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Nutrient dynamics under different ocean acidification scenarios in a low nutrient low chlorophyll system: the Northwestern Mediterranean Sea

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Highlights:
- Mesocosm experiments performed in two Mediterranean sites during two seasons
- Contrasted nutrient stoichiometry in surface waters in summer and winter
- Dissolved organic pool was a large stable fraction of N and P in summer and winter
- CO2 had no effect on nutrient dynamics that was mostly biologically controlled

Keywords: ocean acidification; plankton community; mesocosm experiments; nutrient dynamics; nitrogen; phosphorus; iron; stoichiometry; Oligotrophy; Mediterranean Sea
Abstract

Two pelagic mesocosm experiments were conducted to study the impact of ocean acidification on Mediterranean plankton communities. A first experiment took place in summer 2012 in the Bay of Calvi (France) followed by an experiment in winter 2013 in the Bay of Villefranche (France) under pre-bloom conditions. Nine mesocosms were deployed: three served as controls and six were acidified in a targeted partial pressure of CO$_2$ ($p$CO$_2$) gradient from 450 to 1250 µatm. The evolution of dissolved organic and inorganic nutrient concentrations was observed using nanomolar techniques. The experiments were characterized by a large contribution of organic nutrients to nutrient pools and contrasting in situ conditions with an inorganic N/P ratio of 1.7 in summer and of 117 in winter. In the Bay of Calvi, initial conditions were representative of the summer oligotrophic Mediterranean Sea. While inorganic phosphate concentrations were depleted during both experiments, in situ inorganic nitrogen concentrations were higher in winter. However, nitrate was rapidly consumed in winter in all mesocosms during the acidification phase, leading to a decrease in N/P ratio to 13. During these first mesocosm experiments conducted in a low nutrient low chlorophyll area, nutrient dynamics were insensitive to CO$_2$ enrichment, indicating that nutrient speciation and related biological processes were likely not impacted. During both experiments, nitrate and phosphate dynamics were controlled by the activity of small species that are favored in low nutrient conditions. In contrast to the theoretical knowledge, no increase in iron solubility at high $p$CO$_2$ was observed.
1. Introduction

Since the beginning of the industrial era, the atmospheric carbon dioxide (CO$_2$) level has increased by nearly 40% (from ~280 to 390 ppm in 2011; Hartmann et al., 2013) and may reach between ~450 and ~1000 ppm by 2100 depending on the considered future emission scenario (Collins et al., 2013). As the ocean absorbs about a quarter of total anthropogenic CO$_2$ emissions (Le Quéré et al., 2014), the increase in CO$_2$ dissolved in seawater leads to an increase in seawater acidity (decrease in pH). Surface ocean pH has already declined by approximately 0.1 since the beginning of the industrial era (Orr, 2005) and is expected to decrease an additional 0.06 to 0.32 by the end of this century depending on the considered CO$_2$ emission scenario (Ciais et al., 2013). Although the chemical forms of compounds such as macro- and micro-nutrients in the ocean are controlled by multiple environmental factors including pH, only a restricted number of studies have addressed the impact of ocean acidification on nutrient dynamics and its consequences on biological activity.

Following established theoretical equilibriums of dissolved inorganic phosphate and ammonium (NH$_4^+$) in seawater as a function of pH (i.e. Zeebe and Wolf-Gladrow, 2001), a decrease in surface ocean pH expected to occur this century will lead to a decrease of phosphate (PO$_4^{3-}$) concentrations by up to 80% (and increase in HPO$_4^{2-}$) and an alteration of the NH$_4^+$/NH$_3$ equilibrium. Therefore, ocean acidification could directly affect the chemical speciation of phosphorus and nitrogen with impacts on their bioavailability and stoichiometry.

In addition to pH equilibrium reactions, ocean acidification can impact biological processes and indirectly shift nutrient cycles. In the case of nitrogen, an increase in the partial pressure of CO$_2$ ($p$CO$_2$) could (1) enhance nitrogen fixation (e.g. Hutchins et al., 2007) and (2) reduce the rate of marine nitrification (e.g. Beman et al., 2011). The alteration of the NH$_4^+$ / NO$_3^-$ ratio and the increasing trend toward P-limitation could ultimately impact primary
production and phytoplankton community structure (CIESM, 2008; Hutchins et al., 2009).

For example, Romero et al. (2012) showed that waters rich in NH$_4^+$ favor the growth of small autotrophic species and heterotrophic bacteria while NO$_3^-$ mostly favor diatom growth.

The organic fraction of dissolved nutrients (dissolved organic nitrogen: DON and dissolved organic phosphorus: DOP) can represent a large source of P and N for microorganisms in oligotrophic areas (e.g. Jackson and Williams, 1985; Karl and Bjorkman, 2002; Bronk, 2007). Regarding DOP, most of its compounds cannot be used by phytoplankton directly, unless specific enzymes (e.g. alkaline phosphatase) are produced to hydrolyze DOP into PO$_4^{3-}$ (Holland et al., 2005; Dyhrman and Ruttenberg, 2006). These hydrolysis reactions are sensitive to pH with the consequence that a change in pH could alter the bioavailability of DOP in seawater (Doney et al., 2009).

Furthermore, decreases in ocean pH theoretically increase the proportion of free dissolved trace metals and increase metal solubility (Millero, 1998). This would lead to substantial increases in the total bioavailable fraction of many trace elements and result in toxic concentrations for marine microorganisms (Raven et al., 2005). Several studies have shown that a decrease in ocean pH enhances the solubility of Fe(III), a form of iron that is bioavailable for phytoplankton (e.g. Liu and Millero, 2002; Millero et al., 2009). Iron speciation is largely controlled by organic complexation and photochemical processes (Breitbarth et al., 2010a and ref within). A decrease of pH below 8 favors the iron complexation with organic ligands against the precipitated form Fe(OH)$_3$ (De Rijck and Schrevens, 1998). The effect of ocean acidification on Fe(III)-organic complexation needs further investigations (Hoffmann et al., 2012). Shi et al. (2010) have shown that depending on the nature of the chelating agent, a pH decrease could enhance the solubility of Fe by
maintaining Fe(III) binding and affect the chemical speciation of Fe by favoring Fe chelated by organic compounds rather than its oxy-hydroxide form (Fe(OH)_3).

Ligand production is facilitated by phytoplankton dissolved organic matter (DOM) production. Enhanced release of DOM with increasing pCO_2 (i.e. Engel, 2002) could, as a feedback, increase iron binding and make it more bioavailable for primary production. This has recently been shown in in situ mesocosm experiments conducted as part of the Pelagic Ecosystem CO_2 Enrichment project (PeECE) in Bergen, Norway (Breitbarth et al., 2010b).

Most studies investigating the impact of ocean acidification on pelagic ecosystems have been performed in laboratories but, during the last decade, several mesocosm experiments have been conducted, for instance as part of the PeECE project and the European Project on Ocean Acidification (EPOCA; i.e. Engel et al., 2005; Grossart et al., 2006; Schulz et al., 2008; Riebesell et al., 2013). Mesocosms offer the advantage to follow the response of pelagic communities to an environmental driver under close-to-natural environmental conditions. The above-mentioned mesocosm experiments have been mostly performed in nutrient-replete cold waters. However, a large portion of the open ocean is characterized by low-nutrient, low-chlorophyll (LNLC) conditions. To our knowledge, no ocean acidification mesocosm study has been conducted under these limiting conditions as observed in the Mediterranean Sea.

The Mediterranean Sea is one of the most nutrient-poor waters in the world with a trophic status varying from oligotrophic-mesotrophic in the Northwestern Mediterranean to extremely oligotrophic in the eastern basin (Moutin and Rimbault, 2002; Mermex Group, 2011 and references within). The Mediterranean Sea is in general a sink for atmospheric CO_2 (D'Ortenzio et al., 2008). As this region is relatively more alkaline than the open ocean, it absorbs relatively more anthropogenic CO_2 and is a significant source of CO_2 for the Atlantic
ocean (Ait-Ameur and Goyet, 2006). Long-term data series of carbonate properties and ocean acidification trends in the Mediterranean Sea are scarce. However, current interpolation suggests that pH may have already declined by 0.15 in some areas of the surface Mediterranean Sea (Touratier and Goyet, 2011).

