Differential recruitment of opportunistic taxa leads to contrasting abilities in carbon processing by bathypelagic and surface microbial communities

Marta Sebastián1,2, Irene Forn1, Adrià Auladell1, Markel Gómez-Letona2, M. Montserrat Sala1, Josep M. Gasol1, Celia Marrasé1

1 Departament de Biologia Marina i Oceanografia, Institut de Ciències del Mar, CSIC, 08003 Barcelona, Catalunya, Spain
2 Instituto de Oceanografía y Cambio Global, IOCAG, Universidad de Las Palmas de Gran Canaria, ULPGC, 35214, Gran Canaria, Spain.

Running title: Contrasting patterns in carbon processing in the ocean

Correspondence to Dr Marta Sebastián, Departament de Biologia Marina i Oceanografia, Institut de Ciències del Mar, CSIC, 08003 Barcelona, Catalunya, Spain
Email: msebastian@icm.csic.es
The authors declare no conflict of interest
SIGNIFICANCE STATEMENT

Production of recalcitrant compounds is known to occur during organic matter processing by bacterioplankton, being an important component of the biological pump. Roughly twenty percent of the carbon produced in the sunlit ocean is exported to the deep ocean as dissolved compounds during winter overturn. Recent studies have shown that bathypelagic prokaryotes are metabolically versatile, but whether this versatility translates into a higher ability to process carbon has rarely been explored. To address this issue we performed a transplant experiment to compare the growth, activity and organic matter processing of surface and bathypelagic prokaryotes exposed to the same environmental conditions. We found that incubations with surface prokaryotes led to an accumulation of recalcitrant compounds, which did not occur with bathypelagic prokaryotes, suggesting they were able to process these compounds. These contrasting abilities to process DOM were attributed to the recruitment of a larger number of opportunistic taxa among the bathypelagic assemblages that likely resulted in a broader community capability of substrate utilization. The comparatively higher ability of bathypelagic prokaryotes to use recalcitrant DOC compounds would lead to a lower efficiency in the long-term sequestration of this carbon. Thus, future changes in the intensity of the overturning circulation due to climate change should have an impact on the persistence and fate of DOC in the ocean.

ABSTRACT

Different factors affect the way dissolved organic matter (DOM) is processed in the ocean water column, including environmental conditions and the functional capabilities
of the communities. Recent studies have shown that bathypelagic prokaryotes are metabolically flexible, but whether this versatility translates into a higher ability to process DOM has been barely explored. Here we performed a multifactorial transplant experiment to compare the growth, activity and changes in DOM quality in surface and bathypelagic waters inoculated with either surface or bathypelagic prokaryotic communities. The effect of nutrient additions to surface waters was also explored. Despite no differences in the cell abundance of surface and deep ocean prokaryotes were observed in any of the treatments, in surface waters with nutrients the heterotrophic production of surface prokaryotes rapidly decreased. Conversely, bathypelagic communities displayed a sustained production throughout the experiment. Incubations with surface prokaryotes always led to a significant accumulation of recalcitrant compounds, which did not occur with bathypelagic prokaryotes, suggesting they have a higher ability to process DOM. These contrasting abilities could be explained by the recruitment of a comparatively larger number of opportunistic taxa within the bathypelagic assemblages, which likely resulted in a broader community capability of substrate utilization.

INTRODUCTION

Microbes are the engines driving the earth’s biochemical cycles (Falkowski et al., 2008) and given that a large fraction of the earth’s prokaryotes (i.e. Bacteria and Archaea) occur in the ocean (Whitman et al., 1998; Bar-On et al., 2018), marine prokaryotes play a pivotal role in ecosystem functioning. Although they have traditionally been considered a homogeneous black box, molecular studies and single-cell activity approaches have shown that marine prokaryotes are incredibly diverse, and highly heterogeneous in their levels of activity (Kirchman et al., 2004; Sogin et al., 2006; Alonso-Sáez and Gasol, 2007). These levels of activity are shaped by both the metabolic traits of the prokaryotes
and resource availability, which change drastically along the ocean water column: whereas the euphotic layer is rich in dissolved organic carbon and depleted in inorganic nutrients (e.g. Thingstad et al., 1997), deep ocean waters are nutrient rich but limited by the availability of easily metabolizable organic carbon (e.g. Herndl and Reinthaler, 2013), which is mostly produced in the sunlit ocean.

As a consequence of the decrease in the availability of carbon, prokaryotic abundance and production decreases exponentially with depth (Aristegui et al., 2009; Baltar et al., 2009), and there is a drastic change in community composition and metabolic potential (DeLong et al., 2006; Brown et al., 2009; Sunagawa et al., 2015). For example, bathypelagic prokaryotes harbor more genes devoted to polysaccharide degradation compared to their surface counterparts (DeLong et al., 2006), which may explain the increase in cell specific enzymatic activities and specific uptake rates of polymeric substances towards deep waters (Baltar et al., 2009; Boutrif et al., 2011). In addition, the proportion of prokaryotic cells with high nucleic acids content (HNA cells) also increases with depth (Gasol et al., 2009; Van Wambeke et al., 2011), which might be indicative of larger genomes, in agreement with some genomic (Vezzi, 2005; Lauro and Bartlett, 2008), and global metagenomic observations (Acinas et al., 2019). Large genomes may be the imprint of the pressure for metabolic versatility and an opportunistic life-style (Guieysse and Wuertz, 2012) to cope with the sporadic nature of carbon inputs (Smith et al., 2018), a myriad of diluted organic compounds (Arrieta et al., 2015), and the high proportion of recalcitrant substances (Hansell, 2013; Shen and Benner, 2020) typical of the bathypelagic realm. Indeed, bathypelagic communities have been previously shown to be very malleable in response to carbon starvation (Sebastián et al., 2018), and to harbour opportunistic taxa that can swiftly respond to sudden inputs of organic carbon (Sebastián et al., 2019). However, whether this metabolic flexibility translates into a
contrasting ability to process DOM has been barely explored (but see Boutrif et al., 2011; Shen and Benner, 2018).

Here we performed a multifactorial transplant experiment to compare the growth potential, activity and changes in DOM quality in surface (5 m) and bathypelagic waters (2100 m depth) inoculated with either surface or bathypelagic communities (see Figure S1 for details on the experimental setup). Since growth and DOM utilization in the epipelagic is often limited by the availability of inorganic nutrients, the effect of nutrient additions to surface waters was explored as well. We also focused on the identity and dynamics of opportunistic taxa (i.e. the most responsive taxa) in the different conditions, to shed light onto the role of microbial structure on DOM processing by the surface and bathypelagic communities. Based on the presumably higher metabolic versatility of deep ocean communities, we hypothesized that under the same environmental conditions, bathypelagic prokaryotes would reach higher abundances, and would be more efficient in carbon utilization, than their surface counterparts.

