Contrasting degradation rates of natural dissolved organic carbon by deep-sea prokaryotes under stratified water masses and deep-water convection conditions in the NW Mediterranean Sea

Marc Garel, Christos Panagiotopoulos, Mehdi Boutrif, Daniel Repeta, Richard Sempere, Chiara Santinelli, Bruno Charrière, David Nerini, Jean-Christophe Poggiale, Christian Tamburini

CONTRASTING DEGRADATION RATES OF NATURAL DISSOLVED ORGANIC CARBON BY DEEP-SEA PROKARYOTES UNDER STRATIFIED WATER MASSES AND DEEP-WATER CONVECTION CONDITIONS IN THE NW MEDITERRANEAN SEA

Marc Garel[1], Christos Panagiotopoulos[1], Mehdi Boutrif[1], Daniel Repeta[2], Richard Sempéré[1], Chiara Santinelli3, Bruno Charrière[4], David Nerini[1], Jean-Christophe Poggiale[1] and Christian Tamburini1*[1]

[1]Aix Marseille Univ., Universite de Toulon, CNRS, IRD, MIO UM 110, 13288, Marseille, France
[2]Department of Marine Chemistry and Geochemistry, Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA
[3]C.N.R., Istituto di Biofisica, Pisa, Italy
[4]Univ. Perpignan Via Domitia, Centre de Formation et de Recherche sur les Environnements Méditerranéens (CEFREM), UMR 5110, 66860 Perpignan, France

* Corresponding author. E-mail address: christian.tamburini@mio.osupytheas.fr (C. Tamburini).

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Abstract

Most of the ocean is deep with the majority of its volume (> 80%) lying under a depth greater than 1000 m. Deep-ocean substrates input is mainly supplied as organic matter (in particulate and/or dissolved forms) by physical and biological processes. Bioavailable dissolved organic carbon (DOC) is mainly consumed in surface water by prokaryotes, while most of DOC in the deep ocean is recalcitrant. Deep-sea prokaryotes are known to be adapted to degrade complex substrates. In this study, we investigate the utilization of HMW-DOC on the short temporal scale (10-15 days) by deep-sea prokaryotes maintained at in situ high-pressure conditions. Deep-sea prokaryotic natural assemblages were collected in the Mediterranean Sea in two contrasting hydrological conditions (water column stratification and deep-water formation period conditions). The experimental results were coupled with a cell-quota model, in order to quantify the kinetics of HMW-DOC degradation and its impact on the prokaryotic assemblages under these two contrasting hydrological conditions. The results show that under stratified water conditions autochthonous deep prokaryotic assemblages are able to degrade up to 46.6% of DOC on the timescales of the incubation, when maintained under in situ sampling high-pressure conditions. By contrast, during deep-water convection period condition, DOC is weakly degraded on the timescales of the incubation under in situ high pressure conditions. This study shows that the remineralization rates of DOC are controlled by the prokaryotic communities, which are further driven by the hydrological conditions of the water column.

1 INTRODUCTION

The deep sea, below 1000 m, is one of the largest ecosystems and the largest biome on Earth, yet it is the least well studied and sampled. It is a microbially dominated environment, where prokaryotes drive the most important biological processes. It is characterized by high-hydrostatic pressure, low temperature, high nutrient concentrations and low dissolved organic carbon content (around 33-45 µMC) (Nagata et al., 2010). The main source of carbon and energy is organic matter produced in the euphotic layer and transferred to depth by different processes (Boyd et al., 2019; Dall'Olmo et al., 2016; Hansell et al., 2009; Levy et al., 2013; Siegel et al., 2016). Hence, carbon dioxide and inorganic nutrients are converted by photosynthesis into particulate (POC) and dissolved organic carbon (DOC) in the euphotic zone, and transferred to the deeper ocean by various mechanisms including winter deep convection (Copin-Montégut and Avril, 1993), subduction (Aristegui et al., 2009; Burd et al., 2010; Hansell, 2009; Sohrin and Sempéré, 2005), fragmentation of large aggregates into small particles (Briggs et al., 2020), dissolution of sinking particles (Follett et al., 2014; Smith et al., 1992) and the vertical migration of zooplankton with release of POC/DOC by exudation and defecation (Steinberg et al., 2000; Steinberg and Landry, 2017).

DOC concentration decreases with depth (Hansell et al., 2009) and can be divided into two reservoirs of different biological lability: labile-DOC and recalcitrant DOC (Carlson and Hansell, 2015; Hansell et al., 2009). The recalcitrant fraction can be further divided in semi-labile, semi-refractory, refractory and ultra-refractory DOC (Hansell, 2013). Semi-labile DOC accumulates in the surface ocean where DOC production exceeds DOC consumption and is transferred in the dark ocean where consumption exceeds production (Carlson et al., 1994; Carlson and Hansell, 2015). However, turnover times for semi-labile DOC are largely unconstrained, and the contribution of semi-labile DOC to global prokaryotic heterotrophic production is hard to quantify (Carlson, 2002; Carlson and Hansell, 2015). From a chemical point of view, a major portion of surface high-molecular-weight-DOC (HMW-DOC) is composed of carbohydrates (acyl-polysaccharides) that have remarkably conservative spectrometric and chemical properties throughout the global ocean (Aluwihare et al., 1997a; Benner et al., 1992; Repeta, 2015).

Heterotrophic prokaryotes are one of the main actors of the carbon cycle in marine ecosystems. They i) consume 10 to 50% organic matter produced by primary production in the surface waters (Azam et al., 1983), ii) facilitate particle solubilization (Aristegui et al., 2009; Cho and Azam, 1988; Panagiotopoulos et al., 2002; Sempéré et al., 2000; Tamburini et al., 2009b), iii) produce semi-labile DOC from labile DOC (ranged 0.1 to 0.2 Gt C yr^{-1}) (Fang et al., 2015; Jiao et al., 2010; Tamburini et al., 2003) and finally, iv) account for a large part of heterotrophic respiration in open ocean (20–33.3 Gt C yr^{-1}) (Arístegui, 2003; del Giorgio et al., 1997). However, there are still significant gaps in our knowledge of prokaryotic processes that control transformation, and degradation of the organic matter in the deep sea.

Most studies of DOC degradation have targeted the labile fraction and used simple compounds coupled with radioelements or fluoregenic substrate analogs to measure uptake and turnover time (Hoppe and Ullrich, 1999; Koike and Nagata, 1997; Tamburini et al., 2009a, 2002; Teira et al., 2006a, 2006b). As such very little is known about the degradation of semi-labile or semi-refractory DOC fractions. In addition, most studies have not taken into account the effect of hydrostatic pressure, which is a major parameter acting on prokaryotic metabolisms (Tamburini et al., 2013a). One of the few studies of semi-labile DOC degradation under pressure was made by Boutrif et al., (2011) who used radiolabeled exopolysaccharides (3H-EPS) as a proxy semi-labile DOC and incubated their samples under in situ high-pressure conditions. Using this approach, Boutrif and colleagues were able to show (i) an increase with depth of specific-cell activity assimilating 3H-EPS and (ii) a high contribution of Euryarchaeota in driving the degradation of 3H-EPS in the deep-sea waters of the Mediterranean Sea.

