Differential response of microbial plankton to nutrient inputs in oligotrophic versus mesotrophic waters of the North Atlantic

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ORIGINAL ARTICLE

Differential response of microbial plankton to nutrient inputs in oligotrophic versus mesotrophic waters of the North Atlantic

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Abstract
The effects of inorganic $(DIN + PO_4^{3-})$ and/or organic (glucose + AAs) inputs on phytoplankton and heterotrophic bacteria were assessed, using a microcosm approach, in two contrasting marine environments: an open ocean oligotrophic site (North Atlantic Subtropical Gyre) and a highly productive coastal embayment (Ría de Vigo, NW Spain). Overall, changes in microbial plankton biomass were smaller than those of metabolic rates. The largest increases in primary production, bacterial production and community respiration were measured in response to mixed $(DIN + PO_4^{3-} + glucose + AAs)$ nutrient additions in both sites. Primary production responded to $DIN + PO_4^{3-}$ additions only in oligotrophic waters. The distinct autotrophic responses to nutrient additions measured in these environments were related to the different initial composition of phytoplankton populations and, presumably, also to differences in grazing pressures in both marine ecosystems. Heterotrophic bacteria were limited by organic substrates in both ecosystems, although mixed additions further enhanced bacterial growth in the subtropical gyre. The differences detected in bacterial responses to nutrient additions may be related to differences in nutrient limitations and to the prevalence of different relationships between components of the microbial food web (e.g. coupling between heterotrophic bacteria and phytoplankton and predation pressure) in both environments. We found a more relevant role of inorganic nutrients in controlling the efficiency of bacterial growth in oligotrophic regions as compared with highly productive systems. Our results suggest that organic matter inputs into both ecosystems might result in a tendency towards heterotrophy and in increases in bacterial growth efficiency.

Key words: *Microbial plankton, nutrients inputs, bacteria, phytoplankton, organic nitrogen, microcosms*

Introduction

Human activities (e.g. fossil fuel combustion, and changes in land use) have considerably increased the quantity of reactive nitrogen (Nr) entering the world's oceans in recent decades (Falkowski et al. 1998; Galloway & Cowling 2002; Mathews 2006; Duce et al. 2008). On a global scale, nitrogen input into marine systems has increased two- to threefold from the 1860s and is expected to further increase over future decades (Galloway et al. 2004).

It has been widely demonstrated that nitrogen (N) controls ecosystem productivity over ecological time scales in coastal (Nixon & Pilson 1983; Oviatt et al. 1995) and open ocean (Graziano et al. 1996;

Mills et al. 2004, 2008; Moore et al. 2008) waters. Enhanced productivity eventually results in an accumulation of organic matter which promotes microbial activity and the consumption of dissolved oxygen (Nixon 1995; Cloern 2001; Diaz & Rosenberg 2008; Gruber & Galloway 2008).

The forms of reactive nitrogen that affect aquatic ecosystems include inorganic dissolved compounds (nitrate, ammonium), a variety of dissolved organic compounds (amino acids, urea, and composite dissolved organic nitrogen) and particulate nitrogen. Microbial plankton populations utilize different forms of nitrogen preferentially, and both the magnitude and the composition of the nitrogen

input may modify the structure and metabolism of microbial plankton communities (Antia et al. 1991; Peierls & Paerl 1997; Seitzinger & Sanders 1999; Bradley et al. 2010).

An important fraction of nitrogen inputs into both open-ocean and coastal waters is organic. About one-third of the total quantity of atmospheric nitrogen entering the world's oceans annually is organic and largely bioavailable (>45%) (Peierls & Paerl 1997; Seitzinger & Sanders 1999). It is also known that about 40–50% of nutrients in fluvial inputs are organic (Meybeck 1993).

Nutrient availability in the upper layers of the global ocean depends on the physical and chemical properties of the water column, which widely change through different environments (Longhurst 2006). The rate of nutrient supply into the upper layer of the ocean largely controls the size of dominant phytoplankton species, thereby modifying the structure of the planktonic food web (e.g. Legendre & Le Févre 1989; Kiørboe 1993) and the associated patterns of organic matter circulation (Legendre & Rassoulzadegan 1996; Calbet & Laundry 2004). When nutrient availability is low, the relative importance of small phytoplankton cells is high, total primary producer biomass and photosynthetic carbon fixation are low, and the microbial food web dominates (Azam et al. 1983; Platt et al. 1983; Sherr & Sherr 2000; Fenchel 2008). Larger phytoplankton cells, which dominate as we move towards more eutrophic conditions, have higher potential for growth when nutrients are available (Thingstad & Sakshaug 1990; Agawin et al. 2000; Cermeño et al. 2005).

As a consequence of the contrasting characteristics of the diverse marine ecosystems, the factors limiting productivity of phytoplankton and heterotrophic bacteria in the ocean are likely to change over a variety of spatial and temporal scales (Cullen et al. 1992; Arrigo 2005; Church 2008; Saito et al. 2008). Hence, the microbial responses to inorganic and organic nutrient inputs and the underlying ecological processes are expected to differ in distinct marine ecosystems. However, only a few studies have addressed the effect of inorganic and organic inputs on autotrophic and heterotrophic communities and little is known about the similarities or differences in the responses of open-ocean and coastal microbial communities to the increasing nutrient enrichment of the ocean. Furthermore, no direct comparison of open-ocean vs. coastal autotrophic and heterotrophic responses to the same inorganic and organic nutrient enrichments has ever been published. The present work has two aims: (1) to achieve a better understanding of microbial communities functioning in the ocean through the analysis of their responses

to nutrient inputs; and (2) to compare the responses to qualitatively similar inorganic nutrients and/or organic substrate inputs of microbial communities characteristic from two different environments (i.e. an open-ocean and a coastal location) subjected to increasing nutrient enrichment in a changing world.

Here, we assess the differential effect of inorganic versus organic nitrogen inputs on autotrophic and heterotrophic microbial communities in two contrasting environments: an oligotrophic open-ocean site and a mesotrophic coastal site. We discuss some methodological constraints associated with the experimental approach that must be taken into consideration when comparing results from bioassays implying different enrichment levels. This comparative study allows us to infer some insights into ecological processes regulating the microbial plankton responses to nitrogen loading in coastal and open-ocean ecosystems.

Materials and methods

Survey areas

Sampling sites (open ocean site: 23.12°N, 26.31°W, November 2006, coastal site: 42.24°N, 8.77°W, February 2008) are shown in Figure 1. Vertical profiles down to 300 and 25 m (open-ocean and Ría stations, respectively) of water column temperature, salinity and in-situ fluorescence were obtained with a SBE 9/11 CTD probe and a Seatech fluorometer attached to a rosette sampler.

The Eastern North Atlantic Subtropical Gyre is characterized by strong thermal stratification and nutrient depletion in the upper mixed layer for most of the year, which translates into very low levels of chlorophyll *a* and primary production (Teira et al. 2006; Marañón et al. 2007). This experiment was performed in the framework of the CARPOS project on board B.O. *Hespérides* in November 2006.

The coastal system of the northwestern Iberian Peninsula is characterized by periodic upwelling (usually from March to September) of cold and nutrient-rich North Atlantic Central Waters (NACW) (Nogueira et al. 1997). The Ría de Vigo (Spain), an embayment located in this upwelling area, is a highly productive and dynamic coastal ecosystem (Cermeño et al. 2006). Three experiments were performed in this ecosystem in different seasons in order to cover the temporal variability of microbial community structure and functioning (Martínez-García et al. 2010a). Similar general biological initial conditions and the same patterns and magnitude of response to the nutrient additions were found in the three experiments (response ratios in the experiment shown here are within the same
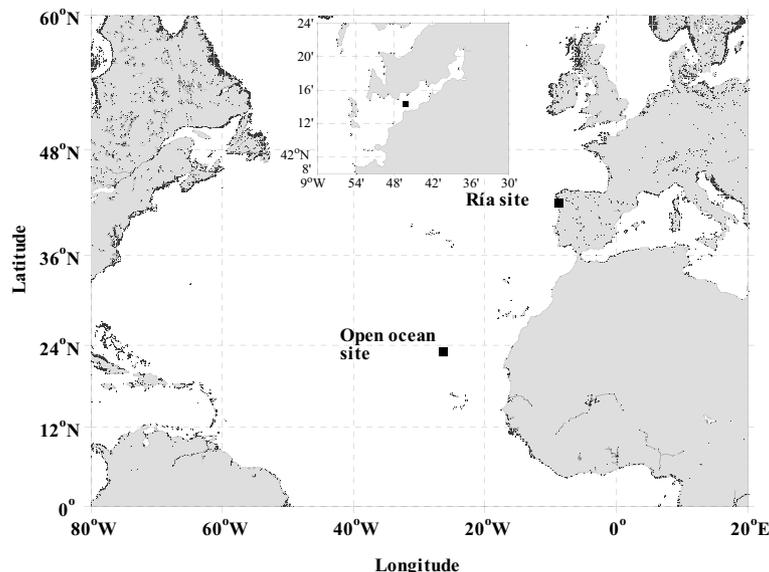
Figure 1. Map of the Atlantic Ocean showing the location of the two sampled stations: in the North Atlantic Subtropical Gyre the open ocean site and in the Iberian margin the Ría site. A detailed map of the embayment in which the Ría site is located has been also included.

range of the other two experiments). In order to simplify the comparison of the two systems, only the experiment performed in winter in the Ría is presented as it was performed in the same season as the one in the open ocean, although under contrasting initial environmental conditions (i.e. water column structure, surface temperature and nutrient concentrations).

