13 Nov. 2015

Dear Editor and Reviewers,

Thank you very much for reviewing our manuscript. We have tired our best to revise the manuscript carefully in line with the comments made by the reviewers.
Our responses to each point suggested by the reviewers are as follows:

Anonymous Referee #1
The manuscript by Jing et al focuses on the diversity of protist communities in the Costa Rica OMZ using pyrosequencing of 18S rRNA and rRNA genes. This work should be of interest to readers of biogeosciences because protists are important, yet understudied members of microbial ecosystems in OMZs. The authors present some interesting data, but the manuscript is very descriptive and the authors should do a better job of searching for the novel findings and re-focusing the manuscript on what the more interesting results mean. This could be achieved by a closer comparison to what has been previously reported for protists in other anoxic and low oxygen habitats. Furthermore, the authors should be a bit more specific about which specific groups they found and how their distributions compare to existing literature. For example, they authors state that they found ciliates but do not go into detail about which genera they found. Ciliates are highly diverse (estimated to contain _4000 species) and so grouping at this level provides for only superficial analysis and discussion. Same applies for dinoflagellates and stramenopiles, for example did you find any MAST stramenopiles or MALV clades? If so which clades and how do they compare to what was found in other OMZs or anoxic waters? I recommend also for the manuscript to be carefully read and edited by an english native speaking colleague, because there are many grammatical and spelling mistakes. Specific comments are below.
Response: We did find Syndiniales from the parasitic dinoflagellates, Stramenopiles and Ciliates in the suboxic and anoxic waters in our study. The revised paper focuses on these major groups and makes a comparison with results in previous related studies, especially for ciliates (Edgcomb et al., 2011 ISME; Orsi et al. 2011 ISME), stramnopiles (Orsi et al., 2011 ISME; Wylezich and Jurgen, 2011 EM) and Syndiniales (Guillou et al., 2009 EM) from anoxic/low oxygen habitats. Related discussion was added accordingly.
We apologize for the rough presentation in the previous submission. Due to a miscommunication, we did not upload the latest (edited) version of the paper and were unable to stop the review process once initiated. We have tried to address carefully all of the points suggested by the reviewer.

Specific comments:
Title: The title as written is grammatically incorrect and should be re-written. Do you Mean "Diversity of protist communities…" ?
Response: Title was modified to "Diversity of protist communities in a marine oxygen minimum zone off Costa Rica".

Abstract: lines 14-16: As written these conclusions are very general and non-specific and do

not focus on the novel aspects of the study (e.g., activity proxies with rRNA).
Please re-write the conclusions to focus on the novel results and conclusions.
Response: This part was re-written as "Reduced community diversity and enhanced parasitic/symbiotic lifestyle in the OMZ, especially in the anoxic core, suggest a strong selective pressure on protistan communities and trophic roles, with implications for altered food web interactions and associated biogeochemical cycling. Our study highlights the advantages of integrated rRNA- and rDNA-based approaches for distinguishing taxonomic presence versus resident active taxa in comprehensive community studies of ocean vertical structure and environmental gradients".

Page 13485, line 5; Page 13494, line 16, and Page 13500, line18: Correct authors name from "Oris" to "Orsi".
Response: All were changed. Sorry for the mistake.

Page 13485: line 23: Typo "picoeuaryotes"
Response: Corrected.

Page 13486: line 14: Is this really the perfect place? Why do you say perfect? There are many OMZs with similar chemical stratification, and some (Arabian Sea) with even thicker OMZ core (1000 m).
Response: This sentence was modified as "OMZs with upper and lower oxic boundary layers around a thick anoxic core, is an ideal location to investigate protistan community composition and function relative to DO variability".

Page 13487: lines 19-23: If you removed the RNA later then you through away a lot of the RNA that is lysed into solution from cells on the filters. This should be acknowledged, and some discussion of how this might have affected the authors results would be good.
Response: Residual RNAlater would inhibit the dissociation of nucleoprotein complexes and subsequently reduce the generation of the RNA. RNAlater were removed by centrifuge with equal volume ice-cold PBS added to reduce the density of the solution. Cells became less fragile when stored at RNAlater and can be centrifuged without lysis. Previous study has shown that the effect of RNAlater preservation is similar to that of freezing for RNA quantification and expression (Mutter et al. Comparative of frozen and RNAlater solid tissue storage methods for use in RNA expression microarrays. BMC Genomics, 2004, 5:88.)

Page 13487: lines 24-26: Please state manufacturer for DNase I and briefly the protocol followed.
Response: Information was added as "DNase I (Invitrogen, Carlsbad, CA) incubating at room temperature for 15 min to eliminate potential DNA contamination. DNase I was inactivated by heating at 65 °C for 10 min with EDTA solution".

Page 13488: line 1-5: Did you ever see amplification from DNA in these RNA controls?
If so how did you proceed?

Response: No, as long as the DNase I digestion was processed carefully according to manufacturer's instruction. If there were PCR products generated from the RNA control, another aliquot of RNA sample was treated with DNase I and the synthesized cDNA was used for PCR again with RNA control.

Page 13489: line 9: Please be more specific what is meant by cut off value of 60. What percent id is this ?
Response: Identity of each OTU was determined through Blast. If the similarity was less than 60% at any taxonomic level, we treated the OTU as unclassified at the higher taxonomic level. That is why the classification of some OTUs could not be taken down to the genus level as the reviewers suggested.

