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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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We greatly appreciate all of reviewers' 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 #1

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This article is a multidisciplinary study. The seasonal evolutions of the dynamics of heterotrophic bacteria in the south Adriatic Pit are studied in the frame of biogeochemistry, phytoplankton and water mass changes. Heterotrophic bacteria are studied through multiple approaches to investigate its physiology and biodiversity. Interpretation of the evolutions of abundances, but also production using both tools (thymidine and leucine) as well as metabolic capacities using Biolog ecoplates, and DGGE techniques for biodiversity, are made in particular in relation with LIW intrusions and a winter convection episode. The study of such simultaneous and varying parameters related to heterotrophic bacterial activity and diversity are scarce, particularly when it is fully analyzed according to regional circulation and water masses. This paper is interesting and should be published. I have, however, many detailed comments that should help to improve the ms.

COMMENT 1: Page 17861 lines 10-20. A scheme showing main events of circulation (with NIG cyclonic and anticyclonic) in the vicinity of both stations p 300 and P 1200 should help the reader.

RESPONSE: The scheme of the concept is rather complex, not easy to report in this paper, and is shown in details in paper by Civitarese et al. (2010), On the impact of the Bimodal Oscillating System (BIOS) on the biogeochemistry and biology of the Adriatic and Ionian Seas (Eastern Mediterranean). Biogeosciences 7, 3987-3997 and available on web site: <http://www.biogeosciences.net/7/3987/2010/bg-7-3987-2010.html>
The characteristics of water which enters the Adriatic Sea from the Ionian Sea via the Strait of Otranto depend on the sense of rotation of the North Ionian Gyre (NIG). If the gyre is of anticyclonic waters entering the Adriatic Sea are influenced both by waters from the Atlantic Ocean (Modified Atlantic Water; MAW) and the ones originating in eastern part of the Mediterranean Sea (Levantine Intermediate Water, LIW and/or Cretaceous Intermediate Water, CIW). If the gyre is cyclonic the Adriatic Sea is influenced exclusively by LIW and CIW. However, the sense of rotation of the NIG does not affect the sense of rotation of the cyclonic gyre around the SAP.

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COMMENT: Line 28 Correct 'autotrophs'...corrected.

COMMENT 2: page 17962 line 24. The frequency of sampling should be given also in this paragraph, event if we have the response on figure 2. If not the paragraph 3.1 is not easy to understand. Were ctd casts made on the same time of the day for all surveys?

RESPONSE: The sampling frequency is included. The sentence now reads: The study was performed at two stations situated on the slope of the SAP, P300 (SAP margin, bottom depth 300-309 m) and P1200 (SAP center, bottom depth 1195-1200 m) (Fig. 1), during five cruises taken on 3 October 2011, 18 February, 29 March, 30 May and 10 September 2012. The deepest sampling depths at both stations were 20 m – 30 m from the recorded bottom depth. CTD castings were performed approximately within the same time (1 h was the biggest shift between two cruises) at each station during all surveys.

COMMENT 3: p 17963. line 3/4 What are the absolute depth of p1200 and P300 and what were the deeper layers, i.e., are they far from the bottom. Is P1200 in the deeper part or centre of the SAP?

RESPONSE: According to the echo-sounder the absolute depths varied from 1195 m to 1200 m (P1200) and 300 m to 309 m (P300). Accordingly, the deepest sampling depths varied between 1163 m and 1169 m and between 286 m and 291 m for P300, respectively, being 20 m - 30 m from the recorded bottom depth. Yes, P1200 is the center of SAP and the closest to its deepest part (~1223-1243 m according to various sources). This is now written in the text (please find this sentence above).

COMMENT 4: line 6. Were nutrients analyzed on board without preliminary fixation? What was the technique used for NH₄? What were the reproducibility and detection limit levels for these nutrients?