The objective of the present study is to assess the effect of ocean acidification on nutrient (Fe, N and P) dynamics in a nutrient-depleted system, limited or co-limited by P and N availability (Tanaka et al., 2011): the Mediterranean Sea. Two mesocosm experiments have been conducted in the Northwestern Mediterranean Sea during contrasting periods in terms of biogeochemical conditions and trophic status. These experiments were conducted in the framework of European Mediterranean Sea Acidification in a Changing Climate project (MedSeA: www.medsea-project.eu). In this paper, we report on the evolution of nutrient concentrations, under both dissolved inorganic and organic forms, and discuss the potential impact of ocean acidification on nutrient dynamics in LNLC ecosystems.
2. Material and methods

2.1. Experimental setup and sampling

The first mesocosm experiment was performed in the Bay of Calvi (BC; Corsica, France) during summer (June-July 2012), and the second experiment was conducted in the Bay of Villefranche (BV; France) during winter (February-March 2013). A complete description of the sites, experimental setup, initial conditions and evolution of the core parameters is given in Gazeau et al. (submitted, this issue-a). Briefly, for each experiment, nine mesocosms (2.5 m in diameter, 12 m depth and ~50 m³ in total volume) were deployed during 20 (BC) and 11 days (BV). Mesocosm bags were made of plastic material (polyethylene mixed with vinyl acetate) with nylon mesh to allow a maximum resistance and light penetration. In order to avoid atmospheric deposition, the top of the mesocosms were covered with UV-transparent ethylene tetrafluoroethylene (ETFE) roofs, except for periods of sampling during which the roofs were partially opened. Three unperturbed mesocosms were used as controls (C1, C2 and C3) and six mesocosms were enriched with CO₂ to obtain a gradient of pCO₂ levels (P1 to P6). Targeted pCO₂ levels were obtained via addition of 75 to >300 L of CO₂-saturated water into mesocosms. A diffusing system was used to ensure a perfect mixing of this CO₂-saturated seawater inside the mesocosms. In order to minimize the stress induced by the addition of large quantities of acid water, the acidification of the mesocosms was performed over the course of 4 days. Control mesocosms presented initial pCO₂ levels of 450 and 350 µatm in BC and BV, respectively. In BC, the six targeted elevated pCO₂ levels were P1: 550, P2: 650, P3: 750, P4: 850, P5: 1000 and P6: 1250 µatm. In BV, the targeted levels were P1: 450, P2: 550, P3: 750, P4: 850, P5: 1000 and P6: 1250 µatm. Mesocosms were anchored in clusters of 3, each cluster containing one control mesocosm accompanied by a medium and a high pCO₂ level (cluster 1: C1, P1, P4; cluster 2: C2, P2, P5
and cluster 3: C3, P3, P6). On June 24th 2012 (in BC) and on February 21st 2013 (in BV), the targeted $p$CO$_2$ levels were reached and the experiments started (day 0). Daily depth-integrated sampling (0 - 10 m) was performed using 5 L Hydro-Bios integrated water samples in the nine mesocosms and in the external environment (OUT) in order to follow the dynamics of each considered parameter in natural free waters.

Seawater samples for total alkalinity ($A_T$; 500 mL) measurements were filtered on GF/F membranes while samples for total inorganic carbon ($C_T$; 120 mL) measurements were immediately inoculated with 20 µL of a saturated solution of mercuric chloride (HgCl$_2$). Both parameters were analyzed within one day of collection. For pigments, 2 L of sampled seawater were filtered onto GF/F membranes. Filters were immediately frozen in liquid nitrogen and stored at -80 °C pending analysis at the Laboratoire d’Océanographie de Villefranche (France). Samples for the determination of heterotrophic prokaryote abundances were collected every second day, immediately fixed with 0.2 µm pre-filtered 25% glutaraldehyde (0.5% final concentration), kept at 4 °C for approximately 30 min, deep frozen in liquid nitrogen and stored at -80 °C until enumeration.

For dissolved inorganic and organic nutrients (NO$_x$, PO$_4^{3-}$, DFe, DON and DOP), bulk seawater samples were collected in 1 L bottles previously rinsed 3 times with sampled seawater. Shortly after sampling, samples were filtered onto 0.2 µm polycarbonate filters (Nucleopore, Whatman) under a laminar flow bench. Filters were cleaned with a 5% hydrochloric acid (HCl) suprapure solution for 30 min, rinsed 20 times with ultrapure water, cleaned again with 1% HCl suprapure solution for 24 h and finally rinsed and kept in ultrapure water in a Teflon vial. After filtration, samples for NO$_x$, PO$_4^{3-}$, DFe, were kept in 60 mL polyethylene bottles, previously cleaned following trace-metal clean procedures (Bruland et al., 1979), and then acidified to pH < 2 with HCl ultrapure, following the recommendations
of Pulido-Villena et al. (2010). Samples for the determination of DON and DOP were not acidified but directly irradiated (see section 2.2.2 and 2.2.3). Data for DON and DOP were not available at the end of both experiments due to an irradiation defect (BC) and bad weather conditions (BV).

2.2. Analytical method

2.2.1. Carbonate chemistry, chlorophyll $a$ and heterotrophic prokaryote abundance

$A_T$ was determined potentiometrically and $C_T$ was determined using an inorganic carbon analyser (AIRICA, Marianda©, Kiel, Germany) coupled to an infrared gas analyser (LI-COR© 6262). More detailed analytical protocols are given in Gazeau et al. (submitted, this issue-a). All parameters of the carbonate chemistry were determined from $C_T$, $A_T$, temperature and salinity using the R package seacarb (Lavigne et al., 2014). Chlorophyll $a$ (Chl$a$) measurements were performed by high performance liquid chromatography (HPLC, following the protocol described in Gazeau et al. (submitted, this issue-b). The abundance of free-living heterotrophic prokaryotes (HP) was estimated by flow cytometry as described in Celussi et al. (in press, this issue).

2.2.2. Dissolved inorganic phosphorus ($PO_4^{3-}$) and dissolved organic phosphorus (DOP)

A Liquid Waveguide Capillary Cell (LWCC), made of quartz capillary tubing, was used to manually analyze $PO_4^{3-}$. The LWCC was connected to a spectrophotometer and measurements were performed in the visible at 710 nm. This spectrometric method is based on Murphy and Riley’s molybdenum blue method and uses two reagents: (1) a mixed reagent composed of an ammonium molybdate solution (40 g L$^{-1}$ in ultrapure water) and an antimony potassium tartrate solution (3 g L$^{-1}$ in ultrapure water) in 5N sulfuric acid solution, and (2) an ascorbic acid solution (18 g L$^{-1}$ in 2/3 of ultrapure water and 1/3 of SDS surfactant solution; Zimmer and Cutter, 2012; Zhang and Chi, 2002). The LWCC was 2.5 m long in order to
decrease the limit of detection to 1 nM. The repeatability and reproducibility standard deviations were around 4.8% and 7%, respectively. Phosphate concentrations ([PO$_4^{3-}$]) were calculated using a calibration method in ultrapure water, following the Beer-lambert’s law, and validated by a standard addition method ($t_{\text{student}} = 0.988, n = 25, p_{\text{value}} = 0.30, \alpha = 0.05$).

To determine DOP concentrations ([DOP]$_{\text{UV}}$), samples were irradiated by ultra-violet light (UV) using the 705 UV Digester (Metrohm). Samples were transferred into 10 mL (a total of 20 mL was irradiated for each sample) quartz tubes and irradiated during 90 min with a mercury vapor lamp, according to the method UV light photooxidation described by Armstrong and Tibbitts (1968). With the standard molybdenum blue method of Murphy and Riley, the concentration of total dissolved phosphorus [TDP] was measured in irradiated samples. [DOP]$_{\text{UV}}$ was then determined by subtracting [PO$_4^{3-}$] from [TDP].

2.2.3. Dissolved inorganic nitrogen (NO$_x$) and dissolved organic nitrogen (DON)

The exact same protocol (and equipment) as described by Louis et al. (2015) was used to determine dissolved inorganic nitrogen concentrations. Briefly, the analysis of NO$_x$ ($=\text{NO}_3^- + \text{NO}_2^-$) was performed by a spectrometric method in the visible at 540 nm, with a 1 m LWCC. This method is based on the colored reaction between NO$_2^-$ and 2 reagents (sulfanilamide and NED) to form a pink azo compound. Previously, samples were passed through a copperized cadmium column to reduce NO$_3^-$ to NO$_2^-$ and determine NO$_x$ concentrations ([NO$_x$]) using a calibration curve in ultrapure water. The limit of detection was ~10 nM and the reproducibility was ~6% (tested using a reference solution, CertiPUR, Merck; $t_{\text{student}} = 0.263, n = 7, p_{\text{value}} = 0.80, \alpha = 0.05$).

