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Marine Landa

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THÈSE DE DOCTORAT

DE L'UNIVERSITÉ PIERRE ET MARIE CURIE

École doctorale des sciences de l'environnement d'Île de France (ED129)

Présentée par : **Marine LANDA-BEZWIERCHY**

Pour obtenir le grade de : **DOCTEUR**

Spécialité : **SCIENCES DE L'ENVIRONNEMENT**

LIEN ENTRE MATIÈRE ORGANIQUE DISSOUTE ET DIVERSITÉ DES COMMUNAUTÉS BACTÉRIENNES HÉTÉROTROPHES MARINES

Directeur de recherche : Ingrid OBERNOSTERER

Co-directeur de recherche : Stéphane BLAIN

Soutenue le 16 septembre 2013 à Banyuls-sur-mer, devant la commission d'examen formée de :

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« An expert is a person who has made all the mistakes that can be made in a very narrow field »

Niels Bohr

REMERCIEMENTS

La thèse, si on résume… mais peut-on résumer la thèse ? La thèse est une expérience, un voyage, une épreuve, tant sur le plan scientifique, intellectuel, qu'humain, social, psychologique. La thèse est un face à face, avec l'inconnu et avec soi-même. Une chose est sûre : la thèse n'est pas un sport solitaire. Alors, merci, gracias, gràcies, thanks, спасибо, dziękuję, grazie, danke, mulţumesc, tack, köszönöm, דאַנק אַ, شكرا :

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À la relecture, Stéphane, je crois que pour le « moins de mots », il faudra repasser !

Foreword

The experiments presented in this manuscript required a considerable amount of work, which was made possible by the combined efforts of many people.

My work consisted in the preparation and set up of the continuous culture experiments in Banyuls, which was accomplished with my supervisors' help. I performed the sampling of the cultures, with the help of Ingrid Obernosterer and Nicole Batailler. I participated in the preparation of the KEOPS2 cruise, and conducted and sampled the on board continuous cultures along with Ingrid Obernosterer. I was also involved in sample collection, filtration, conditioning and storage for the analysis of *in situ* bacterial abundance, diversity and activity during the cruise. I performed DNA extraction and all the bioinformatic analyses required for the analysis of bacterial diversity.

The flow cytometric analyses were performed by Philippe Catala (LOMIC), except for the samples from the on-board continuous cultures that I analyzed.

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GENERAL INTRODUCTION

Bacteria are found in all kinds of ecosystems on Earth, even the most extremes. These microscopic unicellular organisms are far from being trivial: with about 10^{30} bacterial cells on Earth, total bacterial biomass is equivalent to plant biomass (Whitman et al., 1998). The study of the diversity of these organisms is a step towards global comprehension of their role in any kind of ecosystem.

Bacterial diversity and functions in the environment

The term "bacterial diversity" is often used in reference to the number and phylogenetic identity of bacterial taxa. Estimates of total diversity indicate there could be as much as 10^9 different bacterial taxa on Earth (Dykhuizen, 1998), though the likelihood of such a high number is questioned (Schloss & Handelsman, 2004; Pedrós-Alió, 2006). The sequencing of the universal bacterial 16S rRNA gene (Woese & Fox, 1977) has enabled the comparison and classification of bacterial taxa. With this approach, up to 52 phyla have been identified, for most of which no cultured representative is available (Figure 1)(Rappé & Giovannoni, 2003). Whatever the actual number of bacterial taxa is, and whether we can determine it or not, it appears that bacterial phylogenetic diversity is considerable and that most of this diversity remains to be discovered.

From an ecological point of view, it is tempting to make the hypothesis that such a high phylogenetic diversity reflects or results from the different roles bacteria play in their various environments, or the ecological niches they occupy. Microbial ecologists have been searching for associations between bacterial taxa and functional or ecological

traits. In some cases, these associations exist and were easily identified. For instance, autotrophy, or the capacity of performing inorganic carbon fixation, is mostly found in cyanobacteria. In the case of this specific functional trait, phylogeny perfectly matches function. Phylogeny also partly explains the distribution of bacterial taxa in the environment. Marine bacterial populations usually comprise members of a dozen broad phyla among the 52 currently identified (Figure 1), most of which belong to Proteobacteria and Bacteroidetes (Fuhrman & Hagström, 2008).

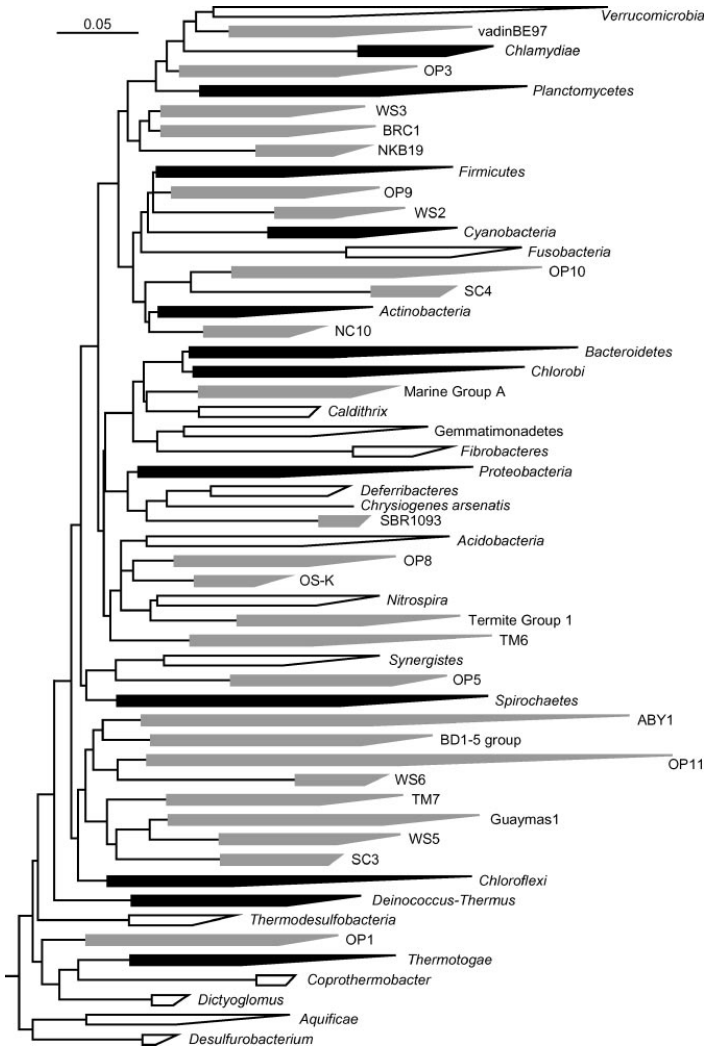


Figure 1. Major phyla of the domain Bacteria, from Rappé & Giovannoni (2003). The 12 original phyla described by Woese (1987), the 14 phyla possessing cultivated representatives and the 26 candidate phyla without cultivated representatives are shown in black, white and gray, respectively.

Similarly, broad groups such as Betaproteobacteria and Actinobacteria are often found dominant in freshwater systems (Glöckner et al., 1999; Humbert et al., 2009). This type of very general distributions of major bacterial groups suggests adaptation to life in saline or to freshwater environments associated to phylogeny at a low resolution (Lozupone & Knight, 2007).

However, the hypothesis that the bacterial groups defined by phylogeny share similar functional and ecological traits contrasts with the existence of many studies demonstrating that phylogenetically close taxa can have different lifestyles and properties (Field et al., 1997; Casamayor et al., 2002; García-Martínez et al., 2002). Bacterial evolution is not restricted to vertical transfer but also consists in horizontal transfer. Concretely, this means that phylogenetically unrelated bacteria can exchange genetic material. This unique feature is a major source of diversity and complicates the relationship between phylogenetic signature and functional capacities (Wiedenbeck & Cohan, 2011). The extent to which functional traits of bacterial groups are linked to phylogeny likely depends on two crucial factors: the phylogenetic level at which the groups are defined, and the functions investigated. Different bacterial groups exhibit various evolution rates, which suggests that different ecological roles might have to be sought for at various phylogenetic levels depending on the groups (Zinger et al., 2012). For instance, the dominance of Alphaproteobacteria, and in particular the SAR11 clade, in marine systems is common knowledge (Morris et al., 2002). However, different SAR11 sub-groups have been associated to distinct geographical distributions (Pommier et al., 2005). This illustrates that the chosen trait, here abundance or geographical distribution, and the level of phylogenetic resolution, here clade or subclade, are critical to find a link between phylogeny and ecological or functional properties.

Marine bacteria and the carbon cycle

Among the many functions of bacteria, a major one is the degradation of organic matter. This function is of particular importance in the ocean, where heterotrophic bacteria obtain the carbon necessary for energy and biomass production mainly from dissolved organic matter (DOM). Oceans cover 70% of the Earth surface and oceanic DOM constitutes a major reservoir of organic carbon, equivalent to the 700 Gt of carbon contained in atmospheric CO_2 (Nagata, 2008). The majority of this huge carbon stock is considered refractory, meaning it is resistant to microbial consumption, and accumulates in the ocean (Bauer, 2002).

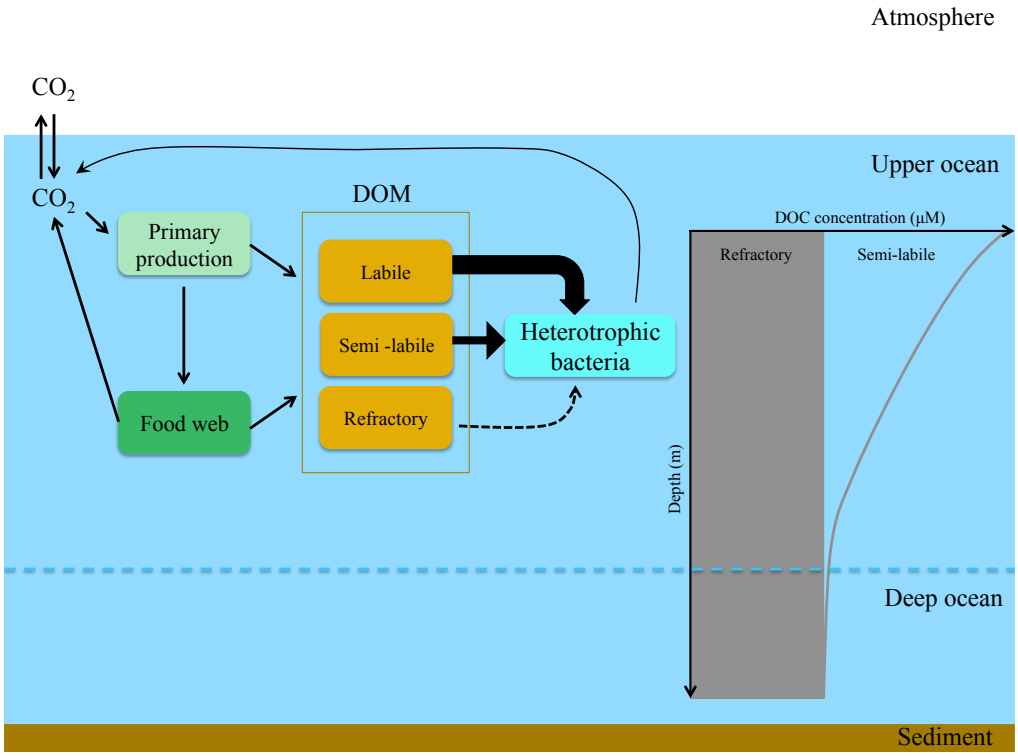


Figure 2. Simplified representation of DOM production and use by heterotrophic bacteria in the upper ocean. The distribution of the semi-labile and refractory DOM in the water column is also indicated. Labile DOM isn't represented because of its low concentration.

The semi-labile and labile DOM pools represent a smaller fraction of the total and yet account for the majority of bacterial production. The turnover times of labile compounds such as amino acids and free sugars is so high, from a few minutes to a few hours, that they are almost undetectable and account for less than 1% of total DOM (Nagata, 2008). Considering that the net oxidation of only 1% of oceanic DOM over a year represents the same flux of carbon as anthropogenic CO_2 emissions (Hedges, 2002), the role of heterotrophic bacteria in the remineralization of DOM renders them key players of the carbon cycle (Figure 2).

In this context, the question of the link between functional traits and phylogeny is a major one. Considering the various origins of oceanic DOM (Moran & Hodson 1994; Nagata, 2000), the complexity and diversity of the compounds constitutive of this carbon pool (Repeta et al., 2002; Benner, 2002; Benner & Kaiser, 2003), the existence of specific traits involved in organic matter degradation among distinct phylogenetic groups could have important repercussions in ecosystem functioning. If so, community composition and diversity would be crucial elements driving the efficiency and outcome of DOM transformation. For this reason, substantial efforts have been made to identify the relationship between the high diversity of marine bacteria and functional traits involved in DOM degradation.

Investigating the roles of distinct bacterial taxa in carbon utilization

From a conceptual point of view, there is no reason to believe that some groups among those commonly found in marine systems cannot use oceanic DOM at all. Dissolved organic carbon is at the base of energy and biomass production in

heterotrophic bacteria, which implies that they are all capable to sense and use it in the environment. However, many studies indicate clear substrate preferences and distinct substrate utilizations for some bacterial taxa, which suggests these taxa are not functionally equivalent. For instance, the examination of the genomic content of several clades within the Bacteroidetes revealed the presence of genes involved in polysaccharide degradation, among others (González et al., 2008; Gómez-Pereira et al., 2012). Microautoradiography coupled to fluorescent in situ hybridization showed that members of this group were in fact actively taking up high molecular weight compounds while Alphaproteobacteria displayed a preference towards glucose and amino acids, and Gammaproteobacteria preferentially used amino acids and ATP (Cottrell & Kirchman, 2000; Alonso-Saéz et al., 2012). However, these substrates preferences were found to vary throughout the year (Alonso-Saéz & Gasol, 2007), suggesting no strict relationship between phylogenetic groups and carbon substrate identity. The response of various phylogenetic groups to more complex DOM sources from different phytoplankton species also indicated distinct but weak preferences of these groups for various DOM sources (Sarmento & Gasol, 2012).

These studies demonstrated the variable capacities of different bacterial taxa to incorporate several labile substrates, however most of them also concluded that all groups were generally able to use all the carbon sources (Gómez-Consarnau et al., 2012). This suggests that despite obvious preferences, bacterial groups can still use a wide range of labile compounds, and that no substrate was utilized by one group exclusively. This indicates two important things. First, that many if not all bacterial groups are capable of generalist carbon uptake, in the sense that most bacterial groups do not depend on a sole substrate for their growth. They seem to be able to use different compounds, an idea supported by several findings (Rink et al., 2007; Mou et al., 2008).

Second, that functional redundancy exists among bacterial groups, at least for the degradation of labile compounds such as amino acids, proteins or carbohydrates.

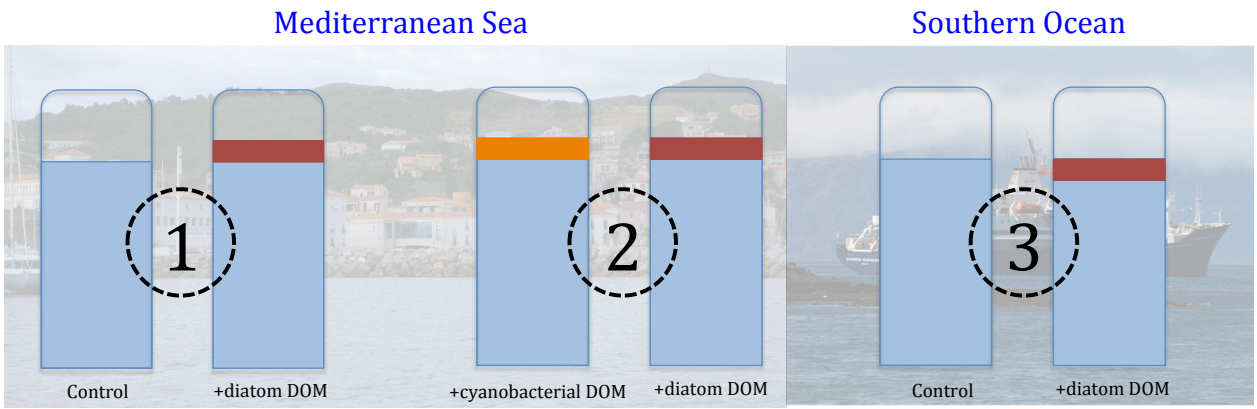
If any bacterial group can incorporate any labile compound and be replaced by any other group, then modifications of the DOM pool such as changes in concentration, availability or chemical composition should have no impact on the structure of natural bacterial communities. Some studies actually report few modifications of bacterial community composition following modification of the DOM pool (Langenheder et al., 2005; Rooney-Varga et al., 2005). However, in most cases, changes in DOM supply and composition led to changes in activity associated to shifts in bacterial community composition (Pinhassi et al., 1999; Arrieta & Herndl, 2002; Kirchman et al., 2004). This is well illustrated in phytoplankton blooms, as revealed by diversity analysis (Fandino et al., 2001; Pinhassi et al., 2004; Tada et al., 2011; Teeling et al., 2012). The exploration of the genes expressed in the context of phytoplankton blooms or of an additional supply of labile DOM in general demonstrated uneven stimulation of phylogenetic groups by DOM and concluded to resource partitioning among the members of the bacterial community (McCarren et al., 2010; Poretsky et al., 2010) that were generally predicted by phylogeny (Rinta-Kanto et al., 2012).

Objectives of the thesis and organization of the manuscript

In light of the various findings reported above, the question of the importance of bacterial community composition for DOM degradation, through the existence of specific functional traits, remains unresolved. The objective of this thesis was to examine the effect of DOM on heterotrophic bacterial diversity and community composition. We

expected that if DOM degradation required the active presence of adapted groups, then different bacterial communities would grow on DOM of various composition and concentration. We chose to grow natural bacterial communities in continuous cultures to stabilize abiotic parameters, and focus on differences in DOM concentration or chemical composition as potential drivers of bacterial diversity in the cultures. The principle and theoretical functioning of continuous cultures are detailed in the Methodology section of this manuscript.

Three independent experiments were conducted, each of them designed to address a specific question (Figure 3).



- What is the effect of an addition of phytoplankton DOM on bacterial diversity?
- What is the effect of phytoplankton DOM of different chemical composition on bacterial diversity?
- What is the effect of an addition of phytoplankton DOM on bacterial diversity?

Figure 3. Simple scheme of the three continuous culture experiments presented in this manuscript, and the questions they were designed to address.

Two experiments were conducted in the Mediterranean Sea and are presented in the first part of this manuscript. The aim of the first experiment was to examine the response of heterotrophic marine bacteria to an addition of phytoplankton-derived

DOM. This experiment and the results and hypotheses that were developed were published in Aquatic Microbial Ecology and are presented in Chapter 1.

The aim of the second experiment was to examine the response of heterotrophic bacteria to phytoplankton-derived DOM of different origin and chemical composition. This experiment and its results and interpretations are presented in Chapter 2, prepared from the revised version of an article submitted to Environmental Microbiology in July 2013.

The second part of this manuscript focuses on the KEOPS2 oceanographic cruise, that took place in the Southern Ocean during October and November 2011 and during which the third continuous culture experiment was conducted. The cruise's general objective was to study the establishment of a phytoplankton bloom in response to a natural iron fertilization event (Blain et al., 2007). One aim of the on-board continuous culture experiment was to investigate the response of heterotrophic bacteria to an addition of phytoplankton-derived DOM in this environment. In parallel, *in situ* bacterial diversity was investigated over the course of the bloom, in various bloom environments. The final objective was to evaluate whether the results from the continuous culture experiment could improve our understanding of the *in situ* bacterial response to bloom conditions. The general context of the KEOPS2 project and the results obtained from the experiment and the *in situ* analysis of bacterial diversity currently constitute unpublished data and are presented in Chapter 3.

In a final "General discussion and conclusion" chapter, the various results collected during this thesis were pooled together and compared, to extract general messages and answers to the questions that were addressed with this work. This section and the General Introduction were translated into French at the end of the manuscript.

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METHODOLOGY

I. EXPERIMENTAL APPROACH

The question of the structuring effect of DOM on bacterial diversity has been subject of research over the past 15 years. Previous studies have addressed this issue by linking changes in bacterial community composition to modifications in DOM matter supply in various aquatic environments (Fandino et al., 2001; Rink et al., 2007; Teeling et al., 2012) or through different experimental approaches. Among those, enrichments in bioassay experiments run in batch mode and in mesocosm experiments, and cross-inoculations are the most common (Docherty et al., 2006; Judd et al., 2006; Tada et al., 2012). The objective of the present work was to address the link between DOM and bacteria from a different angle, using an experimental system that has long been proved useful in microbiology but has rarely been used in this specific context: continuous cultures. A large part of this thesis was dedicated to the preparation, set up, running, sampling and data analysis of three continuous cultures experiments, two in Banyuls-sur-mer and one in the Southern Ocean. The first part of this chapter will shed some lights on the theoretical concepts and practical aspects associated with such an experimental system. Understanding the principle and functioning of continuous cultures is indeed essential to maximize its use and to produce adequate interpretations.

1. Continuous cultures

Continuous cultures, or chemostats, were described for the first time more than sixty years ago (Monod, 1950; Novick & Szilard, 1950). Initially, they were dedicated to the study of microbial growth on a single limiting resource, and their use was rapidly

extended to the study of various aspects of microbial physiology and ecology, such as the competition for resource (Dykhuizen & Davies, 1980; Currie & Kalff, 1984; Füchslin et al., 2012); the response to antibiotics (Cozens et al., 1986; Anwar et al., 1989); predator-prey relationships (Bohannan & Lenski, 1997; Hahn et al., 1999; Massana & Jürgens, 2003) and substrate degradation (Van Hannen et al., 1999; Kisand et al., 2008). The system has also been used for the study of phytoplankton physiology and ecology (Sommer, 1984; Stomp et al., 2004).

A chemostat is an open system where abiotic conditions are maintained stable by continuous input of medium in the culture. As a consequence, microbial populations reach a steady state (Figure 1A), as opposed to batch culture incubations where bacterial populations decline after a while, when resource is exhausted (Figure 1B).

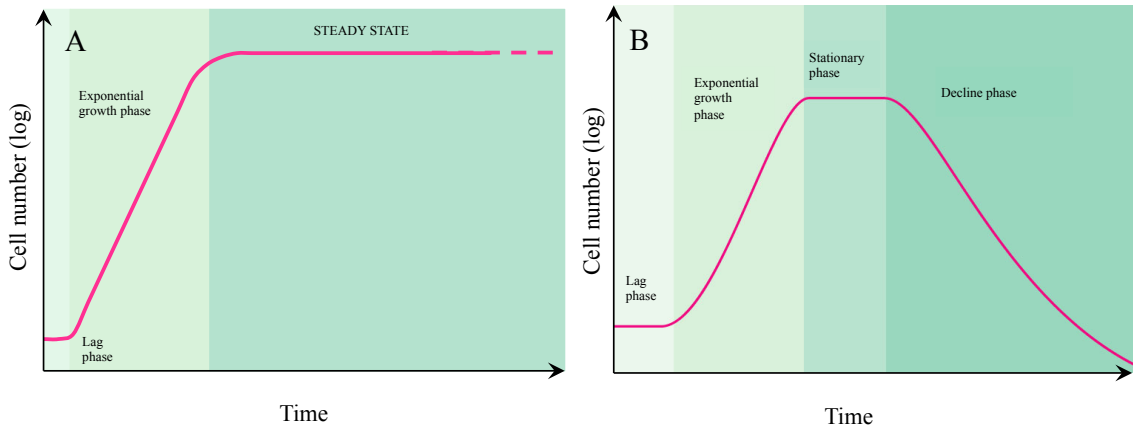

Figure 1. Illustration of bacterial growth stages in a chemostat culture (A) and in a batch culture (B). Adapted from Pearson Education, Inc (2004).

Since its invention, the chemostat has been subject of theoretical research, to describe its functioning and with the aim to provide mathematical explanations for the results obtained by experimentation. In a simple one strain, one limiting substrate

chemostat culture, with an assumed growth efficiency of 1, the dynamics of biomass and substrate concentration can be described as follows (Figure 2):

$$\frac{dS}{dt} = -\mu(S)X + \frac{Q}{V}(S_{\text{in}} - S)$$

and

$$\frac{dX}{dt} = \mu(S)X - \frac{Q}{V}X$$

where:

- S is the concentration of the limiting substrate in the chemostat (mol l^{-1})
- S_{in} is the input substrate concentration (mol l^{-1})
- $\mu(S)$ is the strain's growth rate as a function of substrate concentration (h^{-1})
- X is the biomass (mol l^{-1})
- Q is the flow rate (l h^{-1})
- V is the volume of the culture (l).

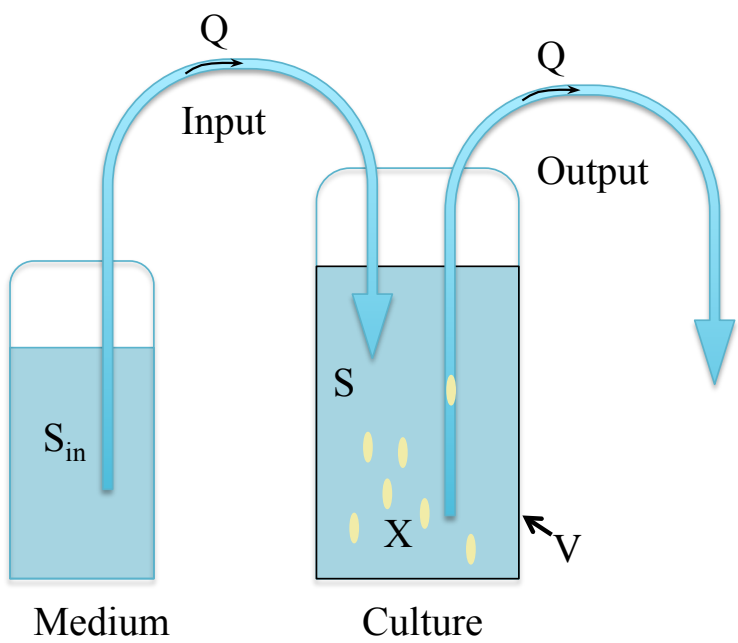

Figure 2. Scheme of the chemostat. Bacterial cells are represented by yellow ovals.

In this model, the substrate concentration in the culture, S, is determined by the amount of substrate supplied by the medium, $\frac{Q}{V}S_{in}$, the loss of substrate through the outflow, $-\frac{Q}{V}S$, and through bacterial utilization, $-\mu(S)X$. The biomass X depends on population growth, $\mu(S)X$, and on the removal caused by the outflow, $-\frac{Q}{V}X$.

When the bacterial population reaches steady state, substrate concentration and biomass are stable. The variation in substrate concentration, $\frac{dS}{dt}$, and in biomass, $\frac{dX}{dt}$, is zero, therefore the equations above are equal to zero. The equilibrium in the chemostat can therefore be expressed as:

$$Xe = S_{in} - Se$$

Where Xe and Se represent biomass (mol l^{-1}) and substrate concentration (mol l^{-1}) in the culture at equilibrium, and

$$\mu(Se) = \frac{Q}{V}$$

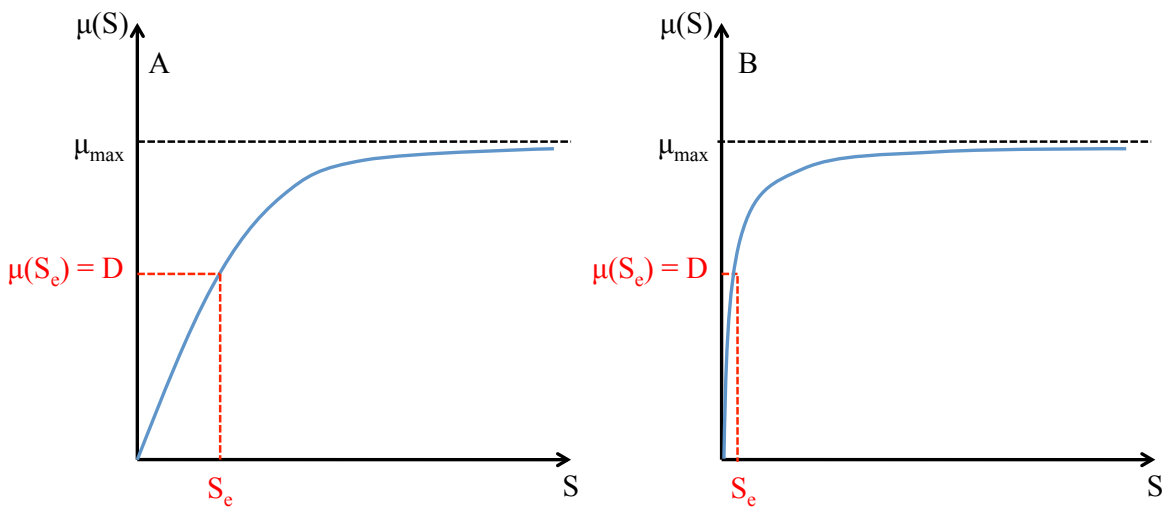

Figure 3. Growth rate as a function of substrate concentration in a simple chemostat model, for a strain of low (A) or high (B) affinity towards the limiting subtrate. On this graph, D represents the dilution rate, D=$\frac{Q}{V}$.

These two equations indicate three major aspects regarding a chemostat culture in steady state:

* Substrate concentration Se is determined by flow rate only, for a given $\mu(S)$ (Figure 3)
* Growth rate is equal to dilution rate.
* Biomass Xe is determined by S_{in} and Se, which depends on the flow rate.

Theoretically, the concentration of substrate remaining in the culture at equilibrium (Se) can be different from zero, when the strain's affinity for the substrate is low (Figure 3A). However, when affinity is high, then Se approaches zero (Figure 3B). In that case, biomass at equilibrium is determined mainly by S_{in}.

From this simple model, many others have been developed to incorporate other aspects of microbial growth: growth efficiency, density-dependent growth, substrate-inhibited growth, competition for resource in multiple species cultures, predator-prey interactions, mortality rates among others (e.g. Wolkowicz & Lu, 1992; Xiu et al., 1997; Ruan et al., 2007; Harmand et al., 2008; Rapaport et al., 2009). In particular, competition for a substrate in a chemostat has been described as illustrated in Figure 4.

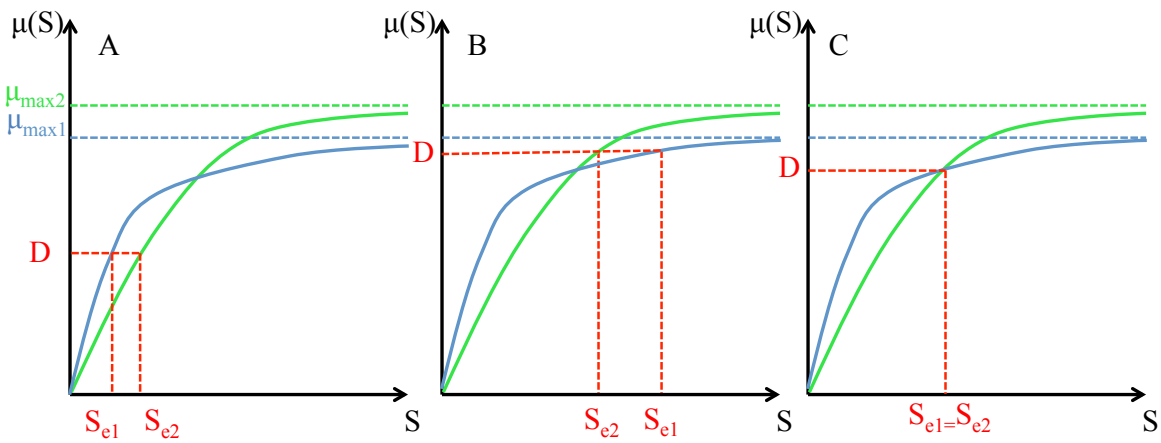

Figure 4. Illustration of competition between two species (blue and green) for the acquisition of a single limiting substrate, at various dilution rates.

According to this model, when two species are in competition for a single limiting substrate, dilution rate (D) determines the species that will outcompete the other, according to their individual growth characteristics. The ecological concept of competitive exclusion (Hardin, 1960) predicts that when environmental parameters are stable, which is the case in a chemostat, only the most efficient species remain after a long period of time. In the case illustrated in Figure 4A, species 1 (blue) is expected to outcompete species 2 (green), because at the chosen dilution rate, it is capable of faster substrate utilization and growth. The use of a higher dilution rate, as illustrated in Figure 4B, will inverse the patterns and favor species 2 (green), for the same reasons. Therefore, competition in a chemostat theoretically selects for the most efficient bacterial group and removes the unsuccessful competitors. However, in some cases, coexistence of two species of equal efficiency is permitted, as illustrated in Figure 4C.

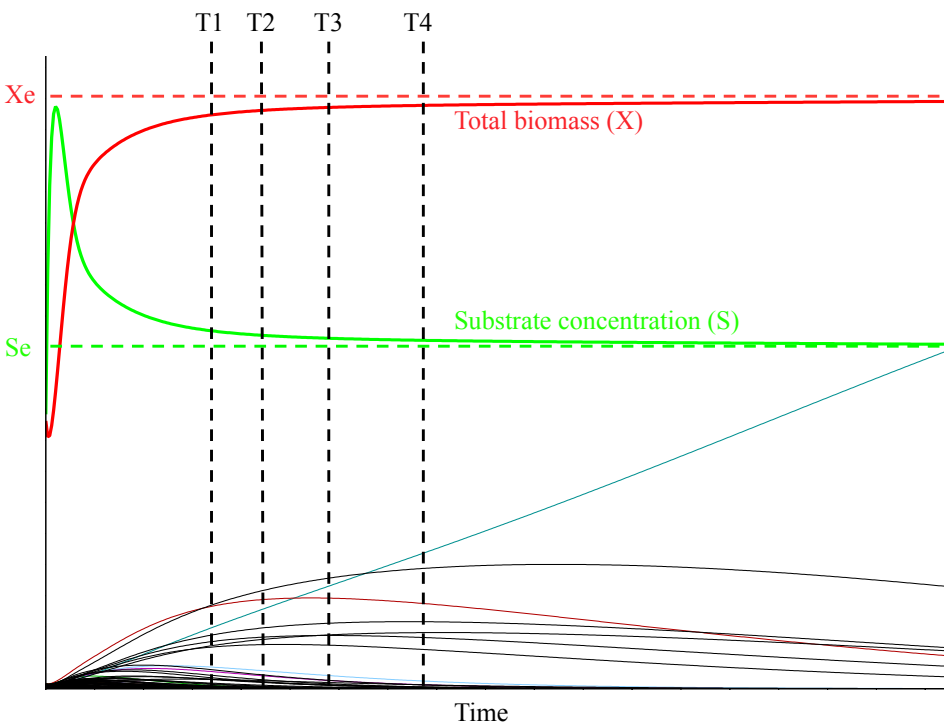

Figure 5. Fluctuations in the relative abundances of individual species over time in a one limiting substrate chemostat initially inoculated with a hundred species, as predicted by a simulation. Each colored line corresponds to the biomass over time of a given species. The figure was provided by A. Rapaport (MODEMIC).

In the case of a chemostat where a high number of species compete for the limiting substrate, the existing models suggest interesting oscillation patterns in the relative abundances of the different species over time (Figure 5). This indicates that dynamic restructuration of communities that can occur for some time before the eventually dominant species outcompetes the others. Such phenomena would lead to the observation of different dominant species at different sampling time points, as illustrated in Figure 5.

The experiments that were carried out during this thesis not only involved multiple species, but also multiple substrates provided in limiting conditions, and were probably characterized by various mechanisms of substrate utilization and competition. For this reason, our experiments represent complex systems and require more complicated models.

Figure 6. Picture of the continuous culture set up used in Banyuls.

The experiments performed during this thesis (Figure 6) were designed to follow the response of natural, complex bacterial communities to various resource conditions. We chose continuous cultures with the objective of maintaining bacterial growth under

steady state ressource conditions over the course of the experiment. Moreover, in a chemostat, growth rate is constrained by flow rate, as demonstrated above. In a multi species system such as the ones we set up, only species with a growth rate equal to or higher than the imposed flow rate can develop and avoid being washed out of the culture. Therefore, this system also constituted a selective tool, in which only species with sufficient growth rate on the substrates provided to them could grow. This set up allowed us to supply communities with a controlled carbon-limited medium, stabilize bacterial biomass, and follow bacterial response to the various tested conditions in terms of growth and biomass build-up, metabolism (enzymatic activities) and diversity. The details regarding each experiment are provided in the respective chapters.

2. Field work

A substantial part of this work consisted in the preparation, participation, and data collection and analysis of the KEOPS2 cruise that took place in October-November 2011 in the Southern Ocean. The main objective was the sampling and analysis of bacterial diversity in the various environments that were visited during the cruise. The cruise details are extensively provided in Chapter 3. A considerable interest of the cruise was the opportunity to link experimentally obtained data with in situ observations, through an on-board continuous culture experiment. The latter was conducted in the same spirit as the first two previously run in Banyuls, but was adapted to the local environmental conditions (e.g. nutrient conditions, temperature). The final objective was to compare the responses of a Southern Ocean winter bacterial community to the experimental and in situ increase of phytoplankton-derived DOM.

II. DIVERSITY ANALYSIS: 454 TAG PYROSEQUENCING

1. Choosing the tool for bacterial diversity analysis

The study of marine bacteria has been hindered for many years by the inadequacy of traditional cultivation methods, which enable the detection of less than 1% of total bacteria in these environments. To circumvent this issue, alternative techniques were developed, benefiting from the significant progresses accomplished in the field of molecular biology over the last thirty years. In contrast with cultivation, the access to bacterial DNA from a water sample is easy and only requires pre-concentration, often performed by collecting the cells on a filter. Therefore, many current methods for marine bacterial community description and analysis rely on bacterial nucleic acids (DNA and/or RNA). These nucleic acids bear huge information about the bacteria they are collected from, and variations in the nucleotide sequence of crucial genes have been used to discriminate between different bacterial groups and infer phylogenetic relationships between them. As a consequence, the sequencing of targeted genes has become a widespread tool in microbial ecology.

Several genes have been identified as good candidates and consequently used to study either phylogenetic or functional microbial diversity. Among them, the gene coding for the 16S ribosomal RNA was chosen at the emergence of these DNA-based classification techniques and remains the gene typically chosen for diversity studies. This essential gene of 1542 bp is found in all bacteria on Earth, and characterized by the alternation of conserved regions, which make ideal targets for amplification primers, and nine variable regions (Baker et al., 2003) (Figure 7).

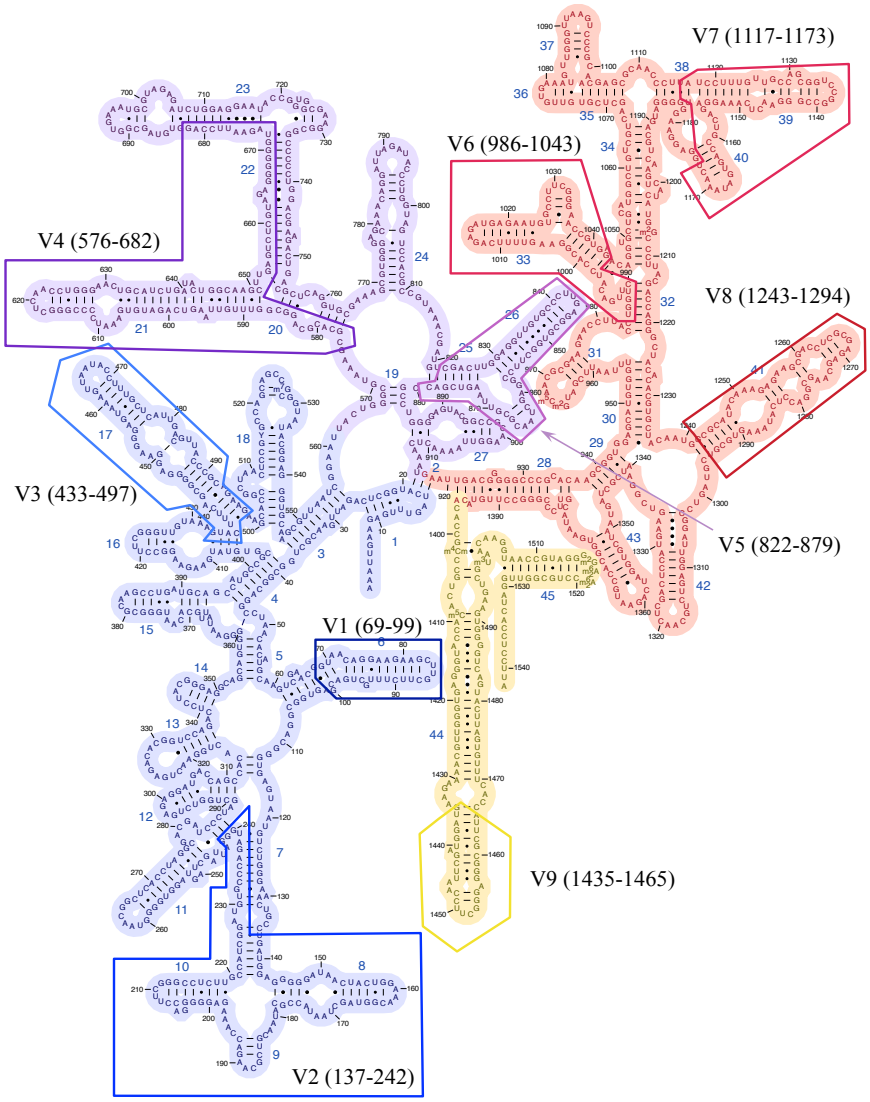

Figure 7. *E. coli* 16S rRNA gene sequence and secondary structure adapted from the Center for Molecular Biology of RNA (University of California). Variable regions are color-coded and represented according to Chakravorty et al., 2007.

The precise nucleotide sequences of these variable regions allow the distinction of bacterial groups at high levels of phylogenetic resolution. Because it has been extensively studied, a huge amount of data is now available, and databases (e.g. NCBI, Greengenes, SILVA, RDP) have been dedicated to the analysis, classification, validation, and storage of thousands of 16S rRNA gene sequences from prokaryotes collected all around the world, including marine environments.

Recently, sequencing technology has undergone rapid and drastic improvements, and traditional tools have given way to next generation sequencing (NGS) technologies. Among them, 454 pyrosequencing is a rapid, high throughput and low cost sequencing technique that produces thousands of sequences of a few hundred bp in a few hours (Margulies et al., 2005). Since its development, this technology has been used for a wide range of applications, including phylogenetic analyses based on key genes. In marine microbial ecology, 454 pyrosequencing has become one of the most common approaches for 16S rRNA gene-based analysis of prokaryote diversity (Zinger et al., 2012).

For all these reasons, a molecular approach based on the diversity of the 16S rRNA gene as a proxy of bacterial diversity, coupled to the efficiency and high throughput of new sequencing technology was a good strategy to meet our objectives. In the context of this thesis work, 454 pyrosequencing of the 16S rRNA gene was performed for the analysis of bacterial diversity both in the continuous culture experiments and the KEOPS2 project.

2. How does pyrosequencing work?

Pyrosequencing is a PCR-based technique that was designed to rapidly produce high amounts of sequence data. Specific primers are used to target the gene of interest, and single strand molecules of DNA are bound to a micro bead using adapters. The mix is prepared so that each bead carries only one DNA template. A first PCR is performed in a micro emulsion, resulting in beads carrying thousands of copies of their initial DNA template (Figure 8A).

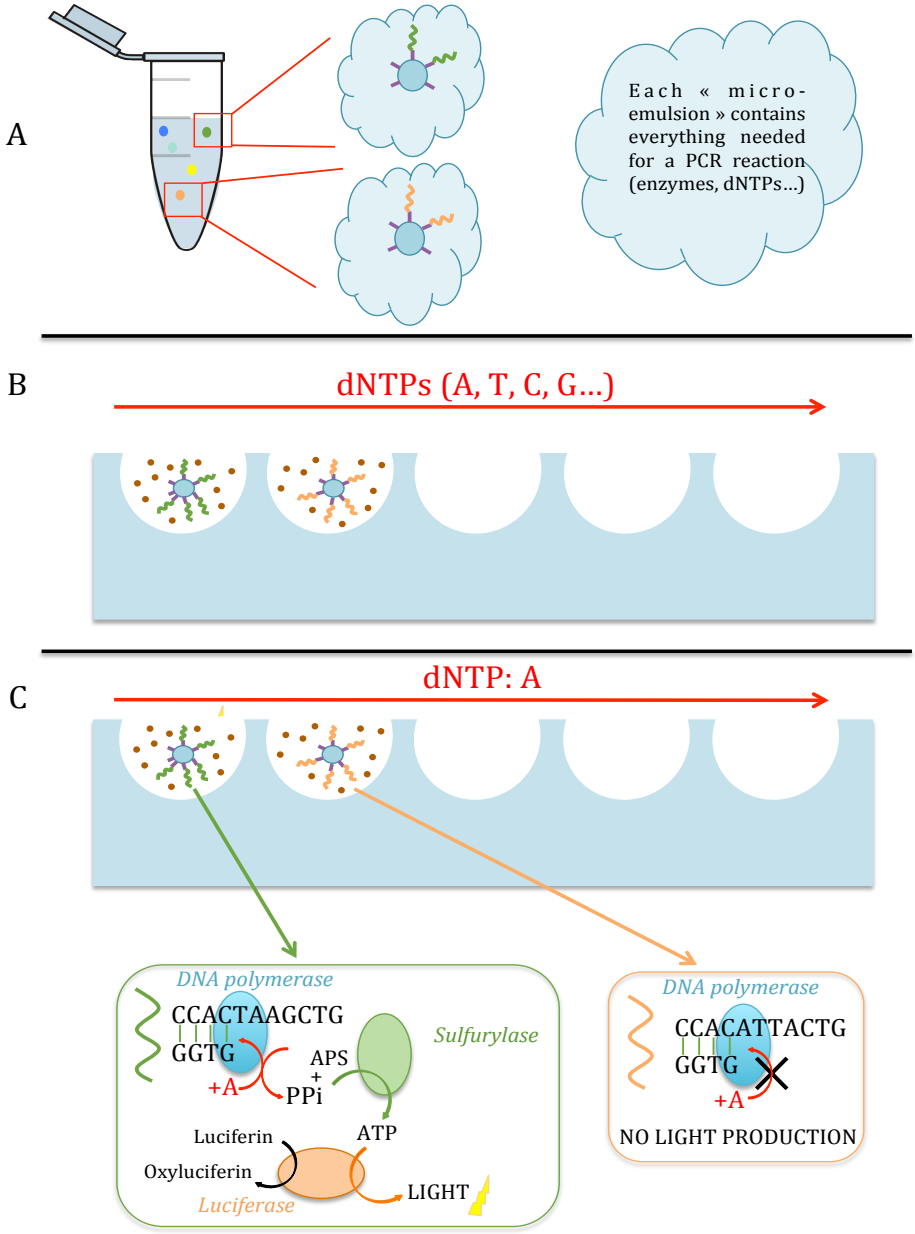

Figure 8. Principle of pyrosequencing.

A: Single strand DNA fragments (green and orange, for two different sequences) bind to micro beads via adapters (purple) in a PCR emulsion and amplification is performed.

B: The DNA-bead complexes are placed into wells of a fibre-optic slide with smaller beads containing enzymes necessary to pyrophosphate sequencing. The four nucleotides are sequentially delivered.

C: Illustration of the detection mechanism occurring when a nucleotide is incorporated (green sequence, left) as compared to an absence of incorporation (orange sequence, right).

The beads are then placed into micro wells, so that each well contains only one bead (Figure 8B). Smaller beads containing enzymes necessary to DNA polymerization and further detection are also placed in the wells. Nucleotides (dNTPs) are then sequentially delivered to the slide. When a nucleotide is incorporated into a sequence, the DNA polymerase releases an inorganic pyrophosphate (PPi) used by sulfurylase to produce ATP. The ATP activates a third enzyme, luciferase, which produces light (Figure 8C). A CCD (charge coupled device) camera detection system records light emission for each well and translates the signal into a peak.

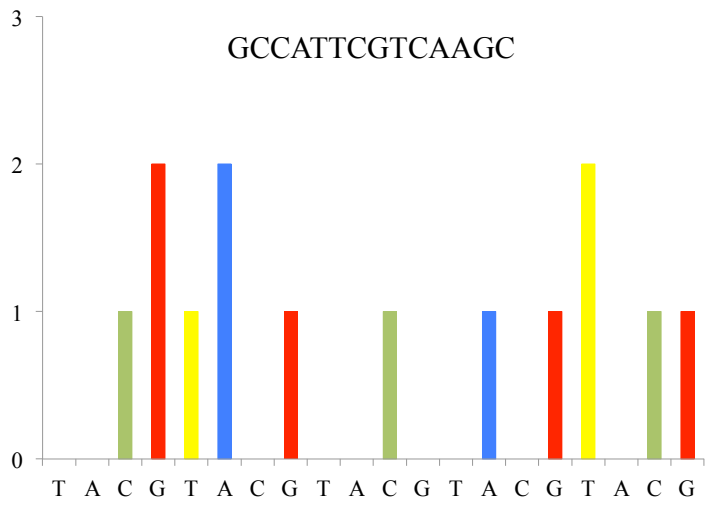

Figure 9. Example of a flowgram obtained for the sequencing of the indicated nucleotide sequence. For each nucleotide provided to the plate, a peak indicates incorporation, and its height is proportional to the number of nucleotides incorporated.

The height of the peak depends on the intensity of light, which depends on the quantity of pyrophosphate released, and therefore, on the number of nucleotides incorporated. A fourth enzyme, apyrase, degrades unincorporated nucleotides and ATP before the following nucleotide pulse is accomplished. At the end of the run, each well, therefore each DNA template is characterized by a flowgram from which the sequence of the DNA

template initially bound to the bead can be reconstructed (Figure 9). The final flowgram obtained for the entire run constitutes the raw data.

3. Raw data processing

Once a flowgram is obtained, a series of bioinformatics manipulations have to be performed to convert raw data into reliable good quality sequences from which diversity analyses can be accomplished. Like for all PCR-based methods, pyrosequencing results can be affected by typical PCR artefacts such as amplification errors and production of chimeric sequences, which can introduce biases in subsequent analyses and lead to diversity overestimations. Other inaccuracies can also result from pyrosequencing itself, which has been shown to cope poorly with long homopolymers and lead to ambiguous sequences. For all these reasons, a substantial amount of sequencing noise and incorrect, bad quality sequences needs to be removed from the raw data. The development, testing and validation of the adequate bioinformatics tools constitute an entire field of research, and many algorithms and entire pipelines have been developed, published and released over the past few years.

Two independent series of runs, for the analysis of the two continuous culture experiments conducted in Banyuls during the first part of this thesis, and for the KEOPS2 project, have been performed by a sequencing platform, and processed separately. Given the time lapse separating the two series of analysis, the tools chosen to process the data were sometimes different, reflecting recent evolution of the bioinformatics pipelines (Table 1). Most of the analyses, however, were performed using the QIIME pipeline

(Caporaso et al., 2010b), following the developing team's recommendations. The most important steps of these analyses are described hereinafter.

Table 1. Major steps in the processing of sequences, and bioinformatics tools used for each independent run

Data processing steps	**Continuous cultures (Chapters 1 & 2)**	**Continuous cultures and in situ diversity (KEOPS2, Chapter 3)**
Denoising	Denoiser (Reeder & Knight, 2010)	AmpliconNoise (Quince et al., 2011) **This step was performed using the Mothur pipeline (Schloss et al., 2009)**
OTU picking	Uclust (Edgar, 2010)	Usearch (=Uclust+Uchime) (Edgar, 2010) **Chimera checking and removal was performed at this step using Uchime, implemented in Usearch**
Choosing a representative sequence	Most abundant	Most abundant
Assigning taxonomy	RDP classifier (Wang et al., 2007) against Greengenes database released in October 2012	RDP classifier (Wang et al., 2007) against Greengenes database released in October 2012
Aligning	PyNast (Caporaso et al., 2010a) using a Greengenes template (DeSantis et al., 2006) **Chimera checking and removal was performed at this step using ChimeraSlayer (Haas et al., 2011)**	PyNast (Caporaso et al., 2010a) using a Greengenes template (DeSantis et al., 2006)
Building a phylogenetic tree	Fasttree (Price et al., 2009)	Fasttree (Price et al., 2009)

a) Denoising

One of the most crucial steps is the identification and removal/correction of sequencing noise. Two different algorithms have been used for the two independent runs (Table 1). Both are based on a flowgram clustering that allows to infer the "true" sequence from similar flowgrams among which some may present an ambiguous signal. Denoiser (Reeder & Knight, 2010), used for the first dataset, constitutes an adaptation of

a "routine" flowgram alignment program (PyroNoise, Quince et al., 2009). AmpliconNoise (Quince et al., 2011), used for the second dataset, is a pipeline that performs both a flowgram clustering (PyroNoise) and a sequence alignement (SeqNoise) to correct errors resulting from PCR as well as pyrosequencing errors.

b) Quality filtering and sample assignment

Another crucial aspect is the removal of sequences that are too short, too long and contain low quality scores. This was performed with the same QIIME script in both cases, and enabled the removal of sequences shorter than 200 bp and containing ambiguous barcode and primer sequences. The same script was used to assign sequences to samples by barcode recognition, after which barcodes and primers were removed.

These two steps considerably reduced each database's size and number of total sequences (Table 2).

Table 2: Number of sequences remaining after each of the first two steps of data cleaning, for each dataset. The order in which each step was performed is indicated with a) and b) for each dataset. The numbers in brackets indicate the percentage of the raw number sequences removed at each step.

	First dataset (18 samples)	Second dataset (73 samples)
Raw number of sequences	264,909	1,583,079
Number of sequences after quality filtering and sample assignment (% removed)	a) 199,968 (25%)	b) 559,041 (14%)
Number of sequences after denoising (% removed)	b) 124,372 (28%)	a) 783,928 (50%)
% total sequences removed	53%	64%

c) Creating OTUs

After denoising, the remaining sequences were clustered into OTUs (Operational Taxonomic Unit) at a sequence similarity threshold of 97%. OTU were picked using the Uclust method and the clustering algorithm "furthest" (Table 1). The algorithm works by picking seed sequences and generate clusters, regrouping sequences under a cluster based on their percent identity with the seed sequence. 3602 OTUs were created for the first dataset. At this stage in the second dataset, chimeric sequences were also identified by combining de novo and reference-based detection with Uchime, implemented in Usearch (Table 1). The sequences identified as chimeras with either detection method were removed and a total of 2078 OTUs was obtained.

As recommended by the QIIME's development team, the rest of the analyses were performed with only one sequence per OTU to reduce dataset size and therefore computational cost. The most abundant sequence found in each OTU was considered the best representative and conserved for further analyses.

d) Assigning taxonomy

The taxonomic identification of OTUs at different phylogenetic levels, from kingdom to genus, was performed using the RDP classifier method (Table 1) (Wang et al., 2007). This program uses sequence segments of eight nucleotides and compares them to a reference database. It then calculates the probability of the observed assignments with a naive Bayesian algorithm, assuming that the sequence segments are completely independent, and provides a confidence value along with each assignment. For the first dataset, the assignment was first performed using the Greengenes database

available at the time, and rerun with the October 2012 release, which was also used for the KEOPS2 dataset.

e) Aligning sequences and constructing a phylogenetic tree

Sequences were aligned using PyNast (DeSantis et al., 2006), an algorithm that aligns each candidate sequence based on the alignment of its closest relative in a reference database. Chimeric sequences were checked and removed after the alignment for the first dataset using ChimeraSlayer (Haas et al., 2011). The aligned sequences were filtered, to remove positions corresponding to gaps in all sequences and determine the positions that are crucial to build a phylogenetic tree.

f) Constructing an OTU table

Finally, an OTU table was constructed for each dataset. The OTU table contains the taxonomic information of each OTU, and its abundance in the different samples. This table enables all subsequent analyses, such as estimations of alpha and beta diversity. Alpha diversity estimates provide information about the diversity within a sample: richness, evenness, phylogenetic diversity; whereas beta diversity provides comparison of diversity between samples, through the calculation of phylogenetic and non-phylogenetic distance matrices. For these analyses, identical sequence numbers in all samples are required. Therefore, each OTU table was randomly subsampled to 3416 and 2930 sequences for the first and the second dataset, respectively.

4. Data analyses

a) Within sample diversity: alpha diversity

A lot of diversity indices have been developed and used in all fields of ecology. Four of them were chosen for the analysis of each dataset. These indices provide mathematical estimations of various aspects of microbial diversity.

* *Chao1*

Chao1 is an index that aims to estimate a community's richness, e.g. the number of species, or OTUs, it contains (Chao, 1987). The major constraint in determining community's richness is the impossibility to achieve the sampling of the total diversity. In most cases, the values obtained for the Chao1 increase with increasing sampling depth (number of sequences), indicating total diversity has not yet been sampled. The Chao1 index provides an indication on the theoretical total number of species/OTUs.

* *Equitability*

The equitability index estimates the evenness of a community. A community with high evenness, where for instance all OTUs display similar relative abundances, is more diverse than a community composed of the same OTUs but with a few dominant ones and the majority of others remaining minor. For the two datasets, the equitability index was calculated using QIIME as follows:

$$E = \frac{H}{logS}$$

where H stands for Shannon's entropy (see below) and S stands for the total number of species. The logarithm to base 2 was used for this calculation.

* *Phylogenetic distance*

The phylogenetic distance index provides an estimation of the phylogenetic diversity within a community. It was calculated using QIIME, and its value corresponds to the minimum total length of all the phylogenetic branches required to span a given community on the phylogenetic tree built with the sequences in all the dataset (Faith, 1992). The higher the index, the more diverse the community is regarding the position of the OTUs in the phylogenetic tree.

* *Shannon*

The Shannon index is a classic diversity metric in ecology (Hill, 1973) and is probably one of the most robust diversity indices (Haegeman et al., 2013). This index considers both the number of species (or richness) and the relative abundance of each of these species (or evenness). It was also calculated using QIIME as follows:

$$H = -\sum_{i=1}^{N} p_i \log p_i$$

where N is the total number of species (or OTUs), and p_i the proportion (or relative abundance) of the ith species. The Shannon index increases with increasing diversity. Again, the QIIME script uses logarithm to base 2 for this calculation.

b) Between sample diversity: beta diversity

The communities developing in the various treatments of the continuous culture experiments and in the distinct sites during KEOPS2 were further compared by the

calculation of distance matrices. Three calculation metrics were used for the two datasets: Bray Curtis, weighted and unweighted UNIFRAC.

* *Bray Curtis*

Bray Curtis (Bray and Curtis, 1957) is a non-phylogenetic metric that allows to determine the dissimilarity between two communities by comparing the relative abundance of the OTUs present in each community.

* *UNIFRAC*

UNIFRAC metrics (Lozupone and Knight, 2005) enable the calculation of distance between communities by taking into account a phylogenetic component, which is given by the phylogenetic tree constructed with the sequences found in these communities. Two UNIFRAC metrics were used in this context. The first one, unweighted, calculates a distance between communities through the differences in the taxonomy of the OTUs, without taking into account their relative abundances. The weighted UNIFRAC, by contrast, uses both the relative abundance of the OTUs and their position on the phylogenetic tree to calculate the overall distance between communities.

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PART ONE

THE EXPERIMENTAL APPROACH: CONTINUOUS CULTURES

INTRODUCTION

The link between heterotrophic bacterial communities and dissolved organic matter (DOM) has been largely investigated in aquatic environments and is now unquestionable (Azam et al., 1994; Pinhassi et al., 1999; Kirchman et al., 2004; Judd et al., 2006; Obernosterer et al., 2010). However, the mechanisms through which the various characteristics of DOM, such as carbon concentration, bioreactivity and composition, influence bacterial populations remain challenging to characterize.

