Final response

Dear Prof. Dr. Yuelu Jiang

We would like to thank you very much for considering our manuscript for publication in Ocean Science and the reviewers Jessica Ray (RC1), John Kirkpatrick (RC2) and Henriette Kolling (SC1) for the helpful comments and recommendations. Please, find below the revisions to our manuscript "Changes in the composition of marine and sea-ice diatoms derived from sedimentary ancient DNA of the eastern Fram Strait over the past 30,000 years". We have revised the manuscript accordingly and provide the answers to those comments (bold), our replies and the changes we made (underlined) point-by-point with line numbers referring to the revised manuscript. In general, we agree with the critiques and re-phrased unclear sections, included new statistical analyses, optimized figures as recommended and provided new supplementary material. Furthermore, the manuscript went through English correction again. As line numbers changed slightly in comparison to the Answers to the reviewer's comments, we provide here the adjusted line numbers after English correction. We hope that our revisions have improved the quality of the manuscript sufficiently for future publication in Ocean Science and are looking forward to receive your decision.

With kind regards and on behalf of the co-authors

Heike Zimmermann

Answers to os-2019-113_RC1

l.127 - Less than 50% of 3 replicates?

We agree that this is confusing and changed the sentence from:

"[…](3) were present at least 3 times among the different replicates, (4) showed taxonomic resolution below the phylum level "Bacillariophyta" and (5) were tagged as "internal" by obiclean in less than 50 % of the different replicates to reduce PCR and sequencing artefacts."

L145-147:

"[…](3) were present at least 3 times among <u>all sequenced PCR products</u>, (4) showed taxonomic resolution below the phylum level "Bacillariophyta" and (5) were tagged as "internal" by obiclean in less than 50 % of <u>all sequenced PCR products</u> to reduce PCR and sequencing artefacts."

l.147 - What was the constraining factor used for CONISS analysis? Please include.

The constraining factor was depth. With regard to your concerns below we decided to omit the CONISS analysis.

l.201 - What are the rare ASVs? A quick perusal of the Table S1 indicates many zeros. Would it be possible or useful to include a supplementary figure showing rank-abundance curves, either for the entire dataset or for the individual samples?

The majority of ASVs are rare (less than 1% per sample). We will provide an additional supplemental file including the rank abundance curves for each depth (see preliminary Fig. 1 below). We decided to also included several stratigraphic diagrams containing the ASVs without grouping as **Suppl. Fig. S1.**

L232-234: The replicates of each sample show some variations (Fig. 3) in the presence and abundance of ASVs, especially for ASVs amounting to less than 1 % per sample (Suppl. Table 2, **Suppl. Fig S2**).

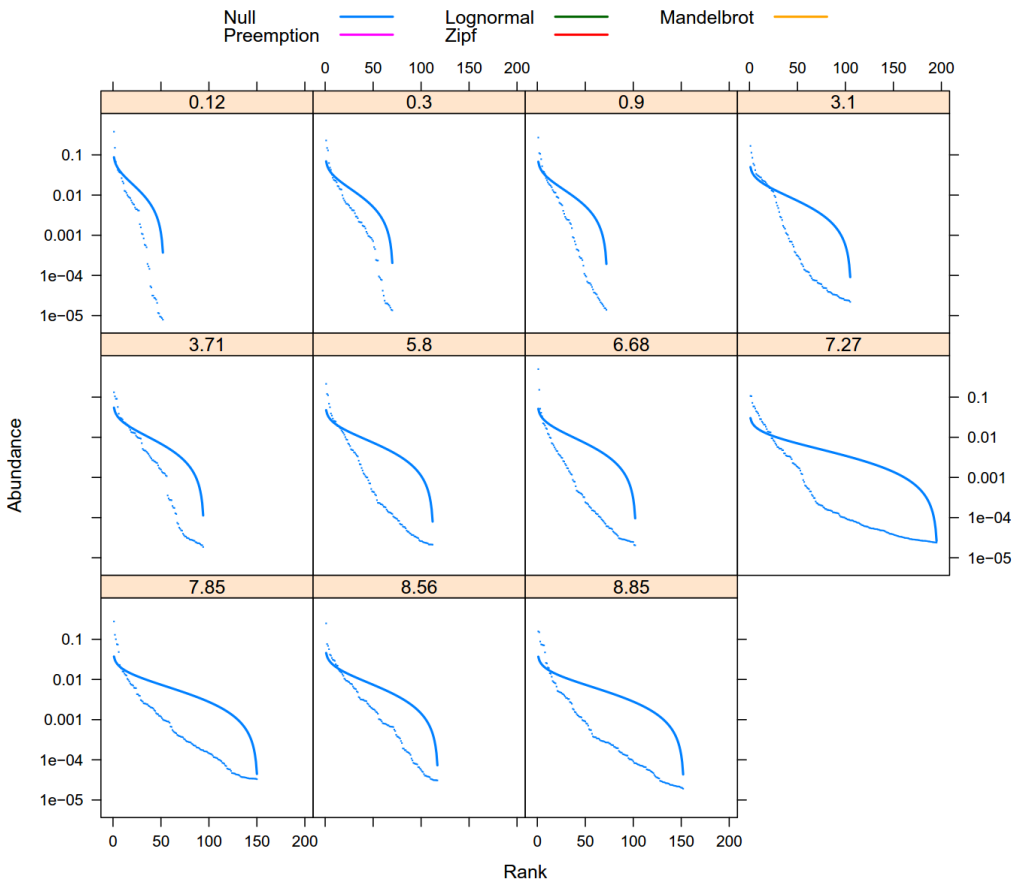


Figure 1: Rank-abundance plot for each sample (depth in m) based on amplicon sequence variants (ASVs).

l.205 - Interesting theory, but why would inhibition only appear in some samples? I think this might be easy to test by a dilution PCR of a few representative samples. Also, if there are differences in the relative amount of diatom DNA in each sedaDNA sample, would it be possible to do a quick qPCR check of rbcL target abundance using the same primer set?

Unfortunately, we could not go back to the lab as work there was reduced to a minimum the past months. We discussed this and decided that it is most likely due to different amounts of diatom template molecules and deleted the part referring to inhibition.

L242-243: It is possible that higher dissimilarities between some replicates are the result of the low amount of template DNA ~~or due to PCR inhibition by compounds that were not sufficiently removed during DNA extraction~~.

l.209 (Section 5) - My first impression from reading the results is that the authors have some difficulty in interpreting diatom community assemblage differences according to the results of the CONISS analysis, i.e. division into five aggregate "zones". In particular because trends in the relative abundance of specific sympagic diatom taxa between different CONISS "zones" are mentioned but not statistically tested. This leads me to question whether CONISS analysis is useful given the present dataset. Were any other analyses attempted to identify discriminant ASVs/taxa? And how did the authors conclude that there are two diatom assemblage reorganizations (l.340) when the CONISS analysis identifies four?

We agree, that this method might not be ideal and removed the CONISS analysis. We have deleted all references to this method and thus slightly adjusted the text.

We also changed the sentences in the abstract and conclusion with this regard:

L21-23: “Taxonomic composition is dominated by cold-water and sea-ice associated diatoms and suggests several re-organizations –after the Last Glacial Maximum, Younger Dryas, after the Early and after the Mid-Holocene.”

L402-404: “The *sed*aDNA record captures substantial temporal change of diatom taxonomic composition and richness with four compositional re-organizations: the first between 20.51 and 15.1 cal kyr BP, the second between 12.8 and 10.1 cal kyr BP, the third between 10.1 and 5 cal kyr BP and the fourth between 5 and 1.6 cal kyr BP.”

l.223 - Could you please elaborate on what is meant by "richness of taxonomic names". According to l.184 different ASVs can be assigned to the same name, so how might this affect the apparent richness? And why use "taxonomic names" for richness calculations when taxonomic rank assignment is not uniform across all ASVs?

With "richness of taxonomic names" we mean grouped ASVs assigned to the same taxonomic name. We compared rarefied taxonomic richness based on (1) ASVs and (2) also for grouped ASVs assigned to the same taxonomic name. While changes of ASV-derived richness vary stronger between the samples in comparison to the changes of ASVs grouped by their taxonomic name, the trends are similar: Richness is highest in the last glacial samples, lower in the deglacial samples and lowest in the Holocene samples.

L258-259: We changed the sentence to:

“Generally, the richness of both ASVs and unique taxonomic names (ASVs grouped based on identically assigned taxonomic names) is higher in samples dated to the last glacial in comparison to those dated to the Holocene (Fig 4).”

Furthermore. we changed the captions of Fig. 4 and 5 to:

“Figure 4: Barplots showing the rarefied (a) number of amplicon sequence variants (ASVs) per sample and (b) ~~number of unique taxonomic names~~ grouped ASVs assigned to the same taxonomic name for each sample with depth (m) of the sediment core MSM05/5-712-2.”

“Figure 2: Taxonomic composition with relative proportions (%) of the 360 detected sequence variants grouped into 75 unique taxonomic names based on identically assigned taxonomic names of sediment core MSM05/5-712-2. Taxonomic names are sorted according to the weighted average with depth.”

l.224 - I am not quite comfortable with the use of "turnover" in the context it is used, since the samples are discrete and therefore a discontinuous representation of time. In my opinion, "turnover" suggests a biological/ecological linkage from one sample to the next, while in this study the samples compared are isolated snapshots in time. Can the authors comment on the choice to use this term?

We agree, that the term is misleading. We chose the term as we see a distinct change in the taxonomic composition, e.g. after the LGM. But since we did not calculate beta-diversity (exactly because we only have a few samples) we changed “turnover” to “shift” in all occurrences.

Fig.5 - I wonder if the reader might find this figure somewhat difficult to interpret given that relative taxon abundance at multiple taxonomic ranks are presented for each sample. Could the authors please the reasoning for presenting the data in this way?

Stratigraphic diagrams are a common representation in paleoecology, for example for pollen or microfossil analysis. As taxonomic resolution differs in different families or genera (for example due to lack of morphological differences or here due to sequence similarity of closely related species in the databse), showing the detected organisms on the lowermost taxonomic level as possible was our goal. Out intentions was to used wa.order="topleft" option in strat.plot, which sorts the taxa according to the weighted average with depth to better visualize the change over time. However, we see your point and will omit the weighted average and make the plot more informative, e.g. by separating centrics from pennates. Again, in the manuscript we want to show the grouped version to make the plot as complete as possible. However, we made a new supplementary file with several figures showing strat.plots of all 360 ASVs.

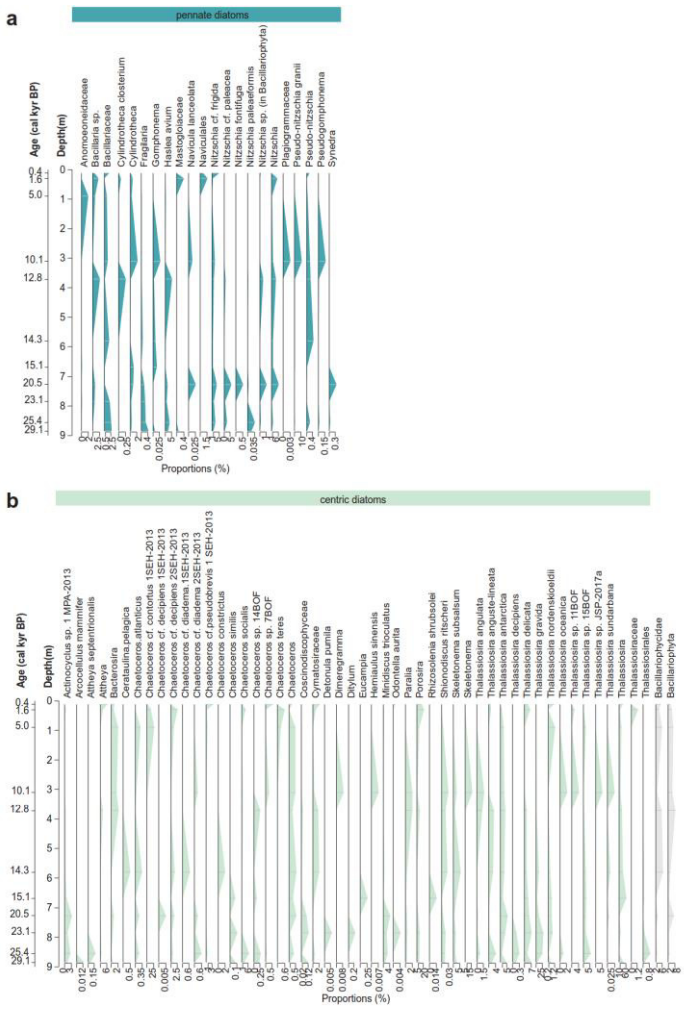


Figure 5: Stratigraphic diagrams with sequences assigned to (a) pennate diatoms (blue) and (b) centric diatoms (green) and higher level sequences assigned between family and phylum level (gray) The taxonomic composition with relative proportions (%) of the 360 detected sequence variants is grouped into 75 unique taxonomic names based on identically assigned taxonomic names of sediment core MSM05/5-712-2.

Table 1 - The Paleoenvironmental conditions descriptions seem somewhat arbitrary. For example, how is "sea-ice retreat" (3.1 m depth) different from "Reduced sea-ice cover allowing spring sea-ice algal and summer phytoplankton productivity" (7.85 m depth).

We agree. We cited the descriptions given in the original research. We decided to omit the table.

l.239 – foraminifer

Changed. Thank you.

Fig.6 - Family-level taxonomy is presented but l.170-174 states that this collation may mask functional differences.

We provided stratigraphic diagrams for all 360 ASVs in the supplement. This is figure was supposed to give an alternative representation of the data on family level in context with IP_{25}. We also sorted the taxa according to centrics and pennates, so that the figure will be easier to understand.

