Deep ocean prokaryotic communities are remarkably malleable when facing long-term starvation

Marta Sebastián[1,2], Jean-Christophe Auguet[3], Claudia Ximena Restrepo Ortiz[3], Maria Montserrat Sala[1], Celia Marrasé[1], Josep M Gasol[1]

[1]Departament de Biologia Marina i Oceanografia, Institut de Ciències del Mar, CSIC. Passeig Marítim de la Barceloneta, 37-49, E08003 Barcelona, Catalunya, Spain.

[2]Instituto de Oceanografía y Cambio Global, Universidad de Las Palmas de Gran Canaria, Parque Científico Tecnológico Marino de Taliarte, s/n. 35214, Telde, Spain

[3]Marine biodiversity, Exploitation and Conservation (MARBEC), UMR CNRS 9190, Université de Montpellier, CC093, Place Eugène Bataillon, 34095 Montpellier Cedex 05

Correspondence to Dr Marta Sebastián, Departamento de Oceanografía Biológica, Instituto de Oceanografía y Cambio Global, Universidad de Las Palmas de Gran Canaria, Las Palmas de Gran Canaria, Spain.

email: marta.sebastian@ulpgc.csic.es

The authors declare no conflict of interest

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.14002

SIGNIFICANCE STATEMENT

The bathypelagic is the largest aqueous habitat on Earth, yet life in this system is challenging from a microbial point of view. Settling particles produced in the sunlit layer of the ocean are considered to sustain most of deep ocean microbial activity, but there is increasing evidence that these particles are not sufficient to meet the energy demands of deep ocean prokaryotes. Although recent genomic studies from isolates and single-amplified-genomes suggest bathypelagic microbes have the potential to be metabolically versatile, almost nothing is known about the compositional responses of microbial communities when facing carbon deprivation. In this study, we explored the long-term (1 year) dynamics of a bathypelagic community in the absence of external inputs of carbon. Despite the lack of external energy supply, we observed a continuous succession of active phylotypes, which was driven by recruitment of taxa from the seedbank (initially rare OTUs). Our results unveil the astounding plasticity of deep ocean microbial communities subjected to organic carbon deprivation, and indicate that there is a large reservoir of microbial diversity that ensures the long-term metabolic potential of deep ocean microbial communities.

SUMMARY

The bathypelagic ocean is one of the largest ecosystems on Earth, and sustains half of the ocean's microbial activity. This microbial activity strongly relies on surface-derived particles, but there is growing evidence that the carbon released through solubilisation of these particles may not be sufficient to meet the energy demands of deep ocean prokaryotes. To explore how bathypelagic

prokaryotes respond to the absence of external inputs of carbon, we followed the long-term (1 yr) dynamics of an enclosed community. Despite the lack of external energy supply, we observed a continuous succession of active prokaryotic phylotypes, which was driven by recruitment of taxa from the seed bank (i.e. initially rare OTUs). A single OTU belonging to Marine Group I of Thaumarchaeota, which was originally rare, dominated the microbial community for ~4 months, and played a fundamental role in this succession likely by introducing new organic carbon through chemolithoautotrophy. This carbon presumably produced a priming effect, because after the decline of Thaumarchaeota, the diversity and metabolic potential of the community increased back to the levels present at the start of the experiment. Our study demonstrates the profound versatility of deep microbial communities when facing organic carbon deprivation.

INTRODUCTION

The oligotrophic bathypelagic ocean is one of the largest ecosystems on Earth, and accounts for 50% of the oceans' heterotrophic activity (Arístegui *et al.*, 2009). This system is characterized by persistently low dissolved organic carbon (DOC) concentrations that sustain reduced prokaryotic abundances (Carlson *et al.*, 2011). Even though it is a rather stable environment, life in the bathypelagic is tough from a microbial point of view (Herndl and Reinthaler, 2013). The DOC pool consists mainly of increasingly recalcitrant compounds that are by-products of microbial metabolism (the so-called microbial pump —Jiao *et al.*, 2010—), and some short-lived (hours to weeks) labile or semilabile DOC that becomes available through the transformation and solubilisation of surface-derived (Arístegui *et al.*, 2009) or buoyant particles (Baltar *et al.*, 2010), or through *in situ* chemolithoautotrophy (Herndl *et al.*, 2005). Sinking particles are considered the main carbon source for bathypelagic communities (Nagata *et al.*, 2000; Hansell and Ducklow, 2003), yet the flux of these particles is intermittent and variable on temporal and spatial scales (Arístegui *et al.*, 2002; Arístegui and Montero, 2005). This frequent carbon scarcity seems to have led to the development of versatile metabolisms, as hinted by increasing findings of potential metabolic flexibility in deep ocean prokaryotes (Tang *et al.*, 2016; Swan *et al.*, 2011; Landry *et al.*, 2017). However, the responses of deep ocean microbes to organic carbon starvation have not been explored.

Most of the knowledge we have about long-term starvation responses comes from studies with a few bacterial strains belonging to the phylum Proteobacteria (Novitsky and Morita, 1977; Soora and Cypionka, 2013; Amy and Morita, 1983; Finkel, 2006). These studies have shown that in the absence of fresh energy

supplies, stable populations persist for months to years, as the result of continuous cycles of life and death. Yet, no studies have dealt with prolonged starvation of natural assemblages, which are characterized by astounding levels of phylogenetic and metabolic diversity (Pedrós-Alió, 2012). This is particularly true for the bathypelagic, which is already an energy limited system. One complexity associated to natural communities is that prokaryotes compete with their neighbors for resources. Another level of complexity is that microbial communities contain a seed bank that can resuscitate upon shifts in environmental conditions (Lennon and Jones, 2011; Gibbons *et al.*, 2013), and that could play an important role in successional dynamics upon starvation.

Here we investigated how a bathypelagic community withstood prolonged starvation in the absence of their main source of carbon, i.e. the surface-derived particle flux. To this aim a parcel (600 L) of water from 2100 m depth was enclosed for 1 year, and we followed the taxonomic and functional dynamics of the prokaryotic community with weekly or by-weekly sampling frequency during the first two months, and monthly after that. We expected to find a rather stable community maintaining basal activities after most labile DOC had been consumed, but instead we observed a dramatic succession of active phylotypes along the year.