RESPONSE: We added the requested details and the text now reads: The samples

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for nutrients: nitrates (NO_3), nitrites (NO_2), phosphates (PO_4) and silicate (SiO_4) were frozen ($-22\text{ }^\circ\text{C}$) and analyzed in laboratory according to Strickland and Parsons (1972). Subsamples for ammonia were fixed immediately after collection onboard with 1M phenol/EtOH and determined in laboratory according to Ivančić and Degobbis (1984). The detection limits and reproducibility for nutrients were as follows: nitrates 0.05 and 0.025 μM ; nitrites 0.01 and 0.01 μM ; ammonia 0.1 and 0.098 μM ; silicates 0.1 and 0.06 μM ; and phosphates 0.03 and 0.03 μM .

New reference: Ivančić, I., and Degobbis, D.: An optimal manual procedure for ammonia analysis in natural waters by the indophenol blue method, *Wat. Res.*, 18, 1143–1147, 1984.

COMMENT 5: Line 10, Because then in the text two water categories were divided according the zero level of Chl a (p 17866 line 17, page 17867 line 11), it should be interesting also to present its detection limit considering a 500 ml volume of water filtered.

RESPONSE: We added the requested limit and the text now reads: The detection limit for chlorophyll a considering the filtered volume of water was 0.01 μgL^{-1} .

COMMENT 6: line 22. Did the authors examine HNA and LNA groups?

RESPONSE: HNA and LNA groups were examined. We analyzed the data in the context of the manuscript, but we did not obtain significant new contribution to described processes. With the manuscript reporting numerous parameters and their interactions, we decided to omit this one.

COMMENT 7: p 17864 lines 5/6. As there were some events of high activities, did the author check for isotopic dilution and specific labeling for both Leu and Tdr techniques?

RESPONSE: No, we didn't check for isotopic dilution and specific labeling. To estimate PHP in February and March we used the conversion factor according to Kirchman (1993). The detailed explanation is given at the answer to p 17873 line 6.

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COMMENT 8: line 8/9 Instead of writing "finished with 100% TCA" , the final concentration of TCA should be given.

RESPONSE: The final concentration of TCA was given. The sentence now reads: After incubation finished with TCA (final conc. 5%), samples were centrifuged

COMMENT 9: line 17. Details how many carbon source per family of molecules presented figure 9. What is the final concentration of carbon in this Biolog plate?

RESPONSE: Details were added to the text, that now reads:".with 31 different carbon sources (in triplicates) belonging to amino acids (6), amines (2), esters (1), carbohydrates (7), carboxylic acids (9), polymers (4) and phosphorylated compounds (2). The final concentration of carbon was not given by manufacturer

COMMENT 10: line 23. It is not clear if each time point of reading is considered in the AWDC formula or only the absorbance of the triplicates when max values were reached. It is not clear how the percentage substrate utilization (fig 9 B C D) and their corresponding error bars are calculated. Figure 9B is too small, mostly impossible to read.

RESPONSE: In AWCD formula only the absorbance of the triplicates was considered when max values were reached. The percentage substrate utilization was calculated as follows: The percentage for each substrate in each sample was calculated from the respective absorbance of each substrate and total absorbance of all substrates. All percentages per substrate groups were summed and divided with number of substrates per group to obtain average per group for each sample (e.g. for AA the sum was divided with 6, for amines with 2 . . . etc). The average and standard deviation per substrate groups utilization was calculated for the groups of samples (e.g. PL at P1200, P300 (Fig. 9b), as well as both stations in PL (Fig 10a) and DL (Fig 10b)). If necessary we will add the above explanation to the text. After your advice we divided Fig 9 in two figs, and we think that now it is readable. At the Fig. 9 and 10 standard deviation and not error is reported as added now in the legend.

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COMMENT 11: page 17866 line 11 Check writing style for units. is it really $\mu\text{g L}^{-1}$, and not $\mu\text{g L}^{-1}$?