13490: line 12: What do you mean by "remarkably higher"? How much higher? You cite the table but would be good to state in the text for readers.
Response: Values were added into the sentence, and now it reads "Richness (Chao1) and diversity (Shannon) indices for the protistan communities were substantially higher for rRNA sequences (Chao1 (2159-4780), Shannon (5.68-6.85)) than those from the rDNA analyses (Chao1 (529-2461), Shannon (4.13-5.62)) (Table 1)".

Page 13490: line 16: A lower protistan diversity in the anoxic water column was also found in the Cariaco Basin (Orsi et al 2011 ISME J).
Response: This reference was added in the discussion.

Page 13490: line 18: What do this coverage values refer to? Is this percent of total sequences? Maybe it is better to report statistics for number of reads corresponding to each OTU?
Response: Coverage values reflect whether the sampling efforts were sufficient to show the real diversity of the sample (Coverage=1-(# of singleton OTUs/number of sequences)). The abundance of OTUs varied substantially, and the distributions of the most abundant 30 OTUs were indicated in Fig. 5a (rRNA) and Fig. 5b (rDNA) at the end of this response.

Page 13490: lines 19-24: I am confused what this sentence is trying to communicate. Please clarify.
Response: This part has been modified as, "The highest coverage was obtained from the core (550 m) of the OMZ (Table 1). These results are consistent with the patterns in the rarefaction curves for rRNA (Fig. 2a) and rDNA (Fig. 2b), which showed that the curve for the 550-m sample flattened earlier compared to those from other depths, and indicated that the rRNA analyses generally required higher sampling effort to assess community composition adequately".

Page 13492: line 6: Did you find diatom rRNA in the OMZ core? If so that would be interesting to report. Edgcomb et al 2011 (ISME J) also found this in the Cariaco Basin and, while controversial, could indicate survival of diatoms in low oxygen waters without sunlight.
Response: Yes, we found Coscinodiscophycea with low abundance in the core of the OMZ in the rRNA level. Coscinodiscophycea are very large centric diatoms that sinking fast. It is

very likely that the existence of this diatom in the core of the OMZ is from the sinking particles.

Page 13492: lines 15-18: Please also comment on whether you found any anaerobic ciliates, and if so which groups you found. For example, did you find any representatives of the Cariacotrichea (Orsi et al., 2011 IJSEM) ? This group has been proposed as an indicator species for low latitude OMZs (Orsi et al., 2012 ISME J) and thus it would be interesting to know if it is also found in the OMZ studied here, or whether it is more restricted to euxinic waters.

Response: Yes, we found seven groups of Ciliphora, and all of those groups were detected from the core of the OMZ at the rRNA level, suggesting that they are anaerobic Cilitates and metabolically active under anoxic waters. All of those groups have been reported previously from anoxic waters (Orsi et al., 2012; Edgcomb and Pachiadaki, 2014).
As for Cariacotrichea, no representatives were found in our samples. However, when we double checked the reference sequences for Blast, there were no Cariacotrichea reference sequences in the NCBI 18S nt database, so we could not exclude their possible existence in our samples

Page 13493: line 6: Define what you mean by "high numbers".
Response: The sentence was modified as "In the rRNA reads, dinoflagellates account for about 80% at 2 and 200 m".

Page 13493: line 8-10: Please specify which groups of ciliates you found (see above comment). This has important implications for the community structure and biogeog-raphy debates, so the discussion would benefit from this a lot.
Response: ***We found seven groups of Ciliphora: Spirotrichea, Litostomatea, Colpodea, Phyllopharyngea, Nassophorea, Prostomatea, Oligphymenophorea. All of those groups were detected from the core of the OMZ in the rRNA level, suggesting that they are either anaerobic Ciliates or could tolerate very low DO and were metabolically active under anoxic conditions. All of those groups have been reported previously from anoxic waters (Orsi et al., 2012; Edgcomb and Pachiadaki, 2014). Related discussion was included in the text as suggested.***

Page 13493: lines 10-11: If you are referring to group I, II, III of Syndiniales please specify that.
Response: Yes, we are referring to Syndiniales group I, II, III, which are parasitic dinoflagellates (Guillou et al., 2009 EM). Syndiniales group I, II, III are now used in the revised text and figures.

Page 13494, line 9 and line 16: Actually the more correct reference for reduced protist diversity in low water column oxygen would be Orsi et al 2011 (ISME J), which validated the observed differences in richness with statistical estimates.
Response: This reference was added as suggested.

Page Page 13494: lines 17-20: To be more specific, I think is more likely due to the fact that the diversity of anaerobic protists is smaller compared to aerobic species (e.g. there are less species of anaerobes). You could read the papers by Wilhelm Foissner as background. For this reason, if you look in a low oxygen environment, diversity will be lower simply because there are less species in existence.

Response: This sentence was modified as suggested. Actually, we are talking about the same thing. As the old microbiological tenet says "Everything is everywhere, but the environment selects". Low-oxygen water is one of the inhospitable environments for many living organisms, which will selectively support certain anaerobic groups rather than the whole community, resulting in a reduced diversity. This is consistent with the comment "lower diversity is observed because fewer species survived in the low oxygen environment".

Page 13495: lines 11-12: You should also read Orsi et al 2011 (ISME J) which found an abundance of hypoxic stramenopiles in the Cariaco Basin, further supporting this point. Edgcomb et al 2011 (ISME J) also found rRNA from stramenopiles in the anoxic waters, but these were from diatoms. The manuscript would benefit from a discussion of these studies as this is highly relevant.

Response: The reference was added.

Page 13495: lines 12-13: How or why do you think this happening?

