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Strong stimulation of N₂ fixation in oligotrophic Mediterranean Sea: results from dust addition in large in situ mesocosms

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Abstract. The response of N₂ (dinitrogen) fixation to contrasted (wet and dry) Saharan dust deposition was studied in the framework of the DUNE project (a DUst experiment in a low-Nutrient, low-chlorophyll Ecosystem) during which realistic simulations of dust deposition (10 g m⁻²) into large mesocosms (52 m³) were performed. Three distinct experimental dust additions were conducted in June 2008 (DUNE-1-P: simulation of a wet deposition, DUNE-1-Q: simulation of a dry deposition) and 2010 (DUNE-2-R: simulation of 2 successive wet depositions) in the northwestern oligotrophic Mediterranean Sea. Here we show that wet and dry dust deposition induced a rapid (24 h or 48 h after dust additions), strong (from 2- to 5.3-fold) and long (at least 4–6 days duration) increase in N₂ fixation, indicating that both wet and dry Saharan dust deposition was able to relieve efficiently the nutrient limitation(s) of N₂ fixation. This means in particular that N₂ fixation activity was not inhibited by the significant input of nitrate associated with the simulated wet deposition (~9 mmol NO₃⁻ m⁻²). The input of new nitrogen associated with N₂ fixation was negligible relative to the atmospheric NO₃⁻ input associated with the dust. The contribution of N₂ fixation to primary production was negligible (≤ 1 %) before and after dust addition in all experiments, indicating that N₂ fixation was a poor contributor to the nitrogen demand for primary production. Despite the stimulation of N₂ fixation by dust addition, the rates remained low,

and did not significantly change the contribution of N₂ fixation to new production since only a maximum contribution of 10 % was observed. The response of N₂ fixation by diazotrophs and CO₂ fixation by the whole phytoplankton community suggests that these metabolic processes were limited or co-limited by different nutrients. With this novel approach, which allows us to study processes as a function of time while atmospheric particles are sinking, we show that new atmospheric nutrients associated with Saharan dust pulses do significantly stimulate N₂ fixation in the Mediterranean Sea and that N₂ fixation is not a key process in the carbon cycle in such oligotrophic environments.

1 Introduction

Over geological time scales, dinitrogen (N₂) fixation is important for the regulation of the fixed nitrogen (N) reservoir in the ocean and thereby sustains ocean productivity (Falkowski, 1997; Tyrrell, 1999). In the modern ocean, N₂ fixation or diazotrophy is now recognized to be the main source of fixed N in the marine environments (Codispoti, 2007; Gruber, 2008; Großkopf et al., 2012) supporting an important part of oceanic primary productivity and organic matter export to the deep ocean (Dore et al., 2002; Karl and Letelier, 2008; Subramaniam et al., 2008). Due to the importance

of N₂ fixation in the global cycling of both nitrogen and carbon (C), information on N₂ fixation and spatial distribution of diazotrophs is increasing (Monteiro et al., 2010; Luo et al., 2012). Nevertheless, despite recent advances, the environmental factors controlling the magnitude of N₂ fixation need to be assessed and a better quantification of their impacts needs to be achieved. In the contemporary ocean, it is assumed that the nutrients limiting N₂ fixation are mainly phosphorus (P) (Sanudo-Wihlemy et al., 2001; Sohm et al., 2008; Ridame et al., 2011), iron (Fe) (Berman-Frank et al., 2001; Kustka et al., 2003; Moore et al., 2009; Jacq et al., 2013) or both (Mills et al., 2004). Recently, it has been shown that trace elements other than Fe could also exert a control on N₂ fixation (Ridame et al., 2011; Ho, 2013).

A large part of oceanic systems is subject to high inputs of aeolian dust aerosols from the great deserts of the world (e.g., Tegen et al., 2004; Jickells et al., 2005). In the past decades, the biogeochemical interest of these aerosols increased when it was realized that aeolian deposition of mineral aerosols represents, on a global scale, the dominant external source of Fe and a major transport pathway for macro nutrients such as P, to the surface of the open ocean (Jickells et al., 2005; Baker et al., 2007; Mahowald et al., 2008). Aeolian dust deposition represents thus a good candidate as a controlling factor in the magnitude of N₂ fixation in low nutrient low chlorophyll (LNLC) environments. Nevertheless, the effects of atmospheric dust-derived nutrients on N₂ fixation in oligotrophic LNLC regions such as the Mediterranean Sea are still poorly understood and quantified.

The Mediterranean Sea is an oligotrophic quasi-enclosed basin strongly impacted by periodic dust storms originating from the Sahara (e.g., Guerzoni et al., 1999; Guieu et al., 2010a). After the spring phytoplankton bloom, the surface mixed layer is isolated from deeper waters by strong stratification becoming macro-nutrient (N, P) depleted and, leading to low primary productivity (Moutin and Raimbault, 2002; Marty and Chiaverini, 2002; Bosc et al., 2004; Lopez-Sandoval et al., 2011). During the whole stratification period, atmospheric inputs are the main source of allochthonous nutrients to the oligotrophic surface waters and the dissolved iron (DFe) concentration is relatively high, most likely due to atmospheric Fe accumulation in surface waters (Sarhou and Jeandel, 2001; Bonnet and Guieu, 2006; Wagener et al., 2010). Direct measurements of N₂ fixation rates in surface waters during stratification periods have shown generally low values ($\leq 0.2 \text{ nmol N L}^{-1} \text{ d}^{-1}$) in the open Mediterranean Sea (Ibello et al., 2010; Bonnet et al., 2011; Ridame et al., 2011; Ternon et al., 2011; Yogev et al., 2011; Rahav et al., 2013a) but relatively high rates have been episodically found (up to $7.5 \text{ nmol N L}^{-1} \text{ d}^{-1}$ in Sandroni et al., 2007). The low rates of N₂ fixation recorded during stratification periods are probably a consequence of the low availability of dissolved inorganic phosphorus (DIP) in the Mediterranean Sea (Pulido-Villena et al., 2010). During the BOUM (Biogeochemistry from the Oligotrophic to the Ultra-oligotrophic

Mediterranean) cruise in the summer of 2008, nutrient addition bottle incubations showed that N₂ fixation was not limited by Fe nor co-limited by Fe and DIP at stations located in the western, central and eastern Mediterranean basins (Ridame et al., 2011). Rather, N₂ fixation was DIP limited at the western and eastern stations. Through the input of new nutrients such as DIP and trace metals to the Mediterranean surface waters (e.g., Ridame and Guieu, 2002; Pulido-Villena et al., 2010; Wuttig et al., 2013), Saharan dust deposition is strongly suspected to play a key role in the control of N₂ fixation. Microcosm experiments performed in the tropical Atlantic and Mediterranean Sea have proven that Saharan dust addition may strongly influence N₂ fixation rates (Mills et al., 2004; Maranon et al., 2010; Ternon et al., 2011; Ridame et al., 2011) as well as the abundance and the distribution of various diazotrophic groups (Langlois et al., 2012).