Experimental design

Surface seawater samples (5–10 m) were collected in 12- or 15-litre acid-clean Niskin bottles and filtered through 150- and 200-μm pore size meshes (open ocean and Ría stations, respectively) to remove larger zooplankton. Subsequently, 12-litre acid-washed polycarbonate bottles were gently filled under dim light conditions.

Following sample collection, nutrients were added to the 12-litre microcosm bottles. The experimental design included duplicate bottles for a series of four treatments: (1) control treatment – no additions made; (2) inorganic addition treatment ($DIN+ PO_4^{3-}$) – 0.5 and 5 μmol l^{-1} nitrate (NO_3^-), 0.5 and 5 μmol l^{-1} ammonium (NH_4^+), 0.1 and 1 μmol l^{-1} phosphate (HPO_4^{2-}) (open ocean and Ría stations, respectively); (3) organic addition treatment (Glucose + AAs): 1 and 5 μmol l^{-1} glucose and 1 and 5 μmol l^{-1} of an equimolar mixture of 18 amino acids (all protein amino acids, except cysteine and tyrosine) (open ocean and ría stations, respectively); (4) mixed addition treatment: combination of inorganic and organic additions. Glucose and amino acids were included, as they are the organic

labile identified substances more abundant in seawater and both atmospheric and riverine inputs have been reported to contain organic carbon and nitrogen (Meybeck 1993; Jacobson et al. 2000; Jurado et al. 2008). The added inorganic nutrients concentrations were 10-fold higher in the coastal experiment than in the open-ocean experiment, in accordance with the differences in the mean initial nutrient concentrations, standing stocks and metabolic rates between both ecosystems (see Initial conditions, Table I and Figure 2). The magnitude of the additions was chosen to be in excess relative to the mean concentrations measured at the surface waters of each ecosystem (Álvarez-Salgado et al. 1996; Marañón et al. 2001).

In both experiments, temperature was maintained within $\pm 0.1°C$ of in-situ values. An in-door incubation chamber and a temperature-controlled incubation room were used in the open-ocean and coastal experiments, respectively. Bottles were illuminated with cool white light from fluorescent tubes (photoperiod 12L : 12D and average PAR was 240 μE m^{-2} s^{-1}). Experiments lasted 3 days

Table I. Initial conditions for each experiment. Sampling depth was 5–10 m at both sites.

Experiment	Ría	Open ocean
Surface Temperature (°C)	13.5	24.7
Surface Salinity	35.0	37.5
Chl a (μg L^{-1})	0.73	0.18
Surface Nutrients (μmol L^{-1})		
NO_3^-	9.6	0.16
HPO_4^{2-}	1.2	0.02
SiO_2	5.4	<0.5
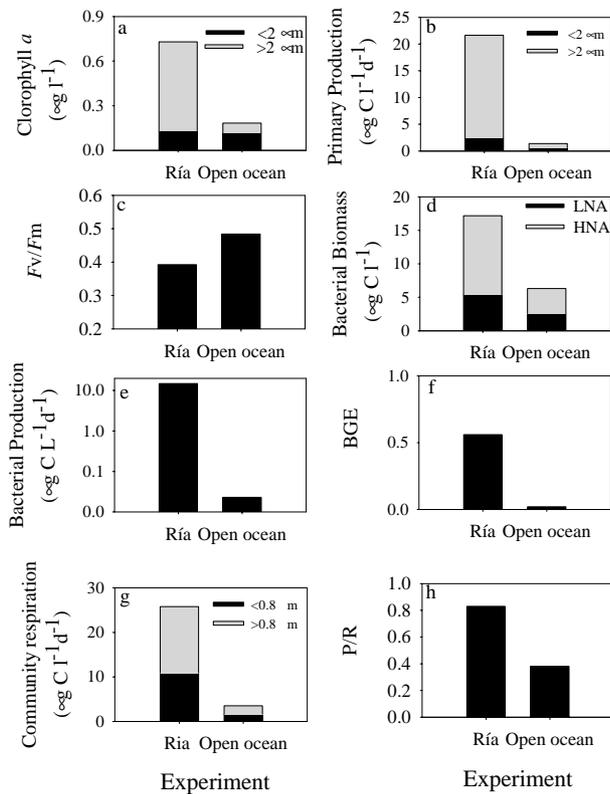
Figure 2. Initial biological conditions at the sampling stations. (a) Size-fractionated chlorophyll a (μg chl $a\,l^{-1}$); (b) size-fractionated primary production (μg C l^{-1} day^{-1}); (c) photosynthetic efficiency (F_v/F_m); (d) heterotrophic bacterial biomass (μg C l^{-1}); (e) bacterial production (μg C l^{-1} day^{-1}); (f) BGE, bacterial growth efficiency; (g) size-fractionated community respiration estimated from in-vivo ETS activity (μg C l^{-1} day^{-1}); (h) P/R, primary production to community respiration ratio.

and samples for size-fractionated chlorophyll a, photosynthetic efficiency, primary production, bacterial heterotrophic production, bacterial and community respiration and flow cytometry were taken every 24 h from the microcosms to monitor changes in microbial community biomass structure and metabolism.

Inorganic and organic nutrients

For the open-ocean experiment the concentration of nitrate and ammonium was determined on-board, on fresh samples with a Technicon segmented-flow auto-analyser and using modified colorimetric protocols that allow a detection level of 2 nmol l^{-1} (Raimbault et al. 1990; Kerouel & Aminot 1997) to be reached. Phosphate concentrations were determined using the standard procedures described in Tréguer & Le Corre (1975). For the Ría experiment, aliquots for inorganic nutrients determination (nitrate, phosphate and silicate) were collected in 50 ml polyethylene bottles and frozen at -20°C until analysis by standard colorimetric methods with

an Alpkem segmented-flow analyser (Hansen & Grasshoff 1983).

Size-fractionated chlorophyll a

Size-fractionated chlorophyll a (chl a) concentrations were measured in 250 and 150 ml water samples (in the open-ocean and the Ría experiments, respectively) which were filtered sequentially through 2- and 0.2-μm polycarbonate filters. After extraction with 90% acetone at 4°C overnight in the dark, chlorophyll a fluorescence was determined, using the non-acidification technique, with a TD-700 Turner Designs fluorometer calibrated with pure chl a.

Photosynthetic efficiency

FRR fluorescence measurements were made using a Chelsea Instruments Fast^{tracka} FRR fluorometer. Fluorescence variables F_0 (initial fluorescence) and F_m (maximal fluorescence) were obtained by fitting the model of Kolber et al. (1998) to the FRR fluorescence using the FRS program. Photosynthetic efficiency (F_v/F_m) was calculated as $F_v/F_m = (F_m - F_0)/F_m$.

Primary production

Four 75-ml acid-cleaned polystyrene bottles (3 light and 1 dark) were filled with seawater and spiked with 555 and 185 kBq (15 and 5 μCi) NaH^{14}CO$_3$ (in the open-ocean and the Ría experiments, respectively). Samples were incubated for 3 and 12 h (in the Ría and the open-ocean experiments, respectively) in the same incubation chamber as the experimental bottles. After the incubation period, samples were sequentially filtered through 2- and 0.2-μm polycarbonate filters under low vacuum pressure (<50 mmHg). Filters were processed to assess ^{14}C incorporation as described in Marañón et al. (2001). The daily primary production rates in the Ría experiments were obtained by applying the photoperiod in the microcosms to the hourly primary production (PP) rates. Gross primary production (GPP) per day, used to compute the PP to community respiration (P/R) ratio, was estimated assuming that (i) phytoplankton respiration amounts to 20% of the carbon fixed during the light period, (ii) the percentage of extracellular release (PER) of phytoplankton is 20% (Marañón et al. 2004).