Surprisingly, few studies directly address the question of an increase in DOM concentration, though this kind of resource modification frequently occurs in aquatic systems. In a lake, variations in the concentration of humic substances in batch cultures led, for the lowest concentrations, to different band patterns on DGGE analyses of bacterial communities present in the cultures after 10 days (Eiler et al., 2003). In their study, the identity but not the number of bands varied with changes in substrate concentration, indicating changes in the composition of the communities in response to varying low carbon concentrations (Eiler et al., 2003). In a soil study, Langenheder & Prosser (2008) found that varying concentrations of benzoate changed the composition of the active fraction of bacterial communities after three days, suggesting distinct bacterial taxa responded to different substrate concentrations. In their study, the relative abundance of the responding taxa increased with increasing substrate concentrations, which resulted in a lower diversity of the active community. However, these authors didn't investigate whether these functional responses were associated with changes in the composition or diversity of total communities (Langenheder & Prosser, 2008).

Other studies have investigated bacterial community response to substrate addition as compared to control conditions, testing changes in concentration as well as substrate identity. Pinhassi et al. (1999) tested the effect of an addition of protein or starch on marine bacterial populations in mesocosms over a period of four days. The authors found no effect of an addition of starch compared to a control treatment, but observed a shift in community composition following protein amendment. Additions of glucose, leucine and yeast extract promoted the growth of distinct bacterial groups after two weeks in a microcosm study performed with marine bacterial communities (Alonso-Sáez et al., 2009). Similarly, Gómez-Consarnau et al. (2012) showed that the addition of single labile compounds such as glucose, amino acids, acetate or pyruvate to batch cultures of marine bacteria stimulated different bacterial groups and led to different community structures after four days. Taken together, these studies indicate that there was an effect of an additional supply of carbon, but that the response of bacterial communities also depended on the nature of the substrates tested.

Therefore the identity, complexity and reactivity of carbon substrates could play a major part in the response of bacterial communities to DOM. This aspect of DOM-bacteria interactions has received considerable attention, in different types of environments and through various experimental approaches. Cross-inoculation studies usually observed a convergence of various bacterial inocula when grown on the same DOM source, suggesting bacterial diversity can be driven by DOM composition (Kirchman et al., 2004; Docherty et al., 2006; Judd et al., 2006). Incubations of lake bacterial populations with various organic substrates such as leachates from algae and maple leaves (Kritzberg et al., 2006) or with algal- and soil-derived DOM (Pérez &

Sommaruga, 2006) led to distinct bacterial community structure. In marine systems, similar observations were made after incubating bacteria with detritus from a green alga or from a cyanobacterium (van Hannen et al., 1999); or with high or low molecular weight DOM (Covert & Moran, 2001). Phytoplankton blooms dominated by distinct species were also found associated with distinct bacterial populations (Pinhassi et al., 2004). However, other studies using similar approaches did not observe such clear responses (Langenheder et al., 2006; Orwin et al., 2006).

Overall, these studies provide evidence that bacterial populations respond to a variation in DOM concentration, composition or both, and propose interesting scenarios to explain their findings, such as the concept of concentration niches (Eiler et al., 2003; Langenheder & Prosser, 2008) or the existence of generalist and specialist behavior within a given bacterial taxon (Gómez-Consarnau et al., 2012; Langenheder & Prosser, 2008). Moreover, the phylogenetic classification of the bacterial groups identified in these studies is consistent with observations of bacterial responses to changes in resource condition in the environment described extensively in the literature (Fandino et al., 2001; Rink et al., 2007; West et al., 2008; Tada et al., 2011). This indicates that these studies were able to grow bacterial taxa that appear to respond to *in situ* modifications of the DOM pool.

For several reasons though, they do not fully elucidate the characteristics of these processes. In batch cultures or mesocosms, environmental parameters such as nutrient concentrations are likely to change over time, which means bacterial populations are subject to a set of constrains and not only to different DOM conditions. This could partly hamper the observation of true DOM effects, especially if other potential shaping factors

such as top down control are also present. In most cases, the substrates used for amendment represent only a fraction of the very large and complex pool of both labile and refractory material that constitutes the DOM typically found in aquatic environments. The fingerprinting methods used in most of these studies to detect changes in community structure do not allow a detailed phylogenetic analysis. Only some aspects of bacterial diversity can be investigated, such as the presence/absence and relative abundance of the dominant phylotypes, but more subtle changes affecting less abundant groups can easily be missed. Providing alpha diversity estimations with these techniques is also complicated (Bent et al., 2007). For all these reasons, the question of how DOM concentration and composition can affect bacterial diversity is still unresolved.

In this context, two objectives of this thesis were to examine the response of marine bacterial communities, first, to an increased supply of DOM, and second, to differences in DOM chemical composition. We wanted to address these questions from a new angle. To reach this objective, we chose to use continuous cultures to maintain natural bacterial communities under stable abiotic conditions over a certain number of bacterial generations. Our experimental design, sampling strategy and analytical tools allowed us to:

- ✓ Make sure DOM was the only parameter responsible for potential differential diversity patterns between the various conditions tested, by keeping other abiotic factors steady.

- ✓ Extensively screen bacterial diversity (alpha-, beta-diversity and phylogenetic characteristics) using 454 pyrosequencing.

- ✓ Study the change of diversity patterns over time, with two time points in each treatment from day 0 (initial community) to day 8 (after three generations) and day 15 (after five generations).

Following this general strategy, the experiments presented Chapters 1 and 2 were designed to address two questions:

- What is the effect of an increased supply of phytoplankton-derived DOM on bacterial diversity? (Chapter 1)

- What is the effect of differences in the chemical composition of phytoplankton-derived DOM on bacterial diversity? (Chapter 2)

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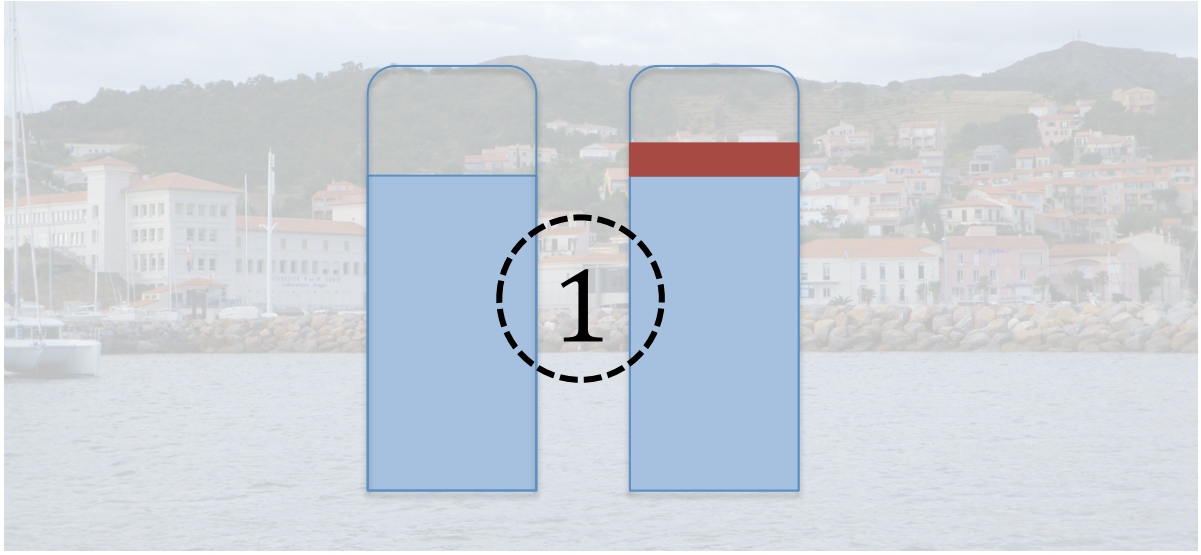
CHAPTER ONE

RESPONSE OF HETEROTROPHIC MARINE BACTERIA TO AN ADDITION OF PHYTOPLANKTON-DERIVED DISSOLVED ORGANIC MATTER



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Changes in bacterial diversity in response to dissolved organic matter supply in a continuous culture experiment

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ABSTRACT: Heterotrophic bacterial metabolism is tightly linked to the availability of dissolved organic matter (DOM), but the relationship between substrate supply and bacterial diversity remains unresolved. The objective of the present study was to determine how an increase in the supply of bioavailable DOM affects bacterial metabolism and diversity. We addressed this issue by growing a natural bacterial community over 5 generations in continuous cultures either on seawater (dissolved organic carbon concentration equal to 74 µM) or on seawater amended with 13 µM of diatom-derived DOM. Bacteria markedly responded to the additional DOM supply, as revealed by 1.6-fold higher bacterial abundances and 10-fold higher cell-specific α- and β-glucosidase activities in the +DOM treatment than in the control throughout the experimental period. Analysis of diversity as determined by tag pyrosequencing of 16S rRNA genes after 3 and 5 bacterial generations showed that the bacterial communities in the +DOM treatment were more similar to each other than to communities in the control. Diversity indices such as richness and phylogenetic distance were higher in the DOM-amended cultures than in the control. *Gammaproteobacteria* dominated the communities selected in our cultures, representing between 87 and 99% of the sequences. The addition of DOM led to a higher relative abundance of several bacterial groups, in particular *Alphaproteobacteria*, *Bacteroidetes*, and *Verrucomicrobia*, which accounted for up to 14% of the sequences, compared to the control in which they represented <5% of the sequences. Our results demonstrate that in this experimental context, an increased supply of bioavailable DOM sustained a higher bacterial diversity.

KEY WORDS: Bacterial diversity · Dissolved organic matter · Continuous cultures · 454 pyrosequencing

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INTRODUCTION

Aquatic environments harbor a vast bacterial diversity (Giovannoni & Rappé 2000, Sogin et al. 2006). The factors that shape this bacterial diversity and in turn how the bacterial community contributes to ecosystem functioning have become a subject of intense research. The variability in bacterial community structure on different temporal and spatial scales has been shown to correlate with changes in biotic and abiotic environmental factors, indicating their selective control of bacterial communities (Ramette 2007, Logue et al. 2012). These studies have further provided insight into the co-occurrence of different phylotypes, suggesting environmentally driven interactions among bacterial groups (Fuhrman et al. 2006, Gilbert et al. 2012). However, the question of how patterns in phylogenetic diversity are linked to patterns in functional capabilities remains unanswered.

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A key role of heterotrophic bacteria in the aquatic environment is the degradation of dissolved organic matter (DOM). Previous studies have provided some evidence that bacterial community composition varies with DOM source, indicating the structuring effect of DOM (Van Hannen et al. 1999, Covert & Moran 2001, Kirchman et al. 2004). Different metabolic capabilities to degrade and utilize DOM among bacterial groups is one of the hypotheses that has been developed to explain these observations, an idea that was first supported by results from fluorescence *in situ* hybridization coupled to microautoradiography (Cottrell & Kirchman 2000, Teira et al. 2004, Vila-Costa et al. 2007). Single-cell analyses have demonstrated that polymeric substances are preferentially degraded by *Bacteroidetes* (Cottrell & Kirchman 2000), a finding that was later confirmed by genomic analyses of several members of this group (Bauer et al. 2006, Gómez-Pereira et al. 2012). Metatranscriptomic analyses of bacterial communities grown on different DOM sources further reveal taxon-specific expression of genes related to carbon metabolism (McCarren et al. 2010, Poretsky et al. 2010, Rinta-Kanto et al. 2012). These studies provide some evidence for a link between resource supply and bacterial community composition, suggesting some specialization of bacterial groups over a range of phylogenetic levels.

If, as suggested by these previous studies, DOM structures bacterial community composition by favoring the development of certain bacterial groups, the way bacterial communities come into contact with DOM might modulate the bacterial response. Aquatic systems receive DOM through different modes and at variable concentrations. River discharges and atmospheric deposition provide episodic pulses of organic matter supply on time scales of hours to days (Naudin et al. 1997, Jurado et al. 2008), in contrast to the overall continuous supply of DOM by phytoplankton. While an episodic pulse of organic matter is likely to have short-term consequences predominantly on the active part of the bacterial community, DOM that continuously supports bacterial growth probably affects bacterial diversity over a longer time scale.

To address the question of how bacterial diversity is linked to DOM supply, we investigated the response of a natural bacterial community to the sustained input of a small amount of bioavailable DOM under constant environmental conditions. We expected an increase in bulk bacterial abundance without any changes in bacterial diversity if the added DOM stimulates all bacterial groups. In contrast, distinct diversity patterns between experimental conditions would be observed if the added DOM stimulates different bacterial groups. Our results show an increase in bacterial diversity, including richness and phylogenetic diversity, in response to the addition of bioavailable DOM.

MATERIALS AND METHODS

Experimental design

To explore DOM–bacteria interactions, we designed an experiment using continuous cultures. Seawater for the continuous cultures was collected in the NW Mediterranean Sea, 5 nautical miles off Banyuls-sur-Mer (42° 28′ 300″ N, 03° 15′ 500″ E; 95 m overall depth). For the medium, 300 l of seawater were collected in September 2009 at 3 m using an All-PFA Double-Bellows Pump (Asti) connected to 50 m PVC tubing. Seawater was 20 µm filtered and stored in 1 polycarbonate (PC) carboy in the dark at room temperature for 6 wk to allow degradation of the readily bioavailable DOM. One day before the start of the experiment, the 300 l were sequentially filtered through 0.4 and 0.2 µm filters using a Sartobran cartridge (150 cm^2, Sartorious), and the filtrate was stored at 4°C in the dark in acid-washed (10% HCl), Milli-Q water rinsed 20 l polycarbonate carboys. For the initial cultures, seawater (10 l) was collected at the start of the experiment on 6 November 2009 at the site described above at 5 m. The seawater was 0.8 µm filtered (PC, Nuclepore), 2 l of the filtrate were used as initial bacterial inoculum, and 8 l were filtered on a 0.2 µm cartridge (Sterivex, Millipore) and stored at −80°C for further analyses.

The exudate of the diatom *Phaeodactylum tricornutum* was used as a source of bioavailable DOM in one of the 2 experimental conditions. The *P. tricornutum* cells were grown in low (15 µM) concentrations of dissolved organic carbon (DOC) artificial seawater amended with nutrients in Guillard's F/2 medium. We did not autoclave the artificial seawater to avoid potential DOC contamination, and thus, the phytoplankton culture was not axenic. As a consequence, the most labile fraction of the DOM was probably degraded in the phytoplankton culture, and the exudate recovered at the end of the exponential growth phase was most likely dominated by semi-labile DOM. The culture was grown at 18°C under continuous light. A total culture volume of 12 l was prepared. Cell growth was followed by measuring the ab-

sorbance at 660 nm. The culture was sequentially filtered through a combusted GF/F filter (90 mm diameter, Whatman) and an acid-washed, Milli-Q rinsed 0.2 µm PC filter (90 mm diameter, Nuclepore). The filtrate was recovered in acid-washed, Milli-Q rinsed 4 l PC carboys and stored at 2°C during the entire experimental period. The DOC concentration in the exudate was 151 µM.

The continuous cultures were run under 2 conditions, each conducted in duplicate. In the control, the initial bacterial community was grown on seawater only, while in the +DOM treatment, the bacterial community was grown on seawater amended with DOM from the *Phaeodactylum tricornutum* culture. At the start of the experiment, the initial bacterial community was diluted 1:10 either with seawater (74 µM DOC concentration) or with a mixture of seawater and phytoplankton-derived DOM (87 µM final DOC concentration) prepared as described above. We added 13 µM of phytoplankton DOC to seawater because in the ocean this bioavailable resource makes up a relatively small fraction of bulk DOM (Benner 2002). The final volume of each of the bacterial cultures was 6.5 l. To ensure that organic carbon was the limiting factor for bacterial growth, nitrogen and phosphorus were added in excess to both the control and the +DOM treatment. The final concentrations in the initial cultures were 94 µM for nitrite and nitrate and 11 µM for phosphate. The input media for each of the 2 conditions consisted of either seawater or a mixture of seawater with phytoplankton-derived DOM. The concentration of inorganic nutrients was identical in both types of media (94 µM for nitrite and nitrate and 11 µM for phosphate). We prepared 4 l of each type of medium every 2 d and kept them in acid-washed PC carboys at 2°C before use. The 2 replicate cultures were supplied by a single PC carboy containing the input medium.

The cultures were run in a closed system consisting of 4 all-glass vessels (15 cm diameter, 50 cm height) connected to the medium by Teflon tubing. The continuous supply of input medium and the output of the cultures were conducted using a peristaltic pump (Gilson). The cultures were bubbled with filter-sterilized air (Sartorios). The bacterial cultures were run as a batch culture until the stationary phase was reached before starting the inflow of input medium and the outflow of culture. The dilution rate was 0.27 d^{-1}, resulting in a generation time of 2.6 d. We selected this dilution rate based on growth rates of 0.3 d^{-1} determined *in situ* for coastal Mediterranean waters (Obernosterer et al. 2010). The continuous cultures were run in a temperature-controlled room (18°C) in the dark.

The cultures were sampled daily during the 15 d experimental period. Samples were taken with glass syringes (Hamilton) connected with a Luer lock to Teflon tubes that reached the bottom of the cultures. This system allowed us to sample without opening the culture containers. The glass syringes were extensively rinsed with Milli-Q water prior to sampling. A total of ~100 ml was sampled every day in each culture, representing <2% of the total volume of the cultures.

Inorganic nutrients and dissolved organic carbon

For inorganic nutrients, subsamples (5 ml) were collected in 20 ml acid-washed polyethylene vials. Concentrations of nitrate (NO_3^-), nitrite (NO_2^-) and phosphate (PO_4^{3-}) were determined immediately after sampling using the automated colorimetric technique on a Skalar autoanalyzer following the protocol of Tréguer & Le Corre (1975). For DOC, triplicate 15 ml subsamples were filtered through 2 precombusted GF/F filters (Whatman) and stored after acidification with orthophosphoric acid (H_3PO_4, final pH 2) in precombusted glass vials with Teflon-lined caps in the dark. DOC analyses were performed by high temperature catalytic oxidation on a Shimadzu TOC-V-CSH analyzer (Benner & Strom 1993). The analytical precision was 1 to 2% (coefficient of variation).

Enumeration of heterotrophic bacteria

For heterotrophic bacterial abundance, duplicate subsamples (1.8 ml) were fixed with formaldehyde (2% final concentration). The samples were incubated at 4°C for 30 min and stored at −80°C until flow cytometric analysis. Samples were thawed and stained with SYBR Green I (Invitrogen–Molecular Probes) at 0.025% (v/v) final concentration for 15 min at room temperature in the dark. Counts were performed with a FACSCalibur flow cytometer (Becton Dickinson) equipped with an air-cooled argon laser (488 nm, 15 mW) (Lebaron et al. 2001).

Enzymatic activities

We measured the hydrolysis of the fluorogenic substrate analogues 4-methylumbelliferyl-α-D-gluco-

pyranoside and 4-methylumbelliferyl-β-D-glucopyranoside (MUF-α-Glc and MUF-β-Glc, respectively, Sigma Aldrich) to estimate potential activity rates of α- and β-glucosidases (Hoppe 1983). We followed a protocol from Van Wambeke et al. (2009) with some modifications. Triplicate subsamples (380 µl) of each culture were incubated with MUF-α-Glc and MUF-β-Glc prepared at 10 mM in methyl cellosolve after determining saturating concentrations. The final concentration of the substrate was 500 µM. The samples were incubated for 8 h in the dark at the same temperature as the cultures. For the 2 enzymes, a blank was prepared each time using sterile seawater and incubated under the same conditions as the samples from the cultures. Incubations were stopped by addition of alkaline (pH 10.5) glycine-ammonium hydroxide buffer (0.05 M glycine + 0.2 M NH_4OH) (10% final concentration). The samples were stored at −20°C until analysis. The samples were thawed at +55°C, and 550 µl of borate buffer (pH 10, Sigma-Aldrich) were added. Fluorescence was determined at 460 nm under 364 nm excitation on a fluorescence spectrophotometer (Hitachi F-2500).

Bacterial diversity

After ~3 bacterial generations (Day 8), samples for bacterial diversity (200 ml) were collected from the outflow in autoclaved PC flasks. Bacterial cells were collected on a 0.2 µm PC filter (47 mm diameter, Nuclepore), and the filters were stored at −80°C until further analysis. At the end of the experiment, corresponding to ~5 bacterial generations (Day 15), samples (1500 to 2300 ml) were collected in autoclaved PC flasks, concentrated on a 0.2 µm cartridge (Sterivex, Millipore) and stored at −80°C until further analysis.

DNA was extracted as described by Laghdass et al. (2011). Briefly, the bacterial cells collected on the filters were lysed using lysis buffer (40 mM EDTA, 50 mM Tris and 0.75 M sucrose) and performing 3 freeze-thaw cycles with liquid nitrogen and a water bath at 65°C. After denaturation and degradation of proteins using sodium dodecyl sulfate (1%) and Proteinase K (0.2 mg ml^{-1}), the DNA was purified using a Qiagen AllPrep DNA/RNA extraction kit according to the manufacturer's instructions. The molecular size and purity of the DNA were analyzed using agarose gel electrophoresis (1%). In total, 7 samples were extracted, and for each sample, a total amount of 600 ng was sent to the Research and Testing Laboratory (www.researchandtesting.com) for 454 tag pyrosequencing.

The bacterial 16S rRNA genes were sequenced using the FLX-Titanium platform as described by Wolcott et al. (2009). The regions V2 and V3 of the 16S rRNA gene were amplified using primers targeting 410 bp between positions 121 and 531 on the *Escherichia coli* reference sequence (Baker et al. 2003). For the 7 samples that were analyzed, a total number of 88 121 sequences was obtained. The raw data are deposited in the Euopean Nucleotide Archive under accession number ERP002553 and can be accessed at www.ebi.ac.uk/ena/data/view/ERP002553. All further analyses were conducted using the Quantitative Insights Into Microbial Ecology (QIIME) pipeline (http://qiime.org) (Caporaso et al. 2010). The sequences were quality filtered, and all sequences shorter than 200 bp or containing ambiguous bases were eliminated from the data set. Sequences were then attributed to each sample according to their barcode sequence. These operations led to the removal of ~25% of the sequences with either bad quality scores or ambiguous barcode sequences. The sequencing errors were corrected among the remaining sequences using the QIIME denoiser algorithm, and barcodes and primers were removed (Caporaso et al. 2010). Operational taxonomic units (OTUs) were identified using the uclust picking method and defined at a 97% sequence similarity level, and only the most abundant sequence was conserved as a representative. Taxonomy was assigned using the RDP classifier program (Wang et al. 2007) with the most recent Greengenes reference data set released in October 2012. The sequences were aligned using the PyNast algorithm (DeSantis et al. 2006, Caporaso et al. 2010). The chimeric sequences were identified and removed with ChimeraSlayer (Caporaso et al. 2010).

A phylogenetic tree was constructed using the FastTree method (Price et al. 2009), and an OTU table was created. To enable comparisons between samples, the OTU table was subsampled to ensure an equal number of sequences in each sample. The resulting OTU table comprised 3416 sequences per sample. All further analyses, such as alpha and beta diversity estimations, were conducted with this OTU table. Phylogenetic differences between samples were tested using Andrew Martin's phylogenetic test (P-test) (Martin 2002, Eckburg et al. 2005). The clustering of samples was checked for robustness with jackknife analyses and for significance with an ANOSIM. All statistics were performed using the QIIME pipeline.

RESULTS

Nutrient conditions and bulk bacterial response in the continuous cultures

The chemical parameters were stable for the 15 d experimental period. The sum of the concentrations of NO_3^- and NO_2^- was 93 ± 2 and 90 ± 3 µM (mean ± SD, n = 15) in the control and the +DOM treatment, respectively. The mean concentrations of PO_4^{3-} (11 ± 1 µM) were not different under the 2 conditions (data not shown). DOC concentrations in the cultures remained stable over time, with mean values of 74 ± 2 and 75 ± 2 µM in the control and the +DOM treatment, respectively (Table 1).

Organic carbon appeared to be the primary limiting factor of bacterial growth in the cultures. Ratios of DOC:(NO_3^- + NO_2^-) and DOC:PO_4^{3-} in the cultures were 0.8 and 7, respectively, throughout the experiment and thus well below the C:N:P ratios of heterotrophic bacteria (45:9:1) (Goldman et al. 1987). Concentrations of DOC in the input media were 74 ± 3 and 87 ± 7 µM for the control and the +DOM treatment, respectively. The difference in the DOC concentrations between the input medium and the DOM-amended cultures demonstrates that bacteria rapidly utilized the added DOC. In the control, DOC utilization was below our limit of detection.

Bacterial abundances increased exponentially in all cultures during the first 24 h when the system was maintained as a batch. After the initial growth phase, bacterial abundances stabilized in 3 out of 4 cultures.

Table 1. Basic biogeochemical parameters in the continuous culture system. Concentrations of dissolved organic carbon (DOC) are given for the input media and the cultures. Mean values ± SD of measurements performed during the 15 d experimental period are given. For the +DOM treatment, the mean value of the replicate incubations was used at each time point

	Control	+DOM
Input medium		
DOC (µM)	74 ± 3 (n = 4)	87 ± 7 (n = 4)
Culture		
DOC (µM)	74 ± 2 (n = 10)	75 ± 2 (n = 10)
Bacterial abundance (10^6 cell ml^{-1})	2.6 ± 0.8 (n = 15)	4.3 ± 0.5 (n = 15)
α-glucosidase activity (amol $cell^{-1}$ h^{-1})	0.13 ± 0.1 (n = 8)	1.17 ± 0.2 (n = 8)
β-glucosidase activity (amol $cell^{-1}$ h^{-1})	0.18 ± 0.1 (n = 8)	1.23 ± 0.2 (n = 8)

In one of the control cultures, bacterial abundance decreased over time, and this replicate had to be discarded for further analysis. Bacterial abundances were 3.9 ± 0.9 × 10^6 and 4.6 ± 0.7 × 10^6 cells ml^{-1} in Replicates 1 and 2 of the +DOM treatment, respectively (mean ± SD, n = 15). The bacterial abundance in each of the replicate +DOM cultures was significantly higher (1.5- and 1.7-fold, respectively; Wilcoxon test, $p < 0.008$) than the bacterial abundance in the control culture (2.6 ± 0.8 × 10^6 cells ml^{-1}) (Table 1). This response of heterotrophic bacteria to the addition of DOM was also reflected in bacterial enzymatic activities. α- and β-glucosidase activities were significantly different between conditions (Wilcoxon test, $p < 0.004$), with roughly 10-fold higher cell-specific activities in the +DOM treatment (Table 1).

Effect of DOM addition on the bacterial diversity

We explored bacterial diversity and composition after 3 and 5 bacterial generations by tag pyrosequencing the 16S rRNA gene and clustering the sequences into OTUs at >97% identity. In total, 507 OTUs were obtained from the clustering of the sequences, and the total number of OTUs for the initial community was 254. In the control, the number of OTUs was 62 and 67 after 3 and 5 generations, respectively. A higher number of OTUs was observed in the +DOM treatment both after 3 generations (118 in Replicate 1 and 119 in Replicate 2) and after 5 generations (80 in Replicate 1 and 109 in Replicate 2). A dendrogram based on a weighted UNIFRAC similarity matrix, using the relative abundance of each OTU and phylogenetic affiliation, illustrates that time was the major factor in determining the differences among the bacterial communities. At each time point, however, the bacterial communities grown on the DOM-amended seawater clustered separately from the bacterial communities grown on seawater alone (Fig. 1). This clustering was robust, as confirmed by jackknife analyses and statistical examination (ANOSIM, $p = 0.01$).

We further compared the dissimilarity between replicate communities and also between the communities in the DOM-amended cultures versus the control, using the P-test (Martin 2002). At both time points, the within-treatment variability between the 2 DOM-amended replicate cultures (7 and 3% after 3 and 5 generations, respectively) was not significant (P-test; $p > 0.08$). In contrast, the dissimilarity between

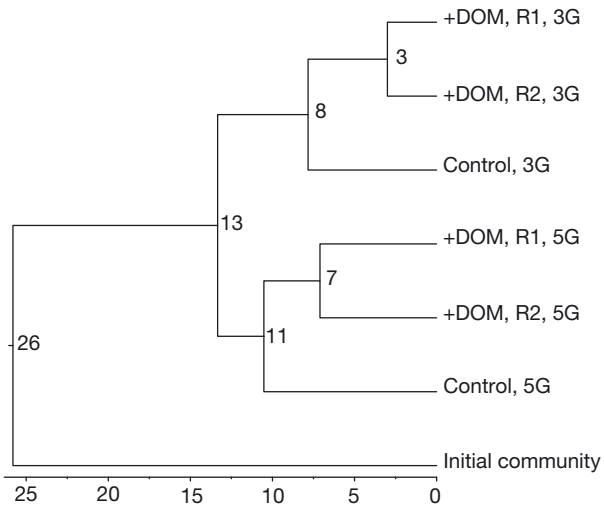

Fig. 1. UPGMA analysis of bacterial communities sampled in the continuous cultures after 3 (3G) and 5 generations (5G). The dendrogram was constructed using the average linkage method on a weighted UNIFRAC similarity matrix. R1 and R2: Replicates 1 and 2 for the +DOM treatment. Values on the scale bar and the nodes indicate the percentage of dissimilarity

the DOM-amended cultures and the control was significant for Replicates 1 and 2 after 3 generations and for Replicate 1 after 5 generations ($p < 0.02$). Taken together, our results indicate that at each time point, the differences in bacterial community structure between the 2 experimental conditions were due to a response to the DOM addition. Interestingly, a dendrogram constructed from an unweighted UNIFRAC similarity matrix, which does not consider the relative abundance of each OTU but considers only its presence or absence and phylogenetic affiliation, revealed an inverse pattern in which communities clustered first according to the experimental condition and then according to time (data not shown). This pattern suggests that the presence of OTUs was mainly determined by the experimental condition, whereas their abundance was mainly determined by time. With either analysis, the data indicate the importance of the DOM addition in structuring bacterial communities in these continuous cultures.

To better understand the changes induced by the DOM addition, we calculated the Chao1 index for bacterial richness and the phylogenetic distance. Overall, the rarefaction curves of the bacterial communities grown in the continuous cultures reached a plateau, indicating that the sampling was deep enough to cover most of the diversity (Fig. 2). The indices for bacterial richness and phylogenetic distance were higher in the DOM-amended cultures than in the control culture at both time points. Richness decreased between the third and fifth generation, but the DOM-amended cultures were still more diverse (Fig. 2a). Phylogenetic distance was almost twice as high in the DOM-amended cultures than in the control culture, with remarkable steadiness over time (Fig. 2b). Overall, these indices were lower in the cultures compared to the inoculum (Chao1: 753; phylogenetic distance: 28).

We investigated the OTUs that were potentially selected by the DOM addition. The identification of an OTU deemed specific to one experimental condition was based on its presence in one condition and its absence from the other. OTUs present in all 3 cultures were considered common. The phylogenetic diversity of the OTUs specific to the +DOM treatment was higher than in the control (Fig. 3). *Alphaproteobacteria*, *Bacteroidetes*, *Verrucomicrobia*, and other groups made up the largest part (50% after 3 and

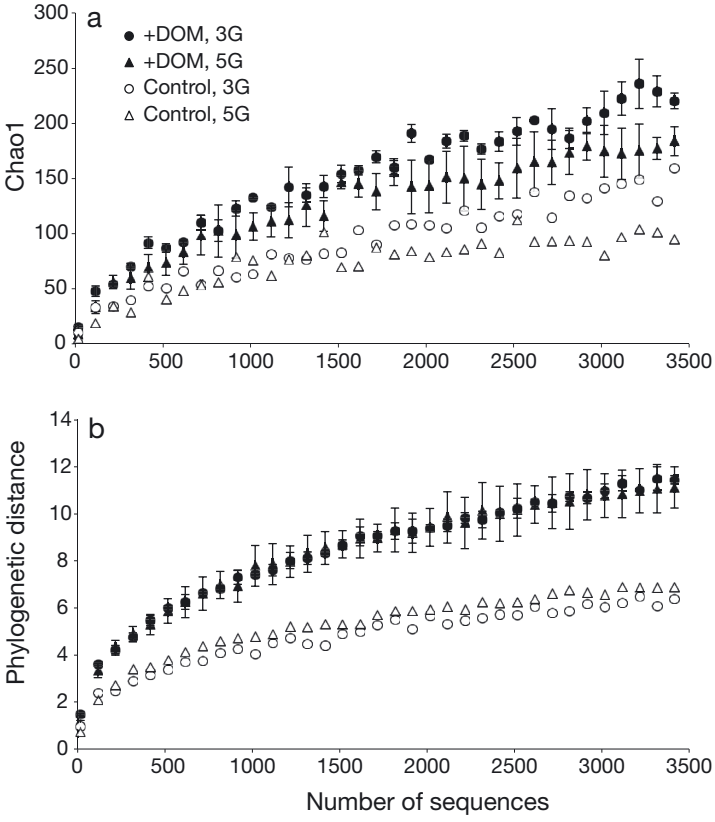

Fig. 2. Rarefaction curves of the diversity indices (a) Chao1 and (b) phylogenetic distance after 3 (3G) and 5 generations (5G). For the +DOM treatment, mean values ± SD are given

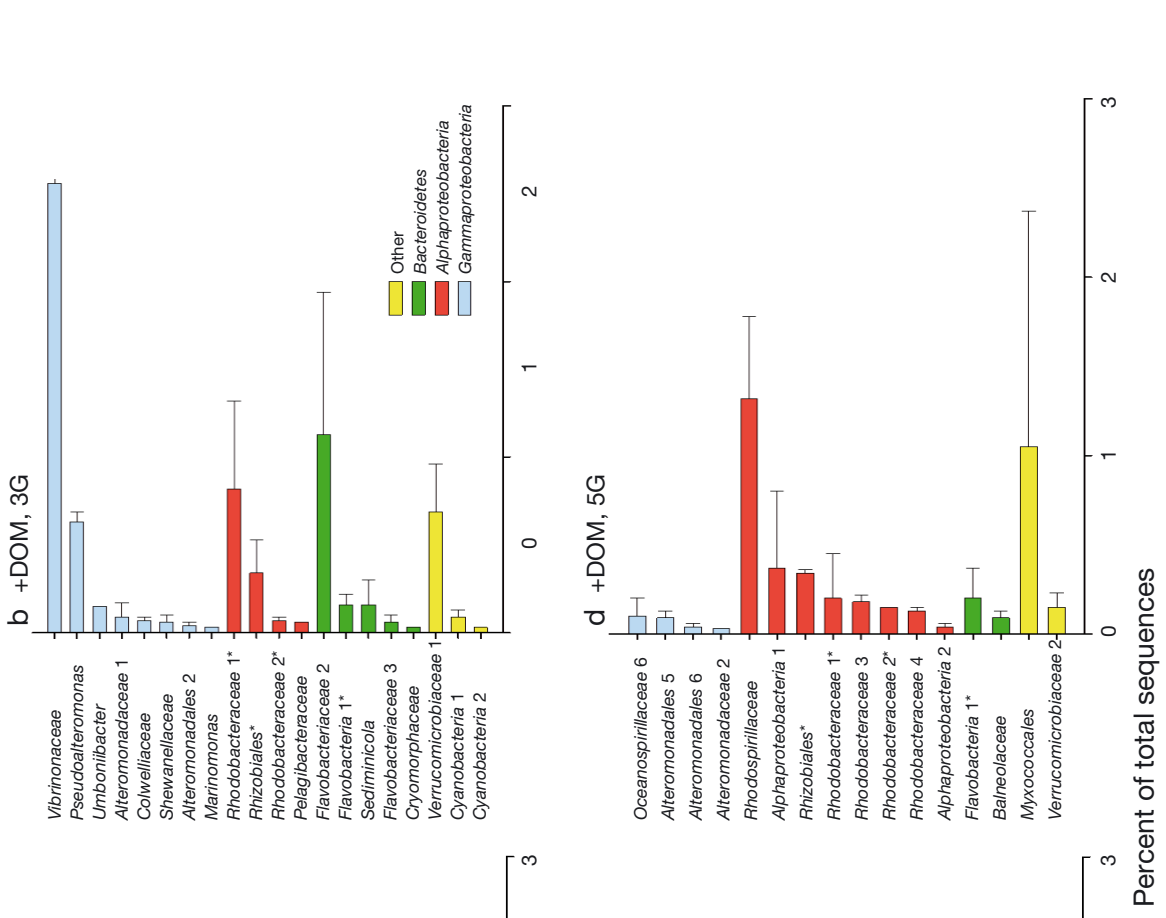

Fig. 3. Percentage of the total number of sequences (3416) of operational taxonomic units (OTUs) specific to (a,c) the control or to (b,d) the +DOM cultures after (a,b) 3 and (c,d) 5 generations (3G and 5G, respectively). For the +DOM treatment, mean values ± SD between replicates are given. The name of each OTU corresponds to its taxonomic assignment. The OTUs with identical taxonomy are differentiated by numbers. OTUs that are marked with an asterisk (*) are those that were found specific at both time points

94 % after 5 generations) of the OTUs specific to the DOM-amended treatment. In contrast, *Gammaproteobacteria* accounted for 99 and 83 % of the control-specific OTUs after 3 and 5 generations, respectively.

Bacterial community composition

As indicated by the clustering of samples (Fig. 1), the communities selected by our experimental system largely differed from the initial community (Fig. 4, see Table S1 in the Supplement at www.int-res.com/articles/suppl/a069p157_supp.pdf). The bacterial communities present in both the control and the +DOM treatment were dominated by *Gammaproteobacteria*, belonging mainly to 3 orders: *Alteromonadales*, *Oceanospirillales*, and *Thiotrichales* (Fig. 4a, Table S2 in the Supplement). However, their relative abundance was lower in the DOM-amended cultures than in the control due to a more pronounced response of other bacterial groups to the culture conditions. The relative contribution of *Alphaproteobacteria* after 3 and 5 generations was 3- and 2-fold higher in the DOM-amended treatment than in the control (Fig. 4b, Table S3 in the Supplement). This pattern was observed for the *Rhodobacterales*, *Rhodospirillales*, and other groups within the *Alphaproteobacteria*. The proportion of sequences belonging to several other bacterial groups, in particular *Bacteroidetes* and *Verrucomicrobia*, was also noticeably enhanced in the +DOM treatment (Fig. 4c,d, Tables S4 & S5 in the Supplement). A maximum of 0.1 % of the sequences detected in a single sample could not be properly characterized even to the phylum level. Overall, the taxonomic affiliation of the sequences retrieved from our cultures confirms the higher phylogenetic diversity in the DOM-amended cultures illustrated in Fig. 2b.

DISCUSSION

Bacterial diversity, including richness and phylogenetic diversity, increased in response to an enhanced supply in bioavailable DOM in our experiment. We chose continuous cultures to provide insight into how a sustained input of DOM under C-limited conditions affects the structure of a natural bacterial community. We were successful in maintaining both stable abiotic conditions and organic carbon as the growth-limiting factor throughout the experimental period. As a selective tool, continuous cultures set a lower limit for the growth rate through the dilution rate (Kovárová-Kovar & Egli 1998), restricting bacterial growth to the groups that are most responsive or adapted to the culture conditions, as reflected by the lower diversity in the cultures compared to the inoculum. Our result thus reflects the adaptation of a bacterial community after 3 and 5 generation times to a small increase in a mixture of organic carbon substrates of varying bioavailability and chemical composition.

Continuous cultures are technically challenging, and the large volumes used for each culture in the present study together with the duration of the experiment made it difficult to set up more than 2 replicate conditions. The patterns in diversity were repeatable for the replicate +DOM cultures and for the samples taken after 3 and 5 generations of each condition, indicating that our conclusions are robust. The observation that the response to the DOM addition in terms of bacterial biomass, metabolism, and diversity was the same over time supports the hypothesis that differences between the +DOM and the control were real rather than due to chance. For all these reasons, we have confidence in the validity of our conclusions, despite the loss of 1 control replicate.

The surprising finding of the present study is the increase in diversity, including richness and phylogenetic diversity, of the bacterial communities in the phytoplankton DOM-amended cultures. We consider 2 possible explanations for this observation. The increase in bacterial richness could be linked to the higher DOC concentrations due to the DOM addition. A previous study using a DNA fingerprinting method found changes in bacterial diversity with increasing DOC (40 to 540 µM) (Eiler et al. 2003). In the present study, the total concentration only partly explains our results because the addition of DOM changed DOC concentrations only slightly (13 µM added to the original 74 µM). By contrast, the phytoplankton DOM most likely had a different chemical composition and bioavailability than the seawater DOM that had been aged for 6 wk prior to the start of the experiment. Carbohydrates contribute substantially to DOM from phytoplankton (Biddanda & Benner 1997, Aluwihare & Repeta 1999). Molecular-level analyses have identified the same individual sugars in phytoplankton DOM and surface DOM; however, their relative contributions and in particular the DOC yields vary greatly between DOM sources (Biersmith & Benner 1998, Aluwihare & Repeta 1999). The higher glucosidase activities are thus likely a response to higher concentrations of oligo- and polysaccharides in our DOM-amended cultures, supporting the idea that DOM composition was different between the condi-

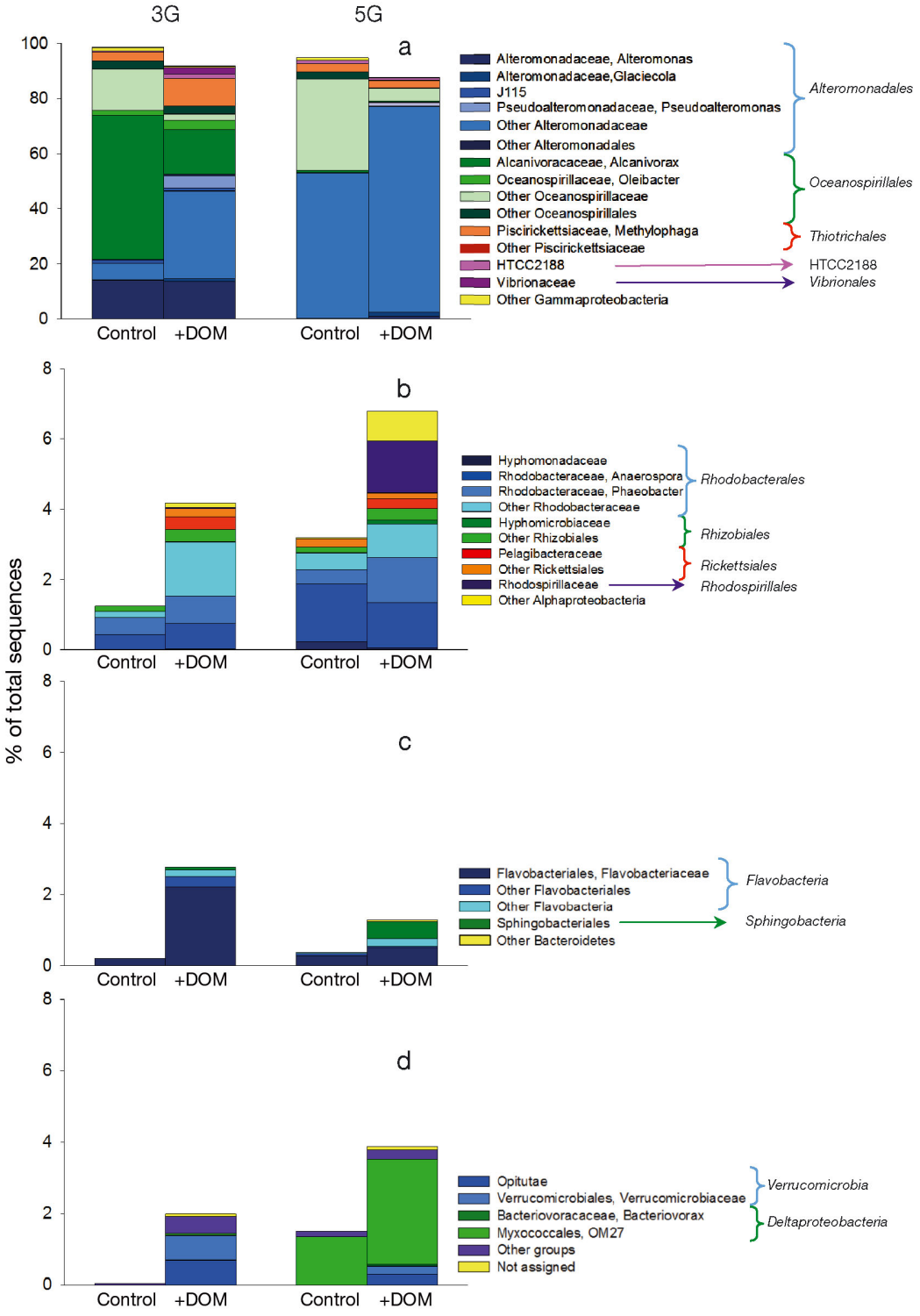

Fig. 4. Percentage of the total made up by the indicated bacterial groups in the communities present in the continuous cultures after 3 and 5 generations (3G and 5G, respectively). Bacterial groups belonging to (a) *Gammaproteobacteria*, (b) *Alphaproteobacteria*, (c) *Bacteroidetes*, and (d) other groups are shown. For (a) *Gammaproteobacteria* and (b) *Alphaproteobacteria*, the different colors correspond to different orders within these classes. For (c) *Bacteroidetes* and (d) other groups, different colors correspond to different phyla and classes. For the +DOM treatment, the mean percentage of the 2 replicates was calculated. Note the different scales on the *y*-axis. Detailed phylogenetic information is given in Tables S2 to S5 in the Supplement

tions. The compounds present in the phytoplankton DOM could act as new niches, based on different chemical composition and higher concentrations, sustaining growth of bacterial groups that were less successful in the culture provided with seawater only. The higher number of OTUs in response to an increase in bioavailable DOM could be due to a direct link between the identity and diversity of the substrates present in the added DOM and functional properties of the selected bacterial groups.

Independent of treatment, *Gammaproteobacteria* represented the majority of bacteria selected by our experimental system. This result is not surprising because *Gammaproteobacteria*, such as *Vibrio* and *Alteromonas*, are frequently observed in high abundances in incubation experiments (Eilers et al. 2000, Pinhassi & Berman 2003, Nelson & Carlson 2012). These previous studies and the present one clearly point to their opportunistic life style, characterized by rapid growth and their ability to quickly take up a range of substrates (Lauro et al. 2009). *Gammaproteobacteria* highly expressed transporter genes linked to broad carbon uptake patterns during an *in situ* phytoplankton bloom (Teeling et al. 2012), and members of this phylum had some of the most diverse transcriptomes for a variety of metabolic processes in a coastal bacterial community (Gifford et al. 2012). The high abundance of *Gammaproteobacteria* in our continuous cultures and their low contribution to the +DOM-specific community indicate that this group responded to the culture conditions rather than to the DOM input. This likely reflects their capability to rapidly adapt to a variety of compounds in the original seawater DOM and also in the phytoplankton DOM.

Rhodobacterales, *Rhodospirillales*, and *Rickettsiales* belonging to *Alphaproteobacteria*, *Flavobacteria* belonging to *Bacteroidetes*, and *Verrucomicrobia* responded most to the DOM addition. These bacterial groups have been shown to respond to *in situ* phytoplankton blooms and blooms simulated in mesocosm studies (Pinhassi et al. 2004, Rink et al. 2007, West et al. 2008). Communities specific to a given diatom culture were mainly composed of *Alphaproteobacteria* and *Bacteroidetes* and to a lesser extent of *Gamma*- and *Betaproteobacteria* (Schäfer et al. 2002, Grossart et al. 2005). The *Roseobacter* clade contributed substantially to the overall carbohydrate uptake during a mesocosm experiment with phytoplankton DOM, as evidenced by highly expressed DOC-related transporter genes (Poretsky et al. 2010). *Bacteroidetes* and in particular *Flavobacteria* were found to produce specific enzymes, such as fucosidases and sulfatases, involved in the degradation of polysaccharides produced during an *in situ* diatom bloom (Teeling et al. 2012). Using stable isotope probing, Nelson & Carlson (2012) observed that OTUs belonging to *Flavobacteria* and *Verrucomicrobia* substantially contributed to the incorporation of a *Synechococcus* exudate. These latter studies suggest a function-related response of bacterial groups to phytoplankton DOM.

The question of how specific the utilization of DOM is to a given bacterial group has been addressed in several previous studies (Malmstrom et al. 2005, Alonso-Sáez & Gasol 2007, Mou et al. 2008, Gómez-Consarnau et al. 2012, Sarmento & Gasol 2012). These studies demonstrated that the potential to utilize a variety of simple, monomeric substrates is widespread among bacterial groups but that affinities for a given substrate might vary (Alonso-Sáez & Gasol 2007, Mou et al. 2008, Gómez-Consarnau et al. 2012). Mixed substrate sources of high biological availability, such as DOM from phytoplankton exudates (Nelson & Carlson 2012, Sarmento & Gasol 2012) or extracellular polymeric substances (Elifantz et al. 2005), are utilized by a diverse bacterial community, perhaps reflecting substrate complexity. By investigating how a natural DOM source selects from a bacterial community in a constantly C-limited system over several generation times, our experiment complements these previous studies. Our results provide a first mechanistic insight into this issue, by investigating the potential adaption of a bacterial community at both the community and the OTU level to a natural DOM source.

Bacterial diversity in aquatic systems is maintained by several bottom-up and top-down factors. Resource competition is common in the oligotrophic ocean, and access to bioavailable organic carbon often limits bacterial growth (Kirchman & Rich 1997, Church et al. 2000, Van Wambeke et al. 2002). We wanted to test the idea of a DOM-stimulated modification of bacterial diversity using an experimental approach that focuses on DOM–bacteria interaction, while maintaining most other potential driving factors constant. The increase in diversity we observed in our experiment might be counteracted in the environment by factors such as top-down control, inorganic nutrient limitation, or a combination of these or other factors. Whether the results from our experiment performed with a bacterial community from the NW Mediterranean Sea are valid for other marine environments and the extent to which different sources of DOM contribute to the maintenance of bacterial diversity should be the focus of future research.

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Changes in bacterial diversity in response to dissolved organic matter supply in a continuous culture experiment

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Supplement. The supplement contains 5 tables each providing a detailed description of the taxonomy of the sequences obtained for the initial community (Table S1) and the continuous cultures (Tables S2 to S5)

Table S1. Description of the initial bacterial community, prior to incubation in the continuous cultures. Percentages of the total sequences belonging to each considered phylogenetic group are given. Phylogenetic affiliation was done using the RDP classifier against the Greengenes database. 'Other' refers to pooled assemblages of groups with low sequence numbers or that were not assigned

Phylum	Class	Order	Family	% of total sequences
Proteobacteria			Ā	77.87
	Alphaproteobacteria		Ā	58.37
		Rhodospirillales	*Rhodospirillaceae*	6.00
		Rickettsiales		48.51
			Pelagibacteraceae	32.73
			Other	15.78
		Other		3.87
	Betaproteobacteria		Ā	0.73
	Deltaproteobacteria		Ā	3.75
		Sva0853		3.48
		Other		0.27
	Gammaproteobacteria		Ā	14.43
		Alteromonadales	Ā	0.88
			Alteromonadaceae	0.41
			Other	0.47
		Chromatiales		1.46
		HTCC2188		2.02
		Oceanospirillales		8.58
			Alcanivoracaceae	0.15
			Halomonadaceae	8.08
			Thiohalorhabdaceae	0.26
			Other	0.49
		Other		1.5
	Other			0.59
Bacteroidetes				0.56
	Flavobacteria			0.47
		Flavobacteriales		0.38
			Flavobacteriaceae	0.29
			Other	0.09
	Other			0.09
	Sphingobacteria	*Sphingobacteriales*		0.09
Actinobacteria				1.90
	Acidimicrobiia	*Acidimicrobiales*		1.70
			OCS155	1.32
			ZA3409c	0.26
			Other	0.12
	Actinobacteria	*Actinomycetales*		0.20
Cyanobacteria	*Synechococcophycideae*	*Synechococcales*	*Synechococcaceae*	13.26
SAR406	AB16			3.28
		Arctic96B-7	A714017	3.10
		Other		0.18
Verrucomicrobia				0.69
	Pedosphaerae	Arctic97B		0.15
	Opitutae			0.18
		Pelagicoccales	*Pelagicoccaceae*	0.03
		Puniceicoccales	*Puniceicoccaceae*	0.15
	Verrucomicrobiae	*Verrucomicrobiales*	*Verrucomicrobiaceae*	0.36
			Other	0.18
Other				2.46

Table S2. Percentage of sequences belonging to phylogenetic groups within *Gammaproteobacteria* after 3 and 5 generations in the control treatment and the replicate +DOM treatments. Phylogenetic affiliation was done using the RDP classifier against the Greengenes database. 'Other' refers to pooled assemblages of groups with low sequence numbers or that were not assigned

			3 generations			5 generations		
			Control	+DOM1	+DOM2	Control	+DOM1	+DOM2
Order	Family	Genus	% of total sequences					
	Sum of *Gammaproteobacteria*		98.54	92.68	91.01	94.82	89.17	86.50
Alteromonadales			21.55	42.33	62.79	53.16	76.99	80.30
	Alteromonadaceae		20.08	37.12	55.62	52.87	75.64	78.86
		Alteromonas	13.93	10.30	16.86	0.06	0.94	1.35
		BD2-13	0	0.03	0.15	0	0	0
		Glaciecola	0.12	0.88	1.46	0.06	0.67	1.90
		HTCC2207	0	0	0	0	0.03	0.09
		Marinobacter	0.20	0	0	0.03	0.03	0.03
		Umboniibacter	0	0.20	0.15	0	0	0.09
		ZD0117	0.06	0.06	0.23	0.03	0	0.26
		Other	5.77	25.65	36.77	52.7	73.97	75.15
	Colwelliaceae		0	0.09	0.09	0	0	0
		Colwellia	0	0	0.03	0	0	0
		Other	0	0.09	0.06	0	0	0
	J115		1.26	0.88	1.26	0.12	0.26	0.23
	OM60		0	0	0	0	0.15	0.06
	Pseudoalteromonadaceae		0.18	3.98	5.21	0.03	0.91	0.97
		Pseudoalteromonas	0.18	3.95	5.18	0.03	0.91	0.97
		Other	0	0.03	0.03	0	0	0
	Shewanellaceae		0	0.09	0.03	0	0	0
	Other		0.03	0.18	0.59	0.15	0.03	0.18
Chromatiales			0	0	0	0.12	0.06	0.03
Enterobacteriales	*Enterobacteriaceae*		0	0	0	0	0	0.06
HTCC2188			0.41	1.76	1.23	1.32	0.38	1.46
	HTCC2089		0	0.09	0.26	0.12	0	0.70
	HTCC2188		0.41	1.67	0.97	1.20	0.35	0.76
	Other		0	0	0	0	0.03	0
Oceanospirillales			72.04	41.86	7.64	36.50	9.48	1.08
	Alcanivoracaceae		52.37	27.87	4.54	0.64	0.64	0.32
		Alcanivorax	52.37	27.87	4.54	0.59	0.64	0.32
		Other	0	0	0	0.06	0	0
	OM182		0	0.03	0	0	0.12	0.06
	Oceanospirillaceae		16.69	9.81	1.43	33.23	8.55	0.53
		Marinomonas	0	0.03	0.03	0	0	0
		Oleibacter	1.61	6.18	0.29	0	0	0
		Oleispira	2.37	0	0	0	0	0
		Neptuniibacter	9.31	1.80	0.79	32.69	0	0.32
		Other	3.39	1.80	0.32	0.54	8.55	0.21
	Oleiphilaceae		0.18	0.09	0.06	0	0	0
	Other		2.81	4.07	1.61	2.63	0.18	0.18
Thiotrichales	*Piscirickettsiaceae*		3.28	5.83	14.14	2.96	1.96	3.37
		Cycloclasticus	0	0	0	0.09	0	0.09
		Methylophaga	3.28	5.83	14.02	2.87	1.96	3.16
		Other	0	0	0.12	0	0	0.12
Vibrionales	*Vibrionaceae*		0	0.41	4.77	0	0	0
		Vibriofortis	0	0.03	0	0	0	0
		Other	0	0.38	4.77	0	0	0
Xanthomonadales	*Sinobacteraceae*		0	0	0	0.09	0	0
Other			1.26	0.50	0.44	0.67	0.29	0.20

Table S3. Percentage of sequences belonging to phylogenetic groups within *Alphaproteobacteria* after 3 and 5 generations in the control treatment and the replicate +DOM treatments. Phylogenetic affiliation was done using the RDP classifier against the Greengenes database. 'Other' refers to pooled assemblages of groups with low sequence numbers or that were not assigned

Order	Family	Genus	3 generations			5 generations		
			Control	+DOM1	+DOM2	Control	+DOM1	+DOM2
			% of total sequences					
	Sum of *Alphaproteobacteria*		1.23	3.10	5.24	3.19	5.50	8.05
Kiloniellales			0	0	0	0.03	0	0.64
Rhizobiales			0	0.23	0.47	0.03	0.41	0.44
	Hyphomicrobiaceae		0	0.03	0	0.03	0.09	0.09
	Other		0	0.20	0.47	0	0.32	0.35
Rhodobacterales			1.08	2.25	3.86	2.75	2.08	5.09
	Hyphomonadaceae		0	0.03	0.03	0.23	0	0.12
		Hyphomonas	0	0	0.03	0.06	0	0
		Maricaulis	0	0	0	0	0	0.09
		Oceanicaulis	0	0	0	0	0	0.03
		Other	0	0.03	0	0.18	0	0
	Rhodobacteraceae		1.08	2.22	3.83	2.52	2.08	4.98
		Anaerospora	0.44	0.53	0.91	1.64	0.41	2.17
		Loktanella	0	0.03	0.03	0	0	0
		Octadecabacter	0	0	0.03	0	0	0
		Phaeobacter	0.47	0.70	0.85	0.41	0.97	1.58
		Thalassobius	0	0	0	0	0	0.03
		Other	0.17	0.97	2.02	0.47	0.70	1.20
Rhodospirillales	*Rhodospirillaceae*		0	0	0.06	0	1.93	1.05
Rickettsiales			0.15	0.56	0.64	0.38	0.32	0.56
	AEGEAN 112		0	0	0.03	0	0	0
	Pelagibacteraceae		0.15	0.26	0.47	0.15	0.15	0.41
	Other		0	0.29	0.15	0.23	0.18	0.15
Sphingo-monadales			0	0	0.12	0	0	0
	Sphingomonadaceae		0	0	0.06	0	0	0
		Sphingopyxis	0	0	0.06	0	0	0
		Other	0	0	0.06	0	0	0
	Other		0	0	0.06	0	0	0
Other			0	0.06	0.09	0	0.76	0.26

Table S4. Percentage of sequences belonging to phylogenetic groups within *Bacteroidetes* after 3 and 5 generations in the control treatment and the replicate +DOM treatments. Phylogenetic affiliation was done using the RDP classifier against the Greengenes database. 'Other' refers to pooled assemblages of groups with low sequence numbers or that were not assigned

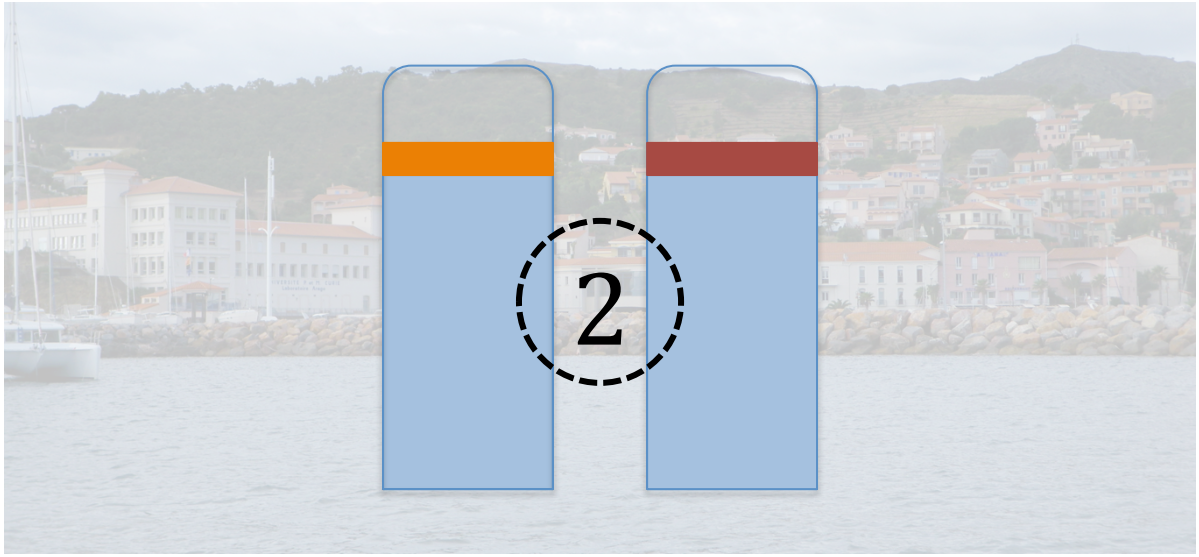
Class	Order	Family	Genus	3 generations			5 generations		
				Control	+DOM1	+DOM2	Control	+DOM1	+DOM2
				% of total sequences					
Sum of *Bacteroidetes*				0.20	3.25	2.28	0.38	1.52	1.05
Bacteroidia	*Bacteroidales*	*Prevotellaceae*	*Prevotella*	0	0	0	0	0.03	0
Flavobacteria				0.20	3.22	2.20	0.38	0.76	0.76
	Flavo-bacteriales			0.20	2.96	2.05	0.35	0.44	0.64
		Cryomorphaceae		0	0.35	0.06	0	0	0
		Flavo-bacteriaceae		0.20	2.58	1.87	0.29	0.44	0.59
			Leeuwenhoekiella	0	0	0.18	0	0	0
			Polaribacter	0	0.06	0	0	0	0
			Sediminicola	0	0.06	0.32	0	0	0.35
			Tenacibaculum	0	0.15	0.06	0	0	0
			Other	0.20	2.31	1.32	0.29	0.44	0.23
		Other		0	0.03	0.12	0.06	0	0.06
	Other			0	0.26	0.15	0.03	0.32	0.12
Sphingo-bacteria	*Sphingo-bacteriales*			0	0.03	0.09	0	0.70	0.26
		Balneolaceae		0	0	0.09	0	0.15	0.06
		Ekhidnaceae		0	0	0	0	0	0.15
		Saprospiraceae		0	0.03	0	0	0.56	0
			Aureispira	0	0.03	0	0	0.53	0
			Other	0	0	0	0	0.03	0
		Other		0	0	0	0	0	0.06
Other				0	0	0	0	0.03	0.03

Table S5. Percentage of sequences belonging to phylogenetic groups within *Betaproteobacteria*, *Deltaproteobacteria*, *Epsilonproteobacteria*, *Verrucomicrobia* and *Cyanobacteria* after 3 and 5 generations in the control treatment and the replicate +DOM treatments. Phylogenetic affiliation was done using the RDP classifier against the Greengenes database. 'Other' refers to pooled assemblages of groups with low sequence numbers or that were not assigned, except for the sequences not assigned at the phylum level

Class	Order	Family	Genus	3 generations			5 generations		
				Control	+DOM1	+DOM2	Control	+DOM1	+DOM2
				% of total sequences					
Other *Proteobacteria*				0	0.21	0.09	1.47	3.46	3.71
Betaproteobacteria	*Methylo philales*	*Methylophilaceae*		0	0	0	0	0.12	0
			Methylotenera	0	0	0	0	0.03	0
			Other	0	0	0	0	0.09	0
Deltaproteobacteria				0	0.12	0	1.35	3.34	3.48
	Bdello-vibrionales	*Bacterio-voracaceae*	*Bacteriovorax*	0	0	0	0	0.03	0.09
	Myxococcales	OM27		0	0.12	0	1.35	3.31	2.61
	Other			0	0	0	0	0	0.79
Epsilon-proteobacteria	*Campylo-bacterales*	*Campylo-bacteraceae*	*Arcobacter*	0	0.03	0	0	0	0
Other				0	0.06	0.09	0.12	0	0.23
Verrucomicrobia				0	0.50	0.88	0	0.09	0.50
Opitutae				0	0	0	0	0	0.33
	Pelagicoccales	*Pelagicoccaceae*	*Pelagicoccus*	0	0	0	0	0	0.18
	Other			0	0	0	0	0	0.15
Verrucomicrobiae	*Verruco-microbiales*	*Verruco microbiaceae*		0	0.50	0.88	0	0.09	0.32
Cyanobacteria				0	0.09	0.20	0	0.12	0
Synecho-coccophycideae	Synechococcales	Synechococcaceae	*Prochlorococcus*	0	0.09	0.20	0	0.12	0
Other				0.03	0.06	0.26	0.15	0.06	0.06
Not assigned				0	0.12	0.03	0	0.09	0.12

CHAPTER TWO

RESPONSE OF HETEROTROPHIC MARINE BACTERIA TO PHYTOPLANKTON-DERIVED DISSOLVED ORGANIC MATTER OF DIFFERENT ORIGIN AND CHEMICAL COMPOSITION



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Phylogenetic and structural response of heterotrophic bacteria to dissolved organic matter of different chemical composition in a continuous culture study

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Summary

Dissolved organic matter (DOM) and heterotrophic bacteria are highly diverse components of the ocean system, and their interactions are key in regulating the biogeochemical cycles of major elements. How chemical and phylogenetic diversity are linked remains largely unexplored to date. To investigate interactions between bacterial diversity and DOM, we followed the response of natural bacterial communities to two sources of phytoplankton-derived DOM over six bacterial generation times in continuous cultures. Analyses of total hydrolysable neutral sugars and amino acids, and ultrahigh resolution mass spectrometry revealed large differences in the chemical composition of the two DOM sources. According to 454 pyrosequences of 16S ribosomal ribonucleic acid genes, diatom-derived DOM sustained higher levels of bacterial richness, evenness and phylogenetic diversity than cyanobacteria-derived DOM. These distinct community structures were, however, not associated with specific taxa. Grazing pressure affected bacterial community composition without changing the overall pattern of bacterial diversity levels set by DOM. Our results demonstrate that resource composition can shape several facets of bacterial diversity without influencing the phylogenetic composition of bacterial communities, suggesting functional redundancy at different taxonomic levels for the degradation of phytoplankton-derived DOM.

