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Protist communities in a marine oxygen minimum zone off Costa Rica by 454 pyrosequencing

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Abstract

Marine planktonic protists, including microalgae and protistan grazers, are an important contributor to global primary production and carbon and mineral cycles, however, little is known about their population shifts along the oxic-anoxic gradient in the water column. We used 454 pyrosequencing of the 18S rRNA gene and gene transcripts to study the community composition of whole and active protists throughout a water column in the Costa Rica Dome, where a stable oxygen minimum zone (OMZ) exists at a depth of 400 ~ 700 m. A clear shift of protist composition from photosynthetic *Dinoflagellates* in the surface to potential parasitic *Dinoflagellates* and *Ciliates* in the deeper water was revealed along the vertical profile at both rRNA and rDNA levels. Those protist groups recovered only at the rDNA level represent either lysed aggregates sinking from the upper waters or potential hosts for parasitic groups. UPGMA clustering demonstrated that total and active protists in the anoxic core of OMZ (550 m) were distinct from those in other water depths. The reduced community diversity and presence of a parasitic/symbiotic trophic lifestyle in the OMZ, especially the anoxic core, suggests that OMZs can exert a selective pressure on protist communities. Such changes in community structure and a shift in trophic lifestyle could result in a modulation of the microbial loop and associated biogeochemical cycling.

1 Introduction

Protists, the unicellular eukaryotes, have a ubiquitous distribution in oceanic waters and are important contributors to global primary production and carbon and mineral cycles. They are a very complex group, spanning all eukaryotic kingdoms but varied substantially in size, shape and motility, and produce upwards of half of the world's oxygen and are the basis of aquatic food webs (Thomas et al., 2012). Protist community diversities in various ecosystems have been remarkably underestimated based on morphological classification previously (Bachy et al., 2013). Until last decade, with the application

BGD
12, 13483–13509, 2015

of various molecular techniques based on 18S rRNA genes, unexpected high diversity of protists have been revealed (López-García et al., 2001; Moon-van der Staay et al., 2001; Not et al., 2007; Guillou et al., 2012; Massana and Pedrós-Aliò, 2008). More recently, next generation 454 pyrosequencing technology has detected both the most abundant and rare protist species (Edgcomb et al., 2011; Oris et al., 2012) and revealed a vast diversity up to several orders of magnitude of those from morphology-based assessment (Bachy et al., 2013), therefore enabling a more realistic/exhaustive picture of the protist community in natural environments.

Protists as an important component of marine ecosystems consist of phototrophs, heterotrophs and mixotrophs. The phototrophic protists with confined distribution to the upper photic zones represent the main primary producers in surface waters (Li, 1994). Comparatively, heterotrophic protists provide a vital link for the cycling of nutrients from the prokaryotic fraction to higher trophic levels in the marine microbial loop (Sherr and Sherr, 1994), and have a much wider distribution throughout the water column. As for mixotrophic protists, they either harbor endosymbionts (Not et al., 2007) or parasitize other eukaryotes (Harada et al., 2007), playing a diverse and complex ecological role. Considering the diverse metabolic and trophic capabilities of protists, their distinct distribution and responses to variations in environmental conditions would be expected.

Low oxygen conditions as a putative stress can reshape local microbial community structure and affect the ecological functions of various groups. As a special niche, anoxic waters has caused significant changes of the community structures of protists (Wylezich and Jürgens, 2011; Orsi et al., 2012) and prokaryotes (Stewart et al., 2012; Kong et al., 2013). Increased diversity of picoeuaryotes in the hypoxic waters compared to those in oxic waters has been observed, and a shift in microbial food webs from grazing to parasitism had been proposed (Rocke et al., 2013). Among the different natural low-oxygen environments, marine oxygen minimum zones (OMZs) characterized by stably depleted dissolved oxygen (DO) concentrations (e.g. $< 20\,\mu M$) occur in the intermediate waters of some parts of the oceans (Ulloa and Pantoja, 2009) representing an ideal place for studying the effects of oxygen on various biological assemblages

BGD
12, 13483–13509, 2015

and processes. The effect of OMZ formation on the communities of marine microbial eukaryotes (Orsi et al., 2012; Parris et al., 2014) have been investigated and anaerobic Ciliates and Dinoflagellates were found as the dominant groups. Nevertheless, the spatial variations of protists, especially the active portion, along the water column with different DO concentrations are still largely unknown.

In our study, a typical marine OMZ in the Costa Rica Dome (CRD) was selected in order to characterize the composition of protists along the oxic-anoxic gradients. The CRD located in the Eastern Tropical North Pacific (ETNP) with a diameter of approximately 300 km, is one of the eastern boundary upwelling systems (Wyrtki, 1984; Fiedler, 2002; Helly and Levin, 2004), and generates a stable OMZ usually found between 400 to 700 m (Wyrtki, 1984). Compared with other marine oxygen deficient systems, the CRD-OMZ is characterized by its permanent, much deeper location and much thicker OMZ layer (300–400 m). This special habitat containing the upper and deeper oxic water layers and the intermediate anoxic water could be a perfect location to investigate the impact of DO on the protist communities and their functions. Therefore, water samples with different DO concentrations from six different depths spanning the aerobic photic zone (20 m), the secondary DO peak (200 m), the upper (400 m) and lower (700 m) oxic-anoxic boundary and the core of OMZ (550 m), as well as the oxygen-rich deep water (2000 m) were collected and studied using high throughput pyrosequencing. For a comparative study, the active protist communities along this oxygen gradient were investigated at rRNA level as well. Our study provides new insights into the ecological significance of marine protist communities in suboxic/anoxic waters.