RESULTS

Experimental initial conditions

As expected, surface seawater had initial inorganic nutrient concentrations one order of magnitude lower than bathypelagic waters (Table 1), but higher dissolved organic carbon (DOC) concentrations. The addition of inorganic nutrients to surface waters (SW + N treatment) resulted in nitrate and phosphate concentrations in the range of the values found in the bathypelagic waters (Table 1). On day 9 of the experiment a combination of carbon-based substances with different lability, including acetate,
glucose, terrestrial humic acids and amino acids (see Table S1), was added to half of the remaining bathypelagic waters (BW+C treatment), yielding DOC values close to the ones found in surface waters (Table 1).

**Dynamics of prokaryotic cell abundance and heterotrophic production**

The abundance of prokaryotic cells reached higher values in the surface water with inorganic nutrients (SW+N, \(\sim 3 \times 10^5\) cells mL\(^{-1}\)) than in the SW and the BW treatments (\(\sim 1.4 \times 10^5\) cells mL\(^{-1}\), Figure 1a), confirming that growth of both bathypelagic and surface communities in the SW treatment was limited by the availability of inorganic nutrients, whereas in the BW treatment the prokaryotes were limited by the availability of organic carbon. This carbon limitation was further proven by the addition of mixed carbon compounds to BW on day 9 of the experiment (see methods and Table S1), which caused a drastic increase in the cell abundance of both surface and bathypelagic communities, reaching values ca 3-fold higher than in the SW+N treatment (\(\sim 8 \times 10^5\) cells mL\(^{-1}\), Figure 1a). This large increase in abundance upon enrichment with some labile compounds as compared to the SW+N treatment, suggests that the DOC present in the surface waters was already partially recalcitrant. No significant differences in cell abundances were observed between surface and bathypelagic communities in any of the treatments (Figure 1a, Table 2).

Likewise, the cell size of surface and bathypelagic prokaryotes was also similar in the SW and BW treatments, and in the same range as the values observed for bathypelagic communities in the SW+N treatment (\(\sim 0.1\) µm\(^3\), Figure 1b, Table 2). However, the size of surface cells in the SW+N treatment notably decreased after 8 days (Figure 1b), reaching 0.08 µm\(^3\) at the end of the experiment. Flow cytometry cytograms showed that the change in cell size coincided with a sharp two-fold decrease in the number of high
nucleic acid (HNA) containing cells (Figure S2), and a shift of the community towards a dominance of low nucleic acid containing cells (LNA). Besides this shift, we also observed the appearance of a population of tiny cells (0.045 \( \mu m^3 \), i.e. microcells) in the surface communities of the SW+N treatment (Figure S2) that was not detected in the bathypelagic communities of this treatment, nor in any community in the other treatments (data not shown).

Leucine incorporation rates (as an estimate of heterotrophic prokaryotic production) of surface prokaryotes peaked on day 2 of the experiment in all treatments, then decreased notably from day 2 to day 3, and afterwards remained stable except for the SW+N treatment, where it continued to decrease until the end of the experiment (Figure 1c). The activity of bathypelagic prokaryotes in the SW+N treatment also increased in day 2, but unlike surface prokaryotes, bathypelagic cells maintained high levels of activity until day 10 of the experiment, when it slightly dropped (Figure 1c). The heterotrophic production of bathypelagic prokaryotes in the SW and BW treatments followed similar trends, increasing until day 10 of the experiment, but decreasing shortly afterwards, and not presenting the day 2 peak observed in surface prokaryotes. Analysis of the cumulative heterotrophic production throughout the experiment showed that it was higher with the bathypelagic prokaryotes than with the surface ones in all water treatments (Figure S3), although these differences were only significant for the SW+N and the BW+C treatments (Table 2).

Dynamics in community structure and diversity

We next explored the taxonomic composition of the communities in the different treatments and how it changed over time. Initial surface communities were dominated by SAR11 bacteria (53% of the reads), whereas bathypelagic communities had a high
proportion of Thaumarchaeota (38% of the reads, Figure 2). In order to remove predators, we prefiltered the sample through a 0.8 µm pore size filter, and then collected the cells onto a 0.2 µm filter. These cells were afterwards resuspended in particle free seawater, counted by flow cytometry, and inoculated in equal amounts in each of the treatments (see methods and Figure S1 for details). Detachment of cells from the filter is never 100% efficient, and the prefiltration step may also remove large prokaryotic cells. Analysis of the community structure after these steps unveiled that there was some change likely due to the removal of large cells (Figure 2), indicated by the reduction in cyanobacterial sequences from the surface communities, which are usually retained by the 0.8 µm filter (Mestre et al., 2017), and the enrichment in Thaumarchaeota, which are preferentially found in the <0.8 µm size fraction (Salazar et al., 2015), in the bathypelagic inoculum. These manipulations resulted in a slight decrease in the Shannon diversity index of the surface inoculum (from 3.1 to 2.6), but not in the bathypelagic inoculum (4.4 and 4.6 in the initial community and the inoculum, respectively). However, a major change in community composition was not observed. In contrast, there was a remarkable shift within the next 40 h in the dominant taxa upon enclosure of the communities. Regardless the water treatment (SW, SW+N, or BW), Gammaproteobacterial taxa dominated the surface assemblages throughout the experiment (Figure 2, upper panel), and they also represented a large proportion of the bathypelagic communities (Figure 2, lower panel). Gammaproteobacteria in all treatments were mostly represented by two taxa (exact amplicon sequence variants, ‘ASV’) of Alteromonadales, asv1 and asv2 (Figure S4, table S2). These two taxa alone summed up ~90% of the surface assemblage sequences on day 2 of the experiment regardless the water treatment (Figure 2, upper panel, Table S2). The contribution of these two taxa to the bathypelagic assemblages was also high but slightly lower, accounting for ~60, ~70%, and 23% in the SW, SW+N, and BW treatment on day
2 of the experiment, respectively. Other gammaproteobacteria like Oceanospirillales also accounted for a noteworthy fraction of the bathypelagic communities, representing around 25% in the SW and SW+N treatments and ~50% in the BW treatment on day 2 of the experiment, but they decreased in abundance with time. Conversely, Oceanospirillales increased in abundance along the experiment in the surface assemblages when transplanted into bathypelagic water (BW treatment), going from 3% of the community on day 2 to ~22% on day 12. Nevertheless, upon carbon addition to the bathypelagic water, the proportion of Oceanospirillales in the surface assemblages decreased again (Figure 2, BW+C treatment).

