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Strong stimulation of N_2 fixation in oligotrophic Mediterranean Sea: results from dust addition in large in situ mesocosms

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Abstract

The response of N_2 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 mesocoms ($52\,m^3$) 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 successive wet depositions) in the north western oligotrophic Mediterranean Sea. Here we show that wet and dry dust deposition induced a rapid (24 h or 48 h after dust additions), strong (2- to 5.3-fold) and long (4 to 6 days duration) increase in N_2 fixation indicating that both wet and dry Saharan dust depositions were able to relieve efficiently the nutrient limitation(s) of N_2 fixation. This means in particular that N_2 fixation activity was not inhibited by the NO_3^- input associated with the simulated wet deposition. The contribution of N_2 fixation to primary production was negligible before (on average 0.4 %) and after (on average 1 %) dust additions in all experiments indicating that N_2 fixation was a poor contributor to the N demand for primary production. Before seedings, new production (NP) was mainly supported by NO_3^- as a source of N as shown by the low contribution of N_2 fixation to NP (on average 3 %). Despite the stimulation of N_2 fixation by dust, the rates remained low, and did not allow to significantly change the contribution of N_2 fixation to NP as a maximum of 10 % contribution was evidenced. A comparison of the responses of N_2 fixation by diazotrophs and CO_2 fixation by the whole phytoplankton community suggests that those metabolic processes were limited or co-limited by different nutrients. The estimated input of new nitrogen (NO_3^-) from simulated wet deposition was much higher than that associated with N_2 fixation. We confirm that although the biogeochemical impact of N_2 fixation seems negligible in the oligotrophic waters of the western Mediterranean Sea, Saharan dust pulses by bringing new nutrients represent a key controlling factor of the magnitude of N_2 fixation rate in the Mediterranean

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Sea and potentially in all LNLC areas impacted by dust deposition such as the tropical Atlantic and Pacific Oceans.

1 Introduction

Over geological time scales, dinitrogen fixation (N_2 fixation) is important for regulating fixed nitrogen concentrations in the ocean and thereby sustaining ocean productivity (Falkowski, 1997; Tyrrell, 1999). In the modern ocean, N_2 fixation or diazotrophy is now recognized to be the main source of fixed nitrogen in the marine environments (134 $Tg\,N\,yr^{-1}$, Eugster and Gruber, 2012) supporting a part of oceanic primary productivity and organic matter export to the deep ocean. Due to the importance of N_2 fixation in both the global cycling of nitrogen (N) and carbon (C), information on N_2 fixation and spatial distribution of diazotrophs is accumulating (Monteiro et al., 2010; Luo et al., 2012). Nevertheless, despite recent advances, the environmental factors controlling the magnitude of N_2 fixation need to be assessed and a better quantification of their impacts to be achieved. In the contemporary ocean, it is assumed that N_2 fixation is mainly limited by either 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 co-limited by both (Mills et al., 2004).

A large part of oceanic systems are subjected to high inputs of aeolian dust aerosols from the great deserts of the world (Tegen et al., 2004; Jickells et al., 2005). Since the past decades, the biogeochemical interest of these aerosols increased when it was realized that aeolian deposition of mineral aerosols represents, at 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 controlling factor in the magnitude of N_2 fixation in LNLC (low nutrient low chlorophyll) environments. Nevertheless, the effects of atmospheric dust-derived nutrients on N_2

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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 phytoplanktonic bloom, the surface mixed layer (SML) is isolated from deeper waters by a strong stratification, and becomes macro-nutrient (N, P) depleted, leading to a low primary production (Moutin and Raimbault, 2002; Marty and Chiaverini, 2002; Bosc et al., 2004; Lopez-Sandoval et al., 2011). During the whole stratification period, atmospheric inputs become therefore the main source of allochtonous nutrients to oligotrophic surface waters and the dissolved iron (DFe) concentration is relatively high, most likely due to atmospheric Fe accumulation in the SML (Sarthou and Jeandel, 2001; Bonnet and Guieu, 2006; Wagener et al., 2010). Direct measurements of N_2 fixation rates in the SML during the stratification period showed generally low values ($\leq 0.2\,\mathrm{nmol\,N\,L^{-1}\,d^{-1}}$) through the open Mediterranean Sea (Ibello et al., 2010; Bonnet et al., 2011; Ridame et al., 2011; Ternon et al., 2011; Yogev et al., 2011) but relatively high rates have been episodically found (up to $7.5\,\mathrm{nmol\,N\,L^{-1}\,d^{-1}}$ in Sandroni et al., 2007). The low rates of N_2 fixation recorded during stratification could be a consequence of the low availability of dissolved inorganic phosphorus (DIP) in Mediterranean Sea. During the BOUM cruise in summer 2008, nutrient additions in bottles have shown that N_2 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, N_2 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 N_2 fixation. Microcosm experiments performed in tropical Atlantic and Mediterranean Sea proved that Saharan dust addition may strongly influence N_2 fixation rate (Mills et al., 2004; Maranon et al., 2010; Ternon et al., 2011; Ridame et al., 2011) as well

