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The effects of nutrient additions on particulate and dissolved primary production in surface waters of three Mediterranean eddies

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Abstract

The effects of additions of nitrogen (+N), phosphorus (+P), alone and in combination, were assessed during three microcosm experiments performed with surface waters of three anticyclonic eddies, located in the Western, Central and Eastern Mediterranean. We examined the effects of nutrient additions on rates of dissolved and particulate primary production and on metabolic rates of the osmotrophic community (phytoplankton and heterotrophic prokaryotes). The experiments were performed in June/July 2008 during the BOUM (Biogeochemistry from the Oligotrophic to the Ultra-oligotrophic Mediterranean) cruise. In all three experiments, particulate primary production was significantly stimulated by the additions of nitrogen (+N, +NP) while no effect was observed with the addition of phosphorus alone. Percent extracellular release (PER) showed an inverse relation with total primary production (PP_{total}), displaying the lowest values (4–8%) in the +NP treatment. Among the three treatments, the +NP had the strongest effect on the community metabolic rates leading to positive net community production values (NCP>0). These changes of NCP were mainly due to enhanced gross community production (GCP) rather than lower respiration rates (CR). In +NP treatments autotrophic production (whether expressed as GCP or PP_{total}) was high enough to fulfil the carbon requirements of the heterotrophic prokaryotes, with phytoplankton and heterotrophic prokaryote production positively correlated. Addition of nitrogen alone (+N) had a smaller effect on community production, resulting in metabolically balanced systems (NCP≈0). Finally, heterotrophic conditions persisted in the +P treatment at the central and eastern stations, and gross production was not sufficient to supply bacterial carbon demand, evidence of a decoupling of phytoplankton production and consumption by heterotrophic prokaryotes.

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1 Introduction

In the ocean, the bulk of organic matter produced by photosynthesis is remineralised through respiration (del Giorgio and Duarte, 2002). The amount respired relative to the amount produced describes the net metabolism of the ecosystem. Net community production (NCP) is then the balance between gross community production (GCP), or simply gross production, and community respiration (CR). When NCP>0, more organic carbon is produced than respired, so the ecosystem is in a state of net autotrophy. When NCP<0, the ecosystem is heterotrophic, in situ respiration exceeds in situ carbon fixation.

Planktonic microbes, particularly heterotrophic prokaryotes, are responsible for most respiration in the water column, especially in the least productive areas (Williams, 1981; Lemée et al., 2002; Gonzalez et al., 2003). Respiration of heterotrophic microbes, the sum of maintenance, growth, and reproduction costs, is supported by the uptake of dissolved organic carbon. A variety of mechanisms within planktonic food webs produce dissolved organic matter, such as phytoplankton exudation and lysis or grazing and release by zooplankton and microzooplankton, viral lysis of heterotrophic prokaryotes, etc. (Jumars et al., 1989; Nagata, 2008).

The dissolved component of primary production (PPd) can represent a significant amount of total primary production (Marañón et al., 2004; Morán and Estrada, 2001,2002) though it is often neglected in primary production measurements which typically estimate only particulate primary production. The portion of total primary production which is excreted as PPd is termed the Percentage of Extracellular Release (PER) and varies greatly depending on environmental conditions. For example, PER increases under conditions of nutrient depletion and/or under low irradiances (Carlson, 2002; Nagata, 2008; Morán et al., 2002).

It is now generally recognized that heterotrophic prokaryotes compete with phytoplankton for mineral nutrients when the heterotrophs are not limited by concentrations of labile organic carbon compounds. Dissolved primary production (PPd) furnishes

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a labile, easily assimilated carbon source for heterotrophic prokaryotes and production is increased under conditions of mineral nutrient limitation (Carlson, 2002; Nagata, 2008). Thus, under conditions of nutrient limitation, phytoplankton produce, in the form of PPd, substrate for heterotrophic prokaryotes whose growth is then potentially limited by the same mineral nutrient restricting phytoplankton growth. As the uptake of organic matter by heterotrophic prokaryotes forms a major carbon-flow pathway, factors controlling the uptake and its variability can dominate overall carbon fluxes and determine the metabolic status of a system (Thingstad and Rassoulgadegan, 1995).

The coupling between phytoplankton and heterotrophic prokaryotes can be explored through a carbon budget relating the total (particulate and dissolved) organic matter produced by photosynthesis and the amount of carbon consumed by heterotrophic osmotroph assemblages, the Bacterial Carbon Demand (BCD). If the ratio of BCD to total primary production is >1 then the autotrophic production of the system is insufficient to support the carbon requirements of the heterotrophic osmotroph assemblages, evidence of spatio-temporal uncoupling between primary production and heterotrophic consumption (Williams et al., 2004; Maixandeau et al., 2005).

Primary production in the Mediterranean Sea, especially in surface waters, is often limited by the availability of the macronutrients nitrogen (N) and phosphorus (P) (Krom et al., 1991; Thingstad and Rassoulgadegan, 1995; Krom et al., 2010). The Mediterranean is probably one of the most oligotrophic seas known and characterized by a west-east increasing oligotrophy gradient in terms of mineral nutrients, biomass and productivity (Krom et al., 1991; Moutin and Raimbault, 2002; Pujo-Pay et al., 2010; Siokou-Frangou et al., 2010). Circulation in the Mediterranean Sea is essentially constrained alongslope, being markedly unstable and generating cyclonic and anticyclonic eddies (Millot, 1999; Hamad et al., 2005). These permanent or semi-permanent subbasin eddies are stable mesoscale features with a lifetime measured in years that transfer, along their drifting motion, waters far from the place of their original formation (Puillat et al., 2002).