Several alphaproteobacterial ASVs belonging to the Rhodobacterales order were also major contributors to the bathypelagic communities in the last time points of the experiment, particularly in the SW+N and the BW+C treatment, where they accounted for 55% and 62% of the communities, respectively (Figure 2, Table S2). Similarly, addition of dissolved organic carbon to the surface communities in bathypelagic waters (BW+C treatment) resulted also in a notable increase in the contribution of Rhodobacterales, represented by a single ASV (Table S2), which accounted for over 30% of the community 2 days after the carbon input.

The reduction in size of surface-derived cells in the SW+N treatment (Figure 1), together with the appearance of the population of tiny cells (Figure S2), was accompanied with a swift change in the community composition from gammaproteobacterial dominated to a larger contribution of Bacteroidetes (Flavobacteria) and Alphaproteobacteria (Figure 2 upper panel, Table S2, Figure S4).

Over the course of the experiment bathypelagic communities displayed overall higher diversity (Shannon index) and evenness than surface communities (Figure 3a). Focusing only on the opportunistic taxa in each of the treatments, which we arbitrarily
defined as those ASVs that were initially rare but got recruited overtime and reached abundances over 5% of the community, we consistently found a higher number of opportunistic taxa in the bathypelagic assemblages in each of the water treatments compared to surface communities (Figure 3b).

Dynamics in hydrolytic activities

To investigate whether these taxonomic changes were accompanied by different physiological capabilities in surface and bathypelagic communities, we explored their enzymatic profiles in the different water treatments. We focused on the activities of $\alpha$-glucosidase (AGase) and $\beta$-glucosidase (BGase), which are involved in the utilization of polysaccharides, leucyl aminopeptidase (LAPase), which degrades the proteinaceous components of DOM, and alkaline phosphatase (APase), involved in the hydrolysis of organic phosphoesters (Figure 4).

Contrary to the expectations of higher per cell hydrolytic rates in bathypelagic prokaryotes than in their surface counterparts, no major differences were observed between specific AGase and BGase activities of the surface and bathypelagic inocula within the different treatments (Figure 4), and both tended to increase towards the end of the experiment. The largest differences were found in the SW+N treatment, where the AGase and BGase activities of surface prokaryotes displayed a maximum on day 2, and decreased afterwards. Overall, both surface and bathypelagic communities showed lower specific AGase, and BGase rates in the BW treatment, probably reflecting lower availability of substrates (Figure 4). Surface prokaryotes displayed higher specific LAPase activity values than their bathypelagic counterparts in the surface water treatments (SW and SW+N) within the first days of the experiment (Figure 4). In the BW treatments, both communities showed similar trends in specific LAPase activities, with
low values throughout the experiment and a maximum at the last time-point. Upon the 
addition of the mixed sources of organic C to the bathypelagic water there was a 
remarkable increase in total hydrolytic activities (Figure S5), which reached similar 
values in both the surface and bathypelagic communities. However, the per cell AGase, 
BGase and LAPase activities in the BW+C treatment were within the range of those 
observed in the rest of the treatments, indicating that there was not an enhancement of the 
cell-specific activities (Figure 4).

In contrast, APase activities showed clear differences between both communities in 
some of the treatments, particularly in surface waters (SW). Addition of inorganic 
nutrients in the SW+N treatment led to a repression of the APase enzymes, and per cell 
specific activities were very low and constant for both communities in this treatment 
(Figure 4). Specific APase activities were higher in the BW treatment than in the SW+N 
treatment, particularly for the surface assemblage (Figure 4, third panel). Addition of 
organic carbon to the BW resulted in the decrease of APase specific activities (Figure 4, 
fourth panel).

DOM processing by surface and bathypelagic communities

To investigate if surface and bathypelagic communities had different abilities at 
DOM processing, we looked at changes in DOM quality by exploring the optical 
properties of chromophoric DOM (CDOM) and, in particular, of its fluorescent fraction 
(FDOM) (see methods for further details). We first focused on the dynamics of 
fluorescent humic-like (peak C in FDOM) and protein-like (peak T in FDOM) substances 
(Coble, 1996) and their ratio (peak C/peak T), which is an indication of the amount of 
recalcitrant versus labile fluorescent material (Baker et al., 2008). Initial values in Peak 
C where higher in BW than in surface waters (SW, SW+N, Figure 5a). However, peak C
increased in all treatments inoculated with surface prokaryotes, reaching values significantly higher than those obtained with bathypelagic prokaryotes in all treatments at the end of the experiment (Wilcoxon test, see Table S3 for the p-values). In contrast, values of peak T were only significantly higher at the final time point of the experiment in the SW and BW+C treatments inoculated with the bathypelagic prokaryotes (Wilcoxon test, Table S3). Peak T considerably increased upon carbon addition to the BW treatment (Figure 5a, right panel), but it rapidly decreased afterwards, indicating that there was a fast consumption of the labile compounds by both the surface and bathypelagic prokaryotes.

The proportion of humic-like versus labile material (peak C/peak T ratio) in the SW treatment was rather constant for both the surface and bathypelagic communities, although it reached significantly higher values with the surface prokaryotes at the end of the experiment (Figure 5a, Wilcoxon test, Table S3). In the SW+N treatment both surface and bathypelagic communities showed opposite trends: whereas the C/T ratio significantly increased with the surface prokaryotes, pointing to a net production of humic substances, the C/T ratio decreased with the bathypelagic prokaryotes. In the BW treatment, the C/T ratio values were high at the beginning of the experiment, but later decreased along the experiment, being this decrease more evident with the bathypelagic inocula than with the surface one (Figure 5a). The organic carbon addition resulted in low C/T ratios due to the large proportion of amino acids added (treatment BW+C, Figure 5a, right panel). At the end of the experiment the C/T ratio of this treatment increased, but this increase was significantly higher with the surface prokaryotes than with the bathypelagic prokaryotes, as in the rest of the treatments (Wilcoxon test, Table S3).

The spectral slope of the chromophoric DOM in the 275-295 nm wavelength range is also an indicator of the quality of the organic matter, with lower values indicating an
increase in aromaticity and/or higher molecular weight (Helms et al., 2008). There was a notable decrease (30%) in this spectral slope from the beginning to the end of the experiment in all the treatments inoculated with surface prokaryotes (Figure 5b), whereas the decrease was much lower (<5%) in those treatments inoculated with bathypelagic prokaryotes, suggesting that surface prokaryotes produced aromatic compounds during the experiment.