Similar to DOP, DON concentrations ([DON]$_{\text{UV}}$) were determined by the irradiation UV method during which DON and all reduced inorganic ions, such as NO$_2^-$, are oxidized to NO$_3^-$. Using the same spectrometric method used to analyze NO$_x$ (see section above), the
concentration of total dissolved nitrogen [TDN] was measured, and [DON]_{UV} was calculated by subtracting [NO₃] from [TDN].

2.2.4. Dissolved iron (DFe)

DFe concentrations were measured by flow injection with online preconcentration and chemiluminescence detection using the exact protocol, instrument, and analytical parameters as described by Bonnet and Guieu (2006). Briefly, our flow injection analysis and chemiluminescence detection (FIA-CL) instrument is based on the method described by Obata et al. (1993). In order to quantify the low DFe concentration [DFe] in seawater, samples (with pH adjusted to ~5) were passed through MAF-8HQ (8-quinolinol-immobilized fluoride containing metal alkoxide glass) resin column to selectively collect and pre-concentrate DFe. After elution of DFe by a HCl solution, the eluent was mixed with a luminol solution (0.74 M), an ammonium hydroxide solution (1 M) and a hydrogen peroxide (H₂O₂) solution (0.7 M). A luminescence reaction occurs between the luminol and H₂O₂, catalyzed with DFe in alkaline conditions. Two distinct seawater reference materials were used to control for analytical accuracy: NASS-5 (North Atlantic Surface Seawater) and D1-508 (SAFe reference seawater) with measured [DFe] of 3.74 ± 0.19 nM and 0.65 ± 0.03 nM respectively in good agreement with consensus values (3.763 ± 0.001 and 0.67 ± 0.04 nM respectively). The repeatability and reproducibility were lower than 10%, and the detection limit was ~20 pM. Data of [DFe] are only available for the experiment in BV.

2.3. Data availability

Data collected during both experiments are freely available on Pangaea, Bay of Calvi (BC): http://doi.pangaea.de/10.1594/PANGAEA.810331 and Bay of Villefranche (BV): http://doi.pangaea.de/10.1594/PANGAEA.835117.
2.4. **Statistical analysis**

In order to test for ocean acidification effects on [NO$_x$], [PO$_4^{3-}$], [DOP]$_{UV}$, [DON]$_{UV}$ and [DFe], the measured concentration in each perturbed mesocosm for each sampling date was subtracted from the average of concentrations in the 3 controls at that time-point. The relative mean deviation (MD, expressed as a %) for each treatment and time-point was obtained by dividing that value by the average concentration in the 3 controls. A simple linear regression model was used to test MD variations as a function of delta pCO$_2$, which corresponds to the difference of pCO$_2$ level between the treatments and the 3 controls at each time-point. A significant linear trend between MD and pCO$_2$ was considered when $p_{value} < 0.05$ (Fisher test). Above this threshold, we assumed that acidification had no linear effect on nutrient dynamics. All regressions were conducted using the R software.
3. Results

3.1. Initial conditions

Table 1 presents the initial conditions (day 0) of $pCO_2$ and $pH_T$ (on the total scale) in the nine mesocosms during both experiments. In BC, the initial $pCO_2$ in control mesocosms was $467 \pm 5$ µatm corresponding to a $pH_T$ of 8.02. At the start of the experiment, perturbed $pCO_2$ levels were close to the targeted levels and ranged from 609 to 1353 µatm (P1 to P6) corresponding to a $pH_T$ gradient from 7.92 to 7.61. In BV, initial $pCO_2$ levels in control mesocosms were lower than in BC, with an average $pCO_2$ of $358 \pm 17$ µatm corresponding to a $pH_T$ of 8.12. Initial $pCO_2$ levels in perturbed mesocosms were close to the targeted levels and ranged from 494 µatm (P1) to 1250 µatm (P6) corresponding to a $pH_T$ ranging from 8.0 to 7.63.

Initial nutrient and Chl$\alpha$ concentrations in BC and in BV inside control mesocosms and outside are presented in Table 2. In BC, [Chl$\alpha$] was higher outside ($0.12 \mu g L^{-1}$) than inside mesocosms ($0.06 \pm 0.01 \mu g L^{-1}$). The same holds true for [PO$_4^{3-}$] (35 vs $22 \pm 3$ nM). In control mesocosms, [DOP]$_{UV}$ was similar to [PO$_4^{3-}$] ($23 \pm 4$ nM), while lower values were measured outside ($\sim 16$ nM). DON$_{UV}$ represented the largest dissolved N pool with concentrations of 2 and $3.6 \pm 2.1 \mu M$ inside and outside control mesocosms respectively, while [NO$_x$] was lower with no difference between outside and inside control mesocosms ($0.05 \mu M$ vs $0.06 \pm 0.01 \mu M$).

In BV, [Chl$\alpha$] was much higher than in BC with initial values of $1.15 \pm 0.06 \mu g L^{-1}$ inside control mesocosms and $0.95 \mu g L^{-1}$ outside. This was also the case for [DOP]$_{UV}$ with values of $45 \pm 1$ nM inside control mesocosms and $48$ nM outside. Regarding [PO$_4^{3-}$], values were lower in BV than in BC and were similar inside and outside control mesocosms ($\sim 10$ nM). [DON]$_{UV}$ were also similar outside and inside control mesocosms at 4.0 and $3.6 \pm 0.7$
µM respectively. In contrast, [NO\textsubscript{x}] was higher in BV than in BC and a difference between OUT and control mesocosms was observed (1.17 \textit{vs} 0.13 \pm 0.03 \mu M). [DFe] was 1.2 nM inside and outside control mesocosms.

3.2. Environmental and experimental conditions during the summer experiment in the Bay of Calvi

In BC, in the perturbed mesocosms, as no further addition of CO\textsubscript{2}-saturated seawater was performed, \(p_{\text{CO}_2}\) levels slightly decreased after the acidification phase over the course of the experiment, especially for high CO\textsubscript{2} mesocosms (P5 and P6). During the experiment, mean \(p_{\text{CO}_2}\) levels were C1: 429, C2: 427, C3: 429, P1: 508, P2: 586, P3: 660, P4: 747, P5: 828, P6: 990 \mu atm. [Chl\textsubscript{a}] did not show any strong temporal trend, although levels slightly increased on day 10 to maximal concentrations of 0.09 \pm 0.003 \mu g L\textsuperscript{-1} on day 14 (Fig. 1).

With regard to the heterotrophic prokaryotes, the initial average abundance (day 0) in all mesocosms was 5.0 \pm 0.3 x 10\textsuperscript{5} cell mL\textsuperscript{-1}. Their density remained relatively constant during the experiment, and ranged from 4.0 x 10\textsuperscript{5} to 6.1 x 10\textsuperscript{5} cell mL\textsuperscript{-1}. The evolution of [PO\textsubscript{4}\textsuperscript{3-}], [NO\textsubscript{x}], [DOP]\textsubscript{UV} and [DON]\textsubscript{UV} in BC in all mesocosms and outside is shown in Figure 2.

After an initial decrease in [PO\textsubscript{4}\textsuperscript{3-}] inside and outside mesocosms between day 0 and day 1, [PO\textsubscript{4}\textsuperscript{3-}] remained low and ranged from 3 to 12 nM inside all mesocosms, and from 7 to 13 nM outside until day 20. Regarding DOP, a decrease was also observed between day 0 and day 1 outside mesocosms and in control mesocosms (no data available for perturbed mesocosms at day 0 except for P2). From day 1 to day 13, [DOP]\textsubscript{UV} varied between 5 and 20 nM with no significant trend, except for P1 where an increase from 8 to 16 nM (linear regression: \(r^2 = 0.89, n = 5, p_{\text{value}} = 0.02\)) was observed.

[NO\textsubscript{x}] increased from 47 \pm 14 nM to 97 \pm 22 nM in all mesocosms between day 0 and day 1, followed by a decrease in both inside and outside mesocosms until day 8 (linear
regression: $r^2 = 0.90$, $n = 8$, $p_{\text{value}} < 0.01$). Then $[\text{NO}_x]$ remained very low (8 ± 6 nM) until day 13 (except for P2 (28 ± 9 nM), and a peak (52 nM) observed for P1). While a gradual increase in $[\text{NO}_x]$ occurred for C2 and P6 from day 16 until the end of the experiment, two peaks were observed on day 16 in P1 and P4 (105 and 124 nM respectively), and a strong increase was observed on day 17 for C1, C3, P2, P3 and P5. At the end of the experiment, $[\text{NO}_x]$ averaged 60 ± 14 nM in all mesocosms. After a high variability of $[\text{DON}]_{\text{UV}}$ between mesocosms on day 0, the average $[\text{DON}]_{\text{UV}}$, inside all mesocosms, varied around 4.8 ± 0.3 µM over the course of experiment. A slight decrease between day 1 and day 13 could be observed in all mesocosms (linear regression: $r^2 = 0.83$, $n = 6$, $p_{\text{value}} = 0.01$).

