Nitrogen and phosphorus recycling mediated by copepods and response of bacterioplankton community from three contrasting areas in the Western Tropical South Pacific (20ºS)

Valentina Valdés[1,2], François Carlotti[3], Ruben Escribano[4, 5], Katty Donoso[3], Marc Pagano[3], Verónica Molina[6] and Camila Fernandez[2, 7, 8]

[1]Programa de Doctorado en Oceanografía, Departamento de Oceanografía, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Concepción, Chile.
[2]UPMC Univ Paris 06, UMR 7621, Laboratoire d'Océanographie Microbienne, Observatoire Océanologique, Sorbonne Universités, Banyuls sur Mer F-66650, France.
[3]Aix-Marseille Univ, Universite de Toulon, CNRS, IRD, OSU PYTHEAS, Mediterranean Institute of Oceanography MIO, UM 110, 13288, Marseille, Cedex 09, France.
[4]Departamento de Oceanografía, Universidad de Concepción, Concepción, Chile.
[5]Instituto Milenio de Oceanografía, Universidad de Concepción, Concepción, Chile.
[6]Departamento de Biología and Programa de Biodiversidad, Facultad de Ciencias Naturales y Exactas, Universidad de Playa Ancha, Playa Ancha, Valparaíso, Chile.
[7]FONDAP INCAR center (15110027), and PFB-31 COPAS Sur Austral Program; Departamento de Oceanografía, Universidad de Concepción, Concepción, Chile.
[8]Centro FONDAP de Investigación en Dinámica de Ecosistemas Marinos de Altas Latitudes (IDEAL), Valdivia, Chile.

Correspondence to: Valentina Valdés (vvaldesc@udec.cl)

Abstract. Zooplankton play a key role in the regeneration of nitrogen and phosphorus in the ocean through grazing and metabolism. This study investigates the role of the organic and inorganic nitrogen and phosphorus compounds released by copepods on biogeochemical processes and on the microbial community composition during the OUTPACE cruise (18 February – 3 April 2015) at three long duration stations (LD). Two LD stations were located in the Melanesian Archipelago region (MA; LD A and LD B) and one in the South Pacific Gyre (SG; LD C), which represent oligotrophic and ultra-oligotrophic regions respectively. At each station, microcosm onboard experiments were performed with locally sampled organisms, comprising a mix of epipelagic copepods fed with their natural food and then incubated along with wild microbial assemblages. In presence of copepods, ammonium and dissolved organic nitrogen showed a significant increase, compared to a control in two situations: in ammonium concentration (rate: 0.29 µmol L^{-1} h^{-1} after 4 h of incubation) in LD C and in dissolved organic nitrogen concentration (rate: 2.13 µmol L^{-1} h^{-1} after 0.5 h of incubation) in LD A. In addition, during the three experiments, an enhanced remineralization (ammonification and nitrification) was observed when adding copepods compared to the controls. A shift in the composition of the active bacterial community was observed for the experiments in LD A and LD B mainly characterized by an increase in Alteromonadales and SAR11, respectively and linked with changes in nutrient concentrations. In the experiment performed in LD C, both groups increased but at different periods

of incubation, Alteromonadales between 1 and 2 h after the beginning of the experiment, and SAR 11 at the end of incubation. Our results in near *in situ* conditions, show that copepods can be a source of organic and inorganic compounds for bacterial communities, which respond to excretion pulses at different time scales, depending on the initial environmental conditions and on their community composition. These processes can significantly contribute to nutrient recycling and regenerated production in the photic zone of ultra and oligotrophic oceanic regions.

1 Introduction

The Tropical South Pacific is considered one of the most oligotrophic regions in the world ocean. Its biogeochemical characteristics include nitrogen and phosphate limitation which modulate the low biological productivity (Moutin et al., 2008). In addition, the strong thermal stratification, enhanced by current global warming, decreases the nutrient supply to the euphotic zone (Polovina et al., 2008). The Western Tropical South Pacific (WTSP) was recently recognized as a hot spot of N_2 fixation (Bonnet et al., 2017) and the input of new nitrogen to the surface ocean through this process sustains over 50 % of the primary productivity (Karl et al., 1997; Carpenter et al., 2004). However, it has been reported that predator-prey interactions can provide substantial amounts of nitrogen and phosphorus, and supply an alternative substrate for phytoplankton and heterotrophic microorganisms stimulating the microbial loop in a variety of areas, contributing to the regenerated and new production (Richardot et al., 2001; Vargas et al., 2007; Arístegui et al., 2014; Valdés et al., 2017a, b).

Metazooplankton metabolism is recognized as one of the main mechanisms controlling concentration, composition and cycling of dissolved organic matter (DOM) in the sea (Sipler and Bronk 2014). Zooplankton may release dissolved organic and inorganic matter through sloppy feeding (Møller, 2004, 2007), leaching of fecal pellets (Hasegawa et al., 2000; Steinberg et al., 2002) and excretion (Saba et al., 2011) and along with bacterial remineralization, it determines the amount of regenerated nitrogen and phosphorus available for phytoplankton production (Steinberg and Landry, 2017). Copepods excrete up to 53% of their body nitrogen per day, mainly in the form of ammonium, followed by urea and amino acids (Bidigare, 1983), thereby recycling much of the nitrogen in the water column (Ikeda et al., 2001; Steinberg and Saba 2008).

The importance of ammonium excretion by mesozooplankton to primary production has been assessed in a variety of areas in the ocean, and the contribution to nitrogen requirements for phytoplankton growth is mainly depending on the productivity of the area (Hernandez-León et al., 2008). Reports indicate that zooplankton could sustain between 40-50% of the nitrogen requirements of phytoplankton in open ocean areas, as in the North Pacific central gyre (Eppley et al., 1973) and this percentage decreases in eutrophic waters, such as upwelling areas (Smith and Whitledge 1977; Peréz-Aragón et al., 2011). Other works indicate that diel vertical migrating zooplankton can sustain between 2% to 19% of the nitrogen requirements by phytoplankton through ammonium excretion at the oligotrophic Sargasso Sea (Steinberg et al., 2002). Furthermore, zooplankton can also excrete substantial amounts of dissolved organic nitrogen (DON), i.e., between 7 to 80%

of the total dissolved nitrogen being released (Miller and Glibert, 1998; Conover and Gustavson, 1999; Steinberg et al., 2002; Steinberg and Saba 2008; Pérez-Aragón et al., 2011). In fact, the composition and quantity of the excreted compounds are highly dependent on the type of food ingested by zooplankton (Miller and Roman, 2008; Saba et al., 2009). Even less studied than nitrogen is the phosphorus release by zooplankton. It has been reported, that one-third of the phosphorus released by zooplankton (amphipods) was in the organic form (Johannes, 1964; Satomi and Pomeroy, 1965). Isla et al. (2004) estimated that mesozooplankton phosphate excretion can sustain 21.7 % of phytoplankton phosphate requirements in oceanic water of the NW Iberian. Titelman et al. (2008) demonstrated that copepod feeding enhances organic phosphorus uptake by bacterial activity in a phosphorus limited system. However, the impact of zooplankton metabolism on the phosphorus recycling in oceanic areas is largely unknown.

The importance of quality and quantity of DOM in structuring bacterioplankton community has been increasingly reported (Alonso-Saez and Gasol, 2007). However, the processes and mechanisms through which nitrogen and phosphorus compounds released by zooplankton can determine and influence the food web and the structure of the microbial community are unclear. Recent research has demonstrated that bacterial community composition experiences changes in response to the addition of DOM (Landa et al., 2013; Sarmento et al., 2013). Studies on the interaction between zooplankton metabolism and bacterial communities are however too scarce. Recent reports demonstrate that the addition of excretory products released by zooplankton (krill) stimulates bacterial growth and production in the Southern Ocean (Arístegui et al., 2014). Specific studies carried out by us in a eutrophic coastal zone off southern/central Chile indicate that the nitrogen excreted by copepods generate a specific response of nitrifying communities and in the active marine bacterioplankton community (Valdés et al., 2017a, b). Despite the advances over the last decades, studies of copepod nitrogen and phosphorus excretion impact on microbial community from oligotrophic and ultra-oligotrophic areas can provide valuable information about of the recycling of this nutrients in larger areas of the ocean.

Herein, we studied the role of organic and inorganic dissolved compounds released by copepods, and its potential effect on their recycling and structuring of the bacterioplankton community during late austral summer in distinct biogeochemical regions of the WTSP.

2 Material and methods

2.1 Study area and sampling strategy

Sampling was conducted in WTSP during austral summer 2015 (18 February -3 April), on board the RV L`Atalante. The transect began west of New Caledonia (18º S-159.9º E) and ended near Tahiti (17.56º S-149.05º W; Fig. 1). Along this transect, two types of stations were sampled: 15 short-duration stations (SD; eight hours duration) dedicated to large-scale description, and three long-duration stations for the Lagrangian studies (LD; approximately eight days duration).

Experiments were conducted in three long-duration stations: LD A (27 February; -19,2º S-164,7º E), LD B (17 March; -18,2º S-189,1º W) and LD C (24 March; -18,4º S-194º W). These stations were chosen along a regional gradient of oligotrophy and are located in two geographic regions: LD A and LD B in the Melanesian archipelago (MA) and LD C in the subtropical gyre (SG). These stations chosen as based on the sea surface chlorophyll-a concentration, which was maximum in LD A and LD B, and minimum in LD C station. More details about the sampling strategy and general biogeochemical and hydrographic characteristics are described in Moutin et al., (2017) and Moutin et al., (this issue). At each sampling station seawater samples were obtained using Niskin bottles (12 L) arranged on a CTD rosette from the Deep Chlorophyll Maximum (DCM). Seawater was collected for chemical and biological initial characterization, including ammonium (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-), phosphate (PO_4^{3-}), chlorophyll- a, DNA and RNA. The DCM depths correspond to 80 m, 34 m and 140 m in LD A, LD B and LD C, respectively. Details of chlorophyll-a methods are available in Dupouy et al., (this issue).

2.2 Mesozooplankton sampling

Samples were collected by vertical hauls of a Bongo net (70 cm mouth diameter) of 120 µm mesh size, equipped with a non-filtering cod-end to obtain undamaged individuals. Mesozooplankton samples were collected at the three LD stations in the upper 100 m depth at a speed of 1 m s^{-1} under night conditions. Live samples were immediately transferred to coolers until sorting at the on-board laboratory.

2.3 Preparation of the microcosm and experimental setup

The experimental design had three steps: (1) Copepod acclimation, (2) feeding and (3) copepod+microbial recycling (Fig. 2). Seawater for incubations (30 L) was collected into clean (10% HCl rinsed) polycarbonate carboys from the DCM. For acclimation (1) and copepod+microbial recycling (3) steps, seawater (22.5 L) was immediately filtered through a 0.7 µm filter (GF/F; Whatman) using a peristaltic pump. The remaining 7.5 L were used in the feeding phase. Seawater for the different steps was maintained in a temperature controlled room (in situ temperature ~25º C) until the beginning of the experiment.