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as the abundance and the distribution of various diazotrophic groups (Langlois et al., 2012).

The microcosm approach presents some limits as the short duration of the experiment (one or two days on average) making results difficult to extrapolate on longer time scale and as the reduced number of measured parameters due to the small incubated volumes implying the study of 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 virus 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 N_2 fixation process sustained by diazotrophic organisms. Here we quantity the impact of contrasted Saharan dust events (wet and dry deposition) on the N_2-fixing activity and estimate the consequences on the primary and new production and implications on the nitrogen pool in the oligotrophic waters of western Mediterranean Sea.

2 Material and methods

2.1 Experiment design and dust characterization

Three distinct experimental dust additions into large mesocosms (52 m^3) were conducted in June 2008 and June–July 2010 in the north western 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 was shown to be 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, three replicate mesocosms (D1, D2 and D3 hereafter referred to as "Dust-meso") entirely designed in plastic were amended with

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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 resulted 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 during 7 days. Each day, three different depths (0.1, 5 and 10 m-depth) were sampled for most parameters in the six mesocosms. 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. The mesocosms were closed systems without lateral advection.

June 2008's experiments: Two distinct seeding experiments were conducted with two types of dust. In the first one (10 to 18 June 2008, hereafter referred to as "DUNE-1-P"), the dust used was previously subjected to physico-chemical transformations that involved HNO_3 and H_2SO_4, 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 realized with the EC dust mixed with 2 L of ultrapure water in order to mimic a wet deposition.

In the second experiment (20 to 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 realized with the non-EC dust mixed with 2 L of unfiltered surface seawater in order to mimic a dry deposition.

June–July 2010's experiments: Two successive seeding experiments (26 June to 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

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the same amount of evapocondensed 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 Dust-meso mixed with 2 L of ultrapure water simulating a wet deposition. It has to be noted that the dust used in DUNE-2-R has not been collected at the same time that the dust used in 2008's experiments (details in Desboeufs et al., 2013).

P, Fe, 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 HNO_3, the N content of EC dust was about 10-fold higher as compared to the non-EC dust (Table 1; Guieu et al., 2010b, 2013). Small difference in the N content in EC dust used in 2008 and 2010's experiments (1.19 ± 0.05 % and 1.36 ± 0.09 %) was observed (details in Guieu et al., 2013).

2.2 N_2 fixation rate

All materials were previously cleaned following trace metal clean procedures. Unfiltered seawater was sampled 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 N_2 fixation rate. Samples were collected in the six mesocosms and outside the mesocosms before dust addition and after seeding. During DUNE-1-P and -Q, 5 mL of $^{15}N_2$ gas (99 at % ^{15}N, EURISOTOP) were added to trace metal clean 4.5 L polycarbonate bottles equipped with septum caps for $^{15}N_2$ uptake determination while during DUNE-2-R, 2.5 mL of $^{15}N_2$ gas were added to 2.3 L polycarbonate bottles. Prior to DUNE-2, intercomparison of N_2 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). Tracer was added to obtain a final enrichment of the N_2 pool of about 10 atom % excess. Bottles were incubated in situ for 24 h at the corresponded sampling depths. Incubations were terminated by filtration onto pre-combusted 25 mm GF/F filters, and filters were stored at −20 °C. Sample filters were dried at 40 °C before analysis. Concentration of particulate

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organic nitrogen (PON) and ^{15}N enrichment of the PON were 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 deviation (SD) was 0.004 µmol L^{-1} for PON and 0.0001 atom% for ^{15}N enrichment. N_2 fixation rates were calculated by isotope mass balanced as described by Montoya et al. (1996). It has to be noted that the N_2 fixation rates measured by the $^{15}N_2$ gas-tracer addition method may have been underestimated due to incomplete $^{15}N_2$ gas bubble equilibration, as recently shown by Wilson et al. (2012).

