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A molecular survey of protist diversity through the central Arctic Ocean

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Abstract The protist assemblage in the central Arctic Ocean is scarcely surveyed despite them being the major primary producers. Elucidating their response to changing environmental variables requires an a priori analysis of their current diversity, including abundant and rare species. In late summer 2011, samples were collected during the ARK-XXVI/3 expedition (RV *Polarstern*) to study Arctic protist community structures, by implementation of automated ribosomal intergenic spacer analysis (ARISA) and 454-pyrosequencing. Protist assemblages were related to the hydrology and environmental variables (temperature, salinity, ice coverage, nitrate, phosphate, and silicate). The abundant (≥1 %) biosphere and rare (<1 %) biosphere were considered separately in the diversity analysis in order to reveal their mutual relationships. A relation between hydrology and protist community structure was highly supported by ARISA and partially by 454-pyrosequencing. Sea ice showed a stronger influence on the local community structure than nutrient availability, making statements on the water mass influence more difficult. Dinoflagellates (Syndiniales), chlorophytes (*Micromonas* spp.), and haptophytes (*Phaeocystis* spp.) were important contributors to the abundant biosphere, while other dinoflagellates and stramenopiles dominated the rare biosphere. No significant correlation was found between the abundant and rare biosphere. However, relative contributions of major taxonomic groups revealed an unexpected stable community structure within the rare biosphere, indicating a potential constant protist reservoir. This study provides a first molecular survey of protist diversity in the central Arctic Ocean, focusing on the diversity and distribution of abundant and rare protists according to the environmental conditions, and can serve as baseline for future analysis.

Keywords 18S rRNA gene · 454-Pyrosequencing · ARISA · Biogeography · Diversity · Phytoplankton

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Introduction

Protists are the major primary producers in the central Arctic Ocean (Caron et al. 2012). The Arctic Ocean promotes the occurrence of species that are specially adapted to the harsh environment (Sakshaug and Slagstad 1991). Local variables, such as multiyear versus annual sea ice or limitations of light and/or nutrients, have the potential to alter the phytoplankton community structure (Li et al. 2009; Tremblay et al. 2009). In particular, picoeukaryotes (0.2–2.0 µm) benefit from the oligotrophic conditions in the Arctic Ocean because of their faster rates of nutrient uptake and reduced metabolic requirements (Grover 1991; Hein et al. 1995). Numerous studies have demonstrated the importance of picoeukaryotes in terms of biomass, production, and diversity, particularly in oligotrophic habitats (Li 1994; Diez et al. 2001; Lopez-Garcia et al. 2001; Moon-van der Staay et al. 2001; Worden et al. 2004; Lovejoy et al. 2006, 2007). Micro- (>20.0 µm) and nanoplanktic (20–2.0 µm) fractions are also significant for the Arctic ecosystem because of their potential to build up great biomass during bloom periods. They are also highly relevant to the carbon and nutrient flux to the deep ocean

(Tremblay et al. 1997; Brown and Landry 2001a, b; Le Borgne et al. 2002).

Studies of the diversity and biogeography of microbial eukaryotic plankton of the central Arctic Ocean are scarce because of the limited accessibility. Investigations of protist communities are consequently patchy with only limited spatio-temporal resolution. Moreover, protist communities in those areas have not been analyzed consistently, due to the application of different tools. Most previous investigations were based on microscopy or flow cytometry. These methods are of limited use for comprehensive assessments of the whole phytoplankton community. Microscopy is well suited for species-specific analyses of the microplankton fraction. The same technique, however, is challenging for the nano- and picoplankton fractions because of their small sizes and paucity of morphological features. In contrast, molecular methods such as the molecular fingerprinting techniques, automated ribosomal intergenic spacer analysis (ARISA), or 454-pyrosequencing are independent of size and morphological features (Caron et al. 2012). Thus, both methods can include all size fractions in surveys of protist communities. Genes coding for the rRNA are particularly well suited for molecular investigations of microbial diversity (Ebenezer et al. 2012). The ubiquity and slow evolutionary rate of the 18S rRNA gene in eukaryotic organisms make it a good marker (Amann and Kuhl 1998; Vaulot et al. 2008). ARISA has frequently been used for quick comparisons of microbial communities. The technique is based on comparison of specific fragment lengths of the intergenic spacer region (ITS) localized between the 18S rDNA and 28S rDNA (Caron et al. 2012). Most studies taking advantage of ARISA focus on the investigation of prokaryotes (Smith et al. 2010). Here, the method is applied for a primary screening of eukaryotes.

454-Pyrosequencing allows high-resolution assessment of microbial communities, given sufficiently deep taxon sampling (Margulies et al. 2005; Stoeck et al. 2010). One caveat is the comparatively high-intrinsic error rate that requires a preprocessing step of raw sequences to enhance the quality (Kunin et al. 2010). The use of 454-pyrosequencing in microbial community studies allowed the first ever consideration of rare species that are suggested to play a key role in ecological buffering and compose the majority of protist diversity (Pedros-Alio 2006; Sogin et al. 2006; Caron and Countway 2009; Stoeck et al. 2010; Caron et al. 2012). Studies focusing on rare bacterial phylotypes have yet to observe evidence of abundance shift under varying environmental conditions even though studies on the whole bacterial community showed a response to abiotic factors (e.g., temperature) and seasonal changes (Galand et al. 2009a; Anderson et al. 2010; Kirchman et al. 2010; Gilbert et al. 2012).

Protist communities in Arctic surface waters are influenced by the light regime, which in turn is influenced by the ongoing sea ice retreat, thinning of multiyear ice or its replacement by annual sea ice (Stroeve et al. 2007). Surface waters are mainly sourced by Atlantic Water (AW), entering through the Fram Strait and Barents Sea, and Pacific Water (PW), entering through the Bering Strait (Rudels et al. 1991; Jones et al. 1998). Both water masses are characterized by specific nutrient signatures. Strong vertical stratification of the Arctic basin can impede upward supply from depth such that surface water is often nutrient limited (Tremblay et al. 2009). The inhabiting protist community is consequently restraint. Different water masses have been reported to host different protist assemblages (Lovejoy et al. 2002). However, molecular studies in the central Arctic Ocean are scarce and to our knowledge based only on clone library sequencing (Bachy et al. 2011).

Considering the ongoing changes in the Arctic Ocean by global warming and their implications, it is crucial to understand the joint role of environmental factors on the protist distribution and to provide detailed data on the prevalent taxa. Hence, the objective of this study is to address the questions: (1) are water masses in the central Arctic Ocean characterized by distinct protist communities? (2) is the ice concentration a driving factor in shaping the protist community in the water column? (3) what is the contribution of the rare biosphere to central Arctic protist communities?

Materials and methods

Study area and sampling program

Samples were collected from August 5 to October 7, 2011, during the ARK-XXVI/3 expedition of the RV Polarstern to the central Arctic Ocean (Fig. 1). Twenty-three stations were sampled for the investigation of the local protist community structure. In parallel, temperature, salinity (S), chlorophyll *a* (Chl *a*) and nutrients were determined for all water samples (Table 1). While temperature and salinity were used from the CTD sensors, Chl *a* and nutrients measurements were analyzed as described below. Melt pond quantity, floe size, ice concentration, ice thickness, and snow thickness were counted by sight to assess the light penetrability (doi:10.1594/PANGEA.803312). Ice thickness was categorized as first-year ice I (0.3–0.7 m), II (0.7–1.2 m), III (>1.2 m) and multiyear ice (>2 m).