Response: Chrysophycea has been known to exhibit both phagocytosis and photosynthesis (Ishida and Kimura, 1986) and some endophytic bacteria have been found from Paraphysomonas and Chromophysomonas (Chrysophyceae) (Preisig ang Hibberd, 1984). In our study, Chrysophycea was confined to the core of the OMZ with low abundance, so they might be associated with sinking aggregates.

Page 13495: lines 19-21: Not all Prorocentrales and Spirotrichea have a mutual symbiotic association with Rhizaria. Please specify which genera you actually found and whether these genera have been shown to live as symbionts.

Response: Blast results show that the dominant species, such as Prorocentrum minimum and Strombidium are not symbiotic, so we have removed the sentence and modified this part.

Page 13495: line 28: You should also read Orsi et al 2013 (Frontiers in microbiology), which found a prevalence of symbiotic relationships between ciliates and bacteria across a geographically broad range of oxygen depleted marine waters.

Response: We read this reference and cited it in our discussion. Symbiotic relationships between prokaryotes and heterotrophic protists, including ciliates, rhizaria and flagellate taxa have been reported (Gast et al 2009, Trends in Microbiology). As for ciliates, they could well adapt to an anaerobic lifestyle due to the evolution of hydrogenosomes (Embley, 2006), and the prevalence of mutualistic symbiotic relationships between ciliates and prokaryotes has been reported from different oxygen-depleted marine waters (Orsi et al., 2012).

Page 13497: lines 24-29: This is an example of a redundant statement that appears multiple times throughout the manuscript. Please edit the text to remove redundancies.
Response: The sentence was simplified as "The reduced community diversity and presence of parasitic/symbiotic trophic lifestyles in the suboxic/anoxic waters of the CRD suggests that oxygen deficiency in the OMZ exerts a selective pressure on the microbial communities that modulates trophic processes and integrated biogeochemical cycles".

Page 13507: Figure 5: Can the authors comment on this apparent complete lack of a linear correlation between the rRNA and rDNA data?
Response: Protist community compositions from both rRNA and rDNA analyses were always plotted together in each figure, so the information conveyed in Figure 5 is redundant, consequently, we deleted this figure.

Figure 6: What do the z scores represent in lay means terms? The arrows point to two different heat blocks, so are the stacked histogram plots showing the average from both blocks? Can you break this up into separate stacked histograms for each block to look at differences between depths?
Response: Z score means (each value-mean of row)/standard dev. All values have been normalized to SD units through the formula z = (x - mean)/sd, to weigh them equally in the clustering. The colors of the heatmap are mapped linearly to the Z-scores, so a color can be interpreted as x standard deviations from the mean.
The arrows are now removed because they were misleading. The figure on the right side is the sum of the red blocks (most abundant groups) shown in each sample.

Anonymous Referee #2
This paper adresses the composition of microbial eukaryotes on a vertical profile in the Coasta Rica dome where there is a pronounced OMZ. The authors used 18S rRNA metabarcoding starting both from DNA and RNA. Unfortunately the 18S region they used is not the most resolutive (V4 or V9 would be more resolutive). The manuscript is fairly descriptive but still provides interesting data. I do not suggest publication at this stage. The paper should be revised in depth and then resubmitted. The major change needed include a better taxonomic treatment. In particular one of the major eukaryotic group (Syndiniales) is never mentioned. I doubt very much it was not present and abundant. Synurophyceae is typically a freshwater group but found there. It could well be present but this will have to be demonstrated. These problems are probably an artifact from the data analysis. All OTUS should assigned to the genus level as much as possible. Also the authors seem not to master fully eukaryotic phylogeny as evidenced by the numerous typos in the taxa as well as the incoherence on some of the level used. Some of the taxonomic assertions are plainly wrong such as the fact that all dinoflagelaltes are photosynthetic (see below). The introduction needs some rewriting as its fairly vague. I would focus much more on what is known about protists in low oxygen regions rather than be very general. In short, the authors should read much more what is known in the taxonomy and oceanographic literature about the taxa they mention and then rewrite the paper.

Response: The V2 region used in this manuscript was proposed to be one of variable regions of 18S rRNA suitable for assessing eukaryotic diversity (Hadziavdic et al., 2014 PLoS ONE 9:e87624). This primer set has been widely used previously and here is applied with pyroseuqncing, which usually has a sequence length restriction. In addition, the identities of different OTUs were further analyzed to be assigned to genus level. However, due to the limited taxonomic resolution of 18S rDNA and the similarity cutoff (60) we applied, some OTUs could not be identified in the genus level and were assigned to the lowest classification levels as possible with confidence.

Those parasitic dinoflagellate groups I, II and III detected from our study were actually Syndiniales, and the names in the text and figures were modified accordingly.

All of our sequences were analyzed by Mothur with the Protist Ribosomal Reference Database (PR2) in 2010, and we now re-analyzed the sequences by Blastn using NCBI nt database, and no Synurophyceae was found there. However, Synurophyceae and Chrysophyceae are closely related, and it is very possible that Synurophyceae was identified as Chrysophyceae in the original analysis. This discrepancy mainly reflects the difference between databases and we believe that our recent analysis with the updated database should be more reliable.

The introduction has been improved as suggested, and more comparisons with previous studies are discussed. The vertical profiles of all physical and oxygen parameters at the sampled station are plotted as Figure 1. Redundancy analysis (RDA) based on major protistan orders together with all abiotic data set as explanatory variables were conducted to find the major factors (Figure S1). Accordingly, we have tried to address carefully all the points suggested by the reviewer.

