

Reply to Referee 2

We thank the Referee for the comment and the acknowledgement of a “well written” paper “illustrated with informative figures”.

The referee comments that next generation sequencing should have been used for the community analysis. We are convinced that advanced fingerprinting methods like ARISA are very valuable and are especially an important contribution to the EPOCA experiment described here as it was carried out in the context of other similar mesocosm experiments (see also answer to referee #1).

In our opinion, next generation sequencing, at the current state of the art, should not be used as a substitute for well-established fingerprinting techniques but rather to create exciting new insights on a deeper level. The resolution of next generation sequencing is very high and the application of metagenomics and –transcriptomics is a promising tool to gain further insights into genetic potential and –activity. However, it should not be neglected that sequencing techniques are still very much the “forefront” of molecular biology and are developing rapidly both in technology and bioinformatical treatment of the data (Lee et al., 2012; Shokralla et al., 2012). Especially the de-noising of the data and therefore the analysis of rare biosphere-diversity are still challenging (Lee et al., 2012) and the fundamental importance of the used primer set was revealed only recently (Klindworth et al., 2012). The datasets created by sequencing are vast and allow the analysis of many different aspects. The focus of the analysis depends to a large degree on the aim and the special interest of the authors of the respective study. These studies, although producing very valuable and unique insights, can rarely smoothly be compared to each other. This is an important aspect, if several similar experiments are carried out over the course of several years to discriminate between regional and general effects of ocean acidification (see also answer to referee #1). Therefore, it is our strong opinion that the advanced fingerprinting approaches like ARISA are still essential to study samples of unknown diversity.

We will revise the English again, as the referee suggests.

References

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M. and Glöckner, F.O.: Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Research*, 1-11, 2012

Lee, C.K., Herbold, C.W., Polson, S.W., Wommack, K.E., Williamson, S.J., McDonald, I.R. and Cary, S.C.: Groundtruthing Next-Gen Sequencing for Microbial Ecology-Biases and Errors in Community Structure Estimates from PCR Amplicon Pyrosequencing. *PLOS ONE*, 7, 9, 1-12, 2012

Shokalla, S., Spall, J.L., Gibson, J.F. and Hajibabaei, M.: Next-generation sequencing technologies for environmental DNA research. *Molecular Ecology*, 21, 1794-1805, 2012