RESULTS AND DISCUSSION

Long-term dynamics of the starved bathypelagic community

Total organic carbon (TOC) and total nitrogen (TN) were quite constant throughout the experiment (Figure 1a), with TOC values within the range reported for the world's bathypelagic regions (35-45 μM, Hansell *et al.*, (2009)).

Heterotrophic prokaryotic production values (estimated as leucine incorporation rates) at the very start of the experiment (Figure 1b) were higher than previously observed values in the bathypelagic (e.g. Baltar *et al.*, 2009), likely due to some confinement effect (see methodological considerations in the Experimental Procedures section). Then it decreased drastically within the first two weeks of the experiment, when most of the labile DOC was likely consumed. During the second month (day 49), heterotrophic production decreased further but remained rather constant for more than a month (day 105, Figure 1b, inset), after which it decreased again down to a minimum value on day 165. Later on, production remained low but displayed a relative maximum on day 278. The values detected at the end of the experiment are within the low range of heterotrophic production values observed in the Mediterranean bathypelagic (Luna *et al.*, 2012).

Prokaryotic cell abundances were generally low, in agreement with previous observations in the deep Mediterranean (e.g. Luna *et al.*, 2012). They presented a relative minimum between days 105-165, and afterwards increased back to the initial values (Figure 1c). Coincident with the initial sharp decrease in leucine incorporation, we observed the presence of a population of small cells (with an estimated carbon content of 6.5 fgC cell^{-1}, instead of the estimated 12.5 fg cell^{-1} of the normal-size cells, Figure S1) that peaked between days 21 and 49 of the experiment. Reduction in size is a well-described response of many bacteria upon carbon starvation, being an important survival strategy for reducing energy costs (Novitsky and Morita, 1976). However, the decline in this population of microcells observed on day 77 of the experiment likely suggests the demise of a large fraction of these cells, which is supported by the strong decline observed in taxonomic diversity (Figure 1d). This indicates that size reduction might be one strategy that

bathypelagic prokaryotes have to cope with carbon scarcity, but some cells in such stage do not seem to withstand starvation for long periods. In fact, prokaryotic species richness decreased to half of the initial values at the end of the experiment (Figure 1d).

In terms of taxonomic composition, there was a striking succession of phylogenetic groups in the rDNA profiles (Figure 2). Alpha- and Gammaproteobacteria dominated the initial community, being the genus *Alteromonas* the major contributor on day 1 (~35% of total reads), but the relative abundance of this genus decreased drastically shortly after (Figure S2). *Alteromonas* is a major player in the drawdown of labile DOC in the ocean (Pedler *et al.*, 2014), and its fast disappearance further supports the idea that labile DOC was rapidly consumed. Unlike most other prokaryotic groups, the relative contribution of Alphaproteobacteria to the community structure remained relatively constant along the experiment (Figure 2), but there was also a marked succession of phylotypes within this group (Figure S3).

On day 77, coincident with the decline in the population of microcells, the contribution of Marine Group I (MCGI) of Thaumarchaeota remarkably increased, peaking on day 133, where it represented more than 70% of the sequences. A single OTU that was rare at the beginning of the experiment (~0.02 of the total DNA reads) accounted for 96% of the MCGI population. These changes in species contributions caused the dramatic drop in taxonomic diversity (Figure 1d). The dominance of Thaumarchaeota lasted until month 6 (day 194), and afterwards the contribution of this phylum notably decreased, and members of Bacteroidetes, Alphaproteobacteria and Planctomycetes became the major contributors to the prokaryotic community (Figure 2).

It has been postulated that PCR-based surveys over-estimate the relative contribution of abundant taxa to the community structure and display bias against the ones present in low proportion (Gonzalez *et al.*, 2012), so in order to test whether the dominance of archaeal sequences was a consequence of the demise of a large fraction of cells of the different bacterioplankton groups or a real 'bloom' of Archaea, we quantified Thaumarchaeota and MCGI 16S gene abundances through quantitative PCR. We observed that the abundance of this phylum was actually higher at the beginning of the experiment (Figure S4), where most Thaumarchaeota OTUs displayed higher relative abundances (Figure S5). Nevertheless, a relative maximum was observed on day 133, when we found the maximum contribution in terms of sequences (Figure S4) and the lowest prokaryotic abundances (Figure 1c). These results suggest that there was not a real bloom, and that the high dominance of Thaumarchaeota sequences was the consequence of a drastic decrease in the abundance of different bacterial phylotypes. The success of these Thaumarchaeota MCGI OTUs likely relies on their ability to fix inorganic carbon (Herndl *et al.*, 2005). Indirect evidence of this comes from the nitrite peak that we observed coincident with the rise in Thaumarcheota relative abundance (Figure S6), because autotrophic Thaumarchaeota obtain the energy to fix inorganic carbon through the oxidation of ammonia to nitrite (Könneke *et al.*, 2005).

We then assessed changes in the fluorescent DOM along the experiment through excitation/emission matrices, which allow to identify protein-like (peak T), and humic-like (peak C and M) substances (Coble, 1996). We found a remarkable signature of protein-like compounds when the relative abundance of

Thaumarchaeota reached its maximum value (Figure S7). This constitutes another indirect evidence supporting the introduction of labile DOM through chemolithoautotrophy. Based on the increase in total prokaryotic cell abundance and the change in community composition observed after the peak of Thaumarchaeota, we hypothesize that these archaeal cells played a central role in DOM cycling in the system and their metabolism by-products enabled growth of bacterial taxa which ended up outcompeting them.

To further explore this idea, we monitored the functional capabilities of the community along the experiment by measuring a suite of hydrolytic enzymes. These enzymes enable microorganisms to utilize complex DOC compounds and high activity values have been reported for deep sea microbial communities (Hoppe and Ullrich, 1999; Baltar *et al.*, 2013, 2009). Esterase activity was high within the first two weeks of the experiment, showed its lowest values when Thaumarchaeota dominated the community, and increased back to the original values when Thaumarchaeota relative abundance decreased and Bacteroidetes became the major contributors to the community (Figure 3a and 2). This enzymatic activity is an estimator of overall potential activity of the microbial enzymes involved in organic matter transformations (Chrost *et al.*, 1999). The rise in esterase activity implies that after one year with no external carbon inputs the microbial community maintained the ability to regain its metabolic potential.

