

# The influence of vent systems on pelagic eukaryotic micro-organism composition in the Nordic Seas

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**Abstract** Deep-sea hydrothermal vents and cold seeps are biological hot spots with chemolithotrophic bacterial production sustaining both benthic and pelagic organisms. Although efforts have been made to understand the diversity and function of the bacterial composition of these systems, first-level consumers, pelagic single cell heterotrophic organisms, which represent an important link between bacterial production and higher trophic levels, remain un-described in hydrothermal vents and seeps of the Nordic Seas. Here, we used a molecular biodiversity assay to investigate the impact of water masses and hydrothermal vents on the eukaryotic micro-organisms surrounding two vents systems, Jan Mayen Vent Field and Loki's Castle, and one cold seep, Håkon Mosby Mud Volcano. The assay generated a total of 482 operational taxonomic units (OTUs) based on a 99 % cut-off value, and the OTUs were grouped according to taxonomic rank. Data analysis using hierarchical clustering and non-metric multidimensional

scaling with class as taxonomic entries suggested that water masses followed by depth was the dominant effect on eukaryotic micro-organism diversity. However, in one of the vent systems, Loki's Castle, the community was different compared to the reference station. Our data suggest that while the total production of vent systems is higher than the surrounding waters, the biodiversity of eukaryotic micro-organisms is more influenced by both water masses and depth.

**Keywords** Protists · 18S rRNA gene · Universal primers · North-east Atlantic · Arctic · Deep-sea · Plume · Hydrothermal vents

## Introduction

Deep-sea hydrothermal vents and cold seep biota are supported by both aerobic and anaerobic metabolism (Hügler and Sievert 2011) through chemolithoautotrophic primary production from prokaryotes (Van Dover 2000; Bennet et al. 2013). These micro-organisms support diverse assemblages of eukaryotes (Bennet et al. 2013), which in turn become the source of energy to higher trophic levels such as mesozooplankton (e.g. Kaartvedt et al. 1992; Burd et al. 2002; Olsen et al. 2013). Indeed, vent and seep systems can have a high productivity, both benthic and pelagic, compared to the surrounding water masses (Van Dover 2000; Wakeham et al. 2001; Hügler and Sievert 2011; Bennet et al. 2013). Hydrothermal vents and cold seeps are found throughout the world's oceans, including the Arctic and the Nordic Seas. Hence, the background for the present study was the discoveries of hydrothermal vents at the Arctic Mid-Atlantic Ridge (AMOR) in the Nordic Seas in 2005 and 2008. These were the Jan Mayen Vent Field

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(2005) and Loki's Castle (2008); in addition, the cold seep Håkon Mosby Mud Volcano was included based on the high methane concentrations in the water column (online resource 2). Since the discovery, several studies (Pedersen et al. 2005, 2010a; Lichtschlag et al. 2010; Schander et al. 2010; Baumberger 2011; Tandberg et al. 2011; Jørgensen et al. 2012; Kongsrud and Rapp 2012; Stensland 2013) have described the geochemistry, benthic fauna and prokaryote sediment community of both the hydrothermal vents and Håkon Mosby Mud Volcano. However, the protist community in these systems remains largely undescribed despite its importance as a link towards higher trophic levels.

The vent fields of the Nordic Seas are geographically located under variable conditions where temperature, salinity, depth and thus prevailing water mass vary. Moreover, the vents have also distinct different physical characteristics, and the Jan Mayen Vent Field has white smoker type chimneys with 270 °C fluids, while the black smokers at Loki's Castle are emitting 350 °C fluids (Pedersen et al. 2010a, b). Furthermore, the Jan Mayen Vent Field has lower concentrations of CH<sub>4</sub> and H<sub>2</sub> compared to the high levels measured in the non-buoyant plume at Loki's Castle (Pedersen et al. 2010b; Stensland 2013). Both vent fields have higher levels of CO<sub>2</sub> due to the magmatic influence compared to sea water (e.g. Pedersen et al. 2010b; Baumberger 2011). At Jan Mayen Vent Field, the concentration varies between 6.2 and 91.7 mmol kg<sup>-1</sup>, while concentration varied between 21.52 and 26.01 mmol kg<sup>-1</sup> at Loki's Castle. In comparison, arctic deep water CO<sub>2</sub> concentration was 2.3 mmol kg<sup>-1</sup> (Baumberger 2011). The Håkon Mosby Mud Volcano is a cold methane seep with no temperature anomaly (Vogt et al. 1999), although the CH<sub>4</sub> concentration is comparable with the hydrothermal vents (online resource 2). Water depth is, nevertheless, the most conspicuous difference, with Jan Mayen shallow vents at approximately 500 m depth, while the Loki's Castle vents are at 2,400 m depth and Håkon Mosby Mud Volcano at 1,300 m depth. The depth could impact the surrounding ecosystem due to variable vertical transport of organic matter from surface production while differential pressure could influence taxa directly, making these three sites fundamentally different as habitats. Indeed, pressure could influence organisms significantly and many taxa cannot maintain their metabolism at the lower Loki's Castle depths (Atkins 2000).

The high levels of CO<sub>2</sub> at the Jan Mayen Vent Field from both the chimney fluid and the diffuse venting are of particular interest and may represent a unique research opportunity as a natural laboratory to study consequences of natural CO<sub>2</sub> leakage. The concentration of CO<sub>2</sub> can be as high as 90 mmol kg<sup>-1</sup> at the base of the plume (Baumberger 2011), which is far higher than the observed range in sea water (81–302 μmol kg<sup>-1</sup>) (Barry et al. 2010). Several

short-term mesocosm studies on the effect of elevated CO<sub>2</sub> levels have demonstrated a limited response of the microbial communities using dissolved CO<sub>2</sub> in concentration up to 3 times the present ocean concentration of CO<sub>2</sub> (Paulino et al. 2008; Riebesell et al. 2008; Troedsson et al. 2013; Niehoff et al. 2013). Despite that the temporal and spatial scale of these mesocosm experiments is challenging, and long-term effects cannot be excluded, the results suggest that large fractions of the microbial communities are resilient to sudden elevated CO<sub>2</sub> levels. On the other hand, taxa at higher trophic levels indicate variable and rapid responses to increased CO<sub>2</sub> levels ranging from negative (Riebesell et al. 2000), negative to neutral (Dupont et al. 2010), or even positive (Troedsson et al. 2013).

