

Dear editor,

We thank the anonymous referees for their thorough comments, which helped improve our manuscript. Please find below our responses carefully addressed to each comment and following the sequence: (i) comment from the referee (in italic), (ii) authors response, (iii) modification made to the manuscript.

## Responses to anonymous referee #2

Comment received and published: 23 October 2014

### General comments

#### **Comment #1:**

*(i) PPS3/3 and gel-cups were not simultaneously deployed. This situation might be a problem because the authors made comparisons based on similar nutrient and plankton scenarios, which is not necessarily true.*

(ii) We are aware of the potential biases that a high patchiness in phytoplankton and zooplankton distributions can cause to the evaluation of particle fluxes collected in gel and standard free-drifting sediment traps. Our Table 1 shows that only one station (E-3), was sampled by a non-synchronised trap deployment. At all other stations, the deployments were either single deployments of gel or PPS3/3 traps (R-2 and F-L), deployments overlapping in time and space (E-1 and E-5) or combined deployments of gel and PPS3/3 traps (A3-2).

At each station, our decision to combine or not the deployments was first constrained by the logistic, but also by the required different durations of deployment for each kind of trap. The PPS3/3 traps, were deployed over an average of 3.5 days to provide sufficient material for the bulk chemical analyses, while the gel traps were deployed over an average of 1.2 days to avoid an overloading of the gels (p 13631, line 6), which would have complicated considerably the image analysis and decrease its accuracy. Most of our separate gel and PPS3/3 trap deployments were in the same temporal and spatial field and likely collected particle fields originating from similar nutrient and plankton ecosystem structures.

Moreover, the core of the informations extracted from the gel or the PPS3/3 were of different nature: qualitative in the case of the gel collection and quantitative in the case of PPS3/3 traps as noted p. 13628 line 20 to p. 13629 line 3. We compared POC fluxes from gel and PPS3/3 traps in the perspective of a method comparison rather than to infer our main conclusions in term of relative importance of the different category of particles in carbon export flux.

Actually, the fluxes collected by the gel and PPS3/3 at every station compared relatively well, considering how far these methods are. This good match tends to suggest that our gel and PPS3/3 traps effectively collected similar flux episodes.

(iii) As also requested by referee#1, we added a sentence p. 13631 line 9 to explain our deployment strategies: "Due to different required deployment duration (shorter for gel traps to avoid overloading, see above), each category of trap was deployed on separate arrays, except at A3-2 (combined deployment; Table 1). All separated deployments of gel and PPS3/3 traps overlapped in time and location (except at station E-3 where they were successive), to maximize the collection of similar particle fields. The arrays had broadly..."

## Comment #2:

(i) *The authors mention that the average trap drift speed was  $8.5 \text{ cm s}^{-1}$  and I wonder whether the authors can provide with consistent evidence and information that this situation did not affect significantly the trap collection efficiency. In the study area the current speed along the slope and shelf break can be significantly higher.*

(ii) - (iii) In p. 13631 lines 16-18, we wrote: "The average trap drift speed of  $8.5 \pm 5 \text{ cm s}^{-1}$  was in the range of horizontal velocities determined by drogued drifter trajectories (Zhou et al., 2014). This is a strong evidence of a limited perturbation due to currents.

Moreover, the limited tilts recorded by the inclinometer (p. 13631 lines 18-20), attest that particle collection efficiency was not reduced by the current speed. This information tends to demonstrate that even in higher current areas that the traps could have potentially met, they always kept a satisfying semi-vertical position ensuring an efficient collection of the sinking flux at a given location and time.

An additional indication is given by the aspect and repartition of the particles embedded in the polyacrylamide gels (fig. 3 and 5): no evidence was found of major particle deformation, heterogeneous collection or gel flow which would have testified of perturbation due to hydrodynamic conditions.