Bacterial heterotrophic production

The [^{3}H]leucine incorporation method (Kirchman et al. 1985), modified as described by Smith & Azam

(1992), was used to determine Leu incorporation rates (LIR). Samples were incubated for 1 and 2 h (in the Ría and the open-ocean experiments, respectively) in the same incubation chamber as the experimental bottles. Dilution experiments (4 in the Ría and 1 in the open-ocean experiments) were performed in order to determine empirical leucine to carbon conversion factors (CF) for the control, the inorganic, the organic and the mixed nutrient treatment, following the methods detailed elsewhere (Calvo-Díaz & Morán 2009). The CFs derived were: 0.092 kg C mol Leu^{-1} in the open-ocean experiment and 2.6 ± 1.1 kg C mol Leu^{-1} for the control, 1.6 ± 0.6 kg C mol Leu^{-1} for the inorganic treatment, 3.3 ± 0.9 kg C mol Leu^{-1} for the organic treatment and 4.4 ± 0.3 kg C mol Leu^{-1} for the mixed treatment in the Ría experiment. Differences between the CFs measured in open ocean and coastal bacterial communities have been previously reported in several studies (Morán et al. 1999; Pedrós-Alió et al. 1999; Sherr et al. 2001; del Giorgio et al. 2011) and may have been increased by nutrient additions in the present investigation. Bacterial growth efficiency (BGE) was calculated as: BP/(BP + BR), where BP is bacterial productivity and BR is bacterial respiration.

In vivo electron transport system (ETS)

ETS activity rate was used as an estimator of community respiration (CR). Size-fractionated in-vivo ETS activity rates were measured using the in-vivo INT method (Martínez-García et al. 2009), which is based on the reduction of the tetrazolium salt 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyltetrazolium chloride (INT) to INTformazan (INT-F) by ETS dehydrogenase enzymes. Four 100- and 250-ml (in the open-ocean and Ría experiments, respectively) dark bottles were filled from each microcosm bottle. One bottle was immediately fixed by adding formaldehyde (2% w/v final concentration) and used as killed-control. Samples were incubated at the same temperature as the microcosm bottles and in dark conditions for 6 and 1 h (in the open-ocean and Ría experiments, respectively). After incubation, samples were fixed and filtered sequentially through 0.8- and 0.2-μm pore size polycarbonate filters. The reduced INT-F was extracted from the filters using propanol and its concentration determined colorimetrically using an Ultra Violet-2401 PC Shimadzu spectrophotometer. BR was operationally defined as ETS activity < 0.8 μm (Robinson 2008). In order to transform ETS activity in carbon respiration, a R/ETS ratio of 12.8 (Martínez-García et al. 2009) and a respiratory quotient (RQ) of 0.8 (Williams & del Giorgio

2005) were used. Daily ETS activity rates were calculated by multiplying the hourly rates by 24.

Flow cytometry

Samples for heterotrophic bacteria (1.8 ml) were preserved with 1% paraformaldehyde + 0.05% glutaraldehyde and frozen at $-80°C$ until analysis on board (NASG) or in the laboratory, within 6 months of collection, with a FACSCalibur flow cytometry (Becton-Dickinson) equipped with a laser emitting at 488 nm. Prior to analysis, heterotrophic bacteria were stained with 2.5 mM DMSO-diluted Sybr-Green I DNA fluorochrome (Molecular Probes). Two groups of heterotrophic bacteria were distinguished by their green fluorescence (FL1, 530 nm) after SybrGreen staining and side scatter (SSC) signals: high (HNA) and low (LNA) nucleic acid content bacteria.

Empirical calibrations specific to these data sets between flow cytometry light scatter (SSC or forward scatter, FSC) and mean cell size [biovolume (BV) or cell diameter], as explained in Calvo-Díaz & Morán (2006), were used: $BV = 0.06 \times FSC + 0.01$; $r^2 = 0.60$, $n = 13$ in the Ría de Vigo; diameter $= 0.79 \times SSC + 0.47$, $r^2 = 0.60$, $n = 16$ in the open ocean, using the sequential filtration method as described in Zubkov et al. (1998) to estimate bacterial BV.

Heterotrophic bacterial biomass was finally calculated by using the allometric relationship of Gundersen et al. (2002): bacterial biomass (fg C cell^{-1}) $= 108.8 \times BV^{0.898}$ for open-ocean waters and that of Norland (1993): fg C cell$^{-1} = 120 \times BV^{0.72}$ for the Ría de Vigo.

Data analysis

In order to compare the effect of different nutrient additions on the standing stocks and rates, we calculated response ratios ($_{RR}$), by dividing the time-integrated value in the addition treatment by the time-integrated value in the control. In the case of standing stocks and P/R ratio, time-averaged values were used. Values presented in this work were integrated (or averaged in the case of standing stocks) from 0 to 72 h incubation. Different amounts of nutrients were added in the two experiments (open-ocean and coastal ocean, see *Experimental design*).

Methodological constraints

Although nutrients were added well above background concentrations, we cannot guarantee that nutrient concentrations in the experimental bottles

just after nutrients were added were above saturation levels in both open and coastal ocean experiments. We are aware that some previous works comparing addition experiments assume saturation levels of the added nutrients to allow the magnitude of the effects promoted by the additions in different experiments to be compared (Downing et al. 1999; Elser et al. 2007). Nevertheless, we decided not to derive conclusions from potentially underestimated response ratios and therefore avoided direct comparisons of response ratios between experiments except when the variable itself is a ratio between variables (i.e. BGE and P/R ratio).

Statistical analysis

A repeated-measure ANOVA (RMANOVA) was conducted to assess time (within subject factor), and treatment (between subject factor, nutrient additions) effects. A Least Significance Difference (LSD) post-hoc test was conducted to assess the effect of the addition treatments on the microbial parameters. For those data sets that did not fit a normal distribution (Kolmogorov–Smirnov test) a log transformation was applied.

Results

Initial conditions

Initial conditions for both experiments are presented in Table I and Figure 2. The Ría station was sampled during an intense winter mixing period with low surface temperature and high surface nutrient concentrations. Chl a concentration and primary production (PP) rate were 0.7 µg Chl a l^{-1} and 21.6 µg C l^{-1} d^{-1}, respectively (Table I and Figure 2). The phytoplankton community was dominated by >2 µm cells ($>80\%$) and the photosynthetic efficiency (Fv/Fm) was 0.4. Bacterial biomass (BB) was 17.2 µg C l^{-1} and was dominated by HNA bacteria (68%) (Table I and Figure 2). The bacterial production (BP) rate was 14.8 µg C l^{-1} day^{-1} (Table I and Figure 2). Community respiration (CR), estimated from in-vivo ETS activity, was 25.8 µg C l^{-1} day^{-1} and BR (<0.8 µm fraction) represented 42% of CR, rendering a high bacterial growth efficiency (BGE) of 0.56. Initial PP to CR ratio (P/R) was 0.8 (Table I and Figure 2).

As expected, in the open-ocean station, surface temperature was higher than in the Ría station, causing strong thermal stratification and much lower nutrient levels than those in the Ría station (one and two orders of magnitude in the case of nitrate and phosphate, respectively). Chl a concentration and PP rate were 0.18 µg Chl a l^{-1} and 1.3 µg C l^{-1} day^{-1}, respectively (Table I and Figure 2). Small phytoplankton (<2 µm) dominated chl a concentration (63%), whereas >2-µm phytoplankton dominated PP (57%). The Fv/Fm was slightly higher (0.48) than that of coastal phytoplankton (Table I and Figure 2). BB was 6.3 µg C l^{-1} and was also dominated by HNA bacteria (59%) and the BP rate was 0.02 µg C l^{-1} day^{-1}. CR was one order of magnitude lower than in the Ría (4.7 µg C l^{-1} day^{-1}), bacteria contributed to 58% of CR and BGE was very low (0.01). The initial P/R ratio was considerably lower than in the Ría (0.3) (Table I and Figure 2).

Responses of coastal microbial communities to nutrient additions

Although nutrient additions did not have a statistically significant effect on autotrophic biomass (chl a) and production (PP) in the Ría experiment (RMANOVA F-test, $p>0.05$), chl a concentration significantly (LSD post-hoc test, $p<0.05$, Table II) increased at the end of the incubation after mixed additions (Figure 3a).