The trends observed in other enzymatic activities likely reflect changes in the quality of the DOM pool and in community traits associated to the shifts in taxonomic composition. α-glucosidase, ß-glucosidase, leu-aminopeptidase and chitobiase activities followed the same pattern as leucine incorporation (Figure

3b-e and 1b), with a drastic decrease within the first two weeks of the experiment and a relative maximum later in the experiment coincident with the increase in the contribution of Bacteroidetes and Planctomycetes (Figure 2) and the rise in esterase activity (Figure 3a). Fucosidase activity, which is involved in the degradation of polysaccharides (Sun *et al.*, 2016), was low at the beginning of the experiment, but displayed a maximum on day 223, when the peak in esterase activity was observed (Figure 3f). Alkaline phosphatase followed a similar trend than bacterial abundance, but values remained relatively high throughout the experiment (Figure 3g), supporting the view that these enzymes play an important role in the acquisition of carbon from dissolved organic phosphorus compounds in the bathypelagic (Hoppe and Ullrich, 1999; Baltar *et al.*, 2009).

Dynamics of the most responsive operational taxonomic units (OTUs)

We next explored the identity and dynamics of the most responsive OTUs along the experiment, i.e. the ones that played the largest role in the taxonomic, and therefore likely in the metabolic, succession. To that end, we focused on those taxa that displayed the largest changes in relative abundances ('responsive' OTUs, see methods). Then we categorized these responsive OTUs as 'pre-bloom', if their mean relative abundances were higher before the peak of Thaumarchaeota (days 1-49, Figure 2), or 'post-bloom', if their relative abundances were higher after day 165, when the Thaumarchaeota population started to decline. We identified 91 OTUs in the 'pre-bloom' pool (73 bacterial and 18 archaeal OTUs, see Supplementary table 1 for further details), which accounted for roughly 95% of all the sequences during the first 49 days (Figure 4a). After this date, their contribution to the total number of sequences markedly decreased, and from day

77 to the end of the experiment they only represented between 10-20% of the total sequences. Interestingly, despite the drastic decrease in their contribution to the community, half of these 'pre-bloom' OTUs were still present at the end of the experiment (Figure 4c), suggesting that they managed to survive but were present in very low abundances. Noticeably, when taking into account all the OTUs that were abundant (representing >1% of the reads), we found that only one *Erythrobacter* OTU (Alphaproteobacteria) was relatively abundant before and after the bloom (data not shown).

The 'post-bloom' pool was composed by 76 OTUs, 75 bacterial OTUs and 1 archaeal MCGI OTU, which was the one that dominated the community during days 77-194 of the experiment (Figure 4b and Figure 2, see Supplementary table 1 for further details). 84% of the 'post-bloom' OTUs were rare (<0.1% relative abundance) during the first month of the experiment, and only 23 out of the 76 OTUs were detected in the original community (Figure 4d). After the first month, the relative abundance of the 'post-bloom' OTUs remarkably increased, and ended up representing over 80% of the reads at the end of the experiment. Hence, the 'post-bloom' OTUs, which accounted only for 7% of the 1117 OTUs detected during the whole experiment, are mostly 'seed' OTUs, i.e., OTUs that transitioned from very rare or dormant to abundant when they found favourable conditions. Microbial seedbanks have been proposed to play a fundamental role in environmental microbial dynamics (Lennon and Jones, 2011; Caporaso *et al.*, 2012; Gibbons *et al.*, 2013; Shade *et al.*, 2014). Yet recruitment of taxa from the seedbank has only been demonstrated in short-term incubation experiments (Baltar *et al.*, 2016; Aanderud *et al.*, 2015). Our results constitute the first demonstration of recruitment from the seedbank over a long-time period in a controlled laboratory

setting. The shift in conditions for these responsive OTUs was likely triggered by the introduction of fresh organic carbon into the system through inorganic carbon fixation by the chemolithoautotrophic Thaumarchaeota (Figure S6 and S7), which dominated the community for almost 4 months. We hypothesize that this freshly produced carbon probably had some sort of 'priming effect' (Kuzyakov *et al.*, 2000), that facilitated the degradation of previously unreactive organic matter.

Both the 'pre' and 'post-bloom' OTUs were composed of an assortment of diverse prokaryotes (Figure 4e,f), yet the composition of these pools was remarkably different. Alphaproteobacteria were major contributors to both pools, but members of the order Rhodobacterales and the SAR11 clade dominated at the beginning of the experiment, whereas Rhodospirillales dominated at the end. Gammaproteobacteria represented ~25% of the OTUs in the 'pre-bloom' pool but accounted for a small fraction of the 'post-bloom' OTUs, and the taxonomic affiliation of the OTUs within this class also differed from the 'pre' counterparts (Figure 4e,f).

The 'post-bloom' pool was also composed by other phyla that had not been detected in the 'pre-bloom' pool, such as Hydrogenedentes, Gemmatimonadetes, Nitrospirae, and Chloroflexi. In addition, members of the Sphingobacteriales and Cytophagales dominated the Bacteroidetes population, whereas this phylum represented a minor fraction of the total community at the beginning of the experiment. The success of these groups in such a carbon-limited system points to complementary alternative metabolisms and the ability to use recalcitrant compounds, as supported by the increase in the hydrolytic activities (Figure 3). Indeed, Bacteroidetes, Gemmatimonadetes, Planctomycetes and Verrucomicrobia have been reported to harbour genes encoding hydrolytic enzymes involved in the

degradation of polymers (Fernández-Gómez *et al.*, 2013; Li *et al.*, 2015). Within the Bacteroidetes, members of the Sphingobacteriales have been observed to have special ability to utilize complex carbon compounds and even predate on other bacteria (McIlroy and Nielsen, 2014). Hydrogenedentes have the potential ability to utilize hydrogen as energy source (Rinke *et al.*, 2013) and degrade complex compounds (Rinke *et al.*, 2013; Nikolaki and Tsiamis, 2013). Nitrospirae obtain energy from nitrite oxidation and members of this phylum have been shown to have extremely versatile metabolisms, which allow them to use CO_2, organic compounds, perform aerobic hydrogen oxidation, and even ammonia oxidation (Koch *et al.*, 2015; Daims *et al.*, 2015). Chloroflexi bacteria are also thought to be metabolically flexible (Yilmaz *et al.*, 2016) and have the potential to use recalcitrant DOM compounds (Landry *et al.*, 2017).