Undamaged individuals were sorted from the live samples and identified using a stereomicroscope at low light. Copepods samples in the three experiments consisted in a mix of the most representative copepods (adults) in the sample (Table S1; More details about zooplankton composition and abundance are available in Carlotti et al., this issue). In the acclimation phase (1), 15 groups of 10 copepods were incubated in 500 mL Nalgene bottles, maintained for 4-6 h in filtered seawater previously filtered (GF/F Whatman) in darkness and at controlled temperature (in situ). In the feeding step (2) copepods were removed from the bottles using a sieve (20 µm) and maintained in new 500 mL polycarbonate bottles (Nalgene) with <150 µm filtered seawater (polycarbonate membrane) for feeding. This step lasted 4 hours and, as the acclimatization phase, took place under controlled temperature (25ºC). Thereafter, each copepod group (15 groups) was placed in a new set of 500

mL polycarbonate bottles (Nalgene) filled with the seawater previously filtered (0.7 µm; Millipore) for the copepod+microbial recycling phase. Also, other 15 bottles without copepods were incubated as control. In total, 30 bottles were used in this step at LD A, whereas additional bottles were included at LD B and LD C, 6 bottles were added at LD B and LD C, 3 with copepods and 3 without copepods as control. The seawater used was filtered to avoid the presence of phytoplankton and small protists, to minimize grazing effect and ensure the presence of natural microbial assemblages. At the end of each step copepods were observed and checked by direct observation to ensure swimming behaviour in the bottles. The percentage of mortality at the end of each experiment at each bottle was less than 20%.

During the copepod+microbial response phase, at time intervals of 0 h (T0), 0.5h (T1: T0+0.5 h), 1 h (T2: T0+1 h), 2 h (T3: T0+2h) and 4 h (T4: T0+4 h) samples were collected for NH_4^+, NO_3^-, NO_2^-, PO_4^{3-}, DON, dissolved organic phosphorus (DOP), bacterioplankton abundance and RNA. The samples for the initial time (T0) were taken once all the bottles were incubated. An additional sampling, corresponding to incubation time of 15 min (T0´: T0+0.25h), was added just for having more measurements of NH_4^+ in the experiments of stations LD B and LD C. Six bottles were sacrificed at each time, three for treatment with copepods and three for control (without copepods). The three bottles sacrificed at each time represented triplicates for treatment and control.

At the end of each observation time, copepod samples were preserved immediately with formalin buffered by sodium borate (5% final concentration) for further zooplankton identification analysis (Table S1).

2.4 Dissolved inorganic and organic nutrients measurements

Nutrient samples (NO_3^-, NO_2^- and PO_4^{3-}) were taken in duplicate in 20 mL HDPE bottles, filtered through 0.7 µm filters (GF/F; Whatman) and poisoned with $HgCl_2$ to a final concentration of 20 µg L^{-1}. Samples were stored at -20 °C and analyzed on board determined by standard colorimetric techniques (Aminot and Kérouel 2007), using a SEAL Analytical AA3 HR system (SEAL Analytical, Serblabo Technologies, Entraigues Sur La Sorgue, France). Samples for NH_4^+ determination (40 ml) were taken in duplicate and analyzed on board using a Jasco FP-2020 fluorometer according to Holmes et al., (1999). Samples for DON and DOP (30 mL) were filtered through pre-combusted (450º C, 6h) GF/F filters (Whatman). Samples were collected in Teflon bottles and analyzed immediately on board by the wet oxidation method (Pujo-Pay and Raimbault, 1994). DON and DOP concentrations were determined by sample oxidation (30 min, 120º C) and corrected for NO_3^-, NO_2^- and PO_4^{3-} concentrations, respectively.

2.5 Bacterioplankton abundance, DNA and RNA extraction

Bacterioplankton abundance was determined by flow cytometry. From each bottle 1,350 µL samples were fixed in sterile cryovials with glutaraldehyde (at 0.1% final concentration). The samples were stored at -80º C until laboratory analysis. Samples were analyzed by flow cytometry (FACScan, Becton Dickinson) at the Observatorire Océanologique de Banyuls

sur Mer, France. The abundance of non-fluorescent picoplankton was estimated from samples previously stained with SYBR green I (Molecular probes) according Marie et al. (1997). DNA samples for the initial characterization of bacterial communities were collected at DCM depth of each sampling site. Samples (9-10 L) were filtered through cellulose ester filters (0.22 µm; Millipore) using a peristaltic pump and stored with RNA later reagent (Ambion) at -20º C until extraction procedures.

DNA was isolated using PowerSoil DNA Isolation Kit (MoBio Laboratories) in accordance with the manufacturer´s specifications following Levipan et al., (2014). DNA was quantified by spectrophotometry (NanoDrop ND-1000 Spectrophotometer). RNA samples were collected for initial characterization of active bacterial community at DCM at each LD station and during each experiment to study the active bacterial community composition. Seawater (100 mL) was filtered using a sterilized syringe and 25 mm swinnex through 0.22 µm hydrophilic PVDF filters (Millipore) and the filters were preserved with RNAlater solution (Ambion) and stored at -80º C until RNA extraction procedures.

RNA samples were extracted using Mirvana kit (AM1560; Ambion) in accordance with manufacturer´s instructions including a mechanical disruption step and homogenization using 200 µm diameter zirconium beads (Low Binding Zirconium Beads, OPS Diagnostic) and homogenized twice at ~3,000 rpm for 30 s by using Mini-Beadbeater-8TM (Biospec Products). In order to remove DNA traces, RNA was treated with TURBO DNA-free kit (Ambion). Finally, concentration and quality (A260/A280 ratio) of RNA extracts as well with DNA extracts were determined by spectrophotometry (NanoDrop ND-1000 Spectrophotometer).

2.6 Analysis of the bacterial community structure

The bacterial community structure was analyzed by Illumina MiSeq sequencing method from 16S rRNA (samples from incubation), using cDNA as template, and 16S rDNA in situ, following Campbell and Kirchman, (2013). cDNA was generated using random primers provided by the ImProm-IITM Reverse Transcription System (Promega). Bacterial 16S rRNA gene libraries from V1-V3 region (27F-519R) were generated at the Molecular Research LP (www.mrdnalab.com, Shallowater, TX, USA).

The 16S rRNA and rDNA gene sequences were processed using Mothur software v1.35.1 (Schloss et al., 2009). Sequencing data sets were curated by quality filtration to minimize the effects of random sequencing errors by eliminating sequence reads <200 bp and trimming of sequences that contained more than one undetermined nucleotide (N) and sequences with a maximum homopolymer length of 8 nucleotides. Chimeric sequences were identified using the Chimera UCHIME algorithm (Edgar et al., 2011) and removed to retain high quality reads.

The 16S rRNA and rDNA gene sequences retrieved were taxonomically classified using the automatic software pipeline SILVAngs available from https://www.arb-silva.de/ (Quast et al. 2013). In LD A station, a total of 178,097 sequences were analyzed, ranging between 10,831 and 19,703 sequences for each library (Fig. S1). In LD B station 219,019 sequences were analyzed from 15,260 to 20,966 for each library, and in LD C 230,805 sequences were analyzed, ranging between 13,699 and 22,104 sequences for each library. In each of the three LD stations, the highest number of sequences correspond to DNA in situ. Libraries were deposited in the European Nucleotide Archive (ENA) under study accession PRJEB21648 with the following run access numbers: ERS1810581-ERS1810616.

The composition was analyzed at Phyla, Class and Order taxonomic level as abundant (>0.5%) and others (<0.5%) in total sequences retrieved from each library. The Chao, Shannon and Evenness indices were calculated using Past3 software. Previously, since the number of sequences per sample was variable, we normalized the different libraries sizes by subsampling routine in Mothur software version 1.36 (Schloss et al., 2009), to reflect the lowest number of sequences encountered (LD A: 10,831; LD B: 15,260 and LD C: 13,699).

2.7 Statistical analysis

Statistical analysis of treatments effects on chemical and biological parameters were performed using a two-way analysis of variance (ANOVA) after checking normality assumptions (Kolmogorov-Smirnov test) and homoscedasticity (Levene´s test). Pairwise multiple comparisons were performed using a Tukey test as a posteriori analysis. Statistical significance was set a $p=0.05$ and analysis was computed using R software. Time point sampled at 15 min was not considered for this analysis, because of too few data.

Changes in bacterial community structure were compared using ordination Bray-Curtis similarities and used to build dendrograms by the unweighted pair group method with arithmetic averages (UPMG). For multivariate statistical analysis, the software package PRIMER v.6 and the add-on PERMANOVA+ was used. The permutational multivariate analysis of variance (PERMANOVA) with fixed factor was applied to investigate the differences of bacterial community composition for treatments and control in both experiments. Principal co-ordinate analysis (PCO) was performed to visualize patterns of the bacterial community in response to the different treatments.

3 Results

3.1 Biogeochemical in situ conditions

Inorganic nutrients concentrations, obtained at the DCM depth, varied substantially between the sampling sites (Table 1). Lower concentrations close to the limit of detection of the methods of NH_4^+, NO_3^-, NO_2^- and PO_4^{3-} were observed in LD B, compared to LDA and LD C. The highest concentrations were observed in LD A, particularly for NO_3^- (1.53 µmol L^{-1}). The

resulting DIN: DIP ratio showed greater values in LD A (9.97 ± 0.70) than LD B and LD C, and the lowest DIN: DIP ratio (1.21 ± 0.11) was estimated for LD C seawater.

3.2 Changes in inorganic nutrients, DON and DOP during the incubations

Inorganic nitrogen and phosphorus concentrations changed substantially over the course of the experiments at the three LD stations (Fig. 3 and 4). Ammonium concentrations (Fig. 3a, e, i) increased significantly (four- and seven-fold) in the presence of copepods in all experiments (ANOVA, LD A: $F_{1,20}$: 93.2, $p<0.001$; LD B: $F_{1,18}$: 61.7, $p<0.001$; LD C: $F_{1,20}$: 108.9, $p<0.001$). The largest and most significant difference in ammonium concentration with respect to the controls was associated with T0 in LD A, T3 in LD B and T4 in LD C (ANOVA and Tukey`s test $p<0.001$; Table S2). Nitrate showed significant differences between the treatment with copepods and controls in LD B and LD C; characterized by notable accumulation in the treatment with copepods (Fig. 3f, j) at T1 in LD B (ANOVA and Tukey`s test $p=0.02$; Table S2), and at T4 in LD C experiments (ANOVA and Tukey`s test $p=0.009$; Table S2). Nitrite (Fig. 3c, g, k) was slightly variable through time in the experiments and no significant difference was detected between copepods and the controls (ANOVA, LD A: $F1,16$: 0.50, $p=0.49$; LD B: $F_{1,18}$: 0.13, $p=0.7$; LD C: $F_{1,20}$: 0.44, $p=0.51$). DON (Fig. 3d, h, l) showed significant differences between the treatment and control in LD A and LD C (ANOVA, LD A: $F_{1,20}$: 8.99, $p=0.007$; LD C: $F_{1,20}$: 29.0, $p<0.0001$), and these differences were associated with the increment in concentration at T1 in LD A and T0 in LD C in the treatment with copepods. At LD B non-significant differences were detected between treatments and controls trough the time (ANOVA, $p>0.05$).

Phosphate in LD A showed a higher concentration during the first hour of incubation, followed by a sharp decrease (from 0.17 to 0.05, approximately; Fig. 4a) in the treatment with copepods as well as control. Despite significant differences in time (ANOVA, $F_{1,20}$: 17.7, $p<0.0001$), treatment versus control differences were not significant (ANOVA, $F_{1,20}$: 4.5, $p=0.06$). Also, no significant differences in phosphate distribution through time and between treatments were found in LD B and LD C experiments (Fig. 4c, e; ANOVA, LD B: $F_{1,18}$: 0.13, $p=0.7$; LD C: $F_{1,20}$: 0.44, $p=0.51$). DOP concentrations (Fig. 4b, d, f) did not vary significantly through time in the three experiments (ANOVA, LD A: $F_{1,20}$: 0.3, $p=0.87$; LD B: $F_{1,20}$: 0.5, $p=0.43$; LD C: $F_{1,20}$: 0.70, $p=0.60$), but significant differences were only observed between the treatment with copepods and control in LD B and LD C (ANOVA, LD A: $F_{1,20}$: 0.43, $p=0.52$; LD B: $F_{1,20}$: 8.92, $p=0.008$; LD C: $F_{1,20}$: 7.94, $p=0.01$).