The objectives of the present study was to explore and describe the eukaryote micro-organism diversity by analysis of water samples from (1) below, (2) within and (3) above the plume at the two hydrothermal vent systems with reference stations (control). The Håkon Mosby Mud Volcano was also included and sampled close to bottom as "below plume", at the depth where the methane gas bubbles was not possible to trace on the echo sounder as "plume" and finally it was sampled above the traces of Methane gas bubbles. No separate reference station was sampled. Håkon Mosby Mud Volcano was included in this study because of the high concentrations of methane and because of the gas plume that rise several hundred metres above the sea floor. These features combined with high temperatures in the sediment contribute to chemosynthesis, which is interesting in context of secondary consumers like protists. The taxa composition from all stations will further be analysed in context of basic physical parameters such as depth, locality and hydrothermal vent versus reference station (control). We used a molecular assay in order to characterize the biodiversity of the pelagic protist community, and based on the physical differences between the Jan Mayen Vent Field, the Håkon Mosby Mud Volcano and Loki's Castle, we explored how hydrothermal conditions, surrounding water mass as well as depth may be important factors for the diversity of the protist community in these systems.

## Materials and methods

### Study area

The samples were collected during the R/V G. O. Sars cruises in 2009 (H2DEEP) and 2011. In 2009, we sampled Loki's Castle (Pedersen et al. 2010a) (73°33.97'N and 008°09.51'E), Håkon Mosby Mud Volcano (Vogt et al. 1997) (72°00.04'N and 014°44.06'E) and a reference station (73°10.37'N and 008°56.52'E) with similar depth to Loki's Castle. Loki's Castle is a black smoker field with

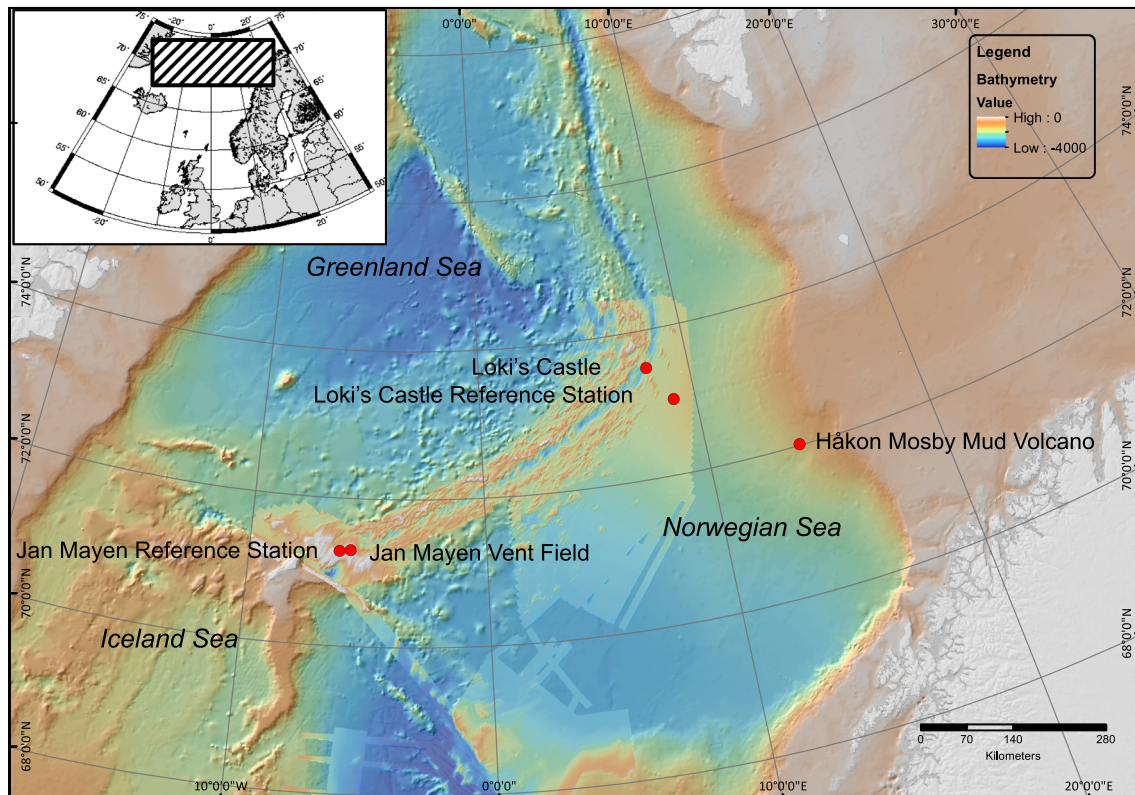
fluid temperatures up to 350 °C, while Håkon Mosby Mud Volcano is a cold-water methane seep. In 2011, we sampled the Jan Mayen Vent Field (71°17.83'N and 005°46.37'W) (Trollveggen) (Pedersen et al. 2005) with a reference station (71°17.15'N and 006°14.20'W). The Jan Mayen Vent Field consists of several high temperature white smokers with fluid temperatures of 260–270 °C (Fig. 1).

In general, the surface water masses of the Nordic Seas can be split into three categories; the warmer and more saline Atlantic water that enters the Norwegian Sea from the south between Iceland and Scotland (Norwegian Atlantic Current), the less saline and colder Arctic water from the Arctic Ocean entering the Greenland Sea through the Fram Strait (East Greenland Current) and finally the Jan Mayen Current, which is an important part of the Greenland Basin cyclonic gyre (Skjoldal 2004; Rudels et al. 2005). The topography is also important in the deeper water circulation (Søiland et al. 2008). It includes continental shelves, slopes, deep-sea mud flats, seamounts, fracture zones and ridges. The Arctic Mid-Ocean Ridge (AMOR), which runs in the central deep parts as an extension of the Mid-Atlantic Ridge (MAR), separates the Greenland Sea Basin from the Norwegian Sea Basin. This topography results in the deep waters of the Greenland Sea

and the Norwegian Sea being cyclonic. However, the Norwegian Sea deep waters consist mainly of water from the Greenland Basin (Greenland Sea Deep Water and Arctic Ocean Deep Water) that enters the Norwegian Sea through gaps in the AMOR, especially in the Jan Mayen Fracture Zone (Skjoldal 2004; Voet et al. 2010).

