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Analysis of the impact of organic pollutants on marine microbial communities

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Doctoral Thesis by compendium of publications

Ph.D. Program in **Marine Sciences**

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“Educating people to understand, to love and to protect the water systems of the planet, marine and fresh water, for the well-being of future generations.”

COUSTEAU, Jacques Yves

“How inappropriate to call this planet Earth when it is quite clearly Ocean.”

CLARKE, Arthur Charles

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LIST OF ABBREVIATIONS

ABC	ATP-binding cassette
ADOC	Anthropogenic dissolved organic carbon
AHA	L-azidohomoalanine
ANOVA	Analysis of variance
APA	Alkaline phosphatase
ARG	Antibiotic resistance genes
BA	Bacterial abundance
BAF	Bioaccumulation factor
BBMO	Blanes bay microbial observatory
BONCAT	Bio-orthogonal non-canonical amino acid tagging
CARD-FISH	Catalyzed reporter deposition fluorescence in situ hybridization
Chit	Chitinase
CNAG	National center for genomic analysis
CTC	5-cyano-2,3-ditolyl chloride tetrazolium
DAPI	4,6-diamidino-2-phenylindole
DCM	Deep chlorophyll maximum
DMS	Dimethylsufide
DMSP	Dimethylsulfoniopropionate
DNA	Deoxyribonucleic acid
DOC	Dissolved organic carbon
DP	Dechlorane plus
EEA	Extracellular enzyme activity
EPA	Environmental protection agency
EPS	Extracellular polymeric substance
FDR	False discovery rate correction
FISH	Fluorescence in situ hybridization
GC/MS	Gas chromatograph coupled to a mass spectrometer
HCB	Hexachlorobenzene
HCH	Hexachlorocyclohexane
HMW	High molecular weight
HNA	High nucleic acid
HPG	L-homopropargylglycine
HTCO	High-temperature catalytic oxidation

IC50	Half-inhibitory concentration
KOW	Octanol-water partition coefficient
LC50	50% Lethal threshold
Leu-amp	L-leucyl aminopeptidase
LNA	Low nucleic acid
LOD	Limits of detection
LOQ	Limits of quantification
LPS	Lipopolysaccharide
MAG	Metagenome-assembled genome
MDA	Multiple displacement amplification
metaG	Metagenomics
metaT	Metatranscriptomics
MGE	Mobile genetic element
mRNA	Messenger ribonucleic acid
MXR	Multiple xenobiotic resistance
NADS	Nucleic acid double staining
NMDS	Non-metric multidimensional scaling
OP	Organic pollutant
OPE	Organophosphate ester
OPEC	Organic pollutants of emerging concern
PAH	Polycyclic aromatic hydrocarbon
PCA	Principal component analysis
PCB	Polychlorinated biphenyl
PCR	Polymerase chain reaction
PFAA	Perfluoroalkyl acid
PFAS	Perfluoroalkyl substance
PFCA	Perfluoroalkyl carboxylic acid
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctane sulfonate
PFSA	Perfluoroalkane sulfonic acid
POP	Persistent organic pollutant
PP	Polypropylene
QSAR	Quantitative structure activity relationships
RNA	Ribonucleic acid
ROS	Reactive oxygen species

rRNA	Ribosomal ribonucleic acid
SAG	Single-cell genome
SDS	Sodium dodecyl sulfate
SPE	Solid phase extraction
TCA	Tricarboxylic acid
TN	Total nitrogen
TOC	Total organic carbon
TON	Total organic nitrogen
UCM	Unresolved complex mixture
UNEP	United nations environment programme
UPLC-MS/MS	Triple-quadrupole mass spectrometer
WWTP	Wastewater treatment plant
β-Glc	β-glucosidase

LIST OF PUBLICATIONS

Cerro-Gálvez, E., Casal, P., Lundin, D., Piña, B., Pinhassi, J., Dachs, J. and Vila-Costa, M. (2019) Microbial responses to anthropogenic dissolved organic carbon in Arctic and Antarctic coastal seawaters. *Environ. Microbiol.* **21***:* 1466–1481.

Cerro-Gálvez, E., Sala, M. M., Marrasé, C., Gasol, J. M., Dachs, J., and Vila-Costa, M. (2019) Modulation of microbial growth and enzymatic activities in the marine environment due to exposure to organic contaminants of emerging concern and hydrocarbons. *Sci. Total Environ.* **678**: 486–498.

González-Gaya, B., Martínez-Varela, A., Vila-Costa, M., Casal, P., **Cerro-Gálvez, E.**, Berrojalbiz, N., *et al*. (2019) Biodegradation as an important sink of aromatic hydrocarbons in the oceans. *Nat. Geosci.* **12**: 119–125.

Karlsson, C., **Cerro-Gálvez, E.**, Lundin, D., Vila-Costa, M., and Pinhassi, J. (2019) Direct effects of organic pollutants on the growth and gene expression of the Baltic Sea model bacterium Rheinheimera sp. BAL341. *Microb. Biotechnol.* **0**: 1–15.

Vila-Costa, M., Sebastián, M., Pizarro, M., **Cerro-Gálvez, E.**, Lundin, D., Gasol, J.M., and Dachs, J. (2019) Microbial consumption of organophosphate esters in seawater under phosphorus limited conditions. *Sci. Rep.* **9**: 233.

ABSTRACT

Increasing amounts of hundreds of thousands of organic synthetic chemicals are currently in use, with many of them intentionally or unintentionally emitted to the environment by human activities. The more recalcitrant fraction of this pollutant mixture reaches marine ecosystems mainly through rivers, continental run-off, and diffuse atmospheric inputs. Once in seawater, it represents the anthropogenic fraction of the dissolved organic carbon (ADOC) pool. However, the total amount of ADOC is unknown, while its effects to ecosystems and detailed composition is largely unknown. Over the past decades, the scientific research effort has focused on the effects of organic pollutants (OPs) in marine biota, especially in oil spills events or under toxicological testing in laboratories, neglecting the importance of the chronic pollution perturbation of the biosphere composition caused by diffusive inputs of large number of pollutants at low concentrations. New interdisciplinary approaches are needed to move from this descriptive and acute level to more holistic approaches taking into account the ADOC pool to improve the understanding of the roles of marine microbes in biogeochemical cycles, and the impact of ADOC chronic pollution on the marine microbial ecosystem. Our aim was to combine functional genomic tools with quantitative biogeochemical approaches under manipulated conditions to determine the bidirectional interaction between marine microbial community structure and function and the ADOC present in coastal seawater. Additionally, it was also intended to perform similar experiments in areas with diverse environmental conditions to elucidate the role of the trophic conditions and levels of pollutants in the response.

In order to fulfil the proposed objectives, several OP amendment experiments were performed with different OP additions and contrasted seawater from the North-Western Mediterranean, the Arctic and the Antarctic. On the one hand, the

effect caused by four families of pollutants individually (hydrocarbons such as alkanes and polycyclic aromatic hydrocarbons (PAHs), and emerging pollutants such as organophosphate esters (OPEs) and perfluoroalkyl substances (PFASs)) was tested in five marine bacterial communities of the NW Mediterranean, and the specific effect of perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) acids (both corresponding to the family of PFASs) in communities from Deception Island (Antarctica). On the other hand, experiments were conducted to observe the effect of an operationally defined ADOC, which consisted of the non-polar extract of *in situ* seawater, to bacterial communities from coastal waters with very different starting environmental conditions, such as Livingston Island (Antarctica), Svalbard (Arctic), Barcelona and Blanes (NW Mediterranean).

The results provided during this thesis are the first reporting the effects on natural marine bacterial communities due to a chronic background pollution in the oceans. They suggest that the baseline ADOC pollution ubiquitously present in the oceans, two orders of magnitude lower than DOC, is modifying bacterial communities and its functionality. ADOC induced the growth of rare taxa, most of them known as pollutants degraders, but also modified the activity of some metabolic pathways from certain taxonomical groups, such as those related to hydrocarbon breakdown and PFOS desulfurization. Consequently, this work provided evidences that ADOC might be changing the dynamics of ocean biogeochemical cycles. The relevance of this perturbation will need to be constrained with future research. At the same time, marine microorganisms are adapted to modulate the concentration and state of incoming pollutants, as an example, we have observed a PFOS decrease in incubations with bacteria from Antarctic waters. However, the bidirectional interaction between ADOC and marine bacteria is closely related with environmental variables and conditions (nutrients availability, water temperature, etc.), as well previous exposure to

pollutants probably facilitating an adaptation of the communities. In terms of the pool of ADOC, the same ADOC perturbation did not result in the same response for marine communities in the Mediterranean, Arctic and Antarctica. The suite of microbial responses are thus taxa and compound specific and besides the growth of the rare biosphere, range from degradation of pollutants, changes in the enzymatic activities, modification of the composition of the cell membranes and surface properties, compound specific stress responses, among others.

Understanding the nature of ocean life and the patterns of its diversity and functionality represents a difficult challenge. Nevertheless, there is an urgency to develop a greater knowledge of marine environments as links between ocean processes and terrestrial anthropogenic life. This thesis has demonstrated that the perturbation of the composition of the organic component of the biosphere, caused by thousands of chemicals at low concentrations, has the potential to affect the major biogeochemical cycles, and this arise as a vector of global change, which is also influenced by human activities.

RESUMEN

En la actualidad, se utilizan cantidades cada vez mayores de cientos de miles de productos químicos sintéticos orgánicos, muchos de los cuales son emitidos por la actividad humana, ya sea de manera voluntaria o involuntaria, al medio ambiente. La fracción más recalcitrante de esta mezcla de contaminantes llega a los ecosistemas marinos principalmente a través de ríos, escorrentía continental y por la entrada difusiva atmosférica. Una vez en el agua de mar, ésta representa la fracción antropogénica de la reserva de carbono orgánico disuelto (llamada ADOC, por sus siglas en inglés). Sin embargo, la cantidad total de ADOC, su composición específica y sus efectos en los ecosistemas son, a día de hoy, bastante desconocidos. En las últimas décadas, el esfuerzo en investigación científica se ha centrado en los efectos de los contaminantes orgánicos (CO) en la biota marina, especialmente en eventos de derrames de petróleo o mediante pruebas toxicológicas en laboratorio, descuidando la importancia de la contaminación crónica y ubicua causada por la entrada atmosférica difusiva de gran cantidad de contaminantes a bajas concentraciones. Es por ese motivo que se necesitan nuevos enfoques interdisciplinarios para pasar de un nivel descriptivo y específico a enfoques más holísticos, teniendo en cuenta la totalidad del ADOC para mejorar la comprensión del impacto de éste en el ecosistema microbiano marino y en la función de los microbios marinos en los ciclos biogeoquímicos. Nuestro objetivo era combinar herramientas genómicas funcionales con cuantificaciones biogeoquímicas en condiciones experimentales para determinar la interacción bidireccional entre la estructura y función de la comunidad microbiana marina y el ADOC presente en el agua de mar costera. A su vez, también se proyectaba realizar experimentos similares en zonas con diversas condiciones ambientales para dilucidar el papel de las condiciones tróficas y los niveles de contaminantes iniciales en la posterior respuesta

microbiana.

Para cumplir con los objetivos propuestos, se realizaron varios experimentos de adición de CO a diferente concentración y composición, así como en varias aguas de mar del Mediterráneo noroccidental, el Ártico y la Antártida. Por un lado, se probó el efecto causado por cuatro familias de contaminantes de manera individual (hidrocarburos como los alcanos e hidrocarburos aromáticos policíclicos (PAH) y contaminantes emergentes como los ésteres de organofosfato (OPE) y sustancias perfluoroalquílicas (PFAS)) en bacterias marinas de cinco comunidades del noroeste del Mediterráneo. Tambié se examinó el efecto específico de los ácidos perfluorooctanosulfonato (PFOS) y perfluorooctanoato (PFOA) (ambos correspondientes a la familia de PFAS) en comunidades de la Isla Decepción (Antártida). Por otro lado, se realizaron experimentos para observar el efecto del ADOC, que consistía en el extracto no-polar de agua de mar *in situ*, en las comunidades bacterianas con condiciones ambientales iniciales muy diferentes, como lo son las de la Isla Livingston (Antártida), Svalbard (Ártico), Barcelona y Blanes (NO Mediterráneo).

Los resultados proporcionados durante esta tesis son los primeros en reportar los efectos en las comunidades bacterianas marinas debido a la contaminación a bajo nivel, pero crónica, de los océanos. Éstos sugieren que la contaminación de ADOC, presente de manera ubicua en los océanos y dos órdenes de magnitud más baja que el DOC (Dissolved Organic Carbon), está modificando las comunidades bacterianas y su funcionalidad. En los experimentos realizados, el ADOC indujo el crecimiento de especies microbianas raras, la mayoría de ellas conocidas como degradadoras de contaminantes, pero también modificó la actividad de rutas metabólicas de ciertos grupos taxonómicos, como los relacionados con la degradación de hidrocarburos y la desulfuración de PFOS. En consecuencia, este trabajo ha proporcionado evidencias sólidas de que

el ADOC debe de estar cambiando la dinámica de los ciclos biogeoquímicos oceánicos. Al mismo tiempo, los microorganismos marinos están adaptados para modular la concentración y el estado de los contaminantes entrantes. Como ejemplo, hemos observado una disminución a lo largo del tiempo de la concentración de PFOS en las incubaciones con bacterias marinas antárticas. Sin embargo, la interacción bidireccional entre ADOC y microorganismos está estrechamente relacionada con las variables y condiciones ambientales (disponibilidad de nutrientes, temperatura del agua, etc.), así como la exposición previa a los contaminantes, probablemente facilitando una mejor adaptación de las comunidades. En términos del ADOC, la misma perturbación con ADOC no resultó en la misma respuesta para las comunidades marinas en el Mediterráneo, el Ártico y la Antártida. El conjunto de respuestas microbianas es, por lo tanto, específico de cada taxón y CO. Dicha respuesta puede verse reflejada en el crecimiento de la biosfera rara, la biodegradación de los contaminantes, los cambios en las actividades enzimáticas, la modificación de la composición de las membranas celulares y sus propiedades de superficie, o una respuesta de estrés específica al compuesto, entre otras.

Comprender la naturaleza de la vida oceánica y los patrones de su diversidad y funcionalidad representa un difícil desafío. Sin embargo, existe la necesidad de agrandar el conocimiento del medio marino como enlace entre los procesos oceánicos y la vida antropogénica terrestre. Esta tesis ha demostrado que la perturbación del componente orgánico de la biosfera, causada por miles de sustancias químicas en bajas concentraciones, tiene el potencial de afectar los principales ciclos biogeoquímicos. Por lo tanto, se debería de tener en cuenta como un vector de cambio global, estrechamente influenciado por la actual actividad humana.

PART I

General introduction

GENERAL INTRODUCTION

Take a moment. Look around. Think about the large amount of consumer products that contain synthetic organic chemicals that surround you and that you use in your daily life. Think of all the ways in which those products may be released into the environment, due to intentional or unintentional human activities. Anthropogenic chemicals will be partially degraded in wastewater treatment plants (WWTP), then dumped into the rivers, where another fraction will get degraded, possibly the compounds with the highest polarity (Gioia and Dachs, 2012). The most refractory pool of organic pollutants will reach the oceans. Is “dilution the solution to pollution” in the oceans? This saying might be no longer true for our oceans that annually receive both atmospheric and terrestrial input of hundreds of thousands of organic pollutants in the so-called process of chronic diffusive pollution (Muir and Howard, 2006; Dachs and Méjanelle, 2010). We must ask, what might be the diverse effects on the biosphere and the marine ecosystem? This thesis aims to answer this question, focused on the effects of organic pollutants, relevant in the marine environment, to the base of the marine food chains. The microorganisms are important not only in terms of biomass (Gasol *et al*., 1997) but also as the main engines of the cycling of nutrients in the oceans (or the biogeochemistry of the oceans), thus the functioning of Earth System.

1. Organic pollutants (OPs)

1.1 Description and legacy

Pesticides, herbicides, flame retardants, pharmaceuticals, surfactants, plastics and plasticizers, hydrocarbons, dioxins, among several hundred thousands of still undescribed organic synthetic chemicals are currently in use or unintentionally emitted by human activities (Daughton, 2005). The current emissions rates and total amount have been increasing during the Anthropocene and have

been detected throughout the environment, even in remote locations far away from the point of release (Galbán-Malagón *et al.*, 2012; Casal *et al.*, 2017). The physicochemical properties of these organic pollutants (OPs) ultimately determine their transport behavior and fate once released into the environment (Wania, 2003, 2006; Lohmann *et al.*, 2007). Common properties of OPs are:

a) High hydrophobicity. The hydrophobic nature and high lipid solubility of these compounds facilitates their pass readily through the phospholipid structure of biological membranes and accumulate in fat deposits, implying a high tendency of the chemical to accumulate in the tissues of living organisms at concentrations orders of magnitude higher than in the surrounding environment. The accumulation mechanisms are due to passive uptake directly from water (bioconcentration), the sum of the passive and active uptakes including consumption of lower trophic level organisms (bioaccumulation), and by the amplification of concentrations when POPs are transferred to higher trophic levels of the food web (biomagnification) (Fig. 1). Concentrations can become magnified at higher levels in the food chain by up to 70,000 times the levels at the base of the food chain (UNEP, 2009).

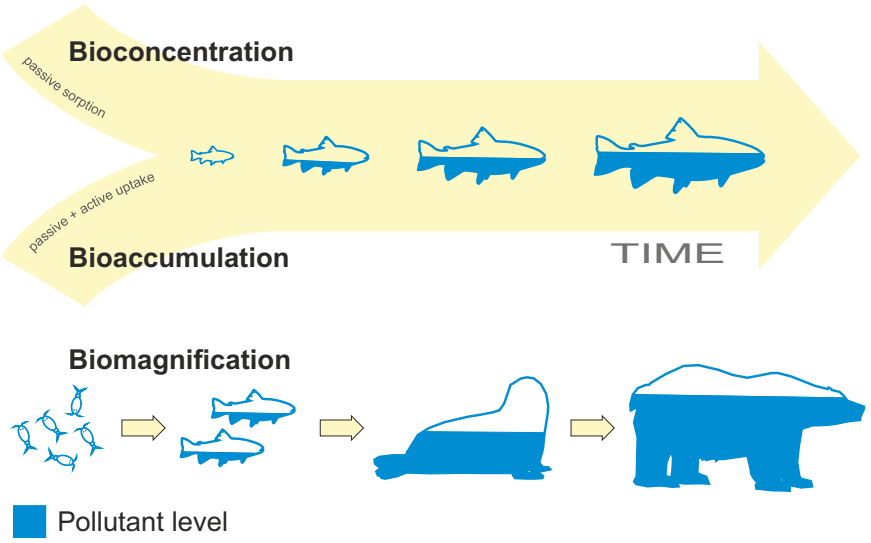


Figure 1. Bioconcentration, bioaccumulation and biomagnification concepts

b) Volatility. Due to their semi-volatile nature, many OPs become widely distributed throughout the globe as a result of their potential for long-range atmospheric transport and subsequent deposition (Wania *et al.*, 1998; Jurado *et al.*, 2007). Consequently, OPs reach regions where they have never been produced or used such as polar pristine environments (Stortini *et al.*, 2009; Galbán-Malagón *et al.*, 2013; Casal *et al.*, 2017).

c) Toxicity. Acute and chronic exposures to OPs, even at extremely low concentrations, can result in toxic, carcinogenic, neurotoxic and/or endocrine disrupting effects to living organisms at lethal and sublethal levels (Tanabe, 2002; Ellis-Hutchings *et al.*, 2006; Vasseur and Cossu-Leguille, 2006).

A fraction of OPs has been classified under the group known as persistent organic pollutants (POPs), which shares an extra fourth criterion:

d) Persistence. They are resistant to environmental degradation through chemical, biological and photolytic processes; hence, their structure remains intact long periods of time (Buccini, 2003; Wong *et al.*, 2005). OPs of global concern are characterized by a high persistence in environmental waters, soils and air. Such persistence implies a large half-life of the chemical in the environment, which allows for enough time for its regional and global transport. For example, even chemicals with low volatility, such as ionizable perfluoroalkyl substances, can then be transported globally by oceanic currents.

The global concern regarding the adverse impact of OPs to the environment, and particularly of POPs, which not only depends on the single individual compounds but also on their numerous co-occurrence, pushed international parties to legislate on the regulation of these compounds. The United Nations Environment Program (UNEP) promoted the adoption in 2001 of the Stockholm

Convention on Persistent Organic Pollutants aiming to eliminate or restrict the production and use of 12 POPs ("The dirty dozen") (UNEP, 2001). Nowadays, this list of pesticides, industrial chemicals and unintentional products has grown (UNEP, 2009). Nevertheless, it is still incomplete and under ongoing revision. Currently, many emerging pollutants with similar physicochemical properties to the ones listed are not found within the POP convention (Lohmann *et al*., 2007; Howard and Muir, 2010). Indeed, the number of synthetic organic chemicals for which we know the environmental fate and effects is a tiny fraction of the total anthropogenic organic carbon. Studies of toxicological effects are addressed for a single or simple mixture of known individual pollutants, which may differ from the effects due to exposure to the overall pool.

1.2 OPs in the ocean

OPs (legacy and emerging) are distributed in the global environment due to a combination of local, regional and long-range transport. This distribution is mainly a result of either the atmospheric transport or the oceanic transport, or a combination of both (Fig. 2). The behavior and fate of the compound in the environment are determined by a combination of: i) their physicochemical properties, ii) the environmental conditions such as temperature, wind speed, or organic matter content, and iii) the relative relevance of primary and secondary sources (Mackay *et al*., 1992). Therefore, although compounds with similar properties tend to move together, there are a number of processes such as cold trapping or the biological pump that induce a fractionation of the chemicals during transport (Wania and Mackay, 1996; Galbán-Malagón *et al*., 2012).

The semi-labile and more recalcitrant fraction of this OP mixture reach and spread aquatic ecosystems by atmospheric or oceanic transport, being the quantification and effects produced by this **anthropogenic dissolved organic carbon (ADOC)** unknown and underestimated to date. Large levels, higher than

previously thought, are being introduced to the marine environment passively, by diffuse atmospheric transport (Dachs *et al*., 2002). For instance, taking polycyclic aromatic hydrocarbons (PAHs) as an example of a model OP family, the atmospheric input to the global ocean was estimated to be at 1.09 Tg per

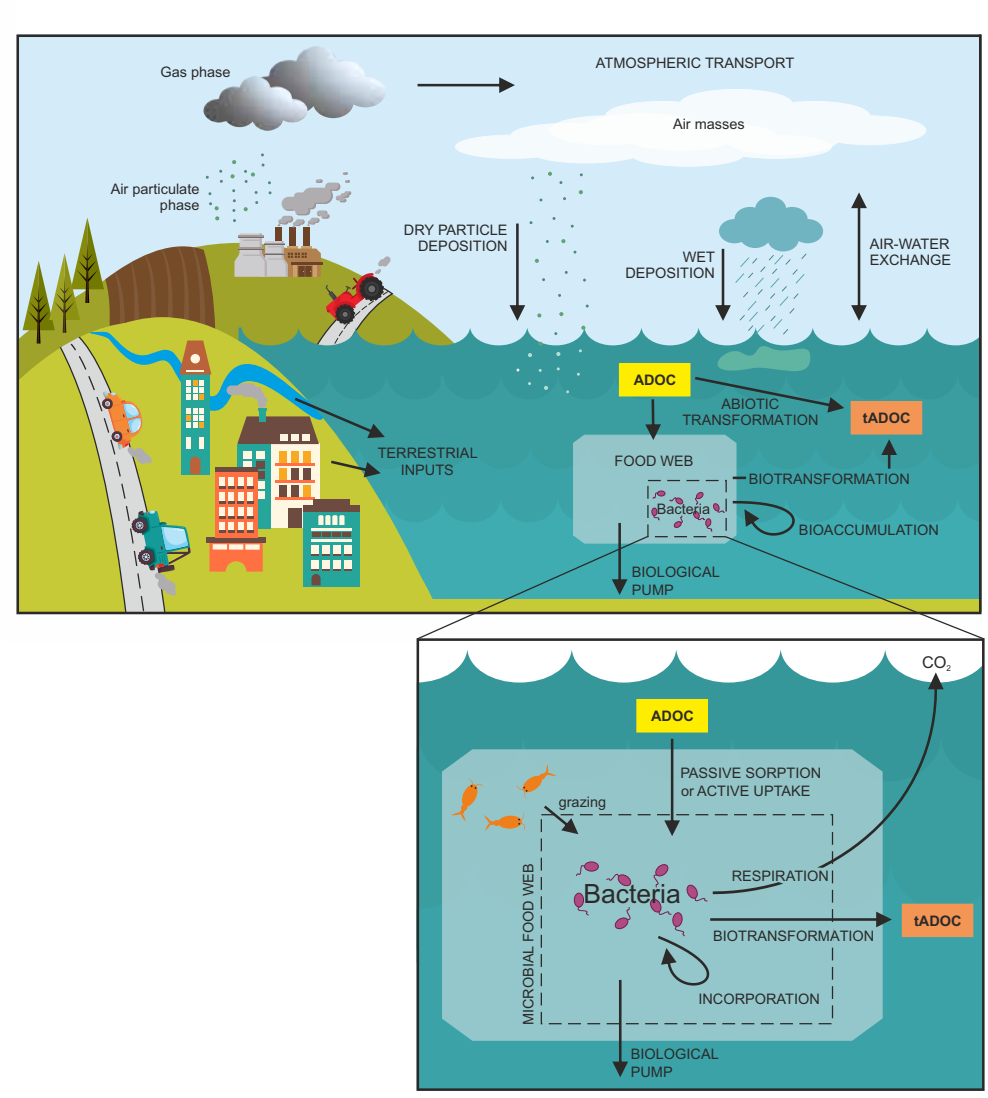


Figure 2. Environmental transport, cycling, and fate of organic pollutants. Processes affecting concentrations of dissolved and particle phase of pollutants in the photic zone of the ocean (adapted from Berrojalbiz et al., 2011a). ADOC: anthropogenic dissolved organic carbon; tADOC: transformed ADOC.

year (González-Gaya *et al*., 2016), being four times higher than the input from the well-known Deepwater Horizon oil spill (Reddy *et al*., 2012). If we consider the total pool of semi-volatile aromatic like compounds (SALC) into the oceans, that is, all those chemicals with similar nature than the identified PAHs, the input represents a relevant carbon deposition to the global ocean, estimated to be around 15% of the oceanic carbon (as CO_2) uptake (González-Gaya *et al*., 2016). In the photic zone of the sea, part of these compounds are removed vertically to deeper layers by sorption to settling organic carbon (the biological pump), limiting the re-volatilization of the compounds into the atmosphere (Dachs *et al*., 2002; Scheringer *et al*., 2004). However, it has been calculated that the integrated sinking flux of PAHs due to the biological pump was two orders of magnitude lower than the atmospheric input (González-Gaya *et al*., 2019), suggesting that the 99% of the PAHs does not settle to the deep ocean and, so, these compounds may suffer from biotic or abiotic transformation or accumulation in the photic zone of the oceans (Fig. 2). PAHs contrast with highly persistent PCBs, for which atmospheric inputs to the ocean are of the same magnitude that settling due to the biological pump (Lohmann and Dachs, 2011). Hydrophobic ADOC tends to bioaccumulate in plankton cells, as a result of their large surface area and significant organic carbon content, producing a constant entry of these compounds into the food webs, with concentrations biomagnifying at higher trophic levels (del Vento and Dachs, 2002; Berrojalbiz *et al*., 2009; Cropp *et al*., 2011). Environmental transformations of ADOC in the photic zone can be subdivided into three processes: i) abiotic oxidation and hydrolysis; ii) photolysis; and iii) biotransformation or respiration (EPA, 2004). However, transformation rates and fate of ADOC are the least characterized vector of the biogeochemistry of these compounds, not only in the oceans but also in other environmental matrices such as soils and sediments. The lack of *in situ* quantifications possibly due to analytical challenges are hampering the correct modeling and risk assessment of

these compounds in the environment (Ottosen *et al*., 2019).

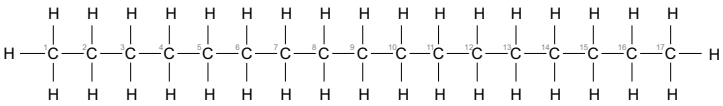
1.3 OPs selected on this study

Five types of sentinel pollutants of high environmental concern in the oceans have been chosen to cover a wide range of physical-chemical properties. We have selected hydrocarbons, which are contaminants internationally recognized as toxic pollutants such as aliphatic and aromatic hydrocarbons (alkanes and PAHs), and two families of organic pollutants of emerging concern (OPEC) (organophosphate esters (OPEs) and perfluoroalkyl acids (PFAAs)) highly relevant in the marine atmosphere. Finally, we have performed exposure experiments with extracted ADOC from seawater that includes both known and unknown compounds, with the aim of accounting realistically for the complex mixture of pollutants in the ocean (see below).

Alkanes

Physico-chemical properties: Alkanes are a group of saturated hydrocarbon molecules, with carbon and hydrogen and single covalent bonds between carbon atoms (Fig. 3). Thus, they tend to be nonpolar and relatively unreactive (Labinger and Bercaw, 2002). They can be linear (n-alkanes), cyclic (cyclo-alkanes) or branched (for example iso-alkanes). In this thesis, we focus only on n-alkanes.

Sources and legal status: These compounds are ubiquitous in the environment and can have both natural and anthropogenic origin. The latter may be due to oil spills, the incomplete combustion of fossil fuels and the entry of refined oils,



heptadecane

Figure 3. Heptadecane, as an example of n-alkane with 17 carbon atoms.

gases and aerosols (Brassell *et al*., 1978). However, some alkanes are produced by phytoplankton, including cyanobacteria (Lea-Smith *et al.*, 2015).

Impact on biota: It has been found that exposure to short chain alkanes causes neurotoxicity and dermal sensitivity, while long chain alkanes have mutagenic and carcinogenic properties (Mckee *et al*., 2015).

Polycyclic aromatic hydrocarbons (PAHs)

Physico-chemical properties: PAHs are organic lipophilic compounds formed by two or more fused benzene rings. The different compounds vary in the number of rings (between 2 and 10), the existence or not of alkyl chains, and fundamentally, in their physical-chemical properties. Naphthalene ($C_{10}H_8$), consisting of two

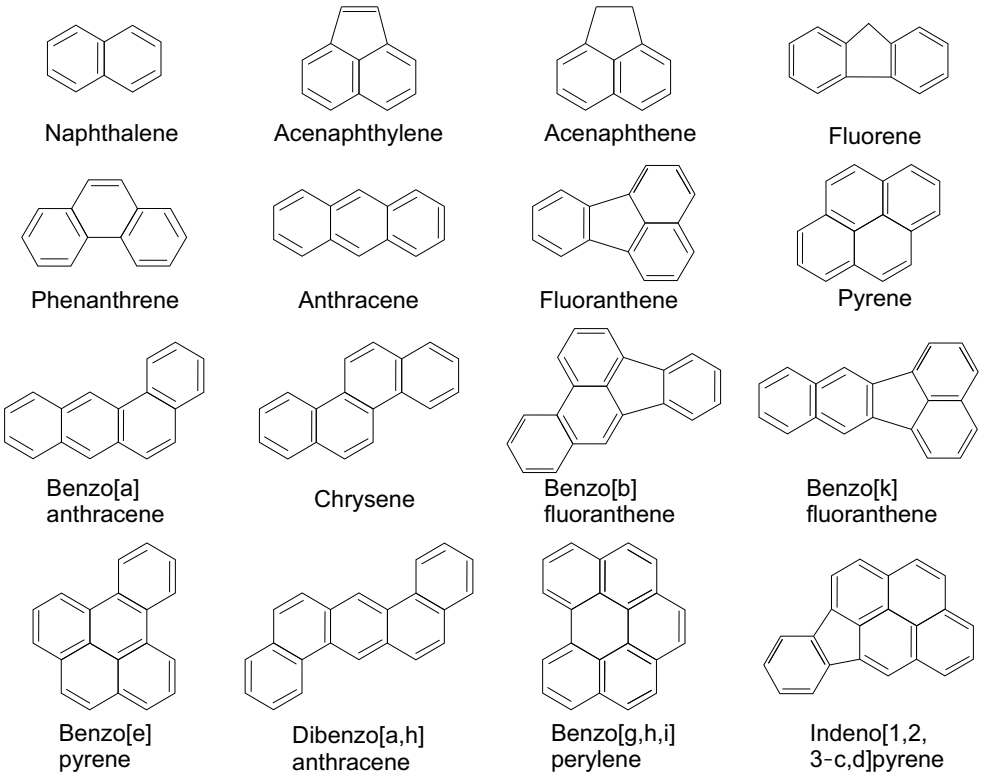


Figure 4. List of Polycyclic aromatic hydrocarbons (PAHs). Selected 16 parent PAHs by the Environmental Protection Agency (EPA) given their toxicity to human health and environment.

fused aromatic rings, is the lowest molecular weight PAH. The aromatic structure of PAHs can include other types of rings, such as five-membered carbon or heteroaromatic rings.

Sources and legal status: These hydrocarbons are mainly originated from the incomplete combustion of organic matter, both anthropogenic (fossil fuels) and natural (forest fires and volcanic eruptions), and are related as well to petrogenic processes. In a minor contribution, there are indicators that suggest a biological origin of PAHs formation, in pigments of fungi, insects and marine organisms (Venkatesan, 1998) or in the degradation of organic matter, mainly diterpenes (Cabrerizo *et al*., 2014). The Environmental Protection Agency (EPA) listed 16 priority PAH compounds based on their toxicity to human health and the environment (Keith and Telliard, 1979; ATSDR, 2009) (Fig. 4), but this list does not include alkylated PAHs or S-containing PAHs, highly abundant in the environment.

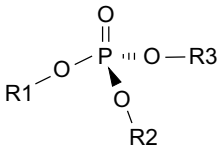
Impact on biota: Due to their great capacity of adsorption to the surface of the particles (Meador *et al*., 1995), and because of its ability to form adducts with DNA, PAHs are highly toxic to organisms, considered mutagenic, teratogenic and carcinogenic (Lehr and Jerina, 1977; Gelboin, 1980) and can alter processes such as cell growth, photosynthesis, respiration and pigment composition (Singh and Gaur, 1988; Cerezo and Agustí, 2015).

Organophosphate esters (OPEs)

Physico-chemical properties: OPEs are organic substances that contain phosphate in their chemical structure (Fig. 5). They are considered organic pollutants of emerging concern (OPEC), not because they are newly synthesized compounds but because they have only been considered for studies and legislation recently (Reemtsma *et al*., 2008).

Sources and legal status: OPEs are synthetic products widely commercialized for many applications, such as to protect or to potentiate the properties of plastics, textiles and other materials as plasticizers or flame retardants (van der Veen and de Boer, 2012). They are used in many cases as substitutes for the polybrominated diphenyl ethers (PBDEs) flame retardants, since PBDEs were banned in the Stockholm Convention in 2009.

Impact on biota: Toxic effects have been detected due to OPEs exposure, for example, TCPP is considered potentially carcinogenic and TCEP is toxic to aquatic organisms and carcinogenic to animals (Lehner *et al*., 2010; van der Veen and de Boer, 2012).



TCEP	Tri(2-chloroethyl) phosphate	**TBEP**	Tributoxyethyl phosphate
TCPP	Tri(chloropropyl) phosphate	**TEHP**	Tri(2-ethylhexyl) phosphate
TDCP	Tri(dichloropropyl) phosphate	**EHDPP**	2-Ethylhexyl diphenyl phosphate
TiBP	Tri-iso-butyl phosphate	**TPhP**	Triphenyl phosphate
TnBP	Tri-n-butyl phosphate	**TCrP**	Tricresyl phosphate

Figure 5. List of Organophosphate esters (OPEs). Generic structure for trimester OPEs and selected compounds in this thesis.

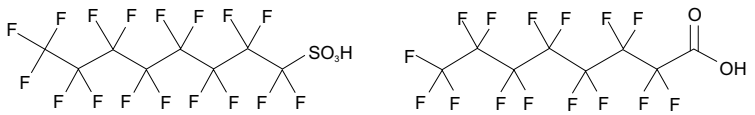
Perfluoroalkyl acids (PFAAs)

Physico-chemical properties: PFAAs are a family of compounds with fluorinated alkyl chains, which makes them highly stable due to the strong fluoride-carbon bond. They are considered OPEC, and consist of two major groups according to head groups, the perfluoroalkyl carboxylic acids (PFCAs) and the perfluoroalkane sulfonic acids (PFSAs). One of the most persistent, bioaccumulative and

biologically active compounds are perfluorooctanoic acid (PFOA, $C_7F_{15}COOH$) and perfluorooctane sulfonate (PFOS, $C_8F_{17}SO_3H$) which belong to the first and second group, respectively (Fig. 6).

Sources and legal status: PFAAs are been widely used for more than 60 years in commercial and industrial products, such as fire-fighting foams, solvents, cosmetics, flame retardants, and in some types of packaging and containers. During the processes of manufacturing, application and residual, PFAAs are released into the environment. The actual presence in the market of some of these compounds is being diminished by the beginning of its regulation. For example, PFOS has been added to the list of the Stockholm Convention in May 2009 (UNEP, 2009), and PFOA are currently being evaluated for listing. However, their precursors and degradation products continue to be produced and released to the environment.

Impact on biota: PFAAs are absorbed in organic matter, accumulate mainly in the liver and in the blood and are excreted very slowly by the kidneys. PFAAs have been commonly detected in human and animal serum from the developed world (Domingo and Nadal, 2017). This ends up affecting the animals´ health, characterized by liver toxicity (Martin *et al*., 2007; Zhang *et al*., 2008), endocrine and metabolism disruption activity (Shi *et al*., 2009; Gao *et al*., 2013), and immunosuppression (DeWitt *et al*., 2008).



PFOS PFOA

Figure 6. Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA).

Anthropogenic dissolved organic carbon (ADOC)

Sources and properties: ADOC comprise hydrocarbons and synthetic organic chemicals. The detailed composition of ADOC is unknown, but it contains tens or hundreds thousand chemicals. Part of them are molecules considered model or sentinel chemicals (PAHs, n-alkanes, etc.), but a large part remains unquantified and uncharacterized (Muir and Howard 2006). For example, in a gas chromatogram of crude oils (Gough and Rowland, 1990) (Fig. 7), resolved peaks appear over a hump of un-resolved compounds, known as unresolved complex mixture (UCM). The UCM in the oceans, that is included in the ADOC, remains poorly characterized chemically, but its mass is hundreds of times higher than the mass of all the resolved peaks (González-Gaya *et al*., 2016; Reddy, 2016). All ADOC chemicals, either if they can be resolved (quantified and identified) or not, will be transported and eventually degraded in the environment depending on their properties, persistence and environmental characteristics. The processes of degradation of organic pollutants occurring during transport from the source to their environmental sinks are intimately coupled with a large diversity of partitioning, advection and aging processes, such as evaporation, dissolution, dispersion, photochemical oxidation, emulsification, microbial degradation, bioaccumulation and adsorption to particulate matter (Zakaria *et al*., 2000). This large diversity of aging processes produce a significant modification of the original composition, generating a great diversity of final transformation products, as not all OPs are fully respired to CO_2. Therefore, ADOC is the sum of all anthropogenic chemicals and their transformation products.

Impact on biota: ADOC is found in marine environments, detected in biota, and they react with organisms, although there is little literature on the subject. The approach used to test the influence of the pool of ADOC compounds on organisms has been done by extracting the OPs from seawater (or sediments). The extract,

containing thousands of chemicals with characteristics depending on the extraction solvent and fractionation scheme, is then used as a mixture of chemicals simulating the mixture found in the environment. Thus, the extract is used as surrogate of ADOC. For example, a recent toxicogenomic approach using seabream hepatocytes has concluded that seawater extracts acted as endocrine disrupting chemicals, inducing gene expression profiles of hormone receptors (Cocci *et al*., 2017). There is also a study proving the lethal effect of water extracts in a zebrafish embryo assay (Carlsson *et al*., 2014), and others focused on the influence on phytoplankton abundance, viability, and gene expression (Echeveste *et al*., 2016; Fernández-Pinos *et al*., 2017; Moeris *et al*., 2019). However, the impact to the heterotrophic microbial compartment remains uncharacterized.

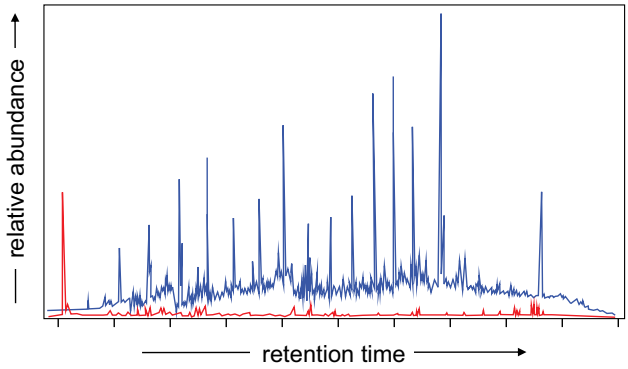


Figure 7. Overlaid chromatogram of a dissolved phase sample of the aromatic fraction. Blue line shows the total ion chromatogram (TIC) obtained by gas chromatography coupled to mass spectrometry (GC–MS) in full scan mode, red line shows the TIC obtained by GC–MS in selected ion monitoring (SIM) mode when targeting the 64 PAHs analyzed. (Adapted from González-Gaya *et al*., 2016).

Individual families of chemicals, such as n-alkanes, PAHs, OPEs and PFAAs are abundant in the marine environment, belonging to ADOC, and because these chemicals can be analyzed quantitatively, they can also be used as surrogate of some of the chemical classes in ADOC. The *in situ* dissolved concentrations of these compounds in seawater range from $1{\cdot}10^4$ to $6{\cdot}10^6$ pg/l for alkanes, 60 to $1{\cdot}10^5$ pg/l for PAHs, 100 to $1{\cdot}10^6$ pg/l for OPEs, 3 to 700 pg/l for PFOA and PFOS, and $6{\cdot}10^4$ to $1{\cdot}10^7$ pg/l for the UCM (for more details see Table 1).

Table 1. Exposure concentration for living marine organisms. Summary of dissolved seawater concentration of selected OP families from some published studies.

Family	Compounds	Location
Alkanes	n-C_{15} — n-C_{32}	Ross Sea (Antarctica)
	n-C_{16} — n-C_{35}	French coast (NW Mediterranean Sea)
	n-C_{14} — n-C_{36}	Black Sea
	n-C_{15} — n-C_{40}	Gulf of Gabès (SE Mediterranean Sea)
	n-C_{12} — n-C_{35}	Malaspina 2010 circumnavigation expedition
PAH	Σ_{15} PAH	Catalan and French coast (NW Mediterranean Sea)
	Σ_{17} PAH	French coast (NW Mediterranean Sea)
	Σ_{19} PAH	Black Sea
	Σ_{19} PAH	Mediterranean and Black Sea
	Σ_{64} PAH	Malaspina 2010 circumnavigation expedition
	Σ_{10} PAH	North–South Atlantic Transect
OPEs	Σ_{8} OPE	Northern Atlantic and Arctic
	Σ_{4} OPE	Yellow Sea and East China Sea
	Σ_{7} OPE	Catalan coast (NW Mediterranean Sea)
	Σ_{10} OPE	Arctic water
PFAAs	PFOA; PFOS	Malaspina 2010 circumnavigation expedition
		Atlantic and Canadian Arctic Oceans
		Arctic Ocean, NW Pacific Ocean and Bering Sea
		Northern Europe, Atlantic and Southern Ocean
		Global survey
		Atlantic Ocean
		North Sea
		Canadian Arctic and sub-Arctic ocean
		Central and South Pacific Ocean and Antarctic region
		North and Baltic seas
UCM	SALCs	Malaspina 2010 circumnavigation expedition
	Aliphatic UCM	Gulf of Gabès (SE Mediterranean Sea)
		Malaspina 2010 circumnavigation expedition
		Black Sea

PAH: polycyclic aromatic hydrocarbons; OPEs: organophosphate esters; PFAAs: perfluoroalkyl acids; UCM: unresolved complex mixture; SALCs: Semivolatile aromatic-like compounds.

Concentration	References
$1.3 \cdot 10^5$ — $2.4 \cdot 10^5$ pg/l	Stortini *et al*., 2009
$9 \cdot 10^4$ — $4.1 \cdot 10^5$ pg/l	Guigue *et al*., 2011
$1.2 \cdot 10^4$ — $5.0 \cdot 10^4$ pg/l	Maldonado *et al*., 1999
$2 \cdot 10^4$ — $6.3 \cdot 10^6$ pg/l	Fourati *et al*., 2018
$5 \cdot 10^4$ — $3.2 \cdot 10^5$ pg/l	Dachs, personal communication
$3.6 \cdot 10^3$ — $3.1 \cdot 10^4$ pg/l	Guitart *et al*., 2007
$4.7 \cdot 10^3$ — $1.5 \cdot 10^5$ pg/l	Guigue *et al*., 2011
108 — 952 pg/l	Maldonado *et al*., 1999
161 — $2.5 \cdot 10^3$ pg/l	Berrojalbiz *et al*., 2011b
$1.9 \cdot 10^3$ — $3.4 \cdot 10^3$ pg/l	González-Gaya *et al*., 2016
58 — $1.1 \cdot 10^3$ pg/l	Nizzetto *et al*., 2008
348 — $8.4 \cdot 10^3$ pg/l	Li *et al*., 2017
$9.2 \cdot 10^4$ — $1.4 \cdot 10^6$ pg/l	Hu *et al*., 2014
$9.5 \cdot 10^3$ — $1.2 \cdot 10^4$ pg/l	Vila-Costa *et al*., 2019
96 — $1.8 \cdot 10^4$ pg/l	McDonough *et al*., 2018
23 — 58 pg/l PFOA; 59 — 742 pg/l PFOS	González-Gaya *et al*., 2014
3 — 259 pg/l PFOA; 9 — 192 pg/l PFOS	Benskin *et al*., 2012
20 — 100 pg/l PFOA; 21 — 60 pg/l PFOS	Cai *et al*., 2012
5.3 — 223 pg/l PFOA; 11 — 232 pg/l PFOS	Ahrens *et al*., 2010
<5 — 439 pg/l PFOA; 1.1 — 78 pg/l PFOS	Yamashita *et al*., 2005
<4 — 229 pg/l PFOA; <10 — 291 pg/l PFOS	Ahrens *et al*., 2009
25 — 300 pg/l PFOA; 12 — 80 pg/l PFOS	Caliebe *et al*., 2004
8 — 182 pg/l PFOA; 24 — 73 pg/l PFOS	Rosenberg *et al*., 2008
<5 — 7 pg/l PFOA; <5 — 22.6 pg/l PFOS	Wei *et al*., 2007
<17 — 90 pg/l PFOA; <14 — 170 pg/l PFOS	Theobald *et al*., 2011
$3 \cdot 10^5$ — $2 \cdot 10^6$ pg/l	González-Gaya *et al*., 2016
$5.4 \cdot 10^6$ — $1.4 \cdot 10^7$ pg/l	Fourati *et al*., 2018
$6.3 \cdot 10^4$ — $5.1 \cdot 10^6$ pg/l	Dachs, personal communication
$2.2 \cdot 10^5$ — $9.3 \cdot 10^5$ pg/l	Maldonado *et al*., 1999

2. Marine microbial communities

Marine microbial communities (consisting of bacteria, archaea, protists, fungi and viruses) are key drivers of biogeochemical cycles in the ocean, such as carbon, nitrogen, phosphorus, sulphur and iron (Gasol and Kirchman, 2018), and constitute about 70% of the total biomass in the sea (Bar-On *et al*., 2018). Traditionally, these very diverse organisms are classified as primary producers (phototrophic or chemotrophic), and heterotrophic secondary producers; but microscopic life in the oceans is much more complex and still poorly understood. This thesis is devoted mostly on prokaryotic organisms and, above all, on heterotrophic bacteria.

Early studies of marine microorganisms, starting at the end of the 19th century, focused on **culturable bacteria**, and isolated pure cultures were necessary in order to identify the taxonomy and test their metabolic capacities (Certes, 1884; Frankland and Frankland, 1894). However, half a century later, it became widely recognized that only a small fraction of the total bacteria present in seawater are cultivable (e.g., Jannasch and Jones, 1959). At the end of the XX century, the advent of molecular tools transformed our capacity to investigate the composition of complex microbial communities. Most of the microbial diversity relied on **PCR** amplification of one gene (typically the 16S, for prokaryotes, or 18S, for eukaryotes, rRNA gene) from environmental samples coupled to clone libraries or denaturing gradient gel electrophoresis (**DGGE**) approaches that provided a view of the taxonomy of the communities from tens to thousands of taxa. From that, the large majority of known marine prokaryotic microorganisms tend to occur in about a dozen generally broad phylogenetic divisions whose relationships can be visualized in phylogenetic trees (Fig. 8). Currently, we take advantage of the affordable high throughput sequencing technology to sequence from thousands to millions of 16S or 18S rRNA genes and refine the trees. However, it is known

that the PCR amplification process may introduce biases in microbial diversity estimates (Acinas *et al*., 2005; Edgar, 2013; Sinclair *et al*., 2015). An alternative to amplicon sequencing has recently become available to microbial ecologists, the possibility to retrieve a number of 16S and 18S reads from **metagenomes** (Logares *et al*., 2014). Additionally, the recent technique called **single-cell genomics (SAGs)** allows retrieval of the genome information of microorganisms one cell at a time (Stepanauskas, 2012). This involves isolating individual cells by flow cytometry and the amplification of its DNA by whole genome amplification. This approach can link novel taxa previously identified with its actual gene repertoire. Both metagenomic and SAGs approaches not only allow to assign taxonomy but allow to know in deep and better definition the potential metabolic capacity of the communities thanks to the high throughput approach. In order

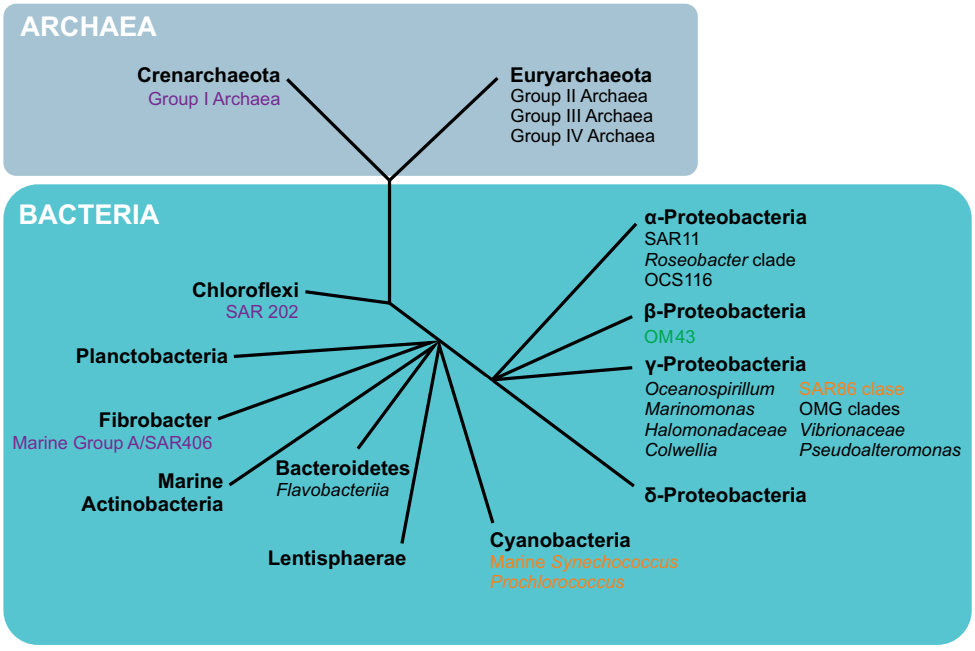


Figure 8. Schematic illustration of the phylogeny of the major Archaea and Bacteria clades, showing only the major marine groups. Purple indicates groups that are mostly found in the mesopelagic and surface waters during polar winter, orange are mostly in the photic zone, and green are mostly coastal. Others seem to be ubiquitous in the seawater. Modified from Giovannoni and Stingl (2005).

to know how bacteria respond to changes in the environment and capture their plasticity to local and global processes, the linkage between diversity and functionality is needed including the distinction between cells that are growing and metabolically active from those that are dormant or dead (Falcioni *et al*., 2008). Many protocols and methodologies have been proposed over the years (see Fig. 9 for examples) but the more recent ones are **metatranscriptomics** (all RNA transcripts from all members of a community), **metaproteomics** (all proteins), and **metametabolomics** (all low-molecular weight metabolites). The factor limiting the omics fields are largely technical, relating to the sensitivity and accuracy of the detection equipment, the quality and quantity of information available in databases, and the limitations of computer storage, processing power, and bioinformatics knowledge. For that reason, the number of scientific publications on the subject decrease the more costly and new the technique is, being metametabolomics a hardly explored discipline in marine ecology.

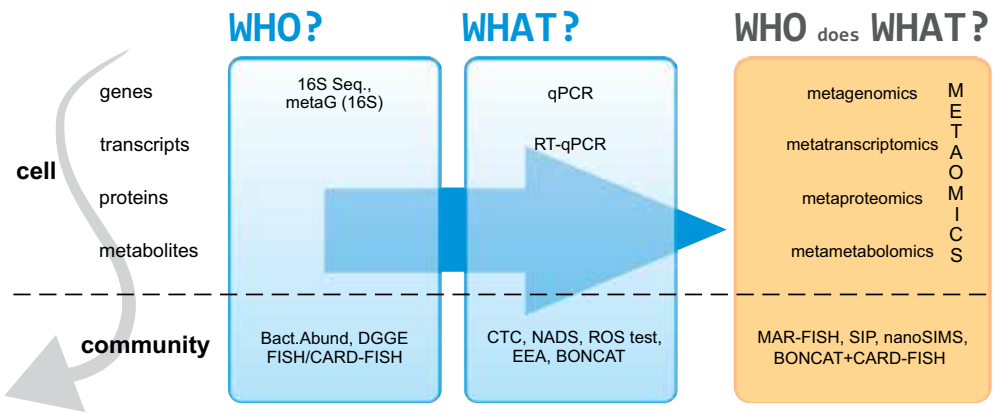


Figure 9. Some of the main approaches available to marine microbial ecologists. DGGE: denaturing gradient gel electrophoresis; FISH: fluorescence in situ hybridization; CARD-FISH: catalyzed reporter deposition fluorescence in situ hybridization; qPCR: quantitative polymerase chain reaction; RT-qPCR: real-time quantitative polymerase chain reaction; CTC: 5-cyano-2,3-ditolyl chloride tetrazolium; NADS: nucleic-acid-double-staining; ROS: reactive oxygen species; EEA: extracellular enzyme activity; MAR-FISH: microautoradiography and fluorescence in situ hybridization; SIP: stable isotope probing; nanoSIMS: Nanoscale secondary ion mass spectrometry; BONCAT: bio-orthogonal non-canonical amino acid tagging.

3. Interaction between OPs and marine microorganisms

Marine microbial communities respond to changes in the composition and concentration of dissolved organic carbon (DOC) (McCarren *et al*., 2010; Mason *et al*., 2012), even if it is a small variations compared to the total DOC (Moran *et al*., 2016). DOC is mostly considered of biogenic origin, whereas the anthropogenic DOC (thus ADOC) is usually explicitly or implicitly neglected, even though it may have an influence on microbial communities and on ecosystems functioning.

Over the past fifty years, the capacity of microorganisms to degrade synthetic organic molecules have been identified and studied with and increasing attention by the scientific community. Some OP-degrading bacteria have been cultured, with a clear bias towards soil bacteria (Goldman, 1972), WWTP and groundwater (e.g. Ng, 2011; Helbling *et al*., 2010, 2012). The assessment of degradation of OPs has been centered to scenarios of bioremediation of contaminated sites (Ying and Kookana, 2003; Benotti and Brownawell, 2009; Muangchinda *et al*., 2017), or as a response to oil spills (Brooijmans *et al*., 2009; Kostka *et al*., 2011; Kimes *et al*., 2014). However, there is relatively little information pertaining to microbial degradation rates at environmental realistic concentrations of anthropogenic compounds in fresh waters (Gröning *et al*., 2007; Knapik *et al*., 2015; Muangchinda *et al*., 2017) and especially in marine or estuarine seawaters (Ying and Kookana, 2003; Benotti and Brownawell, 2009; Kaya *et al*., 2019). Furthermore, few works address the biogeochemical role of biodegradation of OPs at the background concentrations found in the open oceans (Harner *et al*., 2000; Paluselli *et al*., 2018; González-Gaya *et al*., 2019).

Some cultured strains are classified as obligate hydrocarbon degraders belonging to the genera *Oleispira*, *Oleiphilus*, *Thalassolituus*, *Alcanivorax* and *Cycloclasticus* (Head *et al*., 2006; Brooijmans *et al*., 2009), and named as

hydrocarbonoclastic bacteria (HCB, McKew *et al*., 2007). Other microorganisms can degrade OPs without using it as substrate for growth and any energetic benefit through cometabolism (Hazen, 2010; Nzila, 2013). Cometabolism is the process by which a contaminant is fortuitously degraded by a promiscuous enzyme or cofactor during microbial metabolism of another compound. However, it is a mechanism poorly described and still a matter of ongoing research, with largely unknown environmental relevance. Many studies on HCB in the marine environment have been done under oil spills scenarios. For instance, after the catastrophic Deepwater Horizon oil spill in 2010, more than one hundred of research papers have provided information about this episode and the capability of marine bacteria to biodegrade hydrocarbons (for reviews see Kimes *et al*., 2014; Joye *et al*., 2014; King *et al*., 2015; Beyer *et al*., 2016). These studies have demonstrated that multiple HCB operated simultaneously throughout the spill, causing successional dynamics of communities evolving along the changes in hydrocarbons supply. Oil spills favor opportunistic species at short term and enrich methylotrophs and methanotrophs that benefit from methane and other C1 compounds resulting from oil degradation (Kostka *et al*., 2011; Rivers *et al*., 2013; Mason *et al*., 2014). However, the possible biodegradation of hydrocarbon at low concentrations and chronic exposure of other families of the ADOC has been ignored.

In contrast, ADOC may also have a toxic impact on the marine ecosystem, at both biodiversity and ecosystem functioning in a given context, being one of the least-studied stressors in ecology (Lawler *et al*., 2006). OPs cross cell membranes and cause a baseline toxicity to the cells by narcosis, the non-specific intercalation of hydrophobic compound into the phospholipids of the biological membranes, inducing a perturbation of the membrane's permeability, rigidity and efficiency (van Wezel and Opperhuizen, 1995; Cronin *et al*., 2000; Escher *et al*., 2017).

Johnston and colleagues (Johnston *et al*., 2015) performed a systematic review from 264 relevant published studies of the effects of contaminants on marine ecosystems function and concluded that up to 70% of studies proved negative impacts on primary production, reducing productivity and increasing respiration. Most of the experiments were done with phytoplankton and using doping concentrations orders of magnitude higher than those found in the environment, mostly resulting in lethal effects (Head *et al*., 2006; Gilde and Pinckney, 2012; Echeveste *et al*., 2016), but also at sub-lethal effects (Othman *et al*., 2012; Ozhan *et al*., 2014; Everaert *et al*., 2015). The result of these experiments confirm that the smaller phytoplanktonic cells are more sensitive to ADOC than larger algal cells (Echeveste *et al*., 2010a,b), and in some cases, ADOC reduce cells efficiency and abundance, alter cell growing processes, respiration and photosynthesis (Echeveste *et al*., 2010a, 2016; Cerezo and Agustí, 2015; Fernández-Pinos *et al*., 2017; Moeris *et al*., 2018). Recently, Tetu *et al*. (2019) have investigated the effect of plastic leachate exposure on marine *Prochlorococcus* and showed a negative impact on growth and photosynthetic capacity. But the potential effects of contaminants are commonly tested on organisms, or cell lines, in separate tests, neglecting the realistic biotic interactions and processes that would naturally be occurring in the bacterial community of contaminated ecosystems. Indeed, studies that included multiple components of the ecosystem are more likely to find small or no effect of contamination, possibly due to ecological interactions and resilience response of the system (Johnston *et al*., 2015). The understanding of toxic effects will remain patchy until direct measures of whole communities, pollutants and abiotic conditions are undertaken within multiple ecosystems, that is, performing multi-disciplinary ecological studies.

Thus, the interaction between marine microbial communities and the pool of ADOC present in the water column might have an impact in both directions

with positive and negative feedbacks (Fig. 10). ADOC can potentially be toxic affecting negatively the community composition and functionality, on the contrary, can be stimulating through the supply of partially labile dissolved organic matter to the cells. On the other direction of the interaction, microorganisms can alter the pool of ADOC by biodegradation or bioactivation (that is, the metabolic activation of ADOC into a more toxic compound). Due to the lack of data, these interactions are neglected in all current modelling approaches of ADOC cycling in the environment, and despite indirect field evidences, the effects of ADOC on microbial communities and how microbial activities modify ADOC concentrations are virtually uncharacterized.

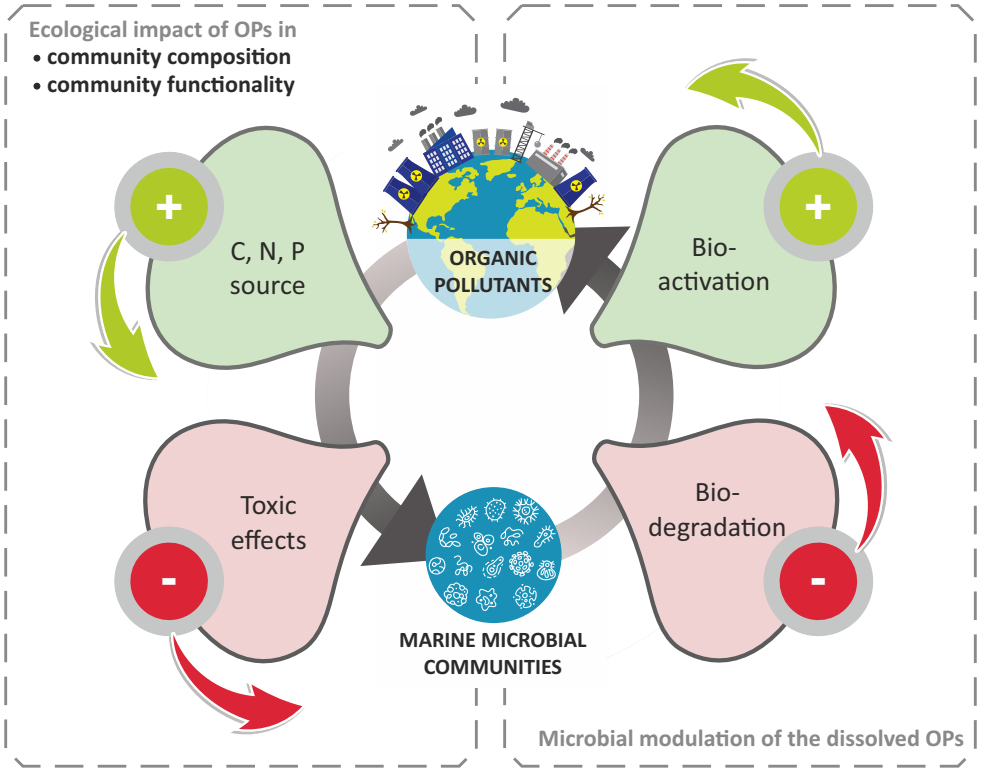


Figure 10. Interaction between marine microbial communities and OPs.

THESIS AIMS

This thesis aimed to explore the impact of background concentrations of ADOC on marine microbial communities in contrasted coastal systems, both in terms of diversity and functionality, comparing communities used to live in different trophic conditions and baseline pollutant concentrations. The novelty lies in the study of the interaction between bacteria and organic pollutants by adding ADOC concentrations in the range or slightly above their environmental variability, in all cases orders of magnitude lower than concentrations used in many toxicological bioassays, or those present in oil-spill scenarios.

The following **hypotheses** were tested in this thesis:

H1: Marine microbial communities are affected by trace amounts of single families or by the complex mixture of pollutants at composition and functional levels. The responses may be taxon-specific.

H2: An original community more adapted to pollutants will be less affected than communities occurring in more pristine systems.

H3: Families of legacy and organic contaminants of emerging concern (OCEC) with contrasted physicochemical properties have differential impacts on the communities.

The working hypothesis of this thesis was that the ubiquitous complex mixture of organic pollutant found in oceans, including unknown and un-characterized pollutants, is affecting in a significant way the composition and cell functioning of marine microbial communities and some of the key ecosystem functions they mediate. Furthermore, microbial activities modify dissolved OP concentrations, thus the role of the microbial pump needs to be included in any OP modeling

approach. **Therefore, the general objective was to analyze the effects of complex mixture of OPs found in seawater at environmentally relevant levels on the metabolic capacity of heterotrophic microbial communities by measuring physiological responses and gene expression profiles of natural coastal communities from contrasted coastal seawaters with varying OP supplies**. The genomic approaches essentially queried the bacterioplankton to identify which components of the OP pool they are capable of transforming, and unmask how mixtures of OPs affect the gene expression of bacterial communities. Although this approach does not provide quantitative measures of OP fluxes, it, indeed, has the potential to offer profound insights into OP-carbon cycling.

The specific **objectives** of this project were to:

1) Determine if OPs of emerging concern (OPEs and PFAAs) and hydrocarbons (n-alkanes and PAHs) have similar effects under a gradient of trophic and pollution conditions (Chapter 1).

2) Determine which microbial groups are more sensitive to OPs, and which ones are more resilient, and relate these responses to their genetic content (all Chapters).

3) Determine the impact of exposure of ADOC compounds to the biogeochemistry of relevant nutrients of the ocean (sulfur and carbon cycle, Chapter 2, 3 and 4).

4) Elucidate if pre-adapted communities have a higher resilience coping with OPs than more pristine communities (Chapter 1, 3 and 4).

5) Assess if surface ADOC concentrations originated by diffusive pollution have an outsized effect on the functioning and structure of polar and Mediterranean marine microbial communities and determine how marine microorganisms cope

with OPs and which key ecosystem processes mediated by bacteria are affected (Chapters 3 and 4).

The long-term aim of this thesis was to be a building block of our understanding on how the anthropogenic perturbation of the organic matter pool influences marine microbial communities and the microbial role in the OPs marine cycle, especially as a driver of the degradation of OPs in the marine environment.

A BRIEF OVERVIEW OF METHODOLOGY

Experiments and sampling sites

In order to address the objectives and test the hypotheses, we performed *in situ* experiments with seawater, combining chemical, molecular and genomic techniques in contrasted sites in exposure experiments. **Chapter 1** summarizes five experiments performed at different sites and depths to test the individual

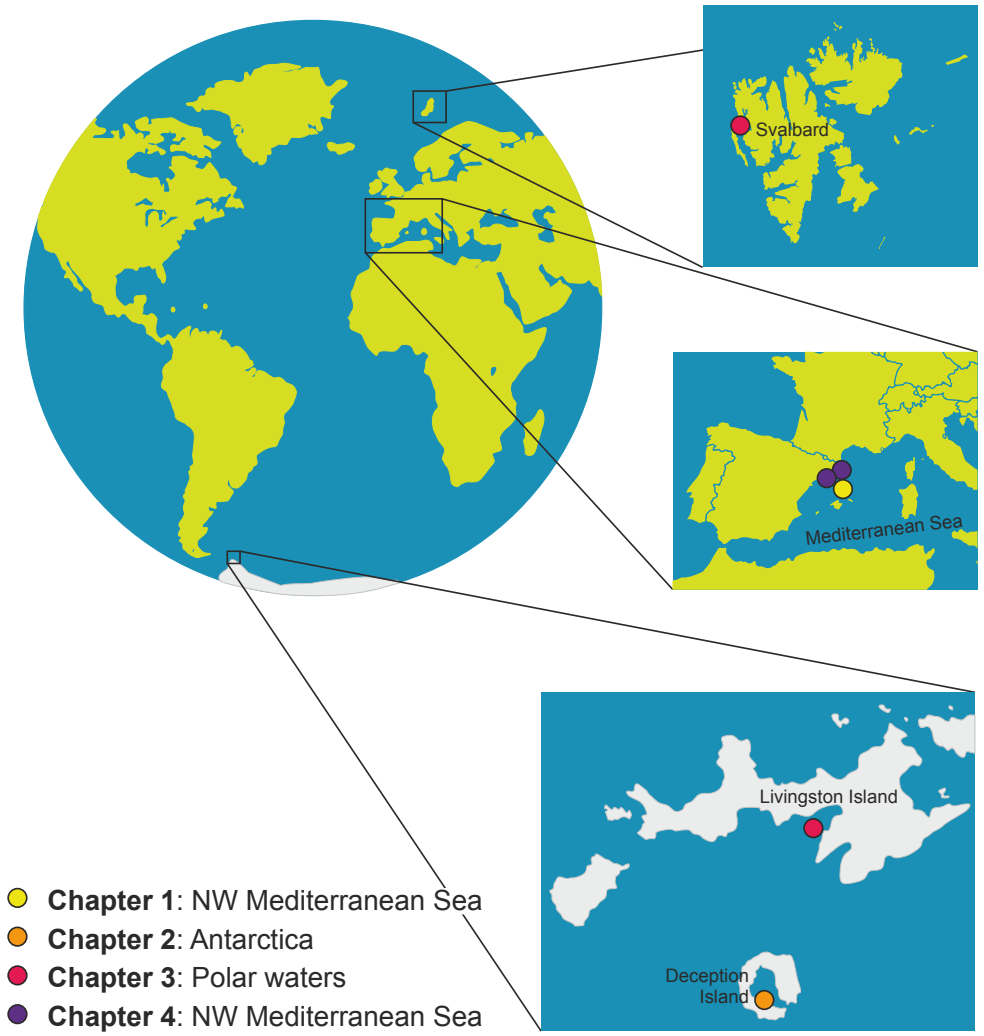


Figure 11. Map of experiment locations.

addition of OP families (n-alkanes, PAHs, OPEs, and PFOS) to the marine microbial communities in a NW Mediterranean transect. In **Chapter 2,** the interaction between a mix of PFOS and PFOA and microorganisms was studied in exposure experiments done in Deception Island (Antarctica) under a range of different concentrations and incubation times.