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In the Mediterranean Sea, heterotrophic prokaryotes were found to be P-limited the east (Zohary and Robarts, 1998; Van Wambeke et al., 2002) or during the stratification period in the west (Thingstad et al., 1998; Alonso-Saez et al., 2008). The CYCLOPS experiment performed recently in the core of the anticyclonic Cyprus eddy in the eastern Mediterranean during the stratified period, provided indications of pure P-limitation for heterotrophic prokaryotes but N and P co-limitation for autotrophic phytoplankton (Thingstad et al., 2005; Zohary et al., 2005). These findings highlighted the complex interrelations of the limiting character of the major macronutrients, both in space and time.

Here we report data on the effects of nutrient enrichment on the communities of three distinct anticyclonic eddies in the different Mediterranean basins. The general objective of these experiments was to define how the structure of the pelagic microbial food web responds to enrichment of the most limiting nutrient (Tanaka et al., 2010). In the present work, our aim was to determine if the metabolic responses to nutrient additions were similar in surface waters of the three anticyclonic eddies and to define the potential key factor that would dictate these responses in each case. For this, we measured particulate (PPp), dissolved primary production (PPd), gross community production (GCP) and community respiration (CR) upon enrichment with N and P added separately and jointly. Through these measurements we further discuss the potential implications relative to metabolic balance and carbon budgets between autotrophic and heterotrophic osmotrophs.

2 Methods

2.1 Experimental set up and sampling

The three microcosm experiments were performed at the cores of 3 anticyclonic eddies, in the Western (station A: 39°5.96′ N–5°21.00′ E), the Ionian (station B: 34°8.20′ N–18°26.70′ E) and the Levantine (station C: 33°37.50′ N–32°39.20′ E) basins

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during the BOUM (Biogeochemistry from the Oligotrophic to the Ultra-oligotrophic Mediterranean) cruise in June–July 2008, on board the French R/V Atalante (Fig. 1). At each station, seawater was collected from 8 m depth within the mixed layer, and 4 series of triplicate 20 l polycarbonate Nalgene bottles (microcosms) were filled. In three of the series, enrichments with addition of NH_4 (+N), PO_4 (+P) and both NH_4 and PO_4 (+NP) were performed, while the fourth series was used as Control where no addition was made. 1.6 µM of NH_4 were added at St. A and B, and 3.2 µM were added at St. C, whereas, 0.1 µM of PO_4 was added at each station, in the respective treatment. The microcosms were incubated in an on-deck flow-through water bath covered with a filter that reduced the incident light by approximately 50% to approximate incident light conditions at the sampling depth. Sampling for determination of a suite of chemical and biological parameters took place on day 0 (prior to the additions), day 2, and at the end of the experiment. At St. A, the experiment lasted 3 days and at St. B and C the experiment lasted 4 days.

2.2 Analytical procedures

2.2.1 Inorganic mineral nutrients

Concentrations of NO_3+NO_2 and soluble reactive phosphorus, referred to as phosphate (PO_4) in this paper, were immediately measured on board with an autoanalyser (Bran+Luebbe autoanalyser II) according to the colorimetric method (Tréguer and Le Corre, 1975). Concentration of NH_4 was also immediately measured on board by fluorometry according to Holmes et al. (1999). The detection limit and the precision were 3 and 2 nM for NH_4, 20 and 20 nM for the NO_3+NO_2, 10 and 10 nM for PO_4, respectively.

2.2.2 Chlorophyll-*a*

Chlorophyll-*a* (chl-*a*) was measured fluorometrically, according to Yentsch and Menzel (1963). For each sample, approximately 0.5 l of seawater was filtered through 0.2 µm

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polycarbonate filters. Filters were kept frozen in the dark until extraction in 90% acetone solution overnight. Measurements were performed on board with a Shimadzu RF5301 spectrofluorometer.

2.2.3 Particulate and dissolved primary production rates

Photosynthetic carbon fixation rates (particulate and dissolved) were estimated by the ^{14}C incorporation method (Steemann Nielsen, 1952) according to Marañón et al. (2004) for the dissolved primary production (PPd) measurements. For each triplicate microcosm of the 4 series (the Control, +N, +P and +NP) three light and one dark 170-ml polycarbonate bottles (Nalgene) were filled with sample water in the morning, around 09:00–10:00 a.m. (LT), inoculated with 20 µCi of $NaH^{14}CO_3$ tracer each and incubated for 4 h in the on-deck flow-through water bath. The incubation period was a compromise between the time needed in order to obtain a significant signal in the dissolved primary production phase, and at the same time, minimize the ^{14}C-labeled DOM assimilation by heterotrophic prokaryotes (Morán and Estrada, 2002). Because of the time constraints of sample treatment, dissolved primary production rate (PPd) was measured only in one of the triplicate microcosms of each series.

At the end of the incubation, two 5-ml aliquots from each light/dark polycarbonate bottle were filtered through 0.2 µm polycarbonate filters (25 mm diameter) using very low vacuum pressure (<50 mmHg). Both the filtrate and the filters were collected for measurements of the dissolved (PPd) measurement and particulate primary production (hereinafter assigned as $PPp_{(5ml)}$). In order to remove excess ^{14}C-bicarbonate, filters were exposed to concentrated HCl fumes for 12 h, while filtrates collected in 20-ml scintillation vials were acidified with 100 µl of 50% HCL and left open overnight in an orbital shaker. Then 10 ml of scintillation fluor were added to the filtrates on board and vials were stored for counting in the laboratory. The rest of the 160-ml sample of the light/dark polycarbonate bottles was also filtered through 0.2 µm polycarbonate filters (25 mm diameter) under low vacuum pressure (<200 mmHg) and filters were put in scintillation vials where 1 ml of 1% HCl solution was immediately added in order to

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remove excess ^{14}C-bicarbonate overnight. These filters were used for measurement of the particulate primary production (PPp) as well. After addition of 4 ml scintillation fluor all vials containing filters were stored for counting in the laboratory in a scintillation counter.

