Metabolic and composition changes in marine bacterial community esposed to variable natural organic matter bioreactivity

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THÈSE DE DOCTORAT
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Spécialité:
Ecologie Microbienne marine
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Présentée par :
Marine BLANCHET

Pour obtenir le grade de :
DOCTEUR de L'UNIVERSITE PIERRE ET MARIE CURIE

Modifications métaboliques et structurelles des communautés de bactéries marines exposées à différentes qualités de matière organique

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Devant le jury composé de :

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"A ship in port is safe; but that is not what ships are built for.
Sail out to sea and do new things."

Grace Hopper

FOREWORD

This thesis was carried out in cotutelle between the University Pierre et Marie Curie (UPMC, France), and the University of Concepcion (UdeC, Chile) in the frame of the International Associated Laboratory Marine Biogeochemistry and Functional Ecology (LIA MORFUN). This LIA links the Observatoire Océanologique de Banyuls sur Mer (OOB, Université Pierre et Marie Curie/CNRS) in France and Universidad de Concepción (UdeC, Concepcion) and Universidad Austral (UACH, Valdivia) in Chile.

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The experiments presented required the combined efforts of many people. The set-up of the experiments, the analysis of the results and the writing of the papers were done in collaboration with my supervisors.

My work consisted in the preparation and set up of the experiments using a microcosm approach. I performed the sampling, conditioning and storage of the different parameters. I did ammonium measurements and the extractions of DNA and the consecutive bioinformatical analysis of obtained sequences.

The sampling of water at each location was performed by Claudia MUNOZ (AI, UdeC) in Chile, Christine SOTIN (AI, Université de Perpignan) at the Rhone River, Eric MARIA (TCE, OOB) and the monitoring service at OOB (SOLA) in Banyuls sur Mer.
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ABBREVIATIONS

BCA	Bacterial Community Activity
BCC	Bacterial Community Composition
BGE	Bacterial Growth Efficiency
BP	Bacterial Production
BR	Bacterial Respiration
C	Carbon
CDOM	Chromophoric (or colored) Dissolved Organic Matter
DFAA	Dissolved Free Amino Acids
DFNS	Dissolved Free Neutral Sugars
DOC	Dissolved Organic Carbon
DOM	Dissolved Organic Matter
DON	Dissolved Organic Nitrogen
DOP	Dissolved Organic Phosphorus
FT-ICRMS	Fourier Transform Ion Cyclotron Resonance Mass Spectrometry
HMW	High Molecular Weight
LDOM	Labile Dissolved Organic Matter
LMW	Low Molecular Weight
OTU	Operational Taxonomic Unit
PE	Priming Effect
POM	Particulate Organic Matter
RDOM	Refractory Disssolved Organic Matter
SLDOM	Semi-labile Dissolved Organic Matter
SRDOM	Semi-Refractory Dissolved Organic Matter
URDOM	Ultra Refractory Dissolved Organic Matter

Contents

Chapter I

General Introduction

I General introduction

I.1 Dissolved organic matter in marine environments: origin and composition

Marine organic matter is a dynamic continuum of particulate (POM) and dissolved organic pool (DOM) defined operationally through filtration. The organic matter that is retained on filters (pore size varying between 0.2 and 0.7 µm according to analysts) constitutes POM, whereas the fraction that can pass through the filters is considered referred to as DOM (Sharp 1973). POM is composed of living (plankton) and dead (detritus) components (Nagata 2008). DOM comprises a continuum of small organic molecules going from low molecular weight (LMW, <1 kDa) such as amino acids and sugars to highly polymeric and colloidal particles of high molecular weight (HMW, >1 kDa). About 75% of the oceanic dissolved organic carbon (DOC) is composed of LMW molecules (Benner 2002).

DOM is the main form (90%) of organic matter in aquatic environments and plays an important role in global biogeochemical cycles. It is one of the largest reactive reservoirs of reduced carbon on Earth (685 Pg C), equivalent to the global atmospheric CO_2 reservoir (780 Pg C) (Hedges 1992, Hansell et al. 2009). DOM in the water column provides substrate for aquatic food webs and its concentration and composition influence the chemical (e.g. availability of nutrients, metal speciation, pollutant toxicity) and physical characteristics (light and heat absorption) of aquatic environments (Stedmon et al. 2003, Aiken et al. 2011). DOM is also the primary substrate supporting bacterial growth and respiration through the microbial loop, which is responsible for much of the carbon and energy flow in aquatic systems.

I.1.1. DOM sources and sinks: implications for bioreactivity

The origins of DOM may determine its complexity and diverse chemical composition. However, despite its importance, the marine DOM reservoir remains a "black box" with less than 10% of known organic molecules identified chemically due to lack of suitable methods.

In the upper ocean, the main sources of DOM are autochthonous and are produced by all the trophic levels of the food web (Nagata 2008) **(Fig I-1).** Primary

production[1] is the ultimate and most important source (Sharp 1973, Fogg 1983) but DOM can also be produced by cell lysis (both viral and bacterial), sloppy feeding by zooplankton, release by prokaryotes and solubilisation of detrital and sinking particles by bacterial and archeal ectohydrolases (Nagata et al. 2000, Jiao et al. 2010).

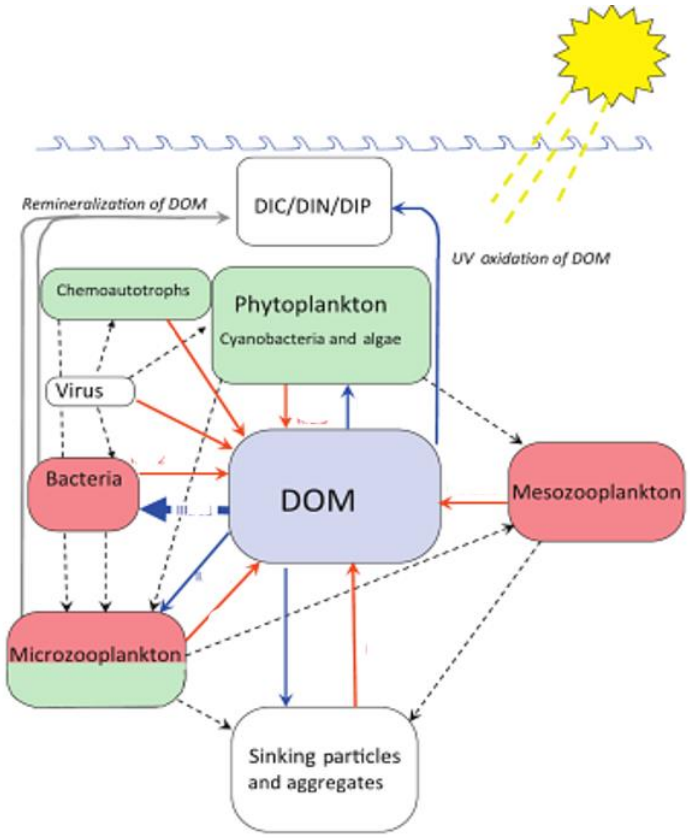

Figure I-1: DOM production and removal processes in marine ecosystems. Solid arrows represent DOM production (red) and DOM consumption (blue). Dashed lines represent food web interactions. From Carlson and Hansell, 2014.

Although river discharge of allochthonous DOM derived from terrestrial material is significant, it accounts only for 1% of global marine primary production (Hedges 1992). DOM from soils is released into rivers when there is soil leaching, which occurs generally during rainy and high discharge periods. Depending on the nature of soils, land use, type of watershed and the regime of the river (Meybeck and Helmer 1989), river DOM can represent about 0.25 Gt C per year (Cauwet 2002). Allochthonous DOM mainly comes from the organic matter from soils or terrestrial plant litter (Thurman 1985) and is thus of terrestrial origin. It is composed of humic

[1] Primary Production is the production of organic carbon from dissolved inorganic carbon, principally through the process of photosynthesis.

matter, bringing lignin and cellulose that are considered as much more recalcitrant to degradation. In coastal areas, "atypical DOM" can also represent an important and rapidly mobilizable fraction. For example, under favourable conditions (high nutrient concentrations), jellyfish can develop quickly and form a bloom (Purcell 2012) that can either released DOM by excretion or during massive die-out events (Yamamoto et al. 2008). In estuaries bordered by dense human populations and industrial development, pollution can also represents a source of DOM (Cauwet 2002).

Because of its diversity of origins, the bulk DOM pool exhibits a broad continuum of biological lability with turnover times going from minutes to millennia. Three major fractions of DOM have usually been distinguished in the literature based on their lifetime[2] (or reactivity): some are remineralized rapidly by heterotrophic microbes and constitute the labile fraction of DOM (LDOM), others are less readily available (semi-labile DOM, SLDOM). However the main part has accumulated over time and comprises the intriguing refractory DOM pool (RDOM). Although this conceptual model has been useful for understanding DOC dynamics and assessing the contribution of this various fractions to biological and biogeochemical processes, its utilization has been limited by the absence of a suitable quantitative description of DOC composition (Carlson and Hansell 2014). In recent years, field campaigns coupled with measurements of bulk DOC and its characterization have greatly improved knowledge on the DOM pool. This has eventually led to the definition of two supplementary major fractions of DOM: semi-refractory (SRDOM) and ultra-refractory DOM (URDOM) (Hansell 2013). The adjective recalcitrant is employed to characterize DOM that accumulates and is resistant to removal, without specifying its reactivity and contains at least the four subfractions: SLDOM, SRDOM, RDOM and URDOM (Hansell 2013).

I.1.1.1 Labile DOM

Only a small part of all DOM is labile, accounting for less than 1% of the bulk DOC pool (Fig I-2a). This pool has a rapid turnover rate (minutes to days) and is composed of compounds such as dissolved free neutral sugars (DFNS), dissolved free amino acids (DFAA) and labile proteins (Benner 2002). Those compounds are present at low concentrations (nanomolar to a few micromolar) in the open ocean, because of

[2] DOM lifetime is defined as the time over which the concentration of the fraction decreases to 1/*e* of its initial value (Hansell 2013)

the tight coupling of their production and consumption rates (Carlson et al. 2002). Their global estimated inventory is 0.2 PgC (Hansell 2013) (Fig I-2b). The main sources of LDOM are phytoplankton excretion, viral lysis and grazing (Nagata 2008) and its global production rate is 15-25 PgC.year^{-1} in the photic zone (Hansell 2013). LDOC is of great biological interest because it provides support for the metabolic energy and nutrients demand of heterotrophic prokaryotes (Carlson and Ducklow 1996). It has been shown that the uptake of DFAA or of glucose alone can support a high fraction and sometimes up to 100% of the bacterial growth in coastal environments (Kirchman 2003). The rapid remineralization of LDOM by bacteria fuels most of the carbon, nutrients and energy fluxes, however most of the resulting inorganic constituents are retained within the upper ocean. Its contribution to the biological pump or carbon sequestration is therefore considered to be minor (Hansell 2013).

I.1.1.2 Semi-labile (SLDOM) and semi-refractory DOM (SRDOM)

DOM with intermediate turnover rates, greater than months but less than centuries (time scale of ocean mixing) was originally described as SLDOM. However a better spatial resolution of DOC and a greater estimation of removal rates has led to divide this pool in two distinct fractions: SLDOM and SRDOM (Hansell 2013, Carlson and Hansell 2014). The vertical gradient of the bulk DOM observed in thermally stratified environments is mostly composed of SLDOM with an inventory of 6 ± 2 PgC and an estimated production rate of 3.4 Pg C year^{-1} (Carlson et al. 2002, Hansell and Carlson 2013). Semi labile DOC accounts for a quarter to a half of the surface DOC pool (Carlson et al. 2002) (Fig I-2a). Its turnover rate ranges from months to years, because it resists rapid microbial degradation, allowing its accumulation in surface waters and its vertical and horizontal export from the region of formation (Hansell 2013). The horizontal export of SLDOM via surface currents provides supplementary allochthonous carbon sources for heterotrophic bacteria (Torres-Valdés et al. 2009). The vertical export of SLDOM to depth via convective mixing or advection (Copin-Montégut and Avril 1993) is estimated at ~1.5 Pg C year^{-1}, making it the most important DOM fraction contributing to carbon export (Hansell 2013). In some oceanic systems, this export can exceed export by sinking particles (Copin-Montégut and Avril 1993). Due to its short life time, vertical SLDOC export is limited to the upper mesopelagic zone (100-500 m) where it provides support for microbial production

(Abell et al. 2000, Hansell and Carlson 2013). The resulting mineralized products can be returned to the air/sea interface within months to years (Hansell and Carlson 2001). The characterized fraction of SLDOM is mostly composed of HMW compounds which include carbohydrates originating from autotrophic and heterotrophic processes (Carlson et al. 2002). These compounds must be hydrolysed to monomers by extracellular enzymes before bacterial uptake. The uncoupling between HMW DOM production by phytoplankton and its heterotrophic consumption can lead to the transient accumulation of HMW SLDOC (Billen 1990).

SRDOM has a turnover time of decades. Its accumulation in the upper ocean is observed in ocean regions that exhibit permanent pycnoclines[3] (Fig I-2a, left). Its inventory reaches 14 ± 2 Pg C (Hansell 2013). Although SRDOM is a minor contributor in the biological pump, vertical export being ~0.34 Pg C $year^{-1}$, its contribution to carbon sequestration is important (Hansell 2013). The composition of SLDOM and SRDOM has not yet been elucidated.

I.1.1.3 Refractory (RDOM) and ultra-refractory DOM (URDOM) pools

The refractory pool is the main fraction of the bulk DOC (630 ± 32 Pg C $year^{-1}$) and is essentially made up of diagenitically altered LMW compounds (Benner et al., 1992; Amon and Benner 1996) (Fig I-2). This finding can seem contradictory because bacterioplankton can only directly take LMW compounds. However, while some LMW compounds are highly available, their majority are resistant to microbial degradation according to the size reactivity continuum model, (Amon and Benner 1996, Sinsabaugh and Foreman 2003). Also the concentration of the individual molecules may be too low for its detection by microbial uptake systems (Kujawinski 2011a). Refractory DOC dominates the deep reservoir of DOC with an average age of 4000 to 6000 years, exceeding the time scale of thermohaline circulation (Benner 2002). Therefore, its distribution in oceanic basins is vertically homogeneous (Fig. I-2a).

The existence of URDOC is imputed to polycyclic aromatic hydrocarbons, also known as thermogenic black carbon. It represents between 2% and 22% of bulk DOC (Dittmar

[3] The pycnocline, situated between the mixed layer and the deep layer, is where water density increases rapidly with depth because of changes in temperature and/or salinity. (from Ocean Motion website: http://oceanmotion.org/)

and Paeng 2009). Black carbon is inert in time scales of ocean mixing and can reside in the DOC pool during 2500- 13900 years before sedimenting (Carlson and Hansell 2014). The primary sources of black carbon are the burning of terrestrial forests and fossils fuels (Hansell 2013).

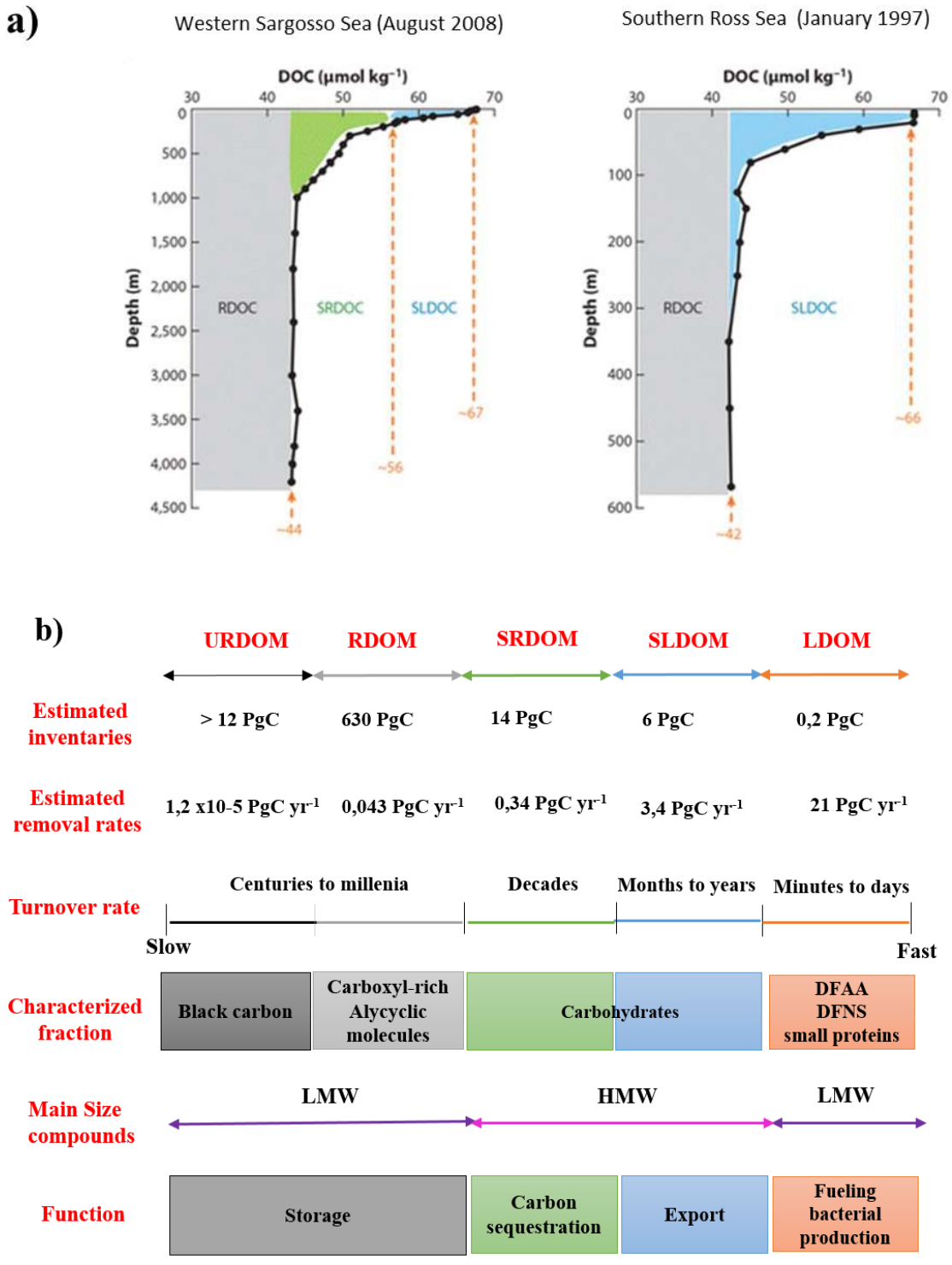

Figure I-2: a) Vertical distribution of the different fractions of DOC in stratified oligotrophic waters (left) and in the southern Ross Sea (right) where pynocline is absent (figures adapted from Hansell, 2013). **b)** Characteristics of the major DOC fractions in the oceans (Adapted from Nagata, 2008 and Hansell, 2013).

I.2 Fate of DOM in coastal environments

I.2.1 General aspects of coastal ecosystems

The coastal ocean is a dynamic and shallow area where rivers, estuaries, ocean, land (e.g. tidal wetlands and continental shelf) and atmosphere usually interact. Although the coastal zone comprises less than 20% of the Earth's surface, it contains 40% of the human population and supplies 90% of the global fish catch (Crossland et al. 2005).

Estuaries are partially enclosed bodies of water, where river water (salinity is 0) and seawater (salinity equal or above 30) meet and mix. Estuaries act like a transition zone between oceans and continents and present strong gradients of biogeochemical parameters (Bauer et al. 2013). They may be defined according to their geological characteristics and also according to its stratification and its inner circulation of water. The data presented in this thesis covers a variety of estuarine and coastal systems including fjords, lagoons, river plumes and coastal upwelling systems.

The river inputs to the coastal ocean associated with its high physical and biological activity makes these areas one of the most dynamic in terms of DOM (Cauwet 2002). Despite the high variability of the depth integrated primary production that can usually represent the main part of DOM production, DOC concentrations vary little throughout the global ocean compared to primary production levels, indicating that several removal processes are occurring.

DOM can undergo a variety of physical-chemical processes in river plumes, estuaries and at the land-ocean interface that will determine the composition and the concentration of DOM reaching the ocean. Some of these processes are abiotic like aggregation and photodegradation. Other processes are biotic such as microbial degradation. Aggregation (including adsorption and flocculation) represents the transformation of dissolved compounds to particles and occurs in estuaries during the mixing of fresh and marine water, where rapid changes in salinity and pH occur (Søndergaard et al. 2003). Then the organic matter can be removed through sedimentation (sinking) or by filter feeding organisms

Below we will comment specifically on biotic DOM processing factors.

I.2.2 Microbial degradation

Despite its refractory characteristics, a large fraction of terrestrially DOM carried by rivers is degraded after mixing with seawater and could in part be explained by the biochemical and compositional changes of DOM occurring in estuaries (Kerner et al. 2003) and the greater diversity of marine bacteria (Stepanauskas et al. 1999).

Marine ecosystems contain $1.2x10^{29}$ heterotrophic prokaryotes (Whitman et al. 1998) which constitutes more than 90% of the living biomass in the sea. A major metabolic strategy in the aerobic area is chemoheterotrophy, where heterotrophic prokaryotes are using OM as both source of carbon and electron donor. However not all the heterotrophic bacteria are chemioheterotrophs. Several groups of bacterioplankton can use sunlight as an alternative source of energy while consuming DOM; they are called photoheterotrophs (Béjà and Suzuki 2008). Heterotrophic bacteria and archaea are the main consumers of DOM and it has been shown that a large fraction of primary production becoming dissolved by various mechanisms in the food web was almost exclusively available to heterotrophic bacteria and archeae (Azam and Hodson 1977, Azam and Malfatti 2007). They rapidly consume fresh LDOM and facilitate its transformation into POM available for grazers and higher trophic levels (trophic link) (Pomeroy and Darwin 2007) or its remineralization into nutrients and carbon dioxide (respiratory sink) (Ducklow et al. 1986) within the microbial loop (Carlson et al. 2007) (Fig.I-3).

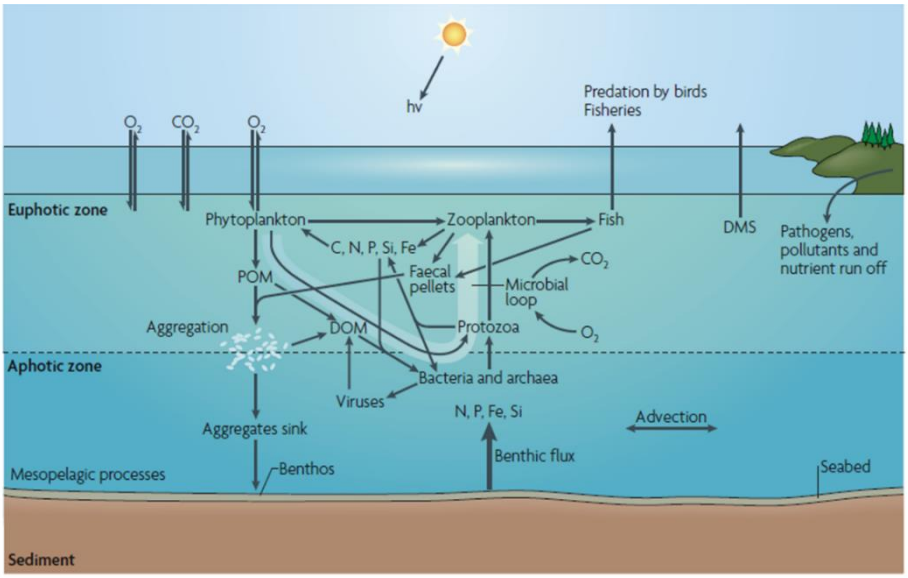

Figure I-3: Microbial structure of a marine ecosystem.

From Azam and Malfatti (2007).

As there are no direct measurements of the DOM flux, there are proxies to figure out which of the two fates of carbon (carbon respiration or biomass production) is most important, such as net bacterioplankton production (BP), respiration (BR) and growth efficiency (BGE).

Bacterial carbon demand (BCD) is the total flux of carbon required to support bacterial growth and include BP and BR (BCD =BP + BR).

BGE helps determining the efficiency by which bacteria convert DOC into new biomass. It can be estimated by calculating the part of the BCD that is used for bacterial production (Giorgio and Cole 1998).

$$BGE = \frac{BP}{BP + BR}$$

The BGE for natural microbial communities usually ranges from 15% in the open ocean to 35% in estuaries (Giorgio and Cole 1998). This means that most of the assimilated carbon is respired (60-99% according to Azam and Malfatti 2007) and released as CO_2. It also implies that little carbon remains available as food and can pass on to higher trophic levels. Although the low growth efficiency estimates indicate that the microbial loop is mainly a sink of carbon, it is also a link by processing DOM or complex detritus, transferring to higher trophic levels otherwise unavailable material and energy (Kirchman 2012).

I.2.2.1 Adaptative strategies of bacterioplankton for DOM utilization

In order to take better advantage of the huge diversity of organic compounds, heterotrophic marine bacteria have developed different behavioural and biochemical strategies to acquire organic matter (Azam and Malfatti 2007) (Fig.4). Marine bacteria inhabit a complex environment, far from being well-mixed and homogeneous, where the concentrations of essential compounds are generally low. Motility can allow bacteria to migrate to more suitable microhabitat in order to increase the uptake of inorganic and organic compounds (Grossart et al. 2001). Some motile bacteria may use chemotaxis[4] to swim toward ephemeral sources rich in LDOC such as primary production, zooplankton excretion and cell lysate (Stocker et al. 2008). Although motility confers many advantages, only a variable fraction of bacteria are motile (5 to 70% depending on the

[4] Chemotaxis is the sensing by bacteria of chemical gradients, and movement up or down a gradient towards or away from a chemical source (Azam and Malfatti 2007).

season and location (Mitchell and Kogure 2006)). This could be due to the fact that some bacteria are too small or not active enough to afford the energetic expense of motility (Kirchman 2012). Some bacteria have the ability to attach to particles and aggregates that constitute substrate-rich microhabitats where they can grow rapidly (Alldredge et al. 1986). The contribution of particle-attached bacteria is only <5% of total bacteria in pelagic oligotrophic ecosystems but can exceed 60% in eutrophic environments (Bell and Albright 1982, Garneau et al. 2009).

I.2.2.2 DOM uptake

Bacteria can only acquire small compounds (<500 Da) through passive or active uptake membrane transport (Kirchman 2012) (Fig.4). Organic compounds larger than 500 Da can be highly bioavailable but must previously be transformed in order to be transported into the cell (Amon and Benner 1996). For this purpose bacteria produce cell-surface-bound hydrolytic enzymes (or ectoenzymes) to hydrolyse HMW compounds (Azam and Malfatti 2007) Specific enzymes are necessary for each polymer, their name being usually containing the polymer name: protease, glucosidase, lipase, phosphatase, chitinase. Nearly all the enzymes are cell-associated in order to avoid the diffusion of the hydrolysis products and avoid other bacteria to "cheat" by using those compounds or the enzymes produced (Kirchman 2012). Hydrolysis of HMW compounds and uptake of resulting LMW compounds are generally coupled processes.

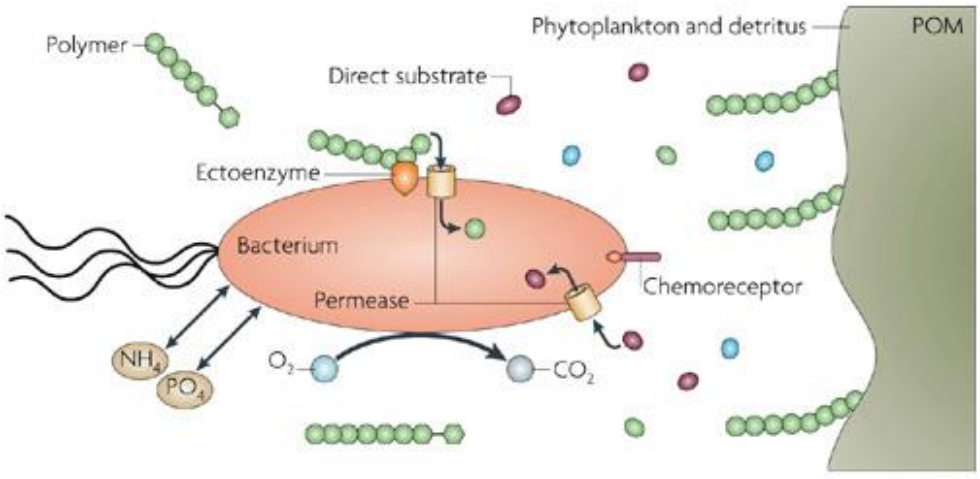

Figure I-4: Adaptative strategies of bacteria in the ocean. From Azam and Malfatti, 2007.

I.2.2.3 Microbial community structure and DOM utilization

The identification of marine bacteria by traditional approaches consists in various biochemical tests such as Gram staining, degradation capacity for key compounds and enzymatic activity. However these techniques have been hindered by the fact that nearly all of those tests rely on phenotype and are observable for culturable microorganisms which only accounts for less than 1% of total microbial biodiversity.

The development of cultivation-independent approaches such as "next-generation" sequencing has revealed an incredibly high taxonomic diversity of prokaryotes in the ocean even if there is significant uncertainty about how much of this diversity is functionally different (DeLong et al. 2006). Those techniques are based on genes, used either for phylogenetic or functional microbial diversity studies. The most used gene for prokaryotes taxonomy and phylogeny is the 16S rRNA gene, which encodes for the 16S rRNA molecule, a component of the 30S small subunit of prokaryotic ribosomes. This gene is a good candidate because it is found in all bacteria and archeae and presents regions of different levels of variability, going from highly conserved to highly variable regions that allow distinguishing microbial groups at a high level of phylogenetic resolution. The classical definition of species is meaningless for prokaryotes because they do not reproduce sexually. Instead they acquire DNA fragments for recombination by different means, including horizontal gene transfer. Thus, many microbial ecologists avoid using the term "species", preferring instead the terms ribotype, phylotype or operational taxonomic unit (OTU). Usually, in the literature they are used to describe microorganisms whose 16S rRNA genes are >97% similar. In this manuscript the term "OTU" will be used.

Of the 50-100 bacterial phyla known in nature, only a dozen are abundant in marine environments and are generally found worldwide (Fig. I-5).The Proteobacteria phylum is found everywhere , but different classes dominate freshwaters and the oceans. In freshwater, *Betaproteobacteria* are more abundant (Glöckner et al. 1999), followed by *Gammaproteobacteria* and *Alphaproteobacteria* whereas in marine waters *Alphaproteobacteria* are usually most abundant, especially the clade SAR11, that constitutes about 33 percent of euphotic zone communities and 25% of mesopelagic communities (Morris et al. 2002) and *Gammaproteobacteria* in second place. The

phylum Bacteroidetes is present in freshwaters and in the oceans, while Actinobacteria is mostly present in freshwaters (Newton et al. 2011) .

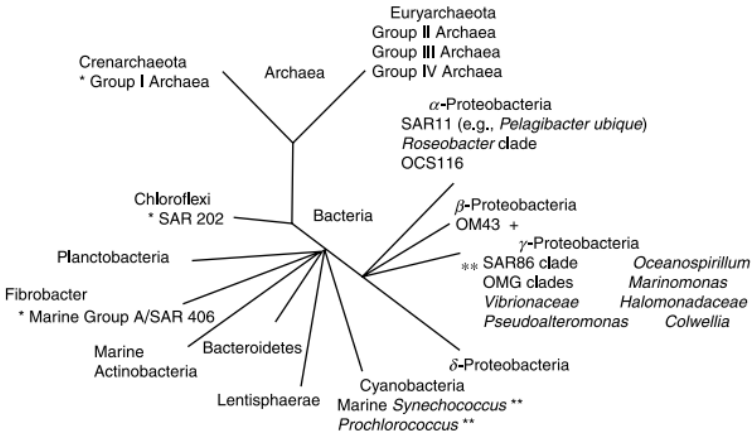

Figure I-5: Schematic illustration of the phylogeny of the major Archaea and Bacteria clades, showing only the major marine groups. Groups with a single asterisk are mostly found in the mesopelagic and surface waters during polar winters (deep mixing), those with two asterisks are mostly in the photic zone, and those with a + are mostly coastal.
From Fuhrman and Hagström 2008

Several studies have shown spatio-temporal variations of bacterial communities' structure, showing the potential coupling between DOM quality/quantity and bacterial community structure. Copiotrophs are adapted to take advantage of rare, nutrient-rich conditions (Lauro et al. 2009). They possess motility and chemotaxis genes and a fast uptake kinetics that allow them to adapt rapidly to newly encountered DOM rich microhabitats (Stoecker, 2012). Their size makes them less competitive at low resources conditions. In contrast, oligotrophs such as *Peligibacter ubique*, member of the clade SAR11, are adapted to life in low nutrient conditions. Their minute size (about 0.4 µm diameter) allows them to maximize uptake of nutrients at low bulk concentrations generally found in the oceans (Lauro et al. 2009, Stocker 2012). They lack several many functional and regulatory genes such as mobility ones, which implies a poor metabolic plasticity and a incapacity for exploiting rich nutrient conditions.

Several studies have reported different responses among major and rare bacterioplankton taxa following the addition of different quantity/quality of DOM (Landa et al. 2013, Dinasquet et al. 2013). Shifts in bacterial community composition are also often reported during phytoplanktonic blooms (Fandino et al. 2001, Pinhassi et al. 2004). Several studies have shown that opportunist copiotrophic bacteria such as

members of the family *Alteromonodaceae*, belonging to *Gammaproteobacteria,* that were rare in the ambient communities were emerging after different LDOC enrichment conditions, suggesting a capacity of copiotrophs for using heterogeneous DOM pools (Nelson and Wear 2014). There are several relationships between the quality of DOM and metabolic and composition changes in the literature but there is still a lack of understanding of why and in which conditions some specific compounds become available for uptake by specific bacteria.

I.2.2.4 Factors limiting DOM uptake

Some factors can limit DOM uptake. As we have seen, not all the compounds are available and can resist to bacterial degradation. The microbial community stucture and the environmental parameters such as temperature and nutrient limitation (Church 2008) can also limit this uptake. This phenomenon coupled with high grazer pressure has been proposed as "malfunctioning microbial loop" to explain and lead to the accumulation of DOC in surface water during productive seasons (Thingstad et al. 1997)

I.2.2.5 Microbial carbon pump

Heterotrophic prokaryotes are not only consumers of DOM, they can also produce DOM. A part of LDOM metabolized by heterotrophic prokaryotes is transformed to a recalcitrant form via the microbial carbon pump, contributing to carbon sequestration (Jiao et al. 2010). Unlike the biological carbon pump which relies on vertical transport of carbon from the euphotic zone to the deep sea, microbial carbon pump operates independently of depth, sequestering carbon in all the water column, including the euphotic zone (Jiao et al. 2010). Other pathways of the microbial carbon pump include the release of bacteria-derived DOM during cell death via lysis or egestion (Jiao and Zheng 2011). Bacterially derived DOC based on D-amino acids biomarkers has reports that about 25 % (165 Pg) of recalcitrant DOC is from bacterial origin (Benner and Herndl 2011).

Refractory organic carbon is a large and important component of the carbon cycle. A small change in this reservoir can have huge effects on levels of atmospheric carbon dioxide, with large implications for climate change. In this context it is important to understand the processes that lead to the preservation of this refractory carbon in the ocean and the ones that can enhance its biodegradation. In the frame of this thesis we have studied two processes: priming effect and UV radiation

I.2.3 Priming effect

A conundrum in oceanography is that although the amount of DOC discharged by rivers can account for the renewal of DOC in global ocean (every 4000-6000 years), riverine DOC accounts for only a small fraction of oceanic DOC **(Fig. 5)** (Bianchi 2011). There is two times more carbon delivered to inland waters (1.9 Pg C by year) than delivered to the ocean (0.9 PgC by year), suggesting that terrestrial DOC is less recalcitrant and more consumed that previously thought (Bianchi et al. 2013).

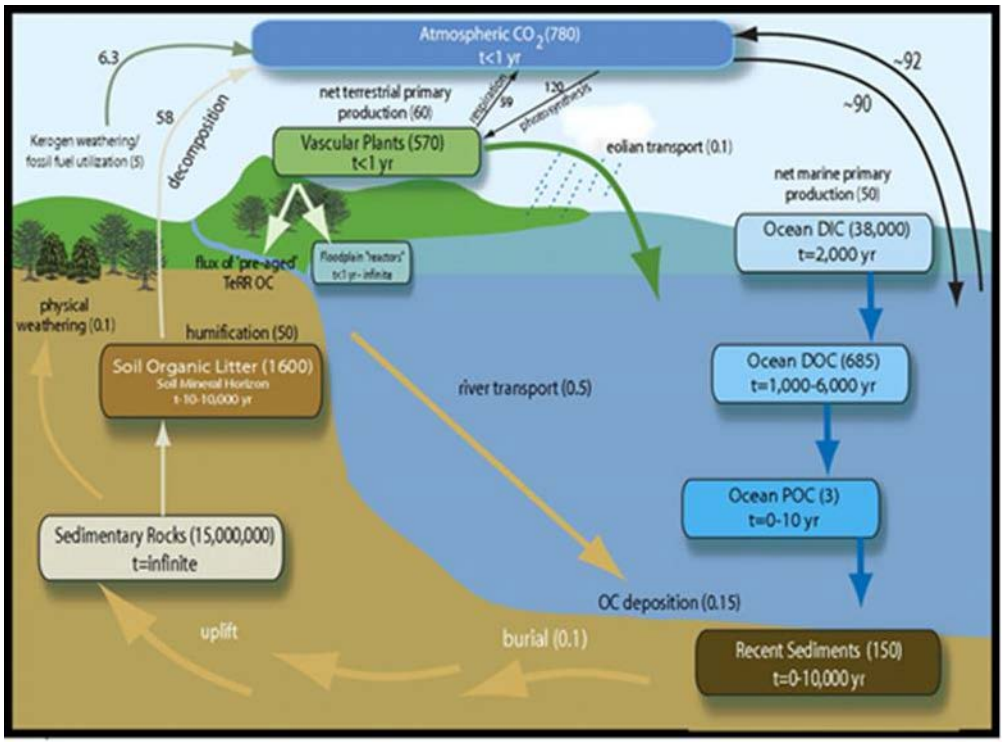

Figure I-6: Estimated inventories (in PgC), fluxes (in Pg C·y^{-1}) and ^{14}C ages associated with the global carbon cycle. From Bianchi, 2011

A process that has been hypothesized to stimulate the mineralization of refractory organic carbon is the priming effect (PE). PE refers to changes in microbial decomposition of recalcitrant organic matter upon the addition of labile organic matter (Bianchi 2011). This phenomenon, that has been repeatedly reported in soils, can be either positive (enhanced mineralization rate of recalcitrant organic matter) or negative. Although the mechanisms involved in PE are not well understood they may be driven by multiple factors such as microbial biomass and composition, chemical structure and availability of organic carbon and nutrient availability and stoichiometry (Fontaine and Barot 2005, Kuzyakov 2010, Guenet et al. 2010).