During the experiments, the resulting DIN: DIP and DON: DOP ratios are shown in Fig. 5. In the LD A experiment the DIN: DIP ratios (Fig. 5a) were lower than Redfield ratios during the first hour of incubation followed by a ratio increment from 12.5 to 32.4 at the end of incubation, only in the treatment with copepods, whereas a decrease in DIN: DIP ratio was observed at the end of incubation in the controls. In the same experiments in LD A, DON: DOP ratio decreased from 25 to 16.4 at the end of the experiment (Fig. 5b). In LD B experiment (Fig. 5c), lower DIN: DIP than Redfield ratio was found through the experiment, suggesting a nitrogen deficiency relative to phosphorus, except at 1 h of incubation when a higher

DIN: DIP ratio was observed (2.6 times higher than Redfield) in the treatment with copepods, linked to a nitrate increment (see Fig. 3f). The DON: DOP ratios were higher than Redfield ratio in the treatment with copepods and control (Fig. 5d). In LD C the DON: DOP ratio (Fig. 5f) indicated a substantial accumulation of nitrogen with respect to phosphorus from the first hour of the incubation in the treatment with copepods, showing an important deviation from the Redfield ratio (> 16:1).

3.3 Changes in bacterioplankton abundance during the experiment evolution

Changes in bacterioplankton abundance are shown in the Fig. 6. Initial experimental conditions showed that bacterioplankton were more abundant in LD B, followed by LD C and LD A experiments. In LD A experiment (Fig. 6a) bacterioplankton presented an increase through time towards the end of the incubation, from $100x10^3$ to $200x10^3$ cell mL^{-1}, showing significant statistical differences between the sampling times (ANOVA, F1,20: 13.5, $p<0.001$) but not between treatments (ANOVA, $F_{1,20}$: 0.004, $p=0.95$). In contrast, in LD B experiment (Fig. 6b), a decrease in bacterioplankton abundance (around $300x10^3$ cell mL^{-1}) was observed between 0.5 to 1 h and between 1 h and 2 h of incubation, in copepod and control treatments, respectively, giving significant differences both between sampling times ($F_{1,16}$: 33.8, $p<0.000$) and treatments (copepods and control; ANOVA, $F_{1,16}$: 7.69, $p=0.014$). In LD C (Fig. 6c), a strong difference (> $200x10^3$ cell mL^{-1}) was observed between sampling times (ANOVA, $F_{1,20}$: 9.93, $p<0.000$) and between treatments and control (ANOVA, $F_{1,20}$: 6.22, $p=0.02$).

3.4 Active bacterial composition and their response during the incubations

The alpha diversity derived from normalized 16S rDNA and rRNA libraries is shown in the Fig. 7. The bacterial taxonomic richness determined (OTU number) and expected on the Chao1 index was higher in DNA in situ compared to the cDNA in situ and the samples related to the experiment. In accordance, Shannon diversity index showed that the bacterial community obtained in situ from 16S rDNA reached higher values compared with the incubated community. The alpha diversity showed slight differences between the treatments with copepods in the three experiments. These differences were characterized by higher values at the beginning of the incubation in the LD A and LD B stations (T1 and T2, respectively; Fig. 7a and 7c) and by higher diversity values at the end of the experiments (T4) in the treatment with copepods in LD C station (Fig. 7b).

The total, in situ bacterial community composition (Fig. 8a) was characterized by a higher contribution of Alphaproteobacteria, mainly by SAR 11, dominating slightly more in LD C, followed by LD B and LD A, respectively, reaching 49.8%, 41.1% and 31.1% of libraries derived from DNA (Fig. 8b). Cyanobacteria were also present in our libraries with a higher contribution in LD C than in LD A and LD B. In addition, a high number of abundant phyla were observed in LD A compared to the other stations. Additionally, the active in situ bacterial composition (Fig. 8c) 16S rRNA was consistent with 16S rDNA, with the predominance of Alphaproteobacteria and SAR11 class followed by the same trend (Fig. 8b and 8c), with higher contribution in LD C followed by LD B and LD A stations.

In general, clustering analyses showed that the active bacterial community composition varied significantly during the incubation compared to the in situ community in the three experiments (Fig. 9). During LD A incubation, the bacteria community composition was characterized by an increase of Gammaproteobacteria from 39% to 68% in T0 and T4 in the copepods treatments (Fig. 9a). Also, a decrease in Cyanobacteria, Chloroflexi and other low frequent taxa was found. During the experiment at LD B, smaller differences in taxa contribution were observed through the incubation. Gammaproteobacteria, increased their contribution through the incubation from 28% at T0 to 32% at T4 (Fig. 9b). In contrast to the other experiments, the incubation at LD C (Fig. 9c) presented a decrease in Gammaproteobacteria contribution and an increase in Alphaproteobacteria. The cluster analysis (Fig. 9) associated with bacterial orders contribution changes during the experiments indicate that the variability was mainly associated to the incubation time, T0, T1 and T2 versus T3 and T4, in LD A and LD B experiments, respectively. However, in LD C experiment the bacterial orders were mainly grouped by treatments without effect of incubation time (with and without copepods; Fig. 9c).

In addition, the greatest dissimilarities, based on SIMPER analysis (Fig. 10; Table S3), between copepod treatments and control in LD A (Fig. 10a) was mainly due to an increase in Alteromonadales and Oceanospirillales in the treatment with copepods, whereas SAR11 and Rhodobacterales increase their relative abundance in controls. At LD B experiment (Fig. 10b), smaller dissimilarities between treatment and control (9.5%) were observed and mainly due to a successive decrease of Alteromonadales contribution and increase of SAR11contribution in the treatment with copepods. In contrast in the LD C station (Fig. 10c), the dissimilarities were mainly due to a larger contribution in Alteromonadales, replaced by SAR 11 at the end of the incubation in the treatment with copepods. The specific order contribution for the three experiments are shown in Fig. S2, S3 and S4, as abundant (>0.5%), semirare (0.5%-0.1%) and rare (<0.1%; Pedrós-Alió, 2012).

PCO analysis with Orders contribution and environmental parameters indicates that in LD A experiment the initial sampling points (T0, T1 and T2) were associated with inorganic compounds (NH_4^+, NO_3^-, NO_2^- and PO_4^{3-}; Fig. 11a) for the treatment with copepods and control. Meanwhile, nitrite concentrations were associated with the last time-points in LD B (Fig. 11b). In LD C experiment (Fig. 11c), no visual association was observed between environmental parameters and the order contribution. In addition, the PERMANOVA main test revealed significant differences among all samples regarding the different experiments (pseudo-F=12.7, p=0.001). However, differences between treatments with copepods and control, and between times not show significant differences (PERMANOVA, pseudo-F=2.1-1.3, p=0.12-0.25).

4 Discussion

The two oceanographic regions in which our experiments were performed presented contrasting trophic conditions. The MA region was characterized by a gradient of nutrient conditions, with the highest and the lowest nitrate concentrations in LD A and LD B, respectively. Both stations were characterized by the presence of *Trichodesmium* spp bloom, with higher N_2 fixation rates (Bonnet et al., 2017; Caffin et al., this issue). According, the satellite data, De Verneil et al., (this issue) suggest

that the low nutrient concentration found in LD B was the consequence of a two months old diazotrophs bloom. In contrast to the MA region, the SG region is characterized by lower nitrate concentrations, higher phosphate concentrations, resulting in lower DIN: DIP ratio. These conditions were reflected in the abundance and composition of zooplankton, and also in the composition of bacterioplankton. In the case of zooplankton, the average abundance in the MA region was 1,212 ind. m^{-3} (sd=334 ind. m^{-3}, highest value 2,017 ind. m^{-3}), whereas in the SG region the average abundance was 665 ind. m^{-3} (sd=213 ind. m^{-3}, highest value 409 ind. m^{-3}; Carlotti et al., this issue). On the other hand, the bacterioplankton community composition revealed a higher diversity in the MA region compared to SG, with a higher dominance of Alphaproteobacteria in both regions, however, SAR 11 increased their contribution in the ultra-oligotrophic area (SG).

4.1 Biogeochemical changes in response to the dissolved compounds released by copepods

During our study, we evidenced that copepods exert an influence on biogeochemical composition during the incubations at contrasting trophic conditions including ultra-oligotrophic (SG) versus oligotrophic conditions (MA), showing significant increase of ammonium and DON accumulations. In addition, also copepods influence DON recycling but differentially at the different stations. In the experiment with copepods carried out in the LD A station, higher DON concentrations were observed at initial times (between 0 and 1 h). This DON was actively consumed between 1 and 2 hours of incubation. However, this higher DON consumption (3.62 µmol L^{-1} h^{-1}; Table S4) was not regenerated, since ammonium did not accumulate with time. In addition, at the same time and until the end of incubation an increase in bacterioplankton abundance was observed (22.8 x 10^3 cell mL^{-1}). Although this increase was non-statistically significant, it has been reported that zooplankton can release highly labile compounds through their metabolism and these compounds can be rapidly assimilated by microbial communities, as heterotrophic bacteria in a variety of marine environments (Arístegui et al., 2014; Vargas et al., 2007). On the other hand, the second experiment carried out in the MA region (at LD B) evidenced a potential nitrification associated with copepods excretion. This because, at the initial time, a decrease in ammonium concentration (between 0.5 and 1 h of incubation) related with nitrate accumulation (highly variable between the triplicates) was observed in the treatment with copepods, but not observed in the control. Nitrifying and heterotrophic communities can rapidly respond to DON and ammonium pulses excreted by copepods in the upwelling area off central Chile, revealed a tight coupling between excretion and the functional microbial groups involved in ammonia oxidation (Valdes et al., 2017a).

In the SG region (at LD C station), the experiment with copepods presented the highest evidence of DON remineralization/ammonification based on the differences in ammonium concentrations between copepods treatments and control up to 0.3 µmol L^{-1}, several times higher than in situ concentration (Table 1). DON consumption was observed through the first 2 h of incubation in the treatment with copepods (Fig. 3; Table S4). In addition, as in LD B, nitrate accumulation was observed at the end of incubation in the treatment with copepods compared to the control. Our results suggest the presence of two processes which could occur in the ultra-oligotrophic station in the SG: the release of DON by copepods stimulating ammonium regeneration by bacteria, and the increment in nitrate concentration strongly linked to the

nitrification process. Thus, DON remineralization could provide additional substrate for microbial and phytoplankton growth in LD C station. In this sense, Raimbault and Garcia (2008) demonstrate that in the very low productive area of South Pacific Gyre, most of the primary production is sustained by active regeneration process, being nitrification active in surface layer and often balances the biological demand for nitrate. These results support the idea that DON excretion by copepods in oligotrophic and ultra-oligotrophic water could play a major role in nitrogen cycling.

On the other hand, DOP showed significantly higher concentrations in the treatment with copepods compared to the control in the three experiments, although not over the whole incubation period but over shorter durations. Our results suggest that copepods could potentially contribute with substantial amount of DOP and provide an alternative source of phosphorus for phytoplankton and microbial loop in this study area. The contribution of DOP by copepods could contribute to maintain the longer bloom in this region (MA), providing an additional source of phosphorus for biomass growth. *Trichodesmium* spp are capable to hydrolyze DOP compounds (Mulholland et al., 2002), thus zooplankton metabolism could potentially supply a significant portion of the cellular phosphorus necessary for their growth.