Particulate and dissolved primary production rates resulting from light and dark incubated samples were calculated from the radioactivity (cpm) measured on filters and in the filtrates, respectively, as shown in the following equation:

$$\mathrm{PPp, PPd(mg\,C\,m^{-3}\,h^{-1})} = \frac{\text{incubated volume}}{\text{filtered volume}} \times \frac{(\mathrm{cpm_{light}} - \mathrm{cpm_{dark}}) \times \mathrm{DIC} \times 1.05}{\mathrm{cpm_{total}} \times h} \quad (1)$$

$\mathrm{cpm_{light}}$, $\mathrm{cpm_{dark}}$=counts per minute measured in the light and dark bottles, respectively; $\mathrm{cpm_{total}}$=counts per minute of the total amount of tracer inoculums; DIC=dissolved inorganic carbon=24 000 mg C m^{-3}; 1.05=correction factor for the lower uptake of ^{14}C as compared to ^{12}C; h=duration of the incubation.

In the microcosms were only PPp was measured, the whole 170-ml sample of the light/dark polycarbonate bottles was filtered and treated as described above for the case of the remaining 160-ml sample. In this case, note that the first term in Eq. (1) that refers to the volumes would be 1.

The percentage extracellular release (PER, %) was calculated as the ratio of dissolved to total primary production (particulate and dissolved) measured simultaneously in the 5-ml aliquots.

$$\mathrm{PER} = \mathrm{PPd} \times 100/(\mathrm{PPp_{(5ml)}} + \mathrm{PPd}) \quad (2)$$

The $\mathrm{PPp_{(5ml)}}$ and the PPp (calculated from the classical procedure of whole 160-ml sample filtration) were tightly correlated (Pearson, r=0.93, p<0.000, n=27), however, the $\mathrm{PPp_{(5ml)}}$ rates were higher (1.5-fold), probably because of the smaller volume used and a potential bias induced by the respective correction of the volume (first term in Eq. 1). For the analysis of results we assumed as the actual particulate primary production rate the one calculated from the whole (or the 160-ml) sample while from the

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PER obtained from Eq. (2) we calculated the corresponding dissolved primary production rate (PPd).

2.2.4 Gross community production, community respiration and net community production

Rates of gross community production (GCP), community respiration (CR) and net community production (NCP) were estimated from changes in the dissolved oxygen concentration during light/dark 24-h incubations, in two of the triplicate microcosms of each series. From each sampled microcosm, twelve replicate BOD (biological oxygen demand) bottles of 125 or 60 ml were filled. From these BOD bottles, four were fixed immediately to measure the oxygen concentration at time 0 (T0), and the rest were incubated in flow-through water on-deck incubators for 24-h under light conditions (4 BOD bottles) or in the dark (4 BOD bottles). The concentration of the dissolved oxygen in the BOD bottles was measured on board by automatic automated high-precision Winkler titration system linked to a photometric end point detector (Williams and Jenkinson, 1982). CR was calculated as the difference in the dissolved oxygen concentration between "dark" incubated samples and the "time 0" samples – providing negative oxygen rates –, NCP was calculated as the difference between the "light" incubated samples and the "time 0" samples, and GCP was calculated as the difference between NCP and the negative CR (Lefèvre et al., 2008). Standard deviations on the rates were calculated from the standard deviation of quadruple samples sets. GCP was converted to carbon units applying a photosynthetic quotient of 1.1 (Laws, 1991).

2.2.5 Heterotrophic prokaryotes

Samples (3.5 ml) were preserved with 2% (final concentration) formol, frozen in liquid nitrogen, and stored at −80 °C until flow cytometric analysis (Troussellier et al., 1995). After thawing at room temperature, measurements for autotrophic and heterotrophic communities were run with a flow cytometer (FACSCan, BD-Biosciences) equipped

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with a 488 nm-15 mW Argon laser. Data acquisition was performed using CellQuest software (BD-Biosciences). SYBR Green I (Molecular Probes) was used to stain heterotrophic bacterial populations, which were discriminated and enumerated by their nucleic acid contents according to their right angle light scatter and green fluorescence (Marie et al., 2000).

2.2.6 Bacterial production

Bacterial production (BP; the term "bacterial" refers thereafter to all heterotrophic prokaryotes, *Eubacteria* and *Archaea*) was measured using the ^{3}H leucine incorporation technique (Kirchman et al., 1997). Briefly, 1.5 ml duplicate samples and a control were incubated with a mixture of L-[4,5-^{3}H] leucine (Perkin Elmer, specific activity 115 Ci mmol^{-1}) and non-radioactive leucine at final concentrations of 16 and 7 nM, respectively. Samples were incubated in the dark at in situ temperature, fixed and treated following the microcentrifugation protocol (Smith and Azam, 1992) as described in detail in Van Wambeke et al. (2010) and using a conversion factor of 1.5 kg C per mole leucine incorporated.

