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Biogeosciences Discussions

Interactive comment on "Role of environmental factors for the vertical distribution (0–1000 m) of marine bacterial communities in the NW Mediterranean Sea" *by* J. F. Ghiglione et al.

J. F. Ghiglione et al.

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Answer to Anonymous Referee #4 Answers to the referee are reported point by point. Changes in the text are located by the number of the corresponding line in the original manuscript.

Answer to specific comments: Answer to specific comment 1: -We propose to remove the last sentence of the abstract (page 2133, lines 24-26: "This study is probably the first example of an analysis employing a complex environmental dataset in combination with microbial community profiles to unravel the mechanisms underneath bacterial assemblages in marine systems.") that may overestimate the originality of our study compared to the existing literature. -We also propose the following modifications throughout

the manuscript: Page 2133, line 4: “Here we show the explanatory power of multivariate statistical analysis” will be changed into “Here we use the explanatory power of direct multivariate gradient analysis”. Page 2134, line 21: “Although indirect gradient multivariate statistical analyses have been used to link microbial community profiling to environmental parameters (Roeling et al., 2001; Edlund et al., 2006), the use of direct gradient analyses like CCA in combination with high-throughput molecular technologies is scarce in spite of the power of this method for this purpose (Ramette, 2007; Rooney-Varga et al, 2005).” Page 2149, lines 5-7: the sentence “To our knowledge, however, no studies have demonstrated the direct influence of environmental parameters on the bacterial community structure of natural environmental gradients” will be removed because it is confusing as the "direct" gets lost in this context. Page 2151, line 15: “Our study is perhaps the first example of such complex biogeochemical dataset” will be changed into “Our study propose a complex biogeochemical dataset” We already acknowledge in the original manuscript that other studies are already published using direct gradient analysis in several environments (see page 2148, lines 11-13: “from marine (Cordova-Kreylos et al., 2006; Klaus et al., 2007; Sapp et al., 2007), lake (Yannarell and Triplett, 2005) and soil (Salles et al., 2004) systems”). As proposed by referee #4, reference to Hannig et al. (2006) will be added in the manuscript (page 2148, line 11) and in the reference section: Hannig, M., Braker, G., Dippner, J., and Jürgens, K. Linking denitrifier community structure and prevalent biogeochemical parameters in the pelagial of the central Baltic Proper (Baltic Sea), FEMS Microbiol. Ecol., 57, 260-271, 2006.

Answer to specific comment 2: As suggested by referee #2, more information will be provided in the revised version of the manuscript concerning the detection limits of the methods used for the physico-chemical parameters and lipids biomarkers in the “Materials and Methods” and “Results” sections: Page 2136, line 19: “Detection limits were 3 nmol l-1 for nitrate and nitrite, 0.02 μmol l-1for phosphate and 5 nmol l-1 for ammonium. To ensure reproducibility in nutrient measurements between analyses, an unique type of in-house standards was used, which was regularly compared to commercial prod-

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ucts (OSIL). Precision was also tested, through the participation in the European intercalibration exercise QUASIMEME (http://www.quasimeme.marlab.ac.uk/)." Page 2143, line 9: "The upper layer (0-40m) was nutrient depleted with nitrate and phosphate concentrations close to the detection limit. Concentrations increased along a nitracline up to maxima (7 and 0.2 μM, respectively) around 80 m depth. Silica varied from 1.0 μM at the surface to 13.5 μM) at 1000 m depth. DOC concentrations varied from 72.8 μM to 38.8 μM from surface to depth." Page 2144, line 16: "Total dissolved lipids varied from 0.4 to 4 μM). Detailed analysis of this fraction of the organic matter is given in Goutx et al. (this issue). Chloroplast lipids (LC) dominated the lipid pool (38.7ś8.5% on average, n=166), which indicated a phytoplankton source for DOM. Triglycerides (TG) were minor lipids ($<$10 % of total lipids)."

Goutx, M., Guigue, C. , Aritio, D. , Ghiglione, J.F., and Andersen, V.: Short term variability of dissolved lipid classes during summer to autumn transition in the Ligurian sea (NW Mediterranean), submitted in this issue.

Answer to specific comment 3: Since individual peaks were not sequenced, we cannot exclude that some peaks may have originated from phytoplankton plastids. A BLAST analysis of each primer or both primers (http://www.ncbi.nlm.nih.gov/blast/) resulted in matching plastid 16S rRNA sequences. However, since seawater was pre-filtered through 3 μm pore size filters, this would have removed larger eukaryotic organisms. Flow cytometry analysis performed at DYFAMED station showed that picoeukaryotes (that could pass through 3 μm-pore-size filters) were on average 603 times less abundant than total bacterioplankton during our sampling period. The percentage of picoeukaryotes among the total cells of the 0.2 to 3 μm fraction size was most of the time less than 1% (mean = 0.22, SD = 0.03), suggesting that plastid peaks may make a minor contribution to the overall CE-SSCP profiles. We propose to add the following sentences: Page 2139, line 3: "pre-filtered through 3 μm -pore-size filters (47 mm, Nucleopore) to remove most eukaryotic organisms (containing platids that complicate the interpretation of CE-SSCP fingerprints) and to prevent clogging of the final filter."

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Page 2146, line 22: “As it was pointed out elsewhere (Riemann et al. 2000, Fandino et al. 2001), we cannot exclude the presence of peaks originating from eukaryotes in the CE-SSCP profiles since the PCR primers used in this study can amplify plastid 16S rRNA genes. However, this should not greatly affect our results, since picoeukaryotes represented less than 1% (mean = 0.22, SD = 0.03) of total cell counts in the fraction 0.2 to 3 μm (data not shown).” Reference to be added in the revised version of the manuscript: Fandino, L.B., Riemann, L., Steward, G.F., Long, R.A., and Azam, F.: Variations in bacterial community structure during a dinoflagellate bloom analyzed by DGGE and 16S rDNA sequencing, Aquat. Microb. Ecol., 23,119-130, 2001 Riemann, L., Steward, G.F., Azam, F.: Dynamics of bacterial community composition and activity during a mesocosm diatom bloom, Appl. Environ. Microbiol., 66,578-587, 2000.

Interactive comment on Biogeosciences Discuss., 5, 2131, 2008.