The phylogenetic composition and metabolic capabilities of marine microbial communities change with depth (Delong et al., 2006; Nagata et al., 2010) as does metabolic capabilities of the microbial communities that regulate carbon export (Giovannoni and Stingl, 2005; Mccarren et al., 2010). Delong et al., (2006) identified a great number of genes, putatively involved in polysaccharide degradation, in deep microbial populations compared to those found in the surface. More recently, using comparative metaproteomics, Bergauer et al., (2018) found that deep-sea microorganisms produce transporters not only for substrate such as amino acids and carbohydrates, but also for osmolytes. These osmolyte transporters increase with depth, reaching 39% of protein sequences identified in the bathypelagic zone. Recently, Saw et al., (2020) describe the enzymatic repertoire of resident mesopelagic and bathypelagic SAR202 that

appear to favor remineralization of recalcitrant DOC. Moreover, under high hydrostatic pressure, piezophile deep-sea microorganisms display unique metabolisms (Campanaro et al., 2005; Lauro and Bartlett, 2008). As an example, piezophilic Photobacterium profondum SS9 have shown its ability to degrade complex organic matter. The regulation of metabolic pathways for the degradation of different polymers such as chitin, pullulan, and cellulose is controlled by pressure, being activated at 28 MPa and turned off at 0.1 MPa (Vezzi et al., 2005). Fieldwork investigation using 3H-labeled extracellular polymeric substance labeled (3H-EPS), done at in situ high pressure conditions, showed that prokaryotes increased their ability to use 3H-EPS with depth (Boutrif et al., 2011).

The Mediterranean Sea is a semi-enclosed sea, with a deep warm temperature (~13°C), and very short ventilation and residence times for deep waters of ~70-126 years (Schlitzer et al., 1991). The NW Mediterranean Sea is one of the few regions in the world's ocean where both dense shelf water cascading and open-sea convection take place (Canals et al., 2006; Durrieu de Madron et al., 2017, 2011; Mertens and Schott, 2002; Santinelli et al., 2010; Tamburini et al., 2013b). These sinking water masses carry POC and DOC, as well as significant numbers of organisms from the surface layer into the deep sea (Avril, 2002; Marshall and Schott, 1999; Martín et al., 2010; Santinelli, 2015; Santinelli et al., 2010; Testor and Gascard, 2006; Vidal et al., 2009). Depending on the preconditioning phase different volumes of surface water are exported (in a more cold and dry winter, more water will sink; Durrieu de Madron et al., 2017), driving a larger amount of dissolved oxygen, semi-labile DOC (Canals et al., 2006; Lefèvre et al., 1996; Powley et al., 2017; Santinelli, 2015; Santinelli et al., 2010) and surface-prokaryotic communities (Luna et al., 2016; Severin et al., 2016; Tamburini et al., 2013a) from surface to deep-sea layers.

This paper investigates the utilization of HMW-DOC on the short temporal scale (10-15 days) by deep-sea prokaryotes maintained at in situ high-pressure conditions. Deep-sea prokaryotic natural assemblages were collected in the Mediterranean Sea in two contrasting hydrological conditions including water column stratification and deep-water formation period conditions. Here, the experimental results were coupled with a cell-quota model (Droop, 1968), in order to quantify the kinetics of HMW-DOC degradation and its impact on the prokaryotic assemblages under these two contrasting hydrological conditions.

2 MATERIALS AND METHODS

2.1 RECOVERING AND PREPARATION OF HMW-DOC

HMW-DOC was obtained from seawater that was drawn from the 600 m depth at the Natural Energy laboratory of Hawaii Authority NEHLA (19° 43' 42.7"N; 156° 03' 33.2" W) in December 2003. Samples were filtered in-line, and the <0.2 µm fraction concentrated using a custom crossflow ultrafiltration system fitted with a GE-osmonics "GE series" membrane. The membrane has a pore size of ~1 nm and nominally retains organic matter of molecular weight >1 kDa (>99% rejection of vitamin B12 in laboratory tests). Seawater (~1800 L) was concentrated daily to 20 L, frozen and returned to the laboratory for further processing. In the laboratory, HMW-DOC concentrates were thawed and filtered through an Ultracel 30kD membrane (Millipore Corp.) to remove viruses and other small particles, reduced in volume to 2 L by ultrafiltration, and desalted by serial (~10x; 2L each) dilution/concentration with ultra-high purity water until addition of a 0.1N $AgNO_3$ solution remained clear. Samples from different concentrates were pooled and freeze-dried to a fluffy white powder that was ~36% carbon. Between 18-21% of the total dissolved organic carbon in seawater was recovered (modified protocole from Repeta and Aluwihare, 2006).

2.2 STUDY SITE AND HYDROLOGICAL CONDITIONS

Water samples were collected at 2000 m depth in August 2008 and April 2010 at the ANTARES study site (42°48'N; 6°10'E) about 40 km offshore the French Mediterranean coast (NW Mediterranean Sea; Tamburini et al., 2013b). The hydrological conditions at this site were monitored over the time period of two and a half years prior to sample collection as part of the cabled ANTARES neutrino telescope and EMSO (European Multidisciplinary Subsea Observatory) observation program (ANTARES – EMSO-LO site), in addition measurements of NW Mediterranean deep convection zone located in the Gulf of Lions (LION Site; Tamburini et al. 2013b). In August 2008, the water column was characterized by a strong summertime stratification, which isolates the Western Mediterranean Deep Water from the upper water masses. In contrast, deep–sea waters sampled in April 2010, were recorded as modified due to a strong deep-water convection (see for details Tamburini et al., 2013b and Durrieu de Madron et al., 2017) causing surface water cooling, vertical mixing and finally homogenization of the water column to the deep that occurred in the Gulf of Lion. These two experiments will be called hereafter stratified (Strat.) and deep-water convection period (Conv.), respectively.

2.3 SAMPLING

Deep–sea water (2000 m) was sampled and kept in high pressure bottles (HPBs) to maintain constantly the ambient high hydrostatic pressure (20 MPa) during all manipulations (sampling and retrieval, incubation, transfer and sub-sampling) corresponding to the in-situ depth conditions (Garel et al., 2019). Before collecting samples, 500µL of the HMW-DOC solution was added in the top-part of HPBs (Figure 1). To assess the effect of pressure on deep-sea prokaryotes, two HPBs were maintained at in situ high-pressure condition (thereafter HP). To determine the effect of HP on our results, two replicates were decompressed and incubated at atmospheric pressure (thereafter DEC). Before use, HPBs (volume capacity 500 ± 20 mL) were thoroughly cleaned as described in Garel et al. (2019). Using this protocol, the organic carbon background (DOC) in the HPBs bottles was ≤ 2 µMC.

2.4 INCUBATION EXPERIMENTS

HMW-DOC solution was amended into the HPBs, just before the CTD cast, to a theoretical final concentration of 120 µMC, and the incubation experiments started once the HPBs came on board (t=0 point). As such, HMW-DOC was incubated with Strat. and Conv. seawater under in situ and atmospheric pressure conditions, in the dark at 13 °C along with the prokaryotic assemblages sampled at 2000 m-depth for 10 and 15 days, respectively. Sub-samples were collected using a piloted pressure generator that maintains the hydrostatic pressure within the HPBs (Garel et al., 2019). Aliquots (in duplicates or triplicates) of 10 – 40 mL were sampled for DOC, sugars, and bacterial abundance measurements.