The microcosm approach presents some limitations as to the short duration of the experiment (one or two days on average) making results difficult to extrapolate on a longer time scale. Also, the number of parameters is reduced due to the small volumes incubated necessitating the study of only one specific process or community. The strategy chosen in the DUNE project was to study the impact of Saharan dust events on the whole ecosystem from viruses to zooplankton over a period of one week and to evaluate the biogeochemical implications associated with this forcing (Guieu et al., 2010b, 2013). The approach applied in DUNE was, for the first time, to perform realistic dust seedings onto large metal-free mesocosms. In this context, the present paper is focused on the N₂ fixation process sustained by diazotrophic organisms. Here we quantify the impact of contrasted Saharan dust events (wet and dry deposition) on the N₂-fixing activity. Based on the measurements of primary production (PP) and on the estimates of new production (NP) presented in a companion paper (Ridame et al., 2013), we estimate the contribution of N₂ fixation to PP and NP and the implications on the N pool in the oligotrophic waters of the western Mediterranean Sea.

2 Material and methods

2.1 Experimental design and dust characterization

Three distinct experimental dust additions into large mesocosms were conducted in June 2008 and June–July 2010 in the northwestern Mediterranean Sea, in the frame of the DUNE project (<http://www.obs-vlfr.fr/LOV/DUNE/index.html>). More precisely, the experiments were realized in the Elbo Bay located in the Natural Preservation Area of Scandola (8.554° E, 42.374° N), which is representative of the LNLC conditions of the open western Mediterranean Sea (Guieu et al., 2010b). The mesocosm experiment design and the accuracy of the strategy developed are fully described in Guieu et al. (2010b). Briefly, six mesocosms (height: 12.5 m,

Table 1. Particulate P, Fe and N (% in weight) in the EC and non-EC dust used during DUNE. Means that were not significantly different for a given chemical element between the different experiments ($p > 0.05$) were labeled with the same letter (in parenthesis).

	DUNE-1-P 10–18 Jun 2008	DUNE-1-Q 20–27 Jun 2008	DUNE-2-R (R1, R2) 26 Jun–9 Jul 2010
Dust treatment	evapocondensed	non-processed	evapocondensed
Simulated deposition	wet	dry	wet
P (%)	0.045 ± 0.015 ^a (A)	0.044 ± 0.009 ^a (A)	0.055 ± 0.003 ^b (A)
Fe (%)	2.31 ± 0.04 ^a (B)	2.28 ± 0.19 ^a (B)	2.26 ± 0.03 ^b (B)
N (%)	1.19 ± 0.05 ^a (C)	0.11 ± 0.01 ^a (D)	1.36 ± 0.09 ^b (E)

^a Guieu et al. (2010b); ^b Desboeufs et al. (2013)

diameter: 2.3 m, surface area: 4.15 m², volume: 52 m³) entirely designed in plastic were deployed. The bags were made of polyethylene mixed with vinyl acetate and the holding structure of PVC and polyethylene. The screw anchors were installed at the sea floor 25–30 m deep. The mesocosms were closed, submerged systems without lateral advection. Three replicate mesocosms (D1, D2 and D3 hereafter referred to as “Dust-meso”) were amended with 41.5 g of mineral dust reproducing a high, but realistic atmospheric dust deposition of 10 g m⁻² (Guieu et al., 2010b). The dust used in the three seeding experiments was from the fine fraction (< 20 µm) of Saharan soils collected in southern Tunisia, which is a Saharan aerosol source area (details in Guieu et al., 2010b; Desboeufs, et al., 2013). Three other mesocosms served as controls (C1, C2 and C3 hereafter referred to as “Control-meso”). The sampling session took place every morning at the same time over the duration of the experiments. Each day, three different depths (0.1, 5 and 10 m depth) were sampled in the six mesocosms using a system of permanent PVC tubing placed at the center of the bags and connected to a Teflon pump. Every 48 h, seawater was also collected outside the mesocosms (hereafter referred to as “out”) at the same depths in order to test the representativeness of data between the Control-meso and out.

June 2008 experiments: two distinct seeding experiments were conducted with two types of dust. In the first one (10–18 June 2008, hereafter referred to as “DUNE-1-P”), the dust used was previously subjected to physico-chemical transformations that involved HNO₃ and H₂SO₄, mimicking atmospheric transport (Desboeufs et al, 2001; and details in Guieu et al., 2010b). This evapocondensed dust (hereafter referred to as “EC dust”) contained on average 0.045 ± 0.015 % of P, 2.31 ± 0.04 % of Fe and 1.19 ± 0.05 % of N, in weight (Table 1). The amendment of the dust mesocosms in the DUNE-1-P experiment was performed with the EC dust mixed with 2 L of ultrapure water in order to mimic a wet deposition event.

In the second experiment (20–27 June 2008, hereafter referred to as “DUNE-1-Q”), non-processed dust was used (hereafter referred to as “non-EC dust”) and contained on average 0.044 ± 0.009 % of P, 2.28 ± 0.19 % of Fe and

0.11 ± 0.01 % of N (Table 1). The amendment of the dust mesocosms in the DUNE-1-Q experiment was conducted with the non-EC dust mixed with 2 L of unfiltered surface seawater in order to mimic a dry deposition event.

June–July 2010 experiments: two successive seeding experiments (26 June–2 July 2010 hereafter referred to as “DUNE-2-R1” and from 3 to 9 July 2010 hereafter referred to as “DUNE-2-R2”, see details in Guieu et al., 2013) were performed with the same amount of EC dust characterized by an average content of 0.055 ± 0.003 % P, 2.26 ± 0.03 % Fe and 1.36 ± 0.09 % N (Table 1; see details in Desboeufs et al., 2013). As in the DUNE-1-P experiment, the EC-dust was sprayed on the Dust-meso mixed with 2 L of ultrapure water simulating a wet deposition event. The dust used in DUNE-2-R was not collected at the same time that the dust used in the experiments of 2008 (details in Desboeufs et al., 2013).

P, Fe, and N contents of the dust: the particulate P and Fe contents of the EC dust (DUNE-1-P and DUNE-2-R) and non-EC dust (DUNE-1-Q) were similar ($p > 0.05$, Table 1). Due to the simulation of cloud water processes that involved HNO₃, the N content of EC dust was about 10-fold higher in comparison with the non-EC dust (Table 1; Guieu et al., 2010b, 2013). Small differences of the N content in EC dust used in the 2008 and 2010 experiments (1.19 ± 0.05 % and 1.36 ± 0.09 %) were observed (details in Guieu et al., 2013).