Water samples were taken in the upper water layer (1–50 m) by a rosette sampler equipped with 24 Niskin bottles (12 l) and a CTD probe. Two liters of subsamples was taken in PVC bottles and filtered on Whatman GF/F

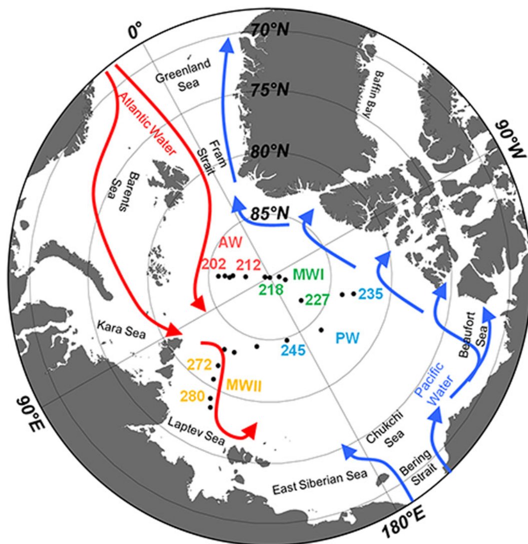

Fig. 1 Study area in the central Arctic Ocean during an expedition of the RV *Polarstern* (ARK-XXVI/3) from August to October 2011. Stations that were analyzed by 454-pyrosequencing are listed and *color* coded according to the water masses: *red* (Atlantic Water); *green* (Mixed Water I); *blue* (Pacific Water); and *yellow* (Mixed Water II)

filters for the Chl *a* and on Isopore Membrane Filters (Millipore, USA) for the protist analysis. Protist cells were collected by sequential filtration of one water sample through three mesh sizes (10, 3, and 0.4 µm) at 200 mbar, in order to obtain a best possible representation of all cell sizes in the molecular approach. The fractionated filtration facilitates a separated amplification in the subsequent PCR step and thus minimizes the danger of under-amplifying picoeukaryotes, due to the limited gene copy number (Zhu et al. 2005). Filters were stored in Eppendorf tubes at −80 °C until further processing. The samples were subjected to ARISA analysis for a quick diversity overview, and a subset of eight samples was analyzed by 454-pyrosequencing for a more comprehensive insight into diversity.

Measurement of Chl *a* and dissolved inorganic nutrients

For measuring Chl *a* concentration, 0.5–2 l of seawater was filtered through Whatman GF/F glass fiber filters and stored at −20 °C. The filters were extracted in 90 % acetone and analyzed with a Turner-Design fluorometer according the method described in (Edler 1979; Evans and O'Reily 1987). Calibration of the fluorometer was carried out with standard solutions of Chl *a* (Sigma, Germany). To quantify the concentration of dissolved inorganic nutrients (nitrate, nitrite, ammonium, phosphate, and silicate), samples were measured directly on board and analyzed according to standard methods (Kattner and Becker 1991; Kerouel and Aminot 1997) with a nutrient analyzer (Evolution III, Alliance Instruments, Austria).

DNA isolation

DNA extraction was carried out with E.Z.N.A^{TM} SP Plant DNA Kit Dry Specimen Protocol (Omega Bio-Tek, USA) following the manufacturer's protocol. Subsequently, the extracts were stored at −20 °C until analysis.

Automated ribosomal intergenic spacer analysis (ARISA)

Initially, equal volumes of DNA isolates of each size class (>10, 10–3 and 3–0.4 µm) were pooled for each sample. The amplification of the eukaryotic ITS1 region was carried out with the fluorescently (dye 6-FAM; 6-carboxy-fluorescein) labeled primer, the primer 1528F (5′-GTA GGT GAA CCT GCA GAA GGA TCA-3′), modified after Medlin et al. (1988), and the primer ITS 2 (5′-GCT GCG TTC TTC ATC GAT GC-3′) (White et al. 1990). The PCR mixture contained 1× HotMasterTaq buffer Mg^{2+} 2.5 mM (5′Prime, USA), 0.4 U HotMaster Taq polymerase (5′Prime, USA), 10 mg/ml BSA, 10 mM (each) dNTP (Eppendorf, Germany), 10 µM of each Primer, and 1 µl of template DNA in a final volume of 20 µl. The PCR amplification was carried out in a MasterCycler (Eppendorf, Germany) under the following conditions: first an initial denaturation step for 3 min at 94 °C succeeded by 35 cycles (denaturation at 94 °C for 45 s, annealing at 55 °C for 1 min, and extension at 72 °C for 3 min) and followed by a final extension at 72 °C for 10 min. The PCR in the analysis was carried out in technical triplicates to buffer potential variances in PCR fragment sizes determined by capillary electrophoresis (ABI 310 Prism Genetic Analyzer, Applied Biosystems, USA).

Data processing of ARISA

The analysis of the electropherograms was carried out with the GeneMapper v4.0 software (Applied Biosystems, USA). To exclude fragments originating from primers or primer dimers, we applied a threshold of 50 bp for peaks. A binning was carried out in R to remove background noises and to obtain sample-by-binned-operational-taxonomic-unit tables (Ramette 2009; R Development Core Team 2008). The resulting data were converted to a presence/absence matrix. Differences in the phytoplankton community structure represented by differences in the respective ARISA profiles were determined by calculating the Jaccard index with an ordination of 10,000 restarts under the implementation of the R package vegan (Oksanen et al. 2011). Nonmetric multidimensional scaling (NMDS) plots were computed, and an ANOSIM was performed to test for significant differences in protist communities according to the varying hydrology [AW, Mixed

Table 1 Hydrological and biological properties at the chlorophyll maximum

Station-ID	Date (month/day/year)	Longitude	Latitude	Depth (m)	S	NO_3	PO_4	Si	*T* (°C)	Ic (%)	It (m)	fl (m)	Mp (%)	Chl *a* (ng/l)	
202*	08/14/2011	59°55.91′E	85°48.19′N	25	33.48	3.61	0.29	1.8	−1.72	90	0.7–1.2	100–500	50	0.32	Atlantic Water
205	08/15/2011	59°15.00′E	86°19.64′N	10	33.28	0.88	0.13	0.98	−1.71	80	>1.2	>2,000	30	0.81	
207	08/16/2011	61°14.23′E	86°42.5′N	25	33.69	2.3	0.26	2.42	−1.68	100	>1.2	100–500	50	0.31	
209	08/17/2011	58°29.30′E	86°59.25′N	14	32.84	1.2	0.23	2.82	−1.73	90	>1.2	20–100	30	0.17	
212*	08/19/2011	59°57.42′E	88°1.10′N	10	32.80	2.65	0.45	5.07	−1.69	100	0.7–1.2	>2,000	40	0.06	
216	08/21/2011	60°42.18′E	89°35.98′N	20	32.16	4.78	0.96	13.29	−1.67	90	>1.2	20–100	50	0.24	
218*	08/22/2011	148°06.72′E	89°57.86′N	20	32.42	5.55	0.99	13.2	−1.69	90	>1.2	100–500	50	0.19	Mixed Water I
220	08/24/2011	116°42′W	89°14.9′N	18	32.18	3.09	0.65	8.48	−1.64	90	>1.2	100–500	20	0.19	
222	08/26/2011	128°15′W	88°44.2′N	10	30.49	0.53	0.55	6.3	−1.62	100	>1.2	20–100	40	0.24	
222	08/26/2011	128°15′W	88°44.2′N	25	31.92	1.13	0.42	5.12	−1.59	100	>1.2	20–100	40	0.17	
227*	08/29/2011	155°02.72′W	86°51.64′N	10	31.02	0.3	0.34	4.12	−1.59	80	>1.2	20–100	40	0.10	
233	09/02/2011	132°21.55′W	83°55.95′N	25	29.95	0.02	0.67	1.58	−1.31	90	0.7–1.2	500–2,000	40	0.04	Pacific Water
235*	09/03/2011	130°02.34′W	83°1.80′N	50	30.75	3.42	0.96	7.28	−1.47	100	0.7–1.2	500–2,000	40	0.11	
239	09/05/2011	164°12.36′W	84°04.41′N	25	30.12	0.58	0.26	3.83	−1.46	100	>1.2	500–2,000	20	0.15	
245*	09/09/2011	166°24.86′E	84°47.67′N	18	30.32	1.3	0.26	5.57	−1.6	100	0.7–1.2	500–2,000	10	0.04	
250	09/11/2011	139°54.35′E	84°23.24′N	25	31.44	1.33	0.26	3.97	−1.54	80	0.3–0.7	100–500	40	0.08	
257	09/13/2011	124°54.20′E	83°19.97′N	10	30.47	0.61	0.19	2.35	−1.65	90	0.3–0.7	20–100	20	0.23	Mixed Water II
259	09/14/2011	117°56.78′E	83°8.92′N	10	30.95	1.04	0.24	3.0	−1.69	70	0.3–0.7	20–100	20	0.85	
260	09/14/2011	114°39.51′E	82°59.65′N	10	31.72	–	–	–	−1.74	70	0.7–1.2	100–500	50	–	
272*	09/19/2011	119°58.14′E	81°46.50′N	10	31.60	0.11	0.14	1.55	−1.64	10	0.3–0.7	< 20	0	0.27	
276	09/20/2011	121°19.8′E	80°38.6′N	10	30.83	0.29	0.18	3.16	−0.09	0	0	0	0	0.19	
280*	09/21/2011	124°07.59′E	79°8.84′N	10	30.54	0.55	0.21	3.66	0.87	0	0	0	0	0.35	
285	09/22/2011	125°48.11′E	78°29.59′N	10	30.55	0.08	0.16	4.85	1.58	0	0	0	0	0.39	
290	09/23/2011	128°09.40′E	77°07.60′N	10	25.66	–	–	–	3.05	0	0	0	0	0.26	