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Introduction

The bacteria-mediated transformation of dissolved organic matter (DOM) is a major flow of carbon and energy in aquatic environments. This process is dependent on the biological availability of DOM, which varies with source and chemical composition (Nagata, 2008). Molecular level analyses have identified amino acids, amino sugars and neutral sugars as molecules that substantially contribute to the bioreactive DOM pool with turnover times of minutes to weeks (Benner, 2002). The question of whether bacterial community composition matters for the degradation of DOM has received much attention over the past years. Substrate preferences and metabolic capacities were revealed for some phylotypes within the diverse bacterial community that processes DOM (Fuhrman and Hagström, 2008; Moran, 2008). Whether functional traits requested for DOM degradation are specific to bacterial phylotypes and how this affects the link between the composition and diversity of DOM and bacterial communities remain largely unresolved.

Phytoplankton is the dominant source of bioreactive DOM in ocean surface waters (Hedges, 1992). Changes in bacterial activity and diversity in response to phytoplankton blooms were reported previously, and these changes were often related to modifications in the DOM supply (Fandino *et al.*, 2001; Arrieta and Herndl, 2002; West *et al.*, 2008; Tada *et al.*, 2011). An idea that emerged from these observations is that shifts in bacterial diversity are a required first step to optimize the degradation of

DOM as its composition changes. These DOM-induced shifts in bacterial diversity imply that bacterial groups with specific metabolic traits are selected for as resources change. Metabolic preferences and substrate affinities have been shown to vary among bacterial groups (Cottrell and Kirchman, 2000; Elifantz *et al*., 2005; Alonso-Sáez and Gasol, 2007). Further, recent experimental and *in situ* transcriptomic studies suggest resource partitioning between different members of the microbial community (McCarren *et al*., 2010; Poretsky *et al*., 2010; Rinta-Kanto *et al*., 2012; Teeling *et al*., 2012).

Bacterial utilization of different DOM sources is, however, not always associated to bacterial diversity patterns. Modifications in DOM supply only slightly affected the structure of bacterial communities in some studies (Kirchman *et al*., 2004; Rooney-Varga *et al*., 2005; Rink *et al*., 2007), indicating that broad bacterial groups are capable of regulating their activities to use a range of substrates, with no or little changes in community structure. The lack of a change implies that these bacterial groups behave as generalists with functional redundancy among the bacterial taxa making up the group and are capable of degrading many components in the DOM pool (Fernández *et al*., 1999; Langenheder *et al*., 2005; Mou *et al*., 2008). Given the variability of the responses observed in these previous studies, the question on the extent to which DOM can act as a driver of bacterial diversity is still open.

In the present study, we addressed the question of whether chemically distinct DOM pools select for bacterial communities that differ in their structure and phylogenetic composition. Using a continuous culture approach, we supplied the different DOM sources at equal dissolved organic carbon (DOC) concentrations over several generation times and simultaneously examined chemical characteristics of the DOM and the taxonomic composition of the bacterial community. These data allowed us to explore whether resource composition is a shaping force on the structure and phylogeny of the bacterial communities selected in the cultures. Our results indicate no phylogenetic response of bacterial groups to either DOM source, but the communities selected by each DOM source presented distinct structural patterns in richness, evenness and phylogenetic diversity.

Results

Concentration of DOC and inorganic nutrients in the continuous cultures

The continuous cultures were supplied with two input media that had almost identical bulk DOC concentrations (75 ± 2 μM for the diatom DOM and 73 ± 3 μM for the cyanobacterial DOM, Table S1).To establish C-limitation, inorganic nutrients were added in excess to each input medium (192 μM for $NO_3^- + NO_2^-$, 20 μM for $NH_4^+$and 24 μM for PO_4^{3-}). Concentrations of $NO_3^- + NO_2^-$ and PO_4^{3-} as well as DOC did not differ between the input media and the cultures, and they remained stable during the 15 days of the experiment (Table S1). Ratios of DOC: ($NO_3^- + NO_2^-$) and DOC: PO_4^{3-} in the cultures were 0.4 and 3, respectively, throughout the experiment, well below the C:N:P ratios of heterotrophic bacteria (45:9:1) (Goldman *et al*., 1987), indicating bacteria were limited by carbon availability rather than inorganic nutrients in these incubations.

Chemical characterization of the two DOM sources

To obtain insights into the chemical composition of the two DOM sources, we analysed total hydrolysable neutral sugars (THNS) and amino acids (THAA). Concentrations and C-normalized yields of both biochemical groups were higher in the diatom DOM than in the cyanobacterial DOM (Table 1). THNS and THAA made up 13% and 18%, respectively, of the DOC pool in the diatom DOM, and 5.6% and 3.5%, respectively, in the cyanobacterial DOM. This pattern was maintained in the input media after mixing each DOM source with aged seawater (Table 1). Relative concentrations (mol%) of individual neutral sugars and amino acids varied greatly between the two media (Fig. 1A and B). In the diatom DOM medium, the relative concentrations of individual sugars were similar, ranging between 10 and 20 mol% (Fig. 1A). By contrast, fucose dominated the THNS pool (56 mol%) in the cyanobacterial DOM medium, whereas the relative concentration of the remaining six sugars was much smaller (5–15 mol%). The two DOM sources also differed in amino acid compositions (Fig. 1B). Sixteen amino acids could be detected in our samples. Glycine was the most abundant amino acid in the two media, but its relative concentration was much higher in the cyanobacterial DOM medium (27 mol%) than in the diatom DOM medium (16%). The cyanobacterial DOM medium was also more

Table 1. Chemical characteristics of the DOM sources, obtained by filtration of a diatom and a cyanobacterial culture, and the input media, obtained after mixing each DOM source with aged seawater. Concentrations of total hydrolysable neutral sugar-C and total hydrolysable amino acid-C and the respective C-yields are given.

	Neutral sugars		Amino acids	
	(μM C)	(% DOC)	(μM C)	(% DOC)
	DOM source			
Diatom	17	13	25	18
Cyanobacteria	4.4	5.6	2.7	3.5
	Input medium			
Diatom DOM	2.7	3.6	4.2	5.6
Cyanobacteria DOM	1.6	2.1	1.7	2.3

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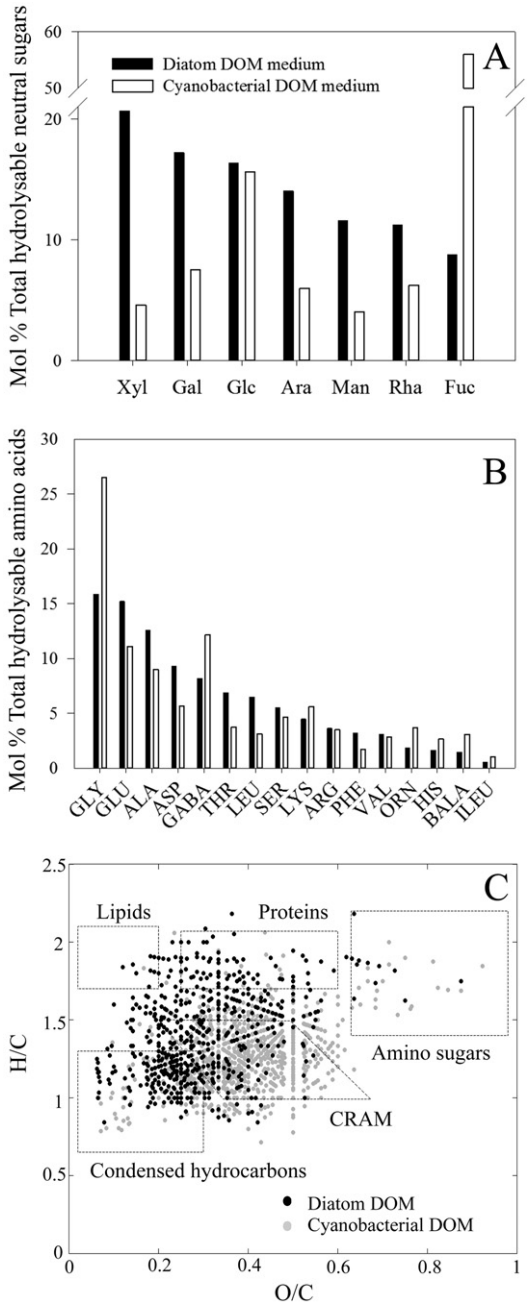

Fig. 1. Chemical characteristics of the input media and the two DOM sources. Composition (mol%) of total hydrolysable neutral sugars (A) and amino acids (B) in the diatom DOM medium and the cyanobacterial DOM medium. van Krevelen diagram showing ratios of H/C and O/C for compounds specific of diatom DOM and cyanobacterial DOM in the PPL-extracted fraction (C). Areas tentatively assigned as carboxylic-rich alicyclic molecules (CRAM), proteins, lipids, condensed hydrocarbons and amino sugars are also shown. Abbreviations for neutral sugars as follows: fucose (Fuc), rhamnose (Rha), arabinose (Ara), galactose (Gal), glucose (Glc), mannose (Man) and xylose (Xyl). Abbreviations for amino acids as follows: glutamic acid (GLU), glycine (GLY), leucine (LEU), alanine (ALA), aspartic acid (ASP), γ-aminobutyric acid (GABA), phenylalanine (PHE), lysine (LYS), arginine (ARG), threonine (THR), serine (SER), valine (VAL), histidine (HIS), ornithine (ORN), β-alanine (BALA) and isoleucine (ILEU).

enriched in γ-aminobutyric acid and β-alanine, the two non-protein amino acids analysed. In contrast, glutamic acid, alanine, aspartic acid, threonine and leucine were more abundant in the diatom DOM medium. A degradation index (DI), calculated based on the amino acid composition of the DOM sources (Dauwe *et al.*, 1999; Davis *et al.*, 2009), was higher in the diatom DOM (2.80) than in the cyanobacterial DOM (-0.02), indicating a more advanced degradation state in the latter. Our results indicate a higher evenness of amino acids and neutral sugars following hydrolysis in the diatom DOM, suggesting a higher diversity of the polymers in this DOM source.

In addition, we determined molecular formulae of compounds in the extracted fraction of each DOM source, accounting for 8% of the total DOM, by ultrahigh resolution mass spectrometry [Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR MS)]. A total of 580 compounds were found exclusively in the diatom DOM, 968 compounds were found only in the cyanobacterial DOM and 1032 molecular formulae were common in both samples. As for THNS and THAA, the FT-ICR MS results indicate differences in the composition of the two DOM sources (Fig. 1C). The diatom DOM was characterized by higher H/C and lower O/C ratios than the cyanobacterial DOM, resulting in the latter in a shift in the van Krevelen diagram towards an area characteristically occupied by carboxyl-rich alicyclic compounds. Compounds found exclusively in the diatom DOM had an overall higher molecular weight (461 versus 438 Da in cyanobacterial DOM) and were richer in sulphur and phosphorus, but diatom DOM had fewer nitrogen compounds than cyanobacterial DOM (Fig. S2). The average distance between each molecular formula in van Krevelen space was computed for both DOM sources (Kim *et al.*, 2003). For cyanobacterial DOM, the average distance was 0.2184 ± 0.0001, whereas the average distance for diatom DOM was 0.2512 ± 0.0001. Therefore, cyanobacterial DOM displayed a higher number of compounds, yet compounds in diatom DOM were ~ 15% more chemically different to each other than the compounds in cyanobacterial DOM.

To test the bioavailability of the two DOM sources prior to the continuous culture experiment, we determined the overall growth rate of a natural bacterial community growing on the diatom and cyanobacterial DOM media in batch mode. Growth rates were only slightly higher in the diatom medium (0.70 ± 0.07 d^{-1}) than in the cyanobacterial medium (0.56 ± 0.06 d^{-1}) but were lower in aged seawater alone (0.31 ± 0.04 d^{-1}).

Abundance of heterotrophic bacteria and nanoflagellates

In all continuous cultures, bacterial abundance increased between day 0 and day 4 and then stabilized at 5.1 ± 0.6

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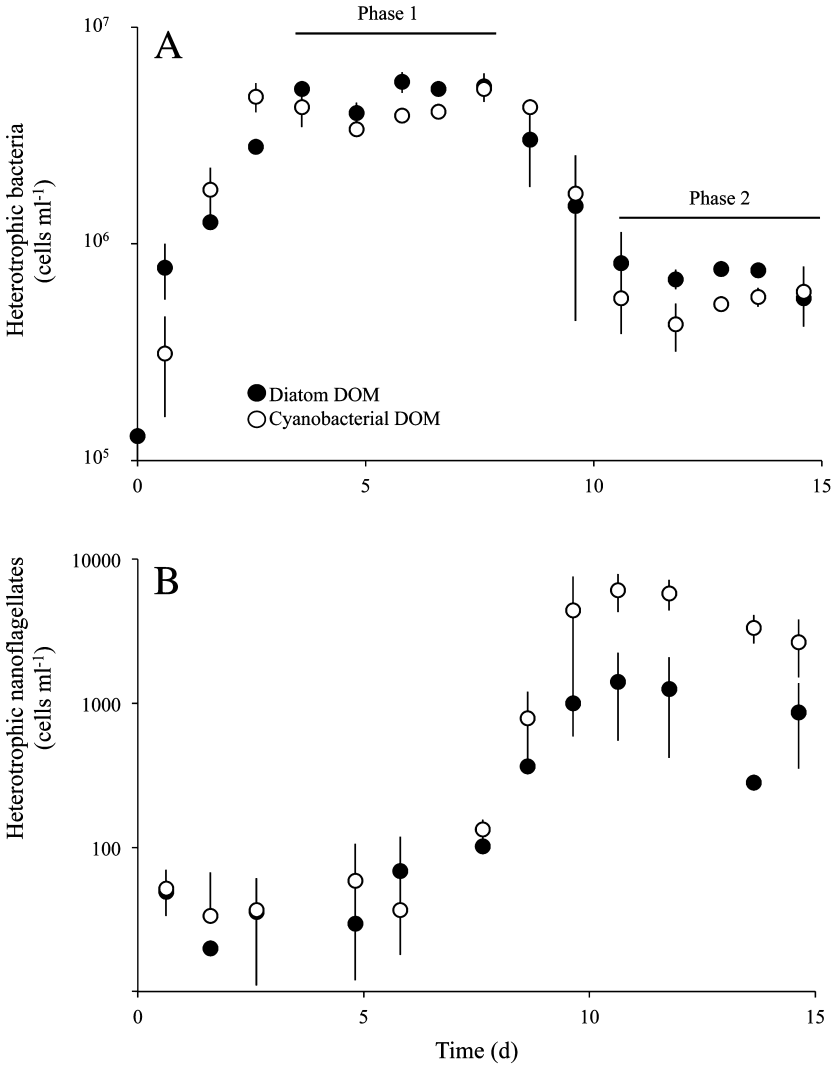

Fig. 2. Abundances of heterotrophic bacteria (A) and heterotrophic nanoflagellates (B) in the continuous cultures supplied with diatom DOM medium and cyanobacterial DOM medium. Mean values ± SD of biological replicates are shown for each time point. Samples for bacterial diversity analyses were taken after phase 1 (three generation times) and after phase 2 (six generation times).

and 4.2 ± 0.7 x 10^6 cells ml^{-1} [mean ± standard deviation (SD), $n = 5$] in the diatom DOM and the cyanobacterial DOM cultures, respectively, between day 4 and day 8 (phase 1, Fig. 2A). Bacterial abundances decreased in all cultures between day 8 and day 11 and then stabilized at 7.5 ± 0.5 × 10^5 cells ml^{-1} in the diatom DOM cultures and at 5.2 ± 0.7 x 10^5 cells ml^{-1} in the cyanobacterial DOM cultures from day 11–15, defined as phase 2. No significant differences in bacterial abundance were detectable between the two treatments during phase 1 or phase 2 (Wilcoxon test, $P > 0.05$) (Fig. 2A).

Decreases in bacterial abundance at day 8 were paralleled by increases in heterotrophic nanoflagellates (HNF) abundance (Fig. 2B). HNF abundance was less than 100 cells ml^{-1} in all cultures during phase 1 and started to increase at day 5, reaching up to 1000 cells ml^{-1} in the diatom DOM cultures and 5000 cells ml^{-1} in the cyanobacterial DOM cultures at day 10. During phase 2 of the experiment, HNF abundance remained stable (Fig. 2B).

Bacterial diversity

We explored bacterial diversity and composition at the end of phase 1 (after three bacterial generation times) and at the end of phase 2 (after six bacterial generation times) by tag pyrosequencing the 16S ribosomal ribonucleic acid (rRNA) gene and clustering the sequences into operational taxonomic units (OTUs) at > 97% identity, leading to a total of 697 OTUs. Statistical analyses performed on a Bray Curtis distance matrix indicated that clustering of the replicates was significant at each time point and treatment [analysis of similarity (ANOSIM), $P = 0.01$]. This tight clustering of the 16S rRNA genes in the replicates indicates overall high replication of the two cultures growing on the same DOM source, and it further suggests that differences

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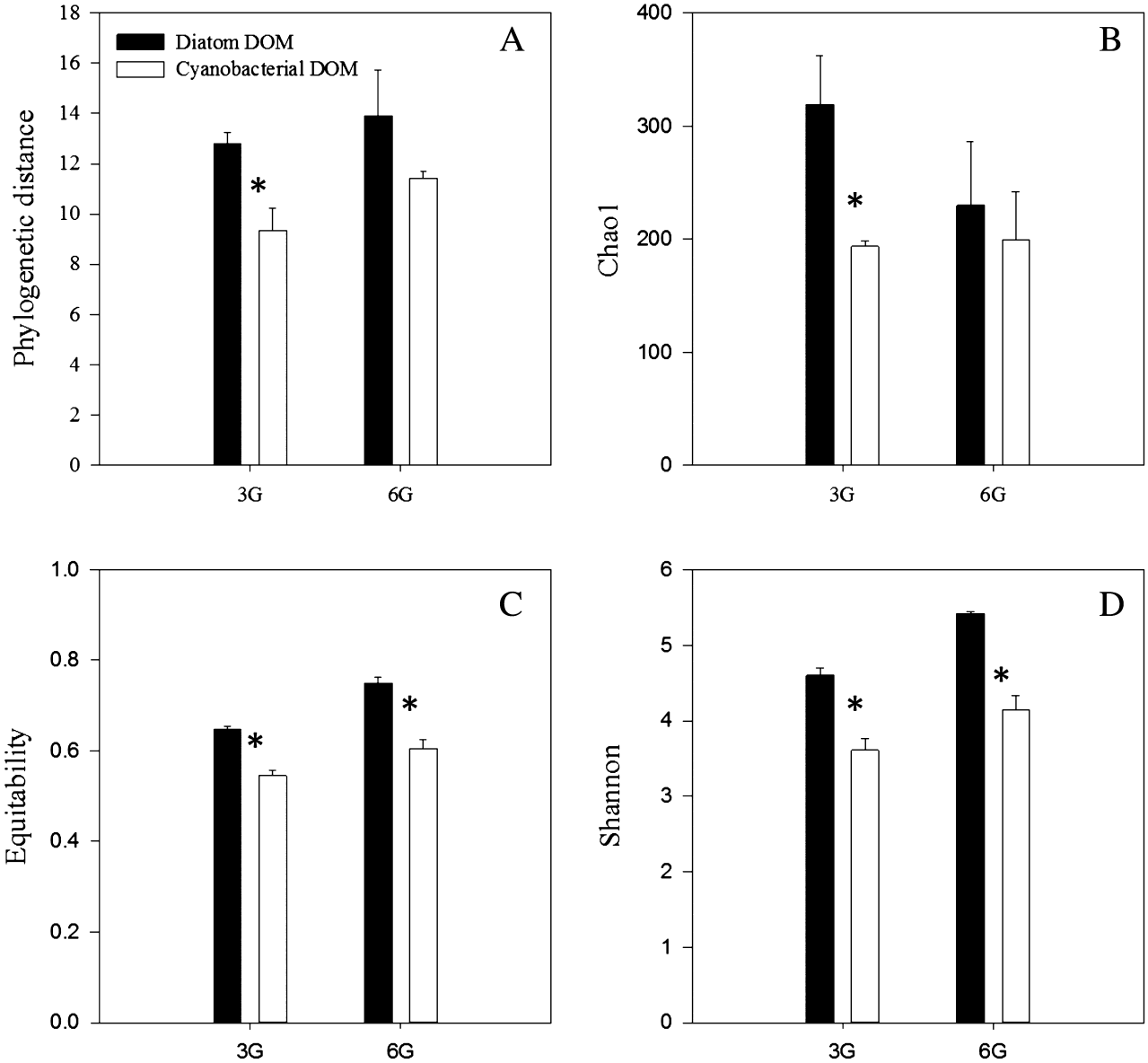

Fig. 3. Diversity indices of bacterial communities in the diatom DOM and the cyanobacterial DOM cultures after three (3G) and six generation times (6G). Mean and SD of biological replicates are given. Asterisk indicates significant differences between treatments ($P < 0.05$).

in the bacterial communities were due to the treatment at each time point. We calculated the phylogenetic distance, Chao1 (a measure of richness), evenness and the Shannon index (which includes both richness and evenness) of the communities in the two treatments (Fig. 3).

All diversity indices were significantly higher for bacterial communities in the diatom DOM cultures than in the cyanobacterial DOM cultures after three generations (Student's *t*-test, $P < 0.05$). After six generations, the indices remained higher in the diatom DOM cultures, and the differences were significant for the evenness and Shannon indices (Student's *t*-test, $P < 0.05$). Consistently with these results, the number of major OTUs (i.e. OTUs accounting for > 1% of the total number of sequences) was higher in the diatom DOM cultures than in the cyanobacterial DOM cultures at both time points (Table 2). It is also interesting to note that three out of the four diversity indices increased in all cultures between the third and sixth generation time (Fig. 3A, C and D), and only the Chao1 decreased in the diatom DOM cultures and remained stable in the cyanobacterial DOM cultures (Fig. 3B).

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Table 2. Number of abundant OTUs (> 1% of total number of sequences) in the replicate cultures (R1, R2), in both replicate cultures of one DOM source (shared – DOM source) and in all cultures (shared – all cultures) after three and six generation times.

	Diatom DOM		Cyanobacterial DOM		Diatom DOM		Cyanobacterial DOM	
	Three generation times				Six generation times			
	R1	R2	R1	R2	R1	R2	R1	R2
Number of OTUs > 1%	19	20	15	14	25	26	12	14
Shared – DOM source	14		6		19		10	
Shared – all cultures	5				9			

Bacterial community composition

The phylogenetic diversity in each bacterial community was further investigated by comparing the relative abundance of major phylogenetic groups (Fig 4 and Tables S3–S5). The communities selected in our cultures were all dominated by *Gammaproteobacteria* (between 47% and 88%) and *Alphaproteobacteria* (between 9% and 37%) and differed substantially from the bacterial community in the inoculum (Table S2). There was a change in the phylogenetic composition of the cultures between phase 1 and phase 2 due to a substantial increase in relative abundances of *Betaproteobacteria*, *Bacteroidetes*, and minor groups (Fig. 4). The proportion of unassigned sequences, which probably belong to poorly characterized phylogenetic groups, was overall very low (< 1%) but increased at the end of the experiment in all cultures (Table S5). The detailed phylogenetic composition of the communities is presented in Tables S3–S5.

Overall, the majority of the OTUs dominating in one culture were also present in at least one of the three other cultures but with different relative contributions (Fig. S3A and B). No pattern was found between the relative abundance of any major OTU and the respective treatment. A phylogenetic test (Martin, 2002) revealed that the communities selected in the four cultures at each time point were different, including those growing on the same type of DOM ($P < 0.05$). Some phylogenetic groups were observed only in one treatment, such as *Glaciecola* and *Thalassomonas* that appear specific to the diatom DOM cultures at the two time points, but with small contributions to the total community (≤ 1% of total number of sequences; Tables S3–S5).

To understand the observed differences in the phylogenetic diversity (Fig. 3A), we calculated the diversity indices within *Gammaproteobacteria*, which accounted for > 47% of total sequences in all cultures at the two time points. We observed that all diversity indices determined for *Gammaproteobacteria* were higher in the diatom DOM cultures than in the cyanobacterial DOM cultures (Fig. S4). Similar analyses could not be applied to other major groups such as the *Alphaproteobacteria* because of their low relative abundance in at least one of the samples.

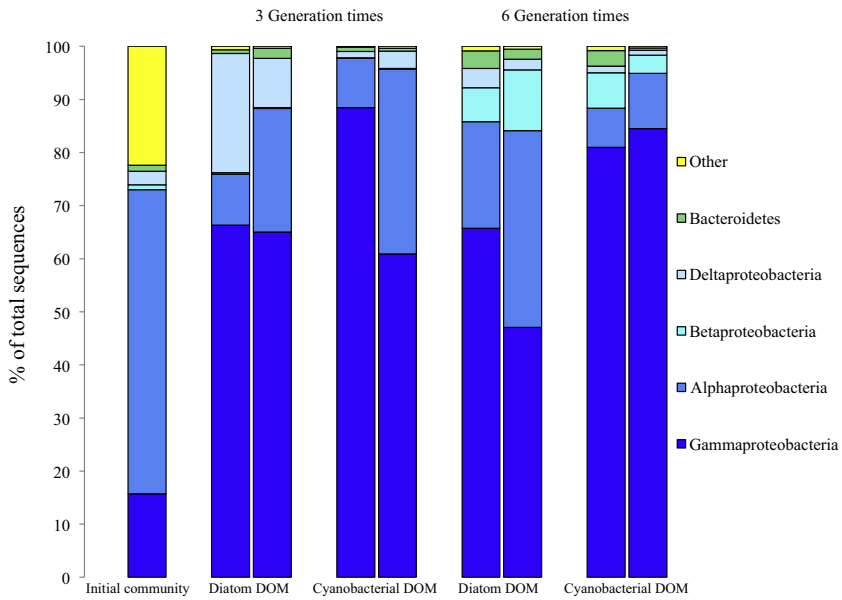

Fig. 4. Contribution of broad phylogenetic groups (% sequences of total number of sequences) to bacterial communities in the initial community, the diatom DOM and the cyanobacterial DOM cultures after three and six generation times. For each treatment, the two replicate cultures are presented.

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Discussion

We designed here an experiment that allowed us to focus on DOM as shaping factor of several facets of bacterial diversity. Our study demonstrates that phytoplankton-derived DOM with different chemical composition does not affect the phylogenetic composition of bacterial communities but can sustain different levels of bacterial diversity. These results suggest that the two distinct DOM pools did not select for functionally specialized OTUs, but the types of substrates present in each of the DOM sources could have provided a different number of niches thereby resulting in a structuring effect on bacterial diversity. These results suggest functional redundancy for the degradation of phytoplankton-derived DOM.

Functional redundancy implies that the metabolic capabilities required to access DOM are widespread among bacterial groups. Our observation supports the hypothesis suggested by Sarmento and Gasol (2012) about overall weak interactions between phytoplankton-derived DOM and bacterial community composition. Using a single cell approach to track the utilization of several radiolabeled phytoplankton exudates, these authors demonstrated that most broad bacterial groups were capable of metabolizing a variety of exudates even though the contribution of each group to total uptake differed among treatments (Sarmento and Gasol, 2012). Similarly, addition of labile monomeric compounds favoured the growth of a few phylotypes that were not restricted to any single broad bacterial group (Alonso-Sáez and Gasol, 2007; Gómez-Consarnau *et al*., 2012). These previous studies and the present study lend support to the notion of overall generalist bacterial behaviour in using labile DOM as suggested by Mou and colleagues (2008). Our study further extends this idea to complex pools of substrates that represent a major source of DOM in the surface ocean.

In contrast to these experimental studies, successions of distinct bacterial populations were recently observed during an *in situ* phytoplankton bloom in the coastal North Sea (Teeling *et al*., 2012) and during mesocosm experiments following the degradation of bioavailable DOM (McCarren *et al*. 2010; Dinasquet *et al*., 2013). The taxon-specific expression of enzymes indicated that distinct bacterial communities accounted for the degradation of phytoplankton-derived DOM during different phases of the bloom (Teeling *et al*., 2012). The amount and composition of DOM produced by phytoplankton varies with growth state (Myklestad *et al*., 1989; Biddanda and Benner, 1997; Barofsky *et al*., 2009). Changes in the composition of DOM over the course of a bloom could be more pronounced than differences in the DOM produced by two phytoplankton species in the same growth phase, with varying effects on bacterial diversity. In our experiment, the DOM sources were supplied continuously, maintaining the composition stable over time. Thus, the DOM used in the present study does not represent the entire spectrum of changes in DOM composition and supply that probably occur during a phytoplankton bloom or during a long-term degradation experiment. Further, major inorganic nutrients rapidly decrease with increasing phytoplankton biomass and in batch-type incubations (Teeling *et al*., 2012). In these cases, the bacterial response is not exclusively driven by DOM but also by the competitiveness among phylotypes in the acquisition of inorganic nutrients (Sebastián and Gasol, 2013). In the present study, inorganic nutrients were continuously supplied in excess to assure that organic carbon limited bacterial activity. How co-limitation by inorganic nutrients affects the phylogenetic response remains to be investigated.

As opposed to the absence of taxonomic responses, the diversity of bacterial communities grown on the two DOM sources differed greatly in our experiment. A few previous studies specifically addressed the question of how DOM could affect bacterial diversity, pointing to a possible link between the chemical composition as well as the number and the complexity of organic compounds, and the richness of the responding bacterial community. According to a DNA fingerprinting approach, the number of ribotypes varied for bacterial communities grown on two sources of phytoplankton detritus (van Hannen *et al*., 1999). These authors observed lower richness for bacterial communities grown on cyanobacterial detritus compared with green algal detritus. Another DNA fingerprinting approach indicated differences in ribotype numbers in soil bacterial communities depending on the added chemical class, such as carbohydrates, fatty acids, aromatic molecules or amino acids (Wawrik *et al*., 2005). Using a stable isotope probing approach in combination with 454 pyrosequencing, Nelson and Carlson (2012) observed that a cyanobacterial exudate was incorporated by a higher diversity of bacteria compared with glucose or gluconic acid. The addition of diatom DOM to seawater increased bacterial richness (Landa *et al*., 2013), whereas single-pulse enrichments with monomeric compounds were not associated with changes in bacterial richness (Gómez-Consarnau *et al*., 2012).

How DOM affects richness and other facets of bacterial diversity is most likely driven by the tools required for the cleavage of the chemically different substrates, their uptake and metabolism by bacteria. Compounds that are readily available, such as labile monomers, could have a minor direct influence on bacterial community structure. In this case, competitiveness in terms of substrate affinity and growth rate is likely the driving factor for the success of the most rapidly responding groups, and thus for potential changes in bacterial diversity. By contrast, compounds

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requiring enzymatic machineries at varying levels of complexity could be more selective, favouring bacteria that possess specific metabolic capabilities. This notion is supported by recent findings that the genetic potential for the uptake of simple compounds and the cleavage of small oligosaccharides is phylogenetically widespread, whereas the potential for polymer degradation appears to be restricted to a few taxonomically distinct clades belonging to all phyla (Berlemont and Martiny, 2013).

In the present study, the diversity of bacterial communities was lower when grown on cyanobacterial DOM. The higher relative concentrations of fucose, glycine, non-protein amino acids, and carboxylic acid-like compounds; the lower amino acid and neutral sugar carbon yields and the DI all suggest that the cyanobacterial DOM was more degraded than the diatom DOM (Cowie and Hedges, 1994; Hedges *et al*., 1994; Dauwe *et al*., 1999; Hertkorn *et al*., 2006; Davis *et al*., 2009). These DOM characteristics can be explained by the fact that our cultures were not axenic and by the longer growth phase of the cyanobacterial culture. The cyanobacterial DOM therefore contained most likely a lower amount of simple substrates, therefore providing less substrate niches and sustaining a reduced number and variety of bacteria. The more degraded cyanobacterial DOM also could have contained a larger fraction of molecules that require specific enzymatic tools. This DOM source could therefore be considered as more selective in that it sustained a reduced number of OTUs in our continuous culture where the residence time limits the interaction between a given compound and the bacterial community.

The absence of specific responses from broad phylogenetic groups to DOM source and composition could be the reflection of resource partitioning taking place at a finer phylogenetic level (Johnson *et al*., 2006; Bhaya *et al*., 2007; Hunt *et al*., 2008). Recent studies have demonstrated that the distribution of genes encoding for enzymes to access abundant biopolymers, such as glycoside hydrolases (Berlemont and Martiny, 2013), chitinase and N-acetyl-glucosaminidase (Zimmerman *et al*., 2013), clustered according to distinct populations within phyla of the sequenced genomes investigated. In the present study, the diversity of *Gammaproteobacteria*, the dominant class in our experiment, was higher in the cultures supplied with diatom DOM than with cyanobacterial DOM, even at identical relative abundance in the two treatments. This could be interpreted as resource partitioning among closely related OTUs within broad bacterial groups.

The higher diversity in the diatom DOM-supported bacterial communities than in the cyanobacterial DOM-supported communities was maintained despite the grazing activity after three generation times. Previous studies demonstrated that grazing pressure can strongly affect bacterial community structure (Simek *et al*., 1997), particularly under different resource conditions (Matz and Jürgens, 2003; Alonso-Sáez *et al*., 2009). In the present study, grazing probably explains the decrease in the relative abundance of dominant groups and OTUs to the benefit of others, resulting in an increase in evenness between the third and sixth generation time. Some of the differences between treatments observed after three generation times, such as the significantly higher richness and phylogenetic diversity in the diatom DOM cultures, were smaller after the appearance of grazers. That the Shannon index remained unchanged supports previous findings by Hahn and Höfle (2001) who suggested that top-down control maybe less important in controlling bacterial diversity in systems where bottom-up control is strong, which is the case in our experiment where bacteria were C-limited.

Understanding how the chemical composition of DOM and the diversity of heterotrophic bacteria affect the interplay between these two key components of the marine C cycle is a major challenge. The present study provides insights to this issue by the concurrent detailed chemical analyses of DOM and bacterial diversity under well-defined experimental conditions. Our results suggest the existence of substrate niches associated to bioreactive DOM, thereby affecting levels of bacterial diversity. The exploitation of these niches requires traits ranging from basic to specific functions, which in turn are weakly associated to phylogeny. These mechanisms driving bacterial community structure are likely to occur also in aquatic systems. With our experimental approach, we focus on the fraction of DOM that turns over on the time scales of days and on bacterial groups capable of maintaining relatively high growth rates. We propose that a structuring effect similar to that observed for labile to semi-labile DOM in the present study can be extended to a larger range of DOM compounds that turn over much slower and possibly involve slow-growing members of the bacterial community. The modifications by grazers of the observed treatment-specific bacterial diversity demonstrate that the effect of resource composition on bacterial diversity can potentially be underestimated. This observation emphasizes the need of experimental approaches to understand the many complex mechanisms co-occurring in natural systems.

Experimental procedures

Experimental design

Continuous cultures were chosen to investigate the response of a natural bacterial community to different DOM sources. Two growth media, each consisting of a mixture of aged seawater and DOM originating from a phytoplankton culture, were inoculated with a natural bacterial community (Fig. S1).

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Bacterial growth was maintained in batch-mode until the stationary phase, and the cultures were then switched to continuous culture mode for the entire experimental duration. The cultures were run in two treatments, defined by input media with different DOM sources. Duplicate cultures were performed for each treatment.

Preparation of media for the continuous cultures

Seawater for the input media and for the natural bacterial community was collected in the NW Mediterranean Sea, 5 nautical miles off Banyuls-sur-Mer (42°28′300 N–03°15′500 E, 95 m overall depth). Roughly 300 l were collected in January 2010 at 3 m using an All-PFA Double-Bellows Pump (Asti) (Cole-Parmer, Hills, IL, USA) connected to 50 m polyvinyl chloride tubing. Seawater was 20 μm filtered and stored in one polycarbonate (PC) carboy in the dark at room temperature for 6 weeks to allow degradation of most of the labile DOM. DOC values decreased from 78 μM at the time of sampling to 63 μM after 6 weeks. The 300 l were then sequentially filtered through 0.4 and 0.2 μm filters using a Sartobran cartridge (Sartorius, Goettingen, Germany) (150 cm^2, Sartorious), and the filtrate was stored at 4°C in the dark in acid-washed (10% HCl), Milli-Q (Millipore, Billerica, MA, USA) water-rinsed 20 l PC carboys.

For the preparation of the two initial growth media and the input media, the seawater was mixed with DOM harvested either from a diatom culture (*Phaeodactylum tricornutum*) or a cyanobacterial culture (*Synechococcus sp. 7803*). These strains were obtained from a culture collection at the Observatoire Océanologique de Banyuls. We used the DOM from phytoplankton cultures as bioavailable sources for bacterial growth, but we did not aim to link the bacterial response to the taxonomic identity of the phytoplankton species that provided the DOM. Therefore, no effort was made to maintain the phytoplankton cultures axenic, and the DOM collected should be considered as phytoplankton-derived DOM processed to some extent by bacteria. For clarity, we refer to the two DOM sources as 'diatom DOM' and 'cyanobacterial DOM' throughout the manuscript.

The phytoplankton cultures were grown in low DOC (15 μM) artificial seawater (ASW) amended with nutrients according to the Guillard's F/2 medium. Absorbance measurements at 660 nm were carried out every day to monitor culture development. To obtain DOM from the phytoplankton cultures, each culture was sequentially filtered through a combusted GF/F filter (90 mm diameter, Whatman, Maidstone, UK) and an acid-washed, Milli-Q-rinsed 0.2 μm PC filter (90 mm diameter, Nuclepore, Whatman, Maidstone, UK). The filtrate was recovered in acid-washed, Milli-Q-rinsed 4 l PC carboys and stored at 2°C during the entire experimental period. DOM was collected in the early stationary phase, which was reached after 5 days in the diatom culture and after 3 weeks in the cyanobacterial culture. Subsamples (20 ml) to determine concentrations of THNS and THAA were stored in high density polyethylene bottles at –20°C until analysis (see below). Concentrations of DOC (see below) were 138 and 78 μM in diatom DOM and cyanobacterial DOM respectively. Each of the DOM sources was mixed with seawater to obtain 15 μM phytoplankton-derived DOC in each treatment. Total DOC concentrations were 75 and 73 μM in the input media prepared with diatom DOM and cyanobacterial DOM respectively.

Continuous cultures

We used a natural bacterial community from seawater (10 l) collected at 5 m at the site described above the first day of the experiment (1 March 2010) as inoculum. After an 0.8 μm filtration (PC, Nuclepore), 2 l were used as initial bacterial inoculum, and the remaining 8 l were filtered on a 0.2 μm cartridge (Sterivex, Millipore, Billerica, MA, USA) and stored at –80°C for further analyses. For the initial bacterial cultures, the 0.8 μm-filtered inoculum was diluted 1:10 with seawater amended with either diatom DOM or cyanobacterial DOM. The final volume of all bacterial cultures was 6 l. To assure that organic carbon was the limiting factor for bacterial growth, nitrate, ammonium and phosphate were added to the initial cultures as well as to the input media. We used large volume continuous cultures to avoid any possible perturbation of the steady state conditions in the cultures because of the daily sampling for biological and chemical analyses (roughly 2% of the total volume). The supply of one culture over six generation times requires large volumes of fresh medium limiting our replication to two per treatment.

The cultures were set up in a closed system consisting of four all-glass vessels (15 cm diameter, 50 cm height) connected to the medium with PTFE tubing (Thermo Scientific, Waltham, MA, USA). The system was run as a batch until the stationary phase was reached before starting the inflow of input medium and the outflow of culture performed by a peristaltic pump (Gilson, Middleton, WI, USA). Four litres of medium was prepared every 2 days and kept in acid-washed PC carboys at 2°C before use. The two replicate cultures were supplied by a single PC carboy containing the input medium, prepared in the same proportions of seawater and diatom DOM or cyanobacterial DOM as the initial cultures, with identical final concentrations of DOC and inorganic nutrients. The dilution rate was 0.28 d^{-1}, resulting in a generation time of 2.5 days. We selected this dilution rate based on growth rates of 0.3 d^{-1} determined *in situ* for coastal Mediterranean waters (Obernosterer *et al.*, 2010). The cultures were bubbled with filter-sterilized air (Sartorious) and run in a temperature-controlled room (18°C) in the dark.

The cultures were sampled daily during the 15 day experimental period. Samples were taken with glass syringes (Hamilton, Reno, NV, USA) connected with a luer-lock to PTFE tubes that reached the bottom of the cultures. This system allowed us to sample without opening the culture containers. The glass syringes were extensively rinsed with Milli-Q water prior to sampling. A total of about 100 ml was sampled everyday in each culture, representing less than 2% of the total volume of the cultures.

Inorganic nutrients and DOC

Triplicate 15 ml subsamples for DOC measurements were filtered through two pre-combusted GF/F filters and stored in the dark after acidification with orthophosphoric acid (H_3PO_4, final pH 2) in pre-combusted glass vials with PTFE-lined caps. DOC analyses were performed by high-temperature

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catalytic oxidation on a Shimadzu TOC-V-CSH analyser (Shimadzu Corporation, Kyoto, Japan) (Benner and Strom, 1993). The analytical precision was 1–2% (CV). For inorganic nutrients, 5 ml subsamples were collected in acid-washed polyethylene vials. Concentrations of nitrate (NO_3^-), nitrite (NO_2^-) and phosphate (PO_4^{3-}) were determined immediately after sampling using the automated colorimetric technique on a Skalar autoanalyser (Skalar Analytical B.V., Breda, The Netherlands) following the protocol of Tréguer and Le Corre (1975).

Analyses of THAA and THNS

THAA in filtered samples were quantified, after acid hydrolysis, by high-performance liquid chromatography using pre-column derivatization and fluorescence detection (Mopper and Lindroth, 1982). Aspartic acid and glutamic acid peaks include the contribution of glutamine and asparagine, respectively, that were deaminated during hydrolysis. Among the 18 amino acids quantified, only 16 with concentrations above detection limit are shown: glutamic acid, glycine, leucine, alanine, aspartic acid, γ-aminobutyric acid, phenylalanine, lysine, arginine, threonine, serine, valine, histidine, ornithine, β-alanine and isoleucine. A DI was calculated using amino acid composition according to the method described by Dauwe and colleagues (1999) adapted to DOM (Peter *et al.*, 2012).

Seven neutral sugars: fucose, rhamnose, arabinose, galactose, glucose, mannose and xylose were analysed according to Skoog and Benner (1997) with modifications. Briefly, samples were hydrolysed in 1.2 mol l^{-1} sulphuric acid and neutralized with a self-absorbed ion retardation resin (Kaiser and Benner, 2000). After desalting with a mixture of cation and anion exchange resins, neutral sugars were isocratically separated with 25 mM NaOH on a PA 1 column in a Dionex 500 system (Thermo Scientific) with a pulsed amperometric detector. Detector settings were analogous to Skoog and Benner (1997). The concentrations of THNS and THAA in the input media were calculated using the values measured in each DOM source and in aged seawater.

Solid-phase extraction and ultrahigh resolution mass spectrometry by electrospray ionization (ESI) FT-ICR MS

Ultrahigh resolution mass spectrometry requires DOM samples at high concentration. Therefore, solid-phase extraction of the two DOM sources was performed on PPL cartridges (Varian) (Agilent, Lake Forest, CA, USA) following the protocol by Dittmar and colleagues (2008). The phytoplankton cultures were filtered as described above. The < 0.2 μm filtrates of the diatom (8.53 l) and the cyanobacterial cultures (5.05 l) were acidified to pH 2, and then each passed over one pre-treated PPL cartridge (6 ml HPLC-grade methanol) at a flow rate of 3 ml min^{-1}. The PPL cartridge was then rinsed with 0.01 N HCl (12 ml) and dried with ultrapure N_2. The extracted DOM was recovered by sequential elution with each 10 ml methanol (HPLC grade) into combusted 20 ml glass tubes at a flow rate of < 2 ml min^{-1}. Elutes were dried in a Genevac Personal Evaporator (Genevac, Ipswich, UK) and then stored at –20°C in the dark until further analyses. For blank determination, the same procedure was performed with ASW. The few organic compounds detected by mass spectrometry in the ASW were subtracted from the two phytoplankton exudates. To determine the recover efficiency, 100 μl of the final 1 ml extract were diluted with 10 ml of Milli-Q water, and DOC concentrations were measured with a Shimadzu TOC-Vcph analyser (Shimadzu Corporation) using potassium hydrogen phthalate as standard for DOC calibration curve. For the two DOM sources, the PPL recovery efficiency was 8% of total DOC.

ESI FT-ICR MS analysis of DOM (in 1:1 water: methanol solution) was performed in negative mode with an Apollo electrospray ion (ESI) source of a Bruker Daltonics 12 Tesla Apex Qe FT-ICR mass spectrometer at the College of Sciences Major Instrumentation Cluster Laboratory, Old Dominion University, Virginia. Samples were internally calibrated using naturally present fatty acids (Sleighter *et al.*, 2008). Only *m/z* values with a signal to noise > 4 were considered. A molecular formula calculator (Molecular Formula Calc version 1.0; NHMFL, Tallahassee, FL, USA) generated formula matches using carbon, hydrogen, oxygen, nitrogen, sulphur and phosphorus. By using the soft ESI technique, the DOM compounds were ionized as intact moieties. The masses of the individual molecules in the complex DOM cocktail were determined with such accuracy that we were able to determine the molecular formulae of 1612 compounds in diatom DOM and 2000 in cyanobacterial DOM. In order to obtain a measure of the chemical differences between the molecules in each sample, we computed the average distance in van Krevelen space between each molecular formula and all other molecular formulae identified in the sample as follows:

$$dist = \frac{1}{N^2}\sum_{j=1}^{N}\sum_{i=1}^{N}\sqrt{(OC_j - OC_i)^2 + (HC_j - HC_i)^2}$$

where N is the total number of molecular formulae identified in each sample, OC_j and HC_j are the normalized ratios of oxygen to carbon and hydrogen to carbon for a given molecular formula, and OC_i and HC_i are the normalized ratios for all other molecular formulae identified in the sample. The implicit assumption is that compounds with similar chemical characteristics will occupy similar regions in the van Krevelen diagram (Kim *et al.*, 2003), leading to relatively small average distances, whereas compounds that are more chemically different to each other will be found farther apart in the diagram, leading to larger average distances.

Enumeration of heterotrophic bacteria and heterotrophic nanoflagellates

For heterotrophic bacterial abundance, duplicate subsamples (1.8 ml) were fixed with formaldehyde (2% final concentration), kept at 4°C for 30 min and then stored at –80°C until flow cytometric analysis as described in Lebaron and colleagues (2001). For the enumeration of HNF, samples (3.8 ml) were fixed with 1% glutaraldehyde (final concentration), incubated for 30 min at 4°C and then stored at –80°C until flow cytometric analyses following the protocol described in Christaki and colleagues (2011).

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Bacterial diversity sampling and DNA extraction

After about three bacterial generations (day 7), 200 ml samples were collected from the outflow in autoclaved PC flasks. Bacterial cells were collected on a 0.2 μm PC filter (47 mm diameter, Nuclepore), and the filters were stored at −80°C for bacterial diversity analysis. At the end of the experiment, corresponding to about six bacterial generations (day 15), samples (500–700 ml) were collected in autoclaved PC flasks, concentrated on a 0.2 μm cartridge and stored at −80°C until further analysis.

DNA was extracted as described in Laghdass and colleagues (2011). Briefly, a lysis step was applied to the filters and cartridges with a lysis buffer (40 mM ethylenediaminetetraacetic acid, 50 mM Tris, 0.75 M sucrose) and three freeze-thaw cycles with liquid nitrogen and a water bath at 65°C. After denaturation and degradation of proteins using sodium dodecyl sulphate (1%) and proteinase K (0.2 mg ml^{-1}), DNA was purified using an AllPrep DNA/RNA extraction kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The molecular size and the purity of the DNA were analysed by agarose gel electrophoresis (1 %). In total, nine samples were extracted, and 600 ng of DNA per sample was sent to the Research and Testing Laboratory (http://www.researchandtesting.com) for 454 tag pyrosequencing.

454 sequencing and analysis

The bacterial 16S rRNA genes were sequenced using the FLX-Titanium platform (Roche, Basel, Switzerland) as described in Wolcott and colleagues (2009). Sequencing was performed on regions V2 and V3 of the 16S rRNA gene. Chosen primers targeted 410 bp between positions 121 and 531 on the *Escherichia coli* reference sequence (Baker *et al*., 2003). For the nine samples that were analysed, a total number of 88 121 sequences was obtained. All further analyses were conducted using the Quantitative Insights Into Microbial Ecology (QIIME) pipeline (http://qiime.org) (Caporaso *et al*., 2010). The sequences were quality filtered, and all sequences shorter than 200 bp or containing ambiguous bases were eliminated from the data set. Sequences were then attributed to each sample according to their barcode sequence. These operations led to the removal of about 25% of the sequences with either bad quality scores or ambiguous barcode sequences. The sequencing errors were corrected among the remaining sequences using the QIIME denoiser algorithm, and barcodes and primers were removed (Caporaso *et al*., 2010). OTUs were identified using the uclust picking method and defined at a 97% sequence similarity level, and only the most abundant sequence was conserved as a representative. These sequence data have been submitted to the DNA DataBase of Japan/European Molecular Biology Laboratory/GenBank databases under accession number ERP002553. Taxonomy was assigned using the Ribosomal Data Project (RDP) classifier programme (Wang *et al*., 2007) with Greengenes reference data set released in October 2012. The sequences were aligned using the PyNast algorithm (DeSantis Jr *et al*., 2006; Caporaso *et al*., 2010). The chimeric sequences were identified and removed with ChimeraSlayer (Haas *et al*., 2011).

A phylogenetic tree was constructed using the FastTree method (Price *et al*., 2009), and an OTU table was created. To enable comparisons between samples, the OTU table was subsampled to ensure an equal number of sequences in each sample. The resulting OTU table comprised 3416 sequences per sample. All further analyses such as alpha diversity estimations were conducted with this OTU table. Indices such as Chao1 (Chao, 1987), phylogenetic distance (Faith and Baker, 2007), Shannon (Hill, 1973) and equitability ($E = H/\log_2 * S$, where H stands for Shannon's entropy and S stands for the total number of OTUs) were calculated. Differences in alpha diversity between treatments were tested statistically using the Student's *t*-test. Phylogenetic differences between samples were tested using Andrew Martin's phylogenetic test (Martin, 2002; Eckburg *et al*., 2005). The clustering of samples was checked for robustness with jackknife analyses and for significance with an ANOSIM. All analyses were performed using the QIIME pipeline.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Fig. S1. Schematic illustration of the experimental set up. The grey-shaded boxes represent the continuous cultures inoculated at the beginning of the experiment with a natural bacterial community, and then supplied with the respective medium. A detailed description of the experiment is provided in the Section 'Experimental procedures'.

Fig. S2 van Krevelen diagrams showing compounds detected exclusively in the diatom DOM and exclusively in the cyanobacterial DOM, and their chemical characteristics. m/z – averaged molecular weight.

Fig S3. A. Heat map representing major OTUs (> 1%) for the bacterial communities in each culture after three generation times. Relative abundance of OTUs is presented as follows: Dark blue – dominant OTU; middle blue – OTUs with relative abundances higher than 5%; light blue – OTUs with relative abundances higher than 1%; Grey – OTUs with relative abundances less than 1%; Dark – OTUs absent from culture. The relative abundance of the dominant OTU was 22% (diatom DOM, R1), 21% (diatom DOM, R2), 46% (cyanobacterial DOM, R1) and 35% (cyanobacterial DOM, R2). The OTU numbers marked in red were also dominant after six generation times.

B. Heat map representing major OTUs (> 1%) for the bacterial communities in each culture after six generation times. Relative abundance of OTUs is presented as follows: Dark blue – dominant OTU; middle blue – OTUs with relative abundances higher than 5%; light blue – OTUs with relative abundances higher than 1%; Grey – OTUs with relative abundances less than 1%; Dark – OTUs absent from culture. The relative abundance of the dominant OTU was 11% (diatom DOM, R1), 9% (diatom DOM, R2), 37% (cyanobacterial DOM, R1) and 25% (cyanobacterial DOM, R2). The OTU numbers marked in red were dominant after three generation times.

Fig. S4. Rarefaction curves of diversity indices determined for *Gammaproteobacteria* after three (left panels) and six (right panels) generation times.

Table S1. Concentrations of dissolved organic carbon (DOC), nitrate + nitrite (NO_3^-+NO_2^-) and phosphate (PO_4^{3-}) in the input media and the cultures during the experimental period. Mean values ± SD are given. Nitrogen was also added in the form of NH_4^+ (20 μM final concentration) to the two input media. For each time point, the average value of the two replicate cultures for each treatment was used to calculate the mean value. n = number of time points for which chemical analyses were done.

Table S2. Phylogenetic characterization of the inoculum. Numbers correspond to the relative contribution of each group to the total community. Phylogenetic affiliation was done using the RDP classifier against the Greengenes database. 'Other' refers to pooled assemblages of groups with low sequence numbers and to non-assigned sequences.

Table S3. Percent sequences belonging to phylogenetic groups within *Gammaproteobacteria* after three and six generation times in diatom and cyanobacterial DOM cultures. Numbers correspond to mean value of the replicates ± SD. Phylogenetic affiliation was done using the RDP classifier against the Greengenes database. 'Other' refers to pooled assemblages of groups with low sequence numbers and to non-assigned sequences.

Table S4. Percent sequences belonging to phylogenetic group within proteobacteria (except *Gammaproteobacteria*, see Table S3) after three and six generation times in diatom and cyanobacterial DOM cultures. Numbers correspond to mean value of the replicates ± SD. Phylogenetic affiliation was done using the RDP classifier against the Greengenes database. 'Other' refers to pooled assemblages of groups with low sequence numbers and to non-assigned sequences.

