

⁶CNRS, UMR 7621, Laboratoire d'Océanographie Microbienne, Observatoire Océanologique de Banyuls-sur-Mer, 66650 Banyuls-sur-Mer, France

⁷Université Pierre et Marie Curie-Paris 6 (UPMC), UMR 7621, 66650 Banyuls-sur-Mer, France

Received: 7 March 2011 – Accepted: 9 March 2011 – Published: 15 March 2011

Correspondence to: C. Ridame (celine.ridame@upmc.fr)

Published by Copernicus Publications on behalf of the European Geosciences Union.

BGD

8, 2629–2657, 2011

Nutrient control of N₂ fixation in the Mediterranean Sea

C. Ridame et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Abstract

A better understanding of the factors controlling N_2 fixation is a pre-requisite for improving our knowledge on the contribution of N_2 fixation in the nitrogen cycling in the Mediterranean Sea. Trace-metal clean nutrient/dust additions bioassays (+P, +PFe, +dust) were performed at three stations located in the western, central and eastern Mediterranean Sea, in summer 2008 as part of the BOUM cruise. The main goals were to investigate the nutrient factor(s) limiting N_2 fixation (uptake of $^{15}N_2$) and to evaluate the potential impact of a Saharan dust event on this biological process during the stratification period. Initially, surface waters at the three stations were DIP-depleted (<10 nM) while the DFe concentrations were relatively high (from 1.2 to 2.3 nM) most likely due to atmospheric iron accumulation in the surface mixed layer. At all stations, Saharan dust input relieved the ambient nutrient limitation of diazotrophic activity as demonstrated by the strong stimulation of N_2 fixation (from x2.3 to x5.3). The highest dust stimulation of N_2 fixation was recorded at the station located in the eastern basin (x5.3). The responses of diazotrophic activity to nutrients addition were contrasted at the sampled stations suggesting a spatial variability of the factor controlling N_2 fixation over the whole basin. At all stations, N_2 fixation was not limited by Fe nor co-limited by P and Fe. At the western station, N_2 fixation was DIP limited while at the eastern one, N_2 fixation was first DIP limited then was limited by one or several chemical element(s) released by dust. Our results demonstrated that a Saharan dust input was able to relieve the successive on-going N_2 fixation limitations. Very interestingly, at the station located in the central basin, N_2 fixation was not limited by the availability of P yet it was strongly stimulated by dust additions (up to x3.1). A chemical element or a combination of several, released by the added dust may have been responsible for the observed stimulations of N_2 fixation. These results indicated that Saharan dust pulses to the surface Mediterranean waters, in addition to P and Fe, could be a source of chemical(s) element(s) that are necessary for metabolic processes and therefore influence rates of N_2 fixation.

Nutrient control of N_2 fixation in the Mediterranean Sea

C. Ridame et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



1 Introduction

The Mediterranean Sea is one of the most oligotrophic areas of the world's ocean (Krom et al., 2004; Bosc et al., 2004) characterized by a strong gradient of nutrients and primary production decreasing eastward (Béthoux et al., 1998; Moutin and Raimbault, 2002; Pujo-Pay et al., 2010; Lagaria et al., 2010). Based on the short dissolved inorganic phosphorus (DIP) turnover times, the Mediterranean Sea can be considered as a Low Phosphate Low Chlorophyll (LPLC) environment (Moutin et al., 2008). Another interesting feature is the unusually high dissolved inorganic N:P ratios of 22–23:1 and 28–30:1 for the western and eastern basins respectively (Marty et al., 2002; Pujo-Pay et al., 2010) which may result from an intense N₂ fixation (Gruber and Sarmiento, 1997; Ribera d'Alcala et al., 2003). This hypothesis is still under debate as recent direct measurements of N₂ fixation rates showed very low values through the open Mediterranean Sea (Ibello et al., 2010; Bonnet et al., 2011) indicating that N₂ fixation may represent a low source of external nitrogen input at annual scale (Krom et al., 2010). At the north-western DYFAMED site, N₂ fixation fluxes ranged from 0.5 to 7.5 nmol N L⁻¹ 12 h⁻¹ over a year, demonstrating a seasonal variability with the highest values recorded in surface during spring and summer (Garcia et al., 2006; Sandroni et al., 2007; Marty et al., 2008). Apart from the extremely high N₂ fixation rate (129 nmol N L⁻¹ d⁻¹) measured in spring in the Eastern basin (Cyprus eddy, Rees et al., 2006), N₂ fixation rates in the Mediterranean Sea are low when compared to other oligotrophic environments (see review in a companion paper, Bonnet et al., 2011).

Most of the Mediterranean studies focusing on diazotrophs reported low abundance of these organisms. While *Trichodesmium sp.* and *Richelia sp.* (symbionts hosted within diatoms) are known to form massive blooms in the Atlantic and the Pacific Oceans (Capone et al., 1997; Carpenter et al., 1999), only low concentrations of these diazotrophic filamentous cyanobacteria have been mentioned in the Mediterranean Sea (Bar-Zeev et al., 2008; Le Moal and Biegala, 2009; Crombet et al., 2011; Yogevev et al., 2011). The presence of smaller unicellular diazotrophs affiliated to Archaea,

BGD

8, 2629–2657, 2011

Nutrient control of N₂ fixation in the Mediterranean Sea

C. Ridame et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Proteobacteria and Cyanobacteria was recently reported in the eastern basin (Man-Aharonovich et al., 2007; Yogev et al., 2011). Within the western basin, at a coastal site, communities of diazotrophic cyanobacteria were largely dominated annually by picoplankton ($<3\mu\text{m}$) as free living cells. Despite their low concentrations throughout most of the year, these organisms were able to reach significant abundance in summer (Le Moal and Biegala, 2009).

The recognition of the importance of N_2 fixation in the global cycling of carbon and nitrogen has recently encouraged intensive studies concerning the major factors that control N_2 fixation and the growth of diazotrophs, particularly for the filamentous diazotroph *Trichodesmium*. From field studies, it appears that in the world ocean N_2 fixation may be limited by either phosphorus (Sanudo-Wihlemy et al., 2001; Moutin et al., 2005; Sohm et al., 2008), iron (Wu et al., 2000; Kustka et al., 2003) or co-limited by both (Mills et al., 2004). Due to the high iron content of the nitrogenase enzyme complex (Raven, 1988; Kustka et al., 2003), N_2 fixation process can be controlled by iron supply. Diazotroph phosphorus limitation could also occur in oceanic area strongly impacted by Fe-rich mineral dust input (Wu et al., 2000). Whether Fe or P is the chemical element ultimately limiting oceanic N_2 fixation remains an open question. In the Mediterranean Sea, the factors controlling N_2 fixation are still poorly known. As the Mediterranean Sea has been described as a phosphate-depleted basin, phosphorus can be logically suspected to be the limiting nutrient for diazotrophic activity.

The Mediterranean Sea is strongly impacted by episodic Saharan dust deposition (e.g. Guerzoni et al., 1999; Guieu et al., 2010a). After the phytoplanktonic bloom, the surface mixed layer (SML) isolated from deeper waters by a strong stratification becomes nutrient-depleted. During the whole stratification period, atmospheric inputs are therefore the main source of allochthonous new nutrients to oligotrophic surface waters. By bringing new nutrients such as DIP (e.g. Ridame and Guieu, 2002; Pulido-Villena et al., 2010) and Fe (e.g. Bonnet and Guieu, 2006) to the Mediterranean surface waters, Saharan dust deposition is strongly suspected to play a key role in the control of N_2 fixation.