#### Field sampling

In order to localize the plume water at Loki's Castle, we used a redox sensor (Eh electrode by Dr. Koichi Nakamura, Japan) connected to a CTD. The plume depth at the Jan Mayen Vent Field was found by temperature anomalies from the CTD and echo sound images (see also online resource 2). The water samples were collected using Niskin bottles. At each vent location, we sampled three depths: deep (below plume), middle (in the plume) and a shallow station (above the plume). At Loki's Castle and the reference station, we sampled at 2,350 m (below the plume), 2,100 m (in the plume) and 450 m (above the plume), and at Håkon Mosby Mud Volcano, we sampled at 1,250, 450, and 150 m, while at Jan Mayen Vent Field and the Jan Mayen reference station, we sampled at 450 m (below the plume), 250 m (in the plume) and 100 m (above the plume). For each sample, 5 L of sea water was first pre-



**Fig. 1** The map is an overview of the Nordic Seas and the localities from this study

filter (sieve) and due to practical problems during the cruise, the 2,009 samples were pre-filtered through a 125- $\mu\text{m}$  mesh sieve, while the 2,011 samples were pre-filtered through a 63- $\mu\text{m}$  sieve. However, the pre-filter data did not yield any distinct pattern alone, nor did the data without pre-filtered sequences, suggesting that the different pre-sieving filters had minor or no effect on the final results and interpretation. The water samples were then further fractionated through 10 and 5  $\mu\text{m}$  pore size 47 mm polycarbonate filters (Millipore<sup>TM</sup>, Merck KGaA Darmstadt, Germany). The pre-filter samples were fixated in 96 % ethanol and stored at  $-80\text{ }^{\circ}\text{C}$ , while the rest of the filters were instantly placed in sterile 1.5 mL cryo-tubes and frozen in liquid nitrogen before storage at  $-80\text{ }^{\circ}\text{C}$  for further analysis. The water samples were further filtered using 0.22- $\mu\text{m}$  Sterivex<sup>TM</sup> filters (Millipore<sup>TM</sup>, Merck KGaA Darmstadt, Germany) to extract prokaryote DNA (unpublished data), and finally, viruses were analysed and described in Ray et al. (2012).

#### DNA extraction, PCR assay and sequencing

Genomic DNA was extracted from the filters using Fast-DNA<sup>®</sup> Spin Kit for Soil (MP Biomedicals, Santa Ana, CA), while the DNeasy Blood and Tissue kit (QIAGEN<sup>®</sup>) was used for the pre-filters. Extraction was carried out according to the manufacturer's instructions. PCR was conducted using the Takara Ex HS taq (Takara BIO, Inc., Japan) with the universal eukaryotic 18S rRNA gene-targeted primers Univ-1131F-7 (5'-AAA CTT AAA GRA ATT GAC GG-3') and Univ-1428R (5'-CTA AGG GCA TCA CAG ACC-3') bp (Troedsson et al. 2008; Hadziavdic et al. 2014) generating a  $\sim 315$ -bp product. The amplification conditions included an initial denaturation cycle (3 min at  $94\text{ }^{\circ}\text{C}$ ), followed by 30 amplification cycles (30 s at  $94\text{ }^{\circ}\text{C}$ , 30 s at  $57.4\text{ }^{\circ}\text{C}$  and 1 min at  $72\text{ }^{\circ}\text{C}$ ). The amplification cycles were followed by a final extension at  $72\text{ }^{\circ}\text{C}$  for 5 min. Amplified gene fragments were visualized by electrophoresis in a 1 % agarose gel. The amplified gene was then ligated into a pCR4-TOPO vector using the StrataClone PCR cloning kit (Stratagene, Agilent Technologies, Inc., Santa Clara, CA) according to the manufacturer's instructions. For sequencing, 96 colonies per original filter fraction were selected and further regrown in Nunclon<sup>TM</sup> delta surface 96-well plates (three plates per depth generating nine plates per locality, in total 45). Each well in this study was filled with 200  $\mu\text{L}$  agar (imMedia<sup>TM</sup> Ampicilin Agar from Invitrogen Corp.) and cooled to room temperature. The plates were shipped to LGC Genomics (<http://www.lgc.co.uk/>) for MTP sequencing (Sanger sequencing) using the commercial T7 primer. All the sequences that derived from the same volume of water (i.e. clone libraries from 5, 10  $\mu\text{m}$  and pre-filters) were pooled

and treated as one sample in the analysis, generating a total of 15 samples analysed, three depths and five localities.

#### Sequence treatment and statistical analysis

Diversity estimates and taxonomical information based on primary DNA structure can vary according to (1) cut-off value used when clustering sequences, (2) target gene (3) variability of each taxonomical group within the particular gene fragment used in the study and (4) the quality of the database used for aligning the new sequences. For each sample (depth and locality), we used Mega 5.0 (Tamura et al. 2011) and FinchTV 1.4.0 (Geospiza<sup>®</sup> Inc.) to inspect and trim sequences for further analyses. SeqNoise from AmpliconNoise (version 1.25) software package (Quince et al. 2011) was used to estimate and remove sequence errors generated in the PCR, and chimeras were removed using Perseus. Sequences were further clustered into operational taxonomic units (OTUs) using the hierarchical complete linkage-clustering algorithm. In this study, we used a 99 % cut-off value in the clustering analysis. The diversity analysis of these samples indicated that we had several groups with different optimal cut-off values. Indeed, the 99 % cut-off value will be accurate for some groups and less accurate for others. However, we chose to maintain a high similarity in the clustering analysis and further evaluated each OTU using NCBI (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). Taxa and rank names for all OTUs were based on the World Register of Marine Species (WoRMS 2013).

