We appreciate the reviewers' interest and thank them for their relevant suggestions and time spent reviewing this manuscript

.REPLY to referee RC1

GENERAL COMMENTS:

- **Q1** It is now generally accepted that minimum detectable uptake rates (N_2 and CO_2) should be determined for every individual incubation experiment, so that rates under their specific detection limit can be reported as such (<DL). Because every sampling site and sampling depth (and sampling time) have their own original substrates concentrations and associated isotope compositions (PN, POC, dissolved N_2 and dissolved inorganic carbon), it makes it important to compute incubation-specific minimum detectable uptake rate, based on the minimum increase in isotope composition detectable by the isotope ratio mass spectrometer. The authors should confirm that all reported N_2 fixation rates are indeed truthful (particularly at depths ≥ 200 m).
  Lines 149-150: The authors should confirm that all reported N_2 fixation rates are indeed truthful (particularly at depths ≥ 200 m), by computing incubation-specific minimum detectable uptake rates, based on the minimum increase in isotope composition (relative to natural abundance), detectable by the isotope ratio mass spectrometer (Fonseca-Batista et al., 2017; White et al., 2020).

Reply to RC1: We agree with the reviewer that some details regarding N2 fixations rates were missing. The following additions (in bold) are now included in the text in MM, section 2.3:

'After collection, 2.3 L of seawater were immediately filtered onto pre-combusted GFF filters to determine natural concentrations and isotopic signatures of particulate organic carbon (POC) and particulate nitrogen (PN). Net N_2 fixation rates were determined using the $^{15}N_2$ gas-tracer addition method (Montoya et al., 1996), and net primary production using the ^{13}C-tracer addition method (Hama et al., 1983). Immediately after sampling, 1 mL of $NaH^{13}CO_3$ (99 %, Eurisotop) and 2.5 ml of 99 % $^{15}N_2$ (Eurisotop) were introduced to 2.3 L polycarbonate bottles through a butyl septum for simultaneous determination of N_2- and CO_2-fixation. $^{15}N_2$ and ^{13}C tracers were added to obtain a ~10 % final enrichment.'.....'After 24 h incubation, 2.3 L were filtered onto pre-combusted 25 mm GF/F filters, and filters were stored at −25° C. Filters were then dried at 40° C for 48 h before analysis. POC and PN as well as ^{15}N and ^{13}C isotopic ratios were quantified using an online continuous flow elemental analyzer (Flash 2000 HT), coupled with an Isotopic Ratio Mass Spectrometer (Delta V Advantage via a conflow IV interface from Thermo Fischer Scientific). **For each sample, POC (in the 0-100m layer) and PN (0-1000m) were higher than the analytically determined detection limit of 0.15 µmol for C and 0.11 µmol for N. Standard deviations were 0.0007 atom% and 0.0005 atom% for 13C and 15N enrichment, respectively. The atom% excess of the dissolved inorganic carbon (DIC) was calculated by using measured DIC concentrations at the LOCEAN laboratory (SNAPO-CO2).** N2 fixation rates were calculated by isotope mass balance equations as described by Montoya et al. (1996). **For each sample, the 13C and 15N uptake rates were considered as significant when excess enrichment of POC and PN was greater than three times the standard deviation obtained on natural samples. According to our experimental conditions, the minimum detectable 13C and 15N**

uptake rates in our samples were 5 nmol C L^{-1} d^{-1} and 0.04 nmol N L-1 d-1 respectively. CO_2 uptake rates were above the detection limit in the upper 0-100m, while N2 fixation was not quantifiable below 300 m depth except at stations 1 and 10 with rates ~0.05 nmol N L^{-1} d^{-1}at 500 m depth.' (see in the submitted version, Results L225-227)

For the sake of clarity, we have symbolized by crosses the N2 fixation rates under detection limit (<0.04 nmol N L^{-1} d^{-1}) on Fig. 2.

- **Q2**

The authors should be more skeptical and critical when comparing high-throughput sequencing of *nifH* gene from different sampling depth, sites and time points- The authors should be more skeptical when comparing relative abundance data from different sampling depth, sites and time points (Gloor et al., 2017): Lines 290-292, 447-450 and 462-463.