3.3. Environmental and experimental conditions during the winter experiment in the Bay of Villefranche

In BV, drops in $p\text{CO}_2$ levels were more important than in BC due to strong winds with mesocosms P1 to P4 showing very similar levels by the end of the experiment. Mean $p\text{CO}_2$ levels were C1: 357, C2: 356, C3: 352, P1: 456, P2: 486, P3: 544, P4: 545, P5: 719, P6: 941 µatm. $[\text{Chl}a]$ averaged 0.98 ± 0.15 µg L$^{-1}$ in the nine mesocosms along the 12-day experiment. Chlorophyll $a$ remained slightly above levels in the surrounding waters for the entire experimental period, except for the last day (day 12) when concentrations increased abruptly outside the mesocosms. As one can see in Figure 1, $[\text{Chl}a]$ increased during the acidification phase, consuming a large proportion of available nutrients (see below), notably $\text{NO}_x$, before the start of the experimental phase (day 0). In all mesocosms, after this initial peak, $[\text{Chl}a]$ gradually decreased until the end of the experiment. While the abundance of the heterotrophic prokaryotes outside the mesocosms remained constant over the experiment (6.3 ± 0.3 x 10$^5$ cell mL$^{-1}$), it increased from day 2 to day 12 to reach 1.1 ± 0.1 x 10$^6$ cell mL$^{-1}$
inside all mesocosms. The initial average density of heterotrophic prokaryotes (day 0) was 7.3 ± 0.4 x 10^5 cell mL⁻¹.

[PO₄³⁻], [NO₃⁻], [DOP]UV, [DON]UV and DFe evolution in BV in all mesocosms and outside are shown in Figure 3. During the acidification phase, the increase in [Chl a] outside and inside mesocosms was concomitant with the decrease in [PO₄³⁻] (from 15 ± 2 to 10 ± 2 nM) and [NO₃⁻]. At day 0, while [NO₃⁻] outside mesocosms remained high (~1 µM), all mesocosms were depleted in NO₃ with an average concentration of 129 ± 30 nM.

No strong variations for [PO₄³⁻] were observed in the mesocosms over the course of the BV experiment (global average: 9 ± 1 nM). [PO₄³⁻] was generally higher outside than inside mesocosms and a peak of 23 nM was reached on day 6 followed by a decrease until the end of the experiment with similar inside and outside concentrations (12 nM at day 12). For the strongest perturbations (P4, P5 and P6), [DOP]UV dropped between day 0 and day 1, while [DOP]UV outside and in P2, P3 and control mesocosms tended to increase. [DOP]UV then remained stable (~44 ± 6 nM) in all mesocosms and outside until the end of the experiment.

Over the course of the BV experiment, [NO₃⁻] was much higher outside (range 889-1618 nM) than inside mesocosms (range 40-682 nM). Inside all mesocosms, [NO₃⁻] was stable until day 3 with an average concentration of 121 ± 50 nM. A general increase in [NO₃⁻] occurred between day 3 and day 9 (linear regression: r² = 0.95, n = 6, p-value < 0.001) with the largest rise observed in P4, P2 and C2. [NO₃⁻] in those mesocosms was the highest measured during the BV experiment (Fig. 4). The lowest [NO₃⁻] was measured in P1 and the standard deviation was the lowest in P6 indicating that [NO₃⁻] in that mesocosm was more stable than elsewhere (Fig. 4).

With respect to DON, concentrations were around 3.5 ± 0.7 µM inside and outside mesocosms and no clear trend could be observed, except for C1 and outside mesocosms.
where $[\text{DON}]_{\text{UV}}$ increased after day 1 until day 10 (linear regression C1: $r^2 = 0.86$, $n = 6$, $p_{\text{value}} = 0.02$; linear regression OUT: $r^2 = 0.82$, $n = 6$, $p_{\text{value}} = 0.03$).

In addition to NO$_x$, PO$_4^{3-}$, DON and DOP, [DFe] data were documented during the experiment in BV. [DFe] were similar outside and inside mesocosms ($2.3 \pm 0.8$ vs. $2.5 \pm 1.2$ nM, respectively). No clear trend was observed over the course of the experiment, although [DFe] slightly increased between day 3 and day 7 in all mesocosms.

### 3.4. Nutrient dynamics vs. $p\text{CO}_2$

Box-plots of inorganic and organic nutrient concentrations during both experiments are shown in Figure 4, and relative MD (see section 2.4) of each perturbed mesocosm during the entire experiments was plotted against delta $p\text{CO}_2$ for each perturbed mesocosm at each time-point, in Figure 5. During both experiments, [PO$_4^{3-}$] dynamics were not significantly related to $p\text{CO}_2$ levels. In addition, there was no significant relationship between $p\text{CO}_2$ and relative MD for PO$_4^{3-}$ (Fig. 5; BC: $p_{\text{value}} = 0.06$; BV: $p_{\text{value}} = 0.69$). The same holds true for [DOP]$_{\text{UV}}$ (Fig. 5; BC: $p_{\text{value}} = 0.97$; BV: $p_{\text{value}} = 0.33$) and [DON]$_{\text{UV}}$ in BC (Fig. 5; BC: $p_{\text{value}} = 0.35$). In BV, despite the significant relationship between relative MD for [DON]$_{\text{UV}}$ and delta $p\text{CO}_2$ ($p_{\text{value}}$ of 0.03), this relationship appears weak considering the large observed deviation between the 3 controls (Figs. 4 and 5). In BC, $p\text{CO}_2$ had no impact on [NO$_x$] (Fig. 5; $p_{\text{value}} = 0.31$). In BV, although box-plots for [NO$_x$] indicate a difference between controls, P2 and P4 on one side and P1, P3, P5 and P6 on the other side (Fig. 4), no significant effect of $p\text{CO}_2$ gradient on NO$_x$ dynamics was observed (Fig. 5; $p_{\text{value}} = 0.36$). Despite the fact that [DFe] was generally lower in CO$_2$-enriched mesocosms compared to controls (Fig. 4), the $p\text{CO}_2$ gradient did not significantly affect the evolution of [DFe] when considering the full duration of the experiment (Fig. 5; BV: $p_{\text{value}} = 0.91$).
4. Discussion

4.1. Representativeness of the environmental settings

The mesocosm experiments presented here focused on the impact of ocean acidification on plankton communities and are the first of such experiments to have been conducted in a low nutrient, low chlorophyll system. Our experimental protocol allowed for quantification of both inorganic and organic nutrient concentrations at nanomolar levels, and therefore allowed us to assess, in detail, temporal nutrient variability and contribution to the functioning of the community. Furthermore, this study provided the opportunity to compare nutrient levels measured at the two sites, with data compiled from the literature and obtained using both conventional (micromolar level) and nanomolar techniques (see Table 3). Finally, this compilation allowed us to put our results in a broader context of seasonal and spatial variability of nutrient levels in the Mediterranean Sea.

In the Bay of Calvi, the low initial in situ [NO₃⁻] (50 nM) was in agreement with [NO₃⁻] < 30 nM measured during a mesocosm experiment conducted in summer 2008 and 2010 in coastal waters of Corsica (Ridame et al., 2014). The low initial in situ [PO₄³⁻] (35 nM) was consistent with data reported in previous studies conducted in the same season (Table 3; 5-20 nM in surface waters). These nitrate and phosphate depleted conditions, observed at the start of this experiment, are representative of summer oligotrophic conditions in the Mediterranean Sea.

In the Bay of Villefranche, the initial in situ [NO₃⁻] (1.17 µM) was very close to the concentration (1.25 µM) measured during the same week at a sampling site located at the entrance of the Bay (Service d'Observation en Milieu LITToral; http://somlit.epoc.u-bordeaux1.fr/fr/). The same range [NO₃⁻ = 0.7-2.3 µM] was also found at that site in surface waters in February 2012 (Thyssen et al., 2014). [PO₄³⁻] can be variable in winter in surface
waters: ~10 nM in this study (but ~50 nM from SOMLIT, close to the detection limit using the conventional analytical method), from 25 to 92 nM using a conventional analytical method (Thyssen et al., 2014) and 7 nM using nanomolar techniques (Louis et al., 2015). Those concentrations were low compared to the ones measured at the open ocean DYFAMED site (43°25’N, 7°52’E): 200-300 nM during the winter-mixing period (January-February) followed by a decrease from 200 to 30 nM during the winter-spring transition (Pulido-Villena et al., 2010). While a NO₃-replete system outside mesocosms was in agreement with conditions usually reported for the Mediterranean Sea in winter, it must be stressed that this was no longer the case at the start of the experiment inside all mesocosms. Instead, a rapid NO₃ uptake by phytoplankton during the acidification phase (4 days before day 0; Gazeau et al., submitted, this issue-a) was concomitant with the increase in biomass in all mesocosms (Figs. 1 and 3). This led to a sharp drop in [NOₓ] from 0.96 to 0.13 µM. Although [NOₓ] outside mesocosms also slightly declined (from 1.6 to 1.2 µM) during that acidification period, [NOₓ] was maintained at a higher level due to a winter-mixing situation (the vertical profile of temperature from SOMLIT indicated a homogeneous water column over 200 m) and/or due to external input via atmospheric wet depositions (Migon, pers. comm.). Therefore, the conditions encountered at the start of the experimental phase were not fully representative of winter conditions for that area.