BGD
12, 13483–13509, 2015

2 Materials and methods

2.1 Sample collection and environmental conditions

Water samples were collected from the OMZ off the Costa Rica coast (8.9845° N, 90.50911° W) during the FLUZiE cruise in June 2010 as described previously (Kong et al., 2013). The sampling station has an annual mean thermocline depth of 35 ~ 40 m (20 °C isotherm depth) (Fiedler, 2002). Seawater hydrographical data was collected by a conductivity-temperature-depth (CTD) rosette system (Sea-Bird Electronics) with attached Niskin bottles. The concentrations of dissolved oxygen, nitrite and ammonium were also measured (Kong et al., 2013).

For DNA/RNA sample collection, 1 L seawater from 6 depths, 20, 200, 400, 550, 700 and 2000 m, representing different parts of the water column described above was filtered through a 0.22 µm pore size polycarbonate filter (47 mm, Millipore) with a low vacuum pressure. Filters for RNA sample collection were immersed in RNA later solution (Ambion) immediately after filtration. All filters were flash frozen and stored at –80 °C until further analysis.

2.2 DNA and RNA extraction and cDNA synthesis

Genomic DNA was extracted from 0.22 µm polycarbonate filters with the PureLink Genomic DNA Kits (Invitrogen, Carlsbad, CA) and eluted into 100 µL TE butter and stored at –80 °C. Total RNA was extracted from 0.22 µm polycarbonate filters with the TRIzol plus RNA purification kit (Invitrogen, Carlsbad, CA). RNA later immersing the filters was removed before the preparation with TRIzol Reagent and extracted RNA was finally eluted in 50 µL elution buffer. rDNA and rRNA concentrations were measured on a NanoDrop 1000 Spectrophotometer (Thermo Scientific).

Before cDNA synthesis, purified total RNA was treated with DNase I to eliminate DNA contamination. Total RNA (up to 200 ng) was then reverse transcribed to cDNA using the SuperScript III first strand cDNA synthesis kit (Invitrogen, Carlsbad, CA).

BGD
12, 13483–13509, 2015

A parallel reaction without SuperScript III RT was used as an RT-PCR negative control. Synthesized cDNA was further digested with 2 U RNase H at 37 °C for 20 min to remove RNA residue and used for subsequent PCR amplification. Non-RT samples were always used as a negative control.

2.3 PCR amplification and 454 pyrosequencing

Both rDNAs and cDNAs were amplified using FastStart High Fidelity PCR system (Roche) with universal primers Euk-82F (5′-GAAACTGCGAATGGCTC-3′) (López-Garcia et al., 2003) and Euk-516R (5′-ACCAGACTTGCCCTCC-3′) (Díez et al., 2001), designed to amplify the complete V2 and V3 domains of all eukaryote 18S rDNA gene. Different MID sequences for pyrosequencing were synthesized together with the forward primer. PCR was carried out with Peltier Thermal Cycler (Bio-Rad) with following cycles: 95 °C for 2 min; 35 cycles of 95 °C for 30 s, 55 °C for 30 s, 72 °C for 40 s; final extension at 72 °C for 7 min.

TriplicatePCR products for each sample were combined and subsequently purified by illustra™ GFX™ PCR DNA and Gel Band Purification kit (GE Healthcare). An amplicon library was constructed and emPCR was conducted to generate millions of copies of sequences linked to each bead, according to the instructions of Rapid library preparation (Roche, 454 Life Science). DNA beads were successfully deposited onto the PicoTiterPlate and sequenced on a GS Junior system (Roche, 454 Life Science).

2.4 Post-run sequence analysis

All forward-oriented reads generated from this study were separated according to their specific multiplex identifiers (MIDs), and quality control was checked in the RDP pyrosequencing pipeline, for example, reads were flagged as low quality when they were less than 300 bp in length, the start of the sequence did not exactly match a primer sequence and when one or more ambiguous bases (N) were present in the sequence (Cole et al., 2009). Chimeras were detected with the Chimera Slayer algorithm imple-

BGD
12, 13483–13509, 2015

mented in the Mothur software package using the 18S rDNA data sets as references. After above filtration, the remaining reads were analyzed with the Mothur software package for alignment, distance calculation and classification (Schloss et al., 2009).

The estimators for richness (Chao1), diversity (Shannon index), coverage and operational taxonomic unit (OTU) numbers were calculated at the cutoff level of 3 % using Mothur's *summary.single* routine. Rarefaction curves were calculated using *rarefaction.single* with 10 000 iterations. Taxonomic identification of each read was carried out with Mothur against the Silva bacteria no-gap reference database at a cutoff value of 60. To estimate similarity among samples, hierarchical cluster analysis was conducted based on a matrix of OTUs in each sample using Bray-Curtis similarity calculated from Mothur and a dendrogram inferred with the unweighted pair-group method with arithmetic means (UPGMA). A heatmap plot depicting the abundance and distribution of OTUs among different samples was generating using the R-package. OTU species affiliations to the red portions of the heatmap were acquired manually using the PR2 database (Guillou et al., 2012). The relationship among samples was determined by Bray distance and the complete clustering method.

2.5 Accession number

All the sequences obtained from this study have been deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under BioSample number of SAMN03839054-SAMN03839065.