Reply to RC1: We appreciate this insightful comment. The authors are well aware of the issues and potential caveates inherent in the analysis of compositional microbiome datasets. However, we do see that the choice of words in the lines mentioned could have been selected more carefully to avoid any speculation of overinterpretation. The following additions (in bold) are now included in the text

Lines 290-292, section 3.2.3 At FAST, no difference in the relative abundances of diazotrophs was recorded between D treatment and the controls at T4. **However, when comparing G treatment relative to D at T4, the relative contribution of NCD was higher (82 % in G vs. 63 % in D) and the relative abundance of UCYN-A was lower** (13 % in G vs. 31 % in D).

447-450 discussion 4.6 **'**Interestingly, despite the decrease in the **relative** contribution of UCYN-A **to the total diazotrophs community** after dust addition, we observed contrasted responses within the UCYN-A group relative to present climate conditions: **the relative abundance of** UCYN-A3 strongly decreased (4.6 % in G vs. 25.4 % in D) whereas **the relative abundance of UCYN-A2 was twice as high (7 % in G vs. 3.4 % in D). Notably, the relative contribution of UCYN-A1 did not appear to be impacted during the dust addition experiment.**

462-463 conclusions 'UCYN-A **might be supporting** extremely high rates of N_2 fixation (72 nmol.L^{-1}.d^{-1}) in the core of an eddy in the Algerian basin influenced by Atlantic waters.

- **Q3**

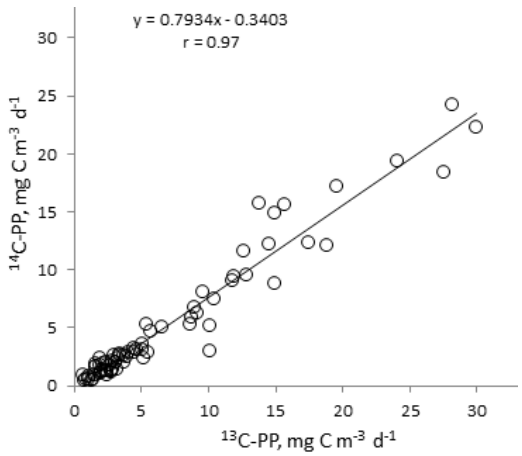
Primary production rates measurements (based on the ^{13}C incubation method), although mentioned all along the manuscript (with relation to corresponding N_2 fixation rates) are not described or discussed. The authors invite the reader to report to the manuscript by Maranon et al. (2020), who used a different methodology (^{14}C incubation technique). The authors should inform the reader (e.g., in the supporting information) about how the results from the two methods compare? Whether they show similar trends across the sampling sites and dust seeding experiments, despite the contrast in gross versus net rate assessments? This would support the authors' decision not to further discuss primary

production in their manuscript and invite the reader to report to Maranon et al. (2020) for more detailed insights. **M&M-Lines 139-140:** The authors should indicate in the supplementary information how consistent the results from the two methods are (^{13}C-PP and ^{14}C-PP). As of now, no other manuscript in the Special Issue describes or discusses the ^{13}C-PP rate measurements. Unless a manuscript comparing the data from the two methods is envisioned, having a brief comparison in the Supplementary Material would support the authors' choice not to discuss ^{13}C-PP further in this manuscript and focus on N_2 fixation and diazotrophic community compositions.

Reply to RC1: For the sake of clarity, we have added this paragraph and this figure in Supplementary Information

'Figure S1: Comparison between ^{13}C-PP and ^{14}C-PP measured in the particulate matter during the dust seeding experiments

In situ samples for ^{13}C-PP and ^{14}C-PP were not systematically measured at the same depths (±10 m) and on the same day; seawater for ^{14}C-PP was collected with the classical rosette (Niskin bottles) (Maranon et al., 2021) while ^{13}C-PP seawater was sampled with the trace metal clean rosette. We therefore chose to use ^{13}C-PP data to estimate the contribution of N_2 fixation to PP because both parameters were measured simultaneously in the same bottle. Nevertheless the shapes of the profiles and trends are similar with both data sets. In addition, ^{14}C-PP (Gazeau et al., 2021b) and ^{13}C-PP were measured in parallel during the dust seeding experiments and the correlation between ^{13}C-PP and ^{14}C-PP values was very strong ($r=0.97$, $p<0.0001$, $n=72$) as shown in the figure below'



