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> Interactive Comment

# Interactive comment on "Picoplankton community structure before, during and after convection event in the offshore waters of the southern Adriatic Sea" by M. Najdek et al.

#### M. Najdek et al.

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Received and published: 31 January 2014

We greatly appreciate all of reviewer's comments and suggestions which have been accepted in revised version of the manuscript. Please find our response letter below.

Interactive comment on "Picoplankton community structure before, during and after convection event in the offshore waters of the southern Adriatic Sea" by M. Najdek et al.

Anonymous Referee #2

Received and published: 9 January 2014





The manuscript reports on results of depth profiles of picoplankton abundance, production, diversity and metabolic capacities in two stations belonging to the South Adriatic Pit sampled 5 times during a year. This period included an intense winter convection episode in February, what gives the paper a special interest. The paper provides a good data set and it is worth publishing. However, I have some comments, specially concerning the representation of results that should be addressed before publishing.

COMMENT 1:P17864 -L5. In such an oligotrophic area, 1.7 ml aliquots for the measurements of prokaryotic heterotrophic production were not close to detection limit?

RESPONSE: To ensure sufficient labeling and increase detection limit due to given sample aliquots, incubation time was prolonged, 2 h for surface (0 - 200m) and 4h for deep waters (400 - 800m) (Azzaro et al., 2012; Ruiz-Gonzales et al., 2012). The missing details were added to M&M section.

New reference: Ruiz-González, C., Lefort, T., Massana R., Simó, R., and Gasol, J. M.: Diel changes in bulk and single-cell bacterial heterotrophic activity in winter surface waters of the northwestern Mediterranean Sea, Limnol. Oceanogr., 57, 29-42, 2012.

COMMENT 2:-L13. There is no need to specify per liter after bacterial abundance.

RESPONSE: Per liter after bacterial abundance was deleted.

COMMENT 3:P17686 -L5. This statement, the influence of NadDW as an increase of T and S in May, is difficult to see in figure 2, specially for temperature. Decrease of S and T in March are due to NadDW at 810-850 m, written in the previous sentence, are also difficult to see. Could you find a better representation of the results?

RESPONSE: To better represent the results on Fig. 2 we inserted small graphs showing these important changes in S and T (800 m - 1200 m) prescribed to NadDW arrival in March and May 2012.

COMMENT 4:P17868 -L5. This is shown in Fig. 5, not in Fig. 6 and 7.

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#### **RESPONSE:** This is corrected

COMMENT 5:P17869 L9. Delete of....deleted

COMMENT 6:P17872 L8. The larger growth of picoeukaryotes was probably stimulated by higher concentration of nitrites. Further explanation and references should be added to support that observation.

RESPONSE: This sentence is rewritten and more proper explanation inserted and now it reads:

The most intense growth of pEu observed in February was generally supported by the highest DIN in PL in comparison to the rest of the year. Although thermohaline properties around two stations were similar; the autotrophic biomass was much higher at P300 combined with much lower DIN in waters around this station in comparison to P1200. The lower DIN might indicate that LIW resided at P300 only short time before water mixing and cooling have started. Moreover, lower DIN could also result from occurrence of very abundant pEu, SYN and HB, which actively assimilate nitrates, NO<sub>3</sub><sup>-</sup> (Allen et al., 2001; Fawcett et al., 2011). This explanation might be additionally supported by higher concentration of nitrites (NO<sub>2</sub><sup>-</sup>) in these waters due to potential involvement of all three groups in NO<sub>2</sub><sup>-</sup> production by NO<sub>3</sub><sup>-</sup> reduction (Lomas and Lipschultz, 2006; Santoro et al., 2003). NO<sub>2</sub><sup>-</sup> accumulation closely paralleling the development of the phytoplankton biomass was also observed in the mixed layer during winter in Red Sea (Al-Qutob et al., 2002). The marked decrease of SYN towards open waters was regularly observed in south Adriatic (Cerino et al., 2012) as well as its dominance in picophytoplankton population (Šilović et al., 2011).

New references: Al-Qutob, M., Hase, C., Tilzer, M. M., and Lazar, B.: Phytoplankton drives nitrite dynamics in the Gulf of Aqaba, Red Sea. Mar. Ecol. Prog. Ser., 239, 233–239, 2002.

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versity and detection of nitrate assimilation genes in marine bacteria, Appl. Environ. Microbiol., 67, 5343–5348, 2001.

Fawcett, S. E., Lomas, M., Casey, J. R., Ward, B. B., and Sigman, D. M.: Assimilation of upwelled nitrate by small eukaryotes in the Sargasso Sea, Nat. Geosci., 4, 717–722, 2011.

Lomas, M. W., and Lipschultz, F.: Forming the primary nitrite maximum: Nitrifiers or phytoplankton?, Limnol. Oceanogr., 51, 2453–2467, 2006.

Santoro, A. E., Sakamoto, C. M., Smith, J. M., Plant, J. N., Gehman, A. L., Worden, A. Z., Johnson, K. S., Francis, C. A., and Casciotti, K. L.: Measurements of nitrite production in and around the primary nitrite maximum in the central California Current, Biogeosciences, 10, 7395–7410, 2013.