2.2 N₂ fixation rate

All materials were previously cleaned following trace metal clean procedures. One sample per depth of unfiltered seawater was collected in the morning at two depths (0.1 m and 5 m depth) during DUNE-1-P and -Q and at 5 m depth during DUNE-2-R for determination of N₂ fixation rates. Samples were collected in the six mesocosms and outside the mesocosms before and after dust seeding. During DUNE-1-P and -Q, 5 mL of ¹⁵N₂ gas (99 atom% ¹⁵N, EURISO-TOP) were added to trace metal clean 4.5 L polycarbonate bottles equipped with septum caps for ¹⁵N₂ uptake determination while during DUNE-2-R, 2.5 mL of ¹⁵N₂ gas were added to 2.3 L polycarbonate bottles. Prior to DUNE-2, intercomparison of N₂ fixation rates measured in both 2.3 and 4.5 L incubated volumes showed fluxes in the same order of

magnitude (variation coefficient < 15 %, unpublished data). Immediately after sampling, ¹⁵N₂ tracer was added to obtain a final enrichment of the N₂ pool of about 10 atom% excess and each bottle was well shaken. Then, the ¹⁵N₂-amended bottles were incubated under in situ conditions on a mooring line, outside the mesocosms for 24 h at the corresponding sampling depths (0.1 m and 5 m depth). Incubations were terminated the following morning by filtration onto pre-combusted 25 mm GF/F filters, and filters were stored at –20 °C. Sample filters were dried at 40 °C for 48 h before analysis. Concentration of particulate organic nitrogen (PON) and ¹⁵N enrichment of the PON were quantified with an isotope ratio mass spectrometer (IRMS, Delta plus, ThermoFisher Scientific, Bremen, Germany) coupled with a C/N analyzer (Flash EA, ThermoFisher Scientific) via a type III interface. Standard deviation was 0.004 μmol L^{–1} for PON and 0.0001 atom% for ¹⁵N enrichment. N₂ fixation rates were calculated by isotope mass balance equations as described by Montoya et al. (1996). The ¹⁵N uptake rates were considered as significant when ¹⁵N excess enrichments of PON were greater than three times the standard deviation obtained on natural samples. According to our experimental conditions and the equations from Montoya et al. (1996), the detection limit for N₂ fixation, calculated from significant enrichment and lowest particulate nitrogen is estimated to be 0.05 nmol N L^{–1} d^{–1}. It should be noted that the N₂ fixation rates measured by the ¹⁵N₂ gas-tracer addition method may have been underestimated due to incomplete ¹⁵N₂ gas bubble equilibration, as recently shown by Mohr et al. (2010). Despite this potential underestimation, the relative changes in N₂ fixation (N₂ fixation_{Dust} / N₂ fixation_{control}) after seeding should not have been affected.

2.3 Complementary data from DUNE companion papers

In this paper, data of chlorophyll *a*, nutrient concentrations (NO₃[–], DIP, DFe), PP and NP are used to characterize the biogeochemical conditions of the seawater before and after seeding. These data are fully described in DUNE companion papers (Wagener et al., 2010; Pulido-Villena, 2010, 2013; Ridame et al., 2013; Guieu et al., 2013; Wuttig et al., 2013) including the methodology and the analytical procedures. Briefly, the chlorophyll *a* concentration was measured after extraction in 90 % acetone on a Turner Trilogy laboratory fluorometer (details in Ridame et al., 2013 and Guieu et al., 2013). Nitrate (NO₃[–]) concentrations were analyzed on 0.2 μm-filtered seawater according to classical methods using the automated colorimetric technique; the detection limit was 30 nM (details in Ridame et al., 2013). DIP was analyzed on 0.2 μm-filtered seawater by spectrophotometry using a long waveguide capillary cell (LWCC); the detection limit was 2 nM (Pulido-Villena et al., 2010, 2013). The concentration of DFe (< 0.2 μm) was measured by flow injection analysis with online preconcentration and chemiluminescence detec-

tion (FIA-CL); the detection limit was 10 pM (details in Wagener et al., 2010). PP (CO₂ fixation) was determined by the ¹³C uptake method after addition of NaH¹³CO₃ (see details in Ridame et al., 2013). This was done simultaneously with a N₂ fixation measurement using the dual ¹³C / ¹⁵N₂ isotopic label technique. As atmospheric deposition constitutes a source of external nutrients to the surface layer, it induces by definition NP. Thus, the increase in PP in the Dust-meso 24 h after seeding observed in DUNE P and R experiments can be associated with NP (NP_{seeding}). We consider that after 24 h, the increase in PP could be partly supported by regenerated nutrients. NP was estimated before seeding and over the course of the experiments in the Control-meso considering that NP represents 15 % of PP during periods of stratification in the western Mediterranean Sea (Marty et al., 2002; Moutin and Raimbaut, 2002; L'Helguen et al., 2002). We estimated the NP in the Dust-meso (NP_{dust}) 24 h after seeding using the following equations:

$$NP_{\text{dust}} = NP_{\text{control}} + NP_{\text{seeding}}, \quad (1)$$

$$NP_{\text{seeding}} = PP_{\text{dust}} - PP_{\text{control}}. \quad (2)$$

The contribution of N₂ fixation to PP was estimated using measurements of PP (reported in the companion paper, Ridame et al., 2013) and average molar particulate C/N ratios calculated for each experiment (7.5 ± 0.4, 7.5 ± 0.5 and 7.8 ± 0.6 respectively over P, Q and R experiments; whole data set in Ridame et al., 2013).

2.4 Statistical analysis

Means of total P, Fe and N contents in the EC and non-EC dust used in DUNE-1-P, -Q and DUNE-2-R were compared using a one-way ANOVA (analysis of variance) and a Fisher least significant difference (LSD) means comparison test (α = 0.05) (Table 1). Means of N₂ fixation rates in the Dust- and Control-meso were compared using a repeated measure ANOVA and a Fisher LSD means comparison test. When assumptions for ANOVA were not respected, means were compared using a Kruskal–Wallis test and a post hoc Dunn's test in XLstat software.