We have also added in the revised version in MM (in bold): *'In situ* 13C-PP will not be discussed in this paper as 14C-PP rates are presented in Maranon et al. (2021) (**see details in Fig. S1**). The *in situ* 13C-PP were used in the present study to estimate the contribution of N2 fixation to PP **as both parameters were measured simultaneously**'

- **Lines 139-140 and 152-153:** Please clarify for the reader that the contribution of N_2 fixation to primary production and to bacterial production where estimated using C:N Redfield ratio (6.6) and ratio from Nagata (1986), respectively.

Reply to RC1: We didn't use the Redfield ratio (6.6) to estimate the contribution of N_2 fixation to PP. Instead, we used the molar C/N ratio measured in the organic particulate matter of our samples by EA-IRMS (L146-147) as on each GFF sample, we measured 4 parameters : particulate carbon and nitrogen, and 13C and 15N isotopic ratios (as mentioned L151).

We have added in the revised version in MM (in bold) 'The *in situ* 13C-PP **and molar C/N ratio in the organic particulate matter in our samples (see below for details) measured simultaneously in our samples** were used to estimate the contribution of N2 fixation to PP **.'**

- **Line 152:** for the sake of clarity, please inform the reader that BP measurements, which methodology has at this stage not yet been described, are complementary data presented in companion manuscripts (Gazeau et al., 2021b; Van Wambeke et al., 2021) and **Lines 152-153:** have the authors considered citing Fukuda et al. (1998) (manuscript with Nagata Toshi himself as co-author), to support their choice of C:N conversion factor. In fact, the cell collection in Fukuda et al., seems more appropriate for bacteria than the GF/F filtration used in Nagata (1986), thereby leading to a more reliable estimate of the bacterial C:N ratio in oceanic settings of 6.8 ± 1.2.

  Reply to RC1: We agree with the reviewer; the choice of a C/N ratio of 6.8 measured in oceanic bacterial assemblages is more appropriate. We have therefore recalculated the contribution of N2 fixation to BP using a molar C/N ratio of 6.8, and modified the contribution (%) of N2 fixation to BP which decreases slightly, in section 4.4 of the discussion (the contribution of N2 Fixation to BP in section 4.5 remains unchanged (from 5.1% to 4.8% so ~5%). The general conclusions (N2 fixation is a poor contributor to BP) remain unchanged.

  Changes in the revised version in MM (in bold) 'As a rough estimate of the potential impact of bioavailable N input from N_2 fixation on BP, **we used the BP rates presented in companion papers (Gazeau et al., 2021b; Van Wambeke et al., 2021), and converted them** in N demand using the molar ratio C/N of **6.8 (Fukuda et al., 1998).'**

  Changes in section 4.4 (in bold) **'**Overall, N_2 fixation was a poor contributor to PP (1.0 ± 0.3 %), as previously shown in the MS (Bonnet et al., 2011; Yogev et al., 2011; Rahav et al. 2013a) and BP (**7 ± 1 %)** except at station 10 where N_2 fixation could support up to 19 % of PP and supply the entire bioavailable N requirements for heterotrophic prokaryotes (**199 % of BP**).'

  - **Line 149:** The authors chose to use of the $^{15}N_2$ bubble addition method for their incubation experiments, which has been shown to underestimate in situ N_2 fixation activity due to incomplete tracer dissolution. The authors clearly stated that. However, to alleviate some of this uncertainty, the authors could consider in the future, sampling the incubation bottles at the end of the experiment (before filtration) to determine the final $^{15}N\%$-N_2 enrichment, which can then be used to compute N_2 fixation rates. Although these rates would likely still underestimate the true activity (due to dissolution kinetics taking place during the 24-hour incubation), they would however reduce the uncertainty and inform on the gap between N_2 fixation rates based on measured versus theoretically estimated ^{15}N-N_2 enrichments.