In this study, the increase in the concentration of the different compounds cannot be attributed only to the excretion process. Firstly, the leaching is one of the possible pathways through which zooplankton can release dissolved compounds, along with the sloppy feeding and excretion. However, it has been demonstrated that the contribution provided by leaching is insignificant compared to excretion, less than 6% in the case of the release of DON, whereas ammonium was undetected (Saba et al., 2011; Steinberg et al., 2000). We did not monitor leaching from fecal pellets produced during our experiments, considering leaching from fecal pellets as negligible, and then DON accumulation could account for up to 1.91 µmol L^{-1} h^{-1} excretion in the LD A experiment. However, the experimental copepod densities were several orders of magnitude higher than those expected in situ (10 copepods for 0.5 L volume, i.e. 20,000 ind. m^{-3}). Consequently, our results might be overestimated, because copepods concentration in our bottles did not reflect the in situ abundance found at each LD station (MA: 1,500 ind. m^{-3} and SG: 500 ind. m^{-3}; Carlotti et al., this issue). However, such concentration was strictly necessary through the experiments to obtain a signal in ammonium concentrations, which were very low in the study area (Table 1). In addition, we cannot estimate excretion rates for all compounds since they appear later in the incubation probably modulated by microbial metabolism. Moreover, running an experiment without microbial influence as control was impossible since most antimicrobial approaches (e.g., autoclaving, adding antibiotics, filtrating) could have negative effect on copepods microbiome and water chemistry affecting also our results.

4.2 Changes in bacterial community composition induced by dissolved compounds released by copepods

During our experiments, we determined the response of microbial community in terms of changes in the abundance and active bacterial composition. Our experiments showed, the bacterioplankton abundance followed the same patterns of

variation in the experiments with copepods and in the controls, for each of the three experiments. Also, we found significant differences between treatments (with and without copepods) and between sampling times, except for LD A, in the bacterioplankton community structure (mainly by Gammaproteobacteria and Alphaproteobacteria). A faster response in Alteromonadales (Gammaproteobacteria) is frequently observed in microcosm studies during incubations, due to their high growth rate and their ability to exploit DOM rapidly when is available (Alonso-Saez and Gasol, 2007; Fuchs et al., 2000; Landa et al., 2013), which is in according with our results. However, the changes in structure associated with the release of dissolved compounds by copepods compared to the controls were not accompanied by an evident increase in bacterioplankton abundance. Several studies have suggested that enrichment experiments tend to enhance the abundance of organism rarely found in nature, but that have opportunistic and copiotrophic qualities that allow them to rapidly adapt to changes in environmental conditions, outcompeting abundant groups in the field (Nelson and Wear, 2014; Pedler et al., 2014; Logue et al., 2016). In contrast, in the experiment carried out in LD B station, a substantial decrease in Alteromonadales was observed in the treatment with copepods compared to the control. The higher contribution through the time was due Alphaproteobacteria, in which SAR11 increased their relative abundance (~8%) at 1 hour of incubation. SAR 11 was the major constituent of 16 rDNA (in situ) and also was active in according to our 16 rRNA (in situ) reads. SAR11 is the most abundant bacterioplankton in the ocean, is well adapted to low concentration of nutrients and specialized in oxidizing many labile and low-molecular-weight compounds produced by other plankton (Giovannoni, 2017). During our incubations, SAR11 increased their contribution only in LD B experiment which coincided with the station with the lowest nutrients concentrations. On the other hand, in the LD C experiment, an increase in Alteromonadales and Vibrionales was observed. Alteromonadales decreased their contribution in the treatment with copepods at the end of the incubation, while increasing their contribution in control. The reverse pattern was observed for SAR11 which increased their contribution at the end of incubation. Based in our experiments, Alteromonadales increase is in coincidence with DON available from copepods excretion during the first 2 h of incubation, afterwards at the end of the experiment nutrients were diminished, and such conditions potentially favoured the versatile SAR 11. Peduzzi and Herndl (1992) observed high monomeric carbohydrate concentration and bacterial activity in experiments where copepods were included. Furthermore, these authors observed that bacterial communities living in oligotrophic areas can be efficient to utilize the newly available substrate source, in according with our results.

Bacterial remineralization of DOM derived from copepods metabolism could be a highly efficient mechanism to maintain the nutrients in the upper layer supporting the phytoplankton and microbial growth. In this sense, the response associated with the different regions of our study suggests that copepods in the MA region could provide DOP as an alternative substrate for phytoplankton and bacterial growth, while the enhanced remineralization by copepods could be more important in the ultra-oligotrophic area of the SG region. However, more studies are necessary to determine the role of zooplankton in the recycling of nutrients in oligotrophic areas. Furthermore, the biogeochemical impact of copepods metabolism may not be limited to the upper layer, as zooplankton can move through the water column by diel vertical migration, promoting the

export of dissolved compounds through their metabolism at deeper layers. We conclude that copepod metabolism can provide substantial amounts of nitrogen and phosphorus (NH_4^+, DON, DOP) which microbial communities can directly use in a short period of time enhancing the bacterioplankton remineralization.

*Acknowledgement***.** This is a contribution of the OUTPACE (Oligotrophy from Ultra-oligoTrophyPACific Experiment) project funded by the French research national agency (ANR-14-CE01-0007-01), the LEFE-CyBER program (CNRS-INSU), the GOPS program (IRD) and the CNES (BC T23, ZBC 4500048836) and by European FEDER Fund under project 1166-39417. The OUTPACE cruise was managed by T. Moutin and S. Bonnet from the MIO (Mediterranean Institute of Oceanography). The authors thank to the crew of the R/V L´Atalante for outstanding shipboard operation. Dr. M. Pujo-Pay (Observatoire Océanologique de Banyuls sur mer), O. Grosso (MIO) and S. Helias (MIO) are thanked for their assistance during the analysis of nutrients. We thank C. Dupouy, T. Moutin and S. Helias for their support in providing Chlorophyll-a and DON-DOP data. This work was supported by the Comisión Nacional de Investigación Científicas y Tecnológicas (CONICYT) through Grants FONDECYT No 1130511 and 1150891 and Instituto Milenio de Oceanografía (IMO) Grant IC120019. Additional support has been provided by FONDAP grants IDEAL and INCAR (15110027 and 15150003, respectively), and CONICYT PIA PFB-31 COPAS Sur austral program. The work was developed in the frame of LIA MORFUN. Valdés and Donoso were funded by CONICYT Scholarship

References

Alonso-Saez, L. and Gasol, J. M.: Seasonal variations in the contributions of different bacterial groups to the uptake of low-molecular-weight compounds in northwestern Mediterranean coastal waters, Appl. Environ. Microbiol. 73, 3528–3535, 2007.

Aminot, A. and Kérouel, R.: Dosage automatique des nutriments dans les eaux marines: méthodes en flux continu, in Méthodes d'analyse en milieu marin, Editions Quae, 2007.

Arístegui, J., Duarte, C. M., Reche, I. and Gómez-Pinchetti, J. L.: Krill Excretion Boosts Microbial Activity in the Southern Ocean, PLoS One 9, e89391, 2014.

Bidigare, R.: Nitrogen excretion by marine zooplankton, p. 385–409. In E. Carpenter and D. Capone [eds.], Nitrogen in the marine environment. Academic Press. 1983

Bonnet, S., Caffin, M., Berthelot, H. and Moutin, T.: Hot spot of N_2 fixation in the western tropical South Pacific pleads for a spatial decoupling between N_2 fixation and denitrification, Proc. Natl. Acad. Sci., 114, E2800–E2801, 2017.

Caffin, M., Moutin, T., Foster, R. A., Bouruet-Aubertot, P., Doglioli, A. M., Berthelot, H., Grosso, O., Helias-Nunige, S., Leblond, N., Gimenez, A. et al.: Nitrogen budgets following a Lagrangian strategy in the Western Tropical South Pacific Ocean: the prominent role of N_2 fixation (OUTPACE cruise), Biogeosciences Discuss, https://doi.org/10.5194/bg-2017-468, in review, 2018.

Campbell, B. J. and Kirchman, D. L.: Bacterial diversity, community structure and potential growth rates along an estuarine salinity gradient. ISME J., 7, 210–220, 2013.

Carlotti, F., Pagano, M., Guilloux, L., Donoso, K. and Valdes, V.: Mesozooplankton structure and functioning across the Western Tropical South Pacific Ocean, Biogeosciences Discuss, https://doi.org/10.5194/bg-2017-573, in review, 2018.

Carpenter, E. J., Subramaniam, A. and Capone, D. G.: Biomass and primary productivity of the cyanobacterium *Trichodesmium* spp. in the tropical N Atlantic Ocean, Deep Sea Res. Part 1 Oceanogr. Res. Pap., 51, 173–203, 2004.

Conover, R. J. and Gustavson, K. R.: Sources of urea in arctic seas: zooplankton metabolism, Mar. Ecol. Prog. Ser., 179, 41r54, 1999.

de Verneil, A., Rousselet, L., Doglioli, A. M., Petrenko, A. A., Maes, C., Bouruet-Aubertot, P. and Moutin, T.: OUTPACE long duration stations: physical variability, context of biogeochemical sampling, and evaluation of sampling strategy. Biogeosciences, 2125-2147, doi: 10.5194/bg-15-2125-2018, 2018.

Edgar, R. C., Haas, B. J., Clemente, J. C., Quince, C. and Knight, R.: UCHIME improves sensitivity and speed of chimera detection. Bioinformatics, 27, 2194–2200, doi: 10.1093/bioinformatics/btr381, 2011.

Eppley, R. W., Renger, E. H., Venrick, E. L. and Mullin, M. M.: A study of plankton dynamics and nutrient cycling in the central gyre of the North Pacific Ocean, Limnol. Oceanogr., 18 (4), 534–551, 1973.

Fuchs, B. M., Zubkov, M. V., Sahm, K. and Amann, R.: Changes in community composition during dilution cultures of marine bacterioplankton as assessed by flow cytometric and molecular biological techniques, Environ. Microbiol., 2(2), 191–201, 2000.

Gardner, W. S. and Paffenhöfer, G. A.: Nitrogen regeneration by the subtropical marine copepod *Eucalanus pileatus*, J. Plankton Res., 4, 725–734, 1982.

Giovannoni, S. J.: SAR11 Bacteria: The Most Abundant Plankton in the Oceans. Annu. Rev. Mar. Sci. 9, 231–255, 2017.

Hasegawa, T., Koike, I. and Mukai, H.: Dissolved organic nitrogen dynamics in coastal waters and the effect of copepods, J. Exp. Mar. Biol. Ecol., 244, 219–238, 2000.

Hernández-León, S., Fraga, C. and Ikeda, T.: A global estimation of mesozooplankton ammonium excretion in the open ocean, J. Plankton Res., 30, 577–585, 2008.

Holmes, R., A. Aminot, R. Kérouel, B. Hooker, and B. Peterson.: A simple and precise method for measuring ammonium in marine and freshwater ecosystems, Can. J. Fish. Aquat. Sci., 56, 1801-1808, 1999.

Ikeda, T., Kanno, Y., Ozaki, K. and Shinada, A.: Metabolic rates of epipelagic marine copepods as a function of body mass and temperature, Mar. Biol., 139, 587–596, 2001.

Isla, J. A., Llope, M. and Andón, R: Size-fractionated mesozooplankton biomass, metabolism and grazing along a 50 N-30 S transect of the Atlantic Ocean, J. Plankton Res., 26, 1301–1313, 2004.

Johannes, R. E.: Uptake and release of dissolved organic phosphorus by representatives of a coastal marine ecosystem, Limnol Oceangr., 9, 224–234, 1964.

Karl, D. M., Letelier, R. M., Dore, J. E., Christian, J. and Hebel, D.: The role of nitrogen fixation in biogeochemical cycling in the subtropical North Pacific Ocean, Nature, 388, 533–538, 1997.

Landa, M., Cottrell, M., Kirchman, D., Blain, S. and Obernosterer, I.: Changes in bacterial diversity in response to dissolved organic matter supply in a continuous culture experiment, Aquat. Microb. Ecol., 69, 157–168, 2013.