3 Results

3.1 OMZ conditions and sequencing statistics

A stable thermocline formed in the sampling station due to the persistent upwelling events as indicated by increased salinity and decreased temperature occurred at the

BGD
12, 13483–13509, 2015

depth of 30 ~ 40 m as described earlier (Kong et al., 2013). Very different DO concentrations were detected at the six typical depths along the vertical water profiles. DO concentration dropped sharply from the surface water with a secondary peak appearing at around 200 m and decreased again to generate a clear OMZ zone from 400 to 700 m with a core at around 550 m (DO ≤ 1.0 µM).

Pyrosequencing generated a total of 26 411 rRNA reads and 41 895 rDNA reads after filtering out low-quality reads according to the applied criteria described in the methodology (Table 1). On average, much more quality reads per sample were obtained at the rDNA level (6983 reads per sample) than at the rRNA level (4402 reads per sample), whilst the latter was classified into much more total OTUs using 97 % sequence similarity as cutoff value. Comparatively, richness (Chao1) and diversity (Shannon) at the rRNA level were remarkably higher than those at the rDNA level (Table 1). Along the vertical profiles, the highest number of total OTUs, Chao1 and Shannon indices at both rDNA and rRNA levels were shown at the depth of 200 m; whilst the lowest diversity indices (Chao1 and Shannon) were found from the core of the OMZ (550 m) at the rRNA level and the surface water (2 m) at the rDNA level.

Higher coverages were shown for rDNA reads (0.87 ~ 0.97) than for rRNA reads (0.70 ~ 0.81). The highest coverage was obtained from the core (550 m) of the OMZ, while the lowest ones were shown at 700 and 400 m at the rRNA and rDNA levels, respectively (Table 1).This was consistent with the patterns demonstrated by the rarefaction curves with 97 % sequence similarity as cutoff value at the rRNA level (Fig. 1a) and at the DNA level (Fig. 1b): the 550 m sample became flat earlier compared with those from other depths; samples at the rRNA level still need more sampling efforts in order to adequately assess community composition.

3.2 Microbial community structure

In total, seven protistan phylogenetic groups, including *Alveolata*, *Cryptophyta*, *Fungi*, *Haptophyceae*, *Rhizaria*, *Stramenopiles* and *Viridiplanctae*, were identified from all the samples at the rRNA level, contrasting to the fact that no *Cryptophyta* was detected at

BGD
12, 13483–13509, 2015

the rDNA level (Fig. 2). *Alveolata* was always the predominant group in different water depths at both the rRNA (65 ~ 75.1 %) and rDNA (82.6 ~ 91.7 %) levels. *Stramenopiles* was the second most abundant phyla in all samples, contributing more to the whole protist community at the rRNA (17.0 ~ 29.8 %) than the rDNA (4.5 ~ 7.0 %) levels (Fig. 2). For the remaining minor groups, *Cryptophyta* was only detected at the rRNA level with less than 1 % of the total community in each sample; *Fungi*, *Haptophyceae*, *Rhizaria* and *Viridiplantae* were much less abundant at both levels. For example, *Rhizaria* was only found at 200 m (0.53 %) at the rRNA level, but undetectable in the surface and deeper waters (700 and 2000 m) at the rDNA level.

Alveolata predominated in all twelve samples and comprised of four phylogentic groups with *Dinophyceae* as the most abundant group. *Dinophyceae* and *Perkinsea* were more abundant at the rDNA level, whilst *Ciliophora* was more numerous at the rRNA level; *Apicomplexa* accounted for less than 1 % in all samples except at 550 m at the rDNA level (Fig. 3a). On the vertical profile, the predominant group *Dinophyceae was* more highly distributed in the surface water (2 m) at both rDNA and rRNA levels, whereas the highest abundance of *Ciliophora* and *Perkinsea* were both found at 400 m at the rDNA level and 2000 m at the rRNA level, respectively.

Within *Ciliophora*, four groups were identified from rRNA reads, but no *Phyllopharyngea* was recovered for the rDNA reads (Fig. 3b). At the rDNA level, surface water (2 m) and deep water (2000 m) were composed exclusively of *Spirotrichea*, which was also predominant in the other water depths; *Litostomata* was only found at 200, 550 and 700 m; whilst *Oligohymenophorea* presented at 200 and 400 m (Fig. 3b). On the other hand, *Spirotrichea* was the predominant ciliate class at the rRNA level, and all four groups were revealed in all samples except for *Phyllopharyngea* which was undetected at 700 m.

As for *Dinophyceae*, the dominant group of *Alveolata*, seven phylogenetic groups were successfully identified with *Gymnodiniales* predominant in all samples. The dominance of *Gymnodiniales* in total *Donophyceae* reads was more pronounced at the rRNA level than at the rDNA level (Fig. 3c). At both rRNA and rDNA levels, *Suessiales*

BGD
12, 13483–13509, 2015

had its lowest presence at the core of OMZ (550 m); whilst the highest presence of *Peridiniales* and *Prorocentrales* were recovered from the deep (2000 m) and surface (2 m) waters, respectively. *Blastodiniales* and *Pyrocystales* were two minor groups and *Gonyaulacales* was only recovered from 200 m at the rRNA level.