To further confirm that the recruited OTUs drove the metabolic succession we analysed the relationships between the 16S rRNA and rDNA sequences of both the 'pre' and 'post' bloom OTUs to infer the activity status of these OTUs (e.g. Campbell *et al.*, 2011; Hugoni *et al.*, 2013). If the prokaryotes present at the end of the experiment were starving or growing under suboptimal conditions, the relationship between their rRNA and rDNA reads would have a smaller slope than the slope of those dominating at the beginning of the experiment. Using this approach, we found that the recruited taxa at the end of the experiment ('post-bloom' OTUs) were as active as the ones prevalent before the bloom (Figure 5). This observation supports the view that the recruited taxa ensured the long-term metabolic potential of the community.

Taken together, our findings show that prolonged starvation in a bathypelagic microbial community led to a consistently active community

maintained by a continuous succession of prokaryotic groups. We hypothesize that Thaumarchaeota played a crucial role in this succession by introducing fresh DOC through chemoautotrophy into the system, and this DOC had a 'priming effect' that enabled the resuscitation of metabolically diverse taxa that ended up dominating the microbial community. While our experimental approach is not a perfect reproduction of what occurs *in situ* (see methodological considerations in the Experimental procedures), it demonstrates the profound malleability of deep-sea microbial populations in the absence of external influences. It has been long assumed that the surface-derived particle flux sustains microbial activities in the bathypelagic but our results show that after one year deprived of surface-derived particles, prokaryotic abundance and diversity displayed similar values to the beginning of the experiment. This provides conclusive evidence of how resourceful deep ocean prokaryotes are when facing organic carbon-limiting conditions, and hints at which phylogenetic groups are likely to thrive in those extreme conditions.

EXPERIMENTAL PROCEDURES

Experimental set up

The bathypelagic water (600 L) was collected from 2100 m in the Northwestern Mediterranean Sea (40º 38' 31.01''N, 2º 51' 1.6''W) in October 2014 during the MIFASOL-I cruise on board the R/V García del Cid. The water was collected at night the last day of the cruise before steaming back to harbour, by multiple deployments of a rosette equipped with Niskin bottles. Water was filtered through a 200 µm mesh to remove large zooplankton and poured into different

thoroughly acid-rinsed 20-L containers. The content of these containers was transferred to a 600 L- HDPE container that had been previously acid-cleaned, rinsed once with distilled water and rinsed 5 times with the sampled water. This container was attached to a plastic pallet, to simplify the handling, and covered with black plastic to prevent exposure of the water to light throughout the handling and the experiment. Once back in the lab, the sampled water was allowed to sit for 24 h before starting the experiment. The container was gently agitated by moving it back and forth using a pallet jack prior to each sampling and samples were syphoned out of the tank by means of an autoclaved and acid-rinsed glass-tube attached to a long silicon tube, with the help of a 60-mL syringe. The lid of the container covered the tube during the sampling, to avoid immigration of foreign taxa. Samples were taken at different time intervals (on days 1, 7, 14, 21, 33, 49, 77, 105, 133, 165, 194, 223, 278, 364) during 1 year. The container was maintained in the dark and at ~17°C, which is only 3°C higher than the typical bathypelagic Mediterranean Sea temperature (Luna *et al.*, 2012). The container was airtight except for the moment of sampling, so a certain gas exchange was allowed. However, given that both oxygen and $C0_2$ are not limiting in bathypelagic waters (Copin - Montégut, 1993; Tanhua *et al.*, 2013) and the low prokaryotic biomass observed along the experiment, we believe this gas exchange did not have any significant effect in the C and O_2 budget.

Methodological considerations

We are aware that our approach has some shortcomings, being the most obvious one the dramatic change in conditions during water recovery, particularly in pressure. Only a few studies have dealt with the effect of changes in hydrostatic

pressure on microbial activity and diversity, but no firm conclusions can be drawn from these studies (Herndl & Reinthaler, 2013, and references therein). It is certainly possible that some pressure-sensitive prokaryotes died during sample collection. However, since we are interested in the long-term response and the whole experiment was carried out under the same pressure, we believe the dynamics observed are independent of this variable.

Confinement of natural assemblages usually leads to changes in community composition and activity (Schäfer *et al.*, 2000; Massana *et al.*, 2001), with *Alteromonas* bacteria playing a main role in these shifts (e.g. Eilers *et al.*, 2000; McCarren *et al.*, 2010). We tried to reduce the confinement effect by performing the incubation with a large volume of water (600 L) (see Ferguson *et al.*, 1984 and references therein). However, the dominance of *Alteromonas* and the high activity observed during the first week of the experiment (Figure 1b), suggest there was some enrichment due to confinement. This issue is inherent to any experimentation with natural communities but we believe mesocosms experiments are still invaluable tools for understanding the functioning of marine ecosystems. Although we acknowledge that the dramatic alterations our parcel of water experienced within the first days probably led to changes in the diversity of the bathypelagic community, our main goal was not to exactly reproduce the bathypelagic environment ex-situ, but to investigate the long-term response of bathypelagic prokaryotes to the absence of external inputs of organic carbon. To further confirm that the OTUs detected in this study are bathypelagic dwelling organisms we searched these OTUs in a trans-Mediterranean bathypelagic survey (HOTMIX cruise, Ortega-Retuerta *et al*. in prep.), and identified 77% of them.

Prokaryotic heterotrophic production and abundance

Prokaryotic heterotrophic production was estimated from the incorporation of tritium-labeled leucine (Kirchman *et al.*, 1985) using the centrifugation procedure. Four replicates of 1.2 ml and two trichloroacetic acid (TCA)-killed controls were incubated with ^{3}H-Leucine at a final concentration of 40 nM. Incubation was performed in the dark at *in situ* temperature for 4 h and stopped with 5% TCA, final concentration. The samples were then kept frozen at -20°C until processing, following (Smith and Azam, 1992).