The stoichiometric nutrient balance [NOₓ] / [PO₄³⁻] can be used to depict the P or/and N (co)-limitation(s) for phytoplankton carbon fixation. In the Mediterranean Sea, below the mixed layer, [NOₓ] / [PO₄³⁻] is close to 22 (e.g. Mc Gill, 1965; Krom et al., 1991; Pujo-Pay et al., 2011) and higher than the Redfield ratio (N / P = 16). In deep waters, estimates are more robust as ratios are calculated based on [PO₄³⁻] and [NOₓ] well above the analytical detection limit (DL) of conventional analytical method (DL: [PO₄³⁻] ≤ 20-50 nM and [NOₓ] ≤ 20 nM).
In surface waters, reported ratios are much more variable: from 6 to 68 in the 0-150 m layer (Table 3). This reflects the difficulty to accurately measure nutrients with conventional methods in severely nutrient depleted waters, with concentrations often below DL during the stratification period (Pasqueron de Fommervault et al., 2015). Estimates of surface water ratios are thus questionable in terms of quality. In surface waters of the Bay of Calvi, using nanomolar techniques, we found an initial in situ $[\text{NO}_x]/[\text{PO}_4^{3-}]$ of 1.7 (Table 3), well below the Redfield ratio. Both low ratio and low nutrient concentrations suggest that this system experienced N and P co-limitation in summer, similarly to what was shown by Tanaka et al. (2011) in Svalbard. In winter in the Bay of Villefranche, initial in situ $[\text{NO}_x]/[\text{PO}_4^{3-}]$ was 177 due to high $[\text{NO}_x]$ and low $[\text{PO}_4^{3-}]$ at the start of the experiment. As mentioned above, NO$_x$ uptake occurred during the acidification phase, causing $[\text{NO}_x]$ to decrease to 129 ± 30 nM and leading to an average $[\text{NO}_x]/[\text{PO}_4^{3-}] = 13$ in all mesocosms. These conditions suggest a potential N and P co-limitation for the plankton community.

During both summer and winter experiments, the contribution of dissolved organic nutrients to the total dissolved phosphate and nitrogen pool was high. This is in agreement with previous studies in the Mediterranean Sea ([DON] / [TDN] = 60-100% and [DOP] / [TDP] = 30-100%; Table 3). Considering that organic forms of nutrients follow the same dynamics as DOM, which are known to accumulate in surface waters in the Northwestern Mediterranean Sea during the stratification period (Copin-Montegut and Avril, 1993), [DON] and [DOP] should peak in summer. In contrast, in our study, initial in situ [DON]$_{UV}$ and [DOP]$_{UV}$ were higher in winter than in summer. The large labile fraction of DOP (60-80%; Aminot and Kerouel, 2004; Lønborg and Alvarez-Salgado, 2012) and the oligotrophic nature of the Mediterranean Sea could explain a high turnover rate of DOP, thus preventing its accumulation in surface waters. Previous studies in the Sargasso Sea (Lomas et al., 2010), as
well as in an oligotrophic subtropical gyre (Steinberg et al., 2001), showed that [DOP] peaked during the winter/spring bloom and declined slowly throughout the summer resulting in enhanced P-stress. This was concomitant with an increase in the alkaline phosphatase activity (APA). Similarly, in the present study, APA was higher in summer than in winter (Celussi et al., in press, this issue), possibly explaining lower [DOP] in summer than in winter. At the start of the experiment in the Bay of Villefranche, in situ [DON]_{UV} and [DOP]_{UV} were close to reported concentrations for the Mediterranean Sea in winter (Table 2; Raimbault et al., 1999; Santinelli et al., 2002). In the Bay of Calvi, in situ [DOP]_{UV} was in the range (0.01 - 0.10 µM) of reported data in summer in surface waters of the Mediterranean Sea (Table 3). With respect to in situ [DON]_{UV}, the observed value of 2 µM was lower than concentrations reported previously for the western basin in winter (from 4.5 to 5.5 µM; Raimbault et al., 1999; Table 3).

In the Bay of Villefranche, initial in situ [DFe] (1.2 nM; Table 1) was lower than values reported in the Bay of Villefranche at the same season by Bressac and Guieu (2013; 3.8 nM at 5 m) and, instead, more similar to winter offshore concentrations (DYFAMED site, 0.81 ± 0.07 nM; Bonnet and Guieu, 2006). The annual [DFe] dynamics in surface waters in the Northwestern Mediterranean Sea are controlled by several factors such as atmospheric deposition, winter mixing, phytoplankton uptake, and particle scavenging. Over an annual time scale, [DFe] ranged from 0.2 to 1.2 nM at DYFAMED site (Bonnet and Guieu, 2006). Minimal values were measured when [Chla] was the highest, as a consequence of biological uptake and removal by the sinking particles. Under stratified conditions, atmospheric deposition results in an accumulation of [DFe] in surface waters that is isolated from deeper layers (Bonnet and Guieu, 2006; Guieu et al., 2010). Because atmospheric inputs, such as Saharan dust deposition, have high interannual variability (Guieu et al., 2014 and ref within),
[DFe] concentrations in surface waters are likely also highly variable from one year to another. This could explain the different [DFe] observed in the Bay of Villefranche at similar season. At the start of the winter experiment in the Bay of Villefranche, [DFe] in surface waters was reflective of [DFe] found in the open western Mediterranean Sea in winter.

4.2. Nutrient dynamics: Biological vs. Acidification effect

During both experiments, results showed that none of the parameters investigated in this study were linearly related to CO₂ enrichment (Fig. 5). These two experiments, conducted in waters depleted in PO₄³⁻, showed low variations in PO₄³⁻ over the experimental period and no significant differences in PO₄³⁻ dynamics over the different pCO₂ conditions. With respect to NO₃, concentrations followed the same temporal trend with no significant differences between control and perturbed mesocosms (Figs. 4 and 5). In the Bay of Villefranche, during the acidification phase, although targeted pCO₂ conditions were not yet reached, a drop in NO₃ observed in all mesocosms suggested that NO₃ uptake by phytoplankton was not impacted by an increase in pCO₂ in the perturbed mesocosms. During the summer and winter experiments, a trend in DON_{UV} and DOP_{UV} was now observed, and concentrations were not affected by acidification (Fig. 5). Although a decrease in pH would favor the solubility of DFe (Breitbarth et al., 2010a), DFe concentrations did not increase with increasing pCO₂ (Fig. 5). As such, it appears that both direct chemical and indirect biological processes (further discussed in this section) that controlled nutrient dynamics during these experiments were independent of pCO₂ conditions.

Previous mesocosm experiments, similar to the ones presented here, focused on the effect of ocean acidification on plankton communities but were performed under nutrient replete conditions using artificial nutrient addition. For instance, in the framework of the Pelagic Ecosystem CO₂ Enrichment (PeECE) project, three land-based mesocosm
experiments were conducted in Bergen (Norway), in 2001, 2003 and 2005 (PeECE I, II and III, respectively). In 2001 and 2003, three treatments represented low (190 µatm), present-day (370-410 µatm) and high (700 µatm) \( pCO_2 \) conditions. In PeECE III, there was one present-day condition and two high \( pCO_2 \) levels (350, 700 and 1050 µatm). After nutrient addition, \([NO_x]_{\text{initial}}\) and \([PO_4^{3-}]_{\text{initial}}\) were 15 and 0.5 µM respectively during PeECE I, 9 and 0.4 µM respectively during PeECE II, and 16 and 0.8 µM respectively during PeECE III. During all three experiments, and in agreement with our results, \( NO_x \) and \( PO_4^{3-} \) uptake were similar in all mesocosms, indicating that phytoplankton nutrient uptake was insensitive to changes in seawater pH (Engel et al., 2005 and 2008; Schulz et al., 2008; Bellerby et al., 2008). As similar increases in DOC, DON and DOP were observed in all PeECE mesocosms, this further suggests that acidification, as observed in our experiments, did not impact the production or consumption of dissolved organic nutrients. In contrast to the lack of \( pCO_2 \) effects on inorganic and organic macronutrients, Breitbarth et al. (2010b) have shown, during the PeECE III experiment and in agreement with previous studies (e.g. Liu and Millero, 2002; Millero et al., 2009), that ocean acidification may lead to enhanced Fe-bioavailability due to (1) an increase in the organically complexed Fe (III) and (2) an increase in Fe(II) concentrations. This is not consistent with data acquired during our experiment in the Bay of Villefranche where [DFe] in high-CO\(_2\) mesocosms were not significantly higher than in low-CO\(_2\) mesocosms.

