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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 #1

Answers to referee #1 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 agree with referee #1 that the “hypothesis” enounced on page 2134, lines 24-27 is more a description of our approach that a hypothesis. This sentence will be removed from the final version of the manuscript. In this paper, it is difficult to compare our data (sampling at four dates during the cruise period) with the large datasets covering different temporal scales (from hours to seasons) in abundance and production of total and particle-attached

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Interactive Discussion

Discussion Paper



Interactive
Comment

bacteria recorded during the same cruise by Mével et al. (submitted in this issue). As proposed by reviewer #1, and since we are not discussing the temporal pattern of the observed vertical distribution in the manuscript, the sentence in page 2135, lines 14-17 will be changed to “As a first objective we sought to determine the vertical distribution of bacterial communities during the late summer - autumn transition period by using multivariate non-parametric statistical methods.”

Answer to specific comment 2: While we think that the reviewer is right in that other studies analyze microbial community profiles using multivariate statistical methods, our study is unique because we analyze a very complex biogeochemical dataset (i.e. here the number of environmental parameters of our study) using direct rather than indirect multivariate gradient analysis. The difference is that the first gives an ordination with an optimal environmental basis showing only those patterns in the species data that can be explained by the available environmental data (being the ordination axes aggregates of the environmental variables that best explain the species data). In order to best explain this in our manuscript we propose the following changes: -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

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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 3: We propose to add information about “bottom-up” and “top-down” control in the introduction and in the discussion sections:

Page 2134, line 21: “More generally, theoretical models and empirical surveys indicate that microbial growth or population size are controlled by resource availability, including nutrient and energy sources (“bottom-up” control) and microbial biomass is controlled by mortality, including factors such as predation or viral lysis (“top-down” control) (Pernthaler, 2005). The relative importance of “top-down” and “bottom-up” controls on bacteria have been poorly investigated (Fuhrman and Hagström, 2008), and our knowledge on the interplay between the different factors involved within “top-down” and “bottom-up” controls is still limited in the field. Protistan predation may often be most influential in limiting the total abundance and biomass of the bacterioplankton, whereas viruses are considered to more profoundly affect prokaryotic community diversity (Pernthaler, 2005). On the other hand, clear bottom-up effects on bacterial

BGD

5, S1404–S1411, 2008

Interactive
Comment

Full Screen / Esc

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Interactive Discussion

Discussion Paper



community structure have been observed in controlled laboratory conditions (Pinassi et al., 1999; Carslon et al., 2002) but few studies demonstrated statistically robust, predictable patterns in the field (Korona et al., 1994). Such complex ecological questions may be addressed by robust statistical analysis. A recent review (Ramette, 2007) underlines that hypothesis-driven technique such as redundancy analysis, canonical correspondence analysis (CCA), or Mantel tests are rarely used by microbial ecologists, despite the fact that high-throughput molecular technologies are now available at the field. For example, this approach allows better understanding of the linkage between bacterioplankton and phytoplankton dynamics in marine coastal environment (Rooney-Varga et al., 2005).”

We propose to remove section page 2134, lines 21-27.

Page 2152, line 5: “In our conditions, resources (“bottom-up” control) as well as other factors such as pressure, temperature, light, O₂ and PAR controlled simultaneously the vertical zonation of bacterial communities in the water column. Unfortunately, the parameters measured in our study do not permit to evaluate the effect of predation by viruses or protists (“top-down” control). There are indications in the literature that bacteria are tightly controlled by protistan predation in highly oligotrophic systems, whereas their growth is limited by competition for nutrients in more productive waters (Strom, 2000; Gasol et al., 2002). On the other hand, virus-induced mortality seems more substantial when bacterial productivity is enhanced or in anoxic conditions (Weinbauer et al., 2004). “Sideways controls” such as competition for resource, allelopathy or syntrophy may also influence bacterial community structure in marine environments and the new insights given by metagenomics approach should allow more thorough investigation of this subject in the near future (Fuhrman and Hagström, 2008; Strom et al., 2008). Numerous factors can regulate microorganism population dynamics, often simultaneously, and our study together with other evidences found in the literature (Fuhrman et al., 2006) underline the importance of relevant statistical analysis for further multidisciplinary investigations to investigate the relative importance of environmental factors in

Full Screen / Esc

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Interactive Discussion

Discussion Paper



predicting the bacterial community dynamics in marine systems.”

We propose to remove section page 2152, lines 5-8.

References to be added in the revised version of the manuscript:

Carlson, C.A., S.J. Giovannoni, D.A. Hansell, S.J. Goldberg, R. Parsons, M.P. Otero, K. Vergin, and B.R. Wheeler. The effect of nutrient amendments on bacterioplankton production, community structure and DOC utilization in the northwestern Sargasso Sea. *Aquat. Microb. Ecol.*, 30, 19 - 36, 2002.

Gasol, J. M., Pedros-Alio, C., and Vaqué, D. Regulation of bacterial assemblages in oligotrophic plankton systems: results from experimental and empirical approaches. *Antonie Van Leeuwenhoek*, 81, 435-452, 2002.

Fuhrman, J.A., and Hagström Å.: Bacterial and Archaeal community structure and its patterns. In: *Microbial Ecology of the Oceans* (2nd edition), edited by Kirchman, D. L., Wiley-Liss, Inc., New York, 45-90, 2008.

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, *Proc. Natl. Acad. Sci., USA*, 103, 13104-13109, 2006.

Korona, R., Nakatsu, C.H., Forney, L.J., and Lenski R.E. Evidence for multiple adaptive peaks from populations of bacteria evolving in a structured habitat. *Proc. Natl. Acad. Sci., USA*, 91, 9037-9041, 1994.