Nutrient control of N_2 fixation in the Mediterranean Sea

C. Ridame et al.

[Title Page](#)[Abstract](#)[Introduction](#)[Conclusions](#)[References](#)[Tables](#)[Figures](#)[◀](#)[▶](#)[◀](#)[▶](#)[Back](#)[Close](#)[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)

In order to understand why the N₂ fixation rates are most of the time low in the Mediterranean Sea, it is important to focus on their environmental controlling factors. As the Mediterranean surface waters are DIP-depleted in summer, the BOUM cruise provides a good frame to investigate the nutrient factors (including Fe) controlling N₂ fixation. The main objectives of this study were: (1) to investigate the role of phosphorus as DIP and Fe in the control of N₂ fixation along a trophic gradient in the Mediterranean Sea; and (2) to evaluate the potential impact of a Saharan dust event on this metabolic activity. A microcosm approach was chosen in this study as nutrient additions experiments have proven to be very useful in identifying potential chemical limitations or co-limitation of biological activity during cruises. A comparison of data collected from both shipboard iron enrichments and in situ enrichments suggested that shipboard experiments provide a robust indication on the limiting chemical factors of the phytoplankton community (Gall et al., 2001).

2 Materials and methods

2.1 Microcosm experiments

Experiments were carried out on board R/V *L'Atalante*, during the European BOUM cruise (Biogeochemistry from the Oligotrophic to the Ultra-Oligotrophic Mediterranean) in the Mediterranean Sea between the 16 June and the 20 July 2008 (<http://www.com.univ-mrs.fr/BOUM/>) (Moutin et al., 2011). The cruise track is shown in Fig. 1. The trace-metal clean nutrient/dust additions experiments were performed at three 5-day drifting stations (see locations on Fig. 1) representative of LNLC (Low Nutrient Low Chlorophyll) conditions: station A located in the western basin (39°11' N, 5°35' E), station B in the Ionian Sea (34°13' N, 18°45' E) and station C in the Levantine Sea (33°63 N, 32°65 E). These three stations were located at the heart of anticyclonic eddies (Moutin et al., 2011).

BGD

8, 2629–2657, 2011

Nutrient control of N₂ fixation in the Mediterranean Sea

C. Ridame et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Nutrient control of N₂ fixation in the Mediterranean SeaC. Ridame et al.

[Title Page](#)[Abstract](#)[Introduction](#)[Conclusions](#)[References](#)[Tables](#)[Figures](#)[◀](#)[▶](#)[◀](#)[▶](#)[Back](#)[Close](#)[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)

Manipulations took place under a laminar flow hood inside a clean container. Materials were first acid-washed (HCl Suprapur). Unfiltered subsurface seawater (8 m depth) was collected within the surface mixed layer (Moutin et al., 2011) with a trace metal clean Teflon pump system. 4.5-liter polycarbonate bottles were filled with seawater and immediately enriched with DIP (dissolved inorganic phosphorus) (+P), DIP and iron (+PFe) and Saharan dust (+dust). Nutrients were added to final concentrations of 30 nM KH₂PO₄ and 2.0 nM FeCl₃. Pre-weighted amounts of the Saharan dust (see “Saharan dust characterization” below) were added to seawater to obtain a final particulate concentration of 1.1 mg L⁻¹. One unamended treatment served as control. Each treatment was performed in triplicate. The microcosms were then placed in an on-deck incubator with circulating surface seawater and covered by a screen to simulate the appropriated irradiance (~50% ambient light level representing the light intensity encountered at 8 m). Over the 48 h-incubation, the bottles were regularly manually stirred. For each station, the incubation started in the morning. Before the nutrient/dust additions, seawater was sampled for initial determination of the N₂ fixation rate, chlorophyll-*a* and concentrations of nutrients including dissolved iron. N₂ fixation was determined between 0 and 24 h and between 24 h and 48 h.

2.2 Saharan dust characterization

A fine fraction of a composite Saharan soil (<20 μm) was used for the dust additions. This dust was shown to be representative of the Saharan aerosol carried over the Mediterranean Sea (Guieu et al., 2002; Ridame and Guieu, 2002). The final concentration of dust in the bioassays was 1.1 mg L⁻¹. Assuming an homogeneous dust distribution within the surface layer (Blain et al., 2004), this dust concentration was representative of a realistic dust deposition of 8 g m² (Loÿe-Pilot and Martin, 1996; Termon et al., 2010a; Guieu et al., 2010b), vertically diluted within the 8 m deep surface layer. The total contents of phosphorus, iron and nitrogen in the dust were on average 0.14% ± 0.01%, 4.97% ± 0.12% and 0.11% ± 0.01% in weight, respectively (Guieu

et al., 2002; Ridame and Guieu, 2002). It has to be noted that the dust used in our experiment was different from the one used by Ternon et al. (2010b).

2.3 Sampling and analysis

N₂ fixation rate—5 mL of ¹⁵N₂ gas (99%, EURISOTOP) were added to 4.5 L polycarbonate bottles equipped with septum caps using a gas-tight syringe for ¹⁵N₂ uptake determination at two selected time points (0 and 24 h). ¹⁵N₂ tracer was added to obtain a ~10% final enrichment. 24 h after on-deck incubation, 4.5 L were filtered onto pre-combusted 25 mm GF/F filters, and filters were stored at -20 °C. Sample filters were dried at 40 °C before analysis. N concentration in the particulate matter as well as ¹⁵N-enrichment was quantified with a mass spectrometer (Delta plus, ThermoFisher Scientific, Bremen, Germany) coupled with a C/N analyzer (Flash EA, ThermoFisher Scientific) via a type III-interface. Standard deviations (SD) were 0.004 μmol L⁻¹ for particulate nitrogen and 0.0001 atom% for ¹⁵N enrichment. N₂ fixation rates were calculated by isotope mass balanced as described by Montoya et al. (1996).

Chlorophyll-a – For initial determination, 2.3 L were filtered on 25 mm GF/F. After extraction in 90% acetone, fluorescence was measured on a Hitachi F-4500 spectrofluorometer, following Neveux and Lantoiné (1993).

Nutrients – For initial determination, samples for nitrate (NO₃⁻), nitrite (NO₂⁻) and phosphate (PO₄³⁻) were immediately analysed on board after collection according to classical methods using the automated colorimetric technique (Wood et al., 1967; Tréguer and Le Corre, 1975), on a segmented flow Bran Luebbe autoanalyser II. The precision of measurements was 20 nM, 5 nM and 5 nM for NO₃⁻, NO₂⁻, and PO₄³⁻ respectively. The detection limits, defined as three times the standard deviation of the blank were 20 nM, 10 nM and 10 nM for NO₃⁻, NO₂⁻, and PO₄³⁻ respectively.

Total dissolved iron: initial dissolved iron (DFe) concentration was measured on filtered seawater (<0.2 μm, Sartobran cartridge filters) by flow injection with online pre-concentration and chemiluminescence detection (FIA-CL) (see details in Ternon et al., 2010b). The detection limit was 12 ± 2 pM and the reagent blank was 35 ± 8 pM.

BGD

8, 2629–2657, 2011

Nutrient control of N₂ fixation in the Mediterranean Sea

C. Ridame et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



2.4 Statistical analysis

For N₂ fixation rate, treatment means were compared using a one-way ANOVA and a Fisher LSD means comparison test ($\alpha = 0.05$). For each incubation (0–24 h and 24–48 h), means that were not significantly different were labeled with the same letter (A, B, C for data at 0–24 h and a, b, c for data at 24–48 h).