More recently, a large off-shore mesocosm experiment has been conducted in the Arctic ocean (Svalbard) following a \( pCO_2 \) gradient ranging from 185 to 1420 µatm. This study has shown that, before nutrient addition (\([NO_x] = 0.1 \mu M \) and \([PO_4^{3-}] = 0.06-0.09 \mu M\)), in agreement with our results, organic and inorganic nutrient dynamics were similar in all \( pCO_2 \) treatments (Schulz et al., 2013). During the phytoplankton growth stimulated by nutrient addition (after day 13, \([NO_x]_{d13} = 5.5 \mu M \) and \([PO_4^{3-}]_{d13} = 0.4 \mu M\)), while the same
dynamics in DON and DOP were observed in all mesocosms, inorganic nutrient uptake ($NO_x$ and $PO_4^{3-}$) was faster at higher CO$_2$ levels indicating a shift in phytoplankton community composition (Schulz et al., 2013), a shift that was not observed during our experiments (Gazeau et al. submitted, this issue-b).

Among the very few bottle experiments focused on the effect of ocean acidification on planktonic communities under low nutrient conditions, results of Yoshimura et al. (2010; $[NO_x]_{initial} = 0.05 \pm 0.02 \mu M$ and $[PO_4^{3-}]_{initial} = 0.25 \pm 0.01 \mu M$) support our conclusion that under limiting conditions, nutrient dynamics are not dependent on $pCO_2$ levels.

In our study, nutrient measurements were performed at nanomolar levels providing greater detail to assess temporal dynamics. As nutrients dynamics did not differ between $pCO_2$ levels, the nine mesocosms can be considered as replicate measurements. As such, daily average nutrient concentrations were calculated (Figs. 6, 7 and 8) providing insight to the relationship between nutrient dynamics and major biological parameters (chlorophyll $a$, particulate organic carbon, organic matter) and processes (production and degradation, heterotrophic enzymatic activities, nitrification). Such stocks and fluxes, measured during the experiments, are presented and discussed in details in companion papers (Gazeau et al., submitted, this issue-b; Maugendre et al., in press, this issue-a; Celussi et al., in press, this issue).

As a brief summary of the companion papers, during the summer experiment in the Bay of Calvi, CO$_2$ enrichment did not have any significant effect on primary production (Maugendre et al. in press, this issue-a), abundance and activity of heterotrophic prokaryotes (Celussi et al., in press, this issue), as well as Chla concentrations and phytoplankton community composition (Gazeau et al., submitted, this issue-b). The summer plankton community was mostly composed of small species such as haptophytes and cyanobacteria.
(Gazeau et al., submitted, this issue-b), dominated by mixotrophs or heterotrophs. While the carbon biomass of autotrophs was very low (from 0.009 ± 0.003 to 0.022 ± 0.009 µmol C L^{-1}; based on compound specific $^{13}$C incorporation; Maugendre et al., in press, this issue-b), mixotrophs represented the most productive group with an increasing biomass over the experimental period (from 0.05 ± 0.01 to 0.08 ± 0.03 µmol C L^{-1}). The low biomass of autotrophs determined by Maugendre et al. (in press, this issue-b) was consistent with the low average [Chl$a$] measured in the Bay of Calvi (from 0.06 to 0.08 µg L^{-1}; Fig. 6). Despite a low autotrophic production, [NO$_x$] decreased by 50% throughout the experiment and [PO$_4^{3-}$] decreased during the first day and then slightly decreased until day 7 (Fig. 6). In such a nutrient-depleted system, haptophytes, considered as mixotrophic, are known to be good competitors for low nutrient resources (Sanders, 1991). The ratio between uptake rates of NO$_x$ (12.8 nmol N L$^{-1}$ d$^{-1}$) and PO$_4^{3-}$ (0.5 nmol P L$^{-1}$ d$^{-1}$), determined from the slope of NO$_x$ and PO$_4^{3-}$ decreases between day 1 and day 7, was around 25. This is close to the typical [NO$_x$] / [PO$_4^{3-}$] in Mediterranean waters below the mixed layer (≈22). Despite a slight decrease in [DON]$_{UV}$, an uptake of DOP was not observed (except between day 0 and day 1). This was unexpected since the gradual increase of the alkaline phosphatase activity (APA) observed until day 12 (on average 169 nM h$^{-1}$; Celussi et al., in press, this issue) should be concomitant with a decrease in [DOP] (i.e., Ruttenburg and Dyrhman, 2002; Mahaffey et al., 2014; Fig. 7). It could be due to either the low [DOP]$_{UV}$ during this experiment and/or a rapid turnover of DOP (Benitez-Nelson and Buesseler, 1999). Towards the end of the experiment, [NO$_x$] raised while [PO$_4^{3-}$] remained stable (Fig. 6). Excluding the external inputs in the mesocosms by UV-transparent roofs covering them, the release of NO$_x$ would result from bacterial remineralization.

In the Bay of Villefranche, as already mentioned, a rapid nutrient uptake by phytoplankton was observed during the acidification phase, concomitant with the increase in
biomass (Fig. 6). After this [Chlα] increase (and [NO₃] drop) in all mesocosms, the biomass of the autotrophic community decreased (from 1.1 to 0.9 µg L⁻¹) while the abundance of heterotrophic prokaryotes increased in all mesocosms (from 7.3 x 10⁵ cell mL⁻¹ to 1.1 x 10⁶ cell mL⁻¹; Fig. 6). From day 3, an increase in [NO₃] occurred in all mesocosms that was likely linked to enhanced heterotrophic prokaryotes activity (Fig. 6). It is possible that the increase in [NO₃] of ~18 nmol N L⁻¹ d⁻¹ was induced by nitrification activity estimated to be ~19 nmol N L⁻¹ d⁻¹ (Al-Moosawi, pers. comm.) although no negative correlation between [NH₄⁺] and [NO₃] could be observed (Fig. 8b). Nitrification could have been preceded by ammonification of the DON pool produced from the degradation of particulate organic matter by heterotrophic prokaryotes. This could explain the negative correlation between particulate organic carbon concentrations ([POC]) and [NO₃] (Fig. 8c), while [DON] remained stable (Fig. 8a; sampling and analytical protocols for POC are described in Gazeau et al., submitted, this issue-b). The decrease in POC could also be due to the phytoplankton cell lysis under experimental stress (e.g. water column trapped in mesocosm). The large amount of released nutrient-rich organic matter resulted in an increase in bacterial abundance and heterotrophic activity. Ultimately, regenerated inorganic nutrients such as NH₄⁺ and NO₃ by nitrification were produced (Al-Moosawi, pers. comm.). While the heterotrophic activity based on the regenerated production was usually higher in summer, the increase in phytoplankton biomass (mostly composed of small species such as cryptophyceae and haptophyceae; Gazeau et al. submitted, this issue-b) at the start of experiment in the Bay of Villefranche allowed an increase in the abundance and activity of heterotrophic prokaryotes (see Celussi et al., in press, this issue, for further details).

Finally, in the Bay of Villefranche, in relation to the increase in heterotrophic activity and nitrification, [DFe] increased significantly (p-value < 0.001) from day 3 to day 7 (Fig. 9a). This could be the result of either an enhancement of Fe solubility by organic ligands exuded...
by phytoplankton or the release of DFe in seawater after cell death (Witter et al., 2000) while 
[Chla] decreased. A negative relationship between [DFe] and [Chla] (p value = 0.001) was 
indeed observed between day 3 and day 7 (Fig. 9b). Although [Chla] continued to decline 
until day 11, [DFe] did not increase after day 7. Finally, other biotic and abiotic processes 
such as scavenging and/or aggregation (Johnson et al., 1997), that were not explicitly studied 
here, could have explained this decrease in [DFe] observed at the end of the experiment.
5. Conclusion

These first ocean acidification mesocosm experiments conducted in coastal LNLC areas allowed for investigation of the effect of ocean acidification on nutrient dynamics using nanomolar techniques. While the summer experiment was fully representative of oligotrophic summer conditions in the Mediterranean Sea, the winter experiment failed to reproduce the mesotrophic conditions typical of the winter time. Instead post-bloom conditions were also encountered, with high concentrations of phytoplankton exudates and low nutrient concentrations.