Guenet et al. (2010) formulated different hypotheses to explain the potential removal of recalcitrant marine DOM. The mechanisms include a) **Co-metabolism** where oxidation of recalcitrant organic matter is a consequence of enhanced microbial activity due to the presence of labile organic matter that supplies energy for the production of ectoenzymes that will degrade recalcitrant organic matter. b) **Net mutualism** between two microbial communities, which involves two distinct communities of heterotrophic microbes. After the addition of labile organic matter, the resulting by-products provide energy that activates the production of hydrolytic enzymes by a second community of microbes that will decompose recalcitrant organic matter. c) **Alternate metabolisms of single community**, where a single population is capable of producing enzymes for the degradation of recalcitrant organic matter thanks to the energy provide by the degradation of labile organic matter.

Initially described in agricultural literature (Löhnis 1926) , PE has been well studied in terrestrial ecosystems and its importance in carbon soil cycling is currently recognized (Blagodatskaya and Kuzyakov 2008). PE has recently been highlighted as a potential important mechanism in aquatic environments and have received a newfound attention (Guenet, Neill, et al. 2010, Bianchi 2011). Those authors suggested the existence of “hotspots” and “hot moments” in aquatic ecosystems where PE could be significant due to the simultaneous presence of refractory and labile organic matter. Coastal areas, river plumes and upwelling systems could be potential hot spot site for PE. In those cases, recalcitrant organic matter (terrestrially derived organic matter) carried by river or coming from deepwater meets autochthonous labile organic matter (high primary production).

I.2.4 Photodegradation of DOM via UV radiation

Natural solar radiation, especially ultraviolet radiation (UV-B [280–315 nm], UV-A [315–400 nm]), has been found to induce chemical transformations of DOM with the production of a variety of photoproducts, including carbon dioxide, carbon monoxide, ammonium, phosphate, and numerous LMW organic compounds (Mopper and Kieber 2002). The light-absorbing fraction of DOM, chromophoric dissolved organic matter (CDOM), from both terrestrial and autochthonous origins, is the primary absorber of sunlight in aquatic ecosystems and plays an important role for most photochemically mediated processes in surface waters (Mopper and Kieber 2002). Although the photomineralization to inorganic carbon represents a loss of DOM potentially available to the microbial community, organic photoproducts can be re-used by bacteria. The photochemical transformations of DOM have contrasting effects on bacterial metabolism by modifying the bioavailability of DOM according to the origin and the initial chemical composition of DOM (Kieber 2000). Figure 7 shows the general trends of bacterial growth changes in response to DOM exposure to UV radiation in relation to DOC-specific absorbance (a proxy of humic substances) and chlorophyll *a* (a proxy of fresh DOM) (Bertilsson and Tranvik 2000). The observed effects of the phototransformations of DOM on bacterial growth suggest an increase of lability for initially refractory substances whereas phototransformations decrease the lability of freshly produced algal carbon. Bacterial growth and bacterial respiration are not necessary modified proportionally by the photochemical transformation of DOM inducing a shift in the bacterial growth efficiency (BGE) (see Abboudi et al. 2008).

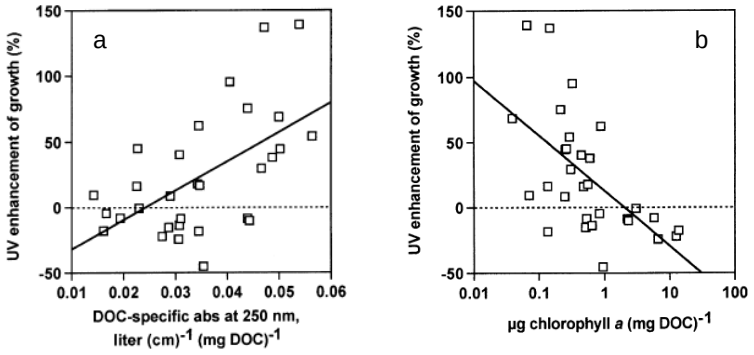

Figure I-7: Effects on bacterial growth of UV treatment of dissolved organic carbon (DOC). The relative UV enhancement of bacterial growth in 30 different lakes (abundance developing in irradiated water as a percentage of abundance in dark controls) in relation to DOC-specific absorbance (a) and chlorophyll *a* to DOC ratio of the initial water samples (b) (Tranvik and Bertilsson 2001).

Besides the metabolic changes induced by DOM phototransformations, different studies have reported that DOM phototransformations can induce a modification of the bacterial structure by selecting the most responsive species to the DOM photoproducts. Judd et al. (2007) firstly observed that sunlight-exposed DOM from lake and stream had a positive effect on BP and caused shifts in bacterial community composition (based on denaturating gradient gel electrophoresis of bacterial-specific 16S rDNA). Pérez and Sommaruga (2007) observed that photodegradation of DOM from different origins (lakes, algae, soil) influenced the activity and the composition of the bacterial communities (based on fluorescent *in situ* hybridization), with an increase in the relative contribution of *Actinobacteria* when DOM was pre-exposed to the solar radiation. Abboudi et al. (2008) shown that photochemical transformation of DOM from coastal lagoon and coastal water induced a shift in the bacterial community as revealed by DNA and RNA fingerprints. Piccini et al. (2009) observed a rapid modification of the bacterial community composition from a coastal lagoon in response to the photodegradation of CDOM in favour of Alpha andBeta*etaproteobacteria*. More recently, Paul et al. (2012) demonstrated that bacterial communities of non-irradiated and UV-irradiated OM from different origins were different and that UV selected for specific members of Alphaproteobacteria, Betaproteobacteria and Bacteriodetes. All these observations can be initiated by the selection of bacterial species more adapted to the use of phototransformed DOM and/or less sensitive to the short-lived reactive oxygen species generated during photochemical reaction (Glaeser et al. 2010). These different observations underline the importance to determine more precisely which bacterial species are stimulated or inhibited by the DOM photodegradation according to its origin.

I.3 Aims and scientific questions of the thesis

The overall aim of the thesis was to study the effect of different labilities in the supply of organic matter can affect bacterial community structure and activity. For this purpose we performed different experimental studies with using microcosm approach articulated around the following questions:

- **Question 1: What are the effects of the addition of highly bioreactive DOM derived from jellyfish on bacterial activities and community structure during a disturbance caused by a jellyfish bloom? Is there a resilience of bacterial functions and diversity when all the LDOM derived from jellyfishes has been consumed? (Chapter II)**

This topic was assessed via the response of natural bacterial communities from a Mediterranean coastal lagoon to the addition of dissolved organic matter (DOM) from the jellyfish *Aurelia aurita.*

- **Question 2: What are the effects of the addition of single or combined DOM sources on bacterial diversity and activity? Is there any evidence of priming effect in marine environments? (Chapter III)**

This topic was assessed via experimental studies designed to explore the existence of the priming effect in contrasting coastal environments showing different types of freshwater inputs (river, amino acids solution or DOM derived from phytoplankton):

  - The input of terrestrially derived organic matter, rich in humic substances carried by the Rhone River in the Mediterranean Sea (France).
  - The input of DOM from lakes and glacier melting into the Baker river fjord complex in Chilean Patagonia. This input involves large volumes of oligotrophic waters and has therefore an effect on local stoichiometric conditions.

- **Question 3: What are the bacterial responses in activity and community composition to photo-oxidation of dissolved organic matter with different initial bioreactivity? (Chapter IV)**

  We studied the response of a Mediterranean bacterial community to the addition of DOM derived from a phytoplankton culture (LDOM) or the Rhone River (recalcitrant) exposed or not to solar radiation.

  The Mediterranean basin is characterized by relatively high solar radiation levels due to its weak cloud cover. Photo-oxidation of DOM can occur at the sea surface, especially in the river plume that may cover extended areas and are associated with high concentrations in riverine DOM and phytoplankton (Joux et al. 2009).

CHAPTER II:

Changes in bacterial community metabolism and composition during the degradation of dissolved organic matter from the jellyfish *Aurelia aurita* in a Mediterranean coastal lagoon

I Changes in bacterial community metabolism and composition during the degradation of dissolved organic matter from the jellyfish *Aurelia aurita* in a Mediterranean coastal lagoon

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Changes in bacterial community metabolism and composition during the degradation of dissolved organic matter from the jellyfish *Aurelia aurita* in a Mediterranean coastal lagoon

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Abstract

Spatial increases and temporal shifts in outbreaks of gelatinous plankton have been observed over the past several decades in many estuarine and coastal ecosystems. The effects of these blooms on marine ecosystem functioning, and particularly on the dynamics of the heterotrophic bacteria are still unclear. The response of the bacterial community from a Mediterranean coastal lagoon to the addition of dissolved organic matter (DOM) from the jellyfish *Aurelia aurita,* corresponding to an enrichment of dissolved organic carbon (DOC) by 1.4, was assessed during 22 days in microcosms (8 liters). The high bioavailability of this material led to (i) a rapid mineralization of the DOC and dissolved organic nitrogen from the jellyfish and (ii) the accumulation of high concentrations of ammonium and orthophosphate in the water column. DOM from jellyfish greatly stimulated heterotrophic prokaryotic production and respiration rates during the two first days, then these activities showed a continuous decay until reaching those measured in the control microcosms (lagoon water only) at the end of the experiment. Bacterial growth efficiency remained below 20%, indicating that most of the DOM was respired and a minor part was channeled to biomass production. Changes in bacterial diversity were assessed by tag pyrosequencing of partial bacterial 16S rRNA genes, DNA fingerprints and a cultivation approach. While bacterial diversity in control microcosms showed little changes during the experiment, the addition of DOM from the jellyfish induced a rapid growth of *Pseudoalteromonas* and *Vibrio* species that were isolated. After 9 days the bacterial community was dominated by *Bacteroidetes*, which appeared more adapted to metabolize high-molecular-weight DOM. At the end of the experiment, the bacterial community shifted towards a higher proportion of *Alphaproteobacteria*. Resilience of the bacterial community after the addition of DOM from the jellyfish was higher for metabolic functions than diversity, suggesting that jellyfish blooms can induce durable changes in the bacterial community structure in coastal lagoons.

Keywords: *Aurelia aurita*, jellyfish, organic matter, heterotrophic bacteria, biodegradation, bacterial growth efficiency, bacterial diversity.

1. Introduction

Bacteria are key organisms in carbon cycling in aquatic ecosystems, acting as a sink (mineralization of dissolved organic carbon to CO_2) or as a link (production of biomass that can be transferred through the microbial food web) (Cotner and Biddanda 2002). The quality and the quantity of organic matter greatly influence bacterial metabolism and community structure (Azam and Malfatti 2007). The major autochthonous source of organic matter in the marine ecosystem comes from the phytoplankton, and a substantial part (10 to 50%) of primary production is channeled through bacteria (Cole et al. 1988). A number of field and experimental studies have indicated that DOM released during phytoplanktonic blooms was associated to important changes in the microbial community diversity and metabolic properties (e.g. McCarren 2010; Sarmento and Gasol 2012). Besides phytoplankton, DOM can be provided by jellyfish (used here to refer to medusa of the phylum Cnidarian and to members of the phylum Ctenophora) that can attain enormous biomasses in marine waters when the conditions are favorable (e.g. high nutrient concentrations) (Pitt et al. 2009, Purcell 2012).

The sudden appearance and disappearance of massive jellyfish blooms is one of the distinct features of this group (Condon et al. 2012). Top-down trophic control of jellyfish populations is low, and jellyfish have been suggested as being a trophic "dead end" (Arai 2005). Nonetheless, bacteria can recycle jellyfish biomass rapidly by different ways. Firstly, jellyfish release dissolved organic matter (DOM) by excretion and mucus production (Pitt et al. 2009). Secondly, during massive die-out events, jellyfish biomass sinks to the seafloor because dead animals have greater density than live animals (Yamamoto et al. 2008). The release rates of total organic carbon by dead jellyfish is significantly higher than that of living jellyfish (Pitt et al. 2009). Depending of the water depth, decomposition of dead jellyfish can occur in the water column or at the sediment surface. Rates of decomposition of both excretion and dead jellyfish biomass result in a large input of nutrients in the marine environment (Pitt et al. 2009; West et al. 2009; Tinta et al. 2010). Recent studies also reported a change in the bacterial structure in response to the use of organic matter from jellyfish for different coastal waters (Tinta et al. 2010; Condon et al. 2011; Tinta et al. 2012; Dinasquet et al. 2013).

Coastal lagoons are semi-enclosed systems occupying approximately 13% of the world's coastline with both marine and fluvial components (De Wit, 2011). The

Mediterranean coast is bordered by a series of coastal lagoons with varying sizes, and some of them are associated with important economic activities (fisheries, aquaculture, tourism, recreation activities) (De Wit, 2011). Jellyfish blooms have been reported in these coastal lagoons and in particular were attributed to the scyphomedusa *Aurelia aurita* (Lo and Chen 2008; Lo et al. 2008; Bonnet et al. 2013). *A. aurita* is widespread in coastal and shelf sea environments around the world, predominantly inhabiting highly eutrophic waters where maximum abundance can reach 300 individuals.m^{-3} (Lo and Chen 2008). Due to the shallow depth of coastal lagoons, dead jellyfish can accumulate rapidly down to the sediment leading to hypoxic and anoxic conditions in these environments (West et al. 2009).

In the present study, we investigated the response of a Mediterranean coastal lagoon bacterial community to the addition of fresh jellyfish biomass (as DOM) of *A. aurita* using a microcosm approach. Our objective was to follow the changes in nutrients, bacterial activities and bacterial community structure during the total degradation of DOM from *A. aurita* in order to determine the resilience of the bacterial community exposed to the disturbance caused by a jellyfish bloom. For our purposes, we measured simultaneously bacterial production and respiration to determine the bacterial growth efficiency. We also combined the use of 454-tag pyrosequencing and DNA fingerprinting based on 16S rRNA genes and culture approach to characterize the bacterial diversity in response to the DOM addition from the jellyfish. To complete previous studies on this topic, we employed longer incubation period (22 days) with four sampling points allowing relevant analyses on the succession of taxa concurrent to the degradation of jellyfish DOM. Moreover, by using an incubation, long enough to reach the complete mineralization of DOC from the jellyfish, it was possible to determine and then compare the resilience of bacterial functions and diversity after jellyfish DOM addition.

2. Material and methods

Preparation of dissolved organic matter from A. aurita

A. aurita jellyfish used in this study were produced in aquarium using *Artemia salina* nauplii as food (Lautan Production, Mèze, France). The jellyfish were less than 4 months old with a diameter around 8 cm. Twelve organisms were crushed with a blender. The homogenate was prefiltered onto 10-µm mesh (Nytex) and then filtered

with a peristaltic pump onto 0.2-µm capsule filter (Polycap TC, Whatman) (previously rinsed with 10% HCl and washed thoroughly with Milli-Q water). The filtrate was recovered in a pre-combusted (450°C, 6 h) glassware bottle before being dispensed in the microcosms (see below).

Preparation of the microcosms and sampling

Water samples were collected in October 2010 from the shoreline of a Mediterranean coastal lagoon (Bages-Sigean, France, [42°36'21" N, 2°53'49" E]). The Bages-Sigean Lagoon covers 38 km^2 with a mean depth of 1.3 m and a maximal depth of 3 m. The catchment area covers greater than 456 km^2 and is drained by three rivers flowing into the lagoon. Water samples (salinity 33‰) were filtered sequentially by gravity onto 250, 100, 50 and 25 µm mesh (Nytex) and then onto 1-µm filter capsule (Polycap TC, Whatman) to remove fine particles. Six 10-L polycarbonate carboys (Nalgene) were filled with 8-L of the filtrated lagoon water. Three carboys received the DOM from *A. aurita* (300 ml) and the three other were used as controls. All the carboys were incubated in the dark at *in situ* temperature (18°C) under magnetic agitation. The caps of the carboys were maintained open to avoid any oxygen limitation. The microcosms were sampled 30 min after DOM addition (T0) and over 22 days (T22). At the last sampling, more than 60% of the initial volume was present in the microcosms.

Nutrients, dissolved organic carbon, nitrogen and phosphorus

Samples (60 ml) for nitrate (NO_3^-), nitrite (NO_2^-) and phosphate (PO_4^{3-}) were stored at -20°C and analyzed within 1 month of collection by colorimetry using a nutrient autoanalyzer (SEAL Analytical AA3HR) (Aminot and Kérouel 2007). Samples (100 ml in duplicate) for ammonium (NH_4^+) were analyzed immediately according to Holmes (1999) with a fluorometer (Jasco). Samples (20 ml in duplicate) for dissolved organic carbon (DOC) were filtered through two pre-combusted (450°C, 6 h) 25-mm GF/F filters; the filtrate was transferred into precombusted glass tubes, poisoned with 85% H_3PO_4 (final pH=2), closed with Teflon lined screw caps and stored in the dark at room temperature until analysis. DOC was analyzed using the high temperature catalytic oxidation (HTCO) technique (Cauwet 1994) using a Shimadzu TOC-V analyzer. Prior to analyses and between each set of samples, an international certified reference sample for DOC concentration was analyzed to check the calibration of the analyzer and its stability over time.

Samples for dissolved organic nitrogen (DON) and phosphorus (DOP) were filtered through 2 pre-combusted (450°C, 6 h) 25-mm GF/F filters (Whatman). Samples were collected directly in Teflon bottles and immediately frozen (-20°C) until analysis. DON and DOP were simultaneously determined by the wet oxidation procedure (Pujo-Pay and Raimbault 1994). DON (±0.1 µM) and DOP (±0.02 µM) concentrations were determined by sample oxidation (30 min, 120°C) corrected for NH_4^+, NO_3^-+NO_2^- and PO_4^{3-} concentrations, respectively.

CDOM fluorescence

The fluorescence properties of colored dissolved organic matter (CDOM) were determined on samples filtered through 2 pre-combusted (450°C, 6 h) 25-mm GF/F filters (Whatman). Fluorescence was measured on a Perkin Elmer LS55 spectrofluorometer using a 1-cm quartz cuvette. Different excitation/emission couples were used to determine protein-like (275/340 nm) and humic-like (320/420 nm) compounds. Fluorescence intensity values were calibrated using the Raman scatter peak of Milli-Q water (Lawaetz and Stedmon 2009).

Virus count

Samples (2 ml) were fixed with 0.02 µm filtered formaldehyde (0.5% final concentration) and stored at -80°C after flash freezing in liquid nitrogen. Samples were filtered onto 0.02-µm filters (Anodisc, 25 mm diameter; Whatman) and virus-like particles (VLP) were stained on the filters using SYBR-Green I and enumerated under a Zeiss Axiophot microscope equipped for epifluorescence microscopy as previously described (Noble and Fuhrman 1998). At least 400 VLP were counted per filter in several randomly selected microscopic fields.

Heterotrophic prokaryotes abundance, production and respiration

Heterotrophic prokaryotic (including Bacteria and Archaea) abundance (HPA) was determined by flow cytometry. Duplicate 3 ml samples in cryovials were preserved with 0.2 µm filtered formalin (2% final concentration). The samples were gently mixed and left in the dark at room temperature for 10 min before quick-freezing in liquid nitrogen and storing at -80°C. The samples were later thawed at room temperature, stained with SYBR Green I (final concentration 0.025% (v/v) of the commercial solution; Molecular Probes Inc., OR) for at least 15 min at 20°C in the dark and analyzed on a flow cytometer (FACScan, Becton Dickinson, San Jose, CA) equipped with a 488 nm, 15

mW argon laser. HP cells were detected on a plot of green fluorescence (515-545 nm) *versus* right angle light scatter (SSC), using the green fluorescence as threshold parameter. Fluorescent beads (1.0 µm; Polysciences Inc., Warrington, PA) were added to each sample analyzed to normalize SSC and green fluorescence. HP growth rate (µ, d^{-1}) was calculated from the following equation $\mu = (\text{Ln } HPT_2 - \text{Ln } HPT_1) / (T_2-T_1)$. Generation time (g, h^{-1}) was determined as $g = (\text{Ln } (2) \times 24) / \mu$.

Heterotrophic prokaryotic production (HPP) was measured by ^{3}H-thymidine incorporation applying the centrifugation method (Smith and Azam 1992). Samples (1 ml in triplicate) were incubated in the dark at 18°C for 1 h with 20 nM [^{3}H]-thymidine (specific activity 83.2 Ci $mmole^{-1}$, Perkin Elmer) in 2 ml microtubes. Incorporations were terminated by the addition of trichloroacetic acid (TCA) to a final concentration of 5%. One killed control was prepared for each assay by the addition of TCA 15 min before the addition of ^{3}H-thymidine. Samples were stored for at least 1 h at 4°C and then centrifuged for 15 min at 12,000 g. The precipitate was rinsed twice with 5% TCA. The precipitates were resuspended in 1.0 ml of liquid scintillation cocktail (FilterCount, Perkin Elmer) and radioactivity determined by liquid scintillation counter (LS 5000CE Beckman). Thymidine incorporation rates were converted into carbon production using the conversion factors of 2.10^{18} cells produced by mole of thymidine incorporated and 20 fg C by cell (Ducklow and Carlson 1992).

Heterotrophic prokaryotic respiration (HPR) was measured at each time point using an oxygen microelectrode (Briand et al. 2004). The microprobes (Unisense, Denmark) are designed with an exterior guard cathode, which results in extremely low oxygen consumption by the electrodes ($4.7–47 \times 10^{-7}$ µmol O_2 h^{-1}). Probes have a response time shorter than 1 s and a precision of 0.05%. The HPR was measured over 4 h to 24 h in duplicate samples for each microcosm, placed in microchambers (2 ml) and immerged in a water bath with controlled temperature (18°C). A specific measurement of the dissolved O_2 concentration was carried out with a minimum of 4 times during incubation in microchambers. HPR was deduced from the linear regression established on these points of measurement. HPR were expressed in mgC m^{-3} d^{-1} using a respiratory quotient of 1 (del Giorgio and Cole 1998). We assume here that most of the respiration measured came from heterotrophic prokaryotes because the water was filtered on 1-µm at the start of the experiment. However, we cannot exclude the growth of protozoa then after, leading to an overestimation of HPR. Bacterial growth efficiency (BGE, %) was calculated from the following equation BGE= HPP/(HPP+HPR).

Bacterial DNA extraction

Samples (500 ml) at T0, T2, T9 and T22 were filtered onto a 0.2 µm Sterivex filter (Durapore, Millipore) and stored at -80°C. For analysis, 840 ml of alkaline lysis buffer (50 mM Tris hydrochloride pH 8.3, 40 mM EDTA and 0.75 M sucrose) was added in the Sterivex. Cell lysis was accomplished by an initial incubation for 45 min at 37°C after adding 50 ml of freshly prepared lysozyme solution (20 mg ml^{-1}), and a second incubation at 55°C for 1 h after adding 100 ml of 10% sodium dodecyl sulfate and 10 ml of proteinase K (20 mg ml^{-1}). Six hundred ml of lysate was treated with 10 µl of a 100 mg ml^{-1} RNase A solution (Qiagen) before DNA extraction with the All Prep DNA/RNA mini (Qiagen) according to the manufacturer's instructions.

Analysis of bacterial community structure by capillary electrophoresis SSCP-based single strand conformational polymorphism (CE-SSCP) analysis

CE-SSCP fingerprinting was performed (i) to follow the changes in total bacterial community structure and (ii) to check the reproducibility between replicate microcosms. DNA was used as a template for PCR amplification of the variable V3 region of the 16S rDNA (*Escherichia coli* positions 329-533), as previously described (Ghiglione et al. 2005). CE-SSCP was performed using the ABI 310 Genetic Analyzer (Applied Biosystems), equipped with a capillary tube (47 cm × 50 mm) filled with a polymer mix composed of 5.6% GeneScan polymer (Applied Biosystems), 10% glycerol and 1× buffer with EDTA (Applied Biosystems). The similarity of the CE-SSCP profiles was assessed using the software SAFUM (Zemb et al. 2007), which normalized the total area of the profiles and mobilities between different runs using an internal standard. SAFUM renders a profile of fluorescence intensity as a function of retention time per sample, thus taking into account the presence and intensity of each individual signal. Ordination of Bray-Curtis similarities among normalized sample profiles was calculated using PRIMER 5 software (PRIMER-E, Ltd., UK).

Analysis of bacterial diversity and community structure by pyrosequencing

Bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP) was performed using the universal bacterial primers guidelines targeting the V1 to V3 hypervariable regions of the bacterial 16S rRNA gene: 27Fmod (5′-AGRGTTTGATCMTGGCTCAG-3′) and 519r (5′-GWATTACCGCGGCKGCTG-3) as described previously (Dowd and al.

2008). Initial generation of the sequencing library was accomplished by a one-step PCR with a total of 30 cycles using the HotStarTaq Plus Master Mix Kit (Qiagen, Valencia, CA) and amplicons originating and extending from the 27Fmod primer. Tag-encoded FLX amplicon pyrosequencing analyses were completed using the Roche 454 FLX instrument with Titanium reagents, and procedures were performed at MR DNA (Shallowater, TX, USA) following manufacturer's guidelines.

Sequences were processed and analyzed using the Mothur software version 1.33 (Schloss et al. 2009) with default settings excluding sequences <200bp. Sequences were denoised using the Mothur implementation of PyroNoise and SeqNoise. Chimeras were removed using Chimera Slayer (Haas et al. 2011). The resulting clean sequences were clustered using operational taxonomic units (OTUs) at a 97% sequence identity level using the UCLUST algorithm (Edgar 2010) and a representative sequence from each OTU was classified using the Ribosomal Database Project (RDP) classifier (Wang et al. 2007) using the SILVA training set. Taxonomic identification of the sequence reads (tags) followed the approach by Sogin et al. (2006) and Huse et al. (2010). All samples were clustered into operational taxonomic unit (OTU) at a distance of 0.03 (Ghiglione and Murray 2012). All OTU and diversity analyses were performed on the randomly re-sampled datasets using Mothur.

Culturable bacterial counts, isolation and 16S rRNA gene sequencing

Culturable heterotrophic aerobic marine bacteria were enumerated during the experiment at T0, T2, T9 and T22 by plating 100 µl of diluted (in sterile seawater) or undiluted samples on marine agar 2216 (MA, Difco, Detroit, Mich.). Cycloheximide (100 mg/L) was added to the media to inhibit fungal growth. All culture were incubated at 25°C in the dark during two weeks before counting the colony forming units (CFU).

The identification of the most representative culturable strains was performed for one of the replicate for each condition after two days of incubation. The colonies were categorized using morphologic characteristics. All the different morphotypes (colony morphology) were picked for two successive sub-culturing steps on MA to ensure purification. One colony of each isolate was then grown in marine broth media (MB, Difco, Detroit, Mich.) during 48h at 25°C under agitation (100 rpm). Each culture was cryopreserved in 5% dimethylsulfoxide or 35% glycerol at -80°C. For genomic DNA extraction, 2 ml of each liquid culture were spun down (10,000 x g, 3 min). DNA extraction, PCR and sequencing were done as previously described (Fagervold et al.

2013). Partial 16S rRNA gene sequences were trimmed manually, double checked and dereplicated using the package Staden-GAP4 (Staden et al. 2003). For bacterial strain identification, each FASTA file was uploaded in Ez Taxon-e (Kim et al. 2012) and compared with the cultured bacterial strain database using BLAST (Basic Local Alignment Search Tool). Sequences were deposited in Genbank (NCBI) under the following numbers: MOLA851-858 and MOLA865-880 from enriched microcosms and MOLA859-864 and MOLA881-887 from control microcosms.

Statistical analyses

Statistical analysis of the effects of treatment on chemical and biological parameters was performed using a one-way analysis of variance (ANOVA) and post-hoc Tukey tests with repeated measures (i.e. microcosms) and assuming homoscedasticity and normality of the data. Statistical significance was set at $p = 0.05$ and analysis was computed using XLSTAT 2014.2 software (Addinsoft).

Bacterial community structures, either as number and area of the peaks in the CE-SSCP profiles or presence and abundance of OTU in the pyrosequencing data, were compared using ordination of Bray–Curtis similarities and used to build dendrograms by the unweighted-pair group method with arithmetic averages (UPGMA). A similarity profile test (SIMPROF, PRIMER 6) was performed on a null hypothesis that a specific sub-cluster can be recreated by permuting the entry species and samples. The significant branch (SIMPROF, $p < 0.05$) was used as a prerequisite for defining bacterial clusters.

The extent of the correlation of bacterial diversity analyzed by pyrosequencing for both conditions with the chemical parameters and the viral abundance was assessed by canonical correspondence analysis (CCA). CCA was performed with MVSP v3.12d software (Kovach Computing Service, Anglesey Wales). Relative abundances of OTUs were transformed with arcsin ($\times^{0.5}$) to normalize the distribution of the data as suggested by Legendre and Legendre (1998).

3. Results

Degradation kinetics of jellyfish dissolved organic matter

The water in the control microcosms (i.e. lagoon water without any addition) was characterized by a high concentration of dissolved organic carbon (DOC, 573 µM), nitrogen (DON, 50 µM) and phosphorus (DOP, 0.7 µM), underlying the eutrophic status of this ecosystem **(Fig. 1a,b,c).** Addition of dissolved organic matter (DOM) from *A. aurita* provided 252 µM DOC, 85 µM DON and 3 µM DOP, increasing significantly (ANOVA, $p<0.05$) their initial concentrations by 1.4, 2.5 and 5.8 times, respectively, compared to the control microcosms. The C:N ratio of the DOM was significantly (ANOVA, $p<0.05$) lower in the enriched microcosms (6:1) compared to the control microcosms (12:1) due to the high protein content of the jellyfish.

The DOC from the jellyfish was consumed at a high rate during the first four days of incubation (57 µM d^{-1}) and then at a lower rate (5.6 µM d^{-1}) (**Fig. 1a**). In contrast, the DOC in the control microcosms was consumed at a constant and low rate (4 µM d^{-1}) during the entire incubation. At the end of experiment, the same concentration of DOC (500 µM) was noted in the control and the enriched microcosms (ANOVA, $p>0.05$). The DON and the DOP concentrations were measured less frequently in the microcosms **(Fig. 1b,c).** The DON concentration decreased by 67 µM during the nine first days and then remained almost constant in the enriched microcosms. In contrast the DOP concentration increased during the first two days and then decreased during the rest of the incubation. For both DON and DOP, we did not observe significant changes in the control microcosms (ANOVA, $p>0.05$). At the end of the experiment, 86% of the jellyfish-derived DON and 57% of the jellyfish-derived DOP were degraded. The C:N ratio of the DOM in the control was close to the enriched microcosms by the end of the experiment (10:1 and 9:1, respectively).

The initial concentrations of NO_3+NO_2 (5.5 µM), NH_4 (6 µM) and PO_4 (0.5 µM) in the control microcosms were low and remained stable during the experiment time (**Fig. 1d,e,f**). The addition of jellyfish biomass led to an enrichment factor by 1.7, 1.0, and 11 for NO_3+NO_2, NH_4 and PO_4, respectively. NO_3+NO_2 concentrations remained stable in enriched microcosms, with the exception of an increase (+ 4 µM) at the end of the experiment (**Fig. 1d**). This increase was only due to changes in NO_2 concentration (data not shown). We observed a continuous increase of NH_4 up to 100 µM at T9, followed by a plateau (**Fig. 1e**). PO_4 concentrations (**Fig. 1f**) evolved in an opposite way to the

DOP concentrations in the enriched microcosms: after a decrease during the first two days, the concentration increased during the rest of the incubation mainly between the days 2 and 9.

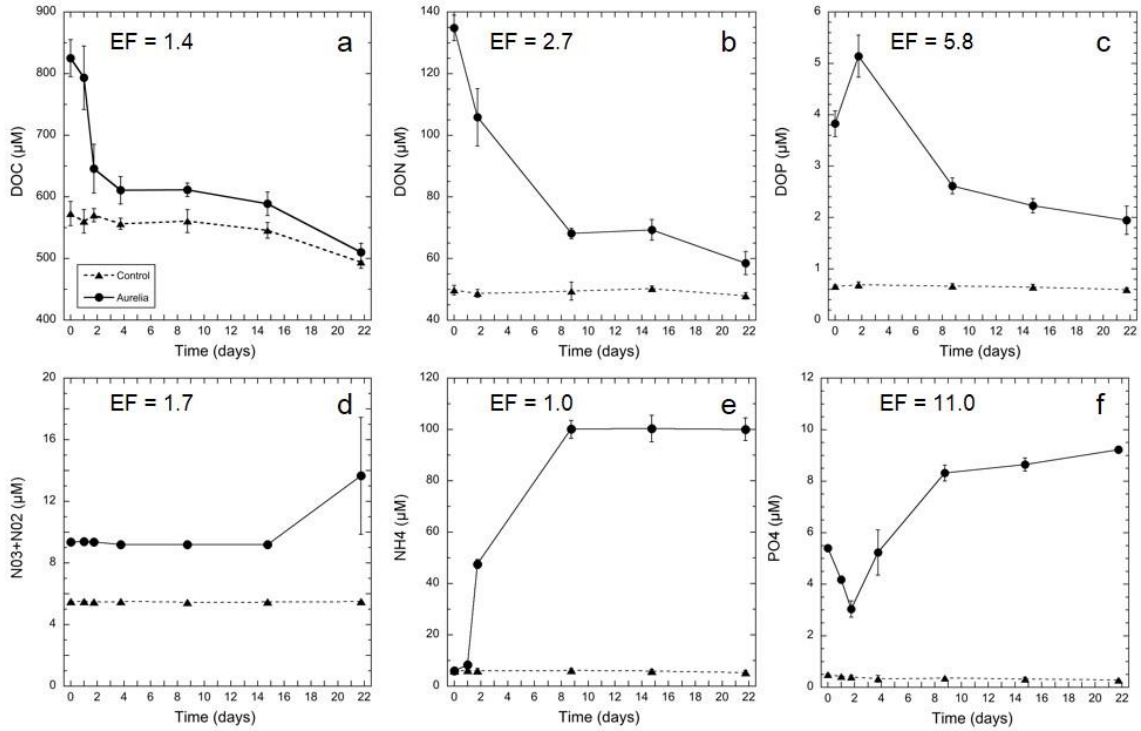

Fig. 1. Dissolved organic carbon (DOC, a), dissolved organic nitrogen (DON, b), dissolved organic phosphorus (DOP, c), nitrates plus nitrites (NO_3+NO_2, d), ammonium (NH_4, e) and phosphate (PO_4, f) concentrations during the incubations. Each point represents three replicates (mean ± standard deviation). The initial enrichment factor (EF) between enriched and control microcosms is indicated for each parameter.

Protein-like and humic-like components of DOM were characterized by their fluorescence properties (**Fig. 2**). Protein-like components measured in the control microcosms remained constant during the experiment (Fig. 2a). In contrast, the protein-like components in the enriched microcosms showed a rapid decrease during the first nine days until reaching the value measured in the control microcosms at the end of the experiment. The concentration of humic-like components (**Fig. 2b**) was identical in both conditions, remaining almost constant during the experiment. However, we noticed a slight but significantly (ANOVA, $p<0.05$) higher concentration of humic-like components in enriched conditions compared to the controls after 15 and 22 days.

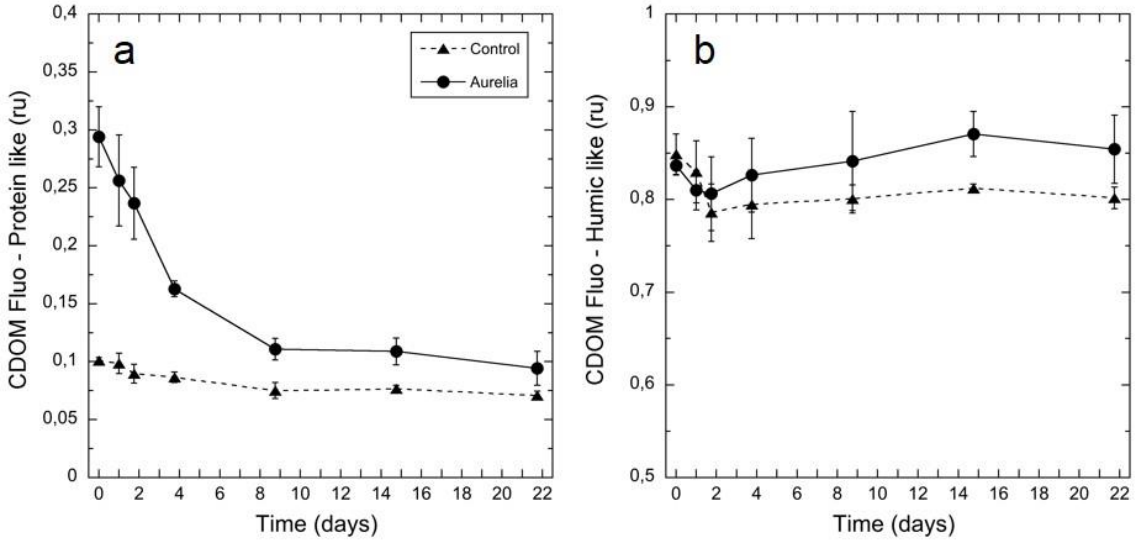

Fig. 2. CDOM fluorescence of protein-like (a) and humic-like substances (b) concentrations during the incubations (ru= Raman Units). Each point represents three replicates (mean ± standard deviation).