3 Results

3.1 Characteristics of the seawater

Statistical analysis of biological and chemical parameters in Table 2 showed no significant difference between Control-, Dust-meso and Out before seeding (see details in Guieu et al., 2010b, 2013). Over the three experiments, chlorophyll *a* (0.07–0.11 μg L^{–1}) and primary production (3.9–5.4 mg C m^{–3} d^{–1}) were initially low as were N₂ fixation

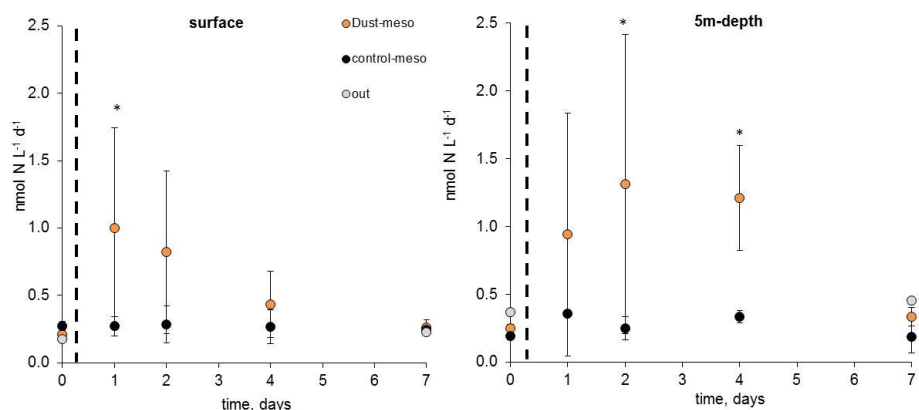


Fig. 1. Mean N₂ fixation rate ($n=3$) in $\text{nmol N L}^{-1} \text{d}^{-1}$ during the DUNE-1-P experiment in the Control-meso (black dot), Dust-meso (orange dot) and Out (grey dot) at surface and 5 m depth. The dotted line represents the time of the dust seeding. Data in the Control- and Dust-meso represent the average and standard deviation of the three replicate mesocosms. Means in the Dust-meso that were significantly different ($p < 0.05$) from the Control-meso are labeled with the * symbol.

rates ($0.20\text{--}0.24 \text{ nmol N L}^{-1} \text{d}^{-1}$) (Table 2). DIP concentrations, ranging between 2 and 5 nM (Table 2) were close to the detection limit (details in Pulido-Villena et al., 2010). The initial NO_3^- concentration was under the detection limit ($< 30 \text{ nM}$) in the DUNE-R experiment. Due to analytical problems, NO_3^- concentrations were not available for P and Q experiments but are strongly suspected to be under the detection limit before seeding as shown in experiment R and in surface waters of the northwestern Mediterranean Sea during stratification (Marty et al., 2002; Pujo-Pay et al., 2011). The initial DFe concentration was higher for DUNE-R (3.3 nM , Table 2) compared to P and Q experiments ($\sim 2.3 \text{ nM}$, Table 2).

Over the duration of the DUNE-1-P experiment, the temperature in the water column of the mesocosms was homogenous and stable (mean $T^\circ = 19.8 \pm 0.5^\circ \text{C}$, Guieu et al., 2010b) while over the course of DUNE-1-Q, temperature increased rapidly (up to 26.0°C at the surface) leading to a strongly marked thermal stratification typical of summer conditions (mean $\Delta T_{0-10\text{m}} = 3.6^\circ \text{C}$ between 21 and 27 June, Guieu et al., 2013). Over DUNE-2-R1, changes in temperature were representative of the transition period between spring (low stratification) and summer (strong stratification) conditions. While at the beginning of the second seeding (DUNE-2-R2) stratification was well established, a destratification followed by a restratification was then observed (details in Guieu et al., 2013). The highest temperature was recorded during DUNE-2-R2 (up to 27.3°C at the surface).

3.2 Response of N₂ fixation to a wet deposition: DUNE-1-P experiment

In surface waters (0.1 m depth), N₂ fixation rates measured in the Dust-meso were higher relative to the Control-meso as shown by a 3.7-fold maximum increase ($p < 0.05$) 24 h after

the EC-dust seeding (Fig. 1). Also, at 5 m depth, N₂ fixation rates were 5.3-fold higher ($p < 0.05$) in the Dust-meso relative to the unamended control, 2 days after seeding. The stimulation of N₂ fixation lasted at least 4 days after seeding. N₂ fixation rates varied between the triplicate dust mesocosms, 24 h and 48 h after addition at both sampling depths with a relative change of only 1.2–1.4 recorded in one of the three dust mesocosms.

3.3 Response of N₂ fixation to a dry deposition: DUNE-1-Q experiment

In surface waters, dust addition led to a significant ~ 2 -fold increase in N₂ fixation rates after 2, 3 and 5 days (Fig. 2). A maximum 5.2-fold stimulation ($p < 0.05$) was observed at 5 m depth 24 h after dust addition. Seven days after seeding, N₂ fixation rates at the surface were of the same order of magnitude in the Control- and Dust-meso. As observed in the DUNE-1-P experiment, the response of N₂ fixation was variable within the group of Dust-meso, particularly in samples collected at 5 m depth.

3.4 Response of N₂ fixation to 2 successive wet depositions: DUNE-2-R experiment

N₂ fixation rates were stable in the Control-meso over the 13 days duration of the R experiment at 5 m depth ($0.22 \pm 0.05 \text{ nmol N L}^{-1} \text{d}^{-1}$) (Fig. 3). Values from outside the mesocosms showed no significant differences from the Control-meso. The first seeding (R1) led to a 3-fold maximum increase in N₂ fixation relative to the Control-meso, three days after dust addition. The second one (R2) induced a 4-fold maximum increase in N₂ fixation relative to the Control-meso, one day after addition (Fig. 3). While the response of the N₂ fixing activity was similar between the three dust mesocosms over R1, a high variability in the magnitude

Table 2. Initial biological and chemical properties of seawater before seeding in experiments DUNE-1-P, DUNE-1-Q and DUNE-2-R (average in Dust-meso, Control-meso and Out). DIP: dissolved inorganic phosphorus, dl: detection limit (30 nM for NO₃[−]), nd: no data. Data for chlorophyll *a*, DIP, NO₃[−] and DFe are the mean concentrations at 0.1, 5 and 10 m depths. Data for primary production and N₂ fixation are the mean rates at 0.1 and 5 m depths for P and Q experiments and at 5 m depth for R experiment.