RESPONSE: Writing style was checked and corrected

COMMENT 12: line 24 The first time a ' \pm ' is cited, the authors should indicate if it is for introducing se or sd. It is not necessary to indicate so much digits when unnecessary (for instance for T it should be 18 ± 4 , or 17.6 ± 4.2 instead of 17.61 ± 4.20 C. Check in the whole text. The worst is page 17888 lines 15-20 (35.64 ± 43.10 pM h⁻¹).

RESPONSE: Mean \pm sd was inserted. The number of digits was reduced throughout whole text, except for salinity.

COMMENT 13: lines 21-24. 'differed significantly... significantly lower...' Add in M&M sections tools used for statistics (comparison of averages, it seems).

RESPONSE: In M&M section new paragraph was added and now it reads... 2.6. Statistical analysis. According to salinity criteria (Vilibić and Orlić, 2002) two groups of water masses were identified: Levantine Intermediate Waters (LIW; $S > 38.75$) and South Adriatic Waters (SAW; $S < 38.75$). Additionally, waters were divided into productive layer (PL) and deeper layer (DL) according to concentration of Chl a above or below the detection limit ($0.01 \mu\text{g L}^{-1}$), respectively. PL refers to a layer of newly produced (labile and biologically utilizable) carbon; nevertheless it was produced or brought by mixing. Differences between the waters, layers and stations were tested by two sample t-test, comparison of means from two groups of each parameter. Differences between cruises and stations in each cruise for all parameters were tested by one-way ANOVA. All data were log or log+1 transformed to comply with assumptions of ANOVA (Supplement Tables S1 and S2).

COMMENT 14: line 25. What is the threshold used for separating water masses affected by LIW or SAW? A criteria of salinity? And when a station where only a part of the water column is influenced by the LIW is the data of the whole water column in

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the 'LIW' category? or divided in two parts? This is important to understand how the averages per type of water mass is calculated, and then compared.

RESPONSE: The water masses LIW and SAW were separated according to salinity criteria (e.g. $LIW > 38.75$, Vilibić and Orlić, 2002). Only the productive layer of the water column of both stations were considered and separated into two groups of waters: LIW ($S > 38.75$) and SAW ($S < 38.75$), as written in the text now (see section 2.6. Statistical analysis)

COMMENT 15: p 17867. line 4. A table should indicate PL depth at all seasons and stations because in the figure 4, it is not easy to guess the 'zero' level' on a log chl scale. It is important, again, because then averages are also compared within and without PL layers.

RESPONSE: Following your suggestions we added a table indicating the PL depth. Table 1. Depth of the productive layer (PL) for the respective cruise and station (please find in Supplement)

COMMENT 16: Line 23 'HB correlated significantly with Chl_a and negatively with po_4 and SiO_4 '. Is it simply an indirect effect of depth? Are these relations still valid when considering only euphotic zone?

RESPONSE: Yes, mentioned correlations were function of depth. The correlations between HB and Chl a in the euphotic zone were still significantly positive due to commensalism between bacteria and phytoplankton. The negative correlation of HB and SiO_4 was still valid for this zone, while for phosphates and DIN it was not. The negative correlation with SiO_4 was probably due to the negative correlation of SiO_4 and Chl a and positive correlation of HB and Chl a, since HB do not use SiO_4 . The relationship of HB and PO_4 is more complex, and even more with DIN (more forms involved), since bacteria can regenerate or compete with phytoplankton for DIN and PO_4 , depending on C:N:P ratio in organic matter. Involvement of many processes, sometimes with inverse effect, (most probably) resulted in not significant correlations in euphotic zone.

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The situation in DL was clearer since in this layer remineralization and accumulation of nutrients strongly prevailed over utilization. The overall negative HB correlation with nutrients was probably due to prevailing of different processes in mentioned zones, ie. nutrients utilization in euphotic zone (low nutrients and high HB) and nutrient regeneration and accumulation in aphotic zone (high nutrients and low HB).