3 Results

3.1 Initial characteristics of the sampled surface waters

Stations A, B and C located at the heart of anticyclonic eddies had contrasting chemical and biological characteristics (Table 1). The initial concentration of chlorophyll-*a* decreased substantially along a west to east gradient (from 45.3 ng L⁻¹ at station A to 13 ng L⁻¹ at station C) (Table 1, Fig. 1). At the three stations, DIP and NO_x (NO₃⁻ + NO₂⁻) concentrations in surface waters were below the detection limit (<10 nM and <20 nM respectively). Dissolved iron concentrations were the highest at station B (2.3 nM) and were in the same order of magnitude at stations A and C (1.2 nM and 1.3 nM, respectively). Initial N₂ fixation rates in surface waters were very similar at the three study sites (Table 1) as demonstrated by the low standard deviation (mean: 0.10 ± 0.02 nmol N L⁻¹ d⁻¹).

3.2 Response of N₂ fixation to nutrients additions

The biological response of diazotrophic activity after nutrients additions was contrasted at the studied stations. At stations A (western basin) and C (eastern basin), DIP additions alone or those associated with Fe, stimulated significantly N₂ fixation relative to the control (Fig. 2, Table 2). At station A, N₂ fixation was 3.2-fold and 5-fold higher in the P and PFe treatments respectively, as compared to the unamended control at

BGD

8, 2629–2657, 2011

Nutrient control of N₂ fixation in the Mediterranean Sea

C. Ridame et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



48 h (Fig. 2). At station C, additions of P and PFe resulted in a final N_2 fixation rate 3-fold and 2.5-fold higher, respectively, than that in the control. At both stations, addition of DIP alone or in combination with Fe had a more pronounced effect on N_2 fixation rate at 48 h (t_{24-48h}) than at 24 h (t_{0-24h}) (Fig. 2). Statistically, the stimulations of N_2 fixation after P and PFe additions at stations A and C were not significantly different (Table 2). Surprisingly, at station B (central basin), N_2 fixation rate was not stimulated by additions of DIP alone or in combination with Fe (Fig. 2).

3.3 Response of the N_2 fixation to Saharan dust additions

Saharan dust additions stimulated significantly N_2 fixation at all the sampled stations with contrasted responses in intensity (Fig. 2, Table 2). The highest stimulation of N_2 fixation was observed at station C (eastern basin) with a 5.3-fold increase relative to the control, at 48 h. Dust addition led to a 2.3-fold and 3.1-fold increase in N_2 fixation at 48 h at stations A and B, respectively. This increase in N_2 fixation was not statistically different between stations A and B. The N_2 fixation stimulation after dust addition was higher at 48 h than at 24 h at stations A and C while it was similar at station B.

4 Discussion

4.1 Environmental conditions versus N_2 fixation

The Mediterranean waters remained strongly stratified at the studied stations representative of summer conditions (Moutin et al., 2011). At the three stations, the DIP and NO_x concentrations in surface waters were extremely low, typical of LNLC environments. N_2 fixation rates in the surface waters of the sampled stations were homogeneous and low (mean: $0.10 \pm 0.02 \text{ nmol NL}^{-1} \text{ d}^{-1}$). These values were consistent with measurements along the BOUM transect (Ternon et al., 2010b; Bonnet et al., 2011) and with previous data over the entire basin (Ibello et al., 2010; Yogev et al., 2011). They were within

BGD

8, 2629–2657, 2011

Nutrient control of N_2 fixation in the Mediterranean Sea

C. Ridame et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Iron – Our results showed that during the summer stratification, Fe did not limit or co-limit with DIP, N₂ fixation at any studied stations. The DFe concentrations at the three stations were relatively high (from 1.2 to 2.3 nM) probably due to atmospheric iron accumulation in the SML (Sarhou and Jeandel, 2001; Bonnet and Guieu, 2006). Our results suggested that a DFe concentration of the order of 1.2 nM and higher did not limit the Mediterranean N₂ fixation. These field observations confirmed those obtained from cultures study (Berman-Frank et al., 2007) in which similar DFe concentrations were not inhibiting the growth and N₂ fixation of unicellular diazotrophic cyanobacteria.

Phosphorus – In spite of DIP concentrations under detection limit (<10 nM) at the three stations, the response of N₂ fixation after DIP additions were contrasted at the studied stations. The microcosms experiments revealed that phosphorus as DIP was a key controlling factor of N₂ fixation in both western (st. A) and eastern basins (st. C). Surprisingly, in the central Mediterranean Sea (st. B), DIP addition did not stimulate N₂ fixation in spite of undetectable DIP concentration before addition. N₂ fixation could be limited by another chemical element or co-limited by DIP and a chemical element different from Fe. The contrasted responses of N₂ fixation to DIP additions at the three sampled stations indicated a spatial variability of the factor controlling the N₂ fixing activity over the whole basin. While phosphorus availability seemed to be the proximate nutrient limiting N₂ fixation in the western (station A) and eastern Mediterranean Sea (station C), it was not the case in the central one (station B).

4.3 Impact of Saharan dust input on N₂ fixation

Results from previous bioassays experiments have shown that a Saharan dust addition was able to increase N₂ fixation in the tropical Atlantic waters (Mills et al., 2004; Maranon et al., 2010). As the Mediterranean Sea is strongly impacted by Saharan dust deposition, we tested the potential impact of such an event on N₂ fixation. Saharan dust additions induced a strong stimulation of N₂ fixation at the three sampled stations with different responses in intensity. The highest dust stimulation of N₂ fixation was recorded at station C located in the eastern basin (x5.3).

Nutrient control of N₂ fixation in the Mediterranean Sea

C. Ridame et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



At the sampled stations A (western basin) and C (eastern basin), Saharan dust additions as well as DIP additions stimulated significantly N_2 fixation. At station A, the stimulation of N_2 fixation was statistically similar in the dust and P treatments (t_{24h} and t_{48h}) (Table 2) while at station C, Saharan dust led to an increase in N_2 fixation significantly much higher than after DIP addition (Table 2): up to 3-fold (+P) versus up to 5.3-fold (+dust) after 48 h.

Our results demonstrate that Saharan dust was a source of bioavailable P and that such a dust input relieves the on-going DIP-limitation of N_2 fixation at western and eastern stations (A and C). Saharan dust is recognized to be a significant source of DIP in oligotrophic environments such as the Mediterranean Sea during the periods of stratification (Ridame and Guieu, 2002). We used a relationship between the percentage of DIP released from Saharan dust in seawater as a function of dust concentration (Ridame and Guieu, 2002), to estimate that about 10 nM of DIP were released by dust in the bottles with dust added which was lower than the DIP addition in the bottles enriched by DIP (30 nM). At the western station (st. A), N_2 fixation was DIP limited alone while at the eastern one (st. C), N_2 fixation was first DIP limited then progressed to limitation by one or several chemical element(s) released by dust. In this way, Saharan dust input was able to relieve the successive on-going N_2 fixation limitations.

One of the most conspicuous results of this study was that in the central Mediterranean Sea (station B) where no DIP limitation was evident, only Saharan dust was able to increase N_2 fixation rates. At this station, N_2 fixation was either: (1) limited by a chemical element other than DIP; or (2) co-limited by DIP and a chemical element different from Fe. Chemical elements released by the dust (in addition to DIP) such as trace metals may be responsible for the observed stimulation. In addition to be a source of iron to the ocean surface waters (see for ex. Jickells et al., 2005), Saharan dust deposition represents a source of trace elements such as Co, Ni, Mo, Mn, Zn, and Cd, which are essential for phytoplanktonic activity (e.g. Morel and Hudson, 1985; Morel et al., 1991). The concentrations of these essential trace elements in seawater are so low as to limit their availability to phytoplankton (Morel et al., 1991).