Nutrients are given in µM. Marked stations (*) were further used for 454-pyrosequencing

S salinity, *T* temperature, *Ic* sea ice concentration, *It* sea ice thickness, *fl* floe size, *Mp* melt pond

Table 2 Analytical process and quality control of 454-pyrosequencing reads (AW: St.202 and 212; MWI: St.218 and 227; PW: St.235 and 245; MWII: St.272 and 280)

Station-ID								
	202	212	218	227	235	245	272	280
Total reads	8,898	24,981	8,557	26,161	70,490	140,918	23,079	35,850
Quality filtering								
<300 bp	1,253	1,959	2,541	3,128	5,013	16,882	4,014	4,614
Homopolymers	785	1,820	126	1,707	5,433	13,300	1,825	2,108
>1 ambiguous N	597	1,971	494	1,796	5,488	10,518	1,354	2,628
Chimeras	2,552	7,485	1,613	1,227	4,987	10,834	1,978	2,536
Nontarget species	0	2,380	14	1,119	5,539	4,101	0	4,140
Final read number	3,711	9,366	3,769	17,184	44,030	85,283	13,908	19,824
Singletons	1,275	4,493	1,428	4,115	13,766	28,166	3,707	8,438
OTUs[a]	164	658	371	764	1,752	3,126	587	1,141
OTUs (abundant)	7	7	10	11	11	12	7	14
OTUs (rare)	157	651	361	753	1,741	3,114	580	1,127
Subsampling	3,711	3,711	3,711	3,711	3,711	3,711	3,711	3,711
Singletons	1,349	1,432	1,392	959	1,103	1,131	902	1,015
OTUs[a]	149	250	299	244	269	276	180	260
OTUs (abundant)	9	8	10	13	11	12	7	14
OTUs (rare)	140	242	289	231	258	264	173	246

[a] *OTU* operational taxonomic unit: 97 % similarity level

Water I (MWI), PW, and Mixed Water II (MWII)]. The same R package was used for a more detailed analysis of the clustering, using the hclust function. A Mantel test (10,000 permutations) was performed to test for a correlation of the protist community structure distance matrix (Jaccard) and the environmental distance matrix (Euclidean). The Mantel test was computed for 22 samples, excluding stations 267 and 290 due to fragmentary environmental factor data. The ade4 R package was applied for the Mantel test (Dray and Dufour 2007). To assess the significance of single environmental variables, a permutation test was calculated using the envfit function of the vegan R package. Subsequently, a PCA of the protist community and the significant environmental factors distances was performed using the same R package.

454-Pyrosequencing

For 454-pyrosequencing, a region (~670 bp) containing the hypervariable V4 region of the 18S rDNA was amplified with the primer set 528F (5′-GCG GTA ATT CCA GCT CCA A-3′) and 1055R (5′-ACG GCC ATG CAC CAC CAC CCA T-3′) (modified after Elwood et al. 1985). Eight samples were analyzed, two from each water mass (Table 2). A PCR for each size fraction was carried out, using the same reaction conditions as described above (ARISA). Equal volumes of the resulting PCR products of each size fraction from each sample were pooled and purified with the Mini Elute PCR Purification Kit (QIAgen, Germany). The purified PCR products were sent to GATC Biotech AG (Germany) for final pyrosequencing with a 454 Genome Sequencer FLX system (Roche, Germany). Sequencing was performed unidirectionally from the adaptor A side of one pool, while the provided quality score was used for a first filtering of low-quality reads.

Quality control and data processing of 454-pyrosequencing

Since 454-pyrosequencing reads can be affected by high error rates (Huse et al. 2007, 2010), raw sequences were processed to increase the quality and to decrease the effects of low-quality reads on diversity estimates. In the course, sequences shorter than 300 bp were excluded to guarantee analysis of the whole V4 region. Sequences longer than the amplified fragment (~670 bp) and with incorrect F-primer sequences were removed as well. Furthermore, after manual screening, sequences with more than one ambiguous base (N) or homopolymers of seven or more successive bases (≥7 bps) were excluded from further analysis. Huse et al. (2007) showed that the removal of sequences with ambiguous bases can reduce the error rate from initial 0.5 to 0.25 %. Homopolymers of 5 bps were found to contribute the highest proportion of induced errors in the GS-FLX system (Behnke et al. 2011). Chimeras were detected and excluded using UCHIME 4.2.40 software (Edgar et al. 2011) that uses the same reference database as for phylogenetic annotation by PhyloAssigner (see below). Based on the most recent critical reviews concerning different denoising pipelines, we processed raw data by applying single

tools rather than loading these data in developed pipelines that can transform finale sequences, inconsistent with the spectrum of errors (Comeau et al. 2013; Gaspar and Thomas 2013). The final processed sequences were clustered into artificial operational taxonomic units (OTUs) or phylotypes by using the software package Lasergene 10 Seqman Pro (DNAStar, USA). A threshold of 97 % was applied to minimize the risk of overestimating the diversity. The application of the 97 % similarity threshold further insures a comparison of our data set with previously published data, generated using the same threshold. Moreover, Kunin et al. (2010) and Behnke et al. (2011) showed that a threshold of 97 % is capable of removing most of the sequencing errors and displaying the original protist diversity. All singletons, defined as an OTU composed of one single sequence that only occurs once in the whole analysis, were removed to evade possible errors induced by the sequencing process. We used consensus sequences of the OTUs in order to further reduce the number of sequencing errors in the diversity analysis. The consensus sequences were placed into a reference tree built up by a selection of 1,200 high-quality sequences from the SSU Ref 108 SILVA database, containing representatives of all main eukaryotic phyla. This involved the use of the bioinformatics pipeline PhyloAssigner that aligns consensus sequences to the reference multiple sequence alignment and assigns them phylogenetic positions in the reference tree using a maximum likelihood algorithm (Vergin et al. 2013). This procedure has the benefit of preserving as much phylogenetic information as possible. Sequences that affiliated with non-protist phyla in the tree under a threshold of 99 % were excluded from further analyses. For a more detailed taxonomic insight into consensus sequences that were placed at lower taxonomic levels (e.g., class or order), reads were additionally aligned with the SILVA aligner (Pruesse et al. 2007) and placed into the ARB reference database tree containing around 50,000 eukaryotic sequences (Ludwig et al. 2004). Based on the limited sequence length obtained by 454-pyrosequencing, we identified phylotypes to the genus but not to the species level. Multiple phylotypes that clustered to the same genus but differed in at least 3 % were numbered. Relative abundances of high-quality reads were calculated and used for discrimination between abundant and rare OTUs that accounted for $\geq$1 and <1 % of the total read number, respectively. For comparison of the sampled diversity by 454-pyrosequencing (rare faction curve analysis), a subsampling of the final processed sequences to the lowest read number (St.202; 3,722 reads) was applied in order to normalize the different sequencing depths. Rarefaction curves were calculated using the freeware program Analytic Rarefaction 1.3. The 454-pyrosequencing sequences were deposited at GenBank's Short Read Archive (SRA) under Accession No. SRA064761.