In total, eight groups of *Stramenopiles* were found from our samples. *Eustigmatophyceae* occupied around 50 % of this phylum (Fig. 3d). *Bacillariophyta* was generally more abundant in the surface waters than in other water depths. *Raphidophyceae* and *Synurophyceae* in addition to *Eustigmatophyceae* had broad distribution in each sample, contrasting to the fact that *Chrysophyceae*, PX-clade and *Pinguiophyceae* were undetectable in the surface (2 m) and deeper (700 and 2000 m) waters at the rDNA level.

3.3 Community comparison

The affiliation and abundance of the top 10 most abundant OTUs at the rRNA and rDNA levels varied with samples (Fig. 4). *Gymnodiniales* was the most abundant group, and was more abundant at rDNA than rRNA levels with the highest abundance present in the surface waters. *Spirotrichea* and *Eumastigochyceae* were two major groups more abundant at the rRNA level. At both rRNA and rDNA levels, the least of *Peridiniales* appeared at the core of OMZ and the highest of *Prorocentrales* occurred in the surface water. *Litostomatea* was almost undetectable at the rDNA level except very little on the surface water; its highest abundance at the rRNA level appeared in the deep water of 2000 m. The remaining groups of *Fungi*, *Haptophyceae*, *Raphidophycea* and *Viridiplantea* occupied less than 5 % of the protist community.

Consistent with the pattern of OTUs, major protist orders recovered from the rRNA and rDNA levels also varied with samples. Although *Gymnodiniales* was always the dominating order, more *Stramenopiles*, *Chattonellales* and *Eustigmatales*, were revealed from the rRNA level, contrasting to the fact that *Alveolata*, particularly *Dinophycea*, *Cilophora* and *Apicomplexa*, were predominant at the rDNA level (Fig. 5). In

BGD
12, 13483–13509, 2015

the core of the OMZ, *Eustigmatales* and *Gymnodiniales* was the dominant order at the rRNA and rDNA levels, respectively.

The heatmap presented in a color-matrix illustrates the distribution of different OTUs (clustering on the *y* axis) among different samples (Fig. 6). The density of OTUs was scaled by color and the phylogenetic composition of the most abundant OTUs (red cells) was highly variable with different samples. At the rRNA level, high number of photosynthetic *Dinoflagellates* appeared at 2 and 200 m and their abundance decreased with depth and was replaced by *Ciliates* in 700 and 2000 m; 400 m was composed of parasitic *Dinoflagellates* (gp I, II, III) and *Ciliates* (Fig. 6a). At the core of 550 m, 48 % of the most abundant OTUs at the rRNA level were identified as parasitic groups I, II and III, or parasitic dinoflagellates. The remaining 52 % consisted exclusively of ciliate or nanoflagellate grazers. At the rDNA level, as the red block demonstrated, *Dinoflagellates* were the most abundant group throughout the water column, but shifted from photosynthetic species in the surface waters to parasitic species gpI, II and III in deeper waters. Phototrophic diatoms and *Prasinophytes* were only present at the surface, while *Ciliates* appeared exclusively in the deep waters of 700, 2000 m and the core of the OMZ (Fig. 6b). *Radiolaria* occurred only at 200 and 400 m, but were not detected at the rRNA level. At the hypoxic 550 m, the most abundant rDNA sequences consisted of many mixotrophic protists such as *Prorocentrum* and *Prasinophytes*. A few diatoms also presented here, most likely silica detritus drifting from surface waters.

UPGMA demonstrated a generally similar clustering pattern regarding to the protist community among different samples at both rRNA and rDNA levels (Fig. 7). Surface water was very different from other water depths and the core of the OMZ formed a distinct branch apparently separating it from the rest of the samples. The rest four samples fell into a single clade, within which shallower water (200 and 400 m) and deeper water (700 and 2000 m) formed two separate clusters at the rRNA level; but the community structure of 700 m was more similar to those of shallower waters (200 and 400 m) rather than to the deepest water (2000 m) at the rDNA level.

BGD
12, 13483–13509, 2015

4 Discussion

Naturally formed oxygen-depleted OMZ ecosystems represent one of the ocean's most extreme environments and are largely unexplored by far. Suboxic/oxicwaters have been proposed as an environmental stress leading to habitat compression, loss of fauna and energy diversion into microbial pathways in the marine ecosystems (Diaz and Rosenberg, 2008). Protists are a very diversified group, comprising all major single celled eukaryotic organisms, and their distribution in the OMZ regions remain largely unknown. Results from our study show that, like metazoan zooplankton, suboxic/anoxic watersin general are not the favorable habitats for protists, indicated by the lowest community diversity and total number of OTUs in the anoxic core of OMZ (550 m). It is noteworthy that the highest diversity of microeukaryotes from the 0.2–1.6 µm size fraction has been reported from the core of OMZ off northern Chile, which could be caused by a contamination of lysed cells from the larger size fraction (Parris et al., 2014). However, the leakage of lysed cells from larger eukaryotes would not affect the diversity estimation at the rRNA level as conducted in our study. Reduced community diversity for metazoans (Levin, 2003) and protists (Oris et al., 2012) under low DO conditions has been reported previously. Together with our findings, it can be summarized that low DO concentration acts as an environmental stress that selectively support certain species assemblages to sustain the ecosystem balance and processes in OMZs.