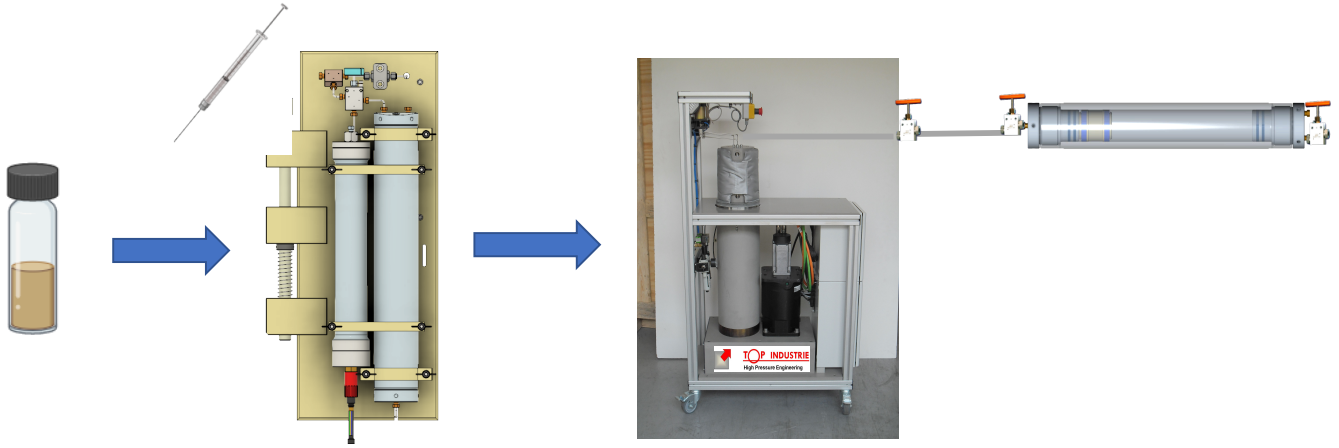
All glassware including glass pipettes and ampoules used for DOC and carbohydrates sampling were combusted at 450 °C for at least 6 h to remove traces of organic compounds. For DOC measurements, 8 mL of sample were collected directly into glass ampoules with 10 µL of H3PO4 85%, flame-sealed, stored in the dark at 4 °C and analyzed within 3 months of sub-sampling. Carbohydrates were collected into cleaned (10% HCl and Milli-Q water) plastic tubes and stored at −20 °C until analyses. Subsamples for microbial abundance were collected into sterile plastic tubes, fixed with formaldehyde (2 - 4% final concentration) and stored at 4 °C for 24 h. Samples were then filtered through 0.22µm-pore-sized polycarbonate filter, dried and stored at −20 °C.

HMW-DOC solution

Before collecting samples, 500 µL of HMW-DOC were added in the top-part of HPBs (500 ± 20 mL)

At each time-point, sub-samples were obtained as described in Garel et al. 2019

Parameters collected in equi-pressure mode at each time-point and at each condition:



2 x 40mL for prokaryotic biomass

2 x 10mL for carbohydrates identification

2 x 8mL for DOC concentration

Initial solution of HMW-DOC
15mg C diluted in 10mL of milliQ water

HPSU described in Garel et al. 2019
- 2 HPSU at *in situ* condition
- 2 HPSU decompressed

PMHP pump allows to maintain *in situ* condition during sub-sampling (see Garel et al. 2019)

Date	Hydrological condition	$[DOC]_{initial}$ (mM) in HMWDOM	$Volume_{added}$ (µL)	$[DOC]_{initial}$ (µM)	Duration of the experiments (d)
August 2008	Strat.	150	500	42	15
April 2010	Conv.	150	500	72	10

Figure 1. Timeline of the HMW-DOC incubation experiment. Before use, high-pressure bottles (HPBs) were thoroughly cleaned as described in Garel et al. (2019). Two experiments were done during stratified (Strat.) and deep-water convection period (Conv.), see text for details. $[DOC]_{initial}$ (µM) is the DOC concentration in the deep-sea water sampled at 2000 m-depth before adding HMW-DOM.

2.5 DOC MEASUREMENTS

DOC was measured by high temperature combustion on a Shimadzu TOC 5000 analyzer (Sohrin and Sempéré, 2005). A four point-calibration curve was performed daily with standards prepared by diluting a stock solution of potassium hydrogen phthalate in Milli-Q water. To avoid the small error associated with day-to-day instrument variability, all samples from a given experiment were analyzed in a single day's run. Procedural blanks were run with acidified sparged Milli-Q water and ranged from 1 to 2 µM C. Blanks were subtracted from the measured values. Deep seawater reference samples (provided by D. Hansell; Univ. Miami) were run daily (43.5 ± 1 µM C, n = 4) to check the accuracy of the TOC analysis. Finally, it is important to note that in our study sub-samples were not filtered, in order to reduce contamination, we therefore consider DOC≈TOC since seawater samples were collected at 2000 m, where POC may be considered as negligible. Experimental errors based on triplicates analyses were estimated to be about 5-8% for DOC.

2.6 DISSOLVED COMBINED NEUTRAL SUGARS (DCNS) DETERMINATION

Aliquots of 8 mL were desalted using dialysis tubes with a molecular weight cut-off of 100-500 Da (Spectra/Por® Biotech cellulose ester) (Engel and Händel, 2011). Before use, dialysis tubes were rinsed with Milli-Q water to remove sodium azide. Before applying the sample, dialysis tubes were rinsed with 1–2 mL of the sample volume. Then, the dialysis tube was filled with 8 mL of the sample and the dialysis was conducted in a 1 L beaker filled with Milli-Q water at 4 °C. Dialysis was achieved after 4-5 h (salinity dropped from 35 to 1–2 g L^{-1}). Prior to sample removal, the tubes were placed into an ultrasonic bath for 5 min. Samples were transferred into pre-combusted glass vials (500 °C, 4 h) and freeze dried. The obtained powder was hydrolyzed with 1M HCl for 20 h at 100 °C (Engel and Händel, 2011; Murrell and Hollibaugh, 2000). As a precaution, we ensured the complete removal of HCl by adding 2–3 mL of Milli-Q water and repeating the freeze-drying. The dried sample was then re-dissolved in 4 mL of Milli-Q water, filtered through quartz wool, and pipetted into scintillation vials and kept at 4 °C until the time of analysis (this never exceeded 24 h). Finally, it is important to note that the current desalination procedure does not allow for the determination of the dissolved free neutral sugars (i.e., sugar monomers present in samples with MW~180 Da) because these compounds are lost and/or poorly recovered during the dialysis step (Panagiotopoulos et al., 2014).

Carbohydrate concentrations in samples were measured by HPAEC-PAD according to (Mopper et al., 1992) and were modified by (Panagiotopoulos et al., 2012, 2001). Briefly, monosaccharides were separated on an-anion exchange column (CarbopacPA-1, Dionex) by isocratic elution (mobile phase 19 mM NaOH) and were detected by an electrochemical detector set in the pulsed amperometric mode (Panagiotopoulos et al., 2014). The flow rate and the column temperature were set at 0.7 mL min^{-1} and 17°C, respectively. Data acquisition and processing were performed using the Dionex software Chromeleon. The recovery yields of the whole procedure (dialysis and hydrolysis) were estimated using standard polysaccharides (laminarin, and chondroitin sulfate) and ranged from 82 to 86% (n = 3). Repeated injections (n = 6) of a dissolved sample previously submitted to dialysis and hydrolysis resulted in a CV of 12 - 15% for the peak area, for all sugars.