Prokaryotic cell abundance was estimated by flow cytometry as described elsewhere (Gasol and Moran, 2015). Prokaryotic cell size was estimated from the side scatter signal using the equation provided by Calvo-Díaz and Moran, (2006). The carbon content of the prokaryotic cells was obtained following Gundersen *et al.*, (2002).

Enzymatic measurements

We monitored the enzymatic activities of Leu-aminopeptidase, α-glucosidase, β-glucosidase, fucosidase, chitobiase, alkaline phosphatase, and esterase using L-leucine-7-amino-4-methylcoumarin, 4-methylumbelliferyl α -D-glucoside, 4-methylumbelliferyl β-D-glucoside, 4-methylumbelliferyl β-D-fucoside, 4-methylumbelliferyl N-acetyl-β-D-glucosaminide, 4-methylumbelliferyl phosphate and 4-methylumbelliferyl butyrate as substrates, respectively (all purchased at Sigma-Aldrich) following the method developed by Hoppe, (1983). Assays were performed as described in Sala *et al.*, (2016). Briefly, each sample (350 µl) was pipetted in quadriplicate into 96 black well plates, and substrates were added to obtain a final concentration of 125 µM. The fluorescence of the 96 well plates was

measured at the beginning and after 5 hours incubation at in situ temperature conditions with a Tecan Infinite 200 microplate reader at 365 nm excitation and 450 nm emission wavelengths. 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).

Total organic carbon and total nitrogen

10 mL water samples were collected in precombusted (450 °C, 24 h) glass ampoules. After adding 50 µL of 25% H_3PO_4 to acidify at pH < 2, the ampoules were heat-sealed and stored in the dark at 4°C until analysis. TOC and TN concentrations were quantified with a Shimadzu TOC-LCSV organic C/N analyzer. Between 3 and 5 injections of 150 µL per replicate were performed. The final organic carbon and nitrogen concentration in each sample was calculated by subtracting a Milli-Q blank and dividing by the slope of daily-made standard curves made from potassium hydrogen phthalate and glycine respectively. Reference samples of the Material Reference Certificate (MRC Batch-13 Lot // 08-13, Hansell Laboratory. University of Miami, RSMAS) were used daily for quality control.

Inorganic nutrients

Samples for inorganic nutrients (10 mL) were kept frozen at −20°C until analysis, which was performed using a CFA Bran + Luebbe autoanalyser following the methods described by Hansen and Koroleff, (2007).

Nucleic acid extraction

Total nucleic acid extractions were performed using the standard phenol-chloroform protocol with slight modifications (Logares *et al.*, 2013). Once extracted, the volume was split in two. One of the aliquots was treated with the

Turbo DNA-free kit (Applied Biosystems, Austin, TX, USA) to remove residual DNA, and PCR reactions were run to check that DNA had been completely removed. DNA and RNA were quantified using a Qubit fluorometer assay (Life Technologies, Paisley, UK). RNA was reverse transcribed using random hexamers and the SuperScriptIII kit (Invitrogen), according to the manufacturer's instructions.

Samples were sent for sequencing at the Research and Testing Laboratory (Lubbock, TX, USA). We used the primers 515F and 926-R (Parada *et al.*, 2016) to amplify the 16S rRNA from the DNA and cDNA pools. Illumina MiSeq 2 x 250 flow cells were used for sequencing following Illumina protocols described elsewhere (Cúcio *et al.*, 2016).

qPCR analyses

The absolute abundance of the Thaumarchaeota phylum and the Marine Crenarchaoetic Group I (MCGI) lineage were followed by quantitative PCR (qPCR) using the primer sets and the PCR program summarized in Hong and Cho, (2015) and Auguet *et al.*, (2011), respectively. Plasmids containing 16S rRNA genes of the Thaumarchaeota phylum and the MCGI lineage were used as standards.

Absolute 16S rRNA gene copies μL^{-1} were quantified in triplicate from DNA extracts and standard DNA dilution curves by quantitative PCR (qPCR, LightCycler 480, Roche). In each qPCR run, amplification of control water samples was done in triplicate in order to confirm lack of exogenous DNA contaminations. DNA distribution and reactive dispensing was done in 384 well plates, using an automatic pipetting system (Echo 525, Labcyte. Inc, CA, USA), that allowed the miniaturization of the assays in a final volume of 1.5 µL (0.5 µL of DNA at 0.5 ng/µL and 1 µL of reactive mix). Reactive mix solutions were prepared mixing each

primer pair (10 µM) with ready-to-use hot start reaction mix LightCycler® 480 probes master (Roche) and molecular grade water to a final primer concentration of 0.8 µM. After quantifications, absolute abundances (nº copies/mL) were calculated. Specificity of reactions was confirmed by melting curve analyses and after looking for unspecific PCR products such as primer dimers or gene fragments of unexpected length in agarose gel electrophoresis.

Data analyses

Computing analyses were run at the Marine Bioinformatics Service of the Institut de Ciències del Mar (ICM-CSIC) in Barcelona. The Illumina sequences were quality filtered following (Logares, 2017). The sequences of both the DNA and cDNA pools were clustered into operational taxonomic units (OTUs) at 97% cutoff using the UPARSE algorithm implemented in USEARCH (Edgar, 2013). Singletons (i.e. OTUs occurring once in just one sample) and chimerical OTUs were removed. The remaining OTUs were taxonomically annotated using BLAST against the SILVA database as reference. OTUs assigned to chloroplasts were removed for subsequent analyses. All raw sequences used in this study are publicly available at the European Nucleotide Archive (ERS1993945 - ERS1993972).

Data treatment and statistical analyses were performed with the Rstudio software using version 1.0.44. The OTU table was randomly subsampled down to the minimum number of reads per sample in order to avoid statistical artifacts due to an uneven sequencing effort among samples. This subsampling was obtained by rarefying the OTU table to the minimum number of reads (26090 reads/sample) using the *rrarefy* function in the *vegan* package (Oksanen *et al.*, 2015). For the sub-

sampled OTU table the sample of cDNA obtained on day 1 of the experiment was excluded, due to the low number of reads it contained (3248).