Effect of jellyfish DOM addition on viral abundance, bacterial abundance and metabolism

Virus abundance remained almost constant during the entire period of the experiment in both controls and enriched microcosms **(Fig. S1).** A small but significant difference was observed between the two conditions at T2 and T4, with higher virus abundance in microcosms enriched with DOM from *A. aurita* (ANOVA, $p<0.05$).

During the three first days, we measured a rapid bacterial growth without lag time after the DOM addition from *A. aurita* (**Fig. 3a**). The bacterial growth rates calculated on the basis of bacterial abundance between T0 and T3 were 1.44 d^{-1} and 0.48 d^{-1} in the enriched and the control conditions, respectively. A sharp decrease in bacterial abundance occurred at T4 in the enriched microcosms, leading to a value identical to the control condition. Then, the bacterial abundance remained almost constant for the rest of the experiment.

A rapid increase in bacterial production (BP) and bacterial respiration (BR) was also observed in the enriched condition during the first days (**Fig. 3b,c**). After 3 days, both activities slowly decreased at a constant and similar rate during the rest of the experiment. After 22 days, the BP and BR were not significantly different between the control and the enriched microcosms (ANOVA, $p>0.05$).

Bacterial growth efficiency (BGE) calculated on the basis of bacterial production and respiration was quite low at the start of the experiment (<1%) (**Fig. 3d**). During the first

9 days, the BGE increased up to 17% in the microcosms enriched with DOM from *A. aurita*. The BGE measured in the control microcosms remained lower than the enriched microcosms until day 15, when both conditions presented the same mean value (13%).

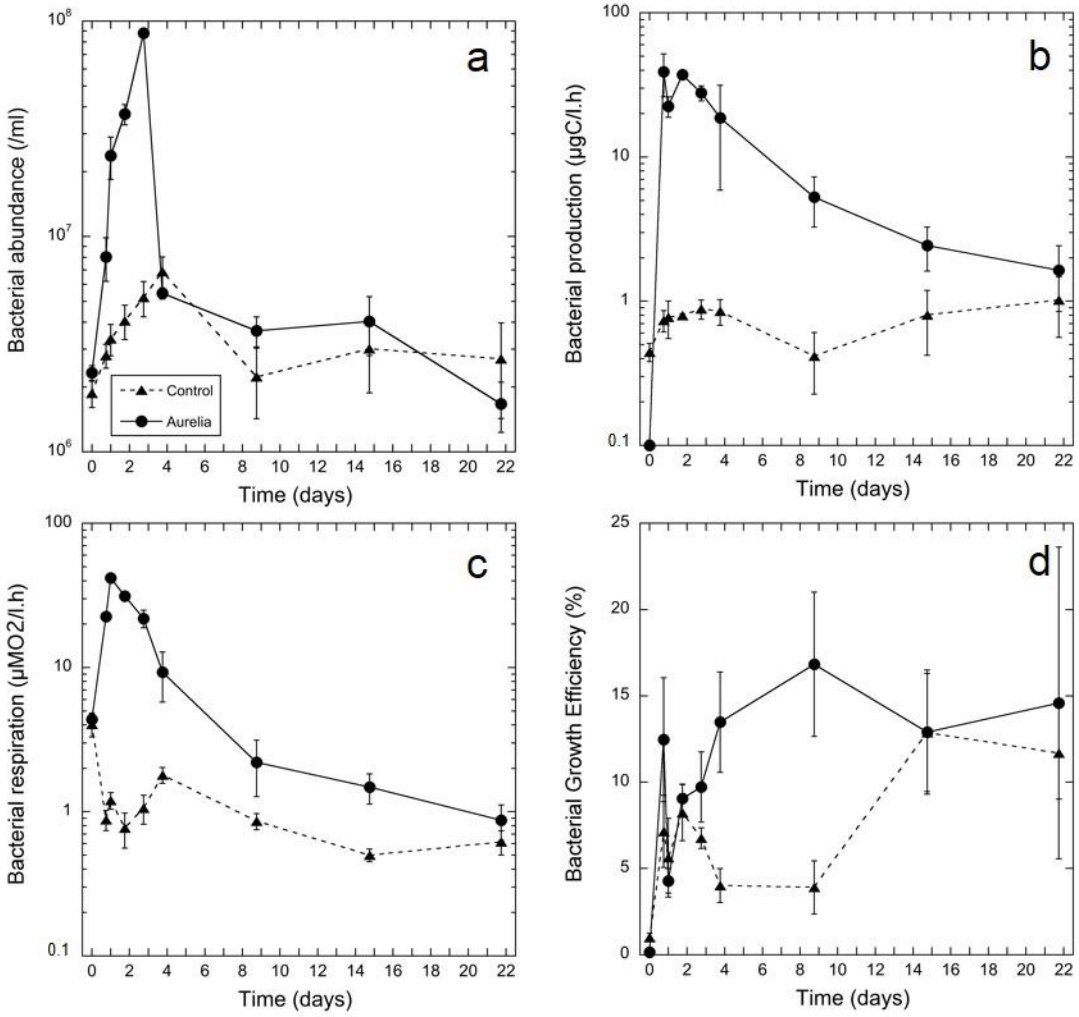

Fig. 3. Bacterial abundance (a), production (b), respiration (c) and growth efficiency (d) during the incubations. Each point represents three replicates (mean ± standard deviation).

Changes in culturable heterotrophic bacteria counts and diversity

At the start of the experiment, culturable bacteria represented only a small part of the total bacteria (0.25%) (**Fig. 4**). After two days of incubation, culturable bacteria accounted for 65% of total bacteria in enriched microcosms, compared to 3% in the control microcosms. The bacterial growth rate calculated on the basis of culturable bacteria counts during this period was 4.8 d^{-1} in the enriched condition compared to 1.92 d^{-1} in the controls. After 9 days, there was a significant difference in the culturable bacteria fraction between the two conditions (2% and 12%, for control and enriched microcosms, respectively) (ANOVA, $p<0.05$). At the end of the experiment, the

culturable bacteria fraction accounted for the same percentage (~10%) in both control and enriched microcosms.

Most of the culturable strains isolated after 2 days from an enriched microcosm, belonged to the *Pseudoalteromonas* and *Vibrio* genera (class of *Gammaproteobacteria*) (Table 1). After 22 days, a clear shift in culturable diversity was observed in this microcosm, with the dominance of *Flavobacteriaceae* (phylum of *Bacteroidetes*) and *Rhodobacteraceae* (class of *Alphaproteobacteria*). Even if the number of bacterial strains isolated from the control microcosm was less important than for enriched microcosm (6 versus 18), the diversity seemed to be more stable during the experiment with a majority of *Rhodobacteraceae*.

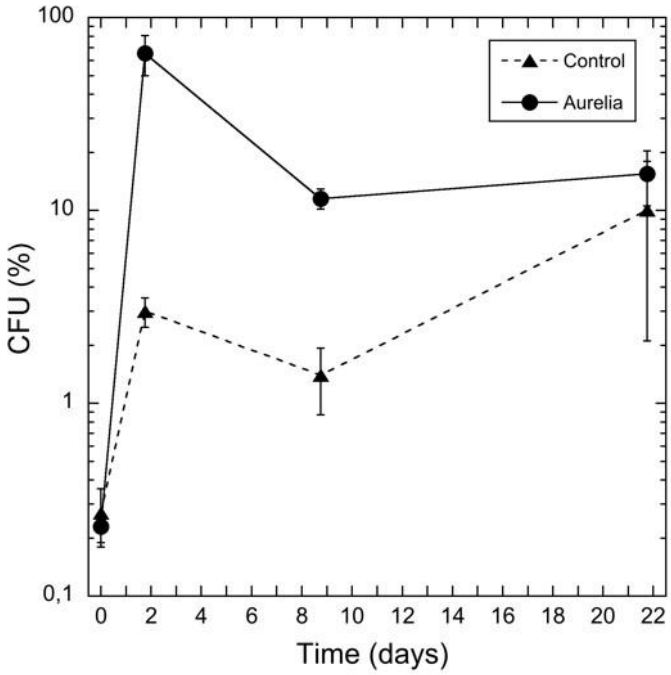

Fig. 4. Percentages of Colony Forming Units (CFU) to total direct counts (flow cytometry) during the incubations. Each point represents three replicates (mean ± standard deviation).

Changes in bacterial community structure induced by Aurelia-derived DOM

A similarity dendrogram based on CE-SSCP data showed a clear separation of the bacterial community diversity into four distinct clusters (**Fig. 5a**). With the exception of the microcosm C2 which presented outliers at T0 and T2, cluster I included the community profiles from control and enriched microcosms at T0, and cluster II grouped together the control microcosms at T2, T9 and T22. The later time points for the enriched microcosm clustered apart from the two clusters into two subclusters comprised of time point T2 (III) and T9, T22 respectively (IV). The bacterial community structure observed in the control microcosms remained closer to the initial bacterial structure than to the enriched microcosms. With the exception of microcosm C2, the replicate microcosms grouped together for a specific condition and time, indicating that the diversity changes observed between conditions or over the time are robust.

To analyze in more detail the composition of the bacterial community, pyrosequencing was performed on one replicate microcosm for each condition at different sampling times **(Fig. 5a in bold)**. A total of 76,027 partial 16S rRNA gene sequences remained after quality controls, yielding on average 9,503 reads per sample (5,080 - 11,600). The number of sequences was normalized to 5,080 per sample (i.e., the lowest number of sequences obtained for a sample). The total of unique OTUs was 2,800 in the whole dataset (at 97% similarity). The rarefaction curves for all samples did not reach a plateau (**Fig. S1**). Hence, our sequencing effort did not cover completely the bacterial diversity. However, the shape of the rarefaction curves was similar for all samples allowing the comparison of these samples. The taxonomic richness based on the Chao1 index was slightly lower in the enriched microcosm at T0 and T2 compared to the control microcosm, and higher at T9 and T22 (Table 2). The diversity based on the inverse Simpson index ($1/\lambda$) ranged from 3.6 to 35.2. The diversity was higher in the enriched condition at T0 compared to the control condition then lower at T2 and T9, and similar at T22. Both conditions showed a transient decrease in diversity at T9 (Table 2).

A dendrogram based on Bray-Curtis similarities using the total number of OTUs was similar to that observed with the DNA fingerprint approach (**Fig. 5b**). The samples at

T0 (with or without the addition of DOM from *A. aurita*) grouped more closely with the samples from the control microcosms at T2, T9 and T22. No significant difference (SIMPROF test, p >0.05) was observed between the samples in the control microcosms at T0 and T9. In contrast, a rapid shift in diversity was observed in the enriched microcosm at T2 (85% dissimilarity). After T9, the diversity tended to stabilize in this microcosm and no significant difference was observed in the bacterial structure at T22 (SIMPROF test, p >0.05).

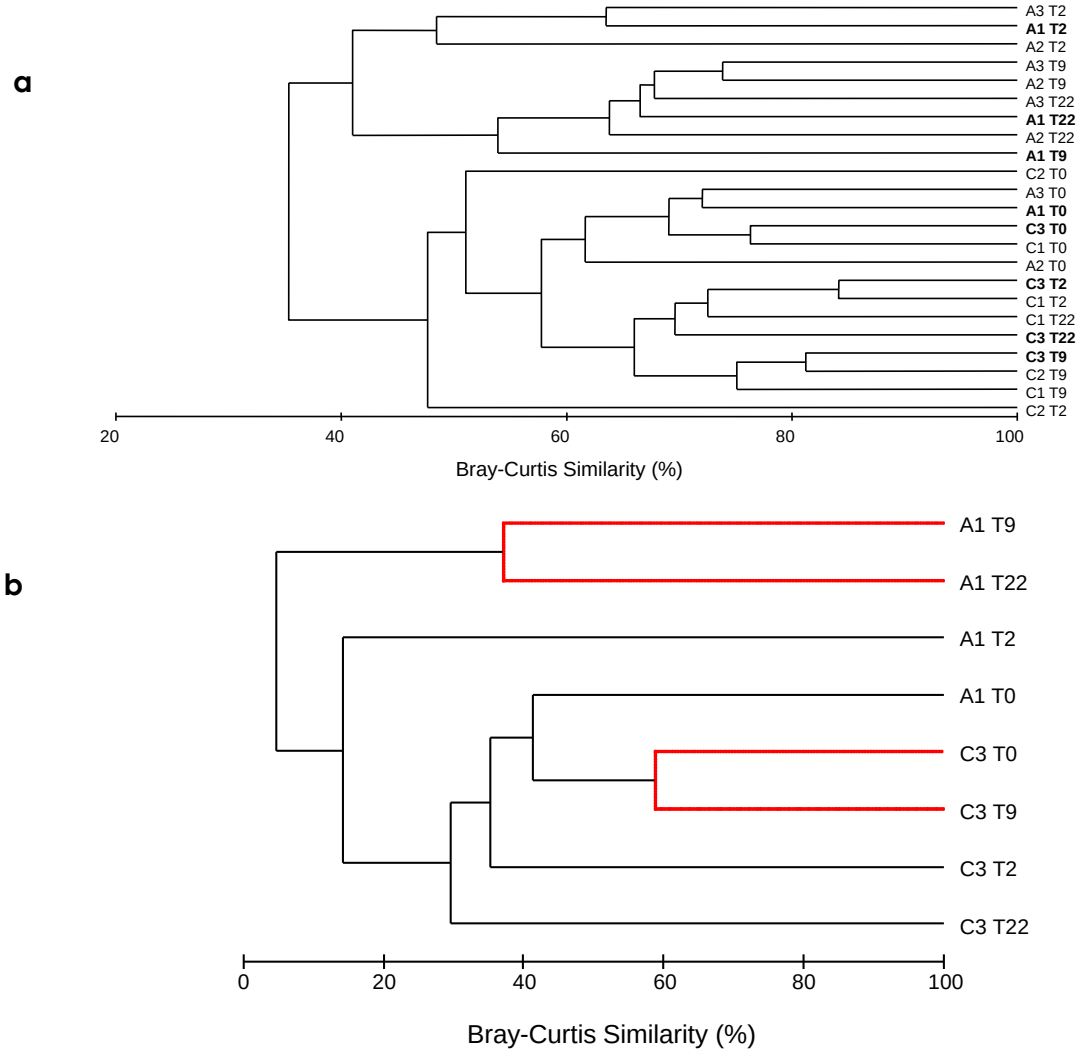

Fig. 5. Dendrograms of similarity based on (a) DNA CE-SSCP fingerprints and (b) OTUs table from the 16S rDNA 454-tag sequences for control microcosms (C1, C2, C3) and enriched microcosms with DOM from *A. aurita* (A1, A2, A3) after 0, 2, 9 and 22 days of incubation (T0, T2, T9, T22). Clustering is on the basis of a distance matrix computed using the Bray–Curtis index of similarity. The dendrogram was inferred with the unweighted pair-group average algorithm (UPGMA). a: The clusters were numbered from I to IV. Samples selected for 454 pyrosequencing are indicated in bold. b: Red branches do not differ significantly (SIMPROF test, p >0.05).

The community composition at the phylum level and proteobacterial subclasses varied between treatments and with time (**Fig. 6**). The changes in abundances described below refer to relative abundances of the sequences and do not refer to absolute abundances of the different bacterial groups. At T0, the enriched microcosm was characterized by a higher proportion of *Actinobacteria* (49% versus 19% in the control microcosm) and by a lower proportion of *Alphaproteobacteria* (16% versus 49% in the control microcosm). The control microcosm showed continuous changes over the experiment. At the end of the experiment, *Alphaproteobacteria* remained the most abundant taxon (67%), whereas the *Actinobacteria* and *Bacteroidetes* tended to decrease (1.4% and 12%, respectively). *Gammaproteobacteria* showed a transient increase at T9 (24%) to reach the initial percentage at the end of the experiment (8%). The bacterial community in the enriched microcosm was characterized by a high proportion of *Gammaproteobacteria* (65%) at T2, followed by a dominance of *Bacteroidetes* (77%) at T9. Finally, at T22, the *Bacteroidetes* decreased (46%) in favor of *Alphaproteobacteria* (36%) and *Plantomycetes* (5%).

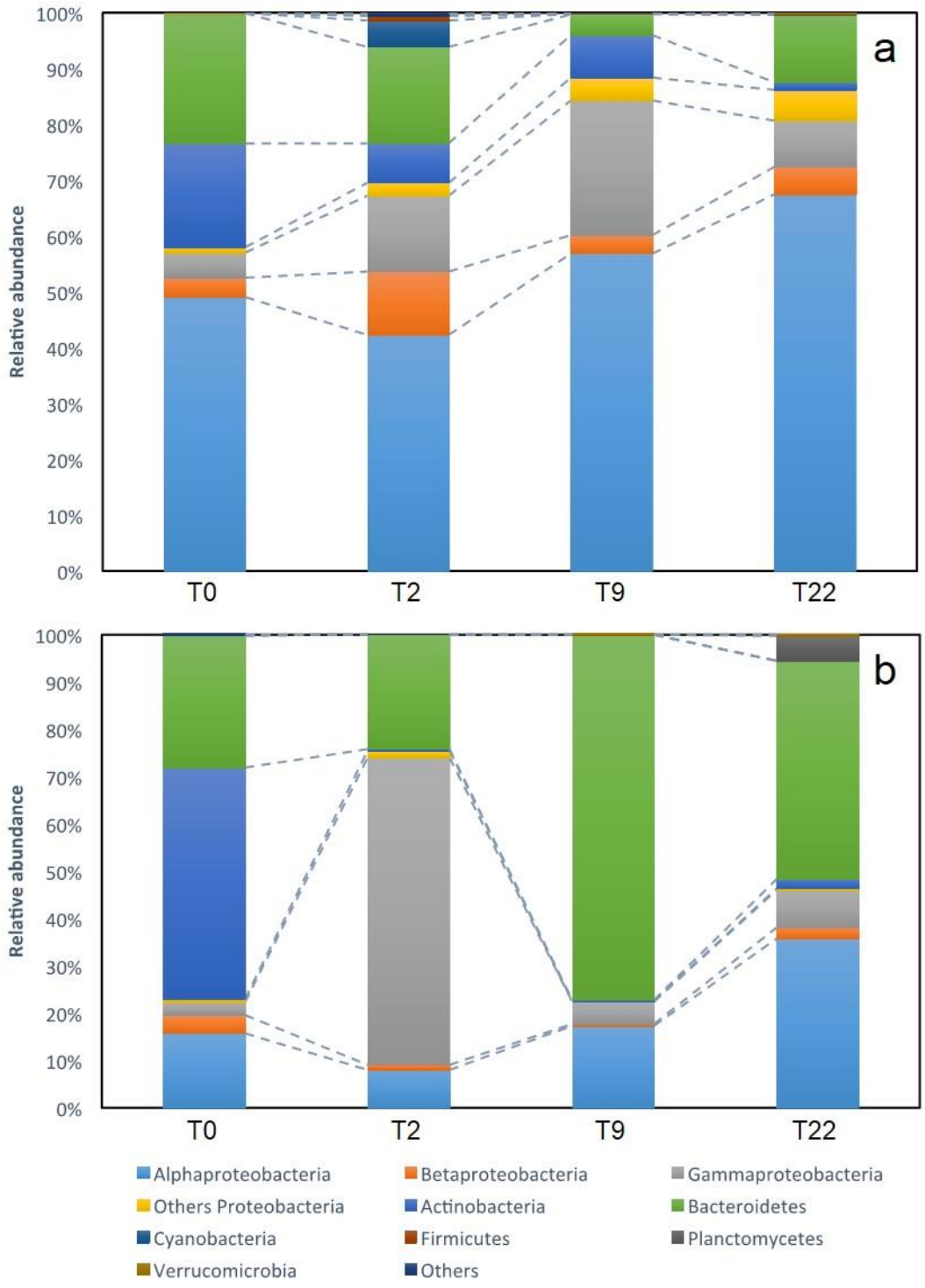

Fig. 6. Cumulative bar charts comparing the main relative phyla and Proteobacteria class abundances for control microcosms (A) and microcosms with DOM from *A. aurita* (B).

Figure 7 shows the taxonomy information for the most abundant OTUs (i.e., present at a percentage higher than 1% in a specific sample). These 71 OTUs represented only 2.5% of the total number of OTUs, however their cumulative abundances represented between 52% and 88% of all the OTUs present in a specific sample. The majority of the *Alphaproteobacteria* sequences in the control microcosm were assigned to SAR11 throughout the experiment. *Actinobacteria* present in both the control and the enriched microcosms at T0 belonged to *Microbacteriaceae*. The bloom of *Gammaproteobacteria* at T2 was affiliated to *Pseudoalteromonas* and *Vibrio* species, and the *Bacteroidetes* at T9 were mainly composed of *Flavobacteriaceae*. At the end of the experiment, the *Alphaproteobacteria* were dominated by *Roseobacter* clade bacteria (RCB) in the enriched microcosms.

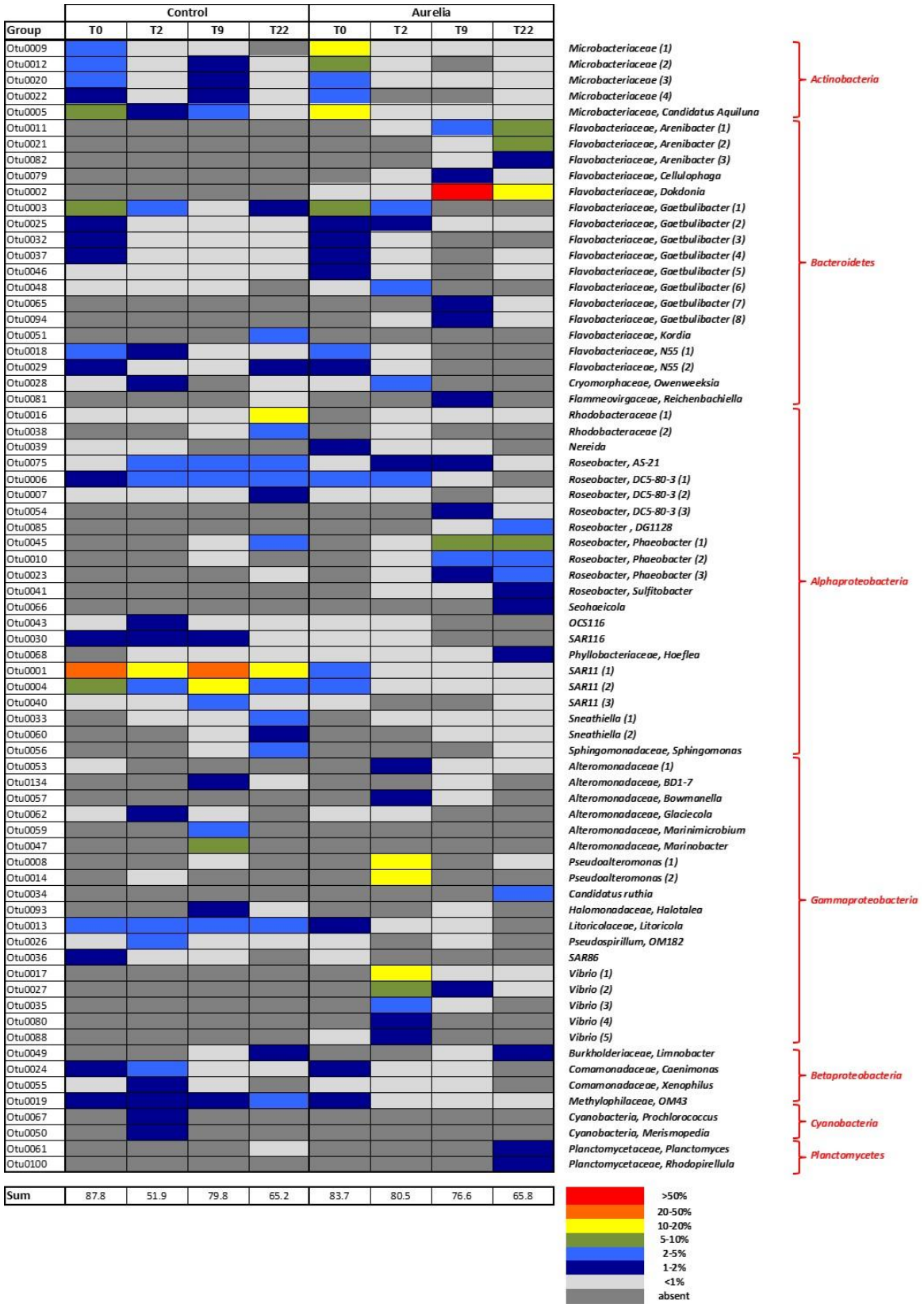

Fig. 7. Heatmap displaying the relative abundances of specific OTUs across the samples. Only OTUs with a contribution higher than 1% in the sample were used. Multiple OTUs with the same taxonomic assignment were numbered sequentially. The contribution of these OTUs to the total OTUs present in each sample is indicated at the bottom.

In order to determine what factors were potentially controlling bacterial community composition, we applied a canonical correspondence analysis (CCA) to the data for both conditions (**Fig. 8**) using the distribution of the dominant OTUs (relative abundance >1%) determined by pyrosequencing. The variance explained by the two first axes represented almost 50 % (26.6% and 21.9% for axis 1 and 2, respectively). The first group comprising all the samples from the control condition and the sample at T0 from the enriched condition was related to none of chemical parameters or viral abundance. The sample at T2 from the enriched condition was isolated from the other clusters and dominated by the OTUs 0008 and 0014 (*Pseudoalteromonas*) and by the OTUs 0017 and 0027 (*Vibrio*). This group was positively structured by DOM and CDOM protein-like components. The third group including the samples at T9 and T22 from the enriched condition was dominated by the OTUs 0002, 0011 and 0021 belonging to the *Flavobacteriaceae* family and OTU0010 belonging to the *Roseobacter* genus. This cluster was positively related to inorganic nutrients, CDOM humic-like components and viral abundance.

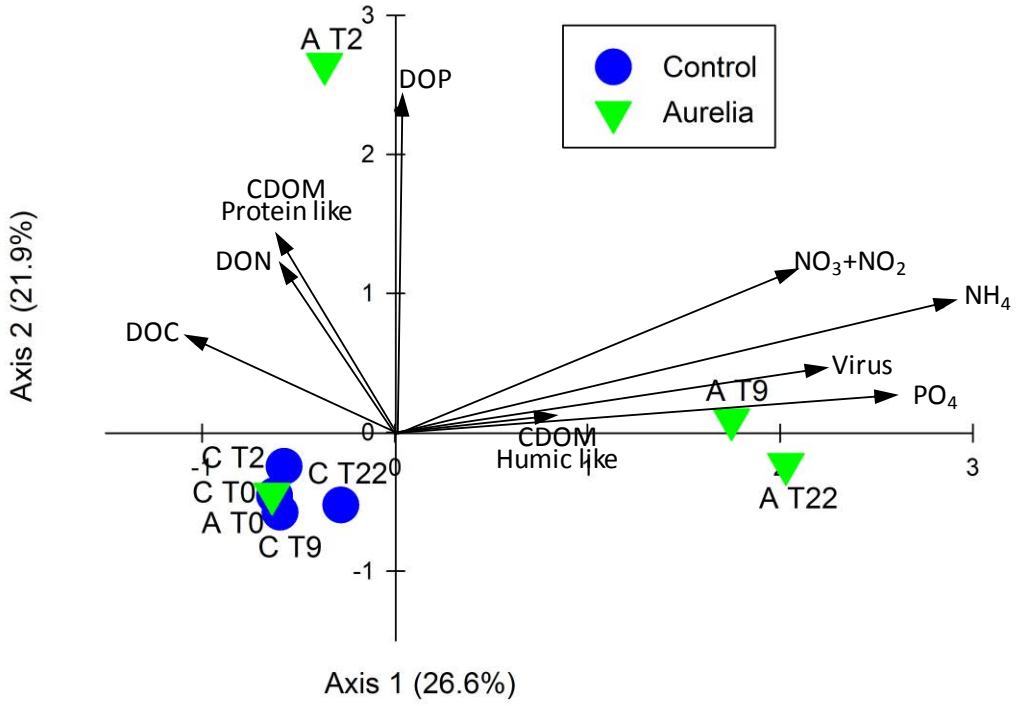

Fig. 8. Canonical correspondence analysis (CCA) of bacterial community structure (OTUs presented on Fig. 7) and chemical factors and viral abundance. The percent of explained variation is shown in brackets. The inflation factors for the analysis are given in Table S1.

4. Discussion

Processing of organic matter from the jellyfish and consequences for the system

If we estimate an average wet weight of 13 g per jellyfish (Lo and Chen, 2008) and apply the relationship between the wet weight and the organic carbon content determined by Schneider (1988) for *A. aurita*, the enrichment in DOM corresponded to the addition of the total biomass from one jellyfish in 11 L (i.e., 91 individuals m^{-3}). This value can be considered as high but not unrealistic (Lo and Chen 2008). After 22 days of incubation, the same DOC concentration was observed in the enriched and control microcosms suggesting that the bacterial community has degraded the entire DOC from *A. aurita*. This result highlights the high bioavailability of the DOM from *A. aurita* jellyfish as already reported by Tinta et al. (2012) and Purcell (2012). The excess of organic and inorganic nitrogen and phosphorus in the enriched microcosms at the end of the experiment suggests that the decrease of bacterial activities were mainly due to limitation by bioavailable carbon. In these conditions, the excess of DON and DOP after degradation of jellyfish DOM can persist in the system until a new pulse of labile organic carbon. The release of nutrients during the decomposition of organic matter from the jellyfish observed in this study and others (e.g., West et al. 2009; Tinta et al. 2010; 2012) might stimulate primary production. However, high concentrations of ammonium, associated to unionized ammonia (NH_3), can be also inhibitory or toxic for some phytoplankton, amphipods, crustacean and fish species (Ferreti and Calesso 2011; Collos and Harrison 2012).

The fact that only 14% DOC was degraded over 22 days in the control microcosm indicates that the bulk of DOC in coastal lagoons is refractory. The priming effect is a process well demonstrated in the case of soils that enhances the microbial decomposition of preexisting refractory organic matter upon addition of labile organic matter (Kuzyakov et al. 2000). During the decline of the bloom, jellyfish biomass can constitute a large reservoir of labile organic matter at the disposal of bacteria that can enhance the priming effect. Nevertheless, there was no evidence that DOM from the jellyfish helped for biodegradation of refractory DOM from the coastal lagoon (i.e., the final DOC and humic-like components concentrations in the enriched treatment were similar and higher, respectively, compared to the control treatment), suggesting that the priming effect did not occur under these experimental conditions. Recent studies on the priming effect in aquatic ecosystems gave contrasting results underlying the need for

further studies on this process (Fonte et al. 2013; Bengtsson et al. 2014; Guenet et al. 2014).

In our study, DOM from *A. aurita* increased the BGE relative to the control condition after 4 days of incubation. In contrast, Condon et al. (2011) observed that the rapid increase of bacterial metabolism in response to the addition of DOM released by a jellyfish was accompanied by a significant decline of BGE by 10% to 15% compared to the control treatment. Dinasquet et al. (2013) observed a similar BGE (30-40%) for bacterial communities exposed or not to DOM from the ctenophore *Mnemiopsis leidyi*. These contrasting observations may result from differences in experimental setup and bacterial communities' composition in these studies. Even if the BGE was stimulated with the presence of DOM from *Aurelia* in our study, its value remained relatively low (<20%) indicating that organic carbon was mainly shunted toward bacterial respiration rather than creation of new biomass. According to Condon et al. (2011), the low value of BGE induced by labile DOM from jellyfish could be explained by a inadequate organic C:N and C:P stoichiometry resulting in nongrowth energy dissipation (i.e., overflow metabolism). It must be underlined that for all these studies, the true value of the BGE remains questionable due to uncertainties in the different conversion factors used for its calculation, including the conversion factor for converting ^{3}H-thymidine uptake into bacterial carbon production (Kirschner et al. 2004) and the respiratory quotient that may change with substrate quality (Berggren et al. 2012). Proteins which were identified as the major constituent of jellyfish are characterized by a RQ of 0.8. By lowering the value of RQ for jellyfish condition to 0.8, BGE was increased by 21% in average (range: 19.5%-24.4%). However, this modification did not change the general shape of the curve and the comparison with the control condition (data not shown). Moreover, the calculation of BGE is complicated because HPP and HPR were measured at different time scales: while the time of incubation of HPP was short (i.e., 1h), HPR required sometimes 24h of incubation. According to del Giorgio et al. (2011), this methodological aspect in activities measurements can introduce a bias for the BGE determination.

Dynamics of bacterial community structure during the degradation of DOM from A. aurita

Interpretation of diversity changes during incubation in microcosms is always questionable due to a potential bottle effect (Massana et al. 2001). The bottle effect may have occurred in our experiment, as observed by the changes in the culturable bacteria fraction in the control microcosms (0.2% at T0 and 10% at T22). However, diversity as measured by pyrosequencing and DNA fingerprinting indicated that the bacterial community structure in the control microcosms did not change as drastically as in the enriched microcosms. In addition, HPP and HPR did not show significant changes over time (ANOVA test, $p>0.05$) in the control incubation. Both observations suggest that even if bottle effect cannot be ruled out, consequences on diversity and functions were minor compared to the changes caused by the increase of DOM. The differences in the bacterial community structure at T0 revealed by pyrosequencing between the control and enriched microcosms may be explained by the introduction of bacteria in the microcosms with the DOM from *Aurelia*, even if the DOM was previously filtered on 0.2 µm. The high percentage of *Actinobacteria* found in these microcosms might be facilitated by the very small size of these bacteria allowing them to escape the filtration (Ghai et al., 2013).

The pyrosequencing and the DNA fingerprint data demonstrate the rapid and profound effect of DOM addition from *Aurelia* on the bacterial diversity changes that persisted until the end of the experiment. Our results confirm the major role played by *Gammaproteobacteria* during the first steps of DOM degradation from jellyfish as observed in other studies (Condon et al. 2011, Tinta et al. 2012, Dinasquet et al. 2013). Surprisingly, the *Pseudoalteromonas* and *Vibrio* species blooming in the enriched microcosms in the first days were not found in the ~5,000 sequences identified for each sample at the start of the experiment, suggesting that they belong to the rare biosphere (i.e., less than 0.1%) (Pedrós-Alió 2012). If we hypothesize that these species represent less than 0.02% (1 sequence on 5,000) of the 2.10^6 bacteria/ml present at the start of the experiment, the theoretical bacterial growth rate for these bacteria during the three first days would be 4.32 d^{-1} (generation time = 3.8 h) to reach the concentration of 9.10^7 bacteria/ml measured in the enriched microcosms after 3 days. This bacterial growth rate is not unrealistic and is comparable to the value calculated on the basis of the culturable count. Tinta et al. (2012) also observed that culturable bacteria isolated

during the degradation of organic matter coming from different jellyfish in Adriatic Sea belonged to *Gammaproteobacteria* with a dominance of strains affiliated to *Vibrionaceae* and *Pseudoalteromonadaceae*. *Vibrio* and *Pseudoalteromonas* species can rapidly outcompete other bacterial species in a context of high concentration of organic matter due to greater metabolic versatility and the presence of multiple copies of rRNA genes (Williams et al. 2011). Changes in bacterial composition may not result only from competition for organic matter, but also from sensitivity of bacterial species for antagonistic compounds. For instance, Titelman et al. (2006) reported that extracts from the scyphomedusa *Periphylla periphylla* can inhibit some bacterial species, including *Actinobacteria*. An antimicrobial peptide, aurelin, exhibiting activity against Gram-positive and Gram-negative bacteria has been also isolated from *A. aurita* (Ovchinnikova et al. 2006). Overall, the diversity index as revealed by pyrosequencing (Table 2) remained unchanged in enriched microcosms at T2. In contrast, Tinta et al. (2012) observed a reduction in the diversity of the bacterial community during the biodegradation of *A. aurita*. This difference could be explained by the lower number of 16S rRNA clone library sequences analysed in the study of Tinta el al. (2012).

Different reasons can be evoked to explain the sudden decrease in bacterial concentration in the enriched microcosms between T3 and T4 (94% less bacteria in one day). Viral infection can induce rapid changes in bacterial abundance in aquatic environments (Berdjeb et al. 2011). However, lytic infection is associated with the release of viruses in the environment. We did not observe significant changes in viral abundance during this period, suggesting that viruses were certainly not the main factor to explain the loss of bacteria observed. Protozoan grazing can be also a cause of bacterial abundance regulation. Most of the heterotrophic nanoflagellates (HNFs) were excluded at the start of the experiment by filtering the water on 1 µm but we cannot exclude the possibility that some smaller HNFs passed through the filter and then proliferated during the experiment. Unfortunately, we did not measure HNF abundance in our experiment to confirm this hypothesis. Even if the role of HNFs cannot be excluded, the bacterial loss rate measured during this period ($3.4\ 10^6$ bacteria/ml.h) seems particularly high (i.e., two orders of magnitude higher than grazing rates values reported in the sea [Jürgens and Massana 2008]); thus grazing by HNF was certainly not the only explanation for the decreasing of bacterial cell numbers. An additional and not exclusive hypothesis, concerns the autolysis of bacteria after cessation of growth.

After the use of the most bioavailable DOM by *Gammaproteobacteria*, the bacterial community shifted towards a dominance of *Bacteroidetes* at T9. These bacteria are specialized in degrading polymeric organic matter compounds, including proteins (Fernández-Gómez et al. 2012). Interestingly, high proportion of *Bacteroidetes* have been reported in a Norwegian fjord where persistent high jellyfish biomass is observed (Riemann et al. 2006) and in association with the ctenophore *Mnemiopsis leidy* (water, tissue and gut) (Dinasquet et al. 2012). In our experiment, structural components of bacterial cells including membranes and peptidoglycan were certainly released in the microcosms during the bacterial decay of *Gammaproteobacteria* whatever the reason (protozoan grazing, viral infection or autolysis) (Nagata et al. 2003). *Bacteroidetes* have the capacities to recycle efficiently this material (Pinhassi et al. 1999; Cottrell and Kirchman 2000). Finally, we cannot exclude also the possibility that *Bacteroidetes* were favoured due to a negative selection by bacterivorous protists or viruses (Berdjeb et al. 2011). At the end of the experiment, there was an emergence of *Planctomycetes* (5% at T22). In aerobic environments, members of *Planctomycetes,* including the genera *Planctomyces* and *Rhodopirellula* identified in our study, have been reported to degrade complex organic matter into simpler compounds (Pizzetti et al. 2011).

