

Dear editor

Thank you for handling our paper

please find below our answers to the reviewer's comments. All the comments were taken into consideration in the revised version of the ms. All the changes made are highlighted in red in the ms to facilitate reading.

Best regards

Urania Christaki

In the Anonymous Referee #1

We thank the reviewer for the positive comments and for accepting our paper.

Anonymous Referee #2

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Protist community composition during early phytoplankton blooms in the naturally ironfertilized

Kerguelen area (Southern Ocean)

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Review

This manuscript consists a very interesting – though quite descriptive - study, which adds up to current knowledge and which is very well written and as concise as it can be. I have minor comments and suggestions for further amelioration of the manuscript, as well as points that I would like them to be clarified.

We thank the reviewer for the time spent on the paper and for all the constructive comments and suggestions. All the comments were addressed and the responses are highlighted in red. All changes in the revised version of the ms have been highlighted in colour to facilitate reading.

Keywords

Line 37: While 18S rRNA and tag pyrosequencing do not need further explanation and facilitate only keyword searches, terms such as “planktonic protists” and “Southern Ocean” are briefly explained in the text, since they provide the highlights and the significance of the study. I would encourage the authors also to explain briefly the “natural iron fertilization” keyword, not only for consistency but mainly because it is the driving force of their study.

We added in L74: “Natural iron-fertilization is an uncommon process in which iron supply of the surface waters from iron-rich deep water is observed. Only two studies referred to natural iron fertilization in the vicinity of Crozet (Pollard et al., 2009) and Kerguelen Islands (Blain et al., 2007). The KEOPS 1 cruise demonstrated that the phytoplankton bloom was sustained by iron supply from iron-rich deep water below, representing natural iron fertilization (Blain et al., 2007). This study also showed that microzooplankton grazing was an important factor for phytoplankton biomass decrease in the bloom area (Brussaard et al., 2008) mainly affecting the small sized phytoplankton population (Brussaard et al., 2008; Christaki et al., 2008).”

Materials and Methods

2.1 Sample collection and DNA extraction

Line 106: The authors state that they pooled all three 47 mm diameter filters in the starting tube of the Power Water kit. Though these tubes are quite larger than e.g. the Power Soil ones, they are still smaller than 15 ml falcon tubes. My personal experience is that it is highly unlikely that all three filters were efficiently beaten. Losses might have occurred from this approach to the extraction and contributed to some of the discrepancies that are speculated later in the manuscript. My suggestion for the future would be to extract separately from each filter and elute them altogether.

The filters were cut in small pieces in the tube before following the extraction protocol, in order to maximize the extraction efficiency. We added in L111: “After pooling together and cutting into small pieces the 10, 3, and 0.6 μ m filters, DNA extractions were carried out...” We also added in L275 of the discussion: “Although the tag pyrosequencing of the 18S rRNA gene has become a routine method in marine microbial diversity studies, it is itself subjected to several limitations, including, DNA extraction and PCR-related biases...”

2.3 Quality filtering and taxonomic affiliations of the sequences

Lines 140-150: In Line 141, the authors state that the dataset provided a representative overview of the diversity based on rarefaction curves that reached a plateau in most of the cases. About 1,000 sequences for R_300m indicate a very undersampled station. However, what concerns me the most is whether these rarefaction curves were generated before or after the removal of the metazoan OTUs, since the adequacy of the rarefaction curves statement precedes the one about the metazoan OTUs removal and the downstream analysis (L145). In any case, the rarefaction curves should reflect the final dataset. Furthermore, I would like to see a Table with the number of the reads before and after the removal for each library, or the number of reads (sequences) in Table 2.

Indeed R_300 produced a low number of sequences. This could be attributed to possible limitations of tag pyrosequencing and DNA extraction efficiency for certain taxonomic groups, as we discuss in L274-276, L288-293.

In the submitted paper, the rarefaction curves were generated before removing all the metazoan OTUs and single singletons. In the revised ms, the rarefaction curves were recalculated with the final dataset excluding metazoan OTUs and single singletons, as the reviewer suggests.

Moreover the number of sequences before and after removing the metazoan reads and the single singletons are now presented in table 2.