Analyses of the responsive OTUs

To analyse which OTUs showed the largest changes in abundance, we calculated the average Euclidean distance of the relative abundance of each OTU between all sampling dates, using the R-script presented in Appendix S1 of Ruiz-González et al. (2015). Those OTUs showing a mean distance >10 were considered 'responsive' OTUs. This value represented an average maximum change of 626 sequences between two sampling dates for any responsive OTU (range 26- 17749).

ACNOWLEDGEMENTS

This work was partially supported by grants DOREMI (CTM2012-34294), HOTMIX (CTM2011-30010/MAR), REMEI (CTM2015-70340-Rf), SUAVE (CTM2014-54926-R), funded by the Spanish Government, and EcoRARE (CTM2014-60467-JIN), funded by the Spanish Government and the European Regional Development Fund (ERDF). We thank Idaira Santos, Raquel Gutierrez and Carolina Antequera for their help during sampling and laboratory analyses. We thank Ramiro Logares for the amplicon data processing, and Clara Ruiz-Gonzalez for her help during data analyses and for comments on a previous version of the manuscript.

REFERENCES

Aanderud ZT, Jones SE, Fierer N, Lennon JT. (2015). Resuscitation of the rare biosphere contributes to pulses of ecosystem activity. *Front Microbiol* **6**. doi:10.3389/fmicb.2015.00024.

Amy PS, Morita RY. (1983). Starvation-survival patterns of sixteen freshly isolated

open-ocean bacteria. *Appl Environ Microbiol* **45**:1109–1115.

Arístegui J, Duarte CM, Agustí S, Doval M, Alvarez-Salgado X a, Hansell D a. (2002). Dissolved organic carbon support of respiration in the dark ocean. *Science* **298**:1967.

Arístegui J, Gasol JM, Duarte CM, Herndl GJ. (2009). Microbial oceanography of the dark ocean's pelagic realm. *Limnol Oceanogr* **54**:1501–1529.

Arístegui J, Montero MF. (2005). Temporal and spatial changes in plankton respiration and biomass in the Canary Islands region: The effect of mesoscale variability. In:*Journal of Marine Systems*, Vol. 54, pp. 65–82.

Auguet JC, Nomokonova N, Camarero L, Casamayor EO. (2011). Seasonal changes of freshwater ammonia-oxidizing archaeal assemblages and nitrogen species in oligotrophic alpine lakes. *Appl Environ Microbiol* **77**:1937–1945.

Baltar F, Arístegui J, Gasol JM, Yokokawa T, Herndl GJ. (2013). Bacterial Versus Archaeal Origin of Extracellular Enzymatic Activity in the Northeast Atlantic Deep Waters. *Microb Ecol* **65**:277–288.

Baltar F, Arístegui J, Sintes E, Van Aken HM, Gasol JM, Herndl GJ. (2009). Prokaryotic extracellular enzymatic activity in relation to biomass production and respiration in the meso- and bathypelagic waters of the (sub)tropical Atlantic. *Environ Microbiol* **11**:1998–2014.

Baltar F, Arístegui J, Sintes E, Gasol JM, Reinthaler T, Herndl GJ. (2010). Significance of non-sinking particulate organic carbon and dark CO 2 fixation to heterotrophic carbon demand in the mesopelagic northeast Atlantic. *Geophys Res Lett* **37**. doi:10.1029/2010GL043105.

Baltar F, Lundin D, Palovaara J, Lekunberri I, Reinthaler T, Herndl GJ, *et al.* (2016). Prokaryotic responses to ammonium and organic carbon reveal alternative CO2 fixation pathways and importance of alkaline phosphatase in the mesopelagic North Atlantic. *Front Microbiol* **7**. doi:10.3389/fmicb.2016.01670.

Calvo-Díaz A, Moran XAG. (2006). Seasonal dynamics of picoplankton in shelf waters of the southern Bay of Biscay. *Aquat Microb Ecol* **42**:159–174.

Campbell BJ, Yu L, Heidelberg JF, Kirchman DL. (2011). Activity of abundant and rare bacteria in a coastal ocean. *Proc Natl Acad Sci USA* **108**:12776–81.

Caporaso JG, Paszkiewicz K, Field D, Knight R, Gilbert JA. (2012). The Western English Channel contains a persistent microbial seed bank. *ISME J* **6**:1089–93.

Carlson CA, Hansell DA, Tamburini C. (2011). DOC persistance and its fate after export within the ocean interior. In:*Microbial carbon pump in the ocean*, pp. 57–59.

Chrost RJ, Gajewski A, Siuda W. (1999). Fluorescein-diacetate (FDA) assay for determining microbial esterase activity in lake water. *Arch fur Hydrobiol* **54**:167–183.

Coble PG. (1996). Characterization of marine and terrestrial DOM in seawater using excitation emission matrix spectroscopy. *Mar Chem* **51**:325–346.

Copin-Montégut C. (1993). Alkalinity and carbon budgets in the Mediterranean Sea. *Global Biogeochem Cycles* **7**:915–925.

Cúcio C, Engelen AH, Costa R, Muyzer G. (2016). Rhizosphere microbiomes of European + seagrasses are selected by the plant, but are not species specific. *Front Microbiol* **7**. doi:10.3389/fmicb.2016.00440.

Daims H, Lebedeva E V., Pjevac P, Han P, Herbold C, Albertsen M, *et al.* (2015). Complete nitrification by Nitrospira bacteria. *Nature*. doi:10.1038/nature16461.

Edgar RC. (2013). UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat Methods* **10**:996–8.

Eilers H, Pernthaler J, Amann R. (2000). Succession of Pelagic Marine Bacteria during Enrichment : a Close Look at Cultivation-Induced Shifts. **66**(11):4634–40.

Ferguson RL, Buckley EN, Palumbo A V. (1984). Response of marine bacterioplankton to differential filtration and confinement. *Appl Environ Microbiol* **47**:49–55.