2.2.7 Bacterial carbon demand

Bacterial carbon demand (BCD) is defined as the amount of bacterial production (BP) plus respiration (BR):

$$BCD = BP + BR \quad (3)$$

We did not directly measure BR in this study but estimated the range of bacterial carbon demand. We assumed bacterial respiration to be bracketed between total community respiration (BCD_{100}=BP+CR) and 50% of it (BCD_{50}=BP+CR/2). These values reflect rates reported for the NW Mediterranean in which BR is found to account for ~75% of CR (Lemée et al., 2002; Gonzalez et al., 2003) and overall an average from open ocean systems of about 50% (Robinson, 2008). The respiratory quotient was

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considered constant for all cases and equal to 0.8 (Lefèvre et al., 2008). In order to compare BCD with the PP_{total} (i.e PPp+PPd), the CR was converted to hourly rates by dividing by 24 while for comparison of BCD with the GCP, the BP was converted to daily rates by multiplying by 24.

2.3 Statistical analysis

For statistical analysis, all data were $\log_{10}$ transformed to meet requirements of homogeneity of variance. For comparisons between the control and the amended microcosms (+N, +P, +NP) at the end of the experiment, a one-way ANOVA and Tukey's HSD analysis (95% confidence level) were performed. For correlation and regression analyses (Model II) between variables the whole data set (day 0, 2 and final) was used.

3 Results

3.1 Initial conditions

The surface waters of the three anticyclonic eddies displayed concentrations of NO_3+NO_2<40 nM, NH_4<50 nM and PO_4 below the detection limit (<10 nM) except from St. C where 30 nM of PO_4 were measured (Table 1). Overall, chl-*a* concentration ranged from 0.03–0.06 mg m^{-3} and PPp rates from 0.09–0.29 mg C m^{-3} h^{-1}, with St. C displaying the lowest and St. A the highest values (Table 2). PPd rates were relatively less variable than PPp rates ranging from 0.02–0.05 mg C m^{-3} h^{-1}, while PER was 9.2 (±4.2)%, 17.7 (±12.4)% and 15.2 (±12.5)% at St. A, B and C, respectively (Table 2). Bacterial abundance and production ranged from 1.79–3.42×10^5 cells ml^{-1} and 0.01–0.03 mg C m^{-3} h^{-1}, respectively. GCP ranged from 0.12–0.92 mmol O_2 m^{-3} d^{-1}, gradually decreasing from the western St. A to the eastern St. C, while the CR ranged from −0.38 to −0.65 mmol O_2 m^{-3} d^{-1}, with St. C displaying the lowest value (Table 2). The NCP, representing the balance between the GCP and the CR, was not significantly

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different from 0 at all three stations (GCP not significantly different from CR, t-test, $p>0.05$), however its value decreased gradually from positive at St. A to net negative at St. C (Table 2).

Thus, while there was a gradient of oligotrophy from site A to C, at all three sites nutrient and chl-*a* concentrations as well as primary production were low and dissolved primary production ranged from about 10 to 20% of total primary production. Rates of bacterial production were approximately 10% of primary production and net community production equalled community respiration.

3.2 Amended microcosms vs. control at the end of the experiment

In all three experiments, the nutrient additions which included nitrogen were associated with significant treatment effects with regard to the autotrophic community. At the end of the experiment, at St. A and B, chl-*a* increased significantly in the +N and +NP microcosms (2 to 5-fold and 5 to 25-fold, respectively) relative to the Control, whereas, at St. C a significant 12-fold increase was observed only in the +NP (Tukey HSD test, $p<0.05$, Fig. 1a). At all stations, no difference in PPp was observed in +P compared to the Control whereas significantly 3-fold higher values were observed in the +N and 16 to 46-fold increases were observed in the +NP (Tukey HSD test, $p<0.05$, Fig. 1b).

Interestingly, PPd response to nutrient additions was not proportional to PPp response. PPd increased significantly only in the +NP at St. A and B (Tukey HSD test, $p<0.05$, Fig. 1b). PER ranged from 9 to 20% and from 4 to 8% in the +N and +NP additions, respectively. The only significant difference with the Control was observed in the +P at St. B where PER reached its highest value (31%, Fig. 1c).

Bacterial production showed a significant 2.3-fold increase in +N at St. B (Tukey HSD test, $p<0.05$) and 4-fold increase in +NP at St. B and C (Tukey HSD test, $p<0.05$, Fig. 1d). In contrast to primary production and chl-*a*, no significant differences of BP were detected between the different microcosms at St. A (ANOVA $p>0.05$).

Gross community production (GCP) at all three sites increased significantly (from 5 to 15-fold) only in the +NP treatment (Fig. 2). Similarly, community respiration (CR),

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increased ~2.7-fold in the +NP at all stations, however this increase was statistically significant only at St. B (Fig. 2). Net community production (NCP) at the end of the experiment was positive in all treatments at St. A and displayed the highest value in +NP (Fig. 2). At St. B and C, NCP was 0 in the +N (0.21±0.64 and −0.06±0.29, respectively), negative in the +P (−0.80±0.34 and −0.75±0.20, respectively) and positive in the +NP treatment (3.52±0.29 and 7.37±1.03, at St. B and C, respectively) (Fig. 2).

Considering all values together, dissolved primary production (PPd) rates were strongly correlated with chl-*a* (Pearson r=0.69, p<0.001, n=33). A significant linear relationship was also found between log(PPp) and log(PPd) with a regression line slope statistically different of the 1:1 line (t-test, p<0.05, Fig. 3).

The phytoplankton assimilation ratio, the chlorophyll-specific rate of primary production (PP_{total}/chl-*a*), at the end of the experiment was 2 to 3-fold higher relative to the Control in the +NP at St. A and B and 1.3-fold higher in the +N and +NP at St. C (Table 3).

