General comments

The paper presents new data of N2 fixation rates measured over the Mediterranean Sea in spring with the new 15N2 enriched seawater method. Also, the authors estimated the contribution of N2 fixation to primary production in both western and eastern basins. Major revisions need to be done to improve the manuscript. First, statistical tests need to be performed before concluding (data on fig 5 a-b and 6). A large part of the manuscript is dedicated to the contribution of heterotrophic diazotrophs versus phototrophs to N2 fixation without phylogenetic analyses. The authors attributed dark N2 fixation to heterotrophic diazotrophs. Nevertheless, it has been shown that unicellular diazotrophic cyanobacteria from group B and C performed N2 fix during the dark period. Consequently, N2 fix measured during the dark period does not reflect specifically the N2 fixing activity of heterotrophic diazotrophs. Based on the data presented in this paper, I suggest to remove parts of the manuscript concerning N2 fixation from heterotrophs versus phototrophs.

"Questions and specific comments"

Introduction

Pg3 l9 : six years

Pg3 l12 : please remove Rahav et al 2012 which is in prep

Pg3 : please add that some studies reported high rates of N2 fix during summer stratification in MS: 7.5 nmol N L^{-1} d^{-1} (Sandroni et al., 2007) and up to 129 nmol N L^{-1} d^{-1} Rees et al., 2006

Pg 3 : line 13 : The DYFAMED site, located in the central zone of the Ligurian Sea, is protected from coastal inputs by the presence of Ligurian Current (eg Marty et al., 2002…)

Pg3 l16: please add information about the diazo group which dominates the diazo community : bacteria ? unicellular cyano ? DDA ?

Pg3 l21:precise if Tricho was observed in open or coastal waters of MS

Pg3 l25 : add “new” in the sentence “the contribution of N2 fixation to new primary production”

Pg4 l1:add results from Sandroni et al 2007 about the seasonal variability of the contribution of N2 fix to NP, in northwestern MS (Sandroni et al 2007 table 1).

MM

Pg4 l14 : X to Y April 2011

Pg4l21:
how did you collect the seawater?
When did you collect the sw (morning ?) ?
“4.6L PC bottles”…are you sure about the volume (4.3?) ?
did you acid washed the material (bottles….) ?
Did you add 15N2 and 13C in the same incubated bottles (precise it) ?
Did you make replicates of bottles for 15N2 and 13C fix (standard deviation on table 2, n=?)?

Pg4 l23 : precise the % of 13C in NaH13CO3 and the volume added in bottles

Pg5 l13 : the incubation period for N2 fix is generally 24h. here you chose a very long incubation time of 48h. please justify this choice. Did you make measurement of N2 fix with an incubation of 24h to check if rates were similar (if done, add these data)? If not, discuss about an eventual underestimation of the rates

P5: add the incubation period for 13CO2 fix ?

Pg5 l1519: unicellular diazo cyanobacteria from group B and C perform N2 fix during the dark period. Consequently, N2 fix measured during the dark period does not reflect specifically the N2 fixing activity of heterotrophic diazo.

Pg5,l22-24: give some details about reproducibility and precision. Add detection limit for particulate C and N.
Precise if you measured the POC or total particulate carbon on the GFF filters

Pg5l25 : not clear. Do you mean that you used the measured POC/PON ratio to convert N2 fixation to primary production ?

P6 l6: add some details about the sampling of nutrients: depth? Filtration ? freezing ?

L12: volume of filtration for chla determination ?

Add a § on statistical analyses on data presented Fig 5A-B, Fig. 6

Results, Figures and tables

Fig 1 and 2 : could be nice to have only one figure with chla data and localization of sampling stations

Table 1 : remove data of nutrients (NO3+NO2) which are below the detection limit
$Si(OH)_4$ **:**add '4'
No data for nanoeukaryote ?