Protists have diverse trophic life styles, and suboxic/anoxic waters acting as a stress could induce the outgrowth of certain bacterivorous mixotrophic and heterotrophic groups due to elevated bacterial abundance at or below the oxycline (Fenchel, 1990; Fenchel and Finlay, 2008; Edgcomb and Pachiadaki, 2014), subsequently causing a shift in protist community composition. Distinct distribution patterns of protist groups throughout the water column as a result of adapting to the vertical oxygen gradient were exhibited in our study. *Alveolata*, particularly *dinoflagellates*, dominated in all depths across oxygen gradients, in agreement with their predominance in other oxic/suboxic regions (Parris et al., 2014). The *Alveolata* in our study are mainly composed of *Gymno-*

BGD
12, 13483–13509, 2015

diniales, which is an order of mixotrophic non-thecate dinoflagellates with the potential of forming blooms (Tester and Steidinger, 1997). *Gymnodiniales* are generally surface-dwelling, and their appearance in the deep waters is likely resulted from the amplification of resting cysts, which act as an adaptive strategy for dinoflagellates to survive through adverse conditions, i.e. the low DO in our case (Anderson, 1984). Another major group of eukaryotic microorganisms, *Stramenopiles* with diverse trophic lifestyles generally occur predominantly in surface waters in spite of their ubiquitous distribution in different geographical locations (Blackwell and Powell, 2000). Like anywhere else, photosynthetic *Bacillariophyta* was most abundant in the surface water in our study. The fact that *Synurophyceae* had the highest abundance in the core of OMZ, where *Chrysophyceae* had its confined distribution, may be a proof of the global distribution of hypoxic *Stramenopiles* (Wylezich and Jürgens, 2011). The latter group possibly acted as the host of some parasitic/symbiotic groups particularly harbored in the anoxic core.

The effect of sustained oxygen depletion on protists was the most obvious in the anoxic core of the OMZ, as it not only led to a reduced microbial diversity, but a shift from grazing food web to a symbiosis/parabiosis-based lifestyle as well. For instance, *Prorocentrales* and *Spirotrichea*, belonging to respective of dinoflagellate orders and ciliate classes, are two major groups in the core of the OMZ in our study, followed by the most abundant group of photosynthetic *Gymnodiniales*. Both *Prorocentrales* and *Spirotrichea* have known mutual symbiotic associations with planktonic *Rhizarians* (Spero and Angel, 1991). It is interesting to find *Apicomplexa*, a special group of *Alveolata*, occurring mainly in the core of the OMZ, as this unicellular and spore-forming protist group is comprised almost entirely of obligate endoparasitic pathogenic protists. Heterotrophic *Ciliophora* and *Perkinsea* seem more abundant in the suboxic waters and the latter is a parasite of bivalve mollusks. The detection of these distinct symbiotic/parasitic protist groups mentioned above in our study is in agreement to the previous reports that the presence of an endosymbiotic lifestyle seems widespread in OMZs (Levin, 2003) and further proves that anoxic conditions at the core of OMZs func-

BGD
12, 13483–13509, 2015

tion as isolated habitats contributing to the development of a high degree of endemic community adaptive to this stress.

In addition to the core of OMZ, the upper and lower boundary of the OMZ acts as an interface with a rich supply of organic matter but less oxygen stress, representing a relatively moderate ecological/physiological threshold for certain microbial groups. Previous studies have demonstrated that the edges of OMZ typically support a high density of zooplankton (Morrison et al., 1999) and that apparent shifts of microbial communities had occurred in the oxic/anoxic interface (Vetriani et al., 2003; Wylezich and Jürgens, 2011). In our study, the edge effect was more apparent in the upper boundary of the OMZ (400 m) as the oxic-suboxic transition interface. *Oligohymenophorea* and *Litostomatea*, two groups of *Ciliophora*, dominated at the upper and lower boundary of the OMZ, respectively. Some members of these two groups have been suggested to possess hydrogenosomes belonging to obligatory anaerobes, while other members are facultative anaerobes and have been reported from different suboxic/anoxic conditions (Lynn, 2008) including OMZs (Parris et al., 2014). In addition, *Rhizaria* only occurred in the upper boundary, and a higher abundance of PX-clade of *Stramenopiles* was found in both the upper and lower boundary of OMZ. The heterotrophic *Rhizaria* together with the dominant mixotrophic *Stramenopiles* and *Alevolata* had been proposed as a SAR supergroup with close phylogenetic affiliations. This highly diverse supergroup has been found to predominate in all depths of the OMZ off northern Chile (Parris et al., 2014). The relatively high nutrient and low oxygen-deficiency tensions in the upper and lower boundaries of the OMZ represent broader transition zones, and therefore might provide more harboring niches for protist communities.

Most eukaryote community studies by far have been based on DNA-derived analysis, which cannot distinguish intact living cells from inactive or lysed cells and extracellular materials of other species. It is problematic especially for protist studies, since many sequences affiliated with metazoans were frequently detected with high abundance due to the high copy numbers of RNA genes in the metazoan cell (Prescott, 1994). Previous RNA-based investigations have revealed a complex and metabolically active marine

BGD
12, 13483–13509, 2015

protist community in anoxic/sulfidic waters in the Black Sea (Wylezich and Jürgens, 2011), however, the difference between the total and active proportions of eukaryotes was not investigated. In our study, both photosynthetic *Cryptophyta* and heterotrophic *Phyllopharyngea* were exclusively found at the rRNA level but undetected at the rDNA level. This highlights the necessity of integrated rRNA- and rDNA-based investigations for a comprehensive community study, since the latter targets the whole complex community and might not be sensitive enough to detect species with low abundance.