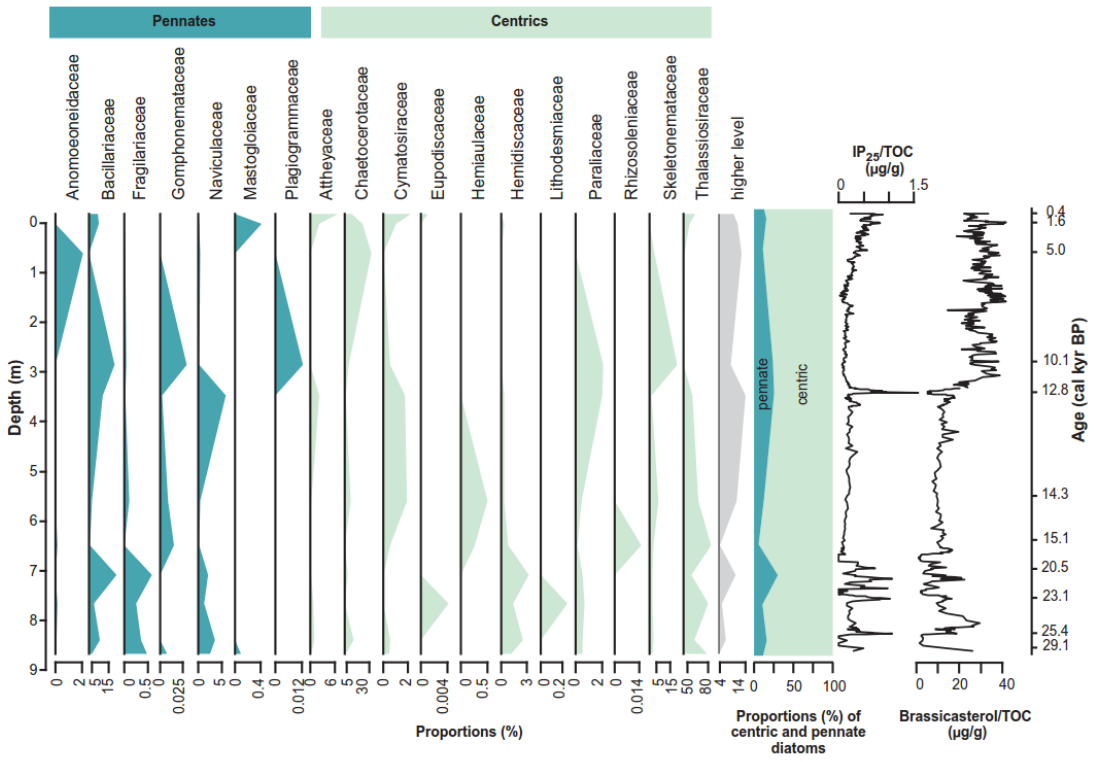


Fig.6 - The double top axes (depth and age) are very helpful, but identify very clear differences in sedimentation rates in the downcore. For example, zone II has higher temporal resolution than zone I. Might not differences in sedimentation rates also affect sedaDNA signal?

Yes, a higher temporal resolution indeed could affect the sedaDNA signal. This could be an additional explanation for the lower richness of zone II in comparison to zone I. However, the distinct shift we see in taxonomic composition is rather an environmental signal. We added the following sentence.

L299-301: “Higher sedimentation rates and thus higher temporal resolution during the Bølling/Allerød phase could have affected the *sed*aDNA signal, yet the distinct shift in taxonomic composition suggests, that this is rather an effect of the changing environmental conditions during this phase.”

l.247 - Again, ambiguous results from CONISS analysis?

Changed

l.336 - "highly detailed taxonomic resolution" depends on what is meant by highly detailed, and what fraction of the data is being referred to. I suggest moderating this statement.

L398: We deleted the word "highly". We mention in this sentence that we refer to the fraction of data resolved on genus and species level. In total 64.4% of our 360 ASVs of the filtered dataset are resolved on genus or species level (see line 163), and we believe this is quite detailed. But

l.320 - Very interesting that N. cf. frigida sticks out as a possible new sea-ice proxy. However, according to Fig.6, this taxon as highest relative abundance in the most recent sample (0.4 kya BP) when sea-ice cover is low. What about Cylindrotheca closterium? Or Haslea avium? Again, I think it would be very helpful if taxon relative abundances were statistically tested in order to identify ASVs/taxa that contribute significantly the observed diatom diversity in different samples.

Around 0.4 kyr BP, which falls in the temporal phase of the Little Ice Age, IP25 values are increasing. So there is sea ice influence. Results of our statistical analysis can be found in the comments of Reviewer 2. We did not find a linear relationship between any of the ASV in our record and IP_{25}. However, we have re-written the section (L357-398) and included the new results.

Answers to os-2019-113_RC2

Methods: Section 2.1: The coring equipment and handling should be described. Was this a piston core? What diameter? How was it handled? Were the cores halved onboard? Did the authors use an archive half or a working half that had previously been used for other sampling activities? Most importantly, how was the core stored – at what temperature? If at -80 C, this should be clearly stated. If not at -80 C, it should be acknowledged that DNA preservation ex situ may be imperfect.

The core was taken in 2007 with a Kastenlot corer (gravity) that has a diameter of 30 x 30 cm. As this is sufficient material, about 1m long subcores are taken and stored at 4°C. The core has to be opened and subsampled on board. However, the subcores had also been used for other sampling activities. We will refer to the supplement of Gersonde et al. (2012) in the manuscript where the procedure is explained in detail.

We will change the sentence to:

L88-91: "The kastenlot core MSM05/5-712-2 (N 78.915662, E 6.767167, water depth 1487 m) was collected from the western continental slope of Svalbard during the cruise of Maria S. Merian (Budéus, 2007) in the eastern Fram Strait in 2007 (Fig. 1). On board, subsections of 1 m length are taken in square plastic boxes as explained in the supplement of Gersonde et al. (2012) and stored at 4°C. Therefore, DNA preservation might be imperfect."

The last sentence changed due to English correction to:

"On board, subsections of 1 m length were placed in square plastic boxes as explained in the supplement of Gersonde et al. (2012) and stored at 4°C. This may have affected DNA preservation."

Do the authors have any tracers or controls for post-coring seawater influence on the sediment core? I am willing to believe that the authors, working in a dedicated lab, were meticulous about their sampling and extraction. Did they use tracer DNA as described by Epp et al.?

At the time of coring (2007), it was not planned to analyze ancient DNA. Hence, no tracers were added. We understand such concerns, but the chance of post-coring contamination deep inside a Kastenlot core seems quite low. A supporting argument is that if we had such contamination, we would not see distinct changes among our samples that are consistent with past climatic changes.

Section 2.2: This is the most significant concern I have about this dataset. The primer set used was designed by Dulias et al. for freshwater lakes. Its specificity for diatoms appears to be very high, as the authors highlight, which is good to address. However, it contains multiple mismatches on both the forward and reverse primers for the taxa noted to contribute to the IP25 proxy (Haslea, Pleurosigma; please see attached "supplement"). (I did not look at other marine taxa, but that should be investigated.)

Please also note the supplement to this comment:

https://www.ocean-sci-discuss.net/os-2019-113/os-2019-113-RC2-supplement.pdf

The primers were designed to function as a general diatom-specific marker by Stoof-Leichsenring et al. (2012), but since then they were mostly applied on lacustrine sediments. We added this information in the introduction:

L78-80: "We used sedaDNA metabarcoding by applying the diatom-specific *rbcL_76* marker (Stoof-Leichsenring et al., 2012) which has already proved successful in low-productivity lakes of northern Siberia (Dulias et al., 2017; Stoof-Leichsenring et al., 2014, 2015), but so far has not been tested on marine sediments."

However, these primers were not tagged for parallel high-throughput sequencing. Tagging and adjustment of PCR conditions was performed by Dulias et al. (2017). We therefore added this information in the methods section:

L118-120: "The PCR reaction mixes and conditions were prepared following the adjusted protocol for tagged *Diat_rbcL_705F* and *Diat_rbcL_808R* primers as described in Dulias et al. (2017) with the exception that 3 µl DNA (DNA concentration 3 ng μL^{-1}) was used as a template."

We agree with your concern. Mismatches in primer binding sites are not ideal, but using DNA metabarcoding always comes with a trade-off. The primer binding sites were chosen to maximize (1) amplification success by having the amplicon short, (2) retrieving as many diatom (pennates and centrics) species as possible with the best resolution possible and (3) it should co-amplify as few non-diatom groups as possible.

Only 3 species are known to produce IP_{25}: *Pleurosigma stuxbergii* var. *rhomboides*, *Haslea spicula* and *Haslea kjellmanii* (Limoges et al., 2018). Of those, only *Pleurosigma stuxbergii* has a reference sequence containing our marker and we detected only 1 mismatch in the forward and one mismatch in the reverse primer-binding site. *Haslea nusanatra* and *Haslea howeana* which you provided in your supplement have a tropical distribution. On the other hand, *Haslea avium*, which was also detected in our study, shows only 1 mismatch in the forward and one mismatch in the reverse primer-binding site. We checked the number of mismatches in the forward and the reverse primers with the 15 *Haslea* spp. reference sequences in our database and found between 1-5 mismatched in forward and 0-2 mismatches

in reverse primers. The number of mismatches *Haslea spicula* and *Haslea kjellmanii* have is certainly in that range. However, most sequences that we retrieve show mismatches: e.g. *Thalassiosira* species mostly have 2-3 mismatches with the forward and 0-2 with the reverse primers. And up to 5 mismatches with the forward and 1 mismatch with the reverse primer can be found for *Chaetoceros socialis* (assigned to GenBank Accession FJ002154).

As a result this dataset should not be considered to be a well-rounded assessment of diatoms in this setting. Any of the increases / decreases in richness, or the absence of certain taxa, or overall assessment of diatom diversity are not valid as we are effectively blinded to many of the taxa known to be present in this location (and presumably throughout the sediment record).

This concern is based on the assumption that the primers were specifically designed for freshwater lakes, which they were not. We agree that absences in our data does not necessarily mean absence in the past.

We included the following sentence:

L230: "The recovery of taxa by *sed*aDNA metabarcoding is prone to false presences or absences. As our study lacks a morphological diatom record, false absences cannot be assessed and true presences cannot be confirmed, which means that absence in our record does not necessarily translate to a true physical absence in the past."

This could perhaps be partially addressed by compiling an alignment of diatom chloroplast rbcL sequences documented to be or have been present at this location and noting how many taxa you would expect to find do or don't have mismatches with the primers. Maybe the authors are aware of this (?). At the end of the day, the detection of the sequences found is real data, and valuable. However, a broad assessment of shifts in diversity and community responses to climate is very problematic.

We agree with your concern. Unfortunately we only know a fraction of the species that occur(red) in the area. The region is overall understudied by molecular surveys and dissolution leads to incomplete microfossil records. Hence, we feel that we cannot provide such an alignment, as it would be incomplete as well. About the mismatches, please have a look 2 questions above. This is a known issue and wobble bases are included in the primers for mitigation of this bias. If it is desired, we can prepare a figure to visualize the primer mismatches.

On a different note, the cycle numbers and annealing temperatures, in my opinion, should be included even if they are included in the given citation. 50 cycles is a very large number. Did the authors ever visualize bands in their extraction or PCR negative controls?

We agree, that 50 cycles are a very large number, but large cycle numbers are not uncommon in ancient DNA studies (up to 65 cycles: (Willerslev et al., 2014) or 45 cycles: (Voldstad et al., 2020)). We point these issues out in lines 210-214. For future studies, reducing cycle numbers to 45 is planned, but we cannot change this for the current project. However, reducing cycle numbers also comes with a trade-off, as this has shown to reduce replicability (Krehenwinkel et al., 2017; Nichols et al., 2018).

L214: We included the reference Nichols et al., 2018.

Yes, after each PCR, we first evaluated bands on an agarose gel. We will include the following sentences:

L120-123: "PCRs were performed with the following settings: 5 minutes at 94°C (initial denaturation), then 50 cycles at 94°C (denaturation), 49°C (annealing) and 68°C (elongation) and a final elongation step at 72°C for 5 minutes. Subsequently the PCR success was checked with gel-electrophoresis."

Line 110 – what version (chemistry)?

We change the sentence to:

L126: "The sequencing library was prepared with the Mid Output kit v 2 according to the Fasteris Metafast protocol for low complexity […]"

Section 2.3: I'm OK with the use of ASVs and I appreciate the authors taking time to explain why they didn't use OTUs. Regarding their reference database, how many references did this method produce? Did these references include diatoms documented in other studies from the waters around Svalbard? The reference database is a frequent scapegoat in the discussion; release 138 is a couple years ago. The taxonomy seems pretty robust to me but it could be updated if need be.

This method produced 2,320 references, and yes, it contained many taxa which are documented in waters around Svalbard, such as *Nitzschia frigida*, *Chaetoceros socialis*, *Thalassiosira antarctica* or *Thalassiosira nordenskioeldii* (to name a few that were also detected in our record).

The ENA/EMBL release 138 was released in November 2018, and thus up-to-date when we started the analyses. The reviewer probably confused this with the GenBank release 138 from 2003.

We changed the sentence to:

L137-139: To generate the reference database for the taxonomic assignment of the sequences we downloaded the EMBL release 138 (released November 2018) and used *ecopcr* (Ficetola et al., 2010) according to the descriptions of Dulias et al. (2017) and Stoof-Leichsenring et al. (2012) containing 2,320 reference sequences.

Going back to the negative controls, on line 122 how many exactly is "the majority" that were singletons?

We provide an additional Supplementary table which contain the negative controls from extractions and PCRs. Furthermore, we will change the sentence to:

L141-142: "Of the 204 different sequence variants detected in extraction and PCR negative controls 83% of their occurrences were singletons and […]"

On line 125, why 10 read counts? How many sequences, and how many ASVs, were removed out of the total using these criteria? As the supplemental table only has the kept sequences, one can't tell.