2.3 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 and a Fisher LSD means comparison test ($\alpha = 0.05$) (Table 1). Means of N_2 fixation rate in 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 has shown no significant difference between Control-, Dust-meso and Out before seedings (see details in Guieu et al., 2010b, 2013). Over the three experiments, chlorophyll *a* (0.07–0.11 µL m^{-1}) and primary production (3.9–5.4 mg C m^{-3} d^{-1}) were initially low as well as N_2 fixation rates (0.20–0.24 nmol N L^{-1} 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 nitrate (NO_3^-) concentration was under detection limit (< 30 nM)

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in the DUNE-R experiment. Due to analytical problem, NO_3^- concentrations were not available for P and Q experiments but are strongly suspected to be under detection limit before seeding as shown in experiment R and in surface waters of the north-western Mediterranean Sea during stratification period (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 (~ 2.3 nM, Table 2).

Over the duration of the experiment DUNE-1-P, the temperature in the water column of the mesocosms was homogenous and stable (mean $T^\circ = 19.8 \pm 0.5\,^\circ$C, Guieu et al., 2010b) while over the course of DUNE-1-Q, the temperature was rapidly increasing (up to 26.0 °C in surface) leading to a strongly marked thermal stratification typical of summer conditions (mean $\Delta T^\circ_{0-10\,m} = 3.6\,^\circ$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 second seeding (DUNE-2-R2) the stratification was well established, a destratification followed by a restrafication was then observed (details in Guieu et al., 2013). The highest temperature was recorded during DUNE-2-R2 (up to 27.3 °C in surface).

3.2 Response of N_2 fixation to a wet deposition: DUNE-1-P experiment

In surface, N_2 fixation rates measured in the Dust-meso were higher as compared to the control as shown by a 3.7-fold maximum increase ($p < 0.05$) 24 h after the EC-dust seeding (mean rate of 1.0 nmol N L^{-1} d^{-1}, Fig. 1). Also, at 5 m-depth, N_2 fixation rate was 5.3-fold higher ($p < 0.05$) in the Dust-meso (mean rate of 1.31 nmol N L^{-1} d^{-1}) as compared to the unamended control, 2 days after seeding. The stimulation of N_2 fixation lasted at least 4 days after seeding. A high variability of N_2 fixation rates was observed within the Dust-meso, 24 and 48 h after addition at the two sampling depths. Indeed, a low change (factor of about 1.2) in N_2 fixation was recorded in one of the three dust mesocosms at these sampling times.

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3.3 Response of N_2 fixation to a dry deposition: DUNE-1-Q experiment

In surface, dust addition led to a significant ~ 2-fold increase in N_2 fixation rate 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 leading to a mean rate of 0.93 nmol N L^{-1} d^{-1} in Dust-meso. Seven days after seeding, N_2 fixation rates in surface were in the same order of magnitude in the Control- and Dust-meso. As observed in the DUNE-1-P experiment, the response of N_2 fixation was variable within the group of Dust-meso in particular in samples collected at 5 m-depth.

3.4 Response of N_2 fixation to successive wet depositions: DUNE-2-R experiment

N_2 fixation rate was stable in the Control-meso over the 13-days duration of the R experiment at 5 m-depth (0.22 ± 0.05 nmol N L^{-1} d^{-1}) (Fig. 3). Values from outside the mesocosms showed no significant differences with those in Control-meso. The first and second seedings led to a ~ 3-fold (mean rate of 0.56 nmol N L^{-1} d^{-1}) and ~ 4-fold (mean rate of 0.97 nmol N L^{-1} d^{-1}) maximum increase in N_2 fixation relative to the Control-meso, three days (R1) and one day (R2) respectively after successive dust additions (Fig. 3). While the response of the diazotrophic community was similar among the three dust mesocosms over R1, a high variability in the magnitude of the response of N_2 fixation was recorded after the second seeding at day 8 among the three dust mesocosms (factor of increase of 2.9, 1.6 and 7.9 for D1, D2 and D3 respectively relative to the Control-meso). Six days after both seedings, N_2 fixation rates in Dust-Meso were still significantly ($p < 0.05$) higher than in the Control-Meso (Fig. 3).