Resilience of bacterial communities

Massive jellyfish blooms can be considered as a disturbance for the aquatic ecosystem. Stability is the general capacity of a community to return to equilibrium after perturbation, and includes components of resistance, recovery and resilience (Pimm 1984). Resistance is a community's ability to remain unchanged when challenged with disturbance. Recovery is a community's ability to return to its pre- disturbance composition or function, and resilience is the rate at which this return occurs (Shade et al. 2011). These different ecological concepts can be applied for a bacterial community exposed suddenly to the organic matter from jellyfish. Our results and others (Condon et al. 2011; Tinta et al. 2012; Dinasquet et al. 2013) showed that the resistance of bacteria is low, with rapid changes occurring in functions and structure when organic matter from a jellyfish is added (exudate or dead biomass). The determination of the recovery and the resilience needs experimental approaches sufficiently long to analyze these concepts. Most of the different studies previously performed on jellyfish biodegradation have used short-term incubations (<9 days) and ended before the complete degradation of organic carbon (dissolved or particulate) from the jellyfish

(e.g., Condon et al. 2011; Tinta et al. 2012). In our study, it was possible to follow the complete degradation of DOC during the 22 days of incubation. Over this incubation we observed the recovery of the bacterial activities (production, respiration) but not for the bacterial structure (as measured by the Bray-Curtis distance), suggesting that resilience was higher for functions than for diversity. Consequently, jellyfish blooms can induce durable changes in chemistry (release of NH_4, DON and DOP) but also in the bacterial community structure of coastal lagoons. Future investigations on jellyfish blooms might be conducted in larger experimental systems (i.e., mesocosms) to measure at longer time scale these effects on different trophic levels by including both pelagic and benthic systems. This more realistic approach will also permit to explore the role of particle-attached bacteria in the jellyfish degradation which was not considered in this study.

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Supplementary information

Table S1. Inflation factor of CCA analysis. A value close to 1 indicates no redundancy with other variables. mc: multicolinearity between variables.

Variable	Inflation factor
NO3 + NO2	mc
NH4	7.84
PO4	mc
DOC	28.81
DON	85.03
DOP	30.77
CDOM Fluo - Protein like	245.5
CDOM Fluo - Humic like	8.07
Virus	6.89

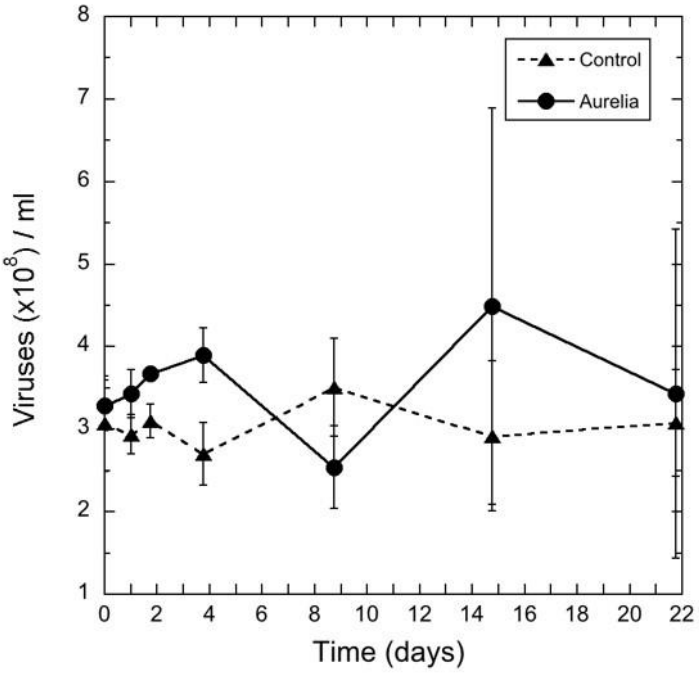

Fig. S1. Virus concentration during the incubations. Each point represents 3 replicates (mean ± standard deviation).

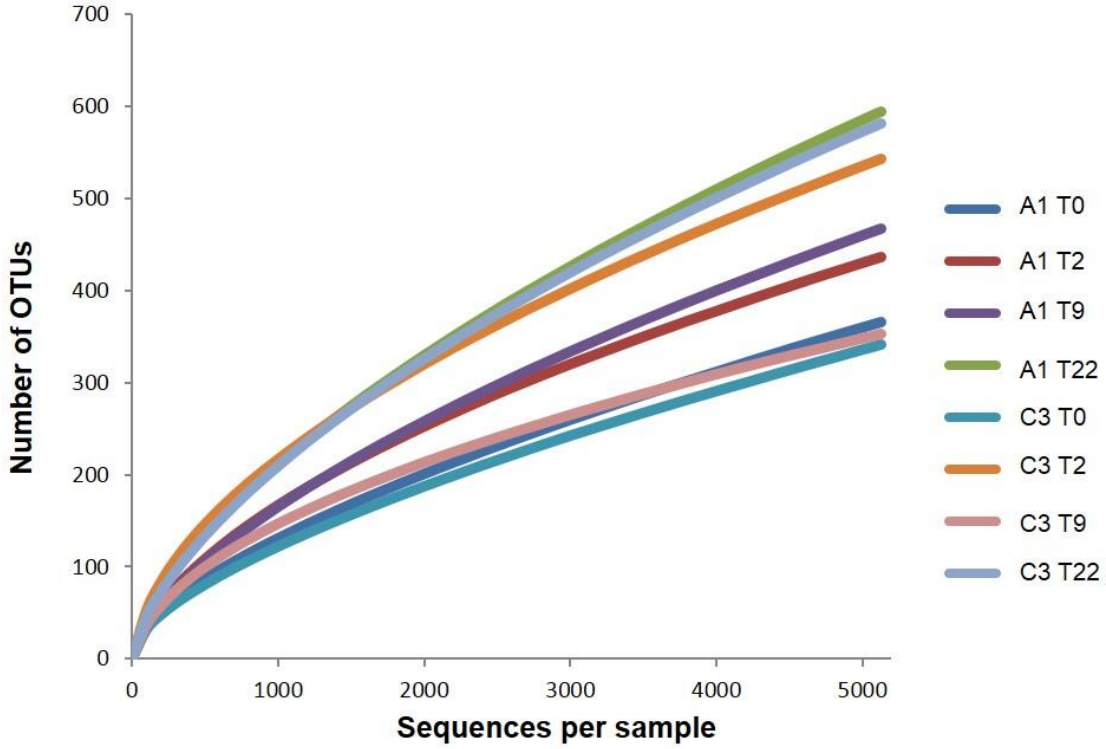

Fig. S2. Rarefaction curves of observed operational taxonomic units (OTU) based on 16S rRNA sequences retrieved from the different samples.

CHAPTER III:

Experimental studies of priming effect in coastal environments.

III Experimental studies of priming effect in coastal environments

This chapter is composed of two papers in preparation correponding to two independent experiments.

III-1 Addition of contrasted dissolved organic matter induce a decoupling between marine bacterial community activity and composition changes without evidence of priming effect

The following draft is in preparation for Aquatic Sciences

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Abstract

Heterotrophic bacterial communities in marine environment are exposed to a heterogeneous mixture of dissolved organic compounds with different bioavailability that may control both their activities and their composition. For instance, coastal environment is a mixing area where recalcitrant allochthonous organic matter from river can encounter labile organic matter from phytoplanktonic bloom. In this study, we incubated a coastal marine bacterial community during 42 days with two sources of dissolved organic matter (DOM): a mixture of amino acids (labile DOM) and a natural riverine DOM (semi labile and refractory DOM). DOM sources were added alone and in combination. Amino acids were actively degraded over 6 days whereas riverine DOM, alone or in combination with amino acids, showed only partial degradation during the incubation (~35%). Addition of amino acids alone or in combination with riverine DOM led to a similar stimulation of bacterial production (BP) and bacterial respiration (BR) compared to the controls conditions, whereas addition of riverine DOM alone did not modify bacterial community activities (BCA) compared to the controls. In contrast, bacterial community composition (BCC) analyzed by 16S rRNA gene pyrosequencing revealed a clear dissimilarity (40%) after 6 days between the controls and the conditions with the riverine DOM alone or in combination with amino acids but not with the addition of amino acids alone. Pyrosequencing data analysis showed that riverine DOM favored some OTUs (e.g., OM43 belonging to *Methylophilaceae*) to the detriment of others (e.g., SAR11 belonging to *Alphaproteobacteria*). Our results show that changes in BCA and BCC can be driven by different types of DOM and thus not necessarily coupled. Moreover, the combination of recalcitrant DOM with labile DOM did not change the microbial decomposition of semi labile and recalcitrant riverine DOM (nor the BCA) suggesting that priming effect did not occur under these experimental conditions.

Keywords: dissolved organic matter, biodegradation, heterotrophic bacteria, coastal waters, bacterial community composition, priming effect.

1. Introduction

Marine dissolved organic matter (DOM) is the largest reservoir of reduced carbon in the oceans with 662 Gt C (1 Gt = $1x10^{15}$ g) (Hansell et al. 2009) equivalent to the carbon as atmospheric CO2 or the terrestrial biota (Druffel et al. 1992, Hedges et al. 1997). Primary production at the surface of the oceans contributes to ~48.5 Gt C (Field et al. 1998). This fresh organic matter is rapidly cycled through the food web in the upper ocean and up to 50% of the primary production is recycled by heterotrophic bacteria (Cole et al. 1988). On the other hand, freshwater inputs to the oceans contribute to the load of 0.25-0.36 Gt of DOC per year (Meybeck 1993, Aitkenhead and McDowell 2000). DOC concentrations have increased in freshwaters in the past decades in response to a combination of declining acid deposition and rising temperatures with possible impacts on coastal marine ecosystems (Evans et al. 2005). Riverine organic matter is a complex and heterogeneous mix of compounds with diverse origin (terrestrial and aquatic), chemical properties and reactivity. Much of the organic matter discharged by larger rivers appears to be soil-derived, highly degraded, nitrogen-poor compounds (Hedges and Benner 1997) and characterized by a high concentration of humic substances, comprising 50-80% of total DOM in freshwater (Aiken et al. 1985). The fate of riverine organic matter in the ocean is still unclear (Hedges and Benner 1997). Based on biodegradation experiments riverine DOM appears as low bioreactive material (Søndergaard and Middelboe, 1995). In contrast, field studies showed that contribution of riverine organic matter to global burial flux seems to be low (i.e. 50% of riverine organic matter introduced to the ocean must be completely remineralized) (Hedges and Benner 1997). These contrasting results can be explained by strong seasonal and regional differences in biodegradability of riverine DOM, photo-degradation processes and flocculation with increased salinity (Hedges and Benner 1997).

Due to inorganic nutrients loading by rivers, coastal zones are productive areas (Cloern et al. 2014) accounting for 10% of global oceanic primary production (Smith and Hollibaugh 1993). For instance, one of the most productive areas of the Mediterranean Sea is the Gulf of Lion, which is influenced by large inputs from the Rhône River. On an annual basis, ~50% of the primary production in the Gulf of Lion can be attributed to continental nutrient inputs (Durrieu de Madron et al. 2011). The fluvial loading of total

organic carbon (TOC) into the Mediterranean Sea comprises 0.08–0.3% of the standing stock of TOC in the whole Mediterranean basin, which is much higher than the average reported for the World Ocean (Smith and Hollibaugh 1993), and highlights the main role played by the rivers in the Mediterranean carbon cycle (Sempéré et al. 2000). The river plume has been hypothesized to be a favorable location for priming effect because riverine recalcitrant organic matter can encounter labile organic matter released during a phytoplanktonic bloom (Bianchi 2011). The priming effect is a process where addition of labile organic matter modifies (generally increases) the microbial degradation of refractory DOM. Priming effect has been repeatedly reported in soils (Blagodatskaya and Kuzyakov 2008) but also more recently reported for aquatic ecosystems (Guenet et al. 2010). The mechanisms involved in priming effect are not well understood but may be driven by multiple factors such as chemical structure and availability of organic carbon, nutrient availability, stoichiometry and microbial composition (Fontaine and Barot 2005, Kuzyakov 2010, Guenet et al. 2010). If confirmed in marine environments, priming effect could contribute to explaining the "missing" terrestrial DOC in the oceans (Bianchi 2011).

In this study we explored the response of a coastal marine bacterial community to the addition of two sources of dissolved organic matter (DOM): a mixture of free amino acids (labile DOM) and a natural riverine DOM (semi labile and refractory DOM). Using a microcosm approach, DOM sources were added alone and in combination. A treatment without DOM addition served as a control of the experiment. Changes in bacterial community activities (BCA, by measuring bacterial production and respiration) and bacterial community composition (BCC, by measuring the diversity of 16S ribosomal RNA genes by pyrosequencing) were measured together with the degradation of DOM during 42 days. We examined the coupling between changes in BCA and BCC for the different DOM additions and we tested the hypothesis of priming effect when DOM sources were combined.

2. Material and methods

Preparation of the amino acids solution and dissolved organic matter from the Rhone River

Rhône River water was collected at the Observatory station of the Rhône river in Arles (SORA) observatory station which is located near the Compagnie Nationale du Rhône (CNR; http://www.cnr.tm.fr/fr/) gauging station using automatic samplers installed at the station (Panagiotopoulos et al. 2012). Samples (~ 40-50 L) were collected on 2nd May 2013 in 10-20 L Nalgene carboys which were previously cleaned with detergent and 2% of HCl for one week. The sampling date corresponded to a high flood event in the Rhone River (4,150 m^3 s^{-1}). Water was centrifuged at the station, transferred to the lab, and further filtered through 5 µm filters (similar molecular sieves used for phytoplankton filtering). River samples were let to stand for 7-10 days at ambient temperature to remove labile organic matter and filtered again on 0.2 µm using a peristaltic pump on a Polycap filter, which was previously cleaned with copious amounts of Milli-Q water. The collected dissolved phase was then frozen and freeze-dried. For this experiment, approximately 19.2 L of Rhône water were lyophilized resulting in a powder with a mass of 4.42 g. The organic carbon content (OC %) was about 1.68% with a C/N ratio of 3.14. At the beginning of the experiment, 996 mg of the lyophilized dissolved organic matter (DOM) was re-dissolved in 1050 mL of Milli-Q water acidified with concentrated HCl solution. The resuspended DOM was then distributed in different microcosms (see below).

A solution of twenty-one L-amino acids (Sigma, 09416-1EA) was prepared in Milli-Q water (see list of amino acids in Table S1). Each amino acid was added using the same molar concentration.

Experimental setup

Surface water sample was collected in July 2013 from a coastal station in the NW Mediterranean Sea (SOLA station, in the Bay of Banyuls-sur-Mer, France, [42°29′N, 3°08′E]. Sample was filtered by gravity on 25-µm mesh (Nytex) to remove large zooplankton and phytoplankton although low phytoplankton biomass have been reported during this period in this area (i.e., < 0.6 µg Chl *a* /L; Abboudi et al. 2008, Bertoni et al. 2011). Twelve pre-combusted (450°C, 6 h) 4L Erlenmeyer flasks were filled with filtrated seawater and supplied with a single or combined DOM sources. Three microcosms received 150-mL of the above-mentioned resuspended DOM

originated from Rhone River, corresponding to the addition of 15 µmol L^{-1} dissolved organic carbon (DOC). Three microcosms were inoculated with 100 µL of the amino acids solution, corresponding to the addition of 9 µmol L^{-1} DOC. Three microcosms received the combined addition of DOM originated from Rhône River and the amino acids solution. The last three microcosms received any addition (control microcosms). To avoid any limitation by nitrogen or phosphorus, NH_4 and PO_4 were added to all the microcosms at 16 µmol L^{-1} and 1 µmol L^{-1} final concentrations, respectively. The microcosms were closed with a cellulose stopper which permitted passive aeration and incubated in the dark at 18°C under magnetic agitation. The microcosms were periodically sampled over 42 days.

Chemical analysis

Samples (20 mL in duplicate) for nitrate (NO_3^-), nitrite (NO_2^-) and phosphate (PO_4^{3-}) were stored at -20°C and analyzed within 1 month of collection by colorimetry using a nutrient autoanalyzer (SEAL Analytical AA3HR) (Aminot and Kérouel 2007). Samples (40 mL in duplicate) for ammonium (NH_4^+) were analyzed immediately according to the Holmes (1999) with a fluorometer (Jasco). Samples (16 ml) for DOC were filtered through 2 pre-combusted (450°C, 6 h) 25-mm GF/F filters, transferred into pre-combusted glass tubes, poisoned with 85% H_3PO_4 (final pH=2), closed with Teflon lined screw caps and were stored in the dark at room temperature until analysis. DOC was analyzed using the high temperature catalytic oxidation (HTCO) technique (Cauwet 1994) using a Shimadzu TOC-V analyzer. Before starting analyses and between sets of samples, an international certified reference sample for DOC concentration (available for the international community) was analyzed to check the calibration of the analyzer and its stability over time.

Samples for dissolved organic nitrogen (DON) and phosphorus (DOP) were filtered through 2 pre-combusted 25-mm GF/F filters (Whatman). Samples were collected directly in Teflon bottles and immediately frozen (-20°C) and stored for later analyses. DON and DOP were simultaneously determined by the wet oxidation procedure (Pujo-Pay et al. 1997). DON (±0.1 µM) and DOP (±0.02 µmol L^{-1}) concentrations, were determined by sample oxidation (30 min, 120°C) corrected for NH_4^+, NO_3^-+NO_2^- and PO_4^{3-} concentrations, respectively.

Excitation-emission matrices

Samples for DOM fluorescence were filtered through 2 pre-combusted 25-mm GF/F filters (Whatman) in 100 mL combusted glass bottles and stored at -20°C until analysis. Excitation-emission matrices (EEM) were obtained using a Perkin Elmer LS55 luminescence spectrometer equipped with a xenon discharge lamp equivalent to 20 kW for an 8-µseg 0 duration. A red sensitive R928 photodiode multiplier worked as a reference detector. The running instructions for the machine were set as follows: scan speed at 250 nm min^{-1}, slit widths for the excitation and emission wavelengths at 10 nm. Measurements were performed at a constant room temperature of 20ºC in a 1 cm quartz fluorescence cell. The Ex/Em wavelengths used for single measurements were those established by (Coble 1996), thus: Ex/Em 280 nm/350 nm (peak-T) as indicator of protein-like substances, Ex/Em 320 nm/410 nm (peak-M) as indicator of marine humic-like substances, Ex/Em 340 nm/440 nm (peak-C) as an indicator of terrestrial humic-like substances and Ex/Em 250 nm/435 nm (peak-A) as an indicator of a group of humic substances with different origins.

Bacterial abundance and activities

Hereafter, the term bacteria will be used for heterotrophic prokaryotes (including Bacteria and Archaea). Bacterial abundance was determined by flow cytometry. Samples (3 mL) in cryovials were preserved with 0.2-µm-filtered formalin (2% final concentration). The samples were gently mixed and left in the dark at room temperature for 10 min before quick-freezing in liquid nitrogen and storage at -80°C. The samples were later thawed at room temperature, stained with SYBR Green I (final concentration 0.025% (v/v) of the commercial solution; Molecular Probes Inc., OR) for at least 15 min at 20°C in the dark and analysed on a flow cytometer (FACScan, Becton Dickinson, San Jose, CA) equipped with a 488 nm, 15 mW argon laser. Bacteria were detected on a plot of green fluorescence (515-545 nm) *versus* right angle light scatter (SSC), using the green fluorescence as threshold parameter. Fluorescent beads (1.0 µm; Polysciences Inc., Warrington, PA) were added to each sample as standards.

Bacterial production (BP) was measured by ^{3}H-thymidine incorporation applying the centrifugation method (Smith and Azam 1992). Samples (1 mL in triplicate) were incubated in the dark at 18°C for 1 h with 20 nmol L^{-1} [^{3}H]-thymidine (specific activity 83.2 Ci $mmole^{-1}$, Perkin Elmer) in 2-mL microtubes. Incorporations were terminated by the addition of trichloroacetic acid (TCA) to a final concentration of 5%. One killed

control was prepared for each assay by the addition of TCA, 15 min before the addition of ^{3}H-thymidine. Samples were stored for at least 1 h at 4°C and then centrifuged for 15 min at 12,000 g. The precipitate was rinsed twice with 5% TCA. The precipitates were resuspended in 1.0 mL of liquid scintillation cocktail (FilterCount, Perkin Elmer) and radioactivity determined by liquid scintillation counter (LS 5000CE Beckman). Thymidine incorporation rates were converted into carbon production using the conversion factors of 2.10^{8} cells produced by mole of thymidine incorporated and 20 fg C by cell (Ducklow and Carlson 1992).

Bacterial respiration (BR) was measured at each time point using an oxygen microelectrode (Briand et al. 2004). The microprobes (Unisense, Denmark) are designed with an exterior guard cathode, which results in extremely low oxygen consumption by the electrodes ($4.7–47 \times 10^{-7}$ µmol O_2 h^{-1}). Probes have a response time shorter than 1 s and a precision of 0.05%. Bacterial respiration was measured over 4 h to 24 h in duplicate samples for each microcosm, placed in microchambers (2 mL) and immerged in a water bath with controlled temperature (18°C). A specific measurement of the dissolved O_2 concentration was carried out a minimum of 4 times during incubation in microchambers. Bacterial respiration was deduced from the linear regression established on these points of measurement. BR were expressed in mgC m^{-3} d^{-1} using a respiratory quotient of 1 (del Giorgio and Cole 1998). We assume here that most of the respiration measured came from bacteria because the water was filtered on 1-µm at the start of the experiment. However, we cannot exclude the growth of protozoa then after, leading to an overestimation of BR. Bacterial growth efficiency (BGE, %) was calculated from the following equation BGE= BP/ (BP+BR).

Bacterial diversity by pyrosequencing

Samples (500 mL) were filtered sequentially onto 3 µm and 0.22 µm pore size polycarbonate filters (Nuclepore). The first filtration was used to eliminate eukaryotes from the sample. The 0.22 µm filters were stored at -20°C. For analysis, frozen filters were cut with sterilized scissors into small strips and vortexed briefly in 840 mL of alkaline lysis buffer (50 mmol L^{-1} Tris hydrochloride pH 8.3, 40 mmol L^{-1} EDTA and 0.75 mol L^{-1} sucrose). Cell lysis was accomplished by an initial incubation for 45 min at 37°C after adding 50 mL of freshly prepared lysozyme solution (20 mg mL^{-1}), and a second incubation at 55°C for 1 h after adding 100 mL of 10% sodium dodecyl sulfate and 10 mL of proteinase K (20 mg mL^{-1}).

Bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP) was performed using the universal bacterial primers guidelines targeting the V1 to V3 hypervariable regions of the bacterial 16S rRNA gene: 27Fmod (5′-AGRGTTTGATCMTGGCTCAG-3′) and 519r (5′-GWATTACCGCGGCKGCTG-3) as described previously (Dowd and al. 2008). Initial generation of the sequencing library was accomplished by a one-step PCR with a total of 30 cycles using the HotStarTaq Plus Master Mix Kit (Qiagen, Valencia, CA) and amplicons originating and extending from the 27Fmod primer for bacterial diversity. Tag-encoded FLX amplicon pyrosequencing analyses were completed using the Roche 454 FLX instrument with Titanium reagents, and procedures were performed at MR DNA (www.mrdnalab.com, Shallowater, TX, USA) following manufacturer's guidelines.

Sequences were processed and analyzed using the Mothur software version 1.33 (Schloss et al. 2009) with default settings excluding sequences <200bp. Sequences were denoised using PyroNoise and chimeras were removed using Chimera Slayer (Haas et al. 2011). The resulting clean sequences were clustered into operational taxonomic units (OTUs) at a 97% sequence identity level using the UCLUST algorithm (Edgar 2010) and a representative sequence from each OTU was classified using the Ribosomal Database Project (RDP) classifier (Wang et al. 2007) using the SILVA training set. Taxonomic identification of the sequence reads (tags) followed the approach by Sogin et al. (2006) and Huse et al. (2010). All samples were clustered into OTUs at a distance of 0.03 (Ghiglione and Murray 2012). All OTUs and subsequent richness and diversity analyses were performed on the randomly re-sampled datasets (5080 sequences by sample) using Mothur.

Statistical analyses

Statistical analysis of treatment effects on chemical and biological parameters were performed using a one-way analysis of variance (ANOVA) and post-hoc Tukey tests with repeated measures (i.e. microcosms). Statistical analyses were performed using XLSTAT 2014.2 software (Addinsoft) and employed an alpha level to 0.05.

Bacterial community structures were compared using ordination of Bray–Curtis similarities and used to build dendrograms by the unweighted-pair group method with arithmetic averages (UPGMA). A similarity profile test (SIMPROF, PRIMER 6) was performed on a null hypothesis that a specific sub-cluster can be recreated by permuting

the entry species and samples. The significant branch (SIMPROF, $p < 0.05$) was used as a prerequisite for defining bacterial clusters. Similarity percentage analysis (SIMPER, Clarke and Gorley 2006) was performed to identify which individual OTUs contributed most to the dissimilarity between grouped samples over-time.

3. Results

Changes in chemical parameters

The initial concentration of DOC in the control microcosms was 64.8 ± 5.5 µM (C treatment) (Fig. 1a). Microcosms were supplied with different sources of DOM, providing respectively 9.5 ± 3.0 µM DOC in the microcosms enriched in amino acids (L treatment), 15.5 ± 4 µM DOC in those enriched with riverine DOM (R treatment) and 26.7 ± 7 µM DOC in the microcosms that have received the combination of both amino acids and riverine DOM (LR treatment). After 6 days, DOC concentrations in L, R and LR condition showed a significant decrease by 15.6 µM, 5.5 µM and 15.2 µM, respectively, whereas DOC remained constant in the controls. During the second period (T6 to T42 days), R treatment is the unique condition where a significant DOC degradation is observed (-7.4 µM).

Addition of amino acids and riverine DOM led to a enrichment of DON in L and R conditions by 1.4 µM and 5.3 µM, respectively, compared to the C condition where DON was undetectable (Fig. 1b). Logically, LR condition showed a slightly higher DON concentration (6.3 µM) compared to the L and R conditions. After 6 days, an important increase of DON concentration was observed in all conditions, with similar concentrations between C and L treatments (17 µM) and between R and LR conditions (24 µM). After 42 days, the DON concentrations measured in the different treatments were closed to those measured at the start of the experiment.

The DOP concentrations measured in the different replicates of each treatment were much more variable than for DOC and DON due to low concentrations (Fig. 1c). We did not observe any significant difference between the treatments over the experiment, with the exception of a higher concentration of DOP in L treatment after 6 days compared to the other treatments.

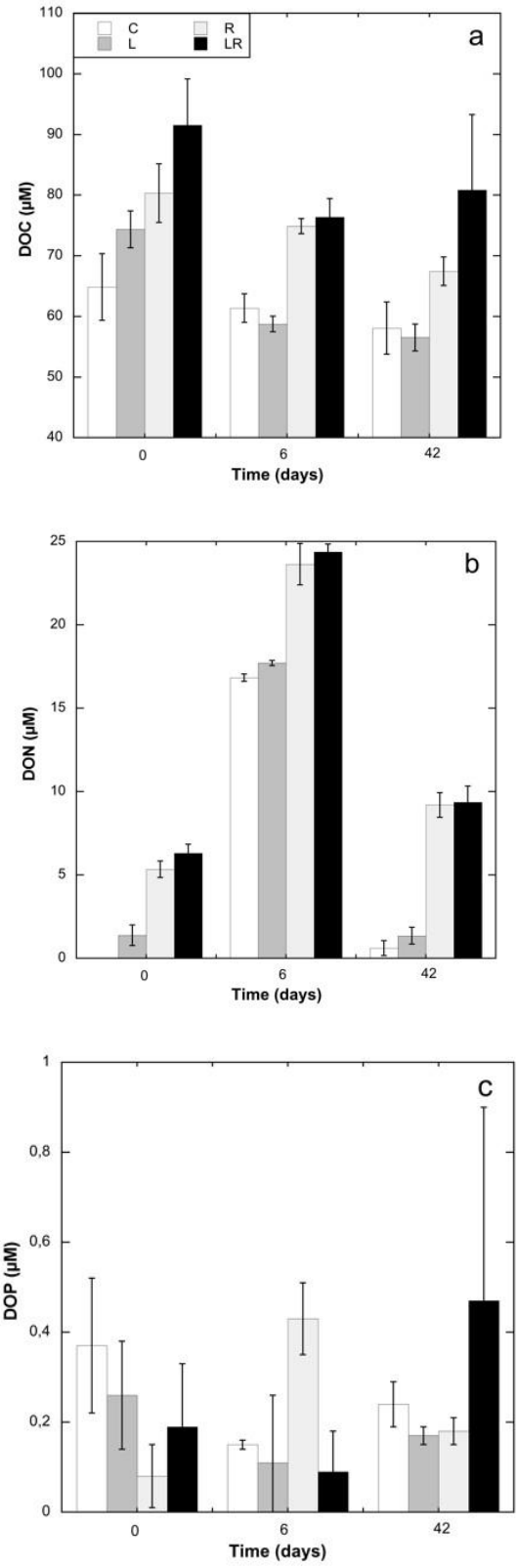

Figure 1. Changes in dissolved organic carbon (DOC, a), nitrogen (DON, b) and phosphorus (DOP, c) concentrations. Mean values ± SD of biological replicates are shown for each time point. C: control, L: addition of amino acids, R: addition of riverine DOM, L+R: addition of amino acids and riverine DOM.

The addition of riverine DOM led to an important increase in NO3- (10 µM) in treatments R and LR, whereas NO3- concentration was undetectable in conditions C and L (Fig. 2a). NO3- concentration remained high in conditions R and LR during all the experiment and a small increase was detectable in conditions C and L (3 µM) at the end of the experiment. NO2- remained undetectable at T0 and T6 for all conditions, before reaching a high and similar concentration by the end of the experiment in all conditions (17 µM) (Fig. 2b). The high concentration of NH4+ measured at T0 for all conditions (i.e., 23 µM) came from the enrichment performed (16 µM) and the natural concentration present in the coastal water (7 µM) (Fig. 2c). NH4+ was actively consumed at the same rate in all conditions and reached 5 µM at T6, and less than 0.1 µM at T42. The concentration of PO4 was similar for all conditions at T0 (1.3 µM) and this was mainly due to the enrichment (i.e., 1 µM) (Fig. 2d). PO4 concentrations decreased slightly in all conditions by the end of experiment with the exception of LR.

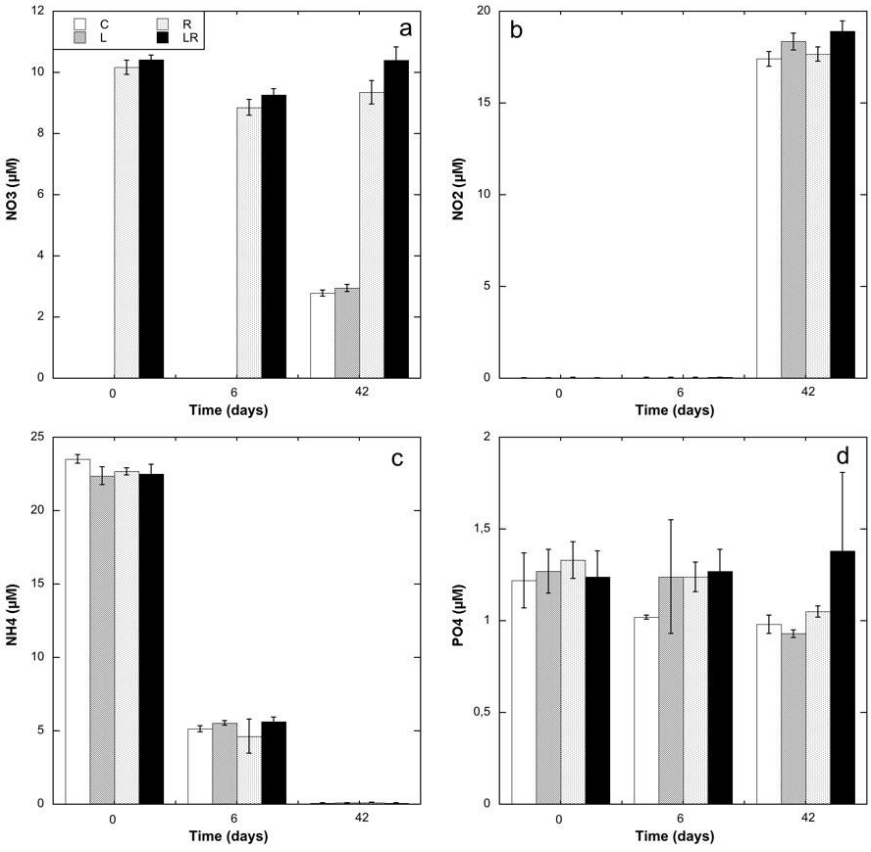

Figure 2. Changes in nitrates (a), nitrites (b), ammonium (c) and phosphates (d) during the incubation experiment. Mean values ± SD of biological replicates are shown for each time point. See Fig. 1 legend for the abbreviations C, L, R, LR.

CDOM was appreciated by its optical properties of absorbance and fluorescence. The addition of riverine DOM led to increasing of absorbance at 350 nm in treatments R and LR (0.69-0.74 m-1) compared to the treatments C and L (0.29-0.38 m-1) (data not shown). The higher concentration of CDOM in treatments R and LR was always observable at T42. Excitations-emission matrices were used to identify major fluorophore groups (Fig. S1). The addition of riverine DOM increased the humic peaks A, C and M by a factor 4 to 5 compared to the control and L treatments (Fig. 3 a,b,c). The fluorescence intensities of humic peaks remained almost constant during the experiment underlying the refractory property of these compounds. The addition of amino acids (L treatment) and riverine DOM (R treatment) contributed to increase the T peak by a factor 3.4 and 1.7, respectively, compared to the C treatment (Fig. 3d). After 6 days, the T peak decreased in L and LR treatments to reach respectively the values measured in the control and the R treatments. No additional changes were observed after 42 days.

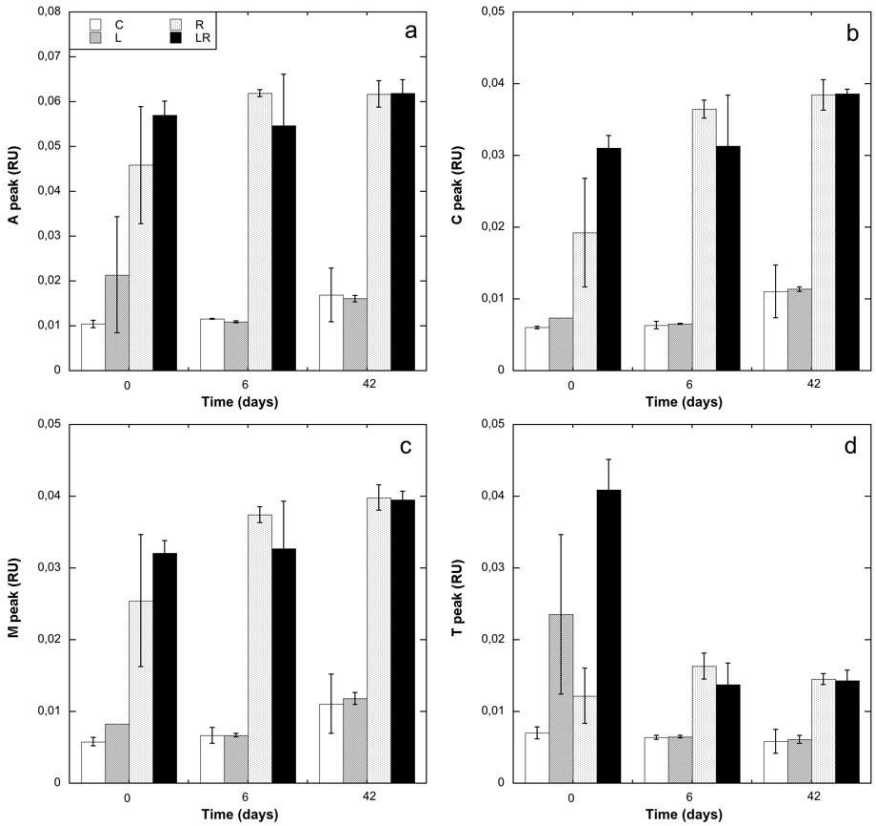

Figure 3. Changes in normalized fluorescence peaks A (UVC humic-like), C (UVA humic-like), M (UVA marine humic-like) and T (protein-like) during the incubation experiment. Mean values ± SD of biological replicates are shown for each time point. See Fig. 1 legend for the abbreviations C, L, R, LR.

Changes in bacterial abundance and metabolism

Bacterial abundance (BA) increased during the first three days in all treatments by a factor 1.7 (Fig. 4). At T2, bacterial abundance was slightly but significantly higher in L and LR treatments compared to the control and R treatments (ANOVA, $p<0.05$). BA decreased sharply in all treatments between T3 and T6 and was following by an increase at T9 in the microcosms enriched in amino acids. Then, it remained almost constant until the end of experiment and no significant differences were observed between treatments (ANOVA, $p>0.05$).

