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Interactive comment on "Presence of *Prochlorococcus* in the aphotic waters of the western Pacific Ocean" *by* N. Jiao et al.

Anonymous Referee #1

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General comments This paper by Jiao and collaborators describes the occurrence of Prochlorococcus cells in the aphotic zone of well mixed areas of the western Pacific Ocean. Although the presence of a few thousands Prochlorococcus cells at depth is potentially interesting, the paper is way too preliminary to be publishable in Biogeosciences in its present state.

Although I tend to believe that the flow cytograms shown in Fig. 1 actually show deep Prochlorococcus cells, there are seemingly no negative control, i.e. analysis of 0.2 μm filtered deep sea water, that would demonstrate for sure that these are not just merely cells released from the flow cell, as it may happen with some flow cytometers after analysis of dense Prochlorococcus populations (as found in the upper lit layer).

But my main concern deals with the molecular part. Although the chosen qPCR ap-

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proach is clever and could have brought nice results, there are too few data shown for the paper to be convincing. The abstract claims the presence of abundant and "active" Prochlorococcus populations in the aphotic zone, while the fact that these populations is active is supported by ONLY ONE (non replicated!) measurement of rRNA per Prochlorococcus HLI cell at 300 m in the Luzon strait (Fig. 3B). All other data shown on this graph (also non replicated!) come from populations retrieved from the lit layer (0-150m depths), not from the aphotic zone. These data are therefore clearly not statistically valid and many more (replicated) data need to be shown to claim that deep Prochlorococcus are "active" in the deep ocean, a claim which to my viewpoint is somewhat doubtful since Prochlorococcus cells certainly cannot photosynthesize (and therefore grow) in the dark, cold waters of the aphotic zone.

Concerning the quantification of the abundance of Prochlorococcus ecotypes, authors seemingly missed one important recent reference (Malmstrom et al. Temporal dynamics of Prochlorococcus ecotypes in the Atlantic and Pacific oceans. ISME J 2010, 4:1252-1264), since they only looked at HLI and LLIV ecotypes, whereas this paper showed the co-occurrence at equal abundances of the low light ecotypes LLI and LLIV populations at depth.

Phylogenetic data shown Figure 4 are also not detailed enough since there is no information on how many environmental sequences were obtained for each clade. A tree clearly showing the novel environmental sequences with a clear indication of their location and depth and how they relate to previously published sequences is absolutely required and solid phylogenetic analyses using different methods and bootstrap support are necessary, not just a mere NJ analysis using MEGA4.

The discussion is not clear about how frequent the vertical mixing events noticed by the authors are at the global ocean scale and thus how frequent is the occurrence of deep Prochlorococcus populations. I am skeptic that this is a very frequent phenomenon, since it is very unlikely that these deep cells may have been missed so long, given the number of cruises all over the world, during which these organisms have been counted

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by flow cytometry (see e.g. Flombaum et al: Present and future global distributions of the marine Cyanobacteria Prochlorococcus and Synechococcus. Proc Natl Acad Sci U S A 2013, 110:9824-9829). Thus, it is most likely that these deep populations are found only in specific areas with strong vertical mixing and are thus globally not very significant.

Specific comments Fig. 3A: The Prochlorococcus abundance as determined by flow cytometry for this specific profile (DC01) should be reminded for easier comparison with molecular data.

There are a number of typos in the text, such as "phynol", "populaitons", "fundmental", "cytomertric", etc.

Interactive comment on Biogeosciences Discuss., 10, 9345, 2013.