Fernández-Gómez B, Richter M, Schüler M, Pinhassi J, Acinas SG, González JM, *et al.* (2013). Ecology of marine Bacteroidetes: A comparative genomics approach. *ISME J* **7**:1026–37.

Finkel SE. (2006). Long-term survival during stationary phase: evolution and the GASP phenotype. *NatRevMicrobiol* **4**:113–120.

Gasol JM, Moran XAG. (2015). Flow Cytometric Determination of Microbial Abundances and Its Use to Obtain Indices of Community Structure and Relative Activity. *Hydrocarb Lipid Microbiol Protoc - Springer Protoc Handbooks* 1–29.

Gibbons SM, Caporaso JG, Pirrung M, Field D, Knight R, Gilbert J a. (2013). Evidence for a persistent microbial seed bank throughout the global ocean. *Proc Natl Acad Sci USA* **110**:4651–5.

Gonzalez JM, Portillo MC, Belda-Ferre P, Mira A. (2012). Amplification by PCR artificially reduces the proportion of the rare biosphere in microbial communities. *PLoS One* **7**. doi:10.1371/journal.pone.0029973.

Gundersen K, Heldal M, Norland S, Purdie DAA, Knap AHH. (2002). Elemental C, N, and P cell content of individual bacteria collected at the Bermuda Atlantic Time-Series Study (BATS) site. *Limnol Oceanogr* **47**:1525–1530.

Hansell DA, Carlson CA, Repeta DJ, Schlitzer R. (2009). Dissolved Organic Matter in the Ocean A Controversy Stimulates New Insights. *Oceanography* **22**:202–211.

Hansell DA, Ducklow HW. (2003). Bacterioplankton distribution and production in the bathypelagic ocean: Directly coupled to particulate organic carbon export? *Limnol Oceanogr* **48**:150–156.

Hansen HP, Koroleff F. (2007). Determination of nutrients. In:*Methods of Seawater Analysis: Third, Completely Revised and Extended Edition*, pp. 159–228.

Herndl GJ, Reinthaler T. (2013). Microbial control of the dark end of the biological pump. *Nat Geosci* **6**:718–724.

Herndl GJ, Reinthaler T, Teira E, Van Aken H, Veth C, Pernthaler A, *et al.* (2005). Contribution of Archaea to total prokaryotic production in the deep atlantic ocean. *Appl Environ Microbiol* **71**:2303–2309.

Hong JK, Cho JC. (2015). Environmental Variables Shaping the Ecological Niche of Thaumarchaeota in Soil: Direct and Indirect Causal Effects. *PLoS One* **10**. doi:10.1371/journal.pone.0133763.

Hoppe H-G. (1983). Significance of exoenzymatic activities in the ecology of brackish water: measurements by means of methylumbelliferyl-substrates . *Mar Ecol Prog Ser* **11**:299–308.

Hoppe HG, Ullrich S. (1999). Profiles of ectoenzymes in the Indian Ocean: Phenomena of phosphatase activity in the mesopelagic zone. *Aquat Microb Ecol* **19**:139–148.

Hugoni M, Taib N, Debroas D, Domaizon I, Jouan Dufournel I, Bronner G, *et al.* (2013). Structure of the rare archaeal biosphere and seasonal dynamics of active ecotypes in surface coastal waters. *Proc Natl Acad Sci USA* **110**:6004–9.

Jiao N, Herndl GJ, Hansell D a, Benner R, Kattner G, Wilhelm SW, *et al.* (2010). Microbial production of recalcitrant dissolved organic matter: long-term carbon storage in the global ocean. *Nat Rev Microbiol* **8**:593–9.

Kirchman D, K'nees E, Hodson R. (1985). Leucine incorporation and its potential as a measure of protein synthesis by bacteria in natural aquatic systems. *Appl Environ Microbiol* **49**:599–607.

Koch H, Lücker S, Albertsen M, Kitzinger K, Herbold C, Spieck E, *et al.* (2015). Expanded metabolic versatility of ubiquitous nitrite-oxidizing bacteria from the genus *Nitrospira*. *Proc Natl Acad Sci* **112**:11371–11376.

Könneke M, Bernhard AE, de la Torre JR, Walker CB, Waterbury JB, Stahl D a. (2005). Isolation of an autotrophic ammonia-oxidizing marine archaeon. *Nature* **437**:543–6.

Kuzyakov Y, Friedel JK, Stahr K. (2000). Review of mechanisms and quantification of priming effects. *Soil Biol Biochem* **32**:1485–1498.

Landry Z, Swan BK, Herndl GJ, Stepanauskas R, Giovannoni SJ. (2017). SAR202 Genomes from the Dark Ocean Predict Pathways for the Oxidation of Recalcitrant Dissolved Organic Matter. *MBio* **8**:e00413-17.

Lennon JT, Jones SE. (2011). Microbial seed banks: the ecological and evolutionary implications of dormancy. *Nat Rev Microbiol* **9**:119–30.

Li M, Baker BJ, Anantharaman K, Jain S, Breier JA, Dick GJ. (2015). Genomic and transcriptomic evidence for scavenging of diverse organic compounds by widespread deep-sea archaea. *Nat Commun* **6**:8933.

Logares R. (2017). Workflow for analysing MiSeq amplicons based on Uparse v1.5. https://doi.org/10.5281/zenodo.259579.

Logares R, Sunagawa S, Salazar G, Cornejo-Castillo FM, Ferrera I, Sarmento H, *et al.* (2013). Metagenomic 16S rDNA Illumina tags are a powerful alternative to amplicon sequencing to explore diversity and structure of microbial communities. Environmental Microbiology, **16**(9):2659-71.

Luna GM, Bianchelli S, Decembrini F, De Domenico E, Danovaro R, Dell'Anno A. (2012). The dark portion of the Mediterranean Sea is a bioreactor of organic matter cycling. *Global Biogeochem Cycles* **26**. doi:10.1029/2011GB004168.

Massana R, Pedros-Alio C, Casamayor EO, Gasol JM. (2001). Changes in marine bacterioplankton phylogenetic composition during incubations designed to measure biogeochemically significant parameters. *Limnol Oceanogr* **46**:1181–1188.