Nutrient control of N_2 fixation in the Mediterranean Sea

C. Ridame et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Beyond iron, little is known about the role of trace metals in the N_2 fixation process and growth of diazotrophs. The concentration of bioavailable molybdenum (Mo), nickel (Ni), zinc (Zn) and cobalt (Co) could all be potential candidates as limiting factors in N_2 fixation and growth of diazotrophs. As a cofactor of the nitrogenase enzyme, Mo is strongly suspected to play a central role in the control of N_2 fixation (Howarth and Cole 1985; Cole et al., 1993; Zerkle et al., 2006). The high levels of sulfate in seawater can competitively inhibit molybdate uptake (MoO_4^{2-}) by N_2 fixers (Howarth and Cole 1985), but it is unclear how important this competition is in nature (Paerl et al., 1987; Paulsen et al., 1991; Tuit et al., 2004). Ni, a component of the hydrogenase enzyme, could be a limiting factor for N_2 fixation (Ho and Hu, 2010). The gene coding for Ni-superoxide dismutase was found in the genomes of most marine cyanobacteria, including unicellular N_2 -fixing cyanobacteria *Crocospaera* (Dupont et al., 2008). Also, the genome of picoplanktonic unicellular diazotrophic cyanobacteria (group A) includes a hydrogenase for nickel-iron uptake (Tripp et al., 2010). The use of HCO_3^- requires carbonic anhydrase dehydration to CO_2 for fixation by Rubisco. Since most of the cellular Zn quota is present in carbonic anhydrase, low levels of dissolved Zn in the ocean (Ellwood and Van den Berg, 2000) suggest its potential to limit the growth of autotrophic N_2 fixers, as has been suggested for diatoms by Morel et al. (1994). Co has also a function in carbonic anhydrase and is a core constituent of vitamin B12 (Raux et al., 2000) involved in central metabolic processes (e.g. Banerjee and Ragsdale, 2003). A recent study showed that the unicellular N_2 -fixing cyanobacteria *Crocospaera* is a major producer of vitamin B12 as compared to the non N_2 -fixing cyanobacteria *Synechococcus* or heterotrophic bacteria suggesting that *Crocospaera* has high Co requirements (Bonnet et al., 2010).

Saharan dust contains Mo, Ni, Zn and Co (Guieu and Thomas, 1996; Eglington et al., 2002) but to what extent the dust input changes their distribution in the water column is not well known in the Mediterranean Sea. Moreover, the bioavailability of those metals, which is probably not directly related to their total dissolved concentrations in the oceans (Ellwood and van den Berg, 2001; Wang et al., 2009; Dupont et al., 2010)

BGD

8, 2629–2657, 2011

Nutrient control of N_2 fixation in the Mediterranean Sea

C. Ridame et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



is also poorly documented. While a very limited database does exist for some of these metals in the Mediterranean Sea (Boyle et al., 1985; Tankere et al., 1995; Vega and Van den Berg, 1997; Morley et al., 1997), there is no data on the bioavailable fraction of these metals for the Mediterranean waters. Identifying and quantifying those metals in atmospheric deposition and how they impact the distribution in the water column is essential for understanding the spatial and temporal distribution of diazotrophs and N_2 fixing activity in the Mediterranean Sea. Clearly many open questions do remain about the metals requirements of the different types of diazotrophs which are highly variable within the diazotrophic community as demonstrated for Fe and Mo in cultured *Crocospaera watsonii* and *Trichodesmium erythraeum* (Berman-Franck et al., 2001; Tuit et al., 2004; Berman-Franck et al., 2007).

4.4 Who was responsible for the different N_2 fixation responses?

The spatial discrepancy in the responses of N_2 fixation could be explained by a spatial variability of the composition of the diazotrophic community. A new marine group of α -proteobacteria affiliated to *Bradyrhizobium* was discovered in surface waters of stations A, B, and C (Le Moal et al., 2010). In addition to *Bradyrhizobium*, other diazotrophic microorganisms were also found at the three stations. While UCYN-A (unicellular diazotrophic cyanobacteria from group A) were detected only at station A (surface waters), γ -proteobacteria and other *Rhizobium* than *Bradyrhizobium* were recovered at station B and C, respectively (Le Moal et al., 2010). The well-known marine diazotrophic cyanobacteria *Trichodesmium* sp., *Richelia* sp., UCYN-B (unicellular diazotrophic cyanobacteria from group B as *Crocospaera* sp.) and UCYN-C (unicellular diazotrophic cyanobacteria from group C as *Cyanothece* sp.) were not detected by either microscopic or molecular methods in surface waters of stations A, B and C (Le Moal et al., 2010). However, their contribution to the diazotrophic activity can not be excluded, as the volumes of seawater used to detect these organisms were 2 to 3 orders of magnitude smaller (Le Moal et al., 2010) than the ones used to measure N_2 fixation rates. Nevertheless, 50% to 100% of the N_2 fixing activity was completed

Nutrient control of N_2 fixation in the Mediterranean Sea

C. Ridame et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



by picoplanktonic diazotrophs (<3 μ m) in the surface layer of the water column (Bonnet et al., 2011). Thus, *Bradyrhizobium*, UCYN-A, *Rhizobium* and γ -proteobacteria were likely the main contributor to the diazotrophic activity in the sub-surface waters of stations A, B and C.

The factors that constrain the growth and N₂ fixing activity of each diazotrophic group are poorly known as few of them have been cultured. Each of these species has likely its own nutrients/metals requirement and strategies to acquire them, as previously demonstrated for P among diazotrophic cyanobacteria (Dyhrman and Haley, 2006; Dyhrman et al., 2006), suggesting that difference in species composition may induce different global diazotrophic community responses. Other factors may also contribute to the different responses of the diazotrophic activity such as the relative abundance of each species and the physiological status within a given group.

5 Conclusions

The controlling factor limiting the diazotrophic activity during summer was shown to be different in the Mediterranean Sea: phosphorus as DIP was shown to be the limiting nutrient in the western and eastern stations while at the central station, DIP could either be not limiting N₂ fixation or co-limiting, with a chemical element different from Fe. The spatial variability of the diazotrophic communities at the three stations, could explain this spatial variability in limitation. At the three stations, Saharan dust alleviated the nutrients stress of diazotrophic activity. One of the most conspicuous results is that a trace element different from Fe present in Saharan dust does impact N₂-fixation. This underlines the importance of Saharan dust deposition on the N₂ fixing activity in the Mediterranean Sea. In the future, a warming atmosphere and surface waters (Somot et al., 2008) could potentially increase the stratification of the surface waters in the Mediterranean Sea. In response to this, the biogeochemical impact of the Saharan deposition events on the diazotrophic community could be more pronounced in particular during periods of stratification.

Nutrient control of N₂ fixation in the Mediterranean Sea

C. Ridame et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Acknowledgements. We thank the captain and crew of the R/V *L'Atalante* for their work at sea. We express our gratitude to Nathalie Leblond, Philippe Catala, France Van Wambeke and Laure Taraud for helping at sea and for analysis. We are grateful to Andrew Lenton for language corrections. This work is a contribution of the BOUM experiment (Biogeochemistry from the Oligotrophic to the Ultraoligotrophic Mediterranean, <http://www.com.univ-mrs.fr/BOUM>). It was funded by the French national CNRS-INSU (LEFE-CYBER) program and the European IP SESAME (Southern European Seas: assessing and Modelling Ecosystem Changes), EC Contract No. GOCE-036949.



The publication of this article is financed by CNRS-INSU.