After testing the interaction between microbial communities and one family of organic pollutants, in Chapters 3 and 4 we analyzed the impact of ADOC, previously extracted from *in situ* seawaters, in four sampling sites, with contrasted trophic and polluted conditions. In **Chapter 3**, experiments were performed in pristine and low polluted polar regions in Svalbard (Arctic) and Livingston Island (Antarctica). In **Chapter 4**, experiments were performed with coastal seawater from NW Mediterranean Sea, Barcelona city (highly polluted and eutrophic) and Blanes Bay (less polluted and rather oligotrophic) (Fig. 11).

Methodological approaches

In general, there are two basic methodological approaches currently being adopted in this thesis; (1) experiments that challenge bacterial communities with **increasing concentrations of OPs**, so-called dose-response experiments, and (2) experiments with addition of OPs concentrations **within environmental ranges.**

Effects of OPs in marine microbial communities during incubational experiments are monitored in both directions, how OPs affect marine bacterial communities but also how the fate of OPs is modified by biota. In order to achieve these results, various chemical and biological techniques have been applied (for more information see Fig. 12 and the methods section in each chapter).

It is important to mention the use of molecular methods in this thesis, such as

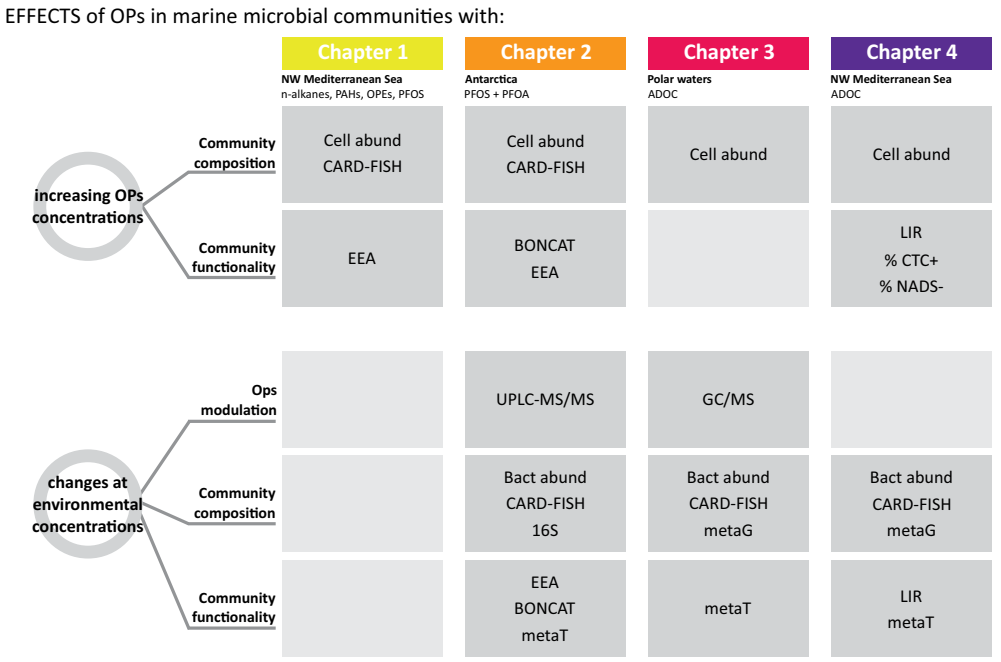


Figure 12. Summary of techniques used in each chapter.

amplicon sequencing of 16S rRNA, metagenomics and metatranscriptomics. The recent advances in molecular approaches have significantly improved our understanding and increased the visibility of many parts of ecological communities. Thus, enabling us to detect early effects or responses at low concentrations of contamination. The set of all the techniques applied in this thesis is briefly described below.

OPs modulation:

- **UPLC-MS/MS**: is a triple-quadrupole mass spectrometer, an instrumental chemical technique that combines the physical separation capabilities of liquid chromatography with the mass analysis capabilities of mass spectrometry. PFOS and PFOA have been quantified with this methodology.

- **GC/MS**: is an instrumental technique, comprising a gas chromatograph (GC)

coupled to a mass spectrometer (MS), by which complex mixtures of semi-volatile chemicals may be separated, identified and quantified. This makes it ideal for the analysis of the hundreds of relatively low molecular weight compounds found in environmental samples. In this thesis, this technique is used for the quantification of n-alkanes, PAHs and OPEs.

Community composition:

- Flow cytometry: Flow cytometry allows to quantify the cell abundance. Heterotrophic prokaryotes cells can split into low and high nucleic acid content (LNA and HNA, respectively).

- CARD-FISH (CAtalyzed Reporter Deposition Fluorescence *In Situ* Hybridization): it allows to quantify the abundance of specific taxonomic groups by hybridization with specific phylogenetic probes and epifluorescence microscope counting.

- 16S (16S rRNA): due to the slow rates of evolution of this region of the gene, the sequencing of 16S ribosomal RNA is highly used for extracting taxonomic information of bacteria and archaea.

- metaG (metagenomics): total DNA from an entire community is sequenced using a next-generation sequencing platform. Resulting sequences are assigned to taxonomic/functional genes using a reference database. Information on taxonomy and potential function can be gained from this technique.

Community functionality:

- EEA (Extracellular Enzyme Activity): is a technique that determined fluorometrically using fluorogenic substrates that emit fluorescence after cleavage of the molecule by the specific enzyme β-glucosidase (β-Glc), L-leucyl aminopeptidase (Leu-amp), alkaline phosphatase (APA) and chitinase (Chit).

- BONCAT (Bio-Orthogonal Non-Canonical Amino acid Tagging): is based on the *in vivo* incorporation of the non-canonical amino acid L-azidohomoalanine (AHA), a surrogate for L-methionine, followed by fluorescent labelling of AHA-containing cellular proteins by azide-alkyne click chemistry. It is used to visualize translationally active cells within complex environmental samples.

- LIR (Leucine incorporation rates): The leucine method for estimating bacterial production consists of measuring the incorporation of radiolabelled leucine (L-[4,5-^{3}H] leucine) into bacterial protein over time.

- %CTC+: the CTC (5-cyano-2,3-ditolyl chloride tetrazolium) is used to quantify the percentage of bacteria actively participating in cell respiration and those that do not relative to the bacterial abundances obtained from cytometry counts.

- %NADS-: The Nucleic-Acid-Double-Staining (NADS) viability protocol is used to enumerate the cells with intact (NADS+) versus damaged (NADS-) membranes, counted by flow cytometry.

- metaT (metatranscriptomics): total RNA (rRNA and mRNA, but only rRNA in our case) from an entire community is sequenced on a next-generations sequencing platform. Resulting sequences are assigned to known active taxonomical and functional genes of the community.

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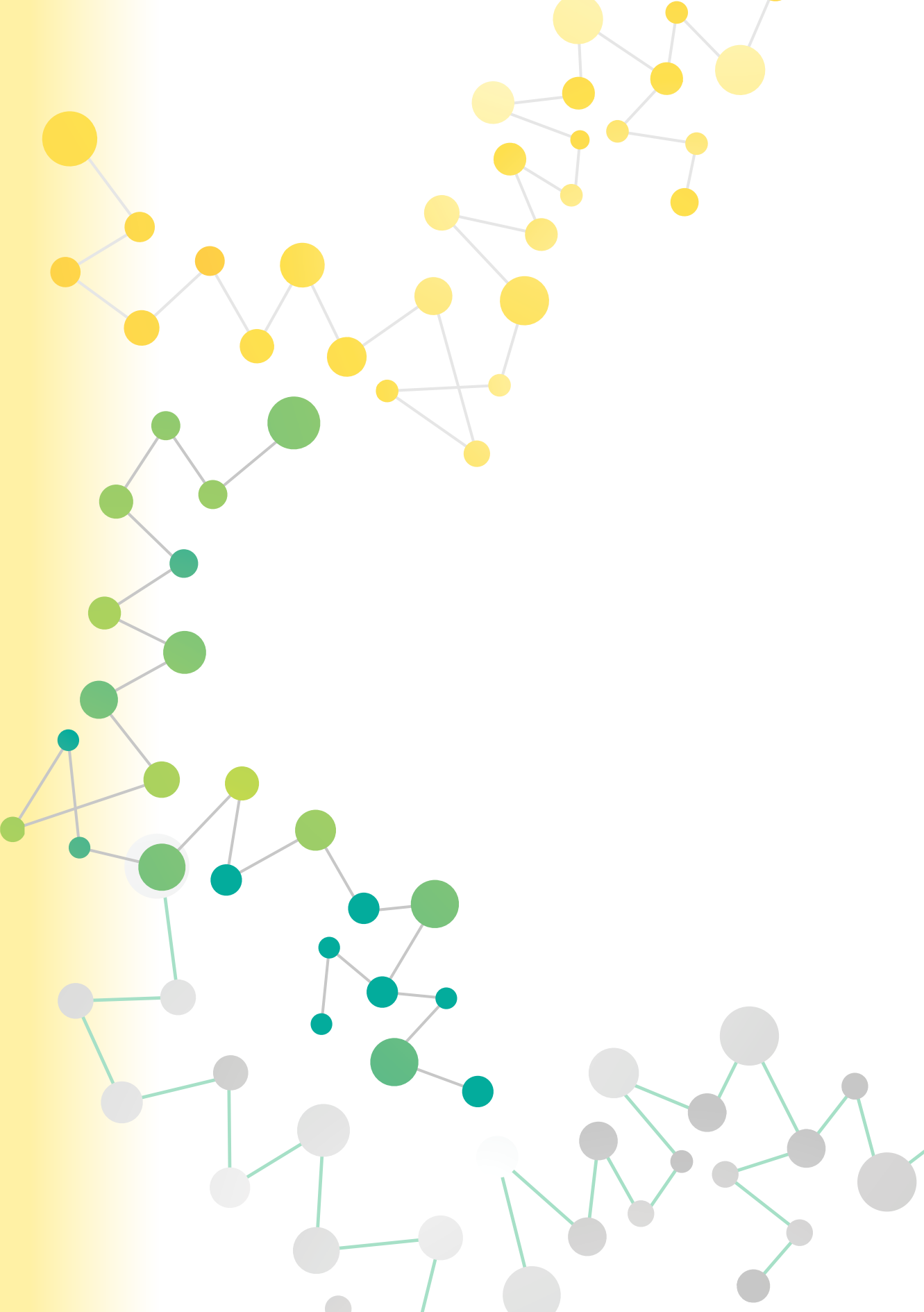
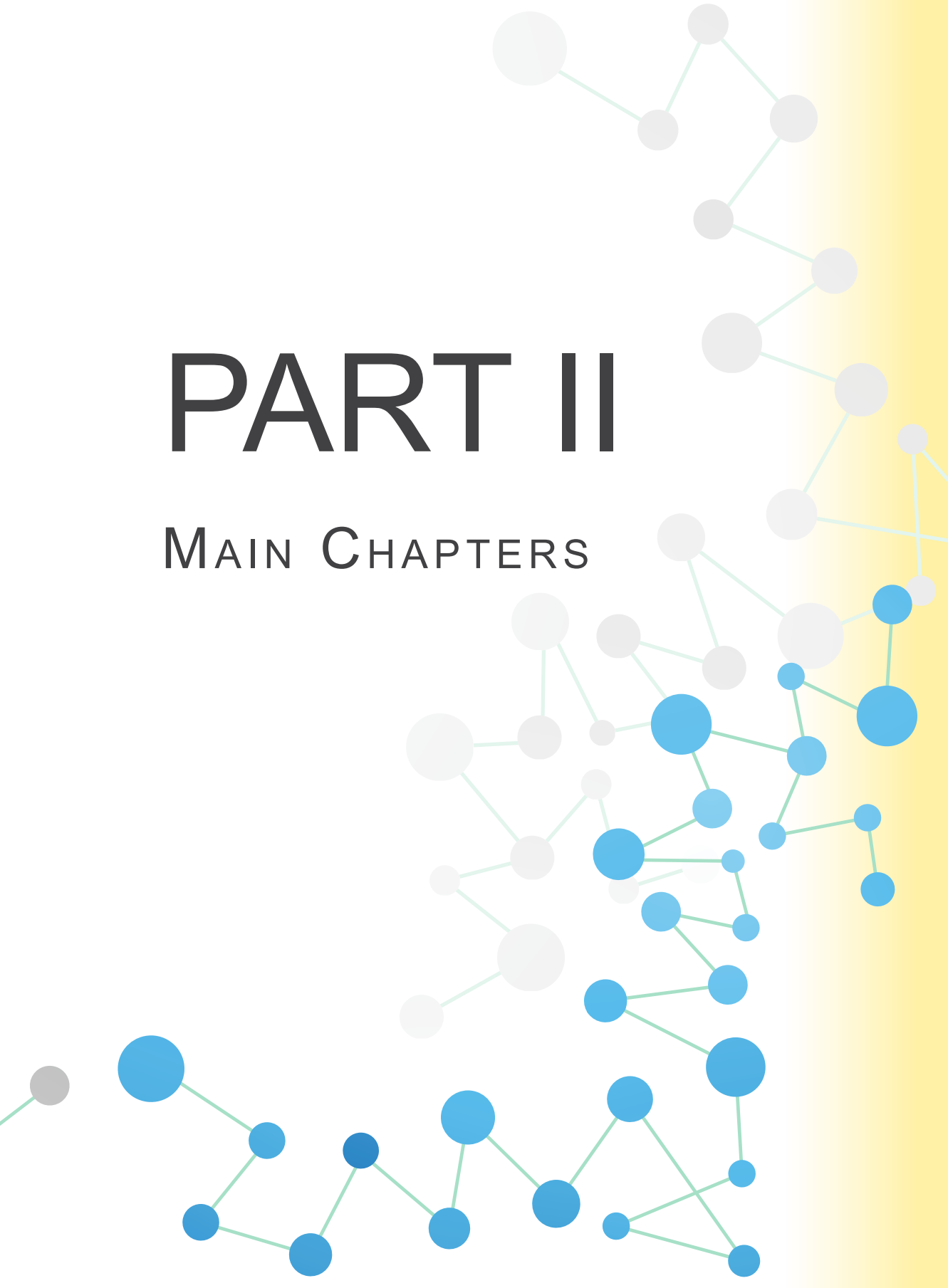


PART II

Main Chapters



Modulation of microbial growth and enzymatic activities in the marine environment due to exposure to organic contaminants of emerging concern and hydrocarbons

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ABSTRACT

Organic pollutants are continuously being introduced in seawater with uncharacterized impacts on the engines of the marine biogeochemical cycles, the microorganisms. The effects on marine microbial communities were assessed for perfluoroalkyl substances, organophosphate esters flame retardants and plasticizers, polycyclic aromatic hydrocarbons, and n-alkanes. Dose-response experiments were performed at three stations and at three depths in the NW Mediterranean with contrasted nutrient and pollutant concentrations. In these experiments, the microbial growth rates, the abundances of the main bacterial groups, measured by Catalyzed Reporter Deposition Fluorescence *In Situ* Hybridization (CARD-FISH), and extracellular enzymatic activities, were quantified. Increasing concentrations of organic pollutants (OPs) promoted different responses in the communities that were compound, organism and nutrient availability (trophic status). The largest differences between OP treatments and controls in the growth rates of both heterotrophic and phototrophic microbial groups were observed in seawater from the deep chlorophyll maxima. Furthermore, there was a compound specific stimulation of different extracellular enzymatic activities after the exposure to OPs. Our results revealed that marine microbial communities reacted not only to hydrocarbons, known to be used as a carbon source, but also to low concentrations of organic pollutants of emerging concern in a complex manner, reflecting the variability of various environmental variables. Multiple linear regressions suggested that organic pollutants modulated the bacterial growth and extracellular enzymatic activities, but this modulation was of lower magnitude than the observed pronounced response of the microbial community to nutrient availability.

INTRODUCTION

Several families of organic pollutants of emerging concern (OPEC) share the properties of persistent organic pollutants (POPs), such as bioaccumulation potential, persistence, long-range transport potential, and toxicity (Sauvé and Desrosiers, 2014), yet they may have not been regulated internationally. These compounds include plasticizers, flame retardants, pharmaceuticals, surface treatments, sealants, etc. Some of them, such as perfluoroalkyl substances (PFASs) and organophosphate ester (OPE) flame retardants and plasticizers, are of special interest in the marine environment due to their persistence, global distribution, and occurrence at concentrations comparable to those of the highly abundant polycyclic aromatic hydrocarbons (PAHs) (Castro-Jiménez *et al.,* 2014; González-Gaya *et al.,* 2014, 2016; Li *et al.,* 2017).

Despite the refractory nature of most POPs, it is widely accepted that some OPs are susceptible to at least partial microbial degradation under favorable environmental conditions (Boyd *et al.,* 2008). Microbial degradation has been identified as one of the main biogeochemical drivers of the occurrence of OPs in the marine water column (Galbán-Malagón *et al.,* 2013; González-Gaya *et al.,* 2019; Vila-Costa *et al.,* 2019). However, both the conditions favoring marine OP biodegradation and the main players in microbial degradation remain unknown. Marine microbial communities are composed by diverse and versatile microorganisms with genetic adaptability against nutrient and pollution stresses (Chakraborty and Das, 2016). However, whereas the availability of inorganic and organic nutrients is recognized as an important factor determining the function of microbial communities (Thingstad *et al.,* 1998, 2005; Sala *et al.,* 2002; Pinhassi *et al.,* 2006; Treusch *et al.,* 2009; Sunagawa *et al.,* 2015), the influence of background pollution on marine microbial communities remains

poorly understood. Importantly, most studies concur that under the selection pressure created by pollutants, some bacteria proliferate better than others (van der Meer, 2006), either because they can degrade the pollutants or because they are resistant or relatively insensitive to the toxic effects (Echeveste *et al.,* 2016; Cerro-Gálvez *et al.,* 2019). Whereas the influence of aliphatic and aromatic hydrocarbons on microbial communities has received extensive attention under marine oil spills scenarios (e.g. Mason *et al.,* 2012), the effects of hydrocarbons at concentrations in the range of their variability in the ocean resulting from diffusive pollution inputs have been overlooked. In addition, little is known about the impact of OPEC except for their contribution to the complex mixture of OPs found in seawater (Hutchinson *et al.,* 2013; Echeveste *et al.,* 2016; Fernández-Pinos *et al.,* 2017; Cerro-Gálvez *et al.,* 2019; Vila-Costa *et al.,* 2019).

OPs, such as n-alkanes, PAHs, OPEs and PFASs have the potential for long-range atmospheric or oceanic transport, some bioaccumulate and biomagnify in biota, and are toxic (Safe, 1994; Berrojalbiz *et al.,* 2011a; Hutchinson *et al.,* 2013). Most PFASs are persistent in the environment, as so far, no field study has demonstrated their degradation (Rivers *et al.,* 2013; Dombrowski *et al.,* 2016; Kleindienst *et al.,* 2016; Wang *et al.,* 2017), but PAHs, n-alkanes and OPEs can be biodegraded in marine waters (Bagby *et al.,* 2017; González-Gaya *et al.,* 2019; Vila-Costa *et al.,* 2019). Their occurrence has been documented for the Mediterranean Sea (Berrojalbiz *et al.,* 2011b; Castro-Jiménez *et al.,* 2014; Brumovský *et al.,* 2016; Vila-Costa *et al.,* 2019) and other oceanic regions (González-Gaya *et al.,* 2014, 2016; Li *et al.,* 2017). Although the influence of hydrocarbons and emerging pollutants at low background concentrations to ecosystem functioning remains unknown, chemical pollution, in general, has been identified as a vector of global change (Rockström *et al.,* 2009).

The northwestern Mediterranean Sea is a highly populated, touristic and industrialized region, and receives a large amount of persistent and non-persistent OPs through river inputs, wastewater discharges, continental runoff and atmospheric deposition (Berrojalbiz *et al.,* 2011a; de Madron *et al.,* 2011; Castro-Jiménez *et al.,* 2012). Concentrations of micropollutants in water show spatial gradients from coastal to off-shore waters, with higher concentrations in shallow coastal waters and low levels in the open sea (García-Flor *et al.,* 2005; Berrojalbiz *et al.,* 2011a; Castro-Jiménez *et al.,* 2013). Particle phase POPs are known to follow a surface to depth depletion pattern (Dachs *et al.,* 1997), even though the levels of OPs in the dissolved phase may show a different vertical distribution as seen in the few data available for the Mediterranean (Berrojalbiz *et al.,* 2011a; Brumovský *et al.,* 2016). The concentrations of OPs in the Mediterranean Sea, especially coastal waters, are higher than in the oligotrophic subtropical oceans, which implies that the resident microbial communities are more habituated to the presence of synthetic chemicals and hydrocarbons. For example, the picocyanobacteria populations of *Prochlorococcus* and *Synechococcus* genera from the Mediterranean have been shown to be less sensitive to OPs than those from the NE Atlantic Ocean (Echeveste *et al.,* 2016). Therefore, addition of OPs could induce different responses depending on the *in situ* concentrations.

Large areas of the Mediterranean Sea are oligotrophic due to phosphorus scarcity including the NW basin (Thingstad *et al.,* 1998, 2005; Marty and Chiavérini, 2010). Additionally, the NW Mediterranean Sea is characterized by a large seasonal variability, typical of temperate regions, with intense mixing process during winter and strong stratification during summer. These conditions result in an annual gradient of nutrient concentration and in small-scale spatial heterogeneity (García-Martínez *et al.,* 2019). The physical, chemical and biological gradients might modify and modulate the variability of the bacterial community structure and their

function as demonstrated in several regions (Alonso-Sáez *et al.,* 2007; Coles *et al.,* 2017). In Mediterranean waters, nutrients, together with organic matter, are well known drivers of this variability (Thingstad *et al.,* 1998; Segura-Noguera *et al.,* 2016). Nevertheless, we hypothesized that other environmental drivers/ stressors like OP availability may also play a role in modulating the dynamics of microbial communities (Echeveste *et al.,* 2010b; Duarte, 2014).

Most of the previous experimental studies testing the effects of organic pollutants on marine microorganisms deal with bioremediation and toxicology, and use doping concentrations orders of magnitude higher than those found in the environment, in most cases resulting in lethal effects (Head *et al.,* 2006; Echeveste *et al.,* 2010b). Unfortunately, the impacts of realistic concentrations of organic pollutants on microbial communities have been historically neglected. This work aimed to fill this lack of knowledge by adding concentrations of OPs in the range or slightly above their environmental variability, in all cases orders of magnitude lower than concentrations used in most toxicological bioassays or those recorded in oil-spill scenarios.

Previously, quantitative structure (chemical) activity relationships (QSAR models) have been proposed to predict the biodegradation of OPs (Escher *et al.,* 2017). These models are widely used in environmental risk assessments (Mackay *et al.,* 2014), as the more recalcitrant pollutants pose a higher risk to ecosystems (Safe, 1994). However, these models have considered only the influence of the chemical structure, but not of the trophic status of the target ecosystem nor the microbial community structure. We aimed to 1) test the responses of marine microorganisms under a gradient of trophic and pollution conditions in the NW Mediterranean Sea in order to discern whether these are compound and/or concentration dependent, and 2) determine the role played by bacterial/ prokaryotic/picoplankton community composition and the ecosystem trophic

status (nutrient availability) modulating this response to exposure to OPs. To this end, we assessed the effects of n-alkanes, PAHs, OPEs and perfluorooctane sulfonate (PFOS) at increasing concentrations in a longitudinal and vertical transect in the NW Mediterranean (at three locations and for three depths). We hypothesized that the composition and function of the microbial communities were affected by small amounts of organic pollutants, and that populations acclimated to live under higher pollutant concentrations (that is, those in the coastal stations) will show a more buffered response to the toxicological effects of OPs and a higher degradation efficiency, as previously postulated (van der Meer, 2006).

MATERIAL AND METHODS

Field sample collection and experimental design

During the MIFASOL-2 sampling cruise on board R/V Garcia del Cid, from 22nd to 25th October 2015, we performed experiments with surface waters (5 m depth to avoid the influence of the ship) at three different locations (Coastal, Mid and Off-shore stations) along a transect between the Catalan coast and the Balearic Islands (NW Mediterranean sea) (Fig. 1, Table 1). Two additional experiments were performed in the Off-shore station with water from the deep chlorophyll maximum (DCM) within the photic zone (65 m depth, station labeled as DCM) and in the bathypelagic zone at 2000 m depth (station labeled as Deep). Experiments were carried out by challenging natural populations separately with four different commercial mixtures of organic pollutants dissolved in acetone (n-alkanes, PAHs, OPEs, and PFOS) at three different concentrations (approximately 6x, 50x and 500x *in situ* concentrations) (see details in Table S1) and a control only with acetone, without OP addition (thus *in situ* concentration). In the case of PAHs and n-alkanes, 500-fold the *in situ* concentrations of the resolved

PAHs and n-alkanes (analyzed as explained below) was representative of the total concentration of hydrocarbons including the unresolved complex mixture (González-Gaya *et al.,* 2016). In the case of OPEs and PFASs, the total amount of anthropogenic organic phosphorus (P) and fluorine (F) remains unknown (McDonough *et al.,* 2018; Pantelaki and Voutsa, 2019), but it is presumably orders of magnitude higher than the amount of PFOS used in the experiments, and the few OPEs added in the treatments with P-containing OPs. Furthermore, high concentrations of PFASs, PAHs, n-alkanes and OPEs have been reported for rivers and coastal waters (e.g. Campo *et al.,* 2015). For PAHs and n-alkanes, the range of concentrations spiked in the experiments is representative of the total hydrocarbons (including the unresolved complex mixture, UCM) found in marine waters from estuaries to open seas (Maldonado *et al.,* 1999; Fourati *et al.,* 2018). The highest concentrations of OPEs and PFOS spiked are higher than those described for the same compounds in the marine environment, but probably not far to the unknown total concentrations of OPEs and PFASs.

Incubations were performed during 48 h in previously baked 40 ml glass vials in the dark and at *in situ* temperature. The collected seawater was added after the volatilization of the solvent in the open vials under the hood at room temperature (approximately 2 h). Experiments were run in duplicates. Samples for quantification of bacterial abundance by flow cytometry were taken at initial time point, and after 4 h, 24 h and 48 h of incubation, and for controls and the treatments at the three different concentrations. Samples for Catalyzed Reporter Deposition Fluorescence *In Situ* Hybridization (CARD-FISH) and bacterial extracellular enzyme activity measurements were obtained at the initial and final time points for the controls and treatments with the highest concentrations.

Analysis of the *in situ* dissolved phase concentrations of organic pollutants

Twenty liters of surface water (1 m depth) were collected with metal carboys at the three stations where experiments were carried out. Seawater was passed through a system composed of a pre-combusted glass fiber filter (GF/F, 0.7µm pore size (Whatmann)) and a XAD-2 column to separate particulate and dissolved phases, respectively. XAD-2 columns were kept at 4°C and processed in the laboratory within 35 days. Organic compounds were extracted from XAD-2 as described before (Berrojalbiz *et al.,* 2011a; Galbán-Malagón *et al.,* 2013; Castro-Jiménez *et al.,* 2016). Briefly, the XAD-2 column was eluted with 200 ml of methanol followed by 300 ml of dichloromethane, adding a surrogate standard to each fraction. The methanol fraction was liquid-liquid extracted with hexane and merged with the dichloromethane fraction. (Berrojalbiz *et al.,* 2011; Galbán-Malagón *et al.,* 2013; González-Gaya *et al.,* 2016). After concentration by rotary vacuum evaporation to 0.5 ml, the extract was fractionated on an alumina column to separate the chemicals into three fractions depending on their polarity (aliphatic, aromatic and polar fractions). The first and second fractions (fractionated by elution using 5 ml of hexane and 12 ml of a mixture of hexane:dichloromethane 1:2, respectively) were concentrated to 50 µl and used to quantify n-alkanes and PAHs. OPEs were analyzed from the second and third fraction (fractionated by elution using 15 ml of dichloromethane:methanol 2:1). All OPs were quantified by gas chromatography coupled to a mass spectrometer (GC–MS). Levels of OPs in the field blanks (XAD columns processed analogously than samples), when present, were significantly lower than in samples, so concentrations were not corrected for blanks. The relative error of measures of OP concentrations in seawater at trace levels using XAD columns is of 15%. Limits of quantification (LOQs) for individual OPs were derived from the field blanks (when present) or the lowest standard of

the calibration curve that could be quantified and are listed in Table S2.

Analytical procedures for nutrients

Sub-samples (15 ml) for dissolved inorganic nutrient (nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+), silicate (SiO_4^{4-}) and phosphate (PO_4^{3-})) were kept at -20ºC until analyses, which were done by standard segmented flow with colorimetric detection (Grasshoff *et al.,* 2009) using a SEAL Analyzer AA3 HR. Total organic carbon (TOC) and total nitrogen (TN) were collected in pre-combusted (24 h, 450°C) glass ampoules. H3PO4 was added to acidify the sample to pH 2 and the ampoules were heat-sealed and stored in the dark at 4°C until analysis. Samples were measured with a SHIMADZU TOC-5000 analyzer, following the high-temperature catalytic oxidation (HTCO) technique (Cauwet, 1994). For the inorganic nutrients analysis, detection limits (defined as three times the standard deviation of ten replicates at 50% diluted samples) were 0.006 µM for NO_3^-, 0.003 µM for NO_2^-, 0.003 µM for NH_4^+, 0.016 µM for SiO_4^{4-}, and 0.01 µM for PO_4^{3-}. The coefficients of variations of all inorganic nutrients were <0.50%. The detection limit was 50 µg/l and 0,356 µM for TOC and TN, respectively. And the coefficients of variations was 1.5% and 3%, respectively.

Cell abundance estimation

Cell abundances were quantified in duplicate aliquots of 1.8 ml taken from each bottle that were fixed with 1% buffered paraformaldehyde solution (pH 7.0) plus 0.05% glutaraldehyde, left at room temperature in the dark for 10 min and stored at -80ºC until processed in the laboratory. Samples were stained using 10x SybrGreen I before being counted in a Becton Dickinson FACSCalibur flow cytometer (BD Biosciences), as described in Gasol and Moran (2015). Briefly, at each time point, a 1.8 ml subsample was fixed with 1% paraformaldehyde solution with 0.05% of glutaraldehyde (pH 7.2), incubated for 10 min at room

temperature in the dark and stored at -80°C. Heterotrophic prokaryotic cells quantification required a previous staining using 10x SybrGreen I. Heterotrophic prokaryotes cells were split into low and high nucleic acid content (LNA and HNA, respectively). Growth rates were calculated based on the slope of cell abundances versus time over the duration of the experiment.

Taxonomical identification by Catalyzed Reporter Deposition Fluorescence *In Situ* Hybridization (CARD-FISH)

Specific taxonomical bacterial groups were detected by CARD-FISH using group-specific phylogenetic probes in the controls and in the treatments with highest addition of contaminants, at the beginning (0 h) and the end of the experiment (48 h). Aliquots of 9 ml water from the experiments were fixed with 1% final concentration of pre-filtered formaldehyde, incubated during 24 h and filtered on a 0.2 µm pore-size polycarbonate filter (25 mm diameter). The filters were kept dried and frozen until their processing in the laboratory. Whole-cell *in situ* hybridizations of sections from the filters were performed as described by Pernthaler *et al.,* (2002) using the oligonucleotide probes listed in Table S3. Filters pieces were counterstained with 4′,6-diamidino-2-phenylindole (DAPI, 10 µg/ml final concentration). Percentages of the positive hybridized cells to total prokaryotic cells were analyzed through epifluorescence microscopy (Olympus BX61) under blue light and UV excitation. Images were acquired using a digital camera (Zeiss camera AxioCam MRm, Carl Zeiss MicroImaging, S.L., Barcelona, Spain) at 630x magnification through the Axiovision software and analyzed using the automated image analysis software ACMEtool. All images (at least 10 fields/ filter) were acquired using 20 ms exposure time.

Extracellular enzyme activity

Extracellular enzymatic activity (EEA) was measured in the controls and in the

treatments with highest addition of contaminants, at the beginning (0 h) and the end of the experiment (48 h). We tested the activities of β-glucosidase (β-Glc), L-leucyl aminopeptidase (Leu-amp), alkaline phosphatase (APA) and chitinase (Chit) to track changes in extracellular hydrolysis of high molecular weight (HMW) compounds rich in carbon (C), nitrogen (N), P and glycosidic bonds respectively (Ayo *et al.,* 2017). EEA was determined fluorometrically using fluorogenic substrates that emit fluorescence after cleavage of the molecule by the specific enzyme, (Hoppe, 1993), following the protocol already described (Hoppe, 1983; Sala and Güde, 1999) but adapted for microplates (Sala *et al.,* 2016). Briefly, in triplicates, 350 µl of sample were mixed with 50 µl of the following substrates: 4-methylumbelliferyl phosphate (APA), 4-methylumbelliferyl β-D-glucoside (β-Glc), 4-methylumbelliferyl N-acetyl- β-D-glucosaminide (Chit) or L-leucine-7-amino-4-methylcoumarin (Leu-amp), all purchased at Sigma-Aldrich, St. Louis, MO, USA. Fluorescence of the samples was measured at 0 and after 24 h incubation in the dark and *in situ* temperature, at 365 excitation and 450 emission wavelengths with a Modulus Microplate (DISMED, Turner BioSystems). The increase in fluorescence in each well was converted into activity using a standard curve prepared with the fluorophores 4-methylumbelliferone (MUF) or 4-methylcoumarinyl-7-amide 4 (Sigma-Aldrich, St. Louis, MO, USA). Specific EEA was calculated by normalizing EEA by bacterial abundance as estimated by flow cytometry.

Statistical analysis

Statistical analysis was performed using R and SPSS. Significant differences between treatments were tested with t-Student tests performed using the ‘t.test’ function and Tukey’s HSD post-hoc test using the “TukeyHSD” with a threshold for the significance set at $P < 0.05$. ANOVA were carried out using the ‘aov’ function. Principal component analysis (PCA) were carried out using the ‘adonis’

function of the 'vegan' package with standardized data (Oksanen *et al.,* 2018). Pearson correlations between microbial growth rates and added concentrations of pollutants corrected by their initial concentration in seawater were calculated using the 'Hmisc' package (Harrell and Dupont, 2008). Multiple parameter linear least-squares regressions were performed with SPSS Statistics version 24.0 (IBM Corporation). The influence of environmental variables on the growth rates of HNA, LNA, *Synechococcus*, Picoeukaryotes, and the bacterial groups (*SAR11*, *Roseobacter*, *Gammaproteobacteria* and *Cytophaga-Flavobacterium-Bacteroidetes* group) was tested separately for each group and OP family (n-alkanes, PFOS, PAHs, OPEs) by assaying the multiple linear regression of the growth rate for the five experiments against nutrient, organic carbon and pollutant concentrations (*in situ* + added) and the interactions of pollutants and nutrients. For the experiments performed at the DCM and at deep waters, the *in situ* pollutant concentrations were assumed to equal the one measured at surface. In addition, each EEA were also regressed against the same variables and the growth rates of the bacterial groups. As HNA, LNA, *Synechococcus* and Picoeukaryotes were measured in controls and for experiments at High, Intermediate and Low concentrations, regressions were performed with 40 measurements. For other variables, regressions were performed with 20 measurements as bacterial groups and EEA were only available for controls and treatments at High concentrations. When independent variables showed a high collinearity, these were not included in the analysis. Details of the significant variables for each regression and uncertainty on the parameters are given in Table S4.

RESULTS

Characterization of initial conditions at each sampling station.

Temperature, chlorophyll a, organic carbon and nutrients

Temperature was maximal in surface waters (20.9°C) and decreased with depth (Table 1). At surface, the concentrations of inorganic nutrients were higher in Coastal waters and decreased towards the Off-shore station where concentrations of NO_3^- plus NO_2^-, SiO_4^{4-} and PO_4^{3-} increased with depth (Table 1). A decreasing gradient of TOC was revealed from Coastal (92.8 µM) to the Off-shore (77.4 µM) samples. Total organic nitrogen (TON) showed the maximum concentration (5.62 µM) at the Mid station and the minimum concentration (3.40 µM) at the Off-shore station. The TOC/TON ratio varied between 26.6 at the Coastal station and 22.8 at the Off-shore station, being the lowest ratio at the Mid station, 14.6. At the Off-shore location, TOC and TON had their maxima values at surface (77.43 µM and 3.54 µM, respectively) and decreased with depth until 47.69 µM and 0.21 µM, while the TOC/TON ratio increased with depth. The highest concentrations of Chl a were recorded at the Coastal station and at the DCM (Table 1). Overall, these results suggest a trophic gradient in surface waters from a more meso-trophic system in the Coastal towards more oligotrophic conditions in Off-shore waters.

Background concentrations of n-alkanes, PAHs and OPEs in surface seawater

The concentrations of the three families of OPs, representative of the aliphatic and aromatic hydrocarbons and P-containing OPs, were quantified by GC-MS in the surface waters (Fig. 1, S1, Table 1). Concentrations of total dissolved PAHs (Σ_{26}PAH) were 24.2, 26.2 and 17.5 ng/l at the Coastal, Mid and Off-shore stations, respectively. The composition profiles of PAHs indicated that low molecular weight

PAHs (2-3 ring PAHs) were abundant in the samples, representing on average 65.5 ± 12.3% of Σ_{26}PAH. The total dissolved concentrations of n-alkanes with 12 to 35 carbon atoms in surface waters were 4600, 1640 and 750 ng/l for the Coastal, Mid and Off-shore stations, respectively. The profile of n-alkanes showed a Gaussian distribution with a maximum at C_{25}, typical of fossil inputs. Above this distribution, there was a slight predominance of n-alkanes with an odd number of carbon atoms over those with an even number of carbons for n-alkanes ranging between C_{30} and C_{35}, indicating n-alkane inputs from terrestrial plants, probably introduced by river inputs or atmospheric deposition (Fig. S1). Most OPEs (TiBP, TnBP, TPhP, EHDP, TEHP, and the chlorinated TCEP and TCPPs) were found in

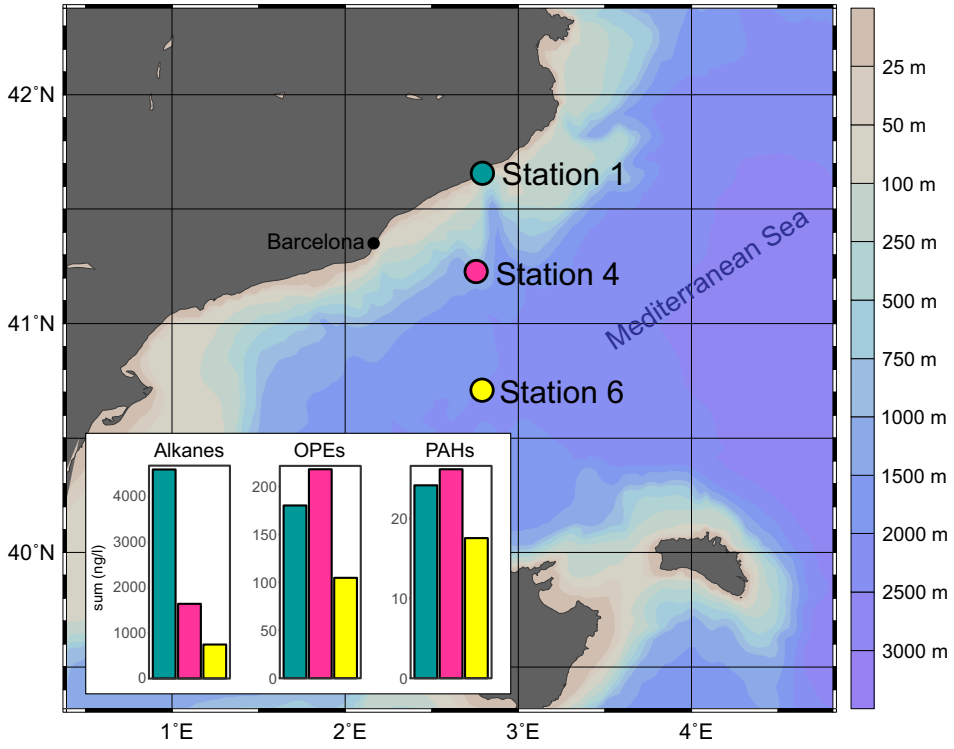

Figure 1. Location of the sampling stations in the Northwestern Mediterranean Sea. Bars indicate the pooled concentration of three families of organic pollutants (n-alkanes, organophosphate esters (OPEs) flame retardants and plasticizers and polycyclic aromatic hydrocarbons (PAHs)) measured in the dissolved phase at the surface (1 m). See details in Table 1.

Table 1. Ancillary information of the sampling sites and concentrations of organic pollutants, nutrients and total organic matter. Concentrations of n-alkanes, polycyclic aromatic hydrocarbons (PAHs) and organophosphate esters (OPEs) flame retardants and plasticizers are the sum of concentrations of individual compounds from each family in the surface water. Perfluorooctane sulfonate (PFOS) concentrations were not measured.

Name	Station	Date	Depth (m)	Latitude	Longitude	Salinity	Temp. (°C)	Chl a (µg/l)
COASTAL	1	10/22/15	5.6	41°39'07.2"N	2°48'06.6"E	38.15	18.24	0.31
MID	4	10/23/15	6.1	41°08'57.5"N	2°49'12.6"E	38.03	19.75	0.14
OFFSHORE / SURFACE	6	10/24/15	5.3	40°38'48.0"N	2°50'49.8"E	37.66	20.90	0.15
OFFSHORE / DCM	6	10/24/15	65	40°38'48.0"N	2°50'49.8"E	38.23	13.84	0.41
OFFSHORE / DEEP	6	10/24/15	1975.4	40°38'48.0"N	2°50'49.8"E	38.49	13.23	nd

NO_3^-: nitrate; NO_2^-: nitrite; NH_4^+: ammonium; SiO_2^{4-}: silicate; PO_4^{3-}: phosphate; TOC: total organic carbon; TN: total nitrogen; TON: total organic nitrogen; nd: no data.

the dissolved phase at the three stations with total OPE concentrations of 180, 218, and 105 ng/l for the Coastal, Mid and Off-shore stations, respectively (Fig. S1). In general, minimum values of dissolved OPs were found in the Off-shore station, in agreement with distance to coast.

Bulk characterization of in situ *microbial communities*

At the initial time point, corresponding to *in situ* conditions, coastal surface seawater had 55% more heterotrophic bacterial cells than the other surface stations and cell concentrations decreased with depth (Fig. S2). HNA cells initially constituted 41-43.8% of the heterotroph cell abundances and this percentage increased with depth (to 55% at 2000 m). The relative abundance of the dominant *SAR11* group (average relative abundance of 22.5 ± 12.3% of DAPI counts) increased from the coast to off-shore, a pattern opposite to that of the *Roseobacter* (1.6 ± 0.2%), consistent with the gradient towards higher oligotrophy in open ocean waters (Fig. S3). *Gammaproteobacteria* (10.6 ± 8.9%) and *Cytophaga-Flavobacterium-Bacteroidetes* group (5.5 ± 2.3%) showed lower abundances in Off-shore waters than at the Coastal site. Percentages of all groups decreased with depth at the

NO_3^- (µmol/l)	NO_2^- (µmol/l)	NH_4^+ (µmol/l)	SiO_4^{4-} (µmol/l)	PO_4^{3-} (µmol/l)	TOC (µmol/l)	TN (µmol/l)	TON (µmol/l)	n-alk (ng/l)	PAHs (ng/l)	OPEs (ng/l)
0.21	0.18	0.93	1.16	0.03	92.80	4.81	3.49	4600	14.7	180
0.19	0.03	0.13	0.95	0.02	80.23	5.84	5.49	1640	18.8	218
0.22	0.02	0.15	0.67	0.03	77.43	3.78	3.40	746	14.5	1045
2.66	0.06	0.01	1.83	0.06	58.93	5.19	2.45	nd	nd	nd
10.53	0.02	0.06	8.82	0.33	47.69	10.76	0.15	nd	nd	nd

Off-shore station, and *Gammaproteobacteria* and *SAR11* groups kept dominating the communities (Fig. S3). Picophytoplankton communities consisted of the picocyanobacteria *Synechococcus* sp. and eukaryotic picophytoplankton as the picocyanobacteria *Prochlorococcus* were present in very low abundances. The targeted picophytoplankton groups showed opposite patterns: *Synechococcus* abundances decreased from Coastal to Off-shore waters and Picoeukaryotes abundances were highest at the DCM of the Off-shore station. As expected, no phytoplanktonic cells were detected in Deep waters (Fig. S2). Extracellular activities of Leu-amp and β-Glc decreased from Coastal to Off-shore waters by 2.5 and 58.8%, respectively. In Off-shore waters, their activity decreased from the surface to values under the detection limit in Deep waters. In contrast, APA and Chit activities increased from Coastal to Off-shore waters and with depth, with the highest values in Deep waters (Fig. S4). PCA analyses of microbial community attributes at initial time points (that is, cell abundances, CARD-FISH counts and enzymatic activities, Fig. S5) distinguished between stations mainly due to the abundance of heterotrophic bacteria (expressed as the sum of HNA

and LNA concentrations, cells/ml). The first principal component, explaining 59% of the variability, distinguished Coastal and Off-shore stations, and surface versus deeper waters. This component had strong positive loadings of APA and negative loadings of bacterial abundances, β-Glc and Leu-amp (Fig. S5). The second principal component, explaining 17% of the variability, accounted for the differences between DCM and the rest of samples. The DCM station was distinguished due to the higher abundance of Picoeukaryotes and the lowest Chit activity.

Responses to OP amendments in the horizontal transect (surface waters)

Changes in bacterial abundances and composition during the incubations

In the surface seawater control bottles (no OP addition), heterotrophic bacteria reached the maximum abundance at the end of the experiment (48 h), increasing by factors of 1.16, 1.30 and 1.55 (for Coastal, Mid and Off-shore, respectively) compared to abundances at initial time (Fig. S2). Significant responses were observed after increasing contaminant concentrations in the treatment bottles compared to the controls, but the responses were heterogeneous and their intensity and direction depended on the site, compound, incubation time and taxonomic group (Fig. 2). No significant differences were observed between cell growth of different groups due to exposure to emerging OPs and hydrocarbons suggesting that the chemical structure is an incomplete parameter to predict the consumption of an OPs in seawater (Escher *et al.,* 2017). This result is surprising as PFOS is thought to be more persistent than OPEs and hydrocarbons. Different response patterns were observed between sites. DCM station showed the highest number of significant differences between treatments and controls for all OPs tested, suggesting that the trophic status and structure of the community play an

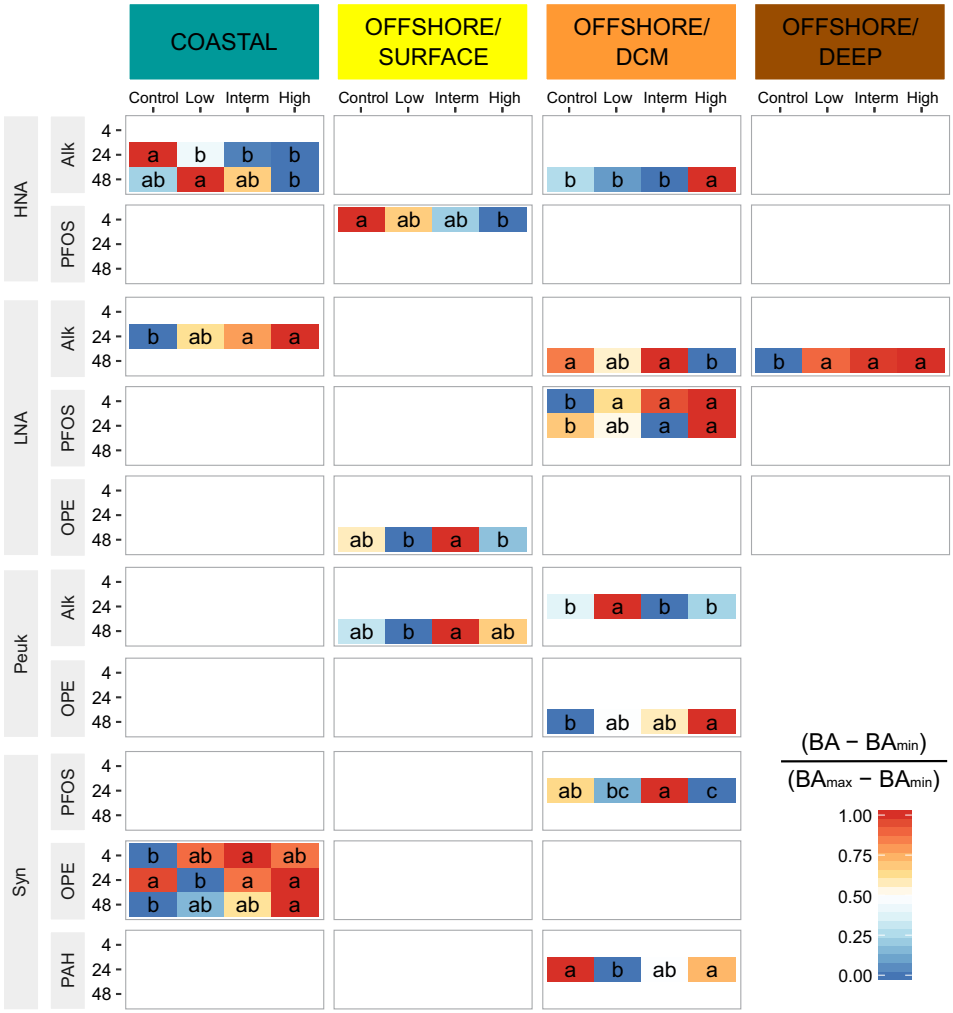

Figure 2. Significant (P < 0.05) differences in cell abundances of heterotrophic bacteria (high nucleic acid content, HNA; low nucleic acid content, LNA) and picophytoplankton (Picoeukaryotes, Peuk: *Synechococcus*, Syn) between Controls (with no pollutant addition) and treatments (additions of Low, Intermediate and High OP concentrations) during different incubation times (4 h, 24 h and 48 h). Differences of mean values from replicates were analyzed using 1-way ANOVA followed by a post-hoc Tukey's HSD test. Red and blue colors indicate maximum and minimum values of each comparison. Notice that only significant differences are represented in this figure. Alk: n-alkane; PFOS: perfluorooctane sulfonate; OPE: organophosphate ester flame retardants and plasticizers; PAH: polycyclic aromatic hydrocarbon; BA: bacterial abundance.

Table 2. Summary of significant multiple linear least-squares regressions of growth rates and extracellular enzyme activities against nutrients, organic carbon and pollutant concentrations and the interactions of pollutants and nutrients for each experiment. Sign in brackets represents the sign of the parameter for that variable (positive or negative). Only variables with parameters significantly different than zero are shown ($P < 0.05$). More detailed information can be found in Table S4.

Exp	Type	Model variable	R^2	*P*	SEE
Alkanes exp	HNA	TN(-), TON(+), Alk(+)	0.878	*<0.0005*	*0.003*
	LNA	NO_2(-), NH_4(+), TN(-), TON(-)	0.582	*<0.0005*	*0.002*
	Syn	NO_2(-), TOC(+), TN(+), TON(-), Alk(+)	0.802	*<0.0005*	*0.001*
	Peuk	NO_3(-), NO_2(+), TOC(-)	0.893	*<0.0005*	*0.003*
	CF	NO_2(-), NH_4*Alk(+)	0.439	*0.007*	*0.041*
	Roseo	TN(-), NO_3*Alk(+)	0.604	*<0.0005*	*0.027*
	SAR11	NH_4*Alk(-), NO_2*Alk(+)	0.299	*0.049*	*0.009*
	APA	EUB(+), Roseo(-), TOC(-), NH_4*Alk(+), NO_2*Alk(-)	0.937	*<0.0005*	*<0.0005*
	β-Glc	EUB(+), NH_4(-), NO_2(+), Roseo(-)	0.696	*0.001*	*<0.0005*
	Chit	NO_2(-), Roseo(-), PO_4*Alk(+)	0.798	*<0.0005*	*<0.0005*
	Leu-amp	NO_3(+)	0.798	*<0.0005*	*<0.0005*
PAH exp	HNA	NO_2(+), NH_4(+), TN(-), TON(+), NO_2*PAH(+)	0.961	*<0.0005*	*0.002*
	LNA	NO_2(-), NH_4(+), TN(-), TON(-)	0.666	*<0.0005*	*0.002*
	Syn	NO_3(-)	0.720	*<0.0005*	*0.001*
	Peuk	NO_2(+), TN(+), TON(-)	0.942	*<0.0005*	*0.002*
	Gamma	NH_4(-), NO_2(+), TN(+)	0.723	*<0.0005*	*0.013*
	Roseo	TN(-), PO_4*PAH(-)	0.810	*<0.0005*	*0.024*
	SAR11	NO_3*PAH(-)	0.470	*0.001*	*0.033*
	APA	Roseo(-), *SAR11*(-), TOC(-), PO4*PAH(-)	0.900	*<0.0005*	*<0.0005*
	β-Glc	NH_4(-), *SAR11*(+), PO_4*PAH(+)	0.552	*0.004*	*<0.0005*
	Chit	PO_4(+), Roseo(-), *SAR11*(-), PO_4*PAH(+)	0.990	*<0.0005*	*<0.0005*
	Leu-amp	NO_2(+), TOC(-), PO_4*PAH(+)	0.876	*<0.0005*	*<0.0005*

Exp	Type	Model variable	R^2	p	SEE
OPE exp	HNA	NO_2(+), TOC(-), TN(+), TON(-)	0.948	*<0.0005*	*0.002*
	LNA	NO_2(-), TOC(+), TN(-), TON(-)	0.651	*<0.0005*	*0.002*
	Syn	NO_3(-), PO_4*OPE(+)	0.708	*<0.0005*	*0.001*
	Peuk	NO_2(+), PO_4(+), TN(+), PO_4*OPE(+)	0.956	*<0.0005*	*0.002*
	CF	NO_2(-), NO_2*OPE(+)	0.379	*0.017*	*0.039*
	Gamma	NH_4(-), NO_2(+)	0.492	*0.003*	*0.020*
	Roseo	NO_3(-), TOC(-), PO_4*OPE(+)	0.654	*0.001*	*0.022*
	APA	EUB(+), Roseo(-), TN(-)	0.865	*<0.0005*	*<0.0005*
	β-Glc	NO_3(-), TOC(-), Roseo(-)	0.502	*0.009*	*<0.0005*
	Chit	NO_3(-), PO_4(+), Roseo(-)	0.994	*<0.0005*	*<0.0005*
	Leu-amp	NH_4(+), TOC(-), NO_3*OPE(-)	0.733	<0.0005	<0.0005
PFOS exp	HNA	TOC(+), TN(-), NO_2*PFOS(-)	0.867	*<0.0005*	*0.003*
	LNA	NO_2(-), TOC(+), TN(-), TON(-)	0.578	*<0.0005*	*0.002*
	Syn	NO_3(-)	0.697	*<0.0005*	*0.010*
	Peuk	NO_2(+), PO_4(+), TN(+), NO_3*PFOS(-), NO_2*PFOS(-), PO_4*PFOS(+)	0.960	*<0.0005*	*0.002*
	CF	NO_2(-), NO_2*PFOS(+)	0.469	*0.005*	*0.038*
	Gamma	NO_2(+), NH_4(-)	0.428	*0.009*	*0.017*
	Roseo	NO_3(-), PO_4*PFOS(+)	0.591	*<0.0005*	*0.024*
	APA	TOC(-), Roseo(-)	0.852	*<0.0005*	*<0.0005*
	β-Glc	NO_3*PFOS(+)	0.465	*0.001*	*<0.0005*
	Chit	NO_3*PFOS(+)	0.514	*<0.0005*	*<0.0005*
	Leu-amp	NO_2(+), TOC(-), NO_2*PFOS(-), NH_4*PFOS(+)	0.832	*<0.0005*	*<0.0005*

Syn: *Synechococcus*; Peuk: Picoeukaryotes; CF: *Cytophaga-Flavobacterium-Bacteroidetes*; Gamma: *Gammaproteobacteria*; Roseo: *Roseobacters*; APA: alkaline phosphatase; β-Glc: β-glucosidase; Chit: chitinase; Leu-amp: L-leucyl aminopeptidase; Alk: n-alkane; PAH: polycyclic aromatic hydrocarbon; OPE: organophosphate ester flame retardants and plasticizers; PFOS: perfluorooctane sulfonate; NO_3: nitrate; NO_2: nitrite; NH_4: ammonium; PO_4: phosphate; TOC: total organic carbon; TN: total nitrogen; TON: total organic nitrogen; SEE: standard error of the estimate.

important role determining the metabolic use of OPs by microbial communities. All the microbial groups presented at least one significant response, as discerned by Tukey tests, due to exposure to a family of OPs (Fig. 2). n-Alkanes promoted the highest significant variability in the abundance of heterotrophic bacteria and picophytoplankton. In Coastal waters, HNA were depleted after the addition of

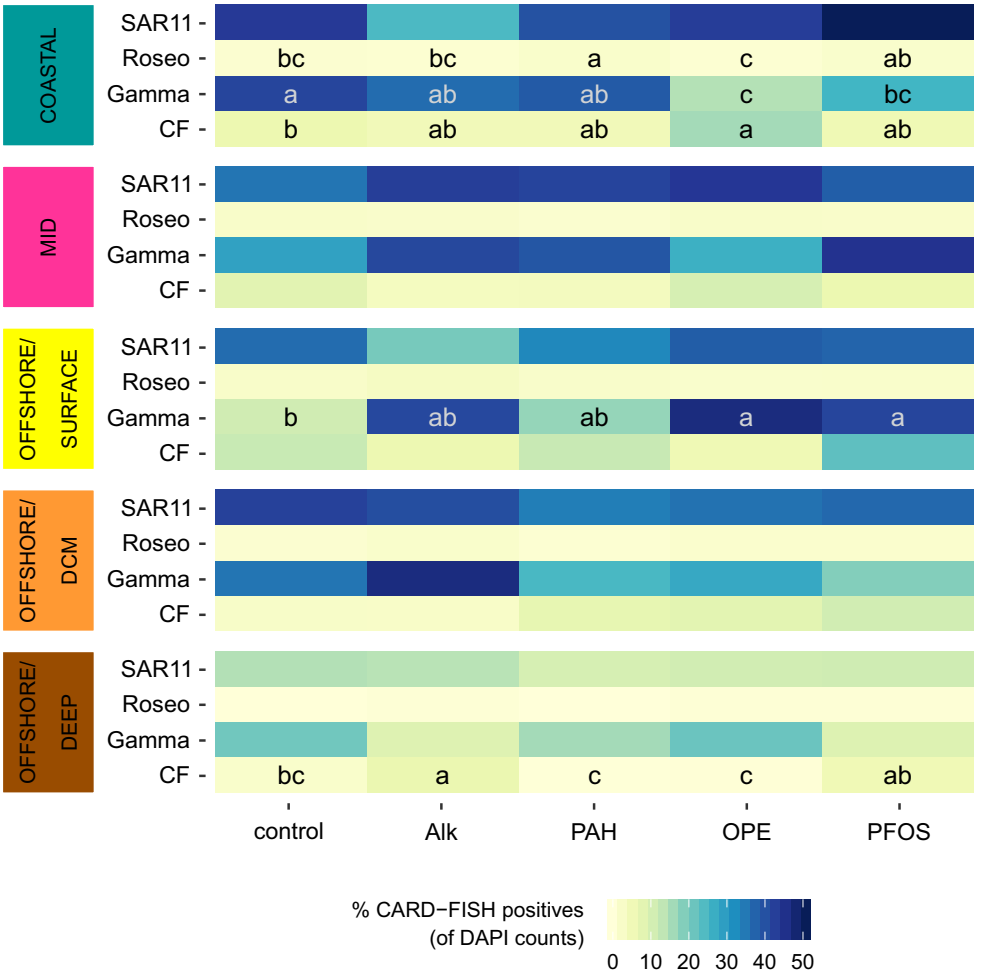

Figure 3. Contribution of each bacterial phylogenetic group (% CARD-FISH) to total heterotrophic community (DAPI-counts) structure after 48 h of incubation of surface waters challenged with the highest concentrations of different OP families. Significant differences ($P < 0.05$) between treatments of mean values from replicates were analyzed using 1-way ANOVA followed by a post-hoc Tukey's HSD test and labeled as different letters in the heatmap. Roseo: *Roseobacters*, Gamma: *Gammaproteobacteria*, CF: *Cytophaga-Flavobacterium-Bacteroidetes*; Alk: n-alkane; PAH: polycyclic aromatic hydrocarbon, OPE; organophosphate ester flame retardants and plasticizers; PFOS: perfluorooctane sulfonate.

n-alkanes, while LNA increased. OPEs stimulated the growth of *Synechococcus* at the Coastal site. The significant bivariate Pearson correlations found between bacterial growth rates and contaminant concentrations (Fig. S6) explained a small percentage of variability. The relationships between growth rates and OP concentrations were greatly strengthened using multiple linear regressions taking into account the concentrations of nutrients, dissolved and particulate organic carbon, and pollutant concentrations (Table 2, S4). The growth rate of HNA was correlated with that of the n-alkanes, PFOS and PAHs and several N forms (Table 2). The growth rates of *Synechococcus* were depended on nutrient conditions (N and P concentrations), n-alkanes and OPEs. Similarly, Picoeukaryote growth rates were significant correlated to PFOS, OPEs and different nutrient concentrations (Table 2). It is noteworthy that PFOS, OPEs and PAHs showed a significant effect in interaction with the concentrations of various nutrients, thus the pollutant effect on microbial communities was influenced by nutrient concentrations.

After 48 h of incubation, the average cell detection with EUB338-II-III probes was 76.7% (SD = 12.0%, n = 48) of all DAPI-stained cells. *SAR11* remained the dominant group in all communities (37.8 ± 11.1%), followed by *Gammaproteobacteria* (32.7 ± 13.1%). *Cytophaga-Flavobacterium-Bacteroidetes* group (8.7± 6.6%) and *Roseobacter* (2.1 ± 0.7%) in all experiments. In Coastal waters, the relative abundance of *Gammaproteobacteria* significantly decreased (Fig. 3) when cells were challenged with the highest concentrations of OPEC, especially OPEs, while the opposite pattern was found in the Off-shore experiment. Significant differences in the percentages of the *Roseobacter* and *Cytophaga-Flavobacterium-Bacteroidetes* groups were found in the Coastal experiments after being challenged with PAHs and OPEs respectively (Fig. 3). Multiple linear regressions showed that growth rates of specific bacterial groups were significantly correlated to inorganic and organic nutrient concentrations (in all groups) and to

OPs (Table 2, S4). Remarkably, *Cytophaga-Flavobacterium-Bacteroidetes* and *Roseobacter* responded to all OP, whereas *SAR11* exclusively to hydrocarbons. In most cases, the influence of OPs was significant when interacting with the availability of nutrients (as surrogate of trophic status).

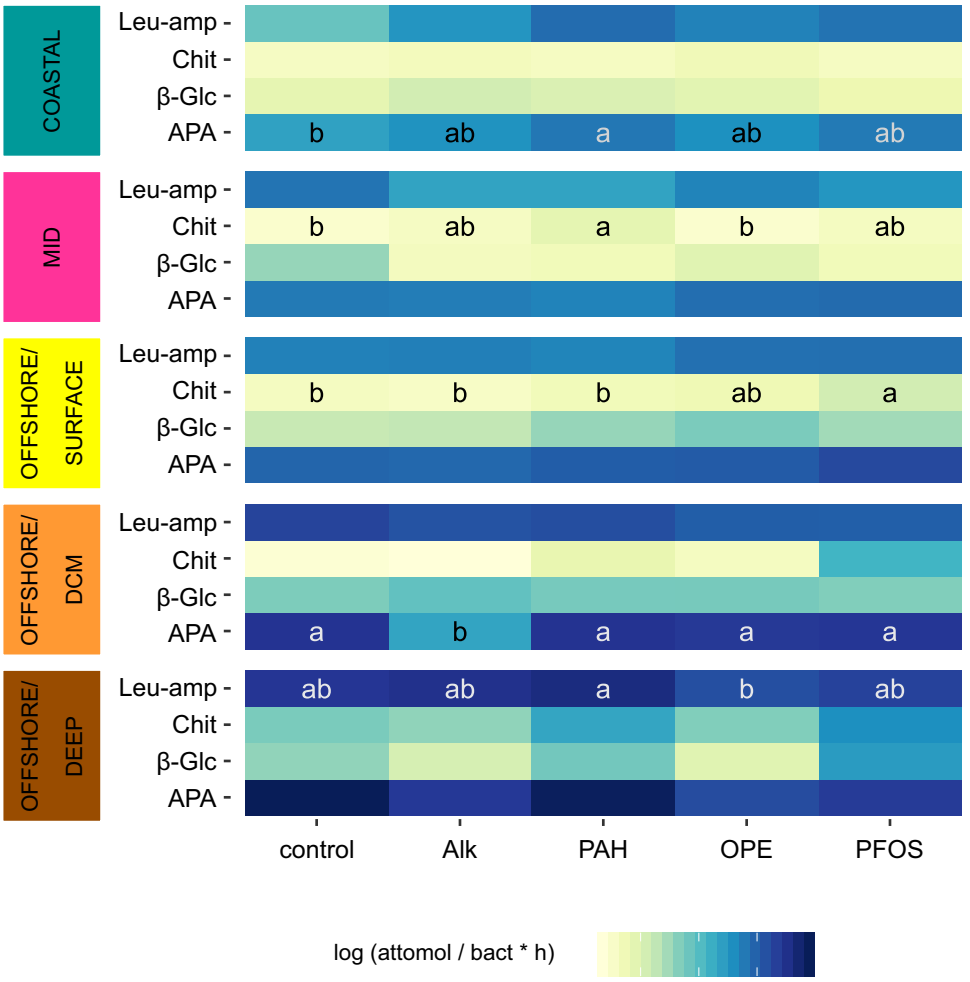

Figure 4. Extracellular enzyme activities (EEA) in the controls (without OP addition) and in the treatment with different OP families at highest concentrations after 48 h. Significant differences ($P < 0.05$) of mean values from replicates were analyzed using 1-way ANOVA followed by a post-hoc Tukey's HSD test and labeled as different letters in the heatmap. EEA are plotted in a logarithmic scale. Notice that EEA values were corrected by bacterial abundances. Leu-amp: L-leucyl aminopeptidase; Chit: chitinase; β-Glc: β-glucosidase; APA: alkaline phosphatase; Alk: n-alkane; PAH: polycyclic aromatic hydrocarbon; OPE: organophosphate ester flame retardants and plasticizers; PFOS: perfluorooctane sulfonate.

Changes in extracellular enzyme activities after OP additions

In general, changes of EEA in OP amendments at highest concentrations after 48 h were more pronounced for Chit activities than for the other enzymes, although differences were also observed between stations (Fig. 4). At the Off-shore station, EEA increased by 1.4-fold after OP additions. The largest increases (2.7-fold) were observed for β-Glc and Chit activities after the addition of OPEs and PFOS, respectively. In contrast, an average decrease (0.5-fold) was experienced in the Mid station in all the extracellular enzymes except Chit activities that were significantly stimulated after n-alkane, PAH and PFOS additions. In Coastal waters, APA activity significantly increased after 48 h of the addition of PAHs and PFOS (Fig. 4). APA, Chit and Leu-amp activities significantly correlated in a multiple linear least-squares regression with PFOS addition (β-Glc, Chit, Leu-amp), PAH addition (all EEA tested), n-alkane addition (APA, Chit) and OPE addition (Leu-amp) together with the bacterial growth rates and organic nutrients (Table 2). In all cases, significant effects of OPs on EEA appeared as interactions between OP and nutrient concentrations.

Responses to OP amendment in the vertical transect at the Off-shore station

The largest number of significant differences in the growth rates between treatments and controls was found in the DCM experiments (Fig. 2). In that layer, n-alkane addition promoted the growth of HNA but decreased LNA and heterotrophic Picoeukaryotes growth rates. Growth of LNA got stimulated after PFOS additions but those inhibited *Synechococcus* growth. In contrast, increasing concentrations of OPEs and PAHs promoted growth of Picoeukaryotes and *Synechococcus* respectively (Fig. 2). In general, the largest increases in EEA were observed for the Chit activities after PFOS additions in the Off-shore experiments, being up to 30.4-fold in the DCM layer (Fig. 4). In the Deep layer,

addition of OPEs significantly decreased Leu-amp activity, and in the DCM layer APA was significantly reduced after n-alkane addition (Fig. 4).

DISCUSSION

The measured OP concentrations in ocean surface waters were of the same order, or up to an order of magnitude higher, than those measured previously in the same region. For instance, concentrations of dissolved OPEs were 10 times higher than the few previous measures available for the Northwestern Mediterranean (10.5 ng/l, Vila-Costa *et al.,* 2019), but comparable to concentrations reported in the Bohai and Yellow Seas (Zhong *et al.,* 2017). The sum of dissolved phase of PAH concentrations was in the order of magnitude than previous studies (4.6 - 41.7 ng/l, Guitart *et al.,* 2004) which is around 10-fold higher than concentrations reported in the same region by other studies (Σ_{19}PAH: 0.16 - 0.81 ng/l, Berrojalbiz *et al.,* 2011a; 1.8 ng/l, Dachs *et al.,* 1997).This is possibly because we quantified a larger number of PAHs and performed the sampling in another season (spring versus fall). Among individual PAHs, phenanthrene was the most abundant compound and the general profile was dominated by low molecular weight PAHs (2-4 rings), in agreement with previous studies (Ehrhardt and Petrick, 1993; Dachs *et al.,* 1997; Tsapakis *et al.,* 2006; Berrojalbiz *et al.,* 2011b). The dissolved concentrations of n-alkanes (0.7 - 4.6 µg/l) in our study were at slightly higher values than those reported in other off-shore surface waters of the Northwestern Mediterranean Sea (n-C_{15} - n-C_{40}: 0.7 - 2.4 µg/l, Marty and Saliot, 1976) and in the Marseille (France) coastal area (n-C_{15} - n-C_{40}: 0.04 - 0.58 µg/l, Guigue *et al.,* 2011, 2014). However, concentrations were similar to those described recently in the Gulf of Gabès (Tunisia) (n-C_{15} - n-C_{40}: 0.02 - 6.3 µg/l, Fourati *et al.,* 2018).

