Interactive comment on "Ecology of aerobic anoxygenic phototrophic bacteria along an oligotrophic gradient in the Mediterranean Sea" by D. Lamy

We would like to greatly thank the reviewers for their thoughtful comments on our manuscript. The comments and suggestions were much appreciated.
Both reviewers had minor comments.
Below a general reply stating about the general changes made on the ms, we answer point-by-point in the issues raised by the reviewer #1 and clearly state how we addressed the comments in the revised ms.

General change
Co-authors were added in the paper due to their relevant contribution to this work: M. Pujo-Pay, L. Oriol and V. Cornet-Barthaux for providing the nutrients and organic matter concentrations and P. Catala and L. Bariat for flow cytometry analyses.
Claude Courties is acknowledged in the revised version of the paper for his contribution to the flow cytometry analyses. Michal Koblížek is acknowledged for his help with detection of AAP bacterial colonies using the infra-red system available in his laboratory.

A companion paper, published in the same special issue in BGD and unveiling the diversity of cultivated and metabolically active AAP during the same Mediterranean survey has been cited in the revised version of the ms (page 4 line 63-65).
"In a companion paper, Jeanthon et al. (2011) reported the isolation of AAP bacteria and unveiled the diversity of the active AAP populations retrieved during the BOUM cruise."

A short paragraph on the relative abundance of AAP bacteria (percent to total prokaryotes) was added in the Results section of the revised version of the ms (page 12 line 247-251).
"Relative abundance of AAP bacteria in subsurface waters did not vary substantially along the longitudinal transect, ranging from 0.4 to 0.9 % of total prokaryote abundance. The relative abundance maxima were at or just above the DCM at all the stations, reaching up to 4% in the western part (station 21 at 50 m) and less than 1% in the eastern part."

Specific comments of reviewer #2

P. 326. L. 1-5. Refer also to their fast growth, which is actually a relevant aspect of these bacteria regarding their role in aquatic food webs.

We agree and we referred to the fast growth of AAP bacterial cells in the revised ms (page 3 line 30).

P. 328. L. 8. Change 'dissolved inorganic phosphate' to 'soluble reactive phosphorous' which is what is being measured by the Rimmelin and Moutin procedure.

This was changed accordingly in the revised ms.

P.336. L. 21. The low phosphate concentrations and short turnover times indicate that the whole microbial community is subjected to P scarcity, not only phytoplankton as mentioned by the authors. This affirmation should be changed.

We agree and the affirmation was changed to "microbial communities experienced conditions were phosphate was scarce." (page 15 line 324).

P. 336. L. 24. Looking at the data presented in Table 1, there are some parameters such as Chla, POP and POC that indeed reflect the increasing west-to-east gradient in oligotrophy as mentioned by the authors. However, it's surprising to me that phosphate concentrations and phosphate turnover time data indicate that cells where more limited by P in the west and in the east, although the Eastern Mediterranean is typically know by its P-limitation. I think this is also the reason why the authors did not observed a strong stimulation on bacterial growth when adding P, but they did when adding N and glucose, and it should be discussed in page 339 (see comment below).

P.339. L.16-22. The initial conditions of the experiments show that in fact bacteria where more P-limited in eastern that in the western stations, and this might explain that there was no stimulation of AAP when adding P. These initial conditions do not reflect the usual conditions of the Med Sea, where the eastern part is one of the more P-limited areas of the world. I would like to see a discussion on this on the last paragraph of page 339.

We agree with the comments of reviewer #2 and we added two different paragraphs in the discussion section.

Page 18 line 408-410: “Despite unusual high phosphate concentrations at station C, *in situ* chemical concentrations indicated more stringent oligotrophic conditions in the eastern basin than in the western basin (Pujo-Pay et al., 2011).”

Page 18-19 line 414-427: “On the basis of our nutrient-addition experiments, we cannot speculate about P-limitation of AAP bacteria since growth of AAP and total prokaryotes were not stimulated by P addition, although higher heterotrophic bacterial activity was observed. Consistent with our results, Tanaka et al. (2011) did not find any indication of P-alone limitation although a clear P-starved status in the Mediterranean basins was observed. Moreover, we observed that net growth of AAP bacteria was enhanced by glucose and nitrogen additions in the eastern basin whereas net growth of total prokaryotes was not stimulated by these additions. This result does not favor the hypothesis that light-derived energy could serve for supporting the nutrient acquisition in a nutrient-depleted environment. The problem may be in trying to deduce the impact of the additions by measuring net growth, which is a function of the bacterial response and mortality factors. However, AAP bacteria and total prokaryotes responded differently in terms of net variation of their abundances, which suggest different growth and mortality controls between these two populations.”

P.338. Discussion. The results show that AAP numbers decrease when increasing oligotrophy, which clearly goes against Kolber’s original paper in 2000, in which it was proposed that AAP would perform better under oligotrophy. This should be clearly reflected in the first paragraph of the discussion (L5-15).

A comment referring to Kolber et al. (2000) was added in the first paragraph of the discussion (page 16 line 356-363).

“Kolber et al. (2000) suggested that phototrophy would give AAP bacteria a competitive advantage over non-phototrophic bacteria when nutrient sources are scarce. Consistent with a global ocean study (Jiao et al., 2007), our data showed the opposite pattern as the maximum AAP bacteria abundance was positively related with the concentration of Chl *a*. Similarly, no clear link between AAP bacterial abundance and oligotrophy was established since abundances of AAP bacteria can be high in estuarine, coastal and shelf waters (e.g. Cottrell et al., 2010; Waidner and Kirchman, 2007).”

Also the authors are right that to see a clear effect of nutrient additions it's better to use experiments where most of mortality sources are reduced by dilution or filtration for example. I wonder why the authors decided to use whole seawater incubations?

Indeed, we use whole seawater and this was specified in the Material and Methods.

Page 9 line 172-174: "Nutrients were added to unfiltered seawater samples in order to obtain a final concentration of 1 μM NH_4Cl + 1 μM $NaNO_3$ (N), 0.25 μM Na_2HPO_4 (P), and 10 μM C-glucose (C)."

Whole seawater samples were used to limit loss of nutrients and labile dissolved organic matter during filtration and to keep attached bacteria.

P. 348. Table. 1. Change the order of the columns to St. A, 17, 9, C. Every other figure is organized with stations from western to eastern Mediterranean and the current order of the table makes it a bit confusing.

The order of the columns in Table 1 was changed to organize it from western (left column) to eastern (right column) Mediterranean as it was also done in the figures.

Page 350. Figure 1. Make a difference between stations sampled and not sampled. The methods sections says that 8 stations where sampled, but looking at this map it seems that the conclusions of the paper are based on all these stations, and I believe it's misleading.

The map (page 32) and its figure caption (page 30 line 686-692) were changed to make a difference between the stations not sampled and those sampled for the assessment of the different AAP bacterial-related parameters and the experiments conducted on the effects of nutrients amendments on the AAP bacterial growth.

Typing errors. P. 335. L 4. There is a comma instead of a dot after stations.

Typing corrections were made in the revised ms.