This is only a first threshold we use for denoising so we do not lose too much information in the beginning. We included the following information in the text:

L148: "Filtering with R reduced the number of read counts from 7,536,449 to 6,199,984."

Section 2.4 (and throughout): Please make sure to always use the term "PCR replicate" or "PCR replication", as these are not sample replicates. PCR replication is useful but the terminology

should be clear as later on it may get confusing for readers who aren't methods geeks and think that these are sample or extraction replicates.

Thank you for pointing this out. We changed this accordingly throughout the manuscript and figure captions.

Section 2.5: Resampling 100 times might be overkill but I guess it can't hurt. The new minimum number of sequence counts doesn't quite make sense to me through – can you clarify "according to descriptions in the preceding paragraph"? (It's not quite 12 *25,601.)

We change the following sentence "For taxonomic composition and richness calculations, we first summed up the sequence counts for each ASV of the replicates belonging to the same sample…"

To:

L164-167: "For taxonomic composition and richness calculations, we combined the PCR replicates of the corresponding sample. This resulted in a new minimum number of sequence counts (300,415 counts) that was used for resampling the dataset 100 times according to descriptions in the preceding paragraph."

Section 3: Line 157 – What do you mean by "outperformed"? Are you referring to the specificity for diatoms? Please clarify.

Thank you for pointing that out. We changed the sentence to:

L187-188: "Possibly owing to a much shorter marker size, the *rbcL_76* marker also surpassed the usually amplified 18S rDNA markers with regard to specificity for diatoms (Coolen et al., 2007; De Schepper et al., 2019; Kirkpatrick et al., 2016)"

Line 164 – "striking proportion" – I don't think it's that striking, in fact, 4.8% seems pretty low!

L194: We deleted the word striking.

Line 177 – can you give a numeric range for the copy number variation in published work?

Unfortunately we cannot give a numeric range, as most papers we found mention this as vaguely as we did. However, we included another reference, where the authors showed that rbcL copy number was significantly different in the 8 tested benthic freshwater diatoms and that this variation is positively correlated with cell biovolume (Vasselon et al., 2018).

We changed the sentence to:

L208-210: "Furthermore, the enrichment of centric diatoms in the *sed*aDNA record could be the result of copy number variation of the *rbcL* gene between different species and cell biovolume (Bedoshvili et al., 2009; Round et al., 1990; Vasselon et al., 2018)."

L81: We also included the reference (Vasselon et al., 2018) here.

Section 4: Here I think again it is important to be clear that the replicates being discussed are PCR replicates of the same template DNA. Also, what happened to the two sample replicates from the deepest sample? How similar or dissimilar were they and does the sample variation exceed the variation found in PCR replication? While two duplicates aren't statistically as useful as a triplicate, they need to be discussed in the context of reproducibility. Doing so would, I think,

strengthen the authors' arguments. This should be addressed in revision (unless I missed it somewhere?).

We already handled PCR replicates, as mentioned above.

In the NMDS plot (see below the improved version of the figure), you can see, that the 3 PCR replicates of the two samples at 8.85 m depth cluster so narrowly, that it is really hard to identify the labels at all. Hence, they were very similar. However, as this is an ordination biplot, we cannot move around the labels. Therefore, we included the colours of the polygons in the caption and hope that this makes it clearer.

The degree of dissimilarity is visualized in the NMDS plot. We agree that more replicates always help to decrease the level of uncertainty and include this statement in the text:

L238-240: "For the oldest sample at 8.85 m depth we additionally processed a sample replicate. The PCR replicates of both sediment samples at 8.85 m depth were highly similar and clustered tightly together in the NMDS plot (Fig. 3). Although a higher number of replicates would improve the robustness of our analysis […]"

We changed the following sentence to make it clear, that the PCR replicates are based on the same template:

L 232-233: "The PCR replicates (different PCR products from the same DNA extract) of each sample show some variations (Fig. 3) […]"

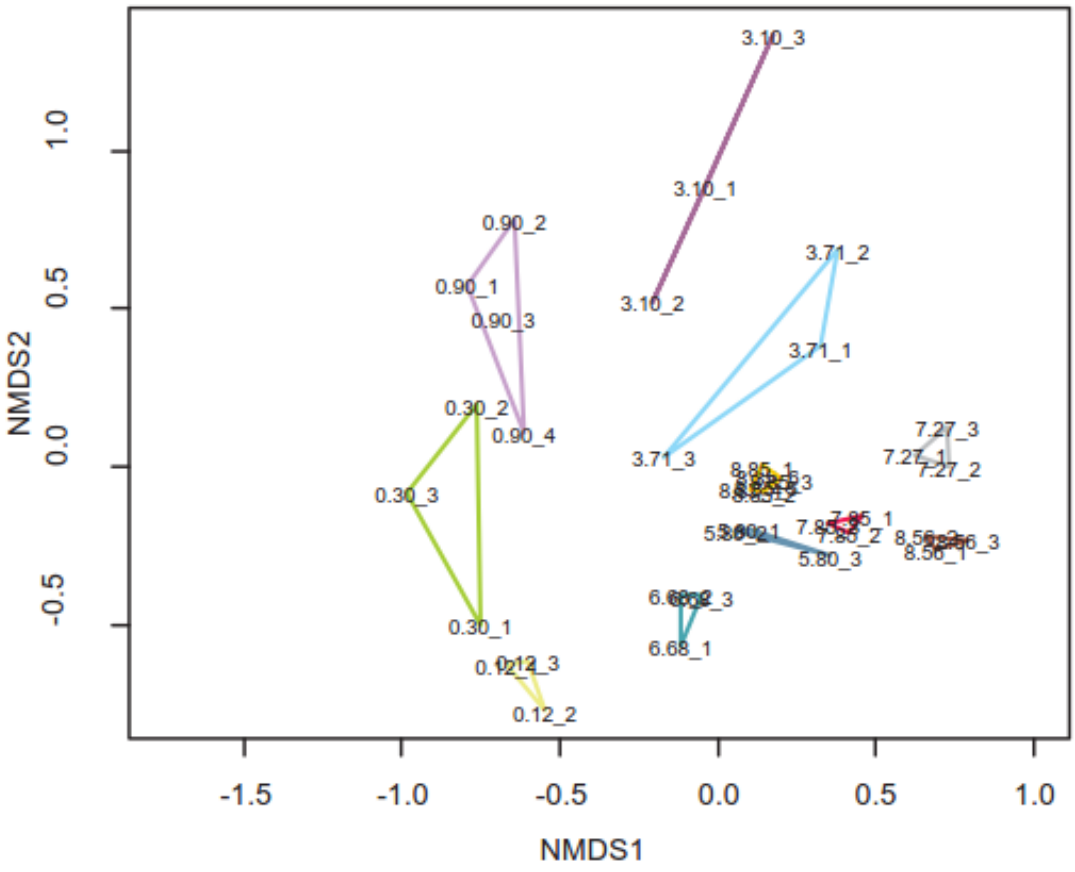


Figure 3: Non-metric multidimensional scaling plot based on the filtered and resampled diatom dataset with the PCR replicates (indicated by an underscore with a number) of a sample (depth in m) linked in a polygon of sample-specific colour: light green = 0.12m, green = 0.3m, light violet = 0.9 m, violet =

3.1 m, light blue = 3.71 m, dark blue = 5.8 m, turquoise = 6.68 m, grey =7.27 m, red = 7. 85 m, brown = 8.56 m, orange = 8.85 m (2 sample replicates with 3 PCR-replicates each).

Section 5: Lines 228-299: This inverse relationship, if significant, is intriguing! Please test statistically for significance.

The inverse relationship between *Chaetoceros* and *Thalassiosira* was tested for significance on family level. The Pearson's correlation efficient is -0.61126971, with a p-value of 0.045701848. Hence, there is a moderate significant negative correlation. We included the information in brackets into the sentence:

L264: "A general trend that can also be observed at the family level is an inverse relationship (r=-0.61, p= 0.046) of the dominant families Thalassiosiraceae and Chaetoceraceae (Fig 6)"

Furthermore, we will include a sentence into the methods section (see answer after the comment regarding section 6).

Lines 242-244... etc: This sort of speculation about how ice conditions may have affected diatom community diversity, etc., is undermined by the primer issue.

Is this concern based on the assumption that the primers were specifically designed for freshwater lakes (which they were not)? Based on the sedaDNA composition (both sympagic and pelagic diatoms were detected) and by taking into consideration the results of other proxies during this time, it is conceivable that conditions were heterogeneous throughout the seasons. We changed the sentence slightly:

L279-282: "It is conceivable that a heterogeneous and dynamic environment produced by winter sea-ice cover with ice-free conditions during summer probably allowed for diverse diatom communities to develop in the different habitats and over the seasons, which is reflected in the highest numbers overall of ASVs in samples dated to this time span."

Due to English correction the sentence changed to:

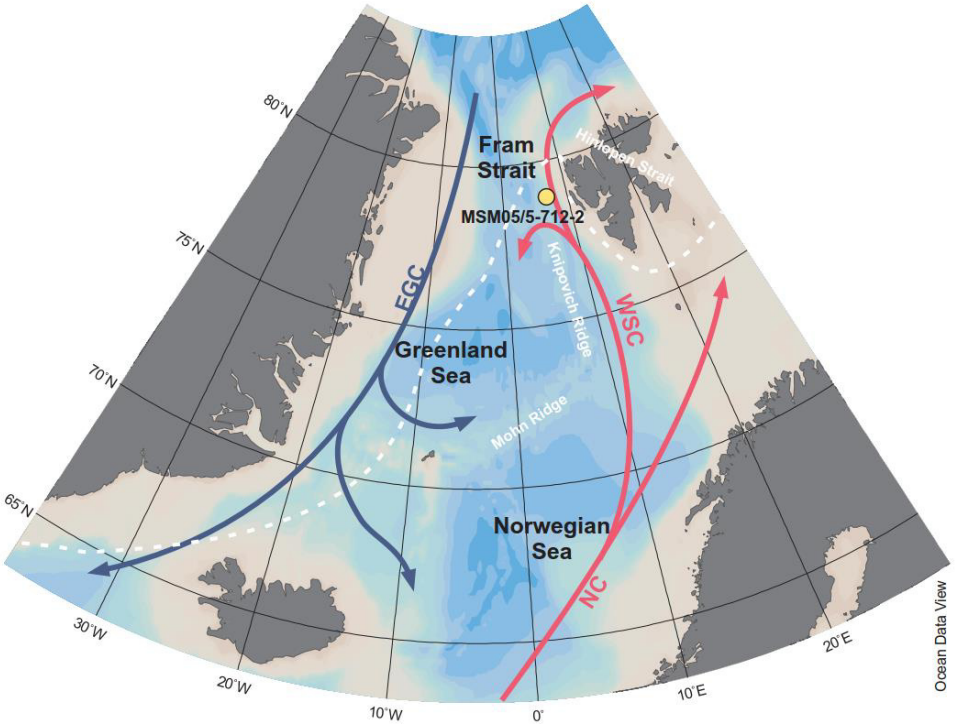
"It is conceivable that a heterogeneous and dynamic environment produced by winter sea-ice cover with ice-free conditions during summer could allow diverse diatom communities to develop in the different habitats and over the seasons, which is suggested by the highest overall numbers of ASVs in samples dated to this time span."

Lines 253 and elsewhere: "Low proportions of Nitzchia cf. frigida..." and similar statements should be reworded, as we do not know the proportion of diatoms. We know the proportions of the sequences found in the dataset after extensive amplification (50 cycles!). Please be careful to keep this distinction clear.

We included the proportions in brackets after each statement.

Line 265: How far away is that (Hinlopen Strait)? In this section in general, there are numerous and interesting comparisons to published records from related locations. It would be appreciated if the proximity of these (in km) were clear for the less-familiar reader. (E.g. also line 276, 278, 291, 304, 323)

We added an annotation of the places mentioned in our text into the map.



Line 274: "peak proportions", "Lower proportions" – can you please quantify. (Also e.g. line 301.)

Yes, we included the proportions in brackets after each statement.

Line 289: Richness and diversity are not the same; please be specific.

We will consistently refer to richness instead of diversity as recommended.

Section 6: Beyond the methods concerns, there are still some interesting points here. These could be strengthened by a statistical analysis testing the correlation between the new data to the published IP25 proxy (lines 319 - 323).

We have tested for significance, first on family level and second on ASV level and will add this information in the text, the plots showing significantly correlated families or ASVs (see Figure below) will be added in the Supplement. As we had to interpolate IP_{25} values for the depths that were analysed in this study, we include a paragraph describing the process in the methods section. We plotted the significantly correlated ASVs (see table below and figure below) and found that they do not show a linear relationship with IP_{25}, which is why we will not include the table in the manuscript. However, some of the patterns are quite interesting (see figures below the table) and we have re-structured section 6 and included new results and parts of the discussion about primer mismatches in Haslea and Pleurosigma.

We included:

L174-179: "For correlation analysis we interpolated IP_{25} values using the methods described in Reschke et al. (Reschke et al., 2019). Therefore, the IP_{25} data were transformed using function *zoo* from the "zoo" package (Zeileis and Grothendieck, 2005) and used in the function *CorIrregTimser* using the package "corit" (https://github.com/EarthSystemDiagnostics/corit). The correlation between Chaetocerotaceae and Thalassiosiraceae as well as between IP_{25} and all ASVs was tested for significance using R function

rcorr (method Pearson) from the package "Hmisc" (Hollander and Wolfe, 1975; Press et al., 1988)(Quelle)."

Table 1: Amplicon sequence variants (ASVs) with significant (p-value) correlation.