In the experiments, we added concentrations within the range of those found in

seawater or higher. In the case of PAHs and n-alkanes, the highest concentrations added (Table 1) were representative of total hydrocarbons found in the ocean when the UCM is considered. For OPEs and PFOS, the highest concentrations were higher than those reported for the open sea, but are comparable to OPE and PFAS concentrations found at the Besòs river mouth and Ebro river estuary, respectively (Cristale *et al.,* 2013; Pignotti *et al.,* 2017). In any case, the total concentrations of OPEs and PFASs in the environment remains unquantified. The highest additions used (from 0.5 to 30 µg/l) are in the low range of those used in previous toxicity studies on marine microorganisms, which were in the order of µg/l to mg/l (Hudak and Fuhrman, 1988; Echeveste *et al.,* 2010b). Rather than looking for toxicity thresholds and recovery times, we detected under which situations hydrocarbons and OPEC at concentrations within the environmental variability influenced the growth rate and enzymatic activities of naturally-occurring marine microorganisms.

The NW Mediterranean microbial communities responded to the different additions of hydrocarbons and OPEC at both functional and taxonomical levels under different trophic conditions (nutrient and carbon concentrations) but without showing major differences between OPEC and hydrocarbons. The polarity of the chemical is directly related to the octanol-water partition coefficient (K_{ow}). Even though it has been suggested that the more hydrophobic compounds have lower potential for degradation (e.g. Gioia and Dachs, 2012), no distinct pattern could be detected between responses to hydrocarbons (more hydrophobic) and to OPEC (more hydrophilic than hydrocarbons). This finding questions the reliability of model approaches based on the chemical structure of the OPs to predict OP degradation in the environment, and urges for *in situ* validation of biodegradability potential, or at least for the inclusion of biological descriptors of the degrading community.

The impact on the communities was dependent not only on the type of organism (heterotrophs vs phototrophs), but also on the different taxonomic groups within these types. Surprisingly, the responses were both positive and negative in the same group when challenged with one compound, and varied depending on the site (Table S4). Thus, this work provides initial experimental evidence that microbial responses to OPs are not only due to the structure, concentration and the metabolic capacities of the consuming microbial consortia (as in Johnsen and Karlson, 2004), but to a complex combination of factors that includes the response to OP exposures, the trophic condition and the identity of the consumer (bacterial group). In fact, such complex interactions among factors, not always additive, have been previously reported in studies that evaluated the drivers regulating the consumption of other organic compounds (Pinhassi *et al.,* 2006; Alonso-Sáez *et al.,* 2007, Vila-Costa *et al.,* 2007). In particular, Pinhassi *et al*. (2006) found, working with Mediterranean communities, that nutrients played a predominant role when examining the microbial response to organic compounds additions. In agreement to the pivotal role of nutrients, the abundances and growth rates of picophytoplankton and heterotrophs in this work were strongly correlated with nutrient concentrations, mostly inorganic N forms (Fig. S6), consistent with their known key role in oligotrophic regions driving the abundance and structure of microbial communities (Gasol and Kirchman, 2018). Conversely, the growth rate of the different bacterial groups did not show strong correlations explaining a high percentage of the variability with the abundance of OPs alone. The influence of OPs modulating the growth rate of the various bacterial groups could only be identified by means of multivariable regression analysis, that is, considering the concurrent influence of nutrients and OPs together. Even though OPs were not the primary driver of growth rates, multiple linear regression between the growth rates and nutrients and pollutants demonstrated that the inclusion of OPs in the fitting regression improved the percentage of the variability explained. This

work shows, for the first time and for experiments carried out in the field, that OPs exert an influence on the growth rates of bacteria (OP parameters were significantly different than zero in the multiple linear regressions as shown in Table S4). Furthermore, the fact that the influence of OPs in most cases raised from significant interactions with nutrient concentrations, indicates that the influence of OPs depends on nutrient concentrations, and thus, on trophic status. These interactions were positive or negative depending on the pollutant family and microbial group (Table S4).

The highest modulation of microbial growth, mostly that of heterotrophic bacteria, appeared in communities challenged with n-alkanes, and to a lower extend with PAHs. PAHs and n-alkanes are major components of oil, and in seawater their effects on microbial communities have been extensively studied during oil spill accidents that represent a pulse introduction of tones of these hydrocarbons in a short period of time (Mason *et al.,* 2012; Reddy *et al.,* 2012). These studies indicate that at high concentrations, aromatic and aliphatic hydrocarbons are able to deeply modify bacterial community structure and functionality, and promote the growth of specific consumers (Suja *et al.,* 2014; Kachienga *et al.,* 2018). However, these results cannot be extrapolated to the effects of OPs at the background levels found in the marine environment. These experiments show that increasing hydrocarbon additions did not always promote bacterial consumption. For instance, increasing n-alkane additions stimulated growth of LNA bacteria in Coastal and Deep waters, and of HNA at the DCM station, but inhibited the growth of HNA in Coastal waters, and of LNA in the DCM, suggesting the dual role of n-alkanes both as source of carbon and as a toxic for the cells. The time frame of the experiments (2 days) did possibly not allow for recovery of the community from a potential acute toxicity (that would select specific consumers), so these differences in responses are either due to *in*

situ community composition, or to environmental status (Hudak and Fuhrman, 1988). Recent studies demonstrate that the microbial carbon pump (Jiao *et al.,* 2011), including biodegradation, is the main process responsible for the fate of background concentrations of PAHs found in seawater, indicating a widespread and ubiquitous occurrence of consumers (González-Gaya *et al.,* 2019). PAHs affected the growth of *Roseobacters* and *SAR11* (Table 2). The lower impact of PAHs than n-alkanes can be due to the higher lability of n-alkanes (Bagby *et al.,* 2017), or to the relative small concentrations of aromatic hydrocarbons tested when compared with TOC, since bacteria utilize a wide range of carbon sources and this capacity is directly dependent on bacterial catabolic capacity (Johnsen and Karlson, 2004). In both amendments, the PAH and n-alkane mixtures used contained 16 and 22 individual hydrocarbons, respectively. However, these artificial mixtures of hydrocarbons lack the UCM found in petroleum derivatives and in the environment, which is 100-1,000 times higher in concentration than individual hydrocarbons (González-Gaya *et al.,* 2016). Even though the experiments at high concentration addition resulted in total PAH and n-alkane concentration similar to those found in the environment when considering the UCM, it is possible that the effects of hydrocarbons may need to be tested by a mixture which composition resembles to a higher degree to the composition of the aliphatic and aromatic UCMs, as suggested in recent work done with *Prochlorococcus* (Fernández-Pinos *et al.,* 2017).

In surface waters, the addition of OPEs increased the contribution of *Cytophaga-Flavobacterium-Bacteroidetes* and decreased the relative abundances of *Gammaproteobacteria* in the Coastal experiment, but significantly increased *Gammaproteobacteria* abundances in the Off-shore station. In agreement with our results, in a Coastal experiment in NW Mediterranean waters, Flavobacteria was the main taxa responding to OPE addition (Vila-Costa *et al.,* 2019), where

it was demonstrated that OPEs could be used as a source of P under P-limiting conditions. Furthermore, previous reports have described the degradation of organophosphorus compounds by Flavobacteria (e.g. Singh, 2009). Our results, however, expand the view of potential OPE-consumers in seawater. The contrasted pattern shown by *Gammaproteobacteria* in coastal versus off-shore station (Fig. 3) indicates that this wide group of bacteria might have components also able to benefit from OPE degradation under more oligotrophic conditions.

The DCM samples showed the highest influence of OPs on microbial activities as discerned by the PCA analysis (Fig. S5). In the more oligotrophic waters (that is, at the Off-shore station), OPEC inhibited heterotrophic bacteria cell growth at surface but not at the DCM. When Chl a was maximal, heterotrophs' growth was stimulated by all OPs tested. This can be explained by higher concentration of nutrients and DOC relieving nutritional limitation of the community, or by the fact that many OPs resemble chemically natural compounds produced by phytoplanktonic cells, such as n-alkanes (Bravo-Linares and Mudge, 2009). One could thus expect a higher metabolic diversity to the bacterioplankton developing at the DCM layer, as it has been observed in open ocean waters (Sunagawa *et al.,* 2015), that would benefit the different enzymes catabolizing PAHs and n-alkanes (mono- and di-oxygenases, Wang and Shao, 2013; Ghosal *et al.,* 2016), OPEs (triesterases, Abe *et al.,* 2014) and PFASs (mostly uncharacterized, Parsons *et al.,* 2008). In agreement with this idea, 16S studies show higher alpha diversities in the DCM than in surface waters (Treusch *et al.,* 2009; Walsh *et al.,* 2016).

The measured EEA integrate several enzymes responsible for the hydrolysis of different high molecular weight (HMW) organic molecules (Ayo *et al.,* 2017). The decreasing trend of β-Glc and Leu-amp from Coastal to Off-shore, totally opposite to the trend shown by APA and Chit, could mirror the higher nutrient limiting conditions of the Off-shore station. Multiple least square linear regressions show

that the four families of OPs influenced several of the EEA activities, but in all cases, the influence of OPs arises as due to an interaction with nutrient availability. Conversely, the linear correlations of EEA activities and OP concentrations were not significant. This result suggests that the effect of pollutants can only be discerned when the influences of nutrients and bacterial community (linked to trophic status) are considered together. Notably, we observed a significant activation of Chit after 48 h of PFOS addition in the Mid and Off-shore experiment and the activation of APA in the Coastal station. The influence of PFOS on microbial activities may be linked to the reported influence of PFOS and other PFASs on cell membrane fluidity and permeability (Fitzgerald *et al.,* 2018). Even though the results obtained by multiple linear regressions of EEA and CARD-FISH bacterial groups show only the variables that were significant (Table 2), these were derived from a limited number of observations (n = 20). Therefore, and especially for the cases when several variables were found to exert a significant effect, caution should be taken when using these regression models for predictive purposes due to potential limitations in the stability of the parameters. The results obtained for the growth rate of the microbial groups (cytometry data) have a higher level of reliability since these are based on a larger dataset (n = 40).

Contrary to previous studies on the toxicological effects of OPs on phytoplankton cells, we did not observe systematic adverse cell effects on phototrophs. Several studies have focused on the toxicity of some families of OPs to phytoplankton, both at the sublethal (Othman *et al.,* 2012; Ozhan *et al.,* 2014; Everaert *et al.,* 2015) and lethal levels (Echeveste *et al.,* 2016, 2010b; Gilde and Pinckney 2012). Echeveste *et al.,* (2010a) highlighted the importance of cell size in determining the phytoplankton sensitivity to PAH toxicity. The LC50 (50% Lethal Threshold) concentration of pyrene and phenanthrene affecting *Synechococcus* and Picoeukaryotes was found to be ca. 100 µg/l (Echeveste *et al.,* 2010a),

concentrations 200 or 20000 times higher than our maximum and minimum addition (500 ng/l and 5 ng/l of pyrene and phenanthrene). Therefore, far from being under a lethal effect, our studies have found that some OPs at low concentrations can even stimulate certain groups of phytoplankton. Some of those have been described as having mixotrophic characteristics (i.e. *Synechococcus*, Vila-Costa *et al.,* 2006).

The present study uncovers a complex response of microbial communities to organic pollutants. The influence of varying concentrations of OPs, at environmentally relevant concentrations, is logically small compared to the influence of the naturally varying concentrations of nutrients. However, the analysis performed using multiple linear regression shows that OPs modulate the growth rates of most bacterial groups and their EEA. Even though the concentrations of OPs used are orders of magnitude smaller than the concentrations of DOC, these seem to be high enough to modulate the activity and structure of the microbial community. Nevertheless, due to the complexity of the responses observed, the detailed elucidation of such modulation of the response will require comprehensive approaches focusing on the multiple stresses (pollutants, nutrients, light...) and combining physiological and genomic responses. Furthermore, it is not possible to assure that populations acclimated to live under higher pollutant concentrations (coastal areas) show a more buffered response to OPs, an issue that will require a more comprehensive future research effort. This work suggests that better estimation approaches of biodegradability could be obtained in the future by refining existing QSAR including relevant microbial information. This model development will require comprehensive assessment of biodegradation under different conditions, something that was beyond the objectives of this work.

CONCLUSIONS

In Mediterranean seawater, bacterial growth and extracellular enzymatic activities were strongly dependent on nutrient conditions, but organic pollutants modulated these activities in a complex manner. Bacterial responses to increasing concentrations of OPs were dependent on the concentration and structure of the compound and the trophic status (nutrients and organic matter) in the marine environment, but also on the taxonomic composition of the degrading microbial community. Among all OPs tested, n-alkanes showed the highest influence on bacterial growth rates but not always in the same direction suggesting these were used as a carbon source but also induced toxic effects. Nevertheless, all the OPs considered affected growth of some bacterial groups. The largest number of significant differences between controls (with no pollutant addition) and treatments was observed in water of the Deep-Chlorophyll Maximum layer (65 m depth). We suggest that the microbial phylogenetic identity and trophic status should receive further attention in assessments and models aiming at understanding and predicting the *in situ* degradation of organic pollutants, and in risk assessments of synthetic chemicals and hydrocarbons in aquatic environments.

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Microbial responses to perfluoroalkyl substances and perfluorooctanesulfonate (PFOS) desulfurization in the Antarctic marine environment

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ABSTRACT

Perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) acids are ubiquitous in the oceans, including remote regions, and are toxic to fish and mammals. The impact to the lowest trophic levels of the food web, however, remains unknown. We challenged natural bacterial communities inhabiting Antarctic coastal waters (Deception Island) with PFOS and PFOA concentrations ranging from 2 ng/l to 600 ng/l and observed that these substrates stimulated growth of part of the microbial community with a taxon-specific response. After 48 h, PFOS was desulfurizated by more than 50% and sulfur metabolism-related genes were significantly enriched in the treatments. Conversely, no significant differences were found between initial and final PFOA concentrations. *Gammaproteobacteria* and *Roseobacter*, two abundant groups of marine bacteria, increased their relative activity. After seven days of incubation, another abundant group, *Flavobacteriia*, became the most active group. Community activities (extracellular enzyme activity and absolute number of transcripts) were higher in the treatments than in the controls, while bacterial abundances were lower in the treatments, suggesting a selection of PFOS and PFOA tolerant community in the exposed treatments. Our results show a direct effect of PFOS and PFOA exposure on the composition and functionality of natural Antarctic marine microbial communities. While no evidence of defluorination of PFOS or PFOA were detected, desulfurization of PFOS suggests a direct link with the sulfur biogeochemistry of the ocean.

INTRODUCTION

Perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) acids are two common perfluoroalkyl acids (PFAAs) used in many applications as surfactants, fire retardants, surface treatments and coating materials (Key *et al*., 1997). PFAAs are ubiquitously detected as environmental contaminants (Buck *et al*., 2011). They are highly persistent due to the high chemical stability of their C-F bonds (~533 kJ mol−1) (Wang *et al*., 2018) and bioaccumulative, resulting in adverse effects to environmental and human health (Van de Vijver *et al*., 2003; Lau *et al*., 2007; Rand and Mabury, 2017). The effect of the exposure to PFOS, PFOA and other PFAAs on the immunologic system, growth, birth weight, fertility, carcinogenesis, lipid metabolism and the thyroid system is a matter of increasing concern, as it has been observed in both fish and mammals (Corsini *et al*., 2014; DeWitt *et al*., 2008; 2016).

PFOS and PFOA are globally transported by oceanic currents and by sea-salt aerosols (Ahrens *et al*., 2010; Benskin *et al*., 2012; González-Gaya *et al*., 2014; Johansson *et al*., 2019). In addition, atmospheric transport of volatile neutral precursors and subsequent oxidation and deposition is also a potential source of PFAAs to remote regions (Martin *et al*., 2006; Del Vento *et al*., 2012; Young and Mabury, 2012). PFAAs have been reported to be ubiquitous in remote areas such as Antarctic waters (Wei *et al*., 2007; Ahrens *et al*., 2010; Zhao *et al*., 2012; Cai *et al*., 2012; Casal *et al*., 2017a). Long-range transport, persistence and toxicity are proprieties that these substances share with other persistent organic pollutants (POPs). PFOS has been the first PFAA to have a global restriction, but not a ban, on its production and use, being added to Annex B of the Stockholm Convention on POPs (Paul *et al*., 2009).

Exposure to PFAAs by microorganisms can originate an increased permeability of

cell membranes, alteration of membrane fluidity (Xie *et al*., 2010; Hu *et al*., 2003), and membrane disruption by PFAA penetration into the membrane bilayer (Nouhi *et al*., 2018). These effects are compound and concentration dependent. A recent study investigating the toxicity of high concentrations of PFAAs to *Escherichia coli* determined a half-inhibitory concentration (IC_{50}) of 10.6 ± 1.0 mg/l for PFOA and 374 ± 3 mg/l for PFOS (Liu *et al*., 2016). At lower concentrations, it has been demonstrated that 10 µg/l of PFOS was sufficient for increasing the sensitivity of the quorum sensing response in *Aliivibrio fischeri* (Fitzgerald *et al*., 2018). Most of these studies have been performed under standardized laboratory experiments using model cultures. Effects might change between individual strains and the mix of species found in microbiomes. In mice gut microbiome, for instance, PFOS exposure caused potentially harmful interactions between PFOS and microbial and animal metabolisms (Lai *et al*., 2018). Unfortunately, no studies have attempted to check the effects of PFAAs on environmental microbiomes.

Despite the high stability of the C-F bound, dehalogenation of fluoroacetate by microorganisms was first reported by Goldman (1965). Some previous studies suggest that PFOS and PFOA dehalogenation is a favorable thermodynamic reaction under reductive conditions, and is thus expected to occur in anaerobic environments such as in soils, sediments or in some wastewater treatment plant (WWTP) compartments. However, few studies have been able to actually detect degradation in field studies. Beškoski *et al*. (2018) assumed that metabolites determined by LC/MS in microbial consortia isolated from river sediments and incubated with PFOS and PFOA were associated with unsaturated monofluorinated fatty acids and hydrocarbons with multiple unsaturated bonds or ring structures. Other studies have observed the decrease of PFOS and PFOA concentrations under incubations with microorganisms isolated from contaminated soils (Yi *et al*., 2016, 2018; Chetverikov *et al*., 2017; Chetverikov and Loginov, 2019), river

sediments (Beškoski *et al*., 2018) and wastewater treatment sludge (Kwon *et al*., 2014; Mejia-Avendaño *et al*., 2015). However, defluorination products of PFOS or PFOA were not detected.

This study aimed to analyze the effects of PFOS and PFOA to pristine remote microorganisms. Given that PFAAs have been introduced in the environment during the last fifty years and their distant resemblance to natural products, we wanted to test whether microbial communities have already evolved the capacity to degrade these compounds, if any potential degradation due to co-metabolism occurs, or if toxic effects were predominant as a microbial response to PFAAs. Dose-response assays and short- *versus* long-term exposure experiments with a mix of PFOS and PFOA were performed under *in situ* conditions with coastal Antarctic microbial communities. Potential biodegradation and sub-lethal effects were detected by using a combination of chemical, physiological, molecular and transcriptomic techniques.

MATERIALS AND METHODS

Description of Experiments

Experiments were carried out in January-February 2017 using surface (0.5 m depth) seawater from Port Foster bay (62°59.386'S, 60°37.115'W), the inner bay of Deception Island (South Shetland Islands, Antarctica). Three experiments were performed by challenging marine natural populations with a mix of PFOA and PFOS (hereafter called PFAAs mix) at different concentrations and exposure times. PFOA and PFOS in the mix were at a 1:0.6 proportion, to mirror natural relative occurrence in seawater as previously reported (Casal *et al*., 2017a). All experiments were run in duplicate.

Dose-response experiments

Microbial communities were exposed at four different concentrations of the PFAAs mix: no addition (control), 2 ng/l (LOW), 60 ng/l (MID) and 600 ng/l (HIGH). Incubations were carried out in polypropylene 50 ml falcon centrifuge tubes that were spiked with the PFAAs mix and let the solvent to evaporate during 2 h under the hood at room temperature before seawater addition. Control tubes were prepared following the same protocol and with the same volume of solvent but without any pollutant addition. Incubations were done in the dark and at *in situ* temperature for 48 h. Changes in community composition were monitored by quantifying bacterial abundance (BA) by flow cytometry at initial time point, and after 3 h, 24 h and 48 h of incubation and by CARD-FISH at initial and final time points. Changes in community activity were monitored by bio-orthogonal non-canonical amino acid tagging (BONCAT) and specific extracellular enzymatic activity (specific EEA) measurements at the initial and final time points.

Short- and long-term exposure experiments

Short-term exposure experiments consisted of three 24 h incubations at two different concentrations of the PFAAs mix (no addition (control), 2ng/l (LOW) and 60ng/l (MID)) in 2 l polypropylene bottles. Long-term exposure experiment consisted of two 6-day incubations under two conditions (no addition (control) and 60ng/l (MID)) in pre-cleaned polypropylene 20 l carboys. Addition of pollutants and incubation conditions were performed as explained above for the dose-response experiment. Changes were monitored after 24, 48, 96 and 144 h. Changes of water PFOS and PFOA concentrations in dissolved phase were quantified by ultra-performance liquid chromatography coupled with a triple-quadrupole mass spectrometer (UPLC-MS/MS) as explained below. Community composition was characterized by cell abundances (flow cytometry), 16S rDNA sequencing and CARD-FISH. Microbial activities were monitored by BONCAT and specific EEA.

Gene expression profiles were analyzed after 24 h and 6 days in all samples, see below.

Analysis of the dissolved phase concentrations of PFOS and PFOA

The extraction method was based on a previously reported procedure (González-Gaya *et al*., 2014; Casal *et al*., 2017a). Briefly, the water samples (2 l) were pre-filtered using glass fiber filters (GF/F, Whatman, O.D. 47 mm, 0.7 µm) and extracted *in situ* by solid phase extraction (SPE) using Waters Oasis® WAX cartridges using a Baker® vacuum station. Prior to extraction, the samples were spiked with a solution containing labelled ($^{13}C_4$-) PFOS and PFOA. Cartridges were kept at −20 °C until their elution in the laboratory as detailed in González-Gaya *et al.* (2014). Extracts were concentrated in precleaned Falcon polypropylene (PP) tubes, transferred to PP vials and dried under a gentle N_2 stream. Samples were reconstituted with 100 µl of an injection standard solution containing $^{13}C_8$-PFOA and $^{13}C_8$-PFOS at 5 pg/µl in 30:70 water:methanol.

PFOS and PFOA identification and quantification was performed by UPLC-MS/MS using a Waters Acquity UPLC system coupled with a Waters XEVO TQS triple-quadrupole operating in the multiple-reaction-monitoring (MRM) mode and using an electrospray ionization (ESI) source (Waters, Milford, MA, USA). PFOS and PFOA separation was achieved using an Acquity UPLC BEH C18 column (1.7 um, 2.1 × 50 mm; Waters) with a gradient mobile phase of water and methanol with a constant 1% of acetonitrile buffer at a flow rate of 400 µl/min. Injection and instrumental conditions, as well as MS/MS parameters for the target compounds can be found in González-Gaya *et al*. (2014).

Quantification was based on a linear eight-point calibration curve (0.05-10 pg/µl). Those samples that showed PFOS or PFOA concentrations above the curve

range were diluted using the injection standard to match the calibration range. Fluorinated materials were avoided during field and lab work. Field and lab blanks covering the whole sampling and analytical procedure were conducted. Field blanks were stored and transported from Antarctica to test for possible external contamination. Limits of detection (LOD) were calculated as the analyte mean concentration corresponding to the sample blanks plus three standard deviations being 0.14 and 0.06 pg/µl for PFOA and PFOS, respectively. Target compounds were identified based on similar retention times and a ratio between the monitored ions of ± 20% of the mean values obtained for the calibration standards. Recoveries of spiked PFAAs were satisfactory for both PFOS (mean % ± SD; 119 ± 33) and PFOA (103 ± 26).

Bacterial abundance (BA)

BA was determined by flow cytometry using a FACSCalibur (Becton Dickinson) flow cytometer, as described elsewhere (Gasol and Moran, 2015). High and low nucleic acid content cells (HNA and LNA, respectively) were counted separately.

Taxonomical identification by Catalyzed Reporter Deposition Fluorescence *In Situ* Hybridization (CARD-FISH)

Specific taxonomical bacterial groups were detected by CARD-FISH using group-specific phylogenetic probes. Aliquots of 9 ml water from the experiments were fixed with 1% final concentration of pre-filtered formaldehyde, incubated during 24 h and filtered on a 0.2 µm pore-size polycarbonate filter (25 mm diameter). The filters were kept dried and frozen until their processing in the laboratory. Whole-cell *in situ* hybridizations of sections from the filters were performed as described by Pernthaler *et al*., (2002) using the oligonucleotide probes listed in Table S1. Filter pieces were counterstained with 4′,6-diamidino-2-phenylindole (DAPI, 10 µg/ml final concentration). Percentages of the positive hybridized cells

to total prokaryotic cells were analyzed through epifluorescence microscopy (Olympus BX61) under blue light and UV excitation. Images were acquired using a digital camera (Zeiss camera AxioCam MRm, Carl Zeiss MicroImaging, S.L., Barcelona, Spain) at 630x magnification through the Axiovision software and analyzed using the automated image analysis software ACMEtool. All images (at least 10 fields/filter) were acquired using 20 ms exposure time.

Bio-orthogonal non-canonical amino acid tagging (BONCAT)

BONCAT was used to determine the percentage of active cells within each CARD-FISH group following the protocol described in Leizeaga *et al*., 2017. Briefly, 9 ml of each sample were incubated during 2 h at *in situ* temperature and dark conditions with 0.5μM of the methionine analog (L-homopropargylglycine, HPG) and cells were fixed with 0.2 μm-filtered formaldehyde (final concentration 1% [v/v]) overnight at 4 ºC. The samples were then gently filtered through a 0.2 μm pore size polycarbonate filter and frozen at -20 ºC until further processing. After thawing, the filters were immersed in 0.1% agarose, dried at 37 ºC for 10-30 min, and then dehydrated with 95% ethanol. Cell walls were permeabilized with lysozyme solution (100 mg lysozyme, 1 ml EDTA 0.5M, 1 ml Tris HCl 1M, 8 ml H_2O) for 60 min at 37 ºC and achromopeptidase (2 μl achromopeptidase, 1 ml buffer (100 μl NaCl 5M, 500 μl Tris HCl 1M, 50 ml H_2O; pH 8)) for 30 min at 37 ºC. Each filter was cut into 1/10 slices using a sterile razor blade. Cu(I)-catalyzed click-chemistry was later performed using azide dye CR110, following Hatzenpichler and Orphan (2015). The CARD-FISH probes were subsequently hybridized as described before, using the Alexa488 labeled tyramide. Filters were mounted with 4,6-diamidino-2-phenylindole (DAPI; Sigma-Aldrich). DAPI, BONCAT, and CARD-FISH-stained cells were visualized by epifluorescence microscopy and images were acquired using the same procedure as described in CARD-FISH section.

Extracellular enzyme activity (EEA)

The activity of four extracellular enzymes (β-glucosidase (β-Glc), L-leucyl aminopeptidase (Leu-amp), alkaline phosphatase (APA) and chitinase (Chit)) was determined using fluorogenic substrates that emit fluorescence after cleavage of the molecule by the specific enzyme (Hoppe, 1983), (Hoppe, 1993), following the protocol described elsewhere (Sala *et al*., 2010; 2016). Briefly, 350 µl of samples in triplicates, were mixed with 50 µl of four different substrates: 4-methylumbelliferyl phosphate (APA), 4-methylumbelliferyl β-D-glucoside (β-Glc), 4-methylumbelliferyl N-acetyl- β-D-glucosaminide (Chit) or L-leucine-7-amino-4-methylcoumarin (Leu-amp) in black microplates. Plates were incubated at 4 ºC and dark conditions. Fluorescence was measured at 0, 15 min and 24 h after incubation, at 365 excitation and 450 emission wavelengths with a Modulus Microplate (DISMED, Turner BioSystems). The increase in fluorescence in each well was converted into activity using a standard curve prepared with the fluorophores 4-methylumbelliferone (MUF) or 4-methylcoumarinyl-7-amide 4. Specific EEA was calculated dividing EEA by bacterial abundance as estimated by flow cytometry.

Genetic biomass collection for metatranscriptomics and taxonomy (rDNA 16S)

At each corresponding incubation time, 2 l of seawater was first pre-filtered through a 3 µm pore-size 47 mm diameter polycarbonate filter and then filtered through a 0.2 µm pore-size 25 mm diameter polycarbonate filter for 20 min to minimize RNA degradation (approx. 1 l). The 0.2 µm filter was placed in 2 ml cryotubes containing 1 ml RNAlater (Sigma-Aldrich, Saint Louis, MO) and stored at -20 ºC until RNA processing in the lab. The remaining volume (approx. 1 l) was filtered using a 0.2 µm pore-size Sterivex filters (Millipore). Sterivex filters were

stored dry at -20 ºC until DNA extraction (see below).

A metatranscriptomic approach was performed following Cerro-Galvez *et al*. (2019a). Briefly, total RNA was extracted, DNA removed, rRNA depleted and mRNA enriched by amplification following the protocol used by Poretsky *et al*. (2010) with minor modifications. Three mRNA standards were synthesized (Gifford *et al.,* 2011) by *in vitro* transcription from plasmids with restriction enzyme: pTXB1 vector (New England Biolabs, Ipswich, MA) with NcoI restriction enzyme (Promega, Madison, WI), pFN18K Halotag T7 Flexi vector (Promega, Madison, WI) with BamHI restriction enzyme (Promega, Madison, WI) and pGEM-4Z (Promega, Madison, WI) with ScaI restriction enzyme (Promega, Madison, WI). These artificial mRNAs were added after RNA extraction but before mRNA enrichment as in Cerro-Gálvez *et al*., 2019a and used as an internal standard (Moran *et al*., 2013; Satinsky *et al*., 2013). Final amplified RNAs were sequenced at the National Center for Genomic Analysis (CNAG, Barcelona, Spain) using Illumina high output mode HS200 2x100bp v4.

Sterivex filters for DNA analysis were filled with lysis buffer (50 mM Tris-HCl, 40 mM EDTA, 0.75 M Sucrose) and incubated at 37°C for 45 min with a 5 mg/ml lysozyme solution. Then, 0.5 mg/mL proteinase K and 100 µl of 10% sodium dodecyl sulfate were added and further incubated at 55°C for 1 h. DNA was extracted twice with phenol-chloroform-isoamyl alcohol (25:24:1), and once with chloroform-isoamyl alcohol (24:1). The resulting solution was concentrated to 200 µl using an Amicon Ultra 10-kDa filter unit (Millipore). Partial bacterial 16S gene fragments of DNA were amplified using a mix of 515F/926R primers (Parada *et al*., 2016) and MyTaq DNA Polymerase. PCR conditions were as follows: 3 min at 94°C, followed by 30 cycles of denaturation at 94°C for 15 s, annealing at 57°C for 20 s, primer extension at 72°C for 15 s and a final extension at 72°C for 10 min. PCR amplification was confirmed by electrophoresis on tris-acetate-EDTA

(TAE) agarose gels. Illumina MiSeq sequencing was of rDNA 16S conducted at the Pompeu Fabra University Sequencing Service.

Bioinformatics

cDNA sequences were quality trimmed and internal standards and any remaining stable RNA was quantified and removed using the Bowtie2 mapping program (Langmead and Salzberg, 2012) against the internal standard sequences and an in-house database of marine bacterial stable RNA sequences (rRNA and tRNA) respectively. Subsequently, read pairs were joined using the PEAR program (https://www.h-its.org/en/research/sco/software). Joined pairs, as well as separate reads not corresponding to joined pairs were aligned to the NCBI RefSeq database (downloaded October 2016) using the Diamond aligner, v0.8.25, (Buchfink *et al*., 2014) in blastx mode with default parameters. The resulting alignments were taxonomically and functionally classified with MEGAN 6.5.10 (Huson *et al*., 2016) and exported for further analysis in R/tidyverse. Sequenced belonging to archaea and eukaryotic cells were excluded due to low abundances. Search for specific functions was performed with Pfam profiles, using HMMER (Eddy, 2011), and by gene name.

A total of 14 metatranscriptomes samples were recovered, ranging in size from 48 to 98 million paired-end reads of 101 bp per dataset (Table S2). Among the close to one billion total reads, 460 million were possible protein-encoding sequences. After quality-trimming, an average of 23.3 ± 9.1% of the protein-coding reads had a functional annotation (based on SEED database and subcategories) and an average of 21.2 ± 4.9% were taxonomically annotated (see below for details on transcript annotation).

16S reads were trimmed, denoised, merged and bimera checked with DADA2 (Callahan *et al*., 2016) version 1.10.0. Taxonomical annotation was performed

with the QIIME 2 naive Bayesian algorithm version 2018.8.0 (Bokulich *et al*., 2018) against the SILVA database (Quast *et al*., 2013) version 132.

Statistical analyses

All data analyses were performed in R software. Significant differences between treatments and controls were analyzed with Student's t-test, non-metric multidimensional scaling (NMDS) or analysis of variance (ANOVA) using the Vegan package (Oksanen *et al*., 2018). The threshold for significance was set at $P < 0.05$. Analysis of differential gene abundances was performed with the EdgeR package (Dimont *et al*., 2015). Temporal variation of PFOS and PFOA in long-term experiments were evaluated by means of linear regression analysis including concentration as dependent variable and time (24, 48, 96, 144 h) as predictor using SPSS Statistics version 25.0 (IBM Corp.). Concentrations were ln transformed when necessary to satisfy normal distribution and variance homogeneity criteria.

RESULTS AND DISCUSSION

Dose-response assays

No statistically significant differences (ANOVA test; $P > 0.05$) were observed in neither community composition nor functional profile between controls and treatments after 48 h with PFAA concentrations up to 600 ng/l (highest concentration tested). This is consistent with previous studies showing significant changes in marine community composition at PFAA concentrations higher than those used in our exposure experiments (>1000 ng/l, Zhang *et al*., 2019; Cerro-Gálvez *et al*., 2019b), but a minor effect of PFAAs on river delta marine community composition when compared with the roles of geochemical parameters and phytoplankton

(Chen *et al*., 2019) when exposed to *in situ* concentrations.

Whereas no trends were distinguished in the structure of the communities, increased BONCAT activities were observed in the *Gammaproteobacteria* and *Roseobacter* groups in treatments with PFAAs. *Gammaproteobacteria* and *Roseobacter* displayed maximum increases of 1.7- and 1.6-fold, respectively, compared with the controls at the MID concentration (60 ng/l of PFAAs mix) (Fig. 1). In agreement with this, these two phylogenetic groups were the main responders to increasing additions of PFOS in Mediterranean marine communities, although *Gammaproteobacteria* showed opposite patterns (decrease and increase in relative abundances) in coastal and offshore communities (Cerro-Gálvez *et al*., 2019b).

The observed decline of EEA of Chit (Fig. 1) with increasing PFAA concentrations is consistent with the activation of *Gammaproteobacteria* and *Roseobacter*. Increased Chit activities were observed in offshore Mediterranean waters at

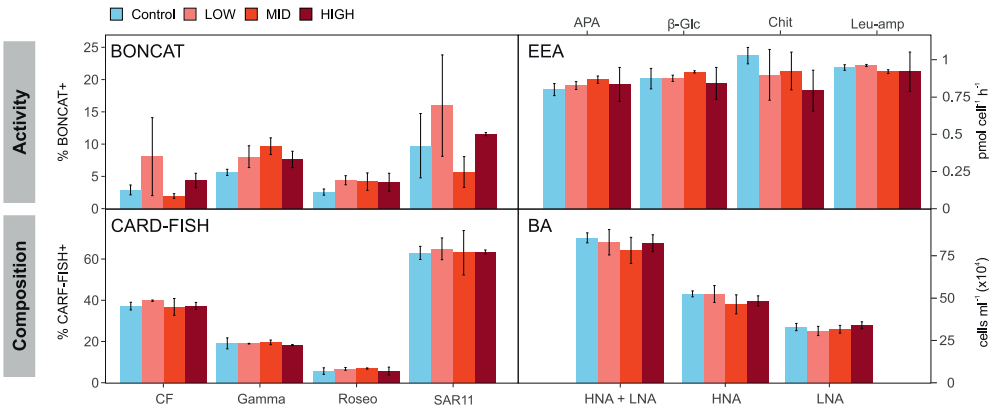

Figure 1. Changes in microbial activities and community composition in dose-response experiments (48 h of PFAAs mix incubation). Notice that the y-axes of EEA plot are modified, absolute EEA rates result from multiplying each value by 10^{-5} for APA, $1.2{\cdot}10^{-6}$ for β-Glc, $1.5{\cdot}10^{-6}$ for Chit, and $1.2{\cdot}10^{-4}$ for Leu-amp. Error bars show standard deviations of replicates. CF: *Cytophaga-Flavobacterium-Bacteroidetes;* Gamma: *Gammaproteobacteria*; Roseo: *Roseobacters*; EEA: specific extracellular enzyme activity; APA: alkaline phosphatase; β-Glc: β-glucosidase; Chit: chitinase; Leu-amp: L-leucyl aminopeptidase; BA: bacterial abundance; HNA: high nucleic acid content; LNA: low nucleic acid content.

high PFOS concentrations concomitant with increased relative abundances of *Flavobacteriia* (Cerro-Gálvez *et al*., 2019b). This group is known to have a high affinity to degrade high molecular weight compounds including biopolymers thus potentially linked to chitinolytic activities (Kirchman, 2002; Veliz *et al*., 2017). Thus, for short-term incubations (48 h), PFAA addition stimulated *Proteobacteria* (mainly *Gammaproteobacteria* and *Roseobacter* within *Alphaproteobacteria*) rather than the other phyla, such as *Flavobacteriia*, without major differences between doses.

LOW and MID concentrations from dose-response bioassays were selected for short-term (24 h) experiments, while MID concentrations were chosen for long-term (6 days) exposure experiments. Responses were detailed at the transcriptional level for short- and long-term experiments, while chemical concentrations were analyzed for long-term experiments at different times of exposure.

Concentrations of PFAAs in long-term experiment

In situ seawater concentrations of PFOS and PFOA were 36 pg/l and 27 pg/l, respectively, comparable to the low concentrations measured in Antarctic coastal seawaters and offshore regions (Wei *et al*., 2007; Ahrens *et al*., 2010; Cai *et al*., 2012; Zhao *et al*., 2012). For example, concentrations are close to those measured in coastal waters from Livingston Island (South Shetland Islands, Antarctica), that averaged 20 ± 35 pg/l of PFOS and 69 ± 39 pg/l of PFOA (Casal *et al*., 2017a).

Dissolved phase concentrations of PFOS at the end of the long-term experiment were significantly lower than in the first measurement (t-test; $P < 0.005$, Table S3). Conversely, PFOA concentrations did not decrease significantly during the 6 days of the long-term experiment (t-test; $P = 0.13$). In this work, PFOS and PFOA were measured in the dissolved phase only. It is well known that when

performing incubation using hydrophobic persistent organic pollutants (POPs) in microcosms (small volumes) there are losses due to adsorption to the bottle walls among others (Fernández-Pinos *et al*., 2017; Cerro-Gálvez *et al*., 2019a). In addition, a fraction of PFOS and PFOA will partition to phytoplankton and bacteria. The bioaccumulation factor (BAF = $C_{Plankton}/C_{Water}$, or plankton-water partition coefficients) for PFOS in Antarctic waters (log(BAF) = 3 ± 0.6) is not significantly different than that for PFOA (log(BAF) = 3.8 ± 0.3) (Casal *et al*., 2017b). The bacteria-water partition coefficients for PFAAs have never been reported before, but are probably higher than those of phytoplankton due to a larger surface to volume ratio of the cells. Therefore, a significant fraction of PFAAs can be sorbed into/onto cells during the time course of the experiments, and this fraction may vary over time due to changes in cell abundance and community composition. Therefore, the concentrations in the dissolved phase measured will not only reflect a potential degradation, but also the partitioning of a variable fraction to cells and bottle walls. An approach to improve the quantification of degradation in field and laboratory experiments is the use of chemical benchmarking (McLachlan *et al*., 2017). With this approach, the degradation of a chemical is measured relative to another chemical of known persistence, and the degradation is then assessed using not the absolute concentrations, but the relative concentrations (ratio of the target analyte over the benchmark chemical). Therefore, the benchmark chemical is used as internal standard of the experimental set up. In this work, we used PFOA as a benchmark chemical and PFOS as the target chemical for which biodegradation was assessed. This is consistent with PFOS showing a significant decrease of concentrations during the long-term assay, while PFOA lacking of a significant decrease. Figure 2 shows the ratio of concentrations of PFOS over PFOA during the time course of the experiment (thus PFOS concentrations in relative abundances), and shows a clear exponential decay. As the ratio of PFOS and PFOA is evaluated, instead of absolute concentrations of

PFOS alone, this approach blocks any variability in dissolved concentrations due to sorption to bottle walls and cells as both chemicals have similar hydrophobicity (similar BAF). Fitting ln(PFOS) *versus* time resulted in a significant regression ($r^2 = 0.42$, $P < 0.05$), with a degradation constant of 0.016 h^{-1}, corresponding to a half-life of PFOS in the long-term experiment of 43 h, that is, a disappearance of more than 50% after 48 h (Fig. 2). Similar fast PFOS degradation rates have been observed in cultures of specific consumers, e.g. *Pseudomonas aeruginosa* strain HJ4 (Kwon *et al*., 2014) and *Pseudomonas plecoglossicida* strain 2.4-D (Chetverikov *et al*., 2017).

In terms of environmental relevance of these degradation rates for the occurrence of PFOS in oceanic waters, it would be surprising that such fast degradation occurs

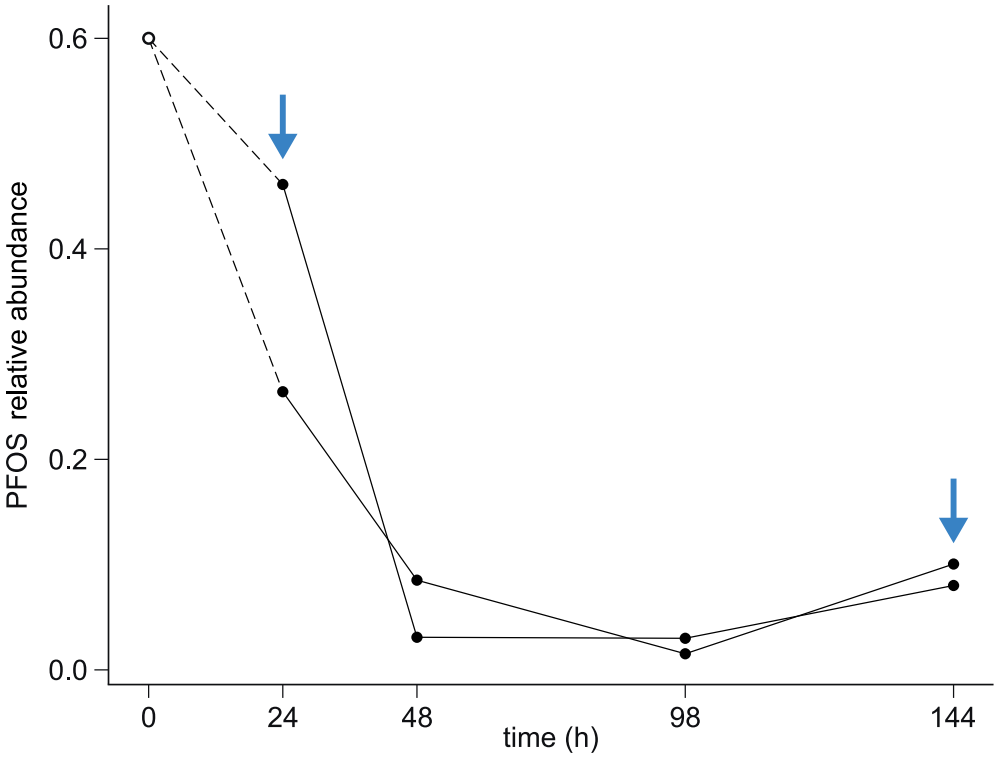

Figure 2. Variation of relative abundance of PFOS in the long-term experiment. Duplicates are represented by separated two lines. Arrows indicate the selected time points for metatranscriptomic sequencing (24 h and 6 days).

in the global oceans, as PFOS is ubiquitously found in seawater (Benskin *et al*., 2010; González-Gaya *et al*., 2014). First, it is possible that Antarctic (or polar) microbial communities are especially suited for such degradation. Secondly, it could happen that the biogeochemical conditions in Antarctic coastal waters favor this process (see discussion below for potential use of sulfur from PFOS). Enzyme mediated degradation rates are dependent on the chemical (substrate) concentration, a consequence of Michaelis-Menten kinetics. In laboratory experiments, it has been shown that pollutant biodegradation is activated by the addition of high concentrations of substrate (Li and McLachlan, 2019) as it could have happened in our spiked treatments. In controls containing field *in situ* concentrations of PFAAs, concentrations of PFOS also 31% decreased during the long-term experiment (not significant), while this decrease was of 85% for the treatments with PFAAs (significant, $P < 0.05$), thus with PFOS concentrations 100-fold those found *in situ*.

The ubiquity of PFOS in oceans provides evidence that it is slowly degraded and remains in the surface ocean for long periods, as estimated previously for tropical and subtropical oceans (González-Gaya *et al*., 2014; Casal *et al*., 2017b). In most oceanic regions, PFOS concentrations are higher than those of PFOA (Wei *et al*., 2007; Ahrens *et al*., 2010; Zhao *et al*., 2012; González-Gaya *et al*., 2014; Casal *et al*., 2017a). The median in the ratio PFOS/PFOA is 4.0 in temperate oceanic waters, with values of 4.2 in the Atlantic, 4.0 in the Indian and 3.8 in the Pacific Oceans (González-Gaya *et al*., 2014). Lower ratios have been observed in Antarctic waters, ranging from 0.3 to 2.2 in several sites including offshore and coastal areas (Wei *et al*., 2007; Zhao *et al*., 2012; Casal *et al*., 2017a). The ratio measured in our samples (1.3) falls in the range of Antarctic samples. These results suggest an active potential of PFOS degradation by polar microorganisms, higher than in temperate seawater (González-Gaya *et al*.,

2014). In order to identify potential PFOS degraders and degradation pathways, we turned to molecular analyses.

Community composition profiles in short- and long-term experiment

In general, bacterial abundances in the treatments were higher than in the controls over the time course of the experiments without distinction between HNA and LNA cells. However, after 6 days, bacterial abundances significantly dropped

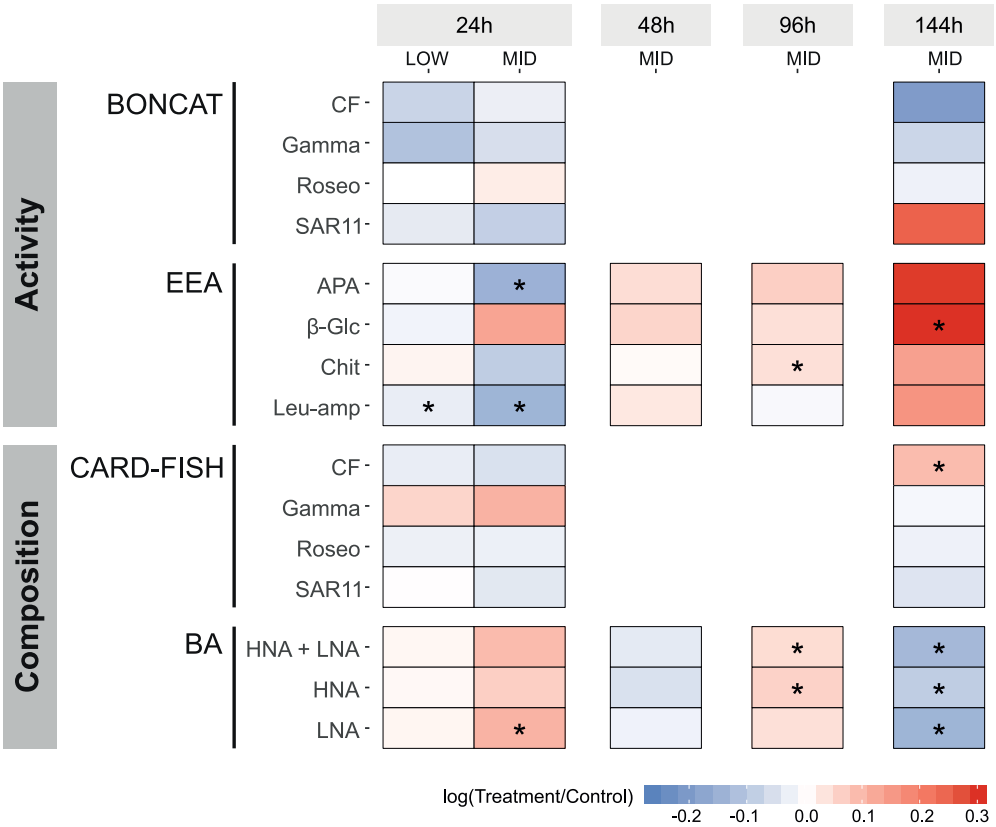

Figure 3. Changes in microbial community activities and composition between controls and PFAA amendments in long-term experiment. Significant differences (t-test; $P < 0.05$) are marked with asterisks. CF: *Cytophaga-Flavobacterium-Bacteroidetes*; Gamma: *Gammaproteobacteria*; Roseo: *Roseobacters*; EEA: specific extracellular enzyme activity; APA: alkaline phosphatase; β-Glc: β-glucosidase; Chit: chitinase; Leu-amp: L-leucyl aminopeptidase; BA: bacterial abundance; HNA: high nucleic acid content; LNA: low nucleic acid content.

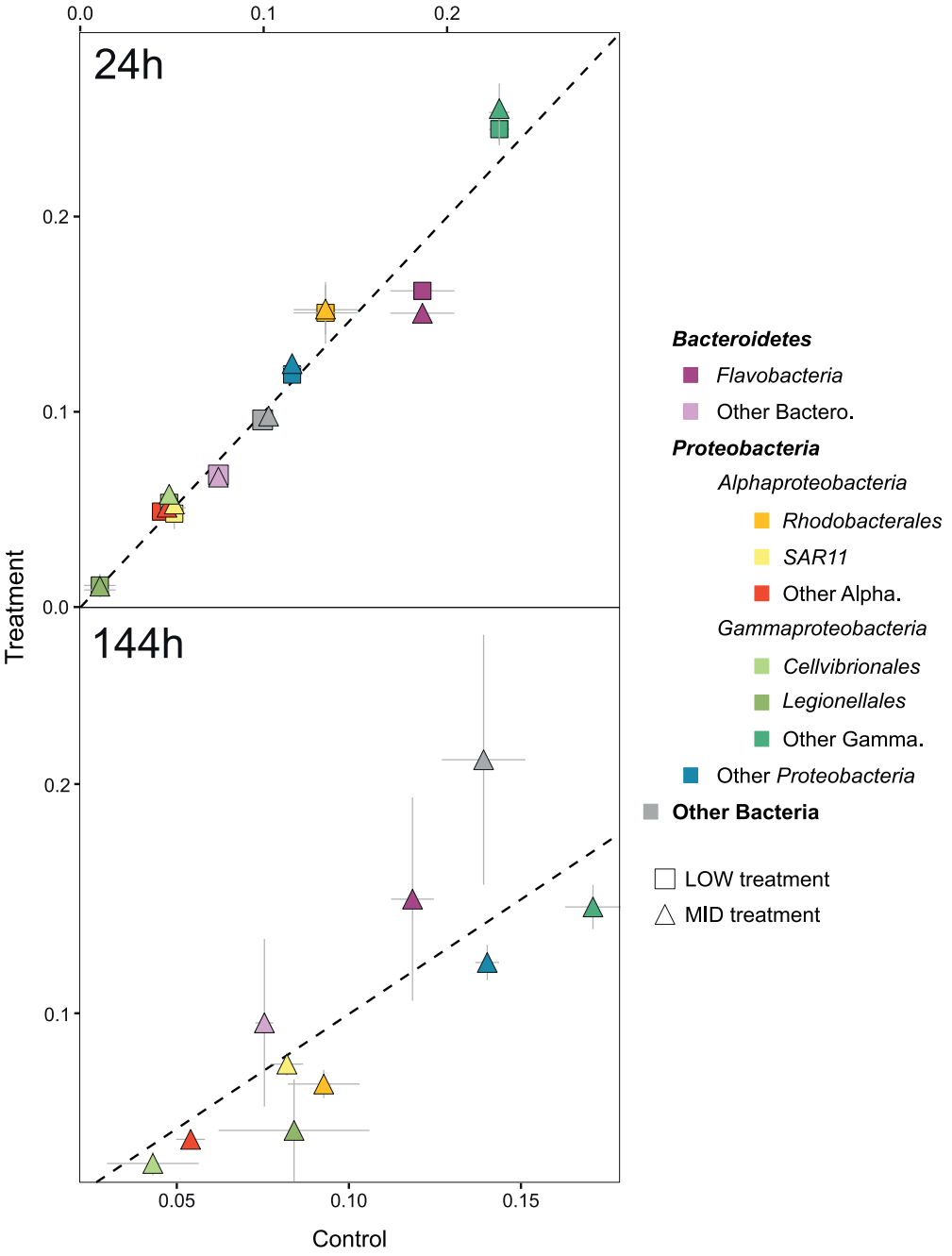

Figure 4. Contribution of each taxonomical group to the total number of transcripts in controls and PFAAs amendments after 24 h (top panel) and 6 days (bottom panel). Data points on the 1:1 line indicate equal contribution to the transcript pool. Values are means of duplicates. Error bars show standard deviations.

in the PFAA exposed communities (Fig. 3). Community composition varied over the time course of the experiments based on 16S rDNA sequencing analyses. Bacterial communities after 6 days of exposure to PFAAs were different from those in the controls (Fig. S1). Although no significant changes were recorded at higher taxonomic levels (Fig. S2), some specific taxa got significantly enriched after 24 h and after 6 days (Table S4). The larger number of taxa increasing in the PFAAs mix enrichments belonged to *Gammaproteobacteria* (specially *Alteromonadales*), and *Flavobacteriia* (mainly *Flavobacteriales*). These results were in agreement with CARD-FISH counts, in which *Gammaproteobacteria* and *Flavobacteriia* increased in relative abundance in the treatments after 24 h and after 6 days, respectively (Fig. 3).

In concordance with our results, the few PFOS and PFOA biodegraders isolated so far belong to the *Proteobacteria* phylum, especially to the *Gammaproteobacteria* class, and the genus *Pseudomonas* (Table S5). Both *Pseudomonas* and *Alteromonas* are closely related genera, common in the marine environment (Baumann *et al*., 1983). In the environment, few communities have been analyzed in relation to PFAA concentrations. In freshwater sediments, *Betaproteobacteria* and *Epsilonproteobacteria* are recognized tolerant classes to high PFAA concentrations (Table S5, Sun *et al*., 2016; Li *et al*., 2017), but these studies did not report degradation of any PFAA. Interestingly, in surface seawaters, *Bacteroidetes* have been reported to have positive correlations to *in situ* concentrations of PFAAs (Chen *et al*., 2019).

Functional profiles in short- and long-term experiment

Generally, specific EEA decreased in the PFAA exposed treatments after 24 h, with significant decreases in Leu-amp and APA (Fig. 3). This decrease after a short exposure, contrast with the EEA increases observed after 6 days,

being significant for β-Glc. This is consistent with the observed changes in communities, but also suggests that potential toxic effects were overcome by *Gammaproteobacteria* after 6 days of exposure, which may have contributed to this response in terms of EEA. The lower abundance of cells but higher specific EEA activities after 6 days suggests the development of a tolerant community, including *Gammaproteobacteria*. These results are in agreement with BONCAT percentages and that absolute number of transcripts that were generally lower in the PFAAs treatments than in the controls after 24 h but higher after 6 days (Fig. 3 and S3). Taxonomy of the metatranscriptomic reads indicated a higher relative abundance of transcripts harbored by *Gammaproteobacteria* and *Rhodobacterales* after 24 h and *Bacteroidetes*, including *Flavobacteriia*, after 6 days (Fig. 4), in agreement with BONCAT trends in dose-response bioassays. Differences in *SAR11* activities (low in metatranscriptomes, high in BONCAT) can be due to the already described divergences between CARD-FISH probes and 16S primers, especially in *SAR11* group from Antarctic waters (Cerro-Gálvez *et al*., 2019a). Individual significantly differentially expressed genes in the treatments than in the controls (EdgeR test; FDR < 0.05) showed a higher transcriptional activity of *Flavobacteriia* after 6 days, accounting for most of the up-regulated genes, along with *SAR11*. Interestingly, as many as 19 individual transcripts in the respiration SEED category increased, indicating a rising of heterotrophic activity by the PFOA tolerant community (Fig. 5). *Bacteroidetes* and *Proteobacteria* have been reported to be the selected resistant bacteria in lab-scale sequencing batch reactor continuously exposed to high PFOA concentrations (in the order of mg/l) (Yu *et al*., 2018).

SEED functional categories “DNA metabolism” and “Sulfur metabolism” got significantly enriched after 24 h (t-test; $P < 0.05$) but not after 6 days (Fig. 5), consistent with the sharp decrease of PFOS concentrations in the short-term

incubation, and suggesting a linkage of PFOS consumption to the sulfur cycle in taxonomic groups able to actively grow on PFOS desulfurization (Fig. 2 and S4). Several significant more than 2-fold increases of genes in different sulfur-related categories in PFAA treatments were observed after 24 h, including genes related to dimethylsulfoniopropionate (DMSP) metabolism and sulfur oxidation (Fig.

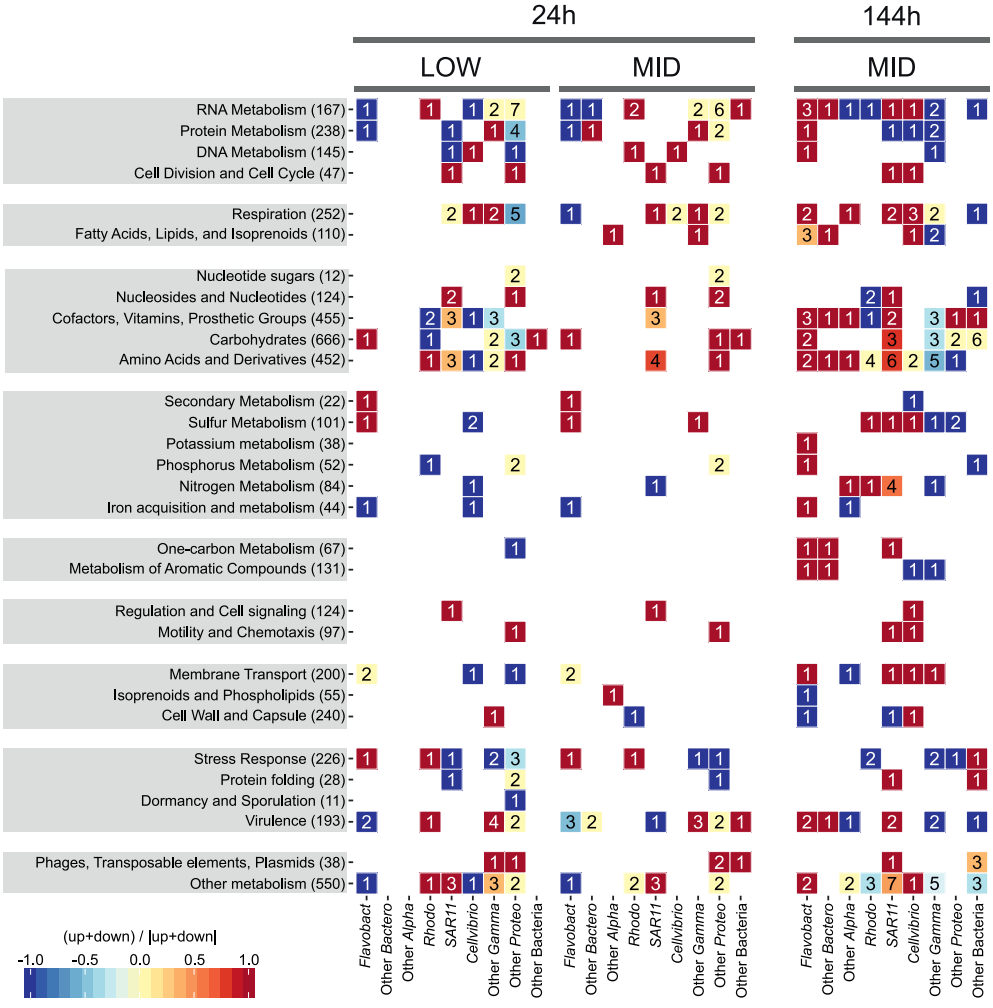

Figure 5. Total number of significantly enriched and depleted transcripts detected by edgeR (FDR < 0.05). Counts are indicated inside each tile. Blue tiles indicate depleted transcripts, red tiles indicate enriched transcripts. Each row corresponds to one SEED category (values in parentheses is the total number of transcripts of that category). Flavobact: *Flavobacteriia*; Other Bactero: Other *Bacteroidetes*; Other Alpha: Other *Alphaproteobacteria*; Rhodo: *Rhodobacterales*; Cellvibrio: *Cellvibrionadales*; Other Gamma: Other *Gammaproteobacteria*; Other Proteo: Other *Proteobacteria*.

6). The higher the concentrations of PFAAs, the higher number of up-regulated sulfur-related genes. Individual significant differentially expressed sulfur-related genes in the PFAA treatments were also more abundant after 24 h than after 6 days (Fig. 7, S5 and Table S6). The enriched genes were classified into three main categories. Firstly, release of sulfite group, encoding lyases of the carbon-SO_3^- bond. Three different gene families were identified potentially encoding this process: sulfonate and alkanesulfonate monooxygenases, potentially forming perfluorooctanol as transformation product, DMSP-related genes, encoding the first step of DMSP degradation, both DMSP lyases (*dddP*, *dddD,* etc.) and DMSP demethylase (*dmdA*), and sulfatase genes which are sulfohydrolases. Secondly, oxidation of sulfite to sulfate mostly encoded by *sox* family genes, showing the use of PFOS as source of reducing power, and a third category including reduction of sulfite to hydrogen sulfide encoded by the bidirectional enzyme sulfite reductase, and incorporation of sulfur-PFOS in bacterial biomass through homocysteine and cysteine metabolism (Fig. 7, S5 and Table S6). Consistent with metatranscriptomes and BONCAT results, most of these genes were encoded by *Gammaproteobacteria*, *Flavobacteriia*, *SAR11* and *Rhodobacterales*. These

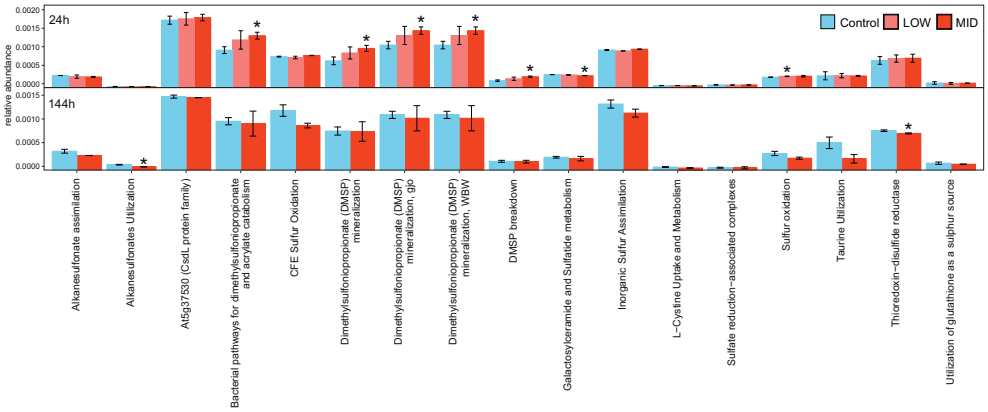

Figure 6. Relative abundance of transcripts belonging to sulfur metabolism SEED subcategories, after 24 h and 6 days of PFAAs incubations. Asterisks indicate significant differences between PFAAs amendment and controls (t-test; $P < 0.05$). Values are means of duplicates. Error bars show standard deviation of duplicates.

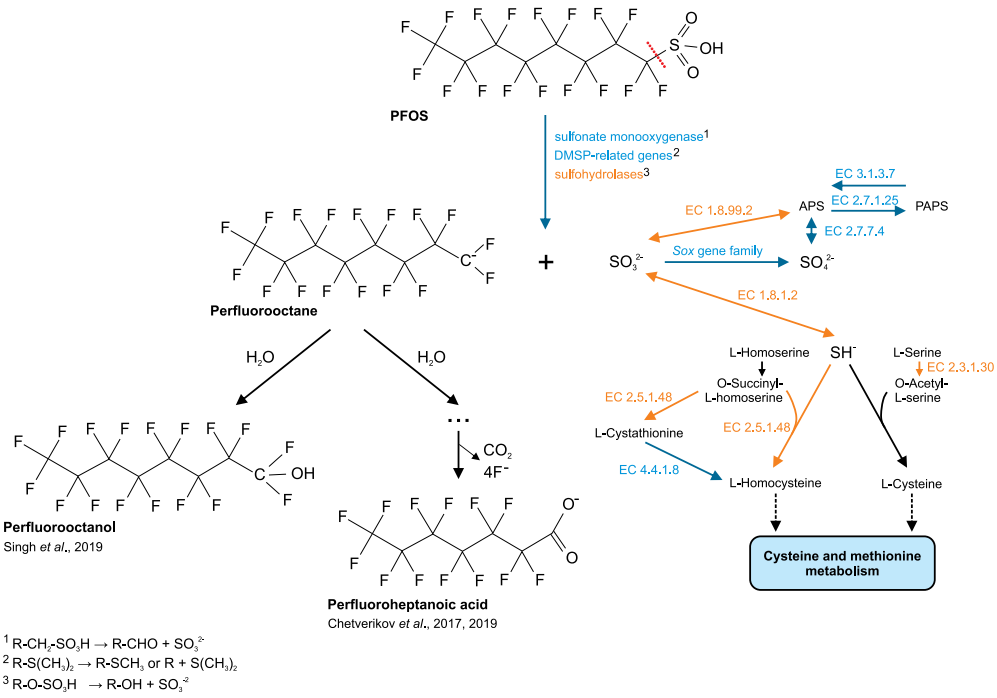

Figure 7. Suggested pathways of bacterial PFOS desulfurization based on enriched transcripts in metatranscriptomes supplemented with chemical pathways suggested in the literature. Significant enriched transcripts (EdgeR test; FDR < 0.05; Table S6) are represented in orange and transcripts >2-fold enriched in PFAAs treatments are labeled in blue (Fig. S3). No evidence of formation of perfluoroheptanoic acid as reported in Chetverikov *et al*. (2017; 2018) was found in this work, while formation of perfluoroctanol was feasible.

groups are known degraders of reduced sulfur in the oceans (so far described mainly for DMSP) and main players in the inorganic sulfur metabolism (González *et al*., 1999; Yoch, 2002; Vila-Costa *et al*., 2006, 2010).

Unfortunately, the absence of identified genes specifically encoding PFOS degradation pathways hampers a direct comparison of our results with previously reported metatranscriptomic data for PFOS degradation. In addition, the fact that differentially expressed genes belonged to the sulfur metabolism SEED category further confirms that degradation was related to desulfurization, but not to defluorination. In fact, no evidence of formation of perfluoroheptanoic acid (Fig.

7) as suggested elsewhere (Chetverikov *et al*., 2017) was found in this work, while formation of perfluoroctanol was feasible. Perfluorooctanol must have a very high Henry's law constant (as polyfluorinated telomers have), and thus was lost by volatilization during the incubations and could not be analyzed. Conversely, perfluoroheptanoic acid was analyzed in controls and treatments (results not shown), but it did not show a significant increase of comparable magnitude with the decrease of PFOS.

The potential linkage between PFOS desulfurization and the sulfur biogeochemical cycle revealed by our data should be further explored. For instance, the few groups identified at sites with high concentrations of PFAAs correspond to species directly related to sulfur metabolism, such as *Thiobacillus* and *Sulfurimonas* in freshwater sediments (Table S5, Sun *et al*., 2016; Li *et al*., 2017).

Potential impact of PFOS desulfurization to the sulfur biogeochemical cycle

Sulfate is at mM concentrations in seawater and bacteria have the capability to reduce it, so that they never get limited by this element (Muyzer and Stams, 2008). However, the use of sulfur available in a reduced form is energetically advantageous. Molecules containing reduced sulfur are hence actively and preferentially consumed in the marine environment due to the energetic advantage they confer to build cell proteins. The main reduced sulfur-containing molecule in the oceans is DMSP, a ubiquitous compound in the upper layers of the water column. It is produced by many species of phytoplankton in the euphotic ocean (Keller *et al*., 1989), where it represents a major pool of reduced sulfur (Bates *et al*., 1994). At the global scale, DMSP and its breakdown product, dimethylsufide (DMS), can supply most of the sulfur and part of the carbon requirements of marine microbial communities (Kiene and Linn, 2000; Simó *et al*., 2009). DMSP-

degradation genes have been identified mostly in *Alphaproteobacteria* (mainly *SAR11* and *Roseobacter*), which can explain the absence of these genes in our treatments where *Flavobacteriia* and *Gammaproteobacteria* harbored most of the transcripts related to sulfur metabolism. Until now, nobody has put into the equation the presence of synthetic compounds containing reduced sulfur in the oceans. However, our results point to a very efficient use of the reduced sulfur from PFOS molecules. The fast desulfurization of PFOS, more than 50% after 48 h, can be a feature of polar environments, specially stressed in Port Foster bay (Deception Island) thanks to the hydrothermal venting activity and underwater volcanism, possibly selecting for microorganisms suited to live in the presence of several forms of sulfur-containing molecules (Somoza *et al*., 2004).

PFOS desulfurization promoted the expression of genes related to sulfur oxidation, sulfur incorporation and DMSP degradation, pointing to the use of PFOS as a reducing power source, sulfur source for bacterial biomass, a direct interference to DMSP cycling in the oceans. Concentrations of PFOS are low in oceanic waters compared with those of DMSP. However, there are other poly and perfluoroalkyl sulfonates in Antarctic waters (Casal *et al*., 2017a), and other anthropogenic organic chemicals containing sulfur such as dibenzothiophenes among others (Casal *et al*., 2018) that substantially increase the concentration of anthropogenic organic sulfur compounds. How widespread this interaction between the unquantified pool of anthropogenic organosulfur compounds and sulfur cycle is, in polar environments, a matter of future research.