Specific remarks

p. 13484 l. 25. I do not think that the Bachy paper is the best reference to support this sentence. Instead he shows that diversity can be OVERestimated by molecular methods. "Until last decade" does not sound right to me

Response: This part was modified as "Most recently, next generation pyrosequencing technology has been used to detect both abundant and rare species (Edgcomb et al., 2011; Orsi et al., 2012; de Vargas et al., 2015), and has demonstrated a vast diversity up to several orders of magnitude higher than fingerprinting (Díez et al., 2001; Wylezich and Jürgens, 2011) and clone library (Edgcomb et al., 2011; Rocke et al., 2013) based assessments".

p.13485 l. 5. Cite de Vargas, C., Audic, S., Henry, N., Decelle, J., Mahe, F., Logares, R., Lara, E. et al. 2015. Eukaryotic plankton diversity in the sunlit ocean. Science. 348:1261605.

Response: This new science paper was cited as suggested.

l. 15. mixotrophic protists are not parasitic (I do not know of any parasitic protist that would be also photosynthetic and feeding on bacteria)

Response: Introduction was rewritten with more information about protists from suboxic and anoxic conditions as the reviewer suggested, and this sentence was deleted.

l. 21 "anoxic waters has caused significant changes"... does not sound right... "can cause" ?
Response: This sentence was modified as "As a special niche, anoxic waters can select for very different community compositions of protists (Wylezich and Jürgens, 2011; Orsi et al., 2012) and prokaryotes (Stewart et al., 2012; Kong et al., 2013).".

p. 13487 l.9. Please provide a supplementary figure with the vertical profiles of all physical and oxygen parameters at the sampled station.
Response: Those parameters were now plotted as Fig. 1 (attached at the end of the response).

p. 13488. l. 7. The region chosen for the metabarcoding is not the best choice for eukaryotes because it is much less variable than other regions such as the V4 and V9 used in almost all other studies.
Response: The primer sets (82F, 516R) targeting V2 region of the 18S rDNA have been widely used in DGGE and clone library studies previously, and recently they were applied with pyrosequencing (Monchy et al, Envicon Microbiol 2011; Monchy et al, PLoS ONE 2012 7(6)e39924; Georges et al, Biogeosciences 2014 11: 5847-5863), which has a sequence length restriction. In addition, recent characterization of the complete 18S rRNA gene (V1-V9) using all eukaryotic sequences present in the SILVA database suggested that V2 together with V4 and V9 were best suited for biodiversity assessment of eukaryotic diversity and yielded the highest taxonomic resolution (Hadziavdic et al., 2014 PLoS ONE 9:e87624. Characterization of the 18S rRNA gene for designing universal eukaryote specific primers).

p. 13489. l. 1 which 18S data sets? Be more precise.
Response: All the sequences were analyzed by Mothur with the Protist Ribosomal Reference Database (PR2). Now we use NCBI nt database.

l. 8. Why assignation was done vs. Silva bacteria while we are dealing with eukaryotes?
Response: All the sequences were analyzed by Mothur with the Protist Ribosomal Reference Database (PR2). Sorry for the mistake. Now we use NCBI nt database.

l. 13. Which R package?
Response: A heatmap plot depicting the abundance and distribution of OTUs among different samples was generated using the gplot package in R* (Gregory et al, 2015).
****Gregory R. Warnes, Ben Bolker, Lodewijk Bonebakker, Robert Gentleman, Wolfgang Huber Andy Liaw, Thomas Lumley, Martin Maechler, Arni Magnusson, Steffen Moeller, Marc Schwartz, Bill Venables (2015) gplots: Various R Programming Tools for Plotting Data. http://CRAN.R-project.org/package=gplots***

p. 13490, a lot of error in spellings. "Haptophyta", "Viridiplantae", "Dinophyceae". Please check all taxonomic names very carefully everywhere! Also only genus and species have to be in italics all other rank names are in normal font.

Response: All the spellings were corrected and italics of those higher rank names were corrected as suggested.

l. 3. "Phylum" (singular) and not "phylae"
Response: Corrected.

l. 25. Among the Dinophyceae you never mention the Syndiniales (what you call latter parasitic group I, II, III and which appears at the OTU level but not in the global assignation). This is often the most abundant group in eukaryotic metabarcodes sets. Please clarify this point.
Response: Those parasitic Dinoflagellates are Syndiniales (Guillou et al, 2008). The labels were changed in the figures and text accordingly.

p. 13492. l. 5 It is interesting that Eustigmatophyceae have been found because they are rarely appear in clone libraries or metabarcoding
Response: We re-analyzed our data using local blast in NCBI and this group does not show up in the local blast. They may have been improperly classified the first time using the Mothur alignment.

l. 13. Please give a more precise assignation of the dominant OTUs possibly down to the genus level.
Response: We re-analyzed our data using local blast in NCBI and assigned each OTU to the lowest classification level as possible, see Fig. S2 (rRNA) and S3 (rDNA). However, due to the limitation of pyrosequencing length and the similarity cut off value (60) applied for classification with confidence, some groups could not be classified down to genus level.

l. 16. Correct "Eumas..."
Response: Corrected.

p. 13493 l. 7. Not all dinoflagelaltes are photosynthetic even within orders that are mostly photosynthetic. Your treatment of the dinos must be much more thorough because at present it is very vague.
Response: We are referring those dinoflagellate dominated in the surface samples but not appeared in deeper water columns. They should include dinoflagellates with different trophic life styles, but we believe that photosynthetic groups should be the majority in the surface. This part was modified accordingly.