DISCUSSION

Many factors affect prokaryotic growth and DOM processing in the ocean, including environmental conditions, the metabolic potential of the prokaryotes, and the quality and quantity of the available resources (Jiao et al., 2010; Arrieta et al., 2015; Carlson and Hansell, 2015), and these factors are known to change drastically along the water column. Our experimental approach enabled us to compare surface and bathypelagic communities under the same environmental conditions, thus focusing only on the effect of the community structure and metabolic capabilities of surface and bathypelagic prokaryotes in community growth and DOM processing.

The lack of significant differences in the maximum cell abundance reached by surface and bathypelagic communities within each of the treatments was surprising given the difference in community structure of the starting communities, and suggests that the environmental conditions, and not the genetic potential of the community, is the factor controlling the biomass yield. This contrasts with a previous study that did find higher biomass yields when surface waters were inoculated with upper mesopelagic communities (250 m) than with surface ones (Carlson et al 2004), although noticeably, in that study the starting cell abundance in the mesopelagic inocula was much lower than the surface one, allowing for a higher prokaryotic growth.
Addition of inorganic nutrients to surface waters stimulated the growth of both the surface and bathypelagic prokaryotic communities (Figure 1, SW+N treatment), as frequently observed in the Mediterranean (Pinhassi et al., 2006) and other oligotrophic regions (Cotner et al., 1997; Mills et al., 2008). However, despite both communities reached similar abundance values, after 8 days surface communities experienced a drastic change in cell size concomitantly with the appearance of a population of microcells (Figure S2). This suggests that by that time a fraction of the surface prokaryotes had become carbon-limited, as miniaturization and/or fragmentation are well-described phenotypic characteristics of growth-arrested cells upon carbon starvation (Novitsky and Morita, 1976, 1977; MacDonell and Hood, 1982; Kolter, 1993). Since this phenotypic response is common to several groups of Gammaproteobacteria (MacDonell and Hood, 1982), we hypothesize that the population of tiny cells originated from the Alteromonadales taxa that dominated the surface communities. 16S rDNA sequencing cannot differentiate between living, dormant or dead cells, so we used Catalyzed Reporter Deposition Fluorescent In situ Hybridization (CARD-FISH) to test this hypothesis, by quantifying the percentage of gammaproteobacterial cells that contained intact ribosomes (or enough ribosomes to be detected) at the end of the experiment (see supplementary methods). While the contribution of Alteromonas to the 16S-based surface community in the SW+N treatment was over 60% at the final time point (Figure 2), Gammaproteobacterial cells only represented 25% of the cells (Figure S6). Furthermore, 50% of the surface community in this treatment did not have enough ribosomes to be detected at the end of the experiment, whereas in the bathypelagic community cells not detected by CARD-FISH represented only 10% (Figure S6). This suggest that under non-limiting conditions for growth (i.e. with enough organic carbon and nutrients) Alteromonas in the surface communities became dormant or dead when the organic
resources they could exploit became limiting. Consistently with our results, Pedler et al. (2014) experimentally demonstrated that a single species of *Alteromonas* could consume all labile carbon present in surface waters in only three days, but was not able to further exploit the remaining carbon. The steady decrease in the heterotrophic production of surface communities after day 4 of the experiment, as opposed to the sustained production that bathypelagic prokaryotes displayed until the end of the experiment, support the view that surface prokaryotes became carbon limited in the SW+N treatment due to the exhaustion of easily metabolizable carbon. These results point to different abilities in resource utilization by the surface and bathypelagic communities.

The higher diversity and evenness observed in the bathypelagic communities compared to the surface ones (Figure 3) are consistent with previous findings in the Mediterranean and elsewhere (e.g. Pommier *et al.*, 2010; Agogué *et al.*, 2011; Ghiglione *et al.*, 2012), although there are also some reports of decreasing values with depth (Brown *et al.*, 2009). *Alteromonas* were the major contributors to both surface and bathypelagic communities in all water treatments as often observed in amended or unamended microcosms with surface and deep ocean communities (Eilers *et al.*, 2000; Schäfer *et al.*, 2000; McCarren *et al.*, 2010; Nelson and Carlson, 2012; Sebastián *et al.*, 2018). *Alteromonas* display the highest growth rates within marine microbial communities and are generally top-down controlled (Ferrera *et al.*, 2011; Sánchez *et al.*, 2017), and as grazers were removed during the experimental setup, the dominance of this genus was somehow expected. *Alteromonas* comprises copiotrophic taxa that have a preference for a particle associated life-style (Acinas *et al.*, 1999; Mestre *et al.*, 2018), and may have a cosmopolitan distribution throughout the water column using these particles as dispersion drivers (Mestre *et al.*, 2018). The fact that we found the same two *Alteromonas* ASVs blooming in both the surface and bathypelagic water treatments (Table S2), might suggest
that these ASV were indeed cosmopolitan taxa. However, 16S rRNA gene sequencing often has poor resolution at defining taxa and may hide different ecotypes within each ASV (VanInsberghe et al., 2020), so it is also possible that our two ASVs represent different species in surface and bathypelagic communities. In order to further explore this, we aligned the V4-V5 region amplified sequences of our two *Alteromonas* ASVs with other known sequences of *Alteromonas* species and analyzed their sequence identity (Figure S7, see methods for further information). We found that *Alteromonas* asv1, our dominant ASV, was 100% identical to sixteen different *Alteromonas* species, and asv2 was 100% identical to four other *Alteromonas* species. This means that we cannot rule out that the ASVs detected in the bathypelagic assemblages represented different species than the surface ones, which may explain why we observed miniaturization in the surface but not in the bathypelagic communities. Nevertheless, other studies have described cosmopolitan distribution of some *Alteromonas* taxa in both sunlit and deep ocean waters (Lopez-Lopez et al., 2005; Ivars-Martinez et al., 2008; López-Pérez et al., 2012). In any case, regardless whether the two ASVs were cosmopolitan taxa or distinct ecotypes, the fact that *Alteromonas* bloomed in all the treatments suggests this genus is responsible for the rapid utilization of a large fraction of the labile carbon throughout the water column, and not only in surface waters (Pedler et al., 2014).

Besides the two dominant *Alteromonas* ASVs, bathypelagic communities harbored several opportunistic ASVs belonging to Rhodobacterales (Figure S4, Table S2). This group of bacteria are also copiotrophs with a preference for a particle associated lifestyle (Li et al., 2015; Mestre et al., 2018; Gómez-Consarnau et al., 2019) and may reach relatively high abundances in the bathypelagic realm (Salazar et al., 2016; Mestre et al., 2018). The increase in bathypelagic Rhodobacterales in late time points of the experiment and the sustained heterotrophic production throughout the experiment, suggest they were
able to exploit the remaining DOM compounds after Alteromonas had exhausted the most labile DOM, pointing to a succession of specialized opportunistic taxa. Thus, Rhodobacterales outcompeted Alteromonas, but perhaps the later could thrive on by-products of Rhodobacterales metabolism, which could also be a plausible explanation of why we did not observe any cell miniaturization in the bathypelagic communities in the SW+N treatment.