Reply to RC1: We are in complete agreement with the reviewer. Such measurement of 15N atom% of the dissolved 15N2 prior to filtration at the end of the incubation period would indeed provide a more accurate N2 fixation rate. We have to develop the measurement of the isotopic ratio of 15N2 under dissolved form with our IRMS. We also chose to use the 15N2 bubble addition method because some studies have shown trace element contamination with the 15N2 enriched water method

- Line 176: please explain what influenced the decision to truncate the reads at 350 bp? (no need to report in the manuscript)

Reply to RC1: we truncated the read at 350bp because the quality decreased for longer reads and we wanted to keep high quality scores. The expected length of the nifH amplicon is 362, therefore the sequence information lost is minimal. From the authors experience this will not impact the taxonomic classification obtained with the employed sequence analysis pipeline.

Results

- **Line 273:** "CV%" not previously defined

Revised to ' The reproducibility between the replicated treatments was good at all stations (**mean coefficient of variation (CV%)** < 14 %).

- **Line 281:** please clarify, "low overall **relative** abundance".

Revised to 'Some of these ASVs had low overall **relative** abundance,'

- **Line 286:** Specify from which condition(s) (Control, Dust and Greenhouse) the average contributions of UCYN-A1 and A3 to the total diazotrophic community were determined from at T0.

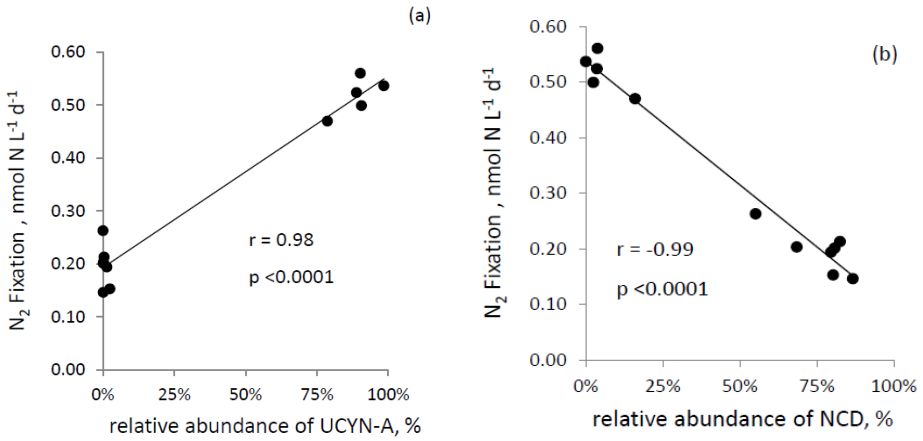
Revised to '(relative abundance of UCYN-A1 and -A3 **in C and D treatments at T0, n=4** : 34 ± 6 % and 45 ± 2 % of the total diazotrophic composition, respectively)'

Discussion:

Lines 320-321, 361-362, 378-379, 401-402, 404, 409 and 455: data not shown, that could be added to the Supplementary Material, with relation to:

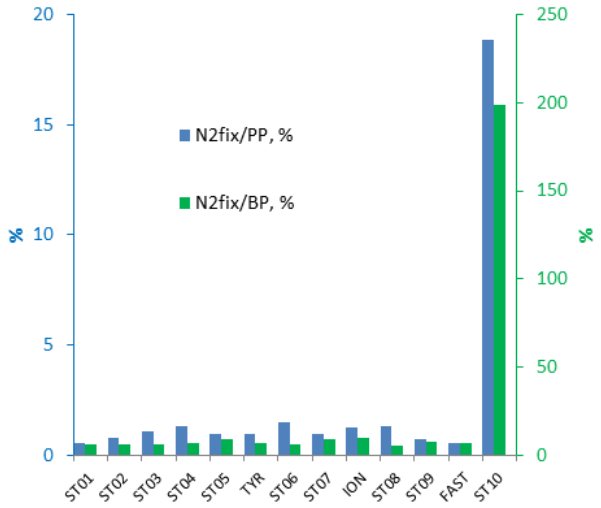
1) correlation between N_2 fixation rates and diazotrophic community composition (for instance, surface N_2 fixation versus UCYN-A and NCD) (Lines 320-321)

Reply to RC1: we have added Fig S8 showing the relationship between surface N_2 fixation and (a) UCYN-A and (b) NCD