2.7 Prokaryotic abundance

Prokaryotic cells were stained by 4, 6-di-amidino-2-phenylindole (DAPI, final concentration 25 µg mL^{-1}). Total cells marked by DAPI were counted using semi-automatic epifluorescence microscope (Olympus B61) and image analyses with biovolume calculation were done as described by Cottrell and Kirchman, (2003). Biovolume (V, Table 1) was converted in carbon content (CC) (µM C) according to the methods described in Posch (2001) using the following equation: $CC = 218 \times V\text{-}0.86$ (Loferer-Krössbacher et al., 1998). Prokaryotes biomass (µg C L^{-1}) is calculated as the product of abundance (DAPI-cells count, Table 1) and mean cellular carbon content (CC). Experimental errors based on duplicates analyses were estimated to be about 3-5%.

2.8 Dynamics of DOC and prokaryotes adjusted to a Droop Model

A cell-quota model (Droop, 1968) was built to represent both the kinetics of HMW-DOC and growth of microbial community. Such model, which has been already reported as much relevant for prokaryote dynamics as well (Eichinger et al., 2010, 2009, 2011) describes here the ability of the deep-sea microbial community to degrade HMW-DOC, store carbon with cell-quota and coupling HMW-DOC, uptake and biomass synthesis. Equations of the model are the following:

$$\begin{cases} \dfrac{dX}{dt} = \mu_{max} X \left(1 - \dfrac{Q_m}{Q}\right) \\ \dfrac{dDOC}{dt} = -k(DOC - DOC_{rec})X \\ \dfrac{dQ}{dt} = k(DOC - DOC_{rec}) - \mu_{max}(Q - Q_m) \end{cases}$$

The model involves three state variables: the biomass of microorganisms denoted as X (µM C), the concentration of the substrate, here HMW-DOC, denoted as DOC (µM C) and the intracellular quota of nutrient, here the carbon, denoted as Q (µM C. µmol cells^{-1}). The growth rate of prokaryotes, µ is the product of the maximum growth rate μ_{max} by an increasing function of the intracellular quota Q. The parameter Q_m is defined as a threshold of intracellular quota, under which growth of prokaryotes is inhibited. The dynamics of concentration of substrate DOC depends on the cell biomass X that degrades substrate with degradation rate k. Only a fraction of DOM is labile and can be degraded by the microbial community present in the closed system, thus we denote by DOC_{rec} the concentration of the recalcitrant fraction of carbon substrate. Using the sampled data $(x_i, doc_i), i = 1, \cdots, n$ in each experimental condition, parameters μ_{max}, k, Qm and DOC_{rec} were estimated by non-linear regression (Nelder-Mead simplex method, Seber and Wild, 2003) when minimizing the sum of squared error (SSE) which measures a distance from the data to the model as follows:

$$SSE(\mu_{max}, k, Q_m, DOC_{rec}) = \sum_{i=1}^{n} \left(\frac{X(t_i) - x_i}{s_x}\right)^2 + \sum_{i=1}^{n} \left(\frac{DOC(t_i) - doc_i}{s_{doc}}\right)^2.$$

This cost function has been constructed to give the same weight between cell biomass X and concentration of the substrate DOC by dividing each distance with its respective standard deviation, s_x for the cell biomass data and s_{doc} for the substrate concentration data. At each step of the minimization process, the trajectories $X(t)$ and $DOC(t)$ were estimated from the model by numerical integration (Euler method) until convergence to the minimum of SSE. Once the parameters were estimated and under some classical statistical hypotheses, bootstrapped data were drawn from the distribution of the model errors (Seber and Wild, 2003, chap. 2). The minimization process has been reconducted 500 times. By this way, a 95% confidence interval for each parameter as well as some interval of variability of the trajectories $X(t)$ and $DOC(t)$ have been evaluated in each experimental condition. By this way, it is easy to compare the dynamical evolution of each state variable between different experimental

conditions and to assess how variability bands are superimposed i.e. how experience are similar (see Figure 2).

2.9 STATISTICAL METHODS

Non-parametric statistical methods were used to test the parameters (growth rates – μ_{max}, degradation rates – k, minimum quota – Q_m and the remaining DOC at the end of the incubation named recalcitrant carbon – DOC_{rec}) obtained using the Droop model after 500 simulations (see above) and to compare incubation (HP versus DEC) and hydrological (Strat. versus Conv.) conditions. Specifically, Kruskal–Wallis and Pairwise Wilcoxon Rank Sum tests (R Core Team, 2017) were used to compare medians between incubation and hydrological conditions. These tests performed on the box-and-whisker plots attests that each parameter estimated is significantly different ($p < 0.05$) between each incubation and each condition (except for DOC_{rec} between Strat_HP and Conv_HP).

3 RESULTS

3.1 BULK AND MOLECULAR COMPOSITION CHARACTERISTICS OF HMW-DOC THROUGHOUT THE EXPERIMENT

At 2000m-depth, DOC concentration significantly increased from 42 µM in December 2009, prior to a deep-water convection period, to 63 µM in March and 72 µM in May 2010 when the new deep water mass occupied the ANTARES site, concurrently with higher oxygen concentration in the bottom waters between March and mid-June 2010 (see for details Tamburini et al. 2013b and Durrieu de Madron et al. 2017). The *in situ* DOC concentrations for Strat. and Conv. experiments were 42 and 72 µM, respectively; after HMW-DOC addition, DOC initial concentrations in HPBs reached 96 ± 16 and 182 ± 46 µM for Strat. and Conv. experiments, respectively. In order to apply the Droop model to compare the kinetics of DOC degradation between each condition, we normalized the raw data of DOC concentrations (Table 1) to a fixed value of 120 µM, corresponding to the expected theoretical final concentration of DOC (Figure 2). Dissolved combined neutral sugars (DCNS) concentrations after HMW-DOC addition were 9 - 11 µMC for Strat. and Conv. experiments under HP conditions, respectively whereas the corresponding values for the DEC experiment were 5 - 12 µMC. These results indicate that sugars account about 5 - 9% of DOC which is in agreement with literature data showing that sugars represent about <10% of DOC (Table 1; (Panagiotopoulos and Sempéré, 2005).

Table 1. Raw data of DOC concentrations before normalization, DAPI-counts, biovolume and sugars relative abundance during incubation experiments during stratified period (Strat.) and deep-water mass convection period (Conv.) conditions. The contribution of the dissolved combined neutral sugars (DCNS) to the dissolved organic carbon (DCNS/DOC) pool is also given. Abbreviations: Fuc.: Fucose, Rha.: Rhamnose.: Ara.: Arabinose.: GlcN.: Glucosamine, Gal.: Galactose, Glc.: Glucose, Man.: Mannose, Xyl.: Xylose. HP: samples maintained under in situ high pressure condition without decompression; DEC: sampled decompressed and incubated at atmospheric pressure (DEC).