Table S5. Percent sequences belonging to other phylogenetic groups after three and six generation times in diatom and cyanobacterial DOM cultures. Numbers correspond to mean value of the replicates ± SD. Phylogenetic affiliation was done using the RDP classifier against the Greengenes database. 'Other' refers to pooled assemblages of groups with low sequence numbers and to non-assigned sequences.

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Supplementary Information

Figure S1: Schematic illustration of the experimental set up. The grey shaded boxes represent the continuous cultures inoculated at the beginning of the experiment with a natural bacterial community, and then supplied with the respective medium. A detailed description of the experiment is provided in the Section "Experimental Procedures".

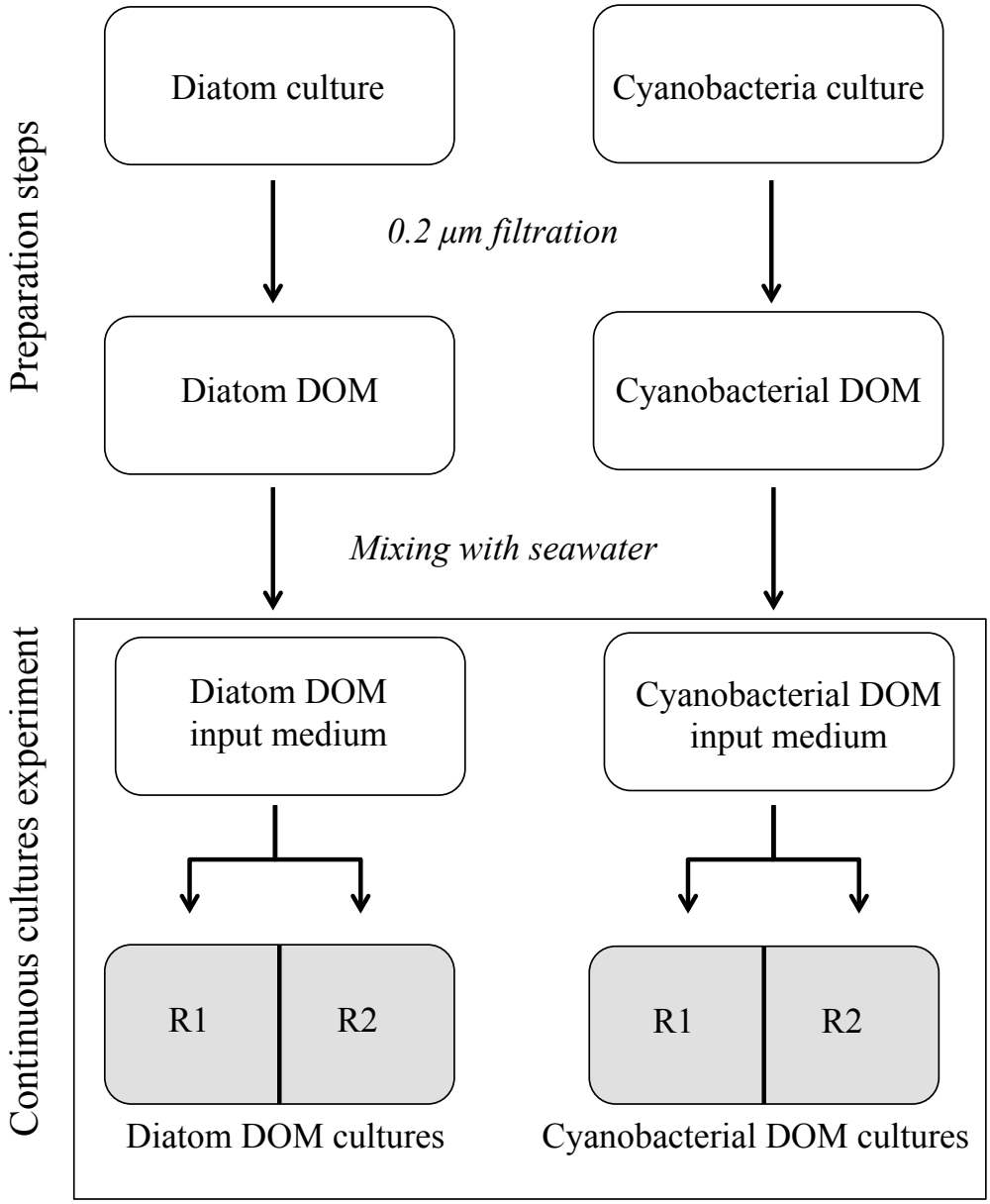
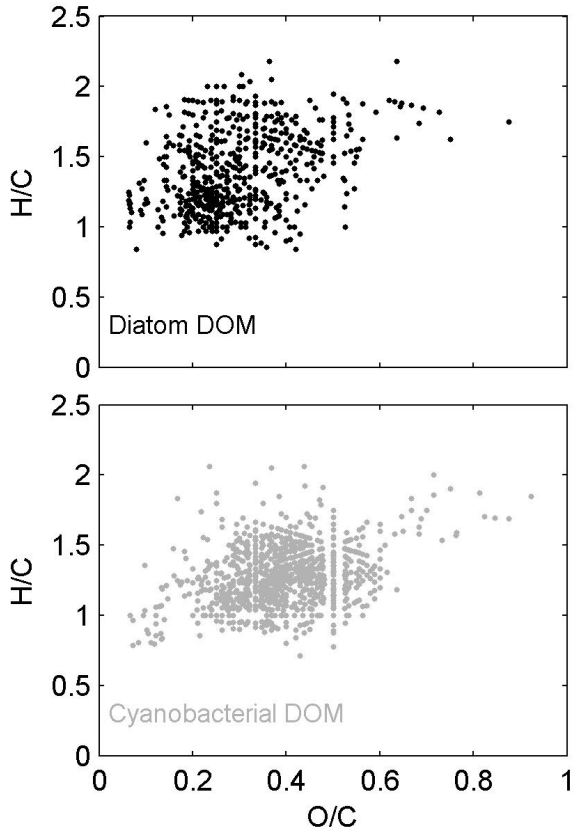


Fig. S2: van Krevelen diagrams showing compounds detected exclusively in the Diatom DOM and exclusively in the Cyanobacterial DOM, and their chemical characteristics. m/z – averaged molecular weight



Average number C = 23.69 ± 6.2; 100% of compounds
Average number H = 32.57 ± 7.08; 100% of compounds
Average number O = 6.81 ± 2.24; 100% of compounds
Average number N = 1.75 ± 1.58; 64% of compounds
Average number S = 0.26 ± 0.47; 24.1% of compounds
Average number P = 0.081 ± 0.32; 6.7% of compounds
Average m/z = 461.3 ± 95.46

Average number C = 20.77 ± 4.91; 100% of compounds
Average number H = 26.6 ± 6.55; 100% of compounds
Average number O = 7.96 ± 2.2; 100% of compounds
Average number N = 2.07 ± 1.32; 84.3% of compounds
Average number S = 0.14 ± 0.36; 13.7% of compounds
Average number P = 0.05 ± 0.252; 4.1% of compounds
Average m/z = 438.36 ± 83.87

Figure S3a :
Heatmap representing major OTUs (>1%) for the bacterial communities in each culture after 3 generation times. Relative abundance of OTUs is presented as follows: Dark blue – dominant OTU; middle blue – OTUs with relative abundances higher than 5%; light blue – OTUs with relative abundances higher than 1%; Grey - OTUs with relative abundances less than 1%; Dark – OTUs absent from culture. The relative abundance of the dominant OTU was 22% (Diatom DOM, R1) ; 21% (Diatom DOM, R2) ; 46% (Cyanobacterial DOM, R1) and 35% (Cyanobacterial DOM, R2). The OTU numbers marked in red were also dominant after 6 generation times.

OTU number	Diatom DOM, R1	Diatom DOM, R2	Cyanobacterial DOM, R1	Cyanobacterial DOM, R2	Taxonomy
225					Bacteriovoracaceae (δ)
772					*Methylophaga* (γ)
2290					*Methylophaga* (γ)
278					*Oleibacter* (γ)
296					Piscirickettsiaceae (γ)
3211					Oceanospirillaceae (γ)
1289					*Alcanivorax* (γ)
1208					Piscirickettsiaceae (γ)
299					Alteromonadaceae (γ)
347					(γ)
126					*Phaeobacter* (α)
1521					Oceanospirillaceae (γ)
1154					Alteromonadaceae (γ)
2279					Oleiphilaceae (γ)
3318					Piscirickettsiaceae (γ)
912					*Thalassobius* (α)
1310					Rhodobacteraceae (α)
127					Rhodobacteraceae (α)
844					*Phaeobacter* (α)
929					Oceanospirillaceae (γ)
2039					Piscirickettsiaceae (γ)
2434					*Phaeobacter* (α)
219					Alteromonadaceae (γ)
220					*Alcanivorax* (γ)
2583					*Alcanivorax* (γ)
1286					*Alteromonas* (γ)
3129					Piscirickettsiaceae (γ)
859					Piscirickettsiaceae (γ)
434					Piscirickettsiaceae (γ)
2974					Oceanospirillaceae (γ)
2291					*Vibriofortis (γ)*

Figure S3b :

Heatmap representing major OTUs (>1%) for the bacterial communities in each culture after 6 generation times. Relative abundance of OTUs is presented as follows: Dark blue – dominant OTU; middle blue – OTUs with relative abundances higher than 5%; light blue – OTUs with relative abundances higher than 1%; Grey - OTUs with relative abundances less than 1%; Dark – OTUs absent from culture. The relative abundance of the dominant OTU was 11% (Diatom DOM, R1) ; 9% (Diatom DOM, R2) ; 37% (Cyanobacterial DOM, R1) and 25% (Cyanobacterial DOM, R2). The OTU numbers marked in red were dominant after 3 generation times.

OTU number	Diatom DOM, R1	Diatom DOM, R2	Cyanobacterial DOM, R1	Cyanobacterial DOM, R2	Taxonomy
772					*Methylophaga* (γ)
2290					*Methylophaga* (γ)
278					*Oleibacter* (γ)
347					(γ)
225					Bacteriovoracaceae (δ)
632					Methylophilaceae (β)
299					Alteromonadaceae (γ)
2718					Sinobacteraceae (γ)
844					*Phaeobacter* (α)
2630					Chromatiales (γ)
2417					Hyphomonadaceae (α)
2544					Oceanospirillales (γ)
2909					Methylophilaceae (β)
1208					Piscirickettsiaceae (γ)
699					Alteromonadales (γ)
2072					Alteromonadaceae (γ)
1316					Cryomorphaceae (CFB)
2100					Pelagibacteraceae (α)
126					*Phaeobacter* (α)
1154					Alteromonadaceae (γ)
564					(α)
1289					*Alcanivorax* (γ)
912					*Thalassobius* (α)
127					Rhodobacteraceae (α)
1310					Rhodobacteraceae (α)
2770					Rhodobacteraceae (α)
220					*Alcanivorax* (γ)
1173					Alteromonadales (γ)
923					Methylophilaceae (β)
1260					Alteromonadaceae (γ)
3453					(α)
1403					Saprospiraceae (CFB)
859					Piscirickettsiaceae (γ)

Figure S4

Rarefaction curves of diversity indices determined for Gammaproteobacteria after 3 (left panels) and 6 (right panels) generation times.

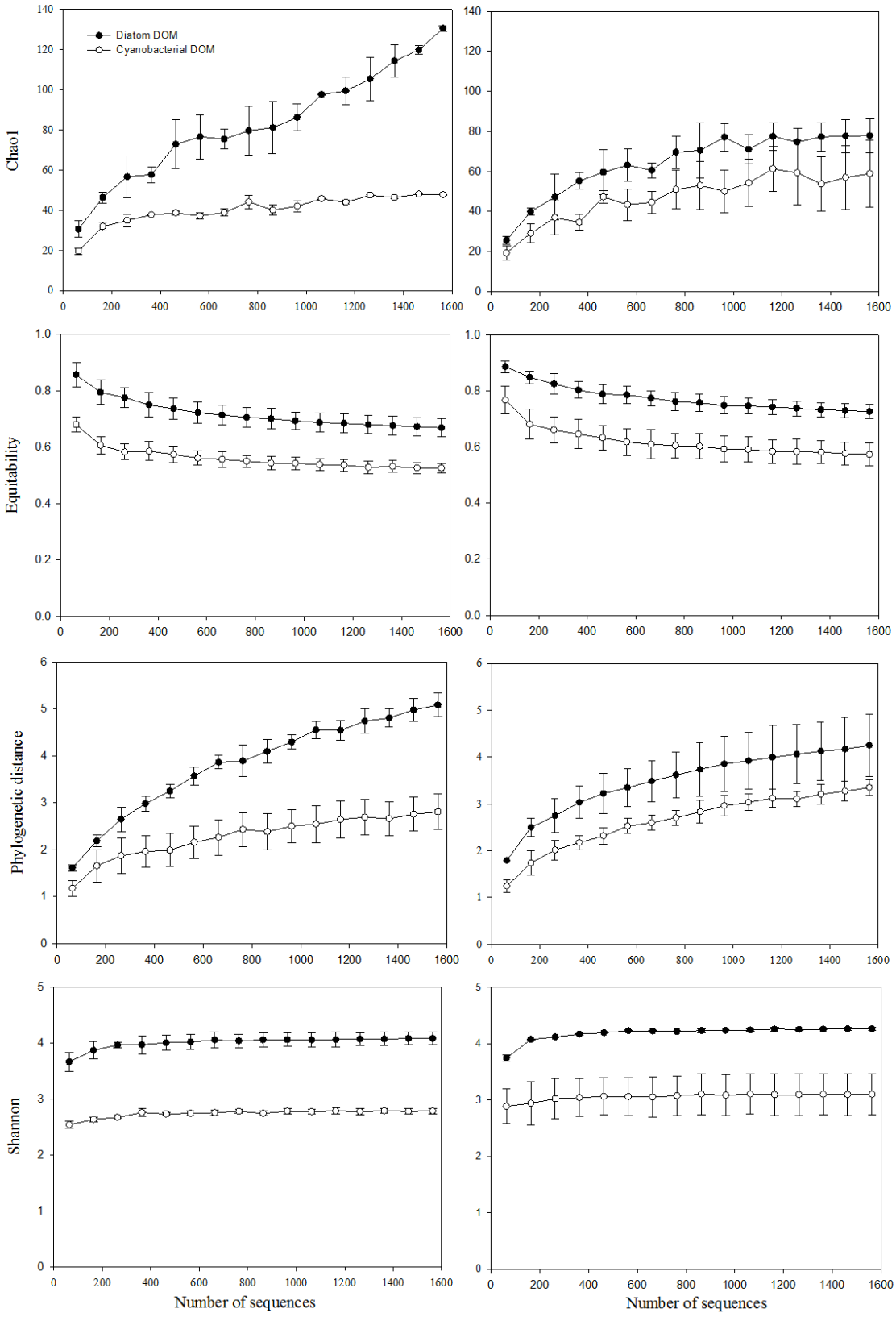


Table S1. Concentrations of dissolved organic carbon (DOC), nitrate+nitrite (NO_3 +NO_2) and phosphate (PO_4^3) in the input media and the cultures during the experimental period. Mean values ± SD are given. Nitrogen was also added in the form of NH_4^+ (20 µM final concentration) to the two input media. For each time point, the average value of the two replicate cultures for each treatment was used to calculate the mean value. n= number of time points for which chemical analyses were done.

	DOC (µM)	NO_3 +NO_2 (µM)	PO_4^3 (µM)
	Input medium		
Diatom DOM	75±2 (n=11)	187±7 (n=11)	21±1 (n=11)
Cyanobacterial DOM	73±3 (n=11)	196±7 (n=11)	26±3 (n=11)
	Cultures		
Diatom DOM	75±2 (n=12)	191±10 (n=12)	21±1 (n=11)
Cyanobacterial DOM	75±2 (n=12)	203±10 (n=12)	26±3 (n=11)

Table S2. Phylogenetic characterization of the inoculum. Numbers correspond to the relative contribution of each group to the total community. Phylogenetic affiliation was done using the RDP classifier against the Greengenes database. 'Other'refers to pooled assemblages of groups with low sequence numbers and to non-assigned sequences.

Phylum	Class	Order	Family	Genus	Initial community
Actinobacteria	Acidimicrobiia	Acidimicrobiales			**4.78**
			OCS155		2.94
			ZA3409c		1.62
			Other		0.21
Bacteroidetes					**1.13**
	Flavobacteriia	Flavobacteriales			1.04
			Cryomorphaceae	*Fluviicola*	0.09
			Flavobacteriaceae		0.55
			Other		0.31
		Other			0.09
	Sphingobacteriia	Sphingobacteriales	Ekhidnaceae		0.09
Chloroflexi					**0.95**
	SAR202				0.28
	TK17				0.67
Cyanobacteria					**2.30**
	Synechococcophycideae	Synechococcales	Synechococcaceae	*Prochlorococcus*	2.20
	Other				0.09
Gemmatimonadetes					**0.46**
Planctomycetes					**0.21**
Proteobacteria					**76.77**
	Alphaproteobacteria				57.30
		Rhizobiales	Hyphomicrobiaceae		0.15
		Rhodobacterales	Rhodobacteraceae		1.07
		Rhodospirillales	Rhodospirillaceae		4.53
		Rickettsiales			49.28
			AEGEAN_112		0.64
			Pelagibacteraceae	*Pelagibacter*	38.35
			Other		10.28
		Other			2.27
	Betaproteobacteria				0.92
		Methylophilales	Methylophilaceae		0.83
			Other		0.09
	Deltaproteobacteria				2.54
		Bdellovibrionales	Bacteriovoracaceae		0.06
		Desulfobacterales	Nitrospinaceae	*Nitrospina*	0.09
		Myxococcales			0.12
		Sva0853			2.05
		Other			0.21
	Gammaproteobacteria				15.70
		Alteromonadales			0.18
		Chromatiales			1.47
		HTCC2188			0.83
		Oceanospirillales			9.37
			Halomonadaceae		8.97
			OM182		0.40
		Thiohalorhabdales			2.88
			Thiohalorhabdaceae		0.83
			Other		2.05
		Xanthomonadales			0.06
		Other			0.92
	Other				0.31
SAR406	AB16				**6.58**
SBR1093					**1.25**
Verrucomicrobia					**0.37**
Other					**0.36**
Non assigned					**4.84**

Table S3. Percent sequences belonging to phylogenetic groups within Gammaproteobacteria after 3 and 6 generations in diatom- and cyanobacterial DOM cultures. Numbers correspond to mean value of the replicates ± SD. Phylogenetic affiliation was done using the RDP classifier against the Greengenes database. 'Other'refers to pooled assemblages of groups with low sequence numbers and to non-assigned sequences.

			3 generations		6 generations	
			Diatom DOM	Cyanobacterial DOM	Diatom DOM	Cyanobacterial DOM
Order	Family	Genus	% of total sequences			
Sum of Gammaproteobacteria			65.66 ± 0.92	74.70 ± 19.49	56.40 ± 13.22	82.74 ± 2.48
Thiotrichales	Piscirickettsiaceae		30.48 ± 1.01	31.71 ± 9.38	19.44 ± 8.14	14.24 ± 5.17
		Methylophaga	10.30 ± 9.83	25.31 ± 18.02	16.54 ± 8.50	13.91 ± 4.87
		Other	20.18 ± 10.85	6.40 ± 8.64	2.90 ± 0.36	0.33 ± 0.29
Oceanospirillales			21.56 ± 4.96	35.31 ± 29.91	13.96 ± 2.05	51.99 ± 0.90
	Alcanivoracaceae	*Alcanivorax*	4.13 ± 0.74	29.57 ± 33.43	4.05 ± 2.93	40.48 ± 6.54
	Oceanospirillaceae		15.82 ± 2.89	5.34 ± 3.32	6.49 ± 4.44	11.28 ± 7.24
		Oleibacter	8.61 ± 2.08	0.96 ± 0.45	5.69 ± 3.68	9.67 ± 6.14
		Oleispira	0.08 ± 0.02	0.11 ± 0.02	0.03 ± 0.05	0 ± 0
		Other	7.13 ± 4.94	4.27 ± 3.74	0.77 ± 0.72	1.61 ± 1.11
	Oleiphilaceae		1.24 ± 1.13	0.10 ± 0	0.37 ± 0.25	0.13 ± 0.09
	Other		0.37 ± 0.20	0.30 ± 0.20	3.05 ± 0.29	0.11 ± 0.11
Alteromonadales			8.05 ± 0.38	5.18 ± 1.01	12.11 ± 3.27	10.10 ± 7.15
	Alteromonadaceae		6.38 ± 0.68	4.21 ± 0.54	7.86 ± 3.00	6.22 ± 4.20
		Alteromonas	0.19 ± 0.05	1.13 ± 1.51	0.11 ± 0.11	0.62 ± 0.65
		BD2 13	2.03 ± 1.38	1.13 ± 1.01	2.30 ± 1.67	2.70 ± 2.41
		Glaciecola	0.59 ± 0.47	0 ± 0	1.08 ± 0.23	0 ± 0
		HTCC2207	0.64 ± 0.59	0.22 ± 0.14	1.64 ± 1.11	0.97 ± 0.47
		ZD0117	1.95 ± 1.40	0.19 ± 0.09	1.67 ± 1.65	1.05 ± 0.59
		Other	0.99 ± 0.99	1.53 ± 1.76	1.05 ± 1.31	0.88 ± 0.07
	Colwelliaceae		0.32 ± 0.32	0.69 ± 0.70	0.41 ± 0.27	0.40 ± 0.56
		Thalassomonas	0.06 ± 0.09	0 ± 0	0.05 ± 0.02	0 ± 0
		Other	0.26 ± 0.23	0.69 ± 0.70	0.37 ± 0.25	0.40 ± 0.56
	J115		0.86 ± 0.72	0.26 ± 0.18	3.03 ± 0.18	3.38 ± 2.30
	Shewanellaceae		0.37 ± 0.07	0.03 ± 0.05	0.57 ± 0	0.05 ± 0.07
	Other		0.13 ± 0.05	0 ± 0	0.22 ± 0.18	0.05 ± 0.02
HTCC2188			4.88 ± 2.57	1.40 ± 1.04	4.08 ± 0.54	5.09 ± 0.34
Chromatiales			0.26 ± 0	0 ± 0	2.57 ± 0.43	0.14 ± 0.07
Xanthomonadales			0.03 ± 0.05	0 ± 0	3.64 ± 0.99	0.91 ± 0.07
Other			0.40 ± 0.02	1.10 ± 1.06	0.61 ± 23	0.27 ± 0.07

Table S4. Percent sequences belonging to phylogenetic group within Proteobacteria (except Gammaproteobacteria, see Table S3) after 3 and 6 generations in diatom- and cyanobacterial DOM cultures. Numbers correspond to mean value of the replicates ± SD. Phylogenetic affiliation was done using the RDP classifier against the Greengenes database. 'Other' refers to pooled assemblages of groups with low sequence numbers and to non-assigned sequences.

			3 generations		6 generations	
			Diatom DOM	Cyanobacterial DOM	Diatom DOM	Cyanobacterial DOM
Order	Family	Genus	% of total sequences			
Sum of Alphaproteobacteria			16.46 ± 9.70	22.03 ± 18.07	28.58 ± 12.04	8.88 ± 2.14
Rhodobacterales			15.31 ± 9.47	21.88 ± 18.00	22.76 ± 11.84	5.49 ± 0.68
	Rhodobacteraceae		14.94 ± 9.04	21.87 ± 18.02	15.04 ± 7.42	3.89 ± 0.90
		Phaeobacter	8.95 ± 6.70	11.34 ± 8.82	5.37 ± 0.47	2.26 ± 0.77
		Thalassiobus	1.52 ± 0.16	1.32 ± 0.97	1.83 ± 1.20	0.45 ± 0.05
		Other	4.48 ± 2.19	9.20 ± 8.23	7.83 ± 5.75	1.18 ± 0.09
	Hyphomonadaceae		0.37 ± 0.43	0.02 ± 0.02	7.70 ± 4.44	1.59 ± 0.23
		Hyphomonas	0 ± 0	0 ± 0	0.19 ± 0.09	0.05 ± 0.02
		Maricaulis	0.03 ± 0	0.02 ± 0.02	1.63 ± 0.14	0.75 ± 0.11
		Other	0.33 ± 0.43	0 ± 0	5.89 ± 4.49	0.80 ± 0.14
	Other		0 ± 0	0 ± 0	0.02 ± 0.02	0 ± 0
Rickettsiales			0.45 ± 0.05	0.08 ± 0.07	2.07 ± 0.59	0.91 ± 0.16
	Pelagibacteraceae		0.41 ± 0.05	0.08 ± 0.07	1.90 ± 0.56	0.86 ± 0.14
	Other		0.03 ± 0	0 ± 0	0.18 ± 0.02	0.05 ± 0.02
Kiloniellales			0.29 ± 0.09	0.02 ± 0.02	0.27 ± 0.38	0.03 ± 0.05
Rhodospirillales	Rhodospirillaceae		0.06 ± 0.05	0.02 ± 0.02	0.61 ± 0.05	0.13 ± 0.09
Rhizobiales			0.03 ± 0	0 ± 0	0.49 ± 0.52	0.75 ± 0.20
	Hyphomicrobiaceae		0.03 ± 0	0 ± 0	0.37 ± 0.38	0.67 ± 0.09
	Other		0 ± 0	0 ± 0	0.13 ± 0.14	0.08 ± 0.11
Sphingomonadales			0 ± 0	0 ± 0	0.11 ± 0.16	0.03 ± 0
Other			0.32 ± 0.14	0.03 ± 0	2.26 ± 0.54	1.55 ± 1.56
Sum of Betaproteobacteria			0.22 ± 0.09	0.13 ± 0	8.92 ± 3.59	5.06 ± 2.28
Methylophilales	Methylophilaceae		0.22 ± 0.09	0.08 ± 0.07	8.77 ± 3.47	4.63 ± 1.85
		Methylotenera	0.03 ± 0.05	0.06 ± 0.09	0.08 ± 0.07	0.57 ± 0.32
		Other	0.19 ± 0.05	0.02 ± 0.02	8.69 ± 3.41	4.05 ± 1.53
Burkholderiales	Comamonadaceae		0 ± 0	0.05 ± 0.07	0.14 ± 0.11	0.43 ± 0.43
		Limnohabitans	0 ± 0	0 ± 0	0 ± 0	0.08 ± 0.07
		RS62	0 ± 0	0 ± 0	0.13 ± 0.14	0 ± 0
		Other	0 ± 0	0.05 ± 0.07	0 ± 0	0.35 ± 0.50
Sum of Deltaproteobacteria			15.87 ± 9.32	2.22 ± 1.47	2.81 ± 1.17	1.08 ± 0.23
Bdellovibrionales	Bacteriovoracaceae		15.87 ± 9.32	2.22 ± 1.47	2.81 ± 1.17	1.08 ± 0.23
Other Proteobacteria			0.41 ± 0.18	0.14 ± 0.11	0.18 ± 0.20	0.02 ± 0.02

Table S5. Percent sequences belonging to other phylogenetic groups after 3 and 6 generations in diatom- and cyanobacterial DOM cultures. Numbers correspond to mean value of the replicates ± SD. Phylogenetic affiliation was done using the RDP classifier against the Greengenes database. 'Other'refers to pooled assemblages of groups with low sequence numbers and to non-assigned sequences.

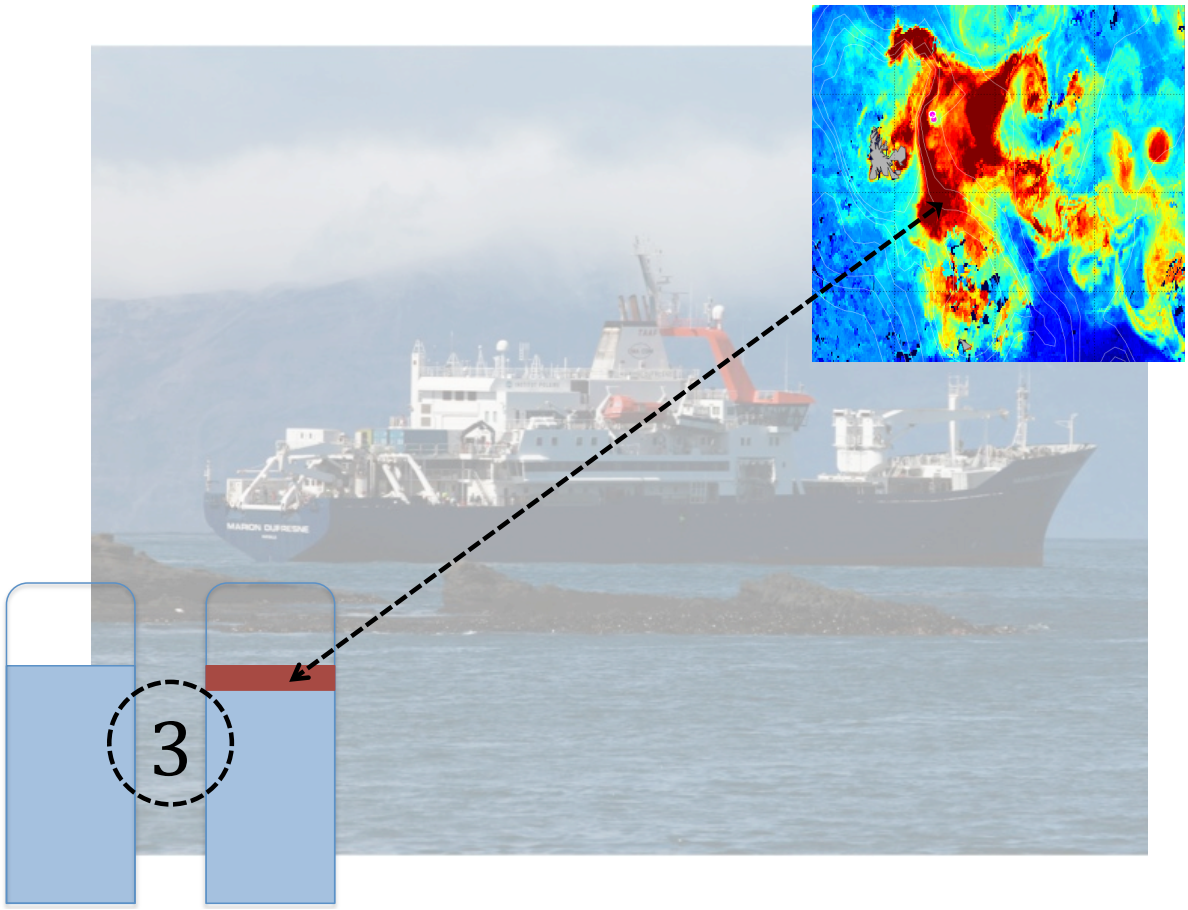
				3 generations		6 generations	
				Diatom DOM	Cyanobacterial DOM	Diatom DOM	Cyanobacterial DOM
Class	Order	Family	Genus	% of total sequences			
Sum of Bacteroidetes				1.31 ± 0.81	0.69 ± 0.20	2.60 ± 1.01	1.71 ± 1.78
Flavobacteriia				1.31 ± 0.81	0.69 ± 0.20	2.58 ± 0.99	0.80 ± 0.59
	Flavobacteriales			0.97 ± 0.79	0.49 ± 0.07	2.55 ± 0.95	0.80 ± 0.59
		Flavobacteriaceae		0.64 ± 0.54	0.30 ± 0.07	1.28 ± 0.45	0.45 ± 0.36
			Polaribacter	0.02 ± 0.02	0.05 ± 0.02	0.08 ± 0.07	0 ± 0
			Sediminicola	0 ± 0	0 ± 0	1.19 ± 0.45	0.35 ± 0.45
			Other	0.62 ± 0.52	0.26 ± 0.05	0.75 ± 0.02	0.10 ± 0.09
		Cryomorphaceae		0 ± 0	0.03 ± 0.05	1.23 ± 1.33	0.32 ± 0.27
		Other		0.33 ± 0.25	0.16 ± 0.05	0.05 ± 0.07	0.03 ± 0.05
	Other			0.32 ± 0.05	0.19 ± 0.14	0.03 ± 0.05	0 ± 0
Sphingobacteria	Sphingobacteriales	Saprospiraceae		0 ± 0	0 ± 0	0.02 ± 0.02	0.91 ± 1.20
Actinobacteria							
Acidimicrobiia	Acidimicrobiales	OCS155		0.02 ± 0.02	0.08 ± 0.07	0.18 ± 0.11	0.11 ± 0.11
Lentisphaerae							
Lentisphaeria	Lentisphaerales	Lentisphaeraceae		0 ± 0	0 ± 0	0.11 ± 0.02	0.03 ± 0
Other				0.05 ± 0.02	0.02 ± 0.02	0.24 ± 0.11	0.37 ± 0.25

PART TWO

COMBINING EXPERIMENTAL TOOLS AND FIELD OBSERVATION: THE KEOPS2 CRUISE

CHAPTER THREE

BACTERIAL DIVERSITY IN THE SOUTHERN OCEAN: CAN RESULTS FROM A DOM ADDITION EXPERIMENT SHED LIGHT ON THE RESPONSE OF BACTERIAL COMMUNITIES TO PHYTOPLANKTON BLOOMS INDUCED BY NATURAL IRON FERTILIZATION ?



I. INTRODUCTION

The role of iron for planktonic metabolism

The Mediterranean Sea is one of the most oligotrophic environments on Earth, where planktonic communities, including heterotrophic bacteria, are usually limited by inorganic nutrients, particularly phosphorus (Thingstad et al., 2005; Pujo-Pay et al., 2011). By contrast, other areas of the world such as the Southern Ocean are characterized by high concentrations of major inorganic nutrients (Figure 1A).

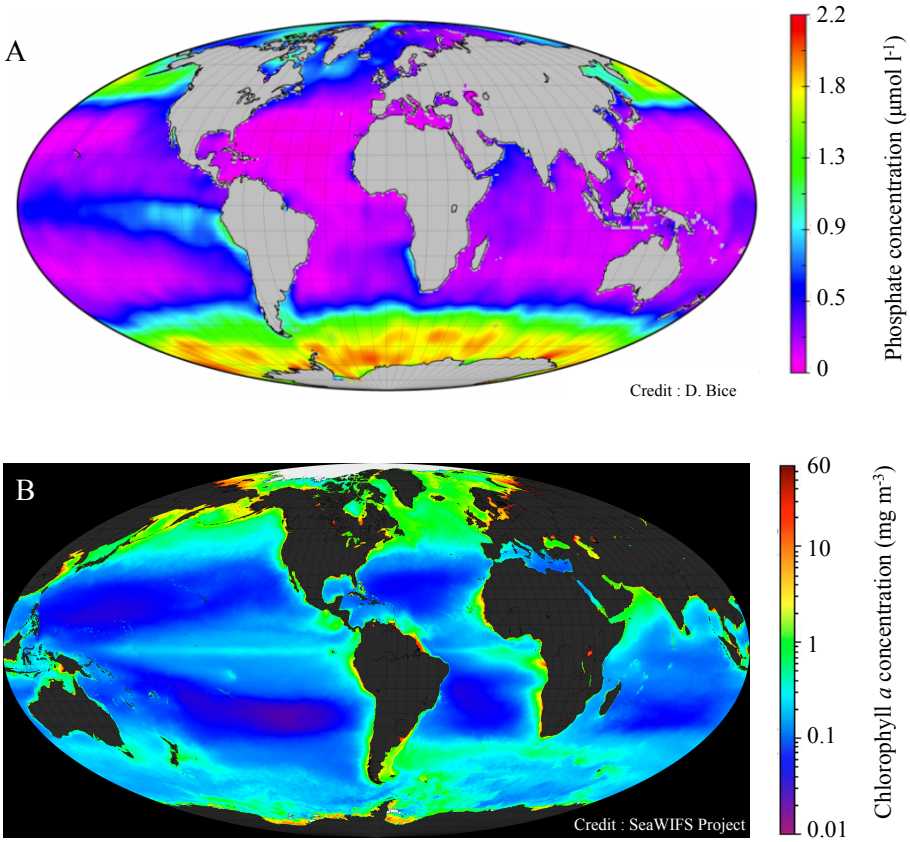


Figure 1. Ocean phosphate (A) and chlorophyll a (B) concentrations. Images were adapted from the College of Earth and Mineral Science website (data from World Ocean Atlas 2009) (A) and from the Earth Observatory website (NASA) (B).

These high concentrations of inorganic nutrients in surface waters go hand in hand with low chlorophyll *a* (chl *a*) content (Figure 1B), because the system lacks a different but nonetheless essential resource: iron (de Baar et al., 1990; Boyd et al., 2000). In these high-nutrient low-chlorophyll (HNLC) systems, the limitation exerted by iron on phytoplankton communities results in low primary production and therefore low nutrient consumption, which explains their accumulation in surface waters.

The three main HNLC regions of the world (Equatorial and Subarctic Pacific Ocean, and Southern Ocean) have been the subjects of many studies that successfully demonstrated the limiting effect of iron on phytoplankton communities, mostly by mesoscale artificial iron enrichment experiments (Boyd et al., 2000; Gervais et al., 2002) but also in naturally iron-fertilized systems (Blain et al., 2007; Pollard et al., 2009). While the constraint exerted by iron concentration and availability was clearly demonstrated for phytoplankton (Coale et al., 2004), the situation is more complex for heterotrophic bacteria. Because of the high cellular iron quotas determined for heterotrophic bacteria, they strongly depend on iron availability (Tortell et al, 1996). However, given the tight link between heterotrophic bacteria and dissolved organic carbon resulting from primary production (Baines & Pace, 1991; Ducklow, 2000), heterotrophic bacteria are also expected to depend on phytoplankton biomass and activity.

In most cases, the bacterial compartment was found to be stimulated by iron addition, and many authors have tried to assess whether bacterial response was direct, that is, induced by increase iron concentration and availability, or indirect and rather due to the release of labile organic carbon concurrent to the phytoplankton bloom. Most studies have led to the conclusion that in these environments, heterotrophic bacteria are

carbon-limited rather than iron-limited (Church, 2000; Cochlan, 2001; Hall and Safi, 2001; Oliver et al., 2004; Hale et al., 2006), even though in some cases a co-limitation by carbon and iron could be demonstrated (Church 2000, Kirchman et al., 2000). This finding makes the iron-limited HNLC regions excellent systems to investigate the link between organic carbon and heterotrophic bacterial metabolism and diversity.

Natural iron fertilization events: the first KEOPS oceanographic cruise

The Southern Ocean is the largest of these HNLC regions. The Kerguelen Islands are the scene of a recurrent diatom bloom event of high intensity and duration (Figure 2). The bloom usually lasts for about three to five months and can cover up to 400 000 km^2. A defining feature of the site is that this bloom is triggered by an iron fertilization naturally induced by the passing of water masses along the Kerguelen plateau (Blain et al., 2007). At the end of winter when light increases in the water column, this natural iron input relieves the limitation of the system and enables primary production and accumulation of phytoplankton biomass, as revealed by both satellite images and *in situ* measurements. Whether carbon or iron, or both, limit heterotrophic bacteria in HNLC waters, this biological compartment is likely to be impacted by events such as the natural fertilization taking place in the vicinity of the Kerguelen Islands and by the subsequent phytoplankton bloom. In particular, the question whether bacterial communities are affected by dissolved organic carbon is highly relevant in these potentially C-limited systems, where the annual phytoplankton bloom triggered by iron fertilization could diminish the bottom up limitation of bacterial growth and activity.

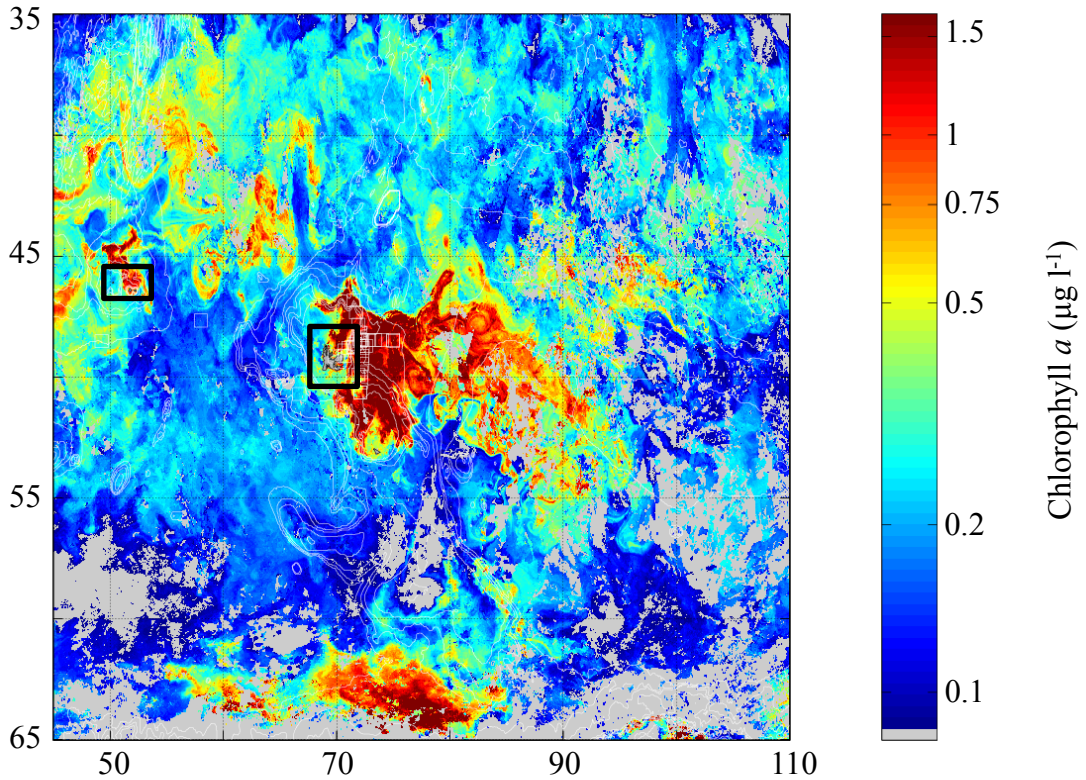


Figure 2. Satellite image of chl *a* showing the local blooms developing in the vicinity of the Crozet Islands (left black square) and the Kerguelen Islands (right black square).
Data come from KEOPS2 and were provided by F. d'Ovidio (LOCEAN-IPSL).

A first KErguelen Ocean and Plateau compared Study (KEOPS) cruise was carried out in January-February 2005, during the decline of the phytoplankton bloom, to understand the ecosystem's response to iron fertilization. This objective was pursued by comparing a station located in the bloom to a station in HNLC waters, unaffected by iron fertilization. This first cruise demonstrated that bacterial production in this area was mainly limited by DOC availability, and that this strong bottom up control was partly relieved by the release of phytoplankton DOM during the bloom (Obernosterer et al., 2008). This indirect stimulation of bacterial communities by iron fertilization was accompanied by major changes in diversity and composition of these communities studied at high phylogenetic resolution (West et al., 2008; Obernosterer et al., 2011).

Only few studies have investigated the diversity component of the response of bacterial communities to these changing environmental conditions. Some authors such as Hutchins et al. (2001), Arrieta et al. (2004) and Thiele et al. (2012) observed only minor changes in bacterial diversity, if any, in response to iron fertilization despite important shifts in phytoplankton community composition and bacterial carbon metabolism. Other studies observed shifts in bacterial diversity in response to iron fertilization, due to the increased contribution to the community of several members of the Bacteroidetes (Agawin et al., 2006) as well as *Roseobacter*-like bacteria (Kataoka et al., 2009). The results from the KEOPS1 cruise were consistent with these studies, and showed that the bacterial groups responding to the bloom conditions belonged to the *Roseobacter* group (NAC-11 7), the Bacteroidetes (Agg58), and the Gammaproteobacteria (SAR92) (West et al., 2008). These findings have been interpreted in part as a response to the release of phytoplankton-derived DOM, implying bacterial taxa that are adapted to the conditions set by the higher DOM supply. This suggests that in these HNLC regions as well as in other marine environments subjected to different constraints, the selective potential of DOM concentration, quality and availability on bacterial taxa could be fundamental.

The objectives of the KEOPS2 cruise

In this context, a second cruise was carried out in October-November 2011 with the goal to study the system at the very beginning of the bloom, and to extend the study area to offshelf iron-fertilized waters. Considering the objectives defined for this thesis, the cruise offered several possibilities.

* First, it constituted an opportunity to follow bacterial diversity over the establishment of a phytoplankton bloom, in a natural environment supposedly characterized by high carbon limitation, and investigate the *in situ* response of the C-limited bacterial community to an increasing primary production and subsequent phytoplankton DOM release. This objective was addressed through the choice, for our study sites, of three water masses undergoing distinct iron fertilization and subsequent phytoplankton bloom events, which were compared to a non-fertilized HNLC site.

* Second, the combination of such data with the data collected during the KEOPS1 cruise would provide elements of comparison between the beginning and the end of a bloom event, and insights into the potential successive adaptive stages of bacterial communities as the bloom develops and decays.

* Third, the cruise offered a unique opportunity to compare environmental data to experimental data, through the performance of an on-board continuous culture experiment similarly to the two experiments presented in the first part of this manuscript. A third and final continuous culture experiment was therefore performed during the cruise, with the objective to study the diversity of a natural local community grown on a continuous supply of *in situ* water amended with phytoplankton-derived DOM compared to no amendment; and further compare these results to the bacterial diversity characterizing the various *in situ* blooms.

II. MATERIAL AND METHODS

1. Sites description

The phytoplankton bloom above the Kerguelen Plateau

The bloom southeast of the Kerguelen Islands represented by station A3 was visited during KEOPS1 (January-February 2005) and KEOPS2 (October–November 2011)(Figure 3). This bloom starts in early October and has an overall duration of 3 to 5 months (Blain et al., 2007), and the spatial extent of the bloom is closely related to the bathymetry of the Kerguelen plateau.

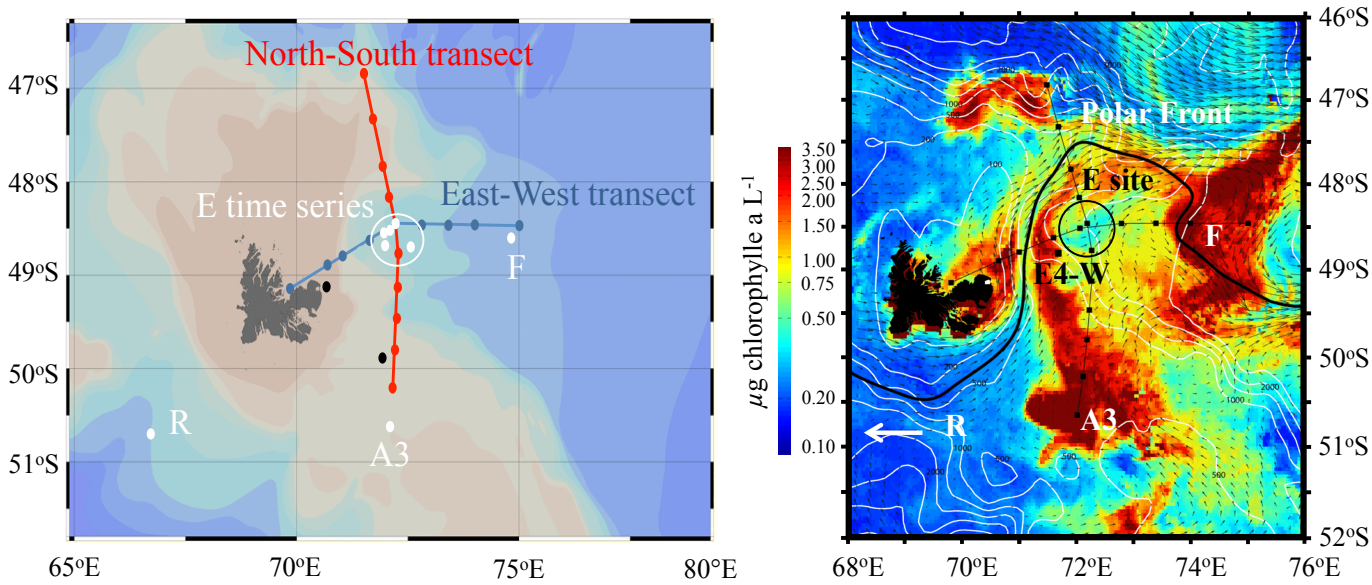


Figure 3. Left: position of the stations visited during KEOPS2. The colors on the map reflect the bathymetry of the site and delimit the Kerguelen plateau (red). Right: Chl *a* concentrations at the five main sites visited during KEOPS2. The map also places the Polar Front and currents direction as determined by Park and D'Ovidio.

During KEOPS1, Station A3, situated in the center of the bloom (527 m depth)(Figure 3 and Table 1) was visited four times, following the bloom during its decline. Two of the 4 visits, described here as A3-3 and A3-4, are considered in this

chapter. During KEOPS2, Station A3 was visited twice, described here as A3-1 and A3-2, during the onset of the bloom. These visits revealed a deep mixed layer (MLD, 160 m), that contrasted with the shallower mixed layer observed at the end of the bloom (52 to 84 m), associated to higher temperatures (Table 1).

Table 1. Brief description of the study sites. MLD : mixed layer depth.

Station	Position	Date	Depth (m)	MLD (m)	Temperature MLD (°C)	Chl *a* MLD (µg l^{-1})
KERGUELEN PLATEAU						
KEOPS2						
A3-1	50°37.88'S-72°04.99'E	Oct 19 2011	527	161	1.7	0.6±0.2
A3-2	50°37.45'S-72°03.32'E	Nov 16 2011	527	165	2.2	2.0±0.3
KEOPS1						
A3-3	50°37.85'S-72°04.26'E	Jan 19 2005	527	52	3.5	1.6±0.5
A3-4	50°37.85'S-72°04.26'E	Feb 12 2005	527	84	3.9	1.0±0.1
OFFSHORE						
KEOPS2						
R	50°23.37'S-66°41.56'E	Oct 27 2011	2526	111	2.1	0.3±0.0
F	48°31.38'S-74°40.07'E	Nov 6 2011	2700	52	4.2	4.0±1.6
E1	48°29.88'S-72°10.66'E	Oct 29 2011	2060	84	2.5	0.9±0.1
E3	48°42.24'S-71°58.21'E	Nov 3 2011	1750	51	2.8	0.6±0.1
E4-E	48°43.00'S-72°34.00'E	Nov 13 2011	2216	77	3.2	1.1±0.0
E5	48°24.71'S-71°53.97'E	Nov 19 2011	1920	35	3.2	1.2±0.1
E4-W	48°45.98'S-71°25.79'E	Nov 11 2011	1390	67	2.5	1.3±0.1

Phytoplankton blooms in offshelf waters.

During KEOPS2, different sites in the naturally iron-fertilized region east of Kerguelen in offshelf waters (> 1300 m depth, Table 1) were visited. Two sites with high phytoplankton biomass, one located north (Station F), one located south of the Polar Front (E4-W) were investigated (Figure 3). Station F is characterized by strong stratification of the MLD (52 m) and a MLD temperature that is higher than at any of the other stations (4.2°C), indicating the influence of subantarctic waters. Source waters of

Station E4-W originate in the South, mixing with Polar Front waters that cross the Kerguelen plateau while traveling north. A series of stations (E1, E3, E4-E and E5) within a complex meander south of the Polar Front were investigated in a quasi-Lagrangian manner (Figure 3). Stations E1, E3 and E5 have close geographical positions, and were visited on Oct. 29, Nov. 3 and Nov. 19, respectively. Station E4-E is located in water masses that result from mixing of the meander with surrounding waters. MLD were variable between sites (35 m – 82 m) and MLD temperatures ranged between 2.5°C and 3.2°C. The reference site (Station R) in HNLC waters is situated west of the plateau. Station R has low phytoplankton biomass throughout the year, and at the time of our visit a deep MLD (111 m) and comparatively low MLD temperature (2.1°C).

2. The on board continuous cultures experiment

Experimental design

During our first visit to Station A3, representative of winter water conditions, we set up a continuous culture experiment to investigate the bacterial response to changes in DOM supply. In the first condition, or control, bacteria were supplied with a low DOC medium, while in the second condition, or +DOM treatment, bacteria were supplied with a low DOC medium amended with phytoplankton-derived DOM.

For low DOC medium preparation, 60 l of seawater from the upper 80 m were sampled under trace-metal clean conditions. The 60 l were collected in acid-washed (10% HCl), Milli-Q water-rinsed polycarbonate (PC) carboys and were immediately 0.2 µm-filtered as described in Chapters 1 and 2. Water for the inoculum was collected at 40 m and 4 l were filtered through a 0.8 µm PC filter (45 mm diameter, Nuclepore). The 0.8 µm filtrate was stored at 6°C for a few hours until the beginning of the experiment. Concurrently, 4.8 l of the < 0.8 µm filtrate were collected on a 0.2 µm cartridge (Sterivex, Millipore), 500 µl of lysis buffer (40 mM EDTA, 50 mM Tris, 0.75 M sucrose) were added, and the cartridge was stored at -80°C for further diversity analysis.

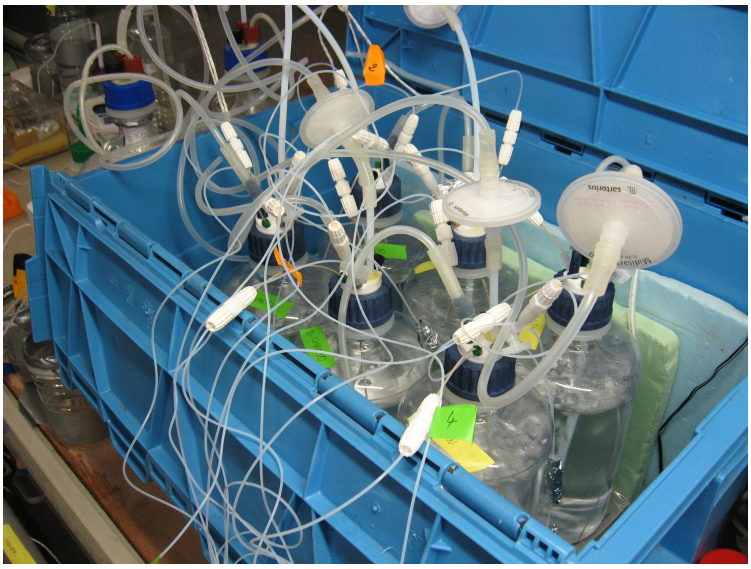


Figure 4. On board continuous cultures set up.

The phytoplankton-derived DOM was prepared in the laboratory before the cruise. A non-axenic strain of the diatom *Chaetoceros debilis*, isolated from the Southern Ocean during the LOHAFEX cruise and provided by the Alfred Wegener Institute (Germany) was cultivated in low DOC (15 µM) artificial seawater amended with nutrients according to Guillard's F/2 medium. The *Chaetoceros* cultures were grown at 4°C at a light:dark cycle of 16h:8h. Prior to the cruise, cells were removed from the

culture by gentle filtration on two acid-washed, Milli-Q water-rinsed 0.2 μm PC filters (90 mm diameter, Nuclepore) placed on top of each other. The filtrate (5 l) was collected in acid-washed, Milli-Q water-rinsed PC carboys and stored at 4°C until the start of the experiment.

The initial cultures (2 l final volume each) were prepared in acid-washed Milli-Q water-rinsed 2 l PC carboys (Figure 4). For the control, the 0.2 μm-filtered seawater sampled at A3 was used as the medium, and for the +DOM treatment the 0.2 μm-filtered seawater was mixed with 0.2 μm-filtered phytoplankton-derived DOM. The final DOC concentration of phytoplankton-DOM was 10 μM. The triplicate cultures of each condition were inoculated with 200 ml of 0.8 μm-filtered seawater, which corresponded to a 1:10 dilution. Inorganic phosphate and nitrate were added to the control to match the concentrations obtained in the +DOM treatment.

The cultures were conducted in a cold room (6°C) in the dark. Each culture was bubbled with filter-sterilized air (Sartorious). A Milli-Q water purge was placed between the source and the filters to avoid potential DOC contamination by air (Figure 5). Cultures were homogenized by stirring. They were run as a batch for a few hours, to allow bacterial growth and avoid washing out the cells. During this time, the two distinct media were prepared in two acid-washed, Milli-Q water-rinsed 4 l PC carboys in the same conditions as the initial cultures but without bacterial inoculum. Each medium was connected to the three replicate cultures of the corresponding condition by Teflon tubings, and the peristaltic pumps (Gilson) were switched on to start the inflow of input medium and the outflow of culture. The imposed flow rate was 0.18 day^{-1}, which was chosen based on the *in situ* growth rates determined during the first KEOPS cruise (Obernosterer et al., 2008) and resulted in a generation time of 5.6 days.

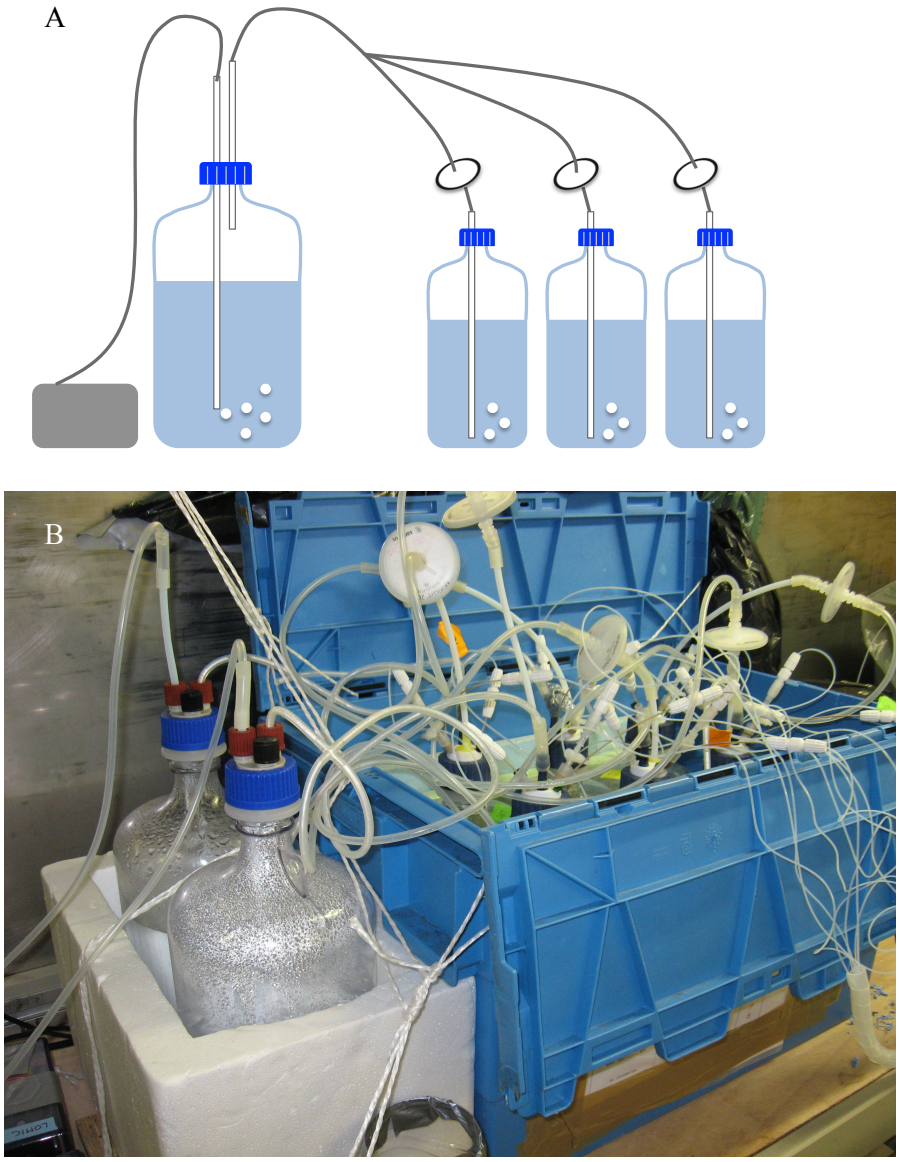


Figure 5. Schematic (A) and picture (B) of the purge system and its connection to the continuous cultures.