Primary production responses to the additions in the Ría paralleled those of chl a (Figure 3b). Phytoplankton community size structure did not change after the additions (i.e. PP due to phytoplankton >2 µm dominated before and after the additions) (Figure 3c). An increase in Fv/Fm was observed in all treatments, including the control (we lack Fv/Fm data from some bottles at t = 48 and 72 h due to saturation of the FRR fluorescence in this experiment) (Figure 3d).

A statistically significant effect of nutrient additions on bacterial abundance (BB), production (BP), respiration (BR) and growth efficiency (BGE) was found (RMANOVA F-test, $p<0.01$).

BB significantly (LSD post-hoc test, $p<0.05$, Table II) increased during the first 48 h of incubation after glucose + AAs and mixed additions (Figure 3e). Maximum BP rates were registered after 24 h incubation (Figure 3f). BP significantly (LSD post-hoc test, $p<0.05$, Table II) decreased after DIN + PO_4^{3-} additions, while it significantly (LSD post-hoc test, $p<0.05$, Table II) increased after glucose + AAs and mixed additions (Figure 3f). The magnitude of response of BP was higher than that of BB. BR significantly (LSD post-hoc test, $p<0.05$, Table II) decreased after DIN + PO_4^{3-} additions in the Ría and increased after glucose + AAs and mixed additions (Figure 3g). BGE significantly (LSD post-hoc test, $p<0.05$, Table II) responded during the first incubation day to glucose + AAs and mixed additions (Figure 3h).

Ría   Open Ocean
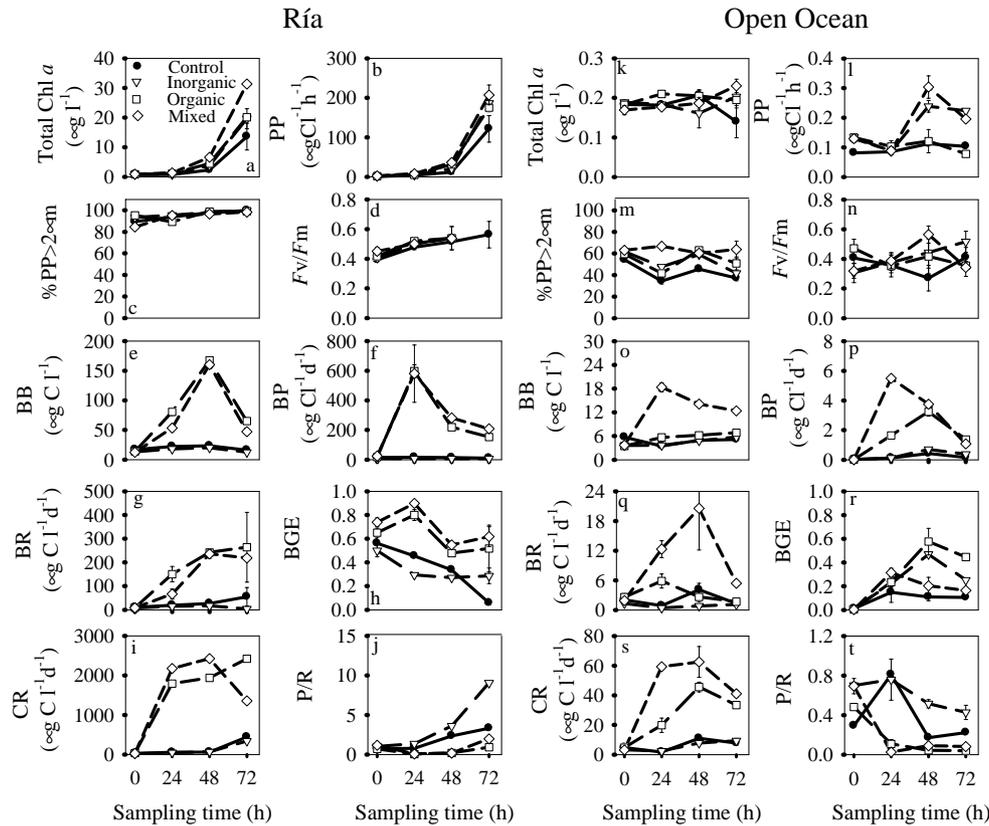
Figure 3. Time course of the mean (a, k) Chl*a*, chlorophyll *a* concentration (µg chl *a* l^{-1}); (b, l) PP, total primary production (µg C l^{-1} h^{-1}); (c, m) %PP >2 µm, percentage of >2 µm primary production; (d, n) *F*v/*F*m, photosynthetic efficiency; (e, o) BB, bacterial biomass (µg C l^{-1}); (f, p) BP, bacterial reduction (µg C l^{-1} day^{-1}); (g, q) BR, ETS activity in the <0.8-µm fraction (µg C l^{-1} day^{-1}); (h, r) BGE, bacterial growth efficiency; (i, s) CR, total ETS activity (µg C l^{-1} day^{-1}); (j, t) P/R, primary production to community respiration ratio, in the Ría and open-ocean experiments, respectively. Control, no addition; Inorganic, inorganic addition; Organic, organic addition; Mixed, mixed addition. We lack *F*v/*F*m data from some bottles at t = 48 and 72 h due to saturation of the FRR fluorescence in the Ría experiment. Note that different scales were used. Error bars represent the standard error from two replicates; where error bars are not visible, they are smaller than the size of the symbol.

Table II. Summary of the effect of the different additions on biological variables (RMANOVA and LSD post-hoc tests): 0, no significant effect; +, significant stimulation $p < 0.05$; −, significant inhibition, $p < 0.05$. Chl *a*, chlorophyll *a* concentration; PP, primary production; %PP >2 µm, contribution of >2 µm to total PP; *F*v/*F*m, photosynthetic efficiency; BB, heterotrophic bacterial biomass; BP, bacterial production; BR, bacterial respiration (BR due to the fraction <0.8 µm); BGE, bacterial growth efficiency; CR, community respiration; P/R, production to respiration ratio.

Variable	Coastal experiment			Open ocean experiment		
	Inorganic	Organic	Mixed	Inorganic	Organic	Mixed
Chl *a*	0	0	+	0	0	0
PP	0	0	+	+	0	+
%PP > 2µm	0	0	0	0	0	+
*F*v/*F*m	0	0	0	0	0	0
BB	0	+	+	0	0	+
BP	−	+	+	0	+	+
BR	−	+	0	−	0	+
BGE	0	+	+	+	+	
CR	0	+	+	0	+	+
P/R	+	−	−	+	−	−

A statistically significant effect of nutrient additions on community respiration (CR) and P/R ratio (P/R = daily GPP/daily CR) was found (RMANOVA F-test, $p < 0.01$). CR significantly (LSD post-hoc test, $p < 0.05$, Table II) increased after glucose + AAs and mixed additions (Figure 3i).

P/R ratios significantly (LSD post-hoc test, $p < 0.05$, Table II) decreased after glucose + AAs and mixed additions and were always significantly (LSD post-hoc test, $p < 0.05$, Table II) higher than the control after DIN + PO$_4^{3-}$ additions (Figure 3j). This can be interpreted as a tendency towards short-term autotrophy after DIN + PO$_4^{3-}$ amendment and towards short-term heterotrophy after glucose + AAs and mixed additions (Figure 3j).

Responses of open-ocean microbial communities to nutrient additions

Nutrient additions did not have a statistically significant effect on autotrophic biomass (chl *a*) and production in the open-ocean experiment (PP)

(RMANOVA F-test, $p > 0.05$). Minor changes in chl a concentration were observed in all treatments (Figure 3k) although significant (LSD post-hoc test, $p < 0.05$, Table II) enhancements of PP rates in the $DIN + PO_4^{3-}$ and mixed treatments were registered (Figure 3l). Significant (LSD post-hoc test, $p < 0.05$, Table II) increases in the relative contribution of phytoplankton > 2 μm to total PP (%PP > 2 μm) were found after mixed additions (Figure 3m). No responses of photosynthetic efficiency (Fv/Fm) to the additions were registered (Figure 3n).

A statistically significant effect of nutrient additions on bacterial abundance (BB), production (BP), respiration (BR) and growth efficiency (BGE) was found in the open-ocean experiment (RMANOVA F-test, $p < 0.01$). Significant (LSD post-hoc test, $p < 0.05$, Table II) increases in BB were only measured after the mixed addition (Figure 3o) and BP significantly increased (LSD post-hoc test, $p < 0.05$, Table II) after glucose + AAs and mixed additions (Figure 3p). The magnitude of response of BP was higher than that of BB.