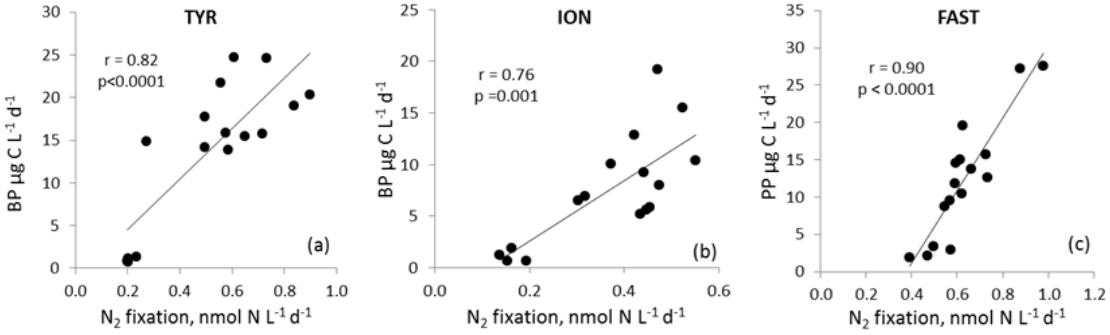


2) contribution of N_2 fixation to PP and BP

L361-362, Reply to RC1: we have added a new figure in SI (Fig S9) showing the contribution of N_2 fixation to PP and BP at the studied stations



L378-379, L401-402, L409 and L455 Reply to RC1: we have added a new figure in SI (Fig S4) showing the relationship between N_2 fixation and (a) BP at TYR, (b) BP at ION, and (c) PP at FAST, during the dust seeding experiments



3) evolution of nutrient concentration in the dust seeding experiments: DIP concentration in Control and Dust experiments at station TYR; requiring citation of the corresponding companion paper (line 404).

L404, section 4.5, we have added (in bold) 'This could explain why DIP concentration in the D treatments became again similar to the controls at the end of this experiment **(Gazeau et al., 2021a)'**.

Lines 331-332: Sentence not clear, please rephrase.

Revised to: 'High **N_2 fixation** rates have previously been observed locally: 2.4 nmol N L^{-1} d^{-1} at the Strait of Gibraltar (Rahav et al., 2013a), ~5 nmol N L^{-1} d^{-1} in the Bay of Calvi (Rees et al., 2017), 17 nmol N L^{-1} d^{-1} in the northwestern MS (Garcia et al., 2006) and 129 nmol N L^{-1} d^{-1} in the eastern MS (Rees et al., 2006).

Line 340: Please explain further why the DFe minimum could not only be the result of uptake by diazotrophs

Reply to RC1: We estimated the theoretical Fe requirement to sustain a N2 fixation of 72 nmol N L^{-1} d^{-1} at 61m, station 10 using a range (min-max) of Fe/C (from 7 to 177 µmol:mol) and associated C/N for diazotrophs (*Trichodesmium*, UCYN) from literature (Berman-Frank et al., 2007; Tuit et al., 2004, Jiang et al., 2018). We found that to sustain this N_2 fixation rate, 0.004 nM to 0.08 nM of DFe are required. Consequently, the minimum in DFe concentration at 61m of 0.47 nM compared to 0.7 to 1.4 nM at the nearby depths, (Bressac et al., 2021) could not be explained solely by the diazotrophs uptake.

We have added in the revised version (in bold): 'It only coincided with a minimum in DFe concentration (0.47 nM compared to 0.7 to 1.4 'nM at the nearby depths, Bressac et al., 2021). **Based on a range of Fe:C (from 7 to 177 µmol:mol) and associated C:N ratios for diazotrophs (*Trichodesmium*, UCYN) from literature (Berman-Frank et al., 2007; Tuit et al., 2004; Jiang et al., 2018), we found that 0.004 nM to 0.08 nM of DFe are required to sustain this N_2 fixation rate. Consequently, the minimum in DFe concentration at 61m could not be explained solely by the diazotroph uptake.**

Line 445: "a decrease in the top-down control on the bacterioplankton which is strongly suspected to increase under future climate conditions" Please explain further why

We rephrased this sentence to **'**The increased contribution of *Pseudomonas* in the G treatment at T0 (before dust addition) reveals a likely positive effect of temperature on the growth of this NCD as an increase in the top-down control on the bacterioplankton was observed after dust seeding under future climate conditions (Dinasquet et al., 2021).'