l. 11 The parasitic groups belong to order Syndiniales... see Guillou, L., Viprey, M., Chambouvet, A., Welsh, R.M., Kirkham, A.R., Massana, R., Scanlan, D.J. et al. 2008. Widespread occurrence and genetic diversity of marine parasitoids belonging to Syndiniales (Alveolata). Environ. Microbiol. 10:3349–65.
Response: The composition of parasitic Syndiniales was further analyzed and plotted (Fig. 7b) and this reference was discussed in the text.

l. 19. Provide references about mixotrophy. Which Prorocentrum and prasinophytes are mixotrophic???
Response: We didn't put it clearly, and this sentence is now modified as "At 550 m depth, the most abundant rDNA sequences are from prasinophytes and mixotrophic Prorocentrum, such as Prorocentrum minimum".

p. 13494. l.12-15. Leakage may also affect rRNA. This really depends on the life time of the rRNA molecule vs the DNA but in contrast to mRNA, rRNA can have a long life time and therefore be found in the leaked material through the filter.
Response: cDNA was synthesized through reverse transcription using mRNA as templates. mRNA is short lived, so studies using cDNA could avoid contamination from dead or lysed cells leaked through the filter. We used the terms of rRNA and rDNA just following other papers (Gremion et al 2003, EM).

l. 26. Dinoflagellates are the dominant group in barcodes studies see: de Vargas, C., Audic, S., Henry, N., Decelle, J., Mahe, F., Logares, R., Lara, E. et al. 2015. Eukaryotic plankton diversity in the sunlit ocean. Science. 348:1261605
Response: This new science paper was cited in the text.

p. 13495. l. 1. Not all Gymnodiniales are mixotrohic by far...
Response: 'mixotrophic' was removed.

l. 4. resting cysts would be expected to be found in the sediments not the water column.
Response: Yes, resting cysts were usually found in the sediments not the water columns. So this part was modified as "Gymnodiniales are generally surface-dwelling species, and their appearance in the deep waters could result from dead cells in surface-derived exported material".

l. 10. Synurophyceae are a freshwater group. Their presence in the OMZ should be demonstrated and discussed with reference to other work on this group;
Response: All of sequences were originally analyzed by Mothur with the Protist Ribosomal Reference Database (PR2) in 2010, and now re-analyzed using local blast in NCBI, and no Synurophyceae was found there. However, Synurophyceae and Chrysophyceae are closely related, and it is very possible that Synurophyceae was identified as Chrysophyceae in the original analysis. The discrepancy mainly reflects the difference between databases and we believe that our recent analysis with the updated database should be more reliable.

l. 21. Apicomplexa are a parasitic group usually not found in marine waters. Please detail exactly which Apicomplexa are found in this profile and discuss with respect to the literature.
Response: There are only one sequence in RNA blast result that is aligned to Aconoidasida (Apicomplexa), but the similarity is only 50%. Therefore, we will not mention it in the revised manuscript.

p. 13496. l. 16. What is the PX-Clade of Stramenopile ? Where found before? Discuss...

Response: The PX-clade of Stremenopiles was also treated as superclass Fucistia, including Chrysomerophyceae, Phaeophycea, Phaeothamniophyceae, Schizocladiophyceae and Xanthophyceae (Kai et al., 2008).

p. 13497 l. 15. As said before many dinos are not photosyntehtic so it is necessary to be much more detailed on what dinos exactly were found, go back to the literature and estimate what is their trophic behavior.
Response: Because those were dinoflagellates not belonging to the parasitic Syndiniales and were more abundant in the surface waters. We assume that they should be composed mainly photosynthetic species. This part was modified accordingly.

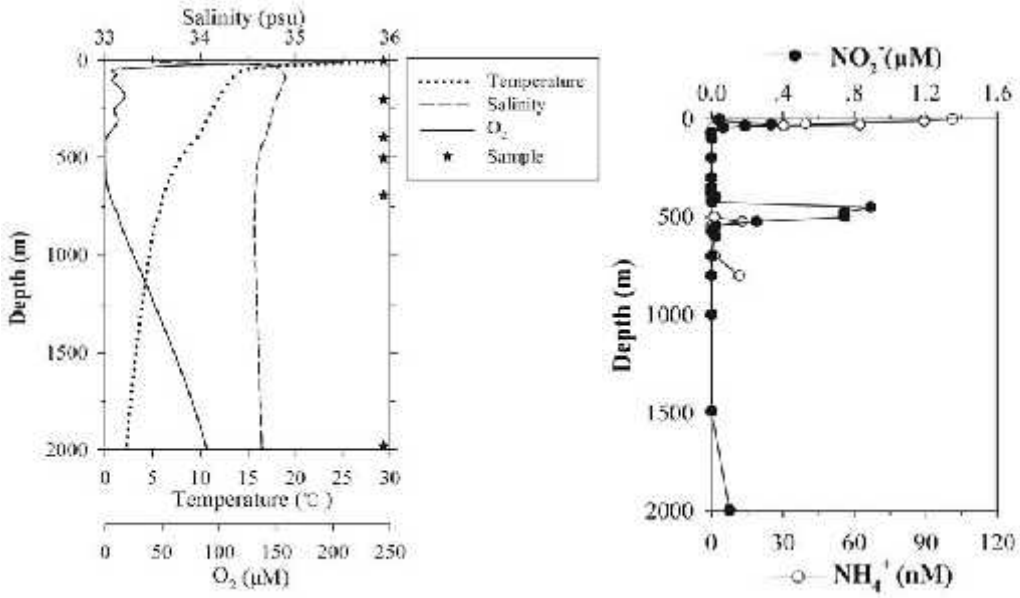
l. 20-30. Please shorten.
Response: This part has been modified as "All of the sampling depths were strikingly different from the surface water, which harbors a large amount of photosynthetic protists. The high percentages of active protists in the OMZ further demonstrate their importance as a major trophic link in the microbial loop under hypoxic conditions (Stoeck et al., 2007). The reduced community diversity and presence of parasitic/symbiotic trophic lifestyles in the suboxic/anoxic waters of the CRD suggests that oxygen deficiency in the OMZ exerts a selective pressure on the microbial communities that modulates trophic processes and integrated biogeochemical cycles".