BR significantly (LSD post-hoc test, $p < 0.05$, Table II) decreased after $DIN + PO_4^{3-}$ additions (Figure 3q). By contrast, BR significantly (LSD post-hoc test, $p < 0.05$, Table II) increased after mixed additions (Figure 3q). BGE significantly (LSD post-hoc test, $p < 0.05$, Table II) increased after $DIN + PO_4^{3-}$ and glucose + AAs additions (Figure 3r).

A statistically significant effect of nutrient additions on community respiration (CR) and P/R ratio (P/R = daily GPP/daily CR) was found in the open-ocean (RMANOVA F-test, $p < 0.01$). CR significantly (LSD post-hoc test, $p < 0.05$, Table II) increased after glucose + AAs and mixed additions (Figure 3s). P/R ratios significantly (LSD post-hoc test, $p < 0.05$, Table II) decreased after glucose + AAs and mixed additions and maintained significantly higher values (LSD post-hoc test, $p < 0.05$, Table II) than the control after $DIN + PO_4^{3-}$ additions (Figure 3t). Consequently, a tendency towards short-term autotrophy after the $DIN + PO_4^{3-}$ treatment and towards short-term heterotrophy after the glucose + AAs and mixed treatments was registered (Figure 3t).

Discussion

Our results indicate that the responses of phytoplankton and heterotrophic bacteria to nutrient additions, both in terms of biomass and metabolism, are different in coastal and oceanic environments. They also suggest that, in general, phytoplankton responses to nutrient amendments are smaller than those of heterotrophic bacteria, irrespective of markedly contrasting initial conditions.
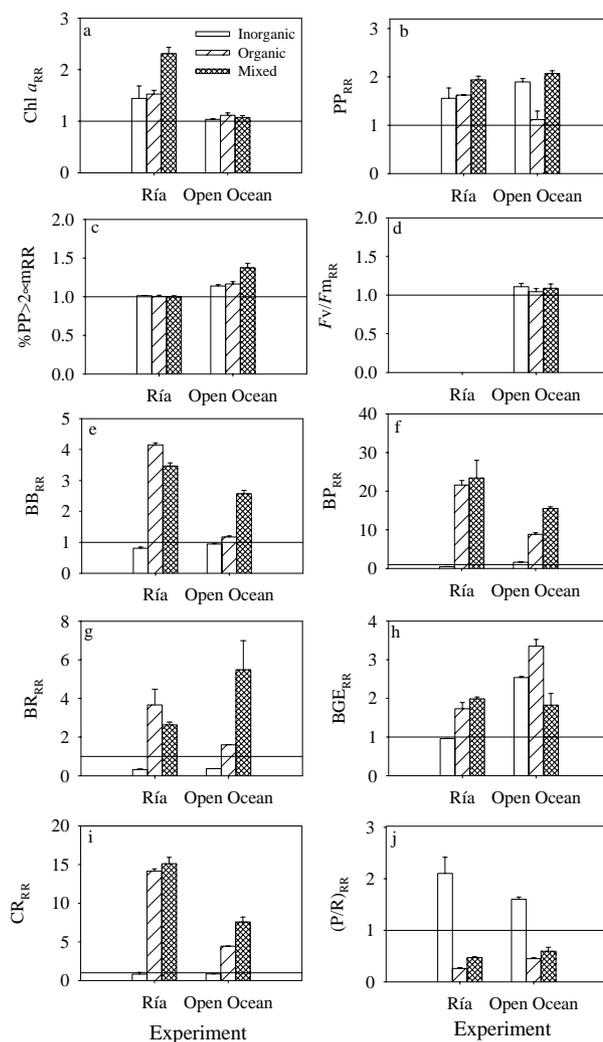
Figure 4. Response ratios of (a) total chlorophyll a concentration (Chl a_{RR}); (b) primary production (PP$_{RR}$); (c) percentage of PP > 2 μm (%PP > 2 μm$_{RR}$); (d) photosynthetic efficiency (FvFm)$_{RR}$; (e) heterotrophic bacteria biomass (BB$_{RR}$); (f) bacterial production (BP$_{RR}$); (g) bacterial respiration (BR$_{RR}$); (h) bacterial growth efficiency (BGE$_{RR}$); (i) community respiration (CR$_{RR}$); (j) primary production to community respiration ratio (P/R$_{RR}$) in microcosms amended with inorganic, organic and mixed nutrients, expressed as a ratio of the time-averaged (or time-integrated) value relative to the time-averaged value (or time-integrated) in the control microcosms. Inorganic, inorganic addition; Organic, organic addition; Mixed, mixed addition. Fv/Fm$_{RR}$ for the Ría experiment were not calculated as we lack Fv/Fm data from some bottles at t = 48 and 72 h due to saturation of the FRR fluorescence in this experiment. Error bars represent the standard error from two replicates. The horizontal line in each graph represents 1 relative to 1 (no change) relative to control. Note that different scales were used.

In the experiment performed in the Ría de Vigo, autotrophic biomass and production were co-limited by $DIN + PO_4^{3-}$ and glucose + AAs (Figure 4a,b). These increases were similar to those registered in similar experiments performed in this area throughout different seasons (Martínez-García et al. 2010a) and might be explained by direct utilization of organic

substrates by the phytoplankton community or by the additional input of inorganic nutrients derived from remineralization by heterotrophic bacteria in the mixed treatments. In contrast, in the open ocean experiment phytoplankton primary production was apparently solely limited by $DIN + PO_4^{3-}$ and no response was observed after the addition of glucose + AAs (Figure 4a,b). Martínez-García et al. (2010b) reported similar responses of primary producers after $DIN + PO_4^{3-}$ and mixed inputs in two similar experiments performed in oligotrophic waters of the central North Atlantic Ocean in the same season. Mills et al. (2004) and Moore et al. (2006, 2008) found higher primary production responses in the subtropical North Atlantic after inorganic (N and P) nutrient additions, possibly due to the higher final concentrations of the nutrients added (twofold higher for N and P). The differences in phytoplankton nutrient limitation in both environments may be related to differences in the phytoplankton community composition, dominated by picoeukaryotes, *Prochlorococcus* and *Synecococcus* in the open-ocean experiment and by diatoms and dinoflagellates in the coastal experiment (data not shown). In this regard, mixotrophy and auxotrophy of coastal phytoplankton communities have been widely reported (Antia et al. 1991; Bronk et al. 2007; Burkholder et al. 2008).

Contrary to our observation in the coastal site, increases in PP were not paralleled by increases in phytoplankton biomass in the open ocean, suggesting substantial grazing pressure at this site (Quevedo & Anadón 2001). Previous addition experiments in oligotrophic areas have related the lack of significant increases in phytoplankton biomass, despite clear increases in primary production after nutrient additions, with top-down processes (Moore et al. 2008; Marañón et al. 2010). In the present work prefiltration (150- and 200-μm pore size mesh in open-ocean and coastal experiments, respectively) of the samples could have released ciliates and heterotrophic flagellates from predators to some extent. In the open-ocean site this may enhance grazing pressure by microbial protists on small phytoplankton and bacteria (Martinez-Garcia et al. 2010b). In contrast, in the coastal experiment a strong grazing control can be ruled out because the phytoplankton community in this experiment was mostly dominated by large phytoplankton, which presumably would not be severely grazed in this 200-μm prefiltered water (Martinez-Garcia et al. 2010a).

The relative contribution of >2-μm phytoplankton cells to PP increased after the additions in the open-ocean experiment (Figure 4c), which was related to the higher storage abilities, photosynthetic efficiencies and maximum potential growth rates of large phytoplankton cells compared to small phytoplankton cells when nutrients concentrations are high (Thingstad & Sakshaug 1990; Agawin et al. 2000; Cermeño et al. 2005). This pattern of response was not evident in the Ría experiment as >2-μm cells initially dominated phytoplankton biomass and production in the coastal experiment (Figure 4c).

Therefore, the distinct autotrophic responses to nutrient additions measured in these environments were related with different composition of phytoplankton populations and also likely with differences in grazing pressures in both marine ecosystems.