Levipan, H.A., Molina, V. and Fernandez, C.: *Nitrospina*-like bacteria are the main drivers of nitrite oxidation in the seasonal upwelling area of the Eastern South Pacific (Central Chile ~36°S): Nitrite-oxidizing bacteria in the seasonal upwelling system off Concepcion, Environ. Microbiol. Rep., 6, 565–573, 2014.

Logue, J. B., Stedmon, C. A., Kellerman, A. M., Nielsen, N. J., Andersson, A. F., Laudon, H., et al.: Experimental insights into the importance of aquatic bacterial community composition to the degradation of dissolved organic matter, ISME J., 10, 533–545, doi: 10.1038/ismej.2015.131, 2016.

Marie. D., Partensky, F., Jacquet, S. and Vaulot, D.: Enumeration and cell cycle analysis of natural populations of marine picoplankton by flow cytometry using the nucleic acid stain SYBR Green I, Appl. Environ. Microbiol., 63, 186–193, 1997.

Miller, C. A. and Glibert, P. M.: Nitrogen excretion by the calanoid copepod *Acartia tonsa*: results of mesocosm experiments, J. Plankton Res., 20, 1767–1780, 1998.

Miller, C. A. and Roman, M. R.: Effects of food nitrogen content and concentration on the forms of nitrogen excreted by the calanoid copepod, *Acartia tonsa*, J. Exp. Mar. Biol. Ecol., 359, 11–17, 2008.

Møller, E. F.: Sloppy feeding in marine copepods: prey-size-dependent production of dissolved organic carbon, J. Plankton Res., 27, 27–35, 2004.

Møller, E. F.: Production of dissolved organic carbon by sloppy feeding in the copepods *Acartia tonsa*, *Centropages typicus*, and *Temora longicornis*, Limnol. Oceanogr., 52, 79–84, 2007.

Moutin, T., Karl, D. M., Duhamel, S., Rimmelin, P., Raimbault, P., Van Mooy, B. A. and Claustre, H.: Phosphate availability and the ultimate control of new nitrogen input by nitrogen fixation in the tropical Pacific Ocean, Biogeoscience. 5, 95-109, 2008.

Moutin, T., Doglioli, A. M., de Verneil, A., and Bonnet, S.: Preface: The Oligotrophy to the UlTra-oligotrophy PACific Experiment (OUTPACE cruise, 18 February to 3 April 2015), Biogeosciences, 14, 3207–3220, 2017.

Moutin, T., Wagener, T., Caffin, M., Fumenia, A., Gimenez, A., Baklouti, M., Bouruet-Aubertot, P., Pujo-Pay, M., Leblanc, K., Lefevre, D., Helias, S., Leblond, N., Grosso, O. and de Verneil, A.: Nutrient availability and the ultimate control of the biological carbon pump in the Western Tropical South Pacific Ocean, Biogeoscience Discuss., https://doi.org/10.5194/bg-2017-565, in review, 2018.

Mulholland, M. R., Floge, S., Carpenter, E. J., and Capone, D. G.: Phosphorus dynamics in cultures and natural populations of *Trichodesmium* spp, Mar. Ecol. Prog. Ser., 239, 45–55, 2002.

Nelson, C. E., and Wear, E. K.: Microbial diversity and the lability of dissolved organic carbon, Proc. Natl. Acad. Sci. U.S.A., 111, 7166–7167, doi: 10.1073/pnas.140575111, 2014.

Pedler, B. E., Aluwihare, L. I., and Azam, F.: Single bacterial strain capable of significant contribution to carbon cycling in the surface ocean, Proc. Natl. Acad. Sci. U.S.A., 111, 7202–7207, doi: 10.1073/pnas.1401887111, 2014.

Pedrós-Alió, C.: The Rare Bacterial Biosphere, Annu. Rev. Mar. Sci., 4, 449–466, 2012.

Peduzzi, P. and Herndl, G. J.: Zooplankton activity fuelling the microbial loop: differential growth response of bacteria from oligotrophic and eutrophic waters, Limnol. Oceanogr., 37, 1087–1092, 1992.

Pérez-Aragón, M., Fernandez, C. and Escribano, R.: Nitrogen excretion by mesozooplankton in a coastal upwelling area: Seasonal trends and implications for biological production, J. Exp. Mar. Biol. Ecol., 406, 116–124, 2011.

Polovina, J. J., Howell, E. A., and Abecassis, M.: Ocean's least productive waters are expanding, Geophys. Res. Lett., 35, L03618, https://doi.org/10.1029/2007GL031745, 2008.

Pujo-Pay, M. and Raimbault, P.: Improvement of the wet-oxidation procedure for simultaneous determination of particulate organic nitrogen and phosphorus collected on filters, Mar. Ecol.-Prog. Ser., 105, 203–203, 1994.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., et al.: The SILVA ribosomal RNA gene database project: improved data processing and web-based tools, Nucleic Acids Res., 41, D590–D596. doi: 10.1093/nar/gks1219, 2013.

Raimbault, P. and Garcia, N.: Evidence for efficient regenerated production and dinitrogen fixation in nitrogen-deficient waters of the South Pacific Ocean: impact on new and export production estimates, Biogeosciences, 5, 323–338, 2008.

Richardot, M., Debroas, D., Thouvenot, A., Sargos, D., Berthon, J. and Dévaux, J.: Influence of cladoceran grazing activity on dissolved organic matter, enzymatic hydrolysis and bacterial growth, J. Plankton Res., 23, 1249–1261, 2001.

Saba, G., Steinberg, D. and Bronk, D.: Effects of diet on release of dissolved organic and inorganic nutrients by the copepod *Acartia tonsa*, Mar. Ecol. Prog. Ser., 386, 147–161, 2009.

Saba, G. K., Steinberg, D. K., and Bronk, D. A.: The relative importance of sloppy feeding, excretion, and fecal pellet leaching in the release of dissolved carbon and nitrogen by *Acartia tonsa* copepods, J. Exp. Mar. Biol. Ecol., 404, 47–56, 2011.

Sarmento, H., Romera-Castillo, C., Lindh, M., Pinhassi, J., Sala, M. M., Gasol, J. M., Marrase, C. and Taylor, G.T.: Phytoplankton species-specific release of dissolved free amino acids and their selective consumption by bacteria, Limnol. Oceanogr., 58, 1123–1135, 2013.

Satomi, M. and Pomeroy, L. R.: Respiration and Phosphorus Excretion in Some Marine Populations, Ecology, 46, 877–881, 1965.

Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., Lesniewski, R. A., Oakley, B. B., Parks, D. H., Robinson, C. J., Sahl, J. W., Stres, B., Thallinger, G. G., Horn, D. J. V. and Weber, C. F.: Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities, Appl. Environ. Microbiol., 75, 7537–7541, 2009.

Sipler, R.E., and Bronk, D.A.: Dynamics of dissolved organic nitrogen, p. 128-213. In D. A. Hansell and C. A. Carlson [eds.], Biogeochemestry of marine dissolved organic matter, second edition. Academic, 2014.

Smith, S. L. and Whitledge, T. E.: The role of zooplankton in the regeneration of nitrogen in a coastal upwelling system off northwest Africa, Deep-Sea Res., 24, 49–56, 1977.

Steinberg, D. K. and Landry, M. R.: Zooplankton and the Ocean Carbon Cycle, Annu. Rev. Mar. Sci., 9, 413–444, 2017.

Steinberg, D. K., Carlson, C. A., Bates, N. R., Goldthwait, S. A., Madin, L. P. and Michaels, A. F.: Zooplankton vertical migration and the active transport of dissolved organic and inorganic carbon in the Sargasso Sea, Deep Sea Res. Part 1 Oceanogr. Res. Pap., 47, 137–158, 2000.

Steinberg, D. K., Goldthwait, S. A. and Hansell, D. A.: Zooplankton vertical migration and the active transport of dissolved organic and inorganic nitrogen in the Sargasso Sea, Deep Sea Res. Part Oceanogr. Res. Pap., 49, 1445–1461, 2002.

Steinberg, D. K. and Saba G. K..: Nitrogen consumption and metabolism in marine zooplankton, p. 1135-1196. In D. G. Capone [eds.], Nitrogen in the marine environment. Academic, 2008.

Stenegren, M., Caputo, A., Berg, C., Bonnet, S., and Foster, R. A.: Distribution and drivers of symbiotic and free-living diazotrophic cyanobacteria in the Western Tropical South Pacific, Biogeosciences 15(5), 1559–1578, doi:10.5194/bg-15-1559-2018, 2018.

Titelman, J., Riemann, L., Holmfeldt, K. and Nilsen, T.: Copepod feeding stimulates bacterioplankton activities in a low phosphorus system, Aquat. Biol., 2, 131–141, 2008.

Valdés, V., Fernandez, C., Molina, V., and Escribano, R.: Nitrogen excretion by copepods and its effect on ammonia-oxidizing communities from a coastal upwelling zone, Limnol. Oceanogr., doi: 10.1002/lno.10629, 2017a.

Valdés, V. P., Fernandez, C., Molina, V., Escribano, R. and Joux, F.: Dissolved compounds excreted by copepods reshape the active marine bacterioplankton community composition, Front. Mar. Sci., 4, 2017b.

Vargas, C. A., Cuevas, L. A., González, H. E., and Daneri, G.: Bacterial growth response to copepod grazing in aquatic ecosystems, J. Mar. Biol. Assoc. UK, 87, 667-674, 2007.

Table 1: Deep chlorophyll-a maximum (DCM) depth, temperature, chlorophyll-a, ammonium (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-), phosphate (PO_4^{3-}) concentration, DIN: DIP ratio, DON and DOP concentrations in the DCM at each sampling site. DON, DOP concentrations was extracted from Moutin et al, (this issue).

	LD A	LD B	LD C
DCM (m)	80	34	140
Temperature (ºC)	25.6	27.5	22.9
Chlorophyll-a (μg L^{-1})	0.29	0.79	0.57
NH_4^+ [μmol L^{-1}]	0.025 ± 0.001	0.01 ± 0.00	0.02 ± 0.000
NO_2^- [μmol L^{-1}]	0.06 ± 0.002	0.02 ± 0.02	0.05 ± 0.004
NO_3^- [μmol L^{-1}]	1.53 ± 0.008	0.05 ± 0.021	0.25 ± 0.012
PO_4^{3-} [μmol L^{-1}]	0.16 ± 0.012	0.03 ± 0.025	0.24 ± 0.008
DIN: DIP	9.97 ± 0.70	4.52 ± 4.07	1.21 ± 0.11
DON [μmol L^{-1}]	4.78	5.88	4.49
DOP [μmol L^{-1}]	0.12	0.18	0.11

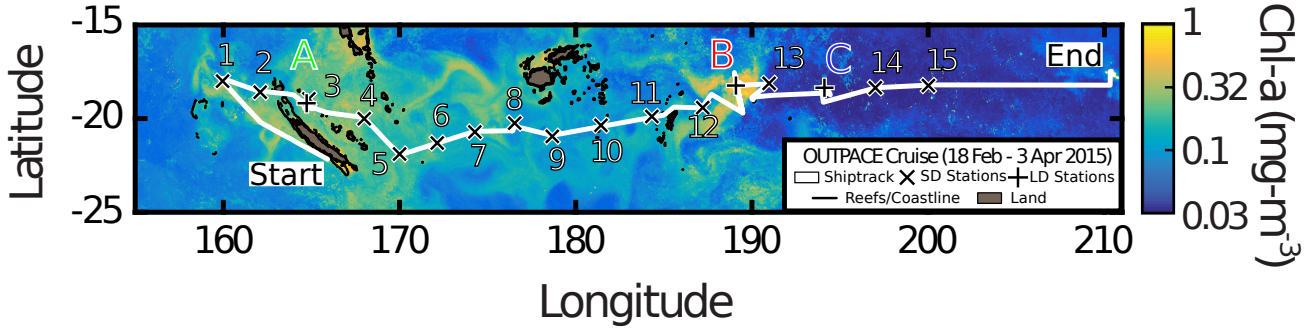

Figure 1: Quasi-Langragian Surface Chlorophyll-a concentration (mg m^{-3}) during the OUTPACE cruise. The satellite data are weighted in time by each pixel's distance from the ship's position for the entire cruise. The white line shows the vessel route (data from the hull-mounted ADCP positioning system). Coral reefs and coastlines are shown in black, land is grey, and areas of no data are left white. The positions of the short (long) duration stations are shown by cross (plus) symbols. Experiments were performed at each long duration station (Figure courtesy of A. De Verneil (02/06/2017)).