	Raw data of DOC (µM-C)	DAPI counts ($x10^5$ cells mL^{-1})	Biovolume (μm^3)	Fuc.	Rha.	Ara.	GlcN.	Gal.	Glc.	Man.	Xyl.	DCNS/DOC	DCNS (µMC)
HMW-DOC in the initial solution				13%	17%	5%	19%	11%	14%	11%	11%		
HP T_0 (Strat.)	111±3.5	0.67	0.05±0.03	0%	1%	1%	2%	16%	63%	8%	10%	8%	9.0
HP T_2 (Strat.)	75±7.1	0.70±0.7	0.07±0.05	2%	2%	1%	3%	20%	55%	9%	8%	8%	5.8
HP T7 (Strat.)	56±4.2	3.98±1.26	0.09±0.06	3%	2%	2%	2%	19%	58%	7%	7%	9%	5.1
HP T_{15} (Strat.)	62±07	6.09±0.09	0.08±0.04	2%	2%	3%	2%	19%	55%	9%	9%	9%	5.8
DEC T_o (Strat.)	81±0.7	0.66±0.25	0.05±0.03	4%	2%	4%	3%	25%	47%	7%	8%	6%	5.2
DEC T_2 (Strat.)	83±0.7	1.43±0.55	0.08±0.03	5%	3%	3%	4%	22%	47%	9%	7%	6%	5.1
DEC T_7 (Strat.)	74±2.1	3.52±0.34	0.07±0.1	5%	3%	4%	3%	21%	47%	9%	8%	8%	4.9
DEC T_{15} (Strat.)	78±2.8	6.03±0.09	0.07±0.1	3%	2%	2%	3%	23%	53%	9%	6%	6%	4.8
HP T_0 (Conv.)	157±21	0.28	0.06±0.04	2%	1%	1%	6%	12%	59%	12%	7%	7%	10.7
HP T_2 (Conv.)	167±27	1.30±0.14	0.05±0.03	2%	2%	2%	5%	12%	60%	11%	6%	5%	9.0
HP T_5 (Conv.)	151±29	2.15±0.07	0.07±0.05	3%	2%	2%	5%	11%	63%	10%	4%	6%	9.7
HP T_{10} (Conv.)	138±20	4.9	0.08±0.06	3%	2%	1%	8%	13%	53%	14%	6%	7%	10.2
DEC T_o (Conv.)	208±57	0.28	0.06±0.03	0%	1%	1%	3%	32%	48%	13%	3%	6%	11.8
DEC T_2 (Conv.)	161±36	1.07±0.05	0.05±0.03	1%	2%	1%	4%	37%	45%	7%	3%	7%	11.4
DEC T_5 (Conv.)	134±30	4.56±0.7	0.06±0.05	2%	2%	2%	5%	22%	52%	9%	5%	8%	10.3
DEC T_{10} (Conv.)	143±28	6.0±0.42	0.08±0.03	3%	3%	3%	5%	27%	46%	8%	6%	7%	10.4

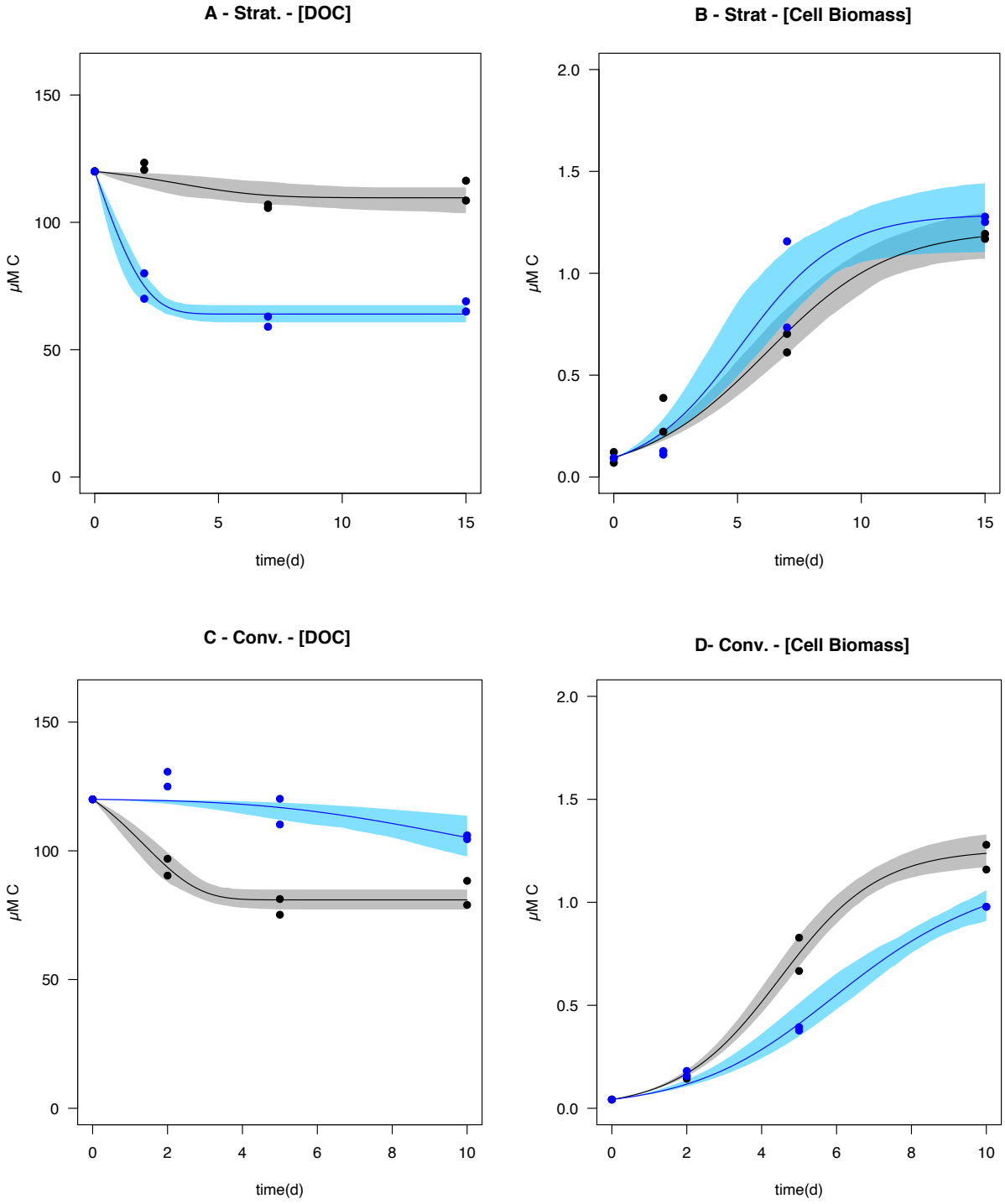


Figure 2. Changes in DOC concentrations (A, C), cell biomass concentrations (B, D) in µMC over time (in days) during stratified period (Strat.) and deep-water mass convection period (Conv.). Blue and black dots are observations respectively for HP and DEC incubations. Blue and black lines are predicted data by model and blue and grey variability bands are 95% confident intervals, respectively for HP and DEC incubations.