Heterotrophic bacteria were limited by organic substrates in both experiments and a secondary limitation by DIN and PO_4^{3-} nutrients was registered in the open-ocean site (Figure 4e,f). The supply of $DIN + PO_4^{3-}$ alone had none and negative effects in BP in the open-ocean experiment and coastal experiments, respectively, suggesting no primary limitation of BP by $DIN + PO_4^{3-}$ in any of the experiments and a probable competition between phytoplankton and bacteria for $DIN + PO_4^{3-}$ in the coastal experiment. Limitation of heterotrophic bacteria biomass and activity by organic carbon in coastal areas is well known (Jacquet et al. 2002; Joint et al. 2002; Davidson et al. 2007). In the open-ocean experiment, the responses of BB and BP were larger in the mixed than in the glucose + AAs treatment, suggesting a secondary limitation by inorganic nutrients: more organic matter was utilized by bacteria in the mixed than in the glucose + AAs treatment, fuelled by the extra $DIN + PO_4^{3-}$ added. The supply of inorganic nutrients has been seen to limit the ability of bacteria to utilize organic matter (Rivkin & Anderson 1997; Thingstad et al. 1997; Gasol et al. 2009; Tanaka et al. 2009). The larger bacterial responses to mixed (including N and P) as compared to glucose + AAs (including N) additions observed in the open-ocean experiment might be explained by two different processes: (a) the previously reported phosphorus limitation in the North Atlantic (Fanning 1992; Mather et al. 2008; Martínez-García et al. 2010b). It is important to note here that because the type of nitrogen substrate added in both treatments is different (i.e. DIN and AAs, and AAs only in the mixed and organic treatments, respectively) caution must be exercised when trying to make conclusions about a single limiting nutrient (i.e. phosphorous). Also (b) the close coupling between heterotrophic bacteria and phytoplankton, i.e. enhanced bacterial growth associated with the release of extra labile photosynthetically produced dissolved organic carbon, increasing offshore (Morán et al. 2002). Therefore, the differences detected in bacterial responses to nutrient additions in the studied environments may be related

to changes in nutrient limitations (i.e. high inorganic nutrient limitation in the oligotrophic site compared to the coastal site) and to the prevalence of different relationships between components of the microbial food web in both environments (i.e. tight coupling between bacteria and phytoplankton in oligotrophic offshore areas). The response of BP was higher than that of BB in glucose + AAs and mixed treatments in both experiments (Figure 4e,f) suggesting an important and similar grazing pressure on bacterioplankton in these two contrasting ecosystems. Previous studies have reported reduced BB relative to BP responses after nutrient additions in oligotrophic and eutrophic environments associated to top-down control processes (Caron et al. 2000). The increase in bacterial predation pressure after glucose + AAs additions has been previously reported and related to enhanced bacterial abundance and cell size that may increase edibility (Alonso-Sáez et al. 2009). BB and BP decreased (response ratios <1) after the $DIN + PO_4^{3-}$ treatment in the Ría experiment (Figure 4e,f). This could be explained by the competition for inorganic nutrients between heterotrophic bacteria and phytoplankton in this experiment.

Organic carbon limitation of BGE in the Ría experiment was shown by marked increases in BGE measured after glucose + AAs and mixed additions but not when adding $DIN + PO_4^{3-}$ alone (Figure 4h). Several authors have also reported negligible effects of inorganic nutrient additions on BGE in coastal systems (Jørgensen et al. 1993; Zweifel et al. 1993; Daneri et al. 1994). In the open-ocean experiment, BGE significantly increased after $DIN + PO_4^{3-}$ additions (Figure 4h), again suggesting a more relevant role of inorganic nutrients in controlling bacterial growth efficiency (Gasol et al. 2009) in oligotrophic versus highly productive systems. On the other hand, the high increase of BGE after glucose + AAs additions in open-ocean areas is probably related to a low quality of the available organic substrates at this site, where the initial BGE was extremely low (0.02; Figure 2). Contrastingly, when mixed additions were performed in the open-ocean experiment no significant increases in BGE were observed as a consequence of the disproportionate increase of BR in the mixed compared to the $DIN + PO_4^{3-}$ and glucose + AAs treatments (Figure 4g). In general, changes in the growth efficiency of heterotrophic bacteria after nutrient additions were more important in the open ocean as compared to the coastal experiment (Figure 4h), as initial oligotrophic populations characterized by low growth efficiency greatly responded to the availability of new nutrients. This finding suggests a higher capacity of starved bacteria

from oligotrophic environments to increase their growth efficiency in response to nutrient inputs compared to coastal bacteria. A higher BGE implies that a higher amount of the carbon processed by bacteria is transformed into biomass, which translates into a higher carbon flow towards higher trophic levels, which may be exported to subsurface waters (Azam et al. 1983; del Giorgio & Cole 2000; Ducklow 2000).

The response of CR to the glucose + AAs and mixed treatments exceeded that of PP in both experiments (Figure 4b,i). This result has been repeatedly observed in similar nutrient addition experiments performed in both areas (Martínez-García et al. 2010a,b). This leads to decreases in the photosynthesis to respiration ratio of microbial planktonic communities after glucose + AAs input in both systems while a tendency towards autotrophy was found after $DIN + PO_4^{3-}$ inputs in both experiments (Figure 4j). It could be expected that the effect of nutrient enrichment on the net metabolism of the planktonic microbial communities in both experiments would differ according to differences in the initial metabolic balance, the phytoplankton nutrient uptake efficiency and the phytoplankton population size structure, among other factors. However, the metabolic balance of these completely different microbial communities showed similar tendencies following the nutrient additions.

In conclusion, the response of phytoplankton and bacteria to $DIN + PO_4^{3-}$ and/or glucose + AAs additions appears to depend not only on the nutrient availability but also on the composition and functioning of microbial food webs. Differences in initial conditions have been shown to shape the responses that could be summarized as: (1) based on the different magnitudes of response of biomass and production, predation pressure over heterotrophic bacteria was inferred to be similar in both systems whereas grazing pressure over phytoplankton appears to be relevant only in the open-ocean experiment; (2) the qualitative differences in phytoplankton nutrient limitation (i.e. inorganic nutrients and/or organic substrates limitation) in both environments may be related to the composition of the contrasting phytoplankton communities and their different nutritional requirements; (3) bacterial responses to nutrient additions appear to be related to a different magnitude of the coupling between bacteria and phytoplankton and the more important inorganic limitation in the open ocean compared to coastal ocean; and (4) surprisingly, despite the different microbial responses and the distinct underlying ecological processes shown to control these responses in the two contrasting marine environments studied, the response pattern

of the metabolic balance to inorganic and/or organic enrichment was similar in both environments.

Acknowledgements

References

Agawin NSR, Duarte CM, Agustí S. 2000. Nutrient and temperature control of the contribution of picoplankton to phytoplankton biomass and production. Limnology and Oceanography 45:591–600.

Álvarez-Salgado XA, Rosón G, Pérez FF, Figueiras FG, Pazos Y. 1996. Nitrogen cycling in an estuarine upwelling system, the Ría de Arousa (NW Spain). I. Short-time-scale patterns of hydrodynamic and biogeochemical circulation. Marine Ecology Progress Series 135:259–73.

Alonso-Sáez L, Unanue M, Latatu A, Azua I, Ayo B, Artolozaga I, et al. 2009. Changes in marine prokaryotic community induced by varying types of dissolved organic matter and subsequent grazing pressure. Journal of Plankton Research 31:1373–83.

Antia NJ, Harrison PJ, Oliveira L. 1991. The role of dissolved organic nitrogen in phytoplankton nutrition, cell biology and ecology. Phycologia 30:1–89.

Arrigo KR. 2005. Marine microorganisms and global nutrient-cycles. Nature 437:349–55.

Azam F, Fenchel T, Field JG, Gray JS, Meyer-Rail LA, Thingstad F. 1983. The ecological role of water-column microbes in the sea. Marine Ecology Progress Series 10:257–63.

Bradley PB, Sanderson MP, Fischer ME, Brofft J, Booth MG, Kerhof LJ, et al. 2010. Inorganic and organic nitrogen uptake by phytoplankton and heterotrophic bacteria in the stratified Mid-Atlantic Bight. Estuarine, Coastal and Shelf Science 88:429–41.

Bronk DA, See JH, Bradley P, Killberg L. 2007. DON as a source of bioavailable nitrogen for phytoplankton. Biogeosciences 4:283–96.

Burkholder JM, Glibert PM, Skelton HM. 2008. Mixotrophy, a major mode of nutrition for harmful algal species in eutrophic waters. Harmful Algae 8:77–93.

Calbet A, Laundry MR. 2004. Phytoplankton growth, microzooplankton grazing, and carbon cycling in marine systems. Limnology and Oceangraphy 49:51–57.

