Sequences of bacterial sulfate reducers, nitrifiers iron oxidizers and iron reducers were identified as well. Analysis of white and green biofilms suggested that sulfide oxidation through chemolitotrophy and phototrophy is highly significant. Hyperspectral analysis showed a tight association between abundant green sulfur bacteria and cyanobacteria in the green biofilms. Together, our findings show that the Dead Sea floor harbors diverse microbial communities, part of which is not known from other hypersaline environments. Analysis of the water's chemistry shows evidence of microbial activity along the path and suggests that the springs supply nitrogen, phosphorus and organic matter to the microbial communities in the Dead Sea. The underwater springs are a newly recognized water source for the Dead Sea. Their input of microorganisms and nutrients needs to be considered in the assessment of possible impact of dilution events of the lake surface waters, such as those that will occur in the future due to the intended establishment of the Red Sea−Dead Sea water conduit.

Appendix B

Diversity of iron oxidizing and reducing bacteria in bioreactors set in the Äspö Hard Rock Laboratory

Danny Ionescu, Christine Heim, Lubos Polerecky, Alban Ramette, Stefan Häusler, **Mina Bižić-Ionescu**,Volker Thiel, Dirk de Beer

In press (2014) in **Geomicrobiology**

Author contributions given in the order of authors:

Conceived and designed the experiments: DI CH. Performed the experiments: DI CH SH **MBI**. Analyzed the data: DI CH LP AR **MBI**. Contributed reagents/materials/analysis tools: VT DdB. Wrote the paper: DI CH LP.

Abstract

Processes of iron mineralization are of great significance to the understanding of Early-Earth geochemistry. Of specific interest are processes at circumneutral pH, where chemical oxidation of Fe can outcompete biological oxidation. To better understand microbially induced mineral formation and the composition of the involved microbial communities, we set up a series of flow-reactors in the Äspö Hard Rock Laboratory, a 3.6 km tunnel that runs under the Baltic Sea. Various aquifers of Fe^{2+}-rich brackish to saline waters penetrate the tunnel through a series of fractions. The reactors were set up with different combinations of light and aeration conditions, and were connected to three aquifers of differing chemical composition and age. Using a combination of 454 pyrosequencing and Catalyzed reporter deposition fluorescence *in situ* hybridization we analyzed the bacterial community from these reactors in two consecutive seasons half a year apart. A general decrease in diversity was observed towards the deep part of the tunnel. Multivariate modeling of the community composition and environmental parameters shows that the overall diversity of the microbial community is controlled by salinity as well as carbon and nitrogen sources. However, the composition of iron oxidizing bacteria is driven by pH, O_2 and the availability of Fe^{2+}. The latter is mostly supplied by Fe^{3+} reduction in the reactors. Thus the reactors form a self-sustained ecosystem. Several genera of known aerobic and anaerobic iron oxidizing bacteria were found. Mariprofundus sp. was found to be dominant in many of the samples. This is the first detection of this marine species in groundwater. The microbial community in the reactors is unique in each site while that in the exposed tunnel is more homogenous. Therefore we suggest that the flow reactors are a good model system to study the non-accessible microbial communities that are likely present in cracks and crevices of the surrounding bedrock.

Appendix C

Mina Bižić-Ionescu

Curriculum Vitae

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EDUCATION:

Apr. **2010**-(Apr. **2014**)

(Jan. **2012**-Oct. **2012 Maternity leave**)

PhD-student at the Max Planck Institute for Marine Microbiology - Department of Molecular Ecology, Bremen, (Germany) and the Leibniz Institute of Ecology and Inland Fisheries - Department of Limnology of Stratified Lakes (IGB) Stechlin (Germany).
Enrolled at the University of Oldenburg and a member of the International Max Planck Research School of Marine Microbiology (MarMic) Bremen, (Germany).