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4 Discussion

Initial characteristics of seawater for all experiments were typical of LNLC environments as depicted by low nutrient concentrations (DIP, NO_3^-) and low phytoplanktonic biomass and production (Table 2). N_2 fixation rates for P, Q and R experiments 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. They were consistent with previous surface measurements in the open western Mediterranean Sea during period of stratification ($\leq 0.2\,nmol\,N\,L^{-1}\,d^{-1}$ 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 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). The low values of N_2 fixation rates in the Control-meso are in agreement with low abundance of picoplanktonic (0.2–3 µm) unicellular diazotrophic cyanobacteria (UCYN) measured before the DUNE seedings (Biegala et al., 2013). In the open Mediterranean Sea, the community of diazotrophic cyanobacteria is indeed mainly dominated by UCYN from picoplanktonic size fraction (Yogev et al., 2011; Le Moal et al., 2011) and Bonnet et al. (2011) reported that up to 100 % of the N_2 fixing activity was found within this size fraction in the western Mediterranean Sea during summer.

4.1 Response of N_2 fixation to dust seedings

All of the dust seeding experiments (P, Q, R1 and R2) induced a significant stimulation of N_2 fixation from 2- to 5.3 fold (Figs. 1–3). The response of diazotrophs was fast as shown by a significant increase 24 h or 48 h after dust addition and long as depicted by an increase in N_2 fixation rate recorded during 4 to 6 days after seedings. In DUNE-1-P and -Q, the magnitude of the stimulation of N_2 fixation after dust addition was similar ($p > 0.05$) at both surface and 5 m depth. Despite the strong increase in N_2 fixation following the simulation of dust events, the rates remained low (maximum rate of $1.31\,nmol\,N\,L^{-1}\,d^{-1}$ in Dust-meso in DUNE-1-P, day 2).

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4.1.1 N_2 fixation versus UCYN abundance

The stimulation of N_2 fixation after dust addition may result from an increase in the picoplanktonic UCYN abundances. In DUNE-1-Q, dust event (dry deposition) led to a strong and significant increase both in N_2 fixing activity (5.2-fold) and in the abundance of picoplanktonic UCYN (5.5-fold on average, Biegala et al., 2013) 24 h after seeding at 5 m-depth. In DUNE-2-R1 and -R2, the significant increase in N_2 fixation after the two successive seedings was associated with a slight increase in the picoplanktonic UCYN abundance (details in Biegala et al., 2013). However, the dust stimulation of N_2 fixation was not necessarily associated with an increase in picoplanktonic UCYN abundance as observed during DUNE-1-P where the N_2 fixation increase 24 h after dust seeding was not correlated with an increase in the picoplanktonic UCYN abundance (Biegala et al., 2013). Although it is tempting to compare directly UCYN abundance and N_2 fixation, one must be careful as the presence of other diazotrophs could contribute to N_2 fixation process. Species composition of diazotrophs has been characterized during DUNE-2-R by Biegala et al. (2013). The authors evidenced the presence of filamentous diazotrophic cyanobacteria (*Richelia)* and non-cyanobacterial unicellular diazotrophs which could, in addition to picoplanktonic UCYN, contribute partially to N_2 fixation. Others aspects as the physiological/genetic specificity among species, the presence of predation and the viral attack may also interfere on either the cellular abundance or the metabolic activity.

In DUNE-1-P and -Q experiments, the increase in N_2 fixation to dust additions was variable among the three dust mesocosms indicating heterogeneity in the magnitude of the response of the diazotrophic activity to the dust input. Such discrepancy could be attributed to a spatial heterogeneity in the distribution of diazotrophs. Biegala et al. (2013) showed a high variability in the abundance of the picoplanktonic UCYN (5 m depth) between the three dust mesocosms at some sampling times in DUNE-P and -Q. During the DUNE-2-R experiment, there was less heterogeneity between N_2 fixation rates in the three dust mesocosms (Fig. 3) and also between picoplanktonic

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UCYN abundances (Biegala et al., 2013) after seeding. Nevertheless, variability of the picoplanktonic UCYN abundance was not systematically associated with variability of N_2 fixation rates.

4.1.2 Pathway of deposition

Both types of dust (EC and non-EC) have been evidenced as 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 the surface waters. Difference in the N content between EC and non-EC dust induced changes in atmospheric input of NO_3^- depending on the pathway of deposition: EC dust 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 experiment) whereas non-EC dust was a negligible source of NO_3^- (Ridame et al., 2013). Despite differences in the atmospheric supply of bioavailable nutrient depending on the type of deposition, both wet (P, R1 and R2) and dry deposition (Q) caused large and similar N_2 fixation increases (no significant difference in the relative changes of N_2 fixation was observed between the four seedings, $p > 0.05$, 5 m depth). By consequence, the pathway of deposition and the type of dust did not influence the magnitude of the stimulation of the N_2 fixing activity.