All matrices were  $[\log(x + 1)]$ -transformed before the non-metric multidimensional scaling (NMDS) and hierarchical clustering analysis using Bray–Curtis similarity resemblance using Primer-E V6 (Clarke and Gorley 2006). Similarity level from the hierarchical clustering analysis was combined with the NMDSplot to distinguish the clusters. The data used in the statistical analyses were based on the raw data with number of reads per OTU.

## Results

#### Sequence data

After cloning samples from five localities, three depths and three fractions (described in methods), we sequenced a total of 4,320 colonies. Sequences that were shorter than 300 bp were discarded in addition to those that were removed after treatment with SeqNoise from the AmpliconNoise (version 1.25) software. After removal, there were left 3,719 sequences ranging from 233 to 269 sequences per sample depth and locality. Fractions were pooled after annotation and before further treatment of the

data since each sample was the same water for all fractions and thus was considered as the same sample. Clustering the sequences at 99 % similarity cut-off (3–5 base pair difference within the clusters) resulted in 482 OTUs. Each OTU was assigned a full rank allocated into five kingdoms, 32 phyla, 74 classes and 121 orders. It was not possible to assign a classification to four sequences, and these remained unidentified. A total of 29 sequences were assigned as unknown kingdom, and 110 sequences were assigned to unknown phyla. For each rank, the number of unknowns increased exponentially (online resource 1).

The number of OTUs ranged from 43 to 83 per sample, and 94 OTUs were shared between two or more samples, while 339 OTUs were only found in one sample. Among those 339 OTUs, 286 were singletons (i.e. only one sequence in the OTU). However, since the biodiversity was estimated using a clone library, the overall sequence coverage is rather low compared to, e.g. next-generation sequencing platforms, and the number of singletons in each OTU is therefore expected to be higher. Nevertheless, single sequence OTUs should be treated with caution. We therefore reduced the number of single sequence taxa substantially by pooling them into lower ranks using NCBI and BLAST. At the class level, the number of single sequence taxa was reduced to 16 singletons (out of 74 classes), i.e. we reduced the single sequence taxa from 60 to 20 %. Similarly, at the level of phylum, we reduced single sequence taxa to three singletons (out of 32 phyla), i.e. ca 10 %. Clustering at lower taxonomical ranks also reduced the proportion of unique taxa (only found in one sample) from 70 % at OTU, 32 % for class and 16 % for phylum level.

#### Taxonomical richness

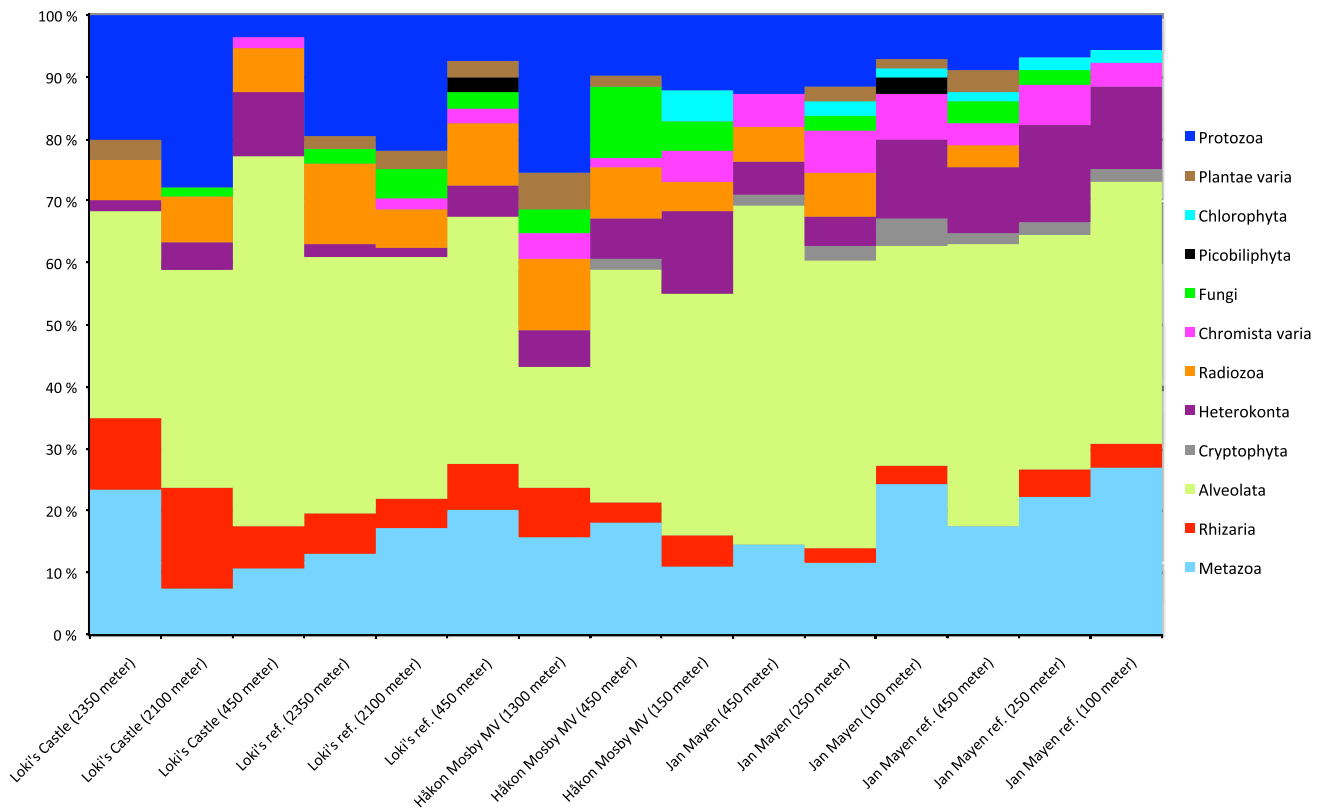
A representative sequence (the most abundant sequence) from each OTU was annotated based on BLAST similarity. A complete taxonomical overview using the World Register of Marine Species (WoRMS 2013) including abundance of OTUs within each group from kingdom to class is given in online resource 1. Orders were included when assignment was possible. Alveolates (Infrakingdom) was the most common group and found abundantly in all samples representing 44 % (range 22–61 %) of the whole dataset, followed by Metazoa/Animalia 17 % (range 7–26 %), Protozoa 13 % (4–28 %), Rhizaria 12 % (range 3–23 %) and Heterokonta 8 % (range 2–16 %). Overall, these groups accounted for 94 % of all taxa and defined the major community compositions in the water column (Fig. 2).