Table 2. Number of OTUs, the richness estimator (S_{chao1}), Simpson and Berger-Parker indices for each sample. Nb of seqs before and after removing metazoan and single singletons sequences

Station	Depths (m)	Nb OTUs	Nb seqs before	Nb seqs after	S_{chao1}	Simpson (1-D)	Berger-Parker
R-2	20	157	5448	4714	198	0.95	0.18
	60	170	6346	1522	218	0.95	0.16
	150	233	4407	1562	390	0.97	0.13
	300	282	1098	950	409	0.99	0.05
F-L	20	186	5586	3028	253	0.76	0.48

	65	508	7305	5730	663	0.98	0.08
	180	265	7818	905	382	0.98	0.05
	300	284	10205	2026	383	0.83	0.40
E-4W	30	173	7151	6108	198	0.85	0.33
	80	209	10977	6674	236	0.92	0.23
	150	191	11989	5771	255	0.94	0.19
	300	97	3178	242	174	0.97	0.08
A3-2	20	215	10666	1803	285	0.93	0.22
	80	200	3866	2118	273	0.98	0.08
	160	181	5986	2022	219	0.95	0.13
	300	330	11590	5662	385	0.94	0.23

Results

3.2 Composition and distribution of protistan assemblages

Lines 185-188: The numbers that the authors report on the NbOTUs/Schao1 ratio and the number of OTUs here do not correspond to Table 2, whether they refer to the sum of OTUs in each station (all depths) or to a specific depth in each of them. Please correct if there is any discrepancy.

The value of NbOTUs/Schao1 ratio reported in the text corresponds to the mean value calculated for all depths and stations. To clarify this we added in L190: “The mean ratio of observed (Table 2) to expected (S_{chao1} , Table 2) OTUs was 75 ± 10 % (mean \pm sd) for all depths and stations.”

Concerning the numbers of OTUs cited in the text, they refer to the sum of OTUs at all depths in each station, including the common OTUs among the different depths. Thus, we added in L192: “The highest number of unique OTUs, considering all depths, was observed...”

3.2.1 High level taxonomic groups

Last Line: This is a more general comment. Station F-L seems to stand out for all metrics (e.g. highest temperature and Chla) and patterns (e.g. Radiolaria very high relative abundance at 300m, but also broader distribution along different depths). What is the significance of this station from an ecological perspective, to be at the Northern side of the Polar Front compared to all other stations? Same in line 239, a much different station.

The F-L station was included in this study because she presented the opportunity to study fertilized waters originating from the North of the Kerguelen Islands. The fertilization process remained the same and constitute the common feature between all the fertilized stations.

Discussion

4.1.1 Phytoplankton

Line 274: Has this survey (Armand et al. 2008) also taken place during blooms? Please clarify to emphasize the relevance.

This study has indeed taken place during KEOPS 1 cruise at the end of the bloom period. To clarify this we added in L279: “...with the 52 diatom taxa morphologically identified in the

Kerguelen area during the KEOPS 1 cruise at the end of the bloom period (Armand et al., 2008).”

Line 282: Were the Fragilariopsis-related OTUs also counted other than observed? Could it also be that they did not consist a considerable fraction that would overcome the overall 1% PCR limitation?

Yes, and they were found to be the four most dominant morphotypes by Sackett *et al.* We changed the text in L285 for more accuracy into: “In particular, *Fragilariopsis kerguelensis*, *Pseudonitzschia* spp., *Eucampia antarctica*, and *Chaetoceros* spp. were found to be the four dominant diatom taxa, via microscopy (Sackett et al., this volume).” Since they were abundant, we offer some other explanations for this in L288-293.

Lines 286-294: My question about the retrieval of unaffiliated sequences in Figures 3 and 4 becomes more relevant here. Please see comments on these figures. Also, could the authors please report the average length of their final high quality sequences?

For the unaffiliated sequences please see response to the following comments.

The average length of the final high quality sequences were added in L190:

“After quality filtering and normalization, 999 unique OTUs, clustering 50 674 sequences (average length: 240 bp), were revealed for the 16 samples.”

Figures 3,4: Was taxonomy assigned to all sequences and at which confidence level?

All the sequences were affiliated at least to one taxon (Table 3) and the average of all the sequence affiliation was 95 ± 3.8 % (mean \pm sd). As the reviewer can see, in some cases the affiliation was to a higher-level taxon (such as Dino-Group I, MAST-3 etc), while in other cases sequences were affiliated to the species level.

It is striking to me that unaffiliated sequences were not retrieved at all. Has taxonomy been subsequently assigned “manually” and on what was it based if so.

All the affiliations presented in the heat-map table were verified manually with both NCBI and PR2. For the rare exceptions where NCBI and PR2 were not in accordance, we kept the PR2 affiliation. To note that the PR2 is a curated database, which doesn’t include unaffiliated sequences. So, all our sequences were annotated to a taxon, whether it was a high-level taxon, or to the species level.

Figure 6: I do not see the asterisks (a) and the dashed lines (b) either.

Asterisks and dashed lines have been now added where appropriate.

Interactive comment on Biogeosciences Discuss., 11, 11179, 2014.