DO concentrations primarily drive the nutrient and energy flow in marine ecosystems and selectively affect the microbial populations. A clear shift from photosynthetic groups in the surface to parasitic protist groups in the deeper waters was observed in our study. It is not surprising to find much more abundance of photosynthetic dinoflagellates in the surface waters, while the abundance of parasitic dinoflagellates and a few ciliates (rRNA level) increased with water depth especially in the OMZ. Ciliates could be the potential hosts for the parasitic Dinoflagellates. On the other hand, the presence of phototrophic Dinoflagellates in the deep waters was possibly dead skeletons sinking down to the water column. In our study, the UPGMA clustering at both rRNA and rDNA levels demonstrates a relative homogeneity of the microbial community in the oxic waters below the euphotic zone, distinct from those in the anoxic core of the OMZ, suggesting that the oxygen gradient presented in the OMZ exerts similar effects on both total and metabolically active protist communities. All the water depths are strikingly different from the surface water which harbors a large amount of photosynthetic protists. The high abundances of active protists uncovered from the OMZ further proved 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 OMZ suggests that the oxygen deficiency occurring in the OMZ exerts a selective pressure on the microbial communities and leads to modulation of different trophic processes and integrated biogeochemical cycles as a result of community adaptation. Protists are capable of many different trophic lifestyles, such as predation, parasitism and symbioses. These

BGD
12, 13483–13509, 2015

are possible survival strategies that regulate the endemic microbial community structure and in turn mediate the biogeochemical cycling in OMZs. Discrepancy between the whole and active protist community suggests that analysis of both rRNA and rDNA are needed to reflect the real ecological roles of marine microbial communities in environmental microbial studies. The vast and relatively unexplored OMZ ecosystems harbor a diverse assemblage of protists and represent a source for genetic diversification and specialization. Therefore, studies focusing on this special environment could help to understand the ecological and evolutionary strategy of microbial adaptations and metabolic processes that prevail in oxygen-deficient marine environments.

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BGD
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BGD
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BGD
12, 13483–13509, 2015

Table 1. Sequencing information and diversity estimates for rRNA and rDNA sequences achieved by pyrosequencing.

Depth	No. of Reads[a]	Total OTUs	Chao1[b]	Shannon[b]	Coverage[b]
rRNA(total reads = 26 411)					
2 m	5569	1786	4354	6.69	0.76
200 m	6570	2209	4884	6.99	0.70
400 m	4126	1441	3303	6.53	0.75
550 m	3745	1013	2229	5.78	0.81
700 m	2496	983	2510	6.11	0.78
2000 m	3905	1137	2386	5.97	0.79
rDNA (total reads = 41 895)					
2 m	2069	746	1377	4.68	0.96
200 m	16 633	1384	2461	5.62	0.87
400 m	1645	360	634	4.22	0.94
550 m	13 000	300	614	4.13	0.97
700 m	6174	687	1254	5.46	0.92
2000 m	2374	357	529	5.00	0.95

Note: [a] trimmed reads that passed quality control; [b] 97 % sequence similarity as cutoff value.

BGD
12, 13483–13509, 2015

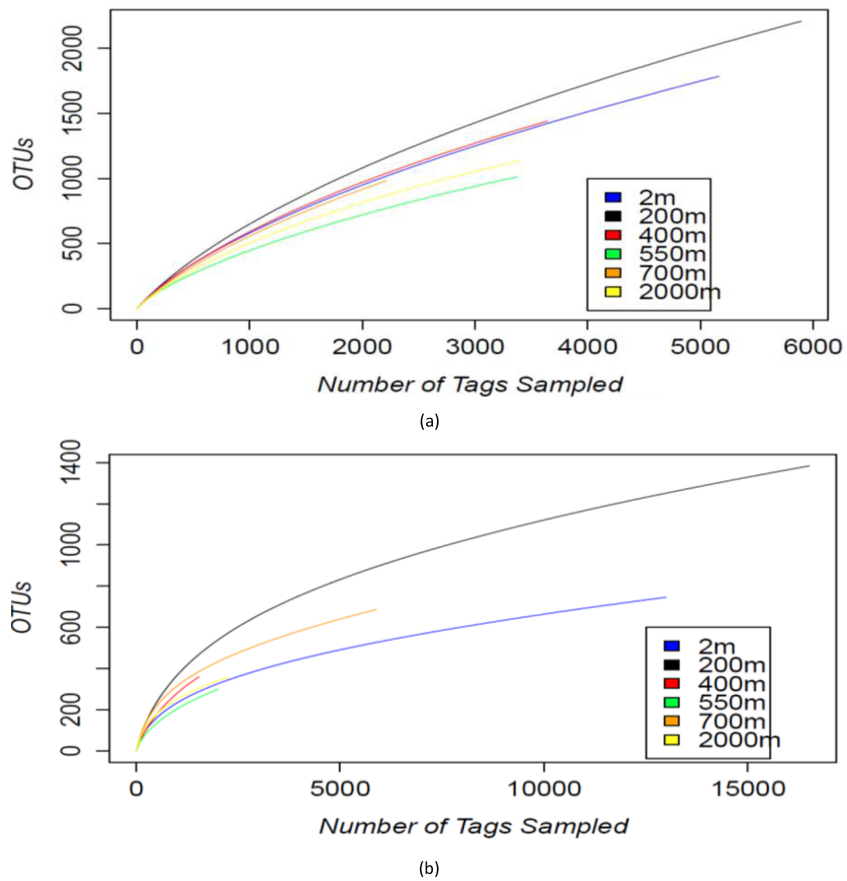

Figure 1. Rarefaction curves for rRNA **(a)** and rDNA **(b)** sequences achieved by pyrosequencing for different water samples collected in the Costa Rica Dome. OTU was defined with 3 % as cutoff value.