Fig. 6. It is impossible to read the labels... Split into 2 subfigures with larger size.
Response: Figure 6 was re-plotted and replaced by heatmaps of the top 30 OTUs from both RNA and DNA analyses.

We sincerely thank the reviewers. We hope that the revised paper is significantly improved and that it could be acceptable for publication in Biogeosciences.

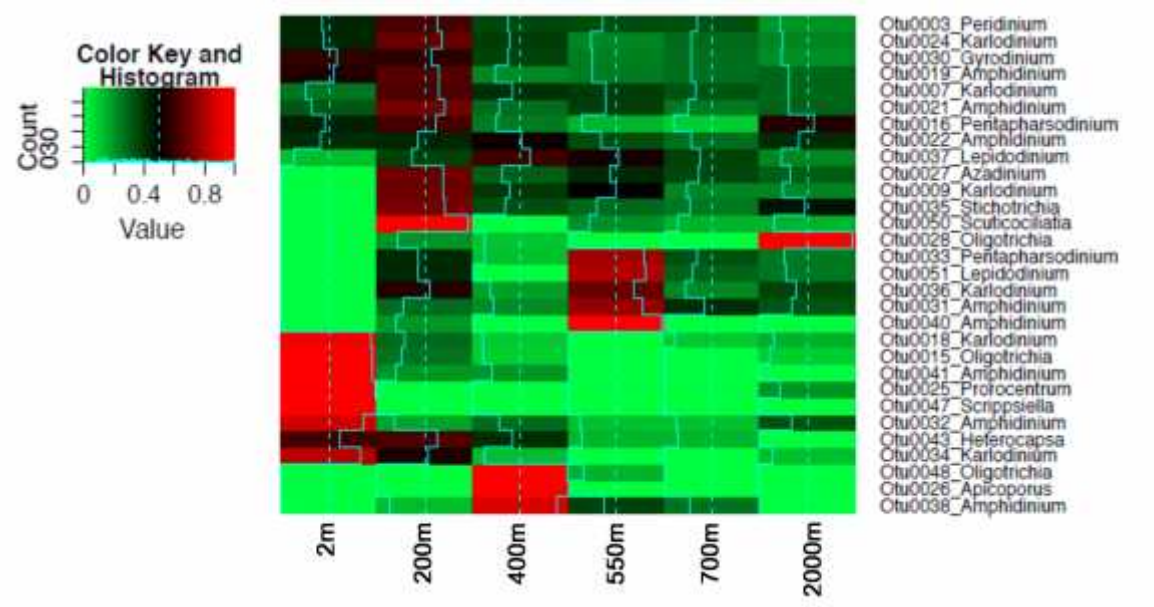
Hongmei Jing and Hongbin Liu on behalf of all co-authors

New Figures:

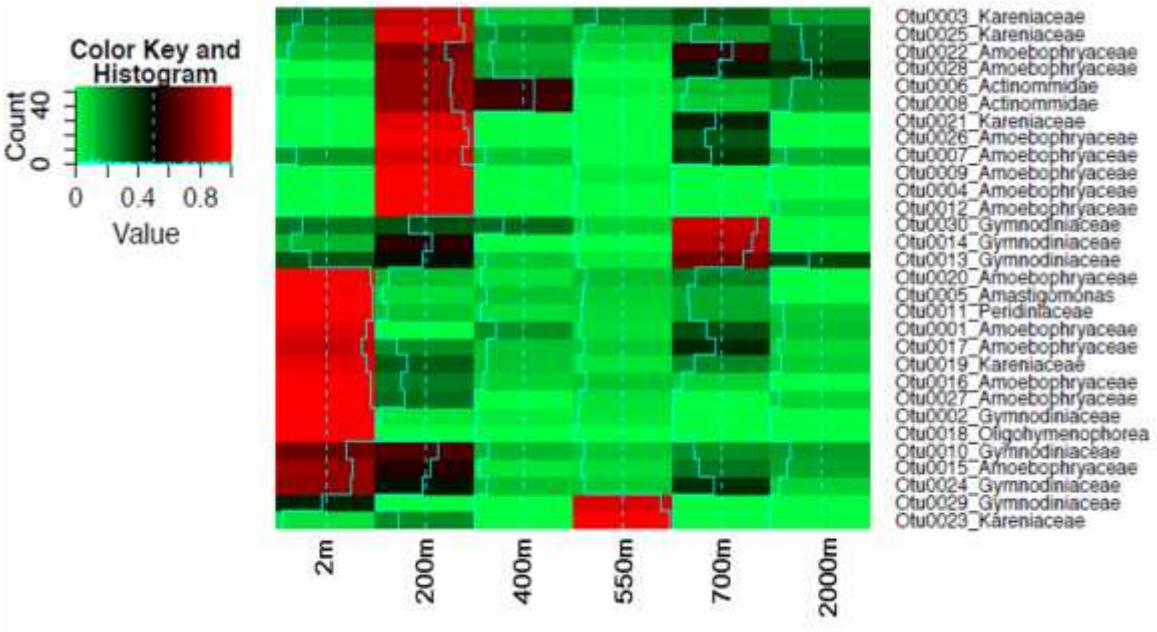


(a) **(b)**

Figure 1. Hydrographic conditions of the sampling stations. The deepest record of ammonium was at 900m.