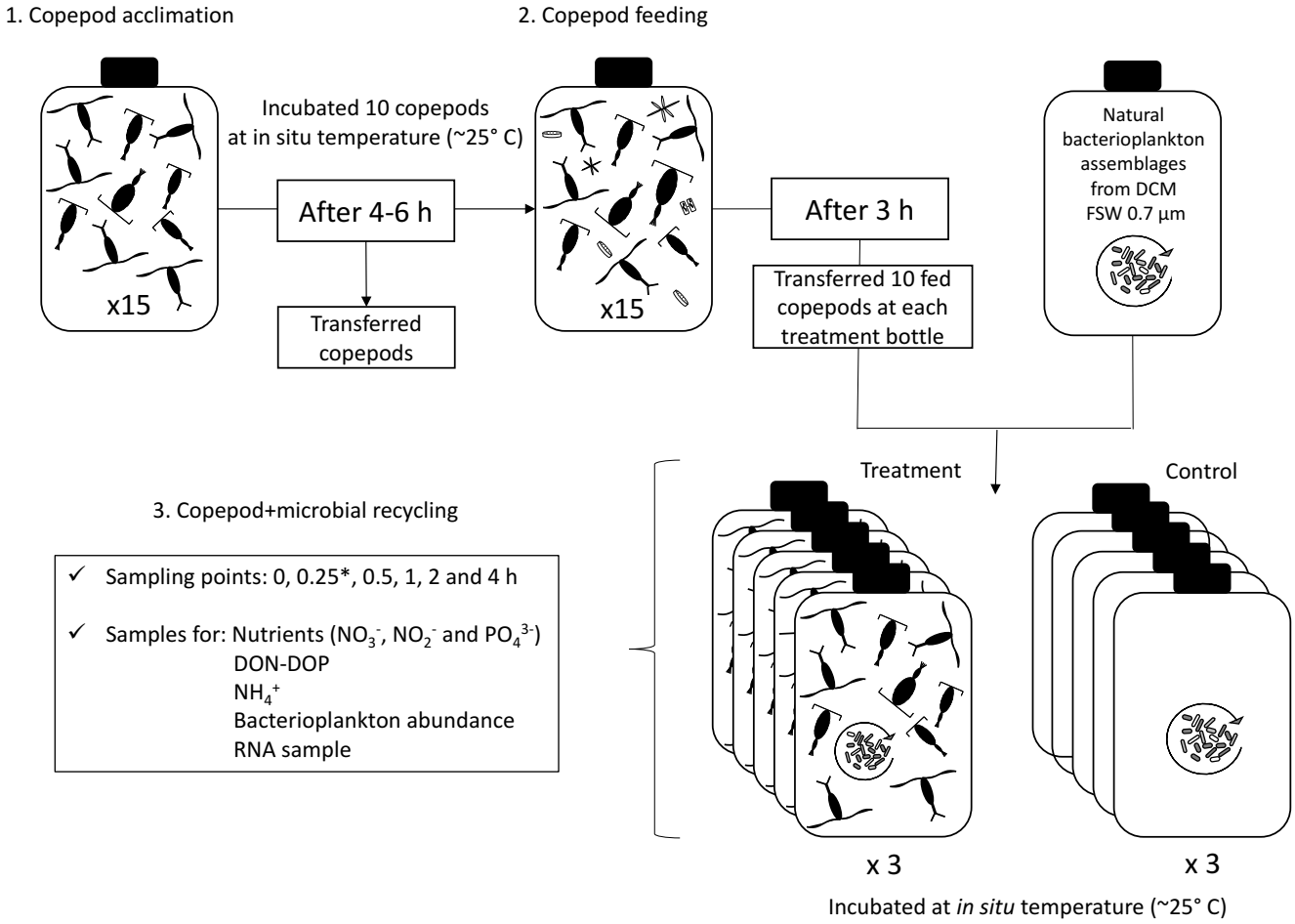

Figure 2: Experimental design. The design consisted of three phases done sequentially. Each bottle with copepods was acclimated for at least 6 hours (phase 1), followed by feeding (phase 2) with the Deep Chlorophyll-a Maximum (DCM) water for 4 hours. Copepod+microbial recycling (phase 3) was studied by adding natural bacterial assemblages from DCM filtered seawater (DCM FSW) to the 15 bottles with fed copepod, and maintaining bottles with natural bacterial assemblages and without copepods (controls). Three bottles with copepods and three control bottles were sacrificed at each of the 5-sampling time. An additional time (0.25 h *) was added for ammonium determination in LD B and LD C.

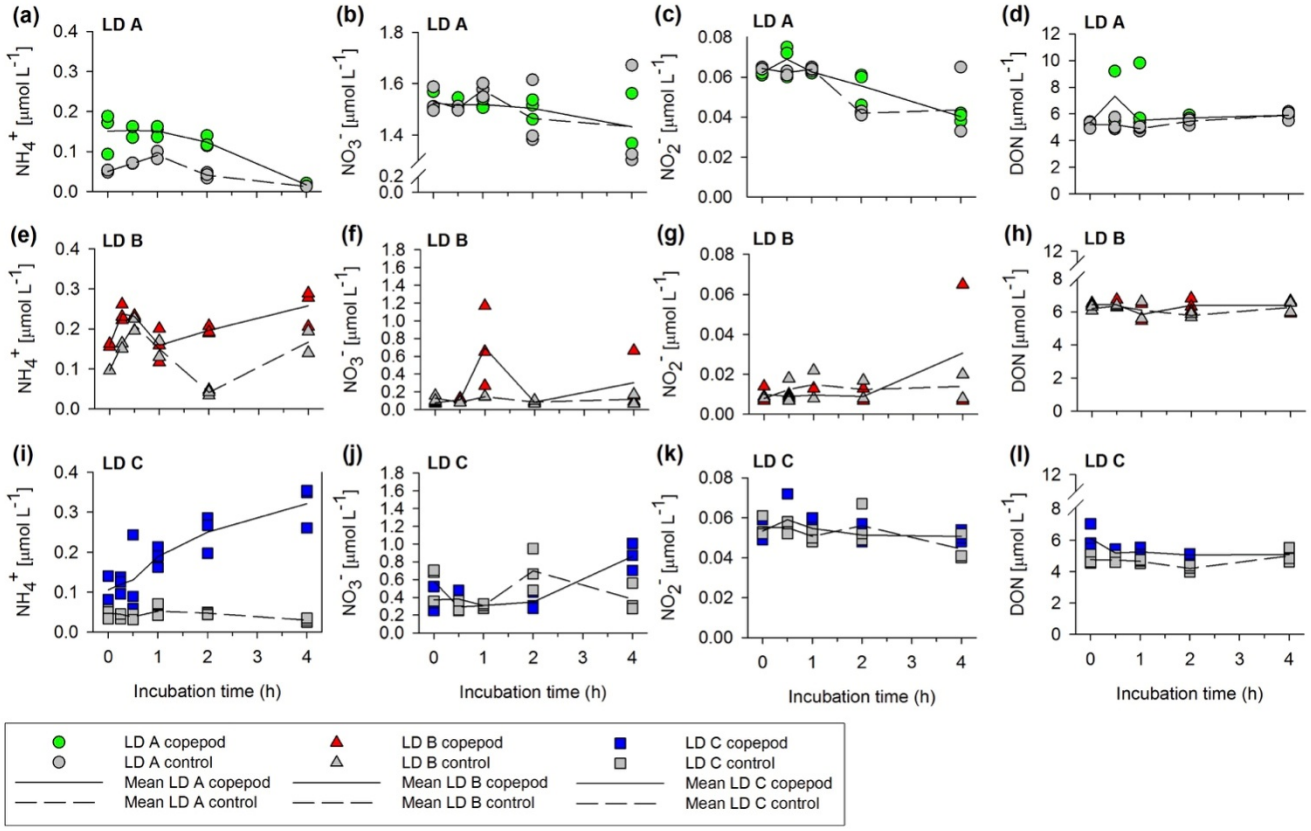

Figure 3: (**a, e, i**) Ammonium (NH_4^+), (**b, f, j**) nitrate (NO_3^-), (**c, g, k**) nitrite (NO_2^-) and (**d, h, l**) dissolved organic nitrogen (DON) variability through the incubation for treatments with copepods and control (without copepods) at each LD experiment.

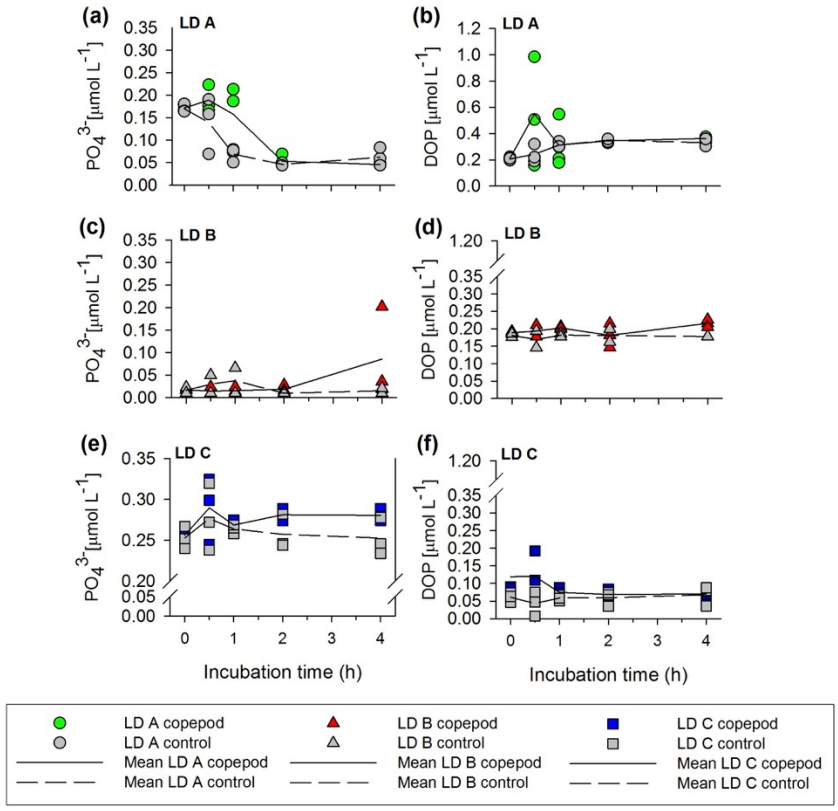

Figure 4: (**a, c, e**) Phosphate (PO_4^{3-}) and (**b, d, f**) dissolved organic phosphorus (DOP) variability through the incubation for treatments with copepods and control (without copepods) at each LD experiment.