However, in the manuscript we did not introduce calculation for euphotic and aphotic zone. They usually coincide with PL and DL, except for February when PL was more extended than euphotic zone. Would it be sufficient if we introduce the following explanation instead of new calculation?

Negative correlations of HB with DIN, PO₄ and SiO₄ were probably due to prevailing of different processes in PL and DL, ie. nutrients utilization in PL (low nutrients and high HB) and nutrient regeneration and accumulation in DL (high nutrients and low HB).

COMMENT 17: Line 24. 'SYN Pro and pEu were detected only in PL'. Is there a particular reason that it should not be systematically the case?

RESPONSE: No, there is no particular reason. This sentence is deleted.

COMMENT 18: p 17868. This paragraph is confusing. It is not always very clear to which data corresponds to the averages. Sometimes PL layers, sometimes DCM, sometime Chl a rich layers...

RESPONSE: The reported averages refer always to PL. However, in some exceptional cases values for other depths were highlighted (eg. DCM or chl a rich layers). We altered sentences trying to be clearer.

...L/T ratios (24.6±43.9), being extremely high (140.6) at the DCM depth (75 m).

... L/T ratios (5.1±6.8). However, when calculated only for 20-100 m, where the highest Chl a values were measured, L/T ratios were extremely low (0.2±0.2).

COMMENT 19: p 17869 lines 1/2. This sentence is not clear. Does Leu and Tdr B

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correlate with HB on one hand, and with Chl a on the other hand, but for a different set of data?

RESPONSE: Correlation of LeuB and TdRB with HB were done for the entire water column (exception were some data sets non comparable, since some data were missing, eg. data for Leucin in May). Correlation with Chl a were performed only in PL. For clearer explanation the new sentence now reads:

Also both ratios significantly positively correlated with Chl a in PL.

COMMENT 20: page 17871 line 10. I cannot not consider that a convection episode, bringing phytoplankton cells in the dark column layer, is extending the 'productive' layer. Phytoplankton in the dark does not make photosynthesis anymore. In addition, due to the dilution effects, integrated data (per m⁻²) should stay the same. At least the term "productive" should be more explicitly defined: Bringing new carbon, labile carbon in the twilight zone etc:::

RESPONSE: You are right and using the term 'productive' layer without an appropriate definition could be misleading. By the term "productive layer" we thought of the layer of newly produced (labile and biologically utilizable) carbon; nevertheless it was produced or brought by mixing. In some periods of the year the depth of the productive layer concur with euphotic zone depth, but during mixing events the productive layer extended deeper, thus not to complicate with different terms in each investigated period we named this layer "productive layer". We followed your suggestion and introduced this explanation in MM section where we the term "productive layer" was mentioned for the first time.

COMMENT 21: p 17873 line 6. PHP is expressed here in carbon units. The authors should add conversion factors used for Leu and Tdr in the M&M sections. In addition, does the values cited here come from Tdr or leu data? Again, it is not clear from which data averaged vales are calculated: whole water column? both stations?

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RESPONSE: The values cited come from Leu data and conversion factor according to Kirchman, 1993 (3.1 kg Cmol⁻¹ of Leu) were used. This information is added to M&M at the end of section “Prokaryotic heterotrophic production”, which now reads:

PHP (nM C day⁻¹) for February and March were calculated from Leu data using conversion factor 3.1 kg Cmol⁻¹ of Leu after Kirchman, (1993). The averaged values included the productive layers for both stations.

We are aware that conversion factors are strongly variable (e.g. Kirchman, 1992 in Mar. Ecol. Prog. Ser. 82, 301-309; Calvo-Diaz et al., 2009 in Appl. Environ. Microbiol. 75, 3216-3221, etc.), especially in low-productivity environment, and should be determined if precise estimations of carbon flux through bacteria were required. However, we used mentioned factor in order to be comparable with the results reported in Azzaro et al (2012) for the same area (P1200), that were calculated with the same factor.