Results

Physical and chemical environment

Twenty-three stations were sampled in the central Arctic Ocean representing four regions with different water masses: AW, MWI, PW, and MWII (Table 1; Fig. 1). Based on the combination of temperature, salinity, and nutrients signatures, stations 202–216 were allocated to AW, stations 218–227 to MWI, stations 233–250 to PW, and stations 257–290 to MWII. AW stations were on average characterized by lowest temperature (−1.69 °C), highest salinity (32.95), nitrate (2.99 µM), and silicate (5.65 µM), while stations in the PW displayed lower salinity (30.51), nitrate (1.33 µM), and silicate (4.45 µM). MWI properties were intermediate between those of AW and PW or similar to either of them. MWII is more clearly distinguishable on account of its lowest salinity (30.29), nitrate (0.48 µM), and silicate (3.09 µM), but highest temperature (−0.16 °C).

The ice concentration was at least 70 % at all stations in AW, MWI, PW and at three stations located in MWII. MWII sea ice concentration was on average ~10 %, including four stations with no ice coverage. Sea ice in the AW and MWI was in general thicker (1.2–2.0 m) than in the PW or MWII (<1.2 m). Floe sizes in AW and PW were >100 m at most of the stations, but mostly <100 m in MWI and MWII. Melt ponds were found in high numbers in the AW (~50 %) and MWI (~40 %) and less commonly in the PW and MWII.

Chl *a* concentrations were generally low (0.04–0.85 µg/l). The highest Chl *a* mean concentration was observed in MWII (0.36 µg/l), while the concentrations in AW (0.19 µg/l) and MWI (0.18 µg/l) were significantly lower and lowest in PW (0.08 µg/l).

ARISA

Twenty-three stations were used for the ARISA, including one station (222) with two depths. ARISA was used to test for significant correlations between protist community structures and water masses and to guide selection of two samples of each water mass for 454-pyrosequencing. The analysis resulted in 260 different fragments of the ribosomal ITS1-region with an average fragment number of 74 per sample, ranging between 46 (St.202; AW) and 107 (St.239; PW). Fragment sizes ranged between 50 and 444 bp. In total, 54 fragments were unique in the analysis, while four fragments were ubiquitous. The similarity between the ARISA profiles of all samples was calculated by the Jaccard index and is presented in a NMDS plot (Fig. 2).

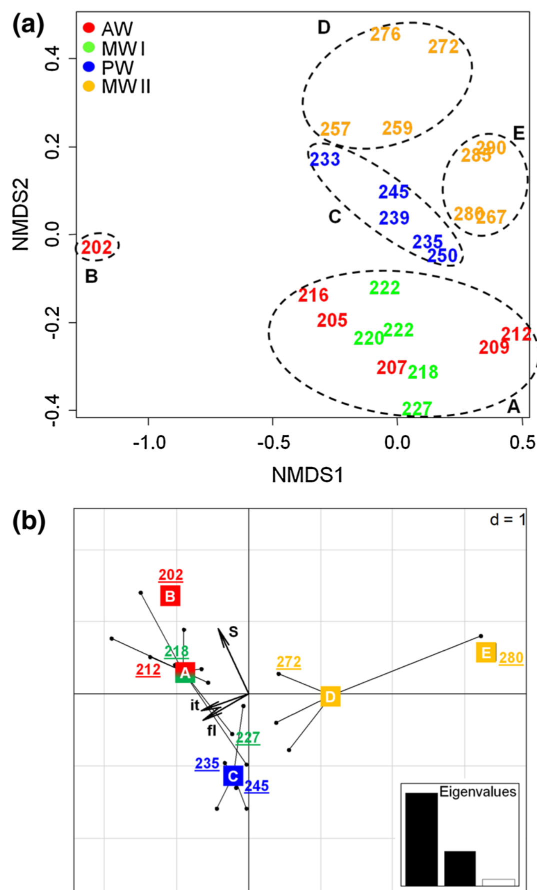

Fig. 2 ARISA. **a** NMDS plot of protist community structure relations computed by the implementation of the Jaccard index (stress = 0.19). **b** PCA of the significant environmental factors and the ARISA grouping. Labeled stations were further analyzed by 454-pyrosequencing. *Color code* as in Fig. 1

The ANOSIM analysis of an a priori water-mass-specific grouping according to the classification in AW, MWI, PW, and MWII showed significant differences among all groups (*R* value 0.35, *p* value 0.001). A more detailed cluster analysis segregated the samples into five different clusters (A–E), in which some clusters were located in proximity (Fig. 2a). Cluster A was composed of samples that originated from AW (St.205–216) and MWI (St.218–227). Cluster B consisted of just one AW sample (St.202) and was considered an outlier because of its highly different ARISA profile. Cluster C included samples from PW, while Clusters D (St.257–276) and E (St.267 and 290) both contained samples from MWII. In principle, the clustering correlated well with the water mass properties at the sampling stations. A Mantel test, including all environmental variables, resulted in a *p* value of 0.07, indicating a nonsignificant similarity between ARISA data and environmental data, at least at the alpha level of 0.05. The fitting of environmental factors to the NMDS ordination, however, resulted in three significant linear correlations with the ranked distances between the samples. These were with salinity, ice thickness, and floe size. Ice thickness presented the strongest correlation (*p* value 0.00) and was followed by salinity (*p* value 0.01) and floe size (*p* value 0.03). Temperature showed an almost significant *p* value of 0.055 and hence must not be disregarded. Nutrients such as nitrate (*p* value 0.08), phosphate (0.3), and silicate (0.18) did not correlate with the NMDS ordination. Figure 2b shows a principal component analysis (PCA) of the three environmental factors that explained most of the differences in the protist community structures. In the PCA, clusters D and E are separated from the other clusters by lower ice thickness and floe size, while clusters A and B are separated by higher salinity.

454-Pyrosequencing

A subset of eight samples was chosen, based on ARISA, for a detailed analysis of the protist community composition. Each water mass was represented by two samples and each cluster by at least one sample. In total, 454-pyrosequencing resulted in an average of 42,366 raw reads per sample (Table 2). Quality filtering left on average 24,634 reads that clustered in 164 (sample 202) to 3,126 (sample 245) OTUs. OTU numbers often correlated with sequencing depth, showing larger OTU numbers at stations with larger read numbers and vice versa. Subsampling to the smallest read number qualified the OTU numbers, showing smaller differences, that range between 149 (St.202) and 299 (St.218) OTUs. The overall OTU decrease mostly influenced the abundance of rare OTUs, while abundant OTUs were little affected. Subsampling showed no clear effect on the distribution of major taxonomic groups for either the abundant or rare biosphere (Fig. S1). Rarefaction curves of each station were created after a subsampling to present the local species richness and to serve as a reference for the covered diversity in a sample (Fig. 3). No saturation of the rarefaction curves was observed, suggesting that the true diversity was not discovered at any station. This finding advises for some caution in protist diversity interpretations. The relative abundance of the major taxonomic group distribution is presented in Fig. 4, once displayed for the abundant biosphere (including OTUs that account for ≥1 % of the total relative abundance at the sampling station) and once for the rare biosphere (including OTUs that account for <1 % of the total relative abundance at the sampling stations).