Based on these two experiments, ocean acidification did not affect the dynamics of nutrients both under inorganic and organic forms: (1) the concentration of dissolved organic nutrients remained stable regardless of $p$CO$_2$ conditions, (2) the expected increase in iron solubility at high $p$CO$_2$ was not observed and (3) the dynamics of NO$_x$ and PO$_4^{3-}$ were controlled by the biological processes independently of $p$CO$_2$ conditions.

Although the use of nanomolar techniques during these experiments facilitated documentation of nutrient dynamics with great precision, the direct measurement of the turnover times of P and N would provide a better view on these dynamics resulting from biological uptake. Such measurement would improve the assessment of ocean acidification impacts on plankton nutrient uptake, particularly in summer, when the turnover time of P is very low (1-2 h; Moutin et al., 2002b). As such, we recommend the measurement of nutrient turnover rates over nanomolar measurements in future mesocosm studies conducted in oligotrophic areas.

This absence of $p$CO$_2$ effects on the bioavailability of nutrients in low nutrient and low chlorophyll waters suggests that there will be no direct positive or negative feedback by the biological pump in response to the rise in atmospheric CO$_2$. However, other changes
projected with climate change, such as the increase in water column stratification, seawater temperature, extreme events occurrence, could induce shifts in the nutrient stoichiometry with important impacts on the diversity of the natural plankton assemblage. The response of marine ecosystems to on-going environmental changes in oligotrophic areas should be tackled using multi-stressor approaches (such as combining ocean acidification, warming and atmospheric deposition) in future investigations.
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Krom, M.D., E.M.S. Woodward, B. Herut, N. Kress, P. Carbo, R.F.C. Mantoura, G. Spyres,


Figure captions

**Figure 1:** Evolution of the concentration in chlorophyll a (Chla) and the abundance of heterotrophic prokaryotes (hetero. prokaryotes) along the course of the experiments in the Bay of Calvi (summer 2012) and Villefranche (winter 2013) outside and inside all mesocosms. The period between day -4 and day 0 corresponds to the acidification phase.

**Figure 2:** Evolution of the concentrations of inorganic and organic nutrients (nitrate + nitrite: NO$_x$, phosphate: PO$_4^{3-}$, dissolved organic nitrogen: DON and dissolved organic phosphorus: DOP) outside (‘OUT’) and inside all mesocosms along the course of the experiment in the Bay of Calvi in summer 2012.

**Figure 3:** Evolution of the concentrations of inorganic and organic nutrients (dissolved iron: DFe, nitrate + nitrite: NO$_x$, phosphate: PO$_4^{3-}$, dissolved organic nitrogen: DON and dissolved organic phosphorus: DOP) outside (‘OUT’) and inside all mesocosms along the course of the experiment in the Bay of Villefranche in winter 2013. The period between day -4 and day 0 corresponds to the acidification phase.

**Figure 4:** Box-plots of nutrient concentrations (dissolved iron: DFe, nitrate + nitrite: NO$_x$, phosphate: PO$_4^{3-}$, dissolved organic nitrogen: DON and dissolved organic phosphorus: DOP) measured during the experiments in the Bay of Calvi (BC) and in the Bay of Villefranche (BV). The statistical profile for “Control” was obtained as the daily mean value of the 3 controlmesocosms (C1, C2 and C3). The box-plot corresponds to 90% of data and the straight dark line represents the median value. The circles are the values considered statistically as outliers.

**Figure 5:** Relative mean deviation (MD; in %) of the concentrations of inorganic and organic nutrients as a function of delta $p$CO$_2$ (µatm) for each mesocosm at each time-point along the
course of both experiments in the Bay of Calvi (BC) and in the Bay of Villefranche (BV). For each sampling date, the measured concentration in each perturbed mesocosm was subtracted from the arithmetic mean of concentrations in the 3 controls. The relative MD was estimated the ratio of this value to the mean concentration in the 3 control and expressed as a percentage. Delta $pCO_2$ represents the difference in $pCO_2$ level between the various treatments and the 3 controls for each time-point. Horizontal red lines correspond to the mean relative standard deviation of the 3 controls for each parameter over the duration of each experiment.

**Figure 6:** Average (all mesocosms) concentrations of chlorophyll $a$ (Chl$a$; green line), nitrate + nitrite ($NO_x$; red line), phosphate ($PO_4^{3-}$; orange line) and heterotrophic prokaryotes (hetero. prokaryotes, grey line) along the course of the both experiments in the Bay of Calvi (BC) and in the Bay of Villefranche (BV). The color areas correspond to the range from minimum to maximum of [Chl$a$], [$NO_x$], [$PO_4^{3-}$] and hetero. prokaryotes measured. The period between day -4 and day 0 corresponds to the acidification phase.

**Figure 7:** Comparison of the evolution of the concentration of dissolved organic phosphorus (DOP) and the alkaline phosphate activity (APA) halfway through the experiment in the Bay of Calvi (BC). Sampling and analytical protocol for APA are described in Celussi et al. (in press, this issue).

**Figure 8:** Nitrate + nitrite ([NO$_x$]) vs dissolved organic nitrogen ([DON]) (a), [NO$_x$] vs ammonium ([NH$_4^+$]) (b), and [NO$_x$] vs particulate organic carbon ([POC]) (c) during the experiment in the Bay of Villefranche (BV). Points represent all mesocosms data between day 3 and day 10. The red line corresponds to the linear regression between [NO$_x$] and [POC] ($r^2 = 0.72$, p-value < 0.001).
**Figure 9:** Box-plot of the dissolved iron concentrations ([DFe]) for all mesocosms along the course of the experiment conducted in the Bay of Villefranche in winter 2013. The red line represents the linear regression of [DFe] (without outlier values) as a function of time between day 3 and day 7 ($r^2 = 0.62$ and $p_{value} < 0.001$) (a). Regression linear between [DFe] and the concentration of chlorophyll $a$ ([Chla]) between day 3 and day 7 ($r^2 = 0.24$ and $p_{value} = 0.001$) (b).
Table 1. Initial (day 0) $p$CO$_2$ (µatm) and pH$_T$ (total scale) conditions inside the nine mesocosms during the experiments in the Bay of Calvi in summer 2012 and in the Bay of Villefranche in winter 2013.

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<td>8.02</td>
<td>7.92</td>
<td>7.85</td>
<td>7.82</td>
<td>7.76</td>
<td>7.66</td>
<td>7.61</td>
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<tr>
<td>Bay of Villefranche</td>
<td>378</td>
<td>347</td>
<td>350</td>
<td>494</td>
<td>622</td>
<td>690</td>
<td>743</td>
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<td>$p$CO$_2$</td>
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<td>8.13</td>
<td>8.13</td>
<td>8.00</td>
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<td>7.87</td>
<td>7.85</td>
<td>7.76</td>
<td>7.63</td>
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</table>
Table 2. Initial (day 0) nutrients (dissolved iron: DFe, nitrate + nitrite: NO$_x$, phosphate: PO$_4^{3-}$, dissolved organic nitrogen: DON and dissolved organic phosphorus: DOP) and chlorophyll a (Chl$_a$) concentrations inside the 3 ‘control’ mesocosms (average and standard deviation calculated from the three independent measurements) for the Bay of Calvi (BC) and the Bay of Villefranche (BV) experiments. (*) When data on day 0 were not available, values have been measured on day 1.