New reference: Kirchman, D.L.: Leucine incorporation as a measure of biomass production by heterotrophic bacteria. In: Kemp PF, Sherr BF, Sherr EB, Cole JJ (eds) Handbook of methods in aquatic microbial ecology. Lewis Publishers, Boca Raton, p 509-512, 1993.

COMMENT 22: Lines 18-21. Indicate where are the statistics showing this.

RESPONSE: (Table 2) was inserted in the text showing significant correlation between T and Leu and between Chl and TdR.

COMMENT 23: Line 25 discussion on L/T. According table S2, only LeuC (specific activity per cell) and L/T are higher in P300, so not absolute rates. The authors argue that presence of Syn should increase the L/T ratio, but line 15 they said that 20 is a balanced ratio. There is not many papers reporting simultaneous Leu and Tdr measurements and more comparison with literature should be done. Are there other reference reporting low values of L/T as they get (<1)? What are the physiological or technical reasons for such low L/T values?

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RESPONSE: According to variations of L/T ratio at station P300 in March (24.6 ± 43.9) average protein and DNA synthesis were more synchronized than at station P1200 (5.1 ± 6.8). The bacterial growth was balanced through water column except for some layers where high L/T ratios (up to 140) increased the average and lead to its high variation (sd). Bacterial abundances and TdR values were not significantly different between stations, but high abundances of cyanobacteria at station P300 may explain different L/T ratios with cyanobacteria being responsible for such vast Leu incorporation.

To our knowledge, L/T ratios <1 were reported in Gasol et al. 2009, Prog. Oceanogr. 83, 189–196, in mesopelagic layer at the station affected by the upwelling filament. Also, very variable but lower L/T ratios were reported by Gasol et al. 1998, Mar. Ecol.-Prog. Ser. 164, 107–12, at DCM depths. The possible physiological reasons for such bacterial response with higher rates of DNA than protein synthesis were due to increase in DOM supply rates (Chl a at DCM depths and vertical C flux in upwelling site) when bacteria first need to adapt their number and then grow in biomass. The other possible reasons could be different carbon conversion efficiencies for both, leucine and thymidine at various depths (or temperature) (Gasol et al., 1998; Ducklow 2000) or differences in incorporation rates between subpopulations in bacterial assemblages, e.g. HNA and LNA (Longnecker et al., 2006).

This paragraph was partly rewritten with more references added

Although both PHP rates increased significantly in March with respect to February, the relationship between the rates quite differed between stations. At P300 the increase in both rates was generally more synchronized (L/T ~ 25) or progressed with increased biomass production (L/T up to 140), whereas at P1200 much higher rates of cell replication than biomass production (L/T ~ 5) was observed, particularly around DCM depths (L/T <1). Consequently, at both stations HB followed the increase in respective autotrophic biomasses that were far more expressed around the SAP. Unbalanced growth of bacteria, when rates of protein synthesis and DNA synthesis are uncoupled, was

usually observed in offshore regions, in deep samples of vertical profiles and during pulses of organic matter supply (Chin-Leo and Kirchman, 1990; Gasol et al., 1998; 2009). Accordingly, L/T ratios from different aquatic habitats varied over a wide range from 0.01 to >200 (Torréton and Dufour, 1996; Gasol et al., 1998; Hoppe et al., 2006; Longnecker et al., 2006; Gasol et al., 2009), with extreme values occurring in more extreme environments. Temperature and resource supply are principal factors that influence bacterial growth and reproduction (Shiah and Ducklow, 1997) but variability of bacterial growth characteristics (as L/T ratio) is influenced by picocyanobacteria, particularly when abundant (Hietanen et al., 2002; Zubkov et al., 2003; Hoppe et al., 2006). Generally, our results showed that T has greater effect on bacterial biomass production (Leu) while Chl a (taken as a measure of substrate supply) affected more strongly cell replication rates (TdR). Thus the higher biomass production rate and increased values of L/T ratios at P300 might be induced by increased temperature of LIW and partly by much higher abundances of SYN. These two factors in addition to generally reduced substrate supply probably led to significantly higher prokaryotic biomass production in waters influenced by LIW. Similar increase in both, L/T ratio and the abundance of cyanobacteria were recorded in the Northern Adriatic during the period of 2003–2008 which coincided with overall increase in S and T in that area (Ivančić et al., 2010). In contrast, at P1200 lower L/T ratios indicated those bacteria maximized reproduction most probably due to extreme increase in Chl a. It was shown that bacteria favor DNA duplication over the protein synthesis in DCM depths where maximal amount of DOM to flow from photosynthesis was expected (Gasol et al., 1998). In addition, extremely low L/T ratios (<1), as we got around DCM, were observed in mesopelagic areas affected by upwelling filament where high bacterial activity (in terms of DNA synthesis rates) was assumed to be sustained by increased vertical flux or by direct intrusion of lateral carbon to mesopelagic waters (Gasol et al., 2009).