BGD
12, 13483–13509, 2015

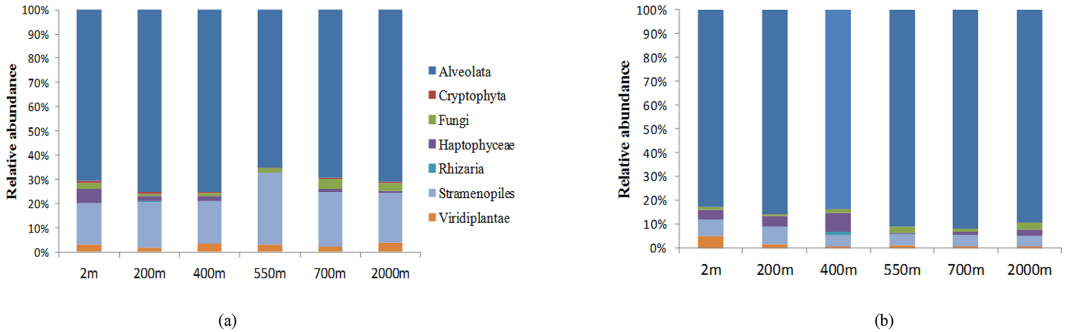

Figure 2. Relative abundance and affiliation of all the rRNA **(a)** and rDNA **(b)** sequences achieved by pyrosequencing for different water samples collected in the Costa Rica Dome.

BGD
12, 13483–13509, 2015

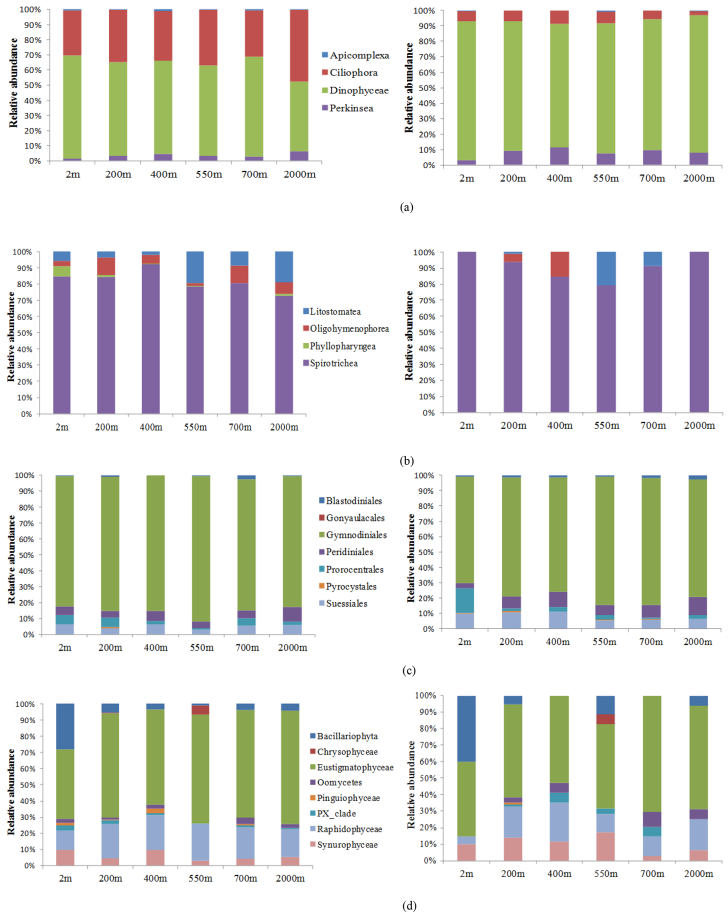

Figure 3. Phylogenetic compositions of *Alveolata* **(a)**, *Ciliophora* **(b)**, *Dinophyceae* **(c)**, *Stramenopiles* **(d)** asseblages based on rRNA (left panel) and rDNA (right panel) sequences achieved by pyrosequencing for different water samples collected in the Costa Rica Dome.

BGD
12, 13483–13509, 2015

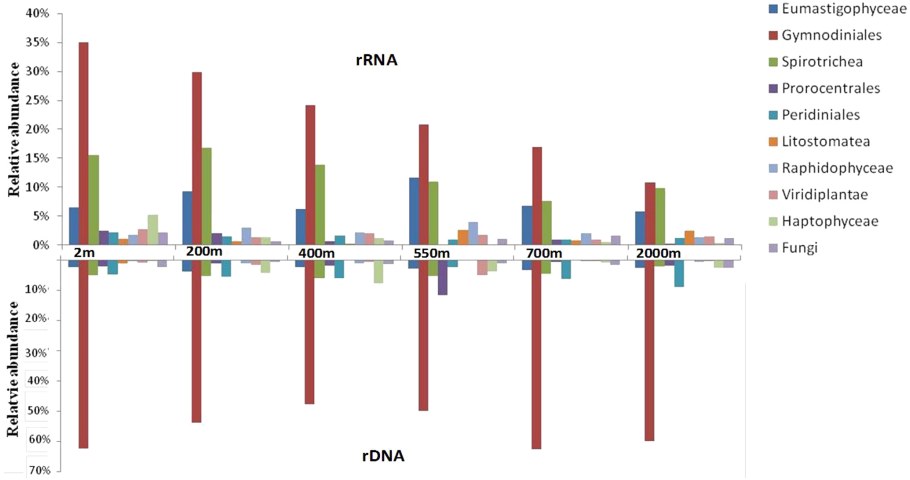

Figure 4. Relative abundance and affiliation of the 10 most abundanct OTUs based on rRNA and rDNA sequences achieved by pyrosequencing for different water samples collected in the Costa Rica Dome.