Before the addition to the HPBs, the analysis of hydrolyzed HMW-DOC indicated the presence of six neutral monosaccarides namely fucose, rhamnose, galactose, glucose, mannose and xylose occurring at approximately equimolar abundance (11-17% of DCNS) as well as arabinose (5% of DCNS) and glucosamine (19% of DCNS). With the exception of glucosamine, which was unusually high in this sample, all the above values agree well with previous investigations and support the idea that these sugars are an important component of acyl-polysaccharides or semi-labile DOM (Aluwihare et al., 1997b; McCarthy et al., 1996; Repeta, 2015; Skoog and Benner, 1997). Although we have not measured in situ background DCNS concentrations at the ANTARES site, data obtained during Conv. conditions (CASCADE cruise 1-23 March 2011) suggested that DCNS represented 1.7% of DOC whereas glucose (49% DCNS), galactose (9% DCNS) and rhamnose (9% DCNS) were among the most abundant monosaccharides. Immediately after HMWDOM addition in HPBs bottles, glucose accounted for 59 to 48% of the DCNS at the beginning of the experiments while at the end it represented 53 - 46% of the DCNS, in HP and DEC incubations respectively, suggesting a selective degradation of this compound during the incubation (Table 1). Galactose was second in abundance accounting for 12 - 32% of the DCNS at the beginning of the experiment and did not show variation throughout the experiment (13-27%), in HP and DEC incubations respectively. With the exception of mannose, the remaining sugars accounted less than 10% of the DCNS pool and again they vary little during the incubation experiment.

3.2 DOC DEGRADATION AND PROKARYOTIC BIOMASS

Key degradation kinetic parameters including DOC degradation rate (k), estimated recalcitrant DOC concentration (DOC_{rec}) as well as maximum growth rate (μ_{max}) and metabolic state of microbial community were estimated in non-steady state and closed conditions by using the Droop model (Figure 2 – Figure 3 and Table 2). Figure 2A compares the kinetics of DOC degradation between observed data (black and blue circles for DEC and HP conditions, respectively) and theorical data (black and blue curves for DEC and HP conditions, respectively) during the stratified period condition. The variability bands around the curves represent 95% confidence intervals showing the statistical difference over time (or not if the variability bands intersect) between incubations. The same method has been used for the cell biomass concentration (Figure 2B).

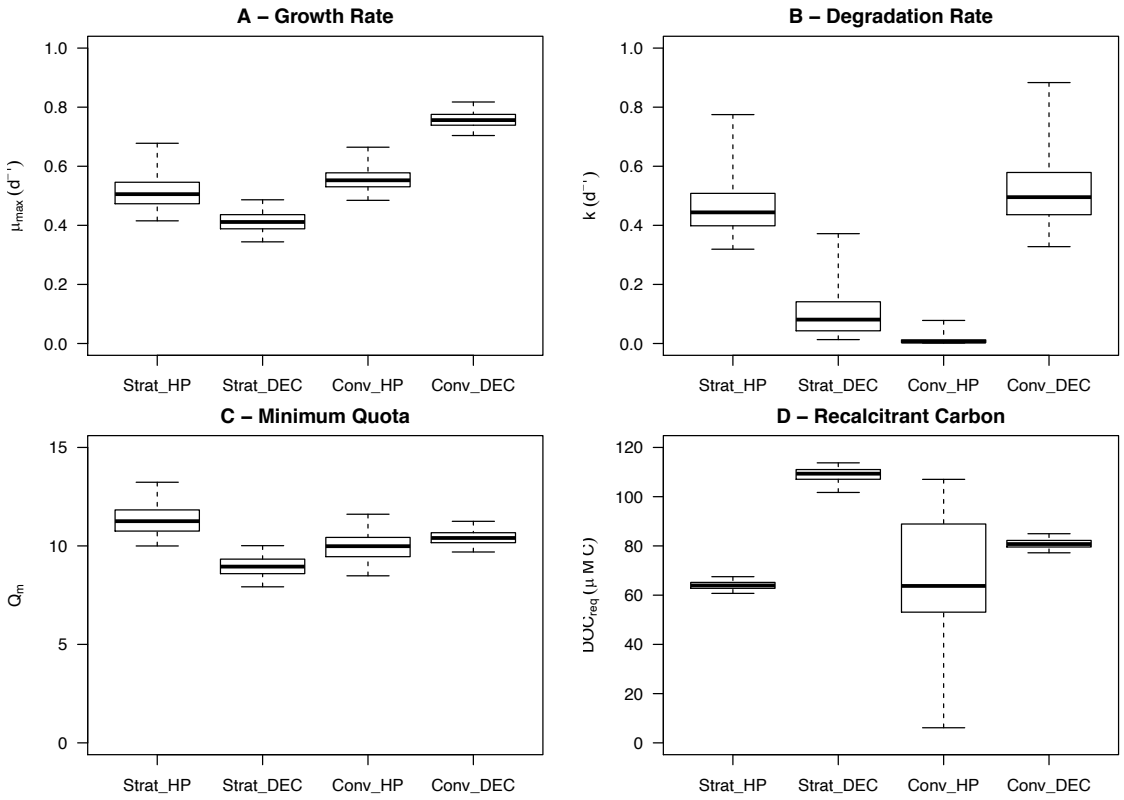


Figure 3. Bootstrapped (with 500 simulations) distribution of parameters estimated by Droop Model during stratified period (Strat.) and deep-water mass convection period (Conv.) conditions maintained under in situ high pressure conditions without decompression of the sample (HP) and decompressed plus incubated at atmospheric pressure conditions (DEC). A) Growth rate (μmax) for both conditions in d-1; B) Degradation constant (k) in d-1; C) Minimum cell-quota (Qm) (a ratio between intracellular concentration of organic carbon and cellular biomass, dimensionless); and D) Concentration of recalcitrant (DOCrec) in μMC on the timescales of the incubations. The horizontal lines are respectively, third quartiles, median and first quartiles. The whiskers correspond to the 95% of the confidence interval.

Results from the Conv. period conditions are given in Figure 2 B and D. Hence, it is possible to directly compare HP and DEC incubations on the same graph and between water masses period conditions, Strat_HP versus Conv_HP, comparing all panels in Figure 2).

During Strat. conditions, DOC degradation was significantly higher in HP than in DEC incubations (Figure 2A). On the timescales of the incubations, 56.0 μM (i-e. 46.6% of the initial concentration) of DOC were degraded in HP incubation whereas only 11.2 μM (i-e. 9.3% of the initial concentration) of DOC were degraded in DEC incubation. Degradation rates (*k*) averaged 0.45 ± 0.12 d^{-1} and 0.11 ± 0.10 d^{-1} in HP and DEC conditions, respectively (Table 2). Growth rate was also higher in HP conditions averaging 0.51 ± 0.07 d^{-1} compared to 0.41 ± 0.03 d^{-1} for DEC incubations (Figure 2B), being closed to growth rates median (Table 2).

By contrast, during Conv. period, DOC degradation rates (*k*) were higher (k = 0.51 ± 0.11 d^{-1}) in DEC than in HP incubations (k = 0.01 ± 0.03 d^{-1}) (Figure 2C). Due to this very low DOC

Table 2. Parameters estimated by cell-quota model and bootstrapped variance and median during stratified period (Strat.) and deep-water mass convection period (Conv.) conditions. μmax is the growth rate in d-1; k is the degradation rate in d-1; Qm is the minimum cell-quota (a ratio between intracellular concentration of organic carbon and cellular biomass, dimensionless); DOCrec is the quantity of residual HMW-DOC in μMC. HP: samples maintained under in situ high pressure conditions without decompression; DEC: samples decompressed and incubated at atmospheric pressure conditions (DEC).