New references: Chin-Leo, G., and Kirchman, D.L.: Unbalanced growth in natural assemblages of marine bacterioplankton, *Mar. Ecol. Prog. Ser.*, 63, 1-8, 1990.

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Hoppe, H-G., Gocke, K., Koppe, R., and Kraus, G.: Changing bacterioplankton growth characteristics on a large spatial scale: oligotrophic versus mesotrophic ocean, *Mar. Ecol. Prog. Ser.*, 323, 21–33, 2006.

Longnecker, K., Sherr, B. F., and Sherr, E. B.: Variation in cell-specific rates of leucine and thymidine incorporation by marine bacteria with high and with low nucleic acid content off the Oregon coast, *Aquat. Microb. Ecol.*, 43, 113-125, 2006.

Torretón, J.P., and Dufour, P.: Bacterioplankton production determined by DNA synthesis, protein synthesis, and frequency of dividing cells in Tuamotu Atoll lagoons and surrounding ocean. *Microb. Ecol.*, 32, 185-202, 1996.

Zubkov, M. V., Fuchs, B. M., Tarran, G. A., Burkill, P. H., and Amann, R. High rate of uptake of organic nitrogen compounds by *Prochlorococcus* Cyanobacteria as a key to their dominance in oligotrophic oceanic waters. *Appl. Environ. Microbiol.*, 69, 1299–1304, 2003.

COMMENT 24: P 17875 line 4. According table S2 Tdr is nit higher at st 1200 in March. Why a low L/T would mean more active bacteria?

RESPONSE: Although in March average TdR at 1200 was about three times higher than at P300, observed difference was not statistically significant due to large variance. From higher TdR in combination with lower L/T ratio in comparison to P300, we presumed that bacteria were more active at P1200. Indeed at P1200 their numbers were markedly lower in February than in March (Fig. 4), while at P300 such increase was not observed (Fig. 4). However, it appears to be too speculative and we deleted this sentence and the following ones about increased grazing activities due to Ref #2 recommendation.

COMMENT 25: Line 15. Sentence unclear “since these two layers matched also in tdr regulation of bacterial function by the same factors”

RESPONSE: This sentence is rewritten and now it reads: Since TdR values matched

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in both layers bacterial function might be regulated by the same factors, i.e. resource quality and availability.

COMMENT 26: Table 1. As the authors transformed some data by using $\log(x+1)$ do they keep all the “zero” data in their regressions (for chlorophyll for instance).

RESPONSE: For regressions with chlorophyll only the pairs of data for productive layer ($\text{Chl} > 0$) were used. Also in other regressions no “zero” data were used (e.g. autotrophic abundances).

COMMENT 27: Figure 4 and Figure 2 have both an interruption of their depth scale but not at the same level, so it is hard to compare trends for the reader.

RESPONSE: Figure 4 and 2 are corrected and now comparable.

COMMENT 28: Figure 4. Why abundances are not homogeneous too along the water column in February, during the convection event?