References

- Banerjee, R. and Ragsdale, S. W.: The many faces of vitamin B12: catalysis by cobalamin-dependent enzymes, *Ann. Rev. Biochem.*, 72, 209–247, 2003.
- Bar-Zeev, E. B., Yogev, T., Man-Aharonovich, D., Kress, N., Herut, B., Beja, O., and Berman-Frank, I.: Seasonal dynamics of the endosymbiotic, nitrogen-fixing cyanobacterium *Richelia intracellularis* in the eastern Mediterranean Sea, *ISME J.*, 2, 911–923, 2008.
- Berman-Frank, I., Cullen, J. T., Shaked, Y., Sherrell R. M., and Falkowski, P. G.: Iron availability, cellular iron quotas, and nitrogen fixation in *Trichodesmium*, *Limnol. Oceanogr.*, 46(6), 1249–1260, 2001.
- Berman-Frank, I., Quigg, A., Finkel, Z. V., Irwin, A. J., and Haramaty, L.: Nitrogen-fixation strategies and Fe requirements in cyanobacteria, *Limnol. Oceanogr.*, 52, 2260–2269, 2007.
- Béthoux, J. P., Morin, P., Chaumery, C., Connan, O., Gentili, B., and Ruiz-Pino, D.: Nutrients in the Mediterranean Sea, mass balance and statistical analysis of concentrations with respect to the environmental change, *Mar. Chem.*, 63, 155–169, 1998.

Nutrient control of N₂ fixation in the Mediterranean Sea

C. Ridame et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Nutrient control of N₂ fixation in the Mediterranean Sea

C. Ridame et al.

[Title Page](#)[Abstract](#)[Introduction](#)[Conclusions](#)[References](#)[Tables](#)[Figures](#)[◀](#)[▶](#)[◀](#)[▶](#)[Back](#)[Close](#)[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)

Blain, S., Guieu, C., Claustre, H., Leblanc, K., Moutin, T., Queguiner, B., Ras, J., and Sarthou G.: Availability of iron and major nutrients for phytoplankton in the northeast Atlantic Ocean, *Limnol. Oceanogr.*, 49, 2095–2104, 2004.

Bonnet, S. and Guieu, C.: Atmospheric forcing on the annual in the Mediterranean Sea. A one year survey, *J. Geophys. Res.*, 111, C09010, doi:10.1029/2005JC003213, 2006.

Bonnet, S., Biegala, I., Dutrieux, P., Slemmons, L. O., and Capone, D. G.: Nitrogen fixation in the western equatorial Pacific, *Global Biogeochem. Cy.*, 23, GB3012, doi:10.1029/2008GB003439, 2009.

Bonnet, S., Webb, E. A., Panzeca, C., Karl, D., Capone, D. G., and Sanudo-Wilhelmy, S. A.: Vitamin B12 excretion by cultures of the marine cyanobacteria *Crocospaera* and *Synechococcus*, *Limnol. Oceanogr.*, 55(5), 1959–1964, 2010.

Bonnet, S., Grosso, O., and Moutin, T.: Planktonic dinitrogen fixation in the Mediterranean Sea: a major biogeochemical process during the stratified period?, *Biogeosciences Discuss.*, 8, 1197–1225, doi:10.5194/bgd-8-1197-2011, 2011.

Bosc, E., Bricaud, A., and Antoine, D.: Seasonal and interannual variability in algal biomass and primary production in the Mediterranean Sea, as derived from 4 years of SeaWiFS observations, *Global Biogeochem. Cy.*, 18, GB1005, doi:10.1029/2003GB002034, 2004.

Boyle, E. A. Chapnick, S. D. Bai X. X., and Spivack, A.: Trace metal enrichments in the Mediterranean Sea, *Earth Planet Sc Lett.*, 74, 405–419, 1985.

Capone, D. G., Zehr, J. P., Paerl, H. W., Bergman, B., and Carpenter, E. J.: *Trichodesmium*, a globally significant marine cyanobacterium, *Science*, 276(5316), 1221–1229, 1997.

Carpenter, E. J., Montoya, J. P., Burns, J., Mulholland, M. R., Subramaniam, A., and Capone D. G.: Extensive bloom of a N₂-fixing diatom/cyanobacterial association in the tropical Atlantic Ocean, *Mar. Ecol. Progr. Ser.*, 185, 273–283, 1999.

Cole J. J., Lane J. M., Marino R., and Howarth R. W.: Molybdenum assimilation by cyanobacteria and phytoplankton in freshwater and salt water, *Limnol. Oceanogr.*, 38, 25–35, 1993.

Crombet, Y., Leblanc, K., Quéguiner, B., Moutin, T., Rimmelin, P., Ras, J., Claustre, H., Leblond, N., Oriol, L., and Pujo-Pay, M.: Deep silicon maxima in the stratified oligotrophic Mediterranean Sea, *Biogeosciences*, 8, 459–475, doi:10.5194/bg-8-459-2011, 2011.

Dupont, C. L. Neupane, K., Shearer, J., and Palenik, B.: Diversity, function and evolution of genes coding for putative Ni-containing superoxide dismutases, *Environ. Microbiol.*, 10(7), 1831–1843, 2008.

Dupont C. L., Buck, K. N., Palenik, B., and Barbeau, K.: Nickel utilization in phytoplankton

- assemblages from contrasting oceanic regimes, *Deep-Sea Res. I*, 57, 553–566, 2010.
- Dyhrman, S. T., Chappell, P. D., Haley, S. T., Moffett, J., Orchard, E. D., Waterbury, J., and Webb, E.: Phosphonate utilization by the globally important marine diazotroph *Trichodesmium*, *Nature*, 439, 68–71, 2006.
- 5 Dyhrman, S. T. and Haley, S. T.: Phosphorus Scavenging in the Unicellular Marine Diazotroph *Crocospaera watsonii*, *App. Environ. Microb.*, 72(2), 1452–1458, 2006.
- Eglinton, T. I., Eglinton, G., Dupont, L., Sholkovitz, E. R., Montlucon, D., and Reddy, C. M.: Composition, age, and provenance of organic matter in NW African dust over the Atlantic Ocean, *Geochem. Geophys. Geosy.*, 3(8), 1–27, 2002.
- 10 Ellwood M. J. and Van den Berg, C. M. G.: Zinc speciation in the Northeastern Atlantic Ocean, *Mar. Chem.*, 68, 295–306, 2000.
- Ellwood M. J. and Van den Berg, C. M. G.: Determination of organic complexation of cobalt in seawater by cathodic stripping voltammetry, *Mar. Chem.*, 75, 33–47, 2001.
- Gall, M. P., Strzepak, R., Maldonado, M., and Boyd, P. W.: Phytoplankton processes. Part 2: Rates of primary production and factors controlling algal growth during the Southern Ocean Iron RElease Experiment (SOIREE), *Deep-Sea Res. II*, 48(11–12), 2571–2590, 2001.
- 15 Garcia, N., Raimbault, P., Gouze, E., and Sandroni, V. : Fixation de diazote et production primaire en Méditerranée occidentale, *C. R. Biologie, Acad. Sci.*, 329, 742–750, 2006.
- Gruber, N. and Sarmiento, J. L.: Global patterns of marine nitrogen fixation and denitrification, *Global Biogeochem. Cy.*, 11, 235–266, 1997.
- 20 Guerzoni, S., Chester, R., Dulac, F., Herut, B., Loye-Pilot, M. -D., Measures, C., Migon, C., Molinaroli, E., Moulin, C., Rossini, P., Saydam, C., Soudine, A., and Ziveri, P.: The role of atmospheric deposition in the biogeochemistry of the Mediterranean Sea, *Prog. Oceanogr.*, 44, 147–190, 1999.
- 25 Guieu, C. and Thomas, A. J.: Saharan aerosols: from the soil to the ocean, edited by: Guerzoni, S. and Chester, R., *The Impact of Desert Dust Across the Mediterranean*, Kluwer A. P., Dordrecht., 207–216, 1996.
- Guieu, C., Loÿe-Pilot, M.-D., Ridame, C., and Thomas, C.: Chemical characterization of the Saharan dust end-member: Some biogeochemical implications for the western Mediterranean Sea, *J. Geophys. Res.*, 107(D15), 4258, doi:10.1029/2001JD000582, 2002.
- 30 Guieu, C., Loye-Pilot, M.-D., Benyaya, L., and Dufour, A.: Spatial variability of atmospheric fluxes of metals (Al, Fe, Cd, Zn and Pb) and phosphorus over the whole Mediterranean from a one year monitoring experiment: biogeochemical implications, *Mar. Chem.*, 120, 164–178,

Nutrient control of N₂ fixation in the Mediterranean Sea

C. Ridame et al.

[Title Page](#)[Abstract](#)[Introduction](#)[Conclusions](#)[References](#)[Tables](#)[Figures](#)[◀](#)[▶](#)[◀](#)[▶](#)[Back](#)[Close](#)[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)

2010a.