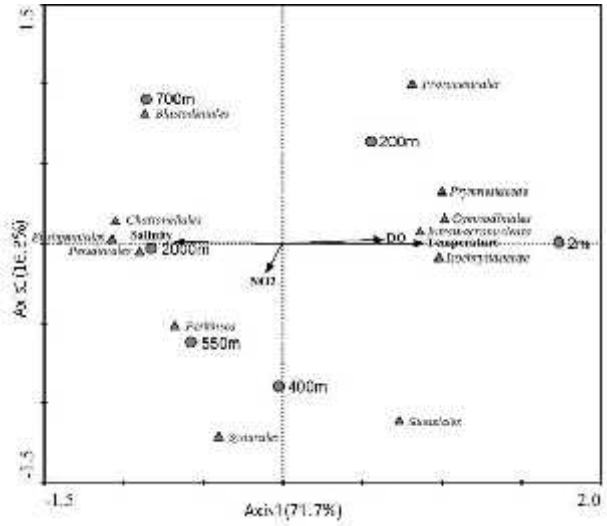


(a)

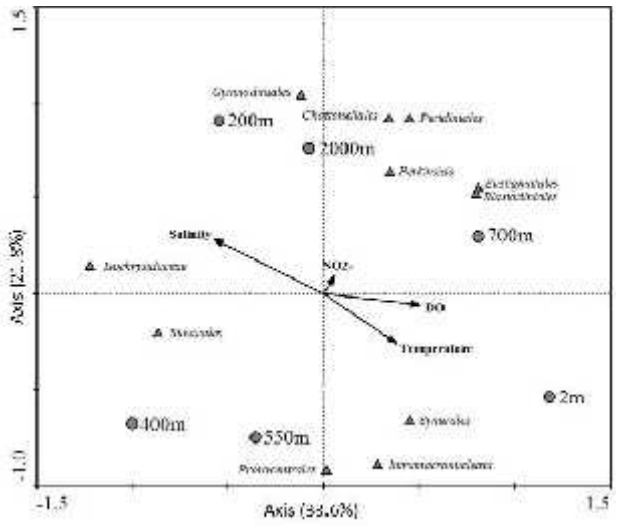


(b)

Figure 5. Heatmaps of the relative abundances and depth distributions of the top 30 OTUs among Costa Rica Dome OMZ samples based on rRNA (a) and rDNA (b) analyses.



(a)



(b)

Figure S1. Redundancy analysis (RDA) for major protistan orders from rRNA (a) and rDNA (b) analyses together with all abiotic data set as explanatory variables. Ammonium with VIF > 20 was excluded.