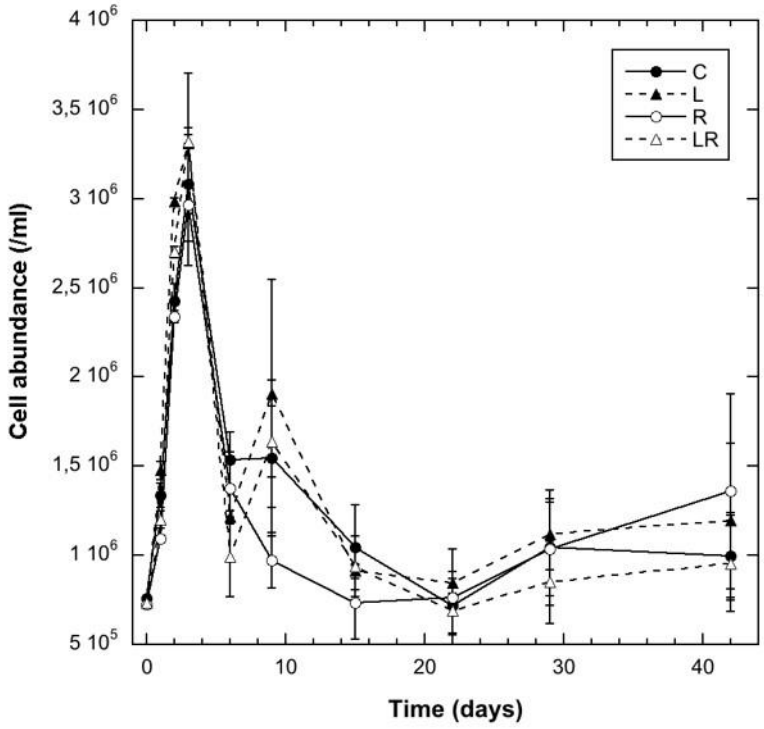

Figure 4. Changes in bacterial abundance during the incubation. Mean values ± SD of biological replicates are shown for each time point. See Fig. 1 legend for the abbreviations C, L, R, LR.

Discrete measurements of bacterial production (BP) and bacterial respiration (BR) were used to calculate the integrated values of both activities on two periods: 0-6 days (5 measurements) and 6-42 days (6 measurements) (**Fig. 5a,b**). During the first period, integrated BP and BR were similar between C and R treatments (ANOVA, $p>0.05$). In contrast, we measured similar and significantly higher integrated BP and BP in L and LR conditions (ANOVA, $p<0.05$). For those treatments, the values increased by a factor 1.5 to 1.7 compared to the values measured in the control treatment. During the second period, no significant differences were measured between the treatments for both

integrated BP and BR (ANOVA, $p>0.05$). The mean BGE was also calculated for the two periods considered (**Fig. 5c**). The values did not differ between treatments for both periods. The values ranged from 14.7% to 19.2% for the first period and from 9.2% to 14.9% for the second period. When the different treatments were combined, the mean BGE values measured during the first period appeared significantly higher than those measured during the second period.

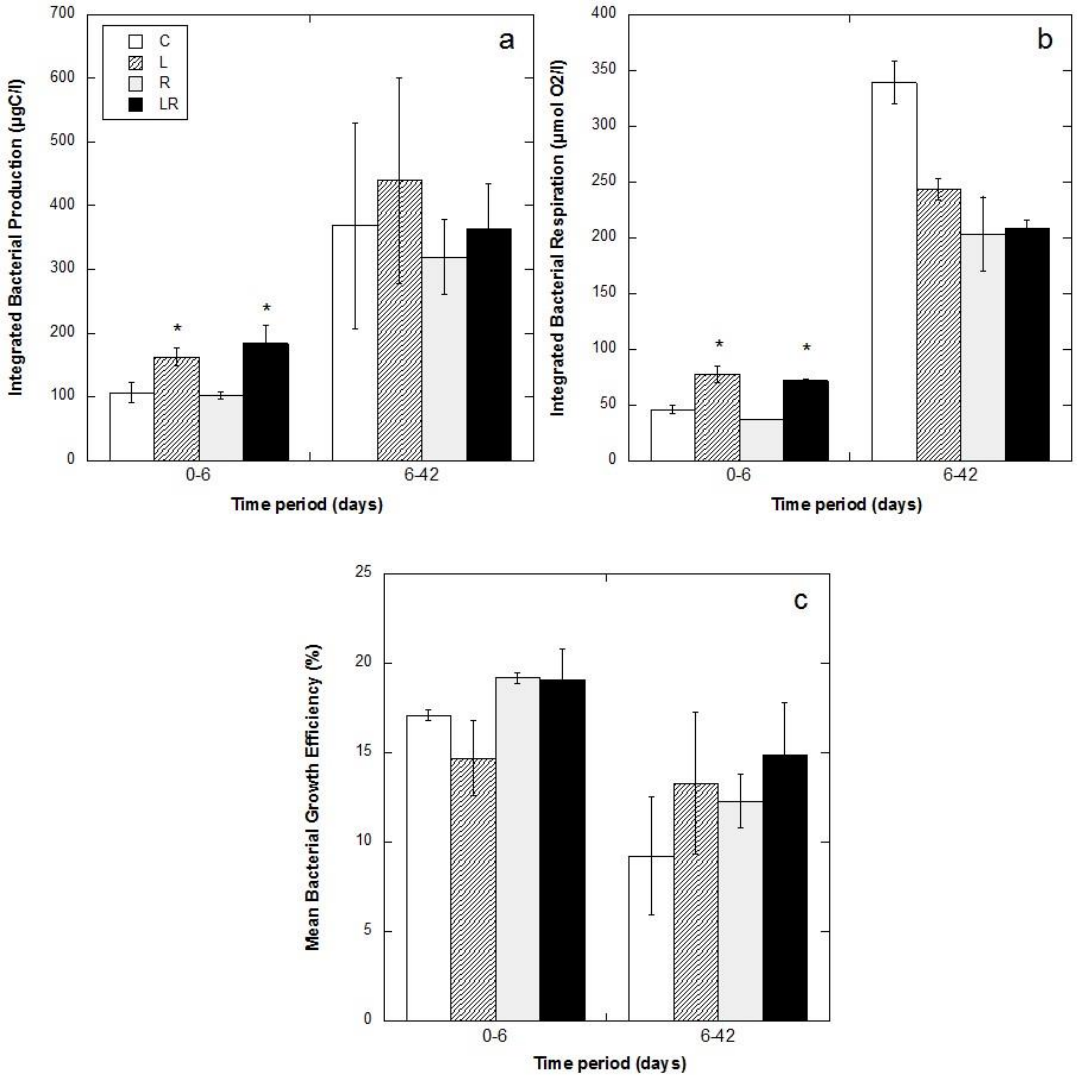

Figure 5. Changes in bacterial integrated production (a), integrated respiration (b) and bacterial growth efficiency (c) during two periods of the experiment. Mean values ± SD of biological replicates are shown for each time point. See Fig. 1 legend for the abbreviations C, L, R, LR.

Changes in bacterial diversity

Bacterial diversity was analyzed in duplicate microcosms for each treatment after 6 days of incubation (T6) and at the end of the experiment (T42). After trimming and quality check a total of 17,3163 partial 16S rRNA gene sequences were obtained, with an average of 10,822 (5,774-15,418) sequences per sample. The sequences were clustered into a total of 2,105 OTUs at >97% similarity (singletons included) and normalized to the number of sequences from the sample with the fewest sequences (i.e., 5,774).

Hierarchical clustering, based on Bray-Curtis similarities revealed a pattern in which bacterial communities were driven first by the sampling time (T0, clusters I and II) and then according to the treatments (subclusters Ia, Ib, IIa, IIb) (**Fig. 6**). Cluster I grouped the samples at T6. Within this cluster, treatments C and L (Ia) were clearly separated from treatments R and LR (Ib) (40% dissimilarity). A small but significantly difference was observed between the treatments R and LR (25% dissimilarity, $p<0.05$, SIMPROF), while treatments C and L were not significantly different ($p>0.05$, SIMPROF). After 42 days of incubation (cluster II), treatments L, R and LR grouped together and were dissimilar at 60% compared to the controls ($p<0.05$, SIMPROF).

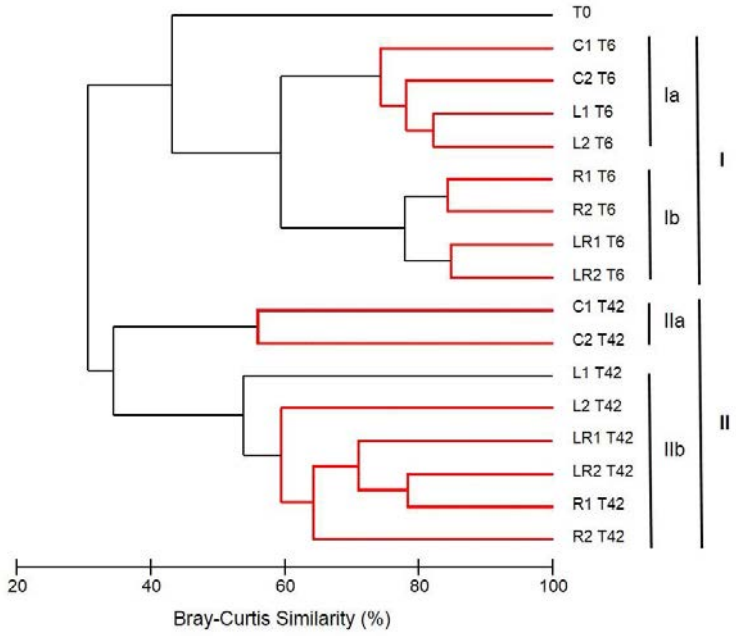

Figure 6. Unweighted pair group method with arithmetic mean (UPGMA) clustering analysis based on Bray-Curtis similarity of 16S rDNA tags. SIMPROF test has been applied to branching structure: red lines indicate red branches in which re-arrangement do not differ significantly ($p>0.05$). See Fig. 1 legend for the abbreviations C, L, R, LR.

The taxonomic richness estimated by the Chao1 index was high at the beginning of the experiment (389), decreased for all conditions at T6 (range: 257-336) and then increased at the end of the incubation (range: 341-367) (**Table 1**). At T6, the richness was significantly higher in the treatment LR compared to the other ones ($p<0.05$). At T42, the different treatments did not show any significant differences in richness ($p>0.05$). The diversity, based on the inverse Simpson index, that includes both richness and evenness, decreased between T0 and T6 for all treatments. At T6, L treatment showed a lower diversity compared to the control, while R and LR treatments showed a higher diversity. At T42, no differences in diversity were observed between the treatments.

Tab. 1 Richness and diversity indices of bacterial communities calculated on randomly picked OTUs normalized to 5774 sequences. Standards deviation between the replicates are shown in parentheses.

		Richness	Diversity
Time (days)	Treatment	Chao 1 index	Inv Simpson index
0	C, L, R, LR	389	9.95
6	C	257 (26)	5.77 (0.41)
	L	274 (5)	4.48 (0.35) *
	R	305 (40)	6.21 (0.00) **
	LR	336 (21) *	6.32 (0.05) **
42	C	341 (24)	6.78 (1.52)
	L	347 (40)	7.47 (2.16)
	R	367 (46)	10.4 (4.60)
	LR	352 (30)	6.93 (0.89)

The bacterial community composition was further investigated by comparing the relative abundance of the major phyla and proteobacterial subclasses (**Fig. 7**). At T0 the bacterial community was dominated by *Alphaproteobacteria* (51%) belonging to the clade SAR11, *Gammaproteobacteria* (29%) and *Bacteroidetes* (14%). As indicated by the clustering of samples (Fig. 6), the major differences observed at T6 were found between grouped C and L treatments and grouped R and LR treatments. For the latter group, there were more *Betaproteobacteria* (30%) and less *Alphaproteobacteria* (35%) than in the control and the L treatments (15% and 52%, respectively). At T42 the main differences were observed between the enriched microcosms and the control microcosms. A high proportion of *Actinobacteria* (28%) was detected in the control

microcosms while this phyla only accounted for less than 2% of total sequences in the enriched microcosms. Conversely, there was higher *Betaproteobacteria* in the enriched microcosms (L, R, LR) (30%) compared to the control (10%). We also observed a substantially higher proportion of *Alphaproteobacteria* in the enriched microcosms (31%) compared to the control (21%).

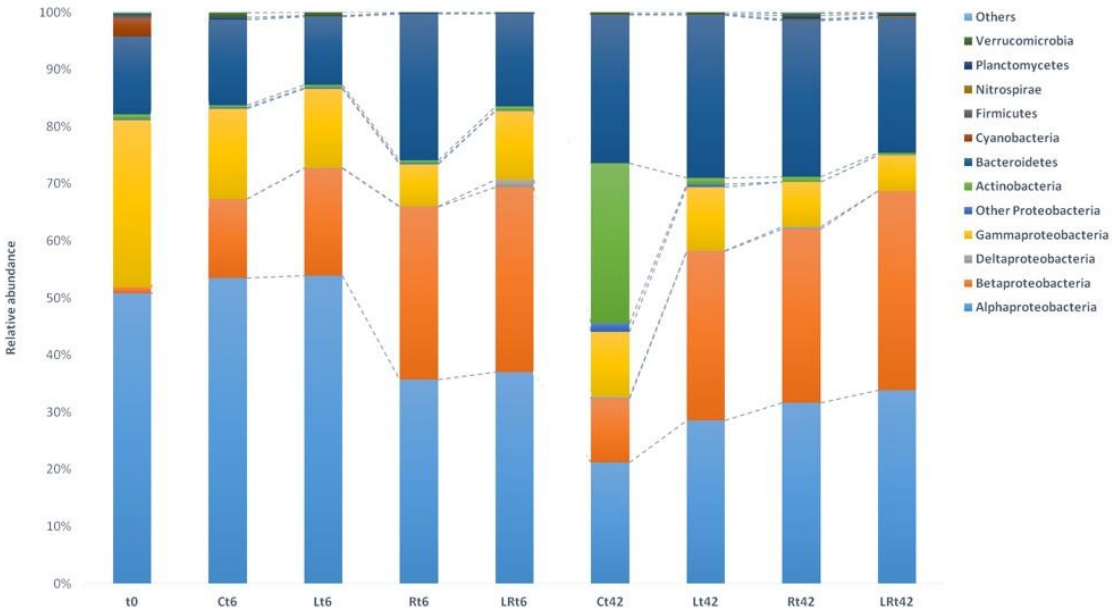

Figure 7. Relative abundance of major bacteria phyla and proteobacteria subclasses expressed as the percentage of total sequences obtained in the sample. The results shown are the average of the duplicate samples for each treatment and sampling time. See Fig. 1 legend for the abbreviations C, L, R, LR.

To explore the response of the bacterial community to the different treatments at T6, we first represented the 720 OTUs at the 3% dissimilarity identified at this sampling time on a Venn diagram (**Fig. S3**). The four treatments, including the control, shared 12.9% of these OTUs. Only 1.9% was shared by the three treatments with a DOM enrichment (i.e. L, R and LR). When the treatments were compared two-by-two, the treatments C and L shared the lowest number of OTUs (16.9%) while the treatments R and LR shared the highest number of OTUs (23.0%). Then, we investigated the OTUs that responded to the different sources of DOM addition and contributed most to the dissimilarities between two different treatments, using the similarity percentage analysis (SIMPER). The 24 OTUs explaining more of 50% of dissimilarity between treatments and their taxonomic affiliation are represented in the **Table 2**. The dissimilarity induced

by the addition of amino acids was low when C and L treatments and R and LR treatments were compared (24.7% and 22.1%, respectively). We also noticed that dissimilarity in these cases was distributed in many OTUs, each of them contributed to a low percentage in the total dissimilarity. On the contrary, the addition of riverine DOM induced a higher percentage of dissimilarity between two conditions from 38.2% to 43.8%. The changes in bacterial structure induced by the riverine DOM were explained mainly by two OTUs. The OTU SAR11 was less abundant in the treatments with riverine DOM (R=22.3%, LR =21.0%) compared to the treatments without addition (C=37.7%, L=43.7%). On the contrary, OTU OM43 belonging to *Methylophilaceae*, was more abundant after riverine DOM addition (R=30.1% , LR =31.2%) than without (C=13.7%, L=16.0%).

Tab. 2 SIMPER analysis showing the contribution and taxonomic affiliation of OTUs explaining 50% of the dissimilarity between treatment. The average abundance of OTUs is expressed as the percentage of total sequences obtained for each treatment. The percentages of dissimilarity between two conditions are indicated at the bottom of the table.

C/L			C/R			C/LR			L/R			L/LR			R/LR				
C	L	Contrib %	C	R	Contrib %	C	LR	Contrib %	L	R	Contrib %	L	LR	Contrib %	R	LR	Contrib %	OTU	
4.45	2.59	3.8	4.45	11.51	9.2	4.45	6.45	2.3	2.59	11.51	11.1	2.59	6.45	4.8	11.51	6.45	11.4	Flavobacteriaceae, Ulvibacter	
0.01	0.76	1.5																Flavobacteriaceae, Zhouia (1)	
0.01	0.61	1.2																Flavobacteriaceae, Zhouia (2)	
0.98	1.19	1.3	0.98	2.44	1.9	0.98	2.52	1.8	1.19	2.44	1.6	1.19	2.52	17,0				Flavobacteriaceae, NS5 (1)	Bacteroidetes
1.38	0.87	1.0							0.87	1.77	1.1							Flavobacteriaceae, NS5 (2)	
1.12	0.4	1.5				1.12	0.18	1.1										Flavobacteriaceae, Cryomorphaceae, Owenweeksia	
1.52	0.44	2.2				1.52	0.19	1.5							0.77	0.19	1.3	Sphingobacteriales, PHOS-HE51	
37.75	43.71	12.1	37.75	22.35	20.1	37.75	20.99	19.1	43.71	22.35	26.5	43.71	20.99	28.2	22.35	20.99	7.5	SAR11	
			0.88	0.03	1.1	0.88	0.01	1,0										SAR116	
0.23	0.87	1.3	0.23	1.71	1.9	0.23	3.56	3.8	0.87	1.71	1,0	0.87	3.56	3.3	1.71	3.56	4.2	Rhodobacteraceae, Nereida	
3.79	2.31	3.4	3.79	2.90	1.6	3.79	0.93	3.3	2.31	2.9	1.1	2.31	0.93	1.7	2.91	0.93	4.5	Rhodobacteraceae, Roseobacter, OCT	Alphaproteobacteria
4.98	2.09	5.9	4.98	3.57	1.8	4.98	1.86	3.6	2.09	3.57	1.8				3.58	1.86	3.9	Rhodobacteraceae, Roseobacter, DC5-80-3	
0.01	1.35	2.7				0.01	3.01	3.4	1.35	0.07	1.6	1.35	3.01	2.1	0.07	3.01	6.7	Rhodobacteraceae, Roseobacter, RGALL	
abs	0.81	1.6							0.81	0.02	1,0	0.81	0.01	1,0				Rhodobacteraceae, Roseobacter, TM1040	
															0.14	0.81	1.5	Rhodobacteraceae, Roseovarius	
13.7	15.98	9.9	13.7	30.06	21.4	13.7	31.92	20.8	15.98	30.06	17.5	15.98	31.92	19.8	30.06	31.92	4.2	Methylophilaceae, OM43	Betaproteobacteria
						0,00	1.07	1.2				0.01	1.07	1.3	abs	1.06	2.4	Desulfuromonadales, GR-WP33-59	Deltaproteobacteria
2.55	2.07	1.6	2.55	0.58	2.6	2.55	0.34	2.5	2.07	0.58	1.8	2.07	0.34	2.2				SAR86	
1.18	1.13	1.3	1.18	2.10	1.2				1.13	2.1	1.2				2.1	1.15	2.1	Alteromonadaceae, SAR92	
															0.44	1.1	1.5	Alteromonadaceae, OM60_NOR5	
4.55	2.67	7.4	4.55	0.55	5.2	4.55	1.98	4.2	2.67	0.55	2.6	2.67	1.98	1.6	0.55	1.98	3.2	Alteromonadaceae, Glaciecola (1)	Gammaproteobacteria
1.71	0.26	3.4	1.71	0.16	2.2	1.71	0.85	1.9							0.16	0.85	1.6	Alteromonadaceae, Glaciecola (2)	
1.03	0.42	1.2	1.03	0.13	1.2													Alteromonadaceae, Glaciecola (3)	
abs	0.54	1.1				abs	1.25	1.4							abs	1.25	2.8	Vibrionaceae, Vibrio (1)	
						abs	1.19	1.4				0.29	1.19	1.1	abs	1.19	2.7	Vibrionaceae, Vibrio (2)	
	24.7			38.2			43.8			40.3			40.3			22.1		Disimilarity (%)	

>20%
10-20%
5-10%
2-5%
<2%

4.Discussion

The aim of this study was to assess the effects of different qualities of DOM in single or combined additions on bacterial community activities (BCA) and bacterial community composition (BCC). The second objective was to explore the possibility of priming effect on the degradation of riverine DOM in presence of easily bioavailable (i.e., free amino acids). Riverine DOM was added after aging to ensure that all labile DOM was consumed. Riverine DOM was also lyophilized to avoid any changes in salinity in microcosms that would be responsible of modifications in bacterial community activities (BCA) and/or bacterial community composition (BCC) (Sjöstedt et al. 2012). A recent study showed that lyophilization had small effects on DOM composition as determined by Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) but these modifications had no effects on the BCA nor the BCC during the biodegradation compared to the addition of concentrated riverine DOM by tangential-flow ultrafiltration (Herleman et al. 2014).

No evidence of priming effect

After 42 days of incubation, a substantial part of the riverine DOC was degraded in treatment R (19% of the initial value) but no degradation was observable after 6 days underlying the semi-labile character of this DOM. In contrast, free amino acids were assimilated rapidly during the first 6 days confirming the labile character of these molecules. The combination of both DOM sources did not induce higher degradation rates of DOC, DON and DOP suggesting that priming effect did not occur in our experiment. Analysis of CDOM fluorescence did not revealed any degradation of humic substances from the riverine DOM in R and LR treatments, suggesting that these molecules are not degradable at the time scale of this experiment even with the presence of readily biodegradable DOM.

Different hypothesis can be proposed to explain the absence of priming effect in our study:

(1) We performed our experiments with the additions of PO_4 and NH_4 in order to avoid any limitation by nitrogen or phosphorus. The concentrations of phosphorus remained in excess during the experiment whereas ammonium was completely used **(Fig. 2).** However, the net release of DON at T6 and NO_3 and NO_2 at T42 suggests that nitrogen

was not limiting in the different treatments. For soil microbiologists, the stimulation of DOM degradation by the simple addition of inorganic nutrients (especially mineral N) is considered as a priming effect (Kuzyakov et al. 2000). Limitation by inorganic nutrients has been demonstrated in many marine environments (e.g., Pinhassi et al. 2006, Ortega-Retuerta et al. 2012, Sebastián and Gasol 2013). In this study, we restricted the priming effect to the observation of a stimulation in DOM degradation after the addition of labile DOM aside from N and P limitations. However, by supplying bacteria with inorganic N and P nutrients, the PE can be reduced. Indeed, when available forms of inorganic nutrients are limited, bacteria can invest in the production of extracellular enzymes to break down more refractory DOM and liberate N and P ("nutrient mining") (Kuzyalov et al. 2010).

(2) The single addition of labile DOM at the beginning of the experiment could be insufficient to trigger the PE. According to the DOC concentrations (**Fig. 1**) and the fluorescence properties of DOM (T peak, **Fig. 3**), free amino acids were completely consumed during the first 6 days. In coastal environment, labile DOM is delivered all along the phytoplankton bloom and can favor the adaptation of bacteria for degrading recalcitrant riverine DOM by fueling continuous energy. Priming effect has been previously observed after a single addition of labile DOM (e.g., glucose) in microcosms containing freshwater and soils (Guenet et al. 2014). However, in this case the concentration of labile DOM added was several magnitudes higher than concentrations observed in aquatic ecosystems.

(3) The addition of labile DOM as dissolved free amino acids (direct substrates) could be inadequate to trigger the PE. By adding bioavailable organic substrates requiring extracellular enzymes for degradation (e.g., proteins or polysaccharides), the simultaneous degradation of riverine DOM degradation would be facilitated. However, this hypothesis has not been confirmed in a recent study where proteinous organic matter from jellyfish did not favor the degradation of recalcitrant DOM from a coastal lagoon during 22 days (Blanchet et al. 2014).

(4) The presence of residual semi labile riverine DOM in our experiment could have interfered with the PE process by delaying the attack of more refractory riverine DOM, therefore making inefficient the addition of labile DOM. However, Koch et al. (2014) observed that addition of glucose (~170 µM C) in 2 years aged seawater did not promote the degradation of background DOC.

(5) The use of elemental composition of DOM (i.e., DOC, DON, DOP) and CDOM fluorescence proxies could be not sufficiently sensitive to demonstrate the occurrence of priming effect. These bulk parameters are exposed to both degradation and production processes. Different studies have demonstrated that bacteria can produce refractory DOM (Jiao et al. 2010) and fluorescent CDOM (Romera-Castillo et al. 2011). This re-working of DOM complicates the observation of priming effect (Bengtsson et al. 2014). ^{13}C or ^{15}N labeled organic molecules have been frequently used for priming effect studies in soils and aquatic to follow more precisely the degradation of recalcitrant or labile material (Bengtsson et al. 2014, Guenet et al. 2014). This approach offers a high sensitivity for tracking the degradation of specific substrates but requires the use of model molecules that do not reflect the complexity of natural DOM.

Bacterial community activities and composition are driven by different DOM qualities

Changes in microbial metabolic functions driven by changes in community composition related to nutrient availability have been reported in several occasions in aquatic ecosystems (Cottrel and Kirchman 2000). We reported here an unusual response of the bacterial community with a decoupling between the changes observed in BCA and BCC. Whereas addition of labile DOM resulted in changes in BCA but not in BCC, the addition of riverine DOM resulted in changes in BCC but not in BCA. The combination of both DOM resulted in changes in BCA identical to those observed with the labile DOM and in BCC identical to those observed with the riverine DOM.

The stimulation of BCA by amino acids was transient underlying the high lability of such molecules. Surprisingly, the bacterial growth efficiency (BGE) remained similar between the different conditions tested when higher BGE was expected after addition of labile DOM (del Giorgio and Cole, 1998). The lowering of BGE in all treatments in the second period of the experiment can be explained by the decrease of the DOM bioavailability along the experiment. In contrast to our results, Farjalla et al. (2009) observed that bacterial production and respiration were higher in the mixture of fresh and accumulated organic matter than expected by the measurements made in single substrates cultures. However, the BGE measured in the mixture was not higher in most of the cases than the value measured with the single addition of fresh DOM. This suggests that when the PE occurs, the additional DOM processed by bacteria is used as efficiently as labile DOM.

One important point of our experimental design is the addition of DOM on an undiluted natural bacterial community. Most of the biodegradation experiments are based on regrowth experiments were bacterial assemblage is diluted (e.g., 1:10) in 0.2-µm-filtered natural water. Generally, the dilution of the bacterial community induces a rapid bacterial growth during the first days of incubation and can affect the BCC (Fuchs et al. 2000). A dominance of Gammaproteobacteria is rapidly observed under these conditions due to their high growth rate their ability to exploit DOM when available (Fuchs et al. 2000, Sjöstedt et al. 2012, Herleman et al. 2014). Inversely, in our study the relative proportion of Gammaproteobacteria tended to decrease during the experiment in the controls and none OTUs overwhelmed the others (**Fig. 7**). By maintaining the natural concentrations of bacteria and grazers at the start of the experiment, we certainly limited the art factual overgrowth of some opportunistic OTUs.

Surprisingly, BCC was modified by addition of riverine DOM but not by amino acids. One consequence of the addition of riverine DOM is the lowering the abundance of SAR11 OTUs at T6 (21% in R and LR vs 38% in the control, Table 2). SAR11 is an important clade of bacteria that dominates bacterioplankton surface community (Morris et al. 2002).

The second major effect is the increase in the abundance of OM43 OTUs. OM43 is a clade of ß-proteobacteria that is commonly found in productive coastal ocean ecosystems and freshwater environments (Rappé et al. 2000). The OM43 clade is related to Type I methylotrophs of the family *Methylophilaceae* (Lidstrom, 2001). They are aerobic, obligate methylotrophs that cannot oxidize methane, but can use C1 compounds (methanol, methylamine, formate) as their source of carbon and energy (Anthony 1982, Giovanonni et al. 2008, Sowell et al. 2011). The abundance of OM43 clade is usually low in marine environment, rarely exceeding 2% of cells (Morris et al. 2006, Sowell et al. 2011). In this study we measured up to 30% OTUs belonging to OM43 in the treatments R and LR at T6 when they represented only 13% in the other treatments. The bloom of OM43 when riverine DOM was added can be explained by high concentrations of C1 compounds naturally present in the Rhone River or produced during the storage of the water sample before the lyophilization step (see Material and methods).

5. Conclusion

We observed in this study that there is no necessary a relationship between BCC and BCA changes after the addition of DOM. BCA was influenced by the addition of labile DOM (amino acids solution) while BCC was driven by the addition of recalcitrant DOM (riverine DOM). We did not observe a change of degradation of the riverine DOM upon addition of labile DOM, suggesting that priming effect did not occur in our experiments. More studies that quantitatively assess PE using stable isotope-based methods and use different type of DOM are needed to uncover its potential presence in aquatic ecosystems.

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Supplementary Information

Table S1. List of the 22 amino acids

Amino acids	MW
L-Alanine	89.09
L-Arginine hydrochloride	210.66
L-Asparagine	132.12
L-Aspartic acid	133.10
L-Cysteine	121.16
L-Cystine	240.30
L-Glutamic acid	147.13
L-Glutamine	146.15
Glycine	75.07
L-Histidine hydrochloride	191.62
L-4-Hydroxyproline	131.13
L-Isoleucine	131.17
L-Leucine	131.17
L-Lysine hydrochloride	182.65
L-Methionine	149.21
L-Phenylalanine	165.19
L-Proline	115.13
L-Serine	105.09
L-Threonine	119.12
L-Tryptophan	204.23
L-Tyrosine	181.19
L-Valine	117.15

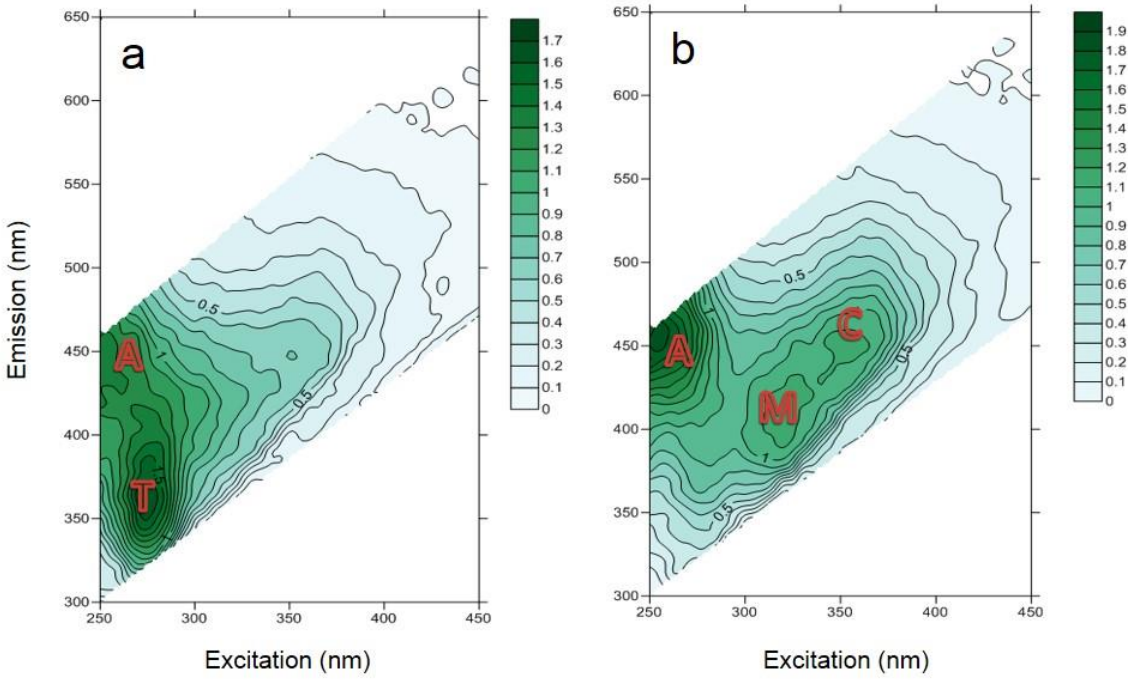

Figure S1. Examples of excitation-Emission spectra for two samples: T0 (a), T40days (b). The A, C, M and T show locations of the respective fluorescence peaks according to Coble, 1986.

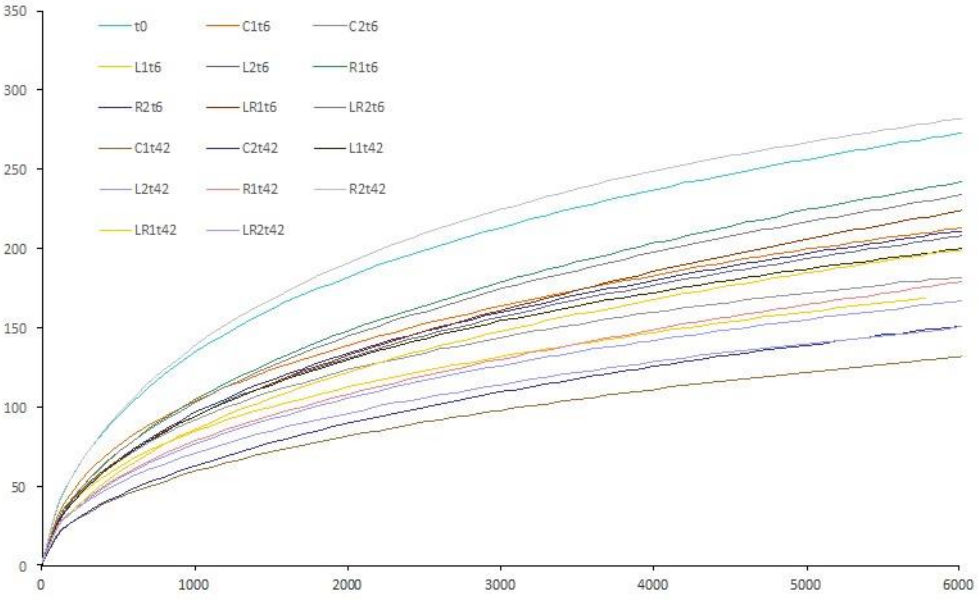

Fig. S2. Rarefaction curves of observed operational taxonomic units (OTU) based on 16S rRNA sequences retrieved from the different samples.

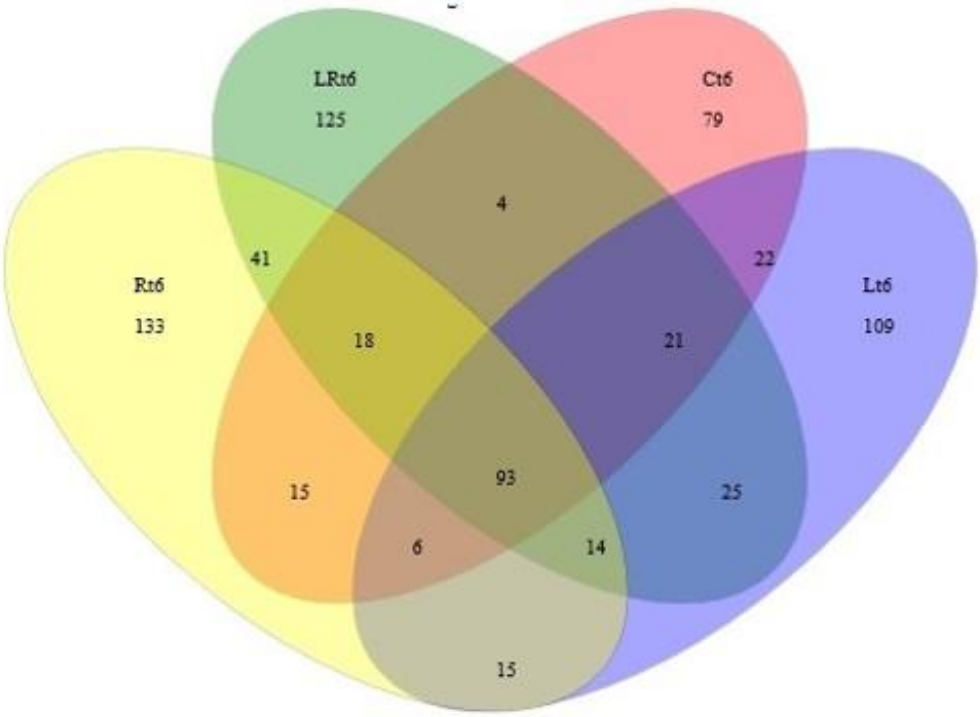

Figure S3. Venn diagram representing the shared and unique OTUs (97%) for each treatment after 6 days of incubation.

III-2 Effect of marine organic matter lability on marine microbial diversity in a Patagonian Fjord

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Abstract

A 50d experiment was setup in a Patagonian fjord (Tortel región, Chilean Patagonia) during late summer 2013. Marine samples were amended with phytoplankton-derived DOM, river-DOM or a combination of both. Concentrations of DOC decreased around 20% during the experiment, particularly during the first 25 days in single-amendment and control treatments but stayed almost unchanged in the combined DOM amendment treatment. Nevertheless, significant differences were detected in microbial community structure independently of the DOM source. Overall we did not observe evidence of positive priming effect in this study area. However, DOC data suggest a possible negative priming effect for the combined DOM source.

1. Introduction

The origin and composition of DOM in marine environments can have significant effects on its resulting biodegradation products (Obernosterer et al., 1999). As DOM composition affects its lability more than DOM size does, cycling of organic compounds in the ocean can be strongly related to ambient stoichiometric conditions (e.g. limitation) (Pete et al., 2010). Although high molecular weight DOM can be rapidly recycled in deep waters, the bulk of DOM is thought to be composed of a mixture of DOM with different labilities that nonetheless cycles very slowly (Amon and Benner, 1994).