Guieu, C., Dulac, F., Desboeufs, K., Wagener, T., Pulido-Villena, E., Grisoni, J.-M., Louis, F., Ridame, C., Blain, S., Brunet, C., Bon Nguyen, E., Tran, S., Labiadh, M., and Dominici, J.-M.: Large clean mesocosms and simulated dust deposition: a new methodology to investigate responses of marine oligotrophic ecosystems to atmospheric inputs, *Biogeosciences*, 7, 2765–2784, doi:10.5194/bg-7-2765-2010, 2010b.

Ho, T. and Hu, C.: Nickel Limitation of Nitrogen Fixation by *Trichodesmium*, Oceans Sciences Meeting, 22–26 February 2010, Portland, Oregon, USA, 2010.

Howarth, R. W. and Cole, J. J.: Molybdenum availability, nitrogen limitation and phytoplankton growth in natural waters, *Science*, 229(4714), 653–655, 1985.

Ibello, V., Cantoni, C., Cozzi, S., and Civitarese, G.: First basin-wide experimental results on N₂ fixation in the open Mediterranean Sea, *Geophys. Res. Lett.*, 37, L03608, doi:10.1029/2009GL041635, 2010.

Jickells, T. D., An, Z. S., Andersen, K. K., Baker, A. R., Bergametti, G., Brooks, N., Cao, J. J., Boyd, P. W., Duce, R. A., Hunter, K. A., Kawahata, H., Kubilay, N., La Roche, J., Liss, P. S., Mahowald, N., Prospero, J. M., Ridgwell, A. J., Tegen, I., and Torres, R.: Global Iron Connections Between Desert Dust, Ocean Biogeochemistry, and Climate, *Science*, 308, 67–71, 2005.

Krom, M. D., Herut, B., and Mantoura, R. F. C.: Nutrient budget for the eastern Mediterranean: Implications for phosphorus limitation, *Limnol. Oceanogr.*, 49(5), 1582–1592, 2004.

Krom, M. D., Emeis, K.-C., and Van Cappellen, P.: Why is the Eastern Mediterranean phosphorus limited?, *Prog. Oceanogr.*, 85, 236–244, 2010.

Kustka, A. B., Sanudo-Wilhelmy, S. A., Carpenter, E. J., Capone, D. G., Burns, J., and Sunda, W. G.: Iron requirements for dinitrogen- and ammonium-supported growth in cultures of *Trichodesmium* (IMS 101): Comparison with nitrogen fixation rates and iron: carbon ratios of field populations, *Limnol. Oceanogr.*, 48(5), 1869–1884, 2003.

Lagaria, A., Psarra, S., Lefèvre, D., Van Wambeke, F., Courties, C., Pujo-Pay, M., Oriol, L., Tanaka, T., and Christaki, U.: The effects of nutrient additions on particulate and dissolved primary production in surface waters of three Mediterranean eddies, *Biogeosciences Discuss.*, 7, 8919–8952, doi:10.5194/bgd-7-8919-2010, 2010.

Le Moal, M. and Biegala, I. C.: Diazotrophic unicellular cyanobacteria in the northwestern Mediterranean Sea: a seasonal cycle, *Limnol. Oceanogr.* 54, 845–855, 2009.

Le Moal, M., Collin, H., and Biegala, I. C.: Intriguing diversity among diazotrophic picoplankton

BGD

8, 2629–2657, 2011

Nutrient control of N₂ fixation in the Mediterranean Sea

C. Ridame et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Nutrient control of N₂ fixation in the Mediterranean Sea

C. Ridame et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



- along a Mediterranean transect: from the origin of plastids to the dominance of rhizobia, *Biogeosciences Discuss.*, 7, 8779–8816, doi:10.5194/bgd-7-8779-2010, 2010.
- Loÿe-Pilot, M.-D., and Martin, J. M.: Saharan dust input to the Western Mediterranean: an eleven years record in Corsica, edited by: Guerzoni, S. and Chester, R., *The Impact of Desert Dust Across the Mediterranean*, Kluwer A. P., Dordrecht., 191–199, 1996.
- Man-Aharonovich, M., Kress, N., Bar-Zeev, E., Berman-Frank, I., and Béjà, O.: Molecular ecology of nifH genes and transcripts in the eastern Mediterranean Sea, *Environ. Microbiol.*, 50, 2354–2363, 2007.
- Maranon, E., Fernandez, A., Mourino-Carballido, B., Martinez-Garcia, S., Teira, E., Cermeno, P., Choucino, P., Huete-Ortega, M., Fernandez, E., Calvo-Diaz, A., Anxelu, X., Moran, G., Bode, A., Moreno-Ostos, E., Varela, M. M., Patey, M. D., and Achterberg, E. P.: Degree of oligotrophy controls the response of microbial plankton to Saharan dust, *Limnol. Oceanogr.*, 55(6), 2339–2352, 2010.
- Marty, J. C., Chiaverini, J., Pizay, M.-D., and Avril, B.: Seasonal and interannual dynamics of nutrients and phytoplankton pigments in the western Mediterranean Sea at the DYFAMED time-series station (1991–1999), *Deep-Sea Res. II*, 49, 1965–1985, 2002.
- Marty, J. C., Garcia, N., and Raimbault, P.: Phytoplankton dynamics and primary production under late summer conditions in the NW Mediterranean Sea, *Deep-Sea Res. I*, 55, 1131–1149, 2008.
- Mauriac, R., Moutin, T., and Baklouti, M.: Accumulation of DOC in Low Phosphate Low Chlorophyll (LPLC) area: is it related to higher production under high N:P ratio?, *Biogeosciences Discuss.*, 7, 7091–7130, doi:10.5194/bgd-7-7091-2010, 2010.
- Mills, M. M., Ridame, C., Davey, M., La Roche, J., and Geider, R. J.: Iron and phosphorus co-limit nitrogen fixation in the eastern tropical North Atlantic, *Nature*, 429, 292–294, 2004.
- Moisander, P. H., Beinart, R. A., Hewson, I., White, A. E., Johnson, K. S., Carlson, C. A., Montoya, J. P., and Zehr, J. P.: Unicellular cyanobacterial distributions broaden the oceanic N₂ fixation domain, *Science*, 327, 5972, 1512–1514, doi:10.1126/science.1185468, 2010.
- Montoya, J. P., Voss, M., Kahler, P., and Capone, D. G.: A simple, high-precision, high-sensitivity tracer assay for N₂ fixation, *App. Environ. Microb.*, 62(3), 986–993, 1996.
- Morel, F. M. M. and Hudson, R. J. M.: *Geobiological Cycle of Trace Elements in Aquatic Systems: Redfield Revisited*, edited by: John Wiley and Sons, Chemical Processes in Lakes, New York, 251–281, 1985.
- Morel, F. M. M., Hudson, R. J. M., and Price, N. M.: What Controls Phytoplankton Production

Nutrient control of N₂ fixation in the Mediterranean Sea

C. Ridame et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



in Nutrient-Rich Areas of the Open Sea?, *Limnol. Oceanogr.*, 36(8), 1742–1755, 1991.