Conclusion:

Lines 462-463: Because cell specific N_2 fixation rates were not determined, this statement should be less affirmative.

Reply to RC1: Please, See our response to general comment in Q2.

Tables and Figures:

- Table 1: Why were some average and standard deviation values not included in the two bottom rows?

The two bottom rows are now filled in the revised version;

Table 1: Integrated N_2 fixation over the surface mixed layer (SML, from surface to the mixed layer depth), from the surface to the base of the euphotic layer (1% PAR depth), over the aphotic layer (1%PAR depth to 1000 m), and from surface to 1000 m at all the sampled stations. Contribution (in %) of SML integrated N_2 fixation to euphotic layer integrated N_2 fixation, and contribution of euphotic layer integrated N_2 fixation to total (0-1000 m) integrated N_2 fixation.

	N_2Fix_{SML} µmolN m^{-2} d^{-1}	$N_2Fix_{euphotic}$ µmolN m^{-2} d^{-1}	$N_2Fix_{aphotic}$ µmolN m^{-2} d^{-1}	$N_2Fix_{0-1000m}$ µmolN m^{-2} d^{-1}	$N_2Fix_{SML}/N_2Fix_{euphotic}$ %	$N_2Fix_{euphotic}/N_2Fix_{0-}$ %
ST01	14.6	42.6	56.5	99.1	34	43
ST02	10.7	36.0	16.0	51.9	30	69
ST03	7.8	58.3	18.1	76.4	13	76
ST04	10.8	46.6	38.5	85.1	23	55
ST05	4.9	46.3	36.1	82.4	10	56
TYR	4.2	38.6	53.0	91.6	11	42
ST06	9.1	34.9	29.8	64.7	26	54
ST07	10.5	43.5	55.4	98.8	24	44
ION	6.2	40.6	56.5	97.1	15	42
ST08	4.3	27.0	12.3	39.3	16	69
ST09	3.4	50.2	43.3	93.5	7	54
FAST	5.9	58.2	35.7	93.8	10	62
ST10	13.7	1908	63.7	1972	1	97
Mean ± std (ST10 excluded)	7.7±3.5	44±9	38±16	81±20	18%±9%	55%±12%
Mean ± std (all stations)	8.2±3.7	187±517	40±17	227±525	17%±10%	59%±16%

- Table 2: Please specify what size fraction (or incubation experiment) is used to compute the C:N (mol/mol) ratio?