## Comment #3:

(i) *Fecal and phytodetrital aggregates were distinguished because fecal matter was more dense and compact and phytodetritus loose and green, which seems to be a rather subjective criteria. Some observations using both light and electron microscopy probably would help to dissipate doubts?. The authors isolated fecal aggregates manually to conduct some tests, but from the text it is not clear enough which kind of test you did to be sure that fecal and phytodetritus-aggregates were correctly classed.*

(ii) Sorry for the confusion. This section needs to be rewritten more clearly to reflect accurately the different steps of our image analysis.

Polyacrylamide gels were studied exclusively using image analysis of light microscopy pictures. A method permitting electron microscopy on marine particles embedded in polyacrylamide gels is unfortunately not yet developed.

All the fecal material, including fecal aggregates and fecal pellets together (not mentioned in the MS and probably source of the confusion), has been isolated manually from the "total" images (displaying all the particles). "Manually" means that fecal particles were isolated with an Image J selection tool based on the assumption that fecal matter is brown and denser as noted p. 13633 lines 16-17 and showed on figure 3.

Two sets of images have been obtained: (1) the images containing only the fecal matter (fecal aggregates + fecal pellets), and (2), the images containing all remaining particles composed mainly of phytodetrital aggregates. Using a combination of shape parameters each category of particle wanted for the flux analysis (fecal aggregates, cylindrical fecal pellets, phytodetrital aggregates) have been selected automatically in these two sets of images through Matlab routines. The objective was to isolate manually particles impossible to separate automatically due to very similar shapes (fecal and phytodetrital aggregates) and group particles easy to separate automatically (fecal aggregates can be easily separated from fecal pellets due to their very different shapes).

(iii) p. 13633 lines 14-20, we rewrote this paragraph: "Because fecal and phytodetrital aggregates had similar complex shapes, automated routines could not separate these particles efficiently. All fecal material was thus isolated manually from all other particles based on the assumption that fecal matter is brown and denser than biologically unprocessed phytoplankton (Ebersbach et al., 2011). From the resulting set of pictures, fecal aggregates were separated easily from cylindrical fecal pellets due to their very contrasted shapes. Tests on the efficiency of our automated selection, conducted on a large sample, showed that ..."

#### Comment #4:

(i) *The high phytodetritus coincide with high abundance of diatoms (as indicate by the biogenic-silica concentration), but no comments on the physiological state of the diatoms we included in the text. Did you notice that diatoms or the other functional groups of the phytoplankton were in relatively bad physiological condition? It would be interesting to have some comments on this situation that have been also reported for upwelling areas (Progress in Oceanography 83: 217-227; 2009).*

(ii)

- PDMPO labelling (Shimizu et al., 2001) on diatoms has been conducted during KEOPS2 (K. Leblanc, MIO, Marseille, France). This fluorescent probe marks the silica actively deposited and thus can be used as an indicator of cell physiological state (Leblanc et al., 2005). The definitive results are not yet available but preliminary analyses show that diatoms at all sites over the Kerguelen plateau showed in general a good level of marking by the PDMPO, suggesting an active silicification during KEOPS2.

- M. Lasbleiz (personal communication, 2014) found a relatively high proportion of alive compared to empty diatom cells, even at very low PAR levels, which tends to suggest a good physiological state at this period of the season: R-2 (116 m, 0.3% PAR): 81%; E-1 (80 m, 0.3% PAR): 70%; E-3 (137 m, 0.01% PAR): 41%; E-5 (110 m, 0.01% PAR): 45%; F-L (52 m, 0.01% PAR): 92%; A3-2 (151 m, 0% PAR): 78%.

Microplankton other than diatoms showed even higher proportions of alive cells: R-2 (116 m, 0.3% PAR): 90%; E-1 (80 m, 0.3% PAR): 85%; E-3 (137 m, 0.01% PAR): 85%; E-5 (110 m, 0.01% PAR): 94%; F-L (52 m, 0.01% PAR): 87%; A3-2 (151 m, 0% PAR): 96%.

These data are preliminary results and cannot be published here.