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Microbial responses to anthropogenic dissolved organic carbon in Arctic and Antarctic coastal seawaters

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ABSTRACT

Thousands of semi-volatile hydrophobic organic pollutants (OPs) reach open oceans through atmospheric deposition, causing a chronic and ubiquitous pollution by anthropogenic dissolved organic carbon (ADOC). Hydrophobic ADOC accumulate in cellular lipids, inducing harmful effects on marine biota, and can be partially prone to microbial degradation. Unfortunately, their possible effects on microorganisms, key drivers of global biogeochemical cycles, remain unknown. We challenged coastal microbial communities from Ny-Ålesund (Arctic) and Livingston Island (Antarctica) with ADOC concentrations within the range of oceanic concentrations in 24 h. ADOC addition elicited clear transcriptional responses in multiple microbial heterotrophic metabolisms in ubiquitous groups such as *Flavobacteriia*, *Gammaproteobacteria* and *SAR11*. Importantly, a suite of cellular adaptations and detoxifying mechanisms, including remodeling of membrane lipids and transporters, was detected. ADOC exposure also changed the composition of microbial communities, through stimulation of rare biosphere taxa. Many of these taxa belong to recognized OPs degraders. This work shows that ADOC at environmentally relevant concentrations substantially influences marine microbial communities. Given that emissions of organic pollutants are growing during the Anthropocene, the results shown here suggest an increasing influence of ADOC on the structure of microbial communities and the biogeochemical cycles regulated by marine microbes.

***** In this chapter, tables and figures numbering corresponds to the original published article ennumeration.

INTRODUCTION

Marine microbial communities can respond to changes in the composition of dissolved organic carbon (DOC) (McCarren *et al.,* 2010; Mason *et al.,* 2012), even if these variations correspond to a small fraction of the total DOC (Moran *et al.*, 2016). DOC can have a biogenic and anthropogenic origin, influencing its composition, quantity and quality. However, the influence of the pool of anthropogenic dissolved organic carbon (ADOC), composed by thousands of organic pollutants (OPs), on microbial communities, has barely been assessed (Dachs and Méjanelle, 2010). Conversely, there are numerous works reporting the effects of simple mixtures of OPs, for which there are commercially available standards (e.g. Echeveste *et al.*, 2010). Most of the known OPs in remote seawaters are hydrophobic, non-polar compounds, and thus part of the lipidic dissolved organic carbon.

ADOC originates from hydrocarbon emissions from fossil fuel use and synthetic organic compounds manufactured and used by humans. In total, around one hundred thousand synthetic organic chemicals are currently in use and commercialized, including agricultural, household and industrial chemicals (Lohmann *et al.*, 2007). Although the total amount of ADOC is unknown, some persistent organic pollutants (POPs) are regulated by international legislation, such as the Stockholm Convention, due to their described adverse impacts on the environment and human health (UNEP, 2009). Chemical pollution remains uncharacterized as a vector of global change (Rockström *et al.*, 2009; Moran *et al.*, 2016). The input of synthetic organic compounds reaching the surface oceans has been increasing during the last century (Dachs and Méjanelle, 2010). OPs reach the global oceans mainly by long range atmospheric transport and deposition (Lohmann *et al.*, 2007; Galbán-Malagón *et al.*, 2012; González-

Gaya *et al.*, 2016), which is a spatially-extended source of “diffusive pollution” supporting baseline concentrations of OPs in the surface ocean (Nash, 2011; Galbán-Malagón *et al.*, 2012; Hung *et al.*, 2016). In polar environments, low temperatures favor the persistence of OPs in the water column. The Arctic has received a greater input of OPs than Antarctica because, historically, most OPs have been produced and used in the Northern Hemisphere (Nash, 2011; Hung *et al.*, 2016).

Microbial communities are likely to play important roles in determining the fate of many ADOC compounds by biodegradation. The study of interactions between microorganisms and OPs has primarily focused on microbial consumption of hydrocarbons in oil spills (Head *et al.*, 2006; Mason *et al.*, 2012). These studies have shown that large inputs of alkanes and polycyclic aromatic hydrocarbons (PAHs) trigger rapid responses in microbial communities and influence the successional dynamics of communities by selecting for specialized degraders (e.g., (Mason *et al.*, 2012; Garneau *et al.*, 2016)). These bacterial groups have the capacity to use alkanes and PAHs as a carbon and energy source (Rivers *et al.*, 2013; Dombrowski *et al.*, 2016). However, the amounts of airborne hydrocarbons reaching the upper ocean are orders of magnitude larger than those from oil spills (González-Gaya *et al.*, 2016). For instance, the diffusive atmospheric deposition of semivolatile aromatic compounds accounts for 15% of the net input of carbon (as CO_2) to the ocean, potentially affecting marine microbial food webs and the oceanic carbon cycle (Galbán-Malagón *et al.*, 2013a; González-Gaya *et al.*, 2016). Still, the ecological impact of such airborne hydrocarbons, and thousands of other synthetic legacy and emerging organic pollutants, remains largely uncharacterized.

Hydrophobic OPs accumulate in planktonic organisms, mainly in the lipids of the membranes, and thereby enter into marine food webs (Wallberg and Andersson,

2000; Galbán-Malagón *et al.*, 2012). They cross cell membranes by passive diffusion and can be absorbed into cytoplasmic structures, become inserted into the membrane phospholipid bilayer (absorption), and/or be adsorbed on the outside of the cell wall (Koelmans, 2014). This uptake of OPs causes a baseline toxicity to the cells by narcosis, the non-specific intercalation of hydrophobic compounds into the phospholipids of the biological membranes, inducing a perturbation of the membrane's permeability, rigidity and efficiency (Van Wezel *et al.*, 1995; Cronin *et al.*, 2000; Escher *et al.*, 2017). Recent studies have shown that hydrophobic OPs have toxicological effects on temperate and polar phytoplankton. The smallest cells, *Synechococcus* and *Prochlorococcus*, were the most sensitive in the subtropical oligotrophic oceans, but diatoms and flagellates also decreased in abundance in the Arctic and Southern oceans (Echeveste *et al.*, 2016). Minimal variation of the aliphatic and aromatic fractions of ADOC inhibited gene expression of photosynthetic genes of *Prochlorococcus* without inducing mortality (Fernández-Pinos *et al.*, 2017). Analysis of community gene expression, metatranscriptomics, has been successfully used to describe effects of oil spills on microbial communities (Moran *et al.*, 2013). However, these methodologies have not been used to study responses of prokaryotes at the ADOC concentrations found in the global oceans.

The central hypothesis of this work is that the low, but ubiquitous, surface ADOC concentrations originated by diffusive pollution have an outsized effect on the structure and functioning of polar microbial communities. We challenged coastal Arctic and Antarctic bacterial communities with realistic ADOC concentrations, previously extracted from seawater, and analyzed changes in community composition, gene frequencies and transcriptional profiles in short-term incubations (24 h). ADOC additions aimed at inducing variations of concentrations within a factor of two, representative of the seasonal and spatial variability of

concentrations observed in Arctic and Antarctic waters (Galbán-Malagón *et al.*, 2012; Galbán-Malagón *et al.*, 2013a; Casal *et al.*, 2018). We assess whether or not this anthropogenic impact can modulate seawater microbial community composition and functioning, and explore bacterial strategies to cope with hydrophobic OPs.

MATERIALS AND METHODS

Description of Experiments

Dose-response of fractions of lipidic DOC on prokaryotic growth

Surface seawater was collected from South Bay at Livingston Island (South Shetlands Islands, Antarctica, 62°39'26.3''S 60°23'18.4''W, 27 January 2015) (Fig. S10). The experiment consisted on adding fractions of lipidic DOC (see below for a description of how this was collected) with different polarity at different concentrations: 1.4-, 2.6- and 8.2-fold of *in situ* concentrations and a control without addition. The effects of the aliphatic (F1), aromatic (F2), polar (F3) fractions were assessed (see below for details), as well as the merged F1+F2, named here as ADOC. Each of these 13 treatments (controls with no additions, and 12 treatments consisting on 3 different concentrations of 4 different ADOC fractions) consisted of 40 ml of seawater incubated in duplicate in the dark in previously baked glass bottles (450°C, 4 h to eliminate traces of organic matter) *at in situ* temperature. In all cases, the fraction of organic chemicals, or solvent for controls, was added to the empty bottles and the solvent let to evaporate before adding the seawater. Abundance of prokaryotic cells was determined after 0, 1, 3, 16, 24 and 48 h of incubation (see below). Growth rates were calculated based on the slope of cell abundances versus time over the duration of the experiment.

Responses of prokaryotic community to ADOC

Two liters of surface seawater from the aforementioned Antarctic site and from Kongsfjorden at Ny-Ålesund (Svalbard Islands, Arctic, 78°56'6.5''N 12°08'30.8''E, 26 June 2014) (Fig. S10) were taken in pre-baked 2 l glass bottles and incubated for 0.5 h or 24 h into pre-baked 2 l glass bottles in duplicates under two conditions: (i) treatment receiving addition of ADOC (F1+F2) dissolved in acetone. These treatments aimed at inducing small changes in the concentrations of hydrophobic anthropogenic chemicals, as those usually observed in oceanic waters. (ii) A control treatment that received the same amount of acetone with no ADOC. In both cases, acetone was let to evaporate for 2 h before adding the seawater. In order to control un-avoidable handling artifacts when working with hydrophobic chemicals, such as sorption to cell walls, volatilization, or un-intended contamination, measurement of a representative family of ADOC compounds, PAHs, were done for the incubation bottles at initial and final times. The addition of ADOC to the treatments resulted in parent PAH exposure concentrations 1.7- and 1.5-fold higher than *in situ* concentrations in the open Arctic and Southern Oceans, respectively. Such measurements in the incubation bottles are extremely important in order to assure the induced levels of hydrophobic chemicals in treatments and controls, as controls (seawater) already contain ADOC and the addition of ADOC aimed at inducing minimal changes in concentrations. Such chemical analyses have been neglected in most previous assessments of hydrocarbon and pollutant effects on microbial communities. The experiment was run with attenuated light with a mesh at *in situ* temperature (1-2ºC). Samples for determining individual OP concentrations in the dissolved phase, abundance of prokaryotic cells, CARD-FISH and nucleic acid collection were taken after 30 min and 24 h of incubation. Therefore, the four bottles sampled after 30 min were different than the four bottles sampled after 24 h of incubation.

Preparation of ADOC

The spike solution used in the experiments was the non-polar fraction of a seawater extract containing the hydrophobic organic compounds present in the surface ocean. Operationally defined ADOC was obtained following the standard protocols for sampling and extraction of hydrophobic and non-polar OPs and hydrocarbons from seawater (Galbán-Malagón *et al.*, 2012; Galbán-Malagón *et al.*, 2013a; González-Gaya *et al.*, 2016; Fernández-Pinos *et al.*, 2017). Briefly, two hundred liters of surface water (4 m depth) was collected during the ATOS-I and ATOS-II sampling cruises in the Arctic (Galbán-Malagón *et al.*, 2012) and Southern Oceans (Galbán-Malagón *et al.*, 2013b). Seawater was pre-filtered on a pre-combusted glass fiber filter (GF/F, Whatmann) and then eluted and concentrated on a XAD-2 absorbent column. The XAD-2 adsorbent was eluted with methanol followed by dichloromethane. The methanol was liquid-liquid extracted with hexane, and the hexane was merged with the dichloromethane and concentrated by rotary vacuum evaporation to 0.5 ml. This extract contained the lipidic DOC, which was fractionated on an alumina column to separate the non-polar and polar compounds using sequentially hexane (fraction F1, aliphatic), dichloromethane:hexane (2:1, fraction F2, aromatic), and dichloromethane:methanol (2:1, fraction F3, polar). Aliquots of F1 and F2 were merged and operationally defined as ADOC, as it contains a mixture of non-polar hydrophobic organic compounds, and these chemicals are known to be anthropogenic (Lohmann *et al.*, 2007), including the unresolved complex mixture (UCM) (White *et al.*, 2013; Farrington and Quinn, 2015) that dominates in terms of mass (González-Gaya *et al.*, 2016). This is analogous to the mixture used previously for assessing the influence of ADOC to phytoplankton (Echeveste *et al.*, 2016; Fernández-Pinos *et al.*, 2017). Compound-specific seawater concentrations of PAHs, PCBs, hexachlorobenzene (HCB), and hexachlorocyclohexanes (HCHs) in the Arctic and Southern Ocean are shown in

Table S5. These chemicals are markers of a much complex mixture present in ADOC. The use of a fraction of seawater extract for which we have previous geochemical knowledge allows obtaining the best experimental approximation to ADOC. It would not be feasible generating a mixture from individual standards of synthetic chemicals and hydrocarbons, since part of the composition of ADOC is unknown and there are no standards even for all the known compounds.

Concentrations of selected ADOC compounds in the experiments

The concentration of characteristic OPs in ADOC were measured prior to their use in the experiments, using the methods previously described (Galbán-Malagón *et al.*, 2012, 2013a; González-Gaya *et al.*, 2016). Furthermore, concentrations of PAHs, chosen as model molecules present in ADOC, were measured in the dissolved phase at the beginning and at the end of the experiment using the filtrate obtained after sampling DNA/RNA material. In addition, n-C_{17} and n-C_{19} alkanes were analyzed as representative of well-known hydrocarbons being both biogenic and anthropogenic. The polar fraction (F3) is structurally complex with many biogenic chemicals, but it contains also some anthropogenic compounds not included in the operationally defined ADOC (merged F1 and F2). Thus, organophosphate esters (OPEs) were analyzed in the incubations as model compounds for polar OPs. Therefore, both n-alkanes and OPEs were not enriched necessarily in the incubations, but showed a variability in their dissolved concentrations due to either microbial mediated processes (degradation, sorption) or manipulation artifacts (sorption to bottle walls…). Analyses were performed following the procedures described elsewhere (Berrojalbiz *et al.*, 2009; Fernández-Pinos *et al.*, 2017; González-Gaya *et al.*, 2019; Vila-Costa *et al.*, 2019). See supplemental information for details.

CARD-FISH and flow cytometric determination of prokaryotic cell abundances

CARD-FISH analyses was performed as described elsewhere (Pernthaler *et al.*, 2004) using the oligonucleotide probes listed on Table S6. Images were acquired using a digital camera (Zeiss camera AxioCam MRm, Carl Zeiss MicroImaging, S.L., Barcelona, Spain) at 630x magnification through the Axiovision software and analyzed using the automated image analysis software ACMEtool. All images (at least 10 fields/filter) were acquired using 20 ms exposure time. Prokaryotic cell abundance was estimated by flow cytometry as described elsewhere (Falcioni *et al.*, 2008). See supplemental material for details.

DNA and mRNA extraction and sequencing

After 30 min ADOC addition, 2 l seawater was pre-filtered through a 3 µm pore-size 47 mm diameter polytetrafluoroethylene filter to exclude grazers and the particle-attached bacteria and free-living bacterial cells were collected on 0.2 µm pore-size 47 mm polytetrafluoroethylene filters under low vacuum pressure. The duration of the filtration step was no longer than 20 min to minimize RNA degradation. Each filter was cut in two halves, one was placed in 1 ml RNAlater (Sigma-Aldrich, Saint Louis, MO) and the other one into 1 ml lysis buffer (50 mM Tris HCl, 40 mM EDTA, 0.75 M sucrose) and stored at -20°C to preserve RNA and DNA respectively.

DNA extraction was performed as described in Vila-Costa *et al.* (2019). A DNA standard (*Thermus thermophilus DSM7039 [HB27]* genomic DNA) was added as internal control at 0.5% of the total mass of extracted DNA to estimate absolute genes numbers. Total RNA was extracted, DNA removed, rRNA depleted and mRNA enriched by amplification as described in Poretsky *et al.* (2010) with the only modification that total RNA was extracted with mirVana isolation kit

(Ambion), after removing the storage reagent by centrifugation. Artificial mRNA was synthesized by *in vitro* transcription from a pGEM-3Z plasmid (Promega, Madison, WI, USA) with ScaI restriction enzyme (Roche, Penzberg, Germany) as described in Gifford and colleagues (2011). This artificial mRNA was added after RNA extraction but before mRNA enrichment as in Rinta-Kanto *et al.*, 2012 and used as an internal standard to calculate absolute transcript abundances (Moran *et al.*, 2013; Satinsky *et al.*, 2013). See supplemental material information for details. Final DNA and amplified RNA were sequenced at the National Center for Genomic Analysis (CNAG, Barcelona, Spain) using Illumina high output mode HS200 2x100bp v4.

Bioinformatics

DNA and cDNA sequences were quality trimmed and internal standards and any remaining stable RNA was quantified and removed using the ERNE mapping program (Del Fabbro *et al.,* 2013) against the internal standard sequences and an in-house database of marine bacterial stable RNA sequences (rRNA and tRNA), respectively. Subsequently, read pairs were joined using the PEAR program (https://www.h-its.org/en/research/sco/software). Joined pairs, as well as separate reads not corresponding to joined pairs were aligned to the NCBI RefSeq database (downloaded October 2016) using the Diamond aligner, v0.8.25, (Buchfink *et al.,* 2014) in blastx mode with default parameters. The resulting alignments were taxonomically and functionally classified with MEGAN 6.5.10 (Huson *et al.,* 2016) and exported for further analysis in R/tidyverse. Sequenced belonging to archaea and eukaryotic cells were excluded due to low abundances. Search for specific functions was performed with Pfam profiles, using HMMER (Eddy, 2011), and by gene name. A list of specific genes and Pfam profiles used is listed in Table S7. Assembly of metagenomic and metatranscriptomic reads was performed with MEGAHIT using default settings (Li *et al.,* 2015) and contigs

coverage was quantified by mapping reads to contigs with Bowtie 2 (Langmead and Salzberg, 2012) and then converting to idxstats files with SAMtools (Li *et al.,* 2009). Metagenome samples resulted in a total of 638 million paired-end reads and an average of 30.6 million reads per sample in a typical length of 101bp. After quality-trimming and filtering of added internal standards, between 32.7 and 39.2% of the potential protein-coding reads were taxonomically annotated and between 9.4 and 15.1% of the protein-coding reads had SEED functional annotations (Table S8). The sequencing of the metatranscriptomes resulted in a total of 853 million reads (53.3 ± 7.1 million raw reads per sample) and remained 397 million possible protein-encoding sequences (30.2 ± 9.7 million per sample). Among these, between 23.2 and 40.4% were taxonomically annotated and between 8.1 and 21.0% were functionally annotated in SEED (Table S8). We observed a significantly lower percentage of identifiable ribosomal RNA in both replicates from Arctic ADOC amendments after 24 h compared to the other samples, indicating the presence of unidentified organisms in these samples (Table S8). To address this question, we assembled all metatranscriptomes – other analyses were based on non-assembled sequence reads – and quantified the relative abundance of contigs in the samples. We observed a significant 5-fold increase of contigs from 200 to 700 bp in the metatranscriptomes of Arctic ADOC amendments compared to the others. The majority of these contigs were annotated as other bacteria and unclassified bacteria. High abundances of unidentified bacteria have been reported previously for Arctic bacterial communities exposed to oil spills (Garneau *et al.,* 2016).

Statistical analyses

All data analyses were carried out in R. Significant differences observed between treatments were analyzed with t-Student test. Permutational multivariate analysis of variance (permanova) was performed with the Vegan package (Oksanen *et*

al., 2018). Analysis of differential gene abundances was performed with the EdgeR package (Dimont *et al.*, 2015). Spearman's rank correlation coefficients were calculated to determine the relationship between the genes/transcripts significantly differentially expressed and the concentrations of individual PAHs, OPEs and alkanes in the treatments and controls, and for 0.5 and 24 h of incubation time (correlations from 8 pairs of measurements).

RESULTS AND DISCUSSION

To analyze the impact of ADOC on microbial communities, we performed two types of experiments. First, we challenged Antarctic bacterial communities with increasing concentrations of different fractions of hydrophobic organic compounds (aliphatic, aromatic and polar lipidic DOC) in order to explore their potential influence to bacterial growth rates. In a second set of experiments, responses of bacterial communities taken from coastal Arctic and Antarctic waters challenged with realistic ADOC concentrations were analyzed by means of molecular and genomic approaches.

Dose-response experiment: exploratory effects of fractions of lipidic DOC with different polarity on bacterial growth rate

OPs belonging to the hydrophobic fraction of DOC (or lipidic DOC) can be separated into 3 fractions of different polarity. The F1 fraction (the most apolar fraction) is mainly anthropogenic and includes linear and branched alkanes, the aliphatic unresolved complex mixture (UCM), polychlorinated biphenyls (PCBs), dechlorane plus (DP), organochlorine pesticides such as hexachlorocyclohexanes (HCHs) and hexachlorobenzene (HCB), and other non-polar OPs (Dachs *et al.*, 1999; Galbán-Malagón *et al.*, 2012; White *et al.*, 2013). A small fraction of F1 is of biotic origin since cyanobacteria and other phytoplanktonic groups produce

linear and branched alkanes, such as n-C_{17} (Lea-Smith *et al.*, 2015). The F2 fraction includes polycyclic aromatic hydrocarbons and other aromatic-like hydrocarbons such as the aromatic UCM (González-Gaya *et al.*, 2016). The F3 fraction (the most polar fraction) is the chemically most complex fraction of lipidic DOC, including many biogenic compounds, but also some OPs such as organophosphate esters plasticizers (OPEs). The absence of previous studies reporting the effects of these fractions on bacterial heterotrophic activities motivated an experiment to determine the dose response of F1 aliphatic, F2 aromatic, F3 polar, and a merged F1+F2 fraction using coastal seawater from South Bay (Livingston Island, Antarctica) in 48 h incubations. F1 and F2 are mostly ADOC, while F3 is not due to high contributions of biogenic chemicals. Nevertheless, its effects were tested for comparative purposes. The contrasting trophic strategies of bacteria with different DNA content and therefore different cytometric signatures (Vila-Costa *et al.*, 2012) motivated an analysis separating growth rates of high nucleic acid (HNA) and low nucleic acid (LNA)-content bacteria in this experiment. Differences in cell growth for the three lipidic DOC fraction amendments were compared to controls.

Enrichment with increasing concentrations of F1 inhibited cell growth at concentrations 2.6-fold higher than the *in situ* (or control) concentrations, but stimulated growth of heterotrophic bacteria at lower concentrations (Fig. 1). LNA bacteria showed slight but notable variations at low concentrations of F2, and an increase of growth rate at high concentrations. HNA showed similar trends as LNA but with a larger variability in the growth rates. The stimulation at lower concentrations might indicate use of compounds in these fractions as carbon or energy sources, whereas at higher concentrations the negative effects on cell growth dominated (Kabelitz *et al.*, 2003; Lea-Smith *et al.*, 2015). Some compounds present in F2 are semi-labile, such as naphthalene and

phenanthrene and can be respired by multiple groups of marine bacteria (Head *et al.*, 2006; Ghosal *et al.*, 2016). The largest variations in growth rate were observed in the treatments with the most polar fraction of lipidic DOC (F3) (Fig. 1). Already at 1.4-fold the *in situ* concentrations, F3 inhibited cell growth respect to the controls for both HNA and LNA, but increased cell growth was observed at higher concentrations, reaching higher rates than in controls for HNA cells (Fig. 1, Table S1). F3 includes a mixture of biogenic and anthropogenic polar compounds such as sterols, ketones, fatty acids, some organophosphate esters (OPEs) flame retardants, and other potentially biologically active and semi-labile

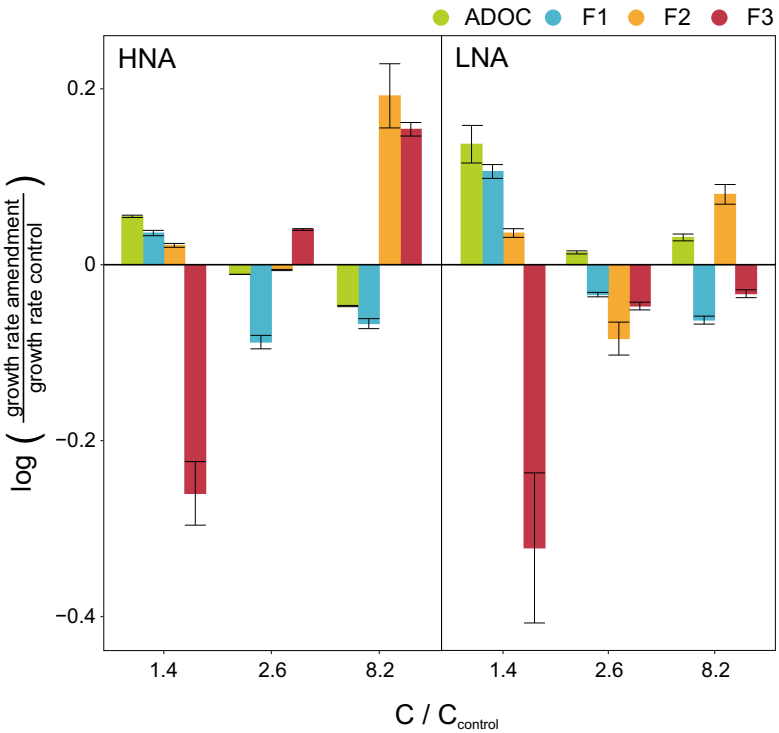

Figure 1. Enrichment factor of growth rate of prokaryotic community microcosms amended with different ADOC fractions (F1 aliphatic, F2 aromatic, F3 polar and F1+F2, named ADOC) under different concentrations. The *x*-axis indicate the enrichment factor in the ADOC amendments. In the *y*-axis, growth rate is reported compared to the non-amended control. The error bars were calculated with error propagation. C, concentration of the ADOC fraction in the amendment; $C_{control}$, concentration of the ADOC fraction in the non amended control; HNA, high nucleic acid cells; LNA, low nucleic acid cells.

molecules. Even though the chemicals present in F3 may affect microbial communities, this fraction of hydrophobic DOC was not considered further in this work since most of the individual chemicals previously reported in this fraction are biogenic polar lipids (Killops and Killops, 2005). The dose response for F1+F2 at the lowest concentrations showed variations of the growth rate consistent with those observed for F1 and F2 separately (Fig. 1). As F1+F2 contains mainly anthropogenic chemicals, this was defined operationally as ADOC, and added to Arctic and Antarctic communities in 24 h incubations in subsequent experiments.

Experiment analyzing the responses of prokaryotic community to ADOC

Concentrations of ADOC compounds

Three characteristic and ubiquitous families of hydrophobic organic compounds (PAHs, n-alkanes and OPEs) were analyzed for the second experiment aiming at assessing the small variations in concentrations of hydrophobic chemicals in treatments and controls. These three families were used as surrogates of chemicals with different hydrophobicity, polarity, and lability. PAHs and alkanes were present in the ADOC added to the treatments, but while PAHs are anthropogenic, n-alkanes such as n-C17 and n-C19 can also have biogenic sources (Lea-Smith *et al.*, 2015). Enrichment of concentrations in treatments versus controls were estimated as the ratio of the mean concentrations in treatments over the mean concentrations in controls. After 30 min of ADOC addition, the increase of concentrations for PAHs in the treatments versus the controls was of 1.7- and 1.5-fold for the Arctic and Antarctica, respectively (Table 1). PAHs can be used as markers of the added ADOC. Therefore, the increase of ADOC in the treatments was of 70% and 50% for the Arctic and Antarctic experiments, respectively. Concentrations of n-C17 and n-C19 alkanes were 1.1- and 1.3-fold the *in situ* concentrations (controls) for the Arctic and Antarctica,

respectively (Table 1); differences not statistically significant). A lack of significant enrichment of OPEs was also observed, consistent with their polarity, and thus not present in the ADOC mixture used in the experiments. OPEs are used here as a marker for polar organic pollutants. OPEs are a widely used family of flame retardants and plasticizers.

It is worth to notice that analysis of OP concentrations in polar waters is extremely challenging, and large volumes of seawater are commonly used (in the order of 100-300L), (Galbán-Malagón *et al.*, 2012, 2013a; Casal, *et al.*, 2018). In this work, only some of the most abundant individual compounds (some PAHs) were above the limits of quantification when analyzed by gas chromatography coupled to mass spectrometry due to the small volume of seawater (2 l) used in the incubations. These concentrations were analyzed from the same bottles where metagenomes and metatranscriptomes were sequenced, using the filtrate after collecting biomass for DNA/RNA material. The average error for the measurements of PAHs at the ultra-trace levels in duplicate bottles was of 16% and 21% for the Arctic and Antarctica, respectively. We did not observe a significant decrease of concentrations of total PAHs, n-alkanes and OPEs larger than the accuracy of the concentration measurements after 24 h of incubation (Table 1; *t*-test, $P > 0.05$). This is consistent with ADOC being persistent enough to reach polar waters by long-range atmospheric transport and deposition. Therefore, ADOC cannot be considered labile, but semi-labile or refractory. Furthermore, degradation is temperature dependent, and the *in situ* seawater temperatures (1-2°C) in Arctic and Antarctica further enhanced chemical's persistence. This is similar to findings in other environments where many families of pollutants in ADOC were shown to be persistent (Lohmann *et al.*, 2007). Still, field experiments performed in tropical seawaters have been able to detect a decrease in concentrations for n-alkanes after 24 h of incubation (Fernández-Pinos *et al.*, 2017). Nevertheless,

it is probable that many of the semi-labile dissolved ADOC components, such as individual n-alkanes, PAHs and HCHs, were being degraded after 24 h of incubation, since microbial degradation of these chemicals is known to occur in the marine environment (Galbán-Malagón *et al.*, 2013a; González-Gaya *et al.*, 2019). We next turned to molecular and genomic approaches to analyze the effects and potential use of ADOC.

Changes in bacterioplankton community composition

Initial Arctic and Antarctic communities were significantly different (permanova, R^2 = 0.68, $P < 0.001$). According to the taxonomy of metagenomic reads at time point 0.5h, the Arctic waters were dominated by *Bacteroidetes* (~32%), mainly members of class *Flavobacteriia*, followed by *Alphaproteobacteria*, mostly accounted for

Table 1. Exposure concentration (ng/l) of selected pollutants [polycyclic aromatic hydrocarbons (PAHs), alkanes and organophosphate esters (OPEs)].

		ARCTIC					
		C05		C24		T05	
		Mean	Error	Mean	Error	Mean	Error
PAHs	Pyrene	100	0	100	0	200	0
	Fluoranthene	100	0	100	0	350	51
	Phenanthrene	850	152	900	202	1,200	0
	Dibenzothiophene	100		100	0	100	0
	Acenaphthene	100	0	100		100	0
	Acenaphthylene	100	0	100		100	0
Alkanes	n-C_{17}	32,350	19,344	36,050	6,819	32,250	8,233
	n-C_{19}	5,550	2,879	4,900	1,313	6,900	1,414
OPEs	TiBP	2,850	758	3,000	202	2,550	455
	TnBP	1,250	152	1,400	303	< LOQ	
	TCEP						
	TCPP-1	45,550	19,647	41,400	5,556	50,050	12,374
	TCPP-2	76,100	21,415	80,150	5,303	76,650	10,960
	TPhP	550	152	< LOQ		500	0
	EHDPP	1,100		< LOQ		1,300	

C: Control; T: ADOC amendment; 05: time point after 30min; 24: time points after 24h;
LOQ: limits of quantification.

by *SAR11* (16-24%) and *Rhodobacterales* (8-12%). Antarctic waters were co-dominated by *Flavobacteriia* (33%), *SAR11* (17%) and *Rhodobacterales* (8%), and also contained a notable proportion of *Gammaproteobacteria* (22%) (Fig. S1). Both environments had bacterial community compositions similar to those earlier described in these regions (Straza *et al.*, 2010; Piquet *et al.*, 2016). There was a good agreement between metagenomics and CARD-FISH approaches (Fig. S2), especially in terms of relative abundance of most groups, supporting metagenomic analysis of community composition.

We found no significant effects of neither ADOC addition nor time on the overall community composition (see permanova results in Table S2). We did detect, however, 58 different taxa in the Arctic and 34 taxa in Antarctica that increased

ANTARCTICA									
T24		C05		C24		T05		T24	
Mean	Error	Mean	Error	Mean	Error	Mean	Error	Mean	Error
100	0								
200	0								
1,300	202	750	253	1,800	505	900	101	1,850	455
150	51	150	51	250	51	250	51	1,250	51
200	101	100	0	100	0	100	0	100	0
100	0	100	0	< LOQ	0	100	0	150	51
45,050	22,476	88,950	26,314	50,500	23,941	62,400	14,950	64,350	11,869
13,250	5,707	14,400	1,515	11,900	2,929	28,100	4,546	16,200	4,647
2,150	253	12,200	2,020	4,150	253	7,900	2,929	7,300	1,111
< LOQ		2,400	202	2,350	51	3,550	51	2,350	152
		4,750	455	5,150	51	< LOQ		5,300	202
42,700	34,446	129,000	20,203	107,850	18,334	69,100	17,072	155,000	38,386
51,050	27,729	284,500	34,850	114,500	2,525	158,400	70,307	215,500	7,576
700		< LOQ		1,000		7,300		900	101
1,200		< LOQ		1,700		1,800		1,800	101

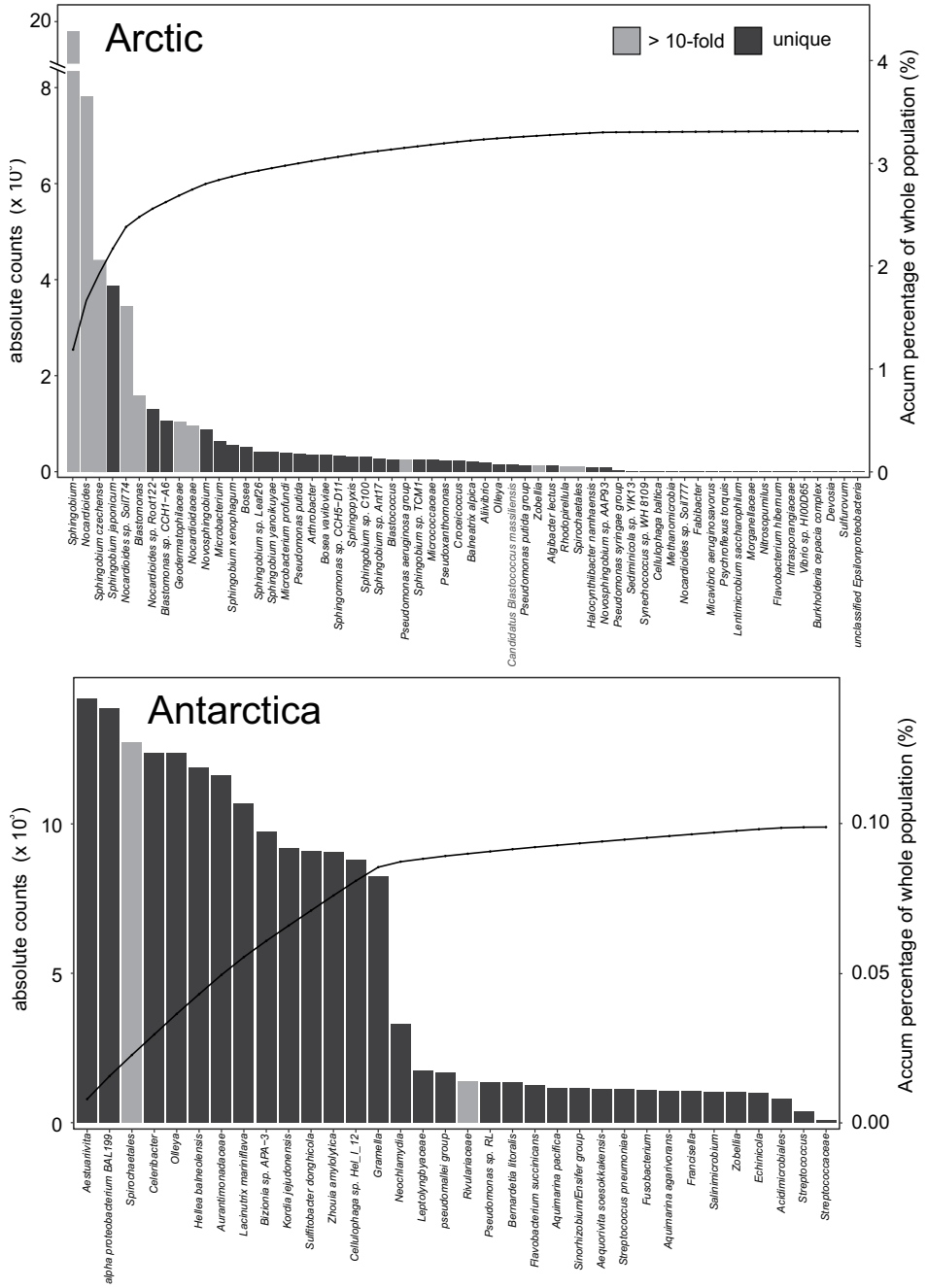

Figure 2. Abundance of the taxa that increased by 10-fold (grey) or were absent in controls (black) after 24 h in the Arctic and Antarctica ADOC amendment. Phylotypes were taxonomically classified at highest resolution. Counts were summarized at each taxonomical level.

their relative abundance more than 10-fold between treatments and controls, or that were unique in ADOC amendments after 24 h (Fig. 2). In the Arctic, these responsive taxa increased their relative abundance from 0.36% (± 0.17%) of the total community in initial waters to 3.31% (± 0.32%) in ADOC amendments after 24 h with a growth from $5.5 \cdot 10^6$ cells/l to $5.4 \cdot 10^7$ cells/l. In Antarctica, at the final time point, they accounted for 0.2% (± 0.16%) of the total community, suggesting a slower or weaker response to ADOC than the one observed in Arctic waters (Fig. S2). Interestingly, in the Arctic, most of the members of the ADOC-enriched taxa corresponded to taxa belonging to the genera *Nocardioides*, *Pseudomonas* and *Sphingobium,* such as *Sphingobium japonicum* UT26 and *Nocardioides* sp. Strain PD653 (Fig. 2, Table S3 for complete list), which are well-known hydrocarbon degraders in cold environments, and known to increase the efficiency of bioremediation processes (Jiao *et al.*, 2016; Yang *et al.*, 2016; Vergeynst *et al.*, 2018). These groups, belonging to the *Alphaproteobacteria* class *Sphingomonadales* and to the phylum *Actinobacteria*, were found to be significantly more abundant after 24 h in ADOC amendments than in the controls (Fig. S3). In Antarctica, OP-degrading bacteria were also detected, such as *Pseudomonas* spp. and *Sulfitobacter* spp. that have been found to be enriched in oil-amendments performed using sub-ice Arctic seawater (Garneau *et al.*, 2016), and in beach sands of the Gulf of Mexico following the Deep Horizon or other oil spills (Rodriguez-R *et al.*, 2015; Joye *et al.*, 2016). Our results indicate that background levels of OPs substantially stimulate the growth of OP-degrading bacteria from the rare biosphere.

Toxic or nutritive? Metatranscriptomic responses to ADOC

We investigated differential transcript abundances between treatments and controls with the EdgeR statistical package (Dimont *et al.*, 2015). As many as 4,450 genes for the Arctic experiment and 2,444 genes for Antarctica (728

transcripts in common) were significantly differentially expressed between treatment and control after 24 h of ADOC exposure. These transcripts belonged to different SEED categories and different phylogenetic groups (Fig. S4). They accounted for 0.13% of total genes expressed in ADOC enrichments in the Arctic and 1.12% in Antarctica. Spearman correlation analyses between the abundance of these transcripts and OP concentrations yield a total of 6,767 and 4,966 significant correlations ($P < 0.05$) in Arctic and Antarctic meta-transcriptomic data, respectively, and were evenly distributed among all SEED categories and taxonomical groups. In many cases, the abundance of one gene or transcript was correlated with more than one OP. This is consistent with the fact that OPs with similar properties (hydrophobicity, lability) co-varied in the experiments, as they do, in fact, in the marine environment (Dachs and Méjanelle, 2010). We observed that the normalization of these numbers by the total transcript or genes harbored by the different taxa (that is, the number of genes correlated / total genes of each taxa), was 100-fold higher in the rare groups *Sphingomonadales* and *Actinobacteria* than in the other groups (Fig. 3). This indicated that these groups, despite accounting for a minor percentage of microbiome gene expression (around 0.345 $\pm$ 0.25%), devoted a higher battery of genes to degradation and other responses to ADOC than the rest of the community. These results are consistent with ADOC use as growth substrate by the increasing rare biosphere in the experiments. The relative abundance of genes/transcripts for each taxa showing correlations with chemicals is similar but lower than that of genes/ transcripts that were differentially expressed between treatments and controls (Fig. 3).

Similarly, there were 714 and 1134 significant correlations between the abundance of specific Pfam for OPs degradation (Table S4) in Arctic and Antarctic metatranscriptomes, respectively, and OP concentrations. These correlations

showed, for example, that transcripts coding for enzymes known to be involved in PAH degradation were correlated with concentrations of 2-4 ring PAHs. The degradation routes of aromatic hydrocarbons are among the best described (Mallick *et al.*, 2011; González-Gaya *et al.*, 2019). Aromatic hydrocarbons are one of the most abundant family of compounds in ADOC, and even though a significant correlation does not demonstrate a cause-effect relationship, the higher expression of Pfam ring hydroxyl A and B in ADOC treatments may be coupled to PAH degradation. Gene expression of genes related to the initial step of PAH degradation (Pfam ring hydroxyl A and B) in the Arctic experiment was also dominated by *Cellvibrionales* (*Gammaproteobacteria*) and unclassified *Gammaproteobacteria* (Fig. 4). Similarly, *Gammaproteobacteria* had a significant increase in transcripts in SEED category of aromatic compounds in

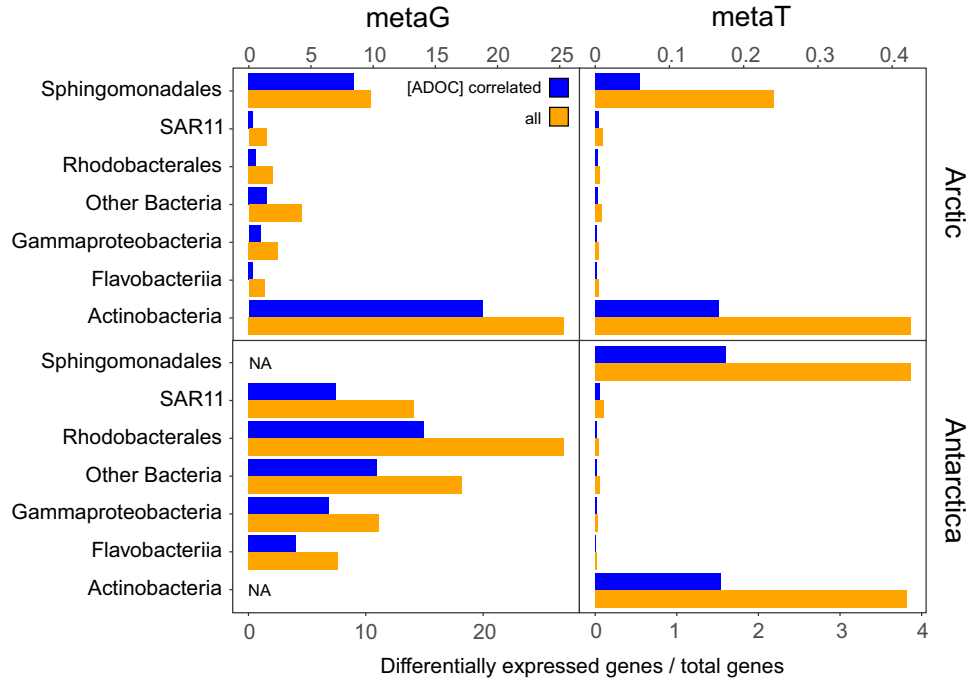

Figure 3. Contribution of genes and transcripts differently present in ADOC treatments versus controls (EdgeR; FDR < 0.05) to the total number of genes harbored or expressed by each taxa (yellow bars). Blue bars represent the subset of genes and transcripts that correlated with one or more of the concentrations of ADOC compounds (Spearman; $P < 0.01$). NA, not detected genes. Results are given in genes per million.

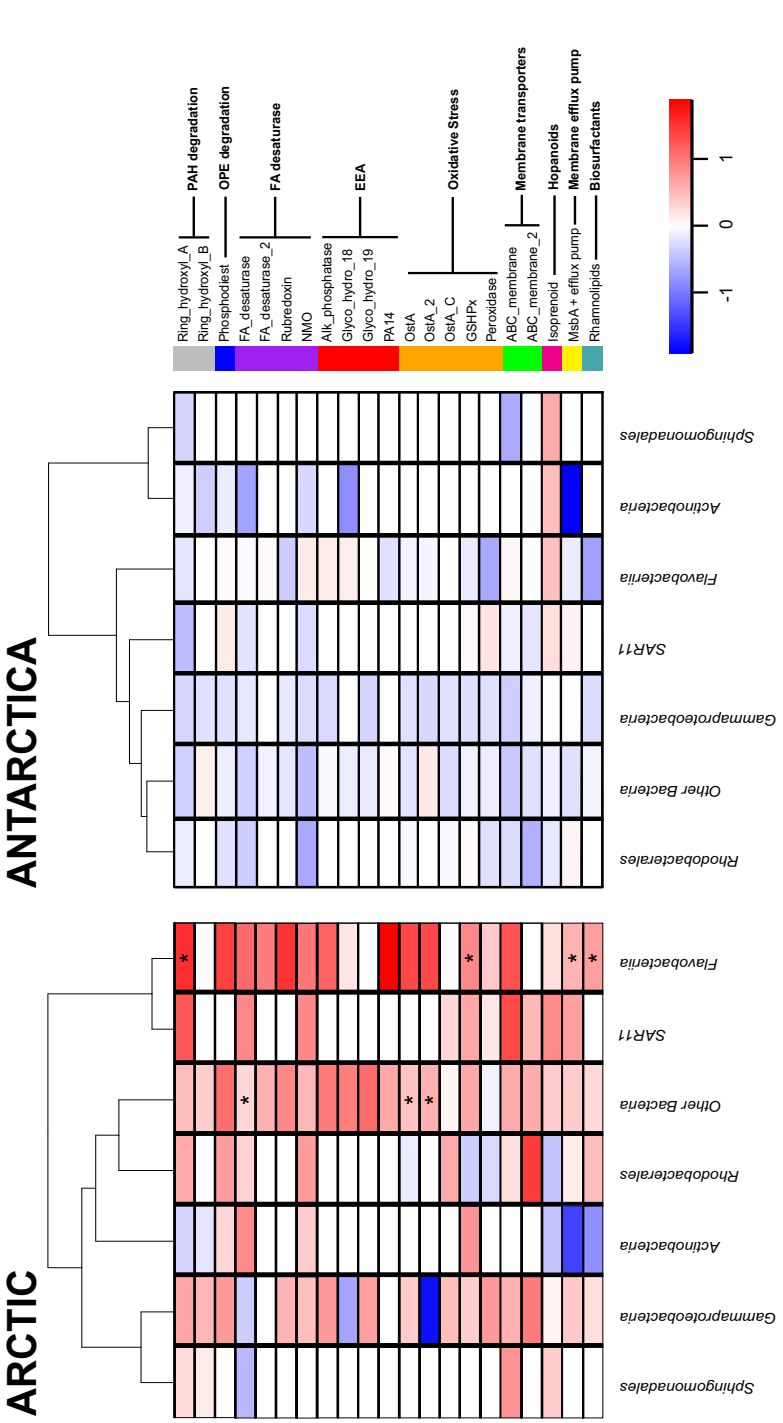

Figure 4. Heatmap of changes in gene expression between controls and ADOC amendments (calculated as natural logarithmic fold change, LFC) after 24 h in Arctic and Antarctic experiment in selected Pfam and genes directly related to the different strategies to cope ADOC effects by bacterial cells (see Fig. 5 and main text). Each column represent a taxonomical group. The color of each tile corresponds to enrichments (red, LFC > 0) or depletions (blue, LFC < 0) of the genes transcriptions. Significant differences (*t*-test; $P < 0.05$) are marked with asterisks. EEA, extracellular enzymatic activity.

ADOC amendments (Fig. S5), in agreement with the reported benefit of some *Gammaproteobacteria* in polar environments, both isolates and wild bacteria, following oil enrichments or oil spill accidents. Additionally, in the Arctic experiment, after 24 h, EdgeR results detected a significantly higher abundance of transcripts encoding the middle step of PAH degradation products (mainly activity of di- and mono-oxygenases) prior the entrance of the oxidation products to the tricarboxylic acid cycle (TCA cycle) in the ADOC amendments compared to controls. These transcripts were harbored by *Actinobacteria*, *Sphingomonadales*, *Flavobacteriia*, *SAR11* treatments after 24 h (Fig. S6, Table S4). Main entrances to the TCA cycle were mostly in form of transcripts encoding enzymes involved in catechol synthesis (through benzoates, for most groups), but also through gentisate (mostly for *Actinobacteria* and *SAR11*) and protocatechuate (*Actinobacteria*, *Sphingomonadales* and *SAR11*) (Fig. S6). In Antarctica, we found a lower number of significantly different expressed transcripts between treatments and controls belonging to PAH degradation pathway compared to Arctic experiment, and they were mostly accounted by *Actinobacteria* (Table S4). These findings suggest that consumption of PAHs from ADOC was carried out by several bacterial groups and that part of it was used to obtain energy through bacterial respiration.

Comparing general SEED categories, in the Arctic, some markers suggested degradation of ADOC compounds other than PAHs. We detected that the activity of some methylotrophic groups increased after 24 h of treatment (Fig. S7). Previous studies have described the selective increase of the abundance of methylotrophs and genes associated to C1 metabolism due to degradation of high molecular weight DOC and in oil spill accidents. Most known chemicals in the ADOC mixture have MW lower than 500 Da. The analyzed PAHs in the mixture have MW lower than 202 Da. Therefore, ADOC has lower MW than that studied in other works (McCarren *et al.*, 2010). The generation of C1 compounds from OPs could be a

fast process (for example demethylation of methyl- and dimethyl-phenanthrenes), giving rise to methylotrophs in the short period of 24 h. Such possibility will require confirmation by further work. *Flavobacteriia* could have had a prominent role related to this since this active group is capable of breaking bonds within a wide range of polymeric complexes and have been associated with PAH degradation, as well as be considered an indicator of pollution. *Flavobacteriia* was the only group that significantly increased the number of transcripts for gliding motility after ADOC amendments (Fig. S5). This has been described as a competitive advantage to access extracellular OPs by bacteria. Furthermore, *Flavobacteriia* also dominated transcripts for OPE degradation (Fig. 4). OPEs have recently been identified as a potential source of organic P in marine microbial communities under nutrient-limited conditions (Vila-Costa *et al*., 2019) and *Flavobacteriia* was identified as the main responder. *Flavobacteriia* had a minimal contribution to the expression of the Pfam for alkaline phosphatase, an activity that has been reported to be reduced when OPEs are used as a P source (Fig. S8; Vila-Costa *et al*., 2019). Altogether, these findings suggest that some components of the ADOC, besides PAHs, were being degraded and can have a broad and varied influence on the heterotrophic metabolism, including respiration, and nutrient scavenging capacity of marine bacteria.

Transcripts related to oxidative stress were detected for all groups, mostly *Gammaproteobacteria, Flavobacteriia* and other bacteria, with a higher abundance in Arctic treatments after 24 h (Fig. 4, S4), indicating a harmful effect of ADOC compounds to the community. PAHs, particularly benzo[a]pyrene, and other hydrophobic chemicals, such as chlorinated aliphatic compounds, can inhibit cell growth through the formation of DNA-PAH adducts (Kabelitz *et al.*, 2003; Ewa and Danuta, 2017). For instance, PAHs have been identified as inducing phytotoxicity to *Prochlorococcus* by reducing DNA synthesis and delaying cell

division (Cerezo and Agustí, 2015). Other studies have found that PAHs cause DNA damage in *E. coli* (Kim *et al.*, 2007). In the upper ocean, the toxicity of PAHs to cyanobacteria is enhanced by UV photosensitization (Echeveste *et al.*, 2011). PAHs, and particularly their oxidized forms (hydroxy-PAHs, quinones), are genotoxic to bacteria, an effect enhanced by the intracellular presence of ROS (Wang *et al.*, 2009). Therefore, UV irradiation may be a key factor modulating the genotoxicity of PAHs.

While PAHs exert a compound specific toxicity, we also found expression responses indicating that non-specific toxicity of the pool of hydrophobic compounds occurred. EdgeR analysis indicated that as much as 10.1% in Arctic and 10.6% in Antarctica of total differentially expressed genes after 24 h of ADOC exposure belonged to cell wall and membrane transport SEED categories, just below SEED categories related to cell growth (carbohydrates, amino acids and protein metabolism) (Fig. S4). For instance, lipid modification in the Arctic and colanic acid biosynthesis in Antarctica were the most differentially expressed genes in the community, indicating reshaping of membranes and cell walls. ADOC concentrations in cell lipids versus water (C_{Lipids}/C_{Water}) are proportional to the octanol-water partition coefficients (K_{OW}), and thus concentrations in membranes are 10^4- to 10^8-fold those in the surrounding seawater (Wallberg and Andersson, 2000; Koelmans, 2014). Our results suggest an overall expression response to ADOC compounds consistent with perturbation of cellular membranes due to the non-specific accumulation of hydrophobic compounds to the cell surfaces and into the membrane lipids that we explored in detail (see below and Fig. 5) (Cronin *et al.*, 2000; Escher *et al.*, 2017; Joye *et al.*, 2018).

Mechanisms of detoxification

We identified three main bacterial strategies to decrease ADOC adverse effects on cells, other than degradation, based on our results and literature search:

(i) modification of membrane rigidity/viscosity and permeability by remodeling membrane composition (Kabelitz *et al.*, 2003; Newman *et al.*, 2016), (ii) export of lipid substances outside the cell (Sharom, 2014), and (iii) production of extracellular polymeric substances (EPSs) and biosurfactants to modify the cell surface (Shao *et al.*, 2017). We detected differences in the use of these strategies between phylogenetic groups. We used these results to create a conceptual framework for bacteria-ADOC interactions in the diverse marine microbial communities (see below and Fig. 5).

The capacity of marine bacteria to change membrane composition is an adaptive mechanism to the non-specific accumulation of hydrophobic compounds at the cell membranes (Fig. 5; Cronin *et al.*, 2000; Escher *et al.*, 2017), as well as a detoxification mechanism (Fig. 5; Wirsen *et al.*, 1987). Remodeling in membrane composition has also been described as a response to other external stressors such as extreme temperatures, pressure, salinities, nutrient scarcity (Carini *et al.*, 2015; Sebastián *et al.*, 2016), and environmental variability (De Carvalho and Fernandes, 2010). The adaptation of cellular membrane composition to modify its rigidity/viscosity and permeability is done through two main mechanisms: (i) by changing the degree of saturation of membrane fatty acids. For example, exudation of an antibiotic chemical by diatoms has been shown to induce bacteria to lower the number of covalent double bonds in order to decrease the rigidity of the fatty acid chain (Newman *et al.*, 2016; Pepi *et al.*, 2017) and (ii) by lowering the number of membrane hopanoids to decrease membrane rigidity (Kabelitz *et al.*, 2003; De Carvalho and Fernandes, 2010). We tracked the possible use of the first mechanism using the Pfam profile FA desaturase (PF00487). This Pfam includes enzymes that could be responsible for both n-alkane degradation and desaturation of membrane lipids. We found FA desaturase hits in our data to correlate negatively with n-alkane concentrations, but positively with many

non-aliphatic OPs, such as PAHs and OPEs. This raises the possibility that FA desaturase transcription was not exclusively related to degradation of aliphatic chains, but may also be responsible for inducing a higher degree of saturation in the membrane's fatty acids in response to ADOC. *Flavobacteriia* and other bacteria dominated the FA desaturase Pfam that was particularly expressed in Arctic ADOC amendments after 24 h (Fig. 4). In order to track the second mechanism, we quantified the number of transcripts of isoprenoid biosynthesis, as hopanoids (triterpenes) are synthesized from isoprene. We observed that *SAR11* showed a higher expression of these genes in Arctic ADOC amendments

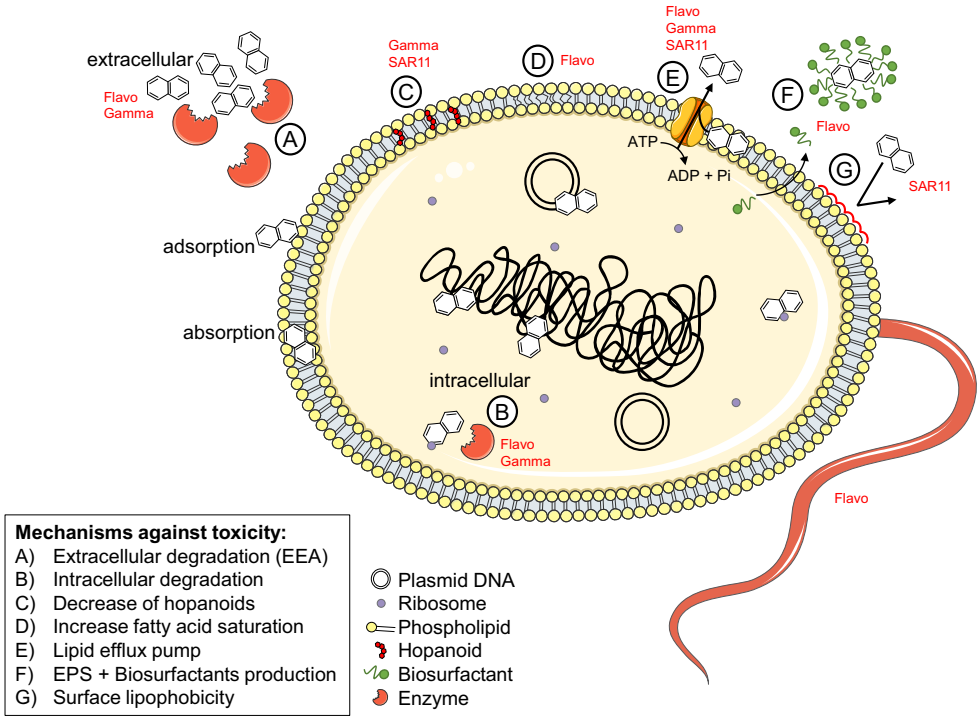

Figure 5. Schematics of the strategies to cope with hydrophobic ADOC of the most abundant bacterial groups. The bacterial groups which have been identified for each pollutant resistance mechanisms are indicated. Flavo, *Flavobacteriia*; Gamma, *Gammaproteobacteria*. Degradation can occur externally or internally to the cell. Efflux of ADOC is another mechanism depleting concentrations inside the cell. The modification of the membrane properties due to accumulation of ADOC in the membrane can be regulated by changes in fatty acid saturation and membrane hopanoids. Lower cell surface hydrophobicity can decrease ADOC accumulation at/into the membrane. Biosurfactants can form micelles with the hydrophobic chemicals, either facilitating degradation or increasing ADOC solubility outside the cell.

after 24 h than in the controls (Fig. 4). It has been described that *SAR11* has a less hydrophobic surface compared with other taxa (Dadon-Pilosof *et al.*, 2017), a trait that allows *SAR11* a free-living strategy and competitive advantages to avoid grazing. Such a cell surface with low hydrophobicity would lower the adsorption of the highly hydrophobic ADOC compounds at the cell surface, and act as a barrier for diffusion of exogenous hydrophobic compounds into the cell through the membrane. We find it reasonable, therefore, that *SAR11* was less perturbed by ADOC adsorption, which conferred a selective advantage within the whole bacterial consortia.

Concentrations of hydrophobic chemicals can be decreased in membranes and the cytoplasm cells by the so called “hydrophobic vacuum cleaner”. A system that exports lipid substances outside the cells (Sharom, 2014). In eukaryotes, these have been identified as p-glycoproteins and other members of the ATP-binding cassette (ABC) transporters, providing multiple xenobiotic resistance (MXR) to the cells (Efferth and Volm, 2017). In prokaryotes, analogues to eukaryotic p-glycoproteins are encoded by *lmrA*, *sav1866* and *msbA* genes (Kerr *et al.*, 2010). Our data showed that expression of the *msbA* gene and genes coding for general ABC transporter multidrug efflux pump after 24 h in the Arctic were higher in ADOC amendments than in the controls for *Flavobacteriia*, *Gammaproteobacteria*, and *SAR11* (Fig. 4; Garneau *et al.*, 2016; Newman *et al.*, 2016; Vergeynst *et al.*, 2018).

Finally, extracellular polymeric substances (EPSs) and biosurfactant production can have direct effects on permeability, electric charge and the degree of hydrophobicity of the cell surface (Shao *et al.*, 2017). In the absence of these organic compounds, the anionic character of lipopolysaccharides (LPS) at the outer membrane (by gram-negative bacteria; De Carvalho and Fernandes, 2010) might confer a lower hydrophobicity to the surface. Surprisingly,

Sphingomonadales were the only taxon showing a higher number of genes for LPS biosynthesis in the cells grown after 24 h in ADOC amendments (Fig. S9). Additionally, EPS and biosurfactants enhance the solubility and bioavailability of hydrophobic compounds by emulsifying. The best characterized biosurfactant are the rhamnolipids (Dobler *et al.*, 2016). We tracked the genes *rmlA* and *rmlB*, which are the bottleneck step during rhamnolipids biosynthesis (Dobler *et al.*, 2016). *rmlA* and *rmlB* were abundant in the metagenome and metatranscriptome for *Flavobacteriia*, *Gammaproteobacteria* and other Bacteria but absent in *SAR11*. Only the differences in *Flavobacteriia* due to ADOC addition were significant (Fig. 4). Even though rhamnolipids have been suggested to facilitate uptake and degradation of organic chemicals (Zeng *et al.*, 2018), a detailed assessment of the interactions of biosurfactants and the other detoxification mechanisms is needed.

Which is the ecological and biogeochemical relevance of ADOC?

To the best of our knowledge, this study describes for the first time the response of natural microbial communities to changes in ADOC concentrations within the factor of two variability found in oceans. Atmospheric deposition of ADOC has been identified as an important source of allochthonous carbon (González-Gaya *et al.*, 2016), with unknown ecological and biogeochemical implications. The vast majority of synthetic organic chemicals currently in use are derived from the use of petroleum distillates, and thus ADOC is old carbon. A recent review identified chemical pollution as one of the several potential processes by which old carbon can enter trophic webs (Guillemette *et al.*, 2017). Our work shows that ADOC, old anthropogenic organic carbon reaching the Arctic and Antarctica by atmospheric transport and deposition, co-shapes microbial communities, and acts as a source of carbon and probably nutrients. Thus, old carbon from ADOC enters the trophic webs.

The additions of ADOC used here were small in relation to the total pools of DOC. In the ocean, the mixture of hydrophobic hydrocarbons and synthetic chemicals is dominated in terms of mass by the aliphatic and aromatic UCM. The concentration of the dissolved UCM is hundreds of times higher than the concentration of total PAHs and n-alkanes (Farrington and Quinn, 2015; González-Gaya *et al.*, 2016). The aromatic UCM ranges from 0.03 to 0.2 μM C (González-Gaya *et al.*, 2016), similar to aliphatic UCM. The UCM is orders of magnitude larger than the sum of resolved compounds and can be used as an estimate of hydrophobic ADOC. Taking into account the measured variability of UCM concentrations in surface seawater, the ADOC added to the incubations was between 0.05 and 1% of the *in situ* DOC in the Arctic (90 μM; Arrieta *et al.*, 2016) and Antarctic waters (60 μM; Ruiz-Halpern *et al.*, 2014). Even though we used low concentrations of ADOC, these environmentally relevant concentrations of anthropogenic compounds changed the composition of the bacterial community, in particular by promoting the growth of members of the rare biosphere. The rare biosphere was apparently related to the consumption of ADOC, a fact that requires further research in terms of the expected ADOC increase in coming decades, and its perturbation of microbial biodiversity. Moreover, there was an increased transcriptional activity among the major taxa to cope with ADOC, in particular in *Flavobacteriia*.

ADOC at ambient concentrations promoted the enrichment of transcripts devoted to cell functioning in the Arctic and, to a lower degree, in Antarctica. We observed an increase of transcripts coding for proteins involved in respiration in some groups (Fig. S4). Future work is needed to confirm that these transcript differences result in an increase of respiration, and how ADOC affects the growth efficiency of bacterial communities, and the overall carbon cycling. This differs from previous reports of negative effects of ADOC on the transcripts of photosynthesis genes of *Prochlorococcus* (Fernández-Pinos *et al.*, 2017). In fact, responses to ADOC

differed for each taxonomical group, with *Flavobacteriia* and *SAR11* being the more responsive groups in the Arctic, and *Flavobacteriia* in Antarctica, probably in part related to different competitive advantages. The original composition of the ADOC used (and original seawater) showed a different composition for the target compounds (Table S5), and such differences might have also been present for the other thousands of chemicals present in ADOC, including the UCM. Such differences in chemical pattern, in addition to concentrations, could also be responsible for the observed differences in Arctic and Antarctic microbial responses.

The accumulation of hydrophobic ADOC in membranes and other cellular lipids induced a suite of responses depending on the physiology of individual phylogenetic groups, and on the metabolic machinery available encoded by their genomes. Even though some compound-specific interactions may occur, for example, formation of DNA-PAH adducts, an important transcriptomic response was consistent with the non-specific accumulation of hydrophobic compounds in the cellular lipids (mainly membranes). The study of the mutual influence of ADOC and microbial communities will require synergistic approaches in environmental chemistry, molecular biology (-omics), and microbiome research. Such research efforts in microbial biogeochemistry of ADOC may contribute to start delimiting the boundaries of the effects of chemical pollution on Earth system functioning. As the number and quantity of anthropogenic chemicals in use has exponentially increased during the Anthropocene, it is feasible that ADOC influences become more pronounced over the next decades.

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Plasticity and perturbation of coastal marine microbiomes exposed to anthropogenic dissolved organic carbon

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ABSTRACT

Chronic pollution by baseline organic contaminants originates irreversible changes in marine ecosystems although these effects are understudied compared to scenarios of acute exposure such as in oil spills. Coastal seawaters receive important doses of organic contaminants and inorganic nutrients resulting from human activities. Whereas eutrophication has been addressed in some studies, the effects of background concentrations of dissolved anthropogenic organic carbon (ADOC), composed of thousands of organic pollutants, to the engines of the marine biogeochemical cycles, the microorganisms, remain less characterized. In this study, we have challenged natural microbial communities with ADOC at environmental relevant concentrations in short-term (48 h) incubations with and without nutrient additions. Dose-response experiments showed that ADOC increased prokaryote leucine incorporation rates at both sites in the absence of nutrient addition, while the increase in growth rates was not significant. In BL, ADOC significantly increased the percentage of active respiring cells, but also the number of “dead” cells, indicating a lower capacity to cope with toxicities by part of the community. Gene expression profiles after 30 min and 24 h indicated that *SAR11* in BCN and *Alteromonas*, mostly *Glaciecola* taxa in BL responded with a suite of responses to ADOC. Metagenomic analysis of community composition indicated a growth of the rare biosphere, mainly in BCN, mostly composed by *Methylophaga* species. On the other hand, genetic mobile elements such as transposons were expressed for some taxa, and transcripts of transposases and a suite of responses known to be indicators to exposure to organic pollutants were correlated. These results suggest that microbial responses to pollution may be related to genetic mobile elements, that eventually may result in a community with higher plasticity.

INTRODUCTION

Chemical pollution is one of the less characterized vectors of global change (Rockström *et al*., 2009), specially under scenarios of chronic pollution such as that due to the myriad of organic pollutants at trace levels found in the marine environment (Dachs and Méjanelle, 2010; González-Gaya *et al*., 2016). The constraining of this vector of global change is especially troublesome as most anthropogenic organic pollutants are unknown or lack appropriate analytical procedures (Muir and Howard, 2006). Anthropogenic dissolved organic carbon (ADOC) in coastal seawater originates from direct and indirect inputs of organic contaminants from rivers, continental run-off, groundwater inputs, atmospheric deposition and marine currents (Schwarzenbach and Gschwend, 2016). Whereas the most polar contaminants are partially biodegraded by microbiomes in waste water treatment plants and during riverine transport (Gioia and Dachs, 2012), in part due to their lower lipophobicity and higher occurrence in the dissolved phase, the most nonpolar fraction of ADOC contains a large number of persistent chemicals that reach coastal seawaters. This nonpolar fraction of ADOC derives from fossil fuels (mostly aliphatic and aromatic hydrocarbons), legacy persistent organic pollutants (POPs), and organic contaminants of emerging concern (OCEC), many of which are still being characterized (Sauvé and Desrosiers, 2014). Individual concentrations of anthropogenic organic compounds are in the order of ppt – ppb. However, the myriad co-occurrence of tens or hundreds of thousands of these micropollutants can reach micromolar concentrations with still uncharacterized effects on the ecosystems (Dachs and Méjanelle, 2010; Cerro-Gálvez *et al*., 2019a). Although some ADOC components, such as some POPs, are regulated by international legislation such as the Stockholm Convention due to their toxicity, persistence, potential for long range transport and bioaccumulation potential, chronic pollution by ADOC remains un-characterized

in terms of Earth System functioning. There is a need of studies characterizing the effects of this pollution to marine microbial communities at environmental concentrations of ADOC (Cavicchioli *et al*., 2019; Cerro-Gálvez *et al*., 2019a), as microbial communities are the engines of planetary biogeochemical cycles.

ADOC pollution at coastal sites is commonly linked to eutrophication. Nutrients shape the composition and functionality of marine microbial communities, and accelerate succession rates. Nutrient availability enhances the genetic transfer between cells by stimulating horizontal gene transfer (Schä *et al*., 2001; Knelman *et al*., 2014; Amos *et al*., 2015). Enhanced HGT within the communities has been related to more resistant microbiomes against pollution, although few studies have addressed this issue for organic chemicals other than antibiotics and antibiotic resistant genes (Subirats *et al*., 2018). On the other hand, eutrophication of coastal waters leads to increased phytoplankton biomasses that capture dissolved contaminants thus lowering exposure concentrations (Dachs *et al*., 2000; Berglund *et al*., 2001; Skei *et al*., 2000) to microorganisms with unknown consequences in terms of natural microbial communities. Interactions of nutrients and hydrocarbons have only been studied under scenarios of oil-spills. A common strategy to mitigate the effects of acute introductions of ADOC, such in oil spills accidents, is nutrient bioremediation: the addition of nutrients relieves the trophic limitation of the communities and stimulate hydrocarbon consumption (Head *et al*., 2006). However, the addition of nutrients seems to stimulate the whole community, and under some circumstances or after a short period of time, hydrocarbon consumption is no longer enhanced (Boyd *et al*., 2008; Sauret *et al*., 2014).