4.1.3 Change in temperature of the water column

Despite strong changes in temperature (from ~ 19 to ~ 27 °C) between experiments, (i) N_2 fixing activity of diazotrophs was not impacted as demonstrated by homogeneous and stable rates in Control-meso over all the experiments and (ii) the magnitude of the response of N_2 fixation to dust events was similar ($p > 0.05$) between all the seeding experiments. Therefore, temperature was probably not a limiting factor of the diazotrophic activity in summer in the northwestern Mediterranean Sea, as also mentioned by Yogev et al. (2011) for the eastern basin. Indeed, the surface temperatures in

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our experiments were within the temperature range where unicellular diazotrophs were usually found (Church et al., 2008; Moisander et al., 2010).

4.2 Contribution of N_2 fixation to primary and new production

The contribution of N_2 fixation to primary production (PP) was estimated using measurements of PP from 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, see Ridame et al., 2013) (Table 3). This contribution was negligible in the Control- and Dust-meso before seedings (0.4 % of PP) remaining negligible after dust seedings in the dust-meso (1 % of PP) for all experiments (Table 3). This indicates that N_2 fixation was a poor contributor to the N demand for PP as also reported in the open western Mediterranean Sea during summer by Bonnet et al. (2011) and in the eastern basin by Yogev et al. (2011). Therefore, assuming that diazotrophs used only dissolved N_2 as a source of nitrogen, the CO_2 fixation from diazotrophs before, as well as after seedings, was negligible as compared to the CO_2 fixation from the whole photosynthetic community.

Based on new production (NP) estimates from Ridame et al. (2013) and on particulate C/N ratios, the contribution of N_2 fixation to NP was on average 3 % in all experiments before dust addition and in the control-meso over the experiments (Table 3). Such low number is in agreement with previous estimates in summer for the western (6–9 %, Bonnet et al., 2011) and eastern Mediterranean Sea (1–2 %, Yogev et al., 2011). By consequence, the essential of the new production (NP) was initially supported by NO_3^- as source of N. Despite the strong increase in N_2 fixation rates, dust addition did not change significantly the contribution of N_2 fixation to new production as a maximum of 10 % contribution was evidenced (Table 3).

Mean N_2 fixation rates were integrated over the mesocosm depth and over the duration of experiments (Table 4). The input of new dissolved nitrogen associated with N_2 fixation in dust-Meso (from 29 to 63 µmol N m^{-2}, Table 4) was at least twice higher than that in control-Meso in all experiments (from 14 to 24 µmol m^{-2}). This input is negligible

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(< 1 %) when compared to the estimated input of new nitrogen (NO_3^-) associated with the wet deposition (EC dust) in the dust-Meso (DUNE-1-P: 7.7 mmol N m^{-2}; DUNE-2-R1 and -R2: 8.4 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. Loÿe-Pilot and Martin 1996), atmospheric deposition is probably the main source of new nitrogen (NO_3^-) during stratification.

4.3 Biogeochemical factors controlling N_2 fixation

Diazotrophs, by their nature, should not be limited by the availability of fixed N in the environment. Due to the high Fe content of the nitrogenase enzyme complex, N_2 fixation process can be controlled by Fe supply. Diazotroph phosphorus limitation could also occur in oceanic areas strongly impacted by Fe-rich mineral dust input (Wu et al., 2000). In DUNE experiments, dust seedings simulating either a dry or wet deposition allowed to relieve the ambient nutrient limitation(s) of N_2 fixation.

Before seedings, DFe concentration ranged from 2.3 to 3.3 nM (Table 2) in the tested waters. 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 concentration. After the DUNE-2-R2 seeding, a transient increase in the DFe concentration in surface of about 2 nM was evidenced (Wuttig et al., 2013) while after DUNE-1-P and DUNE-2-R1 seedings, there was a decrease of about 1 nM due to DFe scavenging on settling dust particles and aggregates in Dust-meso (Wagener et al., 2010; Wuttig et al., 2013). Also a slight decrease in DFe concentration was observed after the DUNE-1-Q seeding (T. Wagener, personal communication, 2013). Despite the increase or decrease in DFe concentration after dust addition, the process of N_2 fixation was 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, Fe was not a controlling factor of N_2 fixation which is in good agreement with results of Ridame et al. (2011) during summer in Mediterranean Sea and confirms that N_2 fixation

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should not be Fe limited in oceanic areas strongly impacted by Fe-rich mineral dust deposition.