The experiment was run for 19 days, during which the cultures were sampled every two to three days. The air purge was renewed every day. Input media were prepared every two days, after an 0.2 µm filtration of the required volume of phytoplankton exudate. Freshly prepared media were sampled for DOC concentration and inorganic nutrients.

Sampling of cultures

Sampling was performed without opening the cultures using glass syringes (Hamilton) connected with a luer-lock to Teflon tubes and glass cubes that plunged to the bottom of the cultures. The glass syringes were extensively rinsed with Milli-Q water prior to sampling. A maximum volume of 50 ml, including syringe cleaning, was taken from the cultures for the various analyses, representing less than 3% of the volume of the cultures. Duplicate 15 ml samples for DOC concentration measurements were filtered through two precombusted GF/F filters (Whatman) into precombusted glass vials containing orthophosphoric acid (H_3PO_4, final pH 2), and stored in the dark until analysis in the home lab. A 1.8 ml sample was taken for bacterial abundance analysis, fixed with formaldehyde (2% final concentration), kept at 4°C for 30 min, and then stored at -80°C to be analyzed back in the laboratory. For the details of the analytic methods, see Chapters 1 and 2. A sample was also taken for the measurement of nitrate (NO_3^-) and phosphate (PO_4^{3-}) concentrations, which were determined on board immediately after sampling using previously described methods (See Chapters 1 and 2).

Samples for bacterial diversity were collected in acid-washed, Milli-Q water-rinsed PC flasks every five days from the outflow of the cultures. The tubes were placed in the flasks at night and between 135 and 216 ml of collected culture were filtered the next morning on 0.2 μm PC filters (Nuclepore), placed in tubes containing 500 μl of lysis buffer (40 mM EDTA, 50 mM Tris, 0.75 M sucrose) and stored at -80°C until the end of the cruise. The last day of the experiment, the volumes filtered for bacterial diversity were ~500 ml for each culture.

3. Sampling and analysis of experimental and *in situ* bacterial diversity

At each station, seawater for bacterial diversity analysis was 25 μm pre-filtered with Nylon screen and collected in 10 l PC carboys rinsed three times with sample water. Seawater was immediately sequentially filtered through 3 μm and 0.8 μm PC filters (45 mm diameter, Nuclepore) and a 0.2 μm cartridge (Sterivex, Millipore). The filters were placed into tubes and 500 μl of lysis buffer (40 mM EDTA, 50 mM Tris, 0.75 M sucrose) were added to the tubes and the cartridges. Samples were stored at -80°C until analysis.

DNA extraction and pyrosequencing

Samples from the continuous culture experiment and from the different sites were further analyzed together. A combined DNA-RNA extraction was performed on the 0.2 μm fractions after lysis and purification steps, as described in Chapters 1 and 2. A random retrotranscription step was performed on RNA for *in situ* samples immediately after extraction, using the SuperScript® VILO™ cDNA Synthesis Kit (Invitrogen) according to the manufacturer's instructions. A PCR was performed on RNA samples to verify their purity.

In total, 73 samples were sent to the Molecular Research DNA laboratory (http://www.mrdnalab.com/) for 454 pyrosequencing of the 16S rRNA gene and transcripts. We used universal bacterial primers 27F-530R, targeting hypervariable regions V1 to V3. Further analyses were conducted with the obtained sequences as described in the Methodology chapter.

III. RESULTS

1. The continuous cultures experiment

1) *Bulk parameters*

Inorganic nutrients and dissolved organic carbon (DOC)

For each experimental condition, nitrate and phosphate concentrations were identical in the input medium and the cultures (Student test, $p>0.05$), indicating bacterial consumption was within the detection limit in all cultures (Table 2).

Table 2. Concentrations of inorganic nutrients and DOC in the input media and the continuous cultures. Mean values ± SD of measurements performed during the 19 d experimental period are given. For the input media, the mean values are based on measurements performed in each of the freshly prepared media over the course of the experiment. For the continuous cultures, the average of the triplicate incubations was used at each time point to calculate the mean value. For DOC concentrations in the cultures, only the stable phase was considered.

	MEDIUM		**CULTURES**	
	Control	+DOM	Control	+DOM
NO_3^- (µM)	39.3±1.5 (n=8)	41.4±1.3 (n=8)	40.3±1.6 (n=9)	41.9±1.3 (n=9)
PO_4^{3-} (µM)	2.1±0.3 (n=8)	2.0±0.5 (n=8)	2.3 ±0.2 (n=9)	1.8±0.1 (n=9)
DOC (µM)	48.9±1.3 (n=7)	48.5±1.6 (n=7)	52.1±1.1 (n=5)	49.6±1.2 (n=6)

In the first days of the experiment, DOC concentrations decreased rapidly in all cultures (Figure 6). From day 7 (in the +DOM treatment) and day 10 (in the control) to the end of the experiment, DOC concentrations remained relatively stable and were significantly higher in the control (52±1, n=5) than in the +DOM condition (50±1, n=6) (Student test, p≤0.05) (Table 2 and Figure 6). However, no significant decrease in DOC could be observed between the media and the cultures in both conditions, indicating that DOC consumption in the cultures was in the range of the detection limit (Table 2). The higher average DOC in the control cultures compared to the input medium also suggests some bacterial production in these incubations.

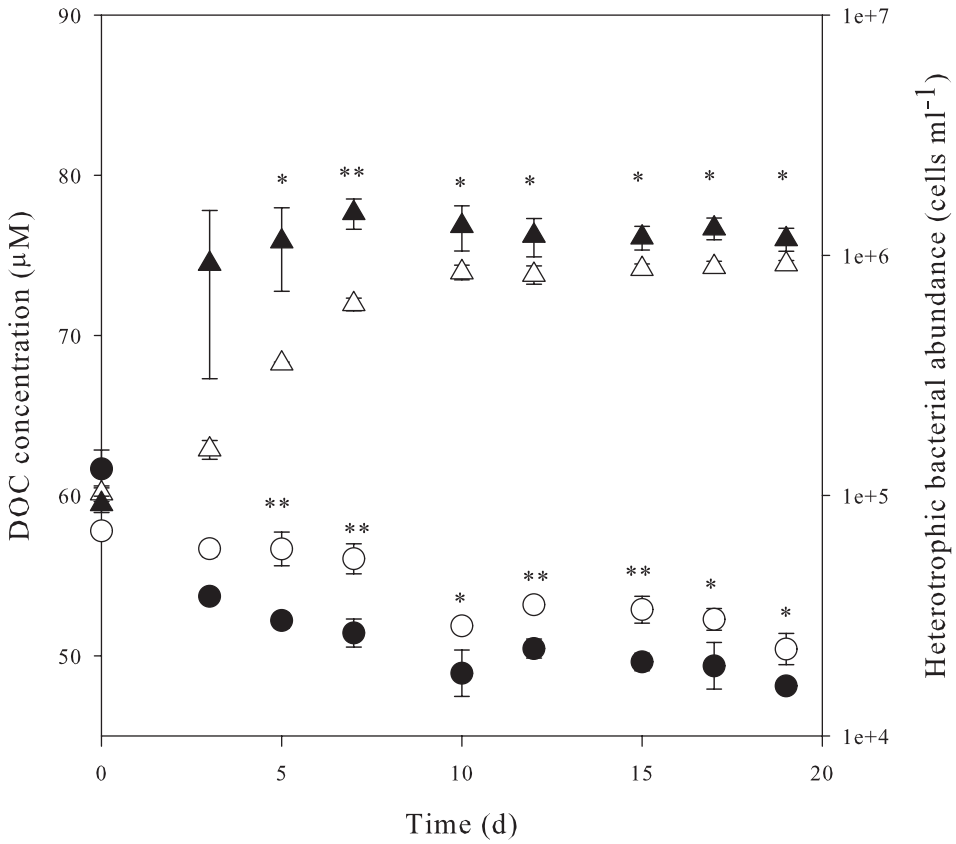


Figure 6. Abundance of heterotrophic bacteria (triangles) and DOC concentration (circles) in the control (white) and the DOM-amended (black) cultures over time. Mean values ± SD of biological triplicates are shown for each time point.

*** and ** indicate significant (p<0.05) and highly significant (p>0.005) differences according to a Student test.**

C:N and C:P ratios were similar in the two treatments (1:1 and 23:1, respectively, data not shown), and below the C:N and C:P ratios of heterotrophic bacteria (5:1 and 45:1) (Goldman et al., 1987), confirming that heterotrophic bacteria were C-limited in these incubations.

Heterotrophic bacterial abundance

In the first days of the experiment, bacterial abundances increased in all cultures (Figure 6). Bacterial abundances stabilized after 5 days in the +DOM treatment, more rapidly than in the control treatment where they were stable after 10 days. Bacterial abundances also reached significantly higher values in the +DOM treatment (1.26±0.1 x 10^6 cells ml^{-1}, n=7) than in the control (8.7±0.3 x 10^5 cells ml^{-1}, n=5) (Student test, $p<0.001$). This result is consistent with the lower DOC concentrations in the +DOM treatment, indicating bacteria benefited from the *Chaetoceros* exudate.

2) Bacterial diversity

Comparison of the communities growing in each treatment

Samples for bacterial diversity were taken after 5, 10, 15 and 19 days (corresponding to 1, 2, 3 and 4 bacterial generation times) and analyzed with 454 Titanium pyrosequencing of the 16S rRNA genes. A clustering of the samples performed with a weighted UNIFRAC distance matrix using an average linkage clustering method indicates a clear effect of DOM addition on bacterial communities (Figure 7), as confirmed by statistical analyses (ANOSIM, p=0.001).

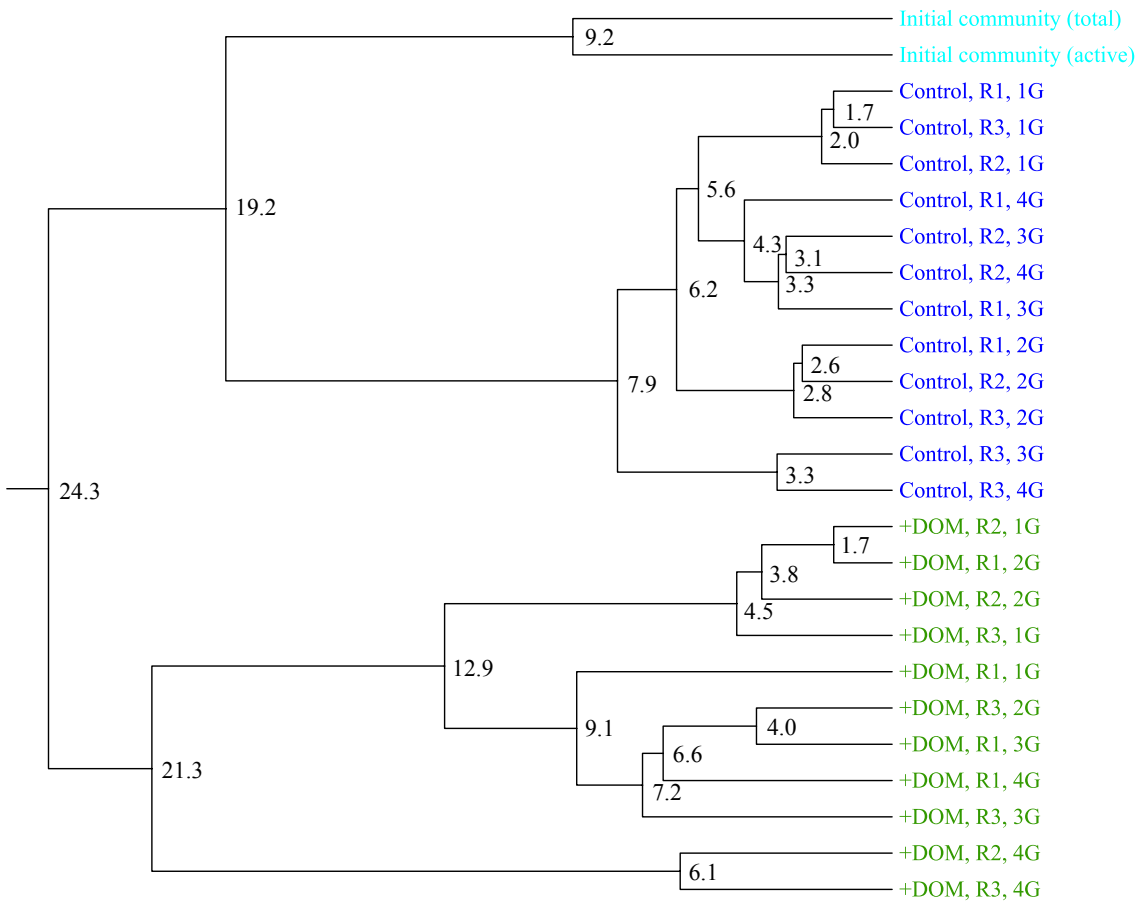


Figure 7. Clustering of the initial community used as an inoculum and the communities sampled in each of the six cultures after 1, 2, 3 and 4 bacterial generation times (G). The numbers indicate the percent dissimilarity at each node. Replicate 2 (R2) at 3G was excluded from the dataset due to its low number of sequences (476).

Samples from the control cultures clustered closer to the initial community used as inoculum (19% dissimilarity) than did samples from the DOM-amended cultures (24% dissimilarity). Overall, bacterial communities developing in each experimental condition were similar to each other. This was particularly the case in the control cultures, where bacterial communities were less than 8% different from each other over the course of the experiment. In the DOM-amended cultures, most communities clustered together with less than 12% dissimilarity, except for two out of three of the

replicate cultures sampled at the end of the experiment, that were 21% different to the other +DOM samples (Figure 7).

Phylogeny of bacterial communities

Overall, communities developing in the DOM-amended cultures were phylogenetically different from those developing in the control cultures (Figure 8), as confirmed by a phylogenetic test ($p \leq 0.05$). The phylogenetic variability between the replicate cultures of each treatment was in 15 out of 20 cases not significant ($p > 0.05$).

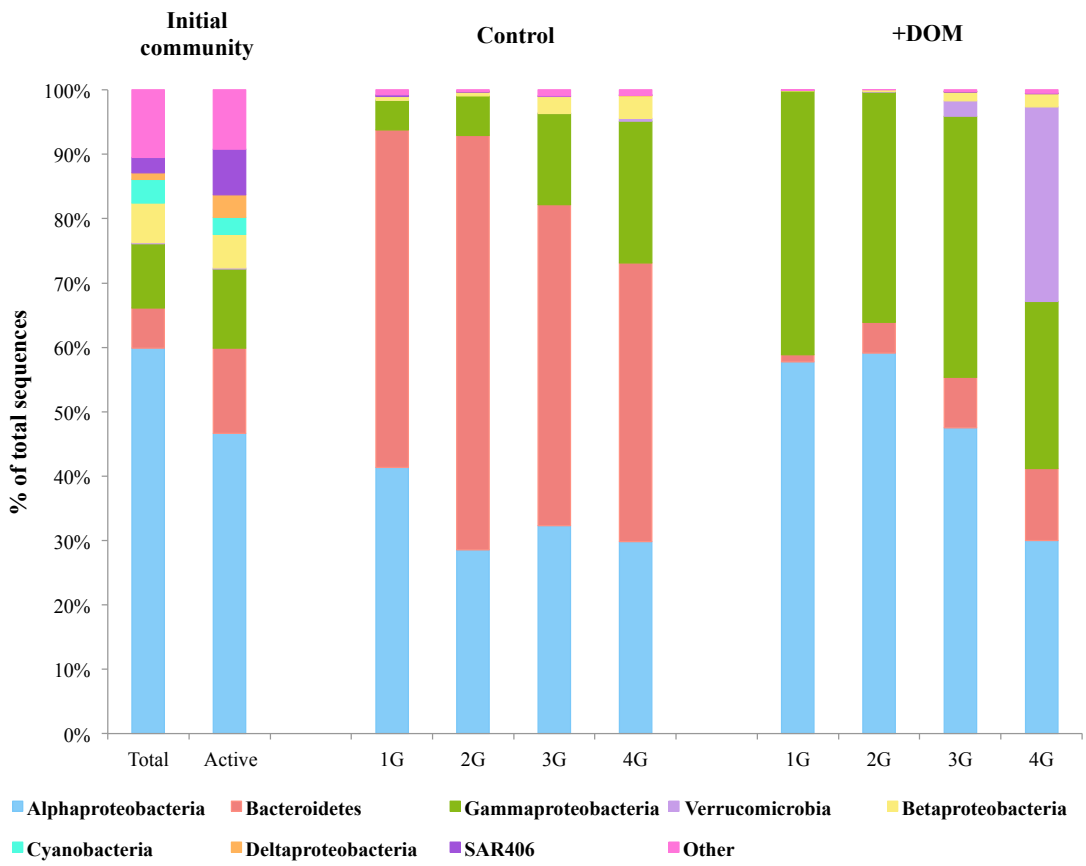


Figure 8. Relative abundances of broad phylogenetic groups in the initial community (total and active) and in the communities sampled in the control and the DOM-amended cultures after 1, 2, 3 and 4 bacterial generation times (G). For the continuous cultures, the mean value calculated from the triplicate cultures is given.

The broad groups developing in the cultures were drastically different between conditions. The DOM-amended cultures were dominated by sequences belonging to the Alphaproteobacteria and Gammaproteobacteria, with a large contribution of Verrucomicrobia at the end of the experiment. By contrast, Bacteroidetes dominated in the control, followed by Alphaproteobacteria, in lower relative abundances than in the +DOM treatment. Some Gammaproteobacteria were also detected in the control, especially at the end of the experiment.

Interestingly, the relative contributions of broad groups to the control did not vary much over time, suggesting little temporal dynamics in the system, at least at this phylogenetic level. In contrast, the relative abundances of broad groups in the DOM-amended cultures were different after 1 and after 4 bacterial generations, indicating the phylogenetic component of the bacterial response to DOM addition was more variable over time (Figure 8).

Diversity patterns within Alphaproteobacteria

The analysis of these groups at a finer phylogenetic level further confirmed that bacteria developing in the cultures were not the same in the control and in the +DOM treatment. Within Alphaproteobacteria, two families exhibited different responses in the two conditions. First, the Pelagibacteraceae or SAR11, that were abundant in the initial community, appeared to maintain high relative abundances in the control (between 20 and 33% of total sequences, Figure 9). By contrast, they only reached between 5 and 13% of total sequences in the DOM-amended cultures, indicating that in this experimental condition they were outcompeted by more successful groups. Second, the Rhodobacteraceae family (containing the group of *Roseobacter*) was found in the two

treatments, with higher abundances in the +DOM treatment. Interestingly, the genera found in the control and in the +DOM treatment were not the same. *Phaeobacter* was the only member of the Rhodobacteraceae developing in the +DOM treatment and accounted for up to 51% of total sequences. This genus was barely detected in the control, where sequences assigned to Rhodobacteraceae mostly belonged to *Thalassobius* (and *Loktanella*), which represented the majority of the Rhodobacteraceae in the initial community. *Thalassobius* was also detected in the DOM-amended cultures but with smaller abundances (Figure 9).

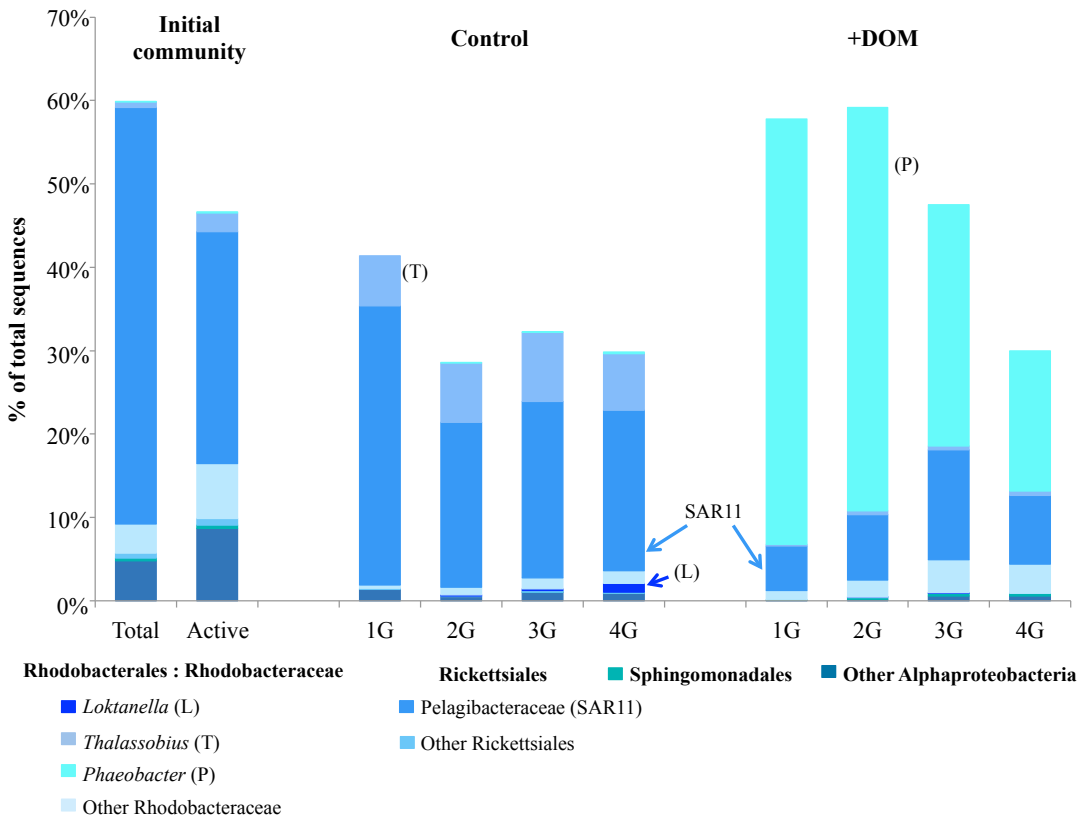


Figure 9. Relative abundances of the different groups within the class of Alphaproteobacteria in the initial community (total and active) and in the communities sampled in the control and the DOM-amended cultures after 1, 2, 3 and 4 bacterial generation times (G). For the continuous cultures, the mean value calculated from the triplicate cultures is given.

A deeper inspection of the genera and families specifically responding to the treatment revealed distinct behaviors within Alphaproteobacteria. At 97% identity, a total of 75 OTUs within the Pelagibacteraceae could be found in the dataset. More than half (39) of these OTUs contributed to ≥ 0.1% of the total community in at least one treatment at one time point (Figure 10). We identified one dominant OTU (OTU 1582) with relative abundances ranging between 3.7% and 22.8% of total sequences. Most of the remaining OTUs had relative abundances ≤1%. The majority of the Pelagibacteraceae OTUs had higher relative abundances in the control than in the +DOM treatment, except for a few OTUs at the end of the experiment (Figure S1).

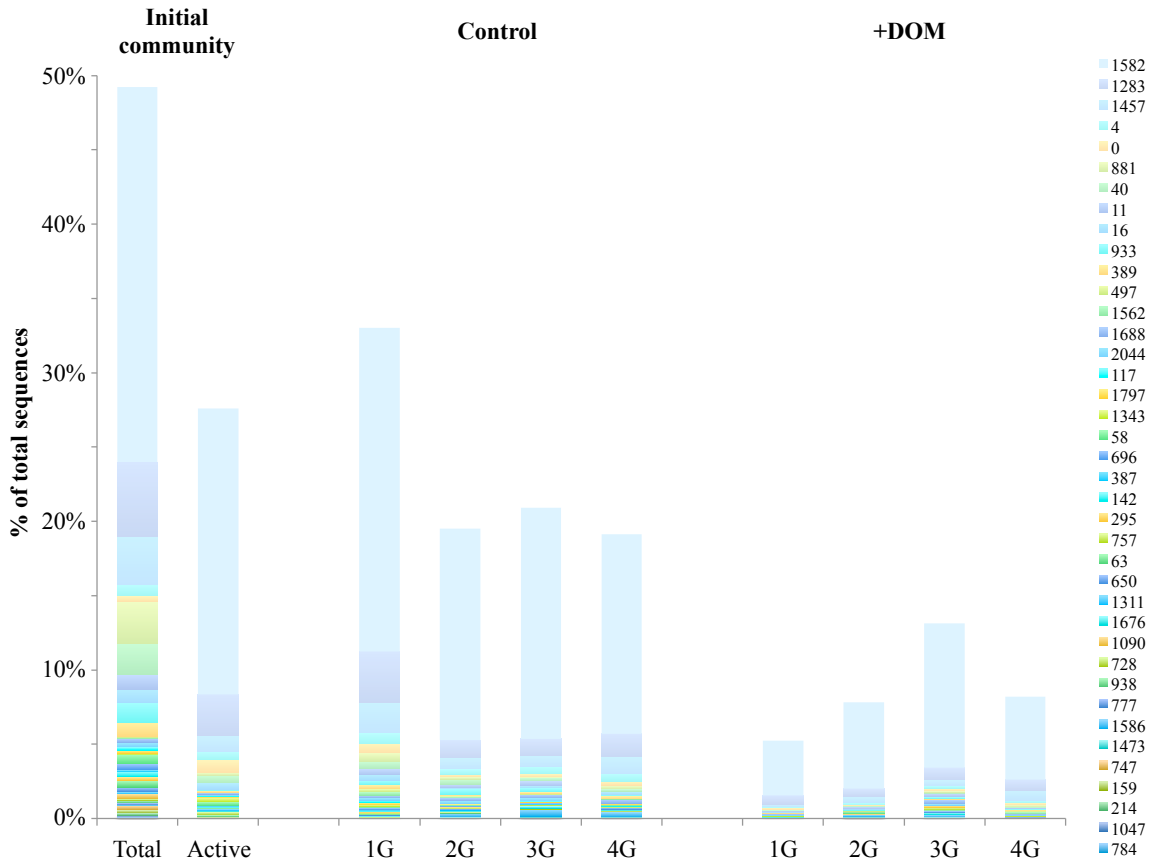


Figure 10. Relative abundances of the OTUs belonging to the Pelagibacteraceae, in the initial community (total and active) and in the communities sampled in the control and the DOM-amended cultures after 1, 2, 3 and 4 bacterial generation times (G). For the continuous cultures, the mean value calculated from the triplicate cultures is given. Only the 39 OTUs representing ≥0.1% of total community in at least one of the conditions and time points are represented. The numbers refer to the OTUs identity in the QIIME-generated OTU table.

Thus, the lower contribution of SAR11 to the communities in the +DOM treatment did not result from the decrease of a few specific OTUs, in particular the dominant one, but was the consequence of a general lack of competitiveness of all the OTUs in this group under the DOM-enriched condition.

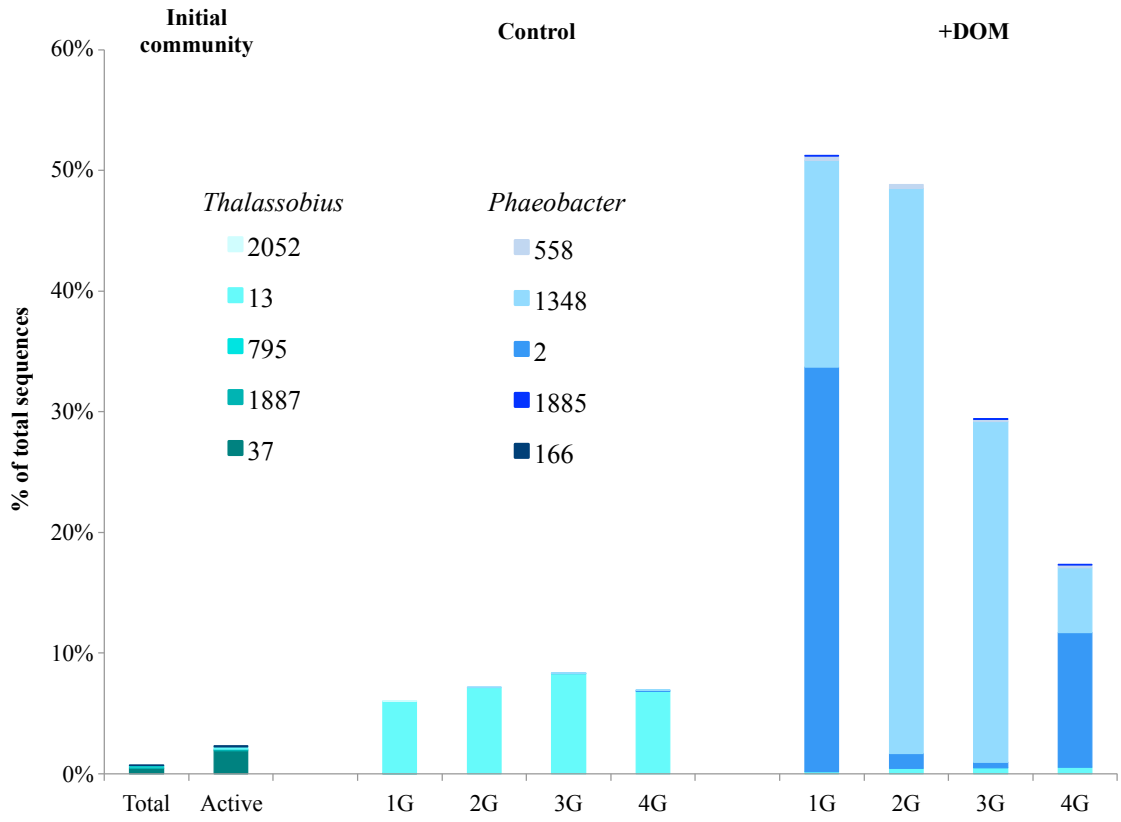


Figure 11. Relative abundances of the OTUs belonging to *Phaeobacter* and *Thalassobius* in the initial community (total and active) and in the communities sampled in the control and the DOM-amended cultures after 1, 2, 3 and 4 bacterial generation times (G). For the continuous cultures, the mean value calculated from the triplicate cultures is given. The numbers refer to the OTUs identity in the QIIME-generated OTU table.

The treatment-induced response of the Rhodobacteraceae was markedly different from that of the Pelagibacteraceae. *Phaeobacter* and *Thalassobius*, the genera with remarkable differences between treatments, were less diverse than the family of

Pelagibacteraceae and were each constituted of 5 OTUs in total. One OTU, identified as *Thalassobius,* was abundant in the control, and it was detected with much lower abundances in the +DOM treatment (Figure 11, OTU 13). By contrast, in the +DOM treatment, 2 OTUs identified as *Phaeobacter* were abundant, making up to 50% of total sequences, and these OTUs were detected in smaller abundances in the control (Figure 11, OTUs 2 and 1348). The other OTUs in the family of Rhodobacteraceae were detected only occasionally in the samples.

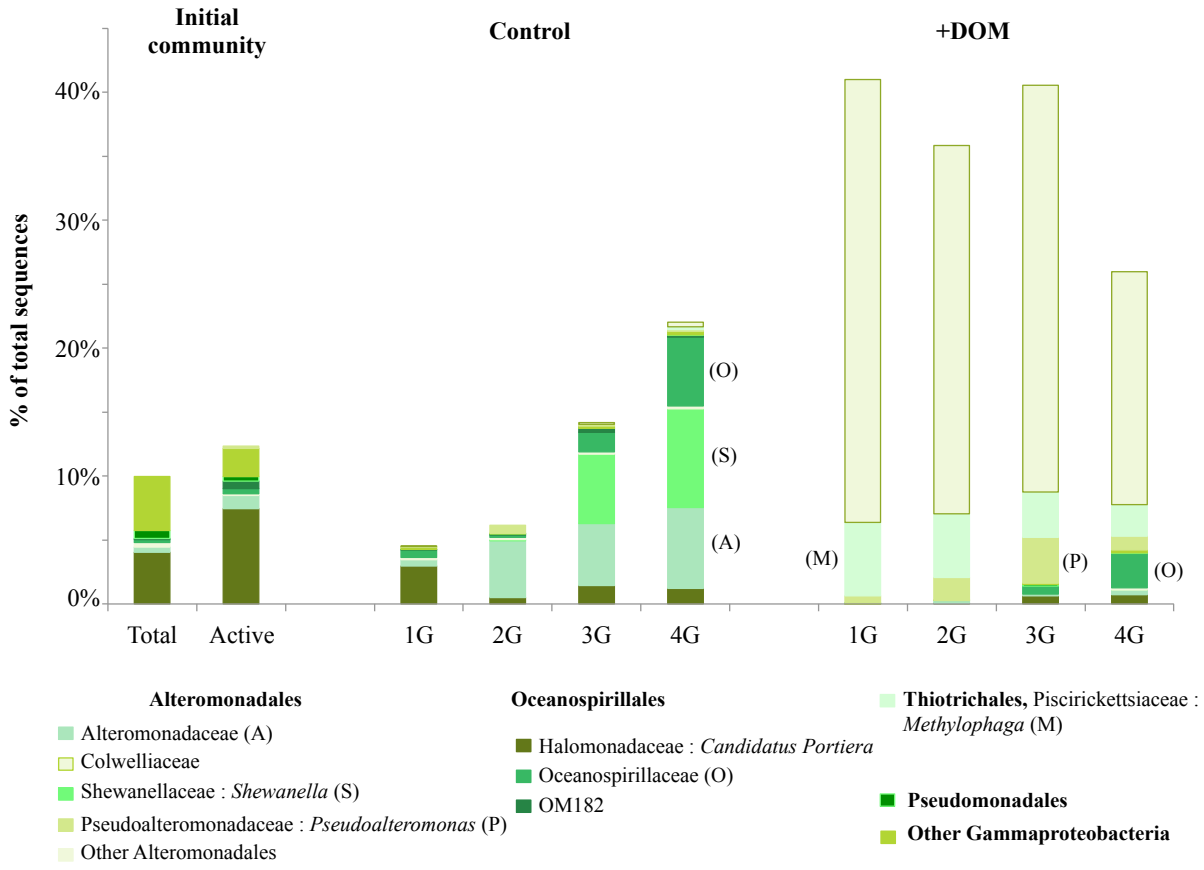


Figure 12. Relative abundances of the different groups within the class of Gammaproteobacteria in the initial community (total and active) and in the communities sampled in the control and the DOM-amended cultures after 1, 2, 3 and 4 bacterial generation times (G). For the continuous cultures, the mean value calculated from the triplicate cultures is given.

Diversity patterns within Gammaproteobacteria

The different orders within the Gammaproteobacteria also presented various response patterns (Figure 12).

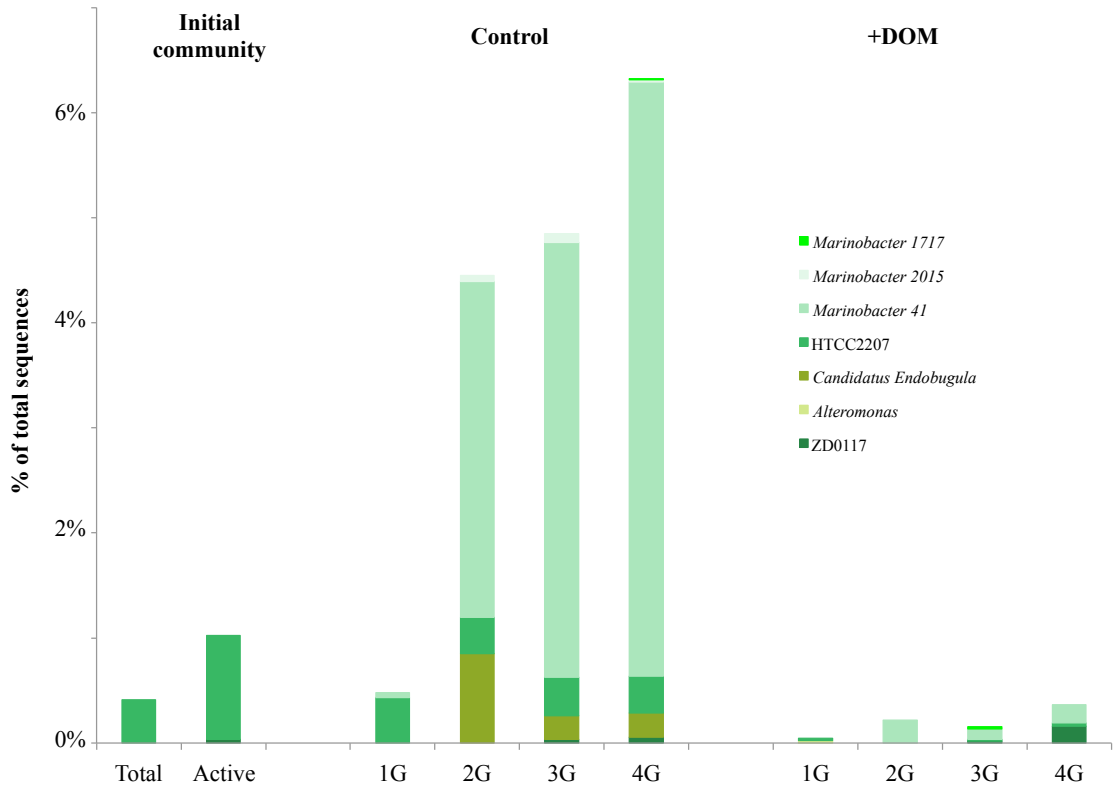


Figure 13. Relative abundances of the different group within the order of Alteromonadaceae, in the initial community (total and active) and in the communities sampled in the control and the DOM-amended cultures after 1, 2, 3 and 4 bacterial generation times (G). For the continuous cultures, the mean value calculated from the triplicate cultures is given. For the genus *Marinobacter*, the three OTUs detected in the dataset are shown. The numbers refer to the OTUs identity in the QIIME-generated OTU table.

Groups within the order Alteromonadales responded differently to the experimental conditions: the family Alteromonadaceae and the genus *Shewanella* were more successful in the control than in the +DOM-treatment, where *Pseudoalteromonas* and the family Colwelliaceae dominated the Alteromonadales sequences and the

Gammaproteobacteria in general. Oceanospirillales were detected in the two treatments, but had higher relative abundances in the control. Interestingly, all the families detected in this order (Halomonadaceae, Oceanospirillaceae and OM182) were more abundant in the control than in the +DOM treatment, suggesting similar ecological niches among the members of this order (Figure 12). By contrast, Pseudomonadales were barely detected in the cultures (< 0.2% of total sequences), indicating this group of Gammaproteobacteria was not successful in these cultures regardless of treatment.

Within Alteromonadales, Alteromonadaceae behaved similarly to the Pelagibacteraceae: three out of the four different genera detected were present in the two treatments and had higher relative abundances in the control (Figure 13). A closer look to the genus *Marinobacter* reveals that 3 OTUs were detected within this group. Only one of them (OTU 41) displayed different relative abundances between treatments (Figure 13).

The detail of the OTUs identified as *Shewanella*, a genus that was enhanced in the control compared to the +DOM treatment, reveals even more pronounced differences (Figure 14A). Of the 5 OTUs constituting the group, only 2 (OTUs 49 and 2003) were abundant in the control, the others had overall low relative abundances. They were completely absent from the +DOM treatment, except for OTU 49, detected in extremely low numbers at the end of the experiment.

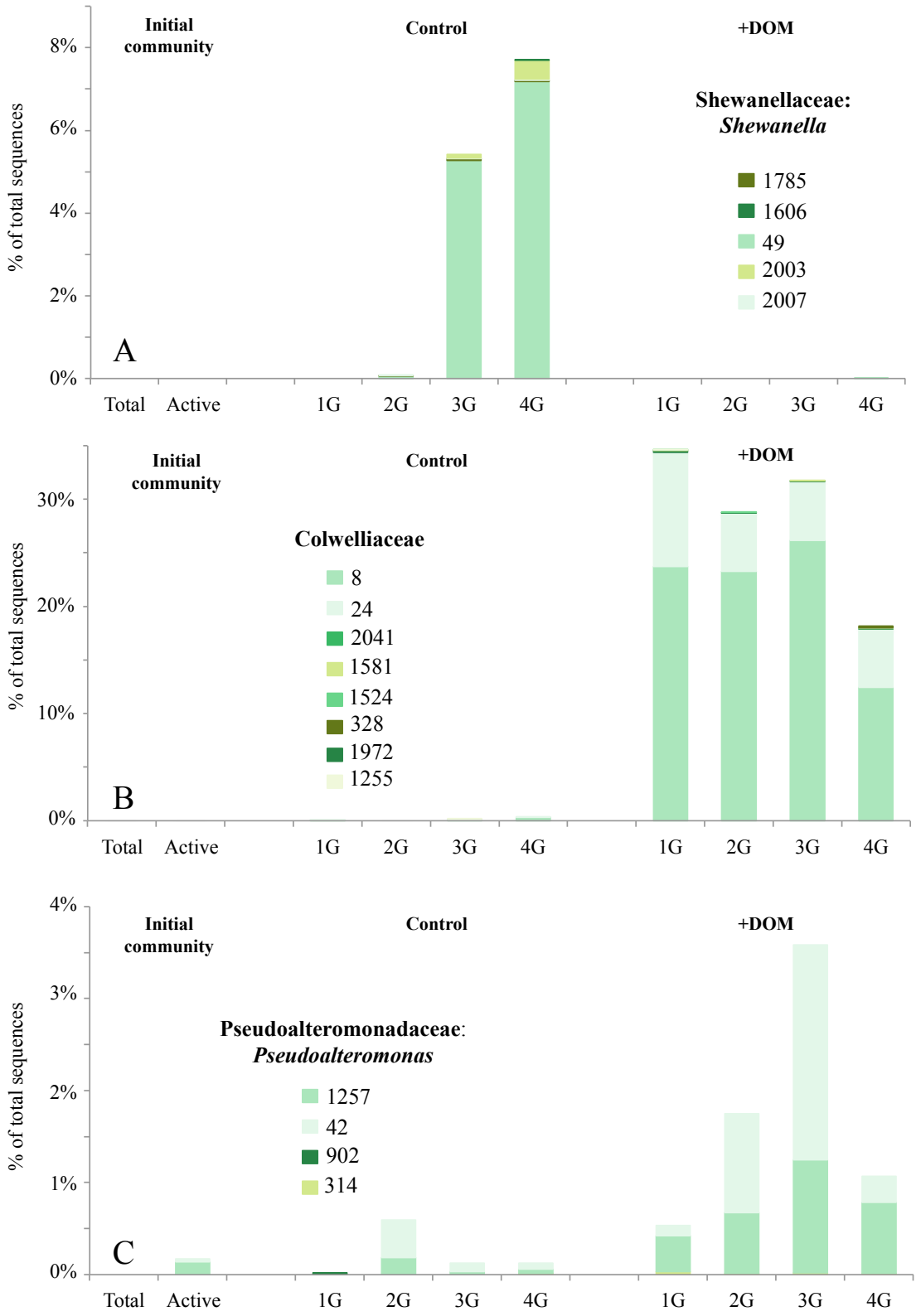


Figure 14. Relative abundances of OTUs belonging to the genus *Shewanella* (A), the Colwelliaceae (B) and the Pseudoalteromonadaceae (C), in the initial community (total and active) and in the communities sampled in the control and the DOM-amended cultures after 1, 2, 3 and 4 bacterial generation times (G). For the continuous cultures, the mean value calculated from the triplicate cultures is given. The numbers refer to the OTUs identity in the QIIME-generated OTU table.

Conversely to the Alteromonadaceae and the Shewanellaceae, two other families in the order of the Alteromonadales were enhanced in the +DOM treatment: the Pseudoalteromonadaceae and the Colwelliaceae (Figure 14 B and C). Eight OTUs were found in the Colwelliaceae, 2 of which were highly abundant in the +DOM treatment, and feebly detected in the control at the end of the experiment (Figure 14B, OTUs 8 and 24). The Pseudoalteromonadaceae presented a pattern similar to the one observed for the *Marinobacter* genus: out of the 4 OTUs detected in the dataset, two were abundant in the +DOM treatment, and also detected in smaller abundances in the control (Figure 14C, OTUs 42 and 1257).

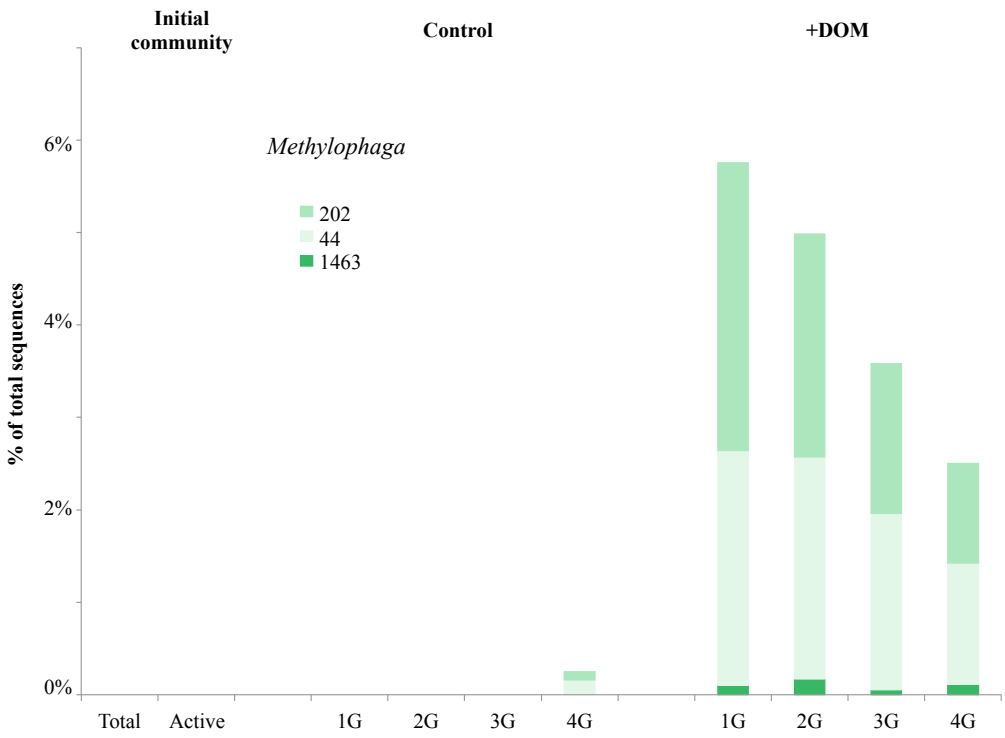


Figure 15. Relative abundances of the three OTUs belonging to the genus *Methylophaga*, in the initial community (total and active) and in the communities sampled in the control and the DOM-amended cultures after 1, 2, 3 and 4 bacterial generation times (G). For the continuous cultures, the mean value calculated from the triplicate cultures is given. The numbers refer to the OTUs identity in the QIIME-generated OTU table.

The order of the Thiotrichales was exclusively found in the +DOM treatment at the exception of the last time point where it became detectable in the control. There were 3 OTUs within this order, all identified as *Methylophaga*, and two of them were equally abundant, the third one remaining minor (Figure 15).

Diversity patterns within the phylum Bacteroidetes

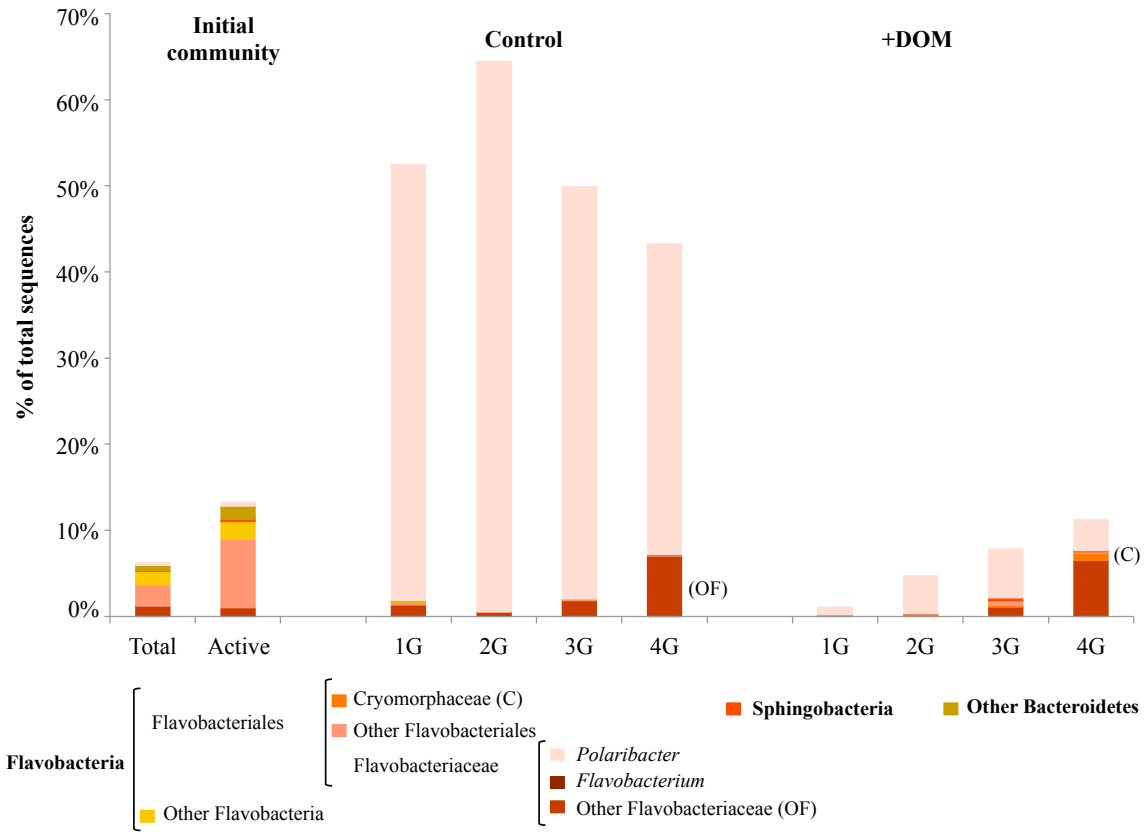


Figure 16. Relative abundances of the different group within the phylum of Bacteroidetes, in the initial community (total and active) and in the communities sampled in the control and the DOM-amended cultures after 1, 2, 3 and 4 bacterial generation times (G). For the continuous cultures, the mean value calculated from the triplicate cultures is given.

Bacteroidetes varied the most among treatments (Figure 16). The genus *Polaribacter* was up to 60 times more abundant in the control than in the +DOM treatment and thereby accounted for most of the differences between the two treatments. Some other sequences, such as those identified as Cryomorphaceae or Sphingobacteria, were enhanced in the +DOM treatment, but in low abundances. Other sequences, such as those identified as Flavobacteriaceae that did not belong to the *Polaribacter* or the *Flavobacterium* genera, were found in similar abundances in the two treatments.

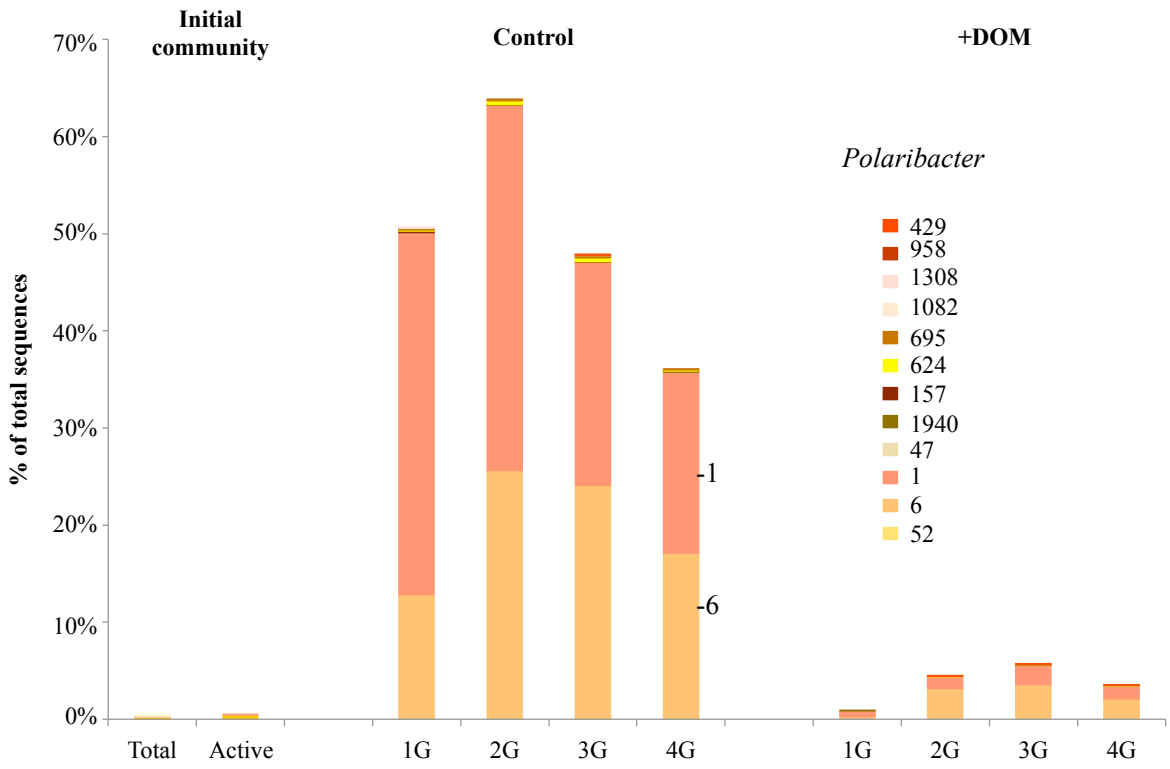


Figure 17. Relative abundances of the twelve OTUs belonging to the genus *Polaribacter*, in the initial community (total and active) and in the communities sampled in the control and the DOM-amended cultures after 1, 2, 3 and 4 bacterial generation times (G). For the continuous cultures, the mean value calculated from the triplicate cultures is given. The numbers refer to the OTUs identity in the QIIME-generated OTU table.

Twelve OTUs were detected within the *Polaribacter* genus, two of them contributing up to 63% of the total community in the control (Figure 17, OTUs 1 and 6). These OTUs were also detected but did not exceed 6% of the total community in the +DOM treatment. The other 10 OTUs were only occasionally detected in any of the cultures.

Diversity patterns of other groups

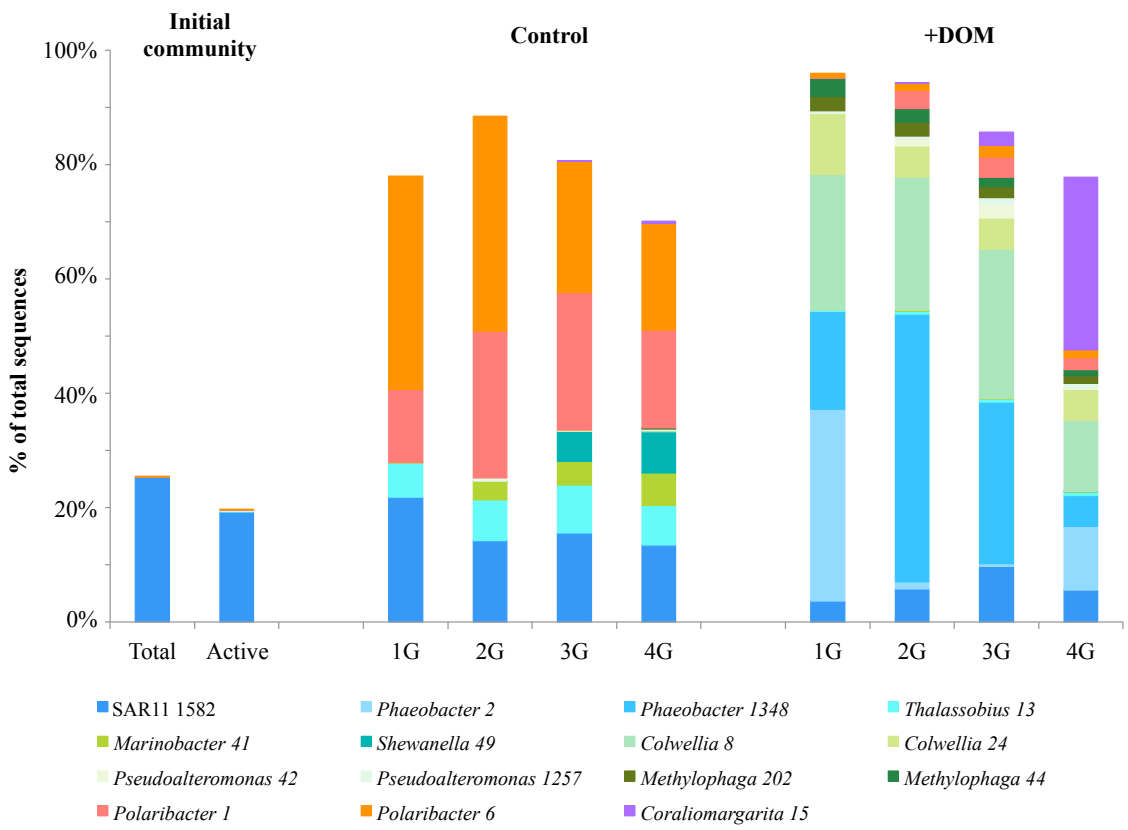


Figure 18. Relative abundances of 15 OTUs displaying major differences between treatments. Relative abundances are shown in the initial community (total and active) and in the communities sampled in the control and the DOM-amended cultures after 1, 2, 3 and 4 bacterial generation times (G). For the continuous cultures, the mean value calculated from the triplicate cultures is given. The numbers refer to the OTUs identity in the QIIME-generated OTU table.

The Verrucomicrobia were detected in the +DOM treatment at the end of the experiment, reaching 30% of the total number of sequences and accounting for <1% of the total community in the control (Figure S2). Interestingly, only one OTU was detected in this phylum.

Overall, phylogenetic differences between treatments were detected in all phylogenetic groups, at a variety of phylogenetic levels, from broad groups to individual OTUs. Only 15 OTUs explained the majority of the differences observed, and accounted for up to 96% of the total number of sequences (Figure 18).