ASV	Correlation coefficient	p-value
Attheya28	0.7542327	0.00732029
Bacillariaceae71	0.6970434	0.01713238
Bacillariophyta157	0.7159116	0.01322211
Bacillariophyta245	-0.6217257	0.0411348
Chaetoceros.cf.pseudobrevis.1.SEH.2013461	0.6619875	0.02649079
Cylindrotheca.closterium558	0.6387489	0.03439247
Gomphonema586	-0.6077406	0.04731908
Haslea.avium589	0.6899115	0.01880586
Nitzschia.cf.frigida709	0.8093591	0.0025435
Thalassiosira1017	-0.673798	0.02301182

The following figure and caption are included as additional supplement.

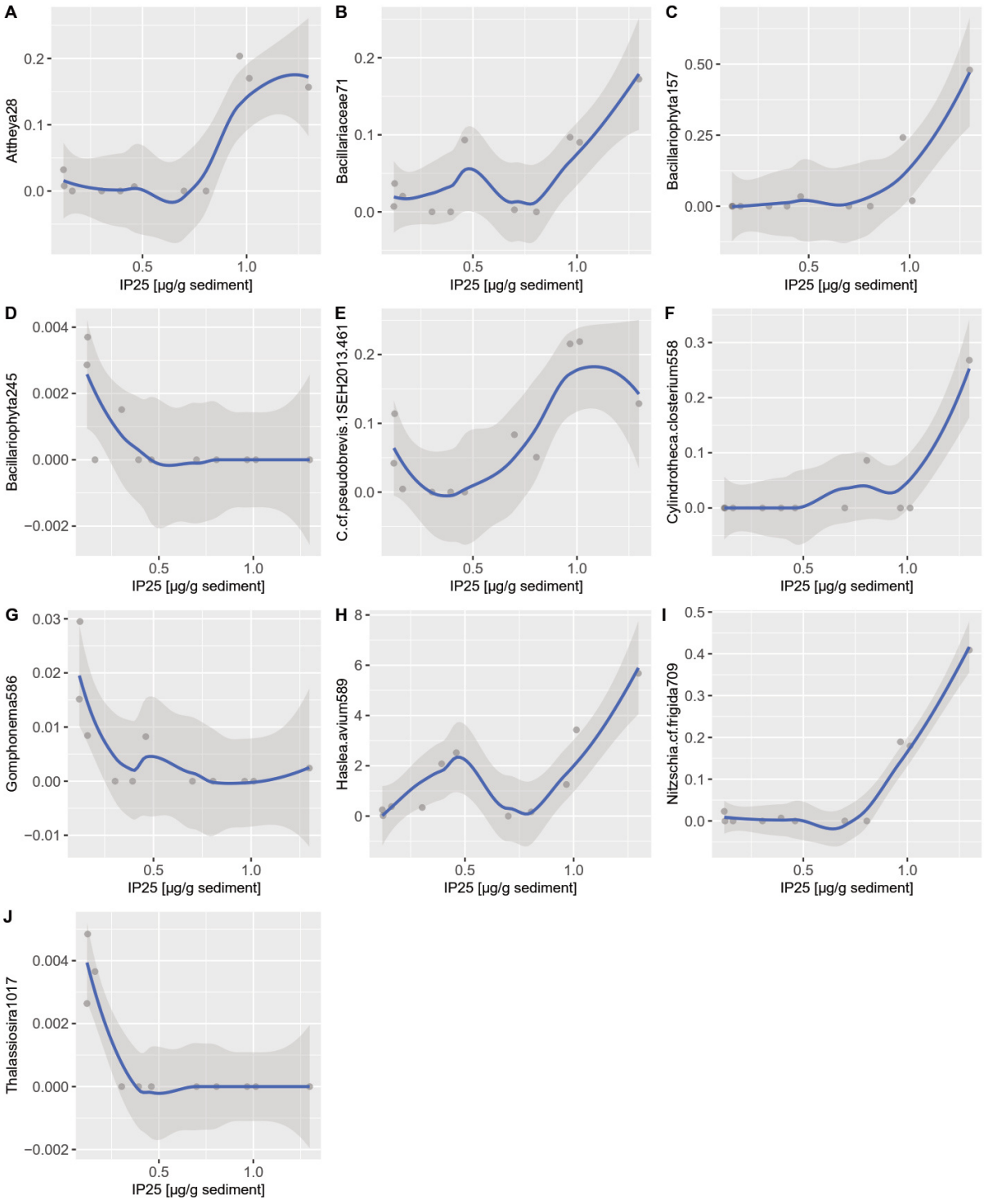


Figure S3: Amplicon sequence variants with significant correlation to interpolated values of the sea ice biomarker IP_{25} with loess smoothing (blue line) and standard error (dark grey area).

Technical corrections: Figure 3: Labels are not readable. Figure 5: This appears upside-down. More importantly, the labels and proportion bars are very, very small; but much of the graph is empty whitespace. Please try making the bars wider. Also, consider organizing the taxa by e.g. rank order (based on proportion) and breaking into 2 or 3 separate figures. Figure 56: Please define "higher level" in the caption.

Fig. 3: As we don't want to manipulate the biplot by spreading apart the labels, we attributed distinct colours and will refer to PCR replicates in the caption. We placed the figure to another answer further above (Figure 3).

Fig. 5: Yes, we split the graph into 2 graphs and included further recommendations from Jessica Ray (Reviewer1). Originally we used a rank order (based on proportion): wa.order="topleft" option in strat.plot, which sorts the taxa according to the weighted average with depth.

We defined higher level in the caption as: "Higher level contains sequences assigned between family and phylum level."

Answers to os-2019-113_SC1

The term "richness" is used very often, and I have difficulties understanding that term. Could you please add some information on this term, as you use it very often.

We included the following sentence:

L162-163: "As a measure of alpha-diversity we calculated richness of (1) ASVs (number of amplicon sequence variants) and (2) unique taxonomic names (number of grouped ASVs that were assigned to the same taxonomic name)."

Abstract: The Abstract is missing some information on the investigated material/samples.

We included the information in the abstract.

L17-18: "By amplifying a short, partial *rbcL* marker on sediment core MSM05/5-712-2, […]"

Introduction: I have the feeling other sea ice proxies should be mentioned. Further, some information on the advantages of the sedaDNA study you present here is missing. It is not clear why sedaDNA may be of advantage for sea ice reconstructions, as the sea ice biomarker IP25 is not as prone to dissolution as microfossils.

We agree. We moved the paragraph further below after we introduced ancient DNA and have re-written it.

L42-45: "Next to microfossil-based reconstructions, the diatom produced sea-ice proxy IP25 (a highly branched isoprenoid alkene with 25 carbon atoms; (Belt et al., 2007)) combined with phytoplankton biomarkers (e.g. brassicasterol, dinosterol; (Volkman, 1986)) permit semi-quantitative reconstructions of past sea-ice distribution (Belt, 2018; Belt and Müller, 2013; Müller et al., 2009; Müller and Stein, 2014; Stein et al., 2012, 2017). However, diatoms […]"

Chapter 6: I feel this chapter needs more detail. The mismatch of IP25 and sedaDNA was to be expected, as you do not investigate the IP25 producers. Could you elaborate more on the reasons for the mismatch of biomarker and sedaDNA record? What about seasonality of N. frigida and IP25 production? From SW Greenland, Krawzcyk et al (2015; Polar Biology) found that N. frigida is most abundant in late winter before the main spring bloom – which is expected to be the main production season of IP25 in Fram Strait. What about habitats (under the ice, inside the ice) between the different species? And finally what are your recommendations for future work or when using sedaDN for sea-ice reconstructions?

We have re-structured section 6 according to comments made by the other reviewers and discussed more about the ecology of *Nitzschia frigida* in this context.

At the end of our conclusions, we added the following recommendation for future work:

L407-410:"Recommendations for future work with sedimentary ancient DNA in the context of sea ice reconstructions include the preparation of reference genomes and a more targeted enrichment, for example of genes that help species to adapt to sea ice and allow them to cope with rapidly changing environmental conditions."

L36 Krawczyk et al., 2017 should be added here

L41 + L382: We added the reference.

L59 & L60 overuse of whether

L69: We exchanged the first occurrence with if.

L82 I find the description of the sea ice condition in the working area confusing.

We changes the sentence to:

L96: "Today, the site is located south of the winter and summer sea-ice margin and is ice-free year round […]"

L82 mentioning past sea ice variability feels wrong here, maybe add this information with some more detail to the introduction

We moved the sentence to L74-76 and changed the sentence to:

"As previous work indicates variability in the past sea-ice cover (Falardeau et al., 2018; Müller et al., 2012; Müller and Stein, 2014; Werner et al., 2011, 2013), samples were chosen according to high, medium, and low concentrations of the diatom produced sea-ice biomarker IP_{25} (Müller et al., 2012; Müller and Stein, 2014) and we expect associated changes in the taxonomic composition."

L86 should say Epp et al. (2019), L116 should say Callahan et al. (2017), L120 should say Dulias et al. (2017) and Stoof-Leichsenring et al. (2012)

We have changed them accordingly.

L134 I do not understand this Quote.

We changed the sentence to:

L154-157: "We resampled the dataset 100 times to the minimum number of sequences available (25,601 counts), then, for each replicate, we calculated the mean number of sequence counts for each ASV across the 100 resampling steps (code available at: https://github.com/StefanKruse/R_Rarefaction; Kruse, 2019)."

L150-159 I feel the information on lake studies has too much detail whereas the information on marine studies is too short as the presented study is marine.

Here we want to show, that only few studies exist that focus on ancient DNA from diatoms. As a couple of weeks ago a study about diatom and foraminiferal ancient DNA was published (Pawłowska et al., 2020) and we will add this to the list at

L68: […] or diatoms in particular (Pawłowska et al., 2020).

We included the underlined statement, to make this clearer:

L78-80: We used *sed*aDNA metabarcoding by applying the diatom-specific *rbcL_76* marker (Stoof-Leichsenring et al., 2012) which has already proved successful in low-productivity lakes of northern Siberia (Dulias et al., 2017; Stoof-Leichsenring et al., 2014, 2015), but so far has not been tested on marine sediments.

L198 This is a major problem. However cannot be changed for your study but I welcome this comment. For future studies the parallel investigation of biomarkers and diatoms in the microfossil and genetic record may be a very promising approach.

Yes, we agree.

Further corrections we made:

1. L4-5: Rüdiger Stein would like his name spelled Ruediger in the author list.
2. We adjusted all µl to µL and replaced division signs such as g/ µL with g μL^{-1}
3. L140: As we prepared Suppl. Table 2, we found that the last column was not included in building the sum of extraction negative controls and we changed it accordingly from 201 to 237 counts.
4. In the comments of SC1 there was some critique about the introduction and we felt, we have to improve the first paragraph of the introduction.

   L28-33:We changed it from:
   "The global climate system is strongly coupled with Arctic sea-ice cover (Goosse and Fichefet, 1999): yet Earth system models display large uncertainties in projections of sea ice, which stresses the need for paleo sea-ice reconstructions that can be used to improve the models (de Vernal et al., 2013). Currently, semi-quantitative reconstructions of past sea-ice distribution can be achieved by combining the diatom produced sea-ice proxy IP_{25} (a highly branched isoprenoid alkene with 25 carbon atoms; (Belt et al., 2007)) and phytoplankton biomarkers (e.g. brassicasterol, dinosterol; (Volkman, 1986)) detected in down-core sediments ."

   to:
   L33-39: "The marine environment is a complex ecosystem in which the distribution of organisms is controlled significantly by abiotic constraints such as sea-surface temperatures (SSTs), salinity, nutrient distribution, light conditions, and sea-ice cover (Cherkasheva et al., 2014; Ibarbalz et al., 2019; Nöthig et al., 2015; Pierella Karlusich et al., 2020). Over the past 30,000 years the subarctic North Atlantic Ocean was subject to frequent sea-ice expansions and contractions (Müller et al., 2009; Müller and Stein, 2014; Syring et al., 2020; Werner et al., 2013), which are expected to have affected the composition of the regional species pool. Diatoms (Bacillariophyta) are unicellular, siliceous organisms that are photoautotrophic and thrive in the euphotic zone of the ocean."
5. L428: We included a sentence to the Acknowledgements:

"We thank Cathy Jenks for English correction."

6. We found two small mistakes in the references and corrected them:

   L504-505: Fetterer, F., Knowles, K., Meier, W. N., Savoie, M. and Windnagel, A. K.: Sea Ice Index, Version 3. Monthly and daily GIS compatible shapefiles of median ice extent, National Snow & Ice Data Center, doi:10.7265/n5k072f8, 2017.

   L518-519: Harrison, W. G. and Cota, G. F.: Primary production in polar waters: relation to nutrient availability, Polar Research, 10(1), 87–104, doi:10.3402/polar.v10i1.6730, 1991.