As the water column was initially DIP depleted and as both wet and dry dust deposition mimicked during DUNE represent a source of new DIP (Pulido-Villena et al., 2010, 2013; Pulido-Villena unpublished data; Losno et al., 2013), the increase in N_2 fixation after dust addition could be rather explained by the increase in the DIP availability. In the western basin, N_2 fixation was previously 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 hypothesize that N_2 fixation during all the DUNE experiments was DIP limited. Saharan dust pulses to the surface Mediterranean waters, in addition to P and Fe, could be also a source of trace elements (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 N_2 fixation (Ridame et al., 2011). As shown in the central Mediterranean basin in summer from bioassay experiments, N_2 fixation increased only after Saharan dust addition (no response to DIP and/or Fe additions) indicating that N_2 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) have clearly shown increase in dissolved manganese after R1 and R2 seedings due to dust dissolution processes. So, we can not exclude that during the DUNE experiments, N_2 fixation was limited or co-limited by DIP and/or a trace element released by dust.

Because N_2 fixation is more energy consuming than NO_3^- reduction, it is strongly suspected that the presence of NO_3^- could inhibit partially N_2 fixation. This hypothesis was validated on the cultured filamentous diazotroph *Trichodesmium* which decreased its N_2 fixing activity (up to −70 %) after 10 µM NO_3^- addition (Holl and Montoya, 2005). However, the response of UCYN could be different as addition of 10 µM of NO_3^- did not change the N_2 fixation rate of the UCYN *Crocosphaera* (Dekaezemaker and Bonnet, 2011). An increase of 9.8 µM in the NO_3^- concentration (equivalent to that used in the culture experiments) was observed in surface (0.1 m) four hours after wet deposition

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in DUNE-2-R2 (Ridame et al., 2013). Despite this strong increase, N_2 fixation rate in Dust-meso was increased suggesting that N_2 fixation activity was not inhibited by the atmospheric input of new nitrogen (NO_3^-).

The pathways (wet or dry) of deposition can induce different responses of biological processes. Bacterial respiration (Pulido-Villena, unpublished data; Pulido-Villena et al., 2013) as N_2 fixation were stimulated after wet or dry deposition while primary production increased significantly only after wet deposition (Ridame et al., 2013). Wet deposition was able to relieve the nutrient limitations of the CO_2 fixation of the whole phytoplanktonic community, the N_2 fixation of diazotrophs and 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 in summer in northwestern Mediterranean Sea (Tanaka et al., 2010) while N_2 fixation was likely limited or co-limited by DIP and/or a trace element other than Fe and bacterial respiration probably limited by DIP (Pulido-Villena et al., 2013).

5 Summary and conclusions

Our experiments demonstrate from original mesocom experiments that atmospheric dust deposition from Sahara may greatly influence N_2 fixation rate in LNLC environments impacted by mineral dust deposition. The response of N_2 fixation to dust event was quantified: N_2 fixation rate can increase up to 5-fold and the stimulation can be observed for a week after deposition. In spite of the increase in N_2 fixation by dust, the rates remained low and dust event did not change significantly the contribution of N_2 fixation to PP and NP. N_2 fixation increased by dust deposition induced an increase in the N pool, but the estimated input of new DIN (NO_3^-) from the wet deposition was much higher than that associated with N_2 fixation. Our study clearly shows that dust event associated with a wet or dry deposition is able to relieve the ambient nutrient limitation(s) of N_2 fixation in oligotrophic environment where ambient dissolved iron concentration

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is likely non limiting. Currently, most of the regional and global biogeochemical models include Fe deposition from mineral aerosols to quantify N_2 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 N_2 fixation in LNLC environments. A better parameterization of DIP supply from aeolian aerosols should improve the representation of N_2 fixation in models in oceanic areas strongly impacted by dust deposition such as the Mediterranean Sea and the tropical north Atlantic.

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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	DUNE-1-Q	DUNE-2-R (R1, R2)
Dust treatment Simulated deposition	10–18 Jun 2008 evapocondensed wet	20–27 Jun 2008 non-processed dry	26 Jun–9 Jul 2010 evapocondensed 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] From Guieu et al. (2010b).
[b] From Desboeufs et al. (2013).