<table>
<thead>
<tr>
<th>Mesocosm Experiment</th>
<th>[PO$_4^{3-}$]</th>
<th>[NO$_x$]</th>
<th>[DOP]</th>
<th>[DFe]</th>
<th>[DON]</th>
<th>[Chl$_a$]</th>
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<tr>
<td></td>
<td>nM</td>
<td>µM</td>
<td>nM</td>
<td>nM</td>
<td>µM</td>
<td>µg L$^{-1}$</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BC</td>
<td>35</td>
<td>0.05</td>
<td>16</td>
<td>2</td>
<td>0.12</td>
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</tr>
<tr>
<td>BV</td>
<td>10</td>
<td>1.17 *</td>
<td>48</td>
<td>1.2 *</td>
<td>4</td>
<td>0.95</td>
</tr>
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<td>Mean control</td>
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<td></td>
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<tr>
<td>BC</td>
<td>22 ± 3</td>
<td>0.06 ± 0.01</td>
<td>23 ± 4</td>
<td>3.6 ± 2.1</td>
<td>0.06 ± 0.01</td>
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</tr>
<tr>
<td>BV</td>
<td>10 ± 2</td>
<td>0.13 ± 0.03</td>
<td>45 ± 1</td>
<td>3.6 ± 0.7</td>
<td>1.15 ± 0.06</td>
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</tbody>
</table>
Table 3. Nitrate + nitrite (NO$_x$), phosphate (PO$_4^{3-}$), dissolved organic and total nitrogen (DON and TDN, respectively) and dissolved organic and total phosphate (DOP and TDP, respectively) concentrations and their ratio reported in the literature for the Mediterranean Sea. Values measured in this study correspond to data outside mesocosms at the beginning of both experiments. Several methods were used to DOP and DON: a) photo-oxidation UV; b) high temperature combustion; c) persulfate wet oxidation; d) UV-peroxide oxidation.

<table>
<thead>
<tr>
<th>Area</th>
<th>Study</th>
<th>Period</th>
<th>Depth</th>
<th>[NO$_x$]</th>
<th>[PO$_4^{3-}$]</th>
<th>[DON]</th>
<th>[DOP]</th>
<th>[NO$_x$]</th>
<th>[DOP]</th>
<th>[DON]</th>
<th>[DOP]</th>
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<tr>
<td>Western basin</td>
<td>This study</td>
<td>June 2012</td>
<td>0-10 m</td>
<td>0.05</td>
<td>0.03</td>
<td>2$^a$</td>
<td>0.02$^a$</td>
<td>1.7</td>
<td>100</td>
<td>98</td>
<td>40</td>
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<td></td>
<td></td>
<td>February 2013</td>
<td>0-10 m</td>
<td>1.17</td>
<td>0.01</td>
<td>4$^a$</td>
<td>0.05$^a$</td>
<td>117</td>
<td>80</td>
<td>78</td>
<td>83</td>
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<td></td>
<td>Coste et al. (1988)</td>
<td>Oct-Nov. 1981</td>
<td>5 m</td>
<td>0.8</td>
<td>0.08</td>
<td>6.3d</td>
<td>0.13d</td>
<td>10</td>
<td>48</td>
<td>89</td>
<td>62</td>
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<td></td>
<td>Aminot and Kerouel (1999)</td>
<td>Sept. 1984</td>
<td>0-15 m</td>
<td>nd</td>
<td>nd</td>
<td>4.0-4.2$^c$</td>
<td>0.08$^d$</td>
<td>nd</td>
<td>55-57</td>
<td>nd</td>
<td>nd</td>
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<td></td>
<td>Rimbault et al. (1999)</td>
<td>Nov. 1997-March 1998</td>
<td>0-160 m</td>
<td>nd</td>
<td>nd</td>
<td>4.5-5.5$^c$</td>
<td>0.06-0.1$^c$</td>
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<td>43-88</td>
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<td>Diaz et al. (2001)</td>
<td>March-April 1998</td>
<td>0-150 m</td>
<td>0.50-1.80</td>
<td>&lt; 0.04</td>
<td>4.32 ± 0.42</td>
<td>0.08 ± 0.02</td>
<td>68.4</td>
<td>75</td>
<td>70-90</td>
<td>&gt; 70</td>
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<tr>
<td></td>
<td>Moutin and Raimbault (2002a)</td>
<td>May-June 1996</td>
<td>0-100 m</td>
<td>1.2 ± 1.4</td>
<td>0.05 ± 0.05</td>
<td>4.6 ± 0.6$^c$</td>
<td>0.08 ± 0.02$^c$</td>
<td>~22</td>
<td>58</td>
<td>-80</td>
<td>62</td>
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<td></td>
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<td>&lt; 30 m</td>
<td>&lt; 0.02</td>
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<td>nd</td>
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<td>nd</td>
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<td>Year</td>
<td>Depth Range</td>
<td>Chlorophyll a</td>
<td>Nitrate</td>
<td>Silicate</td>
<td>Temperature</td>
<td>Salinity</td>
<td>Oxygen</td>
<td>Nitrogen</td>
<td>Phosphorus</td>
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<td>Lucea et al. (2003)</td>
<td>Sept. 1996</td>
<td>0-50 m</td>
<td>&lt; 0.5</td>
<td>&lt; 0.05</td>
<td>5-6</td>
<td>0.02-0.1</td>
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<tr>
<td>Pujo-Pay et al. (2011)</td>
<td>July 2008</td>
<td>0-100 m</td>
<td>0-5.84</td>
<td>0-0.17</td>
<td>4.1-5.5c</td>
<td>0.02-0.09c</td>
<td>29</td>
<td>84</td>
<td>~70</td>
<td>~50</td>
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<td>&lt; 20 m</td>
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<td>&lt; 0.6</td>
<td>&lt; 0.02</td>
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<td>Ridame et al. (2014)</td>
<td>June 2008-2010</td>
<td>0-10 m</td>
<td>&lt; 0.03</td>
<td>0.005 ± 0.003</td>
<td>nd</td>
<td>nd</td>
<td>&lt; 6</td>
<td>nd</td>
<td>nd</td>
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<td>Thyssen et al. (2014)</td>
<td>February 2012</td>
<td>1 m</td>
<td>0.7-2.3</td>
<td>0.025-0.092</td>
<td>nd</td>
<td>nd</td>
<td>15-63</td>
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<td>Eastern basin</td>
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<tr>
<td>Moutin and Raimbault (2002a)</td>
<td>May-June 1996</td>
<td>0-100 m</td>
<td>0.3 ± 0.7</td>
<td>0.01 ± 0.02</td>
<td>4.5 ± 0.5c</td>
<td>0.06 ± 0.03c</td>
<td>42</td>
<td>75</td>
<td>~94</td>
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<td>&lt; 80 m</td>
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<td>&lt; 0.05</td>
<td>&lt; 0.02</td>
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<td>Krom et al. (2005)</td>
<td>May 2002</td>
<td>0-120 m</td>
<td>&lt; 0.001-0.01</td>
<td>&lt; 0.002-0.004</td>
<td>3-11b</td>
<td>0.05a</td>
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<td>~100</td>
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<td>Pujo-Pay et al. (2011)</td>
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<td>0-100 m</td>
<td>0-3.45</td>
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<td>3.5-6.3c</td>
<td>0.01-0.10c</td>
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<td>~90</td>
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<td>Santinelli et al. (2012)</td>
<td>Sept. 2007</td>
<td>0-75 m</td>
<td>0.01-1.47</td>
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<td>2.5-6.9a</td>
<td>0.03-0.07a</td>
<td>23</td>
<td>92</td>
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<td>Jan. 2008</td>
<td>0-200 m</td>
<td>0.2-1.2</td>
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<td>25</td>
<td>82</td>
<td>80-60</td>
<td>50-30</td>
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</tbody>
</table>
Figure 1:

Bay of Calvi

Bay of Villefranche

Figure 2:
Figure 3:
Figure 4:
Figure 5:

Bay of Calvi | Bay of Villefranche

- MD [NO₃] %
- MD [PO₄³⁻] %
- MD [DON] %
- MD [DOP] %
- MD [DFe] %
Figure 6:

Summer (Bay of Calvi)

- [NO$_3$] nM
- [PO$_4$] nM
- Hetero.prokaryotes cell mL$^{-1}$

Winter (Bay of Villefranche)

- [Chla] µg L$^{-1}$
- [NO$_3$] nM
- [PO$_4$] nM
- Hetero.prokaryotes cell mL$^{-1}$

Figure 7:

[DOP] nM

APA nM h$^{-1}$
Figure 8:

- (a) Scatter plot showing [DON] μM vs. Day.
- (b) Scatter plot showing [NH₄⁺] μM vs. Day.
- (c) Scatter plot showing [POC] μM vs. Day. The linear equation is given as:
  \[ y = -74.6x + 1064.6 \]
  - \( p_{\text{value}} < 0.001 \)
  - \( n = 24 \)
  - \( r^2 = 0.72 \)

Figure 9:

- (a) Box plot showing [DFe] nM vs. Day.
  - \( p_{\text{value}} < 0.001 \)
  - \( n = 39 \)
  - \( r^2 = 0.62 \)
  - \( y = 0.53x - 0.39 \)
- (b) Scatter plot showing [Chla] μg L⁻¹ vs. Day. The linear equation is given as:
  \[ y = -4.63x + 6.86 \]
  - \( p_{\text{value}} = 0.001 \)
  - \( n = 39 \)
  - \( r^2 = 0.24 \)