	DUNE-1-P	DUNE-1-Q	DUNE-2-R
Chlorophyll <i>a</i> , µg L ^{−1}	0.11 ± 0.03 ^a	0.08 ± 0.02 ^a	0.07 ± 0.02 ^a
Primary production, mg C m ^{−3} d ^{−1}	5.35 ± 1.11 ^a	4.16 ± 0.38 ^a	3.89 ± 0.46 ^a
N ₂ fixation, nmol N L ^{−1} d ^{−1}	0.24 ± 0.06	0.21 ± 0.05	0.20 ± 0.01
DIP, nM	5 ± 2 ^b	2 ± 0 ^c	5 ± 3 ^d
NO ₃ [−] , nM	nd	nd	< dl ^a
DFe, nM	2.4 ± 0.3 ^e	2.3 ± 0.3 ^f	3.3 ± 0.8 ^g

^a Ridame et al. (2013); ^b Pulido-Villena et al. (2010); ^c E. Pulido-Villena, personal communication, 2013;

^d Pulido-Villena et al. (2013); ^e Wagener et al. (2010); ^f T. Wagener, personal communication, 2013;

^g Wuttig et al. (2013)

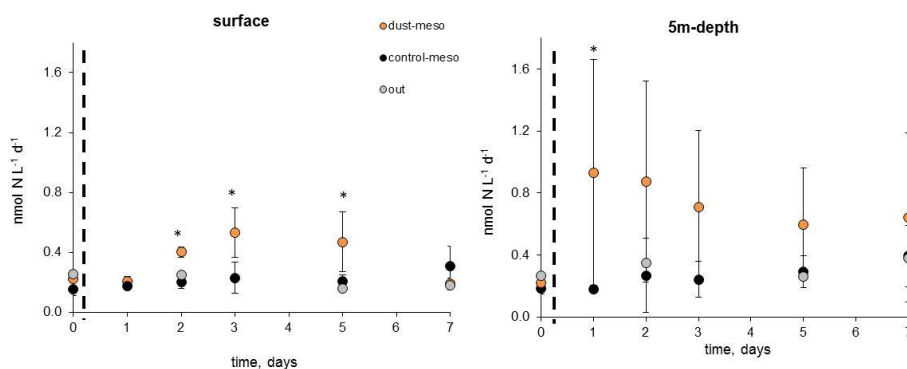


Fig. 2. Mean N₂ fixation rate ($n = 3$) in nmol N L^{−1} d^{−1} during the DUNE-1-Q experiment in the Control-meso (black dot), Dust-meso (orange dot) and Out (grey dot) at surface and 5 m depth. The dotted line represents the time of the dust seeding. Data in the Control- and Dust-meso represent the average and standard deviation of the three replicate mesocosms. Means in the Dust-meso that were significantly different ($p < 0.05$) from the Control-meso are labeled with the * symbol.

of the response of N₂ fixation was recorded after the second seeding at day 8 between the three dust mesocosms (relative changes of 2.9, 1.6 and 7.9 for D1, D2 and D3 respectively). Six days after both seedings, N₂ fixation rates in the Dust-meso were still significantly ($p < 0.05$) higher than in the Control-meso (Fig. 3).

4 Discussion

The initial characteristics of seawater in all experiments were typical of LNLC environments as depicted by low nutrient concentrations (DIP, NO₃[−]) and low phytoplanktonic biomass and production (Table 2). For P, Q and R experiments, N₂ fixation rates were also initially low (< 0.25 nmol N L^{−1} d^{−1}), homogeneous and remained stable over the duration of experiments in the Control-meso. These were consistent with previous surface measurements in the open western Mediterranean Sea during periods of stratification (≤ 0.2 nmol N L^{−1} d^{−1} in Ibello et al., 2010; Bonnet et al., 2011; Ridame et al., 2011; TERNON et al., 2011) and were

within the range of the lowest rates measured with the gas bubble enrichment method in oligotrophic areas of the Atlantic and Pacific oceans (0.1 to 4 nmol N L^{−1} d^{−1} in Mills et al., 2004; Needoba et al., 2007; Bonnet et al., 2009; Fernandez et al., 2010; Rijkenberg et al., 2011). The low values of N₂ fixation rates in the Control-meso are in agreement with the low abundance of picoplanktonic (0.2–3 µm) unicellular diazotrophic cyanobacteria (UCYN) (using TSA-FISH technique; details in Biegala and Raimbault, 2008) measured in the tested waters before seeding for each of the three experiments (I. Biegala, personal communication, 2013). In the open Mediterranean Sea, the community of diazotrophic cyanobacteria is indeed mainly dominated by UCYN (Yogev et al., 2011; Le Moal et al., 2011). Bonnet et al. (2011) reported that up to 100 % of the N₂ fixing activity was found within the < 3 µm size fraction in the western Mediterranean Sea during summer.

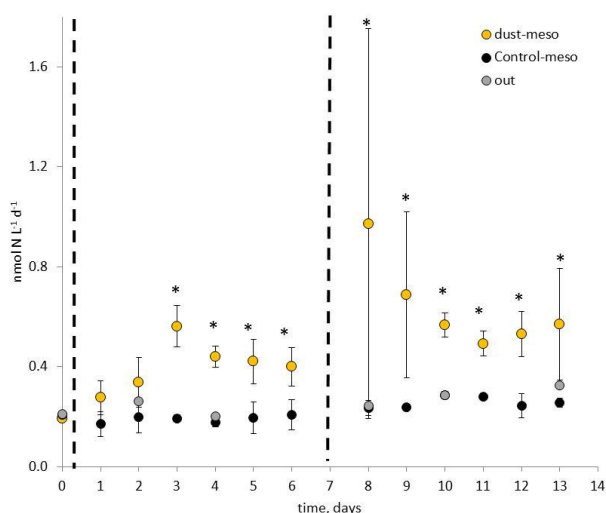


Fig. 3. Mean N₂ fixation rate ($n = 3$) in $\text{nmol N L}^{-1} \text{d}^{-1}$ during the DUNE-2-R1 and -R2 experiments in the Control-meso (black dot), Dust-meso (orange dot) and Out (grey dot) at 5 m depth. The dotted line represents the time of the dust seeding. Data in the Control- and Dust-meso represent the average and standard deviation of the three replicate mesocosms. Means in the Dust-meso that were significantly different ($p < 0.05$) from the Control-meso are labeled with the * symbol.

4.1 Pathway of dust deposition and response of N₂ fixation

All of the dust seeding experiments (P, Q, R1 and R2) induced a significant stimulation of N₂ fixation from 2- to 5.3-fold (Figs. 1–3). The response of diazotrophs was rapid as shown by a significant increase 24 h or 48 h after dust addition and longer as depicted by an increase in N₂ fixation rates recorded for at least 4–6 days after seeding. Despite the strong increase in N₂ fixation following the simulation of dust events, the rates remained low (maximum rate of $1.31 \text{ nmol N L}^{-1} \text{d}^{-1}$ in the Dust-meso in DUNE-1-P, day 2). The relative changes in the N₂ fixation rates were similar after the four seedings ($p > 0.05$, 5 m depth) with no significant variation with depth (surface and 5 m, DUNE-1-P and -Q, $p > 0.05$).