Among the possible sources of marine DOM, phytoplankton is the most conspicuous, and among phytoplankton, diatom-derived DOM can constitute a significant fraction as they are the main contributors to global marine primary production. Riverine DOM on the other hand, represents an important source of DOC (0.25×10^{15} g C y^{-1}) and nutrients for marine waters, although it is often characterized as refractory for microbial degradation. Some of the most important factors influencing the quality of riverine DOM are land use and algal content compared to vascular plant debris. This can vary from 50% of algal production in the Mississippi river to very low rates in the Amazon River (Bianchi, 2011).

A long lasting conundrum of modern biogeochemistry is the 2 fold dichotomy between the input of terrestrial POC and the actual buried POC in marine sediments (Hedges and Keil, 1995). In terrestrial ecosystem, experiments have examined the addition of labile compounds to soils and the resulting release of carbon and nitrogen. This “priming effect” (Bingemann et al., 1953; Kuzyakov et al., 2000) has been studied in terrestrial ecosystems for more than 50 years. Priming can be “real” (whereby soil OM is decomposed) or “apparent” whereby there are changes in microbial biomass turnover but no effects on SOC decomposition.

However, aquatic studies have seldom assessed this issue (Bianchi, 2011). As there is increasing interest in unveiling the effect of DOM composition on microbial cycling and community structure, the concept of priming effect has been transposed to marine environments from terrestrial studies. A rare example of priming in marine sediments showed that addition of algal organic carbon OC induced levels of background

remineralization by as much as 31% (Turnewitsch et al., 2007; van Nugteren and al., 2009). Nevertheless, water column priming studies are scarce and only a few data sets have been generated in marine sediment.

Coastal and fjord systems are strongly influenced by riverine inputs of organic matter, whether it is of particulate (POM) or dissolved (DOM) origin. Chilean Patagonia is one of the main global reserves of freshwater and also one of the most extended fjord areas in the world. Freshwater inputs in this region are mainly governed by rivers such as Baker and Pascua, rainfall and glaciers (which can have significant influence on local oceanographic conditions (Pickard, 1971)). Chilean fjords generally show estuarine circulation and oxic conditions, in contrast with other fjord systems in the northern hemisphere (Pantoja et al., 2011). Freshwater inputs lead to brackish conditions that can oscillate between 1 and 15m in the water column and extend for more than 100 km (Calvete and Sobarzo, 2011). In spite of this massive influence, the effect of riverine waters on local oceanography and biogeochemical cycles is still poorly understood. On the other hand, external sources of carbon (and therefore of DOM) can explain the local uncoupling observed in winter between primary production and community respiration in Reloncavi channel. Moreover, prokaryotes seem to consume most of photosynthetically produced organic matter in this system in spite of small phytoplankters dominance reflected in minimal sedimentation rates. Seasonality in productivity cycles is also important in Chilean Patagonia, as the timing of phytoplankton blooms are controlled by wind patterns and light induced stratification (Montero et al., 2011).

In an attempt to link analytical chemistry, molecular microbiology and bioassays, this study aims to contribute to filling the gaps in our knowledge of OM cycling in aquatic systems by testing the priming hypothesis in the Chilean Patagonian fjord system. We explored the effect of dissolved organic matter on microbial diversity the point of view of its lability. We examined the impact of refractory and labile DOM on community structure under laboratory conditions and tested the occurrence of priming effect in a region highly influenced by river inputs.

2. Materials and Methods

Study area

Experiments were performed as part of the COPAS SurAustral observation program (www.copassuraustral.cl) in March 2013. The study area is the Tortel region in Chilean central Patagonia (47.5°S; 74°W; Fig. 1). The study area is influenced by intense fresh water input, particularly from the Baker River which has the higher record of water volume in Chile (1,133 m s^{-1}) (Pantoja et al., 2011). As most of the Patagonian rivers, the Baker River has its sources in the oriental slope of the Andes Mountain and is fed by a variety of lakes and ice fields. The Baker River follows the east side of the Northern Patagonia Ice Field, and empties into the Baker Channel (closest village Caleta Tortel, Fig. 1). The river forms a delta that divides into two major arms, only one of which can be navigated. The delta effectively filters freshwater and sediment from the continent before it reaches the salty water in the Baker Channel. Even though, its high water input influences the marine ecosystems within the fjord with an important source of allochthonous (terrestrial) and freshwater organic matter in the first meters of the water column.

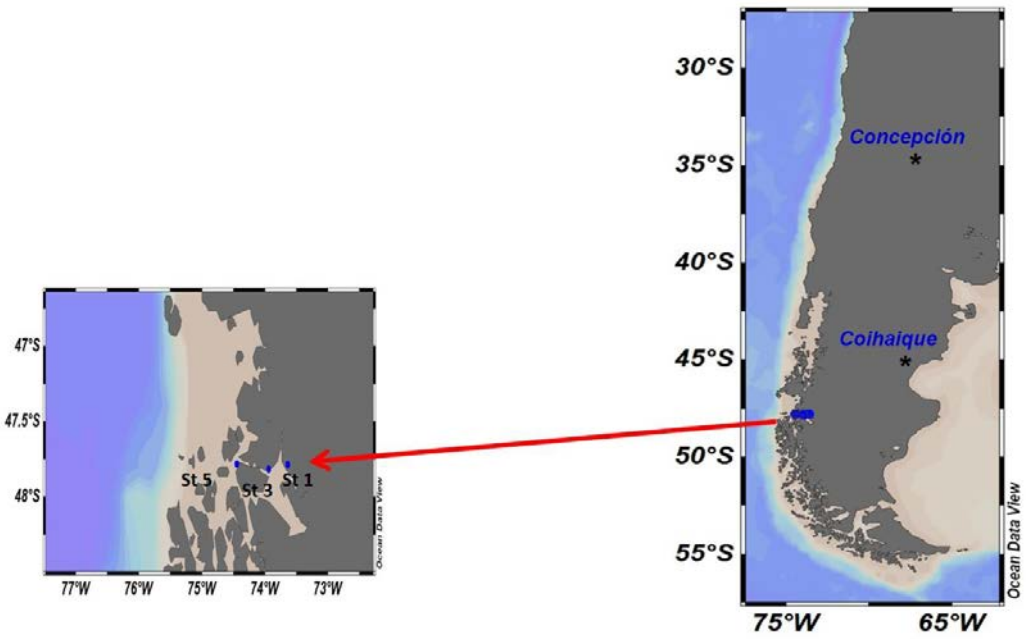

Fig. 1 Study area in central Patagonia. Station 1 close to the river mouth was used as refractory DOM amendment. Station 3 of marine characteristics was sampled for microbial natural communities.

Experimental setup

The experiment was designed to assess the effect of DOM addition on the utilization of DOC and the structure of bacterioplankton community. For doing so, we used river-DOM and DOM exudates of diatom-cultures as amendment for marine fjord water obtained in the study area. Water was retrieved from marine layers (50 L; 100 m depth) at station 3 located in the Martinez Channel, between the Baker river mouth and the Pacific Ocean (Fig. 1). At each station, a CTD cast was performed and nutrient concentrations were determined. After sampling, water for the microcosm experiment was transported at *in situ* temperature to laboratory facilities at the Center for Patagonian Research (CIEP) in Coyhaique where the incubation experiments were setup. Incubations lasted 50 days in dark, *in situ* temperature conditions.

The obtained marine water was filtered by gravity on 25-µm mesh (Nytex). A twelve microcosms system was then set up using 5L polycarbonate carboys. Each carboy was filled with 4-L of the filtrated sea water and amended with a single or combined DOM source as follows (**Table 1**): Three microcosms received 800 mL of the above-mentioned DOM originated from the River ("R"). Three microcosms were inoculated with DOM coming from the phytoplanktonic culture ("L"), corresponding to the addition of 10 µmol L^{-1} DOC. Three microcosms received the combined addition of DOM originated from River and the phytoplanktonic culture ("LR"). Control treatments were set with marine water of st 3 with no addition of DOM ("C").

The labile DOM amendment was obtained from *Skeletonema pseudocostatum* cultures available at Universidad de Concepcion. Cells were grown in Wayne medium and collected at stationary phase. A 2L volume of culture (7.2 x10^5 cell mL^{-1}) was sonicated in subfractions and then filtered through 0.2 µm in order to recover phytoplankton DOM and exudates. An aliquot of resulting DOM was added to two treatments at a final concentration of 10 µmol C L^{-1} ("L" receiving only phytoplankton DOM and "LR" receiving a mixture of river and phytoplankton DOM). This amendment of phytoplankton-derived DOM was performed twice during the experiment, at T0 and after 25 days of incubation.

The refractory DOM amendment was obtained from a freshwater sample (10 L) collected at station 1 (7 m depth), in the estuarine area of Martinez channel, at the Baker river mouth (Fig. 1). The sample was filtered through a 0.2-µm using filter capsules

(Polycap TC, Whatman). The resulting filtrate was recovered as DOM in a pre-combusted glass bottle (450°C, 6 h). An aliquot of resulting DOM was added to two treatments at a final concentration of 10 µmol L^{-1} ("R" receiving only river DOM and "LR" receiving a mixture of river and phytoplankton DOM). Incubations lasted 50 days in dark conditions, with weekly subsamplings. .

Table 1. Summary of incubation conditions per treatment during DOM degradation experiments. River addition of DOM is reported as %dilution in the sample. Phytoplankton DOM addition is reported as DOC concentration amendment.

Treatment	River DOM addition (% dilution)	Phytoplankton DOM addition (DOC µM)
Control [C]	0	0
Phytoplankton DOM [L]	0	10
River DOM [R]	20	0
Mixed DOM [LR]	20	10

Analytical procedures

Samples for nutrient determination were filtered through 0.7 μm filters (GF/F) and stored frozen in duplicate (-20°C) until laboratory analysis. Concentrations of dissolved NO_2^-, NO_3^-, and PO_4^{3-} were determined using standard colorimetric techniques (Grasshoff, 1983). The precision of NO_3^- and NO_2^-, in terms of coefficient variation, was better than ±10% and ± 3%, respectively. Samples for DOC determination were filtered through precombusted 0.7 μm filters (GF/F) and fixed with concentrated H_3PO_4 25% to pH 2. DOC was analyzed using the high temperature catalytic oxidation (HTCO) technique using a Shimadzu TOC-V analyzer (Cauwet, 1994). Samples for bacterial abundance determination were fixed with glutaraldehyde (1% final concentration) and stored frozen (-20°C). Bacteria were counted by flow cytometry after Sybr Green-I staining. High Nucleic Acid (HNA) and low Nucleic Acid (LNA) bacteria were discriminated according to their green fluorescence and counted separately. The discrimination between HNA and LNA cells has been attributed to

different phylogenetic compositions or used as a proxy for active and non-active components of the bacterial community (Gasol et al., 1999).

Bacterial community composition

Samples (500 ml) were filtered sequentially onto 3-µm and 0.22-µm pore size polycarbonate filters (Nuclepore) and stored at -20°C. For analysis, frozen filters were cut with sterilized scissors into small strips and vortexed briefly in 840 mL of alkaline lysis buffer (50 mM Tris hydrochloride pH 8.3, 40 mM EDTA and 0.75 M sucrose). Cell lysis was accomplished by an initial incubation for 45 min at 37°C after adding 50 mL of freshly prepared lysosyme solution (20 mg ml^{-1}), and a second incubation at 55°C for 1 h after adding 100 mL of 10% sodium dodecyl sulfate and 10 mL of proteinase K (20 mg ml^{-1}).

The 16S rRNA gene V1-V3 variable region PCR primers 27Fmod (5′-AGRGTTTGATCMTGGCTCAG-3′) and 16S-4(5′-GCGGCTGCTGGCACG-3′) with barcode on the forward primer were used in a 30 cycle PCR (5 cycle used on PCR products) using the HotStarTaq Plus Master Mix Kit (Qiagen, USA) under the following conditions: 94°C for 3 min, followed by 28 cycles of 94°C for 30 seconds, 53°C for 40 seconds and 72°C for 1 min, after which a final elongation step at 72°C for 5 min was performed. After amplification, PCR products are checked in 2% agarose gel to determine the success of amplification and the relative intensity of bands. Multiple samples are pooled together (e.g., 100 samples) in equal proportions based on their molecular weight and DNA concentrations. Pooled samples are purified using calibrated Ampure XP beads. Then the pooled and purified PCR product is used to prepare DNA library by following Illumina TruSeq DNA library preparation protocol. Sequencing was performed at MR DNA (www.mrdnalab.com, Shallowater, TX, USA) on a MiSeq following the manufacturer's guidelines.

Sequences were joined and processed and using the Mothur software version 1.33, February 2014 (Schloss et al., 2009) and according to the MiSeq standard operating procedure with default settings excluding sequences <150bp. Chimeras were removed using Chimera Slayer (Haas et al., 2011). The resulting clean sequences were clustered into operational taxonomic units (OTUs) at a 97% sequence identity level using the UCLUST algorithm (Edgar, 2010) and a representative sequence from each OTU was classified using the Ribosomal Database Project (RDP) classifier (Wang et al., 2007)

using the SILVA training set. Taxonomic identification of the sequence reads (tags) followed the approach by (Sogin et al., 2006) and (Huse et al., 2010). All samples were randomly resampled to the same size (11,665 sequences per sample) and was performed on operational taxonomic unit (OTU) files clustered at a distance of 0.03 (Ghiglione and Murray, 2012).

All OTU and subsequent richness and diversity analyses were performed on the randomly re-sampled datasets using Mothur. Bacterial community structures were compared using ordination of Bray–Curtis similarities and used to build dendrograms by the unweighted-pair group method with arithmetic averages (UPGMA). A similarity profile test (SIMPROF, PRIMER 6) was performed on a null hypothesis that a specific sub-cluster can be recreated by permuting the entry species and samples. The significant branch (SIMPROF, $p < 0.05$) was used as a prerequisite for defining bacterial clusters. Similarity percentage analysis (SIMPER, (Clarke and Gorley, 2006)) was performed to identify which individual OTUs contributed most to the dissimilarity between grouped samples over-time.

3. Results

Biogeochemical parameters

Initial oceanographic conditions at st 1 and 3 are reported in **Table 2.** Station 1 was representative of freshwater conditions with a low salinity value compared to marine waters. Temperature at 7 m depth was close to 9°C. Nutrient concentrations showed NO_3 + NO_2 values of 4.8 µmol L^{-1}. Station 3 on the other hand was representative of marine waters with higher salinity at 100 m depth (37.8). Temperature at that depth was 8.3°C. Nutrients were consistent in this layer, with NO_2+NO_3 concentrations close to 21 µmol L^{-1} and high NH_4 concentrations (0.48 µmol L^{-1}). DON was also high with values close to 11 µmol L^{-1}.

Table 2. Oceanographic and biogeochemical parameters at 7 m depth of st 3 (natural communities receiving DOM amendments) and st 1 (river discharge samples used as DOM amendment).

	Stations	
	1	3
Lat (°S)	47.59	47.48
Long (°W)	74.27	73.56
Max. depth (m)	3	100
T (°C)	9.0	8.3
PSU	28.1	33.8
02 (ml L-1)	59.3	37.8
NH4 (µM)	nd	0.48±0.06
NO3+NO2 (µM)	4.79±0.1	21.7±1.0
DON (µM)	nd	11.0±1.9

Microcosm incubations showed significant variability in terms of DOC concentrations (**Fig. 2**). After amendment (T0), concentrations of DOC were higher in the phytoplankton amended treatment compared to control conditions (86.5 µmol L^{-1} vs 76.5 µmol L^{-1}). However, low DOC levels in river water led to T0 concentrations below in the river amended treatments that were below control conditions (64.1 µmol L^{-1}). Because of the mixture of labile and refractory DOM, LR treatment also showed lower DOC values than control conditions. These lower values were maintained during the first 15 days of incubation. Meanwhile, treatment L showed a variation of 10 to 20% compared to control conditions (**Fig. 2b**). Oligotrophic conditions in treatment LR were maintained and represented a deficit of almost 30% of DOC compared to control conditions at T15 (**Fig. 2b**). After 25 days of incubation, DOC concentrations in the control and R treatments were low but showed only 10% of difference (52.7 and 48 µmol L^{-1} respectively). Treatments L and LR on the other hand showed similar values (64.8 and 64.2 µmol L^{-1}, respectively) which were due to a mid-incubation addition of phytoplankton DOM (see methods). Values of DOC at T50 were still influenced by the

addition of phytoplankton DOM to the L and LR treatments. While DOC concentrations remained constant in the control and R treatments compared to T25 (51.8 and 43.9 µmol L^{-1} respectively at T50), addition of Phytoplankton-DOM to L and RL resulted in higher concentrations compared to T25 (79.2 and 81.8 µmol L^{-1}). Overall, changes in DOC concentrations were stronger at T25 compared to T50.

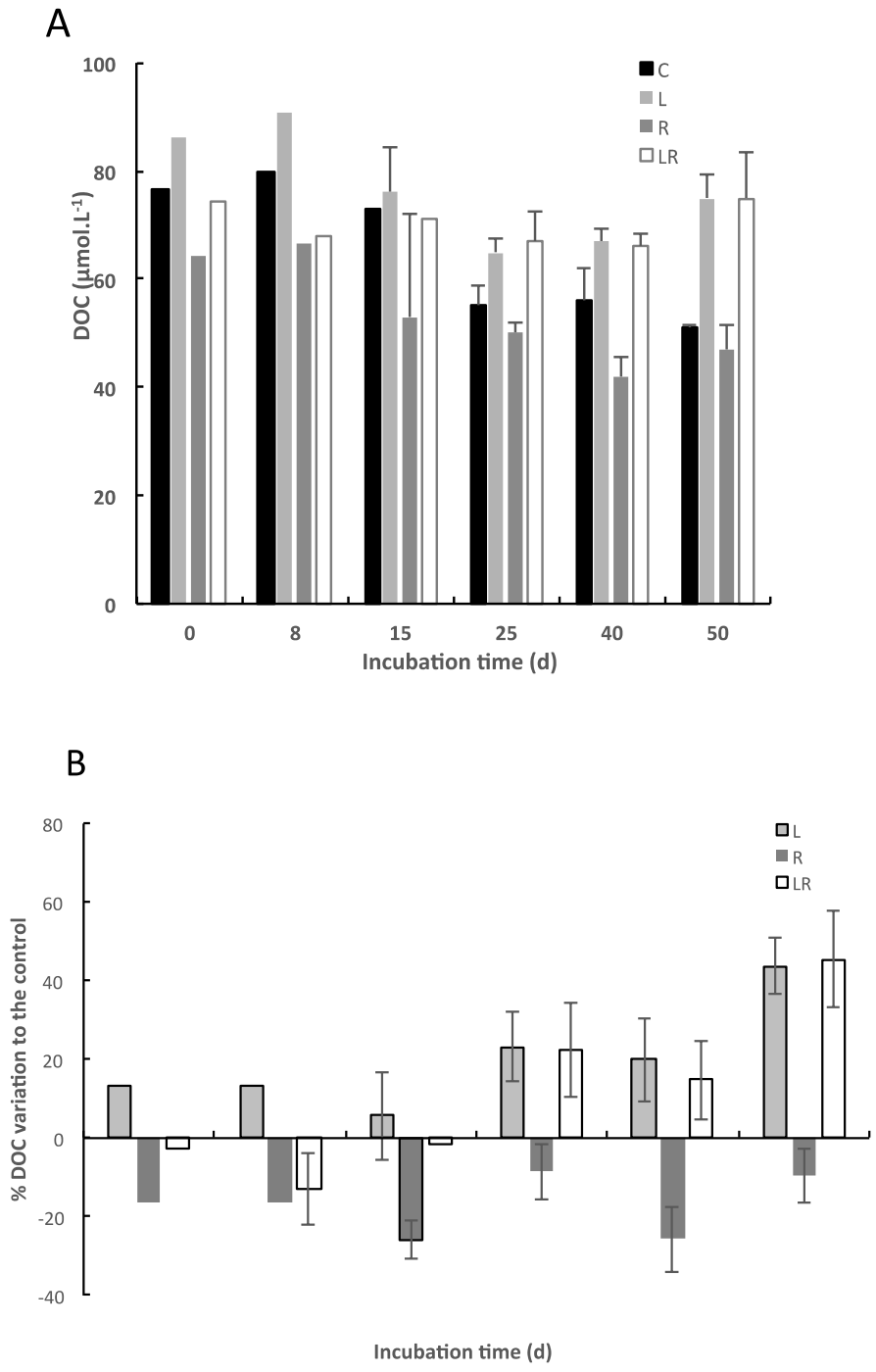

Fig. 2 Changes in dissolved organic carbon A) DOC concentrations and B) % of variability compared to control conditions. Values are reported as mean ± SD of biological replicates (n=3 for DOC at T25 and T50).

DON concentrations were generally lower in control and river treatments compared to phytoplankton amended conditions (**Fig. 3**). Values in the control treatment increased between T25 and T50 reaching 21.5 µmol L^{-1}. This was also the case for treatments L and LR, which reached 28 and 24 µmol L^{-1}, respectively at T50 although this increase is probably due to phytoplankton DOM addition after T25. The lowest DON values were observed in treatment R, with a final concentration of 12 µmol L^{-1} at T50.

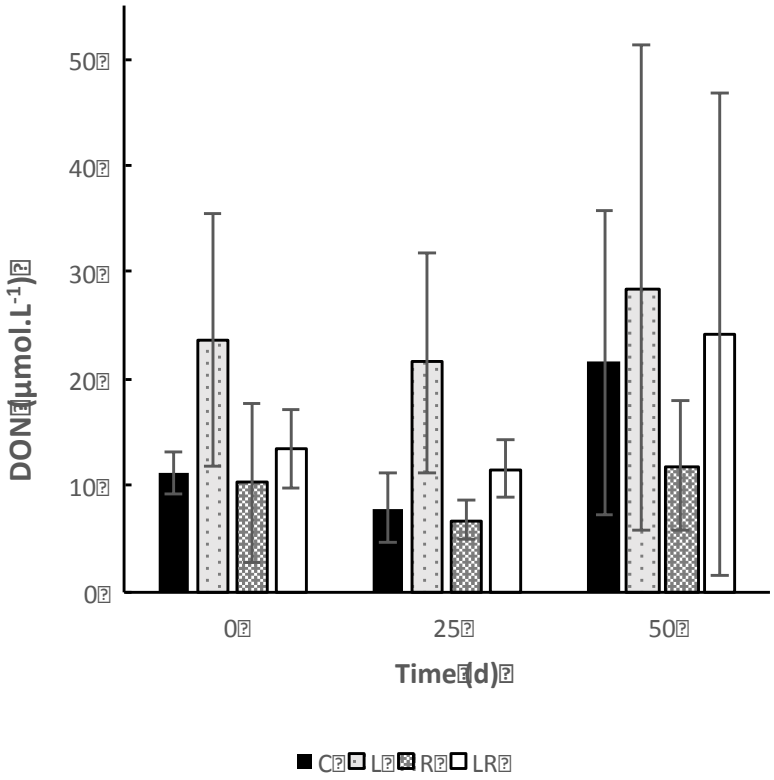

Fig. 3 Changes in concentrations of dissolved organic nitrogen (DON) during the experiment. Mean ± SD of biological replicates (n=3 for DOC at T25 and T50, n=2 for DON).

A general decreasing trend was observed in the carbon to nitrogen (C:N) ratio in organic matter (**Table 3**). Values decreased from 7 to 1.64 between T0 and T50 in the control treatment and from 10.7 to 5.7 in the R treatment. The LR treatment showed a decreasing trend going from 5.8 values at T0 to 1.8 at T50. However, no significant evolution was observed in the L treatment.

Table 3. Evolution of the average C:N ratio calculated from dissolved organic carbon and nitrogen for each treatment during the incubation

Time (days)	C:N ratio per treatment			
	C	L	R	LR
0	7.03 (1.25)	4.33 (2.41)	10.74 (4.26)	5.81 (1.66)
25	7.67 (3.10)	3.35 (1.26)	7.37 (1.26)	6.02 (2.88)
50	1.64 (2.00)	3.73 (1.92)	5.79 (1.92)	1.74 (5.91)

Microbial abundance and diversity

Bacterial abundance (**Fig. 4**) decreased in all treatments during the incubation, reaching the lowest levels in treatment R at T25 (~ $2x10^5$ cell ml^{-1}). Although the control treatment had higher concentrations at T0, values were almost identical in all treatments at T25 (oscillating between 2 and 4 $x10^5$ cell mL^{-1}). An increase was observed between T25 and T50 to roughly half of the initial abundance values (**Fig. 4a**). In general, the proportion of HNA bacteria was higher than LNA in all samples. This suggests that the variability we observed came mostly from the fraction of bacterioplankton that contained viable active cells.

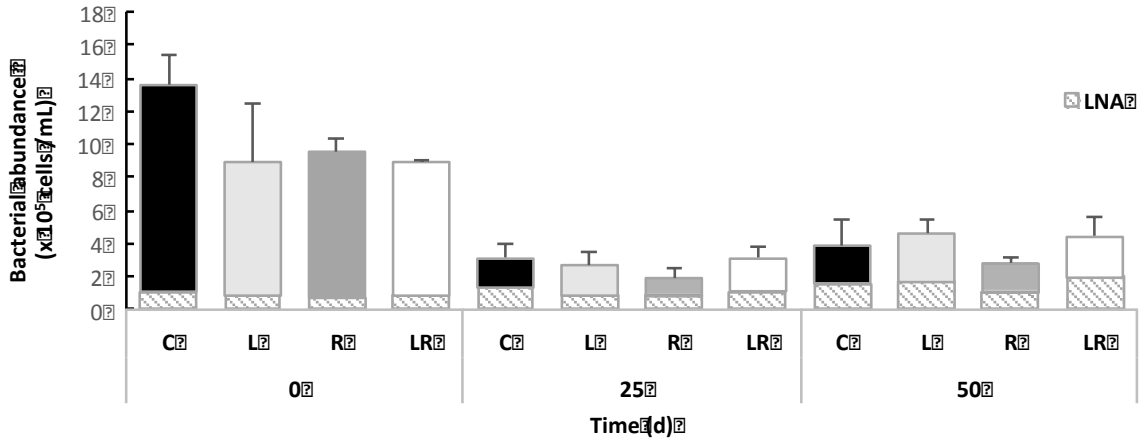

Fig. 4 Bacterial abundance and average cellular characteristics during the experiment. Values are reported as total bacterial and abundance of high nucleic acid containing (HNA) and low nucleic acid containing (LNA) abundances. Error bars represent the SD for 3 replicates.

In order to analyze possible changes in bacterial community composition, we performed Illumina Mi Seq on duplicates for each condition at different sampling times (T0, T25 and T50). After trimming and quality control, we obtained 928,042 partial 16S rRNA gene sequences, with an average of 51,557 (15,716-75,208) reads per sample. The sequences clustered into a total of 6,643 OTUs at >97% similarity (singletons included) and normalized to the number of sequences from the sample with the lowest number of sequences (15,716).

The rarefaction curves did not reach a plateau for all samples (**Fig. S1**), which suggest that our sequencing effort did not cover the entire bacterial diversity. The Chao1 index (**Table 4**), which estimates taxonomic richness, show lower values in the river DOM-influenced microcosms (L and LR). However the inverse Simpson index estimated at T25 shows higher diversity estimates in treatments containing river DOM compared to labile DOM (e.g. LR). Interestingly, T50 shows lower richness and diversity values in R and LR treatments compared to L and the control.

Table 4. Microbial richness and diversity estimates. The calculations were based on randomly picked OTUs normalized to 11,665 sequences.

			Richness	Diversity
Time (days)	Treatment	OTU number	Chao 1 index	Inv Simpson index
0	C, L, R, LR	669	956	6.32
25	C	1254	1164	24.18
	L	1464	1218	15.29
	R	1257	1005	25.66
	LR	547	1013	34.45
50	C	1620	1294	19.79
	L	1484	1168	19.46
	R	715	949	13.73
	LR	800	865	12.56

A hierarchical clustering based on Bray-Curtis similarities for T25 and T50 (**Fig. 5 a and b**) showed a strong influence of the addition of DOM on bacterial community structure. The addition of labile and refractory DOM (LR treatment) resulted in changes significantly different from community modification observed with the addition of a single DOM source regardless of its lability (**Fig. 5a**). The supplementary DOM amendment (phytoplankton exudates after T25) to L and LR treatments did not result in

a net impact on bacterial community structure ($p>0.05$, SIMPROF) and did not show significant differences compared to R and control treatments.

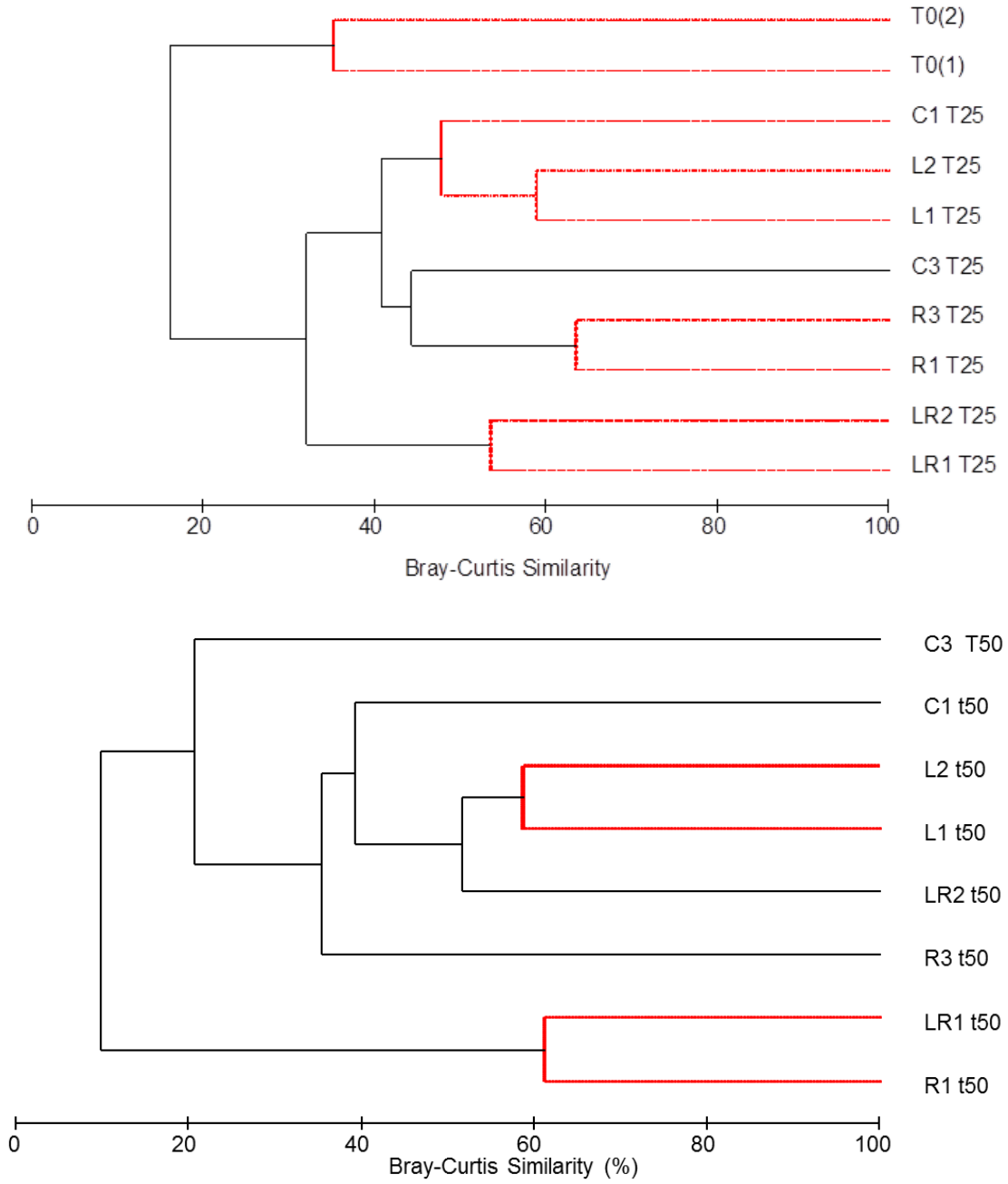

Fig. 5 Unweighted-pair group method with arithmetic mean (UPGMA) dendrogram based on Bray–Curtis similarity of DNA 16S Illumina tags at the beginning of the experiment and after 25 days (a) and 50 days (b) of incubation. Red branches do not differ significantly (SIMPROF test $p>0.05$).

The composition of the bacterial community was further investigated by comparing the relative abundance of the main phyla and proteobacterial subclasses (**Fig. 6**). As seen in **Figures 5**, all treatments showed consistent differences compared to the initial natural communities. At the beginning of the experiment (T0) the bacterial community was dominated by *Gammaproteobacteria* (74%), followed by *Alphaproteobacteria* (10.8%) and *Betaproteobacteria* (5.1%). At T25, the control treatment showed a 30% decrease of *Gammaproteobacteria* while *Alphaproteobacteria* and *Bacteroidetes* increased their abundances to 30 and 13%, respectively. This trend was maintained at T50. The L treatment showed higher abundances of *Alphaproteobacteria* (up to 48% at T50) followed by *Actinobacteria* (up to 21% at T50) compared to the control. The R treatment showed a low contribution from *Gammaproteobacteria* (14%) compared to the control but showed homogeneous abundances of the main groups identified at T25 and T50 (*Gamma*, *Betaproteobacteria* and B*acteroidetes*). The combined LR treatment showed a closer distribution of the major phyla and proteobacterial subclasses compared to the control that R and L. At the end of the experiment, bacterial community structure at LR was closer to R than L. Within *Alphaproteobacteria*, the evolution of groups at T25 showed a large contribution of SAR11 and Rhodobacterales in all treatments. At T50, we observed more *Alphaproteobacteria* in treatment L while abundances of *Rhizobiales* and *Sphingomonadales* were higher in R and LR. Concerning *Gammaproteobacteria*, the abundance of *Enterobacteriales* and *Pseudomonadales* was higher in treatment L compared to R and control for T25 and T50. Overall, less *Bacteroidetes* were observed in all phytoplankton DOM enriched microcosms.

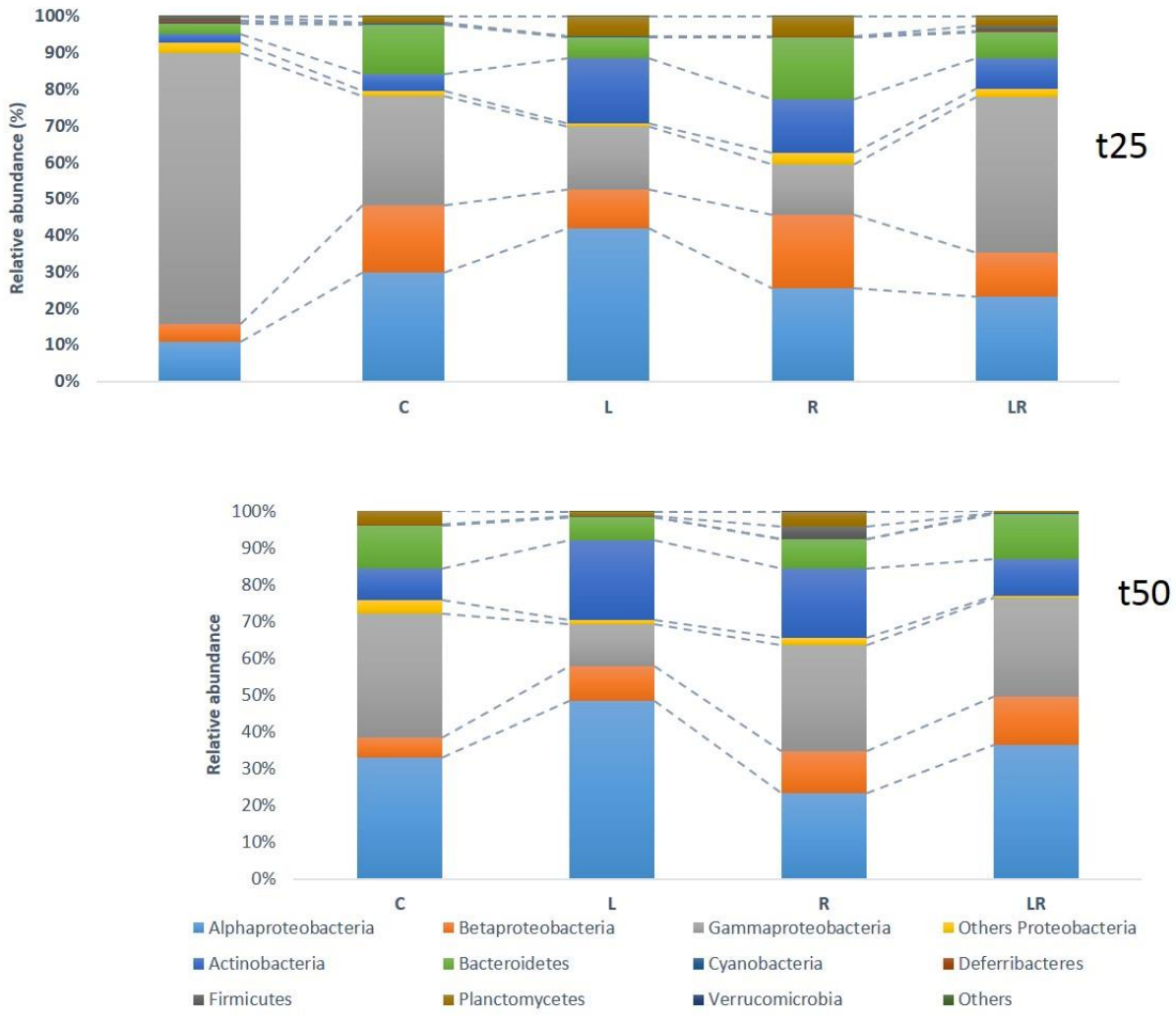

Fig. 6 Relative abundance of major bacteria phyla and Proteobacteria subclasses expressed as the percentage of total sequences obtained in the sample.

As changes induced by the single or combined addition of DOM were different over time, we investigated the OTUs that responded to the different sources of DOM addition and therefore had a higher contribution to dissimilarities between the different treatments. We used the similarity percentage analysis (SIMPER), which allows identifying the OTUs explaining more than 50% of dissimilarity between treatments (**Table S2**).