By looking at higher taxonomical level, Alveolates (predominantly Peridinea) dominated all localities, samples and depths. Rhizaria (cercozoans) was relatively more

common at Loki's Castle, particularly at the deepest samples with vent-affected water (data not presented). A similar trend was found among the protozoans that were also more abundant in the deepest sample at both Loki's Castle stations and the deep sample from Håkon Mosby Mud Volcano. Samples from the Jan Mayen area indicated higher diversity, with the exception of the Håkon Mosby Mud Volcano shallow sample that had similar taxa richness. The increased diversity was predominantly an increase in autotrophic taxa, e.g. heterokonts (diatoms) possibly reflecting a connection to the primary production of the euphotic zone. There was also a trend of autotrophic groups in the samples above 450 m, indicating a strong influence of the euphotic zone on the taxonomic composition in these samples (Fig. 2).

Using Linnean rankers at levels from, e.g., phylum showed further that 78 % of all taxa came from seven phyla; Myzozoa (alveolates) dominated (36 %), while the other six varied between five and 11 %. Myzozoa was represented at all stations by the dinoflagellates Peridinea and Syndinea, the remaining 25 phyla all had low abundances with 1–12 OTUs (0.2–2.4 %) (online resource 1). At the lower classification ranks, seven classes covered almost 60 % of all samples, indicating a similar skew compared to phylum with 16 and three singletons from class and phylum, respectively. Among the abundant groups the dinoflagellate Peridinea dominated with 19 %, almost twice as abundant and diverse as the second most abundant group, which was the protozoan group Diplonemea.

Animalia was dominated by copepods (Maxillopoda) represented by three identified and one unidentified order. Members from the class Maxillopoda was found in all samples (27 OTUs) (online resource 1). Cnidaria constituted seven orders (three classes), while Porifera constituted two classes (Porifera was not possible to distinguish further due to conservative 18S). Gelatinous plankton was found in all samples except the Loki's Castle shallow sample. Porifera was less common, but found at all localities except for the Håkon Mosby Mud Volcano. Protozoa had high abundance with 14 % of all OTUs. However, 52 out of 70 protozoan OTUs were classified as Diplonemea, which has been suggested to be a deep-sea group (Lara et al. 2009). These were found at 15 stations and accounted for 74 % of all Protozoa. The number of reads/sequences cannot be used as a true quantitative measure, but there were relatively more Diplonemea OTUs at 2,350–1,300 m compared to 450–150 m (average of  $11.4 \pm 3.5$  and  $2.7 \pm 1.4$ , respectively) (Protozoa from Fig. 2 and online resource 1). The highest Fungi diversity was found in the eastern parts of the Nordic Seas, but Fungi were also present in lower numbers in the Jan Mayen system. Overall, Fungi represented 3 % of all OTUs. The group of Plantae was a mixture of terrestrial plants and microalgae.



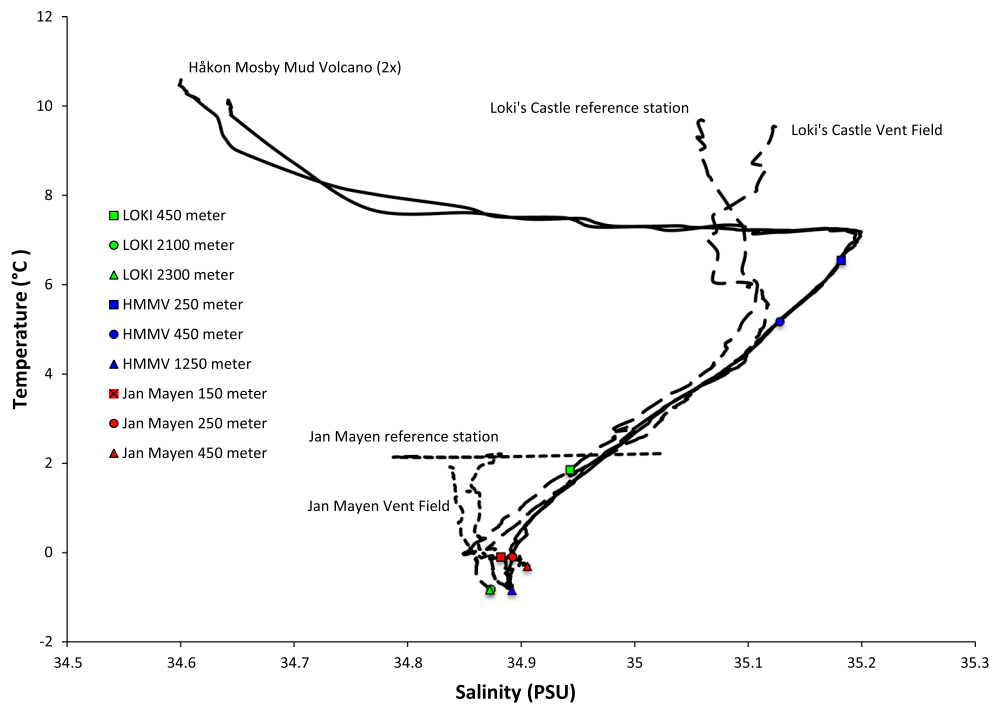
**Fig. 2** Bar chart of the relative abundance of OTUs from each classification rank at all localities and depths. The ranks illustrated in this figure are based on a selection of group names used in eukaryote marine micro-organism studies

The latter was only present in the shallow samples closer to the photic zone. Terrestrial plants on the other hand were found at a larger depth range suggesting a greater vertical transport for these particles compared to microalgae, i.e. detrital particles. Finally, we found a small and recently described phylum, Picobiliphyta (Not et al. 2007; Moreira and López-García 2014). We found representatives in two samples in our study.