Calvo-Díaz A, Morán XAG. 2006. Seasonal dynamics of picoplankton in shelf waters of the southern Bay of Biscay. Aquatic Microbial Ecology 42:159–74.

Calvo-Díaz A, Morán XAG. 2009. Empirical leucine-to-carbon conversion factors for estimating heterotrophic bacterial production: Seasonality and predictability in a temperate coastal ecosystem. Applied Environmental Microbiology 75:3216–21.

Caron DA, Lim EL, Sanders RW, Dennett MR, Berninger UG. 2000. Responses of bacterioplankton and phytoplankton to organic carbon and inorganic nutrient additions in contrasting oceanic ecosystems. Aquatic Microbial Ecology 22:176–84.

Cermeño P, Marañón E, Pérez V, Serret P, Fernández E, Castro CG. 2006. Phytoplankton size structure and primary production in a highly dynamic coastal ecosystem (Ría de Vigo, NW Spain): Seasonal and short-time scale variability. Estuarine Coastal and Shelf Science 67:251–66.

Cermeño P, Marañón E, Rodríguez J, Fernández E. 2005. Large-sized phytoplankton sustain higher carbon-specific photosynthesis than smaller cells in a coastal eutrophic ecosystem. Marine Ecology Progress Series 297:51–60.

Church MJ. 2008. Resource control of bacterial dynamics in the sea. In: Kirchman DL, editor. Microbial Ecology of the Oceans (2nd edition). NY: Wiley-Liss p 335–82.

Cloern JE. 2001. Our evolving conceptual model of the coastal eutrophication problem. Marine Ecology Progress Series 210:223–53.

Cullen JJ, Yang X, Macintyre HL. 1992. Nutrient limitation of marine photosynthesis. In: Falkowski PG, Woodhead A, editors. Primary Productivity and Biogeochemical Cycles in the Sea. NY: Plenum, p 69–88.

Daneri G, Riemann B, Williams PJleB. 1994. In situ bacterial production and growth yield measured by thymidine, leucine and fractionated dark oxygen uptake. Journal of Plankton Research 16:105–13.

Davidson K, Gilpin LC, Hart MC, Foilland E, Mitchell E, Álvarez-Calleja I, Laurent C, et al. 2007. The influence of the balance of inorganic and organic nitrogen on the trophic dynamics of microbial food webs. Limnology and Oceanography 52:2147–63.

del Giorgio PA, Cole JJ. 2000. Bacterial energetics and growth efficiency. In: Kirchman DL, editor. Microbial Ecology of the Oceans (1st edition). NY: Wiley-Liss p 289–326.

del Giorgio PA, Condon R, Bouvier T, Longnecker K, Bouvier C, Sherr E, et al. 2011. Coherent patterns in bacterial growth, growth efficiency, and leucine metabolism along a northeastern Pacific inshore-offshore transect. Limnology and Oceanography 56:1–16.

Díaz RJ, Rosenberg R. 2008. Spreading dead zones and consequences for marine ecosystems. Science 321:926–29.

Downing JA, Osenberg CW, Sarnelle O. 1999. Meta-analysis of marine nutrient-enrichment experiments: Variation in the magnitude of nutrient limitation. Ecology 80:1157–67.

Duce RA, LaRoche J, Altieri K, Arrigo KR, Baker AR, Capone DG, et al. 2008. Impacts of atmospheric anthropogenic nitrogen on the open ocean. Science 320:893–97.

Ducklow H. 2000. Bacterial production and biomass in the oceans. In: Kirchman DL, editor. Microbial Ecology of the Oceans (1st edition). NY: Wiley-Liss p 85–120.

Elser JJ, Bracken MES, Cleland EE, Gruner DS, Harpole WS, Hillebrand H, et al. 2007. Global analysis of nitrogen and phosphorous limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecology Letters 10:1135–42.

Falkowski PG, Barber TR, Smetacek V. 1998. Biogeochemiccal controls and feedbacks on ocean primary production. Science 281:200–06.

Fanning KA. 1992. Nutrient provinces in the sea: Concentration ratios, reaction rate ratios, and ideal covariation. Journal of Geophysical Research 97:5693–712.

Fenchel T. 2008. The microbial loop – 25 years later. Journal of Experimental Marine Biology and Ecology 366:99–103.

Galloway JN, Cowling EB. 2002. Reactive nitrogen and the world: 200 years of change. Ambio 3:64–71.

Galloway JN, Dentener FJ, Capone DG, Boyer EW, Howarth RW, Seitzinger SP, et al. 2004. Nitrogen cycles: Past, present, and future. Biogeochemistry 70:153–226.

Gasol JM, Vázquez-Domínguez E, Vaqué D, Agustí S, Duarte CM. 2009. Bacterial activity and diffusive nutrient supply in the oligotrophic Central Atlantic Ocean. Aquatic Microbial Ecology 56:1–12.

Gruber N, Galloway JN. 2008. An Earth-system perspective of the global nitrogen cycle. Nature 451:293–96.

Gundersen K, Heldal M, Norland S, Purdie DA, Knap AN. 2002. Elemental C, N, and P cell content of individual bacteria collected at the Bermuda Atlantic Time-series Study (BATS) site. Limnology and Oceanography 47:1525–30.

Hansen HP, Grasshoff K. 1983. Automated chemical analysis. In: Grasshoff K, Ehrhardt M, Kermling K, editors. Methods of Seawater Analysis (2nd edition). Weinheim: Verlag Chemie, p 347–95.

Jacobson MC, Hansson HC, Noone KJ, Charlson RJ. 2000. Organic atmospheric aerosols: Review and state of the science. Reviews of Geophysics 38:267–94.

Jacquet S, Havskum H, Thingstad TF, Vaulot D. 2002. Effects of inorganic and organic nutrient addition on a coastal microbial community (Isefjord, Denmark). Marine Ecology Progress Series 228:3–14.

Joint I, Henriksen P, Fonnes GA, Bourne D, Thingstad TF, Riemann B. 2002. Competition for inorganic nutrients between phytoplankton and bacterioplankton in nutrient manipulated mesocosms. Aquatic Microbial Ecology 29:145–59.

Jørgensen NOG, Kroer N, Coffin RB, Hoch MP. 1993. Dissolved free amino acids, combined amino acids, and DNA sources of carbon and nitrogen to marine bacteria. Marine Ecology Progress Series 98:135–48.

Jurado E, Dachs J, Duarte CM, Simó R. 2008. Atmospheric deposition of organic and black carbon to the global oceans. Atmospheric Environment 4:7931–39.

Kerouel R, Aminot A. 1997. Fluorometric determination of ammonia in sea and estuarine waters by direct segmented flow analysis. Marine Chemistry 57:265–75.

Kiørboe T. 1993. Turbulence, phytoplankton cell size and the structure of pelagic food webs. Advances in Marine Biology 29:1–72.

Kirchman D, Knees E, Hodson R. 1985. Leucine incorporation and its potential as a measure of protein synthesis by bacteria in natural aquatic systems. Applied Environmental Microbiology 49:599–607.

Kolber Z, Prasil O, Falkowski PG. 1998. Measurements of variable chlorophyll fluorescence using fast repetition rate techniques: Defining methodology and experimental protocols. Biochimica et Biophysica Acta 1367:88–106.

Legendre L, Le Fèvre J. 1989. Hydrodynamical singularities as controls of recycled versus export production in the oceans. In: Berger WH, Smetacek VS, Wefer G, editors. Productivity of the Ocean: Present and Past. Chichester: Wiley, p 49–63.

Legendre L, Rassoulzadegan F. 1996. Food-web mediated export of biogenic carbon in oceans: Hydrodynamic control. Marine Ecology Progress Series 145:179–93.

Longhurst A. 2006. Ecological Geography of the Sea (2nd edition). San Diego CA: Academic Press, 397 pages.

Marañón E, Cermeño E, Fernández E, Rodríguez J, Zabala L. 2004. Significance and mechanisms of photosynthetic production of dissolved organic carbon in a coastal eutrophic ecosystem. Limnology and Oceanography 49:1652–66.

Marañón E, Holligan PM, Barciela R, González N, Mouriño B, Pazó MJ, et al. 2001. Patterns of phytoplankton size structure and productivity in contrasting open-ocean environments. Marine Ecology Progress Series 216:43–56.

Marañón E, Fernández A, Huete-Ortega M, Mouriño-Carballido B, Cermeño P, Chouciño P, et al. 2010. The degree of oligotrophy controls the response of microbial plankton to Saharan dust. Limnology and Oceanography 55:2339–52.