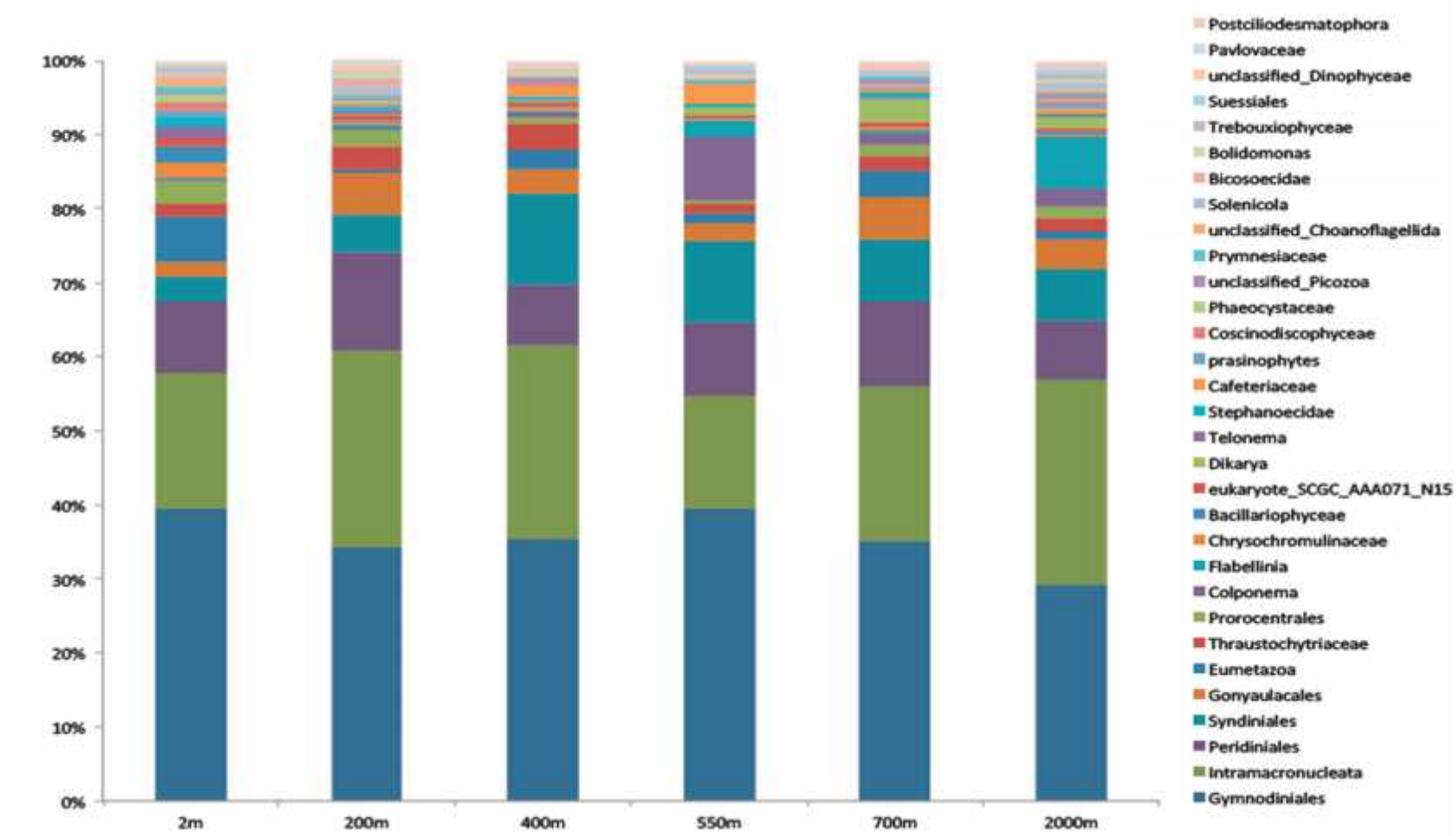


Figure S2. Protistan community compositions from rRNA analyses for different sampling depths in the Costa Rica Dome.

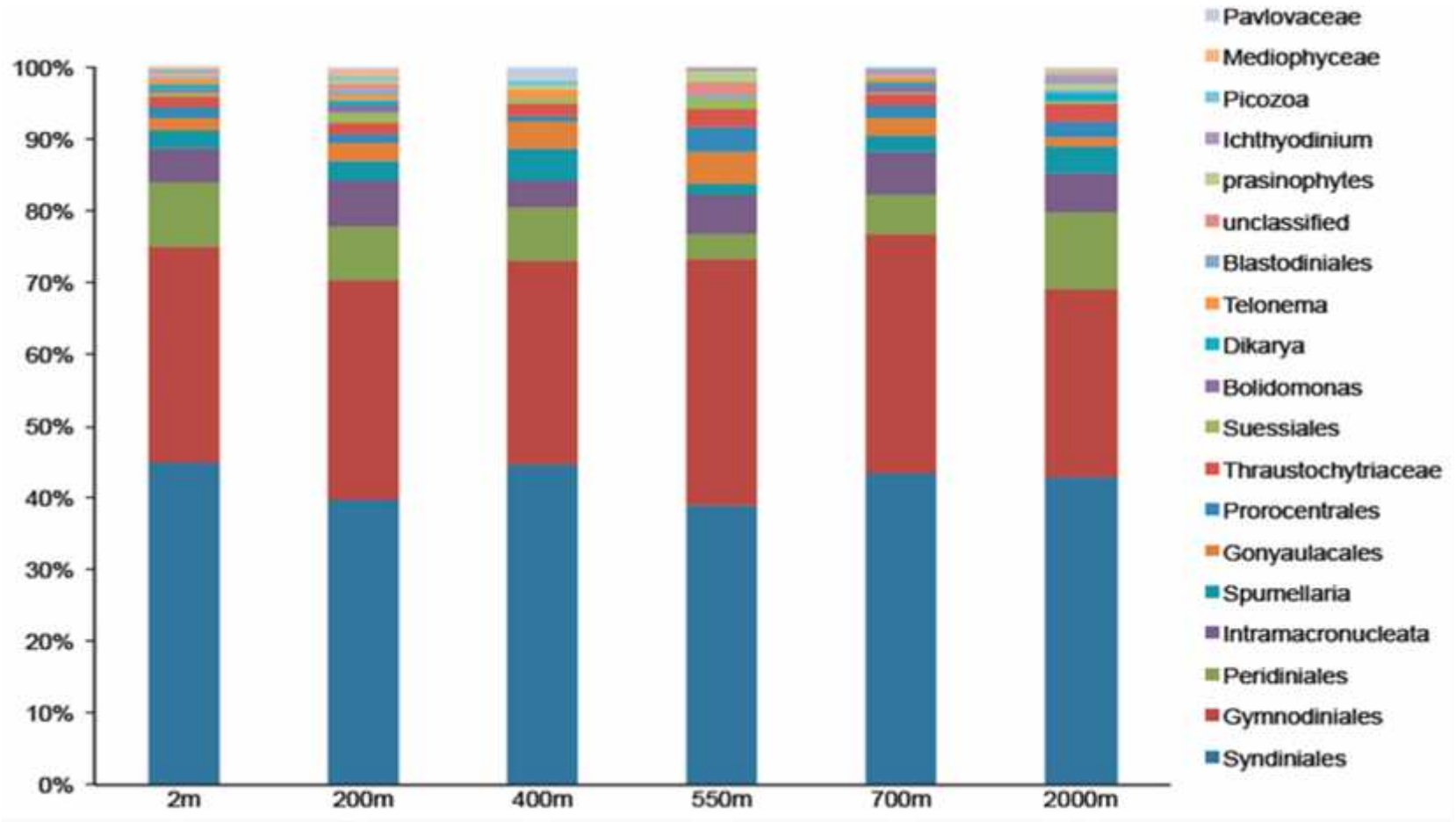


Figure S3. Protistan community compositions from rDNA analyses for different sampling depths in the Costa Rica Dome.