#### Community composition analysis

Using CTD profiles, it was evident that these three different localities represent different water masses (salinity versus temperature plot in Fig. 3). However, the water mass origin are not identified, but distinguished from one another and used as parameter in further analyses. We used hierarchical clustering analysis (Bray-Curtis similarity) and non-metric multidimensional scaling (NMDS) in order to investigate the effect of (1) water mass, (2) depth or (3) chemistry (vent activity, online resource 2) on microbial eukaryote community structure. These analyses were tested at the level of OTU, order, class and phylum. At class level, it was possible to identify three clusters according to the three identified water masses (62 % similarity) (Figs. 3, 4

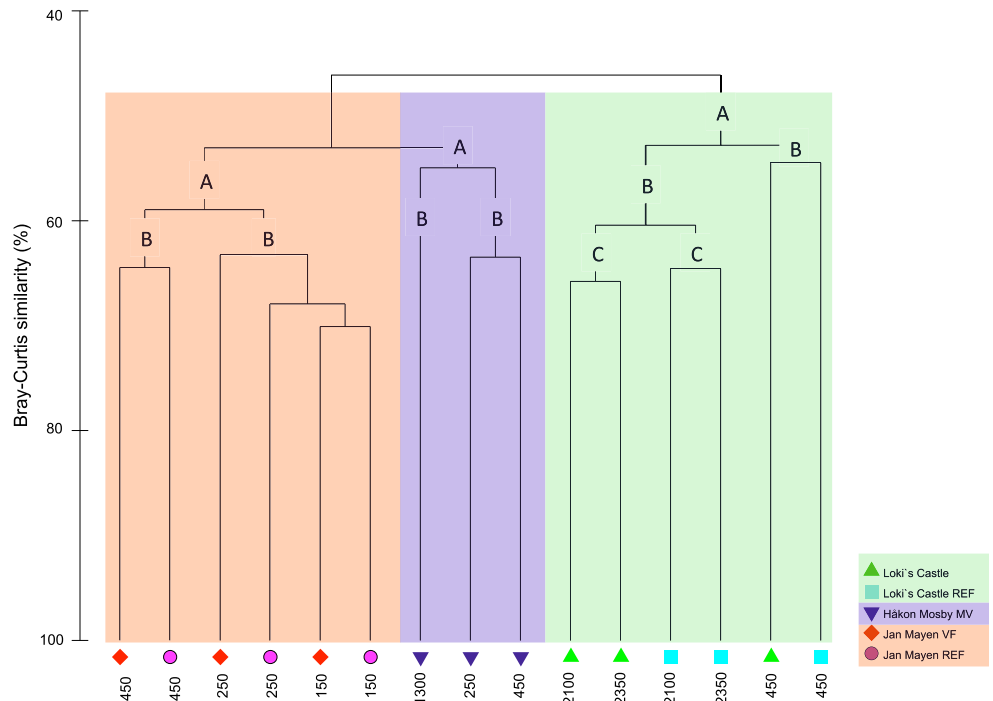
and 5). The three were (1) Håkon Mosby Mud Volcano in the east, (2) Loki's Castle and the reference in the north and (3) the Jan Mayen Vent Field and the reference station in the west (Figs. 4, 5). Inspection of the hierarchical clustering dendrogram in Fig. 4 indicated that the first cluster splits Jan Mayen and Håkon Mosby from Loki's Castle (52 % similarity). The Håkon Mosby and Jan Mayen cluster were split further, resulting in one cluster per type of water mass (62 % similarity) (identified from the CTD data in Figs. 3) (Figs. 4, 5). Within these three water mass-based clusters, the next split (node) was based on depth. The two Jan Mayen deep samples (450 m) clustered together (64 % similarity), but were separate from the others including the medium (250 m) and shallow depth (100 m) Jan Mayen samples. Secondly, the Håkon Mosby deepest sample (1,250 m) was separated from the two shallower (450 and 150 m) Håkon Mosby samples (54 % similarity). The Håkon Mosby Mud Volcano sample did not have a reference station, and no further clustering was therefore possible. Finally, the Loki's Castle shallow samples (450 m) separated from the deep and the medium samples (54 % similarity). However, the deep (2,350 m) and medium (2,100) depths from Loki's Castle sample did not cluster together according to depth, but according to



**Fig. 3** A CTD plot of salinity and temperature at the sampling localities. The salinity vs. temperature plots illustrates the three different profiles that each represents one water mass each. Each