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Changes in the composition of marine and sea-ice diatoms derived from sedimentary ancient DNA of the eastern Fram Strait over the past 30,000 years

Heike H. Zimmermann[1], Kathleen R. Stoof-Leichsenring[1], Stefan Kruse[1], Juliane Müller[2,3,4], Ruediger ~~Rüdiger~~ Stein[2,3,4], Ralf Tiedemann[2], Ulrike Herzschuh[1,5,6]

[1]Polar Terrestrial Environmental Systems, Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research, Potsdam, 14473, Germany
[2]Marine Geology, Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research, 27568 Bremerhaven, Germany
[3]MARUM, University of Bremen, 28359 Bremen, Germany
[4]Faculty of Geosciences, University of Bremen, 28334 Bremen, Germany
[5]Institute of Biochemistry and Biology, University of Potsdam, 14476 Potsdam, Germany
[6]Institute of Environmental Sciences and Geography, University of Potsdam, 14476 Potsdam, Germany

Correspondence to: Heike H. Zimmermann (heike.zimmermann@awi.de)

Abstract. The Fram Strait is an area with a relatively low and irregular distribution of diatom microfossils in surface sediments, and thus microfossil records are ~~underrepresented~~scarce, rarely exceed the Holocene and contain sparse information about past richness ~~diversity~~ and taxonomic composition. These attributes make the Fram Strait an ideal study site to test the utility of sedimentary ancient DNA (*sed*aDNA) metabarcoding. ~~By a~~Amplifying a short, partial *rbcL* marker ~~on~~from samples of sediment core MSM05/5-712-2, resulted in 95.7 % of our sequences ~~are~~ being assigned to diatoms across 18 different families with 38.6 % of them being resolved to species and 25.8 % to genus level. Independent replicates show high similarity of PCR products, especially in the oldest samples. Diatom *sed*aDNA richness is highest in the Late Weichselian and lowest in Mid- and Late-Holocene samples. Taxonomic composition is dominated by cold-water and sea-ice associated diatoms and ~~shows two~~ suggests several re-organizations — ~~one~~ after the Last Glacial Maximum, ~~and another~~ after the Younger Dryas, after the Early and after the Mid--Holocene. Different sequences assigned to, amongst others, ~~to~~ *Chaetoceros socialis* indicate the detectability of intra-specific diversity using *sed*aDNA. We detect no clear pattern between our diatom *sed*aDNA record and the previously published IP_{25} record of this core, although proportions of pennate diatoms increase with higher IP_{25} concentrations and proportions of *Nitzschia* cf. *frigida* exceeding 2 % of the assemblage point towards past sea-ice presence.

1 Introduction

~~The global climate system is strongly coupled with Arctic sea-ice cover (Goosse and Fichefet, 1999); yet Earth system models display large uncertainties in projections of sea ice, which stresses the need for paleo sea-ice reconstructions that can be used to improve the models (de Vernal et al., 2013). Currently, semi-quantitative reconstructions of past sea-ice distribution can be achieved by combining the diatom produced sea-ice proxy IP_{25} (a highly branched isoprenoid alkene with 25 carbon atoms;~~

~~(Belt et al., 2007)) and phytoplankton biomarkers (e.g. brassicasterol, dinosterol; (Volkman, 1986)) detected in down-core sediments .~~ The marine environment is a complex ecosystem in which the distribution of organisms is controlled significantly by abiotic constraints such as sea-~~-~~surface temperatures (SSTs), salinity, nutrient distribution, light conditions, and sea-~~-~~ice coverage (Cherkasheva et al., 2014; Ibarbalz et al., 2019; Nöthig et al., 2015; Pierella Karlusich et al., 2020). Over the past 30,000 years the subarctic North Atlantic Ocean was subject to frequent sea-~~-~~ice expansions and contractions (Müller et al., 2009; Müller and Stein, 2014; Syring et al., 2020; Werner et al., 2013), which are expected to have affected the composition of the regional species pool. Diatoms (Bacillariophyta) are unicellular, siliceous organisms that are photoautotrophic and thrive in the euphotic zone of the ocean. Owing to their sensitive responses to environmental conditions, diatoms are frequently used as indicators for paleoenvironmental reconstructions, to assess, for example, changes in surface water temperatures (Birks and Koç, 2002; Krawczyk et al., 2017; Miettinen et al., 2015), paleoproductivity (Fahl and Stein, 1997; Limoges et al., 2018), and sea-ice distribution (Smirnova et al., 2015; Weckström et al., 2013). Next to microfossil-based reconstructions, the diatom produced sea-ice proxy IP_{25} (a highly branched isoprenoid alkene with 25 carbon atoms; ~~(~~Belt et al., 2007~~)~~) combined with phytoplankton biomarkers (e.g. brassicasterol, dinosterol; ~~(~~Volkman, 1986~~)~~) permit semi-quantitative reconstructions of past sea-ice distribution (Belt, 2018; Belt and Müller, 2013; Müller et al., 2009; Müller and Stein, 2014; Stein et al., 2012, 2017). However,

~~D~~diatoms in northern high-latitudinal regions are less silicified and more prone to silica dissolution (Kohly, 1998; Stabell, 1986) compared to diatoms of the southern polar oceans (Harrison and Cota, 1991). In the Fram Strait – an important area of heat exchange between Arctic and North Atlantic water masses (Untersteiner, 1988) – particularly low and irregular preservation of diatom microfossils prevails in surface sediments (Karpuz and Schrader, 1990; Stabell, 1987). The diatom records are generally underrepresented, contain sparse information about past diversity and taxonomic composition, and rarely exceed the Holocene (Jessen et al., 2010; Koç et al., 2002; Stabell, 1986). This makes the Fram Strait an excellent site to test ancient DNA metabarcoding on a sediment core (Müller et al., 2012; Müller and Stein, 2014).

Ancient DNA is a new proxy that can exploit diatoms as indicators of past marine environmental change (Coolen et al., 2007; De Schepper et al., 2019; Kirkpatrick et al., 2016). Deep-sea sediments have been reported to be rich in DNA with up to 70–90 % of the total DNA pool being extracellular DNA (Coolen et al., 2007; Dell'Anno et al., 2002; Lejzerowicz et al., 2013; Morard et al., 2017), and traces of DNA can be detected in sediments even though microfossils are absent or highly degraded (Boere et al., 2009; Coolen et al., 2009, 2013; Lejzerowicz et al., 2013; Pawłowska et al., 2020)~~(Boere et al., 2009; Coolen et al., 2009, 2013; Lejzerowicz et al., 2013)~~. Therefore, analyses of sedimentary ancient DNA (*sed*aDNA) could be advantageous ~~also~~ in areas of biased preservation due to high silica dissolution rates. Beyond morphological or biogeochemical analyses, ancient DNA can distinguish cryptic species that are morphologically similar (Stoof-Leichsenring et al., 2012), trace temporal changes of intra-specific genetic variation (Epp et al., 2018; Parducci et al., 2012; Zimmermann et al., 2017b), and identify genetic relationships and microevolution (Stoof-Leichsenring et al., 2014, 2015).

Paleogenetic analyses with an emphasis on diatoms have been successfully carried out in various limnic settings ranging from the subarctic (Epp et al., 2015; Stoof-Leichsenring et al., 2014, 2015) to the tropics (Stoof-Leichsenring et al., 2012) and

Antarctica (Coolen et al., 2004). Yet, it is still a relatively underrepresented branch in the marine realm with only a few published studies targeting phytoplankton ancient DNA (Boere et al., 2009, 2011b, 2011a; Coolen et al., 2006, 2007, 2009, 2013; De Schepper et al., 2019; Giosan et al., 2012; Kirkpatrick et al., 2016) or diatoms in particular (Pawłowska et al., 2020). In this study we examine whether if ancient diatom DNA can be retrieved from sediments from the eastern Fram Strait and assess whether it can be used to analyze temporal changes in the taxonomic composition of diatoms. We have three principal objectives: (1) to assess the quality and replicability of the data obtained by *sed*aDNA metabarcoding, (2) to analyze temporal changes of diatom taxonomic composition and richness, and (3) to evaluate diatom *sed*aDNA as a new proxy for sea-ice reconstruction. DNA was derived from distinct samples of the comprehensively analyzed marine sediment core MSM05/5-712-2, covering the major climatic intervals since the Late Weichselian (i.e. the last ~30 kyr BP). As previous work indicates variability in the past sea-ice cover (Falardeau et al., 2018; Müller et al., 2012; Müller and Stein, 2014; Werner et al., 2011, 2013), samplesW were chosen the samples according to high, medium, and low concentrations of the diatom produced sea-ice biomarker IP_{25} (Müller et al., 2012; Müller and Stein, 2014) and as we expect associated changes in the taxonomic composition. We used *sed*aDNA metabarcoding by applying the diatom-specific *rbcL_76* marker (Stoof-Leichsenring et al., 2012) which has already proved successful in low-productivity lakes of northern Siberia (Dulias et al., 2017; Stoof-Leichsenring et al., 2014, 2015), but so far has not been tested on marine sediments. The marker amplifies a short region of the *rbcL* gene, which is located on the chloroplast that is present in diatoms in several copy numbers (Vasselon et al., 2018), thereby increasing the probability of its long-term preservation. The *rbcL* gene has an adequate sequence reference database and was tested as a potential diatom barcode marker with high resolution power (Guo et al., 2015; Kermarrec et al., 2013). Furthermore, it reduces co-amplification of non-photosynthetic bacteria or archaea that are active in subsurface sediments and thus could be preferentially amplified during PCR in comparison to the highly fragmented and damaged ancient DNA.

2 Materials and Methods

2.1 Study site and sample material

The kastenlot core MSM05/5-712-2 (N 78.915662, E 6.767167, water depth 1487 m) was collected from the western continental slope of Svalbard during the cruise of *Maria S. Merian* (Budéus, 2007) in the eastern Fram Strait in August 2007 (Fig. 1). On board, subsections of 1 m length are takenwere placed in square plastic boxes as explained in the supplement of Gersonde et al. (2012) and stored at 4°C. Therefore,This may have affected DNA preservation might be imperfect. The Fram Strait is located between Greenland and Svalbard and connects the Arctic Ocean with the Atlantic Ocean. The study area is influenced by temperate, saline water masses that are transported northwards via the West Spitzbergen Current, which is a continuation of the North Atlantic Current (Aagaard, 1982). Furthermore, the site is located downslope from Kongsfjorden and is thus influenced by one of the major outlets of western Svalbard meltwater (Werner et al., 2013). Today, the site is located south in the vicinity of the winter and summer sea-ice margin and is ice-free year round (Fig. 1)., but previous work indicates variability in the past sea-ice cover (Falardeau et al., 2018; Müller et al., 2012; Müller and Stein, 2014; Werner et al.,

2011, 2013). Age-depth modeling suggests a maximum age of about 30 cal kyr BP for the lowermost core interval (Müller and Stein, 2014). The sampling procedure for ancient DNA analyses followed the protocol for non-frozen sediment cores explained in Epp et al. (2019)(Epp et al., 2019) for the 12 samples taken along the core, of which the depth at 8.85 m was sampled twice to check whether different samples from the same level show similar taxonomic composition and diversityrichness, particularly the oldest sample (Table 1).

2.2 DNA extraction, PCR, and sequencing

The DNA extractions and PCR setups were prepared in a dedicated laboratory for ancient DNA at the Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research (Potsdam, Germany). Total DNA was extracted from 12 samples of approximately 2 g (wet weight) sediment using the same method as described in (Zimmermann et al., (2017a). Each extraction batch included one negative control. The DNA concentrations were measured with the Qubit dsDNA BR Assay Kit (Invitrogen, Carlsbad, CA, USA) on a Qubit 2.0 fluorometer (Invitrogen, Carlsbad, CA, USA). As DNA concentrations were below the detection limit, we concentrated 600 µL of each sample with the GeneJET PCR Purification KIT (Thermo Scientific, Carlsbad, CA, USA) according to the manufacturer's protocol and eluted twice with 15 µL elution buffer. All DNA extracts were stored at -20°C.

We amplified the marker *rbcl_76* (Stoof-Leichsenring et al., 2012), a 76 bp long fragment of the plastid *rbcL* gene using tagged primers *Diat_rbcL_705F* (AACAGGTGAAGTTAAAGGTTCATAYTT) and *Diat_rbcL_808R* (TGTAACCCATAACTAAATCGATCAT) as described in (Dulias et al., (2017). The PCR reactions were set up in small batches, each including up to 9 samples and the corresponding negative control from the DNA extraction as well as a PCR no template control (NTC). For each sample, extraction negative control, and NTC, we performed 3 PCRs with different primer-tag combinations on different days. The PCR reaction mixes and conditions were prepared following the adjusted protocol for tagged *Diat_rbcL_705F* and *Diat_rbcL_808R* primers as described in Dulias et al. (2017)(Dulias et al., 2017) with the exception that 3 µl DNA (DNA concentration 3 ng µL^{-1}) was used as a template. PCRs were performed with the following settings: 5 minutes at 94°C (initial denaturation), then 50 cycles at 94°C (denaturation), 49°C (annealing), and 68°C (elongation), and a final elongation step at 72°C for 5 minutes. Subsequently the PCR success was checked with gel-electrophoresis. All PCR products were purified with the MinElute purification Kit (Qiagen, Hilden, Germany) according to the manufacturer's recommendations. Elution was carried out twice in 10 µL elution buffer. The purified PCR products were mixed in equal concentrations and sent to Fasteris SA sequencing service (Switzerland) who carried out library preparation and sequencing. The sequencing library was prepared with the Mid Output kit v. 2 according to the Fasteris Metafast protocol for low complexity amplicon sequencing and checked by qPCR. The library was sequenced (2 x 150 bp, paired-end) on the Illumina NextSeq 500 (Illumina Inc., San Diego, CA, USA). The sequence data are deposited in the Sequence Read Archive (BioProject: PRJxxxx).

2.3 Bioinformatic processing

The sequences were processed, filtered, and assigned a taxonomic name according to the NCBI taxonomy using the OBITools package (Boyer et al., 2016) with the same bioinformatics parameter settings as described in ~~(~~Dulias et al.~~,~~ (2017). We refrained from clustering sequences into operational taxonomic units and used amplicon sequence variants (ASVs) as recommended by ~~Callahan et al.~~ Callahan et al.~~,~~ (2017)~~(Callahan et al., 2017)~~. The OBITools program *obiclean* can identify ASVs that have likely ~~have~~ arisen due to PCR or sequencing errors. It uses the information of sequence counts and sequence similarities to classify whether a sequence is ~~rather~~ a variant ("internal") of a more abundant ("head") ASV (Boyer et al., 2016). To generate the reference database for the taxonomic assignment of the sequences we downloaded the EMBL release 138 (released November 2018) and used *ecopcr* (Ficetola et al., 2010) according to the descriptions of Dulias et al. (2017) and Stoof-Leichsenring et al. (2012) containing 2,320 reference sequences. ~~(Dulias et al., 2017; Stoof Leichsenring et al., 2012).~~ A total of 7,536,449 sequence counts were assigned to samples, 235 sequence counts to extraction negative controls and 2370~~1~~ counts to PCR negative controls. Of the 204 different sequence variants detected in extraction and PCR negative controls, 83% of their occurrences ~~the majority~~ were singletons (Suppl. Table 1) and most likely artefacts from tag-jumping during library preparation (Schnell et al., 2015). Using R v. 3.5.0 (R Core Team, 2018), we kept only those ASVs (Suppl. Table 2~~1~~) that (1) were assigned a taxonomic name based on 90–100 % similarity to an entry in the reference database, (2) were represented with at least 10 read counts in a replicate, (3) were present at least 3 times among ~~-~~all sequenced PCR products ~~the different replicates~~, (4) showed taxonomic resolution below the phylum level "Bacillariophyta", and (5) were tagged as "internal" by *obiclean* in less than 50 % of all sequenced PCR products ~~the different replicates~~ to reduce PCR and sequencing artefacts. Filtering with R reduced the number of read counts from 7,536,449 to 6,199,984.