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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 NO_3^-), nd: no data. Data for chlorophyll *a*, DIP, NO_3^- and DFe are the mean concentrations at 0.1, 5 and 10 m depths. Data for primary production and N_2 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-R
Chlorophyll *a* ($\mu L\,m^{-1}$)	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^-, µM	nd	nd	< dl[a]
DFe, nM	2.4 ± 0.3[e]	2.3 ± 0.3[g]	3.3 ± 0.8[f]

[a] From Ridame et al. (2013).
[b] From Pulido-Villena et al. (2010).
[c] E. Pulido-Villena,personal communication, 2013.
[d] From Pulido-Villena et al. (2013).
[e] From Wagener et al. (2010).
[f] From Wuttig et al. (2013).
[g] T. Wagener, personnal communication, 2013.

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Table 3. Contribution of N_2 fixation to primary production (PP) and new production (NP) before and after dust seeding in DUNE-1-P, -Q and DUNE-2-R estimated from measurements of PP and average measured particulate C/N ratios (7.5 ± 0.4, 7.5 ± 0.5 and 7.8 ± 0.6 respectively during DUNE-1-P, -Q and DUNE-2-R). New production before seeding and in control-Meso has been considered as 15 % of primary production (Marty et al., 2002) (details in Ridame et al., 2013).

	Before seeding	After seeding
N_2 Fix/PP (%)		
DUNE-1-P	0.4	0.9
DUNE-1-Q	0.4	1.1
DUNE-R	0.5	0.8
N_2 Fix/NP (%)		
DUNE-1-P	2.9	2.4
DUNE-1-Q	3.0	9.8
DUNE-R	3.3	1.8

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Table 4. Input of new nitrogen ($\mu mol\,N\,m^{-2}$) associated with N_2 fixation integrated over the mesocosm and over the duration of experiments in Dust-meso and Control-Meso in DUNE-1-P, -Q and DUNE-2-R1, -R2.

	Control-meso	Dust-meso	Relative change
P	24.3	63.2	2.6
Q	22.6	50.4	2.2
R1	14.3	29.2	2.0
R2	16.2	38.1	2.4

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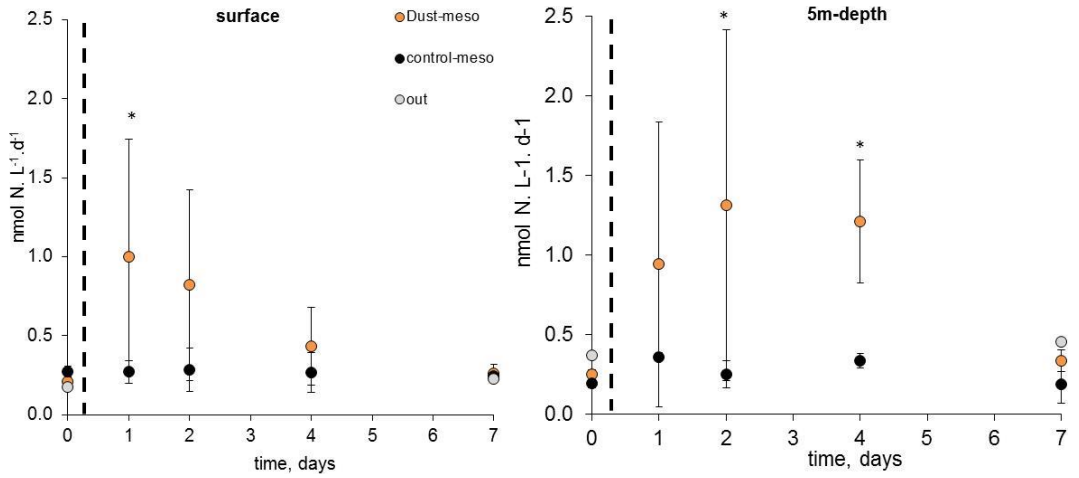

Fig. 1. Mean N_2 fixation rate in nmol N L^{-1} d^{-1} during the DUNE-1-P experiment in Control-meso (black dot), Dust-meso (orange dot) and out (grey dot) at surface and 5 m depths. The dotted line represents the time of the dust seeding. Data in the Control- and Dust-meso represent the average and standard deviation of the three replicate mesocosms. Means in Dust-meso that were significantly different ($p < 0.05$) from Control-meso were labeled with the * symbol.