RESPONSE: Non-homogenous distribution of bacterial abundances during the winter convection might be caused by oscillations in coupling between bacteria and their predators, heterotrophic nanoflagellates. According to the literature in oligotrophic system (Tanaka and Taniguchi, 1999, Mar. Ecol. Prog. Ser., 179, 123-134; Tanaka et al., 2007, Deep Sea Res. I, 54, 1721-1743) it might be supposed that tighter top-down control on bacteria exist in the upper layer while in deeper layers bacteria were more controlled by resources (bottom-up) even during convection. In accordance, during the convection event in 2008 in the investigated area ciliates were not homogeneously distributed in the water, but accumulated in upper layer (Batistić et al., 2012). The increase in HB abundance with depth could be also caused by higher concentration of detritus at the same depths. However, further analysis on microbial interactions in this area is needed to verify these presumptions.

COMMENT 29: Check units in whole text and tables. For DIN for instance, M or mol l-1 but not M l-1. For fluxes for instance, M h-1 or mol l-1 h-1 but not M l-1 h-1.

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RESPONSE: The units were checked and corrected throughout the text and figs.

COMMENT 30: Figure 5. What are the thresholds for separation of LIW and SAW water masses? Does box plots include all seasons and depths? all stations?

RESPONSE: LIW and SAW were separated according to salinity criteria, as explained now in MM section. The box plots include all seasons at both stations and depths in PL since LIW do not intrude in DL.

Figure Captions

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

Fig. 9. in article: Changes of MC (mean AWCD) in productive and deeper layers (A); percentage utilization of substrate groups (AA – amino acids, AMI – amines, C – carbohydrates, CA – carboxylic acids, P – polymers, PC – phosphorylated compounds) at 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 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)

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 2. in article: Vertical distribution of salinity and temperature at stations P300 and

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P1200 during the cruises (3 October 2011, 18 February 2012, 29 March 2012, 30 May 2012, 10 September 2012)

Please also note the supplement to this comment:

<http://www.biogeosciences-discuss.net/10/C8354/2014/bgd-10-C8354-2014-supplement.pdf>

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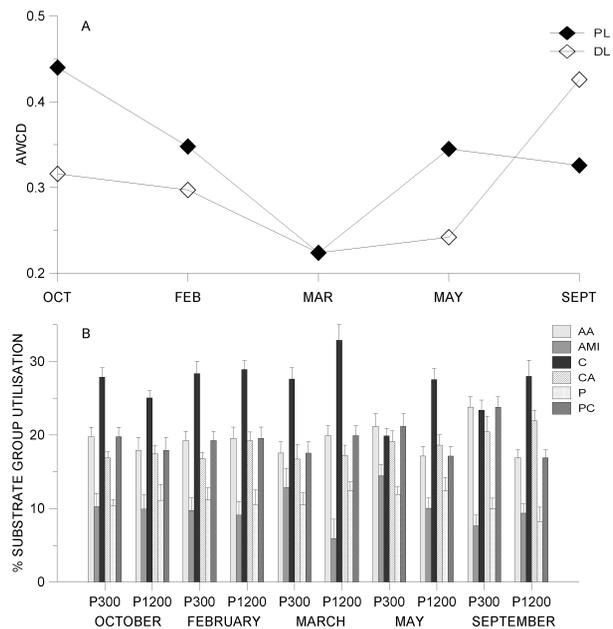


Fig. 1. Fig. 9. in article

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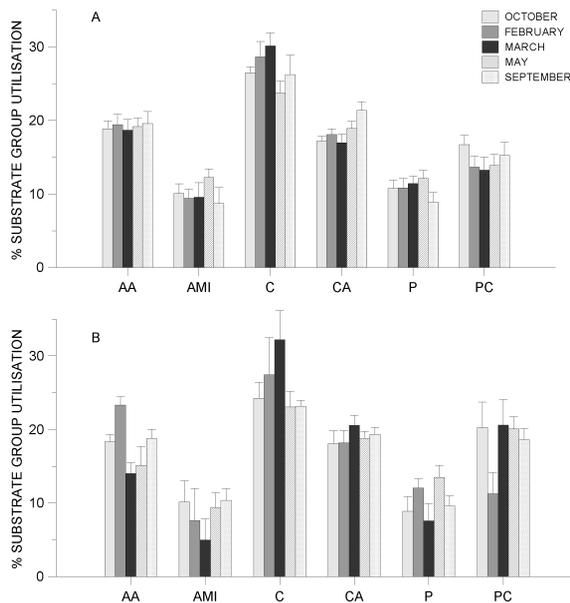