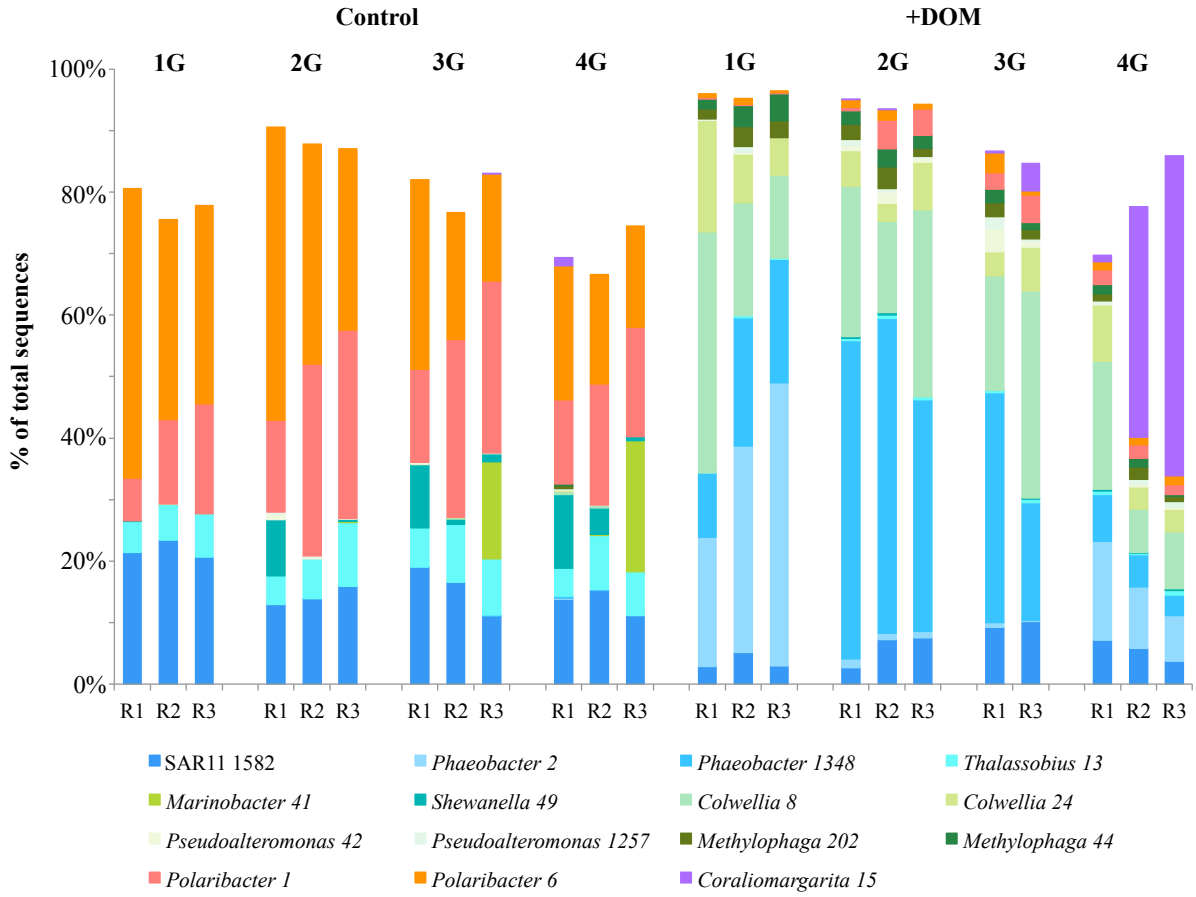


Figure 19. Relative abundances of 15 OTUs displaying major differences between treatments, in the communities sampled in the control and the DOM-amended cultures after 1, 2, 3 and 4 bacterial generation times (G). The value for each replicate culture (R1, R2 and R3) is given. The numbers refer to the OTUs identity in the QIIME-generated OTU table.

In most cases, these OTUs were found in all replicates of a given treatment, in relatively identical relative abundances (Figure 19). However, some variability was observed, especially for a few OTUs such as *Coraliomargarita* 15 and *Marinobacter* 41, that were found in some replicates only, indicating a weak link between the presence of these OTUs and the treatment they were detected in. For the rest of these OTUs, the replicability of their presence and relative abundance among the replicate cultures indicates strong associations to a treatment or a time point (Figure 19).

Diversity levels of total communities and individual broad groups

According to several diversity indices, communities appeared to be more diverse in the control than in the +DOM treatment at the beginning of the experiment, with variable degrees of significance depending on the index and time point considered (Figure S3). However, from day 15 (3 bacterial generations) such differences were no longer observed, indicating that once bacterial abundances had become stable in the two treatments, the communities characterizing each treatment did not display specific diversity patterns. Moreover, after the second bacterial generation, there were more OTUs >1% of the total community in the +DOM treatment (Table 3).

Table 3. Number of OTUs >1% of the total community in the continuous cultures. The numbers correspond to each replicate culture.

Generation times	**Control**	**+DOM**
1	8, 10, 10	7, 7, 7
2	7, 5, 6	10, 11, 8
3	8, 10, 10	11, 10
4	12, 13, 12	16, 15, 10

Interestingly, the calculation of diversity indices for the main broad groups confirmed the differences in these groups' responses to DOM addition. Alphaproteobacteria displayed a pattern similar to the one observed for total communities, with higher diversity in the control than in the +DOM treatment (Figure S4). The pattern was even more pronounced, as the Alphaproteobacteria remained more diverse in the control than in the DOM-amended cultures during the first ten days, after which their diversity in the +DOM treatment increased and reached the levels observed for the control. By contrast, Gammaproteobacteria did not display any difference in diversity between the two treatments (Figure S5). Finally, Bacteroidetes were overall more diverse in the +DOM treatment than in the control, although the difference was not always significant (Figure S6).

2. Bacterial activity and diversity at naturally iron-fertilized sites off the Kerguelen Islands

Bloom stages above the Kerguelen plateau (Station A3)

During KEOPS1 at the late stages of the bloom, the A3 site was visited 4 times (Figure 20). The same station was visited twice during KEOPS2. As confirmed by chl *a* concentrations, the first visit (A3-1) occurred before the start of the bloom, and the second visit, three weeks later (A3-2), occurred during the bloom development. Taken together, the two KEOPS cruises provide key information on the first stages of the Kerguelen bloom (KEOPS2) and the late phase when the bloom begins to collapse (KEOPS1).

Concentrations of chl *a* in the MLD increased from 0.6 µg l^{-1} during our first visit (A3-1) to 2 µg l^{-1} 3 weeks later (A3-2), and chl *a* decreased from A3-3 to A3-4, indicating the onset and decline of the bloom. Bacterial production followed the same pattern at the beginning of the bloom, increasing from 5 to 20 ng C l^{-1} h^{-1} between the first and the second visit. The values determined at the end of the bloom were much higher, between 47 and 128 ng C l^{-1} h^{-1} measured at A3 during KEOPS1. Consistent with bacterial production patterns, bacterial abundances were also overall higher at the end of the bloom than at the beginning (Figure 20).

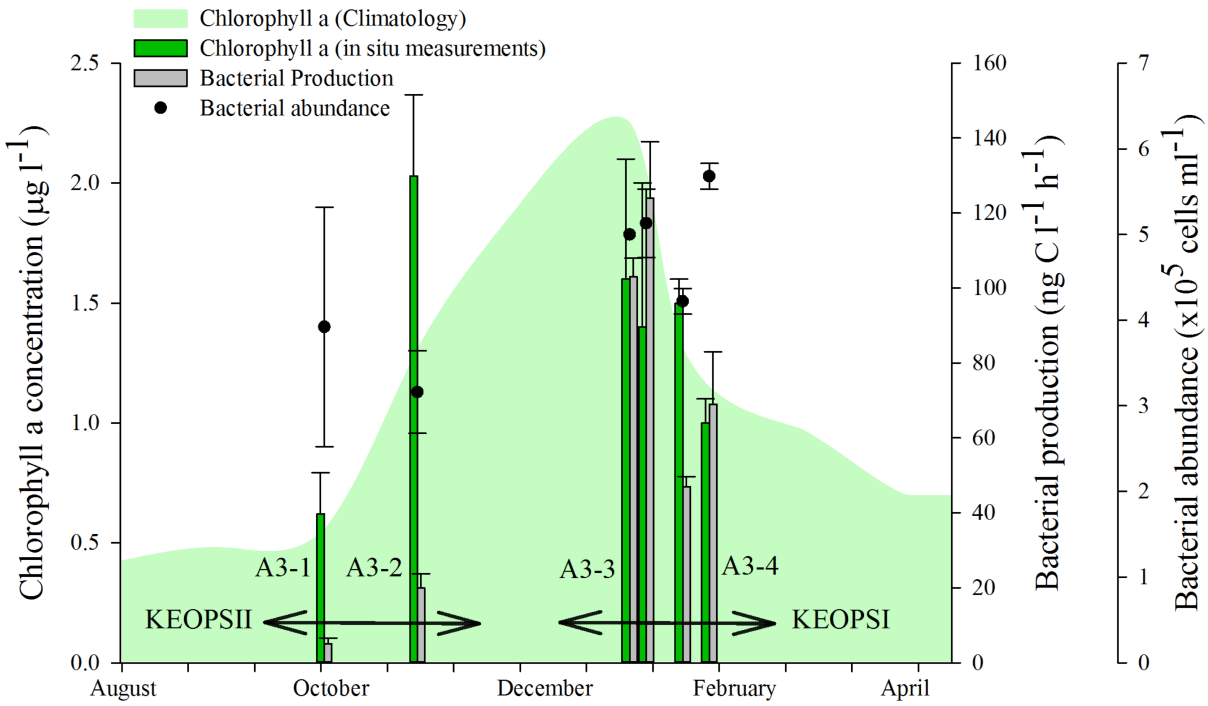


Figure 20. Chl *a* concentration, bacterial production and abundance at station A3, at the beginning (KEOPS2) and the end (KEOPS1) of the phytoplankton bloom above the Kerguelen plateau. For the chl *a* climatology, the mean values calculated from the concentrations determined at 13 m by satellite images for each month over the last 10 years are plotted. For *in situ* chl *a* concentration, bacterial production and bacterial abundance, mean values in the mixed layer are given.
Data come from the KEOPS database and were provided by J. Ras (LOV) for chla , P. Catala (LOMIC) for bacterial abundance and U. Christaki (LOG) for bacterial heterotrophic production.

Biogeochemical features of the study sites

In contrast to bacterial production and consistent with chl *a* concentrations, net primary production at Station A3 was higher at the beginning of the bloom (A3-2) than at the end (A3-3 and -4) (Figure 21).

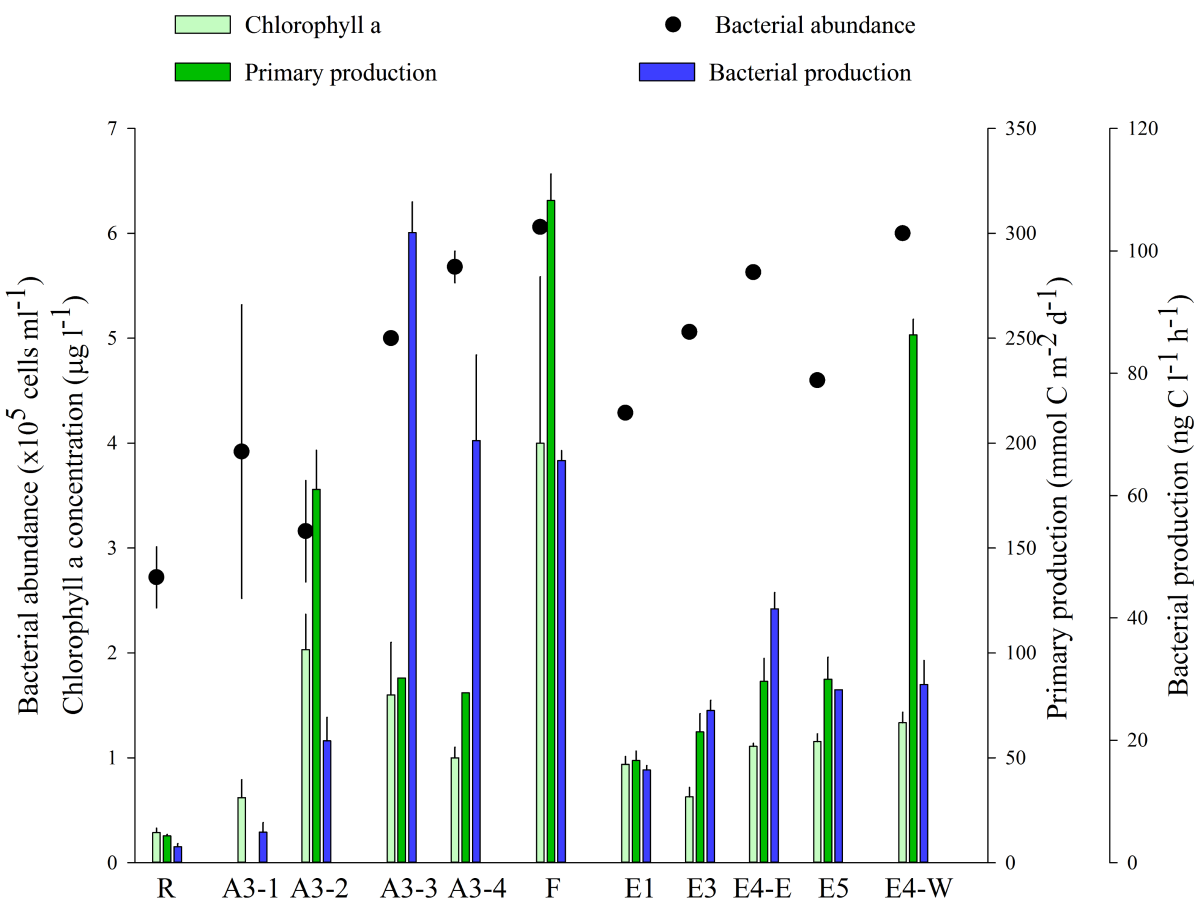


Figure 21. Chl *a* concentration, bacterial abundance, primary and bacterial productions at the different sites visited during KEOPS2, and the two visits to A3 during KEOPS1 (A3-3 and A3-4). The means ± SD in the mixed layer are given, except for primary production, which was integrated over the mixed layer.
Data come from the KEOPS database and were provided by J. Ras (LOV) for chl *a*, B. Griffiths (for KEOPS1) and A-J Cavagna (VUB) (for KEOPS2) for primary production, P. Catala (LOMIC) for bacterial abundance and U. Christaki (LOG) for bacterial heterotrophic production.

In offshore waters, the most productive site was F, also displaying the highest MLD chl a concentrations (4 μg l^{-1}) and bacterial production (66 ng C l^{-1} h^{-1}). The E-sites had chl *a* concentrations and primary production similar to those at A3-3 and A3-4, however bacterial production did not reach the values measured at these stations. Phytoplankton biomass and productivity were clearly enhanced at all iron-fertilized sites as compared to the reference site R. In offshore waters, trends in bacterial heterotrophic abundance and production basically followed those of phytoplankton, with highest values at Station F (Figure 21).

Bacterial diversity

Bacterial diversity was investigated in the surface waters of the different sites by 454 pyrosequencing of the 16S rRNA genes and transcripts. A consensus dendrogram was produced after a UPGMA clustering of the samples based on a weighted UNIFRAC distance matrix and jackknife analyses (Figure 22). As expected, samples from the continuous culture experiment clustered according to treatment and apart from *in situ* samples. Surprisingly though, the control samples were closer to *in situ* samples than to samples from the DOM-amended cultures. Within the branch containing *in situ* samples, some groups were noticeable, such as the samples corresponding to the active communities at the different bloom sites (A3, F and the E sites) that clustered with the total communities found at the end of the A3 bloom (KEOPS1 data). The samples from Stations A3-1 and R clustered together, and they were also similar to some samples from the different bloom sites (E1 and the two E4 stations, A3-2, F). Most *in situ* communities were less than 12% different from each other (9% for the active communities), the most

drastic differences being found between total and active communities (17%) (Figure 22).

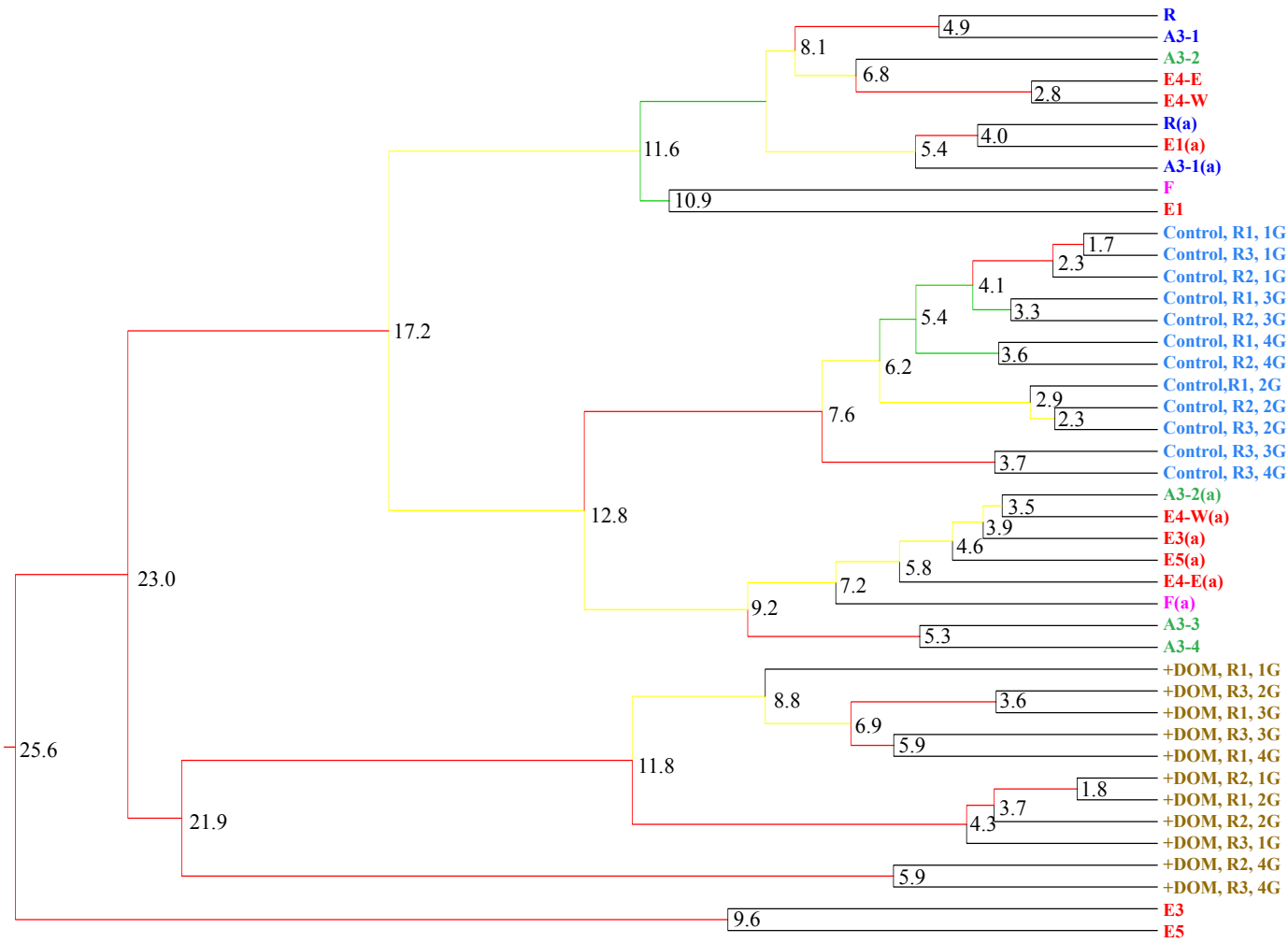


Figure 22. Consensus UPGMA clustering of the samples collected at the different sites and in the continuous culture experiment, according to a weighted UNIFRAC distance matrix. Branch color indicates node strength: 25-50% (green), 50-75% (yellow) and 75-100% (red) according to a bootstrap analysis. Samples were name-colored according to condition: HNLC site and A3 pre-bloom (dark blue), A3 bloom (green), E time series (red), Polar Front bloom (F, pink), experimental control (light blue), experimental +DOM treatment (brown). Active communities obtained from the sequencing of the 16s rRNA transcripts are indicated with an (a). Node numbers indicate the percentage of dissimilarity.

Bacterial community composition

The composition of the communities sampled at the different sites was relatively similar in terms of broad phylogenetic groups (Figure 23).

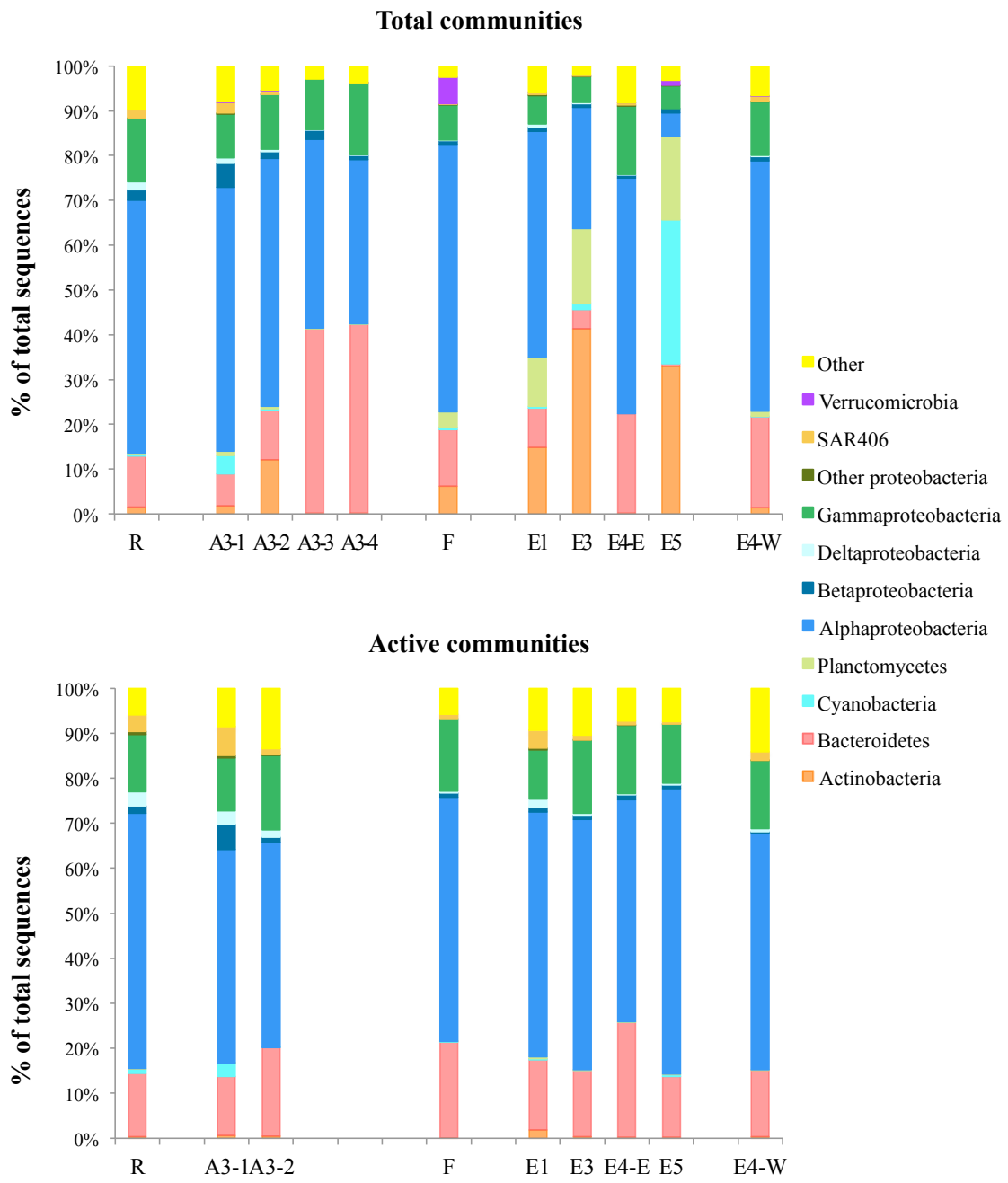


Figure 23. Relative abundances of broad phylogenetic groups in total (top) and active (bottom) communities at the study sites.

At all stations, Alphaproteobacteria largely dominated the communities, followed by Bacteroidetes. However, some differences could be observed, even at such low phylogenetic resolution. Bacteroidetes appeared to be enhanced in bloom conditions, particularly during the late stage (A3-3 and A3-4), compared to HNLC conditions. The E structure was characterized by high abundances of Planctomycetes, which were barely detected at other sites. A phylogenetic test confirmed that in most cases the communities at the various sites were different from each other (Table S1). In contrast to total communities, and as suggested by the percentage of dissimilarity indicated on the dendrogram, the active communities were much more similar to each other among the different sites, at the level of broad phylogenetic groups (Figure 23).

A detailed observation enables a more precise understanding of the bacterial groups responding to the different environmental conditions. The Bacteroidetes especially responded in productive sites such as the late A3 bloom (A3-3 and A3-4) and Stations E4-E and E4-W (Figure 24). Their overall diversity was conserved between sites. However, a few groups had large responses at the end of the A3 bloom, such as *Polaribacter* and sequences belonging to other Flavobacteriaceae. Interestingly, these two groups were also the ones that seemed to be more active at the beginning of the bloom (station A3-2) compared to pre-bloom conditions (A3-1 and R).

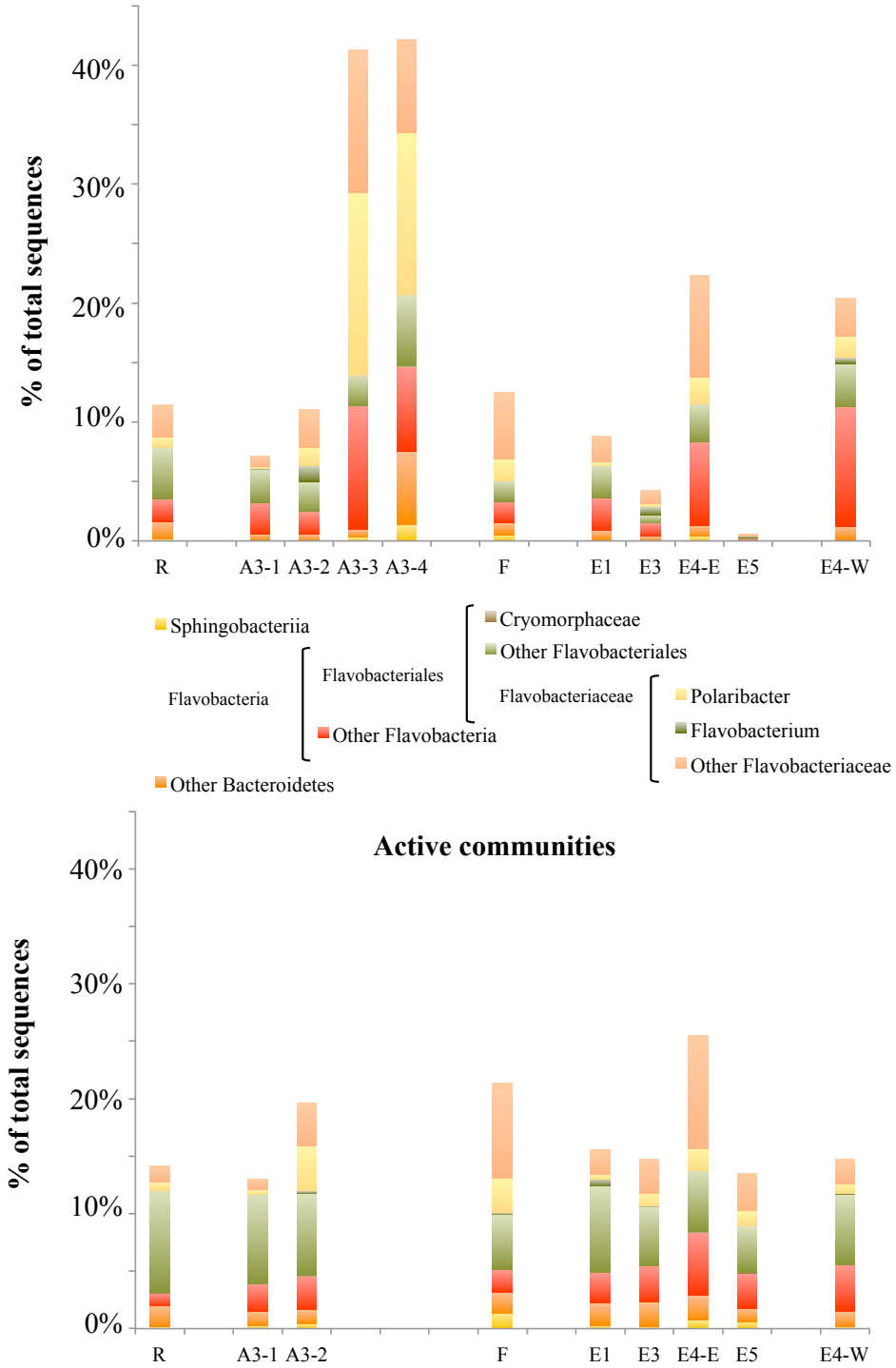


Figure 24. Relative abundances of the different group within the phylum of Bacteroidetes, in total (top) and active (bottom) communities at the study sites.

Overall, all members of this group were less abundant than predicted by their relative contribution to the active community, suggesting their final abundance was controlled by other factors (Figure 24).

The *Polaribacter* response, however, was due to only a few OTUs (Figure 25). Interestingly, the OTU found dominant at the end of the bloom at A3-3 and A3-4 (*Polaribacter* 6) was one of the two *Polaribacter* OTUs responding specifically to the control condition in the continuous culture experiment. In early stages of the bloom, this OTU was detected but was not dominant.

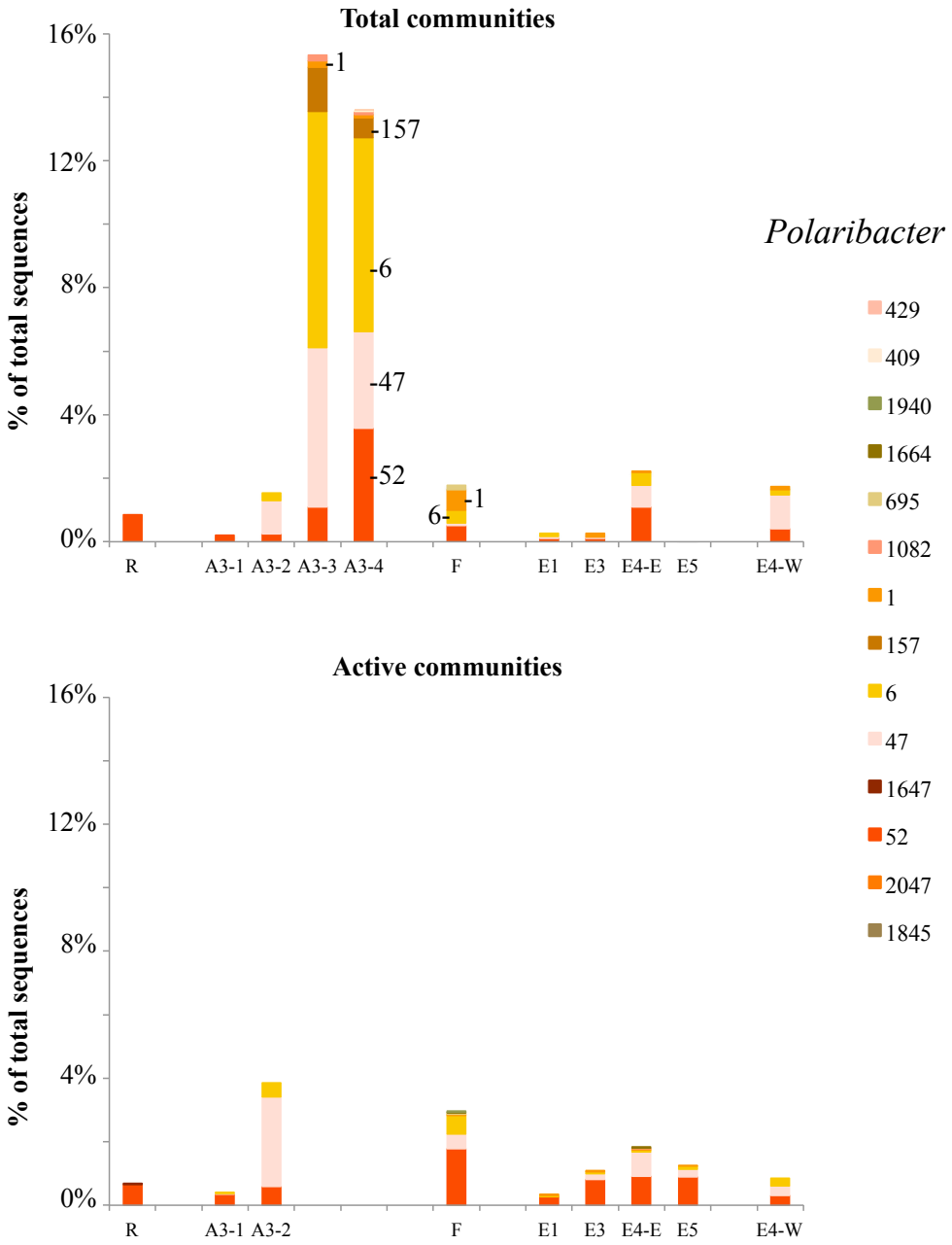


Figure 25. Relative abundances of OTUs belonging to the genus *Polaribacter*, in total (top) and active (bottom) communities at the study sites.

The other *Polaribacter* OTU responding to the experimental control condition (*Polaribacter* 1) was also detected at A3-3 and A3-4, as well as at other stations where the OTU 6 was present. A few other OTUs, such as *Polaribacter* 47 and *Polaribacter* 52, were also enhanced in bloom conditions such as A3, F, E4-E and E4-W, compared to the reference site R. These OTUs were also the most active ones at all sites within the *Polaribacter* genus, which could explain their competitiveness.

Within the 36 OTUs classified as "other Flavobacteriaceae", an identical pattern was observed: a large number of OTUs was present and only a few of them reached high abundances under bloom conditions. These abundant OTUs were the most active within the group, suggesting a strong link between bloom events, their metabolism and their final abundances in a given community (Figure S7).

Within the Alphaproteobacteria, the Pelagibacteraceae (order Rickettsiales) were extremely abundant in all samples, with the exception of the late stage of the A3 bloom, and station E5 despite their important contribution to the active community (Figure 26). As expected, the Pelagibacteraceae were very diverse: 103 OTUs were detected for all samples, 53 of which reached >0.1% of the total number of sequences in at least one sample (Figure S8). A few OTUs were dominant, and among those the most abundant one was OTU 1582. This OTU was also dominant in the continuous culture experiment, particularly in the control. As a matter of fact, this OTU collapsed at station A3-3 and -4, and displayed higher contributions to the active community at HNLC stations than at bloom stations (A3-2, F). The other Pelagibacteraceae OTUs were overall as active as suggested by their relative abundances.

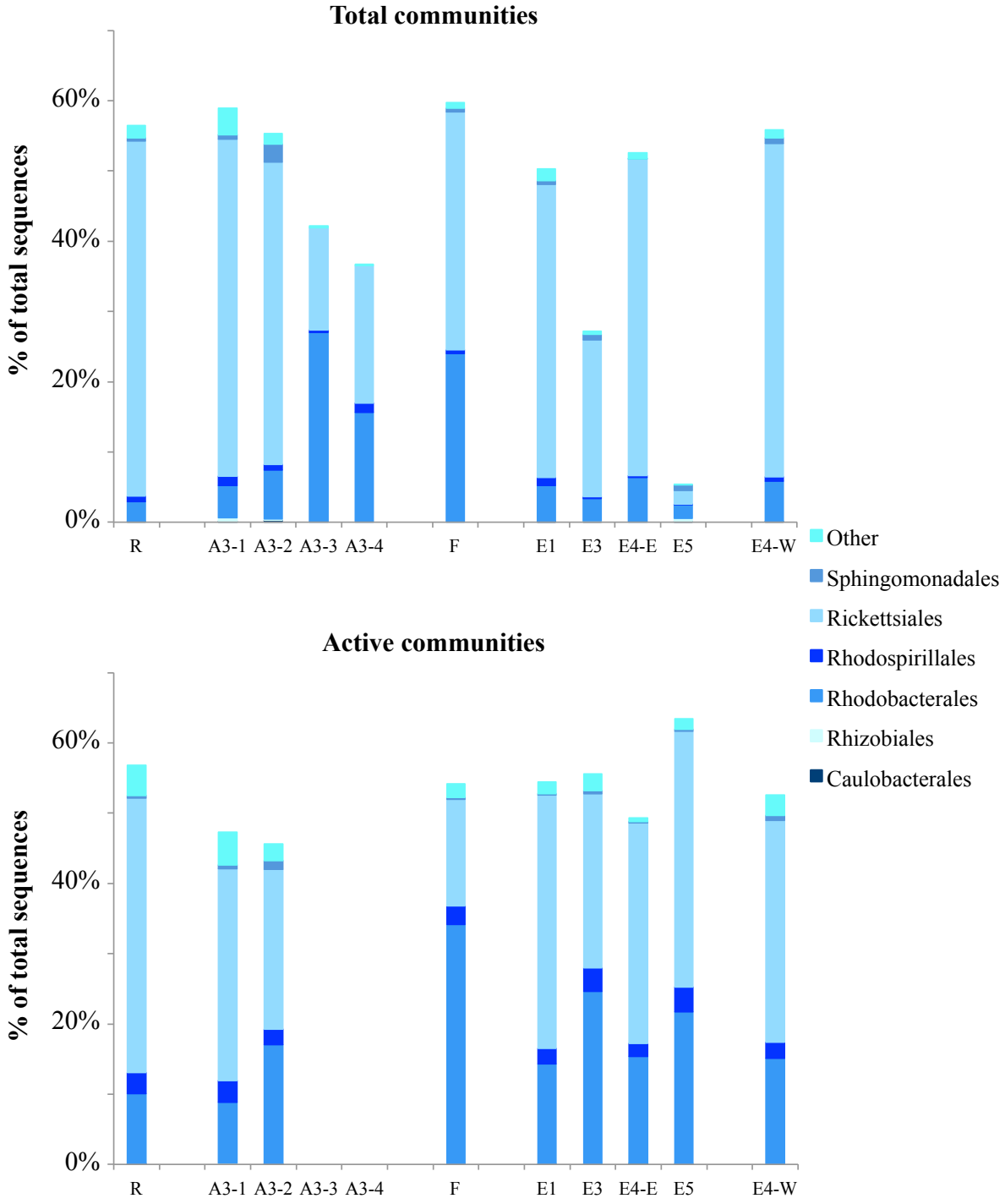


Figure 26. Relative abundances of the different groups within the class of Alphaproteobacteria, in total (top) and active (bottom) communities at the study sites.

Another group within the Alphaproteobacteria that displayed different contributions to the total community among sites was the family of the

Rhodobacteraceae within the Rhodobacterales (Figure S9). Though it was barely detected in the active community, the genus *Phaeobacter* reached 1.3% of the community at station F. Two OTUs were found within this genus at station F and were not detected in any other station. These two OTUs were the ones detected in high abundances in the +DOM treatment of the continuous culture experiments.

The genus *Thalassobius* that grew preferentially in the control in the continuous culture experiment responded to the A3 and the F blooms (Figure 27). The OTU with the largest response to these bloom conditions was OTU 13, the same OTU found in the continuous culture experiment. This OTU was also found to be very active *in situ*, as well as OTU 37 which despite its considerable contribution to the active community did not contribute as much to the total community. Interestingly, this OTU was more abundant in HNLC stations than at bloom sites.

Within the 61 OTUs classified as "other Rhodobacteraceae", 4 presented increased relative abundances in the A3 and the F blooms (Figure S10). These OTUs also presented increased contributions to the active communities at these stations, suggesting a response to environmental conditions. Interestingly, despite their high contributions to the active communities in the E structure, their relative abundances were not as high as in A3 or F. The largest response of this group was observed at station F and was explained by increased abundances of two OTUs, both relative to the total and the active communities. One of them (OTU 71) was found in small relative abundances in the continuous cultures, almost restrictively in the +DOM treatment though a few sequences were also detected in some of the control replicates at the end of the experiment.

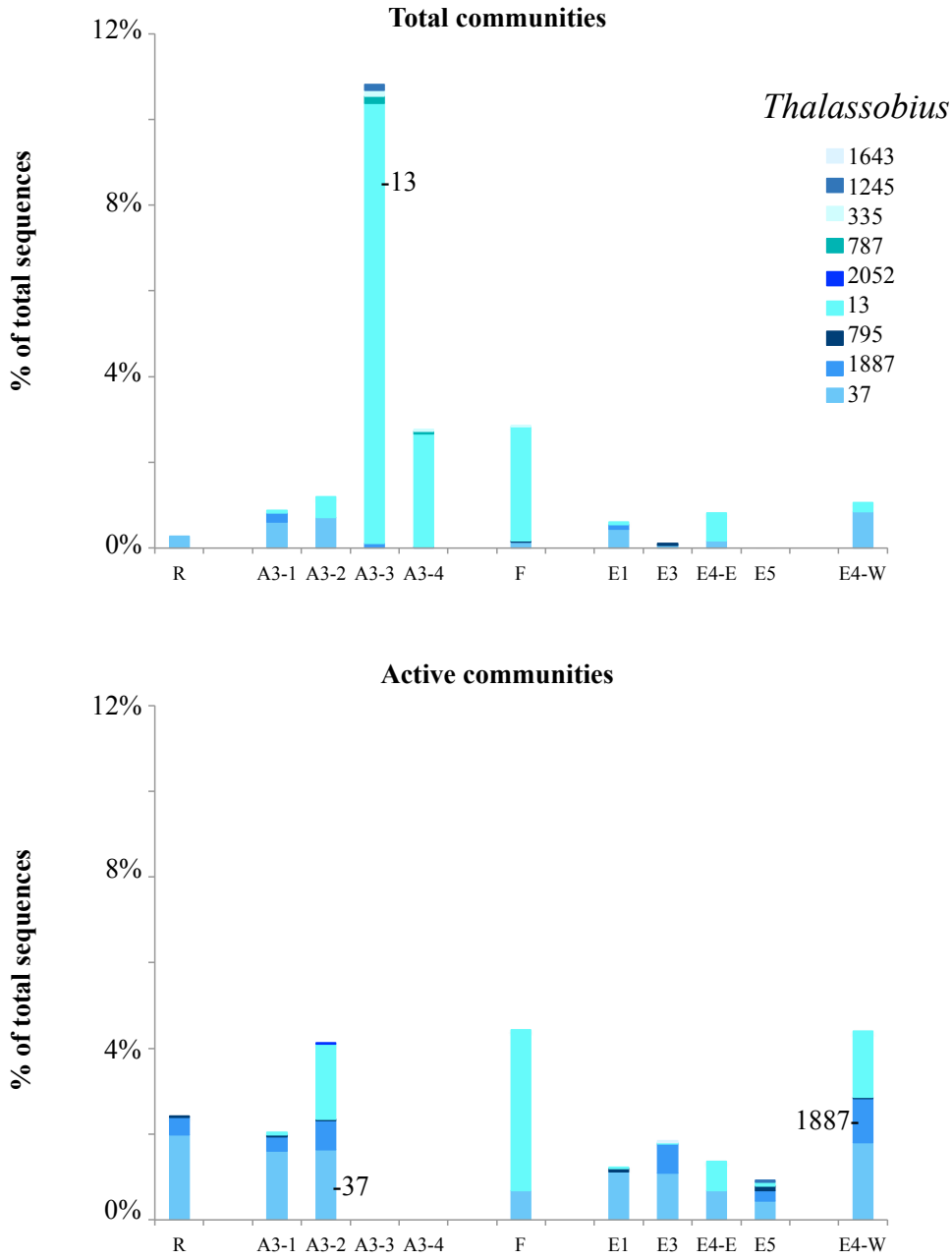


Figure 27. Relative abundances of OTUs belonging to the genus *Thalassobius*, in total (top) and active (bottom) communities at the study sites.

Other groups within the Alphaproteobacteria, such as the Rhodospirillales, had very steady contributions to total and active communities from one site to the next and seemed very little affected by changes in environmental conditions (Figure 26).

The contribution of the Gammaproteobacteria to active communities was much higher, in most stations, than predicted by their relative abundance (Figure 28).

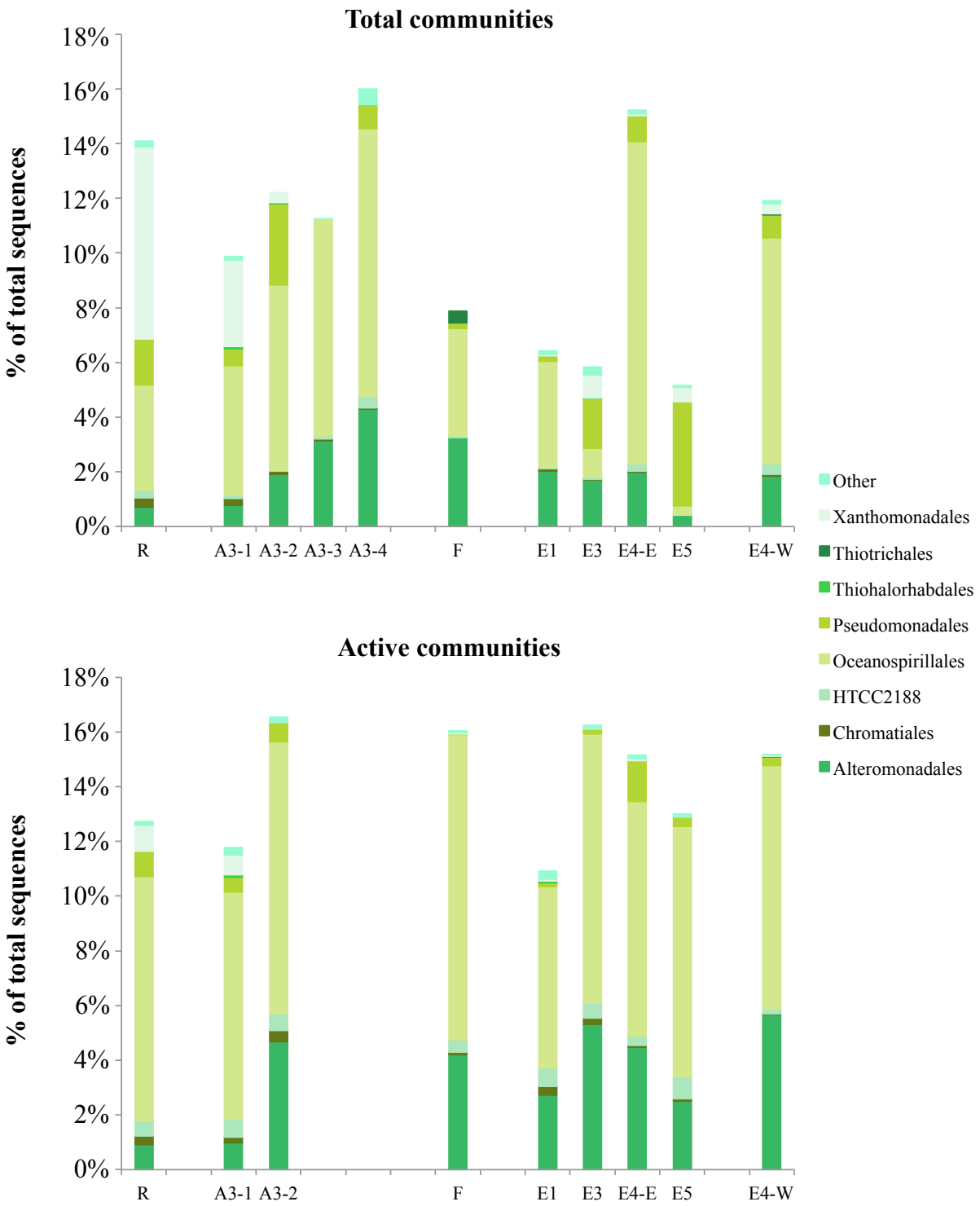


Figure28. Relative abundances of the different groups within the class of Gammaproteobacteria, in total (top) and active (bottom) communities at the study sites.

They also seemed to be more active at bloom sites compared to HNLC stations, though it did not always allow them to become abundant, like at station F. Some groups seemed to collapse under bloom situations and to be more active under HNLC conditions, such as Xanthomonadales. The groups displaying most variations between sites were the Alteromonadales and the Oceanospirillales.

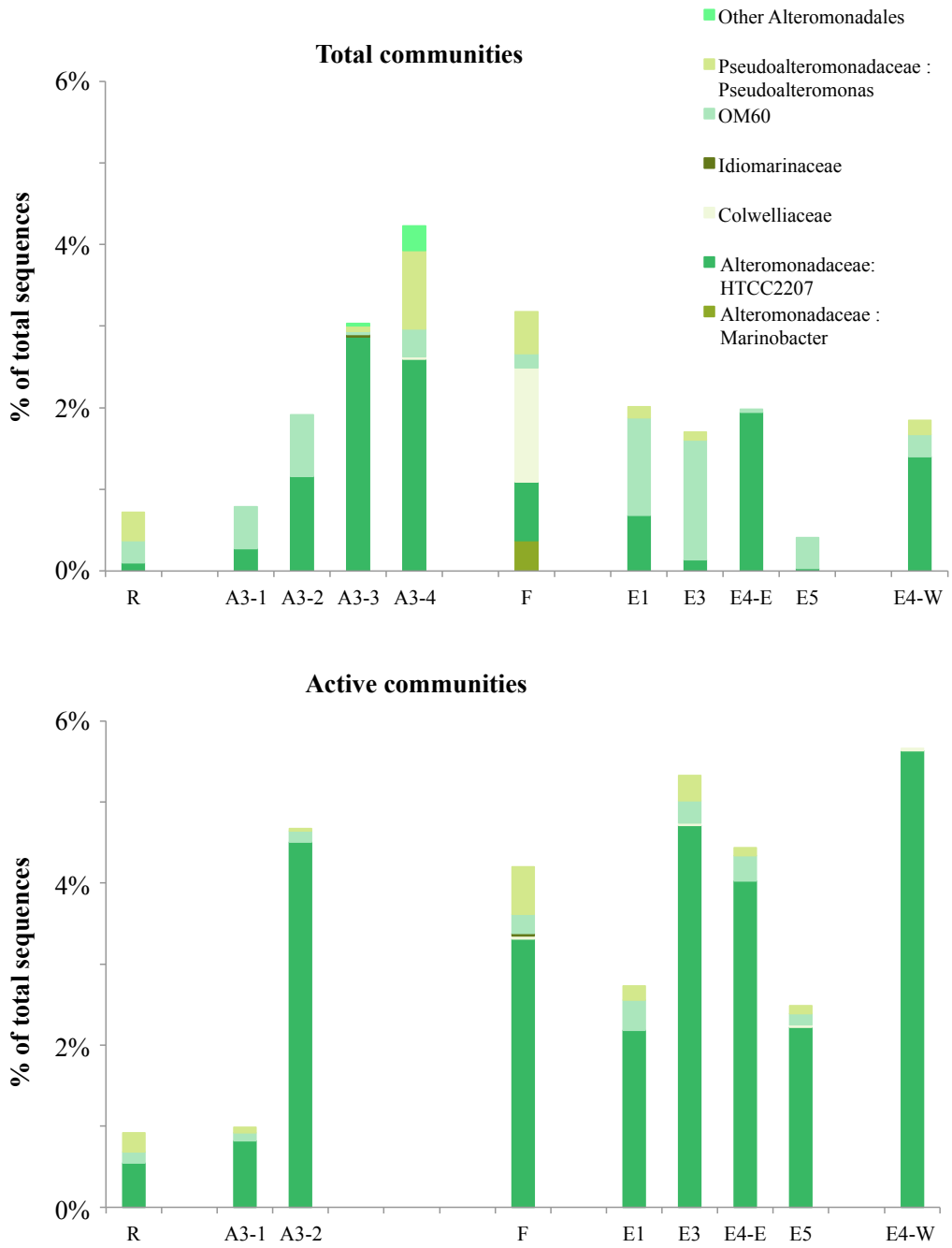


Figure 29. Relative abundances of the different groups within the order of Alteromonadales, in total (top) and active (bottom) communities at the study sites.

The Alteromonadales revealed distinct patterns at the different bloom sites (Figure 29). At A3-2 Alteromonadaceae, and in particular members of the SAR92 clade (HTCC2207), were very active and had high relative abundances as the bloom progressed. At station F, the Colwelliaceae contributed to 2% of the total community, and they were not detected at any other station.

The two Colwelliaceae OTUs detected at station F were, again, the OTUs enhanced in the +DOM treatment of the continuous culture experiment. Surprisingly, their contribution to the active community at F was lower than predicted by their abundance and almost undetectable (data not shown). The genus *Marinobacter*, that was detected in the continuous culture experiment, and was enhanced in the control, was also specifically detected at station F (Figure 29).

Within the group of the Oceanospirillales, the family Halomonadaceae was overall more active, at all sites, than predicted by its relative abundance, which seemed to increase under bloom conditions, except at station F (Figure 30).

Another interesting specificity of station F compared to the other bloom sites was the presence of *Coraliomargarita* OTU 15 belonging to the Verrucomicrobia and detected in high abundance in the +DOM treatment at the end of the continuous culture experiment (data not shown).

A major difference between sites was induced by the large number of Actinobacteria in some stations such as E3 and E5, explaining their position on the dendrogram (data not shown). Surprisingly, the two OTUs within this group making up to 40% of the total communities at these stations contributed to ≤ 1% of the active communities.

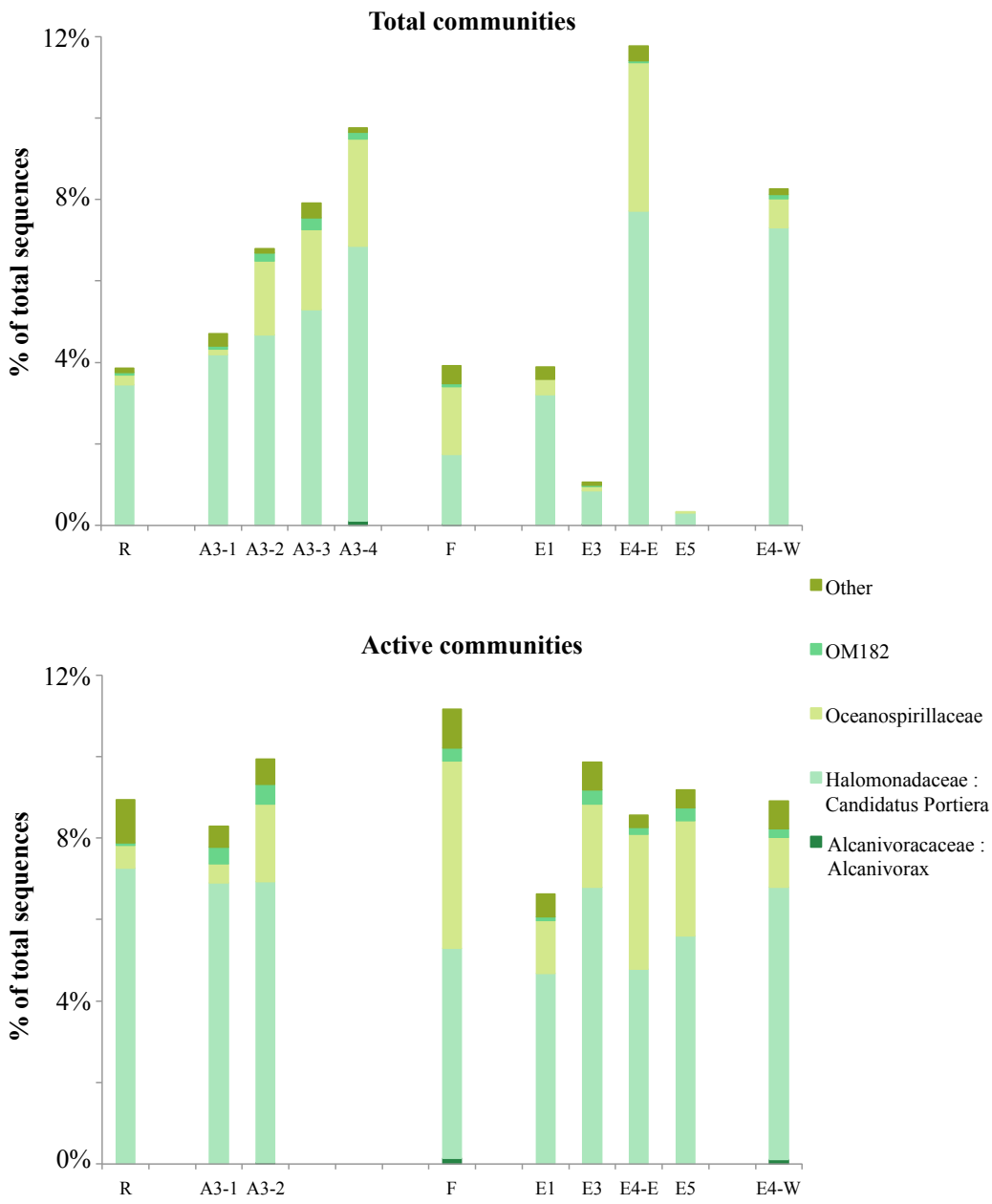


Figure 30. Relative abundances of the different groups within the order of Oceanospirillales, in total (top) and active (bottom) communities at the different study sites.

Though their relative abundances greatly varied between *in situ* conditions and the continuous culture experiment, 14 OTUs displaying a treatment association in the experiment were also detected either at A3 or at F. These OTUs accounted for 37% of the community at F, and 28% of the communities in the A3 bloom (Figure 31).

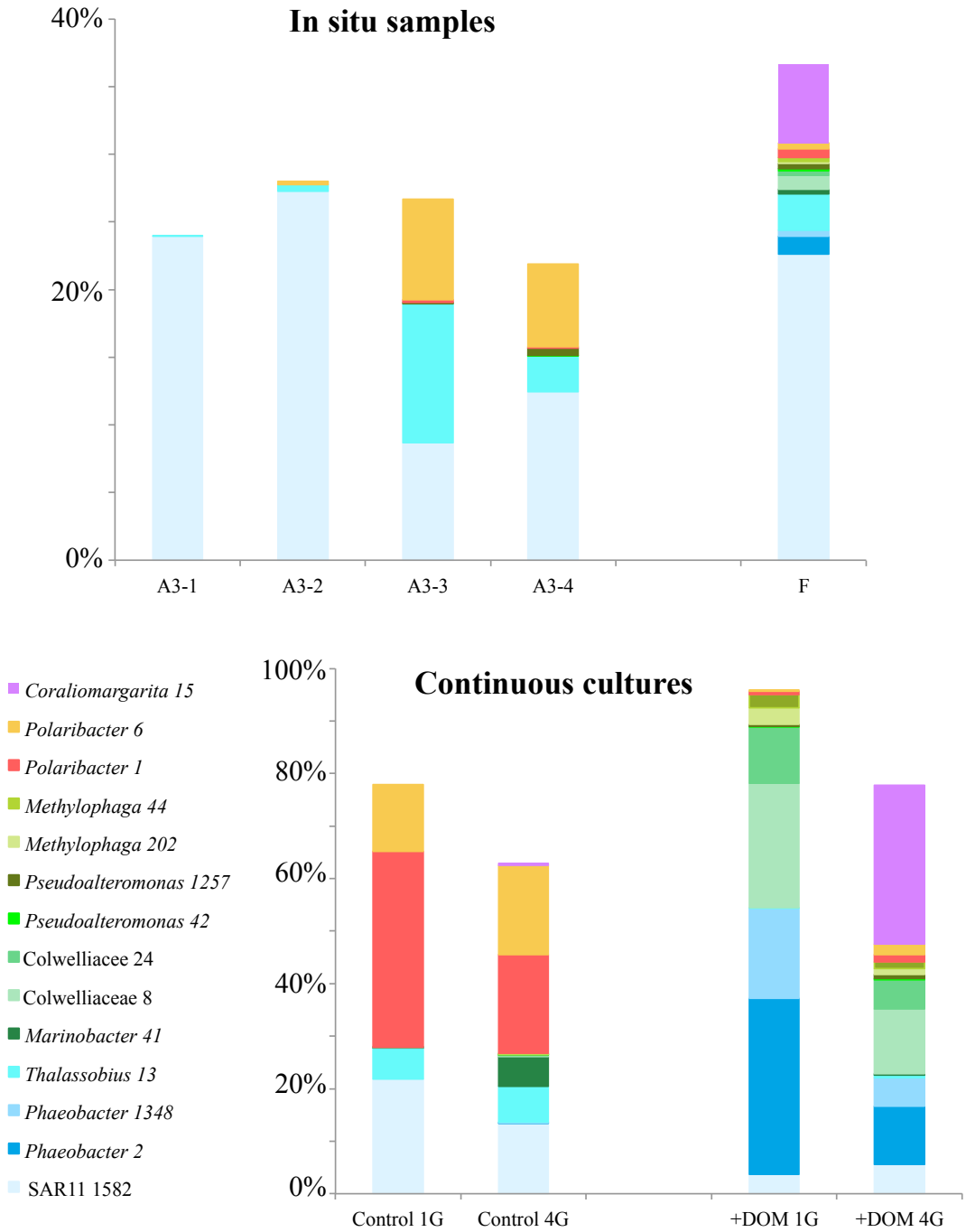


Figure 31. Relative abundances of 14 OTUs specifically responding to either treatment in the continuous culture experiment and detected either at A3 or F.