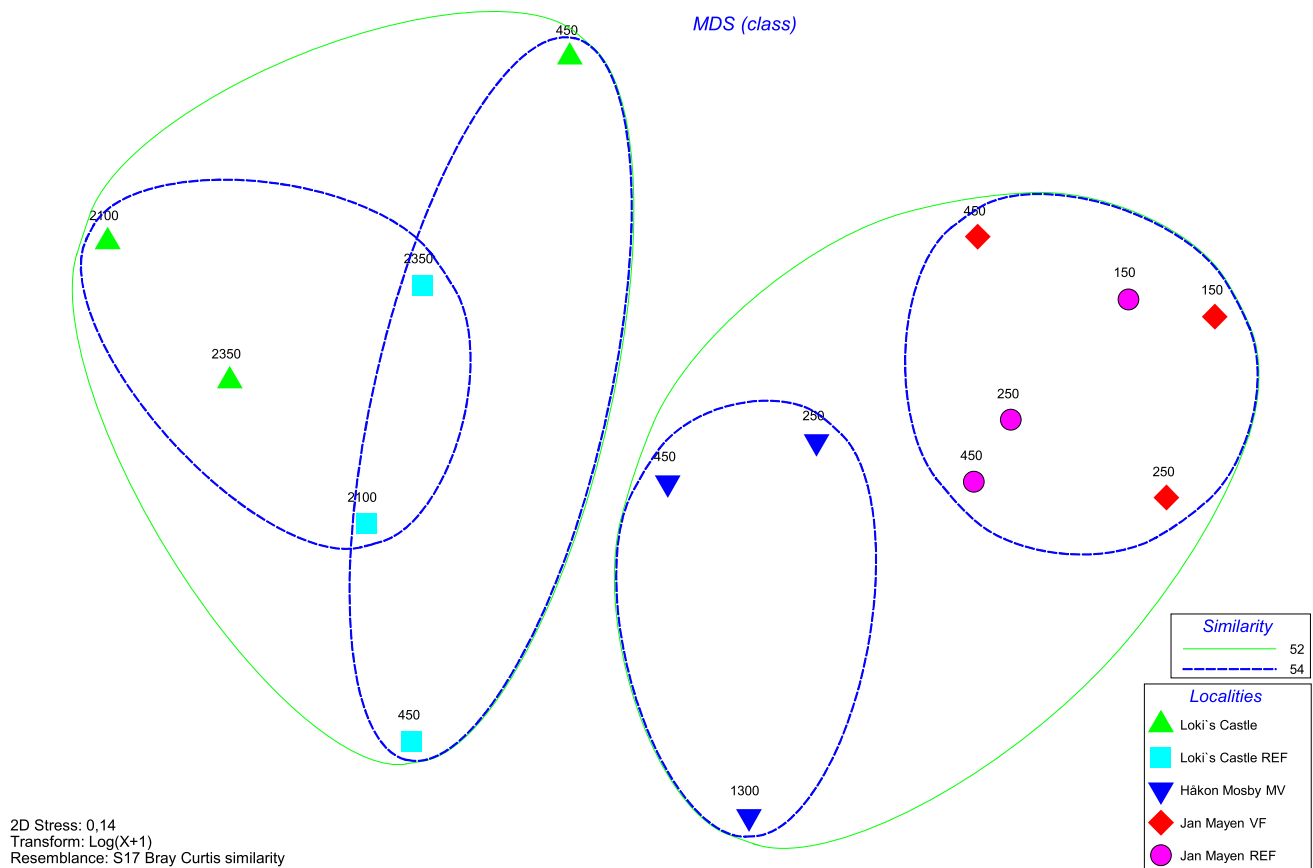
sampling depth and station are indicated in the figure along the CTD profile. The *horizontal dashed line* from the Jan Mayen reference station CTD is due to variation in salinity at the surface

**Fig. 4** The dendrogram, based on class level, shows that samples from specific water masses formed groups together. The water mass clusters are indicated by “A” and colour fields, and “B” indicates separation by depth, and “C” indicates separation by vent and non-vent conditions



locality (64 % similarity). We did not see this result in the Jan Mayen Vent Field samples (Fig. 4). Sea water and plume chemistry have been investigated thoroughly in other studies (Pedersen et al. 2005; Sauter et al. 2006;

Pedersen et al. 2010a; Schander et al. 2010; Baumberger 2011; Tandberg et al. 2011; Jørgensen et al. 2012; Kongsrud and Rapp 2012; Stensland 2013), and further discussion related to chemistry will therefore follow below.



**Fig. 5** A non-metric multidimensional scaling (NMDS) plot yielding clustering of samples, based on class level, according to water masses/localities. The groupings (circles) are based on the dendrogram from Fig. 4

Five groups dominated the three different water masses (Alveolata, Rhizaria, Protozoa, Metazoa and Heterokonta), but the internal proportion within a given sample varied. Almost 50 % of the OTUs at Loki's Castle and reference station were Rhizaria, Protozoa and Metazoa, while these groups constituted 34 and 36 % within the Håkon Mosby Mud Volcano and the Jan Mayen Vent Field and its reference station, respectively. The Loki's Castle and reference station and the Håkon Mosby Mud Volcano shared a large proportion of Rhizaria and Protozoa, but differed from one another with a large Heterokonta portion as well as higher proportion of smaller taxa at the Håkon Mosby Mud Volcano. While the Håkon Mosby Mud Volcano and the Jan Mayen Vent Field and its reference station shared a large Heterokonta proportion and a high amount of smaller taxa, the Jan Mayen Vent Field and its reference station contained a higher proportion of Metazoa and Alveolata. Shallow samples, regardless locality, were more dominated by taxa with phototrophic groups such as Heterokonta, Plantae (debris) and Chlorophyta; Protozoa was on the other hand dominating the four deepest stations (Loki's Castle and the reference station (Fig. 2).

## Discussion

### Cut-off values and data analysis

The species is important as a reference point in studies of impact (natural or anthropogenic) on an ecosystem (Hey 2009). However, the species definition becomes particularly difficult when using short sequences of genomic DNA (barcode) as a reference. A primary DNA sequence at a given similarity cut-off value is therefore regarded as an operational taxonomic unit (OTU) (Schloss and Handelsman 2005; Stackebrandt 2011). Nevertheless, there are, as yet, no universally accepted guidelines to this cut-off value, which ranges from 90 to 99 % (e.g. Stoeck et al. 2007; Caron et al. 2009; Marande et al. 2009; Schnetzer et al. 2011; Edgcomb et al. 2011; Orsi et al. 2011). In our study, we used a modified approach with a highly stringent clustering cut-off value of 99 %. We then individually inspected the annotated taxonomical ranks from family to kingdom using BLAST from NCBI (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). This approach was possible due to the relatively low number of OTUs generated from our clone

library approach and further enabled us to produce a detailed and quality-controlled taxonomy as well as a consistent organization of the data based on WoRMS (2013). The high stringency cut-off generated a large number singleton, which either suggests that the used cut-off value was too high or that the sequence effort was too low (low coverage of the true OTU diversity). We addressed the former by grouping OTUs into higher taxonomic ranks based on the manually quality-controlled taxonomy (online resource 1). The use of higher ranks reduces the taxonomical resolution, and this can be considered to be a weakness of the analysis. However, this is a commonly used practice in many studies due to the complex nature of the dataset and the lack of a universally accepted species cut-off (e.g. Edgcomb et al. 2011). To improve the overall sequence resolution, it is possible to use next-generation sequencing (NGS) platforms. Previous studies comparing Sanger sequencing and NGS found only few differences because the dominant taxa are the same with the two methods (Edgcomb et al. 2011). The main difference between these two approaches is that NGS data generated a higher number of rare taxa. However, it remains to be determined how the additional sequence information from NGS will improve our understanding of biological trends in ecosystems. In our study, we observed increased noise in the analysis with higher resolution. Nevertheless, pooling OTUs into higher ranks, regardless of method, can be considered a taxonomical surrogacy (Bertrand et al. 2006). We argue that the approach used in this study is valid since the correct and true species cut-off for OTU and species definition likely does not exist.