At T25, the dissimilarity between the control and the enriched microcosms was enhanced when the two sources of DOM were added in combination (68% dissimilarity), confirming the pattern observed in Figure 5. Overall, the differences observed were explained by the contribution of OTUs belonging to the subclass

Gammaproteobacteria; which had more OTUs in the LR treatment (combined addition) compared to the controls and single DOM treatments. Interestingly, the same two OTUs belonging to the family *Microbacteriaceae* explained most of the dissimilarity between the treatments L and R and the control but were not observed in the LR treatments.

At T50 the dissimilarity observed between the L treatment and the control was lower (67%) than between the control and R and LR treatments (80% dissimilarity). The dissimilarity between the treatments was in part due to the contribution of OTUs belonging to the subclass *Gammaproteobacteria*. In the R and LR treatments, the genus Pseudomonas contributed the most to the dissimilarity with the control. Here again, the same two OTUs belonging to the family *Microbacteriaceae*, observed at T25 explained most of the dissimilarity between the treatments L and R and the control but were absent in the LR treatment. This suggests that those two OTUs were stimulated by the addition of single DOM source during all the experiment.

4. Discussion

Methodological considerations

During our experiment, a significant fraction of DOC was consumed during the first 25 days of incubation. This led to a supplementary amendment of phytoplankton DOM in treatments L and LR after the T25 subsampling (equivalent to 10 µmol C L^{-1}). However, the evolution of DOC concentrations after this amendment was not significant and concentrations stayed constant until the end of the experiment.

Bacterial abundances also decreased between T0 and T25 but stayed constant between T25 and T50. However, diversity measurements showed little repeatability at T50 between treatments, with distinct groups dominating and significant temporal evolution. Caution is therefore required while interpreting results from T50, which might be influenced by the extent of the incubation period. We therefore only presented diversity dendrograms for T25 and compared them to control and initial conditions.

Although we are confident that our results represent in situ responses to realistic DOM additions, we have to acknowledge the difficulty of characterizing DOM lability in natural environments as our limited analytical capacity prevents us from fully assessing it in this study. Moreover, DOC concentrations for the combined LR treatment were relatively constant during the entire experiment. Based on this, we assume that the second DOM addition to L and LR treatments did not represent a significant

perturbation to the experiment. We also note that treatments R and LR were exposed to lower DOC and nutrient concentrations compared to control and L treatments because they received river DOM coming from the Baker River. Nutrient concentrations and C:N ratios show that the amendment of Baker river DOM led to oligotrophic conditions in terms of DOC at the beginning of the experiment for LR and R.

The organic C:N ratio values obtained during our experiments also suggest significant differences between treatments receiving single and combined DOM sources (**Table 3**). While control and R treatments showed a significant decrease in C:N between T0 and T50 (7.0±1.2 to 3.1±2.0 and 8.3± 4.2 to 4.4±1.9, respectively), treatments L and LR showed a much lower C:N value at T0 (4.4±2.4 and 5±1.6, respectively), which remained almost unchanged over time (3.8±1.9 and 5.8±5.9, respectively at T50). Although unexpected, this opens an interesting line of discussion and the possibility of observing a negative priming effect in marine environments.

DOM degradation and its effect on microbial diversity in Patagonian fjords

In this study we explored the effect of DOM (as assessed by its quality and origin) on bacterial diversity and DOM degradation. We also designed our experiments to test the priming effect hypothesis in a Patagonian Fjord with high levels of primary production. Carbon fixation in the study area was high at the time of sampling, reaching over 18 g C m^{-2} d^{-1} of primary production rates integrated over the first 20 m depth (Daneri G., unpublished data). The stoichiometric conditions of the sampled water parcel at station 3 revealed a slight limitation by phosphorous (N:P ratio 19.3 ± 10.5 µmol L^{-1}) while chlorophyll concentrations in surface waters were high, reaching 6.7 mg Chl*a* m^{-3} (Daneri G., unpublished data).

However, we did not obtain conclusive results on the occurrence of priming effect in this fjord system. Although DOC concentrations decreased in all treatments, changes observed with riverine DOM addition or phytoplankton addition were not significantly different from variations in control conditions (24 µmol L^{-1} variation in C compared to 22 and 16 in L and R, respectively).

Our observations have implications for the ongoing debate on DOM refractability. Indeed, it seems that the quality of DOM is not linked to size fraction but to its origin. It has been shown that efficient release of DON by small size phytoplankton can be

rapidly used by bacteria compared to large size phytoplankton DON (Hasegawa et al., 2000). In freshwater environments, increasing DOM concentration effectively translate into faster degradation rates. Moreover, DOP is more efficiently processed than DON and DOC. However, the main activity corresponds to the bioavailable fraction of DOM (Lonborg et al., 2009). More importantly, the common assumption of river DOC being recalcitrant to degradation has been recently challenged by studies showing high bacterial consumption of river DOC or significant lability in high latitude rivers (Guillemete and del Giorgio, 2011). Our results seem to agree with such observations, although we cannot exclude the occurrence of a negative priming effect at treatments receiving a combined DOM amendment. Indeed, DOC concentrations did not show significant variability in the LR treatment during the entire incubation time while single DOM amendment resulted in decreasing DOC concentrations over time in L and R, at least until T25.

Significant responses were also observed in community structure as a function of the origin and availability of DOM added to each microcosms. Interestingly, combined DOM sources resulted in severe modifications of microbial community dynamics as well as DOC fluxes compared to single-DOM amendments. As a result, treatments with single or combined DOM amendment showed significantly different community structures as seen in **Figures 5 and 6**.

We believe that this features can be due to the response of heterotrophic bacteria to phytoplankton DOM, which is well documented and shows increasing bacterial production, DOM cycling and cell abundances as a function of phytoplankton DOM availability {Obernosterer, 2011 ; Obernosterer, 2008 }. As DOM in marine and freshwater environments is a complex mixture of POC and DOC, it implies the use of transporter genes and specific enzymatic metabolisms. Studies focusing on DOC transporting proteins (i.e., mRNAs) have shown that carboxylic acids, polyamines, and lipids are key substrates in the biologically active pool of coastal DOC. Many bacteria have genes for DOC components commonly found in DOC such as amino acids, whereas other bacteria (e.g., *Roseobacter*, SAR11, Flavobacteriales, and γ-Proteobacteria clades) have genes used for specific components of DOC. Moreover, the role of Archaea and Bacteria in possible priming effects has been seldom studied and was not addressed during our study. However it is an issue that will need to be addressed in the future as Crenarchaeota have been shown to be less active in

assimilating amino acids and glucose, yet twice as active as bacteria in assimilating protein and diatom exudates (Kirchman et al., 2007).

The priming effect of leached DOC stimulating stable DOC consumption was not observed in this study. Overall, priming effect in aquatic ecosystems is currently restrained to freshwater systems and/or the use of extremely labile substrate such as glucose at somewhat unrealistic concentrations (Hotchkiss et al., 2014). Its occurrence in marine environment is not conclusive, as it is the role of stoichiometric conditions n modulating its occurrence.

5. Conclusions and perspectives

During our experiments, changes in DOM composition translated in significant modifications in bacterioplankton community structure. We hypothesize that specialization within the bacterioplankton community for specific DOM compounds occurs at small time frames and influences carbon turnover for the resident bacterial taxa. Also, community dynamics of bacterioplankton can be directly influenced by phytoplankton composition (as well as the presence or absence of key grazing groups (Ratti et al., 2013) which ultimately determine their capacity for degrading DOM compounds. In the case of combined river and phytoplankton DOM, it translated in DOC preservation and probably a negative priming effect, which has seldom been observed in marine environments.

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Supplementary Information

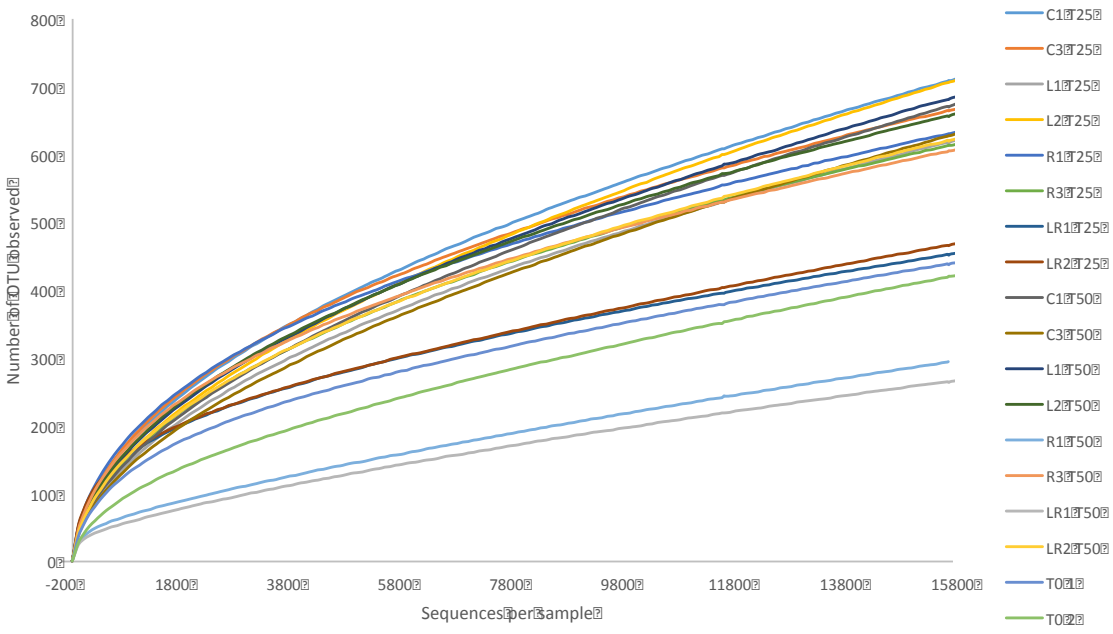

Fig. S1 Rarefaction curves of observed operational taxonomic units (OTUs) based on 16S rRNA sequences retrieved from the different samples

t 25

C/L			C/R			C/LR				
Group C	Group L	Contrib%	Group C	Group R	Contrib%	Group C	Group LR	Contrib%	OTU	
2,60	0,64	1,77	2,60	0,57	1,97	2,60	2,15	1,54	Acidimicrobineae	
0,09	10,65	8,93	0,09	1,68	1,43				Micrococcineae, Microbacteriaceae, Salinibacterium	Actinobacteria
			0,03	7,95	7,13				Micrococcineae, Microbacteriaceae	
						0,07	1,83	1,29	Propionibacteriaceae, Proponibacterium	
1,64	0,43	1,04							Flavobacteriaceae	
2,68	0,47	1,87	2,68	2,66	1,73	2,68	0,53	1,69	Flavobacteriaceae (2)	
2,43	0,56	1,59	2,43	2,41	1,32	2,43	0,95	1,17	Flavobacteriaceae(3)	Bacteroidetes
			0,49	1,74	1,13				Flavobacteriaceae(4)	
			0,03	1,03	0,90				Flavobacteriaceae(5)	
0,01	1,38	1,16							Flavobacteriaceae, NS5	
			0,04	2,31	2,04				Planctomycetaceae	
			0,47	1,49	0,92				Planctomycetaceae, Rhodospirellula (2)	Planctomycetes
0,00	1,54	1,30							Planctomycetaceae, Rhodospirellula	
			0,05	3,51	3,12				Proteobacteria	
									Hyphomicrobiaceae, Ancalomicrobium	
			0,66	2,55	1,71				Rhodobacteraceae	
2,09	7,77	4,80	2,09	3,38	1,16				Rhodobacteraceae, Roseobacter	
0,14	2,50	1,99							Rhodobacteraceae, Roseobacter (2)	Alphaproteobacteria
1,52	2,73	1,21	1,52	1,64	1,02				Rhodobacteraceae, Roseobacter (3)	
8,33	9,57	2,05	8,33	2,98	4,81	8,33	2,94	3,97	SAR11	
						0,08	2,52	1,80	Sphingomonadales	
						0,12	1,68	1,15	Alcaligenaceae, Achromobacter	
						0,08	1,55	1,08	Burkholderiaceae, Burkholderia	
						0,04	1,93	1,39	Burkholderiaceae, Ralstonia	
						0,04	1,46	1,04	Oxalobacteraceae, Herbaspirillum	Betaprteobacteria
2,24	0,38	1,58	2,24	3,03	1,68	2,24	0,67	1,35	Methylophilaceae	
1,86	0,35	1,34	1,86	2,28	1,42	1,86	0,42	1,16	Methylophilaceae, Methylophilus	
8,46	4,15	3,65	8,46	6,85	2,24	8,46	2,76	4,20	Methylophilaceae, OM43	
			0,98	0,07	0,82				Nitrosomonadaceae	
3,81	0,04	3,20	3,81	0,00	3,43	3,81	1,18	2,77	Gammaproteobacteria	
3,35	0,07	2,81	3,35	0,03	2,99	3,35	0,99	2,44	Gammaproteobacteria (2)	
2,73	0,00	2,31	2,73	0,10	2,37	2,73	0,11	1,93	Gammaproteobacteria (3)	
2,70	0,04	2,28	2,70	0,01	2,42	2,70	1,05	1,98	Gammaproteobacteria (4)	
						0,47	3,92	2,55	Alteromonadaceae, Marinobacter	
						1,20	4,08	2,12	Alteromonadaceae, Alteromonas	Gammaproteobacteria
0,16	3,63	2,93				0,16	9,44	6,83	Enterobacteriaceae(100)	
									OM182	
						0,07	2,16	1,55	Alcanivoracaceae, Alcanivorax	
3,10	0,19	2,46	3,10	0,20	2,61	3,10	0,52	2,06	Oceanospirillaceae, Balneatrix	
						0,60	6,63	4,45	Pseudomonadaceae, Pseudomonas	
59,1			55,58			67,87			Dissimilarity (%)	

>5% 2-5% <2%

CHAPTER IV:

Changes in marine bacterial community activities and composition induced by photochemical transformation of dissolved organic matter from diverse origins

IV Effect of photodegradation of contrasted dissolved organic matter sources on bacterial community activity and diversity

This chapter is composed of a draft presenting preliminary results.

Changes in marine bacterial community activities and composition induced by photochemical transformation of dissolved organic matter from diverse origins

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Keywords: dissolved organic matter, photo-oxidation, heterotrophic bacteria, coastal waters, bacterial community composition.

1. Introduction

Natural solar radiation, especially ultraviolet radiation (UV-B [280–315 nm], UV-A [315–400 nm]), has been found to induce chemical transformations of DOM with the production of a variety of photoproducts, including carbon dioxide, carbon monoxide, ammonium, phosphate, and numerous LMW organic compounds (Mopper and Kieber 2002). The light-absorbing fraction of DOM, chromophoric dissolved organic matter (CDOM), from both terrestrial and autochthonous origins, is the primary absorber of sunlight in aquatic ecosystems and plays an important role for most photochemically mediated processes in surface waters (Mopper and Kieber 2002). Although the photomineralization to inorganic carbon represents a loss of DOM potentially available to the microbial community, organic photoproducts can be re-used by bacteria. The photochemical transformations of DOM have contrasting effects on bacterial metabolism by modifying the bioavailability of DOM according to the origin and the initial chemical composition of DOM (Kieber, 2000). The observed effects of the phototransformations of DOM on bacterial growth suggest an increase of lability for initially refractory substances whereas phototransformations decrease the lability of freshly produced algal carbon (Bertilsson and Tranvik 2000). Bacterial growth and bacterial respiration are not necessary modified proportionally by the photochemical transformation of DOM inducing a shift in the bacterial growth efficiency (BGE) (see Abboudi et al. 2008).

Besides the metabolic changes induced by DOM phototransformations, different studies have reported that DOM phototransformations can induce a modification of the bacterial structure by selecting the most responsive species to the DOM photoproducts. Judd et al. (2007) firstly observed that sunlight-exposed DOM from lake and stream had a positive effect on BP and caused shifts in bacterial community composition (based on denaturating gradient gel electrophoresis of bacterial-specific 16S rDNA). Pérez and Sommaruga (2007) observed that photodegradation of DOM from different origins (lakes, algae, soil) influenced the activity and the composition of the bacterial communities (based on fluorescent in situ hybridization), with an increase in the relative contribution of *Actinobacteria* when DOM was pre-exposed to the solar radiation. Abboudi et al. (2008) shown that photochemical transformation of DOM from coastal lagoon and coastal water induced a shift in the bacterial community as revealed by DNA and RNA fingerprints. Piccini et al. (2009) observed a rapid modification of the

bacterial community composition from a coastal lagoon in response to the photodegradation of CDOM in favour of Alpha and *Betaproteobacteria*. More recently, Paul et al. (2012) demonstrated that bacterial communities of non-irradiated and UV-irradiated OM from different origins were different and that UV selected for specific members of *Alphaproteobacteria*, *Betaproteobacteria* and *Bacteriodetes*. All these observations can be initiated by the selection of bacterial species more adapted to the use of phototransformed DOM and/or less sensitive to the short-lived reactive oxygen species generated during photochemical reaction (Glaeser et al. 2010). These different observations underline the importance to determine more precisely which bacterial species are stimulated or inhibited by the DOM photodegradation according to its origin.

The objective of this study was to assess the responses of a bacterial community from coastal waters to photo-oxidation of DOM with different initial bioreactivity. Changes in bacterial abundance, production and diversity were measured in microcosms after the addition of DOM previously irradiated or not. We used for the first time in such studies the pyrosequencing to explore the changes in microbial diversity.

2. Materials and Methods

Preparation and irradiation of DOM

Two different DOM sources were used in two independent experiments.

Rhône river water (10L) was collected on 5th June 2012 at the Observatory station of the Rhône river in Arles (SORA) observatory station which is located near the Compagnie Nationale du Rhône (CNR; http://www.cnr.tm.fr/fr/) gauging station using automatic samplers installed at the station. The flow rate was 2,190 $m^3.s^{-1}$. River samples were filtered through 1.0 µm pore size filter capsule to remove large particles and then through 0.2-µm pore-size filter capsule (Polycap TC, Whatman) previously rinsed with 10% HCl and washed with Milli-Q water. Filtered samples were then distributed into two quartz bottles of 4L. All glassware was precombusted (450°C, 6 h) before use. One bottle was kept to the dark and the other was exposed to simulated sunlight (see below).

The algal extract was obtained from a monoculture of *Chaetoceros* sp. grown in f/2 medium until the stationary phase. The culture was then sonicated in subfractions and then filtered sequentially through 1-µm filter capsule and 0.2-µm and capsule filter

(Polycap TC Whatman) previously rinsed with 10% HCl and washed with Milli-Q water, in order to recover phytoplankton DOM and exudates. Filtered samples were then distributed into two quartz bottles of 4L previously precombusted (450°C, 6 h). One bottle was maintained in the dark and the other was exposed to simulated sunlight (see below).

Samples were exposed during 24 h under a solar simulator Suntest CPS+ (Atlas, GmbH) equipped with a 1-kW xenon lamp, giving an optical output irradiance of 328 W m^{-2} PAR, 43 W m^{-2} UV-A and 2.05 W m^{-2} UV-B as measured with a UV/visible spectroradiometer RAMSES (Trios, Germany). Exposure for 24 h at this solar simulator intensity corresponds to a natural dose measured during two days in summer at the sea surface in the northwestern Mediterranean region (Abboudi et al. 2008). During irradiation, the quartz tubes were maintained at 15°C by submersion in a water bath connected to a cryothermostat. Before and after sunlight exposure, subsamples were collected for DOC and CDOM.

Biodegradation experiment

Surface water samples were collected in June 2012 (Rhone experiment) and in July 2012 (*Chaetoceros* experiment) from a coastal station in the NW Mediterranean Sea (SOLA station, Bay of Banyuls-sur-mer, France [42°29'N, 3°08'E]. Samples were filtered by gravity on 25-µm mesh (Nytex) to remove large zooplankton and phytoplankton. For each experiment, nine precombusted (450°C, 6 h) glass flasks were filled with filtrated seawater and amended with DOM from irradiated or dark treatments. For the Rhone experiment, three microcosms received 800-mL (20% final volume) of irradiated DOM coming from the Rhone (UV treatments) and three microcosms were amended with 800-mL of Rhone DOM maintained in the dark (Dark treatments) corresponding in both cases to the addition of 21 µmol.L^{-1} DOC. The last three microcosms received 800-mL Milli-Q water (control treatments).

For the *Chaetoceros* experiment, three microcosms received 140-mL (3.5% final volume) of irradiated *Chaetoceros* DOM and three others with 140-mL of dark DOM treatment, corresponding to the addition of 20 µmol.L^{-1} DOC. The last three microcosms did not receive any addition (control microcosms).

All the microcosms were closed with a cellulose stopper to allow passive aeration and incubated in the dark at 18°C, under magnetic agitation during 6 to 7 days.

Analytical procedures

Samples for DOC were filtered through 2 precombusted (450°C, 6 h) 25-mm GF/F filters, transferred into precombusted glass tubes, poisoned with 85% H_3PO_4 (final pH=2), closed with Teflon lined screw caps and were stored in the dark at room temperature until analysis. DOC was analyzed using the high temperature catalytic oxidation (HTCO) technique (Cauwet 1994) using a Shimadzu TOC-V analyzer.

The absorbance and fluorescence properties of CDOM were determined on samples filtered through 2 precombusted (450°C, 6 h) 25-mm GF/F filters (Whatman). DOM spectra absorption was determined with a Hitachi U-310 spectrophotometer using a 10-cm cuvette. Absorbance was measured against Milli-Q water as blank. Absorption coefficients a_λ (m^{-1}) were calculated as $a_\lambda = 2.303\ D/L$ where D is the absorbance at the λ wavelength and L is the path length of the absorbance cell in meter.

Fluorescence was assessed on a Perkin Elmer LS55 spectrofluometer using a 1-cm quartz cuvette. Two different excitation/emission couples were used to characterize protein-like (275/340 nm) and humic-like (320/420 nm) compounds. Fluorescence intensity was calibrated using the Raman scatter peak of Milli-Q water (Lawaetz and Stedmon 2009).

Bacterial abundance was determined by flow cytometry. Samples (3 mL) were preserved with 0.2-µm-filtered formalin (2% final concentration) and stored at -80°C. The samples were stained with SYBR Green I (final concentration 0.025% (v/v) of the commercial solution; Molecular Probes Inc., OR) for at least 15 min at 20°C in the dark and analysed on a flow cytometer (FACScan, Becton Dickinson, San Jose, CA). Bacterial production (BP) was measured by ^{3}H-thymidine incorporation applying the centrifugation method (Smith and Azam 1992). Samples (1 mL in triplicate) were incubated in the dark at 18°C for 1 h with 20 nmol L^{-1} [^{3}H]-thymidine (specific activity 83.2 Ci $mmole^{-1}$, Perkin Elmer).

Bacterial community composition

Samples (500 mL) were filtered sequentially onto 3 µm and 0.22 µm pore size polycarbonate filters (Nuclepore). The 0.22 µm filters were stored at -20°C until analysis. For analysis, frozen filters were cut with sterilized scissors into small strips and vortexed briefly in 840 mL of alkaline lysis buffer (50 mmol L^{-1} Tris hydrochloride pH 8.3, 40 mmol L^{-1} EDTA and 0.75 mol L^{-1} sucrose). Cell lysis was accomplished by

an initial incubation for 45 min at 37°C after adding 50 mL of freshly prepared lysozyme solution (20 mg mL^{-1}), and a second incubation at 55°C for 1 h after adding 100 mL of 10% sodium dodecyl sulfate and 10 mL of proteinase K (20 mg mL^{-1}).

Bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP) was performed using the universal bacterial primers guidelines targeting the V1 to V3 hypervariable regions of the bacterial 16S rRNA gene: 27Fmod (5′-AGRGTTTGATCMTGGCTCAG-3′) and 519r (5′-GWATTACCGCGGCKGCTG-3) as described previously (Dowd et al. 2008). Initial generation of the sequencing library was accomplished by a one-step PCR with a total of 30 cycles using the HotStarTaq Plus Master Mix Kit (Qiagen, Valencia, CA) and amplicons originating and extending from the 27Fmod primer for bacterial diversity. Tag-encoded FLX amplicon pyrosequencing analyses were completed using the Roche 454 FLX instrument with Titanium reagents, and procedures were performed at MR DNA (www.mrdnalab.com, Shallowater, TX, USA) following manufacturer's guidelines.

Sequences were processed and analyzed using the Mothur software version 1.33 following the 454 standard operation procedure (Schloss et al. 2009) with default settings. Sequences were denoised using PyroNoise and chimeras were removed using Chimera Slayer (Haas et al. 2011). The resulting clean sequences were clustered into operational taxonomic units (OTUs) at a 97% sequence identity level using the UCLUST algorithm (Edgar 2010) and a representative sequence from each OTU was classified using the Ribosomal Database Project (RDP) classifier (Wang et al. 2007) using the SILVA training set. Taxonomic identification of the sequence reads (tags) followed the approach by Sogin et al. (2006) and Huse et al. (2010) All samples were clustered into OTUs at a distance of 0.03 (Ghiglione and Murray 2012). All OTUs and subsequent richness and diversity analyses were performed on the randomly re-sampled datasets (2,462 sequences by sample for the Rhone experiment and 1,206 for the *Chaetoceros* experiment) using Mothur.

3. Results and discussion

Photochemical transformations of DOM

Two different types of DOM were exposed under a solar simulator: a riverine DOM coming from the Rhone river and a phytoplankton-derived DOM coming from a

monoculture of *Chaetoceros sp*. The initial DOC concentrations were 137 µmol.L^{-1} for the riverine DOM and 800 µmol.L^{-1} for the phytoplankton-derived DOM. During the exposition under the solar simulator we observed a decrease in DOC concentration of the phytoplankton-derived DOM of 16% while there was no changes for the riverine DOM. This photomineralization showed than ther phytoplankton-derived DOM is more photolabile than the riverine DOM (data not shown).

The absorption spectra of riverine DOM (**Fig. 1a**) and phytoplanktonic DOM (**Fig. 1b**) showed the presence of dissolved organic compounds absorbing in UV-B (280-315 nm) and to a lesser extent in UV-A (315-400 nm). Protein-like and humic-like components of DOM were characterized by their fluorescence properties (**Fig. 1c, d**). The value of protein-like components measured at the initial time was more important for the phytoplankton derived DOM (0.55 ru) (**Fig. 1d**) than for the riverine DOM (0.05 ru) (**Fig. 1b**). On the contrary, there were more humic-like compounds in the riverine DOM (0.25 ru) than in the phytoplankton DOM (0.18 ru), underlying their different composition and bioavailability properties. After 24 h of irradiation, a photobleaching occurred in both types of DOM as shown by the decrease in absorption coefficients (especially in UV-B) and fluorescence, while there was no significant effect after dark treatment (**Fig. 1**). Irradiation induced a loss of 50% and 71% in absorbance at 350 nm for riverine DOM and phytoplankton-derived DOM, respectively. Concerning the fluorescent CDOM, the observed decrease was more important for the phytoplankton DOM (62% for the CDOM Fluo-Humic like and 88% for the CDOM Fluo-Protein like) than for the riverine DOM (29% and 40%, respectively) showing than fluorescent CDOM from the phytoplankton DOM was more photolabile than the riverine DOM.

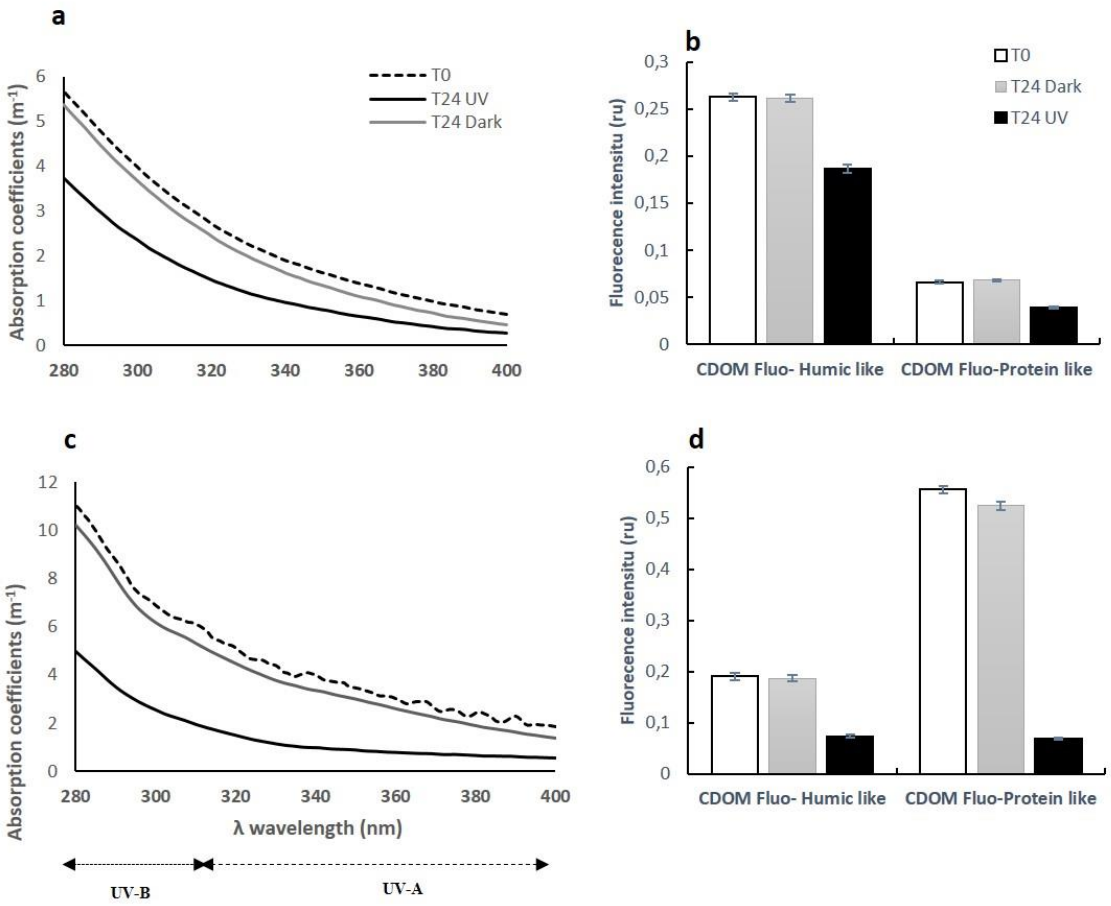

Fig. 1 Changes in absorption (a, c) and fluorescence properties (b, d) of riverine DOM (a, b) and phytoplankton-derived DOM (c, d) before (T0) and after irradiation (T24 UV) or dark treatment (T24 dark) (ru=Raman units). Each value represents the mean of three replicates (± standard deviation).

Changes in DOC concentrations during the biodegradations

The initial concentration of DOC in the control microcosms was 76 ± 2.5 µmol.L^{-1} for the Rhone experiment (Control treatment) (**Fig. 2a**) and 79.9 ± 0.8 µmol.L^{-1} for the *Chaetoceros* experiment (**Fig. 2b**). Microcosms were supplied with different sources of DOM, previously irradiated or not, providing almost the same amount of DOC in all the enriched microcosms (i.e., 22.2 ± 1.3 µmol.L^{-1} for the riverine DOM experiment and 24 ± 2.2 µmol.L^{-1} for the phytoplankton-derived DOM experiment). During the incubation there were higher degradation of DOC in the microcosms enriched with DOM (11 µmol.L^{-1} and 24 µmol.L^{-1} for the Rhône and the *Chaetoceros* experiments, respectively) compared to the controls (8 µmol.L^{-1} and 6.4 µmol.L^{-1}, respectively). However, the rates of DOC degradation was the same in the UV and Dark treatments for both

experiments. These results show the higher bioavailability property of the phytoplankton-derived DOM compared to the riverine DOM.

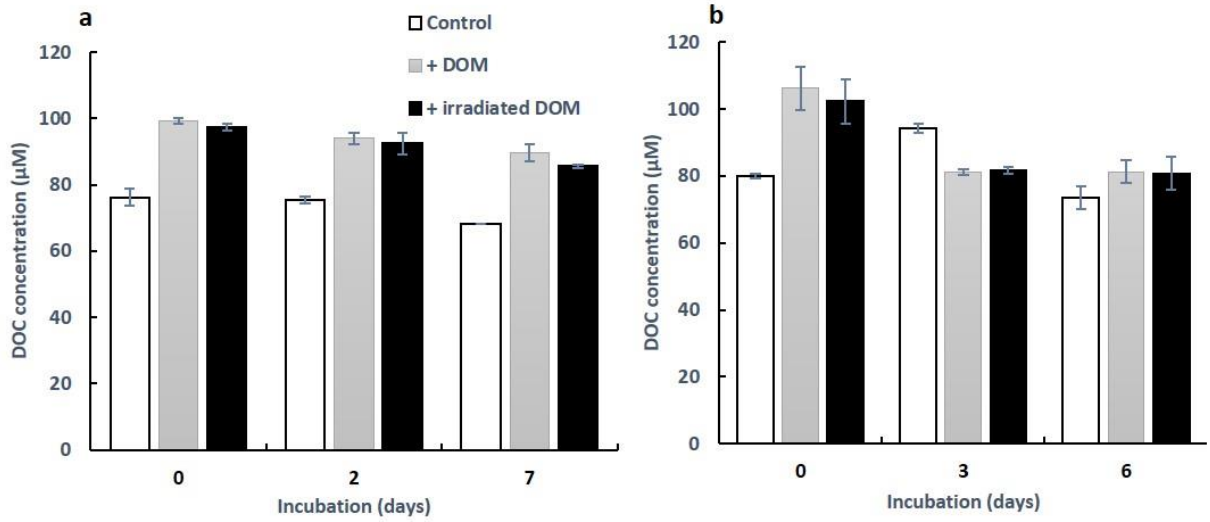

Fig. 2 Dissolved organic carbon (DOC) concentrations during the biodegradation for the experiments with the riverine DOM (a) and the phytoplankton-derived DOM (b). Each value represents the mean of three replicates (± standard deviation).

Changes in bacterial abundance and metabolism during the biodegradations

The addition of riverine DOM and phytoplankton-derived DOM led to the stimulation of bacterial growth and production during the first days of the experiments compared to the controls without DOM addition (**Fig. 3**). The addition of riverine DOM enhanced the bacterial abundance (**Fig. 3a**) and production (**Fig. 3b**) by a factor 2 and 3.6, respectively during the two first days, without significant changes between the two light treatments. After 2 days, a sharp decrease in bacterial abundance was observed in the microcosms enriched with riverine DOM, leading to the same value measured in the control microcosms at T3. The bacterial production decreased more slowly than bacterial abundance in microcosms enriched with DOM, but at the end of the experiment the same bacterial production was measured in all the treatments.

The addition of phytoplankton-derived DOM led also to an increase in bacterial abundance and bacterial production compared to the controls after 2 days (**Fig. 3c,d**). However in this case, a significant difference was observed between the two light treatments with a lower bacterial abundance for the irradiated DOM compared to the

DOM maintained in the dark at T1, T2 and T3. The differences observed between the two light treatments indicate a lowering of DOM bioavailability after irradiation that can be due to cross-linking, humification and polymerization reactions of labile molecules during light exposure (Thomas and Lara 1995, Mopper and Kieber 2002). After 2 days, there was a decrease in bacterial abundance and production in microcosms enriched with DOM until reaching the values of control microcosms at the end of the experiment.

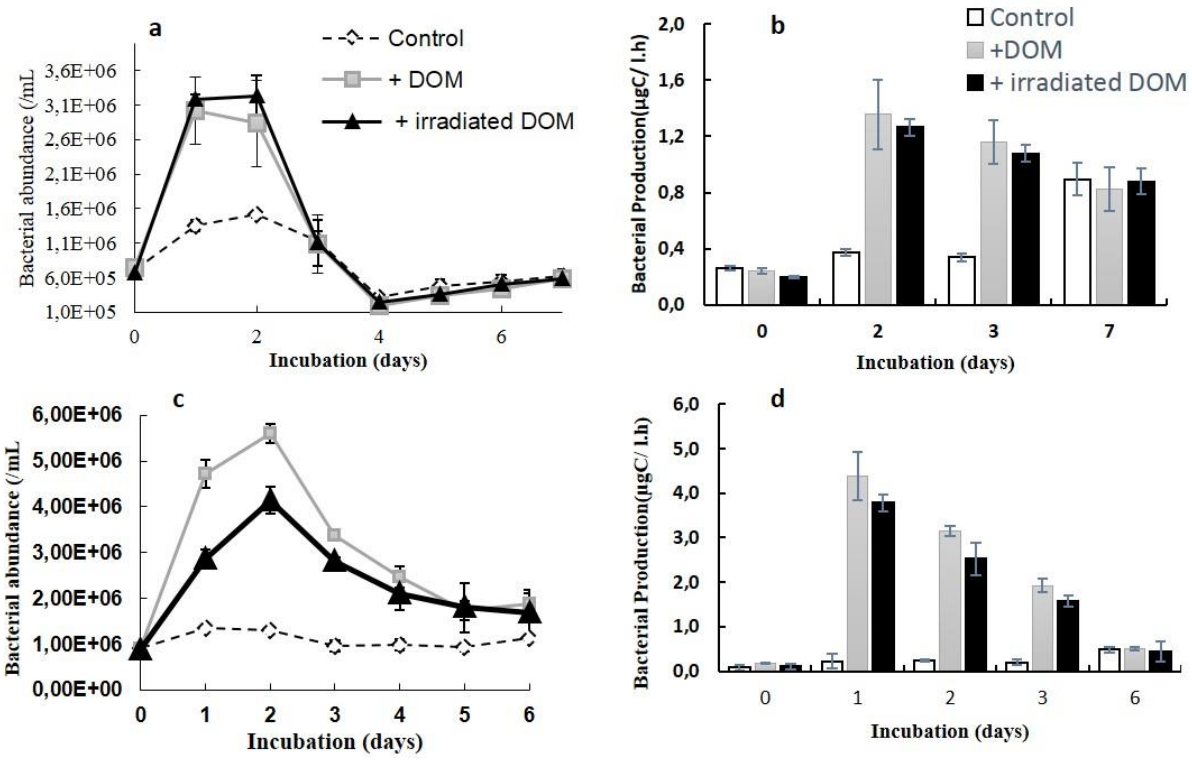

Fig. 3 Changes in bacterial abundance (a,c) and production (b,d) during the biodegradation of the riverine DOM (a,b) and the phytoplankton-derived DOM (c,d). Each value represents the mean of three replicates (± standard deviation).