Diversity of the abundant biosphere

The abundant biosphere accounted for stable proportions within the protist community at all stations. The average contribution was about 67 % with a maximum proportion

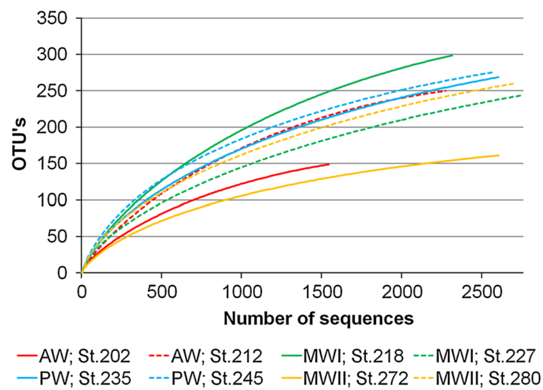

Fig. 3 Rarefaction curves of the 454-pyrosequencing after quality control, subsampling (3,711 reads), and clustering at 97 % similarity level

of 80 % at St.202 and a minimum of 51 % at St.245. A total of 38 different phylotypes accounted for the abundant biosphere in at least one of the eight sampling sites (Table 3). Out of the four water masses, AW (St.202 and St.212) showed the smallest diversity with seven classified abundant phylotypes on average. The diversity increased in the other water masses, showing on average 10.5 phylotypes in the MWI (St.218 and St.227), 11.5 in the PW (St.235 and St.245), and 10.5 in the MWII (St.272 and St.280). Subsampling only affected the average abundant phylotype number in AW (8.5) and MWII (11.5).

Figure 4a shows the distribution of taxonomic protist divisions in the abundant biosphere, while Table 3 shows the diversity of abundant phylotypes. Variability of the five major taxonomic divisions between the sampling sites was assessed by calculating the standard deviations of their contributions, which were around 8 % for haptophytes, 13 % for chlorophytes, 5 % for stramenopiles, 12 % for dinoflagellates, and 1 % for ciliates. Alveolates were the most prominent taxonomic group and divided in dinoflagellates and ciliates. Dinoflagellates were far more abundant than ciliates and contributed on average 30 % to the abundant biosphere, while the average ciliate contribution was fewer than 5 %. Maximum and minimum dinoflagellate proportions were found at St.212 (47 %; AW) and St.272 (9 %; MWII), respectively. A classification of phylotypes on lower taxonomic levels (e.g., genus or species) was difficult for both dinoflagellates and ciliates. Dinoflagellates were represented in the abundant biosphere by *Gymnodinium* sp., *Woloszynskia* sp., and different Syndiniales phylotypes. The widest diversity was found in the order Syndiniales with nine phylotypes that were almost exclusively limited to PW. One phylotype, Syndiniales 2, was abundant at all stations with a highest average proportion in AW (35 %) and a consistent decrease in the following water masses (MWI: 29 %; PW: 16 %; MWII: 12 %). *Gymnodinium* sp.1 was also widely distributed but in smaller average proportions of 1.5–4.0 %.

Chlorophytes formed another dominant taxonomic group with high-read abundances at all sampling sites. The average contribution was 19 % with maximum average proportions in AW (27 %) and MWII (25 %). Three phylotypes, such as *Micromonas* sp.1, *Micromonas* sp.2, and *Pyramimonas* sp., contributed to the abundant biosphere. Only one phylotype, *Micromonas* sp.1, was abundant at all stations. The other phylotypes, *Micromonas* sp.2 and *Pyramimonas* sp., were only present at St.227 (MWI), where *Micromonas* sp.1 showed the smallest presence of 7 %.

Haptophytes accounted on average for 12 % of the abundant biosphere and were consisted of two phylotypes of *Phaeocystis* sp. and two of *Chrysochromulina* sp. *Phaeocystis* sp.2 was distributed more extensively than *Phaeocystis* sp.1, which was only found in the transition zone of MWI and PW. *Phaeocystis* sp.1 contributed the highest percentage in the MWI (St.227; 10 %), while *Phaeocystis* sp.2 was most abundant in the MWI (6 %) and PW (7 %) on average. *Chrysochromulina* sp. showed similar characteristics to *Phaeocystis* sp. by having one phylotype (*Chrysochromulina* sp.1) more widely distributed than the other (*Chrysochromulina* sp.2).

Picobiliphytes and stramenopiles were minor representatives in our “abundant” data set. While picobiliphytes were limited to one station in AW (St.212), showing small proportions of around 1.3 %, stramenopiles were missing in AW but were found in their highest average proportions in MWI (18 %). Out of five stramenopile phylotypes, two were identified to the genus level, as *Dictyocha* sp. and *Fragilariopsis* sp. The distribution of *Dictyocha* sp. was limited to St.280 (9 %), while *Fragilariopsis* sp. display their highest average read abundances in MWI (4 %). Marine stramenopiles (MAST) were represented by two phylotypes (MAST I 1 and 2) that were both limited to one station (St.245).

Diversity of the rare biosphere

The rare biosphere (<1 %) accounted on average for 32 % of all sequences in the protist assemblage. Its maximum and minimum contributions were 46 % at St.245 and 18 % at St.202. A total of 5,311 different phylotypes made up for the rare biosphere. The diversity of these phylotypes differed strongly between water masses. AW presented the least diversity with 404 phylotypes on average. In MWI and MWII, the diversity increased to 557 and 853 phylotypes. Maximum numbers were found in PW (2427). Subsampling decreased average phylotype numbers in the different water masses to 191 phylotypes in AW, 260 in MWI, 261 in PW, and 209.5 in MWII. AW thereby still presented the least diversity in the rare biosphere, while maximum diversity was still observed in the PW.

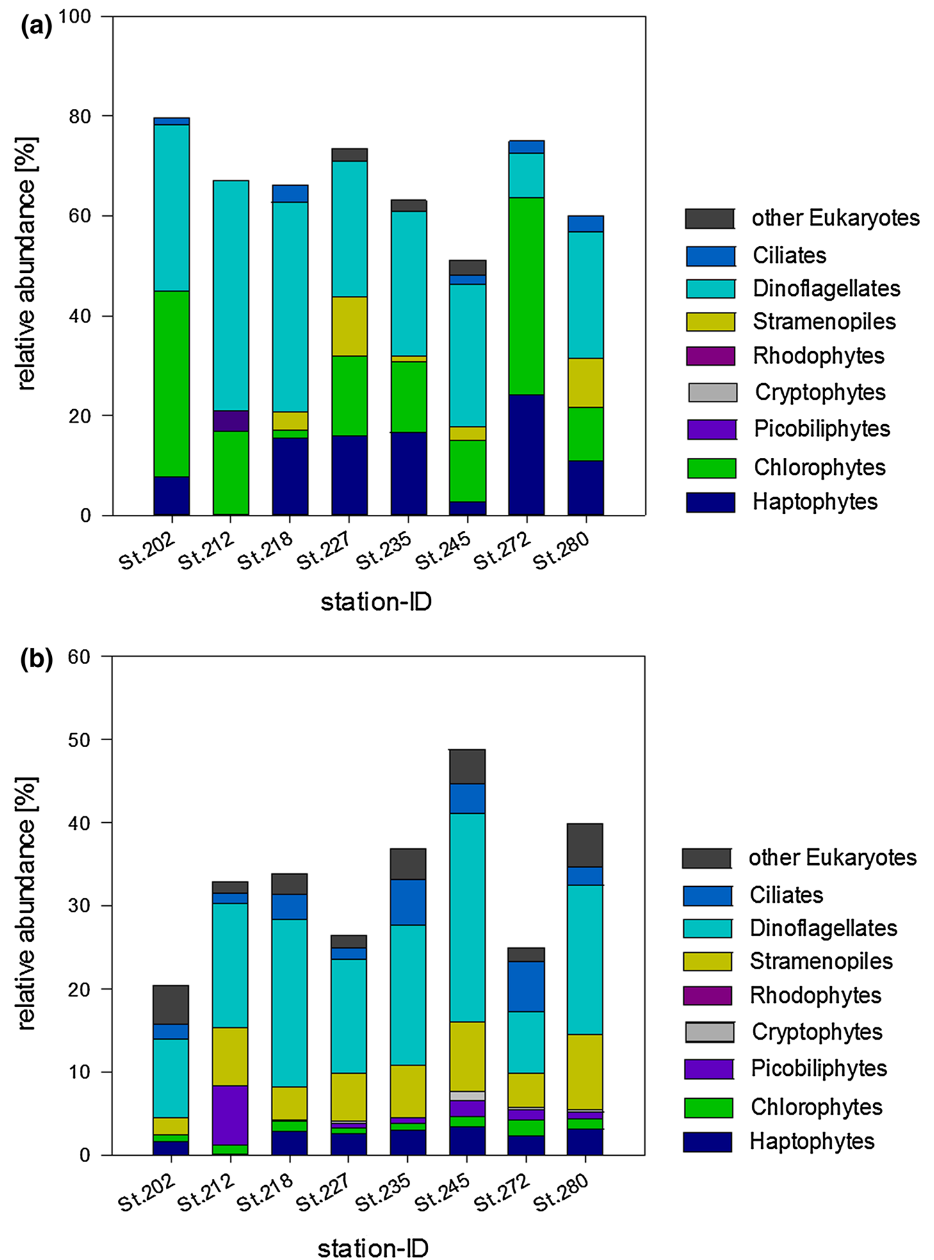

Fig. 4 Relative abundance of major taxonomic groups at the sampling sites (AW: St.202 and 212; MWI: St.218 and 227; PW: St.235 and 245; MWII: St.272 and 280) obtained by 454-pyrosequencing: **a** abundant biosphere (including sequences of ≥1 % abundance at a 97 % similarity level) and **b** rare biosphere (including sequences of <1 % abundance at a 97 % similarity level)