Table 1 : add the surface mixed layer depth for every station

Table 1 : 620 ngC L-1 is wrong for Proch at station 290; it is 62. Check all the data in table 2 please

Fig 3: please add which are the EMS and WMS stations (in particular stx 312 ?)
no figure for picoeukaryote ?

table 2+§results : the spatial variability of the POC/PON ratio could be interesting. Could you add the data in table 2 for the 8 stations?

Fig 5a : please add the data for stations 338 and 339 (4°45W and 7°W)

Fig. 5b –
Calculate the standard deviation of the ratio and add it on the figure
add the ratios for stx 338 and 339
add the results of statistical test
which are the data from station 312 and 304 (same longitude) ?

Fig 5B : give R^2 and n

Fig. 6 : add the standard deviation and results of stat test

Pg7 l15 : remove "0.01 µM" of NO3+NO2 because below detection limit (0.075 µM)

L19: DIP : from 0.01 to 0.24 µM (stx 333)

Add a sentence on the spatial variability of silicic acid

Pg8 l2: Replace' stx 290' by station in the Levantine basin (st. 290)

Pg8: Add a sentence on the spatial variability of nanoeukaryote (Fig. 3C)

Pg8 l18-20: 'The springtime rates of N_2 fixation at all stations were 3–7 folds higher than measurements published previously during summertime' . But the methods used to measure N2 fix (bubbling in summertime and 15N2 enriched sw in springtime) were different as well as the incubation period (24 and 48h). From Wilson et al., 2012 'a 2- to 6-fold increase in the rate of 15N2 assimilation was measured when 15N2-enriched seawater was added to the seawater sample compared to the addition of 15N2 as a gas bubble.'

Pg8 l21 – pg9 l5-8: again you can not exclude the dark N2 fix by unicellular diazo cyanobacteria without phylogenetic analyses

Pg9 l1-2 : give the standard deviation on 0.16 nmol N L-1 d-1 (stx 294) and perform a statistical test to compare the rates at stations 290 and 294

P9 l6: Perform a statistical test on the ratios and add the results on fig 5B- Mention that the ratios determined at stx 290 and 294 are really close to 1 (give the values); I am not sure that ratios at stx 312, 316, 304, 290, 294 will be statistically different.

Discussion

Pg9-§3-3 : this paragraph should be moved in 'discussion'
you used the measured C/N ratio to convert N2 fix in PP in order to estimate to contribution of N2 fix to PP. These C/N ratios are representative of the whole planktonic community and are not specific to diazo organisms. Please discuss about this (and please add the values of C/N in table 2)

Pg10 l17 : a new figure with data of N2 fix and PP could be interesting to show this no correlation in EMS and probably over the whole MS (add R^2).

The authors conclude that no correlation between N2 fix and PP 'suggests that N2 fix is attributed mainly to diazo bacteria'. others explanations should be given. For example we can hypothesize that diazotrophs and non diazotrophic phytoplankton are limited or co-limited by different nutrients.

L25 : remove from the manuscript, results and conclusions from Rahav et al., 2012 which is in preparation; Works cited in the manuscript should be accepted for publication or published already

L28: ANOVA : Have you previously test the homogeneity of variances?

L27 : Is there a correlation between PP and N2 fix in WMS (R^2=?) ?

Pg11 L1-5 : do you have an idea of the order of magnitude of abundance of Richelia in EMS and WMS ? Richelia could also contribute to N2 fix in EMS (L5)

P12 l19-25 : you need the results of the stat test before concluding

Pg12 L29 : Is it possible to convert the nifH transcripts into abundances ?

Pg13 l21 'Higher contribution of N_2 fixation to primary production (4–8 %) was measured in the western basin compared to the eastern basin (_2 %, Fig. 6)'. you need the results of the stat test before concluding

Abstract and conclusions : 'These rates were 3–7 fold higher than N_2 fixation rates measured previously in the Mediterranean Sea during summertime.' I suggest to remove '3-7 fold' because of large differences in methodology (incubation period, 15N2 addition) between rates measured in summer and spring