- Moreover, the generally good physiological state of phytoplankton observed during the early bloom conditions of KEOPS2 as suggested by these results is consistent with the observed limited depletion of nitrates (Dehairs et al., 2014), and silicic acid (Closset et al., 2014).

The present study can be compared with the situation observed by (González et al., 2009), in the Humboldt Current System off Chile. González et al. (2009), suggested that the proportion of carbon exported through euphausiids fecal pellets vs diatoms depends upon the physiological state of phytoplankton. They also suggested that the carbon export mode (i.e. fecal pellets vs. phytoplankton detritus) is related to "the proportion of carbon that effectively sinks (...) compared to the carbon being fixed through GPP" (i.e. carbon export efficiency). A low export efficiency being associated with a diatom-controlled export mode (e.g. average of 32% of sinking organic matter composed of diatoms).

This relationship between the physiological state of phytoplankton and carbon export mode cannot be verified here since, as noted above, most of the phytoplankton presented good physiological states associated to the early-stage of the bloom (limited nutrient exhaustion). Rather, we explain the variations in carbon export modes by a spatio-temporal structuration of plankton communities controlling species-dominance status and trophic interactions.

(iii) We added the following sentences to refer to the work from González et al. (2009):

- p. 13645, lines 3-5: "While this negative relationship has been observed now in several field studies in the Southern Ocean (Savoye et al., 2008; Morris et al., 2007; Jacquet et al., 2011), and elsewhere (e.g. González et al., 2009), the reasons for its existence remain unclear."

- p. 13645, line 8: "..., or increase in DOC export. Phytoplankton physiological state has also been suggested as a possible control of carbon export mode and efficiency (González et al., 2009), although this could not be verified here due to a general good phytoplankton physiological state observed via microscopy over the course of the KEOPS2 study (M. Lasbleiz and K. Leblanc, personal communication, 2014)."

#### Comment #4 (suite):

(i) *Laurenceau et al. suggest that high export efficiency could be mediated by fast-sinking aggregates of heavy silicified, grazing-resistant diatoms. It would be interesting whether the authors make some statements on the relevance of diatoms as triggers of high export efficiency. In other words the relationship between chain-forming diatoms and the efficiency of the biological pump in marine systems.*

(ii) We acknowledge the importance of diatoms in export efficiency and especially the influence of diatom morphology in carbon export efficiency. In this context, we refer to the work conducted by Laurenceau-Cornec et al. (in press, 2014) on the influence of phytoplankton morphology on the sinking velocity of marine snow formed from natural phytoplankton assemblages sampled in the KEOPS2 bloom. This study suggests that the proportion of large chain-forming diatoms without setae vs small setae-forming diatoms strongly influence marine snow structure and consequently sinking velocity and likely export efficiency.

(iii) p. 13646, line 21: we added the following sentence: "... via a control on their structure and excess density (Laurenceau-Cornec et al., in press, 2014). Laurenceau-Cornec et al. (2014) found a strong relationship ( $r^2 = 0.98$ ) between the proportion of small spine-forming diatom cells included in marine snow aggregates (e.g. *Chaetoceros* subgenus *Hyalochaete*), and their sinking velocity, suggesting an important role for phytoplankton morphology on export efficiency."

#### Comment #5:

(i) *The authors speculate that the increase of phytodetrital and fecal aggregates observed at depth in some stations (E-3) could reflect an earlier production event. An analysis of the phytoplankton composition would give insight on this aspect. Whether the composition did not change significantly I did not see reasons to exclude E-3 from Fig. 6.*

(ii) We think a mistake was made here since we did not exclude station E-3 from figure 6. We assume that this comment refers mainly to p. 13642 lines 22-24 but some clarifications are needed. Does reviewer #2 refers to "phytoplankton composition" in term of phytodetritus chemical compounds as possible signature of organic matter degradation state (expected higher in case of an older production event), or in term of phytoplankton community as potential source tracer of the particle field collected in traps?

When writing "...exclude E-3 from Fig. 6" did the reviewer #2 referred actually to the Figure 9 caption (p. 13673): "E-3 was assumed an outlier and was excluded from the best fit calculation..."?