EC and non-EC dust have a similar content of P and Fe (Table 1) as well as of trace metals (Guieu et al., 2010b; Desboeufs et al., 2013). Both types of dust are a source of DIP (Pulido-Villena et al., 2010, 2013; Pulido-Villena unpublished data; Losno et al., 2013) and DFe (Wagener et al., 2010; Losno et al., 2013) to surface waters. N content of EC and non-EC dust was different resulting in changes in the atmospheric input of NO_3^- depending on the pathway of deposition: EC dust – mimicking Saharan dust wet deposition – was a significant source of NO_3^- to the water column (details in Ridame et al., 2013, for results on dissolution experiments and NO_3^- measurements after the DUNE-2-R seeding exper-

iment) whereas non-EC dust – mimicking Saharan dust dry deposition – was a negligible source of NO_3^- (Ridame et al., 2013). However, this difference in the resulting atmospheric supply of new NO_3^- did not affect the response of N₂ fixation as the relative changes in the N₂ fixation rates were similar after wet (P, R1, R2) and dry dust seedings (Q). Thus, the mode of deposition and the type of dust did not influence the magnitude of the stimulation of the N₂ fixing activity in the DUNE experiments indicating that both simulated wet and dry dust deposition events brought a sufficient amount of the nutrient(s) that were limiting N₂ fixation. The similarity in the contents of P, Fe and trace metals between both types of dust could well explain the similarity in the stimulation of N₂ fixation after all the seedings.

Dissolved N₂ constitutes by far the largest pool of nitrogen in the ocean (Gruber, 2008) and it can be used only by diazotrophs for their metabolic processes. Thus, diazotrophs should not be limited by the availability of fixed N in the environment, which represents a major ecological advantage relative to non-diazotrophic phytoplankton in N depleted waters. Moreover, as N₂ fixation is more energy consuming than NO_3^- reduction, the input of NO_3^- could inhibit partially N₂ fixation. This hypothesis has been validated on the cultured filamentous diazotroph *Trichodesmium*, which decreased its N₂ fixing activity (up to –70 %) after a $10 \mu\text{M}$ NO_3^- addition (Mulholland et al., 2001; Holl and Montoya, 2005). However, the response of UCYN could be different as an addition of $10 \mu\text{M}$ of NO_3^- did not change the N₂ fixation activity of the UCYN *Crocosphaera* (Dekaezemaker and Bonnet, 2011). An increase of about $10 \mu\text{M}$ in NO_3^- concentration, equivalent to that used in the culture experiments cited above, was observed in surface waters (0.1 m depth) 5 h after wet deposition in DUNE-2-R (Ridame et al., 2013). Despite this strong increase, N₂ fixation rates in the Dust-meso increased after the wet deposition events (P, R) in a similar manner to those after the simulated dry deposition (Q). This indicates that N₂ fixation activity was not inhibited by the atmospheric input of NO_3^- suggesting that the diazotrophic community was mainly dominated by unicellular diazotrophs.

The stimulation of N₂ fixation after dust addition may result from an increase in the UCYN abundance. In DUNE-1-Q, the dust event (dry deposition) led to a strong and significant increase both in N₂ fixing activity (5.2-fold) and in the abundance of UCYN (I. Biegala, personal communication, 2013) 24 h after seeding at 5 m-depth. In DUNE-2-R1 and -R2, the significant increase in N₂ fixation after the two successive seedings was associated with a slight increase in the UCYN abundance (I. Biegala, personal communication, 2013). However, the dust stimulation of N₂ fixation was not necessarily associated with an increase in UCYN abundance as observed during DUNE-1-P where the N₂ fixation increase 24 h after dust seeding was not correlated with an increase in the UCYN abundance (I. Biegala, personal communication, 2013). Although it is tempting to compare directly UCYN abundance and N₂ fixation, one must be careful

as the presence of other diazotrophs could contribute significantly to the N₂ fixation process. Indeed, although in the Mediterranean Sea the diazotrophic cyanobacteria community is dominated by unicellular organisms (UCYN), low concentrations of filamentous cyanobacteria (*Trichodesmium* and the symbiotic *Richelia*) have been found (Bar-Zeev et al., 2008; Le Moal et al., 2011; Yogeve et al., 2011). Recently, the presence of non-cyanobacterial unicellular diazotrophs such as α - and γ -proteobacteria has been reported in the Mediterranean Sea (Man-Aharonovich et al., 2007; Bar-Zeev et al., 2008; Yogeve et al., 2011; Le Moal et al., 2011) suggesting that N₂ fixation supported by non-cyanobacterial diazotrophs could be significant in the surface Mediterranean waters (Rahav et al., 2013a). It is therefore probable that during the DUNE experiments, non-cyanobacterial unicellular diazotrophs and to a lesser extent filamentous diazotrophic cyanobacteria have, in addition to UCYN, contributed to N₂ fixation. Other aspects such as the regulation of the diazotrophic biomass development by protist grazers, the viral attack and/or single-cell variability in N₂ fixing activity may also explain the larger changes in metabolic rates of N₂ fixation to dust addition relative to those of UCYN cellular abundance. This latter hypothesis is probable as a lack of correlation between *nifH* copy and transcript numbers has been shown previously within the cyanobacterial diazotrophic community (Zehr et al., 2007).

In the tropical Atlantic, Maranon et al. (2010) have shown that although bulk abundance tended to remain unchanged, different groups of phytoplankton and bacterioplankton responded differently to Saharan dust additions thereby inducing changes in the structure of the phytoplanktonic and heterotrophic community. This pattern was also observed for the diazotrophic community after dust events (Langlois et al., 2012; Benavides et al., 2013). After a Saharan dust deposition event in the Canary Islands, the abundance of unicellular diazotrophs increased while that of the filamentous *Trichodesmium* as well as the N₂ fixation rate in the > 50 μ m size fraction were not affected (Benavides et al., 2013). Interestingly, the response within the unicellular diazotrophic community was contrasted: the abundance of the small (< 1 μ m) unicellular diazotrophs (probably from Group A) strongly increased whereas that of medium-sized (1–3 μ m) cells decreased leading to a shift in the structure of the unicellular diazotrophic community. Langlois et al. (2012) found that although abundance of all diazotrophic cyanobacterial and γ -proteobacteria phylotypes increased after Saharan dust additions in the tropical North Atlantic Ocean, the intensity of these increases varied with the largest increases observed for UCYN from Group A. This variability could be explained by variability in the nutrient requirements and mode of acquisition within the diazotrophic cyanobacterial community as demonstrated for Fe and P in cultured unicellular and filamentous cyanobacteria (Tuit et al., 2004; Dyhrman et al., 2006; Berman-Frank et al., 2007; Jacq et al., 2013). Thus, it is likely that in the DUNE experiments, diverse diazotrophs

Table 3. Contribution in percentage of N₂ fixation to PP and NP before and after dust seeding in DUNE-1-P, -Q and DUNE-2-R (see details in Material and methods, Sect. 2).