	Stratified water period								*Deep-water convection period*							
	HP				*DEC*				*HP*				*DEC*			
	μ_{max}	k	Q_m	DOC_{rec}	μ_{max}	k	Q_m	DOC_{rec}	μ_{max}	k	Q_m	DOC_{rec}	μ_{max}	k	Q_m	DOC_{rec}
mean	*0.51*	*0.45*	*11.32*	*64.02*	*0.41*	*0.11*	*8.94*	*108.80*	*0.56*	*0.01*	*9.05*	*65.04*	*0.76*	*0.51*	*10.42*	*80.85*
sd	*0.07*	*0.12*	*0.81*	*1.75*	*0.03*	*0.10*	*0.55*	*3.37*	*0.08*	*0.03*	*1.34*	*29.11*	*0.02*	*0.11*	*0.18*	*1.99*
median	*0.5*	*0.44*	*11.25*	*63.94*	*0.44*	*0.08*	*8.94*	*109.34*	*0.55*	*0.006*	*9.98*	*63.74*	*0.76*	*0.49*	*10.40*	*80.76*

degradation rates in HP incubations, the DOC_{rec} is highly variable (65.04 ± 29.11 µMC, Table 2). This does not allow us to calculate with confidence a percentage of degradation in this case. Prokaryotic growth rates averaged 0.76 ± 0.02 and 0.56 ± 0.08 d^{-1}, for DEC and HP incubations (Figure 2D and Table 2), respectively. In addition, for HP incubation, the community of prokaryotes does not seem to reach a stationary phase in contrast to DEC incubation.

4 Discussion

4.1 HMW-DOC compositional changes during degradation

The monosaccharide composition of the samples after addition of HMW-DOC (samples retrieved at 2000 m at DEC and HP conditions) resembled that generally reported for deep waters (Table 1) (Kaiser and Benner, 2008; Panagiotopoulos et al., 2014; Skoog and Benner, 1997). Our results showed distinct differences in the initial and final sugar composition during the biodegradation experiments for both Strat. and Conv. conditions (Table 1). This finding was more pronounced for glucose (45% decrease of its initial concentration) mannose (27%) and galactose (20%) during Stat. water period under HP incubations than Conv. period also under HP conditions (glucose 14%; mannose and galactose <1%). These results suggest deep-sea prokaryotes under high HP preferentially remove hexoses (e.g. glucose, mannose and galactose) from HMW-DOC similar to results of previous studies carried out in Atlantic and Pacific Oceans (Goldberg et al., 2011; Kaiser and Benner, 2009).

Overall, these results further indicate that neutral sugars can be used as tracers of deep-sea semi-labile DOC and that changes in DOC composition are related to the bioavailability of DOC.

4.2 Prokaryotic degradation of DOC in stratified period condition

In the NW Mediterranean Sea, summertime stratification isolates the upper mixed layer from deeper water masses preventing down-mixing of DOC (Copin-Montégut and Avril, 1993; Santinelli et al., 2013, 2010). Thus, the prokaryotic communities sampled at 2000 m-depth in summertime are well adapted to the deep-sea conditions, due to the stratification (Tamburini et al., 2013a) while surrounded with old and recalcitrant DOC (Santinelli et al., 2010; Santinelli, 2015). Gravitational sinking of particles carrying HMW-DOC in its plume can locally enhance and provide HMW-DOC to free-living deep-sea prokaryotes (Fang et al.,

2015; Follett et al., 2014; Kiørboe and Jackson, 2001; Long and Azam, 2001; Tamburini et al., 2003).

Then, results presented in Carlson et al., (1994), show that stratification sequesters DOC in deep water, leading to an increase of remineralization by deep prokaryotes, that is consistent with our results in Strat_HP. HMW-DOC issue from surface water is more bioreactive than HMW-DOC already present in deep ocean (Carlson et al., 2004). Our results showed that autochthonous deep-sea prokaryotes, during stratified period conditions, are able to use HMW-DOC, at a higher rate (1.7-fold) under *in situ* high pressure conditions than if decompressed and incubated at atmospheric pressure conditions. These results agree with previous investigations of Boutrif et al. (2011) showing higher degradation rates of radiolabeled marine extracellular polymeric substances by deep-sea prokaryotes under HP compared to DEC incubations during stratified period conditions. Overall, the above results are in agreement with other investigations that demonstrated that deep-sea adapted prokaryotes have specific enzymes able to degrade complex organic matter substrates (DeLong and Karl, 2005; Vezzi et al., 2005) and with those of Bergauer et al. (2018) reporting an increase of protein transporter of the microbial community with depth to adapt to the change of OM quality.

Figure 3 summarizes the distribution of parameters estimated by the cell-quota model after 500 simulations (see **Materials and Methods** section) and to compare incubation (HP versus DEC) and hydrological conditions (Strat. versus Conv.). The Kruskal-Wallis and Pairwise Wilcoxon Rank Sum tests (R Core Team, 2017) performed on the box-and-whisker plots attests that each parameter estimated is significantly different ($p < 0.05$) between each incubation (HP versus DEC) in stratified period condition. Under HP conditions Q_m (median Q_m HP = 11.32) was significantly higher than DEC conditions (median Q_m DEC = 8.94). These results indicate that under HP conditions, deep-sea prokaryotes have elevated carbon requirements to grow, or in other words expends more energy for their growth. According to del Giorgio and Cole (1998) , if the supply of organic carbon is scarce, then most of the substrate will be catabolized and used primarily for maintenance energy requirements rather than for growth. On the other hand, the DEC community also sampled at 2000m depth but decompressed may need less organic carbon to grow. Recent results by Arrieta et al., (2015), have incubated at atmospheric pressure conditions, deep seawater with natural community from the Pacific and Atlantic Ocean, have shown that even a low concentration of labile DOC (even of only 2-fold of the natural DOC) could sustain growth after a 10-day incubation. Closed

incubation approaches as well as changes in pressure lead to changes in prokaryotic community composition (Edgcomb et al., 2016; Garel et al., 2019; Ionescu et al., 2015). The prokaryotic community, able to withstand changes in pressure, showed greater catabolism requirements in growing with the available DOC (Q_{in} DEC = 8.94 versus Q_{in} HP = 11.32). However, the HMW-DOC degradation is more efficient under HP than DEC incubations since the DOC_{rec} is significantly higher in DEC incubations (median DOC_{rec} = 109.34 µM) than in HP incubations (median DOC_{rec} = 63.94 µM) on the timescales of the incubations (Figure 3 and Table 2).