Analyses of the optical properties (fluorescence and absorbance) of DOM provided insight into the composition of dissolved organic matter and its processing. Initial values of peak C (humic-like material) and C/T ratio (proportion of humic versus labile material, see methods) were higher in bathypelagic than in surface waters (SW, SW+N, Figure 5a), in agreement with the largely recalcitrant nature of bathypelagic DOM (Hansell, 2013; Catalá et al., 2016; Martínez-Pérez et al., 2017).

Over the course of the experiment, in all treatments inoculated with surface communities we consistently observed a significant increase in the proportion of recalcitrant compounds (C/T ratio) and a net decrease in the S_{275-295} spectral slope of CDOM (Figure 5), which can be associated with a raise in DOM molecular weight or aromaticity (Helms et al., 2008). Conversely, this increase in recalcitrant compounds did not occur in the treatments inoculated with the bathypelagic communities. Production of recalcitrant compounds that persist during long-term incubations is known to occur during organic matter processing by bacterioplankton (Brophy and Carlson, 1989; Ogawa et al., 2001; Ortega-Retuerta et al., 2009; Romera-Castillo et al., 2011; Osterholz et al., 2015). The fact that these compounds did not accumulate with the bathypelagic assemblages suggests that the consumption of these compounds was greater than their production, indicating that bathypelagic prokaryotes were more efficient at the processing of recalcitrant compounds than their surface counterparts. Similarly, Carlson et al. (2004)
found higher DOC drawdown with upper mesopelagic communities (250 m) than with surface ones. In contrast, no differences in DOC drawdown were observed between surface and bathypelagic communities subjected to additions of recalcitrant and plankton-derived DOC (Shen and Benner, 2020). Despite we could not estimate net DOC utilization rates in our study due to the unfortunate contamination of the DOC samples, our observations imply that there is a fundamental difference in the way DOC is processed by bathypelagic and surface prokaryotes.

The lack of net production of recalcitrant compounds by bathypelagic prokaryotes may result counterintuitive with the prevalent view of higher proportion of recalcitrant compounds in the bathypelagic ocean (Hansell, 2013; Catalá et al., 2016; Martínez-Pérez et al., 2017) and the positive relationship usually found between FDOM and apparent oxygen utilization (which integrates respiratory processes) in deep waters (Yamashita and Tanoue, 2008; Jørgensen et al., 2011; De La Fuente et al., 2014; Catalá et al., 2015).

However, it is probably a matter of the temporal scale of our experiment, and recalcitrant compounds would likely end up accumulating also with the bathypelagic prokaryotes once all the compounds they could exploit had been consumed. In fact, previous experiments with bathypelagic prokaryotes have shown both production and consumption of recalcitrant compounds over time (Aparicio et al., 2015), and it has been recently shown that both production and removal of recalcitrant DOC occurs in the deep ocean (Romera-Castillo et al., 2019).

It could be assumed that the bathypelagic prokaryotes are more efficient at hydrolyzing DOC compounds than surface ones given the typical higher per cell enzymatic activities found in the deep ocean (Baltar et al., 2009). However, we did not observe striking differences between surface and bathypelagic communities in the specific enzymatic rates, except for APase activities, which are involved in the hydrolysis
of organic phosphorus compounds. APase induction is mostly controlled by phosphate availability and the internal phosphorus (P) reserves, and it is induced when prokaryotes do not have enough inorganic phosphorus to meet their demands (Hoppe, 2003). The large difference in activities between surface and bathypelagic prokaryotes in the SW treatment indicates that surface prokaryotes, unlike bathypelagic prokaryotes, did not have internal P reserves and relied on alkaline phosphatases to hydrolyze organic P compounds to obtain assimilable P. This is supported by the observation that in the phosphate-rich SW+N treatment the APase values for both communities were similar (Figure 4). Higher values in the BW treatment than in the SW+N treatment are in agreement with findings of high specific APase activities in deep waters (Hoppe and Ullrich, 1999; Baltar et al., 2009), which have been hypothesized to be related to the acquisition of carbon from dissolved organic phosphorus compounds (Hoppe and Ullrich, 1999). Indeed, the fact that we observed a decrease in APase activities upon carbon addition in the BW+C treatment (Figure 4, fourth panel) is consistent with the hypothesis that APases are used to obtain the carbon moiety of the organic phosphorus compounds in deep waters. Despite these differences in APase activities, the per-cell activity rates of enzymes involved in carbohydrate hydrolysis (AGase and BGase) were quite similar among bathypelagic and surface prokaryotes. Thus, overall the main difference we observed between surface and bathypelagic communities was that bathypelagic assemblages were more diverse and seemed to harbor a higher proportion of resourceful taxa (Figure 3), because the number of ASVs accounting for more than 5% each experimental time-point was always higher in the bathypelagic assemblages. It is thus likely that the combined effort of these resourceful taxa resulted in the exploitation of a wider spectra of DOM compounds. Notably, however, the utilization of these compounds may imply a higher energetic cost, because bathypelagic prokaryotes generally displayed
higher cumulative leucine incorporation than the surface ones, while prokaryotic biomasses were similar (Table 2, Figure S3), an indication that this extra leucine is likely catabolized for energy production (see del Giorgio et al., 2011).

It is important to point out that the bathypelagic prokaryotes experienced a dramatic change in conditions during water collection, particularly in pressure, and it is possible that some pressure-sensitive prokaryotes died during the process of water recovery. Increasing efforts are being directed towards the development of devices and approaches that take into account the effect of hydrostatic pressure on the activity of bathypelagic communities (Grossart and Gust, 2009; Edgcomb et al., 2016; Cario et al., 2019; Garel et al., 2019). However, experiments using these devices have shown that respiration and activity is usually lower under atmospheric pressure than under high in situ pressure (Tamburini, Boutrif, et al., 2013; Garel et al., 2019), implying that bathypelagic prokaryotes could even be more efficient at DOM processing than what we have observed here.