N ₂ Fix/PP (%)	Before seeding	After seeding
DUNE-1-P	0.4	0.9
DUNE-1-Q	0.4	1.1
DUNE-R	0.5	0.8
N ₂ Fix/NP _{24h} (%)	Before seeding	24 h after seeding
DUNE-1-P	2.9	1.9
DUNE-1-Q	3.0	11.3
DUNE-R	3.3	1.6

responded differently to the dust additions and that the simulated dust event changed the structure of the diazotrophic community.

In the P as in the Q experiment, the relative changes in N₂ fixation rates varied between the triplicate dust mesocosms (D1, D2, D3) at some sampling times indicating heterogeneity in the magnitude of the response of the diazotrophic activity to the dust input. For example, the relative changes in DUNE-1-P were 4.4, 10.1 and 1.4 respectively in D1, D2 and D3, 2 days after the seeding (5 m depth, Fig. 1). Such a discrepancy could be attributed to a spatial heterogeneity in the composition of the diazotrophic community. The presence of *Trichodesmium* sp. in D3 relative to D1 and D2 could explain the small increase in N₂ fixation after the wet deposition as the input of NO₃⁻ would have inhibited the N₂ fixing activity of *Trichodesmium*. Unfortunately, the phylogenetic characterization of diazotrophs in the tested seawater is currently unavailable. The variability in the relative changes in N₂ fixation rates between the three dust mesocosms could also be due to spatial heterogeneity in the abundance of diazotrophs. Indeed, high variability in the abundance of UCYN (5 m depth) between the three dust mesocosms in DUNE-P or -Q was observed (I. Biegala, personal communication, 2013). During the R experiment, there was less variability in both N₂ fixation rates and UCYN abundance (I. Biegala, personal communication, 2013) within the Dust-meso after seedings.

4.2 Contribution of N₂ fixation to primary and new production

The contribution of N₂ fixation to primary production was negligible in the Control- and Dust-meso before seeding (0.4 % of PP) and remained negligible after dust seeding in the Dust-meso (1 % of PP) for all experiments (Table 3). This indicates that N₂ fixation was a poor contributor to the N demand for PP as also reported in the open western Mediterranean Sea during summer (Bonnet et al., 2011) and in the eastern basin (Yogeve et al., 2011; Rahav et al. 2013a).

Based on new production estimates from Ridame et al. (2013) and on particulate C/N ratios, the contribution of N₂ fixation to NP was estimated to be on average 3 % in all experiments before dust addition and in the Control-meso over the experiments (Table 3). Such a low contribution is in agreement with previous estimates in summer for the western (6–9 %, Bonnet et al., 2011) and eastern Mediterranean Sea (1–2 %, Yogeve et al., 2011). As a consequence, the majority of the new production was initially supported by NO₃[−] as source of N. Despite the strong increase in N₂ fixation rates, dust addition did not significantly change the contribution of N₂ fixation to NP since only a maximum contribution of 10 % was found (Table 3). Recently, it has been shown that the gas bubble enrichment method may underestimate N₂ fixation rates in surface waters by a factor of 2–7 relative to the enriched ¹⁵N₂ seawater method (Mohr et al., 2010; Großkopf et al., 2012; Wilson et al., 2012). The comparison of both methods in the Mediterranean waters demonstrated a 2–3 fold increase in rates using the enriched seawater method relative to the bubble addition method (Rahav et al., 2013b). Assuming a possible 2-fold underestimation of the N₂ fixation rates, the contribution of N₂ fixation to PP would remain negligible before and after seeding (< ~2 %) as well as the contribution of N₂ fixation to NP (< ~6 % before and 24 h after the seedings); the only exception would be 24 h after the Q seeding where N₂ fixation could represent up to about 20 % of NP.

Mean N₂ fixation rates were integrated over the mesocosm depth (12.5 m) and over the duration of experiments (from 6 to 7 days) (Table 4). The input of new dissolved nitrogen associated with N₂ fixation in the Dust-meso (from 29 to 63 μmol N m^{−2}, Table 4) was at least twice as high as that in the Control-meso in all experiments (from 14 to 24 μmol m^{−2}). This input is negligible (< 1 %) when compared to the estimated input of new nitrogen (NO₃[−]) associated with the wet deposition (EC dust) in the Dust-meso (DUNE-1-P: 8.5 mmol N m^{−2}; DUNE-2-R1 and -R2: 9.7 mmol N m^{−2} in Ridame et al., 2013). As wet deposition is the main pathway of Saharan dust deposition over the western Mediterranean Sea (e.g., Loye-Pilot and Martin, 1996), atmospheric deposition is probably the main source of new nitrogen (NO₃[−]) during stratification periods.

4.3 Factors controlling N₂ fixation

Temperature. The surface temperatures in our experiments were within the temperature range where unicellular diazotrophs are usually found (Church et al., 2008; Moisander et al., 2010). Despite strong changes in temperature (from ~19 to ~27 °C) between experiments, (i) the N₂ fixing activity was not impacted as demonstrated by similar and stable rates between P, Q, R1 and R2 in the Control-meso over the course of the experiments ($p > 0.05$) and (ii) the magnitude of the response of N₂ fixation to dust events was similar ($p > 0.05$) between all the seeding experiments. As shown by the small

Table 4. Input of new nitrogen (μmol N m^{−2}) associated with N₂ fixation integrated over the depth of the mesocosm (12.5 m) and over the duration of the experiments in the Dust-meso and Control-meso in DUNE-1-P, -Q and DUNE-2-R1, -R2.

	Duration (days)	Control-meso	Dust-meso	Relative change
P	7	24.3	63.2	2.6
Q	7	22.6	50.4	2.2
R1	6	14.3	29.2	2.0
R2	6	16.2	38.1	2.4

R^2 between temperature and N₂ fixation rates in the DUNE experiments ($R^2 = 0.001$), temperature was probably not a limiting factor of diazotrophic activity during summer in the northwestern Mediterranean Sea as also mentioned by Yogeve et al. (2011) for the eastern basin.

Iron. Due to the high Fe content of the nitrogenase enzyme complex, N₂ fixation can be controlled by Fe supply (Raven, 1988). Furthermore, the high energetic cost of N₂ fixation imposes an additional Fe requirement for increased photosynthetic capacity (Kustka et al., 2003). Diazotroph phosphorus limitation could also occur in oceanic areas strongly impacted by Fe-rich mineral dust input (Wu et al., 2000). In the DUNE experiments, dust seedings simulating both dry and wet deposition were able to relieve the ambient nutrient limitation(s) of N₂ fixation.