Marañón E, Pérez V, Fernández E, Anadón R, Bode A, González N, et al. 2007. Planktonic carbon budget in the Eastern subtropical North Atlantic. Aquatic Microbial Ecology 48:261–75.

Martínez-García S, Fernández E, Álvarez-Salgado XA, González J, Lønborg C, Marañón E, et al. 2010a. Differential responses of phytoplankton and heterotrophic bacteria to organic and inorganic nutrient additions in coastal waters off the NW Iberian peninsula. Marine Ecology Progress Series 416:17–33.

Martínez-García S, Fernández E, Aranguren-Gassis M, Teira E. 2009. In vivo electron transport system activity: A method to estimate respiration in marine microbial planktonic communities. Limnology and Oceanography: Methods 7: 459–69.

Martínez-García S, Fernández E, Calvo-Díaz A, Marañón E, Morán XAG, Teira E. 2010b. Response of heterotrophic and autotrophic microbial plankton to inorganic and organic inputs along a latitudinal transect in the Atlantic Ocean. Biogeosciences 7:1701–13.

Mather RL, Reynolds SE, Wolff GA, Williams RG, Torres-Valdés S, Woodward EMS, et al. 2008. Phosphorus cycling in the North and South Atlantic Ocean subtropical gyres. Nature Geoscience 1:439–43.

Mathews D. 2006. The water cycle freshens up. Nature 439: 793–94.

Meybeck M. 1993. C, N, P and S in rivers: from sources to global inputs. In: Wollast R, Mackenzie FT, Chou L, editors. Interactions of C, N, P and S. Biogeochemical Cycles and Global Change. Berlin: Springer-Verlag, p 163–94.

Mills MM, Moore CM, Langlois R, Milne A, Achterberg E, Nachtigall K, et al. 2008. Nitrogen and phosphorus co-limitation of bacterial productivity and growth in the oligotrophic subtropical North Atlantic. Limnology and Oceanography 53:824–34.

Mills MM, Ridame C, Davey M, La Roche J, Geider RJ. 2004. Iron and phosphorus co-limit nitrogen fixation in the eastern tropical North Atlantic. Nature 429:292–94.

Moore CM, Mills MM, Langlois R, Milne A, Achterberg EP, La Roche J, et al. 2008. Relative influence of nitrogen and phosphorus availability on phytoplankton physiology and productivity in the oligotrophic sub-tropical North Atlantic Ocean. Limnology and Oceanography 53:291–305.

Moore CM, Mills MM, Milnes A, Langlois R, Achterberg EP, Lochte K, et al. 2006. Iron limits primary productivity during spring bloom development in the central North Atlantic. Global Change Biology 12:626–34.

Morán XAG, Estrada M, Gasol JM, Pedrós-Alió C. 2002a. Dissolved primary production and the strength of phytoplankton–bacterioplankton coupling in contrasting marine regions. Microbial Ecology 44:217–23.

Morán XAG, Gasol JM, Pedrós-Alió C, Estrada M. 2002b. Partitioning of phytoplanktonic organic carbon production and bacterial production along a coastal-offshore gradient in the NE Atlantic during different hydrographic regimes. Aquatic Microbial Ecology 29:238–52.

Nixon SW. 1995. Coastal marine eutrophication: A definition, social causes and future concerns. Ophelia 41:199–219.

Nixon SW, Pilson MEQ. 1983. Nitrogen in estuarine and coastal marine ecosystems. In: Carpenter EJ, Capone DG, editors. Nitrogen in the Marine Environment. NY: Academic Press, p 565–648.

Nogueira E, Pérezand FF, Ríos AF. 1997. Seasonal patterns and long-term trends in an estuarine upwelling ecosystem (Ría de Vigo, NW Spain). Estuarine Coastal and Shelf Science 44: 285–300.

Norland S. 1993. The relationship between biomass and volume of bacteria. In: Kemp PF, Sherr BF, Sherr EB, Cole JJ, editors. Handboook of Methods in Aquatic Microbial Bcology. Boca Raton, FL: Lewis Publishers, p 303–07.

Oviatt C, Doering P, Nowicki B, Reed L, Cole J, Frithsen J. 1995. An ecosystem level experiment on nutrient limitation in temperate coastal marine environments. Marine Ecology Progress Series 116:171–79.

Pedrós-Alió C, Calderón-Paz JI, Guixa-Boixereu N, Estrada M, Gasol JM. 1999. Bacterioplankton and phytoplankton biomass production during summer stratification in the northwestern Mediterranean Sea. Deep Sea Research I 46:985–1019.

Peierls BL, Paerl HW. 1997. Bioavailability of atmospheric organic nitrogen deposition to coastal phytoplankton. Limnology and Oceanography 42:1819–23.

Platt T, Rao DVS, Irwin B. 1983. Photosynthesis of picoplankton in the oligotrophic ocean. Nature 301:702–04.

Quevedo M, Anadón R. 2001. Protist control of phytoplankton growth in the subtropical north-east Atlantic. Marine Ecology Progress Series 221:29–38.

Raimbault P, Slawyk G, Coste B, Fry J. 1990. Feasibility of using an automated colorimetric procedure for the determination of seawater nitrate in the 0 to 100 nm range – Examples from field and culture. Marine Biology 104:347–51.

Rivkin RB, Anderson MR. 1997. Inorganic nutrient limitation of oceanic bacterioplankton. Limnology and Oceanography 42: 730–40.

Robinson C. 2008. Heterotrophic bacterial respiration. In: Kirchman DL, editor. Microbial Ecology of the Oceans (2nd edition). NY: Wiley-Liss p 299–336.

Saito MA, Goepfert TJ, Ritt JT. 2008. Some thoughts on the concept of colimitation: Three definitions and the importance of bioavailability. Limnology and Oceanography 53:276–90.

Seitzinguer SP, Sanders RW. 1999. Atmospheric inputs of dissolved organic nitrogen estimulate estuarine bacteria and phytoplankton. Limnology and Oceanography 44:721–30.

Sherr E, Sherr B. 2000. Marine microbes: An overview. In: Kirchman DL, editor. Microbial Ecology of the Oceans (1st edition). NY: Wiley-Liss p 13–46.

Sherr B, Sherr E, Cowles TJ. 2001. Mesoscale variability in bacterial activity in the Northeast Pacific Ocean off Oregon, USA. Aquatic Microbial Ecology 25:21–30.

Smith DC, Azam F. 1992. A simple, economical method for measuring bacterial protein synthesis rates in seawater using 3H-leucine. Marine Microbial Food Webs 6:107–14.

Tanaka T, Thingstad TF, Gasol JM, Cardelús C, Jezberra J, Sala MM, et al. 2009. Determining the availability of phosphate and glucose for bacteria in P-limited mesocosms of NW Mediterranean surface waters. Aquatic Microbial Ecology 56:81–91.

Teira E, Mouriño B, Marañón E, Pérez V, Pazó MJ, Serret P, et al. 2006. Variability of chlorophyll and primary production in the Eastern North Atlantic Subtropical Gyre: Potential factors affecting phytoplankton activity. Deep Sea Research I 52: 569–88.

Thingstad TF, Hagström A, Rassoulzadegan F. 1997. Accumulation of degradable DOC in surface waters: Is it caused by a malfunctioning microbial loop? Limnology and Oceanography 42:398–404.

Thingstad TF, Sakahaug E. 1990. Control of phytoplankton growth in nutrient recycling ecosystems. Theory and terminology. Marine Ecology Progress Series 63:261–72.

Tréguer P, Le Corre P. 1975. Manuel d'analyse des sels nutritifs dans l'eau de mer. Utilisation de l'auto analyseur II, Technicon. Brest: L.O.C. Univ. Bretagne Occidentale, p 59.

Williams PJleB, del Giorgio PA. 2005. Respiration in aquatic ecosystems: History and background. In: del Giorgio PA, Williams PJleB, editors. Respiration in Aquatic Ecosystems. NY: Oxford University Press, p 1–18.

Zubkov MV, Sleigh MA, Tarran GA, Burkill PH, Leakey RJG. 1998. Picoplanktonic community structure on an Atlantic transect from 50°N to 50°S. Deep Sea Research I 45: 1339–55.

Zweifel UL, Norrman B, Hagström A. 1993. Consumption of dissolved organic carbon by marine bacteria and demand for inorganic nutrients. Marine Ecology Progress Series 101: 23–32.

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