McCarren J, Becker JW, Repeta DJ, Shi Y, Young CR, Malmstrom RR, *et al.* (2010). Microbial community transcriptomes reveal microbes and metabolic pathways associated with dissolved organic matter turnover in the sea. *Proc Natl Acad Sci USA* **107**:16420–7.

McIlroy SJ, Nielsen PH. (2014). The family saprospiraceae. In:*The Prokaryotes: Other Major Lineages of Bacteria and The Archaea*, Vol. 9783642389, pp. 863–889.

Nagata T, Fukuda H, Fukuda R, Koike I. (2000). Bacterioplankton distribution and production in deep Pacific waters: Large-scale geographic variations and possible coupling with sinking particle fluxes. *Limnol Oceanogr* **45**:426–435.

Nikolaki S, Tsiamis G. (2013). Microbial diversity in the era of omic technologies. *Biomed Res Int* **2013**. doi:10.1155/2013/958719.

Novitsky JA, Morita RY. (1976). Morphological characterization of small cells resulting from nutrient starvation of a psychrophilic marine vibrio. *Appl Environ Microbiol* **32**:617–622.

Novitsky JA, Morita RY. (1977). Survival of a psychrophilic marine vibrio under long term nutrient starvation. *Appl Environ Microbiol* **33**:635–641.

Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, *et al.* (2015). vegan: Community Ecology Package.

Parada AE, Needham DM, Fuhrman JA. (2016). Every base matters: Assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. *Environ Microbiol* **18**:1403–1414.

Pedler BE, Aluwihare LI, Azam F. (2014). Single bacterial strain capable of significant contribution to carbon cycling in the surface ocean. *Proc Natl Acad Sci USA* **111**:7202–7.

Pedrós-Alió C. (2012). The rare bacterial biosphere. *Ann Rev Mar Sci* **4**:449–66.

Rinke C, Schwientek P, Sczyrba A, Ivanova NN, Anderson IJ, Cheng J-F, *et al.* (2013). Insights into the phylogeny and coding potential of microbial dark matter. *Nature* **499**:431–7.

Ruiz-González C, Niño-García JP, del Giorgio PA. (2015). Terrestrial origin of bacterial communities in complex boreal freshwater networks. *Ecol Lett* **18**:1198–1206.

Sala MM, Aparicio FL, Balagué V, Boras JA, Borrull E, Cardelús C, *et al.* (2015). Contrasting effects of ocean acidification on the microbial food web under different trophic conditions. *ICES J Mar Sci J du Cons* **73**:fsv130.

Schafer H, Servais P, Muyzer G. (2000). Successional changes in the genetic diversity of a marine bacterial assemblage during confinement. *Arch Microbiol* **173**:138–145.

Shade A, Jones SE, Caporaso JG, Handelsman J, Knight R, Fierer N, *et al.* (2014). Conditionally rare taxa disproportionately contribute to temporal changes in microbial diversity. *MBio* **5**:e01371-14.

Smith DC, Azam F. (1992). A simple , economical method for measuring bacterial protein synthesis rates in seawater using 3H-leucine 1. *Mar Microb Food Webs* **6**:107–114.

Soora M, Cypionka H. (2013). Light enhances survival of *Dinoroseobacter shibae* during long-term starvation. *PLoS One* **8**. doi:10.1371/journal.pone.0083960.

Sun C, Fu GY, Zhang CY, Hu J, Xu L, Wang RJ, *et al.* (2016). Isolation and complete genome sequence of *Algibacter alginolytica* sp. nov., a novel seaweed-degrading Bacteroidetes bacterium with diverse putative polysaccharide utilization loci. *Appl Environ Microbiol* **82**:2975–2987.

Swan BK, Martinez-Garcia M, Preston CM, Sczyrba A, Woyke T, Lamy D, *et al.* (2011). Potential for Chemolithoautotrophy. *Science (80-)* **333**:1296–9.

Tang K, Yang Y, Lin D, Li S, Zhou W, Han Y, *et al.* (2016). Genomic, physiologic, and proteomic insights into metabolic versatility in Roseobacter clade bacteria isolated from deep-sea water. *Sci Rep* **6**:35528.

Tanhua T, Hainbucher D, Schroeder K, Cardin V, Álvarez M, Civitarese G. (2013). The Mediterranean Sea system: A review and an introduction to the special issue. *Ocean Sci* **9**:789–803.

Yilmaz P, Yarza P, Rapp JZ, Glöckner FO. (2016). Expanding the world of marine bacterial and archaeal clades. *Front Microbiol* **6**:1524.

FIGURE LEGENDS

Figure 1. Dynamics of an enclosed bathypelagic community during 1-year of starvation. **a)** total organic carbon (TOC) and total nitrogen (TN), **b)** heterotrophic prokaryotic production measured as leucine incorporation, **c)** Prokaryotic abundance (PA), total cells represented as filled circles and population of microcells (see Figure S1), as open circles **d)** diversity and richness estimated with the Shannon index (solid line) and number of OTUs (dashed line), respectively. Error bars represent the standard deviation of the replicates (see methods).

Figure 2. Taxonomic composition (16S-rDNA based) of the prokaryotic community along the experiment, shown at the phylum level except for the Proteobacteria, which are divided into classes.

Figure 3. Temporal dynamics of different enzymatic activities as an indication of the metabolic potential of the microbial communities. Each data point represents the average of four replicates and error bars represent the standard deviation.

Figure 4. Characteristics of the OTUs that displayed the largest changes in abundance during the experiment, i.e. "responsive OTUs" (see text for details). **a, b)** Temporal dynamics of the relative contribution of the 'pre' and 'post-bloom' OTUs to the total number of sequences; solid lines represent only bacterial OTUs, and dashed line all OTUs (archaeal and bacterial). **c, d)** Temporal dynamics of the number of OTUs within the 'pre' and 'post-bloom' pools. **e, f)** Taxonomic affiliation of the responsive OTUs before and after the bloom. Classification was performed at the phylum level except for Proteobacteria, Bacteroidetes and Planctomycetes that

were split into classes or orders depending on their relative contribution.

Figure 5. Relationships between the proportion of 16S rRNA and 16S rDNA sequences in the bacterial 'pre' and 'post-bloom' OTUs (see main text for details).