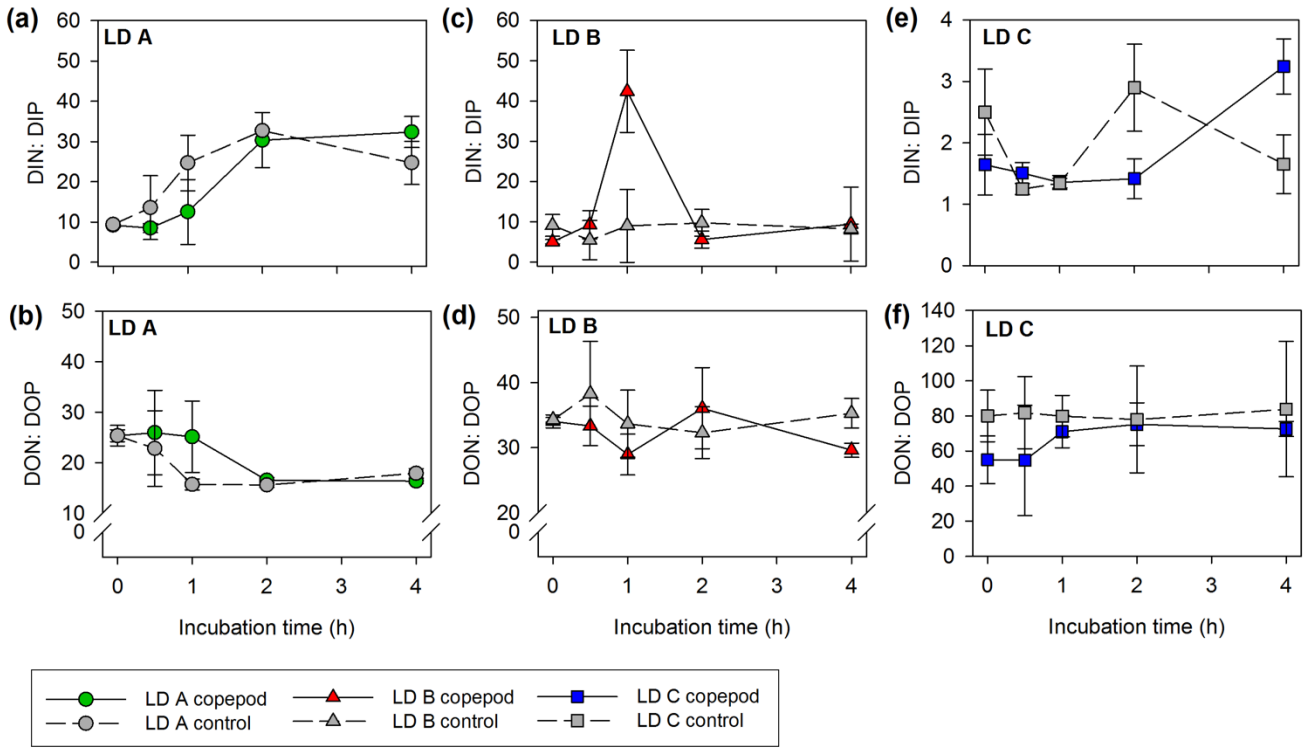

Figure 5: (**a, c, e**) N: P ratios of the inorganic (DIN: DIP) and (**b, d, f**) organic (DON: DOP) nutrients through the incubation for treatments with copepods and control (without copepods) at each LD experiment.

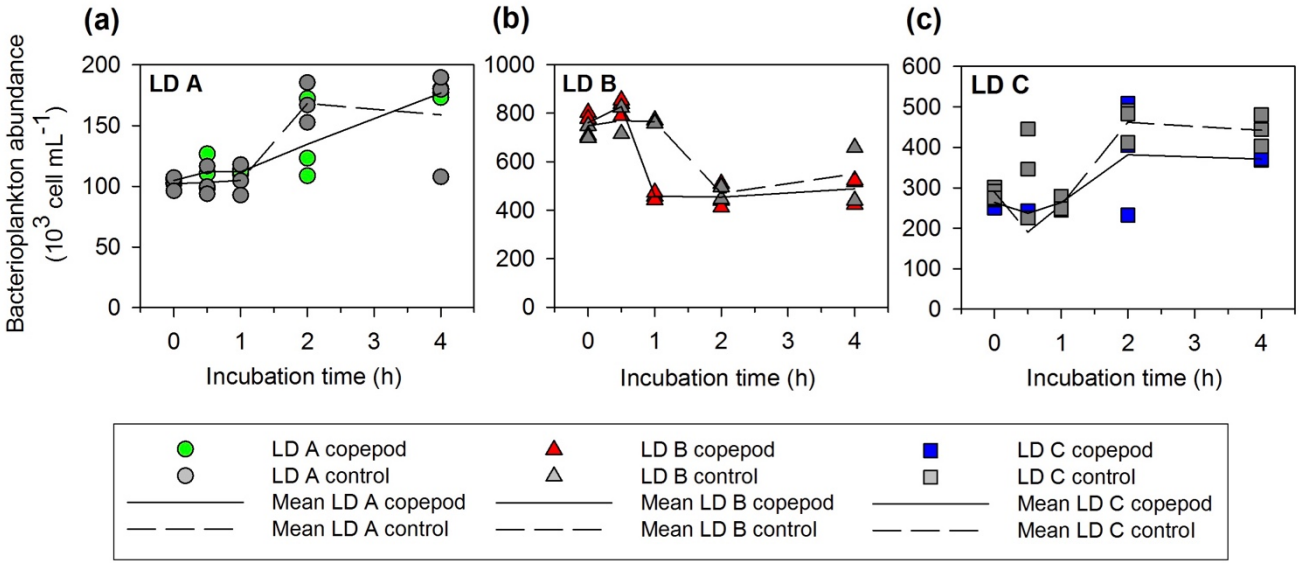

Figure 6: Bacterioplankton abundance through the incubation for treatments with copepods and control (without copepods) at each LD experiment. (**a**) LD A, (**b**) LD B and (**c**) LD C.

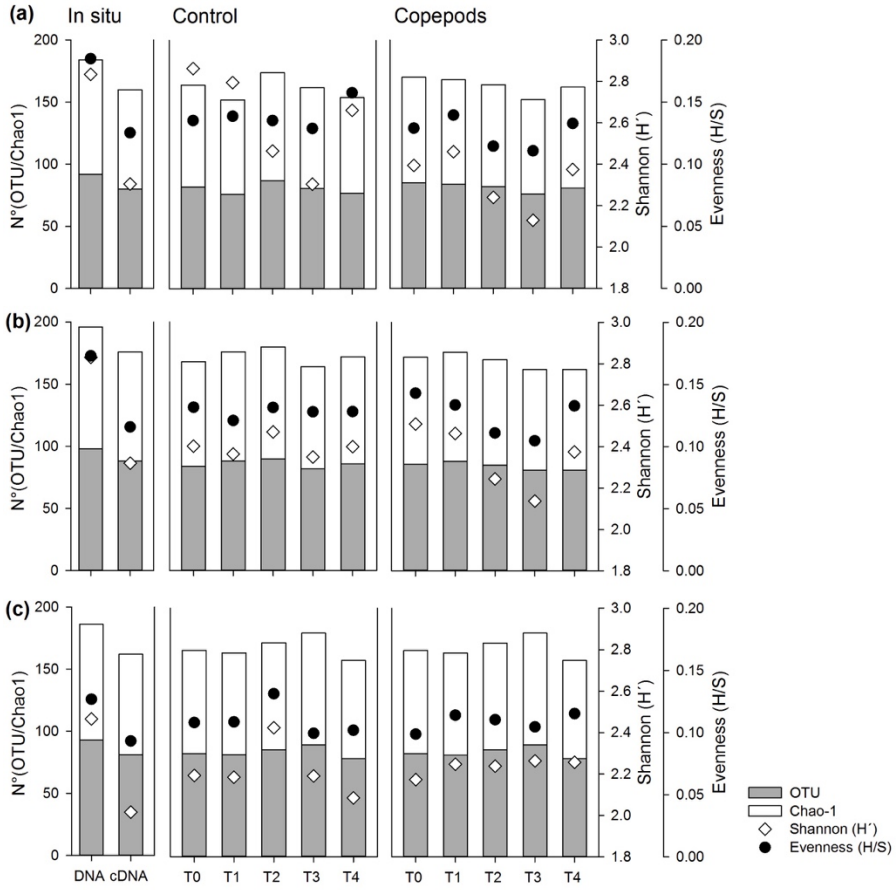

Figure 7: Richness, Chao1, Diversity (Shannon H´) and Evenness from the total and active in situ bacterial community and through the incubation for the active bacterial community at each LD experiment: (**a**) LD A, (**b**) LD B and (**c**) LD C.

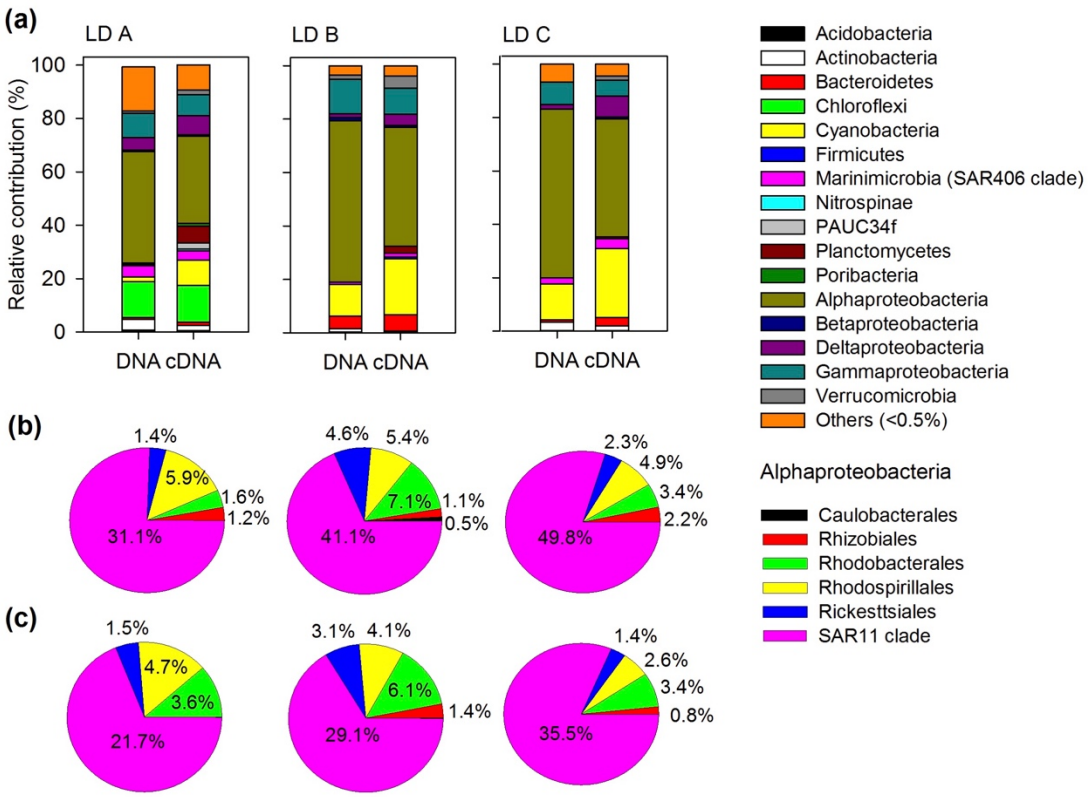

Figure 8: (**a**) Relative abundance of major bacteria phyla and Proteobacteria class at each LD station, with a zoom in Alphaproteobacteria order at initial in situ conditions for the three-sampling site: (**b**) total Alphaproteobacteria (DNA) and **(c)** active Alphaproteobacteria community (cDNA).