Finally, considering all three stations, strong positive correlations between the BP and PP_{total} were found for the +N and +NP treatments only (Table 4).

3.3 Metabolic balance-carbon budget

Assuming bacterial respiration to range from 50 to 100% of CR, the initial range of the BCD:PP_{total} ratio estimations, was <1 at St. A and ranged from 0.6 to 1.6 at St. B and C (Table 5). At the end of the experiment BCD: PP_{total} was always $\ll 1$ in +NP, it varied from 0.4 to 1.4 in +N and it showed an increasing trend in +P from A to C ranging from 0.7–4.8 (Table 5). The estimated BCD:GCP ratio was <1 at St. A for initial and nutrient addition conditions. The same was observed at St. B, with exception of the +P where the ratio was around 1 (Table 5). At St. C, BCD:GCP ratio exceeded 1 at the initial conditions (1.6–3) but remained >1 only in +P at the end of the incubation (1.3–2.2).

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4 Discussion

This is the first study of the effects of N and P additions on particulate and dissolved primary production along with gross community production and community respiration measurements in open oligotrophic Mediterranean waters. Below we discuss the potential implications of nutrient additions relative to metabolic balance and carbon budgets between autotrophic and heterotrophic osmotrophs.

4.1 Effect of nutrient additions on dissolved and particulate primary production

Based on the responses to nutrient additions, the initial autotrophic communities were N-limited at all three sites (see also Tanaka et al., 2010). The significant increase in PPp in the +N and +NP paralleled a chl-*a* increase except in the +N at St. C (Fig. 1a,b). In contrast to PPp, PPd showed a significant increase only at St. A and St. B and only in the +NP treatment. Yet, considering all the results of PPd and PPp together, the two parameters were significantly and positively correlated (Fig. 4). The slope of the log-log linear regression between the dissolved and the particulate primary production was less than and significantly different from 1. This indicates that PPd did not increase proportionally with PPp, resulting in an inverse relationship between PER and total primary production. This is in agreement with other recent studies dealing with PPd in coastal Mediterranean waters (Morán et al., 2002; Teira et al., 2001b), open ocean upwelling regions (Teira et al., 2001a) and oligotrophic Atlantic subtropical gyre waters (Teira et al., 2003).

It should be noted though that PER, by definition, is strongly affected by – even small – changes in PPp and PPd; comparisons between treatments based on PER have a relative value. The inverse relationship of PER with PP_{total} is in agreement with the observation that in excess of both N and P (+NP treatment), PER was minimal, while additions of N or P alone resulted in a higher PER (Fig. 3c). Under ultra-oligotrophic conditions, limitation of a single nutrient and/or co-limitation are in a delicate balance, meaning that the addition of one nutrient will quickly push limitation to another nutrient.

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Consequently, PER variations, in particular in +N and +P treatments, are results of complex effects of the initial conditions with perhaps near co-limitation of N and P (Tanaka et al., 2010).

A potential problem with regard to PER is that measurements are based on the assumption that heterotrophic uptake of dissolved organic carbon produced by phytoplankton is minimized in short time incubations. The 4 h incubations used here are supposed to fulfil this assumption, while in longer incubations of 5–6 h or more, heterotrophic prokaryotes were found to assimilate ∼45% of the excreted carbon (Fernandez et al., 1994; Moran and Estrada, 2002). Interestingly, the initial PER values in this study, ranging 10–20% at the three stations, closely approximated PER values measured in surface waters (∼30% at 12 m) with a 24-h in situ incubation approach (López-Sandoval et al., 2010). This accordance indicates that PER values estimated in the microcosms were reasonable. Finally, PER may also be affected by phytoplankton species composition (Teira et al., 2001b), though this subject is under debate since in one recent study no relation between PER, taxonomic composition and community size structure could be established (López-Sandoval et al., 2010).

The assimilation ratio of phytoplankton, defined as the ratio of primary production normalized to chl-*a*, is an indicator of the photosynthetic capacity of the autotrophic community. The largest increases in the assimilation ratio at all three sites were observed in the +NP treatments (Table 3). At St. A and B the increase of the assimilation ratio in +NP treatment was large relative to that in the +N treatment. In contrast, at St. C similar increases of the assimilation ratio were estimated for the +N and +NP treatments; explicable perhaps by the presence of measurable, though close to quantification limit (Pujo-Pay et al., 2010), phosphates in the surface waters of St. C (30 nM, Table 1).

We found no significant changes in PER with nutrient additions despite the fact that large shifts were evident in the properties of the phytoplankton communities. Nutrients additions led to increases in chl-*a* concentrations, rates of primary production as well as assimilation efficiencies. Our data then suggest that the fraction of primary production

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excreted by phytoplankton in the oligotrophic waters of the Mediterranean is not closely linked to nutrient limitation, at least over the time-scale investigated of a few days.

4.2 Metabolic balance-carbon budget

Comparing the three gyres, although all were oligotrophic, there was a west to east gradient of increasing oligotrophy. This was evidenced in the initial measurements of chl-*a*, PPp and GCP which all decreased from St. A to St. C. Nonetheless, in all three stations there were no significant differences between GCP and CR, indicative of equilibria between GCP and CR for the three sites.

With nutrient additions of both N and P, communities at all three sites became clearly autotrophic with positive values of NCP. At St. A the community was rather autotrophic in all treatments, whereas at St. B and C the system was balanced in +N (NCP≈0), heterotrophic in +P (NCP<0) and net autotrophic in +NP (NCP>0) (Fig. 2). The positive changes of NCP reflected enhanced community production rather than lower respiration rates. Such system shifts with nutrient enrichment to net autotrophy have been reported previously for oligotrophic systems, e.g. in the coastal NW Mediterranean (Duarte et al., 2004) and the North Pacific Subtropical Gyre (McAndrew et al., 2007). As in our experiments, this shows a decoupling of CR and GCP, with GCP displaying faster and larger response to nutrient additions on a time scale shorter than a week, resulting therefore in positive NCP values and shifting the community balance from net heterotrophy, or balanced, to net autotrophy.