Before seeding, DFe concentrations ranged from 2.3 to 3.3 nM in the tested waters (Table 2). Wagener et al. (2010) and Wuttig et al. (2013) have shown that in the DUNE experiments, dust addition induced contrasted changes in the ambient DFe concentrations. After the DUNE-1-P, -Q and DUNE-2-R1 seedings, the DFe concentrations dropped to about 1.5–2 nM (decrease of ~1 nM) due to DFe scavenging on settling dust particles and aggregates in the Dust-meso (T. Wagener, personal communication, 2013; Wagener et al., 2010; Wuttig et al., 2013) while after the DUNE-2-R2 seeding, a transient increase in the DFe concentrations in surface waters of about 2 nM was observed (Wuttig et al., 2013). This increase in DFe was correlated with an increase in the Fe binding ligand concentrations produced by phytoplankton and/or heterotrophic bacteria, which allowed the maintenance of Fe in solution, thereby probably increasing the concentration of bioavailable Fe (Wagener et al., 2010; Wuttig et al., 2013). Despite these changes in DFe concentration after dust addition and the potential changes associated with the bioavailable Fe concentration, the process of N₂ fixation increased after all the dust seedings (P, Q, R1, R2) and the magnitude of this increase was similar in all experiments. This indicates that during the DUNE experiments, the bioavailability of Fe was not a controlling factor of N₂ fixation and that DFe concentrations of the order of 1.5 nM and higher did not limit the N₂ fixation in the western Mediterranean Sea.

This is in good agreement with previous results of Ridame et al. (2011) during summer in the Mediterranean Sea and confirms that N₂ fixation should not be Fe limited in oceanic regions strongly impacted by Fe-rich mineral dust deposition. Luo et al. (2013) concluded that on a global scale Fe distribution (represented by dust deposition) does not influence the spatial patterns of N₂ fixation, but that at regional scales like in the North Atlantic, N₂ fixation is related to Fe-rich mineral dust input. In the northeastern Atlantic, correlations between DFe concentrations, *Trichodesmium* abundance and N₂ fixation have been previously reported (Moore et al., 2009). Culture-based studies have shown that the N₂ fixing activity of *Trichodesmium* was more impacted by Fe limitation than that of UCYN, indicating a much lower Fe requirement for UCYN (Berman-Frank et al., 2007; Jacq et al., 2013). These findings are in good agreement with our results, which highlight the non-limiting role of Fe for N₂ fixation during stratification periods in a region strongly impacted by Saharan dust deposition and where the diazotrophic community is dominated by unicellular diazotrophs.

Phosphorus. As the water column was initially DIP depleted and as both wet and dry dust deposition simulated during DUNE represented a source of new DIP (Pulido-Villena et al., 2010, 2013; Pulido-Villena unpublished data; Losno et al., 2013), the increase in N₂ fixation after dust addition could be rather explained by an increase in DIP availability. In the western basin, N₂ fixation has previously been shown to be significantly stimulated by both DIP and Saharan dust additions in microcosm experiments (Ridame et al., 2011; TERNON et al., 2011). We can therefore hypothesize that N₂ fixation during all the DUNE experiments was first DIP limited. Saharan dust pulses to the surface Mediterranean waters, in addition to P and Fe, could be also a source of trace elements (e.g., Eglington et al., 2002; Wuttig et al., 2013) that are necessary for metabolic processes (e.g., Morel and Hudson, 1985; Morel et al., 1991) and could therefore influence rates of N₂ fixation (Ridame et al., 2011). As shown in the central Mediterranean basin during summer from bioassay experiments, N₂ fixation increased only after Saharan dust addition (no response to DIP and/or Fe additions) indicating that N₂ fixation was either limited by a trace element released by dust different from Fe and DIP, or co-limited by DIP plus a trace element different from Fe released by dust. In DUNE-2-R, Wuttig et al. (2013) clearly showed an increase in dissolved manganese after R1 and R2 seedings due to dust dissolution processes. Thus, we cannot exclude that during the DUNE experiments, N₂ fixation was limited or co-limited by DIP and/or a trace element released by dust.

The pathways (wet or dry) of deposition can induce different responses of biological processes. While bacterial respiration (Pulido-Villena, unpublished data; Pulido-Villena et al., 2013) and N₂ fixation were stimulated after both wet and dry deposition, primary production increased significantly only after wet deposition (Ridame et al., 2013). This could suggest that the increase in N₂ fixation after dust addition

may have been mainly supported by (photo)heterotrophic diazotrophs like proteobacteria (heterotroph) and/or UCYN from Group A (photoheterotroph). Wet deposition was able to relieve the nutrient limitations of the CO₂ fixation of the whole phytoplanktonic community, the N₂ fixation of diazotrophs and the bacterial respiration of heterotrophs. As dry deposition was not a significant source of dissolved inorganic nitrogen (DIN), primary production was likely DIN limited or co-limited by both DIN and DIP (Ridame et al., 2013) as previously shown for the summer in the northwestern Mediterranean Sea (Tanaka et al., 2011) while N₂ fixation was likely limited or co-limited by DIP and/or a trace element other than Fe.

5 Summary and conclusions

Our results from original mesocosm experiments demonstrate that atmospheric dust deposition does greatly influence N₂ fixation rates in low nutrient low chlorophyll environments impacted by dust deposition. The response of N₂ fixation to dust events was quantified: N₂ fixation rates can increase up to 5-fold and the stimulation can be observed for at least one week after deposition. Despite a strong increase in N₂ fixation after the dust deposition events, the rates remained low and N₂ fixation remained a poor contributor to PP and NP. Dust deposition induced increases in N₂ fixation resulting in an increase in the N pool, but the estimated input of new DIN (NO₃⁻) from the wet deposition was much higher than that associated with N₂ fixation. Our study clearly shows that dust events associated with wet or dry deposition are able to relieve the ambient nutrient limitation(s) of N₂ fixation in oligotrophic environments where ambient DFe concentrations are likely non-limiting. Currently, most of the regional and global biogeochemical models include Fe deposition from mineral aerosols to quantify N₂ fixation and do not often consider aeolian input of DIP (e.g., Moore et al., 2006; Coles and Hood, 2007; Monteiro et al., 2011). Here, we have shown that dust deposition through the supply of new nutrients can strongly control N₂ fixation in LNLC environments. A better parameterization of DIP supply from aeolian aerosols should improve the representation of N₂ fixation in models of oceanic areas strongly impacted by dust deposition such as the Mediterranean Sea and the tropical North Atlantic.

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