IV. DISCUSSION

Effect of DOM addition on bacterial diversity

The experimental conditions set up during the on-board continuous cultures favored the rapid establishment of distinct bacterial communities. These communities were dominated by a few OTUs belonging to three broad phylogenetic groups: Bacteroidetes, Alpha- and Gammaproteobacteria. The most abundant OTUs were, however, different between the control, dominated by two *Polaribacter* (Bacteroidetes) OTUs, and the +DOM treatment, dominated by a larger variety of OTUs belonging to Colwelliaceae (Gammaproteobacteria), *Phaeobacter* (Alphaproteobacteria) and Verrucomicrobia. This pattern of higher diversity in diatom-DOM amended cultures is consistent with our previous experiment performed in the Mediterranean Sea (Chapter 1). The differences were already striking after the first bacterial generation, indicating the rapid response of these OTUs to each experimental condition. In most cases, the OTUs that were dominant in one condition were also present in the other but in lower abundances, with a few exceptions such as the *Methylophaga* OTUs detected in the +DOM treatment and absent in the control. Overall, these results show that the OTUs that were competitive in the conditions set by the control treatment were no longer competitive in the conditions set by the DOM addition. This suggests the nature of the resource was a major parameter driving community composition in this experimental context.

Ecology of the groups responding to DOM addition

The OTUs that proved to be the most competitive in the +DOM treatment belonged to the *Phaeobacter*, the *Colwellia*, the *Methylophaga* and the Verrucomicrobia. Most of these groups are not restricted to any particular oceanic region in the world, and several

studies have revealed their presence in polar systems, indicating at least some members of these groups are adapted to such environments (Bowman et al., 1998; Brinkmeyer et al., 2003; Zhang et al., 2008). Recent work on a cultured *Phaeobacter* strain found evidence of a strong capability for the concurrent uptake and use of several carbon substrates, suggesting an efficient opportunistic lifestyle (Zech et al., 2013). Though it is not possible to firmly infer the presence of such functional traits in all *Phaeobacter* ecotypes from their existence in one representative, it is a possible explanation for the dominance of this group in the DOM-amended cultures. The 10 μM of diatom-derived DOM provided in this treatment probably contained easily accessible compounds, such as proteins and polysaccharides (Myklestad, 1995; Aluwihare & Repeta, 1999). This diverse pool of substrates could have provided an advantage to bacteria capable of simultaneous uptake of multiple substrates, such as members of the genus *Phaeobacter*.

Colwellia is known for its strictly psychrophilic lifestyle (Methé et al., 2005). This group is capable of motility (Junge et al., 2003) and production of extracellular polysaccharides (Marx et al., 2009) at very low temperatures. Many biochemical approaches based on culturable representatives have enabled the discovery and characterization of cold-adapted enzymes involved in carbon uptake and metabolism (Huston et al., 2004; Wang et al., 2005; Leiros et al., 2007). All these traits suggest members of this group could be well adapted to life in cold environments, and particularly competitive for carbon uptake, utilization and storage (Methé et al., 2005). These predictive functional characteristics provide reasonable explanation for the rapid response and subsequent high abundances of two *Colwellia* OTUs in the DOM-amended cultures.

The *Methylophaga* are methylotrophic organisms that have been observed at the end of a DOM degradation experiment (McCarren et al., 2010). Their presence in such

systems have been explained by their specific capacities to take up methylated compounds resulting from bacterial degradation of more complex molecules. The diatom-derived DOM used in our amended cultures was collected from a non-axenic diatom culture. It is likely that bacterial degradation processes of organic material had occured, and the methylated degradation products could have created niches for OTUs such as the two *Methylophaga* specifically observed in the +DOM treatment.

Few studies exist on the group of Verrucomicrobia, which considerably limits our knowledge of their potential properties and functional traits. However, the late and intense development of one Verrucomicrobia OTU in two of the triplicate DOM-amended cultures suggests a strong ability, for this OTU at least, to benefit from the carbon compounds available in this treatment. The data indicates this OTU outcompeted the other groups responding strongly to the experimental condition, that are *Phaeobacter* and *Colwellia*, whose relative abundances diminished in the cultures where the Verrucomicrobia developed. This suggests that even if these OTUs were adapted to the conditions set by the addition of DOM, some redundancy for the niche exploitation exists among bacterial groups, and competition is a powerful mechanism in determining the development and persistance of any of these groups. It also suggests that functionally equivalent bacterial groups may need different time scales to adapt to changing environmental conditions, leading to staggered responses.

Ecology of the groups developing in the control

If the OTUs dominating the communities in the DOM-amended cultures can be considered the most competitive in labile DOM uptake, by contrast, the OTUs developing in the control can be regarded as adapted to utilize the relatively less bioavailable carbon pool originating from the pre-bloom « winter water » conditions. When we

collected water for the experiment at A3 the bloom was just initiating, as indicated by the low chl *a* concentrations. The concurrent low bacterial production during this visit at A3 as compared to the other sites in the naturally-fertilized region suggests limitation by organic carbon. Enrichment experiments, demonstrating that carbon stimulated bacterial activity (Figure S11) and the higher bacterial abundances in our +DOM amended continuous cultures support this notion. Bulk DOC concentrations were almost identical among sites, indicating that the quality rather than the quantity of DOM was driving the bacterial bulk responses. For these reasons, it is reasonable to conclude that bacterial communities in the control were provided with overall more refractory DOM.

From that angle, the dominance of two Bacteroidetes OTUs identified as *Polaribacter* in this treatment is consistent with previous work showing that members of this phylum, including a *Polaribacter*, are adapted to the degradation of polymers and high molecular weight compounds (Cottrell & Kirchman, 2000; Bauer et al., 2006; González et al., 2008; Gómez-Pereira et al., 2012).

A remarkable feature of the control cultures was the persistance over time of a dominant SAR11 OTU. In a continuous culture, groups that do not grow are expected to progressively wash out of the culture. The stable relative abundance of the SAR11 OTU suggests it was actively maintaining itself in the system at the imposed growth rate, which implies it was able to utilize the carbon available. This result is consistent with the high abundances of SAR11 in HNLC waters and at A3 prior to the bloom.

A third dominant OTU in the control belonged to the genus *Thalassobius*. Interestingly, this genus is phylogenetically close to *Phaeobacter*, which was abundant in the DOM-amended cultures. This suggests that within the Rhodobacteraceae (or *Roseobacter* clade), niche specialization could occur at high phylogenetic resolution. In this sense, our experiment provides interesting insights into the parameters driving

niche occupation among members of the *Roseobacter* clade. While *Phaeobacter* seemed to be adapted to multiple substrates utilization, *Thalassobius* appears not to be capable of carbohydrate degradation (Arahal et al., 2005). Therefore, it is likely that these two genera, despite their phylogenetic closeness, possess different carbon utilization preferences and abilities, which could explain their response in our cultures but also in environmental conditions.

Bacterial diversity in the various blooms

In situ observations revealed pronounced responses in bacterial community composition in response to phytoplankton blooms. Interestingly, the response was not identical at the different bloom sites. At A3, the OTUs most responsive to bloom conditions were *Polaribacter* (Bacteroidetes) and *Thalassobius* (Alphaproteobacteria). In the bloom developing north of the Polar Front, the Bacteroidetes responded in lesser proportions, in contrast to *Thalassobius* and a few unidentified Rhodobacteraceae OTUs. The time series performed in the meander structure south of the Polar Front revealed high abundances of Actinobacteria and Planctomycetes, as well as dynamic changes in community composition over time.

Polaribacter and Bacteroidetes in general are common found in polar bacterial communities (Bowman et al., 1997; Abell and Bowman, 2005a; Straza et al., 2010) and in the Kerguelen Islands area in particular (West et al., 2008; Ghiglione & Murray, 2012). Two artificial mesoscale iron fertilizations performed in the Northeast and Western Subarctic Pacific (SERIES and SEEDS-II) led to an increase in the proportions of Bacteroidetes, which has been interpreted as a response to the release of organic matter rather than to direct iron input (Agawin et al., 2006; Kataoka et al., 2009). In the present

study, the sites where Bacteroidetes displayed high relative abundances compared to the reference HNLC station R were also the sites where bacterial production was the highest, supporting the idea that Bacteroidetes play crucial roles in organic matter degradation. In particular, the response of Bacteroidetes was mostly observed at the late stage of the bloom. At the very beginning of the bloom, during the second KEOPS2 visit, there were only slight differences in the relative abundance of the Bacteroidetes compared to the first visit, even though primary production was higher and bulk bacterial production indicated a positive response from the community to the changing conditions. This late response of the Bacteroidetes to bloom conditions has already been reported in the literature (Teeling et al., 2012) and has been related to this group's affinity for particulate material and cell debris from senescent phytoplanktonic communities (Abell and Bowman, 2005b). In the present study, their increased abundance in the free-living bacterial community at the end of the bloom could be due to detachment from particles during sample collection and filtration. However, considering their competitiveness in the control of our continuous culture experiment, it could also suggest they are able to use polymers and complex molecules in the dissolved carbon pool and not only on the particulate fraction (Fandino et al., 2001; Kirchman, 2002).

Another group actively responding to bloom conditions was the Rhodobacteraceae, in particular *Thalassobius*. The Rhodobacteraceae have often been observed in bloom situations (González et al., 2000; Rink et al., 2007; Teeling et al., 2012), in temperate systems but also in a bloom developing during the SEEDS-II artificial fertilization (Kataoka et al., 2009). Interestingly, the Rhodobacteraceae OTUs responding at the A3 and F sites were different. The group was not enhanced in the E sites. In the continuous culture experiment, *Thalassobius* was associated to low carbon

concentration and availability whereas *Phaeobacter* was associated to phytoplankton DOM, which was in part confirmed *in situ*. *Phaeobacter* was observed at station F where primary production and probable subsequent DOM release were the highest. *Thalassobius* was also observed at F, but its major response was found at the end of the bloom at station A3-3. This points to its potential adaptation to complex carbon sources such as the ones resulting from senescent phytoplankton cells, as discussed previously for Bacteroidetes. Taken together, our results from the continuous culture and the investigation of bloom sites indicate variable responses of the different members of this group to environmental conditions. This observation agrees with the fact that the Rhodobacteraceae are a very diverse group whose members occupy different ecological niches (Buchan et al., 2005; Brinkhoff et al., 2008).

Under the assumption that bacterial communities were initially C-limited rather than Fe-limited and responded to the additional organic carbon provided by phytoplankton, the differences in bacterial diversity could reflect differences in quality and concentration of DOM at the various sites. Many factors could explain such differences, such as distinct bloom stages, phytoplankton community composition and physiological state at the various sites, variations in the grazing pressure exerted on bacterial and phytoplankton populations and subsequent organic matter release, for instance. The different bacterial productions measured at the sites support the hypothesis of different carbon supply and quality. The question of what factors determined the differences in the bacterial community composition *in situ* will need further investigation.

Combining the results from our continuous culture experiment and *in situ* observations, an interesting remark can be made. Several OTUs that were associated with the control cultures were present at A3 at the decline of the bloom, accounting for

between 22 and 27% of the total community. By contrast, most of the OTUs responding to the DOM addition in the cultures were present at F and accounted for 37% of the community at this site. Thus, with our experimental approach we maintained distinct bacterial communities that each reflected to some extent specific *in situ* communities. An intriguing question was why the bacterial community in our +DOM treatment was more similar to that at F than to that at any other bloom site. A possible explanation could be the high phytoplankton biomass and rates of primary production within a shallow MLD at this site. A consequence of this coupled biogeochemical and physical setting could be an enhanced supply of diatom-derived DOM within roughly 50 m. In comparison, during the beginning of the A3 bloom the MLD reached 160 m. Given that primary production was limited to the upper 60m, this could have lead to the dilution of phytoplankton-derived DOM.

A possible explanation for the differences in bacterial diversity between sites could be due to different bloom stages, and the composition and quality of DOM associated to each stage. Chl *a* concentrations and primary production suggest each bloom site investigated was at a different stage of development. While A3-1 and -2 represent early bloom conditions, and A3-3 and -4 represent the decline of the bloom, the high chl *a* and primary production at station F suggest this station was visited in the peak of the bloom and could represent an intermediate situation between stations A3-1 and 2, and A3-3 and -4. Therefore, differences in bacterial diversity characterizing each system suggest the succession of stage-adapted populations over the course of a phytoplankton bloom, which has often been proposed in the literature (Riemann & Winding, 2001; Teeling et al., 2012). OTUs responding at each phase of a bloom could provide insights into their respective adaptative traits.

Interestingly, the OTUs dominating at the end of the A3 bloom were the ones responding at A3-2 compared to A3-1. This suggests that these OTUs are immediately stimulated by bloom conditions, but need time to reach the high abundances detected at the end of the bloom. This contrasts with the fast response of bacterial groups observed in the continuous culture experiment. Grazing pressure has been shown to reduce the biomass of active groups in natural systems (Gasol et al., 1995; Lebaron et al., 2001) and could have controlled the abundance of the fast-responding OTUs in these iron-induced phytoplankton blooms. The examination of data such as grazers abundance, grazing-induced mortality rates and viral abundances will provide essential information to the global understanding of the composition and activity of bacterial communities in these environments.

In conclusion, our results support the idea that DOM has a structuring effect on communities. At station F, a large proportion of the OTUs found to respond to phytoplankton DOM compounds in the continuous culture experiment could be observed and even reached high abundances. This strongly suggests that the input of phytoplankton at this site impacted bacterial diversity in a way that experimental work helped us detect and understand. These OTUs, however, were not observed in other sites, suggesting that other parameters influenced communities more strongly, or that at these other sites, DOM effect was modulated by environmental conditions.

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Supplementary information

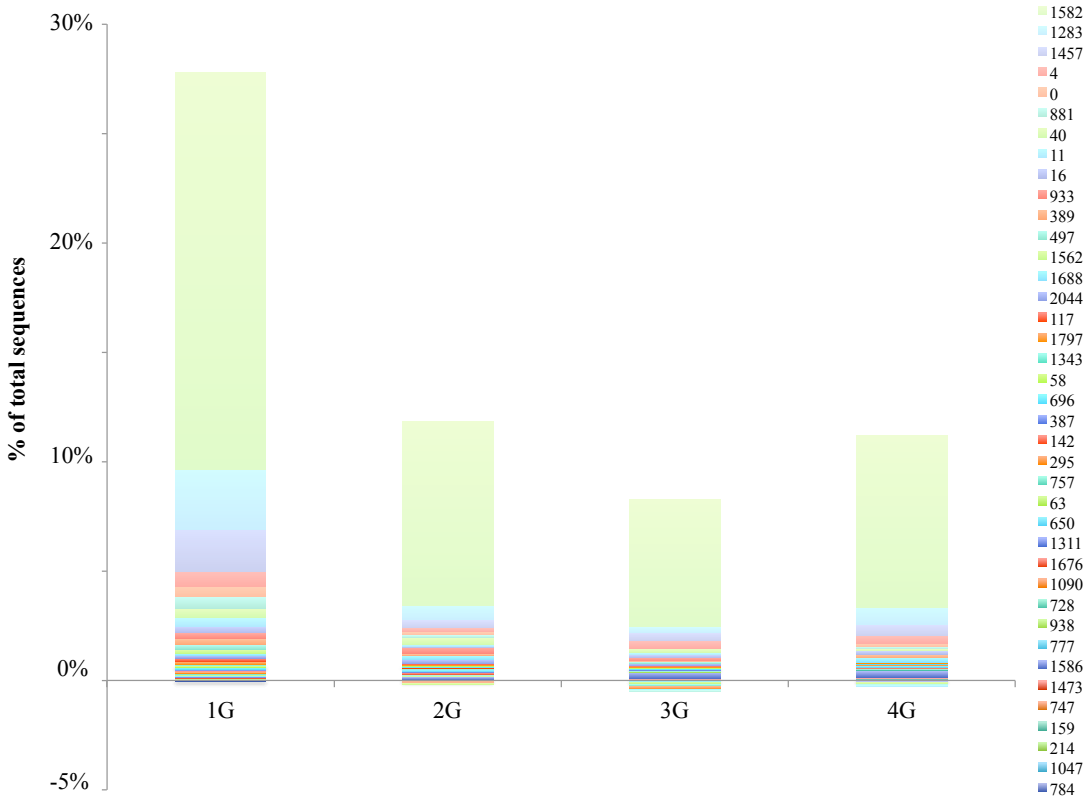

Figure S1. Differences of relative abundance between the control and the +DOM treatment (C-D) for the 39 OTUs plotted in Figure 5. The mean value calculated from the triplicate cultures was used for each experimental condition. The numbers refer to the OTU identity in the QIIME-generated OTU table.

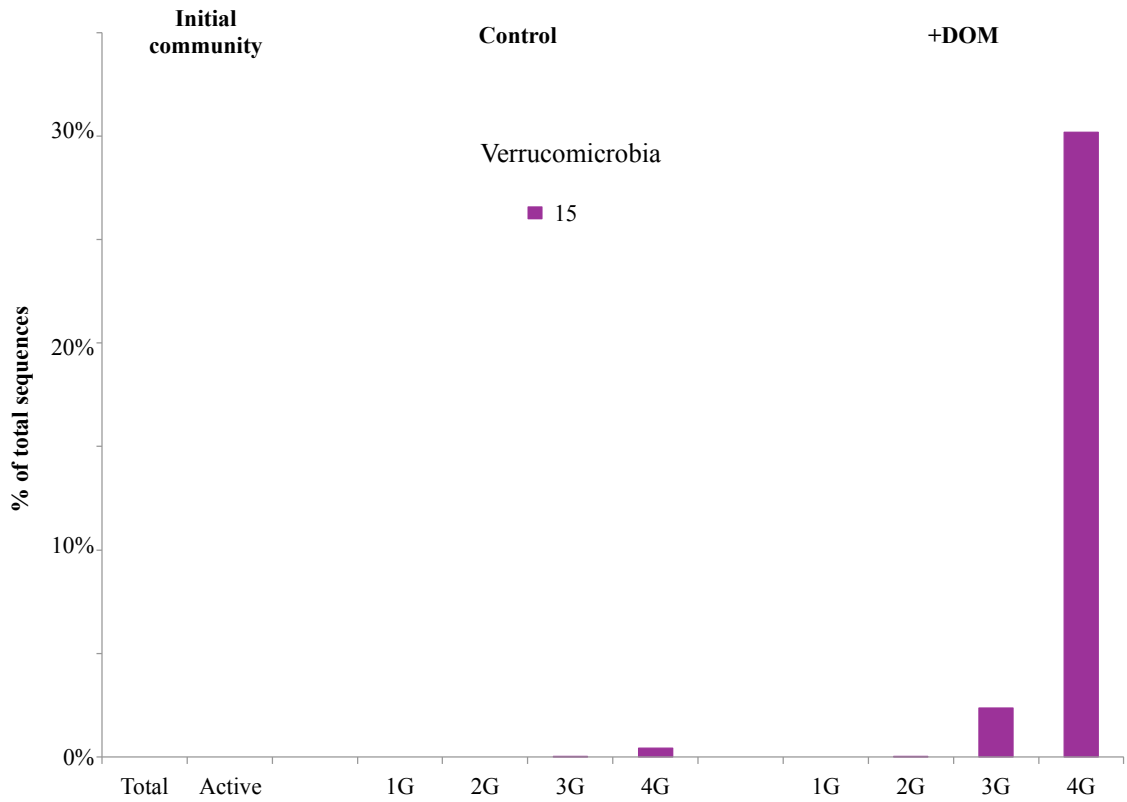

Figure S2. Relative abundances of the OTU belonging to the Verrucomicrobia, in the initial community (total and active) and in the communities sampled in the control and the DOM-amended cultures after 1, 2, 3 and 4 bacterial generation times (G). For the continuous cultures, the mean value calculated from the triplicate cultures is given. The number refers to the OTU identity in the QIIME-generated OTU table.

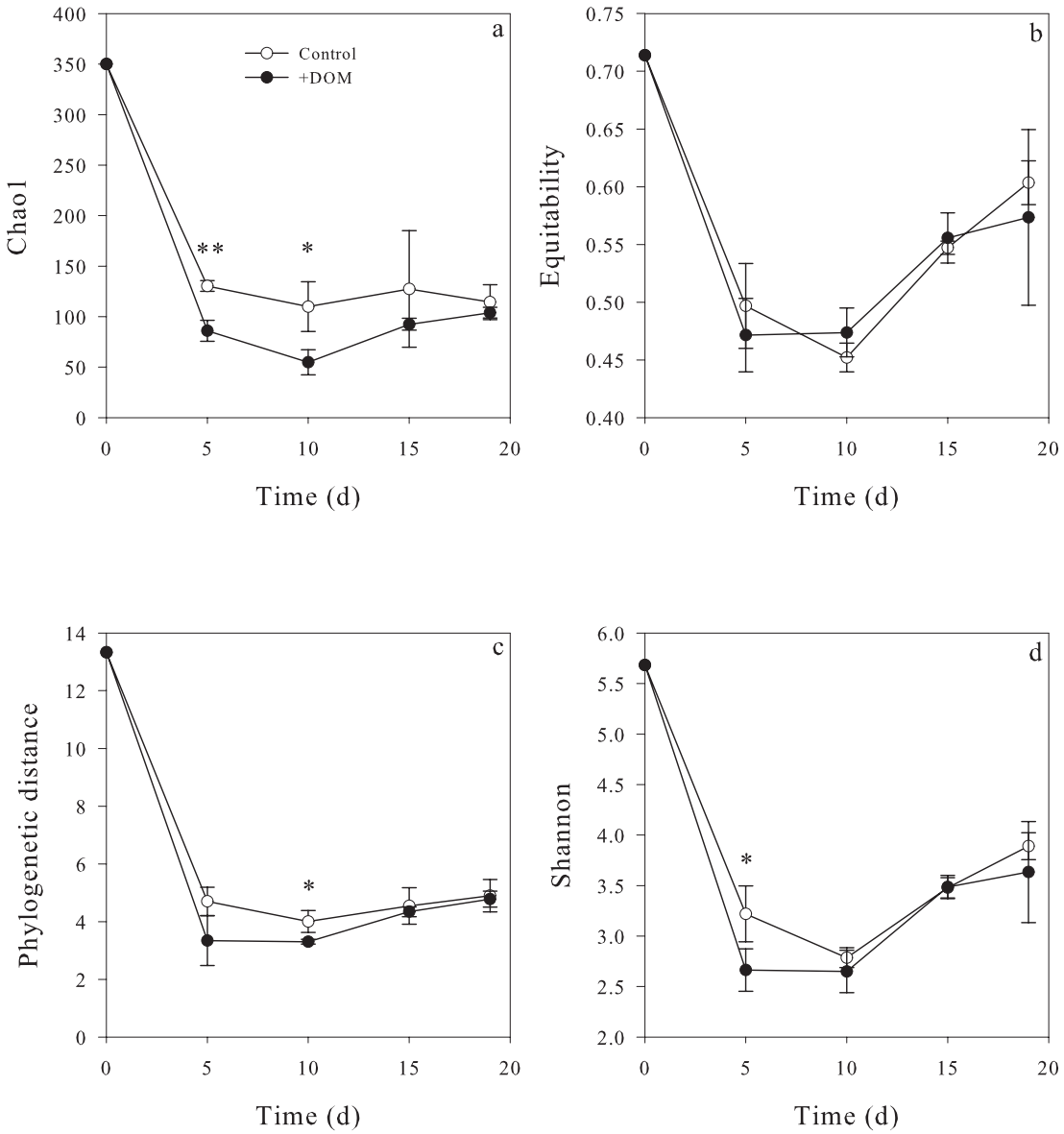

Figure S3. Diversity indices characterizing the initial community (Day 0) and the communities sampled in the continuous cultures over time. Mean values ± SD of biological triplicates are shown for each time point. The calculation was performed with 2930 sequences per sample.

*** and ** indicate significant (p≤ 0.05) and very significant (p≤0.005) differences according to a Student test.**

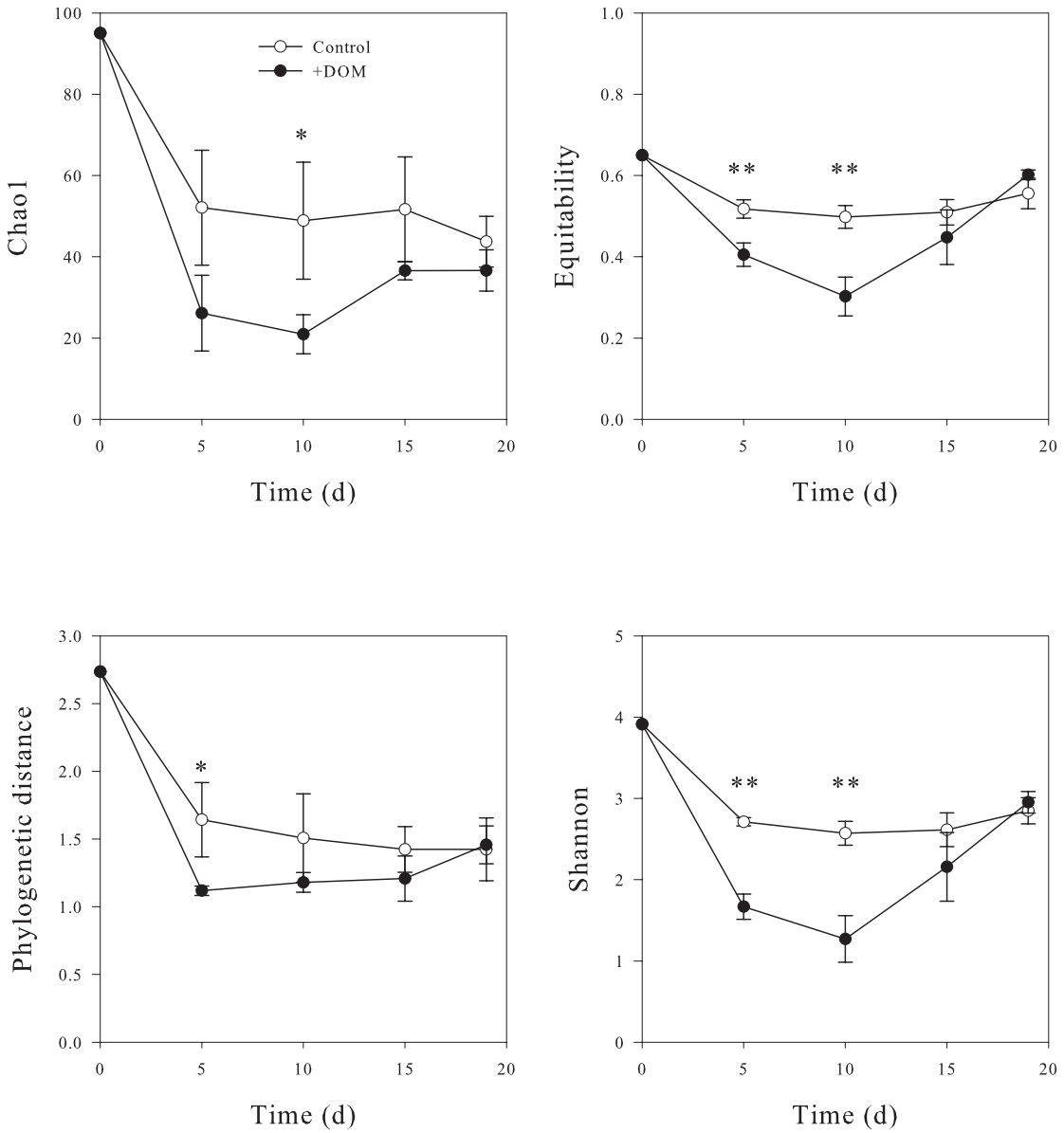

Figure S4. Diversity indices characterizing the initial community (Day 0) and the communities sampled in the continuous cultures over time, restricted to Alphaproteobacteria only. Mean values ± SD of biological triplicates are shown for each time point. The calculation was performed with 565 sequences per sample.
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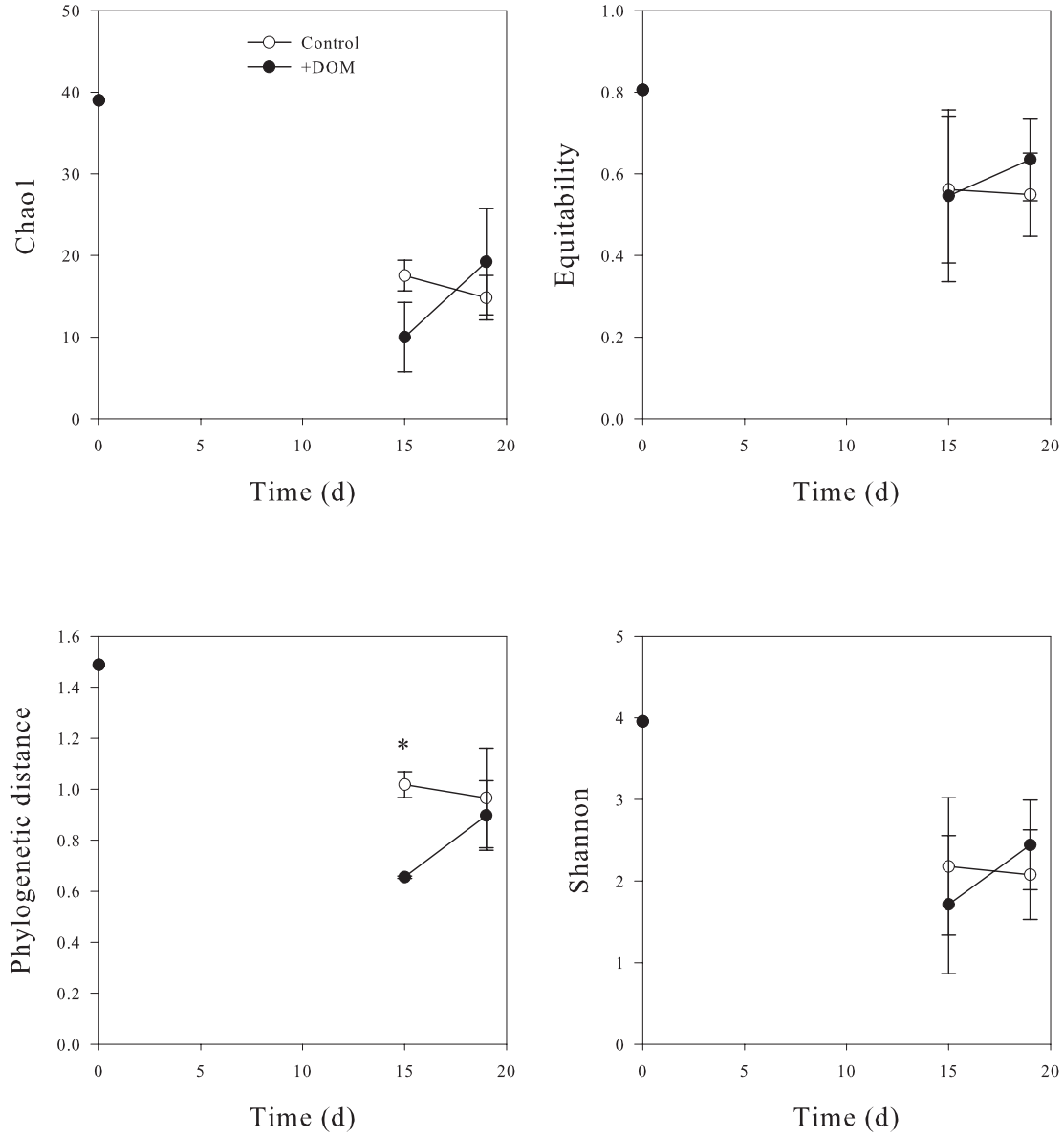

Figure S5. Diversity indices characterizing the initial community (Day 0) and the communities sampled in the continuous cultures over time, restricted to Gammaproteobacteria only. Mean values ± SD of biological triplicates are shown for each time point. The calculation was performed with 157 sequences per sample. Only communities found after 15 and 19 days (3G and 4G) had enough sequences to be analysed.

*** indicates significant differences according to a Student test (p≤ 0.05).**

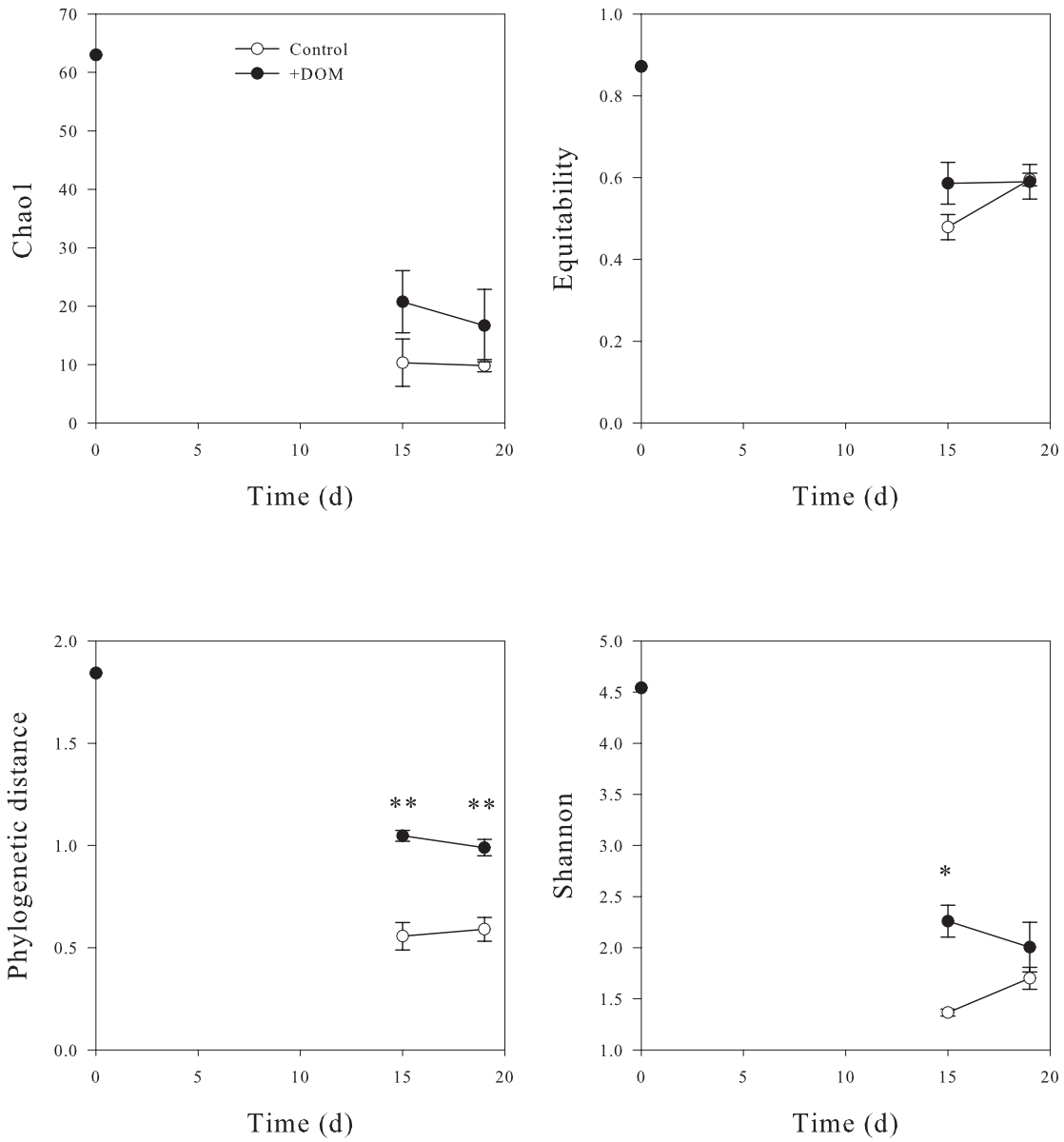

Figure S6. Diversity indices characterizing the initial community (Day 0) and the communities sampled in the continuous cultures over time, restricted to Bacteroidetes only. Mean values ± SD of biological triplicates are shown for each time point. The calculation was performed with 183 sequences per sample. Only communities found after 15 and 19 days (3G and 4G) had enough sequences to be analysed.
*** and ** indicate significant (p≤ 0.05) and very significant (p≤0.005) differences according to a Student test.**

Table S1. Results of the comparison of the phylogenetic composition of the samples using a phylogenetic test. Blue indicates an absence of significance (p>0.05), orange indicates a significant differenrence (p≤0.05). Samples corresponding the active fractions of the communities are indicated with an (a).

	R	R (a)	A3-1	A3-1(a)	A3-2	A3-2(a)	A3-3	A3-4	F	F (a)	E1	E1 (a)	E3	E3 (a)	E4-E	E4-E (a)	E5	E5 (a)	E4-W	E4-W (a)
R																				
R (a)																				
A3-1																				
A3-1(a)																				
A3-2																				
A3-2(a)																				
A3-3																				
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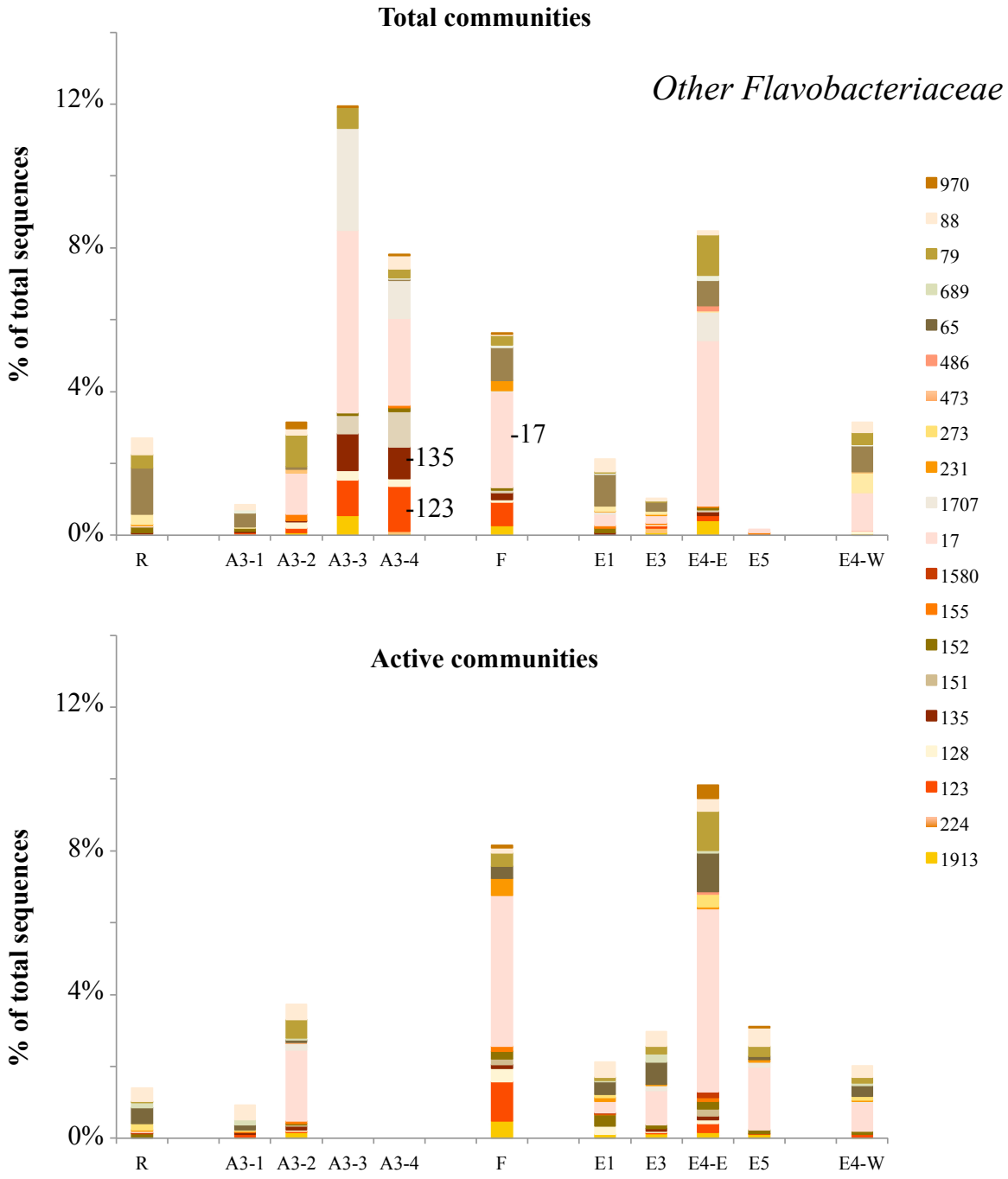

Figure S7. Relative abundances of the OTUs within the Flavobacteriaceae, in total (top) and active (bottom) communities at the study sites. The numbers refer to the OTUs identity in the QIIME-generated OTU table.

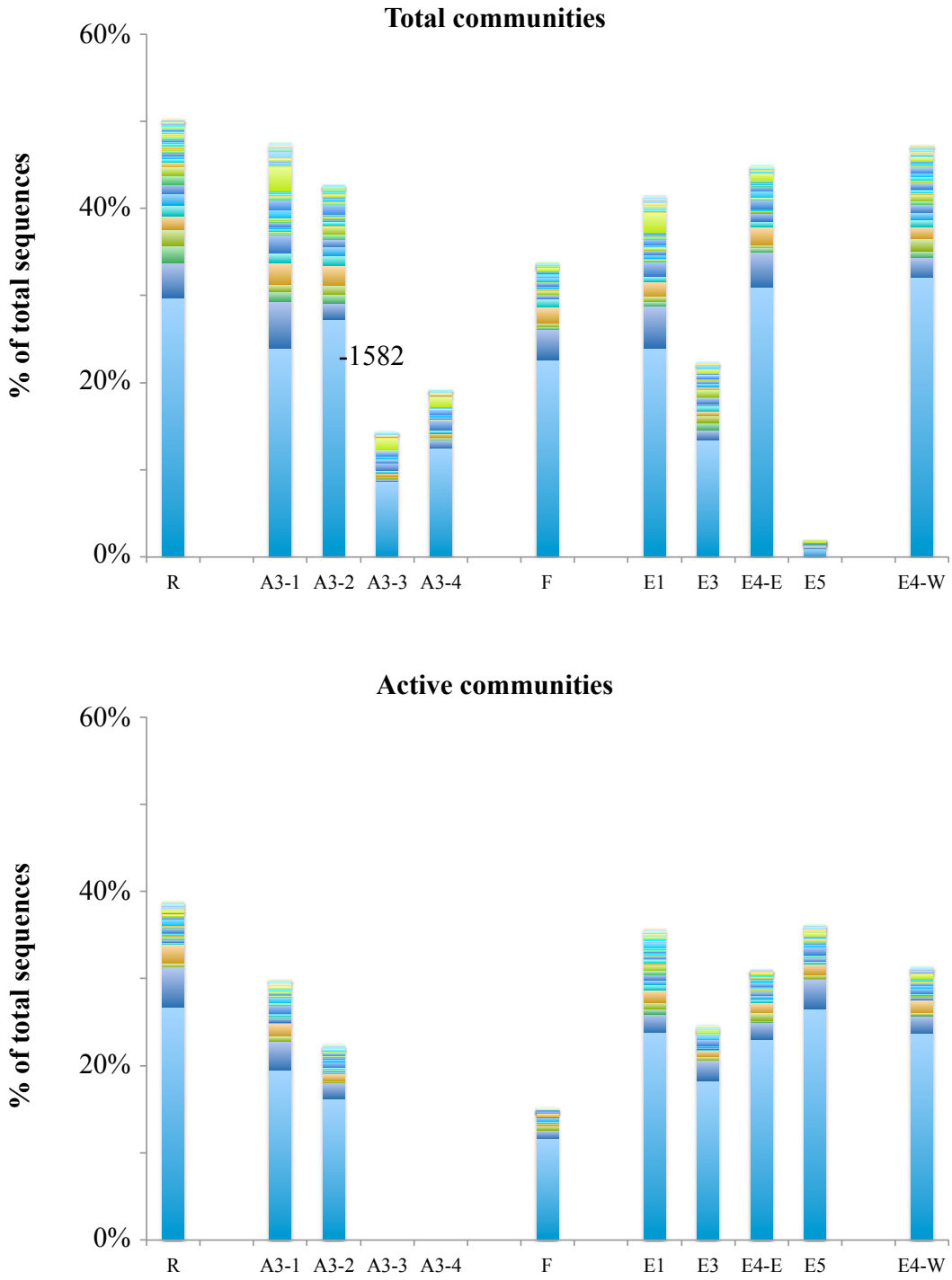

Figure S8. Relative abundances of the OTUs within the Pelagibacteraceae, in total (top) and active (bottom) communities at the study sites. The number refers to the OTU identity in the QIIME-generated OTU table.

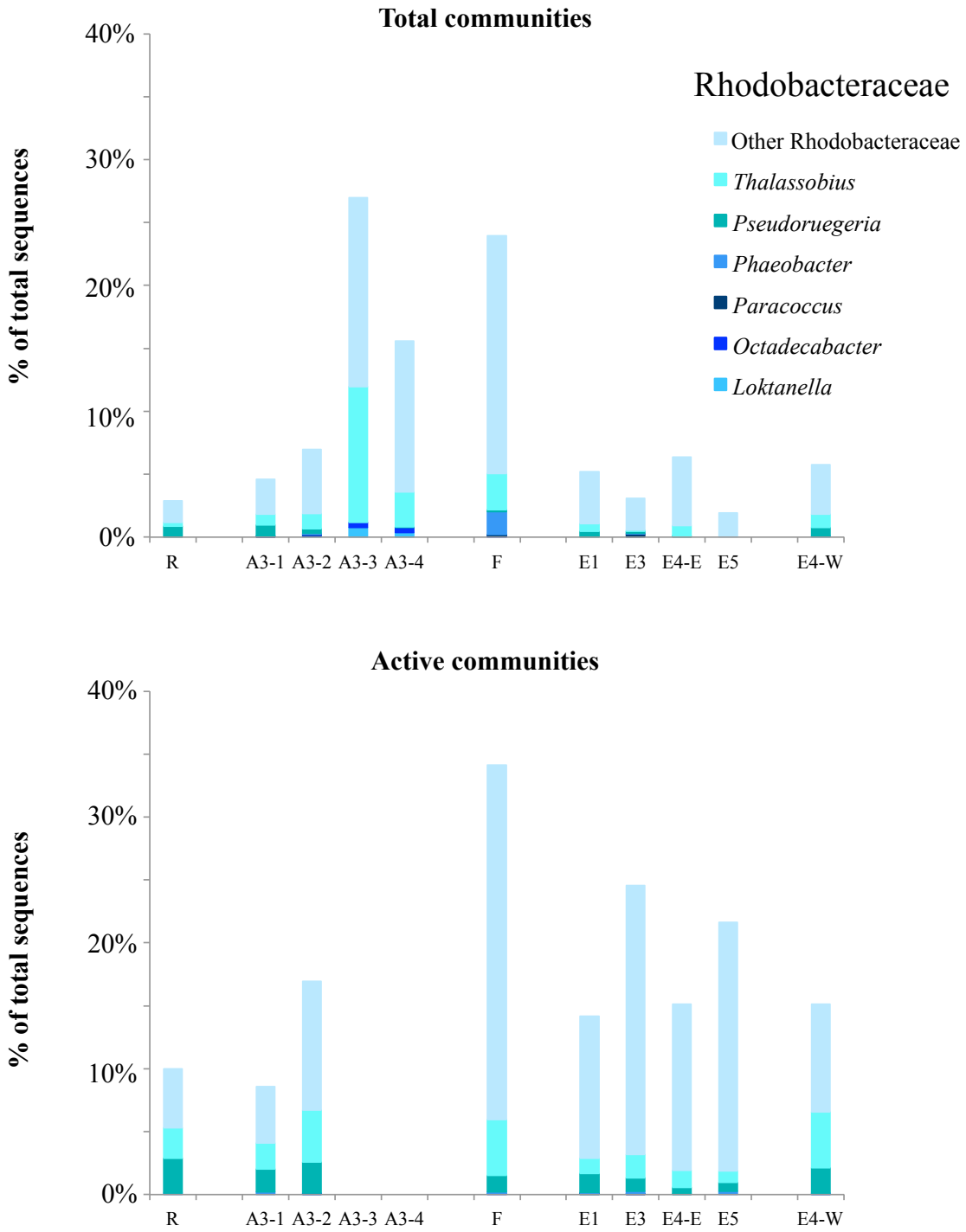

Figure S9. Relative abundances of the OTUs within the Rhodobacteraceae, in total (top) and active (bottom) communities at the study sites.

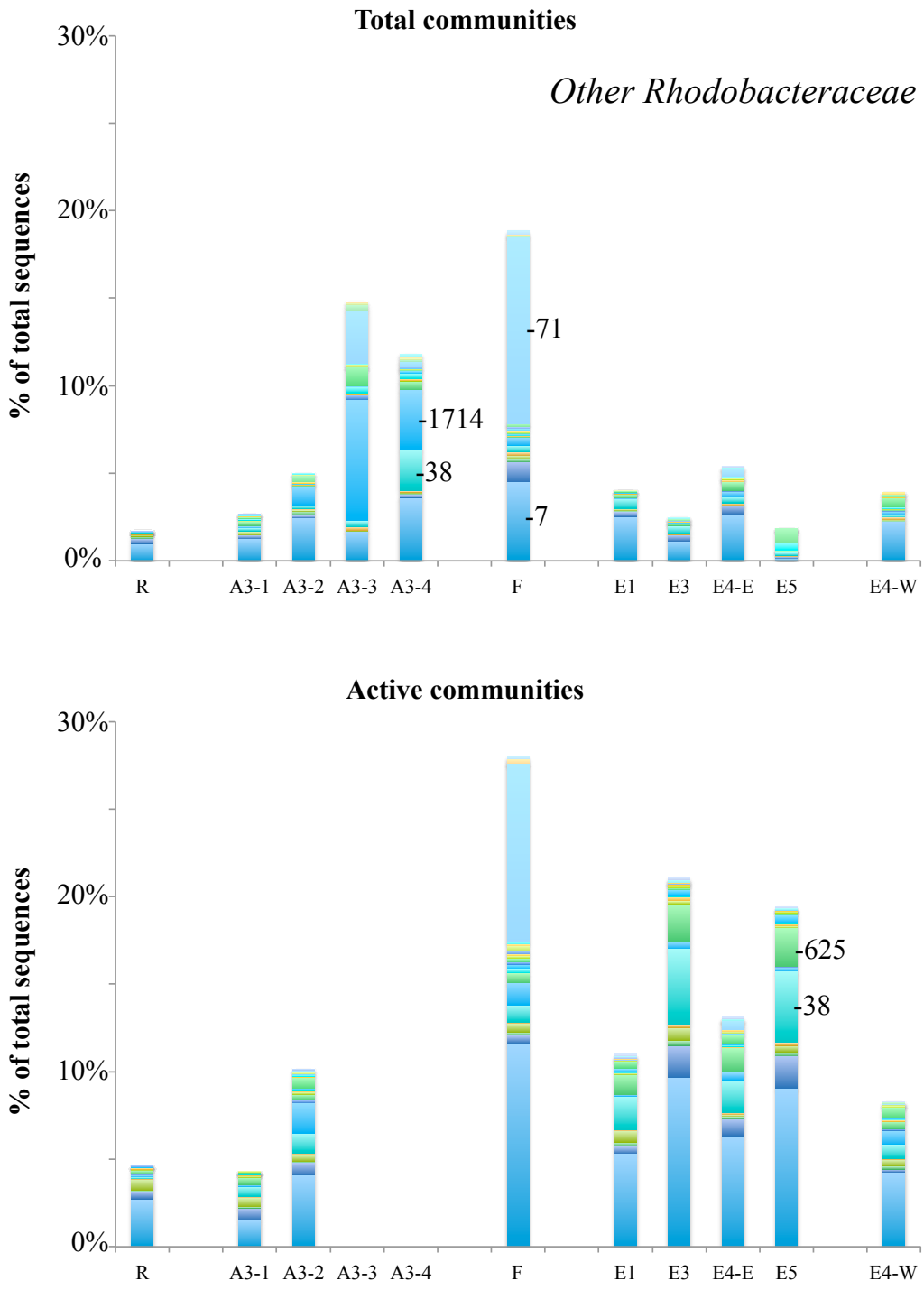

Figure S10. Relative abundances of the OTUs within the Other Rhodobacteraceae, in total (top) and active (bottom) communities at the study sites. Numbers refer to the OTUs identity in the QIIME-generated OTU table.

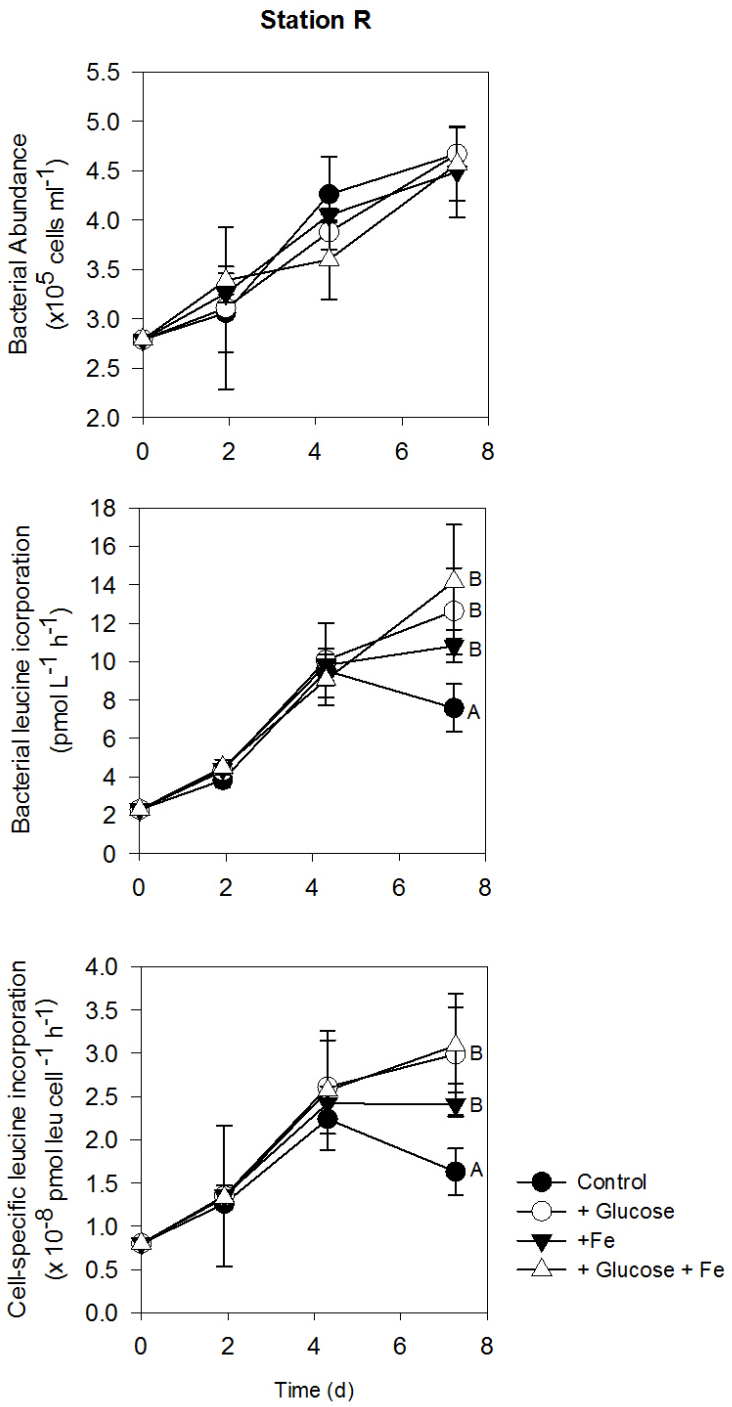

Figure S11. Bacterial abundance (top), leucine incorporation (middle) and cell-specific leucine incorporation in control incubations or in response to enrichments in Glucose (white circles), iron (black triangles) or both (white triangles).

Data were provided by I. Obernosterer.

GENERAL DISCUSSION & CONCLUSION

I. THE CONTINUOUS CULTURE EXPERIMENTS

In this thesis, three continuous culture experiments were conducted in two different environments. Each experiment was designed to address a specific question and the data collected provided elements of response to these questions.

The first experiment was conducted in the Mediterranean Sea, to determine the effect of an additional supply of DOM on bacterial diversity. In this experiment, the two treatments were characterized by differences in concentration as well as origin, and therefore most likely composition, of the organic compounds provided to bacteria in the cultures. Bacterial diversity was overall higher in the amended cultures compared to the control, indicating a higher number and broader phylogenetic diversity of bacterial taxa was selected by the culture conditions (Part one, Chapter 1). We concluded that a higher amount of DOM coupled to a potentially higher proportion of labile compounds constituted a broader resource pool, that sustained more diverse bacterial communities.

The second experiment was also conducted in the Mediterranean Sea, to determine the effect of resource quality on bacterial diversity. Two DOM sources originating from different phytoplankton cultures and presenting different chemical compositions and degradation stages were provided to another bacterial inoculum. One DOM source sustained higher bacterial diversity, especially in the absence of grazers. However, phylogenetic composition of the communities appeared to be unrelated to the DOM source (Part one, Chapter 2). We hypothesized that a higher chemical diversity of carbon compounds could favor diversity in bacterial populations, as opposed to carbon sources containing a reduced chemical variety of compounds, or compounds more difficult to access for bacteria.

The third experiment was conducted in the Southern Ocean, to evaluate the effect of a DOM amendment on bacterial diversity in this contrasting environment. In this experiment, the final carbon concentration was the same in the two tested conditions, but in the amended treatment a fraction of this carbon was from phytoplankton origin, to test the effect of substrate quality rather than potential quantitative effects. We found no structuring effect of DOM addition on the diversity level of total communities. However, the OTUs selected by each condition were different and belonged to distinct phylogenetic groups (Part two, Chapter 3). We hypothesized that in this environment, DOM of phytoplankton origin played a major part in the selection of dominant OTUs.

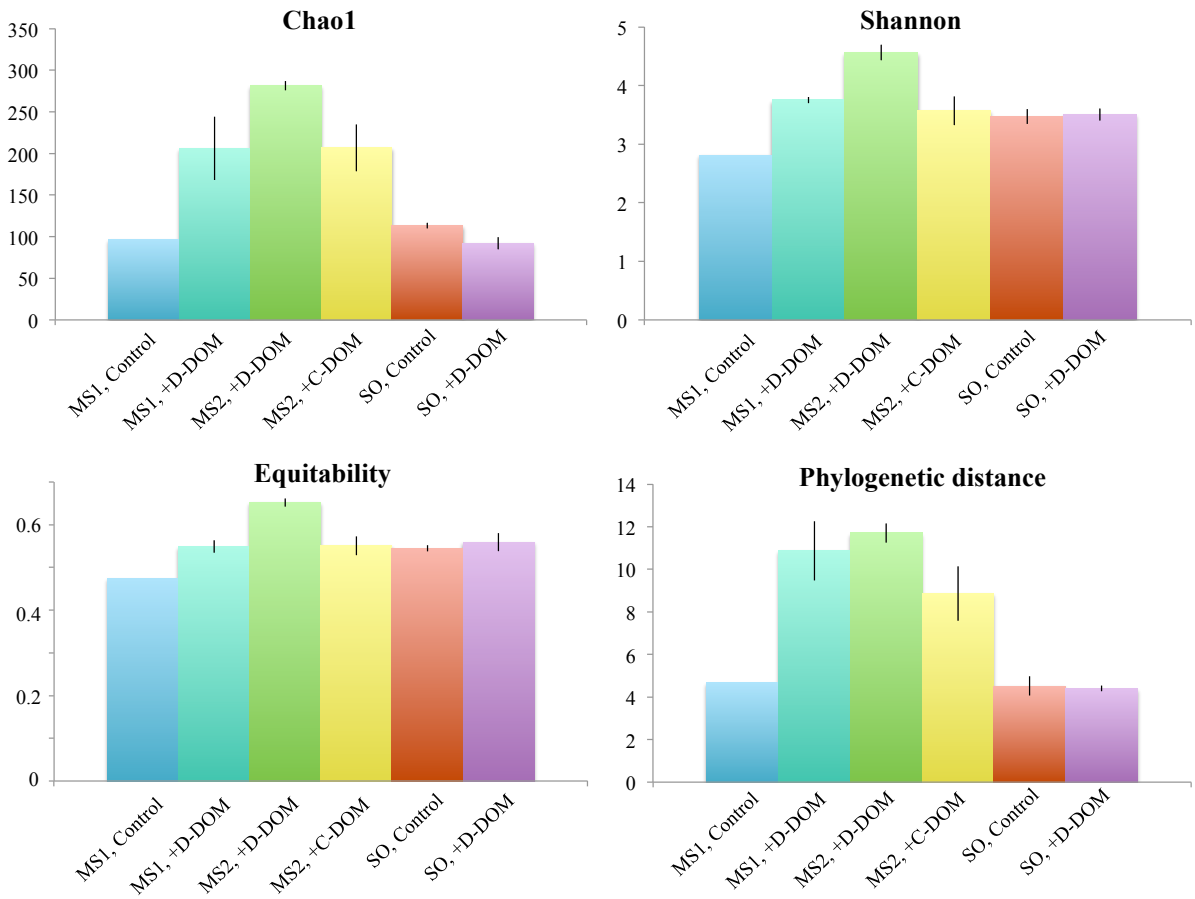

Figure 1. Diversity indices determined for bacterial communities sampled in the three continuous culture experiments conducted in the Mediterranean Sea (MS1 and 2) and the Southern Ocean (SO) after 3 generation times. Datasets were subsampled to 2930 sequences to enable comparison. The mean ± SD of the replicate cultures are indicated for each treatment : Control, diatom-DOM addition (+D-DOM) and cyanobacterial-DOM addition (+C-DOM), except for the unique replicate control in the first experiment.