BGD
12, 13483–13509, 2015

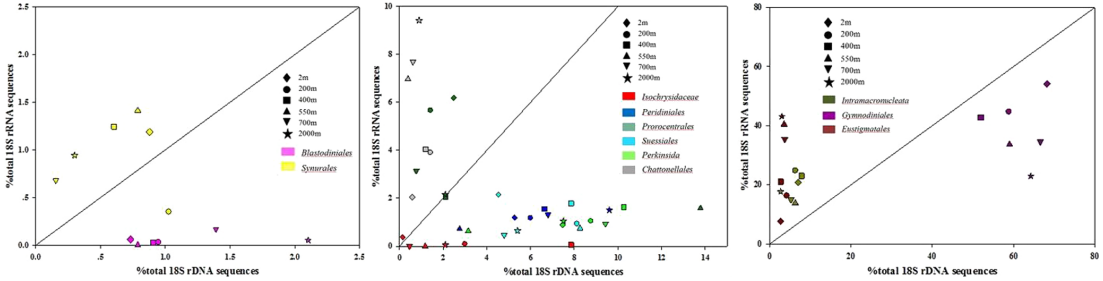

Figure 5. Comparison of major ten orders of protists based on rRNA and rDNA sequences achieved by pyrosequencing for different water samples collected in the Costa Rica Dome.

BGD
12, 13483–13509, 2015

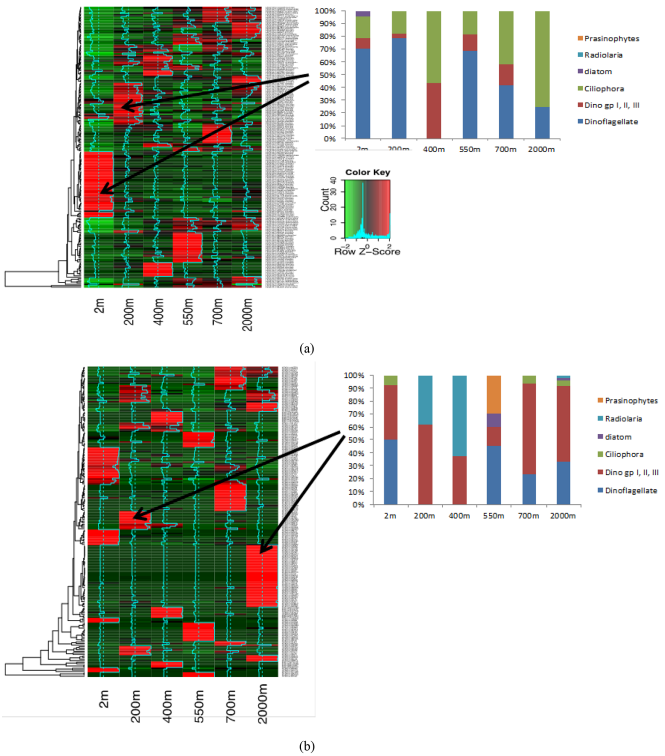

Figure 6. Hierarchical dendrogram illustration for the abundance and distribution of OTUs among different samples at rRNA **(a)** and rDNA **(b)** levels. Red blocks represent the most abundant OTUs with different phylogenetic compositions (right panel). Relative abundance in the form of *z* score ((each value–mean of row)/standard deviation) is indicated by color as shown in the legend (bottom right).

BGD
12, 13483–13509, 2015

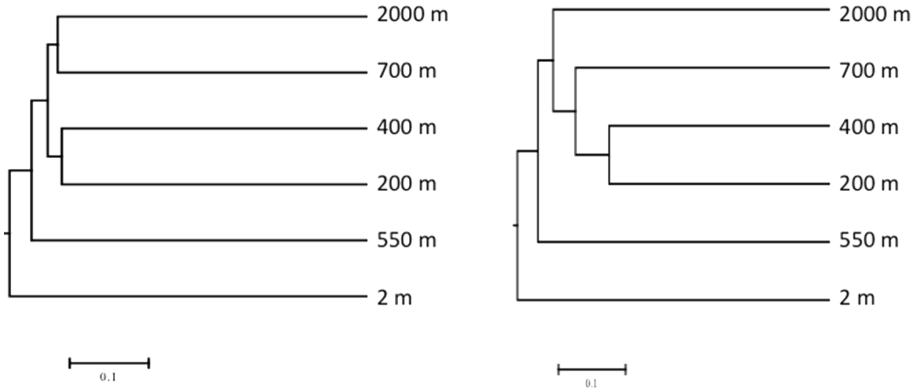

Figure 7. UPGMA demonstating protistcommunity similarities among different samples based on rRNA (left panel)and rDNA sequences (right panel) achieved by pyrosequencing for different water samples collected in the Costa Rica Dome.

BGD
12, 13483–13509, 2015