Fig. 2. Fig. 10. (new)

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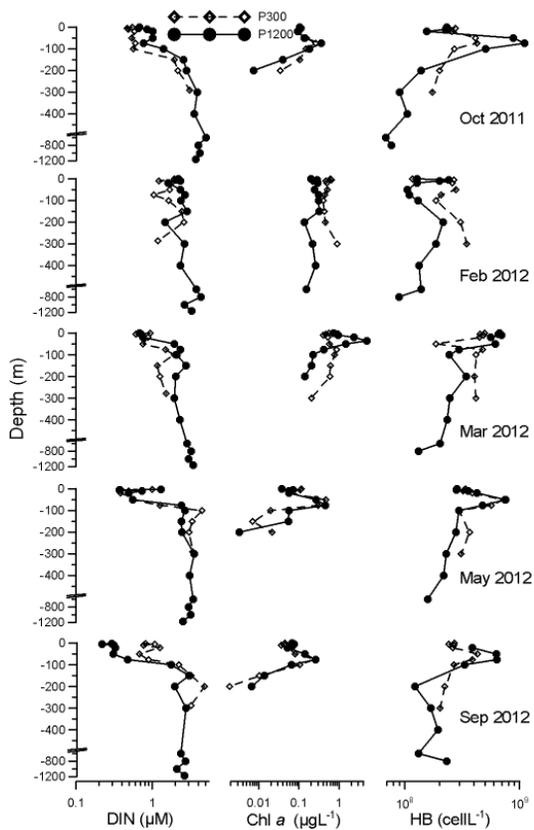


Fig. 3. Fig. 4. in article

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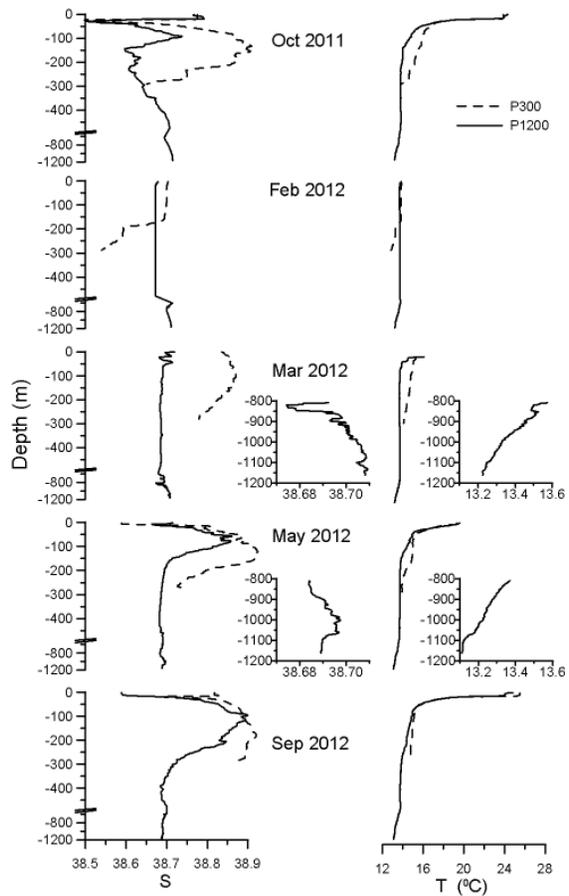


Fig. 4. Fig 2. in article