Changes in bacterial diversity during the biodegradations

Bacterial diversity was analyzed in duplicate microcosms except for the initial point in the *Chaetoceros* experiment. Samples were taken at the beginning of the experiment and after 3 days for the *Chaetoceros* experiment and 7 days for the Rhone experiment. After trimming and quality control, a total of 83,924 partial 16S rRNA sequences with an average of 7,836 sequences per sample (2,462-16,100) were obtained for the Rhone experiment and 11,473 sequences with an average of 7,801 (1,206-17,589) per sample for the *Chaetoceros* experiment. The sequences were the clustered into OTUs at >97%

similarity and normalized to the respective sample with the fewest sequences (i.e 2,462 for the Rhone experiment and 1,206 for the *Chaetoceros* experiment).

Hierarchical clustering based on Bray-Curtis similarities revealed a pattern in which bacterial communities were driven by the DOM enrichment (**Fig. 4**). In the Rhone experiment there was no significant differences between the two light treatments (SIMPROF, p>0.05) (**Fig. 4a**). After 7 days of incubation, UV and Dark treatments were clustering together and were dissimilar at 50% to the controls. In the *Chaetoceros* experiment, after 3 days of incubation the controls were clustering with T0 and presented high dissimilarity with the treatments (75%) (**Fig. 4b**). There was a significant difference between the two light treatment, showing that the photodegradation of phytoplankton-derived DOM had an impact on bacterial community structure.

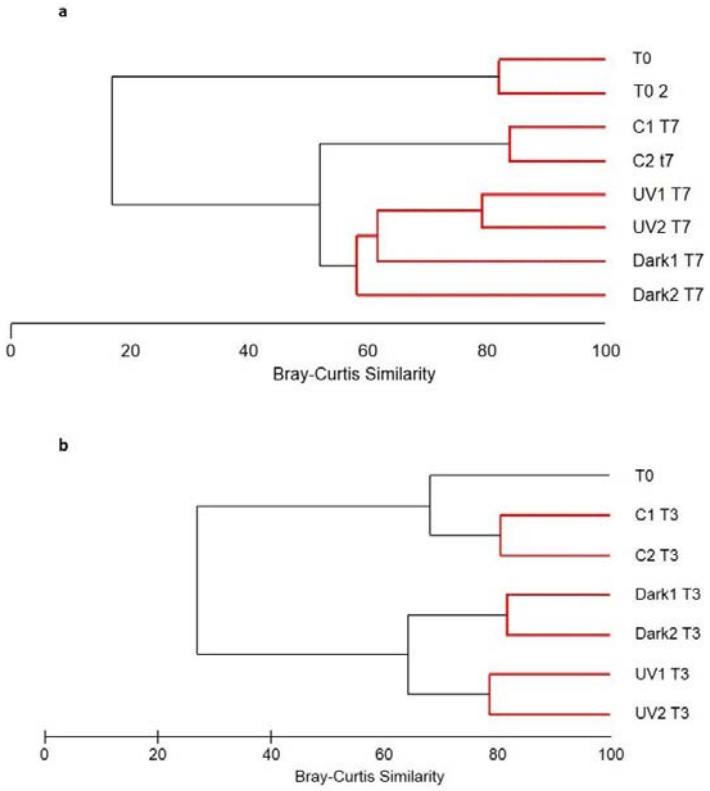

Fig. 4 Dendrograms of similarity based on OTUs table from the 16S rDNA 454-tag sequences for control microcosms (C1, C2) and enriched microcosms with (a) DOM from the Rhone river and with (b) DOM from *Chaetoceros* culture previously photodegraded (UV) or not (Dark) after 0, 3 and 7 days (T0,T3,T7). Clustering is on the basis of a distance matrix computed using the Bray–Curtis index of similarity. The dendrogram was inferred with the unweighted pair-group average algorithm (UPGMA). Red branches do not differ significantly (SIMPROF test, p >0.05). C: control, Dark: microcosms enriched in DOM not photodegraded, UV: micrococosms enriched in DOM previously irradiated.

The bacterial community composition was investigated by comparing the relative abundance of the major phyla and proteobacteria subclasses (**Fig. 5**). The addition of riverine DOM had an impact on bacteria community composition, there was less *Gammaproteobacteria* (19% in the enriched microcosms *vs* 42% in the controls) and more *Alphaproteobacteria* (52% and 60% for the dark and UV treatment respectively *vs* 42% for the controls).

The addition of phytoplankton-derived DOM had an effect on bacterial community composition dependently on the light treatment. There was more *Alphaproteobacteria* (48%) and less *Gammaproteobacteria* (26%) in the light treatment than in the dark treatment (30% and 40%, respectively). Even if no significant in the case of the Rhone experiment, it is worth noting than in both cases there was more *Alphaproteobacteria* in the UV treatment, and more particularly members of the genus *Roseobacter*. Other studies have also shown via DNA fingerprint methods that *Alphaproteobacteria* were favoured in presence of photodegraded OM (Piccini et al. 2009, Paul et al. 2012). Those groups of bacteria could be less sensitive to the reactive species of oxygen generated during the photodegradation or more adapted to use photo-oxidized DOM (Glaeser et al. 2010).

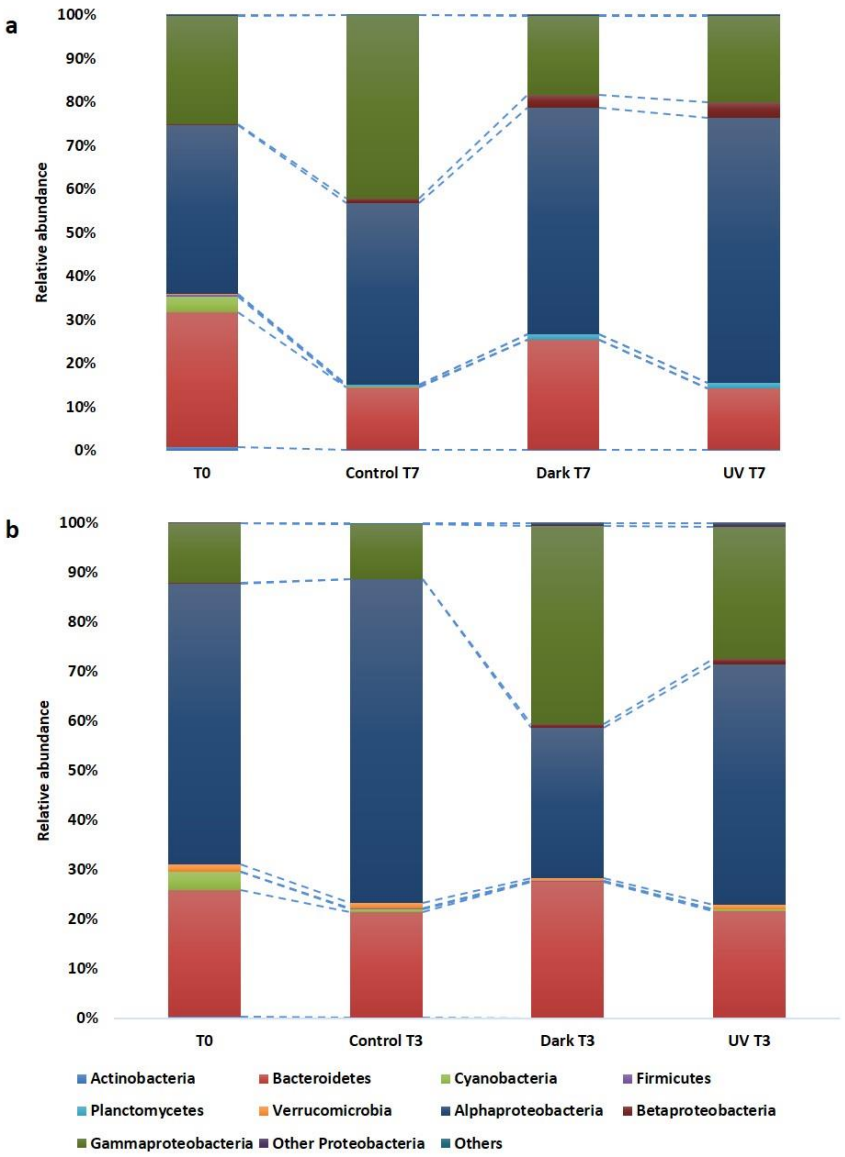

Fig 5: Relative abundance of main bacteria phyla and proteobacteria subclasses expressed as the percentage of total sequences obtained for each light treatment in the Rhone experiment (a) and the *Chaetoceros* experiment (b).

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GENERAL DISCUSSION AND CONCLUSION

V General discussion and conclusion

V-1 General considerations on the experimental approaches and techniques used in my PhD

The overall aim of my thesis was to study how various dissolved organic matter (DOM) sources, presenting different bioreactivity characteristics could affect marine bacterial communities in their activity (BCA) and composition (BCC). A more specific issue of my thesis was to study processes that could enhance the degradation of terrestrial recalcitrant DOM such as priming effect and photooxidation (Chapters III and IV). These processes are difficult to observe *in situ* and experimental studies seem more adapted to address this question. They require relatively long incubation in order to detect significant rates of recalcitrant DOM degradation (week to months for SLDOM) (Moran et al. 2000, Herlemann et al. 2014). Moreover, in Chapter II we employed long incubation periods in order to reach the complete mineralization of DOC from the jellyfish *A. aurita* and determining the resilience of BCA and BCC after a jellyfish bloom. Thus, we carried out several experiments using a batch mode that allows easier replicability compared to a mesocosm approach. Microcosms are closed systems with no outputs except for gases. The systems used in our experiments were not completely closed to allow oxygenation but avoid contamination. Experiments were performed under controlled temperature conditions (corresponding to the *in situ* temperatures) and in darkness to avoid the growth of phytoplankton that could bring a supplementary source of DOM. Experiments were performed in relatively small volumes (4L in Chapters III and IV and 8 L in Chapter II). The medium was not renewed and there is consequently a consumption of the nutrients and formation of by-products, which stay in the microcosms and can affect BCA and BCC. One of the limits of this approach is the "bottle effect". Generally the bottle effect leads to the increase of bacteria belonging to the subclass *Gammaproteobacteria* even in the unamended conditions (Herlemann et al. 2014, Pedler et al. 2014). In our experiments, we inoculated DOM to water containing undiluted natural microbial community to avoid dramatic changes in bacteria community composition during incubations. The control of bacteria by protozoa and virus was maintained in our experiment and can interfere with the response of the bacterial community to the DOM addition.

We characterized our sources of DOM in the different experiments by their concentrations in dissolved organic compounds (DOC, DON, and DOP) and in some cases we used their optical properties (CDOM) as a proxy of DOM quality. We are aware that those techniques limit our understanding on which fraction of DOM was consumed during our experiments.

V-2 Is there an impact of addition of labile organic matter on the degradation of recalcitrant organic matter in aquatic environments?

In terrestrial ecosystems, several studies have shown that the input of labile organic matter may enhance or slow down the degradation of recalcitrant soil organic matter, causing a positive or negative priming effect (PE), respectively (Kuzyakov 2002, Guenet et al. 2010). Recently PE has been hypothesised to occur in aquatic ecosystems, where it could explain the mystery of the missing terrestrial DOC in the ocean. Recent observations in the deep Pacific Ocean have suggested a relatively rapid removal of RDOC in the ocean interior and PE was proposed as one of the potential phenomena involved (Hansell and Carlson 2013).

Initially, we studied the impact of an atypical labile DOM source input caused by a jellyfish bloom on the degradation of recalcitrant compounds presented in a coastal lagoon (Chapter II). Afterwards, in order to uncover the potential occurrence of PE in coastal environments under the influence of river discharge (defined as a "hotspot" for PE by Bianchi (2011)), we performed two experiments (Chapter III a and b) using contrasted riverine DOM as recalcitrant sources. In neither of the above mentioned experiments we did detect a positive PE when bioavailable DOM was mixed with recalcitrant DOM. Indeed, the combination of DOM did not help the degradation of recalcitrant DOM (Mediterranean experiment, Chapter II and III a) or inversely lowered the degradation of recalcitrant DOM (i.e. negative PE, fjord experiment, Chapter III b) .

Over the last few years, several studies have also investigated the potential of PE in various aquatic ecosystems. The results and experimental conditions are listed in **Table V-1**. Considering all the results presented in this table, it appears that PE can be driven by several factors such as the amount of LDOM and the quality and availability of DOM. There is not a straightforward link between the **amount of LDOM addition and the intensity of PE** observed. PE intensity does not increase proportionally with increasing LDOM additions (Guenet et al. 2010, Paterson and Sim 2013). In the

Mediterranean and fjord experiments we added relatively low LDOM concentrations (about 10 µmol C L^{-1}, representing 10% of all the DOC measured at the initial time in the microcosms enriched with combined DOM sources) in comparison to the LDOM amendments reported in the literature (e.g. few $mg.L^{-1}$ and up to 120 $mg.L^{-1}$ of glucose in the experiment of Guenet et al. (2014)). Moreover, Attermeyer et al. (2014) observed than DOC consumption was not related to the frequency of DOC pulse (phytoplankton DOM) during the incubation. Besides the amount, **the quality of organic substances and their availability** to heterotrophic prokaryotes affect the decomposition of DOM pools. Complex organic substrates showed stronger PE than direct energy substrates such as glucose (Fontaine et al. 2003). Those substrates stimulate the production of exoenzymes for their degradation that could be also used to degrade RDOM. In our experiments, we used different types of LDOM, such as free amino acids (Chapter III a) which were consumed during the first days of the experiment as well as more complex substrates such as phytoplankton DOM (Chapter III b) and jellyfish DOM (Chapter II), which require the action of exoenzymes. The phytoplankton-derived DOM was not efficiently consumed in our experiment, showing that it was more recalcitrant than initially thought. In a recent study, Koch et al. (2014) have shown, using a long-term laboratory experiments that after 2 years, only 20% of the algal exudate was consumed while glucose was quickly degraded.

Generally, the detection of PE in aquatic studies was, at least partly, driven by **nutrient addition.** Guenet et al. (2014) observed a higher PE in the eutrophic systems (high nutrient load) compared to the oligo-mesotrophic systems, and other studies have shown that bacterial respiration was enhanced when nutrients and LDOM were added in combination (Carlson et al. 2002, Farjalla et al. 2009). Those results are contrasting with the results obtained in terrestrial studies where PE occur primarily under nutrient limiting conditions (Fontaine et al. 2004, Guenet et al. 2010). It could be explained by the high heterogeneity of soils limiting nutrient mobility and presenting microniches where carbon and nutrients are quite different (Nunan et al. 2003, Lehmann et al. 2008, Guenet et al. 2014). When nutrients are added, microorganisms will preferentially use those labile forms and not invest in energetically costly “mining” of the recalcitrant soil OM and thereby liberate nutrients stored in this OM (Craine et al. 2007). Generally, aquatic and terrestrial OM vary in terms of C:N:P ratios. Soil OM has typically a C:N ratio <20 making it a potentially good source of N while recalcitrant aquatic OM pools

tend to be richer in C, limiting N mining as a potential mechanism involved in aquatic PE (Grimm 2003; Bengtsson et al. 2014).

Considering all the experiments and observations done up to now, it appears that there is a potential for PE in marine environments but it seems premature for the moment to draw a conclusion about its occurrence and its relative importance in aquatic carbon budgets. The addition of LDOM such as glucose at high concentrations that do not reflect natural conditions may compromise the applicability of previous results.

It seems necessary to conduct more extensive studies to better understand coastal DOM processing. Interesting study sites to detect this phenomenon are the hotspots and hot moments defined by Bianchi (2011) where recalcitrant and labile DOM pools meet and mix, such as river plumes and upwelling area. Moreover, it appears crucial to use labelled substrates and measure DOC consumption by stable-isotope method data to see which fraction is consumed upon labile addition.

Table V-1: Synthesis of results on changes in RDOC consumption or respiration after addition of LDOM in various aquatic systems. BCC: bacterial community composition; PE: priming effect. *: Nutrients added at the start of the biodegradation assays

Ecosystem	Exp approach	Incubation time	RDOM origin	LDOM	Nutrients *	BCC analysis	PE	Reference
Arthrobacter strain (isolated from a shallow lake)	Fermenter	10 days	fulvic acids	benzoate	No	No	Yes, co metabolism	de Haan, 1977
Microbial lacustrine community (mesotrophic lake)	Microcosm	8-12 h	monosubstituted phenols	amino acids	No	No	Yes	Shimp and Pfaender 1985)
Marine microbial community (oligotrophic Sargasso Sea)	Microcosm	1-2 months	refractory compounds from the sea	glucose	Yes	Yes	PE suggested	Carlson et al. 2002)
Microbial community from a coastal lagoon	Microcosm	5 days	humic substances from the lagoon	aquatic macrophyte leachate	Yes	No	Yes	Farjalla et al. 2009)
Headwater stream microbial community	Microcosm	42 days	leaf litter	diatoms	Yes	No	Yes	Danger et al., 2013
Lacustrine microbial communities (oligo-meso and autrophic lakes)	Mesocosm	5 weeks	refractory compounds from lakes	glucose/acetate/cellobiose	Yes	No	No	Catalan et al., 2013
Microbial community from a lagoon	Microcosm	5 days	humic substances (extracted from groundwater upwelling)	algal extract	Yes	Yes	Yes	Fonte et al., 2013

Ecosystem	Exp approach	Incubation time	RDOM	LDOM	Nutrients	BCC analysis	PE	Reference
Litter-associated bacteria (freshwater marsh)	Mesocosm	35 days	plant litter	periphytic algal exudates	No	No	Yes	Kuehn, 2013
Lacustrine microbial community (eutrophic lake)	Microcosm	12 days	^{13}C leaf leachate	phytoplankton DOM and exsudates	Yes	Yes	Yes	Attermeyer et al. 2014)
Hyporheic microbial community (biofilm)	Microcosm	21 days	^{13}C willow DOM	glucose/algal extract	Yes	No	No	Bengtsson et al. 2014)
Lacustrine microbial community	Microcosm	40 days	Soil organic matter	^{13}C glucose	Yes	No	Yes	Guenet et al. 2014)
Marine microbial community (Antarctic)	Microcosm	734 days	refractory compounds generated by MCP	^{13}C glucose	Yes	No	No	Koch et al. 2014)
Microbial community from a coastal lagoon	Microcosm	22 days	refractory compounds from the lagoon	jellyfish DOM	No	Yes	No	This study (Chapter II)
Marine microbial community (Mediterranean Sea)	Microcosm	42 days	riverine DOM	amino acids solution	Yes	Yes	No	This study (Chapter IIIa)
Microbial community from a fjord (Chile)	Microcosm	50 days	riverine DOM	phytoplankton DOM and exudates	No	Yes	No	This study (Chapter IIIb)

V- 3 What are the effects of single or combined DOM sources addition on the dynamic of the bacterial community composition?

Marine organic DOM is a complex mixture of organic compounds from different sources, presenting different chemical composition and reactivity. The role of these compounds in supporting bacterial growth or in influencing microbial community structure has traditionally been assessed in aquatic literature primarily in microcosms experiments by adding model compounds (*i.e.* glucose, free amino acids) (Pinhassi et al. 1999, Cottrell and Kirchman 2000, Carlson et al. 2002) or natural DOM (Kirchman et al. 2004, Judd et al. 2007).

In the frame of this thesis, we performed different single or combined additions of various natural DOM photooxidized or not, presenting different bioavailability characteristics (*i.e* riverine DOM, phytoplankton-derived DOM) or model of substrate (free amino acids). We assessed the impact of these different additions on BCC using next generation sequencing techniques: 454 pyrosequencing (Chapters II, III a and IV) or MiSeq Illumina (Chapter IIIb). The response of the main bacterial phyla and Proteobacteria sub-classes are presented in the **Figure V-1**. In all cases, we observed modifications in BCC compared to the initial community even in the unamended microcosms but the effects were more profound in the case of single or combined additions. Overall, we observed at a low phylogenetic resolution level, than the bacteria presented in the initial community and responding to the different treatments were belonging to few phyla and especially to the phyla *Bacteroidetes* and *Proteobacteria* (*Alpha* and *Gammaproteobacteria* mostly). However, the relative abundances of the different clades were different depending on the ecosystem and the origin of DOM. In the PE experiments, we observed a diminution in the relative abundance of *Alphaproteobacteria* after the addition of riverine DOM but not after the addition of amino acids (Mediterranean Sea experiment) or phytoplankton- derived DOM (fjord experiment). This diminution was mostly imputed to the clade SAR11 while *Rhodobacterales* order was less affected. Similarly, we identified less bacteria belonging to the clade SAR11 after the addition of DOM coming from the diatom *Chaetoceros* or from the jellyfish *Aurelia aurita*. In the literature, SAR11 has been extensively studied due to its important abundance in marine ecosystems (Vergin et al. 2013). It has been shown that *Alphaproteobacteria* are important competitors for amino acids in the oceans (Cottrell and Kirchman 2000) and that the SAR11 clade dominates

amino acid uptake in the upper ocean, especially at low concentration (Alonso-Sáez & Gasol 2007, Kujawinski 2011b). Bacteria belonging to the clade SAR11 are classic non motile oligotrophs with a minute size and streamlined genome that make them most successful in open ocean where nutrients are present at low concentrations (Giovannoni et al. 2005, Stocker 2012). On the contrary the members of the *Rhodobacterales* order and more precisely the *Roseobacter* clade are moderate copiotrophs and have a large genome with metabolic flexibility, which allow them to exploit a wide variety of compounds under a range of environmental conditions (Moran et al. 2007, Newton et al. 2010, Seymour 2014). This could explain why the relative abundance of members of *Rhodobacterales* order was less affected after the addition of riverine DOM.

In the jellyfish and *Chaetoceros* experiments we observed an important increase in the relative abundance of *Gammaproteobacteria* during the first days of incubation, mainly explained by the increase of the *Alteromonadales* and *Vibrionales* orders. It has been shown that bacteria belonging to these orders are opportunistic. They have the ability of surviving at low abundance levels in low nutrient area and blooming when favourable rich conditions occur (Yooseph et al. 2010, Nelson & Wear 2014b)). Those bacteria have been suggested to be important player in the cycling of LDOM (Nelson & Wear 2014b) and could be a reason why we only observed them at a high relative abundance at the beginning of the incubation when LDOM is available.

The majority of marine studies in the literature focused on the influence of DOM quality and quantity on BCA and BCC used a single DOM source and did not take account the potential interactive effects between compounds coming from different sources. To our knowledge, only two studies have assessed the impact of mixed sources of DOM on BCC in aquatic ecosystems via fingerprint methods, without identifying specific bacterial groups (Fonte et al. 2013, Attermeyer et al. 2014) .

The addition of combined DOM sources of different origin in the Mediterranean and fjord experiments had contrasted effects but had in both cases a greater impact on BCC than single source amendments. In the Rhone-Mediterranean Sea experiment (Chapter II a), the bacterial diversity and richness, appreciated by the Inverse Simpson and Shannon indices respectively, were higher when the DOM sources were added in combination. This suggests that a greater diversity of compounds due to a combined addition sustain a higher bacterial diversity. The response of BCC was mainly driven by the addition of recalcitrant compounds (Rhone River) resulting in an increase in

Flavobacteria and bacteria belonging to OM43 (*Methylophilales*, *Betaproteobacteria*) and a decrease in SAR11 relative abundance. Inversely, addition of LDOM (amino acids) led to an increase in *Vibrio* bacteria (*Gammaproteobacteria*) that was not observed in the microcosm amended in DOM coming from the river. In the case of the fjord experiment, we observed than the OTUs responding to the combined amendment were different of the OTUs responding to a single DOM addition. This result, associated with the fact than DOC was not consumed, suggest that there was chemical interactions between the two sources of DOM, leading to a less bioavailability and efficient use of DOC by bacteria.

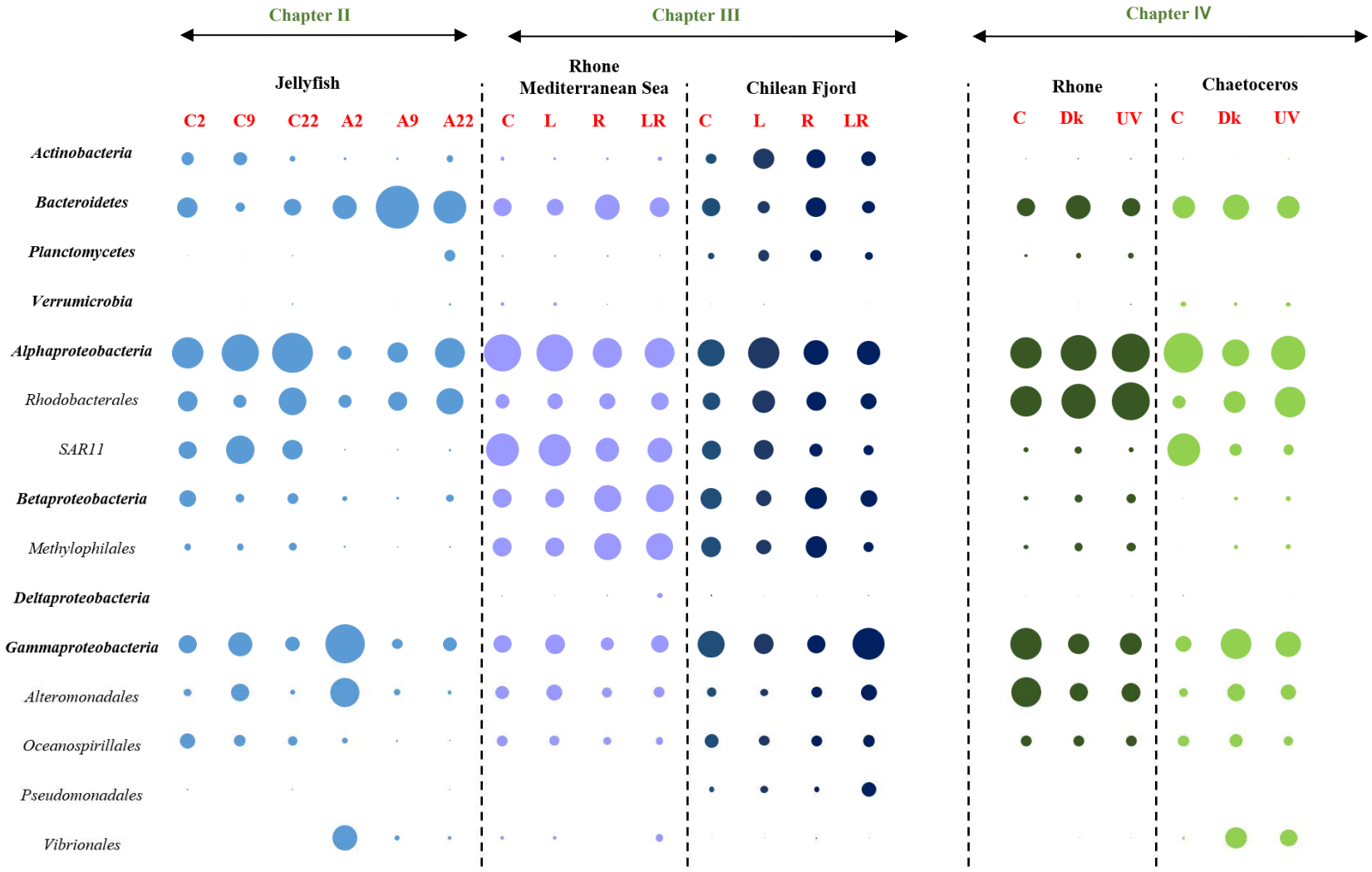

Figure V-2. Major Phyla, Proteobacteria subclasses and specific orders observed in the different studies realized in the thesis. The circle size corresponds to the relative average abundance observed. The different sources of DOM added are indicated in italic. C: control, L: labile DOM, R: recalcitrant DOM, LR:L+R combination, Dk: dark treatment, UV: light treatment. The sampling days are indicated for each experiment.

I.3 V-4 Directions for future research

There are numerous examples of correlation between DOM quality and quantity and changes in microbial metabolism and structure in the literature. However we still lack of understanding why and in which conditions some compounds become available to bacteria uptake. Although there was progress over the last decennia to better define the different DOM pools (**see part I.1.1**), the vast majority of the oceanic DOM pool remains chemically uncharacterized and constitute a barrier to improve our understanding of how heterotrophic prokaryotes contribute to biogeochemical cycles. This lack of knowledge is an obstacle to better understand the type of compounds fuelling bacterial metabolism.

The introduction of Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS) and more recently Orbitrap mass spectrometers is changing our ability to characterize DOM at a molecular level (Koch et al. 2005, Zubarev and Makarov 2013). When those techniques are coupled with electrospray ionization they provide the necessary resolution to identify chemical formulas of thousands of DOM compounds (between 200 and 2000 Da) in a single sample. FTICR-MS can constitute a powerful tool for future research to identify what kind of molecules are photo oxidized by UV radiation or consumed by bacteria. For example Stubbins et al (2010) irradiated water sample from the Congo River during 57 days and performed FTICR-MS before and after irradiation. The fingerprints DOM patterns obtained (**Fig V-3**) has revealed three fractions based on photo-reactivity: the photo-resistant, photo-labile and photo-produced fractions. This study has shown the preferential removal of common tracers of terrestrial DOM such as lignin and a shift in the molecular signature of riverine DOM toward a marine DOM signature. This result reveals the difficulty of tracking terrestrial DOM in the ocean.

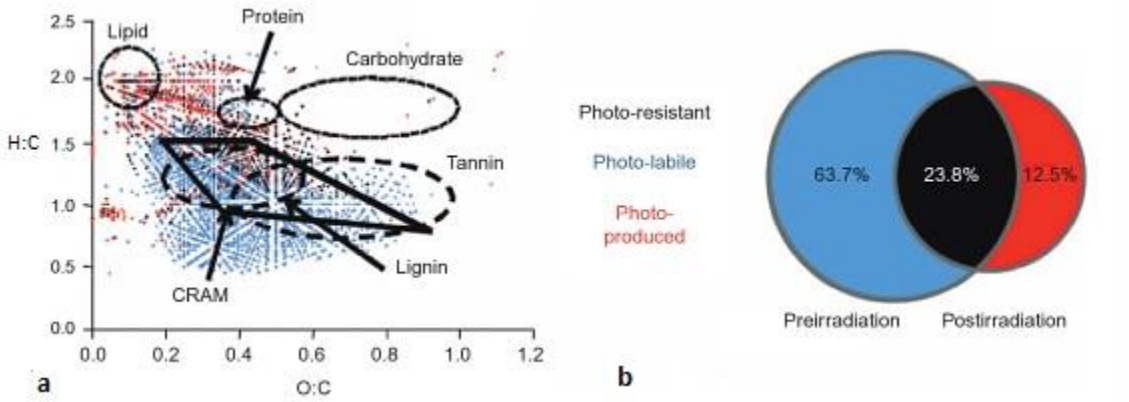

Fig V-3: (a) van Krevelen diagram (blue, red and black data indicate molecular formulae unique to initial Congo water, photo-bleached and those common to both, respectively). Areas assigned as carboxylic-rich alicyclic molecules (CRAM), lignin, tannin, lipid, and protein are delineated. (b) Venn diagram of initial and photobleached Congo River water. Area of overlap in black indicates the photo-resistant molecular formulae present in both samples. The blue area indicates photo-labile formulae unique to the initial sample and the red area indicates photoproducts unique to the irradiated sample. Modified from Stubbins et al. (2010)

Several experiments about priming effect has revealed the interest to use stable-isotope techniques. Some technique such as microautoradiography coupled with fluorescent in situ hybridization, secondary-ion mass spectrometry and stable isotope probing allow to track the bacteria which could incorporate labelled substrates and represent promising approaches to better understand DOM-bacteria interactions. Stable isotope probing coupled with 16S rDNA allows to track the incorporation of labelled ^{13}C or ^{15}N model compounds or more complex substrates (*i.e* algal extract). Integrate studies that include DOM characterization such as FTICR-MS with microbial diversity assessment such as stable isotope probing could favorize our understanding of DOM-prokaryotes interactions and represent a promisingline of research (Kujawinski 2011, Carlson and Hansell 2015). Another way to address the prokaryotes-DOM interactions is to explore the DOM molecules that prokaryotes are equipped to use or uptake. This can be examined using 1) metagenomics tools to get an overview of the metabolic potential by identifiting specific genes or 2) metatranscriptomics and metaproteomics to determine which genes are expressed (Kujawinski 2011).

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ANNEXES

SCIENTIFIC COMMUNICATIONS

Posters:

Blanchet M, Joux F. Effect of ultraviolet radiation on the kinetics of bacterial degradation of riverine organic matter at a coastal Mediterranean site.Symposium"UV radiation and marine ecosystems: current research and strategies for the future". 5-7 December 2012 Universidad de Concepcion, Chile. LIA MORFUN

Blanchet M, Joux F, Pringault O, Catala P, Oriol L, Caparros J, Ortega-Retuerta E, Intertaglia L, Agis, M, Bouvy M. Changes in metabolic activity and diversity of the bacterial community from a Mediterranean coastal lagoon during the degradation of dissolved organic matter from the jellyfish Aurelia aurita . SAME 13 Congress. 8-13 September 2013. Stresa, Italy.

Oral communication:

Blanchet M, Joux F, Catala P, Oriol L, Caparros J, Ortega E, Intertaglia L, Pringault O, Agis M, Bouvy M. Changes in metabolic activity and diversity of the bacterial community from a Mediterranean coastal lagoon during the degradation of dissolved organic matter from the jellyfish Aurelia aurita. Biodiversity and functioning of marine ecosystems workshop. Banyuls sur mer, May 30-June 1, 2012.

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Résumé

En milieu marin, les communautés bactériennes hétérotrophes sont exposées à un mélange hétérogène de composés organiques dissous présentant différents degrés de labilité, qui peuvent contrôler à la fois leurs activités et leur composition. Dans le cadre de cette thèse, nous avons étudié la réponse de communautés bactériennes présentes dans des environnements côtiers contrastés à l'ajout simple ou combiné de différentes sources naturelles de matière organique dissoute (MOD), préalablement photo oxydée ou non et présentant différentes bio réactivités.

Dans un premier temps, nous avons étudié l'impact d'ajout de MOD labile provenant de méduses sur l'activité et la diversité bactériennes d'une communauté d'une lagune côtière méditerranéenne. Nous avons observé que la résilience des communautés bactériennes suite à cet ajout était plus importante pour les fonctions métaboliques que pour la diversité. Ceci suggère que les efflorescences de méduses peuvent entrainer des changements durables de la structure des communautés bactériennes en environnement côtier.

Dans un deuxième temps, nous nous sommes intéressés à la possibilité d'un priming effect (accélération de la dégradation bactérienne de la MOD réfractaire en présence de MOD labile) en milieu côtier. Des expériences d'ajout simple ou combiné de MOD labile et récalcitrante ont été réalisées sur des communautés bactériennes de Mer Méditerranée et d'un fjord de Patagonie chilienne. Dans les deux cas, nous avons observé des changements plus importants de la composition communautaire bactérienne suite à un ajout combiné. Cependant, nous n'avons pas observé une plus forte consommation de MOD récalcitrante suite à l'ajout de composés labiles, ce qui suggère que le priming effect n'a pas eu lieu au cours de nos expériences.

Enfin, nous avons étudié l'impact de la photodégradation de différentes sources de MOD (*i.e.* MOD récalcitrante de rivière, MOD labile provenant de phytoplancton) sur l'activité et la diversité de communautés bactériennes côtières. Nous avons observé que la photodégradation de la MOD issue d'une culture de phytoplancton entrainait à la fois une croissance bactérienne plus faible et une modification de la diversité bactérienne en faveur des Alphaprotéobactéries.

Mots clé : matière organique dissoute, environnements côtiers, priming effect, photooxydation matière organique, bactéries hétérotophes, biodégradation, diversité bactérienne, pyroséquençage

Abstract

Heterotrophic bacterial communities in marine environment are exposed to a heterogeneous mixture of dissolved organic compounds with different bioavailability that may control both their activities and their composition. In the frame of this thesis, we studied the response of different coastal bacterial communities to the single or combined addition of various natural dissolved organic matter (DOM) photo-oxidized or not, presenting different bioavailability characteristics.

Firstly, we studied the effects of the addition of highly bioreactive DOM derived from jellyfish on bacterial activities and community structure in a Mediterranean coastal lagoon. We observed that resilience of the bacterial community after the addition of DOM from the jellyfish was higher for metabolic functions than diversity, suggesting that jellyfish blooms can induce durable changes in the bacterial community structure in coastal lagoons.

Secondly, we investigated the occurrence of priming effect (increase in microbial degradation of refractory DOM upon the addition of labile DOM) on coastal marine environments. Experiments with single or combined additions of recalcitrant and labile DOM sources were performed with a Mediterranean and a Patagonian fjord bacterial communities. In both cases we observed a greater effect of combined addition on bacterial community composition. However we did not observe an increase in recalcitrant DOM degradation of recalcitrant DOM following the addition of labile compounds, suggesting that priming effect did not occur during our experiments.

Finally, we studied the impact of contrasted DOM *(i.e.* recalcitrant riverine DOM, labile phytoplankton-derived DOM) photodegradation on coastal bacterial communities activity and composition. We observed that photodegradation of phytoplankton-derived DOM led to a lower bacterial growth and changes in bacterial community diversity, in favour of Alphaproteobacteria.

Key words: dissolved organic matter, coastal environments, priming effect organic matter photooxydation, heterotrophic bacteria, biodegradation, bacterial diversity, pyrosequencing