Title of thesis: *"Polyphasic comparison of limnic and marine particle-associated bacteria"*

Supervisors:
Prof. Dr. Hans-Peter Grossart (IGB-Stechlin and Potsdam University)
Prof. Dr. Rudolf Amann (MPI-MM-Bremen and Bremen University)
PD Dr. Bernhard M. Fuchs (MPI-MM-Bremen)

Nov. **2009**-Apr. **2010**

Postgraduate research and training in Molecular Ecology (MPI-MM),
Bremen (Germany). This period was aimed at learning methods in molecular microbial ecology.

Aug. **2005**-Aug. **2006**

Academic research at the European Institute for Jewish Studies in Sweden (PAIDEIA) Stockholm (Sweden). Franz Rosenzweig Fellowship for the one-year academic program.

Final thesis title (theoretical): "*Sailing the kosher seas- modern scientific aspects on the biblical criteria of Kosher fishes*".

Oct. **2003**-Jun. **2005**

Diploma thesis (Equivalent to M.Sc.) - University of Belgrade (Serbia) (Faculty of Agriculture). Four semesters of Hydrobiology and ecology
Thesis title (experimental): "*The adaptation of one-year-old juvenile carp (Cyprinus carpio L.) to in-vitro conditions*".
Supervisor: **Prof. Dr. Zoran Markovic**

Oct. **1999**-Oct. **2003** **Pre-diploma (Equivalent to B.Sc.)** - University of Belgrade (Serbia) (Faculty of Biology).

SCIENTIFIC EMPLOYMENT:

Oct. **2006**-Nov. **2009** **Research assistant** at Israel Oceanographic and Limnological Research (IOLR) - Yigal Alon Kinneret Limnological Laboratory (KLL) Migdal (Israel). The lab of **Dr. Werner Eckert**
Project I: Methane evolution in the sediments and water column of Lake Kinneret.
Project II: Sedimentation rates of organic and inorganic matter and of particulate phosphorus in the Jordan Delta.

Jul.-Aug. **2003** and **2004** **Research assistant at the** Institute of Marine Biology (IBM) - Kotor, (Montenegro). The lab for fish biology and ichthyology (**Dr. Aleksandar Joksimovic)**
Project I: Monitoring of Economically important fish species.
Project II: Population dynamics estimation.

PUBLICATIONS:

Bižić-Ionescu, M., Zeder, M., Ionescu, D., Orlić, S., Fuchs, B. M., Grossart, H.-P., & Amann, R. (2014). "Comparison of bacterial communities on limnic versus coastal marine particles reveals profound differences in colonization." In: Environmental Microbiology, pp. 1-36. DOI:10.1111/1462-2920.12466

Ionescu, D., Siebert, C., Polerecky, L., Munwes, Y.Y., Lott, C., Häusler, S., **Bižić-Ionescu, M.**, Quast, C., Jörg Peplies, J., Glöckner, FO., Ramette, A., Rödiger, T., Dittmar, T., Oren, A., Geyer, S., Stärk, HS., Sauter, M., Licha, T., Laronne, B. J., de Beer, D. (**2012**) Microbial and chemical characterization of underwater fresh water springs in the Dead Sea. PloS One, 7(6):e38319
This paper was highlighted in 2011: Story was covered by National Geographic, CNN, BBC, and numerous "popular science" and general magazines and TV-stations worldwide.

Ionescu, D., Heim, C., Polerecky, L., Ramette, A., Häusler S., **Bižić-Ionescu, M.**, Thiel, V., de Beer, D. (**2014**) Diversity of iron oxidizing and reducing bacteria in bioreactors set in the Äspö Hard Rock Laboratory, Geomicrob, (*in press)*

Submitted manuscripts:

Bižić-Ionescu, M., Amann, R., Grossart, H-P. (**2014**) Massive regime shifts and high activity of heterotrophic bacteria in an ice-covered lake. (*submitted* to PNAS)

Manuscripts in prep.