CTD data indicated three distinct water masses (Fig. 3), which we also found to be the strongest factor affecting the biodiversity of the eukaryotic micro-organisms. Initial tests of cut-off values between 97 and 99 % yielded no correlation with these water masses. However, testing higher taxonomic levels (class level) indicated a connection between water mass and taxa composition (Figs. 4, 5). From the 97 % cut-off, we continued to tested family, order, class and phylum. Hierarchical ranks were possible because each OTU was annotated and given at the highest taxonomical level possible. Further, we chose class based on (1) best fit with present environmental variables, (2) 19 out of 32 phyla were possible to identify further to class, while in comparison with 58 out of 74, classes could not be identified further (i.e. maximal resolution at class level), and (3) number of classes was more double the number of phyla. The taxonomic level of class corresponds to a cut-off level of ca 87 % similarity. This is lower than the cut-off value for most environmental DNA studies (e.g. Caron et al. 2009). Indeed, previous studies have explored different cut-off levels ranging from 90 to 100 % (Stoeck et al. 2007; Edgcomb et al. 2011; Orsi et al. 2011;

Hadziavdic et al. 2014), which is necessary for any new primer set used to amplify a fragment of the 18S rRNA gene. However, to our knowledge, this is the first study using the hierarchical rank approach with environmental DNA data.

### Ecological assessment

Although eukaryotic micro-organisms in Nordic Seas plumes have not been studied before, several other studies have focused on the effect of hydrothermal plumes from other parts of the world on both the mesozooplankton (Berg and Van Dover 1987; Burd and Thomson 1995; Burd et al. 2002; Vinogradov and Vereshchaka 2005; Olsen et al. 2013) and the microplankton (Atkins 2000; López-García et al. 2007; Bennet et al. 2013). While hydrothermal vents clearly contribute to increasing the available metabolic energy, how this increased energy affects the biodiversity remains unclear. Atkins (2000) suggested that flagellate protozoans could maintain metabolism within the extreme conditions of hydrothermal vents with high concentrations of, e.g., sulphide in the water column. This conclusion is strengthened by the high protistan diversity described in López-García et al. (2007). Bennet et al. (2013) also found an increase in total organic carbon (TOC) compared to surrounding waters. These studies suggest that vents can be positive for protists, possibly due to increased food availability our data on vent systems in the Nordic Seas did not clearly identify any effect on the microplankton community. Instead, our results suggest that water masses had the strongest effect on the genetic diversity and support the concept that the microplankton community is resilient to hydrothermal fluid venting (Figs. 4, 5). However, one need to view these results with caution due to lack of time series/replicates that reduce the robustness and due to few samples may affect the analysis. Still, hydrological conditions have also been found to be an important factor in the structuring of mesozooplankton (Dvoretzky and Dvoretzky 2013), but also among protists (Yu et al. 2014). Indeed, water depth was identified in our study to affect the microplankton community as the Jan Mayen Vent Field and reference site samples could not be separated. Depth also seemed to be a factor for the microplankton community at Loki's Castle and the Håkon Mosby Mud Volcano. At Jan Mayen, depth was the only factor affecting microplankton diversity, while at Loki's Castle, there was also an effect from the vent, since vent and reference samples separated into two distinct clusters. It is not clear why there was an observed difference between the Loki's reference and vent samples, but this was not observed at Jan Mayen. However, the depth range was less at Jan Mayen and the phototrophic production, "surface signature", may be

higher and therefore have a greater influence on the biodiversity signature above 500 m depth compared to what has been seen below 2,000 m (see Loki's Castle) (Fig. 2). At the depths of Loki's Castle, a much smaller fraction of the surface production reaches the bottom (and the deeper water layers), and therefore, the gradient between vent and non-vent was stronger at Loki's Castle compared to Jan Mayen. The plume chemistry can also have been a factor since Loki's Castle had higher concentrations of energy-rich compounds such as CH<sub>4</sub> and H<sub>2</sub>, compared to the Jan Mayen Vent Field (Stensland 2013). The lower CH<sub>4</sub> and H<sub>2</sub> concentrations at Jan Mayen combined with the influence of surface production could mask any potential effects of the vent signals on the genetic diversity. However, our data indicated that Jan Mayen Vent Field and the reference clustered according to depth alone, suggesting limited effect of the elevated gas levels, including CO<sub>2</sub> even though it was not tested. This supports the concept of resilience of the eukaryotic micro-organism communities (e.g. Paulino et al. 2008; Troedsson et al. 2013), but still both resilience and CO<sub>2</sub> needs further attention in the future.

## Conclusions

Based on our data and multivariate analyses, the signal from the prevailing water mass is stronger than both depth and plume water. Previous studies suggest that the vents have an effect and contribute to increased carbon production (Atkins 2000; Wakeham et al. 2001; Hügler and Sievert 2011; Bennet et al. 2013). However, the effect of vent-specific biodiversity has remained largely unexplored. Motile and migrating plankton can move towards food sources such as hydrothermal plumes. Microplankton on the other hand cannot control their position in the water column and depend on the food source present in the water masses. It is, however, possible that the increased available energy through chemosynthesis induces increased growth in the vent community compared to that at the reference station. The time spent in the vent water with increased metabolic energy (effluent layer) may be important to how much the microeukaryote community potentially will be different from the surrounding water masses. It has also been suggested that, e.g., parasitic organisms can be linked to vents explicitly during their free-living stage (Moreira and López-García 2003) suggesting that some organisms are vent specific. The observed effect of the vent at the Loki's Castle deep samples supports the former hypothesis. Our study demonstrates that research of vent systems needs to integrate oceanographic, physical and chemical water properties as well as geological and biological features in order to understand these complex ecosystems.

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