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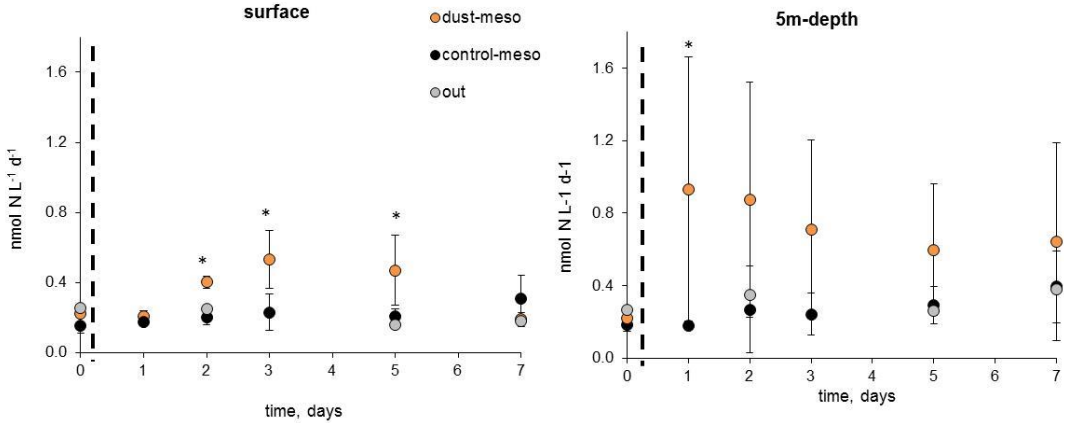

Fig. 2. Mean N_2 fixation rate in nmol $N\,L^{-1}\,d^{-1}$ during the DUNE-1-Q experiment in the Control-meso (black dot), Dust-meso (orange dot) and Out (grey dot) at surface and 5 m depths. The dotted line represents the time of the dust seeding. Data in the Control- and Dust-meso represent the average and standard deviation of the three replicate mesocosms. Means in Dust-meso that were significantly different ($p < 0.05$) from Control-meso were labeled with the * symbol.

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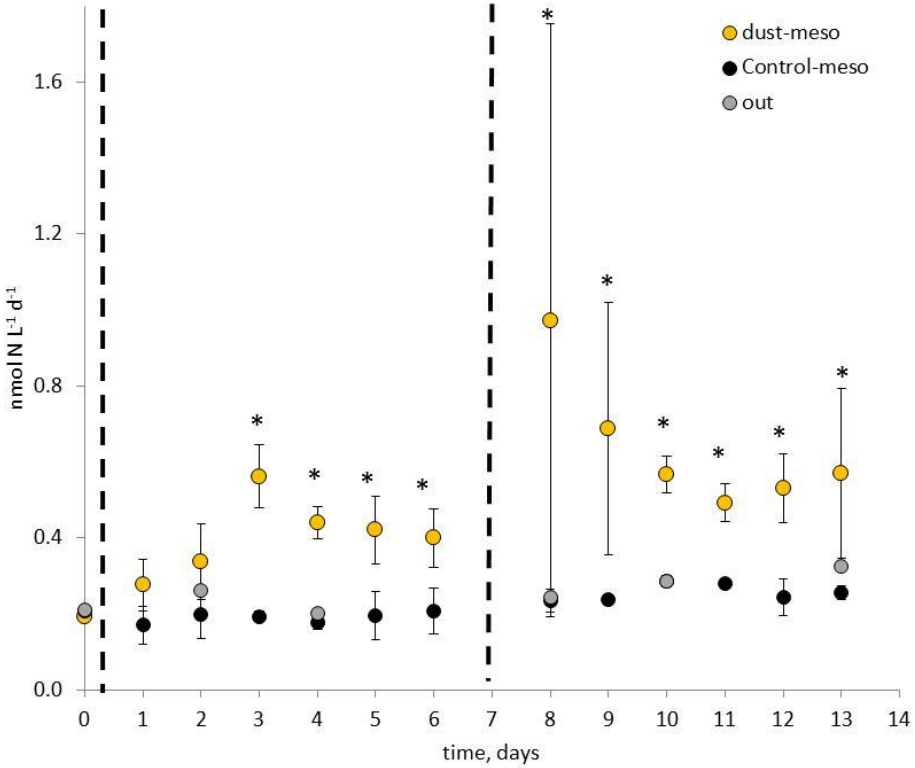

Fig. 3. Mean N_2 fixation rate in nmol N L^{-1} d^{-1} during the DUNE-2-R1 and -R2 experiments in the Control-meso (black dot), Dust-meso (orange dot) and Out (grey dot) at 5 m depth. The dotted line represents the time of the dust seeding. Data in the Control- and Dust-meso represent the average and standard deviation of the three replicate mesocosms. Means in Dust-meso that were significantly different ($p < 0.05$) from Control-meso were labeled with the * symbol.

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