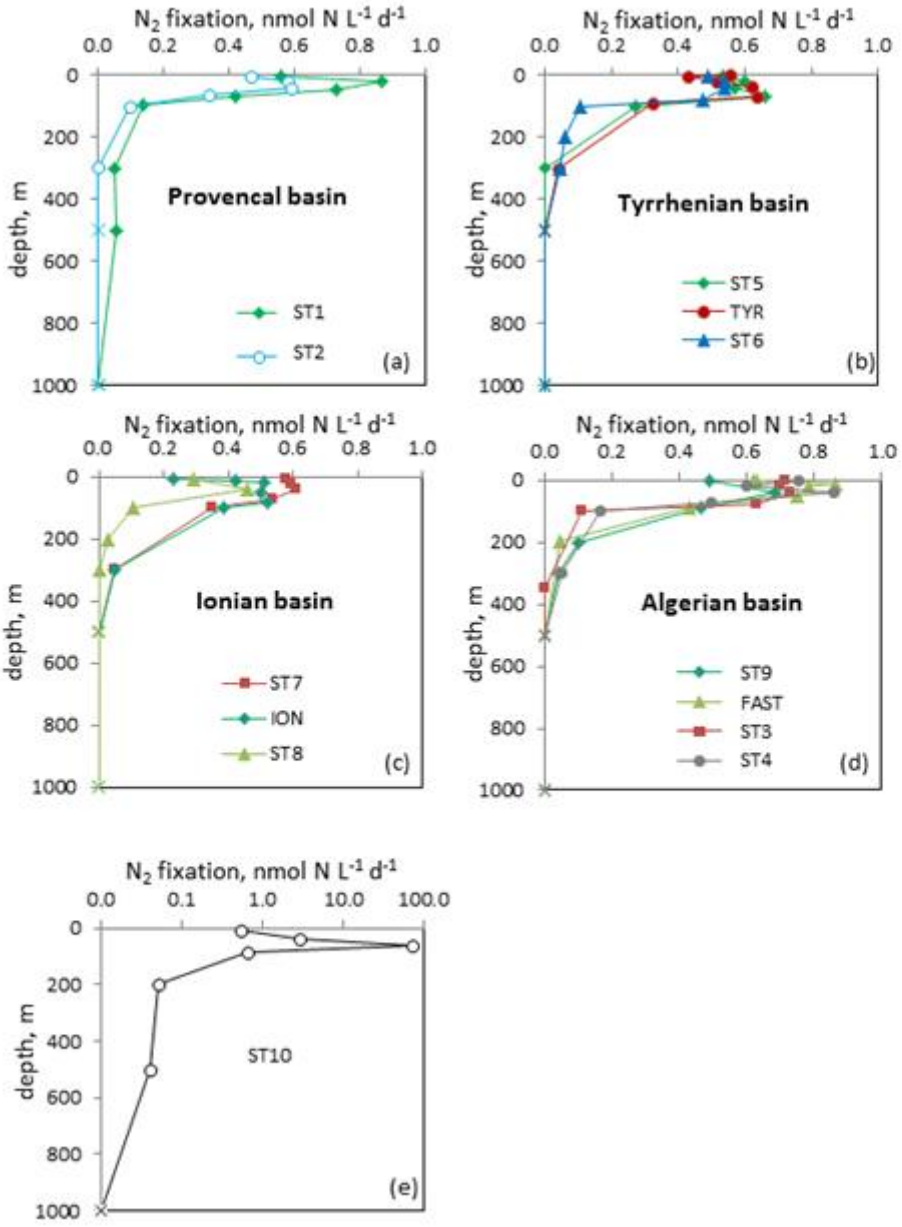
Reply to RC1: the C:N ratio in Table 2 corresponds to the POC:PN ratio calculated from the IRMS measurements of the GFF filters (> 0.7µm).

We have added in the legend of Table 2 (revised version) (in bold) 'Initial physico-chemical and biological properties of surface seawater before the perturbation in the dust seeding experiments at TYR, ION and FAST (average at T0 in C and D treatments, n=4 or data at T-12h in the pumped surface waters, n=1). The relative abundances of diazotrophic cyanobacteria and NCD (non-cyanobacterial diazotroph) are given as proportion of total *nifH* sequence reads. DIP: dissolved inorganic phosphorus, DFe: dissolved iron. **The C:N ratio corresponds to the ratio in the organic particulate matter from IRMS measurements (> 0.7µm)**. Means that did not differ significantly between the experiments (p>0.05) are labeled with the same letter (in parenthesis). '

- Figure 1: Station "TYR" labelled as "TYRR" : This was changed in Fig. 1

- Figure 2: Are data points missing at 1000 m for ST6, ST8 and ST10? Authors should consider breaking the scale of the x-axis (N_2 fixation, nmol N L^{-1} d^{-1}) for station 10. This would improve the readability of the graph, and highlight significant N_2 fixation rates, not only at 61 m.

For the sake of clarity, we have symbolized by crosses the N2 fixation rates under detection limit (<0.04 nmol N L^{-1} d^{-1}) on Fig. 2. N_2 fixation rates at station 10 are now plotted in log scale to improve the readability of the figure



- Figure 6 Please adjust the y-axis to a unique range for all 3 graphs and arrange the graphs side by side.

This has been modified in the revised version

- Figure S5: Please consider dissociating the stations either into separate plots, or even just separated series on the same plot.