Estimates of autotrophic community production were obtained with 2 independent methods: one based on ^{14}C assimilation (PP_{total}), the second was based on O_2 fluxes (GCP). PP_{total} measurements are subject to a number of uncertainties, mainly regarding rapid uptake of the dissolved fraction as already discussed above and O_2 fluxes are particularly difficult to measure in very oligotrophic conditions. Some studies argue that the ^{14}C assimilation measurements during short incubations approximate gross production and that observed discrepancies are due to the omission of the dissolved fraction of primary production (Weger et al., 1989; Gonzalez et al., 2008). Other studies

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regarding phytoplankton metabolism argue that during photosynthesis all CO_2 respired by mitochondria is re-fixed in photosynthesis (Raven, 1972), meaning that photosynthesis uses more O_2 than CO_2 from the ambient environment, since the latter has an internal source, or, in other words, that phytoplankton carbon is expected to be lower than the oxygen fluxes (Marra, 2009; Marra and Barber, 2002). In our study, PP_{total} measured with the ^{14}C assimilation method includes in principle both the dissolved and particulate fractions and corresponds to the maximum hourly primary production rates during the day. When compared with the gross community production daily rate, a close relation was found (Pearson's coefficient r=0.80, n=33, p<0.001) though PP_{total} was generally lower than GCP (Fig. 4). Therefore, the data presented here seem to confirm that PP_{total} estimated during short incubations though somewhat lower, approximates the GCP. Given all the uncertainties discussed above, the good agreement between PP_{total} and GCP in our study supports the idea that the values presented here are likely robust.

The carbon budgets estimated show that compared to initial conditions, nutrient addition led to metabolic shifts in terms of the rates of autotrophic fixation of carbon relative to bacterial carbon demand. In the microcosms where autotrophic conditions were observed (NCP>0, all treatments at St. A, +NP at St. B and C, Fig. 2), the GCP (or equally the PP_{total}) was sufficient to sustain BCD. Whenever the microcosms displayed heterotrophic metabolism (NCP<0, +P at St. B and C, Fig. 2) GCP was not sufficient to supply the BCD. Finally, when the total community was metabolically balanced (NCP≈0, e.g. in the +N), the carbon ratios between the BCD and the GCP generally did not differ from 1 (Table 5). PP_{total} was positively correlated with BP in +N and +NP treatments (Table 4). It is obvious that simple correlations between the two parameters give an indication of a relationship between phytoplankton and heterotrophic prokaryotes (Turley et al., 2000) but do not completely define it, mostly because of the large variations of the BP:PP observed (Conan et al., 1999).

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5 Conclusions

Given that in oligotrophic environments heterotrophic prokaryotes depend at least in part on organic carbon produced by phytoplankton, but also compete with phytoplankton for mineral nutrients, it is often difficult to interpret interrelationships based on observations of PPd, PER and BCD, without detailed knowledge of the underlying mechanisms. Our results though, showed that at St. A nutrient additions revealed a rapid autotrophic potential presenting an autotrophic production largely exceeding the carbon requirements of the heterotrophic prokaryotes in all three combinations of nutrient additions. In St. B and C the limitation of mineral nutrients and the potential competition between autotrophs and heterotrophs could not be relaxed upon addition of N and P alone, presenting autotrophic conditions only in the +NP additions. Our results then highlighted differences between communities of the oligotrophic eddies. The role of osmotroph interactions in the food web functioning and system metabolism in the open oligotrophic Mediterranean waters needs further to be investigated, with respect to the seasonal variability of particular hydrographic features and their trophic status.

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Table 1. Initial (prior to the additions) concentrations (mean±sd of the triplicate microcosms) of mineral nutrients in the enrichments experiments at stations A, B and C. <DL: below detection limits.

Parameter	St. A	St. B	St. C
NO_2+NO_3 (nM)	<DL	37 (±21)	40 (±20)
NH_4 (nM)	34 (±11)	49 (±22)	15 (±5)
PO_4 (nM)	<DL	<DL	30 (±2)

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Table 2. Initial values (mean±sd) of chl-*a*=Chlorophyll-*a*, BA=bacterial abundance, BP=bacterial production, PPp=particulate primary production, PPd=dissolved primary production, PER=percentage extracellular release, GCP=gross community production, CR=community respiration and NCP=net community production at stations A, B and C. For chl-*a*, BA, PPp and BP the standard deviation (sd) was estimated from the triplicate microcosms. For PPd and PER the sd was obtained from the triplicate measurement in a single microcosm while for GCP, NCP, CR the sd was obtained from the quadruple measurements in each of the 2 microcosm (cf. Sect. 2.2.4).