If suggesting an analysis of phytoplankton community or chemical compounds as a tracer of an earlier production event collected in the traps, our data need to be cross-checked with other KEOPS2 data. Unfortunately, PPS3/3 trap results cannot be used here since they provide a bulk chemical composition including all sinking organic and inorganic matter originating from auto- and heterotroph organisms. Similarly, particles collected in gel trap were only analysed in term of particle category statistics and morphologies. A determination of phytoplankton communities (genera and species if possible), collected in the gels, in the form of single or aggregated particles, or even their chemical analysis, are planned but these data are not yet available.

Assuming a steady state and a sinking velocity of the phytodetrital aggregates formed at E-3, of  $\sim 150 \text{ m day}^{-1}$  (based on measurements reported by Laurenceau-Cornec et al., in presse, 2014), a particle field would need  $\sim 1.5$  days to sink from 210 m (second shallowest trap) to 430 m (deepest trap), if neglecting any advection. This, combined with the short deployment time at E-3 (1.02 day), it is very unlikely that the deepest and shallowest traps recorded the similar production event.

(iii) p. 13642, line 22, we added: "... cup variations (Table 4). In addition, if assuming phytodetrital aggregates at E-3 sinking at an average velocity of  $150 \text{ m day}^{-1}$  (based on Laurenceau-Cornec et al., in press, 2014), a particle field would need approximately 1.5 day to sink from 210 m to 430 m, neglecting any advection. A non steady state assumption..."

#### Comment #6:

(i) *Large-size, rare fecal pellets or phyto-aggregates may have a disproportionate high impact in the results and final conclusions. For example, one large pellet could contribute with a large fraction of the total carbon exported and are sometime considered outlier and usually not included in the analysis. How was your criterion on this issue?*

(ii) (Same answer than to referee#1 - general comment#2) We did not exclude the large rare particles from our POC flux estimations. The large rare particles have been excluded only from our spectrum analyses which required a binning of the particles. As noted in page 13634, lines 25-28, the bins containing 5 or fewer particles were not included in the analysis for statistical reasons. All POC flux estimations, however, included all the particles except the very small unidentifiable particles potentially deriving from small gel imperfections (p 13633, lines 21-23). We apologise for this confusion due to a probably too unclear description of our image analysis method. We agree that not including these rare large particles could have led to a significant misestimation of the carbon flux.

(iii) Page 13634, line 26: we changed "... fewer particles were not included in the analysis, ..." to "... fewer particles were not included in the flux spectrum analyses, ...".

#### Comment #7:

(i) *During KEOPS2 a negative relationship between primary productivity and carbon export efficiency was found. So, where did the photosynthetically produced organic matter go in sites with high primary production?. Even though the processes that control export efficiency are beyond the scope of this contribution, this issue is highly relevant and I would ask the authors to provide more antecedents and insights on the possible effect of grazing or other biological/physical processes on the export mode and controls*

(ii) We agree that this question is extremely important. This is the reason why we allocated a large part (almost 40%) of our discussion (p. 13645 line 18 to p. 13648 line 4), to address it. However, acknowledging the high interest of this question and willing to address positively this comment, we put an additional paragraph in our discussion, to explore the possible pathways for the organic carbon produced at station A3 where a high primary productivity was associated with a low export efficiency. This paragraph is based on calculations using our results and results published by Christaki et al. (2014).

(iii) We made the following changes to this section:

- p. 13664, Table 6: the mesozooplankton biomass is now expressed in  $\text{mm}^3 \text{m}^{-3}$  to match with the unit used in Figure 9.

- p. 13646, line 8: the modification of zooplankton biomass unit from  $\text{ind m}^{-3}$  to  $\text{mm}^{-3} \text{m}^{-3}$  modified the ranking of A3-2. We change the sentence to: "The mesozooplankton biomass was high at A3-2".

- p. 13645, line 23: we removed the word "apparent".