Ionescu, D., **Bižić-Ionescu, M.,** Malik, R., Khalili, A., Grossart, H-P. (**2014**) Flow-through roller tanks: A new tool for long term studies of the ecology of aquatic particles. *In prep.*

Bižić-Ionescu, M., Grossart, H-P., Ionescu, D. (**2014**) Macroscopic organic aggregates are hotspots for interactions between colonizing microorganisms as revealed by transcriptomics analysis. *In prep.*

Bižić-Ionescu, M., Ionescu, D., Orlic, S., Amann, R. (**2014**) Free-living and particle-associated microbial communities in the Northern Adriatic Sea along transect from Rovinj to the Po river delta. *In prep.*

Official reports:

Eckert, W., Sulimani, B., **Bižić, M.** (**2010**) Sedimentation rates of organic and inorganic matter and of particulate phosphorus in the Jordan Delta: a key to the perception of erosion processes in the watershed and their relevance to water quality of Lake Kinneret. Annual report to Israel Authority of water and sewage.

Eckert, W., Parparov, A., **Bižić, M.,** Sulimani B. (**2009**) Feasibility study for monitoring dissolved and particulate carbon in Lake Kinneret- Phase II. Final report to the Israel Water Authorities.

Eckert, W., **Bižić, M.** (**2008**) Sedimentation rates of organic and inorganic matter and of particulate phosphorus in the Jordan Delta: a key to the perception of erosion processes in the watershed and their relevance to water quality of Lake Kinneret. Annual report to Israel Authority of water and sewage.

CONTRIBUTION TO CONFERENCES:

Talks:

Feb. **2013** — 3rd International Workshop **"Research in Shallow Marine and Fresh Water Systems"**, Bremen (Germany).
Bižić-Ionescu, M., Zeder, M., Marshall, J., Orlic, S., Amann, R., Fuchs, B. M., Grossart, H-P. A comparison between particle-associated bacteria in Marine and Limnic systems.

Aug. **2012** — 2nd International Workshop on Marine Aggregates (**IWOMA**), Bremen (Germany).
Bižić-Ionescu, M., Zeder, M., Marshall, J., Orlic, S., Amann, R., Fuchs, B. M., Grossart, H-P. Particle-associated bacteria in limnic and marine systems.

Apr. **2011** — Annual Conference of the Association for General and Applied Microbiology (**VAAM**), Karlsruhe (Germany).
Bižić-Ionescu, M., Amann, R., Fuchs, B. M., Grossart, H-P. Aggregate-colonizing microbial communities - a comparison of marine vs. freshwater systems.

May **2011** — **Next generation insight into geosciences and ecology**, Tartu (Estonia).
Bižić-Ionescu, M., Amann, R., Fuchs, B. M., Grossart, H-P. Comparison of bacterial colonization of particles in limnic vs. marine ecosystems.

Posters:

Sep. **2013**

13th Symposium of Aquatic Microbial Ecology (**SAME13**), Stresa (Italy).

Bižić-Ionescu, M., Zeder, M., D. Ionescu, Orlic, S., Amann, R., Fuchs, B. M., Grossart, H-P. Particle-associated bacterial communities are extremely heterogeneous, yet differ profoundly between limnic and marine systems.

Annual Conference of the Association for General and Applied Microbiology (**VAAM**), Bremen (Germany).

Bižić-Ionescu, M., Zeder, M., Marshall, J., Orlic, S., Amann, R., Fuchs, B. M., Grossart, H-P. Comparison of particle-associated microbial communities in limnic and marine systems.

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14th International Symposium on Microbial Ecology (**ISME14**), Copenhagen (Denmark).

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14th International Symposium on Microbial Ecology (**ISME14**), Copenhagen (Denmark).

Bižić-Ionescu, M., Zeder, M., Ionescu, D Marshall, J., Amann, R., Fuchs, B. M., Najdek, M., Orlic, S. Free living and particle associated microbial communities in the north Adriatic Sea along a transect from Rovinj to the Po river delta.

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4th Congress of European Microbiologists (**FEMS**), Geneva (Switzerland).

D. Ionescu, Heim, C., **Bižić-Ionescu, M.,** Quast, C., Peplies, J., Gloeckner, F. O., Thiel, V., Reitner, J., Polerecky, L., de Beer, D. Insights into the community structure and activity of the iron Oxidizing bacteria in the Äspö -hard rock laboratory.

4th Congress of European Microbiologists (**FEMS**), Geneva (Switzerland).