2.4 Reproducibility of PCR replicates

Alpha-d~~D~~iversity estimates, such as richness, depend highly on the sequencing depth. As more taxa, especially rare ones, can be detected with increasing sequencing depth, alpha-diversity is only comparable between samples~~,~~ when it is estimated based on the same number of sequences. Despite efforts to reduce such differences by equimolar pooling of PCR products, the number of sequences generally varies among PCR replicates. Therefore, we analyzed the dissimilarities of PCR replicates as well as one sample replicate (8.85 m depth). We resampled ~~(https://github.com/StefanKruse/R_Rarefaction (Kruse, 2019))~~ the dataset 100 times to the minimum number of sequences available (25,601 counts), then, for each replicate, we calculated the mean number of sequence counts for each ASV across the 100 resampling steps (code available at: https://github.com/StefanKruse/R_Rarefaction; ~~(~~Kruse, 2019~~)~~). This dataset was used to calculate the proportions of each ASV per replicate. The proportional data were used for the Multiple Response Permutation Procedure (MRPP) using the R function *mrpp* on Bray Curtis Dissimilarities to test if dissimilarities within replicates of the same sample are significant. Furthermore, we applied non-metric multidimensional scaling (NMDS) using *metaMDS* to assess which replicates show high and low replicability.

2.5 Taxonomic composition and richness

As a measure of alpha-diversity we calculated richness of (1) ASVs (number of amplicon sequence variants) and (2) unique taxonomic names (number of grouped ASVs that were assigned to the same taxonomic name). For taxonomic composition and richness calculations, we combined the PCR replicates of the corresponding sample. This resulted in a new minimum number of sequence counts (300,415 counts) that was used for resampling the dataset 100 times ~~we first summed up the sequence counts for each ASV of the replicates belonging to the same sample and then resampled the dataset 100 times based on the new minimum number of sequence counts (300,415 counts)~~ according to descriptions in the preceding paragraph. The resampled dataset was used to calculate the relative abundances of each ASV per sample. Finally, ASVs, which were assigned to the same taxonomic name but had different similarities to an entry in the reference database, were summed up to one entry using the R *aggregate* function. ~~Constrained hierarchical clustering (CONISS) with the R function *chclust* followed by *brokenstick* analysis divided the samples into five significant assemblage zones.~~ Stratigraphic diagrams showing temporal changes in taxonomic composition were generated with *strat.plot*. All statistical analyses and visualizations were prepared with R v. 3.5.0 using the packages "vegan" (Oksanen et al., 2011) and "rioja" (Juggins, 2012). For correlation analysis we interpolated IP_{25} values using the methods described in Reschke et al. (2019). We transformed the IP_{25} data using the function *zoo* from the "zoo" package (Zeileis and Grothendieck, 2005) and used in the function *CorIrregTimser* using the package "corit" (https://github.com/EarthSystemDiagnostics/corit) (Reschke et al., 2019). The correlation between Chaetocerotaceae and Thalassiosiraceae as well as between IP_{25} and all ASVs was tested for significance using the R function *rcorr* (method Pearson) from the package "Hmisc" (Hollander and Wolfe, 1975; Press et al., 1988).[22]

3 Marker specificity, taxonomic resolution, and taxonomic coverage

The amplification of the short *rbcL_76* marker permitted the retrieval of diatom DNA from all samples and was highly specific for diatom sequence variants (95.7 %). Only 4.3 % of all assigned ASVs were assigned to other organismal groups: 4 % Bolidophyceae (a closely related sister clade of diatoms with unicellular, siliceous, flagellated algae), 0.2 % Phaeophyceae (brown algae), and 0.1 % Eustigmatophyceae (photosynthetic heterokonts). This exceeds the marker specificity found in previous studies focusing on lake sediment cores (84 % (Stoof-Leichsenring et al., 2012), 88 % (Stoof-Leichsenring et al., 2014), 90 % (Stoof-Leichsenring et al., 2015)) as well as specificity of a previous *in silico* PCR (90.4 % (Dulias et al., 2017)). Possibly owing to a much shorter marker size, the *rbcL_76* marker also surpassed ~~outperformed~~ the usually amplified 18S rDNA markers with regard to specificity for diatoms (Coolen et al., 2007; De Schepper et al., 2019; Kirkpatrick et al., 2016). The initial dataset contained 1,398 ASVs that were systematically filtered to a final diatom dataset containing 360 ASVs (6,199,984 counts) which were further grouped into 75 unique taxonomic names. The majority of diatom sequences are assigned to polar centric Mediophyceae (79.7 %), followed by pennate Bacillariophyceae (14.5 %), while radial centric Conscinodiscophyceae (1.1 %) make up only a small proportion of the dataset. The majority of ASVs are assigned as low as species level (38.6 %) or genus level (25.8 %) (Fig. 2a), ~~Y~~yet, the taxonomic resolution (i.e. the taxonomic assignment of

ASVs) is limited by the incompleteness of the sequence reference database as indicated by the ~~striking~~ proportion of ASVs (4.8%) for which assignment is restricted to phylum level (Bacillariophyta).

In total, 18 different diatom families are represented in the final dataset which is dominated by ASVs assigned to the families Thalassiosiraceae (35 %), Bacillariaceae (15.8 %), and Chaetoceraceae (9.2 %) (Fig. 2b). Particularly dominant ASVs are assigned to *Thalassiosira* (21.2 %) and *Porosira* (10.9 %) (resolution only possible ~~on~~ to genus level) and to *Chaetoceros* cf. *contortus* 1SEH-2013 (6.6 %) – all centric diatoms belong~~ing~~ to the class Mediophyceae. Their dominance is likely the result of high paleoproductivity or of differential overrepresentation caused by preservation and/or technical biases. Indeed, in the Fram Strait, ~~especially~~ *Chaetoceros* and *Thalassiosira* species especially have high productivity in different hydrographical regimes (Gradinger and Baumann, 1991; Lalande et al., 2013) as well as at Kongsfjorden, a major outlet of western Svalbard potentially influencing the coring site (Hodal et al., 2012), and even in micropaleontological studies they are often dominant (Birks and Koç, 2002; Bylinskaya et al., 2016; Oksman et al., 2017, 2019). Next to high paleo-productivity, the preservation of *sed*aDNA could be biased by differential degradation. Heavily silicified and/or spore forming diatoms such as some *Thalassiosira* and *Chaetoceros* species may be less sensitive to dissolution, which might also improve the preservation of DNA over long time periods. Furthermore, the enrichment of centric diatoms in the *sed*aDNA record could be the result of copy number variation of the *rbcL* gene between different species and cell biovolume ~~, as centric diatoms are known to have multiple chloroplasts while pennate diatoms are restricted to either one or a few~~ (Bedoshvili et al., 2009; Round et al., 1990; Vasselon et al., 2018)~~(Bedoshvili et al., 2009; Round et al., 1990)~~. Technical biases often arise during PCR~~s~~ due to mismatches between primer sequences and primer binding sites (Nichols et al., 2018) and the high number of PCR cycles ~~,~~ which is needed to increase the chance of retrieving rare sequences ~~,~~ leads to an over-amplification of already abundant template molecules in comparison to rare ones. While the reduction of cycles could reduce this effect, fewer PCR cycles would reduce replicability (Krehenwinkel et al., 2017; Nichols et al., 2018)~~(Krehenwinkel et al., 2017)~~.

Despite the filtering, several distinct ASVs, (~~for example,~~e.g. *Chaetoceros socialis*) are assigned the same taxonomic name (~~Supplementary~~ Suppl. Table S1, Suppl. Fig. S1). We believe that these represent either different lineages or closely related species so far not included in the database, although we cannot rule out that some ASVs in our filtered dataset might still represent PCR or sequencing artefacts.

~~Regardless of a few~~Despite the current limitations, *sed*aDNA shows much promise. New reference sequences are added to GenBank on a daily basis, due to numerous phylogenetic studies and barcoding projects aiming to improve our systematic knowledge about taxonomic relationships and to archive the molecular inventory of global biodiversity (Degerlund et al., 2012; Li et al., 2015; Luddington et al., 2016). Taxonomic coverage of the reference database can be increased by subjecting diatom strains to single cell sequencing (Luddington et al., 2016; Sieracki et al., 2019). Most importantly, *sed*aDNA allows tracing ASVs through time and eventually relating them to, for example, environmental change, without relying on the state of taxonomic coverage of the reference database.

4 Quality and replicability of the data obtained by *sed*aDNA metabarcoding

A crucial requirement for the interpretation of ancient DNA records is that PCR replicates show similar signals in ~~biodiversity~~ richness and taxonomic composition. The recovery of taxa by *sed*aDNA metabarcoding is prone to false presences or absences. As our study lacks a morphological diatom record, false absences cannot be assessed and true presences cannot be confirmed, which means that absence in our record does not necessarily translate~~s~~ to a true physical absence in the past. Hence, it was important to use independent PCR replicates for each sediment sample and stringent criteria to filter the dataset to remove artefacts introduced by ancient DNA damage, PCR, and sequencing. The PCR replicates (different PCR products ~~of~~from the same DNA extract) of each sample show some variations (Fig. 3) in the presence and abundance of ASVs, especially for ~~rare~~ ASVs amounting to less than 1 % per sample (Suppl. Table 2, Suppl. Figure S2). We tested whether these differences are significant using MRPP ($p = 0.001$, number of permutations 999, observed delta = 0.3683, expected delta = 0.6548), which suggests that PCR replicates of the same sample share significantly lower dissimilarities (average 39.3 %) ~~in comparison~~compared to replicates between different samples (67.5 %). The PCR replicates are highly similar in the oldest samples up to 5.8 m depth and in the youngest sample. For the oldest sample at 8.85 m depth we additionally processed a sample replicate. The PCR replicates of both sediment samples at 8.85 m depth are highly similar and cluster~~ed~~ tightly together in the NMDS plot (Fig. 3). Although a higher number of replicates would improve the robustness of our analysis, ~~This~~ this indicates that reasons other than sample age and associated DNA degradation controlled replicability in this study. It is possible that higher dissimilarities between ~~some~~ PCR replicates are the result of ~~the~~ low amounts of template molecules. ~~DNA or due to PCR inhibition by compounds that were not sufficiently removed during the DNA extraction.~~

5 Temporal change of taxonomic richness and composition

We used *sed*aDNA metabarcoding on samples that, due to the low sample size, represent temporally restricted snapshots of the different climatic intervals since the Late Weichselian. The dataset reveals diatom taxa that have mostly been reported from the Fram Strait in modern surveys (Karpuz and Schrader, 1990; Oksman et al., 2019; von Quillfeldt, 2000) and micropaleontological records (Oksman et al., 2017; Stabell, 1986). We detect typical ice-associated (sympagic) and/or cold-water diatoms (e.g. *Nitzschia* cf. *frigida*, *Cylindrotheca closterium*, *Thalassiosira nordenskioeldii*, *T. gravida*, *T. antarctica*, (Hasle, 1976; von Quillfeldt, 1997; von Quillfeldt et al., 2003), the epiphytic *Attheya septentrionalis* (Poulin et al., 2011) and *Pseudo-nitzschia granii* (Lovejoy et al., 2002)) alongside the temperate to warm-water species *Detonula pumila* (Hasle, 1976) and *Thalassiosira angulata* (Krawczyk et al., 2013; Luddington et al., 2016; Weckström et al., 2014), and some cosmopolitans (*Minidiscus trioculatus*, ~~and~~ *Cerataulina pelagica* (Hasle, 1976)). Beyond marine diatoms, sequences are also assigned to species preferring fresh to brackish water (e.g. *Skeletonema subsalsum* (Hasle and Evensen, 1975), *Nitzschia palea*, ~~and~~ *Nitzschia* . cf. *paleaceae* (Husted, 1930)). Taxonomic composition and richness changes with core depth and fits well into the framework reconstructed by other proxy data (biomarkers (Müller et al., 2012; Müller and Stein, 2014); foraminifers (Werner et al., 2011, 2013, 2016; Zamelczyk et al., 2014); dinoflagellates (Falardeau et al., 2018)).