- p. 13647 line 13, we modified this section and added a paragraph as follows:

"In the low productive systems (e.g. R-2), a direct export can be efficient if processed via fast-sinking aggregates composed of heavy silicified diatoms that are also assumed to be grazing-resistant. In contrast, in the sites of high productivity (e.g. A3-2 and F-L), the export flux can be strongly attenuated if a large fraction of the organic carbon flows toward paths promoting its retention in the surface layer (i.e. grazing, microbial remineralization and biomass accumulation). In the case of high grazing pressure, carbon export is driven mostly via fecal pellets, but these, even if sinking fast, potentially experience coprophagy or coprorhexy (Suzuki et al., 2003; Lampitt et al., 1990; Iversen and Poulsen, 2007), and disaggregation processes facilitating bacterial remineralization (Giering et al., 2014).

At A3, Christaki et al. (2014), proposed a carbon budget integrated over the mixed layer showing the carbon flows through microbial and higher trophic levels for early and late bloom stages. This budget indicates that during KEOPS2,  $2400 \text{ mg C m}^{-2} \text{ d}^{-1}$  were still available for phytoplankton biomass accumulation or export, after subtracting from the Gross Community Production (GCP), the different loss terms due to bacterial, other microplankton and mesozooplankton respiration and virus bacterial lysis. Using our carbon flux value at 200 m and phytodetrital aggregate contributions to this export, the relative fractions of the available

carbon actually used for biomass accumulation or export can be estimated here. The carbon flux at 200 m was  $66 \text{ mg C m}^{-2} \text{ d}^{-1}$  (gel trap results), with 41 % contributed by phytodetrital aggregates (Table 3). This leads to  $27 \text{ mg C m}^{-2} \text{ d}^{-1}$  exported (1.1% of the remaining available carbon) and  $2373 \text{ mg C m}^{-2} \text{ d}^{-1}$  used for biomass accumulation (98.9%).

The same calculations can be made for the late-bloom situation using the values of  $384 \text{ mg C m}^{-2} \text{ d}^{-1}$  for the carbon still available for biomass accumulation or export (Christaki et al., 2014), the KEOPS1 200 m POC flux at A3 ( $62 \text{ mg C m}^{-2} \text{ d}^{-1}$ ; Ebersbach and Trull, 2008) and a 36% aggregate contribution (including both phytodetrital and mixed aggregates; Ebersbach and Trull, 2008). Results lead to  $22 \text{ mg C m}^{-2} \text{ d}^{-1}$  exported (5.7%) and  $362 \text{ mg C m}^{-2} \text{ d}^{-1}$  used for biomass accumulation (94.3%).

These estimations show that the fraction of the carbon available that is exported, is subjected to the largest variations during the season (increased by a factor ~5); while the fraction allocated to biomass accumulation varied comparatively much less (decreased by a factor of ~1.05). It suggests that A3 progressed over the whole season from a retention-dominated to an export-dominated food web system (Wassmann, 1998), possibly related to successions of plankton communities prone to large variations of their export ability, as suggested in this study.

This general picture can be compared ..."

#### **Comment #7 (suite):**

(i) *Could you speculate on the reasons why microzooplankton did not show high numbers in trap samples nor in the water column?*

(ii) In this article, we did not present data on microzooplankton abundance, but made the following statement p. 13632, line 19-21: "A few zooplankton specimens were collected (less than 10 per gel), and were mostly represented by copepods (adult and copepodite stages), appendicularians, foraminifera and radiolarians". We apologise for the confusion, the terms "few" and "10 per gel" concerned the mesozooplankton only. We identified some specimens occasionally in high resolution observations conducted in parallel of our main survey. Due to their small size and generally uniform spherical shape, no automatic counting of these organisms was performed.

(iii) p. 13632, line 19-21, we changed this sentence: "A few mesozooplankton specimens were collected (less than 10 per gel), and were mostly represented by copepods (adult and copepodite stages), and appendicularians. Foraminifera and radiolarians were also occasionally observed."

## REFERENCES

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