Commonly, experiments to analyze microbiome responses to pollutants use exposure concentrations orders of magnitude higher than those encountered in the environment since they are performed from ecotoxicological and

bioremediation perspectives (Megharaj *et al*., 2011; Birrer *et al*., 2017). The handful of previous experiments challenging marine microbial communities with background concentrations of ADOC observed toxicological and inhibitory effects to phototrophs and heterotrophs (Echeveste *et al*., 2016, Fernández-Pinos *et al*., 2017; Cerro-Gálvez *et al*., 2019a). Currently, the impact of hydrophobic ADOC is focused on few families of pollutants or oil extracts, which represent a partial view of ADOC effects. In terms of mass, the unresolved complex mixture (UCM) is an important pool of the ADOC found in seawater (White *et al*., 2013; González-Gaya *et al*., 2016). Some ADOC compounds are prone to partial microbial degradation as long as conditions are favorable (Boyd *et al*., 2008; Head *et al*., 2006). Under aerobic conditions, factors controlling the biodegradation of some organic pollutants are those regulating the consumption of any DOC component (temperature, *in situ* concentrations, bioavailability, nutrient concentrations, presence of consumers, etc.), including the chemical structure of the contaminants and the biological production of biosurfactants (Boyd *et al*., 2008; De Carvalho and Fernandes, 2010; Joye *et al*., 2016; Nogales *et al*., 2011, Cerro-Gálvez *et al*., 2019a). Among these factors, the trade-off between nutrient limitation and contaminants concentrations along with community composition seem to play a crucial role determining rates of consumption and fate and strategies against toxicity (Head *et al*., 2006; Cerro-Gálvez *et al*., 2019a).

The microbial metabolic response to cope against ADOC compounds is diverse in marine microbiomes and involves a suite of physiological strategies in addition to degradation, including the energetic and biosynthetic needs of the cells and stress responses to protect against ADOC toxic effects (Cravo-Laureau *et al*., 2017; Cerro-Gálvez *et al*., 2019a, b). Strategies against toxicity include mechanisms to quench cell envelope stress, since hydrophobic ADOC tends to be sorbed into membranes, and generally the organic matter of the cell, promoting harmful

effects by narcosis (Fernández-Pinos *et al*., 2017; Mitchell and Silhavy, 2019), and other pollutant specific responses (Cerro-Gálvez *et al*., 2019a, b). Therefore, communities can be divided between ADOC-consumers and ADOC-tolerant bacteria. The former are specialized bacteria that use ADOC as a source of C and energy, while the latter are those bacterial groups able to quench the adverse ADOC effects and compete for nutrient resources with the ADOC-consumers (Kostka *et al*., 2011, Cerro-Gálvez *et al*., 2019a). These responses can obviously be related to nutrient availability, which is a limiting factor for large marine regions. ADOC can also be a source of limiting nutrients, as demonstrated for phosphorus (Vila-Costa *et al*., 2019). The versatility of marine microbiomes given by a high genomic plasticity (high abundance of mobile genetic elements, MGE, in the metagenomes) might result in higher potential for ADOC consumption and increased tolerance to ADOC compounds since MGE represent potent means of adaptation (Frost *et al*., 2005; Casacuberta and González, 2013; Vigil-Stenman, 2017). In fact, exposure and pre-exposure of populations to different concentrations of contaminants, modifies the degradation kinetics, and select specific consumers that alter subsequent biodegradation (Aelion *et al*., 1989; Sauret *et al*., 2014, Okere *et al*., 2017). Whether or not environmental relevant concentrations of ADOC are selecting for taxa among ADOC consumers and tolerant bacteria is still unclear for remote environments (Cerro-Gálvez *et al*., 2019a), and never assessed in coastal waters from populated regions.

The Mediterranean Sea is predominantly an oligotrophic sea receiving high anthropogenic pressures (Durrieu de Madron *et al*., 2010). Despite being a marginal sea, concentrations of the different organic pollutants present in ADOC show considerable heterogeneity in their spatial distribution (e.g. Berrojalbiz *et al*., 2011b). Northwestern Mediterranean coast (Catalan coast, Spain) encompasses significant biological and physicochemical gradients including high variability in

concentrations of micropollutants due to proximity of urban areas (Garcia-Flor *et al*., 2005; Berrojalbiz *et al*., 2011b; Sánchez-Ávila *et al*., 2012; Castro-Jiménez *et al*., 2013).

In order to get a mechanistic understanding of the *in situ* strategies within microbial communities to cope with the hydrophobic fraction of ADOC in coastal systems, and the role played by nutrients and genome flexibility in their response to pollutants, we performed experiments at two coastal sites from the NW Mediterranean with contrasting anthropogenic pressures in terms of pollutants and nutrients. The hypothesis to test was if microbial communities previously exposed to higher concentrations and more frequent pulses of organic contaminants and higher nutrient concentrations, presumably with higher plasticity, devote a higher percentage of their activity to consume these compounds than a more pristine and oligotrophic community. The latter may devote more genes quenching toxic effects, but such an exposure to ADOC may drive a dissemination of plasticity in the community.

METHODS

Sampling site description

Experiments were performed with seawater collected off-Barcelona harbor and at the Blanes Bay Microbial Observatory (BBMO) (Fig. 1). The off-Barcelona harbor site (BCN) is representative of a polluted eutrophic coastal site (yr-average Chla > 1.65 µg/l; Arin *et al*., 2013). BCN receives freshwater inputs from highly polluted Besòs and Llobregat rivers, run-off from the city of Barcelona, and there are tons of accumulated legacy organic pollutants in the sediment from past uncontrolled release of sewage sludge that can be re-suspended during storm events. Approximately 60 km north from Barcelona, Blanes Bay (BL, NW Mediterranean,

BBMO) is representative of a rather oligotrophic coastal site moderately affected by human influences with low terrestrial inputs of nutrients (yr-average Chla < 0.8 µg/l; Gasol *et al*., 2012).

Concentration of ADOC and preparation of ADOC spike solutions

As much as 200 l of surface seawater was collected from off-Barcelona (BCN) harbor (26th May 2015; 41°22'16.4"N 2°11'23.3"E) and from a site located 50 km far from BCN (6th June 2015; 41°06'48.0"N 1°47'38.4"E) using several 20 l pre-cleaned metal carboys. Concentration of three characteristic and ubiquitous families of hydrophobic organic compounds belonging to ADOC (organophosphate esters (OPEs) flame retardants and plasticizers, polycyclic aromatic hydrocarbons (PAHs), and n-alkanes were analyzed using the methods previously described (Berrojalbiz *et al*., 2011a, González-Gaya *et al*., 2016). Briefly, seawater was concentrated on a XAD-2 adsorbent and eluted with dichloromethane and methanol, after concentration, extracts were fractionated on an alumina column using solvents of different polarity. Concentrations of 24 n-alkanes, 64 PAHs and 10 OPEs were quantified by GC-MS as described elsewhere (Cerro-Gálvez *et al*., 2019b). The 24 n-alkanes identified and quantified were a series from nC_{12} to nC_{35}. The 64 target PAHs were naphthalene, methylnaphthalenes (sum of two isomers), dimethylnaphthalenes (sum of six isomers), trimethylnaphthalenes (sum of seven isomers), acenaphthylene, acenaphthene, fluorene, dibenzothiophene, methyldibenzothiophenes (sum of three isomers), dimethyldibenzothiopenes (sum of five isomers), phenanthrene, methylphenanthrenes (sum of four isomers), dimethylphenanthrenes (sum of seven isomers), fluoranthene, pyrene, methylpyrenes (sum of five isomers), dimethylpyrenes (sum of eight isomers), benzo[*g,h,i*]fluoranthene, benzo[*a*]anthracene, chrysene, methylchrysenes (sum of three isomers), benzo[*a*]pyrene, perylene, and dibenzo[*a,h*]anthracene.

The 10 OPEs were triisobutyl phosphate (TiBP), tri-n-butyl phosphate (TnBP), tris(2-chloroethyl) phosphate (TCEP), tris(1-chloro-2-propyl) phosphate (TCPPs, 3 isomers), tris[2- chloro-1-(chloromethyl)ethyl] phosphate (TDCP), triphenyl phosphate (TPhP), 2-ethylhexyl diphenyl phosphate (EHDPP), and tris(2-ethylhexyl) phosphate (TEHP).

The most non-polar fractions, F1 and F2 contain hydrophobic hydrocarbons and synthetic organic compounds with a large contribution of an anthropogenic UCM (White *et al*., 2013; González-Gaya *et al*., 2016; Cerro-Gálvez *et al*., 2019a). Both fractions were merged as representative of ADOC, and used as spike solution in the experiments. This mixture of nonpolar ADOC is similar to that used in previous works (Echeveste *et al*., 2010, 2016; Fernández-Pinos *et al*., 2017; Cerro-Gálvez *et al*., 2019a).

Experiments with natural communities

Coastal seawater was collected from the surface (0.5 m depth) at a site close to Barcelona harbor mouth (16th June 2015, 41°22'16.4"N 2°11'23.3"E, "BCN"), as representative of a polluted eutrophic coastal site, and Blanes Bay (22nd June 2015, BBMO, 41°40'13.5"N 2°48'00.6"E, "BL"), as representative of a rather oligotrophic site with moderated pollution (Fig. 1). Responses to ADOC additions were analyzed in two experiment types. (1) *Dose-response experiment*. Marine microbiomes were challenged with four different exposure concentrations of ADOC (1x, 7.5x, 40x and 240x *in situ* concentrations). ADOC spike solution was added to 40 ml glass tubes (previously baked, 450°C, 4 h) in the treatments. The same volume of solvent with no ADOC was added to the controls. Solvent was let to evaporate under the hood for 2 h before seawater addition. To test the importance of nutrient availability, we tested two different trophic conditions: an enrichment with P, N and C (0.6 μM $NaH_2PO_4 \cdot H_2O$, 2

μM $(NH_4)_2SO_4$ and 24 μM glucose final concentration; "with nutrients"), to stimulate bacterial growth and prevent any potential nutrient limitation, and a control ("without nutrients"). Samples were incubated in duplicate in the dark at *in situ* temperature for 48 h. Monitoring of abundance of prokaryotic cells, leucine incorporation rates (as proxy of bacterial production), the percentage of damaged or dead cells (NADS-), and the abundance of actively-respiring bacteria (CTC+) were performed after 0, 4, 24 and 48 h of incubation (see below). (2) *Bacterial responses to ADOC.* A second set of experiments using the same seawater consisted on adding 7.5x ADOC concentrations dissolved in acetone to 2 l pre-baked glass bottles (treatments), and only the solvent (acetone) to control bottles. The solvent was let to evaporate for 2 h before adding the seawater. The collected water was added to the glass bottles and incubated at in situ temperature and dark conditions for 24 h. The experiment was run in duplicate. We collected and preserved samples for molecular analyses as described above at 0.5 and 24 h. Additional samples were taken at same time points to analyze bacterial concentration and production, and nutrient concentrations.

Flow cytometric determinations

(1) *Prokaryotic cell abundance.* In order to quantify prokaryotic cell abundance, we collected duplicates of 1.8 ml from each bottle and fixed it with 1% buffered paraformaldehyde solution (pH 7.0) plus 0.05% glutaraldehyde, left it at room temperature in the dark for 10 min and stored it at -80°C. Prokaryotic cell abundance was later estimated by flow cytometry (Gasol and Morán, 2015). (2) *Abundance of membrane-compromised cells (NADS).* The Nucleic-Acid-Double-Staining (NADS) viability protocol was used to enumerate the cells with intact versus damaged membranes (Falcioni *et al*., 2008). NADS+ (assumed to be live, with intact membranes) and NADS- (assumed to be inactive, with comprised cell

membranes, named as “dead”) were counted by flow cytometry. (3) *Actively-respiring bacteria (CTC).* To quantify the abundance of highly respiring bacteria, we follow a protocol using 5-cyano-2,3-ditolyl tetrazolium chloride (CTC) described elsewhere (Sieracki *et al*., 1999). Briefly, fresh sample aliquots (0.4 ml) received 5 mM CTC (from a fresh stock solution, 50 mM) and were incubated for 90 min in the dark at room temperature. CTC+ cells were enumerated by flow cytometry.

Bacterial production, by [^{3}H]Leucine incorporation rates (LIR)

Heterotrophic bacterial production was estimated from [^{3}H]Leucine incorporation using the method described by Kirchman *et al*. (1985), with the modifications suggested by Smith and Azam (1992). For each sample, triplicate aliquots (1.2 ml) and a trichloroacetic acid-killed control were incubated with 40 nM [^{3}H]Leucine for 1 h in the dark. Bacterial production is shown as [^{3}H]Leucine incorporation in pmol leucine l^{-1} h^{-1}.

Inorganic nutrients concentrations

Samples (10 ml) were kept frozen at -20°C until analysis of dissolved inorganic nutrient concentration (nitrite (NO_2), nitrate (NO_3), ammonium (NH_4) and phosphate (PO_4)) were done. Measurements were performed by Continuous Flow Analysis (CFA) on a Bran+Luebbe (currently Seal Analytical) following Hansen and Koroleff (2007).

Nucleic acids extraction and sequencing

After 0.5 and 24 h incubations of the second experiment, each bottle was pre-filtered through a 3 µm pore-size 47 mm diameter polytetrafluoroethylene filter and bacterial cells were collected on 0.2 µm pore-size 47 mm polytetrafluoroethylene

filter under low vacuum pressure. The duration of the filtration step was no longer than 15 min to minimize RNA degradation. Each filter was cut in two halves, one was placed in 1 ml RNAlater (Sigma-Aldrich, Saint Louis, MO) and the other one into 1 ml lysis buffer (50 mM Tris HCl, 40 mM EDTA, 0.75 M Sucrose) and stored at -80°C to preserve RNA and DNA respectively. DNA extraction was performed following the protocol described in Cerro-Gálvez *et al*. (2019a). To estimate absolute genes counts, we added a DNA standard (*Thermus thermophilus DSM7039 [HB27]* genomic DNA) that functioned as internal control at 0.5% of the total mass of extracted DNA. mRNA was extracted and amplified as described in Poretsky *et al*. (2010) with the modification of the use of mirVana isolation kit (Ambion) to extract the total RNA. Artificial mRNA was synthesized by in vitro transcription from a pGEM-3Z plasmid and used as internal standard at 0.5% final concentration in order to calculate absolute transcript abundances (Moran *et al*., 2013; Satinsky *et al*., 2013). Resulting DNA and amplified RNA were sequenced at the National Center for Genomic Analysis (CNAG, Barcelona, Spain) using Illumina high output mode HS200 2x100bp v4.

Bioinformatics

DNA and cDNA sequences were quality trimmed and internal standards and any remaining stable RNA was quantified and removed using the ERNE mapping program (Del Fabbro *et al*., 2013) against the internal standard sequences and an in-house database of marine bacterial stable RNA sequences, respectively. Subsequently, read pairs were joined using the PEAR program (https://www.h-its.org/en/research/sco/software). Joined pairs, as well as separate reads not corresponding to joined pairs were aligned to the NCBI RefSeq database (downloaded October 2016) using the Diamond aligner v0.8.25 (Buchfink *et al*., 2014) in blastx mode with default parameters. The resulting alignments were taxonomically and functionally classified with MEGAN 6.5.10 (Huson *et al*., 2016)

and exported for further analysis in R/tidyverse. Search for specific transposases was performed with Pfam profiles, using HMMER (Eddy, 2011). The list of specific Pfam profiles used is listed in Brazelton *et al.* (2009). Assembly of metagenomic and metatranscriptomic reads was performed with MEGAHIT (Li *et al*., 2015) and contigs was quantified by mapping reads to contigs with Bowtie 2 (Langmead and Salzberg, 2012) and then converting to idxstats files with SAMtools (Li *et al*., 2009). Metagenomic analyses resulted in a total of 341 million paired-end reads and an average of 28.4 million reads per sample in a typical length of 101bp. After quality-trimming and filtering of added internal standards, between 12.8 and 17.6% of the potential protein-coding reads were taxonomically annotated and between 32.5 and 47.9% were functional annotated in SEED (Table S1). The sequencing of the metatranscriptomes resulted in a total of 949 million paired-end reads, 59.3 million raw reads per sample. After removal of rRNA, tRNA and internal standard reads, 369 million possible protein-encoding sequences remains, 23 million per sample. Among these, between 16.5 and 30.3 were taxonomical annotated and between 35.5 and 47.9 were successfully annotated to a SEED functional protein and category.

Statistical analyses

Data treatment and statistical analyses were performed with the R Statistical Software. Significant differences between treatments were analyzed with t-Student tests performed using the “t.test” function with a threshold for the significance set as $P < 0.05$. Principal component analysis (PCA) and Permutational multivariate analysis of variance (permanova) were carried out using the “vegan” package with standardized data (Oksanen *et al*., 2018). Analysis of differential gene abundances was performed with the “EdgeR” package (Dimont *et al*., 2015).

RESULTS AND DISCUSSION

Initial characterization of sampling sites

Level of pollution. Three model families of ubiquitous organic pollutants (64 PAHs, 24 n-alkanes and 10 OPEs) were used as surrogates of the hydrophobic ADOC to characterize the level of pollution in both sampling sites. On average, concentrations of dissolved PAHs, OPEs and n-alkanes in BCN waters were 21-, 5- and 4-fold higher than those in BL, respectively (Fig. 1, Table S2). The higher level of pollution in BCN waters is consistent with the BCN site receiving large

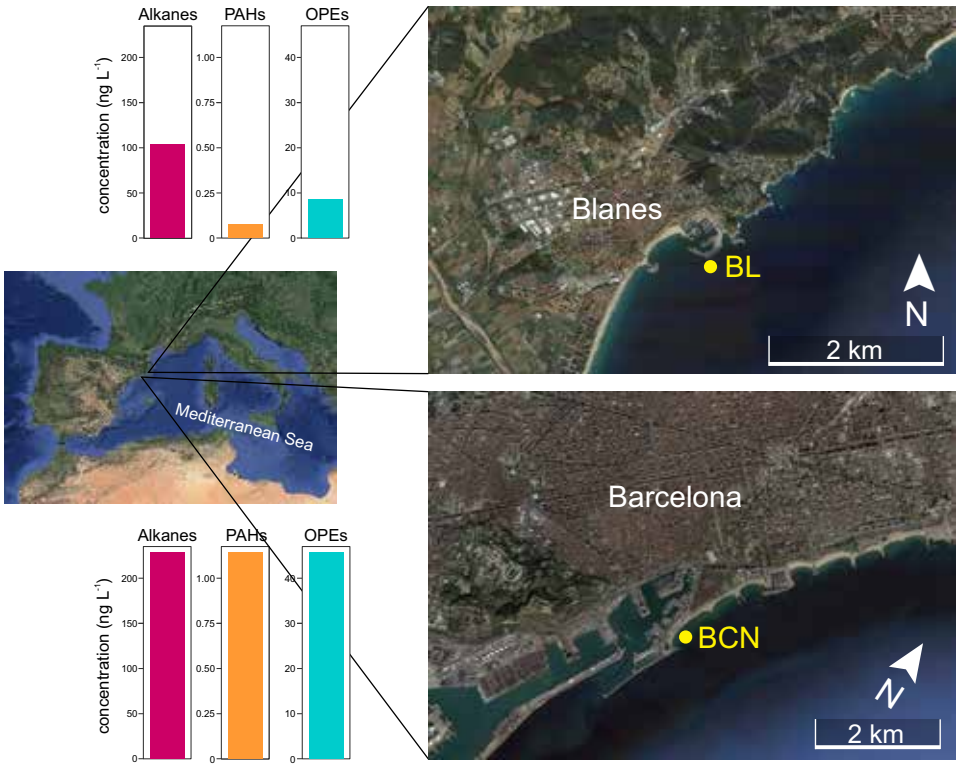

Figure 1. Location of the sampling site of the seawater used for the experiments in the Northwestern Mediterranean Sea. Bars indicate the pooled concentration of three families of organic pollutants (organophosphate esters (OPEs) flame retardants and plasticizers, polycyclic aromatic hydrocarbons (PAHs), and alkanes) measured in the dissolved phase at the surface (1 m). See details in Table S2.

anthropogenic inputs from anthropogenically impacted Besòs and Llobregat rivers, atmospheric inputs from adjacent urban and industrial regions, wastewater treatment plant effluents, run-off from the city of Barcelona, high shipping traffic, and tons of accumulated legacy organic pollutants in the sediment from past uncontrolled release of sewage sludge that can be re-suspended during storm events (Tolosa *et al*., 1996; García-Flor *et al*., 2005; Berrojalbiz *et al*., 2011b; Sánchez-Ávila *et al*., 2012). Measured concentrations at both sites were within the order of magnitude of concentrations previously measured in the same region. Specifically, the dissolved concentrations of n-alkanes in our study (n-C_{12} – n-C_{35}: 240 ng/l in BCN and 110 ng/l in BL, see Table S2 for details) were similar or slightly lower values than those reported in the NW Mediterranean Sea (n-C_{15} – n-C_{40}: 40 - 4600 ng/L, Marty and Saliot, 1976; Guigue *et al*., 2014; Cerro-Gálvez *et al*., 2019b) and in the Gulf of Gabès (Tunisia) (n-C_{15} – n-C_{40}: 20 - 6300 ng/, Fourati *et al*., 2018). The sum of PAH concentrations in the dissolved phase (Σ_{64}PAH: 1.2 ng/l in BCN and 0.078 ng/l in BL) were lower than those reported in coastal seawaters from the NW Mediterranean (Σ_{15}PAH: 3.6 - 30.7 ng/l, Guitart *et al*., 2007; Guigue *et al*., 2014), but within the same range than in the open NW Mediterranean (Σ_{19}PAH: 0.16 - 0.81 ng/l, Berrojalbiz *et al*., 2011a). Among individual PAHs, phenanthrene was the most abundant compound (0.21 ng/l in BCN and 0.028 ng/l in BL) and the general profile was dominated by low molecular weight PAHs (2-4 rings), in agreement with previous studies (Ehrhardt and Petrick, 1993; Dachs *et al*., 1997; Tsapakis *et al*., 2006; Berrojalbiz *et al*., 2011b; Cerro-Gálvez *et al*., 2019b). Concentrations of Σ_{10}OPE (46 ng/l in BCN and 8.4 ng/l in BL) were in the range of those previously measured in NW Mediterranean Sea (Cerro-Gálvez *et al*., 2019b; Vila-Costa *et al*., 2019).

Level of nutrients

Concentrations of nutrients were 8-fold higher in BCN than in BL (Table 1).

Average concentrations of NO_2+NO_3 were 3.6 ± 0.01 µM in BCN, and 0.6 ± 0.4 µM in BL. NH_4 concentrations were 8.2 ± 1.4 and 1.3 ± 0.5 µmol/l in BCN and BL, respectively, while PO_4 concentrations were 0.5 ± 0.05 and 0.04 ± 0.00 µM. These nutrient concentration indicate a higher eutrophication in BCN than in BL waters as previously described (Arin *et al*., 2013; Gasol *et al*., 2012).

Table 1. Concentration (in µmol/l) of inorganic nutrients from Barcelona (BCN) and Blanes (BL) experiments.

		NO_2^- + NO_3^-	NH_4^+	PO_4^{3-}
BCN	C05	3.56 ± 0.01	8.21 ± 1.40	0.46 ± 0.05
	T05	4.99 ± 0.07	6.27 ± 1.17	0.23 ± 0.00
	C24	2.71 ± 0.79	4.00 ± 0.20	0.16 ± 0.00
	T24	2.95 ± 0.68	4.87 ± 0.74	0.17 ± 0.02
BL	C05	0.59 ± 0.42	1.26 ± 0.49	0.04 ± 0.00
	T05	0.42 ± 0.02	0.14 ± 0.10	0.05 ± 0.03
	C24	0.21 ± 0.05	0.61 ± 0.76	0.04 ± 0.01
	T24	0.14 ± 0.01	3.27 ± 1.15	0.03 ± 0.00

NO_2^-: nitrite; NO_3^-: nitrate; NH_4^+: ammonium; PO_4^{3-}: phosphate; C: Control; T: ADOC amendment; 0.5 = time point after 30min; 24 = time points after 24 h.

Characterization of the microbiomes

Abundance of heterotrophic cells were 2.2- ± 1.3-fold higher in BCN than in BL for both HNA and LNA cells (Table 2). At both sites, heterotrophic bacterial community was dominated by HNA cells (77.7 ± 3.2% in BCN, 65.5 ± 5.9% in BL). Physiological traits of the communities showed a higher abundance of active cells in BCN than in BL. Specifically, significant higher leucine incorporation rates were observed in BCN than in BL (442.6 ± 27.1 pmol Leu/l·h and 36.28 ± 3.6 pmol Leu/l·h, respectively). The percentage of highly respiring cells (CTC+) (6.5 ± 0.2% and 2.7 ± 0.2% in BCN and BL, respectively), and the proportion of "dead" cells (as determined by NADS protocol) (in BCN 1.2-fold greater than in BL) followed the same pattern. *S*ignificant different composition was observed in both initial microbial communities (PERMANOVA analyses, R^2 = 0.82, P = 0.002) according to metagenomic reads. *Proteobacteria* was the

Table 2. Biological parameters analyzed from the control bottles of dose-response experiment during the 48 h of incubation. Significant differences between control bottles with and without nutrients addition are represented with an asterisk (t-test; $P < 0.05$).

			Nutrients -		Nutrients +		
		Timepoint (h)	mean	sd	mean	sd	
BCN	GR	4	0.0362	0.0083	0.0322	0.0016	
		24	0.0354	0.0025	0.0847	0.0012	*
		48	0.0216	0.0115	0.0480	0.0010	
	HNA	0	3262185	81308	-	-	
		4	1148558	34836	1140472	11214	
		24	2453931	188628	8827115	223323	*
		48	2733586	1422849	5546308	169804	
	LNA	0	933558	74179	-	-	
		4	304996	13405	289609	2086	
		24	491990	10283	781805	42644	
		48	1076527	572745	7040535	778744	*
	% CTC+	0	6.5	0.2	-	-	
		4	6.5	0.2	7.0	0.3	
		24	5.3	1.7	26.5	9.4	
		48	6.9	3.0	30.2	4.6	*
	% NADS dead	0	10.7	0.9	-	-	
		4	10.1	0.2	14.5	1.5	
		24	6.6	1.0	2.4	0.7	*
		48	3.9	0.3	13.3	11.7	
	LIR	0	442.6	27.1	-	-	
		4	1554.8	128.9	1767.7	22.3	
		24	970.8	142.0	3873.9	159.2	*
		48	1095.2	19.9	4575.0	234.6	*
BL	GR	4	0.0610	0.0024	0.0659	0.0014	
		24	0.0184	0.0020	0.0687	0.0030	*
		48	0.0256	0.0060	0.0503	0.0020	
	HNA	0	1272199	41954	-	-	
		4	322333	18503	337570	3721	
		24	602661	20945	2552133	203986	*
		48	1623625	355675	4323619	296142	*
	LNA	0	669110	19205	-	-	
		4	354912	23005	353334	105	
		24	222355	61207	213513	3287	
		48	230139	175204	1620110	276950	*
	% CTC+	0	2.7	0.2	-	-	

	4	3.3	1.0	3.4	0.1	
	24	9.1	0.9	56.2	0.2	*
	48	9.5	0.0	48.9	1.4	*
% NADS dead	0	8.9	2.0	-	-	
	4	7.5	1.4	34.1	1.8	*
	24	18.5	4.3	13.6	1.7	
	48	10.6	2.8	9.6	1.6	
LIR	0	36.3	3.6	-	-	
	4	34.8	10.1	34.0	5.6	
	24	946.9	241.8	1441.9	207.8	
	48	657.2	156.8	779.5	111.1	

GR: growth rates; HNA: high nucleic acid content; LNA: low nucleic acid content; LIR: leucine incorporation rates.

dominant bacterial phylum in BL and BCN microbial communities (76% and 68%, respectively). *Bacteroidetes* was the next most abundant group (11% in BL and 22% in BCN) in both samples. At class level, *Alphaproteobacteria* was the most abundant (53% in BL and 31% in BCN) but at order level, the most abundant groups differed between locations. *SAR11* accounted for a higher percentage (up to 31%) in BL whereas order *Rhodobacterales* was the most dominant (up to 12%) in BCN. The contrasted relative abundance of the frugal *SAR11* in BL and the copiotrophs *Rhodobacterales* in BCN also reflects the different trophic status of the waters. The relative abundance of MGE transposons was taken as a proxy of the genomic plasticity of the microbial communities and their potential for adaptation to environmental changes (Frost *et al*., 2005, Vigil-Stenman *et al*., 2017). Transposons were significantly more abundant in BCN than in BL metagenomes (Fig. 2). The higher abundance of MGE is consistent with the BCN microbial community being pre-exposed to higher pollution, nutrients levels, as well changing conditions including anthropogenic environmental stresses. Thus, environmental and genomic data pointed to a more plastic community in BCN used to live under more episodes of high POP concentrations, while in BL, the lower abundance of MGE is consistent with a lower pre-exposure to changing anthropogenic stressors and presumably a less resilient community in BL.

Effect of nutrient addition without ADOC additions

Nutrient addition in the controls increased LIR by 4.2-fold in BCN and 1.2-fold in BL after 48 h of incubation (Table 2). Significant higher percentages of CTC-respiring cells were observed in nutrient amended controls in both sites after 48 h. Cell growth of heterotrophic cells (LNA and HNA) doubled in the nutrient amended controls in both sites, and were especially higher for LNA cells after 48 h (Table 2). The percentage of dead or no viable cells (NADS-) decreased along the incubation, showing a higher decrease trend in nutrient amendments in BL (from 34 to 9.6%) than in BCN (from 10.8 to 5.0%). These results show a notable degree of limitation by nutrient availability at both sites in agreement with previous studies that showed prominent P-limitations in NW Mediterranean coastal waters (Sala *et al*., 2002; Pinhassi *et al*., 2006). Nutrient addition included addition of inorganic N and P forms plus labile organic C. Given the higher *in situ* concentrations of inorganic nutrients in BCN seawater, the higher increase of LIR in BCN than in BL seawaters and the similar increases of % of CTC-respiring

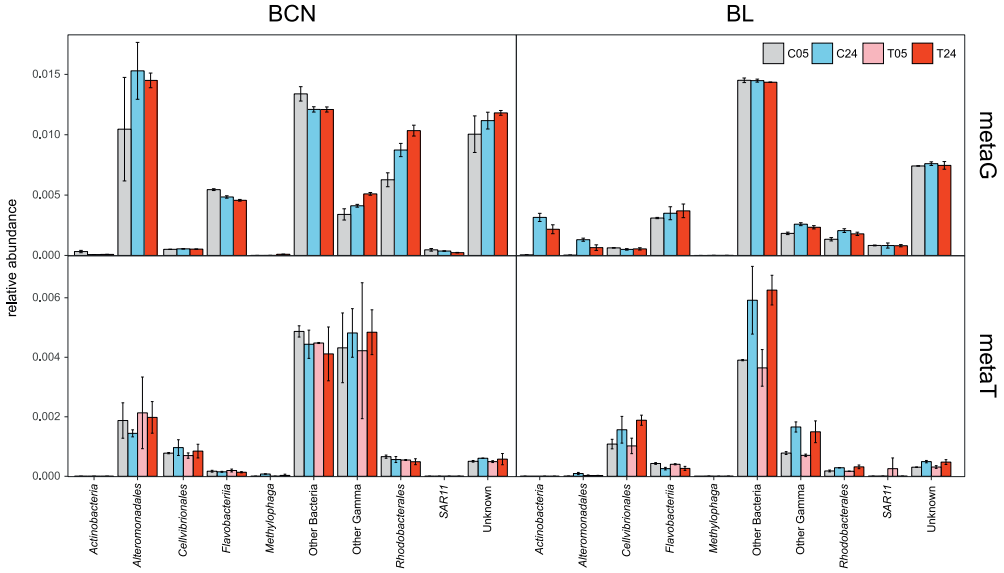

Figure 2. Relative abundance of transposases (Pfam profiles) in Barcelona (BCN) and Blanes (BL) metagenomes (metaG) and metatranscriptomes (metaT).

cells, these results suggest a higher limitation of labile C to build biomass in BCN microbiomes than in BL.

Responses to ADOC and nutrient additions

The dose-response experiment of ADOC treatments amended with and without nutrients originated significant changes in physiological traits of the microbiomes, especially in absence of nutrients (Fig. 3). Increasing concentrations of ADOC promoted a steady increase of heterotrophic prokaryotes growth rates after 48 h of ADOC additions under low nutrient availability at both sites (Fig. 3). Enhanced growth rates after ADOC additions were more notable in HNA than in LNA cells (Fig. S1). Similarly, bacterial production as given by LIR increased for high ADOC additions and low nutrient availability at both sites (Fig. 3 and S2). At BL, the percentage of highly respiring cells (CTC+) followed a similar trend than LIR, increasing steady from low values similar to controls at 7.5x ADOC addition

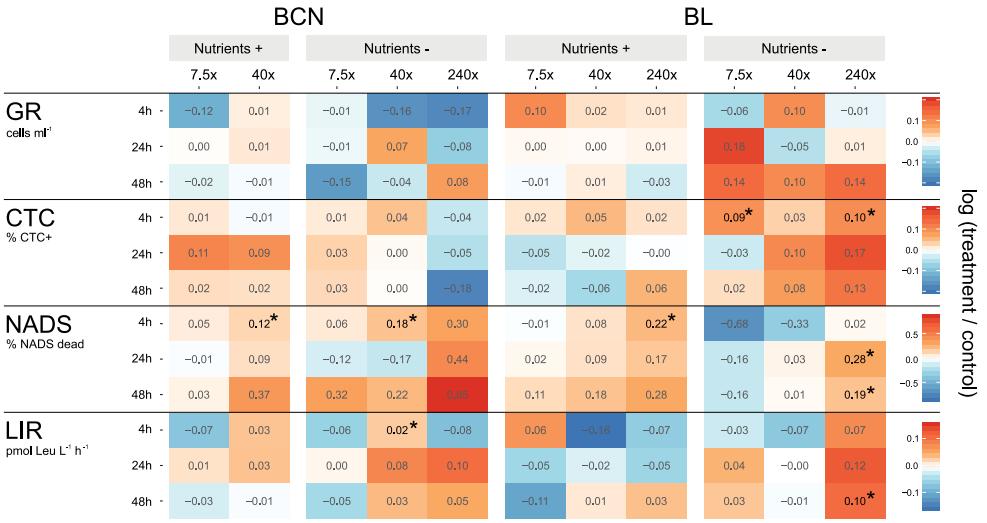

Figure 3. Heatmap of changes in biological measurements between controls and ADOC amendments (calculated as logarithmic of change) in the experiments. Significant differences (t-test; $P < 0.05$) between each treatment and control are marked with asterisks. GR: growth rates, LIR: leucine incorporation rates.

(7.4% of CTC+ cells) to higher numbers at 240x additions (12.7% of CTC+ cells) under low nutrient availability (Fig. 3 and S3). BCN communities did not show significant differences of CTC+ percentages between treatments and controls at any time or trophic condition. The percentage of CTC+ and LNA and HNA abundances, leucine rates and growth rates were positively correlated among them in the experiments (Fig. S4). The percentage of 'membrane-compromised cells' (NADS-) relative to the total number of bacterial cells steady increased along to increased ADOC additions in BL, irrespective of nutrients availability. A similar pattern in NADS- increases with ADOC, but of lower magnitude, was observed in BCN experiments (Fig. S5). The percentage of NADS- cells was positively correlated to growth rates ($N = 54$, Pearson's $r = 0.49$, $P < 0.05$; Fig. S3). These results suggest that ADOC compounds were mostly used as a substrate to growth by at least part of the microorganisms when nutrient availability was low, that is, under nutrient-limiting conditions. As ADOC additions also increased the number of no viable cells, especially in BL, ADOC may have a toxicological effect on prokaryotes in addition to be a source of carbon and nutrients.

The efficient use of hydrocarbons (prominent component of ADOC) as source of C and energy has been assigned to specialized microorganisms called hydrocarbonoclastic bacteria (HCB) (Mallick *et al*., 2011; Ghosal *et al*., 2016, Joye *et al*., 2016). These specialist degraders also show specificities according to the compound and can be distinguished whether they consume aliphatic or aromatic hydrocarbons, for instance (Head *et al*., 2006). Culture and mesocosm studies show that HCB are able to growth on hydrocarbons or oil extracts at low nutrient concentrations, although specific metabolic needs have been detected among different strains (Leys *et al*., 2005; Head *et al*., 2006; Ortmann *et al*., 2019). Furthermore, for most hydrocarbons, the organic carbon is mostly respired to CO_2 to be used as energy source rather than incorporated into

biomass, thus ADOC-degrading specialists would have reduced N and P needs when compared to bacteria degrading other DOC compounds (Bacterial Growth Efficiencies > 60%, Leys *et al*., 2005; Boyd *et al*., 2008). Additionally, the current application of activity-based approaches to capture active microbiomes in field studies (Singer *et al*., 2017) is expanding the number of marine microorganisms known to be able to benefit from hydrocarbon consumption that those listed as HCB (e.g. Rivers *et al*., 2013; Dombrowski *et al*., 2016). Nutrient addition might stimulate the whole community and not specifically ADOC degraders (Boyd *et al*., 2008). Our results show that under nutrient limiting conditions ADOC compounds are more efficiently used as a source of C for building microbial biomass. This suggests other chemicals than hydrocarbons supporting bacterial growth and a potential pool of specialists on a wide range of ADOC which can use it as C and energy source. ADOC use confer these specialists a competitive advantages over may other microorganisms under nutrient limiting conditions.

ADOC additions increased the number of no viable cells, especially in BL but not in BCN. As BCN communities have been pre-exposed to higher concentrations of hydrocarbons and synthetic chemicals (ADOC), these communities are used to live exposed to higher background concentrations of ADOC. Such larger pre-exposure of BCN bacterial communities to ADOC and other anthropogenic pressures have conferred them a higher plasticity (higher abundance of MGE, Fig. 2) to adapt to changing pools of ADOC. Apparently, bacterial populations from BCN had a higher capacity to quench the toxicities of ADOC compounds.

On the other hand, pre-exposed bacteria has a higher capacity for biodegradation of ADOC. Culture and field studies show that adaptation of the communities to pollutants is a selective factor that increase their resilience to toxicants and allow a more efficient removal of pollutants (Barkay and Pritchard, 1988; Echeveste *et al*., 2011; Ortmann *et al*., 2019). For example, pre-exposure to hydrocarbons has

been shown to modify the time lag phase, rates and extent of degradation (Boyd *et al*., 2008; Okere *et al*., 2017). Pre-exposure of populations to low and high levels of contaminants may therefore impact subsequent biodegradation (Aelion *et al*., 1989). Hwang *et al*. (1989) suggested that different enzyme systems may be responsible for contaminant biodegradation at different concentrations and that enzymes with different kinetics are expressed depending on contaminant concentration and pre-exposure. In terms of the kinetics described by the Michaelis-Menten equation, a higher adaptation to environmental variables is related to a lower value of the Michaelis constant (Km) (Gunderson and Stillman 2014; Ng *et al*., 2015). At low concentrations of substrate (ADOC compounds), an enzyme with lower Km value with have a faster kinetics than another with a higher values of Km, or analogously, a given enzyme of a bacterial population with lower Km values, will be able to degrade faster the substrate than another bacterial population with a higher Km for the same enzyme-substrate. At low concentrations of substrate, differences in reaction rates can be important even for the same Vmax due to the non-linear nature of the Michaelis-Menten kinetics. In this sense, a pre-exposure to ADOC by communities from the BCN site, may have adapted enzymatic responses with lower Km, facilitating the use of certain ADOC compounds as a substrate. Such hypothesis will need future research using certain bacterial groups and specific pollutants families with different pre-exposure scenarios. The proliferation of ADOC-degradative genes among the community, induction and depression of ADOC-related enzymes, and the presence of a sub-population able to metabolize or co-metabolize ADOC compounds, either native or mutants (Ojo, 2007; Haritash and Kaushik, 2009) are features of a microbial population pre-exposed and adapted to pollutants. MGE, such as transposases play an important role in bacterial plasticity and the higher amount of transposases (Fig. 2) may confer BCN communities a higher capacity to cope efficiently and with a short lag phase with ADOC (see below).

These strategies were further analyzed in the communities by means of omic's approaches.

Changes in composition of the communities challenged with background ADOC concentrations

Metagenomic signatures responding to the effects of low concentrations of ADOC were studied after 0.5 and 24 h in incubations without nutrient additions, thus under nutrient limitation.

Treatments were done with a nominal increase of concentrations of x7.5 *in situ* concentrations. Therefore, the OP concentrations in the treatments fell in the

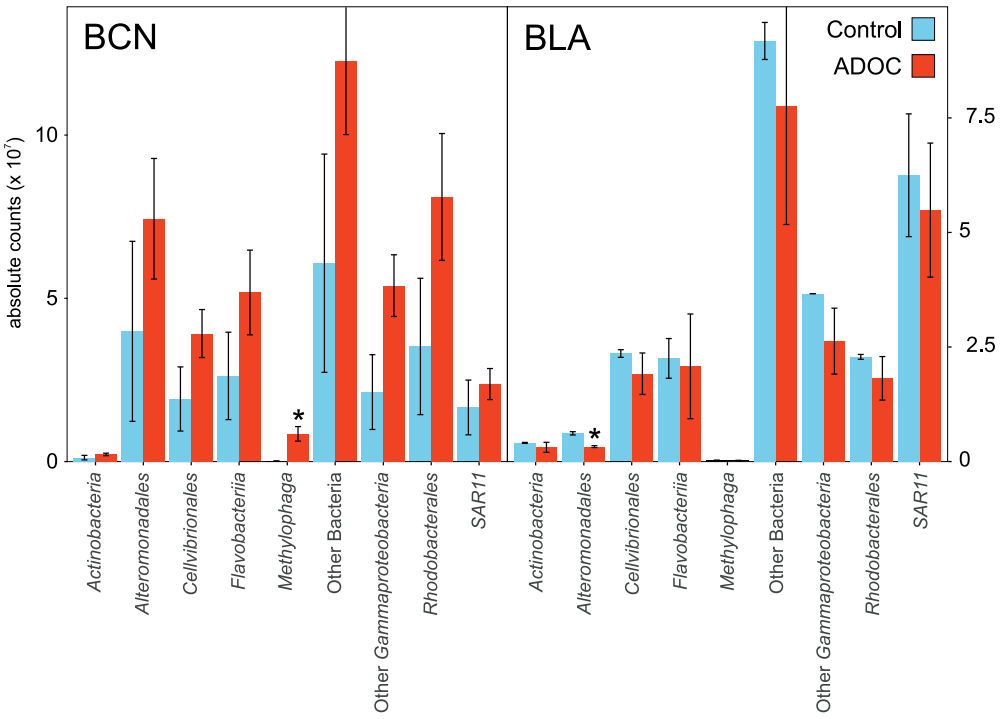

Figure 4. Taxonomical affiliation of Barcelona (BCN) and Blanes (BL) metagenomes, 24 h after treatment. Asterisks indicate significant differences between ADOC amendment and controls (t-test; $P < 0.05$). Values are means of duplicates. Error bars show standard deviation. ADOC: ADOC amendment.

range of 0.6 – 8.9 ng/L for Σ_{32}PAH, 63 – 343 ng/L for Σ_{10}OPE and 802 – 1767 ng/L for Σ_{24}alkanes (n-C_{12} – n-C_{35}). These concentrations are similar to those previously measured in the dissolved phase of the Mediterranean Sea (Guigue *et al*., 2011; Fourati *et al*., 2018; Cerro-Gálvez *et al*., 2019b; Vila-Costa *et al*., 2019). Furthermore, as these ADOC chemicals are hydrophobic and semi-volatile, it is known that real exposure concentrations are lower than nominal (spiked) concentrations. Due to adsorption to bottle walls, partitioning to cells, losses by volatilization etc, ADOC exposure concentrations were approximately 2-fold the *in situ* concentrations, as observed in previous analogous experiments where some surrogate OPs for ADOC were measured in the treatments (Fernández-Pinos *et al*., 2017; Cerro-Gálvez *et al*., 2019a). Thus, cells were exposed to low ADOC concentrations, and within the range of environmental variability.

In these incubations, we observed a decrease of PO_4 and NO_2+ NO_3 concentrations in the treatments in BL after 24 h. In BCN, concentrations remained close to the controls (Table 1). Contrastingly, LIR increased in ADOC enrichments in BCN but not in BL compared to the controls (Fig. S6). Bacterial abundances in controls and treatments remained similar at both sites for the duration of experiment (24 h, Fig. S7).

Addition of ADOC promoted a notable change of community composition in BCN. Principal component analyses (PCA) indicated that *Gammaproteobacteria* and *Rhodobacterales* were the most responding groups to ADOC at expenses of *SAR11* and *Flavobacteriia* (Fig. S8). In BL, no significant differences were observed after 24 h between controls and ADOC treatments, although a notable depletion of *Alteromonadales* and Other *Gammaproteobacteria* gene abundances were detected (Fig. 4). An analysis of the transposases (by Pfam profiles) in the BCN and BL communities shows these were higher in BCN than in BL (Fig. 2). Similarly, highly impacted regions of the inner Baltic sea have

been shown to have higher abundances of transposases, than in the adjacent marine waters (Vigil-Stenman *et al*., 2017). Transposases are thought to play an important role in bacterial plasticity. Transposase gene counts from the BCN site were associated with *Gammaproteobacteria*, *Rhodobacterales, Alteromonadales* and other bacteria groups. Conversely, in BL, the abundance of transposases associated with *Alteromonadales* was lower than in BCN.

After 24 h of incubation, there were a number of genes that changed their relative abundance in the metagenomes (Fig. 5), and some of these are used for sustaining the cell, while other are functional genes with clear role as a microbial response to ADOC (Cerro-Gálvez *et al*., 2019a). Most of these genes showing different abundance after exposure to ADOC were correlated with the relative abundance of transposases in the metagenomes (Fig. 6). With the exception of *Methylophaga* and other *Gammaproteobacteria*, there was no increase of the relative abundance of the other different taxa, which suggest that during the exposure to ADOC, there was a dissemination of genes known to be used as response to ADOC (stress response, membrane transport, metabolism of phosphorus, fatty acids and isoprenoids, etc.) (Cerro-Gálvez *et al*., 2019a). Similarly, exposure to biofilm to waste water treatment waters has been shown to induce a concurrent dissemination of antibiotic resistance genes (ARG) and MGE (Subirats *et al*., 2018).

The analyses of the taxa that increased in abundance more than 10-fold in ADOC treatments than in controls, or that were absent in controls but present in the treatments after 24 h, revealed the growth of mostly *Methylophaga* species and other *Gammaproteobacteria* taxa coming from the rare biosphere in BCN (Fig. 7). They accounted from 0.12% of total community in initial waters that increased to 2.06% in ADOC treatments after 24 h. In agreement, most of the significantly differently abundant genes determined by EdgeR that got enriched

in the ADOC treatments were accounted by *Methylophaga* in BCN. Specifically, *Methylophaga* genus were 70-fold more abundant in treatments than in controls (Fig. 4). Similar increases were observed for other described ADOC-degrading genus such as *Alcanivorax* (2.1-fold increase), *Nocardioides* (2.1-fold increase) and *Pseudomonas* (2.3-fold increase). In BL, ADOC-stimulated taxa belonged to different groups, mostly *Actinobacteria* and *Flavobacteriia*, but their contribution to the total populations remained low (from 0.09% to 0.11% of total cells) after 24 h (Fig. 7).

Increases of specialized ADOC-consumers from the rare biosphere following ADOC pulses have been observed both at low concentrations in polar waters (Cerro-Gálvez *et al.*, 2019a) and high concentrations following oil spill accidents

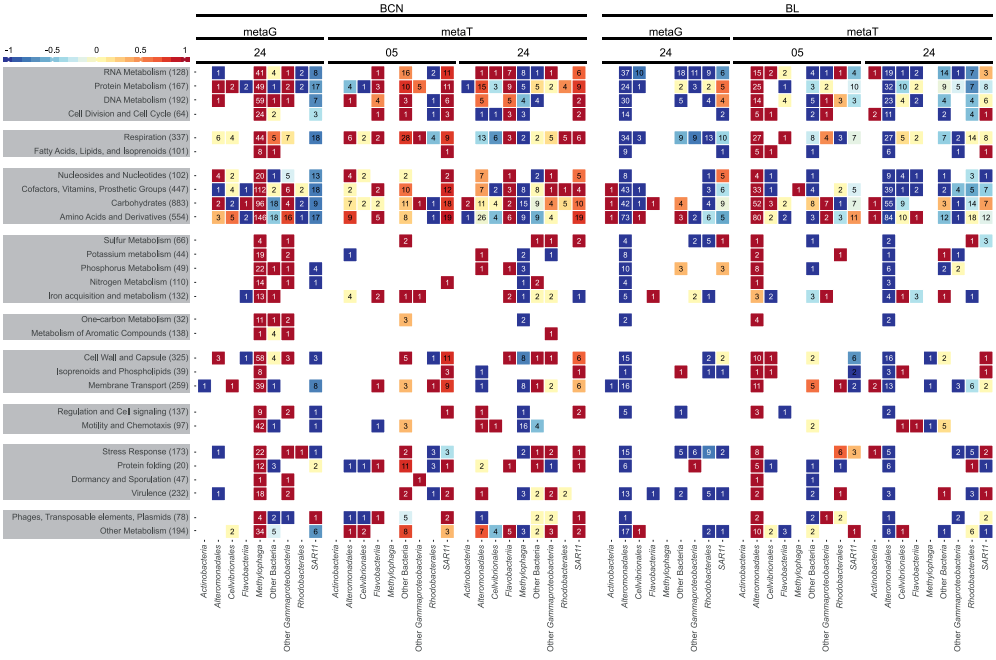

Figure 5. Summary of significant up- and down-regulated transcripts detected by edgeR (FDR < 0.05). Rows correspond to SEED categories and the value in parentheses is the total number of transcripts that belong to that category. Numbers indicate the total transcripts that are significantly increased or depleted for each SEED and taxonomical category

(Teira *et al*., 2007; Hazen *et al*., 2010; Kleindienst *et al*., 2016). *Methylophaga* has been identified as an efficient hydrocarbon degrader in seawater (Mishamandani *et al*., 2014) and it can become enriched following oil spills (Kleindiesnt *et al*., 2016). In the plume originated after Deep Water Horizon oil spill in the Gulf of Mexico, methylotrophs in general got enriched in the plume community following increases of ADOC-degrading specialists (Mason *et al*., 2012; Joye *et al*., 2016). This can be explained by removal of methyl- group from alkylated hydrocarbons, for example methyl phenanthrenes, which are abundant in the ocean (González-Gaya *et al*., 2016) and were abundant in the original ADOC (Table S2). This demethylation could be fast, and thus with the potential to trigger a methylotrophic transcripts after 24 h. Previous

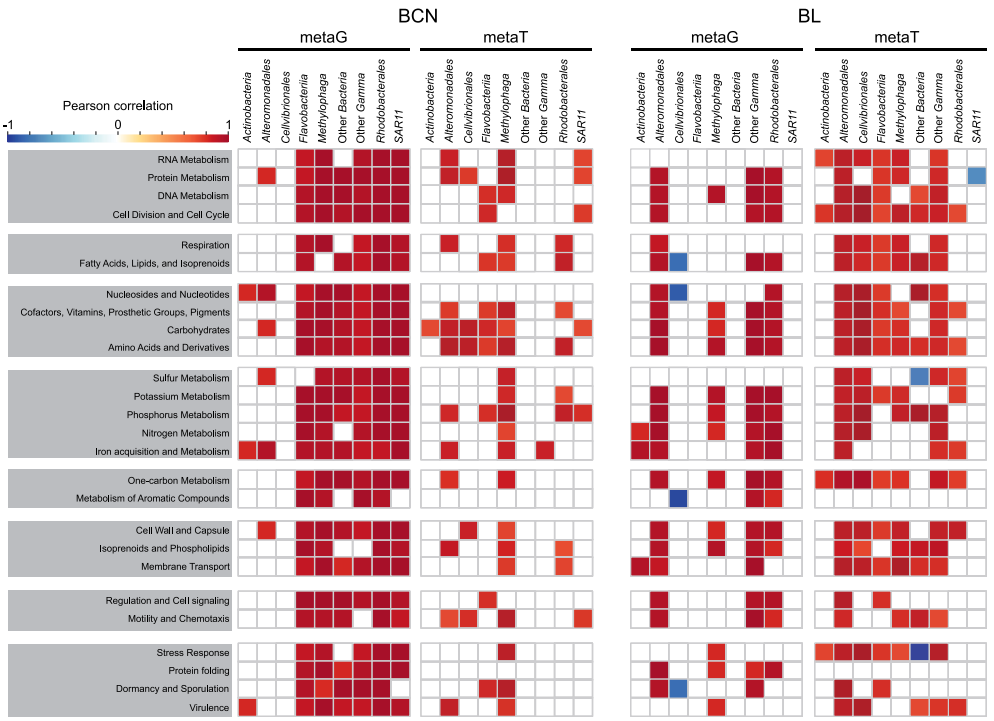

Figure 6. Summary of Spearman correlation between transposases and significant up- and down-regulated transcripts detected by edgeR (FDR < 0.05). Rows correspond to SEED categories

work has described C1 chemicals (and methylotrophs) after 24 h in the Arctic (Cerro-Gálvez *et al*., 2019a) and after several days when high MW DOC is degraded (McCarren *et al*., 2010).

Gene expression profiles after ADOC additions

Many of the differently expressed genes enriched after 30 min and 24 h of ADOC addition (*SAR11* and *Flavobacteriia* in BCN and *Alteromonadales*, mostly the genus *Glaciecola,* in BL) belonged to groups that decreased in relative abundance in the metagenomes after 24 h of incubation (Fig. 4 and 5). Therefore, this genetic response is not related to growth, but to a microbial response due to exposure to ADOC different than consumption of ADOC. In agreement, these groups accounted for most of the depleted EdgeR genes in the metagenomes (Fig. 5). An

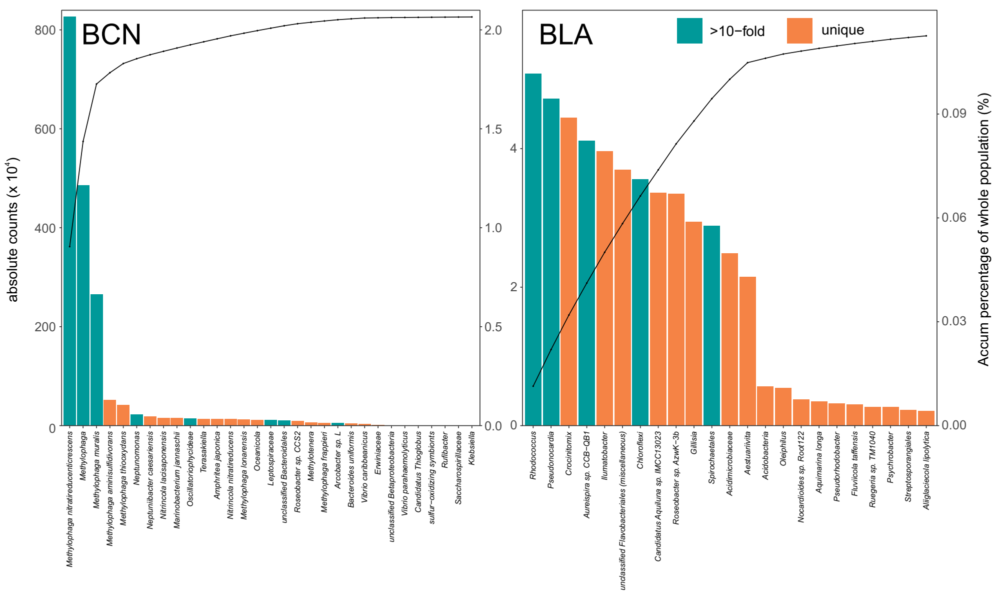

Figure 7. Abundances in the taxa that increased by 10-fold (green) or were absent in controls (orange) after 24 h of incubation in the Barcelona (BCN) and Blanes (BL) ADOC amendment. Phylotypes were taxonomically classified at highest resolution. Counts were summarized at each taxonomical level.

important number of transcripts related to cell wall and membrane got enriched in these groups in agreement with previous studies on genomic responses to ADOC (Cerro-Gálvez *et al*., 2019a) and with the role of hydrophobic ADOC compounds to perturb cell membrane's permeability, rigidity and efficiency (Van Wezel *et al*., 1995; Cronin *et al*., 2000; Escher *et al*., 2017). Genes related to oxidative stress were enriched in some taxa only. Significantly, enriched transcripts at both sites were mostly related to heterotrophic activities of the cells, including respiration, although responses were taxon-specific. In contrast, activity of the growing *Methylophaga* group could not be captured after 30 min and transcripts got significantly depleted after 24 h. This apparent contradictory result (activation of genes for cell growth but no actual increase in abundance after 24 h and *vice versa*) can be explained by the distinction between ADOC tolerant versus ADOC-degrading community (Rodríguez-Blanco *et al*., 2010; Sauret *et al*., 2014), as well by the response in terms of MGE of *Methylophaga*. ADOC-tolerant community include those taxonomical groups that harbor anti-toxicant strategies other than direct degradation of ADOC to cope the adverse effects of ADOC compounds that allow them to compete with ADOC-degraders for the available nutrients. In the case of *Methylophaga*, there was a significant correlations between the transcripts of transposases and the transcripts of most of SEED categories (Fig. 6), including stress response, membrane transport, isoprenoids and cell walls, all SEED categories known to be related to microbial responses to ADOC (Cerro-Gálvez *et al*., 2019a). As consumers of C1 chemicals, *Methylophaga* may not have the capacity to degrade themselves the ADOC compounds, but only methyl groups liberated by other bacteria. Such combination of opportunistic character, with the apparent capacity to express transposons together with most cellular functions may have conferred a special competitive advantage to *Methylophaga* under exposure to ADOC.

SAR11 had a low abundance of transposon genes, but these were as well correlated with the genes that increased due to exposure to ADOC in BCN (not in BL). Contrasting with *Methylophaga*, genes differentially expressed after ADOC exposure were generally not correlated with the transcripts of transposons (Fig. 6). The strategy of *SAR11* seems to be related to tolerance to hydrophobic chemicals, but exposure to ADOC did not resulted in an expression to MGE. For instance, *SAR11* has a less hydrophobic cell surface compared to other taxa (Dadon-Pilosof *et al*., 2017) that lower the adsorption to ADOC compounds thus lowering the possibility of narcosis. This selective advantage of *SAR11* has been observed in polar waters (Cerro-Gálvez *et al*., 2019a).

Most of the active *Alteromonadales* in BL corresponded to *Glaciecola sp*., a group that dominated the phenanthrene-tolerant community in NW Mediterranean coast, but not the phenanthrene-degrading community (Sauret *et al*., 2014). These authors observed that *Glaciecola* accounted for most of the ^{12}C-fraction after stable-isotope probing (SIP) incubations adding ^{12}C- and ^{13}C-phenanthrene in the less polluted sites (Marseille and Banyuls), with similar levels than BL bay. Our studies agree with these previous results and show that *Glaciecola* cell activation can be observed at much lower ADOC concentrations (from mg/l in Sauret *et al*. (2014) to ng/l in our study). One characteristic of the ADOC-growing community, mainly observed in oil spill scenarios, is the long incubation times (from 7 to 15 days) required to be detected (e.g. Garneau *et al*., 2016, Ghosal *et al*., 2016, Joye *et al*., 2016). Nevertheless, an analysis of MGE for *Alteromonadales*, show that even transposons were not among the most abundant, these showed significant correlations with most of SEED categories, including those related to genetic responses to ADOC such as stress response, motility, membrane transport, isoprenoids, cell wall, as well central cellular functions (Fig. 6). In this case, as in *Methylophaga*, the generalized correlations between transposases

and expression of most SEED categories suggest an ongoing adaptation to ADOC exposure. The short time of our study might have prevent the actual grow of *Alteromonadales* (*Glaciecola)* in BL metagenomes.

A surprising trend is the dissimilarity between metagenomes and metatranscriptomes. Not only because those groups growing in gene abundance show lower transcripts (and the opposite), but also due to the fact that transposon genes are generally correlated with genes in BCN, but to a lower degree in BL. An on the other hand, transcripts of transposons are generally correlated with transcripts of differentially expressed genes in BL, but to a lower degree in BCN. As far as we know, this is the first time that both transposons are measured using both metagenomes and metatranscriptomes under experimental conditions of an environmental stress (ADOC in this case). The results obtained suggest that adapted communities, such as in BCN, show transposons correlated with the genes responding to the stress. Conversely, less adapted communities, such as in BL, are the transcripts of transposons generally correlated with the differentially expressed transcripts. Future work is needed to confirm this observations. In this sense, the development of higher amount of transposases may be a strategy to develop plasticity to a new environmental pressure (ADOC in this case). Such strategy had been previously developed due to pre-exposure for the BCN bacterial community. Previous studies have shown that concurrent dissemination (and correlation) of MGE and tolerance genes such as ARG (Subirats *et al*., 2018).

As far as we know, this is the first study that shows that correlation between MGE (transposases) and the functions known to respond to stress, in this case exposure to ADOC. These correlations between the transcripts were especially abundant for the community that has a lower pre-exposure to ADOC, and lower transposases abundance. As there were no significant changes of the bacterial

community in BL, we suggest that these concurrent trends of MGE and cellular functions are the result of dissemination of the mechanisms to cope with ADOC among the bacterial community in BL. Future research will need to confirm these observations and the detailed mechanism of adaptation to ADOC and how it affects evolution if microbiomes during the Anthropocene.

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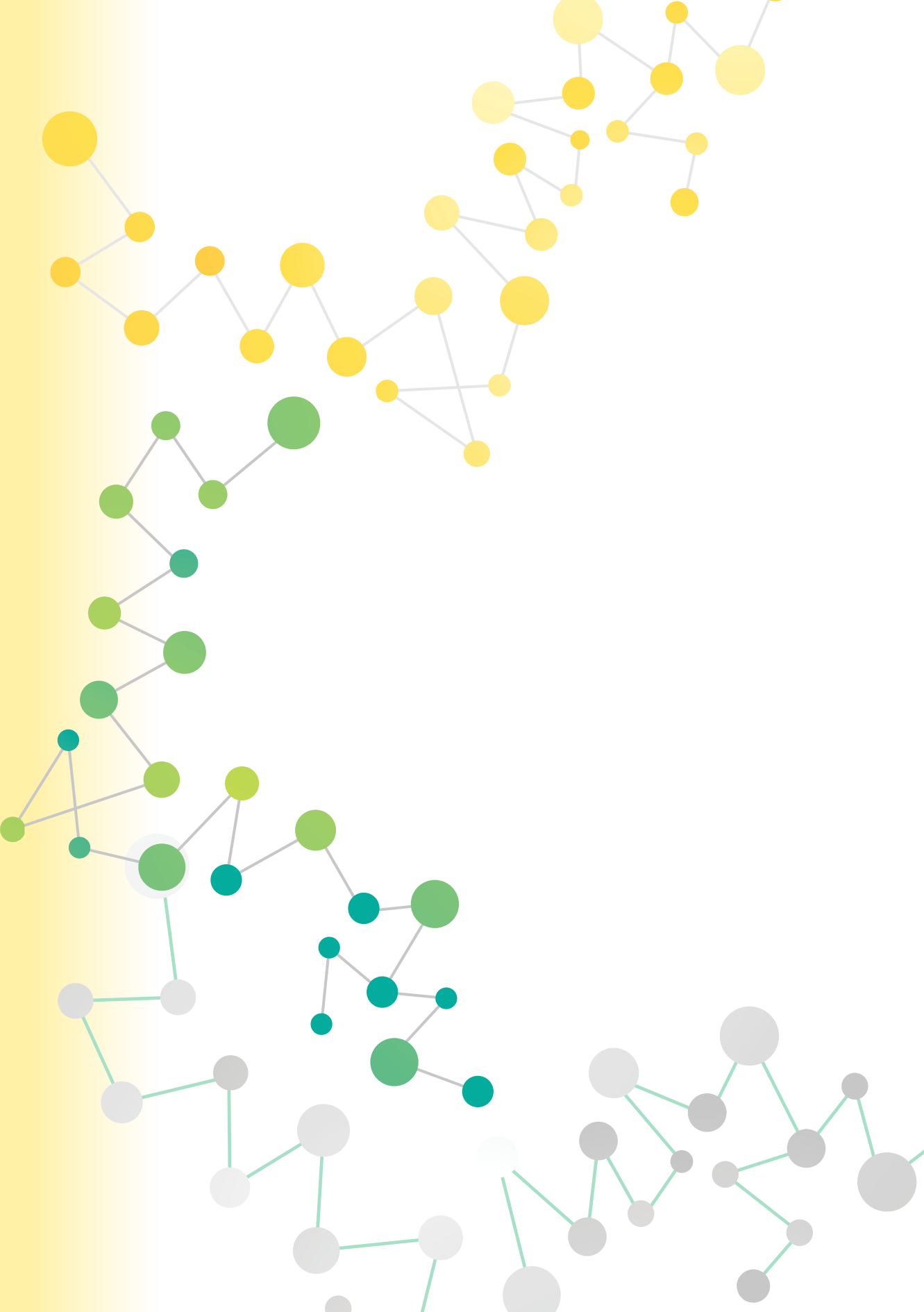

PART III

Epiloge

GENERAL DISCUSSION

The work presented in this thesis is a contribution to build a better understanding of the interactions between the low, but chronic concentrations of organic pollutants (OPs) originated by diffusive pollution, and the marine microorganisms. One of the major novelties and challenges of this study was joining two disciplines that to date were commonly separated, marine ecology and environmental chemistry, towards a genomic microbial biogeochemistry of OPs. On the one hand, pollutants reaching the oceans has been studied in terms of toxicological implications (Tanabe, 2002; Vasseur and Cossu-Leguille, 2006; Desforges *et al*., 2018) and mainly under acute events, such as oil spills (King *et al*., 2015; Beyer *et al.*, 2016). However, diffusive pollution had not been considered as a driver of change in marine communities, perhaps based on the mistaken old rule that "dilution is the solution to pollution". On the other hand, pollutants has been addressed mainly from a biogeochemical, fate and transport, perspective (Dachs *et al*., 2002; Muir and Howard, 2006; Lohmann *et al*., 2007), neglecting the activity of the microbial communities as a potential modulator of the fate and cycling of pollutants in the marine environment (Galbán-Malagón *et al*., 2012; 2013). Another important strength of this thesis was the use of a complex mixture (and not always individual families of pollutants) in exposure experiments with concentrations that were in the range of their environmental variability (and not orders of magnitude larger such as in ecotoxicological and bioremediation approaches), so far only applied to phytoplanktonic communities (Echeveste *et al*., 2010, 2016; Fernández-Pinos *et al*., 2017). Furthermore, rather than working with bacterial cultures, this thesis has performed experiments with the complexity associated to work with marine microbiomes (Stocker, 2012) in highly contrasted seawater environments (geographically, trophic conditions, temperature, etc.). Finally, the fact that most of the effects of diffusive pollution are at sub-lethal

level and close or below the detection limit of classical techniques, commonly used to characterize microbial responses to external perturbations, has forced to put more effort to develop and apply state-of-the-art high throughput sequencing techniques such as metagenomic and metatranscriptomic approaches. This approach include specific laboratory protocols, sequencing, bioinformatic analyses and multivariate statistics to interpret the big data sets generated.

The evidences from this study suggest that the baseline ADOC pollution ubiquitously present in the oceans, two orders of magnitude lower than DOC, is modifying bacterial communities in terms of structure and function, and in consequence, the dynamics of ocean biogeochemical cycles. At the same time, the microorganism inhabiting the oceans are capable of modulate the occurrence and fate of incoming pollutants. However, this bidirectional interaction is closely

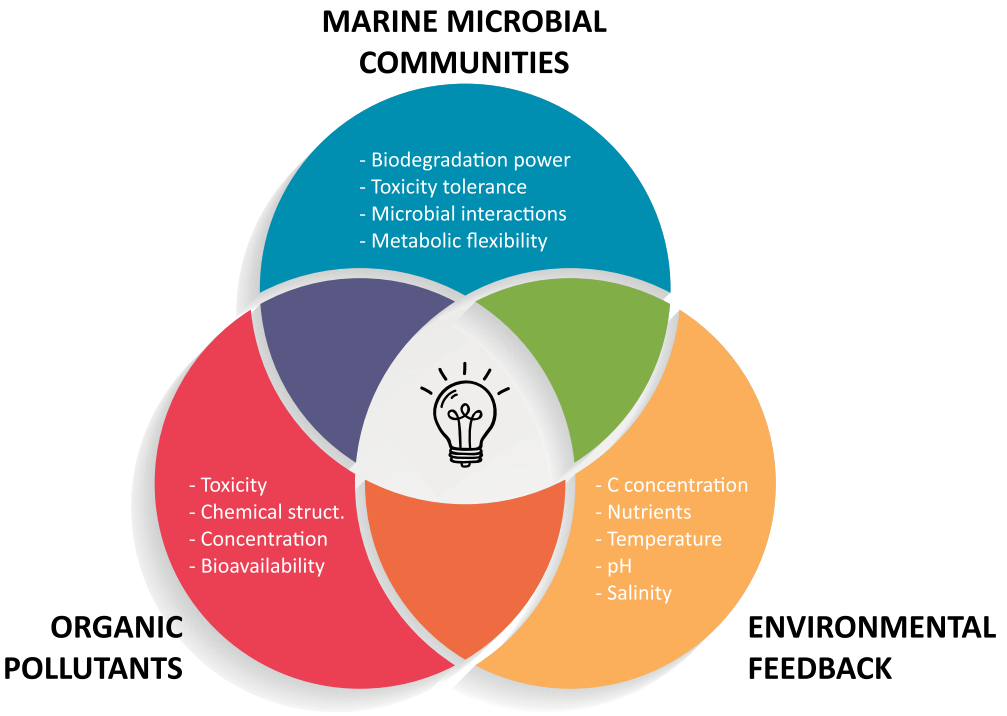

Figure 1. Interaction between organic pollutants and marine bacteria.

related with environmental variables and conditions (nutrients, etc), through a three way feedback. These relationships are modified according to the intrinsic characteristics of each party. Taken all chapters of this thesis together, the results found after the ADOC amendments of marine microbial communities are summarized below (Fig. 1).