Generally, the richness of both ASVs and unique taxonomic names (ASVs grouped based on identically assigned taxonomic names) is ~~slightly~~ higher in samples dated to the ~~Late Weichselian~~last glacial in comparison to those dated to the Holocene (Fig 4). A shift of diatom *sed*aDNA composition ~~Taxonomic turnover~~ is captured with some ASVs being predominantly abundant in Late Glacial samples (e.g. those assigned to *Thalassiosira gravida*, *Minidiscus trioculatus*, and *Nitzschia* cf. *paleacea*) whereas others are mainly present in Holocene samples (e.g. *Chaetoceros* cf. *contortus* 1 SHE-2013, ~~and *Chaetoceros* C.~~ cf. *pseudobrevis* 1 SHE-2013) (Fig. 5). This shift ~~turnover~~ is also strongly reflected at the family level (Fig. 6). A general trend that can also be observed at the family level is an inverse relationship ($r = -0.61$, $p = 0.046$) of the dominant families Thalassiosiraceae and Chaetoceraceae (Fig 6). ~~Constrained hierarchical clustering (CONISS) followed by brokenstick analysis divided the samples into five significant assemblage zones corresponding to the Late Weichselian (including the last glacial maximum (LGM)), the deglacial (Heinrich Stadial 1, Bølling/Allerød and Younger Dryas) and the Early, Mid and Late Holocene (Fig. 5).~~

~~Zone I comprises the s~~Samples dated to the Late Weichselian and the last glacial maximum (LGM) ~~and is~~ are characterized by highest overall richness with regard to ASVs (Fig.4). The samples contain high proportions of sympagic taxa (*Thalassiosira gravida* (4.8–28.1 %), *T. antarctica* (4.9–17.9 %), *T. delicata* (3.1–7.5 %), *Chaetoceros socialis* (0.3–7 %), *Nitzschia* cf. *frigida* (0.9–3.3%), *Porosira* (6–10.4 %)) as well as littoral (*Haslea avium* (1.3–3.4 %)), oceanic ~~taxa~~ (~~*Haslea avium* and~~ *Minidiscus trioculatus* (1.4–4.4 %)), and brackish to freshwater taxa (*Nitzschia* cf. *paleacea* (0.3–5.9%), ~~and~~ *Skeletonema subsalsum* (1–2.6 %)) (Fig. 5). Overall, ASVs assigned to the families Thalassiosiraceae, Bacillariaceae, and Naviculaceae (Fig. 6) dominate these samples. The relatively h~~H~~igh proportions of sympagic taxa, especially of *Nitzschia* cf. *frigida*, in samples dated to the Late Weichselian and LGM ~~(zone I)~~ are in accordance with previously reconstructed cold sea-surface conditions based on high proportions of the polar planktic fora~~m~~ainifer *Neogloboquadrina pachyderma* (Zamelczyk et al., 2014), low dinocyst concentrations with a dominance of phototrophic taxa (Falardeau et al., 2018), and moderate concentrations of both~~,~~ the sea-ice proxy IP_{25} and the phytoplankton biomarker brassicasterol (Müller and Stein, 2014). It is conceivable that a ~~A~~ heterogeneous and dynamic environment produced by winter sea-ice cover with ice-free conditions during summer ~~probably~~ could allow~~ed~~ ~~for~~ diverse diatom communities to develop in the different habitats and over the seasons, which is ~~reflected in~~suggested by the highest overall numbers ~~overall~~ of ASVs in samples dated to this time span. *Thalassiosira* and *Chaetoceros* are also abundant in the LGM microfossil assemblage identified further south at the Knipovich Ridge (eastern Fram Strait), although species of the genera *Rhizosolenia* and *Coscinodiscus* are strongly represented there as well (Bylinskaya et al., 2016).

~~CONISS assigned samples dated to the deglacial phase to zone II, despite their representation of extremely different climatic intervals. The samples thus show highly variable taxonomic composition.~~ Samples taken from Heinrich Stadial 1 and Bølling/Allerød phases ~~samples~~ are characterized by high proportions of ASVs assigned to *Thalassiosira* (35.1–65.3 %), *T. antarctica* (6.8–9.8 %), and *T. delicata* (4.1–5.3 %), and abundant *Skeletonema subsalsum* (2.1–5.6 %), whereas ~~*Porosira* is not detected and~~ proportions of *Thalassiosira gravida* (3.4–11.3 %), *Nitzschia* cf. *frigida* (0.6–0.7 %), *Haslea avium* (0.2–0.4 %), and *Minidiscus trioculatus* (0–0.7%) are lower compared to the LGM. The taxonomic composition of the sample

dated to Heinrich Stadial 1 suggests a partial re-organization of the diatom *sed*aDNA composition, which took place either gradually or abruptly sometime between 20.5 and 15.1 cal kyr BP. The l~~L~~ow~~er~~ proportions of *Nitzschia* cf. *frigida* and other cold-water and sea-ice diatoms in samples corresponding to Heinrich Stadial 1 and Bølling/Allerød correspond well to low IP_{25} and moderate brassicasterol concentrations in these samples, reflecting a reduction of the sea-ice cover (Müller and Stein, 2014). In the Bølling/Allerød sample the relatively high abundance of sequences assigned to the brackish~~ to~~ freshwater preferring diatom *Skeletonema subsalsum* (Hasle and Evensen, 1975) might be explained by elevated meltwater discharge from Svalbard. Low surface-water salinity was inferred previously from the dinocyst record of this core about a century earlier, possibly resulting from melting of the Barents Sea ice-sheet (Falardeau et al., 2018). Higher sedimentation rates and thus higher temporal resolution during the Bølling/Allerød ~~phase~~ could have affected the *sed*aDNA signal, yet the distinct shift in taxonomic composition suggests~~.~~ that this is rather an effect of the changing environmental conditions during this phase.

The Younger Dryas sample exhibits moderate proportions of *Thalassiosira antarctica* (11.8 %) and *T. delicata* (2.3 %) and is marked by the presence of the sympagic diatom *Cylindrotheca closterium* (0.3 %) and relative increases in *Porosira* (9.1 %), *Haslea avium* ~~*Thalassiosira antarctica*~~ (5.7 %), and *Nitzschia* cf. *frigida* (1.3 %). Higher proportions of sympagic diatoms in this sample point towards colder conditions in comparison to the Bølling/Allerød sample and the presence of sea ice. Severe and extended sea-ice cover at the coring site are indicated by heterotrophic species in the dinocyst record (Falardeau et al., 2018), peak concentrations of IP_{25}, and very low brassicasterol concentrations (Müller and Stein, 2014). A diatom microfossil record from Hinlopen Strait northwest of Spitzbergen detects first diatom occurrence during the Younger Dryas (10.8 ^{14}C kyr BP) with more than 30 % sea-ice associated species yet with a different taxonomic composition in comparison to our record (Koç et al., 2002).

~~Zone III encompasses t~~The Early Holocene sample, which is marked by peak proportions of several families such as Skeletonemaceae (19.6 %) and Bacillariaceae (18.3%) and considerable increases of Chaetocerotaceae from 6.5 to 32.4 % combined with a strong decrease of Thalassiosiraceae from 57.8 to 44.4 % and Naviculaceae from 5.7 to 0.2 %, point~~sing~~ towards a second partial re-organization of the taxonomic composition between 12.8 and 10.1 cal kyr BP. In particular, the sample is dominated by sequences assigned to *Skeletonema* (16.8 %), *Thalassiosira* (14.5 %), and *Pseudo-nitzschia granii* (11.4 %). *Thalassiosira angulata*, a species associated with low sea-ice concentrations (Oksman et al., 2019) and temperate water masses (Weckström et al., 2014), displays peak proportions (1.7 %) in this sample. Lower proportions of sympagic diatoms in this sample are in accordance with the sea-ice retreat reconstructed from low IP_{25} and high brassicasterol concentrations (Müller and Stein, 2014) and high proportions of the subpolar planktic foraminifers *Turborotalita quinqueloba* in another core further northwest (Werner et al., 2016). The diatom composition recorded by *sed*aDNA is also quite different to what has been found in microfossil records from the Fram Strait and the Greenland, Iceland, and Norwegian Seas, where first diatom microfossils are recorded between 13.4 and 9 cal kyr BP (Koç et al., 1993; Schröder-Ritzrau et al., 2001). Southwest of Svalbard, diatom-rich sediments dated to 10.1 and 9.8 cal kyr BP are attributed to the inflow of Atlantic surface water and a retreat of the Polar Front (Jessen et al., 2010; Stabell, 1986) and are composed mostly of *Coscinodiscus* spp., *Rhizosolenia*, and *Paralia sulcata* (Jessen et al., 2010; Rigual-Hernández et al., 2017; Stabell, 1986). In concordance with this,

sequences assigned to *Paralia* have highest proportions in the Early Holocene sample. *Coscinodiscus* is likely resolved to class level as sequences (Coscinodiscophyceae), and thus are present in our record albeit only sparsely. *Rhizosolenia* was not detected in this sample, which could be explained by poor DNA preservation or regional differences of the past diatom communities between the two coring sites.

~~Zone IV comprises t~~The sample dated to the Mid-Holocene~~, which~~ is marked by ~~a~~ low diatom richness and by peak proportions of the families Chaetoceraceae (32.4 %) and Anomoeneidaceae (2.1 %). It is dominated by sequences assigned to *Chaetoceros* cf. *contortus* 1SEH-2013 (27 %), *Thalassiosira* (23.3 %), and *T. antarctica* (9 %), accompanied by abundant *Porosira* (7.9 %) and *Skeletonema* (3.9 %). The relatively low richness suggests a loss of diversity between the Early and Mid-Holocene. The Mid-Holocene sample is characterized by a diatom composition that cannot be clearly related to sea ice, yet the low richness is supported by low diatom concentrations in a sediment core from Mohn Ridge (Koç et al., 1993) and low phytoplankton productivity (Müller et al., 2012). The near absence of *Nitzschia* cf. *frigida*, however, does not match with the reconstructed strong sea-surface cooling and sea-ice growth from lower proportions of *Turborotalita quinqueloba* (Werner et al., 2011, 2013), low concentrations of $CaCO_3$, and high concentrations of IP_{25} and ice-rafted detritus (Müller et al., 2012).

~~Zone V includes t~~The samples dated to the Roman Warm Period and the Little Ice Age (Late Holocene)~~. Richness in this sample is on~~contain~~have a similar richness ~~level~~ to that of the Mid-Holocene sample. ~~It~~ This phase is characterized by cold-water and ice-associated taxa with peak proportions of *Porosira* (15.1–22.9 %) accompanied by significant proportions of *Chaetoceros* cf. *contortus* 1SEH-2013 (3.6–14.9 %), *Attheya* (2.3–6.7 %), *Thalassiosira antarctica* (1.4–4.7%), *T. delicata* (4.2–6.3 %), and *Chaetoceros socialis* (0.9–3.6 %). Richness is lowest in the youngest investigated sample which was dated to the Little Ice Age. The sample is characterized by a peak of *Chaetoceros* cf. *pseudobrevis* 1SEH-2013 (3.9 %), *Attheya* (6.7 %), and the sympagic diatoms *Porosira* (15.1 %), *Thalassiosira nordenskioeldii* (1.1 %), and *Nitzschia* cf. *frigida* (5.3 %). The Late-Holocene samples are characterized by elevated proportions of sympagic taxa in comparison to the Mid-Holocene sample which is in agreement with Neoglacial cooling (increasing IP_{25} concentrations and moderate to high concentrations of brassicasterol (Müller et al., 2012), increases in ice-rafted detritus and the dominance of polar planktic foraminifers (Werner et al., 2011)). Diatom microfossil records located southwest of Svalbard display an increase in diatom abundance since approximately 1.5 cal kyr BP (Rigual-Hernández et al., 2017; Stabell, 1986). The microfossil record of Rigual-Hernández et al. (2017) is mostly composed of *Chaetoceros* resting spores which matches the increase of Chaetocerotaceae in our *sed*aDNA data. The composition of the record published by Stabell (1986) is more similar to Rigual-Hernández et al.'s (2017) Early-Holocene diatom maximum with *Coscinodiscus* spp., *Rhizosolenia*, and *Paralia sulcata*. Differences between composition and diversity of our samples and the records of Rigual-Hernández et al. (2017) and Stabell (1986) suggest either regional differences or differential preservation of microfossils and *sed*aDNA between the sites.

6 Potential of diatom *sed*aDNA as a proxy for sea-ice distribution

The *sed*aDNA record generally contains a high proportion of sequences assigned to cold-water and ~~diatoms~~ sea-ice associated diatoms, such as *Nitzschia* cf. *frigida*, *Thalassiosira antarctica*, and *T~~halassiosira~~. nordenskioeldii* (Hasle, 1976; Poulin et al., 2011; von Quillfeldt, 1997; von Quillfeldt et al., 2003). Furthermore, pennate diatoms, which often dominate bottom ice layers (Van Leeuwe et al., 2018), display higher proportions in samples dated to the LGM, the Younger Dryas, and the Late Holocene with moderate ~~to~~ IP_{25} concentrations (Fig. 6). Among ASVs, *Nitzschia* cf. *frigida* (ASV 709), *Attheya* (ASV 28), and Bacillariophyta (ASV 154) show increased proportions with IP_{25} exceeding 0.8 µg ~~/~~g^{-1} organic carbon (Supple~~mentary~~. Fig.~~ure~~ S3). In contrast, Bacillariophyta (ASV 245), *Gomphonema* (ASV 586), and *Thalassiosira* (ASV 1017) are detected only in samples with IP_{25} of less than 0.5 µg ~~/~~g^{-1} sediment.