COMMENT 7:P17875 L5. The explanation that the higher bacterial metabolic activity in station P1200 is due to grazing seems too speculative since the authors do not provide any data, so I recommend to delete the sentences from L5 to L10.

RESPONSE: The speculative explanation is deleted.

COMMENT 8:Discussion. February and March samplings are very different, also in terms of bacterial abundance and production. However, bacterial composition appears to be very similar in the productive or in the deep layer between both months. A paragraph discussing on that should be included in the discussion.

RESPONSE: The included paragraph reads:

The coupling between bacterial community composition and carbon metabolism was found in some marine studies (Fuhrman et al., 2006; Alonso-Sáez et al., 2007). Furthermore, Fuhrman et al (2006) demonstrated the repeatable temporal patterns in distribution and abundance of bacterial taxa, which was significantly influenced by a range of abiotic and biotic factors. However, BCC appeared to be similar in PL and DL between February and March in spite of apparent differences in abiotic factors, bacterial

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abundance, production and MC between two months. This observation could be explained by "adjustment scenario" where change in community composition involves shifts in the relative abundance and activity of the existing phylotypes (Comte and del Giorgio, 2011). Since such bacterial communities generally have high degree of metabolic/functional plasticity, BCC could remain stable under quite different environmental conditions. The lack of relationship between BCC and carbon metabolism, as we observed, generally suggests a high level of functional redundancy in the bacterial assemblage in carbon processing, at least at the phylogenetic resolution level analyzed by DGGE (Alonso-Sáez et al., 2008).

New references: Alonso-Sáez, L., Arístegui, J., Pinhassi, J., Gómez-Consenau, L., González, J. M., Vaqué, D., Agusti, S., and Gasol, J. M.: Bacterial assemblage structure and carbon metabolism along the productivity gradient in the NE Atlantic Ocean, Aquat. Microb. Ecol., 46, 43-53, 2007.

Alonso-Sáez, L., Vázquez-Domínguez, E., Cardelús, C., Pinhassi, J., Sala, M. M., Lekunberri, I., Balagué, V., Vila-Costa, M., Unrein, F., Massana, R., Simó, R., and Gasol, J. M.: Factors controlling the year-round variability in carbon flux through bacteria in a coastal marine systems, Ecosyst., 11, 397-409, 2008.

Comte, J., and del Giorgio, P. A.: Composition influences the pathway but not the outcome of the metabolic response of bacterioplankton to resource shifts, PLoS ONE, 6(9), 2011, e25266. doi:10.1371/journal.pone.0025266.

Fuhrman, J. A., Hewson, I., Schwalbach, M. S., Steele, J. A., Brown, M. V., and Naeem, S.: Annually reoccurring bacterial communities are predictable from ocean conditions, PNAS, 103, 13104-13109, 2006.

COMMENT 9: Figures and Tables:

-Citation of figures. Figure is not cited in the text and Figure 3 citation appears before Figure 2.

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RESPONSE: Fig. 1 is now cited in the text; M&M, Sampling and environmental parameters. Also now Fig.2 is cited before Fig.3

COMMENT 10:-Units should appear inside parenthesis and not after a /. This is the case for depth and temperature in fig. 2, and all axes in figures 4, 5, 6 and 7.

RESPONSE: All units are now in parenthesis on Figs. 2, 4, 5, 6 and 7.

COMMENT 11:-Figure 3. The dates of the satellite images on surface chlorophyll a (24 February and 26 March) are not very close to the dates of sampling in those months: 12 Feb and 12 Mar. It would be more informative for the manuscript if you could choose images closer in time to the sampling dates.

RESPONSE: This confusion arose from incorrect figures legends where the year of cruises appeared before month. The correct sampling dates were: 3 October 2011, 18 February 2012, 29 March 2012, 30 May 2012 and 10 September 2012. This is corrected in the legends of Figs. 2 and 4, 6, 7 and 9. Accordingly, the satellite images correspond to the situation six day after the February (24 February) cruise and three days before the March (26 March) cruise.

Please note that satellite images closer to the sampling dates were not used since images for those days either did not exist or their quality was very low.

COMMENT 12:-Figure 5. It seems hard to believe that SAW and LIW have similar L/T ratios since they have opposite values for Leu (C or B), lower in SAW, and TdR, lower in LIW. I think the authors should check the calculation of the ratio.

RESPONSE: The calculation of the ratio was checked, it was correct; however for the SAW data plot wrong values were drown into Fig.5. The L/T ratios for LIW were (2.03-140.6, 15.29±24.96) and for SAW (0.12-72.62, 11.26±14.01). This was corrected and for that reason we checked all calculations for Tables and Figures.

COMMENT 13:-Figure 6 and 7. I recommend to change the axes of the graphs, with the months in X, and with vertical columns. As it is now, it looks like a depth profile.

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RESPONSE: The axes are changed as advised.

COMMENT 14:-Figure 8. The addition of a column with different colours (or black and white) corresponding to PL or DL would help to better visualize the results.