Pollutant-bacteria interaction in the marine environment. What does it depend on?

a) Organic pollutants

ADOC as a neglected factor of marine ecology. In this work, the concept of a pool of anthropogenic OPs has been named for the first time as ADOC. This was done to stress the occurrence of an anthropogenic component of DOC, which has not been identified before as a driver of the major biogeochemical cycles through marine bacteria. Only the effects of certain families of OPs on bacteria were addressed previously to this work (Hudak and Fuhrman, 1988; Lu *et al.*, 2012; Cerezo and Agustí, 2015). The influence of varying concentrations of ADOC, at environmentally relevant concentrations, is logically small compared to the influence of the naturally varying concentrations of nutrients. Nevertheless, even though ADOC is orders of magnitude smaller than the concentrations of DOC, the analysis performed during this thesis conclude that ADOC modulate microbial communities. Therefore, ADOC is a neglected carbon input that should be considered as a factor in the ocean ecology and major biogeochemical cycles.

ADOC composition and concentrations determine bacterial responses. After comparing the impact of different families of compounds, as well as the ADOC mixture, it can be concluded that both the nature of the compound and its dose are key in the bacterial response. As seen in **Chapter 1**, experiments done with the same bacterial community had different responses to different doses

of the same compound, and in fact, the response was due to the interaction of pollutants with nutrients. Pollutants behave like any other organic compound of nature, in which depending on the concentration in which it is found it may range from being a beneficiary compound for the biota to a lethal one. For this reason, it is important to distinguish between two different scenarios, the case of oil spills with punctual and acute inputs of ADOC, leading to high transient concentrations, and on the other hand, chronic atmospheric deposition and/or continental inputs, which may be low in terms of magnitude but an input temporally persistent and geographically ubiquitous. Many studies have been done in oil spills situations, demonstrating its effects on microbial communities (Kimes *et al*., 2014; Joye *et al*., 2014; King *et al*., 2015; Beyer *et al.*, 2016). Contrasting with these previous studies, this thesis has shown for the first time the relevance of low concentrations for a myriad of chemicals and the feasibility of studying the atmospheric diffusive pollution and its impact.

b) Marine microbial communities

Species-specific responses to ADOC. In general, the impact on the communities was dependent not only on the type of organism (heterotrophs vs phototrophs), but also on the different taxonomic groups within these types. Differences can be at individual level, making it more challenging to observe with typical biological techniques and encouraging us to use genomics techniques. By DNA sequencing, in **Chapter 2** we demonstrated that two taxa increased its relative abundance after 24 h of the PFAAs amendment: *Gammaproteobacteria*, possibly in charge of the PFOS biodegradation, and *Flavobacteriia*, known to have positive correlation (tolerance) with PFAAs contamination. In **Chapter 4**, after 24 h of ADOC treatment, *SAR11* presented a relative increase in the Barcelona experiment, while in Blanes was *Alteromonadales*, mostly the genus *Glaciecola*, increasing its relative abundance. Metatranscriptomic profiles were

necessary when responses were not observed at composition level, but in the specific activity. Thus, in Arctic and Antarctic experiment ADOC addition elicited transcriptional responses in multiple microbial heterotrophic metabolism in ubiquitous groups such as *Flavobacteriia*, *Gammaproteobacteria* and *SAR11*. Taken together, these findings suggest a key role of *Gammaproteobacteria*, Flavobacteriia and *SAR11* in either tolerance or degradation of OPs.

Different microbial strategies to cope with ADOC. Under a perturbation of ADOC addition, some bacteria may use these compounds as a nutrient source but others will activate mechanisms of detoxification. Using an ADOC component as a nutrient source could lead to the complete respiration of the chemical to CO_2, or be a partial degradation producing some transformation products (such that observed for desulfurization of PFOS). In the **Chapter 3**, we identified and described three main bacterial strategies to prevent or moderate ADOC adverse effects other than degradation: 1) modulation of membrane rigidity and permeability by changing its composition, 2) active exportation of substances outside the cell, and 3) production of extracellular polymeric substances (EPSs) and biosurfactants. These strategies could end in the acquisition of the microbial community of a better adaptation and agility of actuation under future ADOC pressures.

Growth of the rare taxa. Results demonstrate that ADOC exposure changed the composition of microbial communities though the stimulation of the growth of the rare taxa. In the Arctic experiment, most of this rare taxa belonged to documented OPs degraders (*Nocardioides*, *Pseudomonas* and *Sphingobium*), and in Barcelona experiment, it was mostly composed by Methylophaga species. However, in Antarctic and Blanes experiment, OP-degrading bacteria were also detected, but with a weaker or slower increase, suggesting a dependence on other external factors such as nutrient conditions or adaptation due to pre-exposure to

pollutants. Microbial communities and their environment are very complex and it is common to find different responses under the same perturbation. The growth of the rare biosphere can be a biomarker of marine pollution, a subject that can only be explored by means of metagenomic or metataxonomic approaches

c) Environmental feedback

The trophic status of the community and environmental conditions as a crucial factor. Additional to the community structure, the ADOC concentration and the metabolic capacities, the trophic status (nutrients and organic matter) also determine the bacterial response to ADOC. Our experiments concluded that the responses were both positive and negative in the same taxonomical group when challenged with one family of compounds, and varied depending on the trophic conditions of each experiment. At some point, nutrient limitation may provoke an alert response of the population and the activation of metabolic pathways possibly less efficient but capable of providing necessary compounds. This was shown in **Chapter 4**, in Barcelona and Blanes experiments, ADOC compounds were mostly used a substrate to growth by at least part of the microorganisms when nutrient were not added, that is, under nutrient-limiting conditions. Another case in which there is a difference of response according to the conditions of the environment is in the growth of the rare biosphere, seen only in two of the four experiments done with ADOC exposures.

GENERAL CONCLUSIONS

1. Perfluoroalkyl substances (PFASs) and organophosphate ester (OPE) flame retardants and plasticizers, organic pollutants of emerging concern (OPEC), elicited similar responses in marine microbial communities than polycyclic aromatic hydrocarbons (PAHs) and n-alkanes at concentrations in the range of their environmental variability.

2. Organic pollutants (OPs) modulated the extracellular enzymatic activity and the growth rates of marine bacteria.

3. The responses were dependent on the structure and concentrations of the pollutant, but also on the trophic status and phylogenetic identity of the main bacterial degraders, following complex patterns.

4. The half-life of PFOS in Antarctic experiment was of 43 h, while sulfur metabolism-related genes were significantly enriched in the treatments, suggesting a desulfurization of the compound. On the other hand, no significant decrease of PFOA was found after 6 days.

5. Two taxa increased after 24 h of the PFAAs amendment: *Gammaproteobacteria*, possibly in charge of the PFOS biodegradation, and *Flavobacteriia*, known to have positive correlation (related to tolerance) with PFAAs contamination.

6. Bacterioplankton communities did not experience major changes in its overall composition after 24 h of ADOC exposures.

7. Background levels of ADOC substantially stimulate the growth of the rare biosphere with taxa known to be OPs degraders. This response was more important in the experiments performed in the Arctic and Barcelona than in Antarctica and Blanes.

8. Responses in bacterial functionality to ADOC differed for each taxonomical group, with *Flavobacteriia* and *SAR11* being the more responsive groups in Arctic, *Flavobacteriia* in Antarctica, *Alteromonadales* in Barcelona, and *Cellvibrionales* and *Rhodobacterales* in Blanes.

9. Microbial communities responded with a suite of responses when exposed to ADOC besides degradation. These response are taxa and site specific and include modification of the membrane prop-erties (decreasing the number of hopanoids, changing the degree of fatty acid saturation, activation of channels of exportation outside the cell and production of extracellular poly-meric substances).

RECOMMENDATIONS FOR FUTURE RESEARCH

After this thesis, several questions about the marine bacterial responses to chronic pollution of ADOC still remain to be answered. However, it has also raised many new questions and recommendations in need of further investigation:

Characterization of ADOC. It is important to quantify the concentration and characterize the unknown organic compounds being part of the ADOC present in the environment, in order to help to understand its impact and toxicity mechanism. A detailed study of microbial response under a scenario of effect-direct assessments of multiple fractions of ADOC could be a fruitful approach.

Ecological impact of chronic exposure of ADOC. In the experiments of this current thesis, low concentrations of ADOC, at environmentally relevant concentrations, changed the composition of the bacterial community and its biodiversity, in particular promoting the growth of members from the rare biosphere that are presumably related with the biodegradation of pollutants. In addition, the cumulative and persistent toxic effect of the pollutants is likely to cause long-term changes in the population, a fact that should be monitored with temporal studies in areas with a progressive increase of pollution.

Biogeochemical impact. Little is known about the impact that ADOC and its biodegradation can produce on the biogeochemical cycles of the oceans. ADOC being consumed resulted in an increase of marine bacterial respiration and production, affecting the overall carbon cycling. It has also been demonstrated that specific families of OPs can alter biogeochemical cycles. For instance, OPEs could be used as a source of P under P-limiting conditions, affecting the P-cycle, and the desulfurization of PFOS suggested a direct link with de S-cycle of the oceans. Further research in this direction should be accompanied by a better

characterization of the anthropogenic perturbation of the organic- P, S and N present in the marine environment.

Need of inclusion of physiological traits of microorganisms in environmental risk assessments. Recently, the field is moving towards blending realistic conditions: complex mixtures instead of families or individual pollutants, complex communities instead of selected culturable species (chosen for their ease of cultivation and not for their ecological relevance), low and chronic pollutant exposure instead of a high acute input. The new understanding of this thesis should help to improve predictions of the impact of ADOC to microbiomes and provide the following insights for future investigation for the robust development of marine bacteria as next generation risk indicators in the marine environment. Thanks to their ubiquitous presence and the rapid responses following changes in their chemical environment, microbiomes can be taken as biosensors of pollution and their genes potential markers of exposures to individual or complex mixtures of contaminants (Caruso *et al*., 2016; Sutcliffe *et al*., 2019). The growth of the rare biosphere can be used as a biomarker of marine pollution, a subject that can only be explored by means of metagenomic or metataxonomic approaches (Joye *et al*., 2016). In fact, exposure to pollutants in gut microbiomes is detected by the presence or absence of certain strains (Claus *et al.*, 2016), but whether or not a similar approach can be taken using environmental microbiomes remains under study (Tlili *et al*., 2015).

Adaptation of microbial communities to OPs. The issue of adaptation, plasticity and tolerance of microbial communities needs an ambitious approach in order to quantify its relevance and influence on the interactions of OPs, microbial communities and major biogeochemical cycles. The present thesis showed that responses differ depending on the pre-exposure to ADOC in the community.

Development of gene-inclusive biogeochemical cycles of OPs and nutrients. Current biogeochemical modelling of OPs do not include the structure and function of microbial communities. Such information could be included by using the information capture by metagenomes and metatranscriptomes, at least for some families such as PAHs, n-alkanes with relatively know metabolic patterns. On the other hand, OPs could be included in the models aiming at modeling the biogeochemical cycles of nutrients in the ocean.

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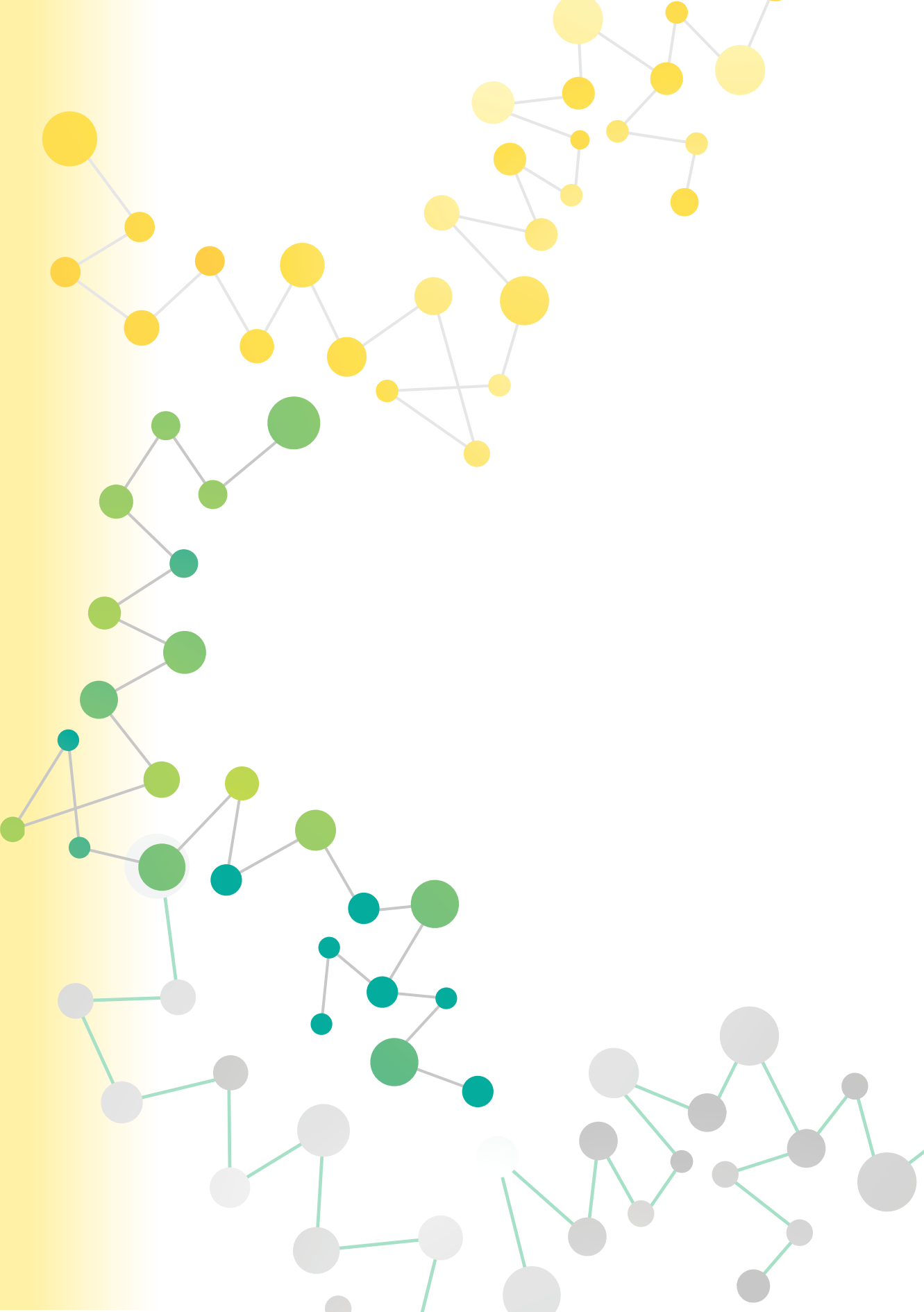


PART IV

Annex

Supporting information of **Chapter 1**

SUPPLEMENTARY TABLES

Table S1. List of PFOS, PAHs, OPEs and n-alkanes added to the four sets of experiments. Concentrations of organic pollutants were added to treatments at low, mid and high levels as indicated (in ng/l, amount added for each individual chemical). In parenthesis (and bold) it is indicated the enrichment factor (total concentration in treatment / total concentration in control). The enrichment factor is not available for PFOS as it was not analyzed at the sampling stations.

Treatment	Conc. and enrichment factor				Mix	Compounds
	Control	Low	Interm	High		
PFOS	-	10	100	1000	MPFOS	Sodium perfluoro-1-[1,2,3,4-13C4]octanesulfonate.
PAHs	0 **(1)**	5 **(5)**	50 **(37)**	500 **(365)**	mix of PAH	Acenaphthene, Acenaphthylene, Anthracene, Benzo(a)anthracene, Benzo(b) fluoranthene, Benzo(k) fluoranthene, Benzo(g,h,i) perylene, Benzo(a)pyrene, Chrysene, Dibenz(a,h) anthracene, Fluoranthene, Fluorene, Indeno(1,2,3,c,d) pyrene, Naphthalene, Phenanthrene, Pyrene.
OPEs	0 **(1)**	100 **(7)**	1000 **(60)**	10000 **(591)**	mix of OPEs	EHDPP: 2-ethylhexyl diphenyl phosphate; TCEP: tris(2-chloroethyl) phosphate; TCrP: tricresyl phosphate (4 isomers); TDCP: tris[2-chloro-1-(chloromethyl)ethyl] phosphate, TiBP: triisobutyl phosphate, TnBP: tri-n-butyl phosphate, TPhP: triphenyl phosphate.
Alk	0 **(1)**	300 **(6)**	3000 **(49)**	30000 **(477)**	mix of Alkanes	n-Decane, n-Undecane, n-Dodecane, n-Tridecane, n-Tetradecane, n-Pentadecane, n-Hexadecane, n-Heptadecane, n-Octadecane, n-Nonadecane, n-Eicosane, n-Heineicosane, n-Docosane, n-Tricosane, n-Tetracosane, n-Pentacosane, n-Hexacosane, n-Heptacosane, n-Octacosane, n-Nonacosane, n-Triacontane, n-Hentriacontane, n-Dotriacontane, n-Tritriacontane, n-Tetratriacontane, n-Pentatriacontane.

Table S2. Limits of quantification (LOQ) for the target organic pollutants analyzed in seawater. LOQ were derived from the field blank (when present), or from the lowest standard that could be quantified from the calibration curve.

Family	Compound	LOQ (ng/l)
Alkanes	Dodecane	0.0084
	Tetradecane	0.0072
	Pentadecane	0.0151
	Hexadecane	0.0376
	Heptadecane	0.0560
	Octadecane	0.0794
	Nonadecane	0.0428
	Eicosane	0.1215
	Heneicosane	0.1286
	Docosane	0.2492
	Tricosane	0.3446
	Tetracosane	0.5211
	Pentacosane	0.6613
	Hexacosane	0.8200
	Heptacosane	0.7978
	Octacosane	0.9097
	Nonacosane	0.9958
	Triacontane	1.0107
	Hentriacontane	1.0332
	Dotriacontane	1.0738
	Tritriacontane	0.9748
	Tetratriacontane	1.0029
	Pentatriacontane	0.9549
PAHs	Naphthalene	0.0001
	Methylnaphthalenes	0.0001
	Dimethylnaphthalenes	0.0001
	Trimethylnaphthalenes	0.0001
	Acenaphthylene	0.0001
	Acenaphthene	0.0001
	Fluorene	0.0455
	Dibenzothiophene	0.0001
	Methyldibenzothiophenes	0.0001
	Dimethyldibenzothiopenes	0.0001

Family	Compound	LOQ (ng/l)
	Phenanthrene	0.3030
	Methylphenanthrenes	0.0001
	Dimethylphenanthrenes	0.0001
	Trimethylphenanthrenes	0.0001
	Tetramethylphenanthrenes	0.0001
	Anthracene	0.0001
	Dibenzo[a,h]anthracene	0.0330
	Fluoranthene	0.1080
	Pyrene	0.1355
	Methylpyrenes	0.0001
	Dimethylpyrenes	0.0001
	Benzo[g,h,i]fluoranthene	0.0001
	Benzo[a]anthracene	0.0001
	Chrysene	0.0225
	Methylchrysenes	0.0001
	Benzo[g,h,i]perylene	0.0001
OPEs	TiBP	0.0631
	TnBP	0.0005
	TCEP	0.0005
	TCPP-1	0.0005
	TCPP-2	0.0005
	TCPP-3	0.0005
	TPhP	0.0005
	EHDPP	0.0769
	TEHP	0.0005

Table S3. List of oligonucleotide FISH probes used in CARD-FISH analyses.

Probe name	Target organisms	Reference
EUB 338-II-III	Most Bacteria	(Amann *et al.*, 1990; Daims *et al.*, 1999)
Alf968	Alphaproteobacteria	(Neef, 1997)
Ros537	Alphaproteobacteria; *Roseobacter*	(Eilers *et al.*, 2001)
SAR11-441R	Alphaproteobacteria; Sar11 cluster	(Morris *et al.*, 2002)
Gam42a	*Gammaproteobacteria*	(Manz, W. *et al.*, 1992)
CF319a	Cytophaga–Flavobacterium–Bacteroidetes group	(Manz, W. *et al.*, 1996)

Table S4. Significant multiple linear least-squares regressions of growth rates (cell abundances and CARD-FISH) and extracellular enzyme activities against nutrients, organic carbon and pollutant concentrations and the interactions of pollutants and nutrients for each experiment. Only variables with parameters significantly different than zero were kept in the multiple linear regressions ($P < 0.05$).

Exp	Growth rate	R^2	Adj R^2	SEE	Model P	Indep. variable	Coeff	β-Coeff	Coeff P
Alk	HNA	0.878	0.867	0.003	<0.0005	Alkanes	3.807E-9	0.131	0.031
						TN	-0.002	-0.540	<0.0005
						TON	0.002	0.458	<0.0005
	LNA	0.582	0.534	0.002	<0.0005	NO_2	-0.123	-2.105	<0.0005
						NH_4	0.019	1.871	<0.0005
						TN	-0.001	-0.682	<0.0005
						TON	-0.001	-0.591	0.002
	Syn	0.802	0.769	0.001	<0.0005	Alkanes	2.386E-9	0.279	0.002
						NO_2	-0.035	-0.975	<0.0005
						TOC	1E-10	2.027	<0.0005
						TN	0.001	0.713	<0.0005
						TON	-0.001	-0.772	<0.0005
	Peuk	0.893	0.883	0.003	<0.0005	NO_3	-0.001	-0.512	<0.0005
						NO_2	0.156	1.266	<0.0005
						TOC	-0.001	-1.308	<0.0005
	CF	0.439	0.373	0.041	0.007	NO_2	-0.674	-0.818	0.002
						NH_4 * Alk	1.484E-7	0.539	0.029
	Roseo	0.604	0.558	0.027	<0.0005	TN	-0.016	-0.974	<0.0005
						NO_3 * Alk	1.1E-8	0.572	0.008
	SAR11	0.299	0.217	0.009	0.049	NH_4 * Alk	-8.067E-8	-1.520	0.022
						NO_2 * Alk	3.359E-7	1.235	0.057
	APA	0.937	0.814	<0.0005	<0.0005	EUB	0.006	0.379	0.008
						Roseo	-0.002	-0.814	<0.0005
						TOC	-3.64E-06	-0.673	<0.0005
						NH_4 * Alk	6.85E-10	1.431	<0.0005
						NO2 * Alk	-3.27E-09	-1.333	<0.0005
	β-Glc	0.696	0.615	<0.0005	0.001	EUB	1E-10	0.573	0.015
						NH_4	-7.43E-06	-1.482	0.003
						NO_2	3.45E-05	1.221	0.011
						Roseo	-2.73E-05	-0.636	0.006
	Chit	0.798	0.760	<0.0005	<0.0005	NO2	-4.689E-6	-0.251	0.045
						Roseo	-2.06E-05	-0.727	<0.0005
						PO_4 * Alk	7.444E-12	0.417	0.002

Exp	Growth rate	R^2	Adj R^2	SEE	Model P	Indep. variable	Coeff	β-Coeff	Coeff P
	L-amp	0.798	0.787	<0.0005	<0.0005	NO_3	8.8E-6	0.893	<0.0005
PAH	HNA	0.961	0.955	0.002	<0.0005	NO_2	0.079	-0.564	<0.0005
						NH_4	0.011	0.459	<0.0005
						TN	-0.002	-0.655	<0.0005
						TON	0.002	0.390	<0.0005
						NO_2 * PAH	2.001E-6	0.076	0.050
	LNA	0.666	0.628	0.002	<0.0005	NO_2	-0.069	-1.409	<0.0005
						NH_4	0.011	1.281	<0.0005
						TN	-0.001	-1.109	<0.0005
						TON	-0.001	-0.615	<0.0005
	Syn	0.720	0.710	0.001	<0.0005	NO_3	-0.002	-0.848	<0.0005
	Peuk	0.942	0.936	0.002	<0.0005	NO_2	0.085	0.725	<0.0005
						TN	0.005	0.541	<0.0005
						TON	-0.005	-0.715	<0.0005
	Gamma	0.723	0.671	0.127	<0.0005	NH_4	-0.119	-1.886	<0.0005
						NO_2	0.630	1.768	<0.0005
						TN	0.005	0.513	0.002
	Roseo	0.810	0.788	0.242	<0.0005	TN	-0.015	-0.694	<0.0005
						PO_4 * PAH	-1.955E-5	-0.294	0.039
	SAR11	0.470	0.441	0.033	0.001	NO_3 * PAH	-1.158E-6	-0.686	0.001
	APA	0.9	0.873	<0.0005	<0.0005	Roseo	-0.001	-0.622	<0.0005
						SAR11	-0.002	-0.623	<0.0005
						TOC	-3.05E-06	-0.435	0.001
						PO_4 * PAH	-9.45E-08	-0.646	<0.0005
	β-Glc	0.552	0.468	<0.0005	0.004	NH_4	-2.194E-6	-0.417	0.027
						SAR11	3.066E-5	0.725	0.007
						PO_4 * PAH	1.83E-09	0.782	0.004
	Chit	0.990	0.987	<0.0005	<0.0005	PO_4	1.994E-9	0.445	<0.0005
						Roseo	-1.19E-05	-0.176	0.009
						SAR11	-2.13E-05	-0.263	<0.0005
						PO_4 * PAH	1.99E-09	0.445	<0.0005
	L-amp	0.876	0.852	<0.0005	<0.0005	NO_2	1E-10	0.375	0.004
						TOC	-2.456E-6	-0.899	<0.0005
						PO_4 * PAH	1.861E-8	0.326	0.006
OPE	HNA	0.948	0.942	0.002	<0.0005	NO_2	1E-10	0.546	<0.0005
						TOC	-0.001	-0.418	<0.0005
						TN	0.001	0.221	0.033

Exp	Growth rate	R^2	Adj R^2	SEE	Model P	Indep. variable	Coeff	β-Coeff	Coeff P
						TON	-0.046	-0.339	<0.0005
	LNA	0.651	0.611	0.002	<0.0005	NO_2	-0.51	-0.996	<0.0005
						TOC	1E-10	1.658	<0.0005
						TN	-0.001	-0.421	0.009
						TON	-0.002	-1.136	<0.0005
	Syn	0.708	0.688	0.001	<0.0005	NO_3	-0.002	-0.862	<0.0005
						PO_4 * OPE	4.955E-7	0.332	0.003
	Peuk	0.956	0.949	0.002	<0.0005	NO2	0.103	0.790	<0.0005
						PO4	0.338	0.611	<0.0005
						TN	0.001	0.126	0.005
						PO_4 * OPE	6.964E-7	0.118	0.009
	CF	0.379	0.306	0.039	0.017	NO_2	-0.538	-0.707	0.007
						NO_2*OPE	5.83E-6	0.607	0.018
	Gamma	0.492	0.432	0.02	0.003	NH_4	-0.133	-1.716	0.002
						NO_2	0.55	1.261	0.019
	Roseo	0.654	0.589	0.022	0.001	NO_3	-0.013	-1.503	<0.0005
						TOC	-0.001	-0.643	0.04
						PO_4 * OPE	2.78E-06	0.702	0.02
	APA	0.865	0.839	<0.0005	<0.0005	EUB	0.007	0.402	0.019
						Roseo	-0.002	-0.807	<0.0005
						TN	-2.77E-05	-0.602	0.001
	β-Glc	0.502	0.409	<0.0005	0.009	NO_3	-6.03E-07	-1.448	0.003
						TOC	-1.44E-07	-1.388	0.002
						Roseo	-2.63E-05	-0.541	0.025
	Chit	0.994	0.993	<0.0005	<0.0005	NO_3	-2.33E-07	-0.775	<0.0005
						PO_4	1.67E-05	1.664	<0.0005
						Roseo	-5.48E-06	-0.156	<0.0005
	L-amp	0.733	0.683	<0.0005	<0.0005	NH_4	4.84E-05	0.534	0.018
						TOC	-2.66E-06	-1.379	<0.0005
						NO_3 * OPE	-4.96E-11	-0.454	0.012
PFOS	HNA	0.867	0.856	0.003	<0.0005	TOC	1E-10	0.540	<0.0005
						TN	-0.002	-0.481	<0.0005
						NO_2 * PFOS	-2.909E-5	-0.128	0.049
	LNA	0.578	0.530	0.002	<0.0005	NO_2	-0.034	-0.713	<0.0005
						TOC	1E-10	1.108	0.003
						TN	-0.001	-0.686	<0.0005
						TON	-0.001	-0.940	0.002

Exp	Growth rate	R^2	Adj R^2	SEE	Model P	Indep. variable	Coeff	β-Coeff	Coeff P
	Syn	0.697	0.686	0.010	<0.0005	NO_3	-0.001	-0.835	<0.0005
	Peuk	0.960	0.951	0.002	<0.0005	NO_2	0.099	0.861	<0.0005
						PO_4	0.289	0.595	<0.0005
						TN	0.003	0.314	<0.0005
						NO_3 * PFOS	-2.420E-6	-0.211	0.048
						NO_2 * PFOS	-3.447E-5	-0.211	0.003
						PO_4 * PFOS	1E-10	-0.388	0.002
	CF	0.469	0.407	0.038	0.005	NO_2	-0.661	-0.829	0.001
						NO_2 * PFOS	1.00E-10	0.54	0.022
	Gamma	0.428	0.361	0.017	0.009	NH_4	-0.101	-1.64	0.005
						NO_2	0.429	1.24	0.027
	Roseo	0.591	0.543	0.024	<0.0005	NO_3	-0.008	-0.976	<0.0005
						PO_4 * PFOS	1.00E-10	0.628	0.005
	APA	0.852	0.834	<0.0005	<0.0005	TOC	-2.12E-06	-0.396	0.001
						Roseo	-0.002	-0.689	<0.0005
	β-Glc	0.465	0.436	<0.0005	0.001	NO_3 * PFOS	1.23E-09	0.682	0.001
	Chit	0.514	0.487	<0.0005	<0.0005	NO_3 * PFOS	1.79E-09	0.717	<0.0005
	L-amp	0.832	0.788	<0.0005	<0.0005	NO_2	1.00E-10	0.479	0.007
						TOC	-2.37E-06	-1.206	<0.0005
						NO_2 * PFOS	-6.67E-07	-1.13	0.004
						NH_4 * PFOS	1.29E-07	1.114	0.006

Syn: *Synechococcus*; Peuk: Picoeukaryotes; CF: *Cytophaga-Flavobacterium-Bacteroidetes*; Gamma: *Gammaproteobacteria*; Roseo: Roseobacters; Alk: alkanes; APA: alkaline phosphatase; β-Glc: β-glucosidase; Chit: chitinase; L-amp: L-leucyl aminopeptidase; Alk: alkanes; PAH: polycyclic aromatic hydrocarbons; OPE: organophosphate ester flame retardants and plasticizers; PFOS: perfluorooctane sulfonate; NO3: nitrate; NO2: nitrite; NH4: ammonium; PO4: phosphate; TOC: total organic carbon; TN: total nitrogen; TON: total organic nitrogen; SEE: standard error of the estimate.

SUPPLEMENTARY FIGURES

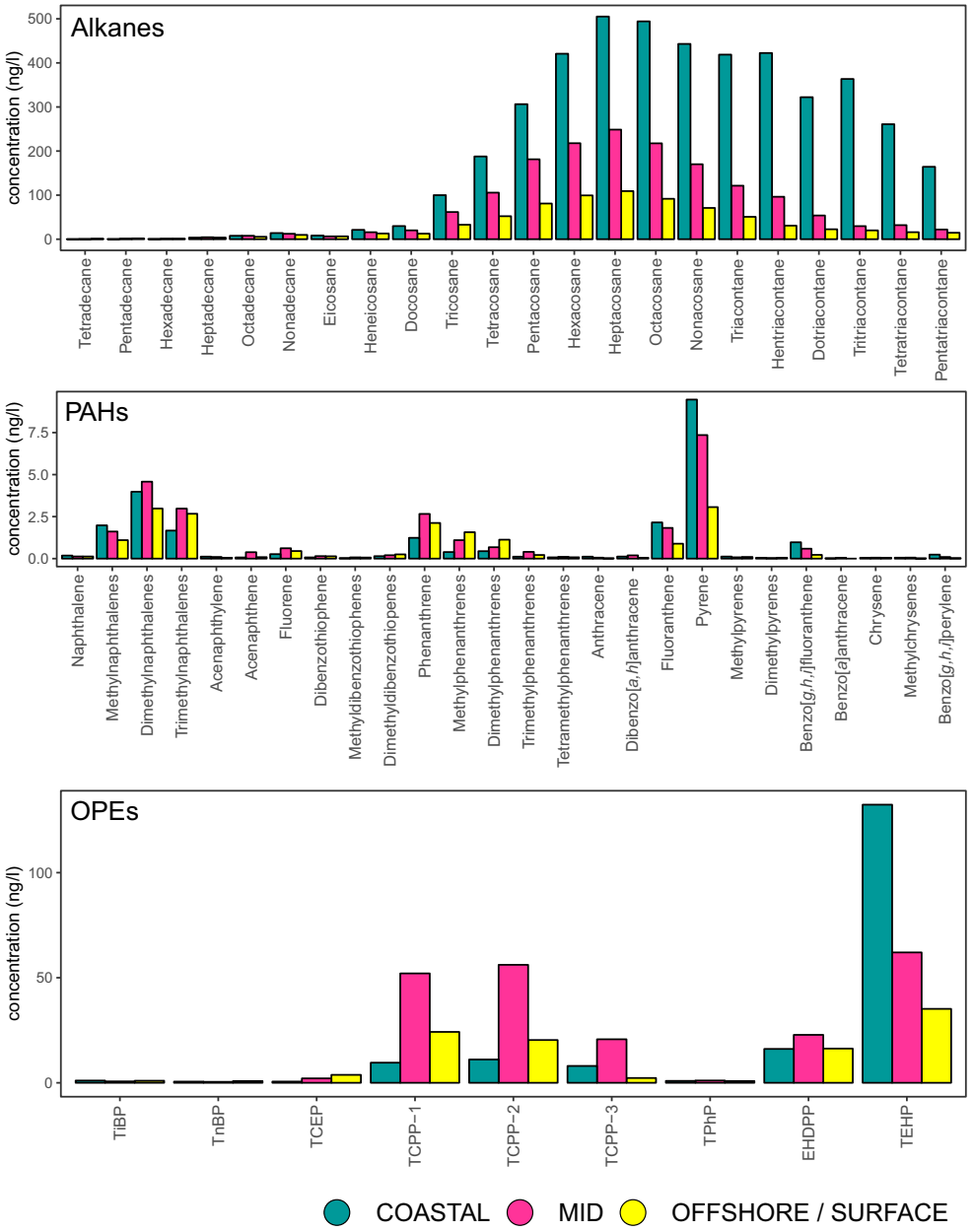


Figure S1. Concentration of alkanes, polycyclic aromatic hydrocarbons (PAHs) and organophosphate esters (OPEs) flame retardant and plasticizers in the initial surface waters.

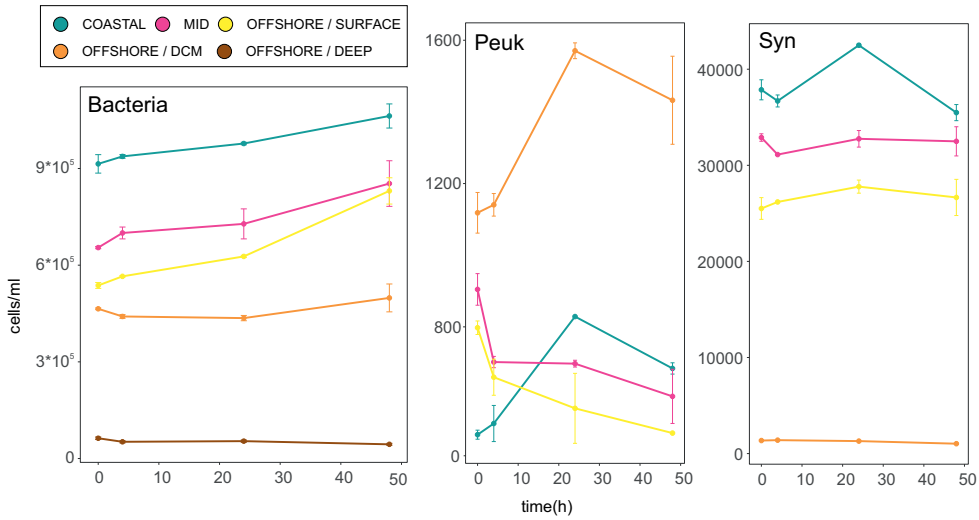


Figure S2. Mean cell abundances from replicates of heterotrophic bacteria and picophytoplankton in the controls of the experiments (with no OP addition) quantified by flow cytometry. Error bars represent standard deviation of replicates. Peuk: Picoeukaryotes; Syn: *Synechococcus*. Bacteria is the sum of HNA and LNA cells.

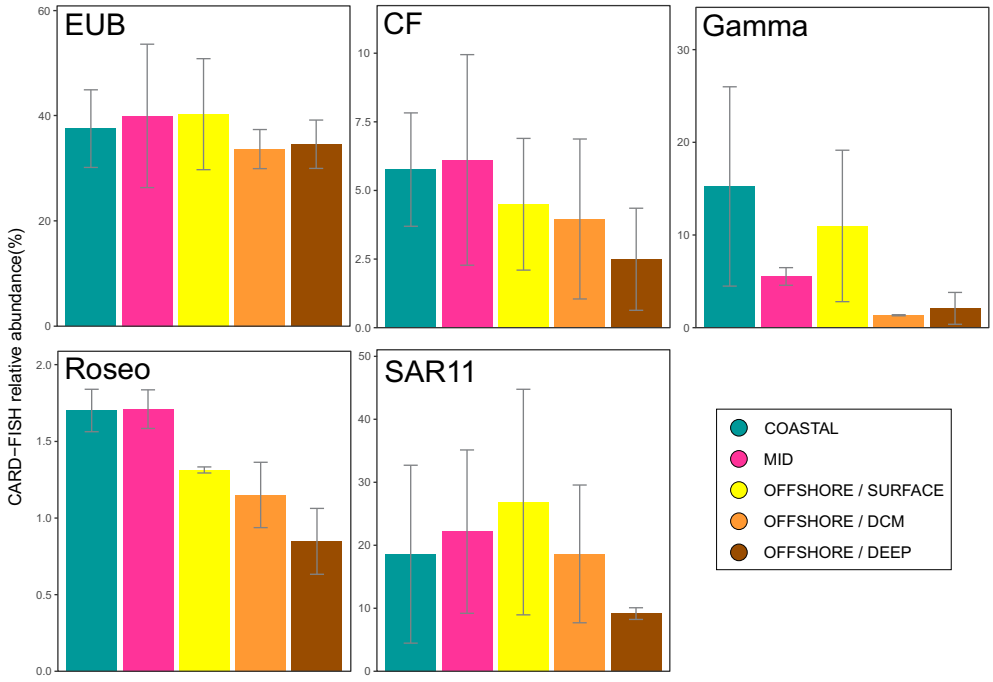


Figure S3. Bacterial community composition of initial surface waters quantified by CARD-FISH (shown as percentage of total DAPI counts). Error bars represent standard deviation of replicates. EUB: *Eubacteria*; CF: *Cytophaga-Flavobacterium-Bacteroidetes*; Gamma: *Gammaproteobacteria*; Roseo: *Roseobacter*.

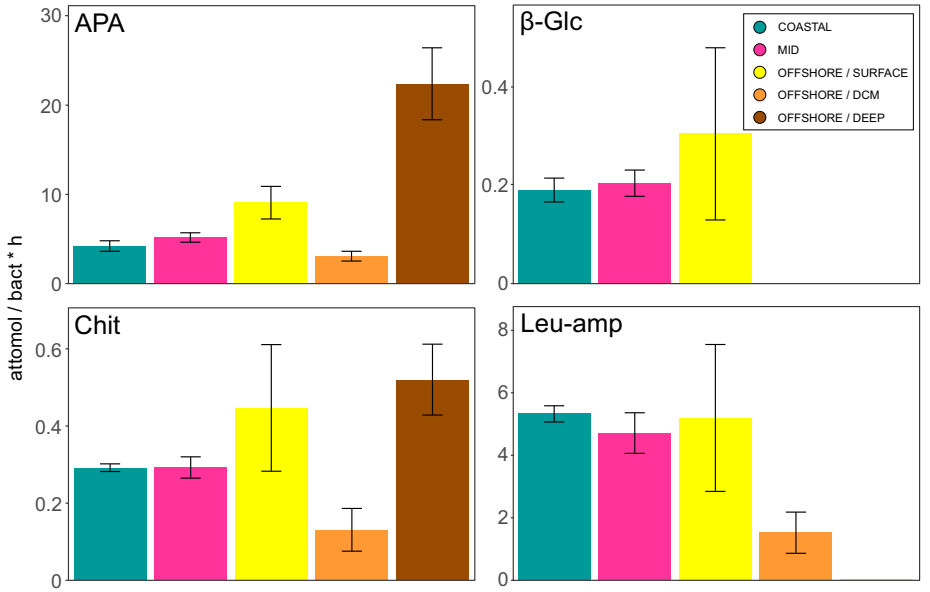
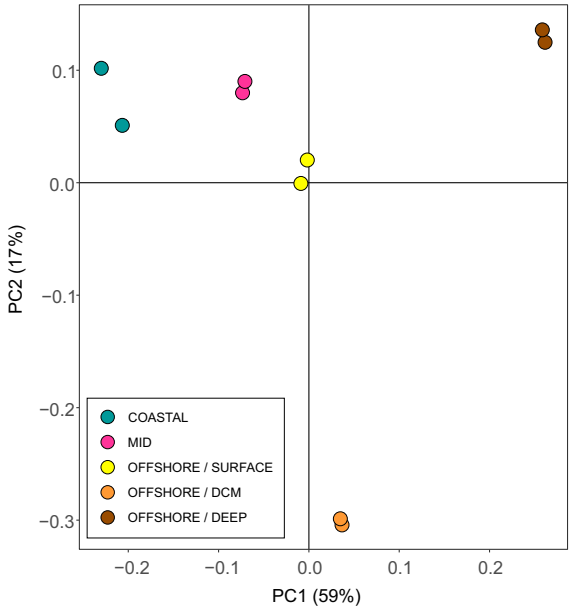


Figure S4. Cell-specific extracellular enzyme activities in the initial time point for each sampling site. APA: alkaline phosphatase; β-Glc: β-glucosidase; Chit: chitinase; Leu-amp: L-leucyl aminopeptidase. Standard deviations of the replicates (n = 4) are denoted by the error bars.



	PC1	PC2	PC3
HNA + LNA	-1.4001	0.0275	-0.1072
HNA	-1.3917	-0.1493	-0.1190
LNA	-1.3896	0.1623	-0.0909
Peuk	-0.7212	-1.0793	0.5201
Syn	-1.2173	0.5972	0.3058
APA	1.1882	0.7487	-0.0334
β-Glc	-1.2138	0.3166	-0.5622
Chit	0.7054	1.1432	0.3569
Leu-amp	-1.2525	0.3813	0.4888

Figure S5. Principal component analysis (PCA) of standardized biological descriptors (extracellular enzyme activity, picophytoplankton and bacterial abundances) at the initial time of each sampling site. Values in parentheses show the % of total variation associated with each principal component. The table shows the component matrix and Eigenvalues for the analysis.

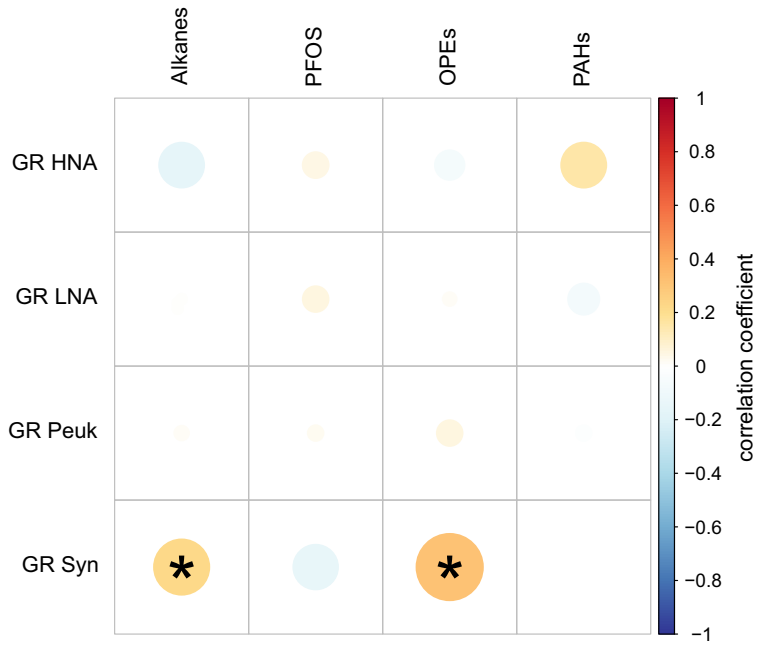


Figure S6. Pearson's correlations between microbial growth rates (GR) at 48 h and added concentrations of pollutants corrected by their initial concentration in seawater. Color and size are proportional to the correlation coefficient, being red for positively correlated and blue for negatively correlated. Significance ($P < 0.05$) is represented with an asterisk. Peuk: Picoeukaryotes; Syn: *Synechococcus*; PFOS: perfluorooctane sulfonate; OPE: organophosphate ester flame retardants and plasticizers; PAH: polycyclic aromatic hydrocarbons.

SUPPLEMENTAEY REFERENCES

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Supporting information of **Chapter 2**

SUPPLEMENTARY TABLES

Table S1. List of oligonucleotide FISH probes used in CARD-FISH analyses.

Probe name	Target organisms	Reference
EUB 338-II-III	Most Bacteria	(Amann *et al.*, 1990; Daims *et al.*, 1999)
Ros537	Alphaproteobacteria; *Roseobacter*	(Eilers *et al.*, 2001)
SAR11-441R	Alphaproteobacteria; Sar11 cluster	(Morris *et al.*, 2002)
Gam42a	*Gammaproteobacteria*	(Manz, W. *et al.*, 1992)
CF319a	Cytophaga–Flavobacterium–Bacteroidetes group	(Manz, W. *et al.*, 1996)

Table S2. Metatranscriptome dataset from the experiments

	Short-term experiment					
	0 A	**0 B**	**C144 A**	**C144 B**	**MID144 A**	**MID144 B**
Total joined reads	76,088,950	53,996,102	68,949,104	87,345,790	47,915,138	72,516,228
Mean internal standard reads	502,075	261,300	490,319	807,205	256,421	338,569
Standard normalization factor	173,737	373,662	12,478	10,181	27,036	57,158
rRNA reads	28,820,356	15,920,461	40,918,461	50,310,582	27,948,656	36,128,179
% rRNA	37.9	29.5	59.3	57.6	58.3	49.8
Possible proteins	45,136,451	36,881,311	26,063,619	33,831,285	18,901,510	34,890,957
% potential protein-coding	59.3	68.3	37.8	38.7	39.4	48.1
SEED annotated proteins	1,195,724	5,824,605	4,153,942	5,015,761	4,379,140	5,005,738
% SEED annotated proteins	2.6	15.8	15.9	14.8	23.2	14.3
taxonomical annotated proteins	4,333,945	5,522,115	5,051,480	6,253,719	4,776,261	6,832,386
% taxonomical annotated proteins	9.6	15.0	19.4	18.5	25.3	19.6

C: control treatment; LOW and MID: PFAAs mix treatment; 0: initial time point; 24 time point after 24 h; 144: time point after 6 days; A and B: replicates.

Table S3. Concentration of perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) acids in the long-term experiment during the incubations.

		Treatment A	**Treatment B**	**Control A**	**Control B**
PFOS (pg/l)	Amendment / 0 h	36000.0	36000.0	34.6	34.6
	24 h	5819.3	9448.8	0.0	0.0
	48 h	36.0	907.5	23.4	6.1
	96 h	985.1	483.5	24.8	19.8
	144 h	2581.7	1713.2	31.1	8.4
PFOA (pg/l)	Amendment / 0 h	60000.0	60000.0	27.0	27.0
	24 h	12618.2	35756.0	26.6	9.0
	48 h	1163.3	10648.2	283.6	10.4
	96 h	32828.2	31604.3	40.3	43.0
	144 h	32198.9	17037.1	29.1	12.2

A and B: replicates.

Long-term experiment							
0 A	**0 B**	**C24 A**	**C24 B**	**LOW24 A**	**LOW24 B**	**MID24 A**	**MID24 B**
92,344,000	59,265,962	55,604,894	61,015,760	62,782,988	97,803,752	58,540,278	94,097,442
1,310,310	486,940	552,802	734,669	848,783	1,516,984	955,046	1,646,558
17,100	54,347	65,751	38,569	31,186	22,060	29,492	19,720
50,275,354	29,153,781	25,378,822	27,701,972	29,200,559	48,681,708	26,538,292	47,840,785
54.4	49.2	45.6	45.4	46.5	49.8	45.3	50.8
37,064,008	28,228,971	28,103,632	30,178,311	30,332,006	43,132,758	28,446,370	39,913,844
40.1	47.6	50.5	49.5	48.3	44.1	48.6	42.4
11,101,074	9,628,638	8,567,572	8,536,946	9,768,003	12,207,834	8,376,331	10,654,813
30.0	34.1	30.5	28.3	32.2	28.3	29.4	26.7
9,344,084	7,820,130	7,406,936	5,861,405	7,579,479	9,265,260	6,527,748	8,546,299
25.2	27.7	26.4	19.4	25.0	21.5	22.9	21.4

Table S4. List of taxa with significantly different (FDR < 5 or |logFC| > 5) abundances between treatment and control based on cDNA 16S sequencing.

Experiment	Comparison	logFC	logCPM	FDR	Phylum
short-term exp	MIDvsCONTROL (24h)	-1.00	13.78	0.03	Bacteroidetes
short-term exp	MIDvsCONTROL (24h)	-0.78	15.89	0.03	Bacteroidetes
short-term exp	MIDvsCONTROL (24h)	7.31	7.32	0.56	Proteobacteria
short-term exp	MIDvsCONTROL (24h)	-6.59	7.00	0.93	Bacteroidetes
short-term exp	MIDvsCONTROL (24h)	5.68	7.10	1	Bacteroidetes
short-term exp	MIDvsCONTROL (24h)	-6.09	6.30	1	Bacteroidetes
short-term exp	MIDvsCONTROL (24h)	6.99	6.05	1	Proteobacteria
short-term exp	MIDvsCONTROL (24h)	-7.34	5.75	1	Bacteroidetes
short-term exp	MIDvsCONTROL (24h)	-5.69	5.99	1	Proteobacteria
short-term exp	MIDvsCONTROL (24h)	6.26	5.55	1	Proteobacteria
short-term exp	MIDvsCONTROL (24h)	-5.77	5.41	1	Proteobacteria
short-term exp	MIDvsCONTROL (24h)	-5.92	5.31	1	Proteobacteria
short-term exp	MIDvsCONTROL (24h)	5.96	5.37	1	Actinobacteria
short-term exp	MIDvsCONTROL (24h)	-5.60	5.25	1	Cyanobacteria
short-term exp	MIDvsCONTROL (24h)	5.68	5.31	1	Bacteroidetes
short-term exp	MIDvsCONTROL (24h)	-5.19	5.47	1	Proteobacteria
short-term exp	MIDvsCONTROL (24h)	5.10	5.41	1	Proteobacteria
short-term exp	LOWvsCONTROL (24h)	6.08	7.32	1	Proteobacteria
short-term exp	LOWvsCONTROL (24h)	6.78	7.10	1	Bacteroidetes
short-term exp	LOWvsCONTROL (24h)	-7.59	6.52	1	Bacteroidetes
short-term exp	LOWvsCONTROL (24h)	-7.34	5.75	1	Bacteroidetes
short-term exp	LOWvsCONTROL (24h)	6.62	6.05	1	Proteobacteria
short-term exp	LOWvsCONTROL (24h)	-5.49	6.02	1	Cyanobacteria
short-term exp	LOWvsCONTROL (24h)	5.66	6.07	1	Proteobacteria
short-term exp	LOWvsCONTROL (24h)	-5.92	5.31	1	Proteobacteria
short-term exp	LOWvsCONTROL (24h)	-5.60	5.48	1	Proteobacteria
short-term exp	LOWvsCONTROL (24h)	5.53	5.67	1	Proteobacteria
short-term exp	LOWvsCONTROL (24h)	5.63	5.46	1	Bacteroidetes
short-term exp	LOWvsCONTROL (24h)	-5.60	5.25	1	Cyanobacteria
short-term exp	LOWvsCONTROL (24h)	5.53	5.50	1	Actinobacteria
short-term exp	LOWvsCONTROL (24h)	-5.19	5.47	1	Proteobacteria
short-term exp	LOWvsCONTROL (24h)	5.15	5.41	1	Proteobacteria
short-term exp	LOWvsCONTROL (24h)	5.05	5.22	1	Bacteroidetes
long-term exp	MIDvsCONTROL (24h)	8.80	9.82	0.00	Proteobacteria
long-term exp	MIDvsCONTROL (24h)	6.47	7.32	1	Proteobacteria
long-term exp	MIDvsCONTROL (24h)	-5.80	7.24	1	Proteobacteria

Class	Order	Family	Genus	Species
Flavobacteriia	Flavobacteriales			
Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Polaribacter	
Gammaproteobacteria	Alteromonadales	Colwelliaceae	Colwellia	rossensis
Flavobacteriia	Flavobacteriales	Cryomorphaceae	Brumimicrobium	
Flavobacteriia	Flavobacteriales	Cryomorphaceae	Crocinitomix	
Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Dokdonia	
Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Psychrobacter	marincola
Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Dokdonia	donghaensis
Gammaproteobacteria	Chromatiales	Granulosicoccaceae	Granulosicoccus	
Gammaproteobacteria	Alteromonadales	Alteromonadaceae	Paraglaciecola	
Alphaproteobacteria	Rhizobiales	Methylobacteriaceae	Methylobacterium	
Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Sulfitobacter	litoralis
Actinobacteria	Actinomycetales	Nocardioidaceae	Nocardioides	
Cyanobacteria				
Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Gillisia	
Alphaproteobacteria	Caulobacterales	Hyphomonadaceae	Algimonas	
Alphaproteobacteria	Caulobacterales	Hyphomonadaceae		
Gammaproteobacteria	Alteromonadales	Colwelliaceae	Colwellia	rossensis
Flavobacteriia	Flavobacteriales	Cryomorphaceae	Crocinitomix	NA
Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Lacinutrix	jangbogonensis
Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Dokdonia	donghaensis
Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Psychrobacter	marincola
Cyanobacteria	Family_II	GpIIa		
Gammaproteobacteria	Alteromonadales	Psychromonadaceae	Psychromonas	kaikoae
Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Sulfitobacter	litoralis
Gammaproteobacteria	Alteromonadales	Psychromonadaceae	Psychromonas	arctica
Gammaproteobacteria	Alteromonadales	Colwelliaceae	Colwellia	aestuarii
Sphingobacteriia	Sphingobacteriales	Chitinophagaceae	Ferruginibacter	
Cyanobacteria				
Actinobacteria	Actinomycetales	Microbacteriaceae		
Alphaproteobacteria	Caulobacterales	Hyphomonadaceae	Algimonas	
Alphaproteobacteria	Caulobacterales	Hyphomonadaceae		
Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Lutibacter	
Gammaproteobacteria	Alteromonadales	Colwelliaceae	Colwellia	hornerae
Gammaproteobacteria	Alteromonadales	Colwelliaceae	Colwellia	rossensis
Gammaproteobacteria	Alteromonadales	Alteromonadaceae	Paraglaciecola	arctica

Experiment	Comparison	logFC	logCPM	FDR	Phylum
long-term exp	MIDvsCONTROL (24h)	6.02	6.49	1	Bacteroidetes
long-term exp	MIDvsCONTROL (24h)	5.49	6.30	1	Bacteroidetes
long-term exp	MIDvsCONTROL (24h)	5.95	5.99	1	Proteobacteria
long-term exp	MIDvsCONTROL (24h)	-6.29	5.69	1	Fusobacteria
long-term exp	MIDvsCONTROL (24h)	-5.25	5.83	1	Proteobacteria
long-term exp	MIDvsCONTROL (24h)	5.55	5.54	1	Firmicutes
long-term exp	MIDvsCONTROL (24h)	-5.16	5.50	1	Actinobacteria
long-term exp	MIDvsCONTROL (48h)	8.96	9.82	0.00	Proteobacteria
long-term exp	MIDvsCONTROL (48h)	8.04	8.92	0.01	Proteobacteria
long-term exp	MIDvsCONTROL (48h)	7.86	8.89	0.01	Proteobacteria
long-term exp	MIDvsCONTROL (48h)	7.27	8.85	0.02	Proteobacteria
long-term exp	MIDvsCONTROL (48h)	-0.83	13.93	0.03	Cyanobacteria
long-term exp	MIDvsCONTROL (48h)	6.99	7.00	1	Bacteroidetes
long-term exp	MIDvsCONTROL (48h)	5.38	7.10	1	Bacteroidetes
long-term exp	MIDvsCONTROL (48h)	6.04	6.49	1	Bacteroidetes
long-term exp	MIDvsCONTROL (48h)	-5.71	6.30	1	Marinimicrobia
long-term exp	MIDvsCONTROL (48h)	5.30	6.52	1	Bacteroidetes
long-term exp	MIDvsCONTROL (48h)	5.50	5.54	1	Firmicutes
long-term exp	MIDvsCONTROL (96h)	8.46	8.89	0.02	Proteobacteria
long-term exp	MIDvsCONTROL (96h)	6.39	8.98	0.13	Proteobacteria
long-term exp	MIDvsCONTROL (96h)	6.27	7.00	1	Bacteroidetes
long-term exp	MIDvsCONTROL (96h)	5.37	7.32	1	Proteobacteria
long-term exp	MIDvsCONTROL (96h)	5.21	7.10	1	Bacteroidetes
long-term exp	MIDvsCONTROL (96h)	5.20	6.49	1	Bacteroidetes
long-term exp	MIDvsCONTROL (144h)	-6.30	8.17	1	Proteobacteria
long-term exp	MIDvsCONTROL (144h)	9.42	7.06	1	Proteobacteria
long-term exp	MIDvsCONTROL (144h)	5.81	7.32	1	Proteobacteria
long-term exp	MIDvsCONTROL (144h)	-5.18	7.35	1	Bacteroidetes
long-term exp	MIDvsCONTROL (144h)	7.76	6.49	1	Bacteroidetes
long-term exp	MIDvsCONTROL (144h)	7.87	6.05	1	Proteobacteria
long-term exp	MIDvsCONTROL (144h)	6.87	6.30	1	Marinimicrobia
long-term exp	MIDvsCONTROL (144h)	-7.15	6.00	1	Proteobacteria
long-term exp	MIDvsCONTROL (144h)	6.35	5.67	1	Proteobacteria
long-term exp	MIDvsCONTROL (144h)	6.25	5.63	1	Proteobacteria
long-term exp	MIDvsCONTROL (144h)	5.90	5.64	1	Actinobacteria
long-term exp	MIDvsCONTROL (144h)	6.16	5.38	1	Proteobacteria
long-term exp	MIDvsCONTROL (144h)	5.46	5.24	1	Proteobacteria

Class	Order	Family	Genus	Species
Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Tenacibaculum	ovolyticum
Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Dokdonia	
Gammaproteobacteria	Chromatiales	Granulosicoccaceae	Granulosicoccus	
Fusobacteriia	Fusobacteriales	Fusobacteriaceae	Psychrilyobacter	
Gammaproteobacteria	Alteromonadales	Psychromonadaceae	Psychromonas	
Bacilli	Bacillales	Bacillaceae_1	Bacillus	
Actinobacteria	Actinomycetales	Microbacteriaceae		
Gammaproteobacteria	Alteromonadales	Colwelliaceae	Colwellia	hornerae
Gammaproteobacteria	Alteromonadales	Pseudoalteromonad.	Pseudoalteromonas	
Gammaproteobacteria	Alteromonadales	Colwelliaceae	Colwellia	
Gammaproteobacteria	Alteromonadales	Colwelliaceae	Colwellia	aestuarii
Flavobacteriia	Flavobacteriales	Cryomorphaceae	Brumimicrobium	
Flavobacteriia	Flavobacteriales	Cryomorphaceae	Crocinitomix	
Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Tenacibaculum	ovolyticum
Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Lacinutrix	jangbogonensis
Bacilli	Bacillales	Bacillaceae_1	Bacillus	
Gammaproteobacteria	Alteromonadales	Colwelliaceae	Colwellia	
Gammaproteobacteria	Thiotrichales	Francisellaceae	Francisella	
Flavobacteriia	Flavobacteriales	Cryomorphaceae	Brumimicrobium	
Gammaproteobacteria	Alteromonadales	Colwelliaceae	Colwellia	rossensis
Flavobacteriia	Flavobacteriales	Cryomorphaceae	Crocinitomix	
Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Tenacibaculum	ovolyticum
Gammaproteobacteria	Arenicellales	Arenicellaceae	Arenicella	
Gammaproteobacteria	Oceanospirillales	Oceanospirillaceae	Oleispira	
Gammaproteobacteria	Alteromonadales	Colwelliaceae	Colwellia	rossensis
Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Maribacter	
Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Tenacibaculum	ovolyticum
Gammaproteobacteria	Alteromonadales	Colwelliaceae	Colwellia	aestuarii
Gammaproteobacteria	Alteromonadales	Moritellaceae	Moritella	
Gammaproteobacteria	Alteromonadales	Colwelliaceae	Colwellia	aestuarii
Gammaproteobacteria	Alteromonadales	Pseudoalteromonad.	Psychrosphaera	
Actinobacteria	Actinomycetales			
Gammaproteobacteria	Thiotrichales	Piscirickettsiaceae		
Gammaproteobacteria	Pseudomonadales	Moraxellaceae		

Table S5. Summary of microorganisms selected from the literature as reported to have the ability to degrade (biodegraders) or tolerate (tolerants) high concentrations of PFOS and PFOA.

Bacteria	Compound	Site	References
Biodegraders			
Pseudomonas plecoglossicida 2.4-D (*Gammaprot.*)	PFOS	soil contaminated by waste from petrochemical production	(Chetverikov *et al.*, 2017)
Pseudomonas aeruginosa strain HJ4 (*Gammaprot.*)	PFOS	wastewater treatment sludge	(Kwon *et al.*, 2014)
Pseudomonas parafulva YAB-1 (*Gammaprot.*)	PFOA	soil near a perfluorinated compound production plant	(Yi *et al.*, 2016, 2018)
Ensifer adhaerens strain M1 (*Alphaprot.*)	PFOS & PFOA	soil of storage and testing of fire-fighting equipment	(Chetverikov and Loginov, 2019)
Tolerants			
Proteobacteria, *Epsilonprot.*, *Thiobacillus* (*Betaprot.*), and *Sulfurimonas* (*Epsilonprot.*)	PFOA	river sediment	(Sun *et al.*, 2016)
Thiobacillus thioparus and *Thiobacillus denitrificans* (*Betaprot.*)	PFOA	river sediment	(Li *et al.*, 2017)
Fluviicola (*Bacteroidetes*), *Limnohabitans* (*Betaprot.*), *Sediminibacterium* (*Bacteroidetes*), and *Polynucleobacter* (*Betaprot.*)	mostly PFOA	surface seawater of a delta	(Chen *et al.*, 2019)
Bacteroidetes, *Proteobacteria*, and *Acidobacteria*	PFOA	lab-scale sequencing batch reactor	(Yu *et al.*, 2018)

Table S6. List of transcripts with significantly different (FDR < 5) abundances between treatment and control based on metatranscriptomic sequencing.