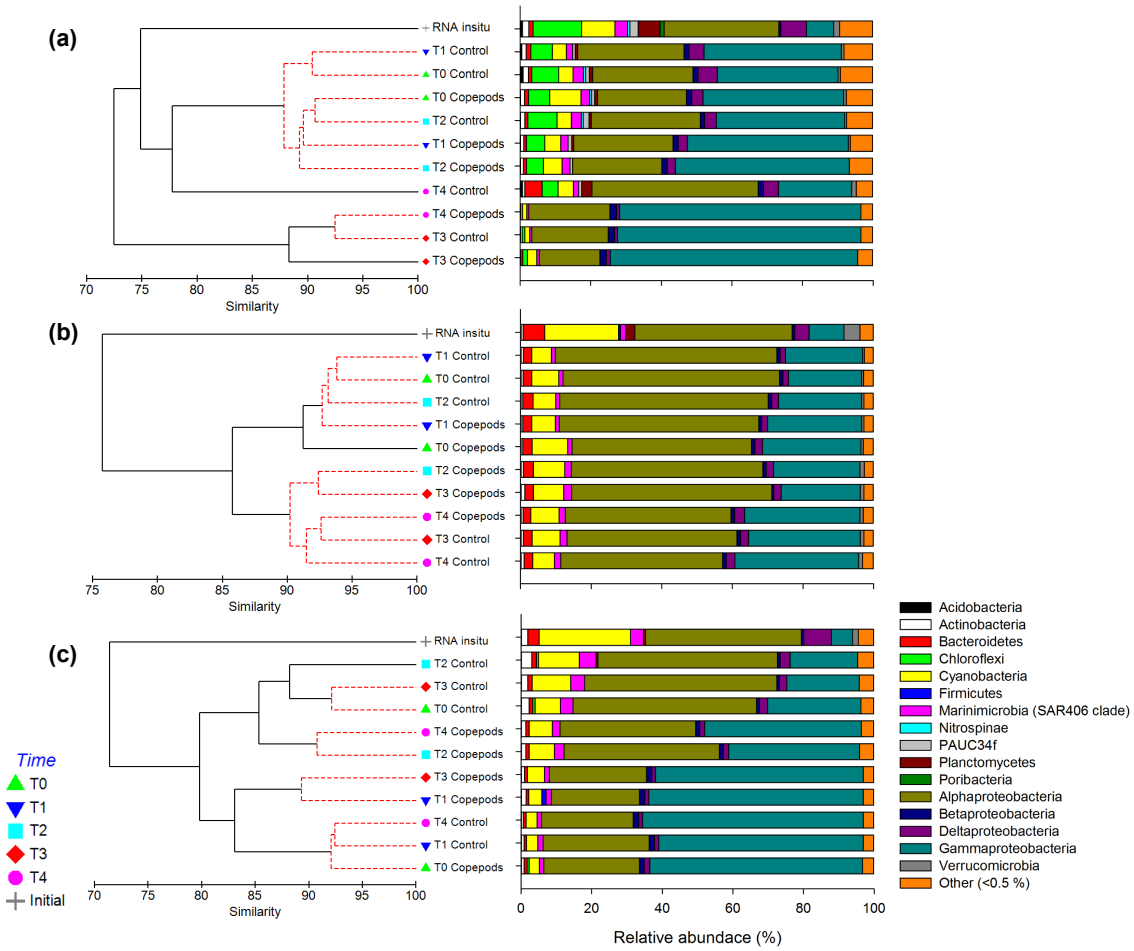

Figure 9: Similarity dendrograms based on the 16S rRNA and relative contribution of the abundant bacteria phyla and Proteobacteria subclasses for the initial in situ community, treatments with copepods and controls (left panel) through the incubation for the three experiments (**a**) LD A, (**b**) LD B and (**c**) LD C. Clustering in one the basis of a distance matrix computed using the Bray-Curtis index of similarity. The dendrogram was inferred with the unweighted pair-group average algorithm (UPGMA). Bacterial communities in the samples connected with red branch lines are not significantly different (SIMPROF test, $p<0.05$).

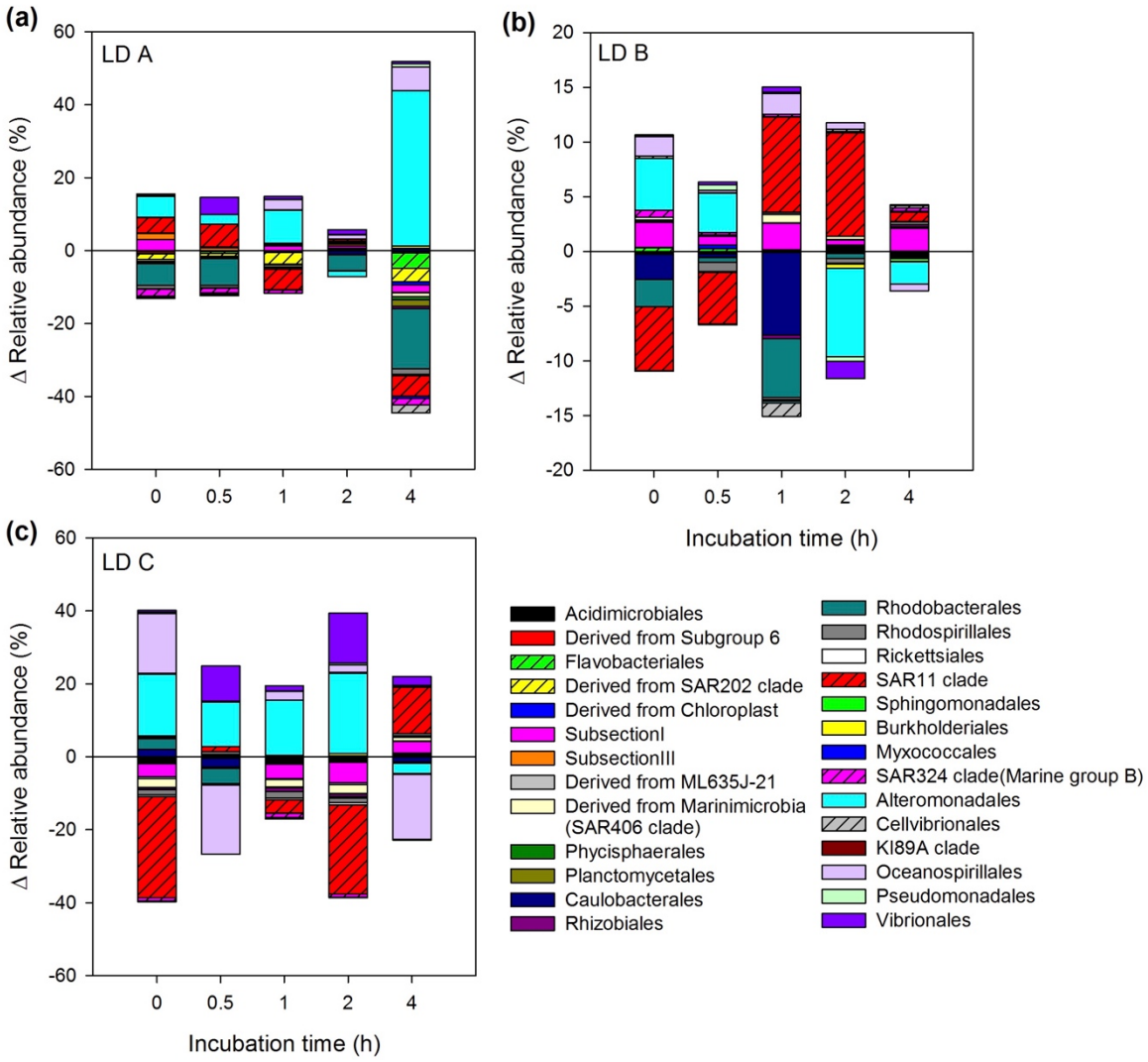

Figure 10: Contribution of active Orders through the incubation based on the SIMPER results. List of the OTUs explaining 50% of the dissimilarity observed through the experiment between treatment with copepods (top) and control (bottom) for each LD experiment: (**a**) LD A, (**b**) LD B and (**c**) LD C.

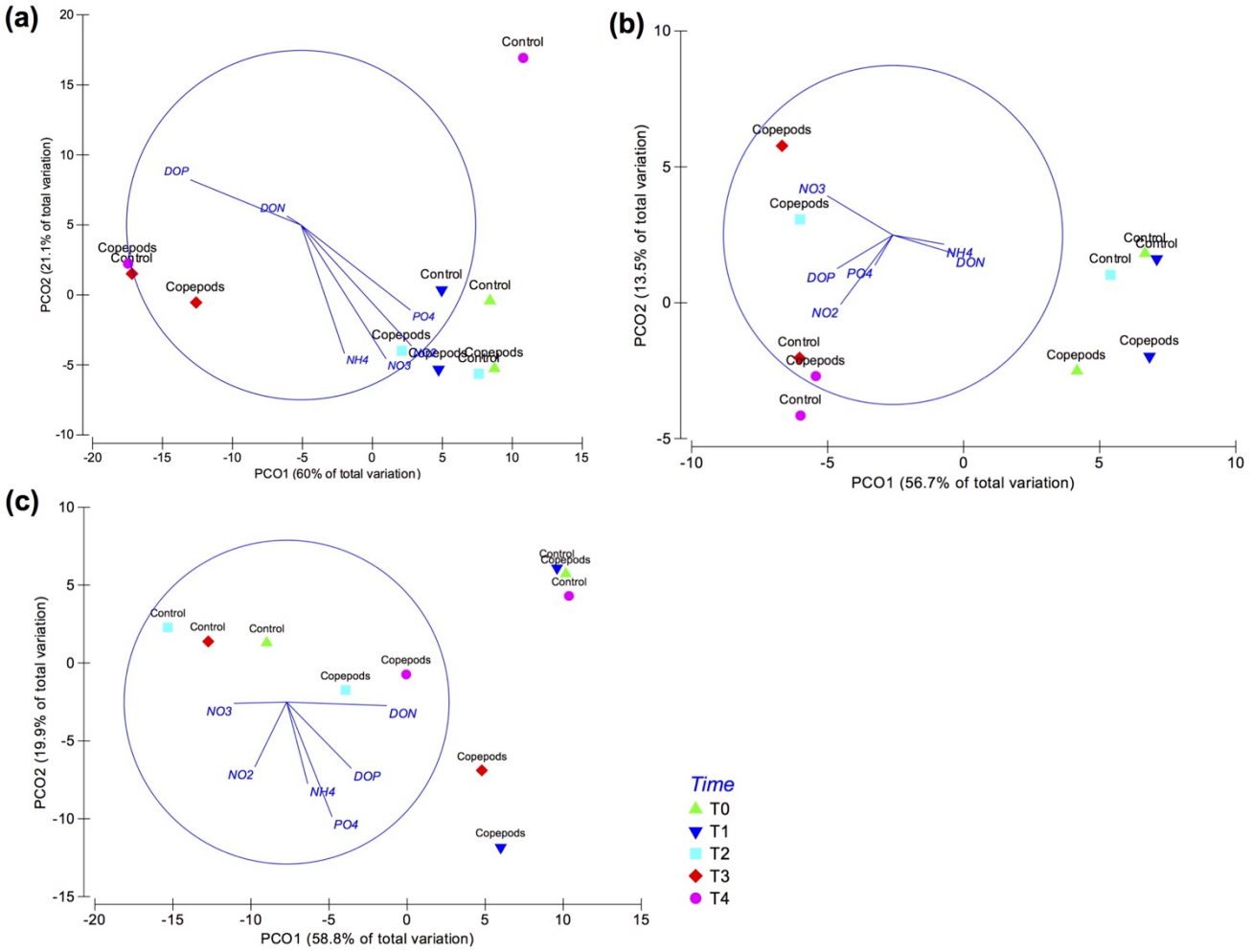

Figure 11: Principal component ordination based on Bray-Curtis similarity at order taxonomic level, of treatment with copepods and control (**a**) LD A, (**b**) LD B and (**c**) LD C. Vectors indicate the best environmental variables (normalized transformed) correlated with ordinations and vector lengths correspond with the correlation values.