4.3 Prokaryotic degradation of DOC in deep-water convection period condition

During deep-water convection event, the story is completely different. Under that condition, bioavailable surface DOC can be carried to the deep microbial community with the mixing of surface and deep Mediterranean seawaters. Tamburini et al. (2013b) reported that the concentration of DOC at 2000m increases from ~42 µM under stratified conditions, to ~72 µM under deep-water period condition. These values are in agreement with those reported by Santinelli et al. (2010) in the Gulf of Lions in May 2005, that show a marked increase in DOC by deep convection event. During deep-water convection events, deep seawater is amended with ~30 µM labile or semi-labile DOC originating from the surface. Moreover, in this specific case, the prokaryotic communities were composed of both prokaryotes from surface water mixed with prokaryotes from deep waters (Tamburini et al., 2013b), surface seawater includes a larger proportion of surface prokaryotic cells, which are not adapted to *in situ* HP conditions (Egan et al., 2012; Tamburini et al., 2013a). Another study of Turley (1993) has shown that prokaryotes from surface water are affected by increasing hydrostatic pressure, i.e decrease of protein synthesis, during sinking of particles, thus preserving particles to reach the seabed. A study of Grossart and Gust (2009), using shallow-water bacterial strains submitted to increasing pressure, indicates that strains from the surface respond individually to pressure exposure and appear to rapidly reduce cell division. In the same vein, our series of experiments increasing hydrostatic pressure of surface prokaryotic communities lead in generally to a decrease in the activities of surface prokaryotic communities with increasing pressure (Tamburini et al., 2006, 2009b; Riou et al., 2018). As a consequence, on the timescales of the incubations, DOC degradation rates in the HP incubations (median k = 0.006 d^{-1}) are significantly lower than in the DEC incubations (median k = 0.49 d^{-1}) during the deep-water convection event (Kruskal-

Wallis and Wilcoxon Signed Rank tests, p <0 .05) (Figure 3 and Table 2). Due to low degradation rate during the deep-water convection under HP incubation (Figure 2C and Figure 3B, Conv_HP), the highest variability is found, by the Droop model, on the estimation of DOC_{rec} (Figure 3D, Conv_HP).

Similarly, growth rates are lower when incubated at HP conditions (median μ_{max} = 0.55 d^{-1}) than when decompressed and incubated at atmospheric pressure conditions (median μ_{max} = 0.76 d^{-1}) (Figure 3 and Table 2, Kruskal-Wallis and Wilcoxon Signed Rank tests, p < 0.05). Moreover, in the HP incubations, the minimum cell-quota estimation is relatively high with the highest variability (Q_m HP = 9.05 ± 1.34, mean ± s.d., see Table 2), slightly lower that DEC conditions (Q_m DEC = 10.42 ± 0.18). In the HP incubations of our experiments during deep-sea convection event, degradation rates appear to be inhibited or not visible. The production and removal of recalcitrant DOC can be hypothesized to occur, as shown in Romera-Castillo et al., (2019), sustaining the growth rates estimated in the HP incubations. In contrast, after decompression (DEC incubations), surface prokaryotes carried out in the deep-sea water and incubated at atmospheric conditions are able to partially degrade the DOC available. However, the DOC_{rec} at the end of incubation during the deep-water convection event remained relatively high in DEC incubations. In their experiments conducted with surface and mesopelagic prokaryotic consortium, Carlson et al. (2004) found high DOC degradation and biomass yields in surface waters inoculated with upper mesopelagic consortium than with surface ones. They suggested that mesopelagic prokaryotes adapted to use recalcitrant DOC are in competition for inorganic nutrients with surface prokaryotes that are adapted to use labile DOM, they may grow at slower rates thereby limiting semi-labile DOC remineralization.

4.4 DOC DEGRADATION IN STRATIFIED VERSUS DEEP-WATER CONVECTION PERIOD CONDITIONS

Changes of the *in situ* hydrostatic pressure conditions during sample retrieval and incubation impact the degradation of HMW-DOC due to the change of prokaryotic community (Egan et al., 2012; Tamburini et al., 2013a). Recent studies have demonstrated that decompression of deep seawater samples very likely involves a shift of the prokaryotic community (Edgcomb et al., 2016; Garel et al., 2019). Even if the shift of the prokaryotic community is true for the both incubations (HP and DEC) due to the 2-weeks incubation time

(Lee and Fuhrman, 1991; ZoBell and Anderson, 1936), to make statements about DOC degradation in the deep ocean, HP incubation conditions are more realistic.

Follett et al., (2014) have shown that in the deep ocean, 10 - 30% of the deep DOC reservoir is semi-labile and supported by a flux of ~1 Gt C year $^{-1}$ carbon introduced by sinking particles. During stratified period conditions, the deep seawaters are sequestered for up to century, and carbon is introduced principally by gravitational sinking of particles (Le Moigne, 2019; Siegel et al., 2016). Moreover, autochtonous prokaryotes adapted to *in situ* high pressure conditions (Boutrif et al., 2011; DeLong and Karl, 2005; Tamburini et al., 2013a; Vezzi et al., 2005) seem able to degrade up to 46.6% of the added DOC (according to Figure 2A; Table 2). Finally, it is more and more obvious that some deep prokaryotes exploit environments where the most abundant energy resources are sinking organic particles or recalcitrant dissolved organic matter (DOM). As an example, SAR202 group III dominate bathypelagic waters presumably indicating that molecules, such as recalcitrant organic compounds, susceptible to oxidation by FMNOs (flavin-dependent monooxygenases) become one of the few remaining harvestable DOM resources at these depths (Saw et al., 2020). However, during the deep convection event periods, surface prokaryotes are carried deeper into the water column, mixing with the pressure adapted community. The result is a lower degradation observed under *in situ* HP incubations, (Figure 2C; Table 2). In this experiment, we hypothesize that adapted deep-sea bacteria grew by using mainly the surface semi-labile DOC transported by deep water formation. Semi-labile DOC can stay in deep waters for several weeks in agreement with Santinelli et al. (2010). These authors estimated DOC removal rates to be 14.4 µM C year^{-1}, or 0.04 µM C d^{-1} in deep waters recently ventilated (and thus characterized by high DOC concentrations). This removal rate is in agreement with our results, the estimate of the degradation rate in the HP incubations during Conv. experiments, being apparently very low (0.01 ± 0.03 d^{-1}), the production and removal of recalcitrant DOC can be hypothesized to occur as discussed previously.

5 CONCLUSION AND PERSPECTIVES

Our results demonstrate that the degradation of DOC under stratified and deep convective mixing conditions depends on the origin and the history of the prokaryotic communities, which were controlled by the origin of the water masses in the NW Mediterranean Sea. Depending on the hydrological conditions, the proportion of allochthonous and

autochthonous prokaryotes in deep-sea microflora can vary, with surface bacteria increasing in deep-sea waters during deep-sea convection (Luna et al., 2016; Severin et al., 2016). Degradation of HMW-DOC is less effective during deep-sea convection than during stratified period conditions, where adapted deep-sea prokaryotes probably have a specific metabolism to degrade HMW-DOM. This study must be considered in the context of climate change, as the stratification of the surface layers of the Mediterranean Sea is expected to intensify and thus limit the vertical transfer of both dissolved oxygen and DOC by winter convection of surface waters (Adloff et al., 2015; Powley et al., 2016).

The interactions between the DOC and the deep-sea prokaryotic communities are still unclear, and future experiments should include measurements of oxygen consumption throughout the incubation and the dissolved inorganic matter (DIC) concentration, key indicators of the microbial remineralization. Furthermore, a better understanding of the metabolic abilities of deep-sea prokaryotes, at a cellular scale, and under *in situ* conditions of pressure and temperature, are needed. Since dense water formation occurs in other regions worldwide, a steering of deep pelagic ecosystem activity similar to that observed in the NW Mediterranean Sea should occur there too.

Genomic and transcriptomic analysis will be required in further studies to highlight the succession of different prokaryotic communities and metabolic pathways involved in degradation of the different pool of dissolved organic matter.

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