Our findings further support the idea that bathypelagic communities are extremely versatile, as hinted by experimental (Boutrif et al., 2011; Sebastián et al., 2018, 2019), genomic and metagenomic evidence (Vezzi, 2005; DeLong et al., 2006; Acinas et al., 2019), and can use compounds that are resistant to surface communities. Although it had already been hypothesized that organic compounds that accumulate in the surface ocean may serve as substrates for mesopelagic populations (Carlson et al., 2004, 2011), our work indicates that this may also be the case for bathypelagic prokaryotes. DOC is exported to deep layers of the ocean through winter mixing, or during processes of deep-water formation (Carlson et al., 1994; Hansell and Carlson, 2001; Hopkinson and Vallino, 2005; Treusch et al., 2009; Santinelli, 2015), being this export an important component of the biological pump (Copin-Montégut and Avril, 1993; Hopkinson and Vallino, 2005;
Carlson et al., 2011). In the NW Mediterranean, where the water for this experiment was collected, deep water formation occurs episodically and seasonally as a consequence of both dense shelf water cascading and open-sea convection, which may last several days (Béranger et al., 2009). During this process, fresh organic matter is conveyed to deep ocean prokaryotic communities (Tamburini, Canals, et al., 2013; Severin et al., 2016), and sometimes the convection is so intense that resident deep water is completely replaced by newly formed deep water, yielding high DOC concentrations in the bathypelagic (Luna et al., 2016). The comparatively higher ability of bathypelagic prokaryotes to use these DOC compounds points to a lower efficiency in the long-term sequestration of this exported carbon, which has implications for the global carbon cycle. Thus, future changes in the intensity of the overturning circulation due to climate change should have an impact on the persistence and fate of DOC in the ocean.
**METHODS**

*Experimental set up*

Water was collected on September 29th 2014 from the surface (5 m, ~100 L) and the bathypelagic (2100 m, ~60 L) of the Northwestern Mediterranean Sea (40º 38’ 31.01”N, 2º 51’ 1.6”W) during the MIFASOL-I cruise on board the R/V García del Cid. The water was taken at night the last day of the cruise before steaming back to harbour, filtered through a 200 µm mesh to remove large zooplankton and poured into different thoroughly acid-rinsed 20-L containers. Once in the laboratory, 18 L of water from each depth were gently filtered through a 0.8 µm pore size filter, and prokaryotic cells were collected onto a 0.2 µm polycarbonate filter to generate the prokaryotic inocula for the transplant experiments (see Figure S1 for details on the experimental set-up). The remaining water was 0.2 µm filtered to produce cell-free water for the different treatments, described below. Filtration was carried out gently to avoid disruption of cells that could release carbon compounds. Prior to filtering, 3 L of miliQ water were flushed through the system to minimize organic carbon contamination. Cells collected from the surface and bathypelagic were resuspended in 0.9 L of their corresponding 0.2 µm filtered seawater and prokaryotic abundance was quantified through flow cytometry and then diluted so that the starting inoculum was the same in all treatments (5x10^4 cells mL⁻¹, that represented 1.1 ± 0.02 µg C L⁻¹ (average ± SE)). Four 20-L carboys containing 0.2 µm filtered surface seawater (‘SW’) and two 20-L carboys containing 0.2 µm filtered bathypelagic seawater (‘BW’) were prepared and inoculated with either surface (Sp) or bathypelagic (Bp) prokaryotic cells. Inorganic nutrients were then added to one of the SW carboys containing Sp and to another containing Bp to yield the SW+N treatments. The amount of inorganic nutrients added was enough to reach similar concentrations to
the ones usually found in the Mediterranean bathypelagic (see Table 1). After prokaryotic
cells and inorganic nutrients (when appropriate) had been added to the 20-L containers,
that volume was divided into two replicates and deposited in the 10-L containers. The
experiment was performed in the dark, with surface water treatments being kept at room
temperature (~20°C) and bathypelagic water kept at 16°C, which is ca. 3 degrees higher
than the usual Mediterranean bathypelagic temperature. On day 9 of the experiment, the
remaining water volume in the BW treatment was divided in half into two containers and
carbon (mixed sources, including glucose, acetate, terrestrial humic acids and amino
acids, see Table S1 for details) added to one of the containers. This treatment was named
BW+C, and aimed to explore the short-term response of the communities developing in
bathypelagic water to a sudden input of carbon of different levels of lability, with total
DOC concentration values closer to the ones observed in the surface waters (Table 1).

Total organic carbon

Ten-mL water samples were collected in precombusted (450 °C, 24 h) glass
ampoules. After adding 50 µL of 25% H₃PO₄ to acidify at pH < 2, the ampoules were
heat-sealed and stored in the dark at 4°C until analysis. TOC 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 concentration
in each sample was calculated by subtracting a Milli-Q blank and dividing by the slope
of daily-made standard curves created using potassium hydrogen phthalate. 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. Only data
on the initial TOC samples that were measured immediately after collection are available,
because many of the samples got contaminated during storage due to a defective batch in
the glass vials where the samples were collected.

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

Flow cytometry analyses

Prokaryotic cell abundance was estimated by flow cytometry as described elsewhere
(Gasol and Morán, 2015). The average cell biovolume was estimated using the relative
FL1 signal following Gasol and del Giorgio (2000) and an in-house calibration between
Syto13 and SybrGreen. This calibration was performed by staining cells with both dyes
and correlating the fluorescence signal (standardized to the fluorescent signal of the same
plastic beads) of both dyes.

The carbon content of the prokaryotic cells was computed using the allometric
relationship of Norland et al., (1993): pg C cell⁻¹ = 0.12 pg (µm³ cell⁻¹)⁰.⁷, and the biomass
by multiplying the carbon content by the abundance of cells.

Prokaryotic heterotrophic production

Prokaryotic heterotrophic production was estimated from the incorporation of tritium-
labelled leucine, which measures protein synthesis (Kirchman et al., 1985). Four
replicates of 1.2 ml and two trichloroacetic acid (TCA)-killed controls were incubated
with ³H-Leucine at a final concentration of 40 nM. Although 40nM may seem high for
oligotrophic regions and could stimulate production, previous experiments showed this
concentration is saturating and was chosen to estimate potential activity in order to facilitate comparison between treatments. 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). Briefly, samples were thawed and centrifuged for 10 min at 12000 x g. Supernatant was removed and 1mL TCA 5% was added to the tubes and mixed by vortexing. Samples were again centrifuged for 10 min at 12000 x g, the supernatant aspirated, and 1mL of liquid scintillation cocktail (Optimal HiSafe) was added to the each of the tubes. The tubes were then placed into 20-mL scintillation vials, stored in the dark for at least 24h, and radio-assayed on a Beckman scintillation counter. Conversion of leucine to carbon units was done with the theoretical factor 1.5 kg C mol Leu⁻¹ (Simon and Azam, 1989).