Figure 4b shows the distribution of taxonomic protist divisions in the rare biosphere, while Table 4 shows the diversity of rare phylotypes. In most cases, the variability of the major five taxonomic divisions was smaller for the rare than for the abundant biosphere. Haptophytes varied by 1 %, chlorophytes <1 %, stramenopiles 2 %, dinoflagellates 6 %, and ciliates 2 %. The taxonomic composition reflected the taxonomical structure of the abundant biosphere. Reads of alveolates and particularly of dinoflagellates were again most abundant. Rare dinoflagellates contributed on average 16 % to the total protist community. Maximum and minimum proportions were found in PW at St.245 (25 %) and in MWII at St.272 (7 %). Ciliates occurred at all sampling sites with a mean contribution of 3 %. A classification of rare phylotypes was difficult because the majority could not be exactly assigned at the genus level. Therefore, a section of precisely identified phylotypes (phylotypes that match with sequences in the database), ranging between 0.1 and 0.99 % in relative abundance, is given in Table 4. The selection of rare phylotypes comprised four different dinoflagellates of which two (Woloszynskia sp.1 and 2) were also found in the abundant biosphere. The other phylotypes were classified as *Pelagodinium* sp. and *Prorocentrum* sp., whose greatest abundance was in MWI (St.218).

Table 3 Phylotypes of the abundant biosphere (≥1 %)

	Station-ID							
	202	212	218	227	235	245	272	280
Chlorophytes								
Micromonas sp.1	37.1	16.9	1.6	7.2	14.2	12.4	39.5	10.7
Micromonas sp.2	r	–	r	6.3	r	r	r	r
Pyramimonas sp.	–	–	–	2.5	r	r	r	r
Haptophytes								
Phaeocystis sp.1	r	–	r	10.4	2.5	1.5	r	r
Phaeocystis sp.2	3.3	r	8.5	2.9	12.4	1.15	2.0	3.9
Chrysochromulina sp.1	4.5	–	6.6	2.6	1.7	r	r	1.2
Chrysochromulina sp.2	–	–	–	r	–	r	22.4	6.0
Picobilliphytes								
Picobilliphyte 1	–	1.9	–	–	–	–	–	–
Picobilliphyte 2	–	1.1	–	r	r	r	–	r
Picobilliphyte 3	–	1.0	–	r	r	r	r	–
Stramenopiles								
Stramenopile 1	–	–	r	6.8	r	r	r	r
MAST I 1	–	r	r	r	r	1.9	r	r
MAST I 2	–	r	r	r	r	1.0	r	r
Dictyocha sp.	–	–	r	r	r	r	r	8.8
Fragilariopsis sp.	r	r	3.6	5.1	1.0	r	r	1.1
Dinoflagellate								
Gymnodinium sp.1	4.3	3.5	5.1	2.8	1.5	r	1.7	2.4
Gymnodinium sp.2	r	r	1.3	r	r	r	r	1.7
Gymnodinium sp.3	–	r	1.3	r	1.0	r	r	–
Woloszynskia sp.1	r	–	–	r	r	r	r	1.4
Woloszynskia sp.2	1.1	r	–	r	–	–	–	r
Syndiniales 1	r	1.1	r	r	r	r	r	r
Syndiniales 2	28.0	42.1	34.4	24.4	21.3	9.9	7.0	17.4
Syndiniales 3	r	–	r	r	4.0	r	r	r
Syndiniales 4	–	r	r	r	r	10.3	–	r
Syndiniales 5	–	–	r	r	–	5.3	–	1.0
Syndiniales 6	–	–	r	–	1.3	r	–	r
Syndiniales 7	–	–	r	–	r	1.0	–	r
Syndiniales 8	–	–	–	–	r	1.8	–	r
Syndiniales 9	r	–	r	r	r	–	r	1.2
Ciliates								
Strombidium sp.1	r	r	1.7	r	r	r	r	r
Strombidium sp.2	–	–	–	r	r	r	1.4	–
Ciliate 1	–	r	1.6	r	r	r	r	r
Ciliate 2	1.3	–	r	r	r	r	r	r
Ciliate 3	r	–	r	r	r	r	r	1.3
Ciliate 4	–	–	–	r	–	r	1.2	–
Ciliate 5	–	–	r	r	r	2.0	–	1.9
Undefined eukaryote 1	–	–	r	2.5	–	r	–	–
Undefined eukaryote 2	–	r	r	r	2.2	3.0	–	r

Relative contributions are presented in percent, while (r) refers to the occurrence in the rare biosphere and (–) to no occurrence at all (AW: St.202 and 212; MWI: St.218 and 227; PW: St.235 and 245; MWII: St.272 and 280)

Chlorophytes accounted for an average percentage of 1 %. One phylotype, *Pyramimonas* sp., was missing in AW but was present in rare proportions in PW and MWII.

Haptophytes contributed on average 2.4 % to the rare biosphere in the protist community. However, no phylotype matched a representative in the database.

Table 4 Phylotypes of the rare biosphere (<1 %)

	Station-ID							
	202	212	218	227	235	245	272	280
Chlorophytes								
Pyramimonas sp.	–	–	–	a	<0.1	0.3	0.2	0.4
Stramenopiles								
Stramenopile 1	–	–	0.4	a	0.1	0.9	0.4	0.1
MAST I 1	–	0.8	0.1	0.2	<0.1	a	0.1	0.3
MAST I 2	–	0.4	0.2	<0.1	0.3	a	0.2	0.5
MAST III 1	–	–	–	0.3	0.9	0.9	–	0.7
Dictyocha sp.	–	–	0.7	0.2	0.6	<0.1	0.2	a
Florenciella sp.	–	–	0.1	0.2	0.4	0.3	0.3	0.2
Diadesmis sp.	–	0.2	0.1	–	–	–	–	–
Fragilariopsis sp.	<0.1	0.3	a	a	a	0.1	<0.1	a
Telonema sp.	0.4	–	0.9	0.5	0.7	0.9	<0.1	0.7
Dinoflagellates								
Woloszynskia sp.1	0.2	–	–	0.4	<0.1	<0.1	0.3	a
Woloszynskia sp.2	a	0.2	–	<0.1	–	–	–	0.2
Pelagodinium sp.	–	<0.1	0.8	0.6	<0.1	<0.1	0.1	<0.1
Prorocentrum sp.	0.6	0.7	0.5	0.2	0.3	0.3	0.2	0.2

A selection of rare phylotypes that were recovered in the database and represented by proportions of 0.1–0.99 %. Relative contributions are presented in percent, while (a) refers to the occurrence of the phylotype in the abundant biosphere and (–) to no occurrence at all (AW: St.202 and 212; MWI: St.218 and 227; PW: St.235 and 245; MWII: St.272 and 280)

Stramenopiles demonstrated a mean share of 5.8 %. MASTs were represented by three phylotypes. While MAST I 1 and 2 were also recovered in the abundant biosphere, MAST III 1 was only present in the rare biosphere with its maximum proportions in PW (0.8 %). In contrast, the rare biosphere's proportions of MAST I 1 and 2 varied between <0.1 and 0.8 %. Diatoms also displayed a greater diversity in the rare biosphere. Besides *Fragilariopsis* sp., two additional phylotypes were recovered that affiliated to *Florenciella* sp. and *Diadesmis* sp. *Diadesmis* sp. occurred in the transition zones of AW (St.212) and MWI (St.218), while *Florenciella* sp. was missing in AW and presented its highest proportion in PW (0.4 %) and MWII (0.3 %).