Parameter	St. A	St. B	St. C
chl-*a* ($mg\,m^{-3}$)	0.06 (±0.00)	0.05 (±0.00)	0.03 (±0.00)
BA ($cells \times 10^5\,ml^{-1}$)	3.28 (±0.32)	3.42 (±0.39)	1.79 (±0.06)
BP ($mg\,C\,m^{-3}\,h^{-1}$)	0.02 (±0.00)	0.03 (±0.00)	0.01 (±0.00)
PPp ($mg\,C\,m^{-3}\,h^{-1}$)	0.29 (±0.02)	0.23 (±0.01)	0.09 (±0.03)
PPd ($mg\,C\,m^{-3}\,h^{-1}$)	0.03 (±0.02)	0.05 (±0.03)	0.02 (±0.01)
PER (%)	9.2 (± 4.2)	17.7 (±12.4)	15.2 (± 12.5)
GCP ($mmol\,O_2\,m^{-3}\,d^{-1}$)	0.92 (±0.39)	0.78 (±0.36)	0.12 (±0.90)
CR ($mmol\,O_2\,m^{-3}\,d^{-1}$)	–0.63 (±0.38)	–0.65 (±0.30)	–0.38 (±0.92)
NCP ($mmol\,O_2\,m^{-3}\,d^{-1}$)	0.29 (±0.41)	0.13 (±0.32)	–0.26 (±0.22)

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Table 3. Phytoplankton assimilation ratio, defined as the ratio of PP_{total} (sum of particulate and dissolved primary production) to chl-*a* (PP_{total}/chl-*a*, mg C mg chl-a^{-1} h^{-1}), in initial samples and at the end of the experiments at stations A, B and C.

	St. A	St. B	St. C
Initial	5.3	5.6	3.6
Control	4.9	11.5	8.1
+N	6.1	6.8	10.4
+P	6.2	8.8	6.1
+NP	16.1	21.4	10.3

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Table 4. Pearson correlation coefficients between bacterial production (BP) and total particulate primary production for the Control, +N, +P and +NP additions pooled from the three eddies.

Treatment	BP-PP_{total}
Control	0.335, p=0.51, n=6
+N	0.702, p=0.03, n=9
+P	0.224, p=0.56, n=9
+NP	0.787, p=0.01, n=9

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Table 5. BCD:PP_{total}=ratios of the bacterial carbon demand (BCD) to total primary production for the initial conditions, and at the end of the experiment, in the amended microcosms with nitrogen (+N), phosphorus (+P), and nitrogen and phosphorus (+NP) additions. GCP was converted to carbon units applying a photosynthetic quotient (PQ) of 1.1 (Laws, 1991). BCD:GCP=ratios of the bacterial carbon demand (BCD) to gross community production (GCP) for the same samplings.
Within the parenthesis the lower value in each case is an estimation of the ratio assuming bacterial respiration to be half the community respiration (BCD_{50}=BP+CR/2) while the higher value is based on the assumption that bacterial respiration equals community respiration (BCD_{100}=BP+CR).

	St. A	St. B	St. C
		BCD:PP_{total}	
Initial conditions Amended microcosms at the end of the experiment	<1 (0.4–0.7)	< or >1 (0.6–1.2)	< or >1 (0.8–1.6)
+N	<1 (0.4–0.7)	< or >1 (0.7–1.4)	<1 (0.4–0.7)
+P	< or >1 (0.7–1.2)	>1 (1.4–2.7)	>1 (2.6–4.8)
+NP	≪1 (0.1–0.2)	≪1 (0.1–0.2)	≪1 (0.2–0.3)
		BCD:GCP	
Initial conditions Amended microcosm at the end of the experiment	<1 (0.3–0.6)	<1 (0.4–0.8)	>1 (1.6–3)
+N	<1 (0.5–0.9)	<1 (0.5–0.9)	< or >1 (0.7–1.2)
+P	<1 (0.5–0.8)	< or >1 (0.7–1.4)	>1 (1.3–2.2)
+NP	≪1 (0.2–0.4)	≪1 (0.3–0.6)	≪1 (0.2–0.3)

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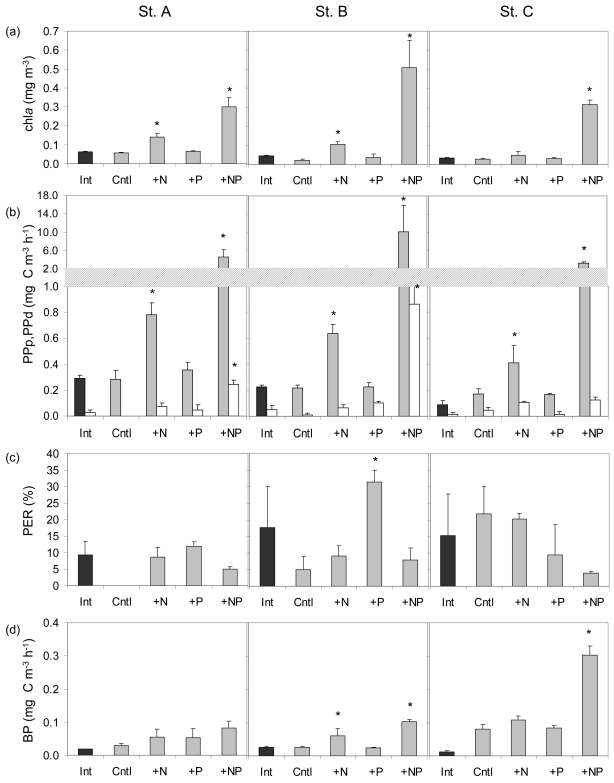

Fig. 1. Values of studied parameters measured in the microcosms, at the initial conditions (Int, black bars) and at the end of the experiment in the Control (Cntl), nitrogen (+N), phosphorus (+P), and nitrogen and phosphorus (+NP) series **(a)** chl-*a* **(b)** particulate (grey bars) and dissolved (white bars) primary production **(c)** percentage extracellular release (PER) **(d)** bacterial production. Missing values of PPd and PER in the Control in **(b)** and **(c)** denote that measurements were below detection limit. Chl-*a*, PPp and BP figures are modified from Tanaka et al. (2010). * Denotes significant difference with the control.