Enzymatic activities

Leu-aminopeptidase (LAPase), α-glucosidase (AGase), β-glucosidase (BGase), and alkaline phosphatase (APase) activities, were estimated using the following fluorogenic substrates: L-leucine-7-amino-4-methylcoumarin, 4-methylumbelliferyl α-D-glucoside, 4-methylumbelliferyl β-D-glucoside, and 4-methylumbelliferyl phosphate, 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 quadruplicate into 96 black well plates, and substrates were added to obtain a final concentration of 125 µM. This concentration has been found to be saturating in previous experiments and was thus chosen to estimate potential activities, to facilitate comparison among treatments. The fluorescence of the 96 well plates was measured with a Tecan Infinite 200 microplate reader at 365 nm excitation and 450 nm emission wavelengths, at the beginning and after 5 hours of incubation at in situ temperature.
temperature conditions. Activity was derived from the increase in fluorescence in each well over time, using a standard curve prepared with the fluorophores 4-methylumbelliferone (MUF) or 4-methylcoumarinyl-7-amide 4 (Sigma-Aldrich).

Optical Characterization of DOM

The optical properties of colored dissolved matter (CDOM) and of its fluorescent fraction (FDOM) provide information about the origin and lability of DOM. We inspected these properties to evaluate possible changes in DOM lability in the different treatments. Samples for FDOM characterization were collected on day 0, day 9 and day 13 of the experiment. FDOM was measured using a Perkin Elmer LS55 luminescence spectrometer provided with a xenon discharge lamp equivalent to 20 kW for an 8-µs duration. A red sensitive R928 photodiode multiplier operated as a reference detector. Samples were measured in quadruplicate in a 1 cm acid-cleaned quartz cell at a constant room temperature. Quartz cells were rinsed with sample water before analyses. We focused on the detection of humic-like substances that fluoresce at 440 nm when excited at 340 nm (peak-C, Coble, 1996), which are recalcitrant compounds produced in situ by marine microorganisms (Castillo et al., 2010; Romera-Castillo et al., 2011; Jørgensen et al., 2014) and have been related to respiration processes in the ocean (De La Fuente et al., 2014). We also looked at peak-T (Coble, 1996), which relates to protein like substances with Excitation/Emission wavelengths of 280 nm/350 nm and is used as a tracer of biologically labile DOM (Yamashita and Tanoue, 2003). The humic-like vs protein-like ratio (peak-C/peak-T) was then used as a proxy of DOM lability. Fluorescent data was normalized to Raman units (R.U.) according to Lawaetz and Stedmon, (2009).

Samples for CDOM were taken at the beginning and the end of the experiment. CDOM absorption was measured in a Varian Cary spectrophotometer equipped with a 10
cm quartz cell. The spectra were collected between 250 and 750 nm at constant room
temperature using Milli-Q water as blank. The absorption spectra was calculated as in
Romera-Castillo et al., (2013). Following Helms et al. (2008), CDOM spectral slopes
were obtained in the 275-295 nm wavelength range ($S_{275-295}$) using liner regressions of
the natural log-transformed absorption spectra. This range was selected because it
provides information on the molecular weight and the aromaticity of the substances, with
lower slopes as the molecular weight and the aromaticity increase (Helms et al., 2008).

*Nucleic acid extraction*

Samples for nucleic acid extraction (0.5-1 L) were filtered through 47-mm 0.2 µm
polycarbonate filters with a peristaltic pump, and filters were stored frozen until
extraction. Total nucleic acids were extracted using the PowerWater DNA isolation Kit
(MO BIO Laboratories, Carlsbad, CA) following the manufacturer instructions. DNA
was quantified using a Qubit fluorometer assay (Life Technologies, Paisley, UK). The
V4-V5 region of the 16S rRNA gene was amplified with the primers 515F-Y and 926R
(Parada et al. 2016) and sequenced in an Illumina MiSeq platform using 2x250bp paired-
end approach at the Research and Testing Laboratory facility (Lubbock, Texas, USA;

*Data analyses*

Computing analyses were run at the Marine Bioinformatics Service of the Institut de
Ciències del Mar (ICM-CSIC) in Barcelona. Primers and spurious sequences were
trimmed using cutadapt (Martin, 2011) using the following parameters: --discard-
untrimmed --minimum-length=32. DADA2 v1.8 was used to differentiate exact sequence
variants (Callahan et al., 2016). DADA2 resolves ASVs (amplicon sequence variants) by
modelling the errors in Illumina-sequenced amplicon reads. The approach is threshold
free, inferring exact variants up to 1 nucleotide of difference using the quality scores
distribution in a probability model. After filtering through DADA2, 76.4% of the total
reads (mean 64644, min 25135 max 124930) were retained for further analyses.

Taxonomic assignation was performed using the function ‘assignTaxonomy’ against
SILVA v.132 (Quast et al., 2012; Yilmaz et al., 2014) through the RDP naive Bayesian
classifier method described in Wang et al., (2007). The ASV table was randomly
subsampled down to the minimum number of reads per sample using the \texttt{rrarefy}
function in the \texttt{vegan} package (Oksanen et al., 2019). All raw sequences used in this study are
publicly available at the European Nucleotide Archive (ERX4135557-ERX4135620).

Data treatment and statistical analyses were performed with the R (version 3.3.2) and

\textit{Statistical analyses}

We used ANOVA followed by Tukey’s Honestly Significant Difference (HSD) test to
explore difference between biotic variables (prokaryotic abundance, prokaryotic biomass,
contribution of HNA cells, cumulative production) in the different treatments (‘Sp SW’,

Paired Wilcoxon signed rank tests were performed to test if the optical properties of DOM
in each individual water treatment (‘SW’, ‘SW+N’, ‘BW’, ‘BW+C’) were different
between surface and bathypelagic communities.

\textit{Analysis of Alteromonas sequences}

We explored in detail the possible species assignment of the two most abundant
\textit{Alteromonas} ASVs in our dataset, in order to elucidate whether a single ASV could
represent more than one species. To this end, we extracted all the sequences defined as
Alteromonas species from SILVA release 138 (Quast et al., 2012). Sequences were trimmed with cutadapt v1.14 (Martin, 2011) to only keep the V4 and V5 region analyzed in this study. The resultant sequences were aligned with the two ASVs with DECIPHER R package v2.14 (Wright, Erik, 2016), obtaining the nucleotide distances. The results were displayed using a hierarchical clustering heatmap of these distances with the pheatmap R package v1 (Kolde, 2015).