Cryptophytes and rhodophytes were present at most of the stations with mean proportions of 0.2 % and <0.1 %, respectively. However, phylotypes could not be identified in more detail or were below the selection limit (0.1 %).

Discussion

Protist community structure and water masses

One aim of this study was to investigate whether water masses in the central Arctic Ocean are characterized by distinct protist communities. Previous studies observed that distinct protist assemblages were linked to distinct water masses in open water (Lovejoy et al. 2002; Hamilton et al. 2008; Lovejoy and Potvin 2011), implying that hydrographical structure can be considered as a proxy for protist distribution. However, the influence of water mass might be less in the central Arctic Ocean because of the additional role that sea ice plays there. To address this possibility, we combined ARISA and 454-pyrosequencing for correlation of protist diversity and community structure differences with different hydrographical properties.

ARISA profiles clustered on the basis of water mass and were significantly associated with salinity, ice thickness, and floe size. Salinity can be used to discriminate between low saline PW and higher saline AW. The grouping of protist community structures, in one AW/MWII cluster, one PW cluster, and two MWII clusters, suggests a correlation between protist community composition and hydrographic regime in the central Arctic Ocean. This finding is in line with other studies that reported a biogeography of microbial communities (archaea, bacteria, and protists) that is often correlated with local water mass properties (Gradinger and Baumann 1991; Lovejoy et al. 2002; Hamilton et al. 2008; Galand et al. 2009a, b, c; Lovejoy and Potvin 2011).

An association between water mass and protist diversity was also reflected in the results of 454-pyrosequencing, albeit less pronounced than in those of ARISA. Indeed, 454-pyrosequencing-derived differences in relative abundance of major taxonomic divisions in both the abundant and rare biosphere demonstrate no evidence for water mass influence. One reason for this might be the limited sample number in the 454-pyrosequencing, including two samples of each water mass. However, some of these samples showed quite different community structures in the ARISA results. Therefore, it is more likely that the correlation

between water mass and protist diversity is just not reflected at higher taxonomic level. Consistent with this view, a previous study on the reflections of species composition (mollusks) at different taxonomic levels revealed that either genera or families may be used as an effective taxonomic level for detecting spatial differences in species diversity (Terlizzi et al. 2009). For this reason, we extended our investigation from a preliminary taxonomic group-based analysis to a comprehensive phylotype-specific analysis of the abundant and rare biosphere.

Ubiquitous phylotypes address environmental changes solely in terms of abundance shifts and not in terms of presence/absence patterns. Two phylotypes in the abundant biosphere, Syndiniales 2 and *Micromonas* sp.1, displayed such a water mass association by varying in relative contributions. *Micromonas* has often been reported to contribute significantly to Arctic protist assemblages (Lovejoy et al. 2002, 2006, 2007; Potvin and Lovejoy 2007, 2009; Lovejoy and Potvin 2011). Phylogenetic analysis revealed five distinct clades, in which one (CCMP 2099) was predominantly found in the Arctic Ocean (Slapeta et al. 2006; Lovejoy et al. 2007). This phylotype affiliated to our dominant *Micromonas* sp.1 phylotype, which was particularly abundant in AW and MWII. In contrast, *Micromonas* sp.2 affiliated to a clade composed of samples (e.g., CCMP1545) that originated from the English Channel (Slapeta et al. 2006; Lovejoy et al. 2007). The observations that minimum proportions of *Micromonas* were limited to MWI and that similar proportions were recovered in both PW stations point to an influence of water mass on their distribution profile.

Other phylotypes displayed more distinct water mass relations based on patterns of presence and absence. *Fragilariopsis* sp., a prominent Arctic species, was rare in AW but most abundant in MWI, where AW and PW mix and provide an adequate nutrient regime. One potential candidate might be *F. cylindrus*, one of the most significant cold water diatoms in the polar oceans that can be dominant in both sea ice and the water column (von Quillfeldt 2004; Mock et al. 2006). *Pyramimonas* sp., Stramenopile 1, and *Dictyocha* sp. were completely absent from AW. We observed that abundant phylotypes occurring in smaller percentages (e.g., *Phaeocystis* sp.1 and *Chrysochromulina* sp.2) often presented a clearer water mass preference than the ones with higher percentages (e.g., *Phaeocystis* sp.2 and *Chrysochromulina* sp.1). The question emerges if the apparently stronger water mass association of low-abundance phylotypes is a consequence of missing these phylotypes during DNA amplification, or if they really represent endemic species with low abundances. Protistan endemism occurs widely, particularly in polar regions (reviewed in Foissner 2006). Hedlund and Staley (2004) hypothesized that less abundant microbes are more likely candidates for endemism than more abundant taxa. Conversely, widely distributed and more abundant phylotypes could be characterized by increased tolerances and/or beneficial adaptations to local constraints (e.g., low temperature and light intensity). No water mass relation was found for Syndiniales phylotypes. This order is assumed to be composed of parasitic organisms, reported in a great variety of marine hosts, including dinoflagellates, radiolarians, ciliates, crabs, or copepod eggs (Groisillier et al. 2006; Guillou et al. 2008). For this reason, we assume that no correlation was found because Syndiniales distribution is more controlled by host availability than water mass properties.

Statements on the distribution of rare phylotypes are difficult to make because most phylotypes could not be characterized at lower taxonomic levels. Indeed, most sequences were not recorded in the database because many phylotypes still do not exist in culture, a prerequisite for proper identification. In this study, we looked at a selection of rare phylotypes, complying with two requirements, i.e., that phylotypes had to be recovered by the database and had to show a minimum contribution of 0.1 % to the protist community. We often observed water mass associations in phylotypes that were exclusively found in rare abundances. Marine stramenopiles are important members of the rare biosphere (Massana et al. 2006a). MAST III 1 was exclusively rare and presented a water mass correlation by virtueof its absence from the first three sampling stations (AW and MWI (218)). Other new phylotypes, e.g., *Florenciella* sp. and *Diadesmis* sp., also showed distributions that were correlated with water mass attributes. To date, investigations on the distribution of the rare biosphere have more closely focused on prokaryotes than on eukaryotes (Sogin et al. 2006; Galand et al. 2009a). As a consequence, questions regarding the distribution of rare protists remained unanswered. The hypothesis of Baas-Becking (1934) “everything is everywhere, but the environment selects” is still a controversial discussion topic (Foissner 1999; Finlay 2002; Finlay and Fenchel 2004; Lachance 2004; Galand et al. 2009a). Galand et al. (2009a) analyzed the distribution of the rare bacterial biosphere and detected a biogeography that opposed Baas-Becking’s dictum. Our results are based on a limited sample size making it impossible for us to make robust statements on the distribution of the rare biosphere. The distribution profiles of single phylotypes, however, suggest to some extent a biogeography of rare protists.

In summary, the implementation of ARISA hints at different water-mass-related protist community structures that, however, are not thoroughly supported by the results of 454-pyrosequencing. Nevertheless, a more detailed examination within the abundant and rare biospheres revealed some distinct distribution patterns, leading to the

conclusion that protist diversity is coupled with water mass in the central Arctic Ocean, only less pronounced than elsewhere. We therefore suppose that other environmental parameters, such as ice thickness and floe size, further influence protist communities and their association with water masses.