Morel, F. M. M., Reinfelder, J. R., Roberts, S. B., Chamberlain C. P., Lee, J. G., and Yee, D.: Zinc and Carbon Co-Limitation of Marine Phytoplankton, *Nature*, 369, 740–742, 1994.

Morley, N. H., Burton, J. D. Tankere, S. P. C., and Martin, J.-M.: Distribution and behaviour of some dissolved trace metals in the western Mediterranean Sea, *Deep-Sea Res. II*, 44(34), 675–691, 1997.

Moutin, T. and Raimbault, P.: Primary production, carbon export and nutrients availability in western and eastern Mediterranean Sea in early summer 1996 (MINOS cruise), *J. Mar. Syst.*, 33–34, 273–288, 2002.

Moutin, T., Van Den Broeck, N., Beker, B., Dupouy, C., Rimmelin, P., and Le Bouteiller, A.: Phosphate availability controls *Trichodesmium* spp. biomass in the SW Pacific Ocean, *Mar. Ecol. Progr. Ser.*, 297, 15–21, 2005.

Moutin, T., Karl, D. M., Duhamel, S., Rimmelin, P., Raimbault, P., Van Mooy, B. A. S., and Claustre, H.: Phosphate availability and the ultimate control of new nitrogen input by nitrogen fixation in the tropical Pacific Ocean, *Biogeosciences*, 5, 95–109, doi:10.5194/bg-5-95-2008, 2008.

Moutin, T., Van Wambeke, F., and Prieur, L.: Introduction to the biogeochemistry from the Oligotrophic to the Ultraoligotrophic Mediterranean (BOUM) experiment, *Biogeosciences Discuss.*, in preparation, 2011.

Needoba, J. A., Foster, R. A., Sakamoto, C., Zehr, J. P., and Johnson, K. S.: Nitrogen fixation by unicellular diazotrophic cyanobacteria in the temperate oligotrophic North Pacific Ocean, *Limnol. Oceanogr.*, 52, 3311–3321, 2007.

Neveux, J. and Lantoiné, F.: Spectrofluorometric assay of chlorophylls and phaeopigments using the least squares approximation technique, *Deep-Sea Res. I*, 40(9), 1747–1765, 1993.

Paerl, H. W., Crocker, K. M., and Prufert, L. E.: Limitation of N₂ fixation in coastal marine waters: Relative importance of molybdenum, iron, phosphorus, and organic matter availability, *Limnol. Oceanogr.*, 32(3), 525–536, 1987.

Paulsen, D. M., Paerl H. W., and Bishop P. E.: Evidence that molybdenum-dependent nitrogen fixation is not limited by high sulfate concentrations in marine environments, *Limnol. Oceanogr.*, 36(7), 1325–1334, 1991.

Pujo-Pay, M., Conan, P., Oriol, L., Cornet-Barthaux, V., Falco, C., Ghiglione, J.-F., Goyet, C., Moutin, T., and Prieur, L.: Integrated survey of elemental stoichiometry (C, N, P) from the Western to Eastern Mediterranean Sea, *Biogeosciences Discuss.*, 7, 7315–7358,

doi:10.5194/bgd-7-7315-2010, 2010.

Pulido-Villena, E., Rérolle, V., and Guieu, C.: Transient fertilizing effect of dust in P-deficient LNL surface ocean, *Geophys. Res. Lett.*, 37, L01603, doi:10.1029/2009GL041415, 2010.

Raimbault, P. and Garcia, N.: Evidence for efficient regenerated production and dinitrogen fixation in nitrogen-deficient waters of the South Pacific Ocean: impact on new and export production estimates, *Biogeosciences*, 5, 323–338, doi:10.5194/bg-5-323-2008, 2008.

Raux, E., Schubert, H. L., and Warren, M. L.: Biosynthesis of cobalamin (vitamin B₁₂): a bacterial conundrum, *Cell. Molec. Life Sci.*, 57, 1880–1893, 2000.

Raven, J. A.: The Iron and Molybdenum use Efficiencies of Plant Growth with Different Energy, Carbon and Nitrogen Sources, *New Phytologist*, 109(3), 279–287, 1988.

Rees A. P., Law, C. S., and Woodward, E.M.: High rates of nitrogen fixation during an in-situ phosphate release experiment in the eastern Mediterranean Sea, *Geophys. Res. Lett.*, 33, L10607, doi:10.1029/2006GL025791, 2006.

Ribera d'Alcala, M., Civitarese, G., Conversano, F., and Lavezza, R.: Nutrient ratios and fluxes hint at overlooked processes in the Mediterranean Sea. *J. Geophys. Res.*, 108(C9), 8106, doi:10.1029/2002JC001650, 2003.

Ridame, C. and Guieu, C.: Saharan input of phosphate to the oligotrophic water of the open western Mediterranean Sea, *Limnol. Oceanogr.*, 47(3), 857–869, 2002.

Sandroni, V., Raimbault, P., Migon, C., Garcia, N., and Gouze, E.: Importance of diazotrophy and dry atmospheric inputs of nitrogen in nutrient budget of the western Mediterranean Sea, *Deep-Sea Res. I.*, 54, 1859–1870, 2007.

Sanudo-Wilhelmy, S. A., Kustka, A. B., Gobler, C. J., Hutchins, D. A., Yang, M., Lwiza, K., Burns, J., Capone, D. G., Raven, J. A., and Carpenter, E. J.: Phosphorus limitation of nitrogen fixation by *Trichodesmium* in the central Atlantic Ocean, *Nature*, 411(6833), 66–69, 2001.

Sarthou, G. and Jeandel, C.: Seasonal variations of iron concentrations in the Ligurian Sea and iron budget in the Western Mediterranean Sea, *Mar. Chem.*, 74, 115–129, 2001.

Sohm, J. A., Mahaffey, C., and Capone, D. G.: Assessment of relative phosphorus limitation of *Trichodesmium* spp. in the North Pacific, North Atlantic, and the north coast of Australia, *Limnol. Oceanogr.*, 53(6), 2495–2502, 2008.

Somot, S., Sevault, F., Déqué, M., and Crépon, M.: 21st century climate change scenario for the Mediterranean using a coupled atmosphere-ocean regional climate model, *Global Planet Change*, 63, 112–126, 2008.

Tanaka, T., Thingstad, T. F., Christaki, U., Colombet, J., Cornet-Barthaux, V., Courties, C.,

BGD

8, 2629–2657, 2011

Nutrient control of N₂ fixation in the Mediterranean Sea

C. Ridame et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Nutrient control of N₂ fixation in the Mediterranean Sea

C. Ridame et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Grattepanche, J.-D., Lagaria, A., Nedoma, J., Oriol, L., Psarra, S., Pujo-Pay, M., and Van Wambeke, F.: N-limited or N and P co-limited indications in the surface waters of three Mediterranean basins, *Biogeosciences Discuss.*, 7, 8143–8176, doi:10.5194/bgd-7-8143-2010, 2010.

5 Tankere, S. P. C., Morley, N. H., and Burton, J. D.: Spatial and temporal variations in concentrations of trace metals in the regions of the Straits of Sicily and Gibraltar, *Wat. Pollut. Res. Reports*, 32, 205–219, 1995.

Ternon, E., Guieu, C., Loÿe-Pilot, M.-D., Leblond, N., Bosc, E., Gasser, B., Miquel, J.-C., and Martín, J.: The impact of Saharan dust on the particulate export in the water column of the North Western Mediterranean Sea, *Biogeosciences*, 7, 809–826, doi:10.5194/bg-7-809-2010, 2010a.