Experiment	Comparison	logFC	logCPM	FDR
long-term exp	MIDvsCONTROL (144h)	-7.85	3.92	0.00
long-term exp	MIDvsCONTROL (144h)	2.20	8.83	0.00
long-term exp	MIDvsCONTROL (144h)	1.86	7.38	0.00
long-term exp	MIDvsCONTROL (144h)	1.77	8.28	0.00
long-term exp	MIDvsCONTROL (144h)	1.60	8.42	0.00
short-term exp	MIDvsCONTROL (24h)	1.24	8.99	0.02
long-term exp	MIDvsCONTROL (144h)	-8.83	2.63	0.00
long-term exp	MIDvsCONTROL (144h)	-8.39	2.94	0.00
long-term exp	MIDvsCONTROL (144h)	-4.91	4.36	0.00
long-term exp	MIDvsCONTROL (144h)	6.80	4.68	0.01
long-term exp	MIDvsCONTROL (144h)	2.48	6.71	0.00
long-term exp	MIDvsCONTROL (144h)	2.29	8.43	0.00
short-term exp	MIDvsCONTROL (24h)	1.92	6.71	0.00

SEED3	Taxon
2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-succinyltransferase	unclassified Pelagibacteraceae
3-ketoacyl-CoA thiolase (EC 2.3.1.16)	Flavobacteriaceae
3-ketoacyl-CoA thiolase (EC 2.3.1.16)	Flavobacteriales
3-ketoacyl-CoA thiolase (EC 2.3.1.16)	Bacteroidetes
3-methylmercaptopropionyl-CoA dehydrogenase (DmdC)	unclassified Porticoccaceae
3-methylmercaptopropionyl-CoA dehydrogenase (DmdC)	Gammaproteobacteria
3-methylmercaptopropionyl-CoA dehydrogenase (DmdC)	Oleispira antarctica
Acetate permease ActP (cation/acetate symporter)	unclassified Pelagibacteraceae
Acetyl-coenzyme A synthetase (EC 6.2.1.1)	Candidatus Thioglobus singularis
Acetylornithine aminotransferase (EC 2.6.1.11)	Gammaproteobacteria bacterium
Acriflavin resistance protein	Polaribacter
Acriflavin resistance protein	Flavobacteriaceae
Acriflavin resistance protein	Polaribacter

Experiment	Comparison	logFC	logCPM	FDR
long-term exp	MIDvsCONTROL (144h)	-2.62	4.79	0.04
long-term exp	MIDvsCONTROL (144h)	-4.55	4.18	0.03
long-term exp	MIDvsCONTROL (144h)	5.91	4.66	0.00
long-term exp	MIDvsCONTROL (144h)	2.74	5.72	0.00
short-term exp	MIDvsCONTROL (24h)	-3.78	3.96	0.00
long-term exp	MIDvsCONTROL (144h)	-7.96	2.58	0.02
short-term exp	LOWvsCONTROL (24h)	-2.81	3.94	0.04
long-term exp	MIDvsCONTROL (144h)	-1.21	9.83	0.01
short-term exp	LOWvsCONTROL (24h)	4.93	3.18	0.01
short-term exp	MIDvsCONTROL (24h)	4.83	3.18	0.01
short-term exp	MIDvsCONTROL (24h)	2.15	6.47	0.00
short-term exp	LOWvsCONTROL (24h)	2.06	6.47	0.00
short-term exp	LOWvsCONTROL (24h)	-7.76	3.86	0.00
long-term exp	MIDvsCONTROL (144h)	2.06	5.62	0.02
long-term exp	MIDvsCONTROL (144h)	1.79	5.84	0.05
short-term exp	LOWvsCONTROL (24h)	-1.75	6.25	0.02
short-term exp	MIDvsCONTROL (24h)	-2.17	5.75	0.00
short-term exp	MIDvsCONTROL (24h)	-3.24	4.86	0.00
short-term exp	LOWvsCONTROL (24h)	-5.47	4.86	0.00
short-term exp	LOWvsCONTROL (24h)	-3.38	4.74	0.00
long-term exp	MIDvsCONTROL (144h)	4.39	4.20	0.04
short-term exp	LOWvsCONTROL (24h)	7.35	5.17	0.00
short-term exp	MIDvsCONTROL (24h)	7.19	5.17	0.00
short-term exp	LOWvsCONTROL (24h)	9.35	3.35	0.00
short-term exp	LOWvsCONTROL (24h)	1.90	7.35	0.00
short-term exp	MIDvsCONTROL (24h)	1.60	7.35	0.01
long-term exp	MIDvsCONTROL (144h)	2.25	8.22	0.00
long-term exp	MIDvsCONTROL (144h)	1.98	9.15	0.00
short-term exp	LOWvsCONTROL (24h)	3.64	4.31	0.00
short-term exp	MIDvsCONTROL (24h)	3.42	4.31	0.01
long-term exp	MIDvsCONTROL (144h)	3.42	4.98	0.02
short-term exp	LOWvsCONTROL (24h)	-2.28	4.86	0.04
short-term exp	LOWvsCONTROL (24h)	7.53	2.36	0.01
long-term exp	MIDvsCONTROL (144h)	5.57	3.64	0.00
short-term exp	MIDvsCONTROL (24h)	-3.17	4.23	0.01
short-term exp	LOWvsCONTROL (24h)	-3.27	4.23	0.01
short-term exp	MIDvsCONTROL (24h)	1.92	7.19	0.00
short-term exp	LOWvsCONTROL (24h)	1.65	8.03	0.00

SEED3	Taxon
Acyl carrier protein	Flavobacteriaceae
Adenosine deaminase (EC 3.5.4.4)	Planktomarina temperata
Adenylosuccinate synthetase (EC 6.3.4.4)	Candidatus Pelagibacter
Adenylylsulfate reductase alpha-subunit (EC 1.8.99.2)	Candidatus Pelagibacter
ADP compounds hydrolase NudE (EC 3.6.1.-)	Candidatus Thioglobus singularis
Alkaline phosphatase (EC 3.1.3.1)	Terrabacteria group
Aminomethyltransferase (glycine cleavage system T protein) (EC 2.1.2.10)	Vibrionaceae
Ammonium transporter	Pelagibacteraceae
Arylsulfatase (EC 3.1.6.1)	Formosa
Arylsulfatase (EC 3.1.6.1)	Formosa
ATP synthase B chain (EC 3.6.3.14)	Candidatus Pelagibacter
ATP synthase B chain (EC 3.6.3.14)	Candidatus Pelagibacter
ATP synthase beta chain (EC 3.6.3.14)	Methylophilales bacterium
ATP synthase delta chain (EC 3.6.3.14)	marine gamma proteobacterium
ATP synthase delta chain (EC 3.6.3.14)	Pelagibacteraceae
ATP synthase epsilon chain (EC 3.6.3.14)	Pelagibacteraceae
ATP synthase F0 sector subunit a	unclassified Porticoccaceae
ATP synthase F0 sector subunit a	Methylophilales bacterium
ATP synthase F0 sector subunit a	Methylophilales bacterium
ATP synthase F0 sector subunit b	Methylophilales bacterium
ATP synthase gamma chain (EC 3.6.3.14)	marine gamma proteobacterium
Beta-glucosidase (EC 3.2.1.21)	Formosa
Beta-glucosidase (EC 3.2.1.21)	Formosa
Beta-lactamase (EC 3.5.2.6)	Erwinia amylovora
Beta-ureidopropionase (EC 3.5.1.6)	Proteobacteria
Beta-ureidopropionase (EC 3.5.1.6)	Proteobacteria
Butyryl-CoA dehydrogenase (EC 1.3.99.2)	Flavobacteriales
Butyryl-CoA dehydrogenase (EC 1.3.99.2)	Flavobacteriaceae
Carbamoyl-phosphate synthase large chain (EC 6.3.5.5)	Candidatus Pelagibacter ubique
Carbamoyl-phosphate synthase large chain (EC 6.3.5.5)	Candidatus Pelagibacter ubique
Cell division protein FtsI [Peptidoglycan synthetase] (EC 2.4.1.129)	Gammaproteobacteria bacterium
Chaperone protein DnaJ	Candidatus Pelagibacter
Chaperone protein DnaK	OM43 clade
Chaperone protein DnaK	Cyanobacteria
Chaperone protein DnaK	Methylophilales bacterium
Chaperone protein DnaK	Methylophilales bacterium
Chitin binding protein	Terrabacteria group
Chitin binding protein	Bacillales

Experiment	Comparison	logFC	logCPM	FDR
short-term exp	LOWvsCONTROL (24h)	-2.81	4.30	0.04
short-term exp	MIDvsCONTROL (24h)	8.54	3.43	0.00
short-term exp	LOWvsCONTROL (24h)	7.48	3.43	0.00
short-term exp	LOWvsCONTROL (24h)	-2.95	4.39	0.01
short-term exp	LOWvsCONTROL (24h)	2.01	5.64	0.02
long-term exp	MIDvsCONTROL (144h)	4.30	3.43	0.01
long-term exp	MIDvsCONTROL (144h)	-1.62	6.87	0.01
long-term exp	MIDvsCONTROL (144h)	-1.97	5.73	0.02
long-term exp	MIDvsCONTROL (144h)	-2.48	6.69	0.00
short-term exp	LOWvsCONTROL (24h)	-2.77	5.58	0.00
short-term exp	MIDvsCONTROL (24h)	3.42	4.04	0.01
short-term exp	LOWvsCONTROL (24h)	3.12	4.04	0.03
long-term exp	MIDvsCONTROL (144h)	1.68	6.53	0.04
long-term exp	MIDvsCONTROL (144h)	1.94	7.84	0.00
short-term exp	LOWvsCONTROL (24h)	1.38	7.84	0.03
short-term exp	MIDvsCONTROL (24h)	1.37	7.84	0.03
long-term exp	MIDvsCONTROL (144h)	-4.95	4.80	0.00
short-term exp	MIDvsCONTROL (24h)	-2.93	4.16	0.03
long-term exp	MIDvsCONTROL (144h)	-3.28	4.16	0.01
short-term exp	MIDvsCONTROL (24h)	5.32	3.27	0.01
long-term exp	MIDvsCONTROL (144h)	2.71	4.69	0.04
short-term exp	LOWvsCONTROL (24h)	1.50	7.54	0.01
short-term exp	MIDvsCONTROL (24h)	4.70	3.96	0.00
short-term exp	LOWvsCONTROL (24h)	3.89	3.96	0.02
short-term exp	LOWvsCONTROL (24h)	-6.74	3.12	0.01
long-term exp	MIDvsCONTROL (144h)	-1.31	9.00	0.01
short-term exp	LOWvsCONTROL (24h)	8.49	3.15	0.00
long-term exp	MIDvsCONTROL (144h)	7.76	3.19	0.01
short-term exp	MIDvsCONTROL (24h)	7.57	3.15	0.00
short-term exp	MIDvsCONTROL (24h)	6.63	3.63	0.01
short-term exp	LOWvsCONTROL (24h)	2.11	6.41	0.00
short-term exp	MIDvsCONTROL (24h)	1.88	6.41	0.00
short-term exp	MIDvsCONTROL (24h)	-1.97	5.67	0.02
short-term exp	LOWvsCONTROL (24h)	-2.58	6.23	0.00
short-term exp	MIDvsCONTROL (24h)	-5.94	6.23	0.00
long-term exp	MIDvsCONTROL (144h)	-7.16	3.53	0.03
short-term exp	LOWvsCONTROL (24h)	-7.48	2.84	0.00
short-term exp	MIDvsCONTROL (24h)	-7.48	2.84	0.00

SEED3	Taxon
Choline dehydrogenase (EC 1.1.99.1)	Vibrionaceae
Choline-sulfatase (EC 3.1.6.6)	Formosa
Choline-sulfatase (EC 3.1.6.6)	Formosa
Chorismate synthase (EC 4.2.3.5)	Candidatus Pelagibacter
Cobalt-zinc-cadmium resistance protein CzcA	Rhodobacteraceae
Coenzyme PQQ synthesis protein B	OM43 clade
Cold shock protein CspE	Gammaproteobacteria
Cyclohexadienyl dehydratase (EC 4.2.1.51)(EC 4.2.1.91)	Proteobacteria
Cyclohexadienyl dehydratase (EC 4.2.1.51)(EC 4.2.1.91)	Rhodobacteraceae
Cysteine desulfurase (EC 2.8.1.7)	marine gamma proteobacterium
Cytochrome c heme lyase subunit CcmF	Candidatus Thioglobus singularis
Cytochrome c heme lyase subunit CcmF	Candidatus Thioglobus singularis
Cytochrome c oxidase polypeptide I (EC 1.9.3.1)	unclassified Gammaproteobacteria
Cytochrome c oxidase polypeptide II (EC 1.9.3.1)	Gammaproteobacteria bacterium
Cytochrome c oxidase polypeptide II (EC 1.9.3.1)	Gammaproteobacteria bacterium
Cytochrome c oxidase polypeptide II (EC 1.9.3.1)	Gammaproteobacteria bacterium
Cytochrome oxidase biogenesis protein Cox11-CtaG	Candidatus Thioglobus singularis
DEAD-box ATP-dependent RNA helicase CshA (EC 3.6.4.13)	Colwelliaceae
DEAD-box ATP-dependent RNA helicase CshA (EC 3.6.4.13)	Colwelliaceae
Deoxyribodipyrimidine photolyase (EC 4.1.99.3)	Planktomarina temperata
Dihydrolipoamide dehydrogenase of pyruvate dehydrogenase complex	Candidatus Thioglobus singularis
Dihydropyrimidine dehydrogenase [NADP+] (EC 1.3.1.2)	Proteobacteria
DNA polymerase III beta subunit (EC 2.7.7.7)	marine gamma proteobacterium
DNA polymerase III beta subunit (EC 2.7.7.7)	marine gamma proteobacterium
DNA topoisomerase I (EC 5.99.1.2)	Methylophilales bacterium
DNA-binding protein HU-beta	Gammaproteobacteria
DNA-directed RNA polymerase beta subunit (EC 2.7.7.6)	Moraxellaceae
DNA-directed RNA polymerase beta subunit (EC 2.7.7.6)	Sphingobacteriaceae
DNA-directed RNA polymerase beta subunit (EC 2.7.7.6)	Moraxellaceae
DNA-directed RNA polymerase beta subunit (EC 2.7.7.6)	Microbacteriaceae
DNA-directed RNA polymerase beta subunit (EC 2.7.7.6)	OM43 clade
DNA-directed RNA polymerase beta subunit (EC 2.7.7.6)	OM43 clade
DNA-directed RNA polymerase beta subunit (EC 2.7.7.6)	Bacteroidales
DNA-directed RNA polymerase beta subunit (EC 2.7.7.6)	Methylophilales bacterium
DNA-directed RNA polymerase beta subunit (EC 2.7.7.6)	Methylophilales bacterium
DNA-directed RNA polymerase beta subunit (EC 2.7.7.6)	Sphingomonadales
DNA-directed RNA polymerase beta subunit (EC 2.7.7.6)	Tenacibaculum
DNA-directed RNA polymerase beta subunit (EC 2.7.7.6)	Tenacibaculum

Experiment	Comparison	logFC	logCPM	FDR
long-term exp	MIDvsCONTROL (144h)	-7.98	2.21	0.05
long-term exp	MIDvsCONTROL (144h)	-9.93	3.62	0.00
short-term exp	MIDvsCONTROL (24h)	4.45	3.60	0.00
long-term exp	MIDvsCONTROL (144h)	-8.97	2.74	0.00
short-term exp	LOWvsCONTROL (24h)	-3.70	5.87	0.00
short-term exp	LOWvsCONTROL (24h)	-3.16	7.24	0.00
long-term exp	MIDvsCONTROL (144h)	7.44	2.91	0.04
long-term exp	MIDvsCONTROL (144h)	-1.82	6.32	0.02
short-term exp	LOWvsCONTROL (24h)	4.34	3.38	0.04
short-term exp	LOWvsCONTROL (24h)	-4.15	3.73	0.03
long-term exp	MIDvsCONTROL (144h)	1.62	9.12	0.00
long-term exp	MIDvsCONTROL (144h)	1.17	9.26	0.02
long-term exp	MIDvsCONTROL (144h)	-8.55	2.39	0.01
long-term exp	MIDvsCONTROL (144h)	2.14	7.16	0.00
long-term exp	MIDvsCONTROL (144h)	1.77	9.28	0.00
short-term exp	MIDvsCONTROL (24h)	-1.84	7.16	0.00
long-term exp	MIDvsCONTROL (144h)	3.02	4.08	0.04
long-term exp	MIDvsCONTROL (144h)	1.81	6.58	0.01
long-term exp	MIDvsCONTROL (144h)	2.24	5.74	0.04
long-term exp	MIDvsCONTROL (144h)	1.48	8.36	0.00
long-term exp	MIDvsCONTROL (144h)	7.27	3.22	0.03
long-term exp	MIDvsCONTROL (144h)	-1.82	6.41	0.04
long-term exp	MIDvsCONTROL (144h)	-8.69	2.80	0.00
short-term exp	LOWvsCONTROL (24h)	9.16	5.87	0.00
short-term exp	MIDvsCONTROL (24h)	7.26	5.87	0.00
long-term exp	MIDvsCONTROL (144h)	-9.81	5.87	0.00
long-term exp	MIDvsCONTROL (144h)	1.48	8.07	0.01
long-term exp	MIDvsCONTROL (144h)	-1.81	6.41	0.03
long-term exp	MIDvsCONTROL (144h)	1.81	7.44	0.00
short-term exp	MIDvsCONTROL (24h)	-6.36	3.41	0.02
long-term exp	MIDvsCONTROL (144h)	-6.54	4.66	0.00
short-term exp	MIDvsCONTROL (24h)	7.74	3.00	0.00
short-term exp	LOWvsCONTROL (24h)	7.31	3.00	0.00
short-term exp	LOWvsCONTROL (24h)	-4.12	3.68	0.01
short-term exp	MIDvsCONTROL (24h)	-2.70	5.38	0.00
long-term exp	MIDvsCONTROL (144h)	1.70	7.13	0.01
long-term exp	MIDvsCONTROL (144h)	4.19	4.87	0.00
long-term exp	MIDvsCONTROL (144h)	-1.60	7.52	0.01

SEED3	Taxon
DNA-directed RNA polymerase beta subunit (EC 2.7.7.6)	PVC group
DNA-directed RNA polymerase beta subunit (EC 2.7.7.6)	Oleispira antarctica
DNA-directed RNA polymerase omega subunit (EC 2.7.7.6)	Rhodobacterales bacterium
Enoyl-CoA hydratase (EC 4.2.1.17)	Oleispira antarctica
Exodeoxyribonuclease I (EC 3.1.11.1)	Candidatus Pelagibacter ubique
Ferric siderophore transport system, biopolymer transport protein ExbB	unclassified Porticoccaceae
Ferrochelatase, protoheme ferro-lyase (EC 4.99.1.1)	Candidatus Pelagibacter ubique
Fe-S oxidoreductase-like protein in Rubrerythrin cluster	Gammaproteobacteria
Glucose dehydrogenase, PQQ-dependent (EC 1.1.5.2)	unclassified Gammaproteobacteria
Glucose-6-phosphate 1-dehydrogenase (EC 1.1.1.49)	Methylophilales bacterium
Glutamate synthase [NADPH] large chain (EC 1.4.1.13)	Rhodobacteraceae
Glutamate synthase [NADPH] large chain (EC 1.4.1.13)	Pelagibacteraceae
Glutamate synthase [NADPH] large chain (EC 1.4.1.13)	Oleispira antarctica
Glutamate synthase [NADPH] putative GlxC chain (EC 1.4.1.13)	Candidatus Pelagibacter ubique
Glutamate synthase [NADPH] putative GlxC chain (EC 1.4.1.13)	Pelagibacteraceae
Glutamate synthase [NADPH] putative GlxC chain (EC 1.4.1.13)	Candidatus Pelagibacter ubique
Glutamate synthase [NADPH] small chain (EC 1.4.1.13)	Pelagibacteraceae
Glutamate synthase [NADPH] small chain (EC 1.4.1.13)	Alphaproteobacteria
Glycolate dehydrogenase (EC 1.1.99.14), FAD-binding subunit GlcE	Proteobacteria
Heat shock protein 60 family chaperone GroEL	Candidatus Pelagibacter ubique
Branched-chain amino acid transport system permease protein LivH	Candidatus Thioglobus singularis
Ketol-acid reductoisomerase (EC 1.1.1.86)	Rhodobacterales
L-lactate dehydrogenase (EC 1.1.2.3)	PVC group
L-proline glycine betaine binding ABC transporter protein ProX	Rhodobacteraceae bacterium SB2
L-proline glycine betaine binding ABC transporter protein ProX	Rhodobacteraceae bacterium SB2
L-proline glycine betaine binding ABC transporter protein ProX	Rhodobacteraceae bacterium SB2
Malate dehydrogenase (EC 1.1.1.37)	Pelagibacteraceae
Malonate-semialdehyde dehydrogenase (EC 1.2.1.18)	Proteobacteria
Methylmalonate-semialdehyde dehydrogenase (EC 1.2.1.27)	Pelagibacteraceae
Molybdenum cofactor biosynthesis protein MoaB	Candidatus Pelagibacter ubique
Monoamine oxidase (1.4.3.4)	marine gamma proteobacterium
Na(+)-translocating NADH-quinone reductase subunit A (EC 1.6.5.-)	OM43 clade
Na(+)-translocating NADH-quinone reductase subunit A (EC 1.6.5.-)	OM43 clade
Na(+)-translocating NADH-quinone reductase subunit A (EC 1.6.5.-)	Methylophilales bacterium
Na(+)-translocating NADH-quinone reductase subunit B (EC 1.6.5.-)	Polaribacter irgensii
Na(+)-translocating NADH-quinone reductase subunit E (EC 1.6.5.-)	Flavobacteriaceae
Na+/H+ antiporter NhaA type	Candidatus Pelagibacter ubique
N-Acetyl-D-glucosamine ABC transport system, sugar-binding protein	Proteobacteria

Experiment	Comparison	logFC	logCPM	FDR
short-term exp	LOWvsCONTROL (24h)	-5.31	3.36	0.01
long-term exp	MIDvsCONTROL (144h)	2.36	5.10	0.04
long-term exp	MIDvsCONTROL (144h)	7.99	2.81	0.01
short-term exp	MIDvsCONTROL (24h)	1.54	7.96	0.00
short-term exp	LOWvsCONTROL (24h)	-7.16	2.58	0.01
long-term exp	MIDvsCONTROL (144h)	1.07	12.16	0.00
short-term exp	LOWvsCONTROL (24h)	7.88	2.26	0.01
long-term exp	MIDvsCONTROL (144h)	-8.74	4.82	0.00
long-term exp	MIDvsCONTROL (144h)	-3.60	4.08	0.01
short-term exp	MIDvsCONTROL (24h)	2.36	5.85	0.00
short-term exp	MIDvsCONTROL (24h)	5.45	7.31	0.00
long-term exp	MIDvsCONTROL (144h)	1.84	8.83	0.00
long-term exp	MIDvsCONTROL (144h)	-9.13	7.31	0.00
short-term exp	MIDvsCONTROL (24h)	6.32	3.59	0.02
short-term exp	LOWvsCONTROL (24h)	-3.73	4.83	0.00
short-term exp	MIDvsCONTROL (24h)	-4.14	4.83	0.00
short-term exp	MIDvsCONTROL (24h)	4.21	3.97	0.00
short-term exp	LOWvsCONTROL (24h)	3.85	3.97	0.02
long-term exp	MIDvsCONTROL (144h)	3.46	5.12	0.00
long-term exp	MIDvsCONTROL (144h)	2.33	6.62	0.00
long-term exp	MIDvsCONTROL (144h)	1.47	8.02	0.01
long-term exp	MIDvsCONTROL (144h)	3.03	4.52	0.05
short-term exp	LOWvsCONTROL (24h)	8.66	3.60	0.00
short-term exp	MIDvsCONTROL (24h)	8.43	3.60	0.00
short-term exp	MIDvsCONTROL (24h)	-2.43	5.45	0.00
short-term exp	LOWvsCONTROL (24h)	-2.98	5.45	0.00
long-term exp	MIDvsCONTROL (144h)	3.42	4.78	0.01
long-term exp	MIDvsCONTROL (144h)	3.08	4.62	0.02
short-term exp	LOWvsCONTROL (24h)	-5.56	2.82	0.01
long-term exp	MIDvsCONTROL (144h)	3.99	4.91	0.00
long-term exp	MIDvsCONTROL (144h)	3.02	4.64	0.00
long-term exp	MIDvsCONTROL (144h)	7.07	5.60	0.00
long-term exp	MIDvsCONTROL (144h)	-9.63	3.89	0.00
short-term exp	LOWvsCONTROL (24h)	7.40	2.60	0.01
short-term exp	MIDvsCONTROL (24h)	7.11	2.60	0.02
long-term exp	MIDvsCONTROL (144h)	3.10	4.10	0.03
short-term exp	LOWvsCONTROL (24h)	-6.58	2.70	0.04
short-term exp	LOWvsCONTROL (24h)	-7.12	3.32	0.00

SEED3	Taxon
NAD(P) transhydrogenase subunit beta (EC 1.6.1.2)	Rhodobacterales bacterium
NADP-dependent malic enzyme (EC 1.1.1.40)	Polaribacter
Oligopeptide ABC transporter, periplasmic oligopeptide-binding protein OppA	unclassified Porticoccaceae
O-succinylhomoserine sulfhydrylase (EC 2.5.1.48)	Pelagibacteraceae
Peptidyl-tRNA hydrolase (EC 3.1.1.29)	Methylophilales bacterium
Phage major capsid protein	Bacteria
Phage tail fiber protein	Moraxellaceae
Phenylalanyl-tRNA synthetase beta chain (EC 6.1.1.20)	marine gamma proteobacterium
Phospho-N-acetylmuramoyl-pentapeptide-transferase (EC 2.7.8.13)	Candidatus Pelagibacter ubique
Phosphoribosylformylglycinamidine synthase, synthetase subunit	Pelagibacteraceae
photosystem II protein D1 (PsbA)	Terrabacteria group
photosystem II protein D1 (PsbA)	Cyanobacteria
photosystem II protein D1 (PsbA)	Terrabacteria group
Phytoene dehydrogenase (EC 1.14.99.-)	SAR116 cluster
Polyribonucleotide nucleotidyltransferase (EC 2.7.7.8)	Methylophilales bacterium
Polyribonucleotide nucleotidyltransferase (EC 2.7.7.8)	Methylophilales bacterium
Porphobilinogen synthase (EC 4.2.1.24)	Candidatus Pelagibacter
Porphobilinogen synthase (EC 4.2.1.24)	Candidatus Pelagibacter
Probable Co/Zn/Cd efflux system membrane fusion protein	Polaribacter
Probable Co/Zn/Cd efflux system membrane fusion protein	Flavobacteriaceae
Probable low-affinity inorganic phosphate transporter	Flavobacteriaceae
Protoporphyrin IX Mg-chelatase subunit H (EC 6.6.1.1)	Cyanobacteria
Pyrophosphate-energized proton pump (EC 3.6.1.1)	OM43 clade
Pyrophosphate-energized proton pump (EC 3.6.1.1)	OM43 clade
Pyrophosphate-energized proton pump (EC 3.6.1.1)	Methylophilales bacterium
Pyrophosphate-energized proton pump (EC 3.6.1.1)	Methylophilales bacterium
Recombination inhibitory protein MutS2	Flavobacteriia
Rhodanese-like domain protein	Polaribacter irgensii
Riboflavin kinase (EC 2.7.1.26)	Rhodobacterales bacterium
Ribonuclease E inhibitor RraA	unclassified Porticoccaceae
Ribosome hibernation protein YhbH	Fluviicola taffensis
Ribulose bisphosphate carboxylase large chain (EC 4.1.1.39)	Cyanobacteria
Ribulose bisphosphate carboxylase large chain (EC 4.1.1.39)	Terrabacteria group
RNA polymerase sigma factor RpoD	OM43 clade
RNA polymerase sigma factor RpoD	OM43 clade
RNA polymerase sigma factor RpoD	Candidatus Pelagibacter ubique
RNA polymerase sigma factor RpoH	Methylophilales bacterium
RNA polymerase sigma factor RpoH	Vibrionaceae

Experiment	Comparison	logFC	logCPM	FDR
short-term exp	MIDvsCONTROL (24h)	3.94	5.74	0.00
short-term exp	LOWvsCONTROL (24h)	3.46	5.74	0.00
short-term exp	MIDvsCONTROL (24h)	1.34	8.47	0.02
long-term exp	MIDvsCONTROL (144h)	-6.35	5.51	0.02
short-term exp	LOWvsCONTROL (24h)	3.59	5.17	0.00
short-term exp	MIDvsCONTROL (24h)	3.53	5.17	0.00
long-term exp	MIDvsCONTROL (144h)	-4.69	4.00	0.02
long-term exp	MIDvsCONTROL (144h)	3.70	5.14	0.00
short-term exp	LOWvsCONTROL (24h)	7.68	2.32	0.01
long-term exp	MIDvsCONTROL (144h)	3.53	4.14	0.00
long-term exp	MIDvsCONTROL (144h)	-1.94	8.46	0.00
long-term exp	MIDvsCONTROL (144h)	-2.12	7.38	0.00
long-term exp	MIDvsCONTROL (144h)	7.98	4.47	0.00
long-term exp	MIDvsCONTROL (144h)	8.22	5.07	0.00
short-term exp	LOWvsCONTROL (24h)	-4.36	5.07	0.00
long-term exp	MIDvsCONTROL (144h)	-8.03	4.65	0.00
short-term exp	MIDvsCONTROL (24h)	-11.00	5.07	0.00
short-term exp	MIDvsCONTROL (24h)	6.83	2.91	0.02
short-term exp	LOWvsCONTROL (24h)	6.80	2.91	0.02
long-term exp	MIDvsCONTROL (144h)	1.18	9.12	0.03
long-term exp	MIDvsCONTROL (144h)	3.19	4.72	0.03
short-term exp	LOWvsCONTROL (24h)	-7.66	4.42	0.00
short-term exp	MIDvsCONTROL (24h)	9.08	3.96	0.00
short-term exp	LOWvsCONTROL (24h)	9.08	3.96	0.00
short-term exp	MIDvsCONTROL (24h)	2.45	5.39	0.00
short-term exp	LOWvsCONTROL (24h)	-9.28	3.24	0.00
short-term exp	MIDvsCONTROL (24h)	-9.28	3.24	0.00
short-term exp	LOWvsCONTROL (24h)	-1.49	6.91	0.05
short-term exp	LOWvsCONTROL (24h)	-4.99	3.09	0.04
long-term exp	MIDvsCONTROL (144h)	-8.23	2.14	0.04
short-term exp	MIDvsCONTROL (24h)	8.16	3.21	0.00
short-term exp	LOWvsCONTROL (24h)	8.16	3.21	0.00
short-term exp	MIDvsCONTROL (24h)	-3.72	5.00	0.00
short-term exp	LOWvsCONTROL (24h)	-4.78	5.00	0.00
long-term exp	MIDvsCONTROL (144h)	1.17	10.24	0.01
long-term exp	MIDvsCONTROL (144h)	-1.82	7.32	0.00
long-term exp	MIDvsCONTROL (144h)	-1.12	10.08	0.01
long-term exp	MIDvsCONTROL (144h)	-1.39	8.30	0.01

SEED3	Taxon
RNA polymerase sigma factor RpoH-related protein	Planktomarina temperata
RNA polymerase sigma factor RpoH-related protein	Planktomarina temperata
RNA polymerase sigma factor RpoH-related protein	Rhodobacteraceae
RNA polymerase sigma factor RpoH-related protein	Rhodobacterales bacterium
S-adenosylmethionine synthetase (EC 2.5.1.6)	Candidatus Pelagibacter
S-adenosylmethionine synthetase (EC 2.5.1.6)	Candidatus Pelagibacter
S-adenosylmethionine synthetase (EC 2.5.1.6)	Candidatus Thioglobus singularis
S-adenosylmethionine:tRNA ribosyltransferase-isomerase (EC 5.-.-.-)	Polaribacter irgensii
Serine acetyltransferase (EC 2.3.1.30)	Moraxellaceae
Sulfite reductase [NADPH] hemoprotein beta-component (EC 1.8.1.2)	Rhodobacteraceae
Taurine-binding periplasmic protein TauA	Proteobacteria
Taurine-related protein	Betaproteobacteria
Threonine dehydratase biosynthetic (EC 4.3.1.19)	Candidatus Thioglobus singularis
TonB-dependent receptor	Cellulophaga
TonB-dependent receptor	Cellulophaga
TonB-dependent receptor	Sphingomonadales
TonB-dependent receptor	Cellulophaga
Transaldolase (EC 2.2.1.2)	OM43 clade
Transaldolase (EC 2.2.1.2)	OM43 clade
Transcription termination protein NusA	Flavobacteriaceae
Transketolase (EC 2.2.1.1)	Cyanobacteria
Transketolase (EC 2.2.1.1)	Methylophilales bacterium
Translation elongation factor Tu	Moraxellaceae
Translation elongation factor Tu	Moraxellaceae
Translation elongation factor Tu	Bacteroidia
Translation elongation factor Tu	Flavobacterium
Translation elongation factor Tu	Flavobacterium
Translation initiation factor 2	Candidatus Pelagibacter ubique
Translation initiation factor 2	Methylophilales bacterium
Translation initiation factor 3	Oleispira antarctica
tRNA (Guanine37-N1) -methyltransferase (EC 2.1.1.31)	OM43 clade
tRNA (Guanine37-N1) -methyltransferase (EC 2.1.1.31)	OM43 clade
tRNA (Guanine37-N1) -methyltransferase (EC 2.1.1.31)	Methylophilales bacterium
tRNA (Guanine37-N1) -methyltransferase (EC 2.1.1.31)	Methylophilales bacterium
Ubiquinol--cytochrome c reductase, cytochrome B subunit (EC 1.10.2.2)	Alphaproteobacteria
Uracil-xanthine permease	Rhodobacterales
Various polyols ABC transporter, periplasmic substrate-binding protein	Proteobacteria
Various polyols ABC transporter, periplasmic substrate-binding protein	Rhodobacterales

SUPPLEMENTARY FIGURES

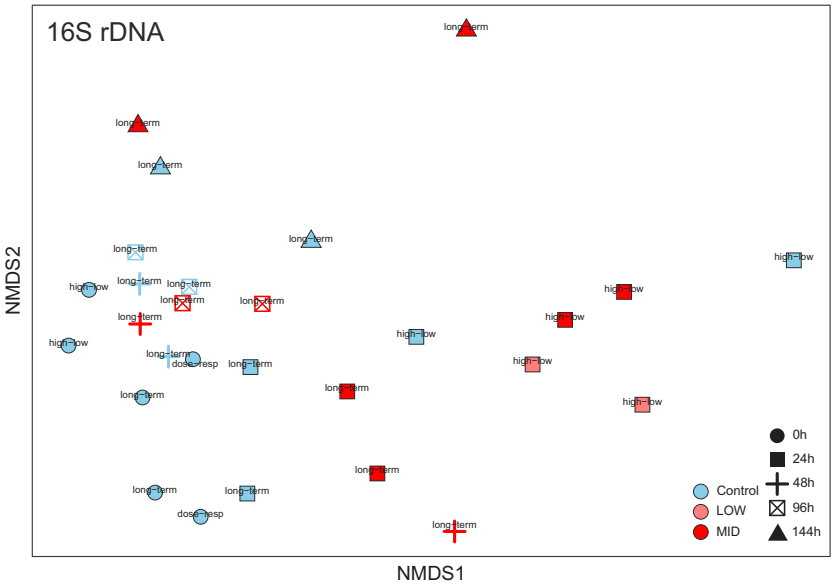


Figure S1. Non-metric multidimensional scaling (NMDS) plot showing the similarities of sample 16S rDNA composition in dose-response, short term and long term experiments. The colour of each point corresponds with the treatment condition and the shape with the timepoint.

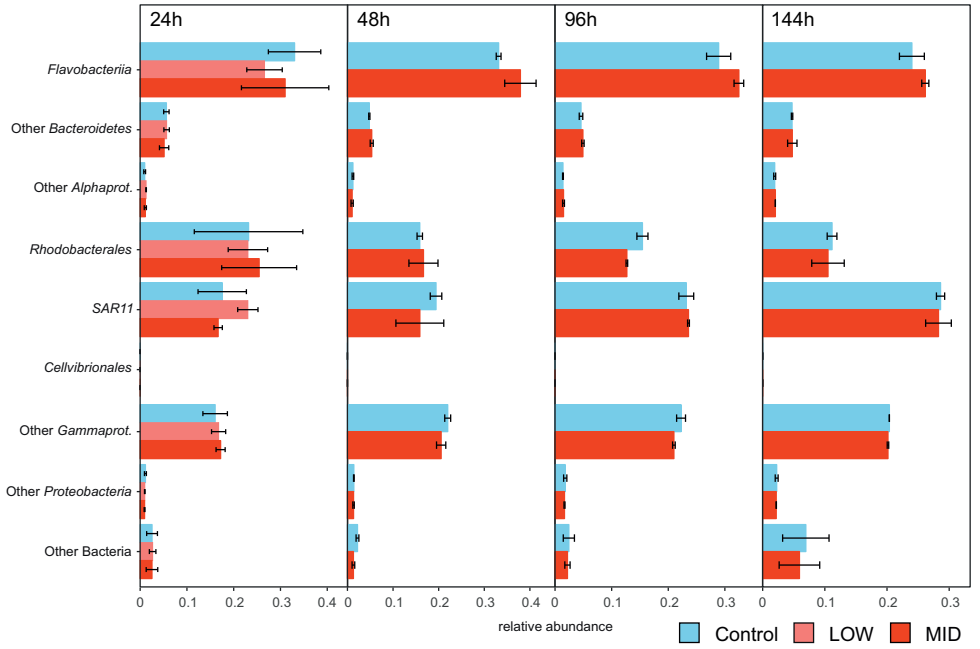


Figure S2. Relative abundance of each taxonomic affiliation by 16S rDNA.

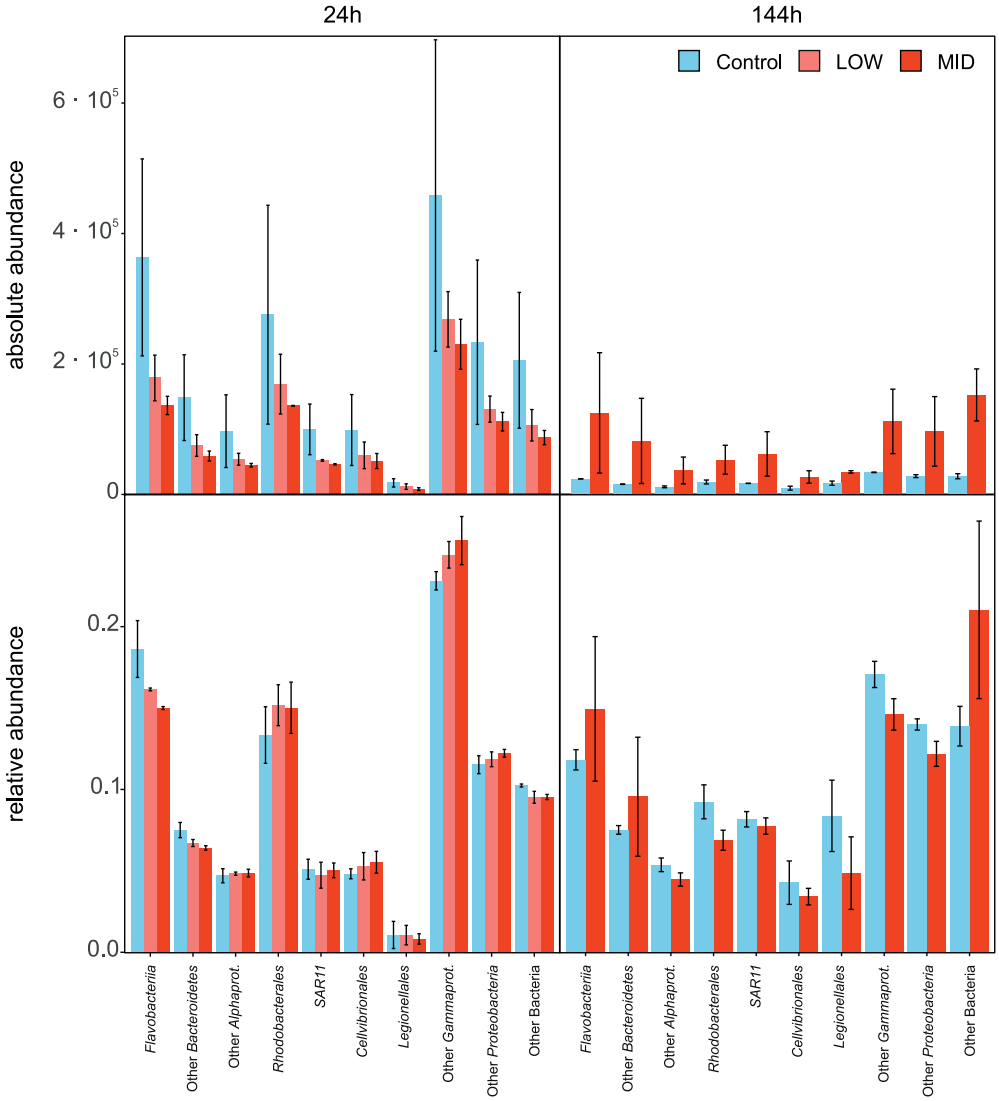


Figure S3. Absolute and relative abundance of each taxonomical group in metatranscriptomic profiles after 24h and 6 days of the PFAAs incubations.

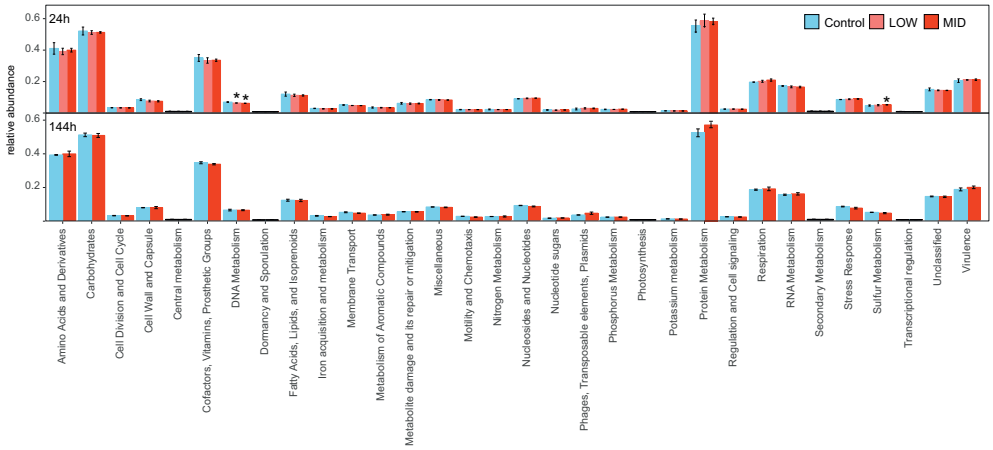
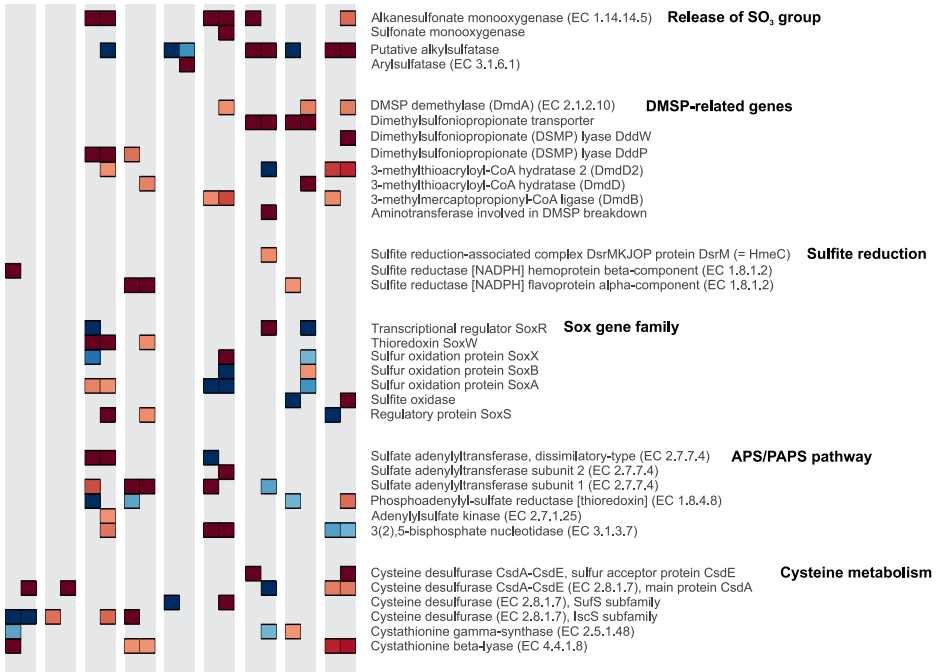


Figure S4. Relative abundance of general SEED categories in the metatranscriptomes of short- and long-term experiment. Asterisks indicate significant differences between treatments and controls (t-test; P <0.05). Values are means of duplicates. Error bars show standard deviation.



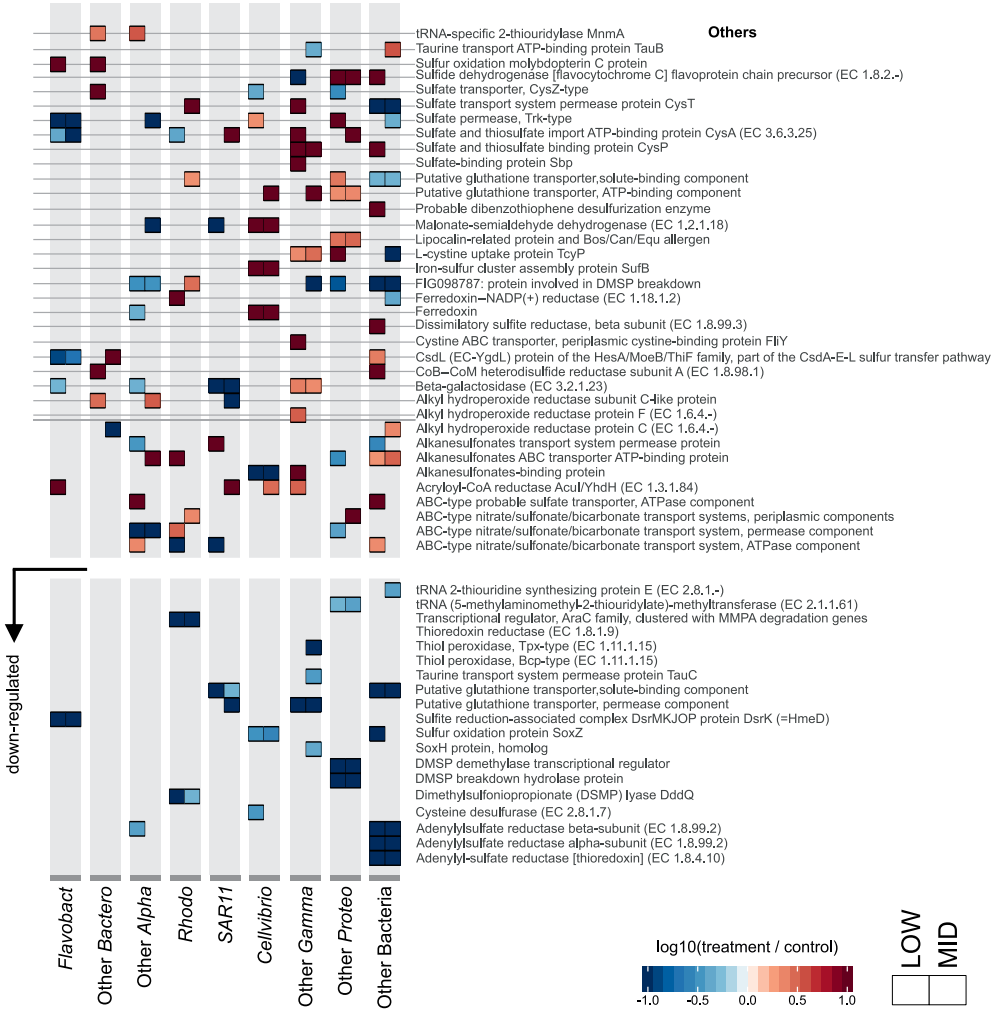


Figure S5. Heatmap of changes in sulfur metabolism transcritps between PFAAs amendments and control after 24 h of incubation. Notice that it is only represented when differences are greater than double or less than half.

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Supporting information of **Chapter 3**

SUPPLEMENTARY MATERIAL

Material and Methods

Analysis of concentration of organic pollutants in the dissolved phase

Concentrations of a selected group of model molecules present in ADOC such as PAHs, n-alkanes and organophosphate esters (OPEs) were measured in the dissolved phase at the beginning and at the end of the experiment. After filtration of water on 0.2 µm Teflon filters for nucleic acid sampling, the filtrate was concentrated on a solid-phase extraction Oasis HLB 6cc cartridge (Waters, Montevideo, Uruguay) with 500 mg sorbent, using a vacuum manifold. The SPE cartridges had been previously conditioned with 3 ml of hexane followed by 3 ml of dichloromethane/hexane (2:1), 3 ml dichloromethane/methanol (2:1) and 3 ml of HPLC-grade water. A surrogate standard mix was added at the Oasis cartridges and, then, eluted with 5 ml of hexane, 5 ml of dichloromethane/hexane (2:1) and 5 ml of dichloromethane/hexane (3:1). In order to purify and extract any aqueous residual from the sample, a glass funnel filled with 50–60 g of anhydrous sodium sulfate was used. Concentration of the extract until 100 µl was performed by vacuum rotary evaporation and nitrogen stream. PAHs, OPEs, and n-alkanes were quantified by gas-chromatography coupled to mass spectrometry as described elsewhere (Berrojalbiz *et al.*, 2009; Fernández-Pinos *et al.*, 2017; Vila-Costa *et al.*, 2018).

CARD-FISH

Subsamples of 45 ml were fixed with formaldehyde (1% final concentration) for 24 h, filtered on a 0.2 µm pore-size polycarbonate filter (47 mm diameter) and kept dried and frozen until processed. Whole-cell *in situ* hybridizations of sections from the polycarbonate filters were performed as described by Pernthaler et al. (2004) (Pernthaler *et al.*, 2004) using the oligonucleotide probes listed on Table

S2. All probes were purchased from biomers.net (Ulm, Germany). Filters were permeabilized with lysozyme (10 mg/ml, 37°C, 60 min) and achromopeptidase (60 U/ml, 37°C, 30 min) before hybridization. Hybridizations were carried out at 35°C overnight and specific hybridization conditions were established by addition of formamide to the hybridization buffers (45% for Alf968 and Sar11, 55% for the other probes). After hybridization, the signal was amplified with Alexa 488-labeled tyramide for 15 min at 46°C. CARD-FISH preparations were counter-stained with 4,6-diamidino-2-phenylindole (DAPI, 1 µg/ml) and visualized by epifluorescence microscopy (Olympus BX61) under blue light and UV excitation. Percentages of the positive hybridized cells to total prokaryotic cells and cell sizes were calculated by semi-automatic counts. Images were acquired using a digital camera (Zeiss camera AxioCam MRm, Carl Zeiss MicroImaging, S.L., Barcelona, Spain) at 630x magnification through the Axiovision software and analyzed using the automated image analysis software ACMEtool. All images (at least 10 fields/filter) were acquired using 20 ms exposure time. Area and perimeter were measured of 100-500 cells to calculate the biovolumes following Massana et al. 1997 (Massana *et al.*, 1997).

Flow cytometric determination of prokaryotic cell abundances

Subsamples of 1.8 ml for quantification of abundances of prokaryotes were fixed with 1% buffered paraformaldehyde solution (pH 7.0) plus 0.05% glutaraldehyde, left at room temperature in the dark for 10 min, flash-frozen in liquid nitrogen and stored at -80ºC. Prokaryotic cell abundance was estimated by flow cytometry as described elsewhere (Falcioni *et al.*, 2008).

DNA and RNA

Sample collection. After 30 min, 2 l seawater was pre-filtered through a 3 µm pore-size 47 mm diameter polytetrafluoroethylene filter and bacterial cells were collected on 0.2 µm pore-size 47 mm polytetrafluoroethylene filter under low

vacuum pressure. The duration of the filtration step was no longer than 20 min to minimize RNA degradation. Each filter was cut in two halves, one was placed in 1 ml RNAlater (Sigma-Aldrich, Saint Louis, MO) and the other one into 1 ml lysis buffer (50 mM Tris HCl, 40 mM EDTA, 0.75 M Sucrose) and stored at -20°C to preserve RNA and DNA respectively.

DNA extraction and sequencing. The half filter corresponding to DNA extraction was incubated at 37°C for 45 min and 300 rpm with a 5 mg/ml lysozyme solution in lysis buffer. Then, 0.5 mg/ml proteinase K and 100 µl of 10% sodium dodecyl sulfate (SDS) was added and further incubated at 55°C for 1 h and 300 rpm. DNA was extracted twice with phenol-chloroform-isoamyl alcohol (25:24:1; pH 8), and once with chloroform-isoamyl alcohol (24:1). Genomic DNA from the aqueous phase was then precipitated adding 1/10 vol of ammonium acetate 4 M and 1 vol of isopropyl alcohol. After 20 min of incubation at -20°C, the precipitate was centrifuged for 10 min and the supernatant was removed. The obtained DNA pellet was washed with 1 ml of 70% ethanol and finally suspended in 50 µl of water. A DNA standard (*Thermus thermophilus DSM7039 [HB27]* genomic DNA) was added as internal control at 0.5% of the total mass of extracted DNA. Final DNA were sequenced at National Center for Genomic Analysis (CNAG, Barcelona, Spain) using Illumina high output mode HS200 2x100bp v4.

RNA extraction and sequencing. Total RNA was extracted with mirVana isolation kit (Ambion), after removing the storage reagent by centrifugation. Artificial mRNA was synthesized by in vitro transcription from a pGEM-3Z plasmid and used as internal standard. Internal standard was added at 0.5% final concentration of total extracted RNA in order to calculate transcript abundances (Moran *et al.*, 2013; Satinsky *et al.*, 2013). Residual genomic DNA was removed using the TURBO DNase (Ambion). Eukaryotic and prokaryotic ribosomal RNA was removed enzymatically using MICROBEnrich (Ambion) and MICROBExpress (Ambion)

according to the manufacturers' recommendations. The MessageAmp II-Bacteria Kit (Ambion) was used to linearly amplify RNA. Samples were sequenced using the HiSeq Illumina high output mode HS200 2x100bp v4 at CNAG (Barcelona, Spain).

SUPPLEMENTARY TABLES

Table S1. Growth rates (in h^{-1}) of prokaryotic community microcosms amended with different ADOC fractions (F1 aliphatic, F2 aromatic, F3 polar and F1+F2, named ADOC) under different concentrations. Standard errors from duplicates are depicted between brackets.

HNA								
$C/C_{control}$	**ADOC**		**F1**		**F2**		**F3**	
1	0.0229	(±0.0008)	0.0224	(±0.0044)	0.0189	(±0.0042)	0.0222	(±0.0001)
1.4	0.0260	(±0.0011)	0.0244	(±0.0003)	0.0199	(±0.0012)	0.0122	(±0.0039)
2.6	0.0223	(±0.0002)	0.0183	(±0.0008)	0.0186	(±0.0028)	0.0244	(±0.0015)
8.2	0.0205	(±0.0001)	0.0192	(±0.0003)	0.0293	(±0.0111)	0.0317	(±0.0037)

LNA								
$C/C_{control}$	**ADOC**		**F1**		**F2**		**F3**	
1	0.0141	(±0.0039)	0.0163	(±0.0027)	0.0144	(±0.0045)	0.0161	(±0.0005)
1.4	0.0193	(±0.0044)	0.0208	(±0.0009)	0.0157	(±0.0001)	0.0077	(±0.0047)
2.6	0.0145	(±0.0001)	0.0151	(±0.0006)	0.0119	(±0.0048)	0.0144	(±0.0031)
8.2	0.0151	(±0.0010)	0.0141	(±0.0002)	0.0173	(±0.0013)	0.0149	(±0.0046)

C: concentration of the ADOC fraction in the amendment; $C_{control}$: concentration of the ADOC fraction in the non amended control; HNA: high nucleic acid cells; LNA: low nucleic acid cells.

Table S2. Results from the PERMANOVA of the metagenomic data from Arctic and Antarctic experiments by location, treatment and sampling time point.

	Df	**SumsOfSqs**	**MeanSqs**	**F.Model**	**R2**	**Pr(>F)**
location	1	0.041217	0.041217	23.217000	0.687200	0.001
Sample type (T or C)	1	0.002898	0.002898	1.632600	0.048320	0.180
Sampling time point (0.5 vs 24h)	1	0.001660	0.001660	0.935200	0.027680	0.360
Residuals	8	0.014202	0.001775		0.236790	
Total	11	0.059978			1.000000	

Table S3. List of taxa that increased in abundance by 10-fold in Arctic ADOC amendment after 24h already described in previous studies. The column of "interaction to ADOC" defines if the taxon was found in a polluted environment (presence) or if degrading activity was detected (degradation).

Order	Family	Genus	Taxon
Actinomycetales	*Intrasporangiaceae*	-	*Intrasporangiaceae*
	Microbacteriaceae	*Microbacterium*	*Microbacterium*
			Microbacterium profundi
	Micrococcaceae	*Arthrobacter*	*Arthrobacter*
	Micrococcineae	*Micrococcaceae*	*Micrococcaceae*
	Nocardioidaceae	-	*Nocardioidaceae*
		Nocardioides	*Nocardioides*
			Nocardioides sp. Root122
			Nocardioides sp. Soil774
			Nocardioides sp. Soil777
Geodermatophilales	*Geodermatophilaceae*	-	*Geodermatophilaceae*
		Blastococcus	*Blastococcus*
		Blastococcus	*Candidatus Blastococcus massiliensis*
Bacteroidales	*Lentimicrobiaceae*	*Lentimicrobium*	*Lentimicrobium saccharophilum*
Flavobacteriales	*Flavobacteriaceae*	*Algibacter*	*Algibacter lectus*
		Cellulophaga	*Cellulophaga baltica*
		Flavobacterium	*Flavobacterium hibernum*
		Olleya	*Olleya*
		Psychroflexus	*Psychroflexus torquis*
		Sediminicola	*Sediminicola sp. YIK13*
		Zobellia	*Zobellia*
Synechococcales	*Synechococcaceae*	*Synechococcus*	*Synechococcus sp. WH 8109*
Planctomycetales	*Planctomycetaceae*	*Rhodopirellula*	*Rhodopirellula*
-	-	*Micavibrio*	*Micavibrio aeruginosavorus*
Rhizobiales	*Bradyrhizobiaceae*	*Bosea*	*Bosea*
			Bosea vaviloviae
	Hyphomicrobiaceae	*Devosia*	*Devosia*
Rhodobacterales	*Rhodobacteraceae*	*Halocynthiibacter*	*Halocynthiibacter namhaensis*

Interaction to ADOC	References
degradation	(Wang *et a*l., 2016)
presence	(Schippers *et a*l., 2005)
degradation	(O'Loughlin *et al.*, 1999; Westerberg *et al.*, 2000; Camargo *et a*l., 2004)
degradation	(Delgado-Balbuena *et a*l., 2016)
degradation	(Takagi *et a*l., 2009)
nd	-
nd	-
nd	-
nd	-
presence	(Prabagaran *et a*l., 2007)
presence	(Thompson *et a*l., 2017)
nd	-
presence	(Kappell *et a*l., 2014)
presence	(Wang *et a*l., 2014)
nd	-
presence	(Lage and Bondoso, 2011)
nd	-
presence	(Jiao *et a*l., 2016)
presence	(Kumar *et a*l., 2008)
nd	-

Order	Family	Genus	Taxon
Sphingomonadales	*Erythrobacteraceae*	*Croceicoccus*	*Croceicoccus*
	Flammeovirgaceae	*Fabibacter*	*Fabibacter*
	Sphingomonadaceae	*Blastomonas*	*Blastomonas*
			Blastomonas sp. CCH1-A6
		Novosphingobium	*Novosphingobium*
			Novosphingobium sp. AAP93
		Sphingobium	*Sphingobium*
			Sphingobium czechense
			Sphingobium japonicum
			Sphingobium sp. Ant17
			Sphingobium sp. C100
			Sphingobium sp. Leaf26
			Sphingobium sp. TCM1
			Sphingobium xenophagum
			Sphingobium yanoikuyae
		Sphingomonas	*Sphingomonas sp. CCH5-D11*
		Sphingopyxis	*Sphingopyxis*
Burkholderiales	*Burkholderiaceae*	*Burkholderia*	*Burkholderia cepacia complex*
-	*-*	*-*	*unclassified Epsilon proteobacteria*
		Sulfurovum	*Sulfurovum*
Enterobacteriales	*Morganellaceae*	*-*	*Morganellaceae*
Oceanospirillales	*Oceanospirillaceae*	*Balneatrix*	*Balneatrix alpica*
Pseudomonadales	*Pseudomonadaceae*	*Pseudomonas*	*Pseudomonas aeruginosa group*
		Pseudomonas	*Pseudomonas putida*
		Pseudomonas	*Pseudomonas putida group*
		Pseudomonas	*Pseudomonas syringae group*
Vibrionales	*Vibrionaceae*	*Aliivibrio*	*Aliivibrio*
		Vibrio	*Vibrio sp. HI00D65*
Xanthomonadales	*Xanthomonadaceae*	*Pseudoxanthomonas*	*Pseudoxanthomonas*
Spirochaetales	*-*	*-*	*Spirochaetales*

nd: not defined in the literature.

Interaction to ADOC	References
degradation	(Huang *et al*., 2015)
presence	(Wang *et al*., 2014)
presence	(Gupta *et al*., 2009)
degradation	(Degtjarik *et al*., 2013)
degradation	(Leys *et al*., 2005)
presence	(Jindal *et al*., 2013)
degradation	(Mahenthiralingam *et al*., 2005)
degradation	(Vogt *et al*., 2011)
nd	-
nd	-
degradation	(Wasi *et al*., 2013)
nd	-
nd	-
degradation	(Choi *et al*., 2013)
nd	-

Table S4. List of significantly up-regulated (red color) and down-regulated (blue color) transcripts from Metabolism of Aromatic Compound SEED category, in Arctic and Antarctic treatment after 24 h ADOC addition.

SEED2	SEED3	Actino	Flavo	Gamma	Sphingo	Other Bact	Rhodo	SAR11
		ARCTIC						
4-Hydroxyphenylacetic acid catabolic pathway	4-hydroxyphenylacetate 3-monooxygenase (EC 1.14.13.3)							
4-Hydroxyphenylacetic acid catabolic pathway	4-hydroxyphenylacetate 3-monooxygenase, reductase component (EC 1.6.8.-)							
Anaerobic Aromatic Degredation (benzoate)	4-hydroxybenzoyl-CoA reductase, gamma subunit (EC 1.3.99.20)							
Anaerobic Aromatic Degredation (benzoate)	Acetyl-CoA acetyltransferase							
Anaerobic Aromatic Degredation (benzoate)	Benzoate-CoA ligase (EC 6.2.1.25)							
Anaerobic Aromatic Degredation (benzoate)	Benzoyl-CoA reductase subunit BadD (EC 1.3.99.15)							
Anaerobic Aromatic Degredation (benzoate)	Rrf2 family transcriptional regulator							
Anaerobic Aromatic Degredation (benzoate)	transcriptional regulator, Crp/Fnr family							
Anaerobic benzoate metabolism	Glutaryl-CoA dehydrogenase (EC 1.3.99.7)							
Aromatic Amin Catabolism	Aldehyde dehydrogenase (EC 1.2.1.3), PaaZ							
Aromatic Amin Catabolism	Nitrilotriacetate monooxygenase component B (EC 1.14.13.-)							
Aromatic Amin Catabolism	Phenylacetaldehyde dehydrogenase (EC 1.2.1.39)							
Benzoate catabolism	Benzoate 1,2-dioxygenase (EC 1.14.12.10)							
Benzoate catabolism	Benzoate 1,2-dioxygenase beta subunit (EC 1.14.12.10)							
Benzoate catabolism	Muconate cycloisomerase (EC 5.5.1.1)							
Benzoate catabolism	Muconolactone isomerase (EC 5.3.3.4)							
Benzoate degradation	1,2-dihydroxycyclohexa-3,5-diene-1-carboxylate dehydrogenase (EC 1.3.1.25)							
Benzoate degradation	Benzoate 1,2-dioxygenase alpha subunit (EC 1.14.12.10)							
Benzoate degradation	Benzoate transport protein							
Benzoate degradation	Ortho-halobenzoate 1,2-dioxygenase alpha-ISP protein OhbB							
Benzoate degradation	Ortho-halobenzoate 1,2-dioxygenase beta-ISP protein OhbA							
Benzoate degradation	benzoate-specific porin							

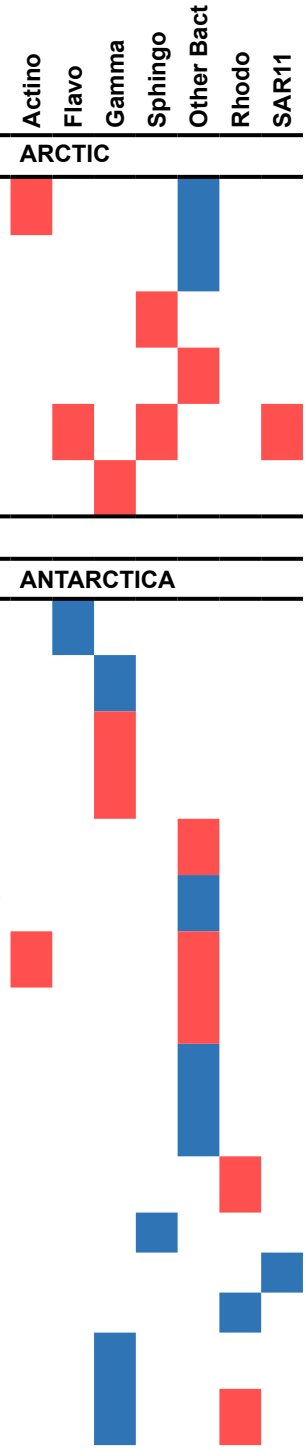
SEED2	SEED3	Actino	Flavo	Gamma	Sphingo	Other Bact	Rhodo	SAR11
		ARCTIC						
Benzoate transport and degradation cluster	2-keto-4-pentenoate hydratase/2-oxohepta-3-ene-1,7-dioic acid hydratase (catechol pathway)							
Benzoate transport and degradation cluster	4-hydroxybenzoyl-CoA reductase, beta subunit (EC 1.3.99.20)							
Benzoate transport and degradation cluster	Benzoate transport, inner-membrane translocator precursor							
Benzoate transport and degradation cluster	Benzoyl-CoA oxygenase component A							
Benzoate transport and degradation cluster	Benzoyl-CoA oxygenase component B							
Benzoate transport and degradation cluster	Hydroxybenzoyl-CoA reductase subunit							
Benzoate transport and degradation cluster	P-hydroxylaminobenzoate lyase							
Biphenyl Degradation	2,3-dihydroxybiphenyl 1,2-dioxygenase							
Biphenyl Degradation	2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoate hydrolase (EC 3.7.1.-)							
Biphenyl Degradation	Biphenyl dioxygenase alpha subunit (EC 1.14.12.18)							
Biphenyl Degradation	Biphenyl-2,3-diol 1,2-dioxygenase (EC 1.13.11.39)							
Biphenyl Degradation	Large subunit naph/bph dioxygenase							
Catechol branch of beta-ketoadipate pathway	3-oxoadipate CoA-transferase subunit A (EC 2.8.3.6)							
Catechol branch of beta-ketoadipate pathway	3-oxoadipate CoA-transferase subunit B (EC 2.8.3.6)							
Catechol branch of beta-ketoadipate pathway	Catechol 1,2-dioxygenase 1 (EC 1.13.11.1)							
Catechol branch of beta-ketoadipate pathway	Muconolactone isomerase (EC 5.3.3.4),putative							
Catechol branch of beta-ketoadipate pathway	mandelate racemase/muconate lactonizing enzyme family protein							
Central meta-cleavage pathway of aromatic compound degradation	1,2-dihydroxynaphthalene dioxygenase							
Central meta-cleavage pathway of aromatic compound degradation	2-hydroxymuconic semialdehyde hydrolase (EC 3.7.1.9)							
Central meta-cleavage pathway of aromatic compound degradation	2-polyprenylphenol hydroxylase and related flavodoxin oxidoreductases							
Central meta-cleavage pathway of aromatic compound degradation	4-oxalocrotonate tautomerase (EC 5.3.2.-)							

SEED2	SEED3	Actino	Flavo	Gamma	Sphingo	Other Bact	Rhodo	SAR11
		ARCTIC						
Central meta-cleavage pathway of aromatic compound degradation	Intradiol ring-cleavage dioxygenase (EC 1.13.11.1)							
Central meta-cleavage pathway of aromatic compound degradation	Protein involved in meta-pathway of phenol degradation							
Central meta-cleavage pathway of aromatic compound degradation	Protocatechuate 4,5-dioxygenase alpha chain (EC 1.13.11.8)							
Central meta-cleavage pathway of aromatic compound degradation	Protocatechuate 4,5-dioxygenase beta chain (EC 1.13.11.8)							
Chlorobenzoate degradation	2-chlorobenzoate 1,2-dioxygenase beta subunit (EC 1.14.12.13)							
Chlorobenzoate degradation	Putative benzaldehyde dehydrogenase oxidoreductase protein (EC 1.2.1.28)							
Cresol degradation	4-cresol dehydrogenase [hydroxylating] flavoprotein subunit (EC 1.17.99.1)							
Gallic acid utilization	Gallate dioxygenase (EC 1.13.11.57)							
Gallic acid utilization	Gallate permease							
Gentisate degradation	2-polyprenyl-6-methoxyphenol hydroxylase and related FAD-dependent oxidoreductases							
Gentisate degradation	Gentisate 1,2-dioxygenase (EC 1.13.11.4)							
Gentisate degradation	Maleate cis-trans isomerase (EC 5.2.1.1)							
Gentisate degradation	Putative n-hydroxybenzoate hydroxylase							
Gentisate degradation	Salicylate hydroxylase (EC 1.14.13.1)							
Gentisate degradation	putative 4-hydroxybenzoyl-CoA thioesterase							
Homogentisate pathway of aromatic compound degradation	Possible 3-(3-hydroxy-phenyl)propionate hydroxylase (EC 1.14.13.-)							
Homogentisate pathway of aromatic compound degradation	Transcriptional regulator, IclR family							
Hydroxyaromatic decarboxylase family	Hydroxyaromatic non-oxidative decarboxylase protein B (EC 4.1.1.-)							
Hydroxyaromatic decarboxylase family	Hydroxyaromatic non-oxidative decarboxylase protein D (EC 4.1.1.-)							
Naphtalene and antracene degradation	2-hydroxychromene-2-carboxylate isomerase							
Naphtalene and antracene degradation	Naphthalene 1,2-dioxygenase system ferredoxin--NAD(+) reductase component (EC 1.18.1.3)							

SEED2	SEED3	Actino	Flavo	Gamma	Sphingo	Other Bact	Rhodo	SAR11
		ARCTIC						
Naphtalene and antracene degradation	naphthalene dioxygenase ferredoxin							
Phenol hydroxylase	Phenol hydroxylase, P4 oxygenase component DmpO (EC 1.14.13.7)							
Phenylacetyl-CoA catabolic pathway (core)	Phenylacetate-CoA oxygenase, PaaI subunit							
Phenylacetyl-CoA catabolic pathway (core)	Phenylacetate-coenzyme A ligase (EC 6.2.1.30) PaaF							
Phenylpropanoid compound degradation	Lignostilbene-alpha,beta-dioxygenase and related enzymes							
Phenylpropanoid compound degradation	Phenylpropionate dioxygenase and related ring-hydroxylating dioxygenases, large terminal subunit							
Phenylpropanoid compound degradation	Probable VANILLIN dehydrogenase oxidoreductase protein (EC 1.-.-.-)							
Phenylpropanoid compound degradation	Putative 3-alpha-hydroxysteroid dehydrogenase/carbonyl reductase oxidoreductase protein (EC 1.1.1.50)							
Phenylpropanoid compound degradation	Putative phthalate 4,5-dioxygenase oxygenase subunit (OhpA2)							
Phenylpropanoid compound degradation	Vanillate O-demethylase oxygenase subunit (EC 1.14.13.82)							
Phenylpropanoid compound degradation	vannilate transporter VanK							
Protocatechuate branch of beta-ketoadipate pathway	3-oxoadipate enol-lactone hydrolase/4-carboxymuconolactone decarboxylase							
Protocatechuate branch of beta-ketoadipate pathway	4-carboxymuconolactone decarboxylase (EC 4.1.1.44)							
Protocatechuate branch of beta-ketoadipate pathway	Pca regulon regulatory protein PcaR							
Protocatechuate branch of beta-ketoadipate pathway	Protocatechuate 3,4-dioxygenase alpha chain (EC 1.13.11.3)							
Salicylate and gentisate catabolism	AreB (Aryl-alcohol dehydrogenase) (EC 1.1.1.90)							
Salicylate and gentisate catabolism	Fumarylacetoacetate (FAA) hydrolase (EC 3.7.1.5)							
Salicylate and gentisate catabolism	putative facilitator of salicylate uptake							
Salicylate and gentisate catabolism	regulator protein,AreR							
Salicylate and gentisate catabolism	regulator protein,SalR							
Salicylate and gentisate catabolism	salicylate esterase							
Toluene 4-monooxygenase (T4MO)	Toluene-4-monooxygenase, subunit TmoA							

SEED2	SEED3
Toluene 4-monooxygenase (T4MO)	Toluene-4-monooxygenase, subunit TmoC
Toluene 4-monooxygenase (T4MO)	Toluene-4-monooxygenase, subunit TmoF
Toluene degradation	toluenesulfonate zinc-independent alcohol dehydrogenase
n-Phenylalkanoic acid degradation	enoyl-CoA hydratase, R-specific
p-Hydroxybenzoate degradation	P-hydroxybenzoate hydroxylase (EC 1.14.13.2)
p-cymene degradation	p-cumic aldehyde dehydrogenase (CymC) [EC:1.2.1.3]

SEED2	SEED3
4-Hydroxyphenylacetic acid catabolic pathway	4-hydroxyphenylacetate 3-monooxygenase (EC 1.14.13.3)
4-Hydroxyphenylacetic acid catabolic pathway	4-hydroxyphenylacetate symporter, major facilitator superfamily (MFS)
4-Hydroxyphenylacetic acid catabolic pathway	Transcriptional activator of 4-hydroxyphenylacetate 3-monooxygenase operon, XylS/AraC family
Anaerobic Aromatic Degredation (benzoate)	2-hydroxycyclohexanecarboxyl-CoA dehydrogenase (EC 1.1.1.-)
Anaerobic Aromatic Degredation (benzoate)	4-hydroxybenzoyl-CoA reductase, gamma subunit (EC 1.3.99.20)
Anaerobic Aromatic Degredation (benzoate)	Acetyl-CoA acetyltransferase
Anaerobic Aromatic Degredation (benzoate)	Cyclohex-1-ene-1-carboxyl-CoA hydratase (EC 4.2.1.17)
Anaerobic Aromatic Degredation (benzoate)	Rrf2 family transcriptional regulator
Aromatic Amin Catabolism	Phenylacetaldehyde dehydrogenase (EC 1.2.1.39)
Benzoate catabolism	Benzoate 1,2-dioxygenase beta subunit (EC 1.14.12.10)
Benzoate catabolism	Catechol 1,2-dioxygenase (EC 1.13.11.1)
Benzoate catabolism	Muconate cycloisomerase (EC 5.5.1.1)
Benzoate degradation	Benzoate transport protein
Benzoate degradation	Benzoylformate decarboxylase (EC 4.1.1.7)
Benzoate degradation	Ortho-halobenzoate 1,2-dioxygenase alpha-ISP protein OhbB



SEED2	SEED3	Actino	Flavo	Gamma	Sphingo	Other Bact	Rhodo	SAR11
		ANTARCTICA						
Benzoate degradation	Ring hydroxylating dioxygenase, alpha subunit (EC 1.14.12.13)							
Benzoate transport and degradation cluster	2-keto-4-pentenoate hydratase/2-oxohepta-3-ene-1,7-dioic acid hydratase (catechol pathway)							
Benzoate transport and degradation cluster	Benzoate transport, inner-membrane translocator precursor							
Benzoate transport and degradation cluster	Benzoyl-CoA oxygenase component B							
Benzoate transport and degradation cluster	benzoate degradation ring-cleavage hydrolase							
Biphenyl Degradation	Biphenyl-2,3-diol 1,2-dioxygenase (EC 1.13.11.39)							
Biphenyl Degradation	Large subunit naph/bph dioxygenase							
Biphenyl Degradation	biphenyl-2,3-diol 1,2-dioxygenase III-related protein							
Catechol branch of beta-ketoadipate pathway	Muconolactone isomerase (EC 5.3.3.4),putative							
Central meta-cleavage pathway of aromatic compound degradation	2-hydroxymuconic semialdehyde hydrolase (EC 3.7.1.9)							
Central meta-cleavage pathway of aromatic compound degradation	Catechol 2,3-dioxygenase (EC 1.13.11.2)							
Central meta-cleavage pathway of aromatic compound degradation	Intradiol ring-cleavage dioxygenase (EC 1.13.11.1)							
Central meta-cleavage pathway of aromatic compound degradation	Protocatechuate 4,5-dioxygenase beta chain (EC 1.13.11.8)							
Chloroaromatic degradation pathway	Beta-ketoadipyl CoA thiolase (EC 2.3.1.-)							
Chlorobenzoate degradation	Putative benzaldehyde dehydrogenase oxidoreductase protein (EC 1.2.1.28)							
Gallic acid utilization	Gallate permease							
Gentisate degradation	Gentisate 1,2-dioxygenase (EC 1.13.11.4)							
Gentisate degradation	Salicylate hydroxylase (EC 1.14.13.1)							
Phenylacetyl-CoA catabolic pathway (core)	Phenylacetate-coenzyme A ligase (EC 6.2.1.30) PaaF							
Phenylacetyl-CoA catabolic pathway (core)	Phenylacetic acid degradation protein PaaN, ring-opening aldehyde dehydrogenase (EC 1.2.1.3)							
Phenylpropanoid compound degradation	Chlorogenate esterase							

SEED2	SEED3	Actino	Flavo	Gamma	Sphingo	Other Bact	Rhodo	SAR11
		ANTARCTICA						
Phenylpropanoid compound degradation	Phenylpropionate dioxygenase and related ring-hydroxylating dioxygenases, large terminal subunit							
Phenylpropanoid compound degradation	Probable VANILLIN dehydrogenase oxidoreductase protein (EC 1.-.-.-)							
Phenylpropanoid compound degradation	Putative phthalate 4,5-dioxygenase oxygenase subunit (OhpA2)							
Protocatechuate branch of beta-ketoadipate pathway	Protocatechuate 3,4-dioxygenase alpha chain (EC 1.13.11.3)							
Salicylate and gentisate catabolism	putative facilitator of salicylate uptake							
Toluene 4-monooxygenase (T4MO)	Toluene-4-monooxygenase, subunit TmoA							
Toluene 4-monooxygenase (T4MO)	Toluene-4-monooxygenase, subunit TmoC							

Table S5. Compound-specific concentrations (pg/l) of hexachlorobenzene (HCB), hexachlorocyclohexanes (HCHs), polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) in the Arctic and Southern ocean seawater used as spike solution in the experiments. The mean LOQ was of 0.007 pg/l for organochlorine compounds (PCBs, HCH and HCB), and of 0.01 pg/l for PAHs.