RESPONSE: The symbols corresponding to PL or DL were added.

COMMENT 15:-Figure 9. As far as I know, Biolog Ecoplates do not contain amides but amines, the authors should check and correct if needed. The standard differentiation of Ecoplate substrates into categories includes the categories listed in the paper, but instead of phosphorylated compounds, phenolic compounds. I wonder if the abbreviation PC could correspond to phenolic instead of phosphorylated compounds.

RESPONSE: Yes, we agree, amines should stand instead of amides, this is corrected. The differentiation of Ecoplate substrates into categories was made according to Garland and Mills (1991) where Glucose-1-Phosphate and D,L- $\alpha$ -Glycerol Phosphate were listed within category phosphorylated compounds (they used the term chemicals instead of compounds), although some authors categorize them as carbohydrates (since they may belong to both categories). We categorized phenolic compounds (2-hydroxy benzoic acid and 4- hydroxy benzoic acid) as carboxylic acids (as they may belong to both categories).

COMMENT 16:-Quality of the figures should be improved. For example, the text in fig. 3 is very difficult to read. Also fig. 9 has a poor quality.

RESPONSE: The text from Fig. 3 was placed into legend to avoid mentioned difficulties. The legend now reads: Fig. 3. Satellite image (MODIS Aqua Chlorophyll\_a map, processed by GOS-ISAC (Rome) – CNR) of surface chlorophyll a ( $\mu$ gL<sup>-1</sup>) distribution in the Adriatic Sea on 24 February 2012 and 26 March 2012.

Please note that, to increase readability, Fig. 9 was divided in two Figs: Fig. 9. and Fig. 10.

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#### **Figure Captions**

The following are the full captions of the corrected figures, numbered as in the article.

Fig 2. in article: Vertical distribution of salinity and temperature at stations P300 and P1200 during the cruises (3 October 2011, 18 February 2012, 29 March 2012, 30 May 2012, 10 September 2012)

Fig. 4. in article: Vertical distribution of dissolved inorganic nitrogen (DIN), chlorophyll a (Chl a) and heterotrophic bacteria abundance (HB) at stations P300 and P1200 during the cruises (3 October 2011, 18 February 2012, 29 March 2012, 30 May 2012, 10 September 2012)

Fig. 5. in article: Dissolved inorganic nitrogen (DIN), phosphates (PO4), silicates (SiO4), chlorophyll a (ChI a), abundances of picoeukaryotes (pEu), Synechococcus (SYN), Prochlorococcus (Pro), heterotrophic bacteria (HB), bulk and cell-specific prokaryotic rates for; leucine (LeuB and LeuC) and thymidine (TdRB and TdRC) incorporation rates and their ratios (L/T) in Levantine intermediate water (LIW) affected and non-affected waters, southern Adriatic waters (SAW)

Fig. 6. in article: Dissolved inorganic nitrogen (DIN), phosphates (PO4), silicates (SiO4), chlorophyll a (ChI a), abundances of picoeukaryotes (pEu), Synechococcus (SYN) and Prochlorococcus (Pro) in productive (PL) and deeper layers (DL) during the cruises (3 October 2011, 18 February 2012, 29 March 2012, 30 May 2012, 10 September 2012)

Fig. 7. in article: Heterotrophic bacteria (HB), bulk and cell-specific prokaryotic rates for; leucine (LeuB and LeuC) and thymidine (TdRB and TdRC) incorporation and their ratios (L/T) in productive (PL) and deeper layers (DL) during the cruises (3 October 2011, 18 February 2012, 29 March 2012, 30 May 2012, 10 September 2012)

Fig.8. in article: Cluster analysis dendrogram of DGGE banding pattern (Gel Compare,

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v. 4.1, Applied Maths, Kortrijk, Belgium) performed calculating the Pearson correlation similarity coefficient for BC sampled in PL and DL (October 2011 - September 2012)

Fig. 9. in article: Changes of MC (mean AWCD) in productive and deeper layers (A); percentage utilization (mean  $\pm$  sd) of substrate groups (AA – amino acids, AMI – amines, C – carbohydrates, CA – carboxylic acids, P – polymers, PC – phosphorylated compounds) mat stations P1200 and P300 (B) during the cruises (3 October 2011, 18 February 2012, 29 March 2012, 30 May 2012 and 10 September 2012)

Fig. 10. (new): Changes in percentage utilization ( $mean \pm sd$ ) of substrate groups (AA – amino acids, AMI – amines, C – carbohydrates, CA – carboxylic acids, P – polymers, PC – phosphorylated compounds) in productive layer (A); and deeper layers (B) during the cruises (3 October 2011, 18 February 2012, 29 March 2012, 30 May 2012 and 10 September 2012)

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Fig. 1. Fig. 2. in article

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Fig. 2. Fig. 4. in article

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Fig. 3. Fig. 5. in article

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Fig. 4. Fig. 6. in article

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Fig. 6. Fig. 8. in article

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Fig. 7. Fig. 9. in article

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Fig. 8. Fig. 10. (new)