The analysis of the three experiments combined provides additional information to the individual results of each of these experiments. In the Mediterranean Sea, the levels of diversity varied between experiments. However, the diversity level in the amended cultures was always higher than in the control culture, with any of the two DOM sources, for the four indices we used (Figure 1). This was particularly striking for richness and phylogenetic diversity.

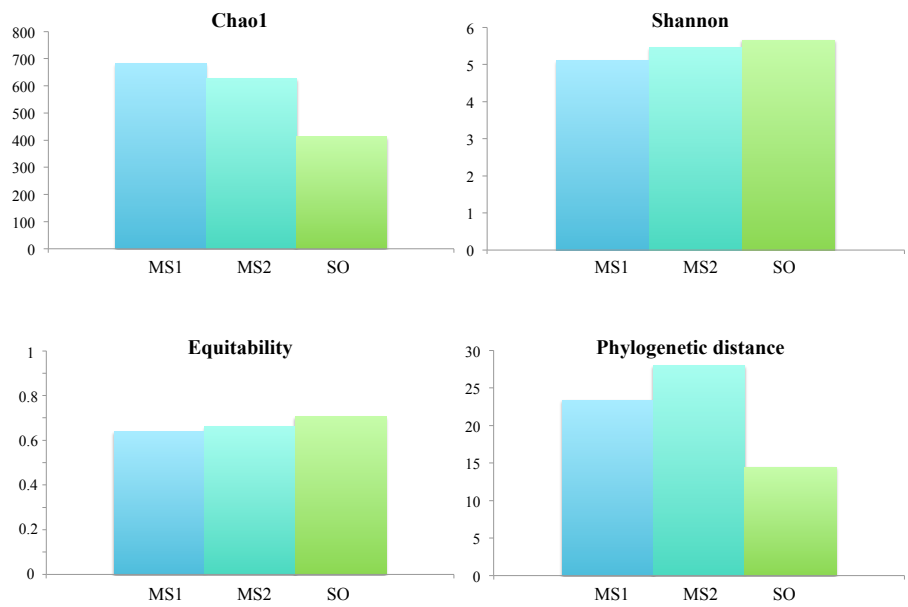

Figure 2. Diversity of the inocula used for each continuous culture experiment in the Mediterranean Sea (MS1 and 2) and in the Southern Ocean (SO). Datasets were subsampled to 2930 sequences to enable comparison.

In comparison to the results from the Mediterranean Sea, the richness and phylogenetic diversity of the communities in the Southern Ocean experiment were lower, which probably reflects the lower values determined for the inoculum (Figure 2). Different algorithms were used for the analysis of the two independent datasets, which complicates comparisons. However, this should not account for the patterns observed

between samples within a given dataset. In this case, the low richness of the inoculum in the Southern Ocean is not aberrant and could be due to the environment itself, where diversity was suggested to be overall lower than at lower latitudes (Ghiglione et al., 2012). It could also reflect the season when we conducted the experiment. Another study by Ghiglione & Murray (2012) revealed that October was the month when the microbial community in the coastal region of the Kerguelen Islands was the least diverse. For evenness and the Shannon index, the communities from the Southern Ocean experiment maintained diversity levels similar to the ones observed in the two Mediterranean Sea experiments, which was also the case for the inoculum (Figure 2). In general, the diversity of the inoculum could play a significant role in the outcome of such experiments, an idea supported by other studies (Langehender et al., 2006).

Bacterial diversity was always higher in the cultures amended with phytoplankton-derived DOM than in the non-amended cultures, which suggests that this effect of DOM on bacterial diversity could be robust, at least in this environment. The experiment in the Southern Ocean led to slightly different results, suggesting different selection mechanisms in the cultures. No differences in diversity levels were observed between the control and the +DOM treatment. A possible explanation to this is that the increase in bacterial diversity observed in the first experiment was the result of increased carbon concentration rather than its origin and composition. In the Southern Ocean experiment, there was no increase in final carbon concentration following phytoplankton DOM addition. However, the diversity of the dominant OTUs was higher in this treatment than in the control for the Southern Ocean experiment. These OTUs were also different from the ones dominating the control cultures. Therefore, the diversity of OTUs responding the most to the DOM amendment was higher than the diversity of OTUs benefiting the most from the carbon available in the control

conditions. From that angle, the addition of phytoplankton DOM in the Southern Ocean experiment also increased levels of diversity but affected the dominant OTUs only, in contrast to the results from the first experiment carried out in the Mediterranean Sea, where the increase in diversity was due to less abundant OTUs. Interestingly, we also observed a higher diversity of dominant OTUs in the diatom-DOM treatment from the second experiment performed in the Mediterranean Sea, and interpreted this as a consequence of different quality of the two DOM sources. This observation in the Southern Ocean could be further evidence for the distinct characteristics of the two experimental conditions, and their effect on bacterial diversity.

A major reason for the different responses to phytoplankton DOM addition in these two environments could be the nutritional status of the inoculum. Data from the literature strongly indicate that bacterial community in the Mediterranean Sea, and at our sampling site in particular, are P-limited throughout the year, including in November and March, when we conducted the two experiments (Pinhassi et al., 2006; Laghdass et al., 2012). To ensure limitation by organic carbon in the continuous cultures, these P-limited communities were grown in inorganic nutrients-enriched media. Therefore, the limitation due to the absence of phosphate in the environment was relieved at the beginning of the experiment, and replaced by a strong C-limitation. The literature reports changes in bacterial diversity and activity as a result of nutrients addition in the Mediterranean Sea (Lebaron et al., 2001; Schäfer et al., 2001; Pinhassi et al., 2006) as well as in other marine environments (Sipura et al., 2005), and especially when coupled to organic nutrients addition (Carlson et al., 2002; Øvreås et al., 2003; Teira et al., 2011). This does not challenge the validity of the differences in bacterial diversity observed between the different DOM conditions; however it could mean that the communities in these incubations also reacted to the increased supply of initially

limiting phosphate. A third experiment was performed in the Mediterranean Sea to test this hypothesis but due to carbon contaminations issues it was not analyzed in depth.

By contrast, the initial community used in the Southern Ocean experiment was not limited by phosphate or nitrate, but more likely by organic carbon (Obernosterer et al., 2008). Therefore, the factor initially limiting bacterial growth in the environment was maintained when starting the experiment. It is likely that this different limitation status of the inocula explains the different responses observed. This hypothesis finds support in the observation of high proportions of Gammaproteobacteria in the Mediterranean Sea experiments, whereas this group reached smaller relative abundances in the Southern Ocean experiment. Gammaproteobacteria have been suspected to have high phosphorus requirements (Sebastián et al., 2012) and be among the most responsive groups to enrichment experiments (Øvreås et al., 2003; Teira et al., 2011; Sebastián & Gasol, 2013), which could explain their competitiveness in the first Mediterranean Sea experiment compared to the Southern Ocean experiment. This group is also believed to be more affected by grazing than other groups, because of its rapid adaptation to environmental conditions and subsequent high growth rates and cell size (Lebaron et al., 2001; Beardsley et al., 2003; Ferrera et al., 2011). If true, this could explain why they reached smaller abundances in the second Mediterranean Sea experiment, where grazers were detected, and actively predating bacteria; compared to the first one of which they were absent.

These experiments successfully demonstrated the bacterial response to various characteristics of the organic resource, and also pointed out the variability of bacterial response to phytoplankton DOM. Our results also suggest that the impact of DOM on

bacterial diversity can be affected by environmental conditions, such as inorganic nutrients conditions for instance. This is supported by the observation of distinct communities in the various KEOPS2 blooms. At a larger scale, this could explain the variability of the results provided by the many studies addressing the question of the link between resource and diversity. In the future, more experimental studies should be designed and performed to improve our understanding of the parameters influencing the relationship between substrate identity, quality and concentration and bacterial populations. These experimental studies should be coupled to *in situ* observations and analyses of bacterial communities' responses to their continuously changing environment.

II. PHYLOGENY AND FUNCTIONAL TRAITS

The question of the link between phylogeny and functional traits is a major one in microbial ecology. The degradation of organic resource being one of bacteria's main roles in the environment, studying the link between this resource and the diversity of bacteria degrading it is one way of addressing the question of groups' traits.

The experiments conducted during this thesis tend to support the idea that functional traits are only weakly linked to phylogeny, at least when it comes to carbon uptake and degradation. All major marine groups seemed able to take up carbon within the complex and diverse pool of compounds derived from phytoplankton. Evidence that different groups display different uptake efficiencies or preferences towards various substrates are abundant in the literature (Cottrell & Kirchman, 2000; Gómez-Consarnau et al., 2012; Alonso-Sáez et al., 2012; Sarmento & Gasol, 2012). However, it seems that the expression of these traits is partly regulated by environmental conditions (Alonso-

Sáez & Gasol, 2007), which could explain why these studies generally concluded to weak links between the groups and their preferred substrates.

The role of the environment in the determination of the bacterial groups involved in the cycling of organic matter is likely as important as the characteristics of the groups themselves. As an illustration, several studies have suggested that some bacterial taxa perform well on high substrate concentration whereas others perform better at low substrate concentration (Eiler et al., 2003; Füschlin et al., 2012). This indicates that all these groups are capable of carbon uptake but that the characteristics of the environment (in this case, substrate concentration) eventually determine which groups end up, in a particular situation, benefiting from the resource. The effect of phosphate versus carbon limitation on the inocula used in our three continuous culture experiments also supports this hypothesis.

A crucial point in the investigation of the links between community composition and DOM degradation is the phylogenetic level at which community composition is examined. The continuous culture experiment performed in the Southern Ocean revealed different competitive advantages for two genera in the Rhodobacteraceae, *Thalassobius* and *Phaeobacter*, confirmed by their distinct responses to *in situ* bloom conditions. Conversely, the *in situ* data showed that within a given group taken at high phylogenetic resolution, such as *Thalassobius* or *Polaribacter*, different OTUs displayed distinct responses to a given environmental condition. This suggests either that phylogenetically close OTUs are not redundant, or that they are in competition for their ecological niches. This emphasizes the need to pursue the investigation of the diversity of taxa existing within the broad phyla, to better understand their ecology and the role they play in the cycling of organic matter among other functions.

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Les bactéries sont retrouvées dans tous les écosystèmes de la planète, y compris les plus extrêmes. Ces organismes unicellulaires microscopiques sont loin d'être insignifiants : avec un nombre de bactéries sur Terre estimé à 10^{30}, la biomasse bactérienne totale avoisine celle des plantes (Whitman et al., 1998). L'étude de la diversité de ces organismes est une étape clé vers la compréhension globale de leur rôle dans les écosystèmes.

Diversité bactérienne et fonctions dans l'environnement

Le terme de « diversité bactérienne » est fréquemment employé en référence au nombre et à l'identité phylogénétique des taxons bactériens. Les estimations de la diversité bactérienne totale proposent jusqu'à 10^9 taxons différents sur Terre (Dykhuizen et al., 1998), bien que la vraisemblance d'un tel chiffre soit discutée (Schloss & Handelsman, 2004 ; Pedrós-Alió, 2006). Le séquençage du gène de l'ARN ribosomal 16S, gène bactérien universel (Woese & Fox, 1977), a permis la comparaison et la classification des taxons bactériens. Cinquante-deux phyla bactériens ont pu être identifiés par cette approche, dont la plupart ne possèdent pas de représentant cultivé (Figure 1) (Rappé & Giovannoni, 2003). Quel que soit le nombre réel de taxons, et que l'on puisse ou non le déterminer avec précision, la diversité phylogénétique des bactéries est considérable et reste à découvrir.

D'un point de vue écologique, il est tentant d'émettre l'hypothèse qu'une telle diversité phylogénétique reflète ou découle des différents rôles que jouent les bactéries dans leur environnement, ou des niches écologiques qu'elles occupent. Les écologistes microbiens tentent depuis des années d'établir un lien entre les taxons bactériens et

l'existence de traits fonctionnels ou écologiques. Dans certains cas, ces liens existent et sont facilement identifiables.

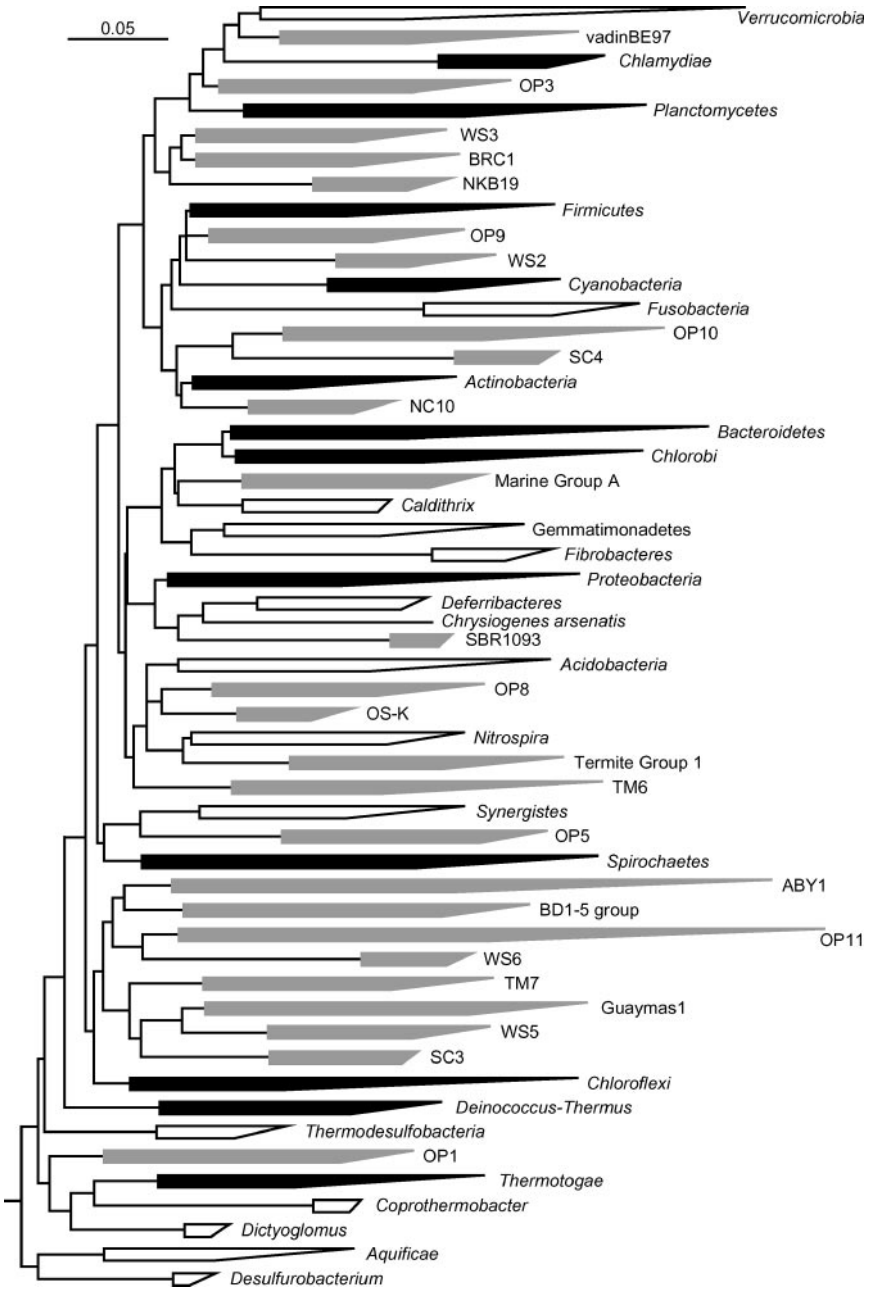

Figure 1. Phyla majeurs du domaine des Bactéries, tiré de Rappé & Giovannoni (2003). Les 12 phyla initialement décrits par Woese (1987), les 14 phyla comprenant des représentants cultivés et les 26 phyla « candidats » sont représentés en noir, blanc et gris, respectivement.

Par exemple, l'autotrophie, ou la capacité à fixer le carbone inorganique, est retrouvée quasiment exclusivement chez les cyanobactéries. Dans le cas de ce trait fonctionnel, la phylogénie et la fonction sont parfaitement corrélées. La phylogénie permet également d'expliquer en partie la distribution des taxons bactériens dans l'environnement. Les populations bactériennes marines comprennent des membres appartenant généralement à une douzaine de taxons parmi les 52 identifiés à ce jour (Figure 1), notamment les Protéobactéries et les Bacteroidetes (Fuhrman & Hagström, 2008). Les eaux douces, quant à elles, sont généralement dominées par des groupes comme les Betaprotéobactéries et les Actinobactéries (Glöckner et al., 1999 ; Humbert et al., 2009). Cette distribution des groupes bactériens suggère que l'adaptation de ces derniers à la vie en eau douce ou salée est liée à la phylogénie, même à faible résolution (Lozupone & Knight, 2007).

Cependant, l'hypothèse selon laquelle les bactéries appartenant à un même groupe phylogénétique possèdent des caractéristiques fonctionnelles et écologiques similaires est mise à mal par l'existence d'un grand nombre de travaux qui démontrent que des taxons phylogénétiquement proches font preuve de modes de vie différents (Field et al., 1997 ; Casamayor et al., 2002 ; García-Martínez et al., 2002). Chez les bactéries, l'évolution se fait par transfert latéral aussi bien qu'horizontal, ce qui implique que des bactéries sans lien phylogénétique puissent partager du matériel génétique. Cette particularité est une source importante de diversité et complique la relation existant entre la signature phylogénétique et les capacités fonctionnelles d'un groupe (Wiedenbeck & Cohan, 2011). L'étroitesse du lien entre phylogénie et traits fonctionnels des groupes bactériens dépend vraisemblablement de deux paramètres cruciaux : le niveau phylogénétique auquel les groupes sont définis, ainsi que les fonctions étudiées. Les différents groupes bactériens présentent des taux d'évolution distincts, ce qui

suggère que les diverses niches écologiques occupées par les membres de ces groupes sont à chercher à des résolutions phylogénétiques variables (Zinger et al., 2012). Par exemple, il est admis que les Alphaprotéobactéries, et en particulier le clade des SAR11, dominent les communautés bactériennes des systèmes marins (Morris et al., 2002). En revanche, certains sous-groupes des SAR11 possèdent des distributions géographiques spécifiques (Pommier et al., 2005). Ceci illustre que le trait observé, dans ce cas l'abondance d'un groupe ou sa distribution géographique ; ainsi que le niveau phylogénétique auquel on se place, ici le clade ou le sous-clade, sont prépondérants dans la recherche des liens existant entre phylogénie et propriétés écologiques et fonctionnelles.

Les bactéries marines et le cycle du carbone

Parmi les nombreuses fonctions des bactéries, la dégradation de la matière organique est fondamentale. Cette fonction prend toute son importance en milieu marin, où les bactéries hétérotrophes obtiennent le carbone nécessaire à la production d'énergie et de biomasse essentiellement à partir de la matière organique dissoute (MOD). Les océans couvrent 70% de la surface du globe, et la MOD océanique constitue un énorme réservoir de carbone organique équivalent aux 700 Gt de carbone contenu dans le CO_2 atmosphérique (Nagata, 2008). La majorité de ce stock considérable de carbone est dit réfractaire, ce qui signifie qu'il est résistant à la consommation microbienne et s'accumule dans l'océan (Bauer, 2002). La matière labile et semi-labile représente une fraction moins importante de l'ensemble de la MOD et alimente malgré tout une part importante de la production bactérienne. La vitesse de recyclage des composés labiles comme les acides aminés ou les sucres libres est si élevée (de quelques

minutes à quelques heures) que ces molécules sont quasiment indétectables et représentent moins de 1% du stock de MOD (Nagata, 2008). Dans la mesure où l'oxydation nette sur un an de seulement 1% de la MOD océanique représente un flux de carbone équivalent aux émissions anthropogéniques de CO_2 (Hedges, 2002), le rôle des bactéries hétérotrophes dans la reminéralisation de la MOD en fait des acteurs clés du cycle du carbone (Figure 2).

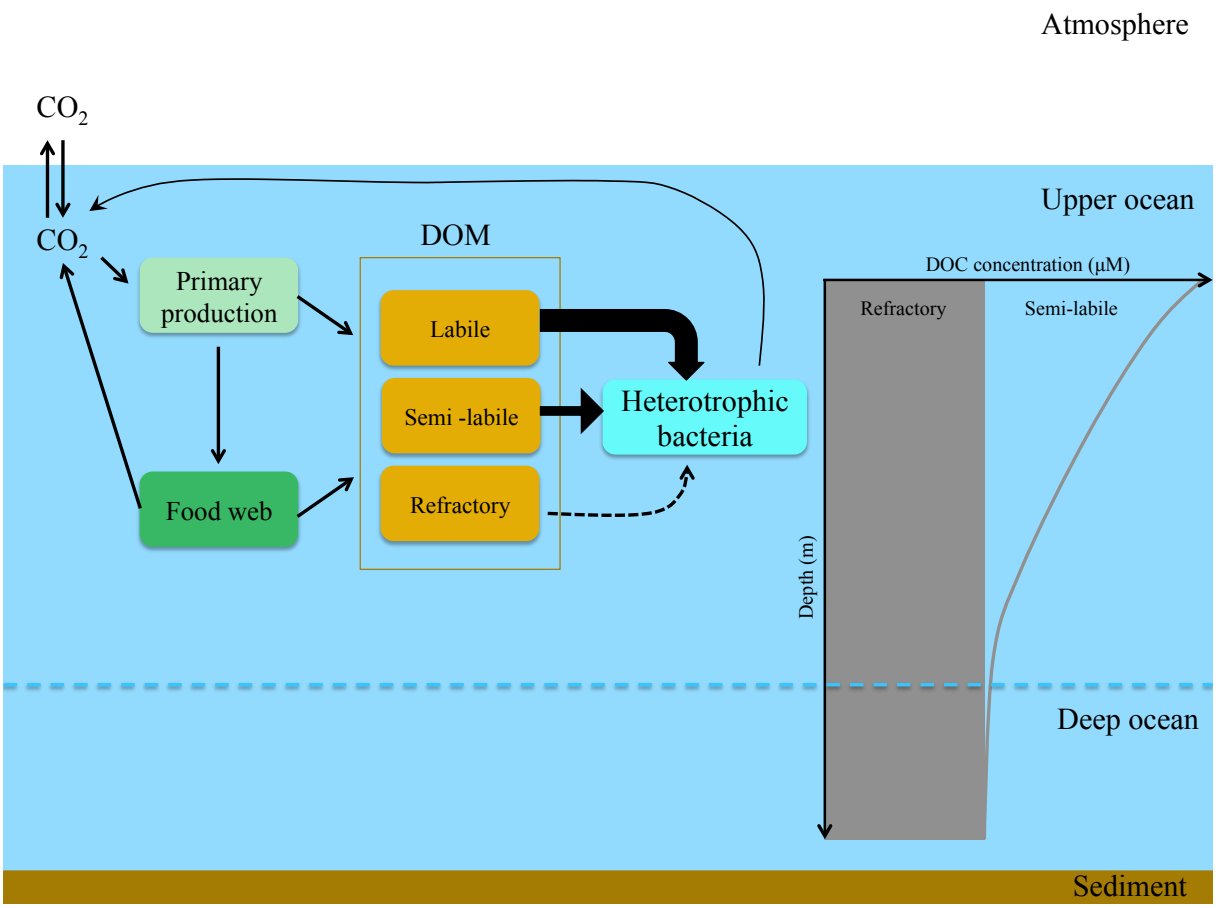

Figure 2. Schéma simplifié des voies de production de la MOD et son utilisation par les bactéries hététrotrophes dans la couche pélagique. La distribution de la MOD semi-labile et réfractaire dans la colonne d'eau est indiquée à droite. La MOD labile n'est pas représentée en raison de sa très faible concentration.

Dans ce contexte, la question du lien entre la phylogénie des bactéries et leurs traits fonctionnels est prépondérante. Compte tenu des diverses origines de la MOD océanique (Moran & Hodson, 1994 ; Nagata, 2000), de la complexité et de la diversité

des composés constitutifs de ce pool de carbone (Repeta et al., 2002 ; Benner, 2002 ; Benner & Kaiser, 2003), l'existence chez les divers groupes bactériens de traits spécifiques impliqués dans la dégradation de la MOD pourrait avoir des répercussions importantes sur le fonctionnement de l'écosystème. Dans ce cas, la composition et la diversité des communautés bactériennes sont des éléments déterminants du point de vue de l'efficacité et du résultat des transformations de la matière organique. Pour cette raison, des efforts considérables ont été réalisés pour caractériser la relation existant entre la grande diversité phylogénétique des bactéries marines et les traits fonctionnels impliqués dans la dégradation de la MOD.

Le rôle des différents groupes bactériens dans l'utilisation du carbone organique

Sur le plan conceptuel, il n'y a pas lieu de penser que certains groupes, parmi ceux que l'on trouve classiquement en milieu marin, sont incapables d'utiliser la MOD océanique. Le carbone organique dissous se trouve à la base de la production d'énergie et de biomasse des bactéries hétérotrophes, ce qui implique qu'elles sont toutes capables de le détecter dans l'environnement et de s'en servir. Cependant, de nombreux travaux montrent de nettes préférences des groupes pour certains substrats, ainsi que des efficacités variables d'utilisation de ces substrats. Ces données suggèrent que ces groupes bactériens ne sont pas équivalents sur le plan fonctionnel. Par exemple, l'étude du contenu génomique de plusieurs membres des Bacteroidetes a permis de révéler la présence de gènes impliqués dans la dégradation des polysaccharides, entre autres (González et al., 2008 ; Gómez-Pereira et al., 2012). La combinaison des méthodes de microautoradiographie et d'hybridation fluorescente *in situ* a montré que les membres de ce groupe étaient effectivement très actifs vis-à-vis de l'incorporation des composés à

haut poids moléculaire, tandis que les Alphaprotéobactéries préféraient le glucose et les acides aminés, et les Gammaprotéobactéries utilisaient surtout les acides aminés et l'ATP (Cottrell & Kirchman, 2000 ; Alonso-Saéz et al., 2012). Ceci étant, ces préférences des groupes pour certains substrats varient avec la saison (Alonso-Saéz & Gasol, 2007), ce qui suggère un faible couplage entre les groupes phylogénétiques et l'identité chimique des composés qu'ils dégradent. La réponse de divers groupes bactériens à des sources de MOD plus complexes, provenant de plusieurs espèces phytoplanctoniques, indique également des préférences dans l'ensemble faibles de ces groupes pour les différentes sources de matière (Sarmento & Gasol, 2012).

Ces études ont démontré la variabilité d'incorporation des substrats labiles parmi plusieurs groupes bactériens, mais la plupart d'entre elles concluent que tous les groupes sont globalement capables d'utiliser l'ensemble des sources de carbone testées (Gómez-Consarnau et al., 2012). Ceci suggère qu'en dépit des préférences qu'ils affichent, les groupes bactériens sont aptes à utiliser une large gamme de composés labiles, et qu'aucun substrat n'est utilisé de manière exclusive par un groupe. Ceci amène à deux conclusions fondamentales. Premièrement, la plupart des groupes bactériens, sinon tous, affichent un comportement généraliste vis-à-vis de la ressource, dans le sens où aucun d'entre eux ne dépend d'un substrat unique pour croître. Ils semblent capables d'utiliser diverses molécules, ce qui est confirmé par plusieurs résultats expérimentaux (Rink et al., 2007 ; Mou et al., 2008). Deuxièmement, la redondance fonctionnelle existe chez les bactéries marines, en tout cas en ce qui concerne la dégradation des composés labiles tels que les acides aminés, les protéines et les hydrates de carbone.

Si tous les groupes sont en mesure d'incorporer n'importe quel substrat labile et d'être remplacés, dans un écosystème, par un autre groupe, les modifications du pool de MOD comme des changements de concentration, de biodisponibilité ou de composition

chimique des substrats devraient rester sans effet sur la structure des communautés bactériennes. Certaines études montrent en effet de faibles modifications de la composition des communautés bactériennes suite à des changements de MOD (Langenheder et al., 2005 ; Rooney-Varga et al., 2005). Cependant, dans la plupart des cas les changements dans l'apport et la composition de la ressource sont accompagnés de modifications du niveau d'activité des communautés associées à des restructurations de la composition des communautés bactériennes (Pinhassi et al., 1999 ; Arrieta & Herndl, 2002 ; Kirchman et al., 2004). Ceci est particulièrement illustré par les analyses de diversité effectuées lors d'efflorescences phytoplanctoniques (Fandino et al., 2001 ; Pinhassi et al., 2004 ; Tada et al., 2011 ; Teeling et al., 2012). L'examen des gènes exprimés dans le contexte de ces efflorescences, ou dans le cadre d'une augmentation de l'apport en MOD labile de manière générale a montré une stimulation différentielle des groupes phylogénétiques, ce qui a mené à la conclusion que la ressource était répartie parmi les membres des communautés bactériennes (McCarren et al., 2010 ; Poretsky et al., 2010), souvent en fonction de leur phylogénie (Rinta-Kanto et al., 2012).

Objectifs de la thèse et organisation du manuscrit

À la lumière de ces divers résultats, la question du rôle de la structure des communautés dans la dégradation de la MOD, via l'existence de traits fonctionnels spécifiques des groupes, demeure en suspens. L'objectif de ce travail de thèse était d'examiner l'effet de la MOD sur la diversité et la composition des communautés bactériennes hétérotrophes. Ce travail s'est basé sur l'hypothèse que si la dégradation de la MOD nécessite la présence active de groupes bactériens adaptés, alors des MOD de concentration et de compositions variables devraient engendrer des communautés

bactériennes différentes. Pour cela, des communautés bactériennes naturelles ont été maintenues en culture continue de manière à stabiliser l'ensemble des paramètres abiotiques et déterminer si des différences de concentration ou de constitution chimique de la MOD jouent des rôles déterminants sur la diversité des communautés obtenues dans ces cultures. Le principe et le fonctionnement théorique des cultures en continue font l'objet d'explications détaillées dans la section Méthodologie de ce manuscrit.

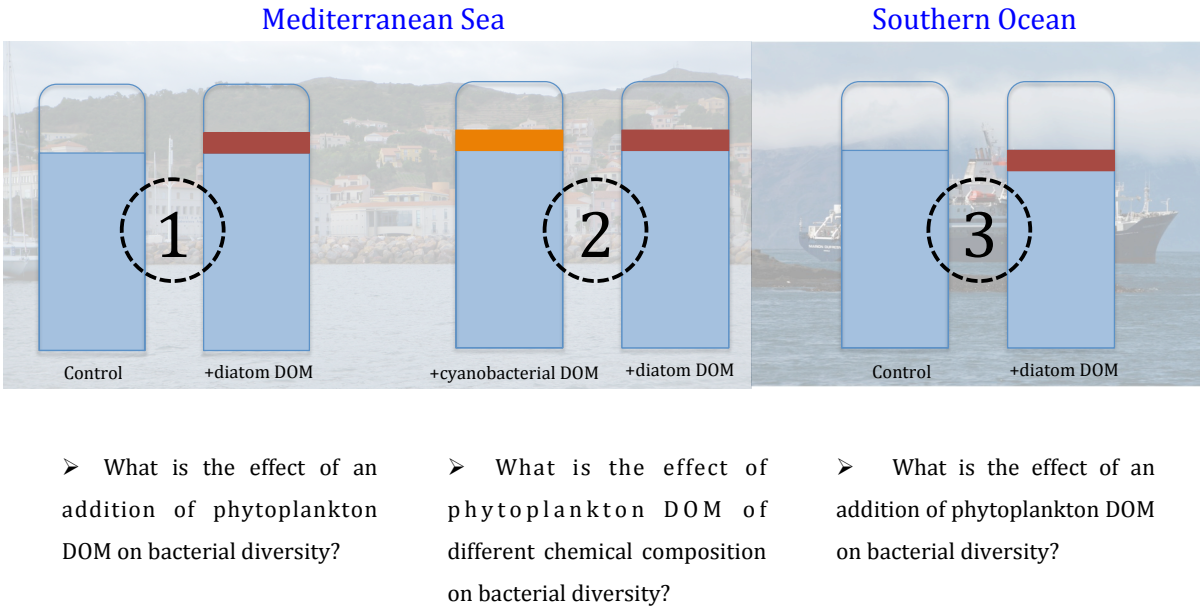

Figure 3. Schéma simplifié des trois expériences de cultures en continu présentées dans le manuscrit, et les questions dont elles faisaient l'objet.

Trois expériences indépendantes ont été réalisées, chacune d'entre elles ayant été conçue pour répondre à une question spécifique (Figure 3). Deux expériences ont été menées en mer Méditerranée et sont présentées dans la première partie de ce manuscrit. L'objectif de la première expérience était d'examiner la réponse des bactéries marines hétérotrophes à un ajout de MOD phytoplanctonique. Cette expérience, les résultats obtenus et les hypothèses proposées pour les expliquer ont fait l'objet d'une

publication dans le journal Aquatic Microbial Ecology et sont présentés dans le Chapitre 1.

L'objectif de la deuxième expérience était d'étudier la réponse des bactéries marines hétérotrophes à des MOD phytoplanctoniques d'origine et de composition chimique différentes. Cette expérience, les résultats obtenus et les interprétations tirées sont présentés dans le Chapitre 2, préparé à partir de la version révisée du manuscrit soumis à Environmental Microbiology en juillet 2013.

La seconde partie de ce manuscrit s'intéresse à la campagne océanographique KEOPS2, qui s'est déroulée dans l'océan Austral en octobre-novembre 2011 et au cours de laquelle la troisième expérience en culture continue a été menée. L'objectif général de la campagne était d'étudier la mise en place d'une efflorescence phytoplanctonique en réponse à un événement naturel de fertilisation en fer (Blain et al., 2007). Le but de l'expérience en culture continue réalisée à bord était d'examiner la réponse des bactéries hétérotrophes à une addition de MOD phytoplanctonique dans cet environnement. En parallèle, la diversité bactérienne *in situ* a été analysée dans diverses efflorescences phytoplanctoniques et au cours du temps. L'objectif final était de regarder si les résultats obtenus grâce à l'expérience de culture continue pouvaient contribuer à améliorer la compréhension de la réponse *in situ* des bactéries aux conditions d'efflorescence phytoplanctonique. Le contexte général de la campagne et les résultats obtenus à partir de l'expérience en culture continue et des analyses de la diversité bactérienne *in situ* constituent un ensemble de données non publiées présentées dans le Chapitre 3.

I. LES EXPÉRIENCES DE CULTURE CONTINUE

Dans le cadre de cette thèse, trois expériences de culture continue ont été réalisées, dans deux environnements contrastés. Chaque expérience avait pour objectif de répondre à une question spécifique, et les données obtenues ont permis d'apporter des éléments de réponse à ces questions.

La première expérience de culture en continue a été menée en mer Méditerranée pour étudier l'effet d'un apport de MOD sur la diversité bactérienne. Dans cette expérience, les deux conditions testées différaient par leur concentration en carbone organique dissous (COD) ainsi que par son origine et probablement, par voie de conséquence, par sa composition. La diversité bactérienne déterminée dans les cultures enrichies en MOD était globalement plus élevée que dans le contrôle, signifiant qu'un plus grand nombre et une plus grande diversité phylogénétique de taxons bactériens ont été sélectionnés par ces conditions de culture (Partie un, Chapitre 1). Ces données ont permis de conclure qu'une quantité plus importante de MOD, couplée à une proportion plus élevée de composés labiles, représentait un ensemble de ressources plus important et a permis la mise en place de communautés bactériennes plus diversifiées.

La deuxième expérience a également été réalisée en mer Méditerranée, pour déterminer l'effet de la qualité de la ressource sur la diversité bactérienne. Un second inoculum bactérien a été alimenté avec deux sources de MOD provenant de cultures phytoplanctoniques différentes et caractérisées par des compositions chimiques et des états de dégradation distincts. L'une de ces sources a permis l'obtention de communautés bactériennes plus diversifiées que l'autre, particulièrement en l'absence de prédateurs. Cependant, la composition phylogénétique des communautés s'est

établie sans lien apparent avec la source de MOD fournie aux bactéries (Partie un, Chapitre 2). L'hypothèse a été proposée qu'une plus grande diversité chimique des composés carbonés agissait comme un facteur de diversité des populations bactériennes, au contraire des sources de carbone de faible diversité chimique ou contenant des composés plus difficiles d'accès aux bactéries.

La troisième expérience a eu lieu dans l'océan Austral, pour déterminer l'effet d'un ajout de MOD sur la diversité bactérienne dans cet environnement. Cette fois, les concentrations finales en carbone organique dissous étaient identiques dans le contrôle et dans les cultures enrichies, dans lesquelles une fraction du carbone disponible pour les bactéries était d'origine phytoplanctonique. Cette configuration a permis de tester l'effet de la qualité plutôt que de la quantité de substrat sur la diversité bactérienne. Lors de cette expérience, l'ajout de MOD n'a pas eu d'effet sur le niveau de diversité des communautés bactériennes, en revanche les OTUs sélectionnées par chaque traitement étaient différentes et appartenaient à des groupes phylogénétiques distincts (Partie deux, Chapitre 3). Dans cet environnement, la MOD phytoplanctonique a joué un rôle majeur dans la sélection des groupes dominants.

La comparaison des résultats des trois expériences permet d'approfondir l'analyse. En mer Méditerranée, les niveaux de diversité obtenus dans les cultures varient entre les deux expériences. Cependant, le niveau de diversité atteint dans les cultures enrichies en MOD était toujours supérieur à celui obtenu dans le contrôle, pour toutes les sources de MOD utilisées et les différents indices de diversité calculés (Figure 4). Cette observation est particulièrement vraie lorsque l'on s'intéresse aux indices de richesse et de diversité phylogénétique.

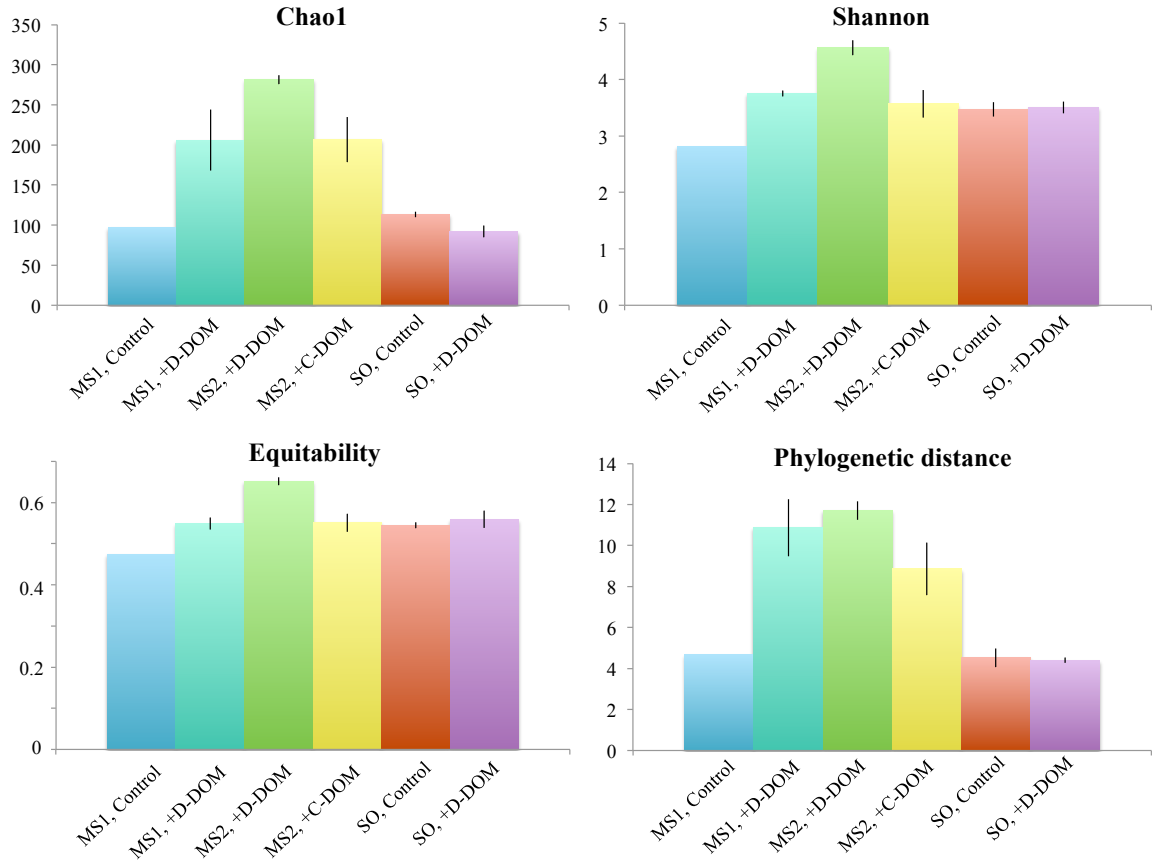

Figure 4. Indices de diversité des communautés bactériennes issues des trois expériences de culture continue réalisées en mer Méditerranée (MS1 et MS2) et dans l'océan Austral (SO), après trois temps de génération. Le jeu de données a été sous-échantillonné à 2930 séquences par échantillon pour pouvoir les comparer. La moyenne ± l'écart type calculés à partir des réplicats sont représentés pour chaque traitement : contrôle, ajout de MOD de diatomée (+D-DOM) ou de cyanobactérie (+C-DOM), sauf pour le réplicat unique du contrôle de la première expérience.

En comparaison, ces mêmes indices se sont avérés plus faibles dans les cultures réalisées dans l'océan Austral, ce qui reflète probablement les valeurs plus faibles déterminées pour l'inoculum (Figure 5). Le fait que des algorithmes différents aient été utilisés pour l'analyse des deux jeux de données indépendants complique la comparaison, mais ces divers algorithmes n'expliquent pas, à eux seuls, les tendances observées au sein d'un même jeu de données. Dans le cas présent, la faible richesse de l'inoculum utilisé pour l'expérience dans l'océan Austral n'est pas aberrante et reflète certainement l'environnement lui-même, où la diversité pourrait être plus faible qu'à

d'autres latitudes (Ghiglione et al., 2012). La saison pendant laquelle l'expérience a été réalisée pourrait aussi jouer un rôle dans le niveau de diversité observé : une étude de Ghiglione et Murray (2012) a révélé que la diversité de la région côtière des îles Kerguelen était la plus faible en octobre comparé au reste de l'année. Dans les cultures, les indices de Shannon et d'uniformité présentaient des valeurs similaires dans les expériences en mer Méditerranée et dans l'Austral, ce qui était également le cas pour les inocula (Figure 5). De manière générale, la diversité de l'inoculum pourrait jouer un rôle majeur dans les résultats de ce type d'expériences, une idée qui est alimentée par certains travaux (Langenheder et al., 2006).

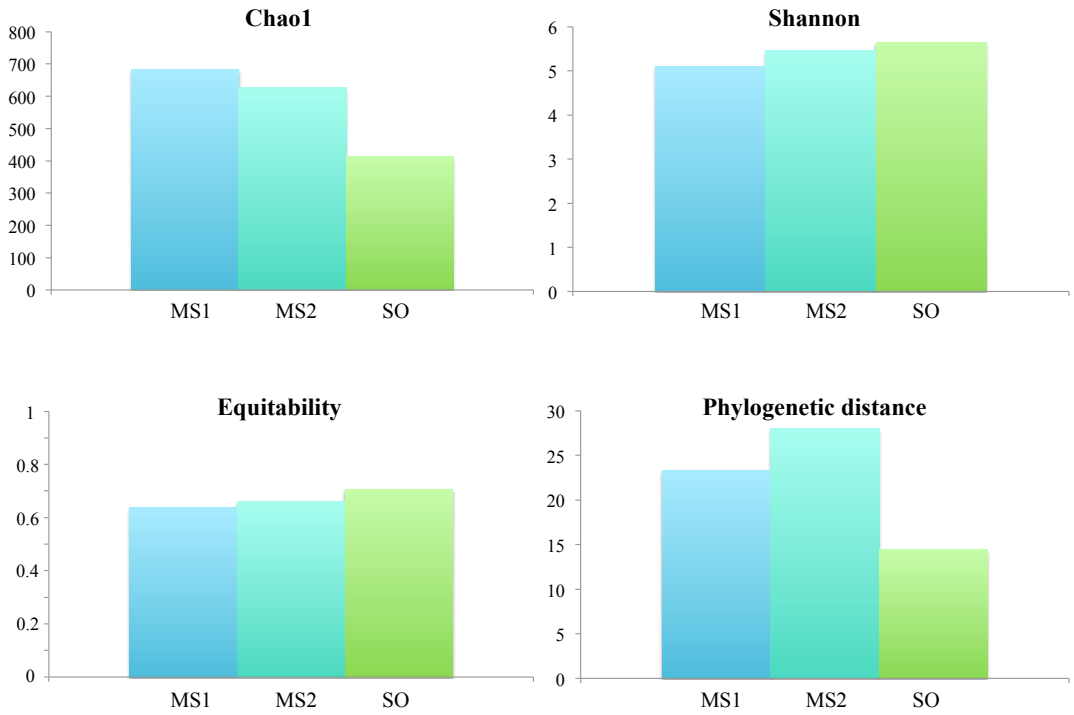

Figure 5. Diversité des inocula utilisés pour chaque expérience en culture continue, en mer Méditerranée (MS1 et MS2) et dans l'océan Austral (SO). Les jeux de données ont été sous-échantillonnés à 2930 séquences par échantillon pour permettre la comparaison.

L'augmentation de diversité induite par l'ajout de MOD phytoplanctonique, en comparaison avec des cultures non-enrichies (contrôle) a été observée de manière répétée en mer Méditerranée, ce qui suggère un effet robuste de la MOD sur la diversité bactérienne dans ce milieu. L'expérience menée dans l'océan Austral a conduit à des résultats différents, ce qui porte à croire que d'autres mécanismes de mise en place de la diversité se sont produits dans ces cultures. Les niveaux de diversité observés étaient les mêmes dans le contrôle et dans l'enrichissement en MOD. Une explication possible serait que l'augmentation de la diversité observée dans les premières expériences serait le résultat de l'augmentation de la quantité de carbone fournie aux bactéries plutôt que liée à son origine ou sa composition. Dans l'expérience réalisée dans l'océan Austral, la quantité finale de carbone fournie était identique dans les deux traitements. La diversité des OTUs dominantes était plus élevée dans les cultures enrichies que dans les cultures contrôles. Ces OTUs n'étaient d'ailleurs pas les mêmes dans les deux traitements. Ceci indique que les OTUs répondant le plus à l'ajout de MOD étaient plus diversifiées que celles se développant sur les composés carbonés présents dans la condition contrôle. Vue sous cet angle, l'addition de MOD phytoplanctonique dans l'expérience réalisée dans l'océan Austral a également entraîné une augmentation des niveaux de diversité dans les cultures, mais cette fois au niveau des groupes dominants uniquement, ce qui contraste avec l'expérience réalisée en Méditerranée où l'augmentation de diversité concernait des OTUs moins abondantes. De manière intéressante, une augmentation de la diversité des groupes bactériens dominants a également été observée dans les cultures enrichies en MOD issue de diatomée dans la seconde expérience menée en Méditerranée. Ce résultat avait été interprété comme la conséquence des différences qualitatives entre les sources de MOD utilisées. L'observation de ce résultat dans l'expérience de l'océan

Austral constitue un argument supplémentaire soutenant l'hypothèse que les caractéristiques chimiques de la MOD affectent la diversité bactérienne.

Une raison majeure expliquant les différences de réponse à l'ajout de MOD phytoplanctonique dans les cultures pourrait se trouver dans l'état nutritionnel de l'inoculum. Les données de la littérature indiquent que les communautés bactériennes en mer Méditerranée sont la plupart du temps fortement limitées par le phosphate, en particulier au niveau du site échantillonné et aux périodes de l'année durant lesquelles les deux expériences ont été menées, en novembre et en mars (Pinhassi et al., 2006 ; Laghdass et al., 2012). Pour garantir la limitation par le carbone au cours des expériences, les milieux de culture ont été préparés avec des quantités importantes de nutriments inorganiques. En conséquence, la forte limitation des communautés bactériennes due à la déplétion en phosphate dans l'environnement a été levée dès le début des expériences, et remplacée par une limitation par le carbone. La littérature fait état de changements dans l'activité et la diversité bactérienne suite à des enrichissements en sels nutritifs en Méditerranée (Lebaron et al., 2001 ; Schäfer et al., 2001 ; Pinhassi et al., 2006) ainsi que dans d'autres milieux (Sipura et al., 2005), tout particulièrement lorsque ces ajouts sont couplés à des ajouts de matière organique (Carlson et al., 2005 ; Øvreås et al., 2003 ; Teira et al., 2011). Cela ne remet pas en cause la validité des observations qui montrent des réponses variables des bactéries aux deux conditions testées au sein d'une même expérience, mais peut en revanche indiquer que les communautés ont également réagi à l'apport du phosphate qui était initialement limitant. Une troisième expérience avait été réalisée en mer Méditerranée pour tester cette hypothèse, mais n'a pas été menée à terme en raison de fortes contaminations en carbone organique.

De manière opposée, l'inoculum utilisé pour l'expérience dans l'océan Austral n'était pas limité par le phosphate, mais plus vraisemblablement par le carbone (Obernosterer et al., 2008). En conséquence, le paramètre qui limitait la croissance bactérienne en milieu naturel est resté limitant lors du démarrage de l'expérience. Ces différences dans l'état de limitation des communautés bactériennes expliquent sans doute en partie les différences de réponse de ces communautés aux conditions de culture. Cette hypothèse est soutenue par l'observation de fortes proportions de Gammaprotéobactéries dans les expériences menées en mer Méditerranée, tandis que ce groupe ne représentait qu'une faible fraction des communautés dans l'expérience de l'océan Austral. Il est probable que ce groupe possède de forts besoins en phosphate comparé à d'autres (Sebastián et al., 2012), ce qui expliquerait ses réponses marquées dans les expériences d'enrichissement en sels nutritifs (Øvreås et al., 2003 ; Teira et al., 2011 ; Sebastián & Gasol, 2013) ainsi que leur compétitivité dans la première expérience menée en mer Méditerranée. Ce groupe est aussi suspecté d'être plus sensible à la prédation que les autres, en raison de sa capacité à rapidement s'adapter à des changements de conditions environnementales, qui lui confère de forts taux de croissance et une taille cellulaire importante (Lebaron et al., 2001 ; Beardsley et al., 2003 ; Ferrera et al., 2011). Si confirmé, cela pourrait expliquer pourquoi au cours de la deuxième expérience réalisée en mer Méditerranée, durant laquelle des prédateurs des bactéries se sont développés dans les cultures, les Gammaprotéobactéries représentaient une fraction plus faible de la communauté que dans la première expérience, dont les prédateurs étaient absents.

Ces expériences ont permis de mettre en évidence une réponse des bactéries à diverses caractéristiques de la ressource organique, ainsi qu'une certaine variabilité de

cette réponse. L'ensemble de ces résultats suggère que l'impact de la MOD sur la diversité bactérienne est modulé par les conditions environnementales, telles que les conditions de nutriments inorganiques par exemple. Cette hypothèse est soutenue par l'observation de communautés différentes dans les diverses efflorescences étudiées lors de la campagne océanographique KEOPS2. À grande échelle, cette hypothèse explique aussi la variabilité des résultats des nombreuses études qui se sont penchées sur la question du lien entre ressource et diversité bactérienne. À l'avenir, les approches expérimentales visant à comprendre les paramètres qui influencent la relation entre l'identité, la qualité et la concentration du substrat et les populations bactériennes seront d'un grand apport. Ces études devront être couplées à des observations *in situ* et des analyses détaillées des réponses des communautés bactériennes à leur environnement perpétuellement en changement.

II. PHYLOGÉNIE ET TRAITS FONCTIONNELS

La question du lien entre phylogénie et traits fonctionnels est fondamentale en écologie microbienne. La dégradation de la ressource organique étant l'un des rôles majeurs des bactéries dans l'environnement, l'étude du lien entre la ressource et la diversité des bactéries qui la dégradent permet de se pencher sur la question des traits fonctionnels des groupes.

Les expériences réalisées au cours de cette thèse soutiennent dans l'ensemble l'idée que les traits fonctionnels sont faiblement liés à la phylogénie, en ce qui concerne l'incorporation et la dégradation des composés carbonés. Tous les grands groupes marins semblent capable de récupérer le carbone contenu dans l'ensemble complexe et diversifié de composés provenant du phytoplancton. La littérature regorge de preuves

que certains groupes possèdent des préférences pour des substrats, ou des différences dans leur efficacité à dégrader ou incorporer ces substrats (Cottrell & Kirchman, 2000 ; Gómez-Consarnau et al., 2012 ; Alonso-Sáez et al., 2012 ; Sarmento & Gasol, 2012). Cependant, il semble que l'expression de ces traits spécifiques à certains groupes soit en partie régulée par les conditions environnementales (Alonso-Sáez & Gasol, 2007), ce qui est en adéquation avec la conclusion à laquelle parviennent la plupart de ces études sur la faiblesse du lien existant entre les groupes bactériens et leurs préférences ou particularités vis-à-vis des différents substrats.

Le rôle de l'environnement dans la détermination des groupes impliqués dans le recyclage de la matière organique est probablement aussi important que les caractéristiques mêmes de ces groupes. À titre d'exemple, plusieurs études suggèrent que certains groupes sont plus performants à faible concentration de substrat tandis que d'autres se surpassent à forte concentration (Eiler et al., 2003 ; Füschlin et al., 2012). Ceci indique que ces groupes sont tous capables d'utiliser le substrat en question, mais que ce sont les caractéristiques de l'environnement, en l'occurrence la concentration de ce substrat, qui détermine au final les groupes qui bénéficient de la ressource présente. L'effet d'une limitation en phosphate ou en carbone sur les inocula utilisés pour les trois expériences en culture continue illustre ce phénomène.

Un point crucial dans la recherche de liens entre la composition des communautés bactériennes et la dégradation de la MOD est le niveau phylogénétique auquel la composition de ces communautés est étudiée. L'expérience de culture en continue réalisée dans l'océan Austral a montré des avantages compétitifs différents pour deux genres des Rhodobacteraceae, *Thalassobius* et *Phaeobacter,* confirmés par leurs réponses distinctes aux conditions d'efflorescence *in situ*. À l'inverse, les données *in situ* ont également montré qu'au sein d'un groupe phylogénétique étudié à haute

résolution, comme dans le cas des *Polaribacter* ou de *Thalassobius*, différentes OTUs ne répondaient pas de la même manière à une condition environnementale donnée. Cela suggère que les OTUs proches sur le plan phylogénétique ne sont pas fonctionnellement équivalentes, ou qu'elles sont en compétition pour la même niche écologique. Ces observations montrent à quel point l'étude de la diversité des taxons existant au sein des grands phylas est nécessaire à la compréhension de leur écologie et des rôles qu'ils jouent dans la dégradation de la matière organique.

Abstract

In the marine environment, heterotrophic bacteria strongly depend on dissolved organic matter (DOM). How the composition, concentration and availability of DOM affect the structure and diversity of bacterial populations remains however an open question. In this thesis, we performed three continuous culture experiments in the Mediterranean Sea and the Southern Ocean, to address three questions: 1) How does an increase in DOM affect bacterial diversity? 2) What is the phylogenetic and structural response of heterotrophic bacteria to chemically distinct DOM? 3) Can changes in DOM supply explain changes in bacterial diversity during an *in situ* phytoplankton bloom? To address these questions, we grew natural bacterial communities in various DOM conditions and examined bacterial diversity by 454 Tag pyrosequencing of 16S rRNA genes. In the Mediterranean Sea, we observed that an increase in the supply of DOM resulted in an increase in bacterial richness and phylogenetic diversity, and in moderate changes in the phylogenetic composition of bacterial communities. By contrast, two phytoplankton DOM sources that varied in their chemical composition sustained bacterial communities with different levels of diversity but did not affect their phylogenetic composition. In the Southern Ocean, the addition of diatom-derived DOM increased the diversity of dominant groups and this was coupled to taxonomic shifts. These bacterial groups were also observed *in situ*, associated with a phytoplankton bloom induced by natural iron fertilization (KEOPS2). Taken together, our results demonstrate the shaping effect of DOM on bacterial diversity, both in experimental studies and in situ.

Key words: Bacterial diversity; phytoplankton DOM; continuous cultures; pyrosequencing, Mediterranean Sea; Southern Ocean.

Résumé

En milieu marin, les bactéries hétérotrophes dépendent de la matière organique dissoute (MOD). L'effet des caractéristiques de cette matière sur la structure et la diversité des populations bactériennes demeure flou. Durant cette thèse, trois études en culture continue ont été réalisées en mer Méditerranée et dans l'océan Austral pour répondre à trois questions : 1) Quel est l'effet d'une augmentation de MOD sur la diversité bactérienne ? 2) Quelle est la réponse phylogénétique et structurelle des bactéries à des MOD de composition chimique différente ? 3) Les modifications du « pool » de MOD engendrées lors d'un bloom de phytoplancton peuvent-elles expliquer les changements de diversité bactérienne observés lors de ces évènements? Pour répondre à ces questions, des communautés bactériennes naturelles ont été maintenues dans diverses conditions de MOD, et la diversité bactérienne a été étudiée par pyroséquençage du gène de l'ARNr 16S. En mer Méditerranée, un ajout de MOD a induit une augmentation de la richesse et de la diversité phylogénétique des communautés bactériennes, ainsi que des modifications phylogénétiques mineures. En revanche, deux sources de MOD chimiquement distinctes ont engendré des communautés bactériennes présentant des niveaux de diversité différents, sans impact sur leur phylogénie. Dans l'océan Austral, l'ajout de MOD de diatomée a augmenté la diversité et modifié la taxonomie des groupes dominants. Ces groupes ont été observés *in situ*, associés à un bloom induit par une fertilisation naturelle en fer (KEOPS2). Ces résultats démontrent l'effet structurant de la MOD sur la diversité bactérienne dans un contexte expérimental et dans l'environnement.

Mots clés : Diversité bactérienne ; MOD phytoplanctonique ; cultures continues ; pyroséquençage ; mer Méditerranée ; océan Austral.