Family	Compound	ANTARCTICA	ARCTIC
PAHs	**Phenanthrene**	300	8.7
	Anthracene	18	1.3
	Methylphenanthrenes	550	6.8
	Dimethylphenanthrenes	140	4.6
	Dibenzothiophene	36	nd
	Methyldibenzothiophenes	190	nd
	Pyrene	110	3.9
	Fluoranthene	150	2.4
	Benzo[a]anthracene	6.6	nd
	Chrysene	6.1	2.0
	Benzo[b+k]fluoranthene	1.9	nd
	Benzo[a]pyrene	3.3	2.4
	Benzo[e]pyrene	0.3	nd
	Indeno[1,2,3-cd]pyrene	40	3.1
	Dibenzo[a,h]anthracene	53	2.6

Family	Compound	ANTARCTICA	ARCTIC
PCBs	**PCB 18**	0.041	0.019
	PCB 17	0.050	0.0065
	PCB 31	0.062	0.016
	PCB 28	0.048	0.010
	PCB 33	0.044	0.011
	PCB 52	0.060	0.013
	PCB 49	0.018	0.11
	PCB 44	< LOQ	0.0092
	PCB 99/101	0.449	0.057
	PCB 87	< LOQ	0.35
	PCB 110	0.21	0.26
	PCB 82	0.052	0.0041
	PCB 151	0.027	0.012
	PCB 149	0.11	<LOQ
	PCB 118	0.10	<LOQ
	PCB 153	0.075	0.0053
	PCB 132/105	0.040	0.0048
	PCB 138	0.076	0.079
	PCB 158	0.0067	0.053
	PCB 187	0.045	0.015
	PCB 183	0.0027	0.063
	PCB 128	0.0067	0.00060
	PCB 177	0.0027	0.011
	PCB 171/156	0.032	0.081
	PCB 180	0.025	0.0089
	PCB 191	< LOQ	0.00050
	PCB 169	< LOQ	0.022
	PCB170	< LOQ	0.0051
	PCB 201/199	< LOQ	0.00050
HCB and HCHs	**HCB**	0.40	1.3
	α-HCH	0.26	0.97
	β-HCH	0.17	< LOQ
	δ-HCH	0.37	< LOQ
	γ-HCH	1.1	0.42

LOQ: limits of quantification; nd: no determined.

Table S6. List of oligonucleotide FISH probes used in CARD-FISH analyses.

Probe name	Target organisms	Reference
EUB 338-II-III	Most Bacteria	(Amann *et al.*, 1990; Daims *et al.*, 1999)
Alf968	Alphaproteobacteria	(Neef, 1997)
Ros537	Alphaproteobacteria; *Roseobacter*	(Eilers *et al.*, 2001)
SAR11-441R	Alphaproteobacteria; Sar11 cluster	(Morris *et al.*, 2002)
Gam42a	*Gammaproteobacteria*	(Manz, W. *et al.*, 1992)
CF319a	Cytophaga–Flavobacterium–Bacteroidetes group	(Manz, W. *et al.*, 1996)

Table S7. List of specific Pfam domains and genes. EEA: extracellular enzymatic activity; LPS: lipopolysaccharides.

Bacterial strategy	Pfam ID	Name	Description
PAH degradation	PF00848	Ring_hydroxyl_A	Ring hydroxylating alpha subunit (catalytic domain)
	PF00866	Ring_hydroxyl_B	Ring hydroxylating beta subunit
OPE degradation	PF01663	Phosphodiest	Type I phosphodiesterase / nucleotide pyrophosphatase
FA desaturase	PF00487	FA_desaturase	Fatty acid desaturase
	PF03405	FA_desaturase_2	Fatty acid desaturase
EEA	PF00301	Rubredoxin	Rubredoxin
	PF03060	NMO	Nitronate monooxygenase
	PF00245	Alk_phosphatase	Alkaline phosphatase
	PF00704	Glyco_hydro_18	Chitinase
	PF00182	Glyco_hydro_19	Chitinase
	PF07691	PA14	beta-glucosidases
Oxidative Stress	PF03968	OstA	OstA-like protein
	PF13100	OstA_2	OstA-like protein
	PF04453	OstA_C	Organic solvent tolerance protein
	PF00255	GSHPx	Glutathione peroxidase
	PF00141	peroxidase	Peroxidase
Membrane transporters	PF00664	ABC_membrane	ABC transporter transmembrane region
	PF06472	ABC_membrane_2	ABC transporter transmembrane region 2

Bacterial strategy	Name	Description
Membrane efflux pump	Msba	Lipid A export ATP-binding/permease protein MsbA
	Efflux pump	ABC transporter multidrug efflux pump
Hopanoids	Isoprenoids	Isoprenoid biosynthesis
Biosurfactants	Rhamnosyltransferase	Rhamnosyltransferase
LPS	LPS	Lipopolysaccharide biosynthesis

Table S8. Metagenome and metatranscriptome datasets from Arctic and Antarctic experiments.

metaG	ANTARCTICA				
	C0.5A	C0.5B	C24A	C24B	T24A
Total joined reads	26,923,088	35,152,012	32,404,204	47,678,196	31,192,048
Internal standard reads	176,518	220,567	197,595	271,990	197,750
Standard normalization factor	6.9	11.0	6.9	17.0	10.0
Possible proteins	26,338,349	34,534,094	31,723,840	47,093,789	30,568,206
% potential protein-coding	97.8	98.2	97.9	98.8	98.0
SEED annotated proteins	3,289,665	3,247,553	4,797,129	6,192,279	4,168,119
% SEED annotated proteins	12.5	9.4	15.1	13.1	13.6
taxonomical annotated proteins	9,378,699	11,566,540	12,430,799	16,275,815	11,149,714
% taxonomical annotated proteins	35.6	33.5	39.2	34.6	36.5

metaT	ANTARCTICA						
	C0.5A	C0.5B	T0.5A	T0.5B	C24A	C24B	T24A
Total joined reads	51,017,366	52,783,126	40,487,742	55,096,188	49,370,456	62,386,818	55,001,270
Internal standard reads	1,899,554	1,696,635	1,312,267	1,518,082	1,700,608	970,898	2,070,425
Standard normalization factor	7,909	12,160	10,473	10,696	8,195	12,525	9,373
rRNA reads	26,094,806	27,282,430	20,104,510	27,630,922	21,807,302	31,727,172	24,823,314
% rRNA	51.1	51.7	49.7	50.2	44.2	50.9	45.1
Possible proteins	20,502,178	22,088,968	17,427,190	23,024,252	21,961,776	27,314,872	25,292,074
% potential protein-coding	40.2	41.8	43.0	41.8	44.5	43.8	46.0
SEED annotated proteins	2,553,670	3,201,797	3,167,901	3,356,599	4,622,934	4,299,118	4,071,816
% SEED annotated proteins	12.5	14.5	18.2	14.6	21.0	15.7	16.1
taxonomical annotated proteins	5,753,188	6,870,759	6,311,476	6,822,459	8,649,055	9,059,430	8,213,475
% taxonomical annotated proteins	28.1	31.1	36.2	29.6	39.4	33.2	32.5

C: Control; T: ADOC amendment; 0.5: time point after 30min; 24: time points after 24h.

	ARCTIC					
T24B	C0.5A	C0.5B	C24A	C24B	T24A	T24B
29,386,400	18,752,278	15,554,632	43,294,476	21,712,268	26,054,952	39,609,150
229,550	50,961	50,059	84,538	85,423	107,898	119,872
17.4	180.2	261.0	150.0	127.0	124.9	101.7
28,816,320	18,457,805	15,177,085	42,994,672	21,313,218	25,637,453	39,245,909
98.1	98.4	97.6	99.3	98.2	98.4	99.1
3,573,934	2,511,094	1,990,176	5,647,750	2,807,485	3,410,412	5,206,520
12.4	13.6	13.1	13.1	13.2	13.3	13.3
9,417,132	6,943,589	5,476,705	15,696,266	7,905,464	9,854,777	14,802,815
32.7	37.6	36.1	36.5	37.1	38.4	37.7

	ARCTIC							
T24B	C0.5A	C0.5B	T0.5A	T0.5B	C24A	C24B	T24A	T24B
62,464,898	57,155,082	57,798,118	63,595,486	58,529,986	39,971,642	48,162,736	49,081,330	49,708,754
2,815,829	1,303,400	1,644,326	1,445,969	1,533,122	671,192	1,307,875	628,313	503,255
12,541	30,839	16,199	29,601	23,449	65,078	14,304	131,322	153,570
31,099,252	21,057,244	30,758,986	23,253,848	30,486,716	17,729,036	26,630,962	13,470,594	11,619,592
49.8	36.8	53.2	36.6	52.1	44.4	55.3	27.4	23.4
26,725,470	31,025,378	21,929,716	36,459,120	22,182,104	17,683,438	16,556,014	32,472,818	34,869,308
42.8	54.3	37.9	57.3	37.9	44.2	34.4	66.2	70.1
4,955,753	4,523,904	2,182,545	6,566,131	2,572,983	2,216,484	1,340,370	5,082,476	4,096,502
18.5	14.6	10.0	18.0	11.6	12.5	8.1	15.7	11.7
10,010,216	10,972,328	6,151,850	14,714,935	6,838,925	5,583,466	3,839,503	12,713,406	12,572,333
37.5	35.4	28.1	40.4	30.8	31.6	23.2	39.2	36.1

SUPPLEMENTARY FIGURES

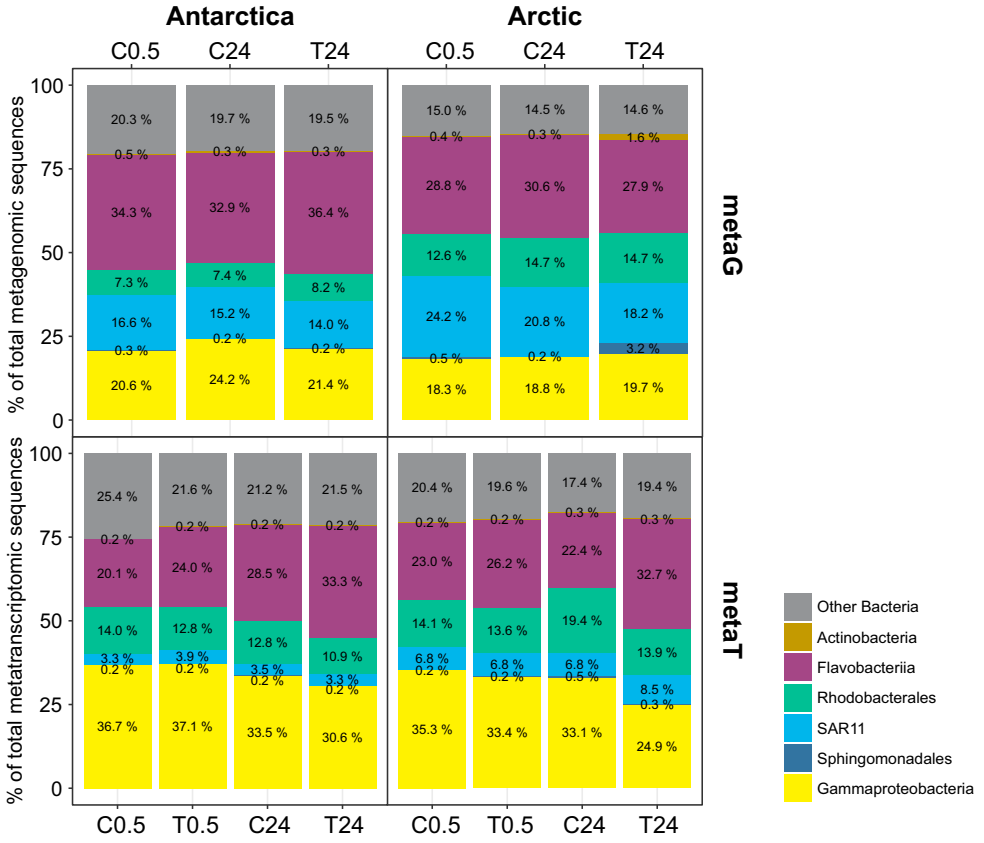


Figure S1. Relative abundance of major taxonomic groups in Arctic and Antarctic metagenomes (metaG) and metatranscriptomes (metaT). C: Control; T: ADOC amendment; 0.5: time point after 30min; 24: time points after 24h.

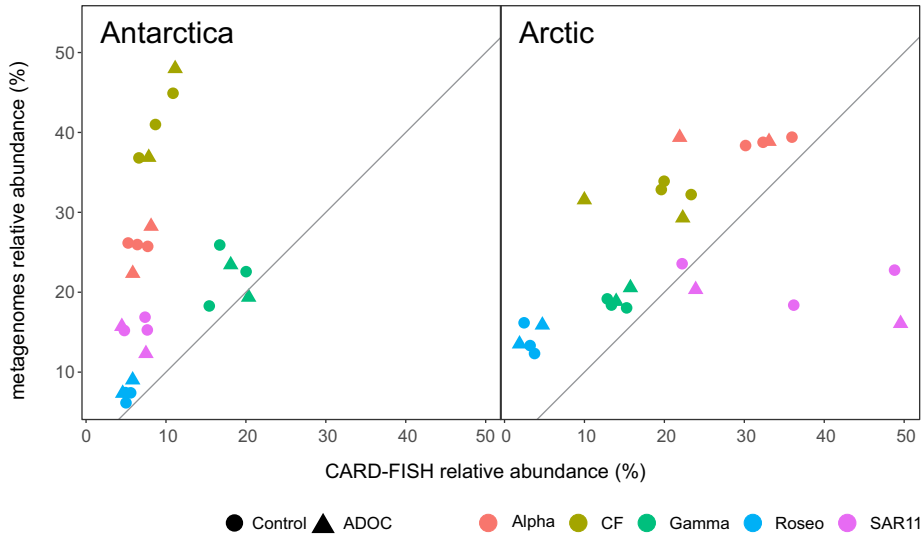


Figure S2. Comparison between the relative abundance of metagenomic reads and CARD-FISH counts (% of DAPI-stained cells) for the main taxonomic groups. ADOC: anthropogenic dissolved organic carbon treatment; Alpha: *Alphaproteobacteria*; CF: *Cytophaga–Flavobacterium–Bacteroidetes* group; Gamma: *Gammaproteobacteria*; Roseo: *Roseobacter*.

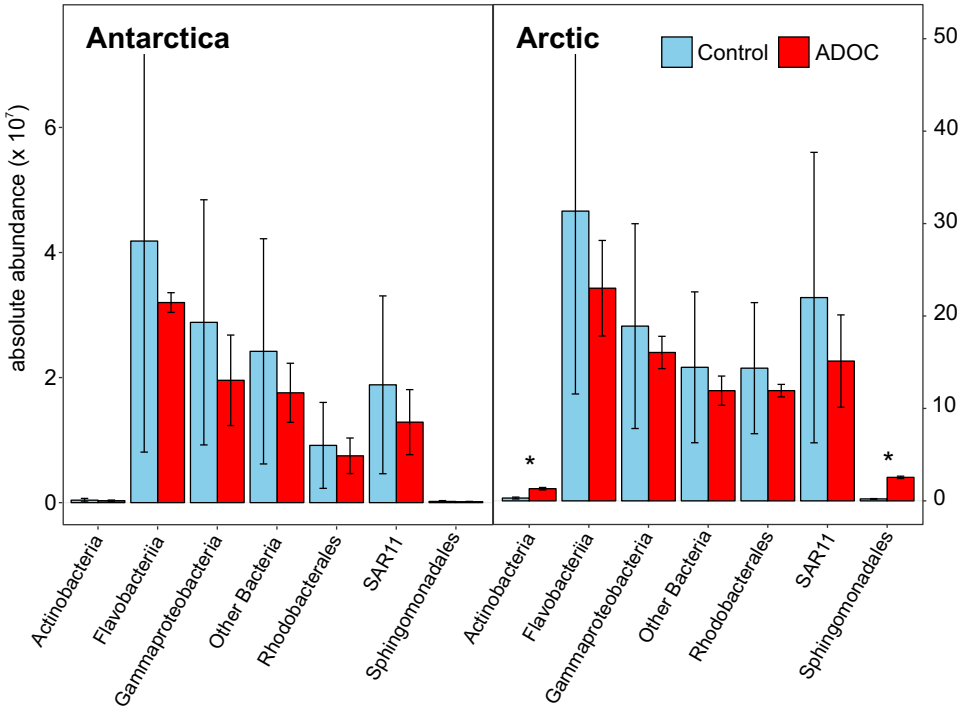


Figure S3. Taxonomical affiliation of Antarctic and Arctic metagenomes, 24 h after treatment. Asterisks indicate significant differences between ADOC amendment and controls (*t*-test; $P < 0.05$). Values are means of duplicates. Error bars show standard deviation. ADOC: ADOC amendment.

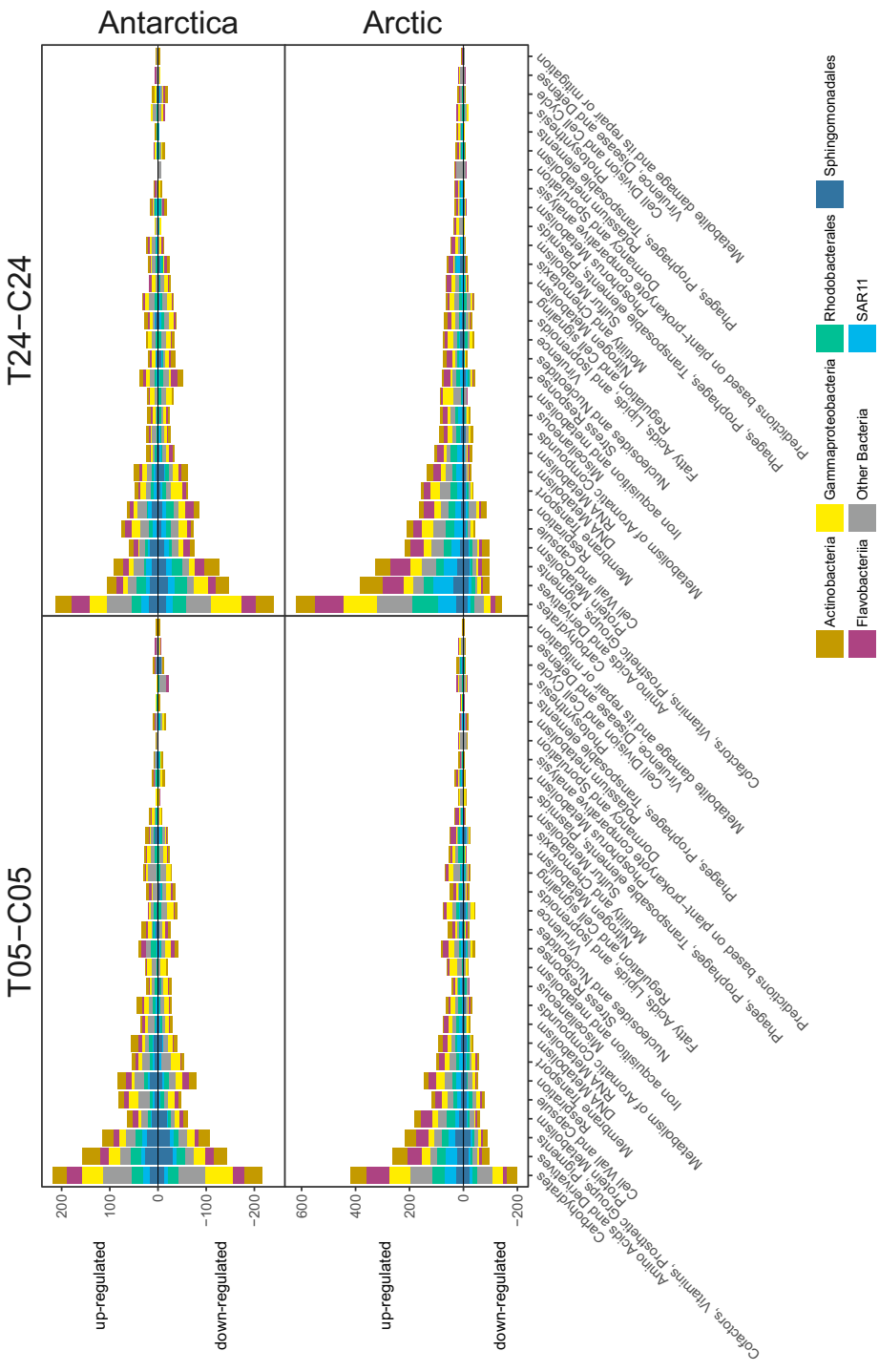


Figure S4. Up-regulated and down-regulated gene expression between controls and ADOC amendments after 0.5 (T05-C05) and 24 h (T24-C24) in Arctic and Antarctica. Bars indicate the numbers of or down-regulated transcripts detected by edgeR (FDR < 0.05).

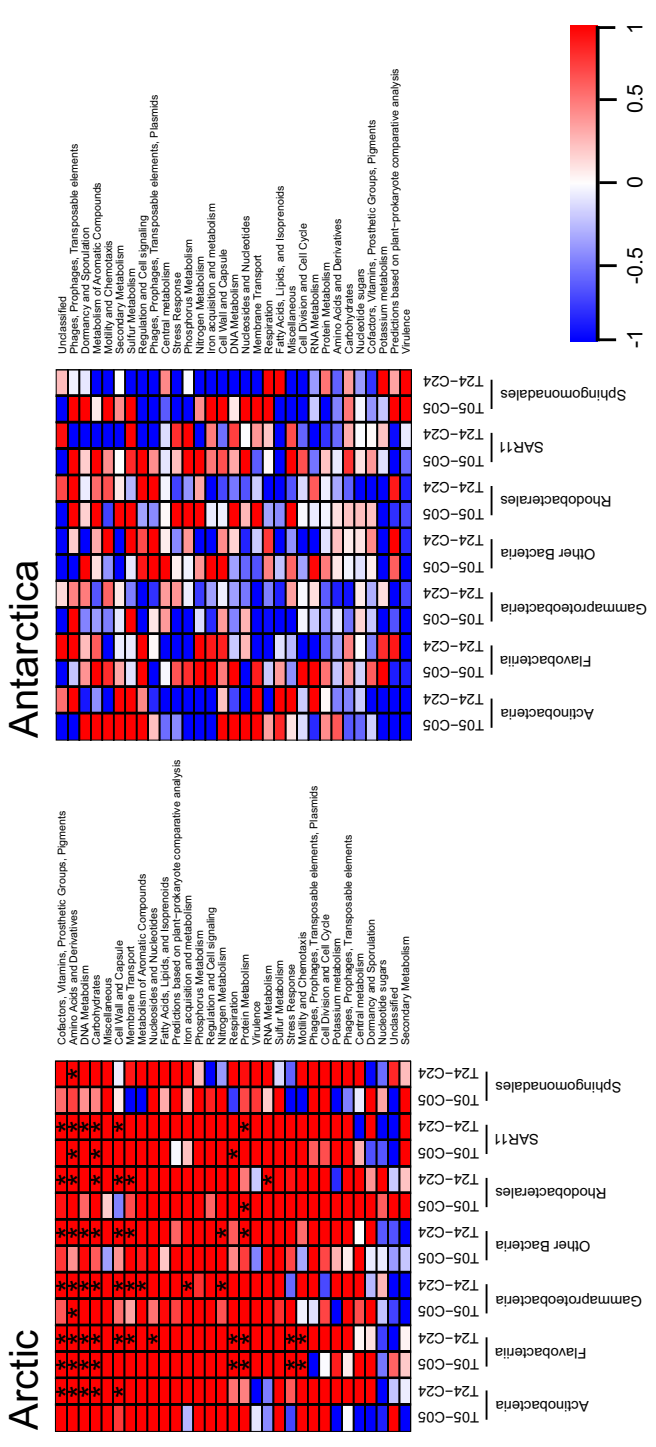


Figure S5. Heatmap of changes in gene expression between controls (C) and ADOC amendments (T) after 0.5 (T05-C05) and 24 h (T24-C24) in Arctic and Antarctic experiments for the main taxonomic groups, expressed both as logarithmic fold changes (treatment versus controls, LFC). Rows represent each SEED category, each column represent a taxonomical group and the color of each tile corresponds to enrichments (red, LFC > 0) or depletions (blue, LFC < 0) of the genes transcription. Significant differences are indicated by asterisks (*t*-test; $P < 0.05$).

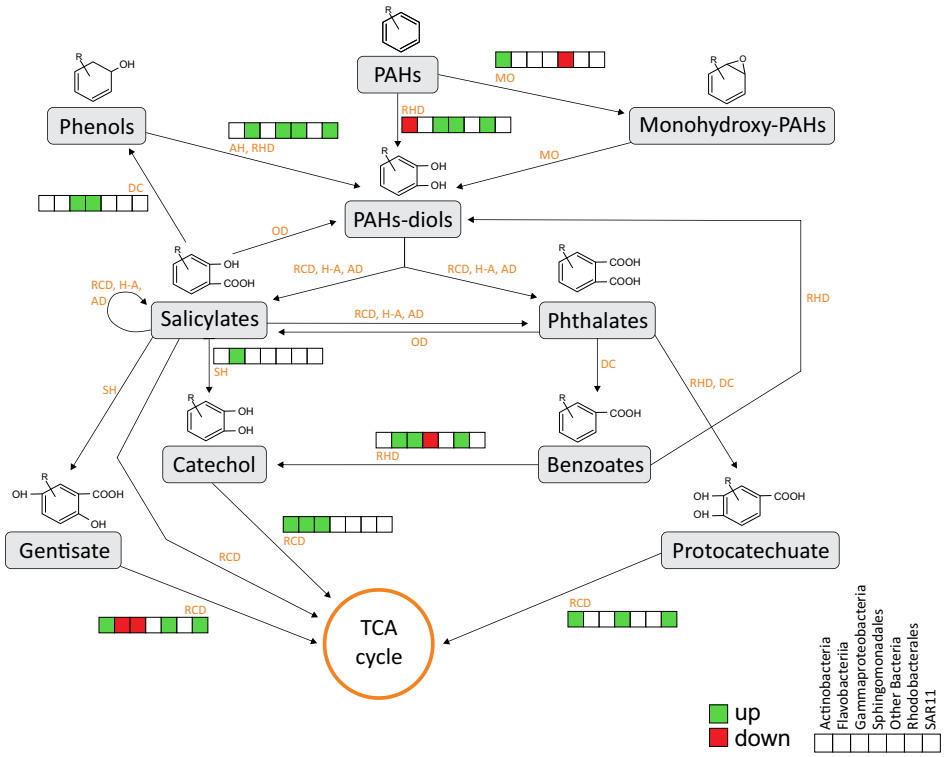


Figure S6. Scheme of the main degradation routes of PAHs, modified from Mallick *et al.* 2011. Significantly up-regulated (green) and down-regulated (red) transcripts in Arctic treatment after 24 h ADOC addition are labeled for each taxonomical group. Specific genes can be found in Table S4. Several transcripts encoding the same route are summarized per taxon. RHD: ring-hydroxylating dioxygenases; OD: oxidative decarboxylase; MO: monooxygenase; AH: aromatic hydroxylase; SH: salicylate hydroxylase; RCD: ring cleavage dioxygenase; H-A: hydratase-aldolase; AD: aldehyde dehydrogenase; DC: decarboxylase.

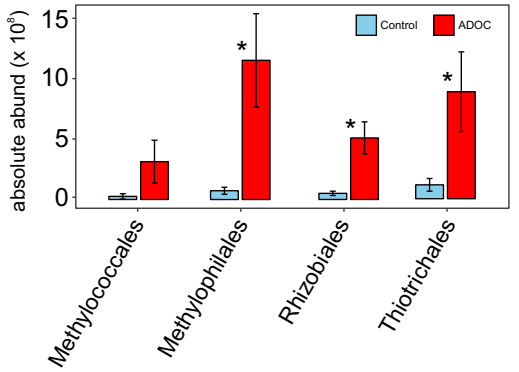


Figure S7. Transcript abundances of methylotrophic groups after 24 h in Arctic experiment. Error bars show standard deviation of duplicates. ADOC, ADOC amendment. Significant differences are indicated by asterisks (t-test; $P < 0.05$).

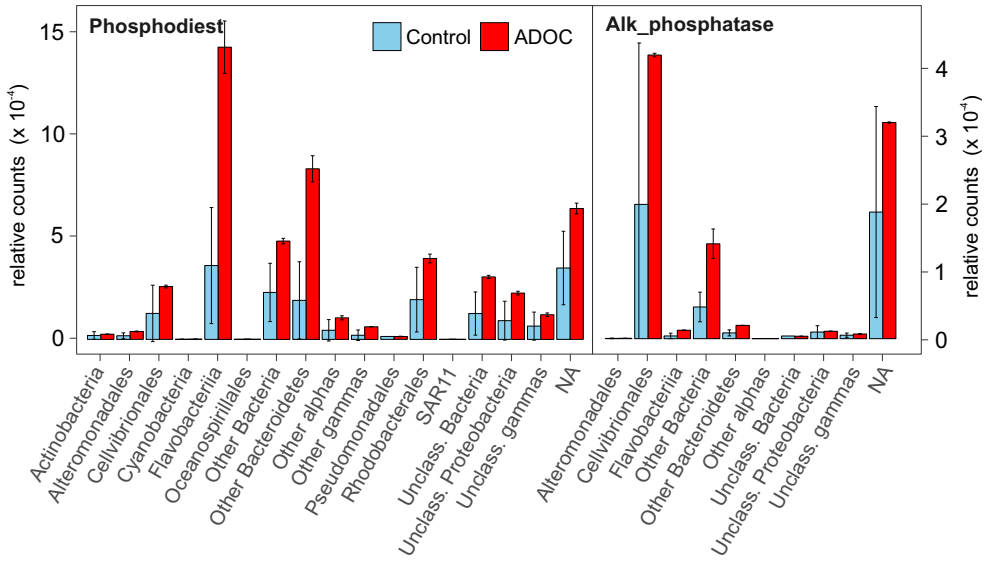


Figure S8. Taxonomical affiliation of relative abundances (of total reads) of Type I phosphodiesterase (PF01663) and Alk_phosphatase (PF00245) Pfam domains in Arctic experiment after 24 h of ADOC addition. Error bars show standard deviation of duplicates.

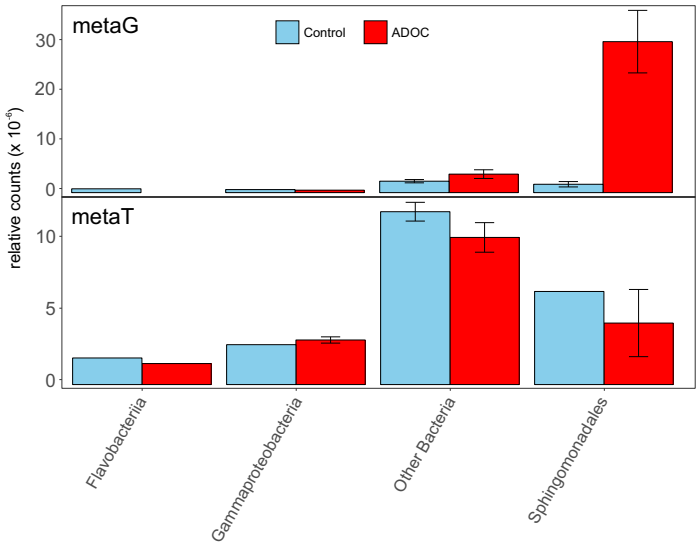


Figure S9. Relative abundances (percentage of total reads) of LPS (SEED category "lipopolysaccharide biosynthesis") in metagenomes (metaG) and metatranscriptomes (metaT) of Arctic experiment after 24 h. Error bars show standard deviation of duplicates. ADOC: ADOC amendment.

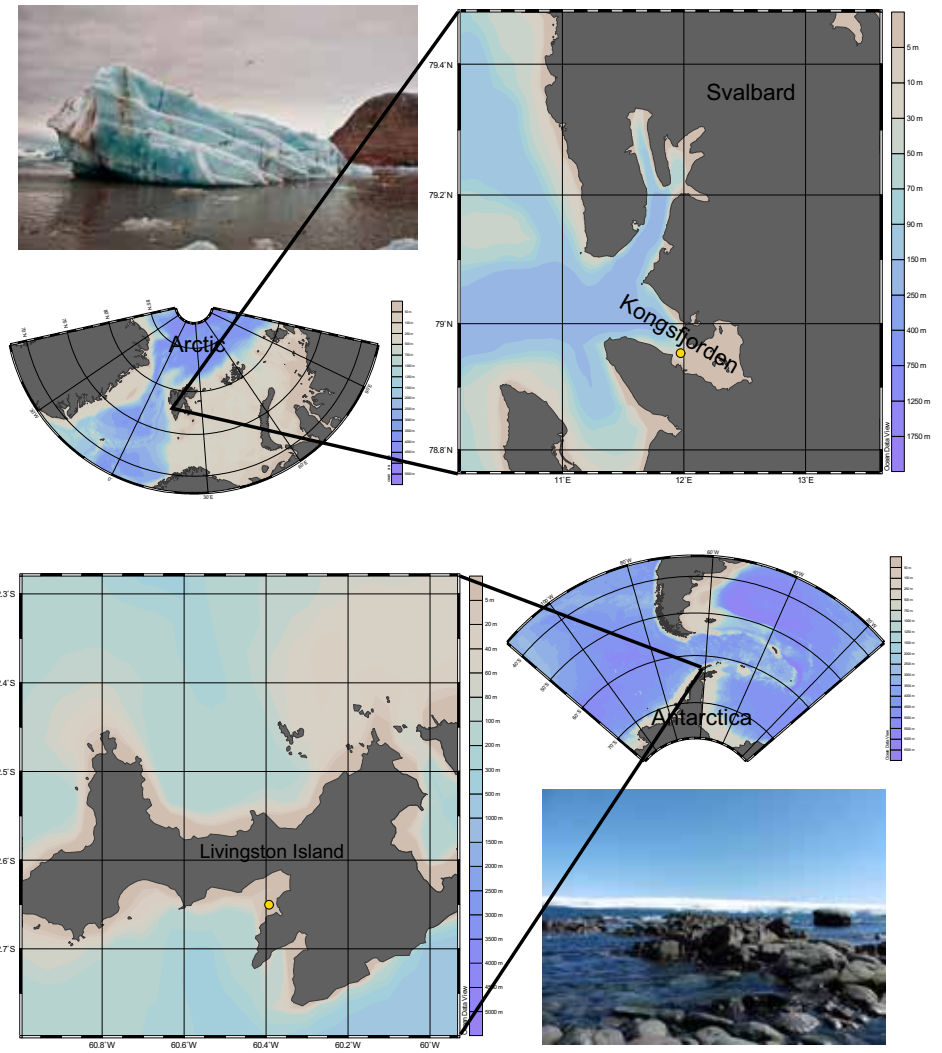


Figure S10. Location of the sampling site for the seawater used for the experiments. Experiments were performed *in situ* at South Bay (Livingston Island, Antarctica) and Kongsfjorden (Ny Ålesund, Arctic).

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Supporting information of **Chapter 4**

SUPPLEMENTARY TABLES

Table S1. Metagenome and metatranscriptome datasets from Barcelona and Blanes experiments.

metaG	BARCELONA				
	C0.5A	C0.5B	C24A	C24B	T24A
Total joined reads	18,687,614	23,448,372	20,405,304	41,201,326	23,808,270
Internal standard reads	123,940	94,708	124,987	223,881	127,769
Standard normalization factor	36.9	30.1	54.8	11.4	76.4
Possible proteins	18,312,937	23,070,517	20,124,576	40,722,497	23,454,584
% potential protein-coding	98.0	98.4	98.6	98.8	98.5
SEED annotated proteins	2,781,887	3,655,843	3,408,756	6,598,355	4,127,096
% SEED annotated proteins	15.2	15.8	16.9	16.2	17.6
taxonomical annotated proteins	7,582,770	9,964,833	9,321,372	17,987,432	11,240,625
% taxonomical annotated proteins	41.4	43.2	46.3	44.2	47.9

metaT	BARCELONA						
	C0.5A	C0.5B	T0.5A	T0.5B	C24A	C24B	T24A
Total joined reads	71,196,214	65,749,050	80,443,490	59,187,424	45,460,596	49,141,324	72,055,396
Internal standard reads	3,333,633	1,733,772	2,855,576	1,836,766	1,715,675	1,402,204	2,081,764
Standard normalization factor	8,237	15,701	9,099	13,642	18,707	19,240	12,868
rRNA reads	38,549,874	35,077,320	41,627,924	30,912,998	23,877,182	26,128,926	40,196,870
% rRNA	54.1	53.4	51.7	52.2	52.5	53.2	55.8
Possible proteins	26,225,946	26,607,844	31,902,212	24,528,872	17,841,666	19,942,618	27,769,144
% potential protein-coding	36.8	40.5	39.7	41.4	39.2	40.6	38.5
SEED annotated proteins	5,829,599	7,034,102	6,727,065	7,427,642	4,051,560	5,409,757	6,567,605
% SEED annotated proteins	22.2	26.4	21.1	30.3	22.7	27.1	23.7
taxonomical annotated proteins	11,436,811	12,949,186	13,428,986	12,842,386	7,814,300	9,759,938	12,082,093
% taxonomical annotated proteins	43.6	48.7	42.1	52.4	43.8	48.9	43.5

C: Control; T: ADOC amendment; 0.5: time point after 30min; 24: time points after 24h.

		BLANES					
T24B	C0.5A	C0.5B	C24A	C24B	T24A	T24B
20,349,270	28,629,172	29,408,022	27,582,978	39,349,270	39,120,946	28,745,756
116,148	121,795	136,780	120,845	185,546	172,068	124,764
69.8	11.0	12.8	38.8	25.9	16.2	37.9
19,943,113	28,272,006	29,027,193	27,009,787	38,985,838	38,740,810	28,395,316
98.0	98.8	98.7	97.9	99.1	99.0	98.8
3,417,074	3,627,340	4,136,437	3,985,255	6,259,450	5,567,487	4,290,304
17.1	12.8	14.3	14.8	16.1	14.4	15.1
9,337,833	9,196,484	10,420,386	10,504,366	16,177,666	15,724,605	10,991,565
46.8	32.5	35.9	38.9	41.5	40.6	38.7

	BLANES							
T24B	C0.5A	C0.5B	T0.5A	T0.5B	C24A	C24B	T24A	T24B
69,498,944	56,039,906	58,886,780	70,649,934	45,376,792	47,592,808	49,519,480	52,384,250	56,282,260
2,063,560	2,700,576	2,710,566	4,878,764	2,063,275	1,496,070	1,605,104	1,568,920	1,763,148
12,782	2,352	2,447	1,649	3,796	7,362	6,339	6,653	7,338
36,769,092	30,385,222	34,508,474	44,095,244	24,258,582	24,436,358	27,506,742	26,867,308	28,349,502
52.9	54.2	58.6	62.4	53.5	51.3	55.5	51.3	50.4
28,777,260	21,211,720	20,412,994	19,970,666	17,250,244	20,105,176	19,286,648	22,540,612	24,482,494
41.4	37.9	34.7	28.3	38.0	42.2	38.9	43.0	43.5
7,661,793	3,973,147	3,910,150	3,291,962	3,171,908	5,160,610	4,525,547	5,590,791	5,546,360
26.6	18.7	19.2	16.5	18.4	25.7	23.5	24.8	22.7
13,935,397	8,309,550	8,027,193	7,637,533	6,579,092	9,637,850	8,563,266	10,427,887	10,773,189
48.4	39.2	39.3	38.2	38.1	47.9	44.4	46.3	44.0

Table S2. Seawater dissolved phase concentrations of n-alkanes, polycyclic aromatic hydrocarbons (PAH) and organophosphate esters (OPE) flame retardants and plasticizers in Barcelona (BCN) and Blanes (BL).

Family	Compound	Concentration (ng/l)	
		BCN	BL
n-alkanes	Dodecane	0.148	< LOQ
	Tridecane	< LOQ	< LOQ
	Tetradecane	0.178	0.010
	Pentadecane	0.329	< LOQ
	Hexadecane	1.575	< LOQ
	Heptadecane	2.109	0.269
	Octadecane	3.527	0.558
	Nonadecane	1.282	0.207
	Eicosane	2.630	0.594
	Heneicosane	0.571	0.164
	Docosane	1.911	0.537
	Tricosane	1.182	0.301
	Tetracosane	2.596	0.935
	Pentacosane	2.164	0.862
	Hexacosane	4.735	2.043
	Heptacosane	9.043	2.438
	Octacosane	15.992	7.929
	Nonacosane	22.732	9.647
	Triacontane	29.232	13.629
	Hentriacontane	28.245	13.400
	Dotriacontane	29.664	14.347
	Tritriacontane	27.686	14.022
	Tetratriacontane	25.615	13.854
	Pentatriacontane	22.494	11.191
PAHs	Naphthalene	0.0125	0.0052
	Methylnaphthalenes	0.0020	< LOQ
	Dimethylnaphthalenes	0.0168	< LOQ
	Trimethylnaphthalenes	0.0531	0.0233
	Acenaphtylene	0.0040	< LOQ
	Acenaphtene	0.0032	0.0002
	Fluorene	0.0193	0.0031
	Dibenzothiophene	0.0074	0.0003
	Methyldibenzothiophenes	0.0072	0.0003

	Dimethyldibenzothiopenes	0.0302	< LOQ
	Phenanthrene	0.2116	0.0280
	Methylphenanthrenes	0.1147	0.0030
	Dimethylphenanthrenes	0.0993	0.0035
	Fluoranthene	0.2309	0.0050
	Pyrene	0.1405	0.0056
	Methylpyrenes	0.0232	< LOQ
	Dimethylpyrenes	0.0132	< LOQ
	Benzo[ghi]fluoranthene	0.0184	< LOQ
	Benzo[a]anthracene	0.0099	< LOQ
	Chrysene	0.0451	0.0001
	Methylchrysenes	0.0493	< LOQ
	Benzo[a]pyrene	0.0407	< LOQ
	Perylene	0.0337	< LOQ
	Dibenzo[a,h]anthracene	0.0018	< LOQ
OPEs	TiBP	1.705	0.287
	TnBP	1.346	0.171
	TCEP	2.780	0.479
	TCPP-1	13.866	2.705
	TCPP-2	11.684	2.506
	TCPP-3	11.932	1.659
	TDCP	1.336	0.289
	TPhP	0.097	< LOQ
	EHDPP	0.515	0.125
	TEHP	0.407	0.178

LOQ: Limit of quantification

SUPPLEMENTARY FIGURES

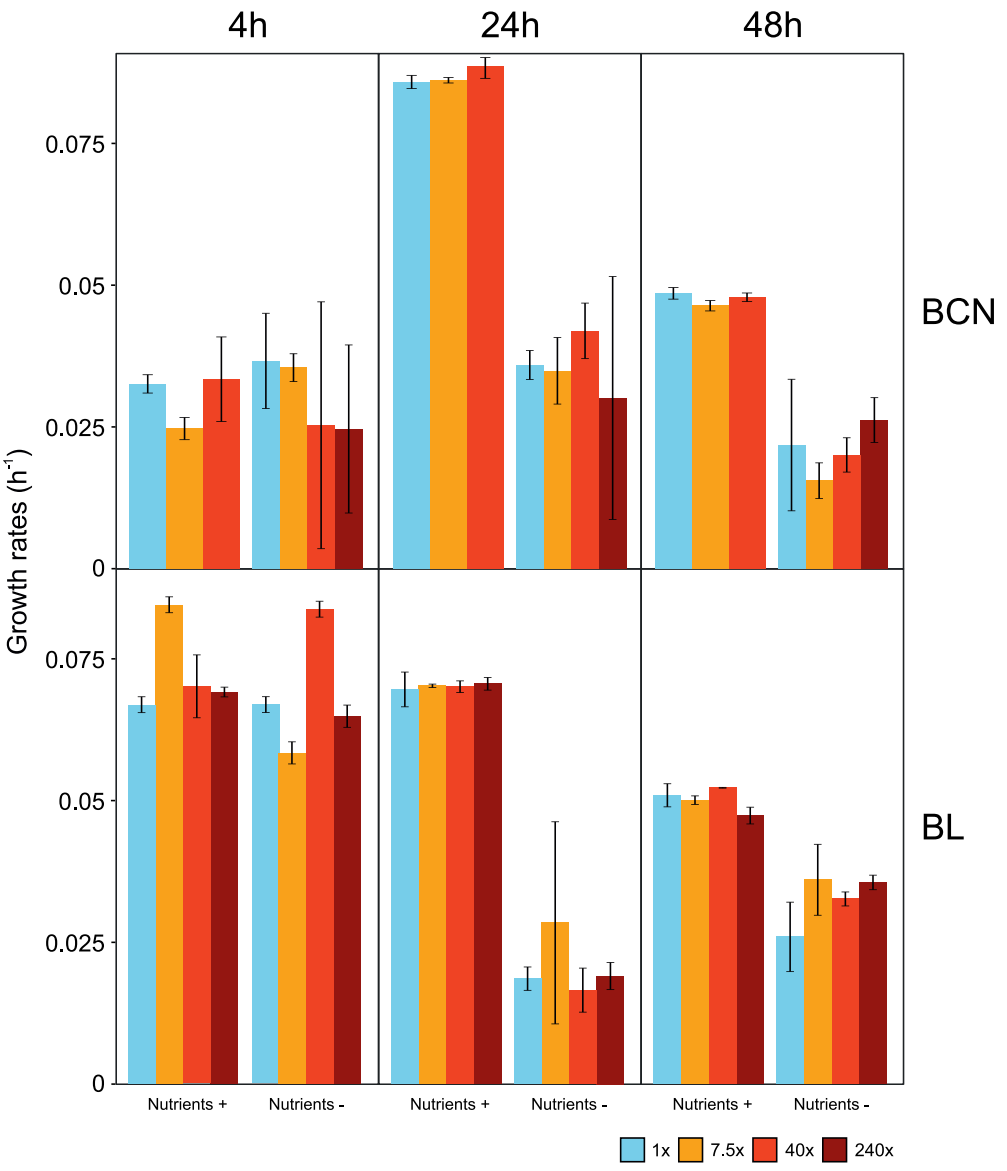

Figure S1. Growth rates (in h^{-1}) of prokaryotic community amended with different ADOC concentrations after 4 h, 24 h and 48 h, and comparing between with and without nutrients addition. Error bars represent standard deviation of replicates.

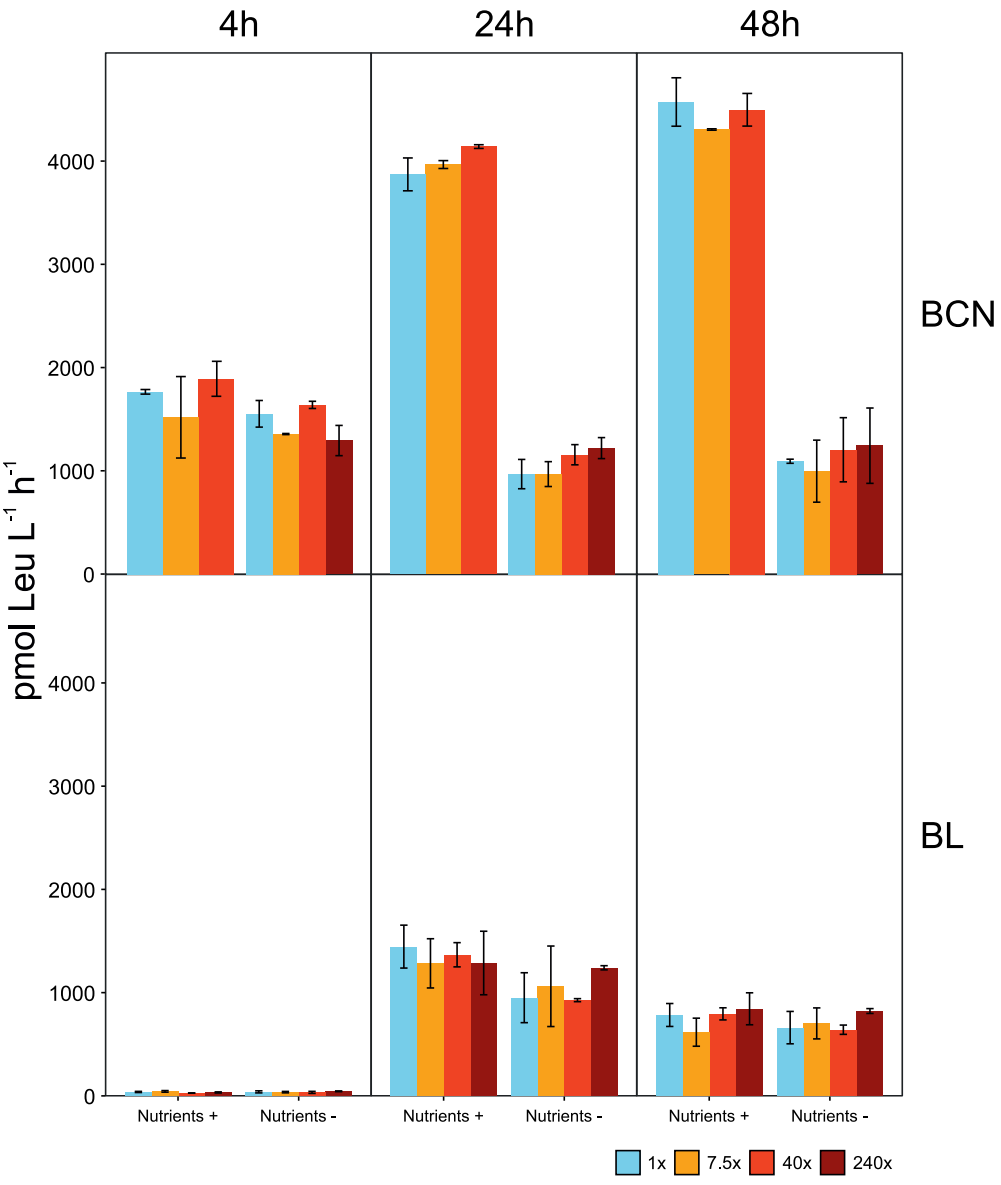

Figure S2. Bacterial production (measured as [^{3}H]Leucine incorporation) of bacterial community amended with different ADOC concentrations after 4 h, 24 h and 48 h, and comparing between with and without nutrients addition. Error bars represent standard deviation of replicates.

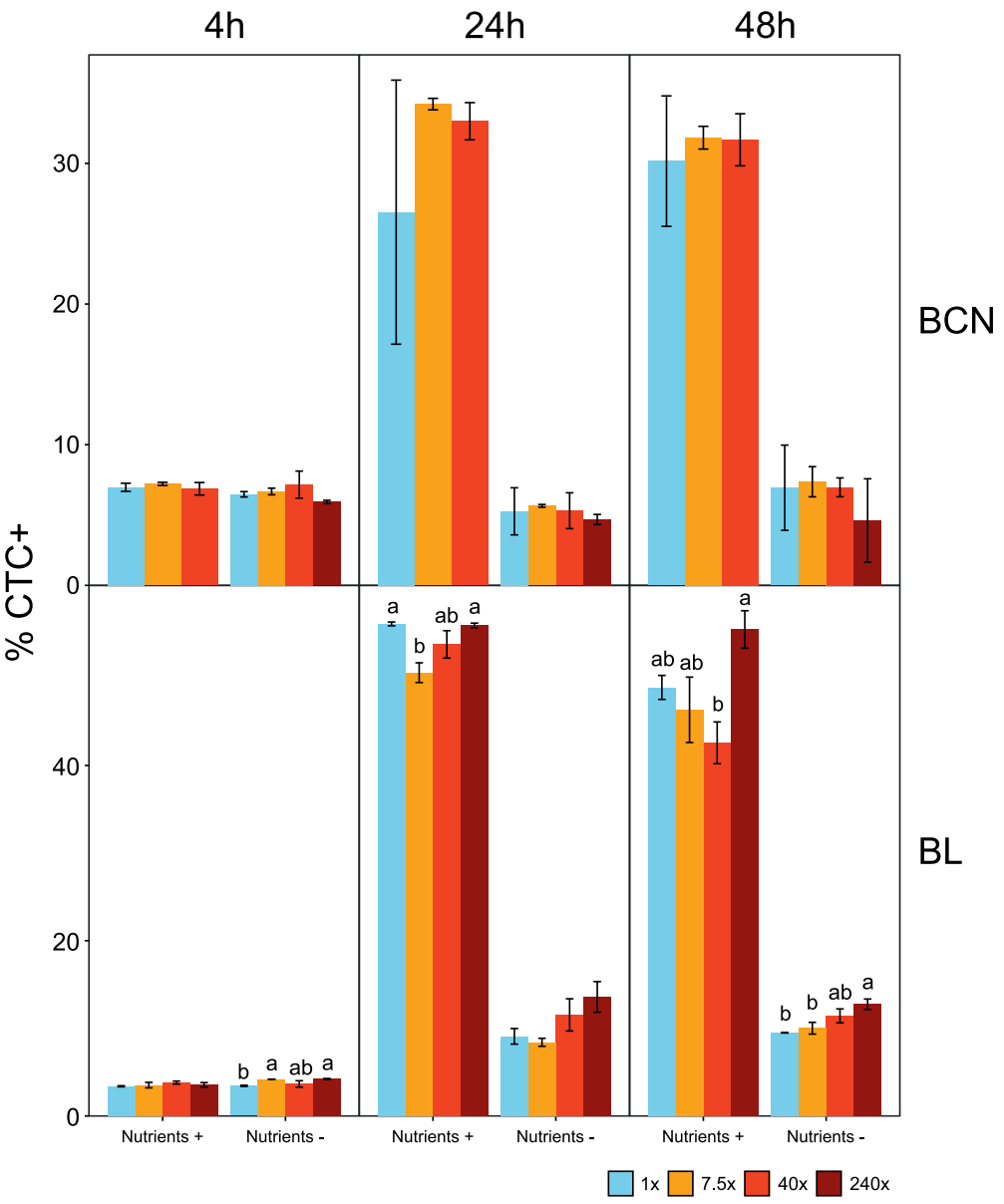

Figure S3. Percentage of actively-respiring bacteria (% CTC+) in the dose-response experiment after 4 h, 24 h and 48 h ADOC amendment, and comparing between with and without nutrients addition. Error bars represent standard deviation of replicates. Differences of mean values were analyzed using 1-way ANOVA followed by a post-hoc Tukey's HSD test and labeled significances ($P < 0.05$) with different letters.

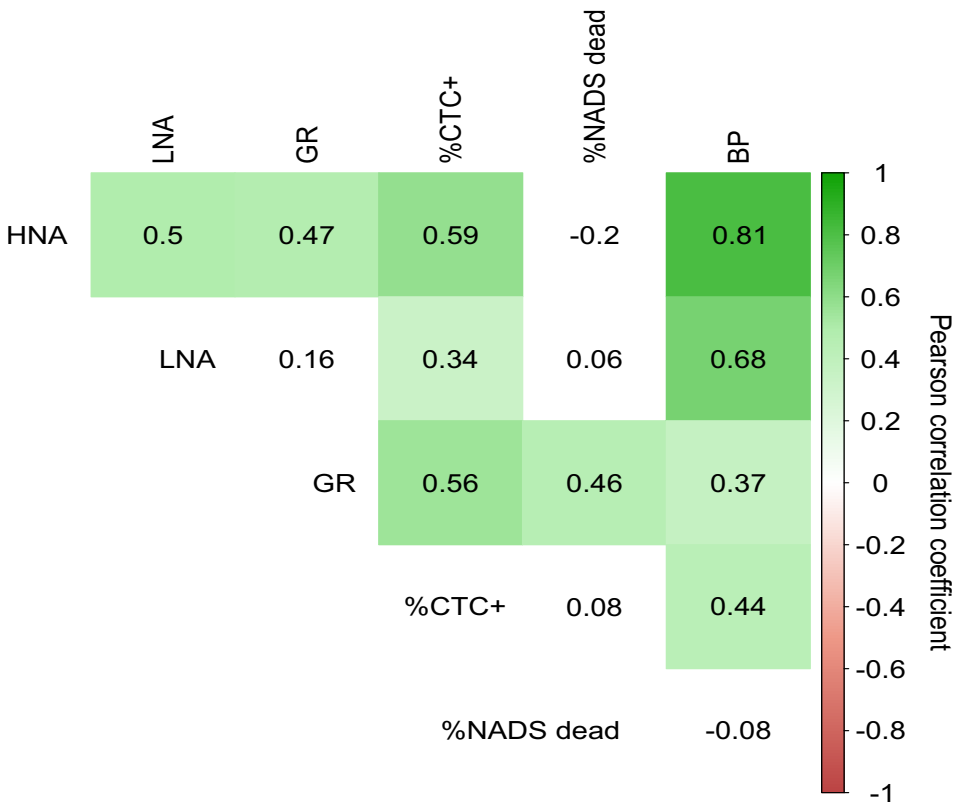

Figure S4. Pearson's correlations between microbial growth rates (GR) at 48 h and added concentrations of pollutants corrected by their initial concentration in seawater. Number is the correlation coefficient and color is proportional to it, being green for positively correlated and red for negatively correlated. Non-significant correlations ($P > 0.05$) are represented as white color.

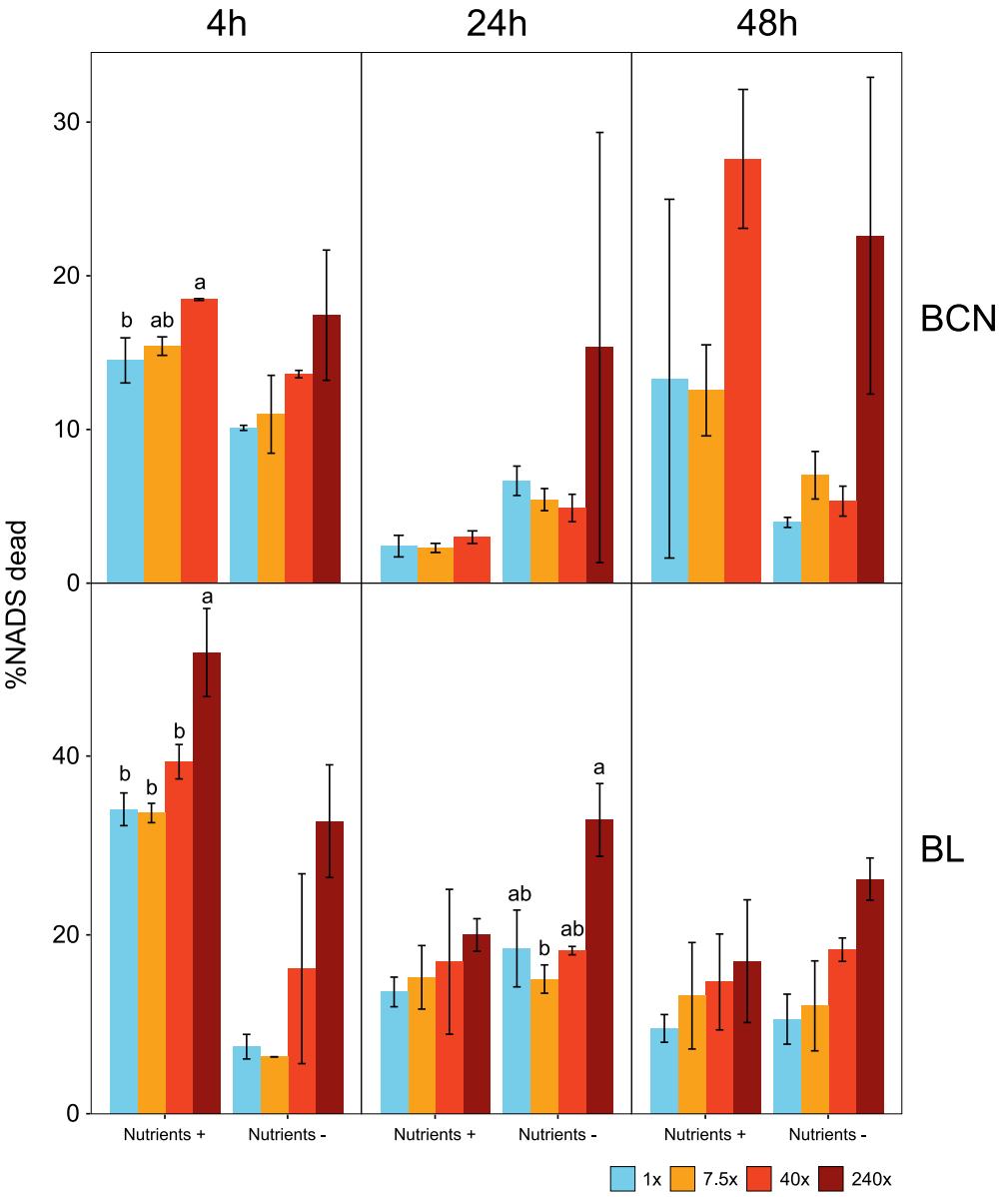

Figure S5. Percentage of damaged or dead cells (% NADS-) in the dose-response experiment after 4 h, 24 h and 48 h ADOC amendment, and comparing between with and without nutrients addition. Error bars represent standard deviation of replicates. Differences of mean values were analyzed using 1-way ANOVA followed by a post-hoc Tukey's HSD test and labeled significances ($P < 0.05$) with different letters.

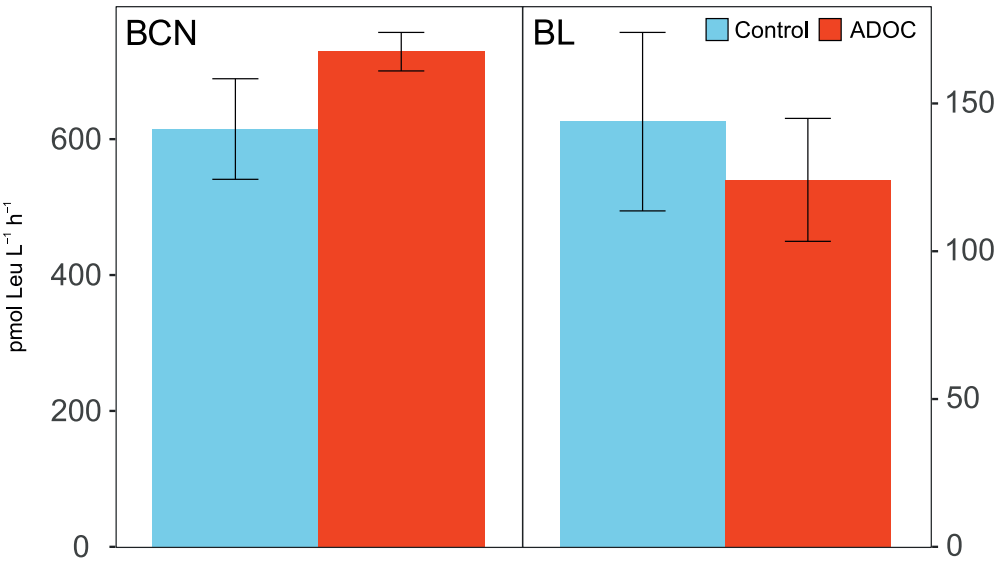

Figure S6. Bacterial production, measured as leucine incorporation rates, of the experiment after 24 h. Error bars represent standard deviation of replicates.

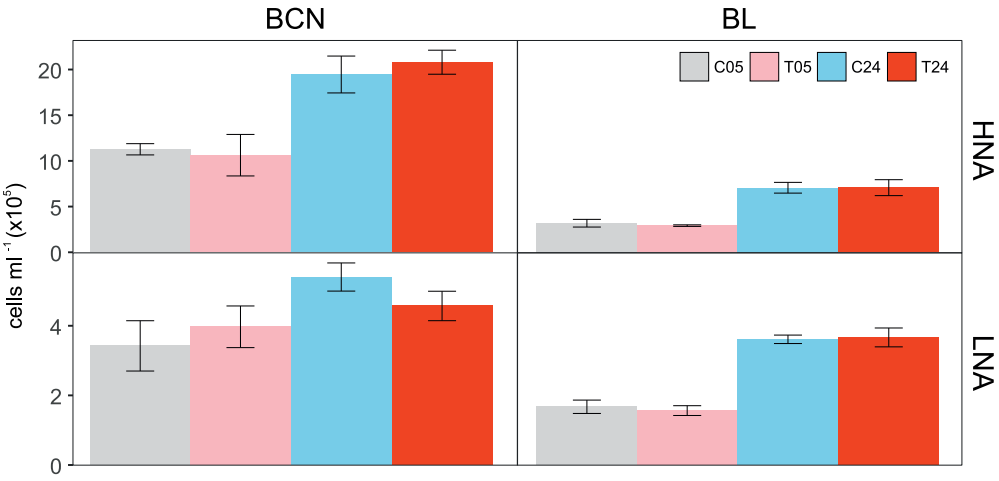

Figure S7. Cell abundances of heterotrophic bacteria in the experiments quantified by flow cytometry. Error bars represent standard deviation of replicates. HNA: high nucleic acid content; LNA: low nucleic acid content; C: control; T: ADOC amendment; 05: time point after 30min; 24: time points after 24h.

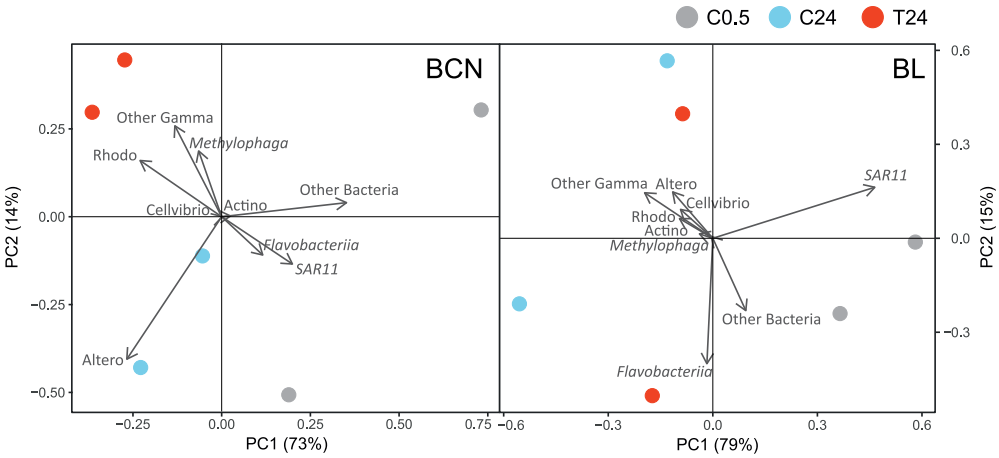

Figure S8. Principal component analysis (PCA) of metagenomic data in the ADOC amendment experiment. Values in parentheses show the % of total variation associated with each principal component. C: Control; T: ADOC amendment; 0.5: time point after 30 min; 24: time points after 24 h; Actino: *Actinobacteria*; Altero: *Alteromonadales*; Cellvibrio: *Cellvibrionales*; Gamma: *Gammaproteobacteria*; Rhodo: *Rhodobacterales*.