Pernthaler, J.: Predation on prokaryotes in the water column and its ecological implications, *Nature*, 3, 537-546, 2005.

Pinhassi, J., Azam, F., Hemphälä, J., Long, R.A., Martinez, J., Zweifel, U.L., and Hagström, Å.: Coupling between bacterioplankton species composition, population dynamics, and organic matter degradation. *Aquat. Microb. Ecol.*, 17, 13-26, 1999.

Rooney-Varga, J.N., Giewat, M.W., Savin, M.C., LeGresley, M., and Martin, J.L. Links

BGD

5, S1404–S1411, 2008

Interactive
Comment

Full Screen / Esc

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between phytoplankton and bacterial community dynamics in a coastal marine environment. *Microb. Ecol.*, 49, 163-175, 2005.

Strom, S.L. : Bacterivory: interactions between bacteria and their grazers. In: *Microbial Ecology of the Oceans*, edited by Kirchman, D. L., Wiley-Liss, Inc., New York, 351-386, 2000.

Strom, S.L.: Microbial ecology of ocean biogeochemistry: a community perspective. *Science*, 320, 1043-1045, 2008.

Weinbauer, M.G., and Rassoulzadegan, F.: Are viruses driving microbial diversification and diversity? *Environ. Microbiol.* 6, 1-11, 2004.

Answer to specific comment 4: The answers to these questions have been discussed in detail in the response to the comments by U. Stingl (Referee). We propose to add new information about CE-SSCP:

Page 2135, line 14: "In a recent paper (Rodriguez et al., in press), we show that CE-SSCP does not miss any known major group detected by a clone library of the same sample and that this technique is a reasonably quantitative measure of the relative abundance of the only taxon we could independently measure (*Prochlorococcus*, by flow cytometry)."

Page 2147, line 9: "Molecular fingerprinting techniques in general may be restricted in giving information about microbial diversity, since they results in 30 to 40 peaks (or bands) in marine waters. This is due to co-migration of PCR-products from different species within the same peak (or band), as depicted by several authors (Wintzingerode et al., 1997; Schmalenberger and Tebbe, 2003). Similarly, Schauer et al. (2000) mentioned the subjectivity in deciding whether a very weak DGGE band is a real band or a background artefact. In our study, this problem was, at least partly, overcome by taking into account the presence or absence of individual CE-SSCP peaks and the relative contribution of each peak to the total surface area of the pattern. The

BGD

5, S1404–S1411, 2008

Interactive
Comment

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Discussion Paper



advantage of analysis fingerprinting data this way has been recently enlightened by numerical simulation (Loisel et al., 2006). This study underlines that fingerprinting patterns contains extractable data about diversity although not on the basis of a number of bands or peaks, as is generally assumed to be the case, and can be considered as an “image” of the whole microbial ecosystem free of inventory (cloning) limitation.”

The vertical distribution of bacterial communities has been discussed in more details with regards to reviewer comments.

Page 2148, line 3: “This is also consistent with genotypic and phenotypic properties of stratified *Prochlorococcus* “ecotypes” for example, that are suggestive of depth-variable adaptation to light intensity and nutrient availability (Rocap et al., 2003). However, none of these studies tested for the statistical significance of vertical trends (autocorrelation), nor did they test for the predictability of vertical pattern from environmental parameters, so we do not know whether these studies show spatial patterning or predictability comparable to what we observed. Recent metagenomic approaches showed that vertical zonation of taxonomic groups was concomitant with functional gene repertoires and metabolic potential (DeLong et al., 2006) and future efforts are needed to interpret the observed genomic pattern as a function of contextual environmental parameters (Ramette, 2007).”

References to be added in the revised manuscript: DeLong, E.F., Preston, C.M., Mincer, T., Rich, V., Hallam, S.J., Frigaard, N.U., Martinez, A., Sullivan, M.B., Edwards, R., Brito, B.R., Chisholm, S.W., and Karl, D.M.: Community genomics among stratified microbial assemblages in the ocean’s interior. *Science*, 311, 496-503, 2006.

Loisel, P., Harmand, J., Zemb, O., Latrille, E., Lobry, C., Delgenès, J.P., and Godon, J.J. : Denaturing gradient electrophoresis (DGE) and single-strand conformation polymorphism (SSCP) molecular fingerprintings revisited by simulation and used as a tool to measure microbial diversity, *Environ. Microb.*, 8, 720-731, 2006.

[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)[Discussion Paper](#)

Rocap, G., Larimer, F.W., Lamerdin, J., Malfatti, S., Chain, P., Ahlgren, N.A., Arellano, A., Coleman, M., Hauser, L., Hess, W.R., Johnson, Z.I., Land, M., Lindell, D., Post, A.F., Regala, W., Shah, M., Shaw, S.L., Steglich, C., Sullivan, M.B., Ting, C.S., Tolonen, A., Webb, E.A., Zinser, E.R., Chisholm, S.W.: Genome divergence in two *Prochlorococcus* ecotypes reflects oceanic niche differentiation. *Nature*, 424, 1042-1047, 2003.

Rodríguez-Blanco, A., Ghiglione, J.F., Catala, P., Casamayor, E.O., and Lebaron, P.: Spatial comparison of total vs. active bacterial populations by coupling genetic fingerprinting and clone library analyses in NW Mediterranean Sea, *FEMS Microb. Ecol.*, in press.

Wintzingerode, F.V., Göbel, U.B., and Stackebrandt, E.: Determination of microbial diversity in environmental samples: pitfalls of PCR-based rRNA analysis, *FEMS Microbiol. Rev.*, 21, 213-229, 1997.

[Interactive comment on Biogeosciences Discuss., 5, 2131, 2008.](#)

BGD

5, S1404–S1411, 2008

[Interactive
Comment](#)

[Full Screen / Esc](#)

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