This figure has been modified in the revised version (see below)

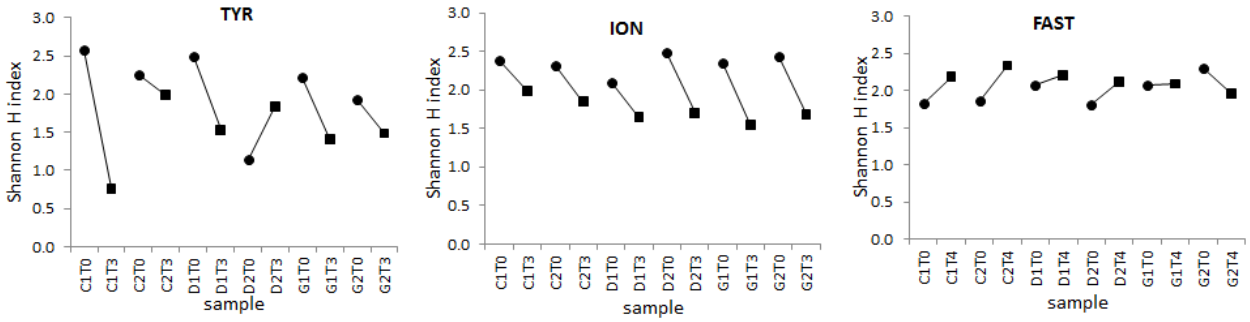


Figure S5: **Changes in the general diversity trends visualized by Shannon H index, during the dust seeding experiments at TYR, ION and FAST between initial time (dot) and final time (square) connected by a line to indicate directional change in diversity following each incubation experiment.** Shows that for TYR and ION the diversity decrease from T0 to Tend whereas the opposite is true for FAST

TECHNICAL CORRECTIONS:

Lines 24-25: "strong longitudinal gradient increasing eastward" → **corrected**

Line 72: "enhance" instead of "enhanced" → **corrected**

Line146: space missing between "and" and "^{13}C" → **corrected**

Line 153: Adapt reference "Nagata, 1986" instead of "Nagata et al., 1986" → **replaced by Fukada et al., 1998**

Line 158: replace "following" by "as follows"→ **corrected**

Line 159: For the sake of clarity, the variable Tx could be removed from the formula, since the term cancels itself being in both the numerator and denominator. On the other hand, "$N_2FIXATION_T$" could be replaced by "$N_2FIXATION_{Tx}$"→ **corrected**

Line 244: replace "as" by "or"→ **corrected**

Line 318: Add in the parentheses "(**in** this and previously published studies)".→ **corrected**

Line 337: delete "and", to read "… take place, combined with…"→ **corrected**

replace "high stocks" by "higher stocks"→ **corrected**

Line 349: "whole diazotrophic community in the euphotic zone" instead of "the whole diazotrophs"→ **corrected**

Reference Table S1 at the end of the sentence → **added**

Line 384: Data reported here do not support an increase of diazotrophs abundances, so consider replacing "obviously" by "likely".→ **completely agree**

Line 398: replace "**to** dust seeding" by "**by** dust seeding → **corrected**

Line 406: please clarify, “heterotrophic prokaryotes, NCD, and photoautotrophs” had to compete for dust-derived DIP
Revised to **‘Consequently, diazotrophs as well as non diazotrophs (heterotrophic prokaryotes and photoautotrophs) could all uptake the dust-derived DIP reducing then potentially the amount of DIP available for each cell that could explain the lower stimulation of N_2 fixation relative to TYR’**

Line 407: “the lower stimulation” instead of “the lowest”→ **corrected**
Line 477: “UCYN-A remain” instead of “remained”→ **corrected**
Line 480: “are expected” instead of “would expect”→ **corrected**

References added in the revised version:

Berman-Frank, I.A., Quigg, A., Finkel, Z. V, Irwin, A.J., Haramaty, L. (2007) Nitrogen-fixation strategies and Fe requirements in cyanobacteria. Limnol. Oceanogr. 52, 2260–2269.

Fukuda, R., Ogawa, H., Nagata, T., & Koike, I. (1998). Direct determination of carbon and nitrogen contents of natural bacterial assemblages in marine environments. Applied and Environmental Microbiology, 64(9), 3352–3358. https://doi.org/10.1128/aem.64.9.3352-3358.1998

Tuit, C., Waterbury, J., Ravizza, G. (2004) Diel variation of molybdenum and iron in marine diazotrophic cyanobacteria. Limnol. Oceanogr. 49, 978–990. doi:10.4319/lo.2004.49.4.0978