ACKNOWLEDGEMENTS
This work was partially supported by grants DOREMI (CTM2012-34294), HOTMIX (CTM2011-30010/MAR), EcoRARE (CTM2014-60467-JIN), ANIMA (CTM2015-65720-R) and MIAU (RTI2018-101025-B-I00), funded by the Spanish Ministry of Science and also by Grup Generalitat 2017SGR/1568. MS was supported by a Viera y Clavijo contract funded by the ACIISI and the ULPGC. Bioinformatics analyses were performed at the MARBITS platform of the Institut de Ciències del Mar (ICM; http://marbits.icm.csic.es). We thank Clara Ruiz González and three anonymous reviewers for their comments on a previous version of the manuscript.

The authors declare no conflict of interest

REFERENCES


Agogué, H., Lamy, D., Neal, P.R., Sogin, M.L., and Herndl, G.J. (2011) Water mass-


Preprint of paper published in Environmental Microbiology


FIGURE LEGENDS

**Figure 1.** Abundance (a), cell size (b) and heterotrophic production (c) of surface prokaryotes (Sp, open circles) and bathypelagic prokaryotes (Bp, filled circles) inoculated in surface waters (SW, left panel), surface waters with nutrients (SW+N, second panel), and bathypelagic waters (BW, third panel). The asterisk represents the moment when mixed sources of organic carbon were added to a subsample of the bathypelagic waters treatment (see methods). The small panel on the right represents how this carbon-enriched BW treatment evolved (BW+C). Note the change in scale in the BW+C for cell abundance. Each data point represents the average of two replicates and error bars represent the range of values.

**Figure 2.** Taxonomic changes in the different treatments along the transplant experiment.
Initial: community at the moment of sampling. Inoculum: starting community (after prefiltration through a 0.8µm filter, collection of cells onto a 0.2µm filter and resuspension). SW: surface waters, SW+N: surface waters with added nutrients, BW: bathypelagic waters, BW+C: bathypelagic waters with added labile carbon.

**Figure 3.** Boxplot of a) Shannon and Evenness of surface and bathypelagic communities over the course of the experiment. b) the number of taxa (ASV) that were rare in the starting community but represented more than 5% of the community each of the time-points of the experiment, here considered as opportunistic taxa. Sp: surface prokaryotes, Bp: bathypelagic prokaryotes, SW: surface waters, SW+N: surface waters with added nutrients, BW: bathypelagic waters.

**Figure 4.** Temporal dynamics of specific enzymatic activities of surface prokaryotes (Sp, open circles) and bathypelagic prokaryotes (Bp, filled circles) inoculated in surface waters (SW, left panel), surface waters with nutrients (SW+N, second panel), and bathypelagic waters (BW, third panel). The asterisk represents the moment when mixed sources of carbon were added to a subsample (half of the remaining volume) of the bathypelagic waters treatment (see methods). The small panel on the right represents how this carbon-enriched BW treatment evolved (BW+C). Each data point represents the average of two biological replicates and error bars represent the range of values.

**Figure 5.** Characterization of the dissolved organic matter (DOM) a) fluorescent DOM: Humic-like substances (C peak, upper panel), protein-like substances (T peak, middle panel) and C/T peak ratio (an indication of the amount of recalcitrant versus labile
material, lower panel). Each data point represents the average of two biological replicates (with four technical replicates each) and the error bars represent the range of values. b) Percent change in the spectral slope of the chromophoric DOM in the 275-295 nm wavelength range from the beginning to the end of the experiment. A decrease in the slope is indicative of an increase in aromaticity. Sp: surface prokaryotes, Bp: bathypelagic prokaryotes, SW: surface waters, SW+N: surface waters with added nutrients, BW: bathypelagic waters, BW+C: bathypelagic waters with added carbon.
Figure 1
Figure 2
Figure 3
Figure 4
Figure 5
Table 1. Inorganic nutrients and total organic carbon concentrations at the beginning of the experiment. SW: Surface water, SW + N: Surface water enriched with N and P, BW: Bathypelagic water. BW+C: Bathypelagic water with added carbon (see methods for details). Values in parenthesis represent the range of values.

<table>
<thead>
<tr>
<th></th>
<th>Nitrate (µM)</th>
<th>Phosphate (µM)</th>
<th>TOC (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SW</td>
<td>0.4 (0.3)</td>
<td>0.05 (0.01)</td>
<td>78 (2.3)</td>
</tr>
<tr>
<td>SW + N</td>
<td>13 (0.4)</td>
<td>0.5 (0.03)</td>
<td>78 (3.8)</td>
</tr>
<tr>
<td>BW</td>
<td>9.7 (0.7)</td>
<td>0.4 (0.06)</td>
<td>47 (3.9)</td>
</tr>
<tr>
<td>BW + C</td>
<td>--</td>
<td>--</td>
<td>63.8 (4.3)</td>
</tr>
</tbody>
</table>
Table 2. Comparison of the biological properties among the treatments. Values for prokaryotic abundance (PA), biomass and %HNA (proportion of high nucleic acid prokaryotes) represent the average of the values once saturation in cell abundance was reached (from day 6 till the end of the experiment). Cumulative heterotrophic production (PHP) is also shown (average and range of values for the two replicates). Different letters in each row represent values that are significantly different from each other (as analyzed by ANOVA followed by Tukey’s Honestly Significant Difference (HSD) test, p<0.01). Significant differences between surface and bathypelagic communities within each water treatment are highlighted in bold for clarity.

<table>
<thead>
<tr>
<th></th>
<th>SW</th>
<th>SW+N</th>
<th>BW</th>
<th>BW+C</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PA (x10^5 cells mL^-1)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sp</td>
<td>1.2a</td>
<td>1.2a</td>
<td>3.0b</td>
<td>2.8b</td>
</tr>
<tr>
<td>Bp</td>
<td>1.6b</td>
<td>1.6b</td>
<td>7.6c</td>
<td>8.6c</td>
</tr>
<tr>
<td><strong>Biomass (µgC L^-1)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sp</td>
<td>2.83a</td>
<td>2.89a</td>
<td>6.42b</td>
<td>6.65b</td>
</tr>
<tr>
<td>Bp</td>
<td>3.78a</td>
<td>3.78a</td>
<td>18.1c</td>
<td>20.7c</td>
</tr>
<tr>
<td><strong>%HNA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sp</td>
<td>89a</td>
<td>87a</td>
<td>59a</td>
<td>94a</td>
</tr>
<tr>
<td>Bp</td>
<td>92a</td>
<td>92a</td>
<td>99a</td>
<td>99a</td>
</tr>
<tr>
<td><strong>Cum. PHP (µgC L^-1)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sp</td>
<td>131±15a</td>
<td>202±12a</td>
<td>211±24b</td>
<td>394±12b</td>
</tr>
<tr>
<td>Bp</td>
<td>111±20a</td>
<td>203±26a</td>
<td>208±26a</td>
<td>336±12b</td>
</tr>
</tbody>
</table>