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4th Congress of European Microbiologists (**FEMS**), Geneva (Switzerland).

Bižić-Ionescu, M., Fuchs, B.M., Amann, R., Grossart, H-P. Comparison of bacterial colonization of particles in limnic vs. marine ecosystems.

TEACHING EXPERIENCE:

Mar. **2012** — **Teaching assistant**
Marine Microbiology course for students from the Hebrew University of Jerusalem (Israel) and the University of Osnabrueck (Germany).
Location: Max-Planck Institute for Marine Microbiology, Bremen (Germany).
Course Director: **Prof. Dr. Karlheinz Altendorf.**

Aug-Sep **2012** — **Teaching assistant**
Molecular Microbial Ecology (FISH-based) course for students from the University of Bremen (Germany) and the international group of students.
Location: Center for marine research - Rudjer Boskovic Institute, Rovinj (Croatia).
Course director: **PD. Dr. Bernhard M. Fuchs.**

Aug-Sep **2010** — **Teaching assistant**
Molecular Microbial Ecology (FISH-based) course for students from the University of Bremen (Germany) and the international group of students. Location: Max-Planck Institute for Marine Microbiology, Bremen (Germany) and the marine station Helgoland (Germany).
Course Director: **PD. Dr. Bernhard M. Fuchs.**

2012 (6 weeks) — **Student supervision**
Graduate student **Josephine Rapp** (MarMic Program).
Project: Bacterial diversity in Arctic algal aggregates.

2011 (8 weeks) — **Student supervision**
Undergraduate student **John Marshal** (University of Bremen (Germany)).
Project: microbial diversity in the two basins (South-West, SW and North-East, NE) of the artificially divided Lake Grosse Fuchskuhle in North-Eastern Germany.

2011 (6 weeks) — **Student supervision**
Graduate student **Kin Ovanesov** (MarMic Program).
Project: The active groups of particle-associated bacteria on POM aggregates from the Gulf of Aqaba.

2011 (6 weeks) — **Student supervision**
Graduate student **Laura Gallego** (MarMic Program).
Project: Comparison of particle–associated bacteria in two freshwater basins.

REVIEWER ACTIVITY	FEMS Microbiology letters
EXTRACULLICULAR COURSES	Course in Marine Biotechnology; ARB course; Metagenomics course; Course in Statistics; R course.
SOFT SKILL COURSES	Self and time management; Leadership seminar; Conflict management.

RESEARCH GRANTS AND SCHOLARSHIPS:

Apr. **2010**-Apr. **2014**	German Academic Exchange Service (**DAAD**) - PhD scholarship.
Jun. **2010**	Association of European marine biological laboratories (**ASSEMBLE marine**) research grant. Location: Interuniversity Institute for Marine Sciences, Eilat (Israel). Project: Bacterial communities on particulate organic matter macroaggregates.
Aug. **2005**-Aug. **2006**	One-year **Franz Rosenzweig fellowship** program for Jewish studies in Stockholm (Sweden)

LANGUAGES:

Serbian	Native
English	Fluent (Academic level)
Hebrew	Good
German	Moderate

METHODOLOGICAL EXPERIENCE:

Molecular microbial ecology:
DNA and RNA work; Fingerprinting (DGGE, ARISA); Fluorescence *in situ* hybridization (FISH, CARD-FISH); Microscopy (Laser scanning confocal and epifluorescence).

Biogeochemistry:
Gas-chromatography; Elemental CHN analysis; Total dissolved inorganic & organic carbon analysis; Sequential phosphorus fractionation analysis; O_2, pH, H_2S microsensors.

Population dynamics estimation in ichthyology:
Length-weight relationship; Growth; Mortality; Biomass estimation; Tools selectivity; MSY (maximum sustainable yield); Fish age determination and growth rates by otoliths.

Additional experience:
Underwater sampling (marine snow, cores); On-board sampling (lakes, sea).

7. ACKNOWLEDGMENTS

"If I have seen further it is by standing on the shoulders of giants"

Sir Isaac Newton (1675)

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