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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 #2**

Received and published: 12 July 2013

This article presents evidence that *Prochlorococcus* is present in deep samples (>300 m) at a number of locations in the western Pacific. The authors also discuss some physical mechanisms which can be responsible for the transport of *Prochlorococcus* to deep waters. It is also stated that these deep populations are metabolically active, but no measurements of metabolic activity (e.g. uptake or release of compounds) have been conducted. While some of the observations presented here are useful, I find that the authors greatly exaggerate their biogeochemical implications. The frequent use of the term ‘abundant’ to refer to the deep *Prochlorococcus* is misleading, as the authors use it to convey a sense of importance - which however should be based on carbon biomass, not cell density. My overall recommendation is that this article requires major revision before publication in *Biogeosciences*.

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## Specific comments

The Introduction seems to work on the basis that photoautotrophs should be confined to the euphotic layer. However, what is confined to the euphotic layer depth is photoautotrophic growth. In the absence of a perfect physical barrier, it should not come as a surprise that some photoautotrophic cells are present well below the euphotic layer. Downward water movement is bound to result in a downward particle transport, and this will affect all particles of a given size, irrespective of whether they are living or dead particles, or whether they are photoautotrophic, mixotrophic or heterotrophic cells. The question then is to assess how globally important are these events of fast vertical transport.

The authors refer often (in the Introduction and the Discussion) to the modelling results of Richardson and Jackson (2007) as supporting evidence for the importance of picophytoplankton for deep export. However, these modelling results have not been, as far as I can tell, substantiated by direct, sea-true data.

I concur with reviewer #1 that some methodological precautions should have been taken, such as running blank samples through the flow cytometer to make sure that no contamination from surface samples is contributing to the cell abundances measured in deep samples.

Table 1 indicates that *Prochlorococcus* was present at depths as large as 1500 m but no abundances are shown. Vertical profiles of abundance are given only for the Luzon strait. However, it would be helpful to see a plot showing all pairwise depth and abundance data, using different symbols to distinguish regions.

The results presented in the current manuscript, when converted into carbon biomass data (which is the relevant currency for biogeochemistry, not cell abundance) do not seem to support the view that picophytoplankton are key players in carbon export to the deep ocean. Assuming a mean *Prochlorococcus* carbon biomass of 30 fgC per cell (Heldal et al 2003 L&O 48(5), 1732–1743), a mean deep abundance of, say, 5000

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cell/mL (Fig. 2) translates into ca.  $0.15 \mu\text{gC/L}$  of organic carbon. If surface chl *a* in the studied region was, say,  $0.2 \mu\text{g/L}$ , one can have a surface phytoplankton C biomass of around  $20 \mu\text{gC/L}$ . Add to this the non-phytoplanktonic material (detritus, bacteria, heterotrophic protists) and one easily reaches a POC concentration of  $40\text{--}50 \mu\text{gC/L}$ . The authors should examine the biogeochemical relevance of the observed *Prochlorococcus* taking into account that these deep cells may represent, in terms of carbon,  $<0.5\%$  of surface stocks.

The authors assert that *Prochlorococcus* cells were viable, but no actual study of cell viability has been done. If those cells have recently (e.g. a few days) been transported to deep waters, they may still retain properties of actively growing cells such as possessing pigments and rRNA (incidentally, it must be noted that the only deep sample for which rRNA/rDNA data is given corresponds to 300 m). This, however, does not mean that those populations were actively growing under the harsh conditions they were experiencing (e.g. low temperature and absence of light). It may well be that they were just dying but not quite dead yet. These issues could easily be tackled in the laboratory, by taking exponentially growing cells, transferring them to conditions of low temperature and darkness, and then monitoring the evolution of cell abundance, pigment and RNA content and, importantly, ability to fix  $\text{CO}_2$  or uptake dissolved substrates. Hot temperatures can obviously be very destructive, but there seems to be nothing surprising in a photoautotrophic microbe resisting low temperatures and darkness for a few days, and then being able to resume active growth upon transfer to favorable conditions.

P 9356 When comparing the deep to surface *Prochlorococcus* ratio to general estimates of the f-ratio the authors seem to extrapolate their observations to the global ocean. This, however, would require that the mechanisms of rapid vertical transport discussed here are widespread in the ocean - which seems highly unlikely and has not been demonstrated by the authors.

Related to the above, the mechanisms of downward particle transport should operate

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also for larger cells. If *Prochlorococcus* is carried to the bottom, so must be other cells - only that, their size being larger and their abundance being smaller, conventional sampling methods will not detect them. My point is that finding picophytoplankton cells at great depths does not necessarily reinforce the importance of the microbial carbon pump - unless these downward water movements selectively transport picoplankton, leaving behind the larger cells.

Fig. 4 is used as evidence that both HL and LL ecotypes are present in 'all the water depths' (p. 9351, bottom para.). However, this figure does not specify sampling depths for the deep samples – it just indicates > 200 m depth. More specific information should be given here, by indicating exactly the presence/absence of each ecotype at different depth ranges, e.g. 200-400 m, 400-600, 600-800, etc.

Related to the Figure above, it seems strange that no *Synechococcus* sequences were found, not even in the euphotic layer. Flow cytometry data (for instance, papers by Zubkov et al. from the AMT cruises) indicate that *Synechococcus* is always present when *Prochlorococcus* is present (but not the other way around). Some comments on this should be included - perhaps I am misreading the Figure and Syn sequences were indeed found.

Additional basic hydrographic information is needed. At a minimum, temperature and chl a (or fluorescence) profiles should be shown for all studied regions. Estimates of surface suspended POC concentrations would be required, to place in context the significance of deep *Prochlorococcus* in terms of organic carbon.

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

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