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The effects of nutrients additions on primary production rates

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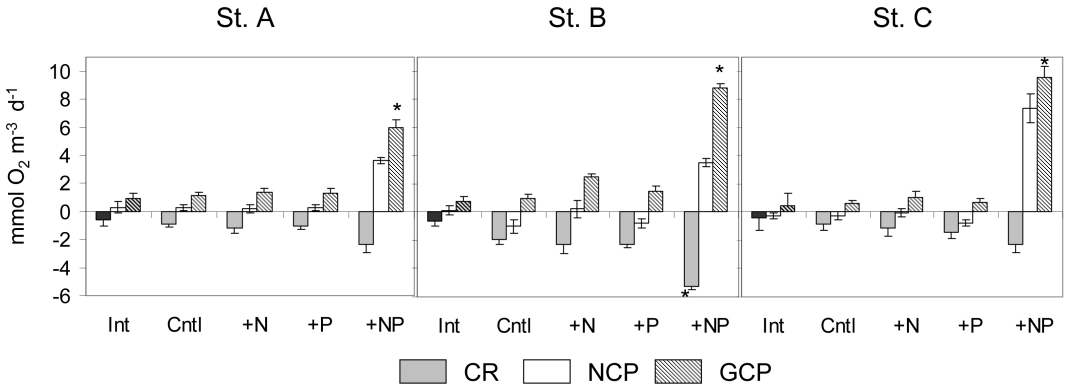

Fig. 2. Gross community production (GCP), community respiration (CR) and net community production (NCP) initial (Int) and at the end of the experiment in the Control (Cntl), the nitrogen (+N), phosphorus (+P) and nitrogen and phosphorus (+NP) additions. * Denotes significant difference with the control.

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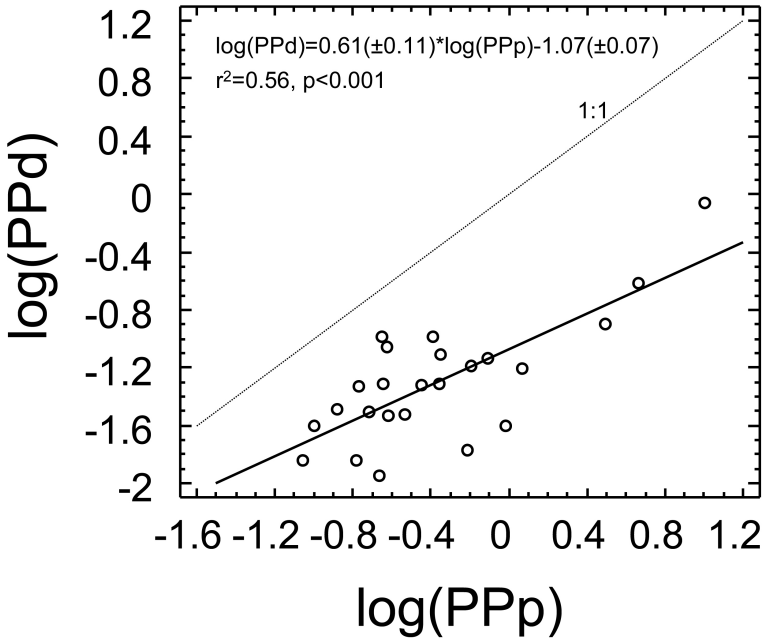

Fig. 3. Linear regression (Model II) of log-transformed particulate (PPp, mg C m^{-3} h^{-1}) and dissolved primary production (PPd, mg C m^{-3} h^{-1}) combining all measurements realized during this study.

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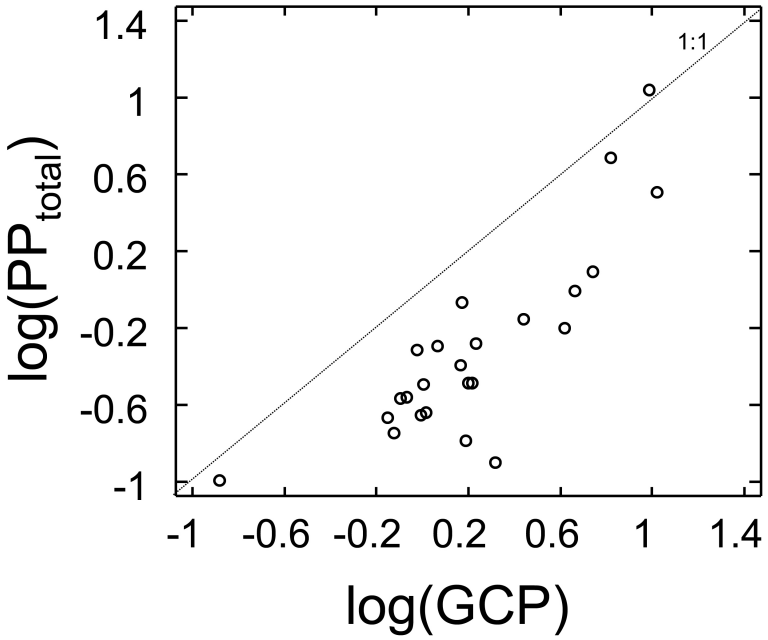

Fig. 4. Log-transformed total primary production (particulate and dissolved, PP total, mg C m^{-3} h^{-1}) and gross community production (GCP, mg C m^{-3} d^{-1}). GCP was converted to carbon units by applying a photosynthetic quotient (PQ) of 1.1 (Laws, 1991). The line represents the 1:1 relationship.

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