Protist community structure and ice coverage

Mundy et al. (2005) made general assumptions that physical factors, particularly light irradiance, strongly influence protist community structure. Light irradiance was only indirectly assessed by local sea ice conditions in this study. Protist community structure variances strongly correlated with physical sea ice factors, such as ice thickness and floe size. Most of the eight stations showed an ice concentration of at least 80 % (except St.272 and St.280). The analysis of major taxonomic divisions presented a relatively stable distribution along the stations, pointing to a strong controlling effect of sea ice concentration on the protist assemblage.

Dinoflagellates dominated the protist community structure at most sampling sites except St.202 and St.272, where chlorophytes (*Micromonas* spp.) accounted for the majority. Previous studies reported the prominence of heterotrophic dinoflagellates in the central Arctic Ocean, contributing up to 40 % (Sherr et al. 1997; Rat'kova and Wassmann 2002; Richardson et al. 2005). These systems consisted of pico- and nanoflagellates supported by low regenerated nutrient concentration (Azam et al. 1983; Landry et al. 1997; Ardyna et al. 2011). This study repeats those observations. Picoplankton abundance was found to correlate positively with increasing sea ice concentration (Booth and Horner 1997). *Micromonas* is a prominent picoplankton species (Not et al. 2004; Slapeta et al. 2006; Lovejoy et al. 2007; Foulon et al. 2008) that was abundant at all our sampling sites. However, its maximum proportions were recorded at stations that were highly (St.202) and sparsely (St.272) ice covered. Sea ice concentration and light availability are inversely linked. Based on the low ice concentration and long-term favorable light conditions, we assume a post-bloom scenario at St.272 in which microplankton cells (e.g., diatoms) were grazed or sunk into deeper water layers. In fact, periods outside short blooming events are often dominated by nano- and picophytoplankton (Not et al. 2005). The low nutrient concentrations at St.272 further confirm this assumption. A post-bloom situation at St.202 might also explain the great abundance of *Micromonas* cells in AW. AW reaches well into the Arctic Ocean (Schauer et al. 2002, 2004; Holliday et al. 2008, 2009; Beszczynska-Möller et al. 2012) and so acts as a conveyor belt for protist communities. At high latitudes, phytoplankton blooms occur when sea ice breaks up (Leu et al. 2011). In summer 2011, the Arctic Ocean was characterized by low sea ice concentrations, implying an early phytoplankton bloom timing. This earlier bloom may have allowed time for a post-bloom community to have drifted to the sampling site by mid-August.

The stramenopiles constitute another important taxonomic group in the Arctic Ocean (Lovejoy et al. 2006) and include light-dependent and light-independent types. The abundance of these types should therefore respond different to varying sea ice concentrations. Autotrophic genera in this study, such as *Fragilariopsis* and *Dictyocha,* were marginally distributed in the abundant biosphere and showed their greatest contributions at light-rich stations (i.e., those with small floes, numerous melt ponds, or no ice coverage). A high abundance of melt ponds is suggested to positively affect the light climate in surface waters and to favor algal growth (Gradinger 1996; Perovich et al. 1998; Mundy et al. 2009). The great percentage of *Fragilariopsis* sp. under favorable but still limited light conditions is unsurprising. Ice algae possess a high photoacclimation potential that allows them to grow even under low-light regimes (Kirst and Wiencke 1995; Smetacek and Nicol 2005). Moreover, *Fragilariopsis* sp. has been observed to possess a great tolerance to changing salinity (Sogaard et al. 2011), facilitating active growth in surface water following sea ice release.

Heterotrophic stramenopiles such as the MAST group were found in maximum percentages at St.245, which was characterized by the worst light conditions (large floes and few melt ponds). *In situ* experiments have shown that MAST cell abundance can even increase under zero light conditions (Massana et al. 2006b), which is particularly advantageous in the central, permanently ice-covered Arctic Ocean. Parasitic protists, as members of the Syndiniales group, seemed to cope best with the unfavorable light conditions in the central Arctic Ocean but started decreasing under low or absent ice conditions. The great variety of marine hosts (Groisillier et al. 2006; Guillou et al. 2008) makes phylotypes of Syndiniales independent of a good light regime.

In summary, sea ice coverage strongly influenced the protist assemblage in the central Arctic Ocean by favoring heterotrophic and small protists, e.g., dinoflagellates and chlorophytes, over stramenopiles, e.g., diatoms.

Contribution of the rare biosphere to the protist communities

Numerous studies have focused on the diversity, distribution, and function of the rare biosphere (Pedros-Alio 2006, 2007; Sogin et al. 2006; Caron et al. 2009; Galand et al. 2009a). In particular, the distribution of rare species has led to many discussions of whether cosmopolitan distribution

might be a consequence of high dispersal and low loss rates (Pedros-Alio 2006). A biogeographical distribution is proposed, when rare phylotypes inhabit an area according to ecological mechanisms equivalent to those that account for abundant species (Martiny et al. 2006; Kirchman et al. 2009). We observed a high genetic diversity in the rare biosphere. However, only a limited number of phylotypes could be identified in detail due to the absence of representatives in the database. A prerequisite for increasing the hit rate in databases is the generation of clonal cultures, which are extremely difficult to establish. The use of PhyloAssigner at least allowed a classification of rare phylotypes into major taxonomic groups. In this study, the rare biosphere was characterized by a more diverse diatom and dinoflagellate assemblage, in which, for example, diatoms were increased by *Diadesmis* sp. and *Florenciella* sp. and dinoflagellates by *Pelagodinium* sp. and *Prorocentrum* sp. The greater diversity of the rare biosphere has been reviewed repeatedly (Sogin et al. 2006; Caron and Countway 2009; Galand et al. 2009a). A comparison with the abundant biosphere revealed that rare phylotypes can be occasionally abundant at stations characterized by specific environmental conditions. For instance, this phenomenon has been observed for *Woloszynskia* spp., marine stramenopiles (MAST), and *Fragilariopsis* sp. We therefore hypothesize that rare protist taxa can and do become dominant following appropriate changes in environmental conditions.

We have to acknowledge that a realistic classification of the whole rare biosphere is very difficult because environment, timing, sequencing depths, and data processing can strongly influence the detection of rare species (Caron et al. 2012). In our study, for example, the lowest sequencing depth corresponds with the greatest number of unrecovered phylotypes, despite these being widely distributed at the other stations. Hence, the distribution and diversity of rare phylotypes must be interpreted with caution when sequencing depths are low because one cannot distinguish whether the rarity of an OTU is real or an artifact of undersampling. In particular, this applies to phylotypes with relative abundances near the defined threshold. Furthermore, we have to keep in mind that part of the rare biosphere can be composed of dead cells, resting stages, or extracellular DNA (Pedros-Alio 2006; Patterson 2009; Caron et al. 2012). The ecological role of the rare biosphere is still under discussion. Caron and Countway (2009) hypothesized that the rare biosphere can play an important role in ecosystem functioning at any time and that important activities do not require high species abundances. Several studies have suggested an ecological role as a backup or seed reservoir that enhances the biological buffer capacity to environmental changes (Sogin et al. 2006; Caron and Countway 2009). A constant distribution of major taxonomic groups within the rare biosphere, as observed in this study, has not been referred so far. One explanation for the stable distribution may rely on the assumption that the low abundance of rare phylotypes provides a perfect refuge from grazing mortality (Fenchel and Finlay 1983; Pernthaler 2005; Pedros-Alio 2006) and thus leads to stable compositions.

In summary, we observed that the rare biosphere is an autonomous system without significant correlations to the abundant biosphere or water masses. The relative contribution of major taxonomic divisions in the rare biosphere was unexpectedly uniform, which may support the hypothesis of a seed-reservoir function. Since the rare biosphere is assumed to be composed of species whose individual requirements do not fit the current environmental factors (Caron et al. 2009; Pedros-Alio 2012), it is likely that similar conditions in a study area result in similar compositions of the rare biospheres. Hence, we assume that similar habitats provide their rare protist community in constant taxonomic compositions in the case that the rare biosphere does provide a backup function (Sogin et al. 2006). To what extent the observations in the Arctic Ocean and their interpretation also applies to other habitats will need to be analyzed in following studies.

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