Ternon, E., Guieu, C., Ridame, C., L'Helguen, S., and Catala, P.: Longitudinal variability of the biogeochemical role of Mediterranean aerosols in the Mediterranean Sea, *Biogeosciences Discuss.*, 7, 8087–8121, doi:10.5194/bgd-7-8087-2010, 2010b.

15 Tréguer, P. and Le Corre, P.: Manuel d'analyses des sels nutritifs dans l'eau de mer, Laboratoire d'Océanographie Chimique. Université de Bretagne Occidentale, Brest, 110 pp., 1975.

Tripp, H. J., Bench, S. R., Turk, K. A., Foster, R. A., Desany, B. A., Niazi, F., Affourtit, J. P., and Zehr, J. P.: Metabolic streamlining in an open-ocean nitrogen-fixing cyanobacterium, *Nature*, 1–5, doi:10.1038/nature08786, 2010.

20 Tuit, C., Waterbury, J., and Ravizza, G.: Diel variation of molybdenum and iron in marine diazotrophic cyanobacteria, *Limnol. Oceanogr.*, 49(4), 978–990, 2004.

Vega, M. and Van den Berg, C. M. G.: Determination of cobalt in seawater by catalytic adsorptive cathodic stripping voltammetry, *Anal. Chem.*, 69, 874–881, 1997.

25 Wang, D., Aller, R. C., and Sañudo-Wilhelmy, S. A.: A new method for the quantification of different redox-species of molybdenum (V and VI) in seawater, *Mar. Chem.*, 113, 250–256, 2009.

Wood, E. D., Armstrong, F. A., and Richards, F. A.: Determination of nitrate in sea water by cadmium copper reduction to nitrite, *J. Mar. Biol. Ass. UK*, 47, 23–31, 1967.

30 Wu, J., Sunda, W., Boyle, E. A., and Karl, D. M.: Phosphate depletion in the western north Atlantic Ocean, *Science*, 289, 759–762, 2000.

Yogev, T., Rahav, E., Bar-Zeev, E., Man-Aharonovich, D., Stambler, N., Kress, N., Béjà, O., Mulholland, M. R., Herut, B., and Berman-Frank, I.: Is dinitrogen fixation significant in the Levantine Basin, East Mediterranean Sea?, *Environ. Microb.*, article first published online:

18 January 2011, doi:10.1111/j.1462-2920.2010.02402.x, 2011.
Zerkle, A. L., House, C. H., Cox, R. P., and Canfield, D. E.: Metal limitation of cyanobacterial N₂ fixation and implications for the Precambrian nitrogen cycle, *Geobiology*, 4, 285–297, 2006.

Discussion Paper | Discussion Paper | Discussion Paper | Discussion Paper | Discussion Paper

BGD

8, 2629–2657, 2011

Nutrient control of N₂ fixation in the Mediterranean Sea

C. Ridame et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Nutrient control of N₂ fixation in the Mediterranean Sea

C. Ridame et al.

Table 1. Initial chemical and biological properties of the surface seawater used for the incubations experiments. [DIP] (Dissolved Inorganic Phosphorus) and [NO_x] (NO₃⁻ + NO₂⁻) were under detection limit at all stations.

	Station A	Station B	Station C
Temperature (°C)	24.46	26.96	25.24
salinity	37.43	38.63	39.60
Chlorophyll- <i>a</i> (ng L ⁻¹)	45.3	22.0	13.0
N ₂ fixation, nmol N L ⁻¹ d ⁻¹	0.10	0.08	0.09
DFe, nM	1.2	2.3	1.3

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Nutrient control of N₂ fixation in the Mediterranean Sea

C. Ridame et al.

Table 2. Results of the statistical comparison (ANOVA, FISHER test) between the different treatments (control, +P, +PFe, +dust) at the end of the experiment for N₂ fixation (t_{24-48h}). NS: the difference between the two treatments is not statistically different; S: the difference between the two treatments is statistically different.

	A	B	C
P vs. Control	S	NS	S
PFe vs. control	S	NS	S
dust vs. control	S	S	S
P vs. PFe	NS	NS	NS
P vs. dust	NS	S	S
PFe vs. dust	NS	S	S

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Nutrient control of N₂ fixation in the Mediterranean Sea

C. Ridame et al.

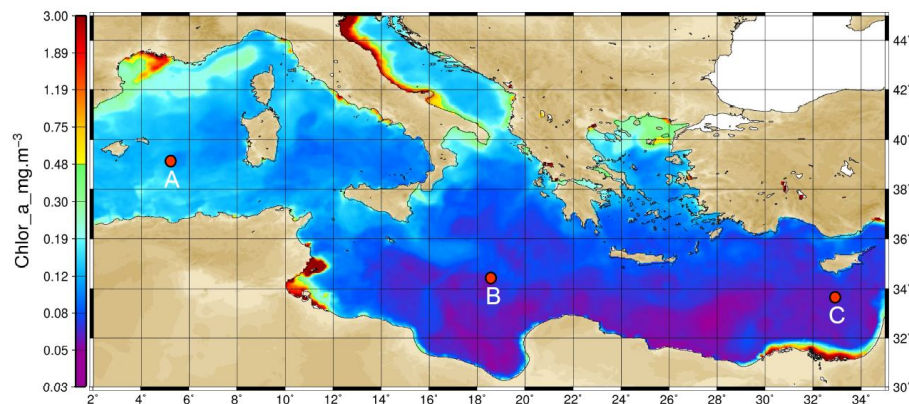


Fig. 1. Locations of the stations A, B, C where microcosm experiments were performed and satellite chlorophyll concentration (MODIS) during the BOUM cruise.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Nutrient control of N₂ fixation in the Mediterranean Sea

C. Ridame et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

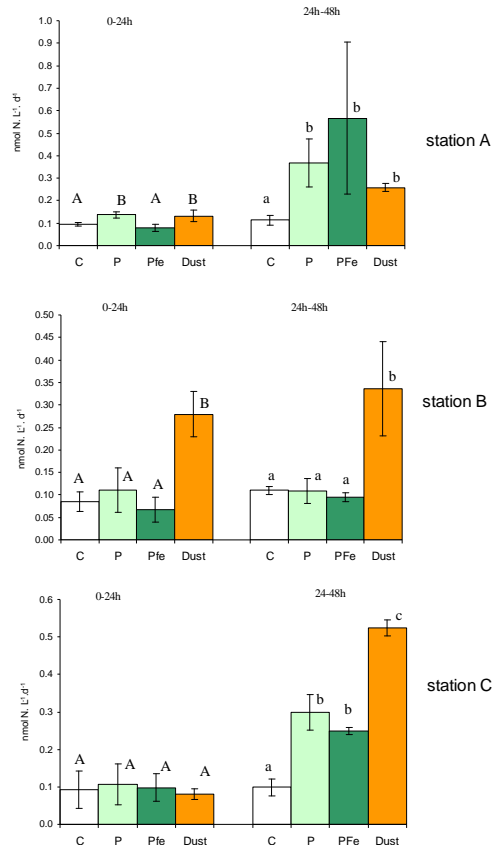


Fig. 2. N₂ fixation rate in control (C), P, PFe and dust treatments during incubation times (0–24 h and 24–48 h) at stations A, B and C. Treatment means were compared using a one-way ANOVA and a Fisher LSD means comparison test ($\alpha = 0.05$). For each incubation (0–24 h and 24–48 h), means that are not significantly different are labeled with the same letter (A, B, C for analysis at 0–24 h and a, b, c for analysis at 24–48 h).