The sea-ice proxy IP_{25} is produced ~~only~~ by only a few known ice-associated diatoms: *Haslea kjellmanii* (Cleve) Simonsen, ~~*Haslea crucigeroides* (Hustedt) Simonsen and/or *Ha~~slea~~*~~. *spicula* (Hickie) Lange-Bertalot, and *Pleurosigma stuxbergii* var. *rhomboides* (Cleve in Cleve and Grunow) Cleve (Brown et al., 2014; Limoges et al., 2018)~~(Brown et al., 2014). ~~. We detected none of the known producers~~, ~~as either the marker did not allow for this resolution~~ due to the incompleteness of the reference database (for the species of *Haslea*) and/or because the DNA of these species was not preserved in sufficient quantities as these species are low in abundance (Brown et al., 2014). Only for *Pleurosigma stuxbergii* is there a publicly available reference containing our marker region ~~is publicly available~~ and is included in our database, whereas 15 references ~~of~~for non-IP_{25} producing *Haslea* species are available in our database. Hence, an absence in our record does not mean an absence in the past communities. Sequences assigned to *Haslea avium* in our sedaDNA record do not show a linear relationship with IP_{25}, but are nevertheless present in most samples. ~~Nevertheless, the *sed*aDNA record generally contains a high proportion of sequences assigned to sea ice associated and cold water diatoms and displays an increase in pennate diatoms, which often dominate bottom ice layers (Van Leeuwe et al., 2018), with increasing IP_{25} concentrations (Fig. 6).~~ Our data, in combination with other proxy data from this core, suggest that sequences assigned to *Nitzschia* cf. *frigida* could be a useful indicator of past sea-ice distribution in *sed*aDNA records. Samples with low concentrations of the sea-ice proxy IP_{25} (< 0.4 µg ~~/~~g^{-1} total organic carbon) ~~exhibit~~ have less than 2 % of sequences assigned to *Nitzschia* cf. *frigida*, whereas samples characterized by moderate to high (> 0.8 µg ~~/~~g^{-1} total organic carbon) concentrations of IP_{25} display highly variable proportions without a clear relationship. *Nitzschia frigida* (Grunow) forms arborescent colonies (Medlin and Hasle, 1990) and is often abundant from late winter in the bottom layer of ~~in~~ nearshore first-year ice and in multi-year ice in the Arctic pack-ice zones (Krawczyk et al., 2017; Melnikov et al., 2002; Olsen et al., 2017; Poulin et al., 2011; von Quillfeldt et al., 2003)~~,~~ b~~B~~ut it can also be found in the water column during vernal under-ice or ice-edge blooms (Hasle and Heimdal, 1998; Olsen et al., 2017). Recently, De Schepper et al. (2019) used *sed*aDNA metabarcoding on a core from the East Greenland Sea and identified the Mediophyceae OTU_5051 which was significantly correlated to the IP_{25} concentrations. The class Mediophyceae is the most diverse and dominant group in our dataset and contains several sea-ice associated taxa, such as~~, for example~~, *Thalassiosira antarctica*, *T. nordenskioeldii*, and *Porosira glacialis*. Yet their tendency to prevail in both sea ice and open water might be responsible for the non-linear

relationship with IP_{25}~~indistinct patterns~~ observed in our record~~, especially in comparison to biomarker signals~~. This is supported by Weckström et al. (2013) who find no specific response of sea-ice diatom microfossil composition to either IP_{25} concentrations or observational sea-ice data in the Labrador Sea.

7 Conclusions

For the first time in a marine environment, our study targets high-resolution, diatom-specific sedimentary ancient DNA using a DNA metabarcoding approach. We show that diatom DNA is preserved with substantial taxonomic richness in the eastern Fram Strait over the past 30,000 years even though diatom microfossils are recorded in the Svalbard region only since the Younger Dryas. This highlights the advantage of our approach for paleoenvironmental reconstructions aiming to identify drivers of community-level taxonomic composition and diversity, especially in regions known for their irregular and poor diatom microfossil preservation, such as the Fram Strait. The *rbcL_76* marker is highly diatom specific and provides ~~highly~~ detailed taxonomic resolution, mostly at genus and species level. The shortness of this marker is a strong advantage that leads to adequate replicability and high quality as diversity patterns do not show conspicuous signs of bias by age-associated DNA fragmentation. The *sed*aDNA record captures substantial temporal change of diatom taxonomic composition and richness with ~~two~~ four compositional re-organizations: the first between 20.51 and 15.1 cal kyr BP, ~~the LGM and Heinrich Stadial 1 and~~ the second between 12.8 and 10.1 cal kyr BP, the third between 10.1 and 5 cal kyr BP, and the fourth between 5 and 1.6 cal kyr BP ~~the Younger Dryas and the Early Holocene~~. Our record extends diatom compositional and diversity information back to the Late Weichselian as microfossil records in the Fram Strait are rare and extend only as far as the Younger Dryas due to poor preservation. Increasing proportions of pennate diatoms are associated with increased IP_{25} concentrations, and sympagic diatoms are present, but with no clear pattern with regard to biomarker signals. Recommendations for future work with sedimentary ancient DNA in the context of sea-ice reconstructions ~~involve~~include the preparation of reference genomes and a more targeted enrichment, for example of genes that help species to adapt to sea ice and allow them to cope with rapidly changing environmental conditions.

Supplement

~~**Supplementary Table S1:** Processed and taxonomically assigned sequencing output containing 1,398 amplicon sequence variants, their taxonomic information, and their read counts for each PCR replicate.~~

Code/Data availability

The sequence data are deposited in the Sequence Read Archive (BioProject XXX). The rarefaction script is available at https://github.com/StefanKruse/R_Rarefaction (Kruse, 2019).

Author contributions

H.H.Z., K.R.S.L., and U.H. conceived and designed the study; H.H.Z. performed experiments and data analysis; K.S.L. sampled the core and provided laboratory equipment; S.K. wrote resampling R script; R.S., R.T., J.M. and U.H. provided the framework for the study (samples, funding); H.H.Z. wrote the paper that all co-authors commented on.

Competing interests

The authors declare that they have no conflict of interest.

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Table 1: List of samples of core MSM05/5-712-2 with corresponding age (Müller et al., 2012; Müller and Stein, 2014), covered time interval and the reconstructed paleoenvironmental conditions including references.

Depth (m)	Age (cal kyr BP)	Time interval	Paleoenvironmental conditions
0.12	0.4	Late Holocene, Little Ice Age (LIA)	Sea-ice margin (Müller et al., 2012; Werner et al., 2011)
0.3	1.6	Late Holocene, Roman Warm Period	Sea-ice margin (Müller et al., 2012; Werner et al., 2011)
0.9	5	Mid Holocene	Sea-surface cooling and sea-ice extension (Müller et al., 2012; Werner et al., 2016)
3.1	10.1	Early Holocene	Sea-ice retreat (Falardeau et al., 2018; Müller and Stein, 2014; Werner et al., 2016)
3.71	12.8	Younger Dryas (YD)	Severe and extended sea-ice cover (Falardeau et al., 2018; Müller and Stein, 2014)
5.8	14.3	Bølling/Allerød (B/A)	Sea-ice retreat (Falardeau et al., 2018; Müller and Stein, 2014)
6.68	15.1	Heinrich Stadial 1 (H1)	Sea-ice retreat (Falardeau et al., 2018; Müller and Stein, 2014)
7.27	20.5	Last Glacial Maximum (LGM)	Sea-ice cover allowing spring sea-ice algal productivity (Falardeau et al., 2018; Müller and Stein, 2014; Zamelczyk et al., 2014)
7.85	23.1	LGM	Reduced sea-ice cover allowing spring sea-ice algal and summer phytoplankton productivity (Falardeau et al., 2018; Müller and Stein, 2014; Zamelczyk et al., 2014)
8.56	25.4	LGM	Reduced sea-ice cover allowing spring sea-ice algal and summer phytoplankton productivity (Falardeau et al., 2018; Müller and Stein, 2014; Zamelczyk et al., 2014)
8.85	29.14	Late Weichselian	Significant drop from previously high sea-ice concentration (Müller and Stein, 2014; Zamelczyk et al., 2014)

735

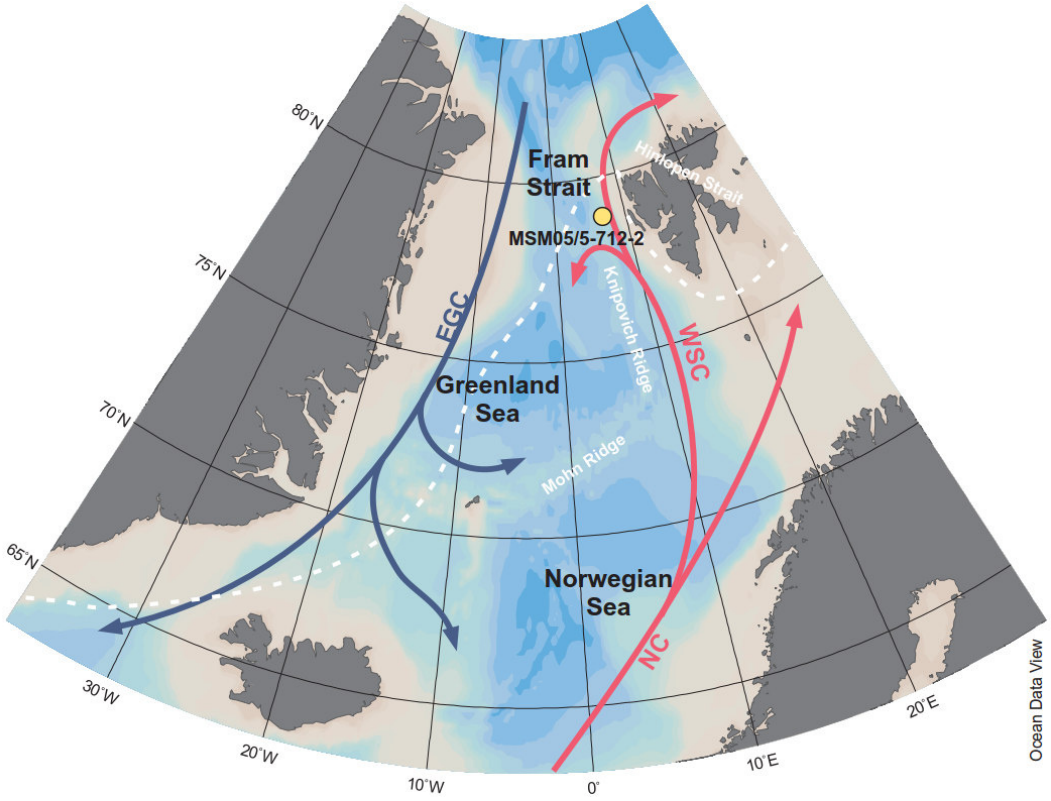
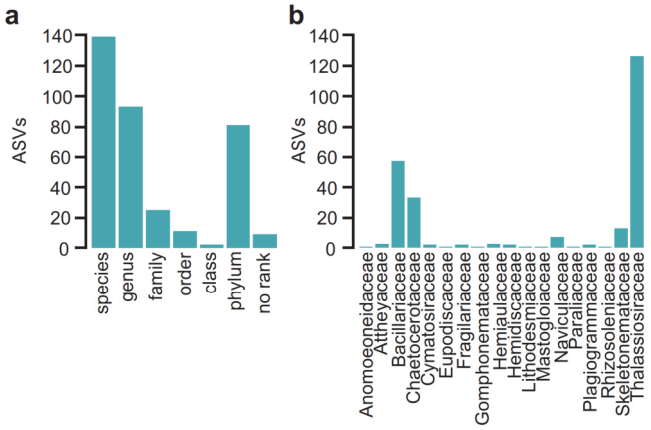


Figure 1: Map showing the coring site of MSM05/5-712-2 with bathymetric data derived from Ocean Data View (Schlitzer, 2002) and median March sea-ice extent from 1981-2010 (white, dashed line; https://nsidc.org/data/seaice_index/archives [accessed 06.08.2019] (Fetterer et al., 2017)).



740

Figure 2: Number of diatom amplicon sequence variants (ASVs) assigned (a) to different taxonomic levels and (b) to different families.

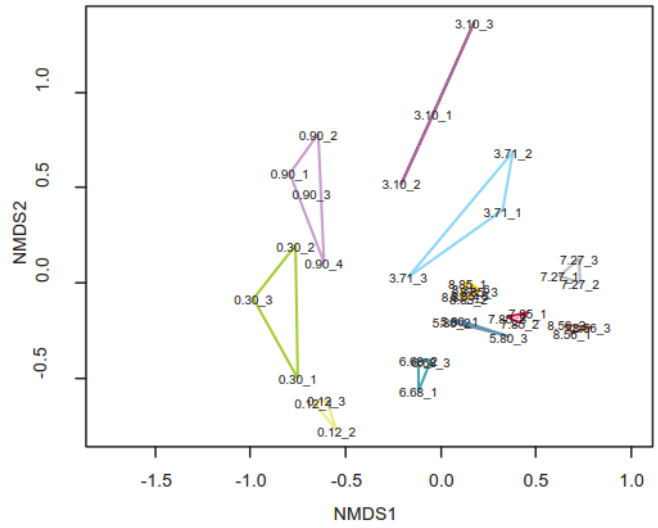


Figure 3: Non-metric multidimensional scaling plot based on the filtered and resampled diatom dataset with the PCR replicates (indicated by an underscore with a number) of a sample (depth in m) linked in a polygon of sample-specific colour: light green = 0.12m, green = 0.3m, light violet = 0.9 m, violet = 3.1 m, light blue = 3.71 m, dark blue = 5.8 m, turquoise = 6.68 m, grey =7.27 m, red = 7. 85 m, brown = 8.56 m, orange = 8.85 m (2 sample replicates with 3 PCR-replicates each).~~.~~

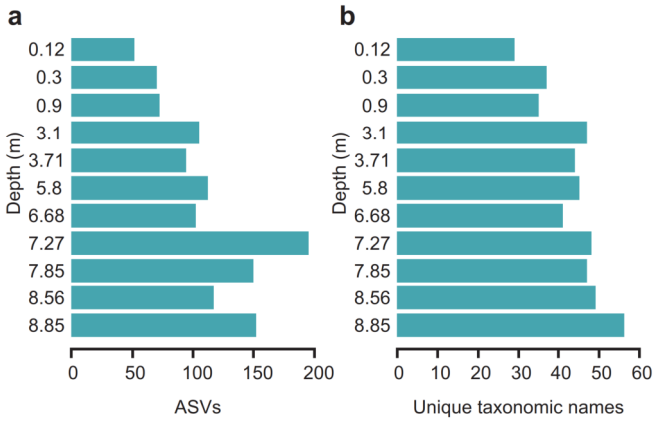
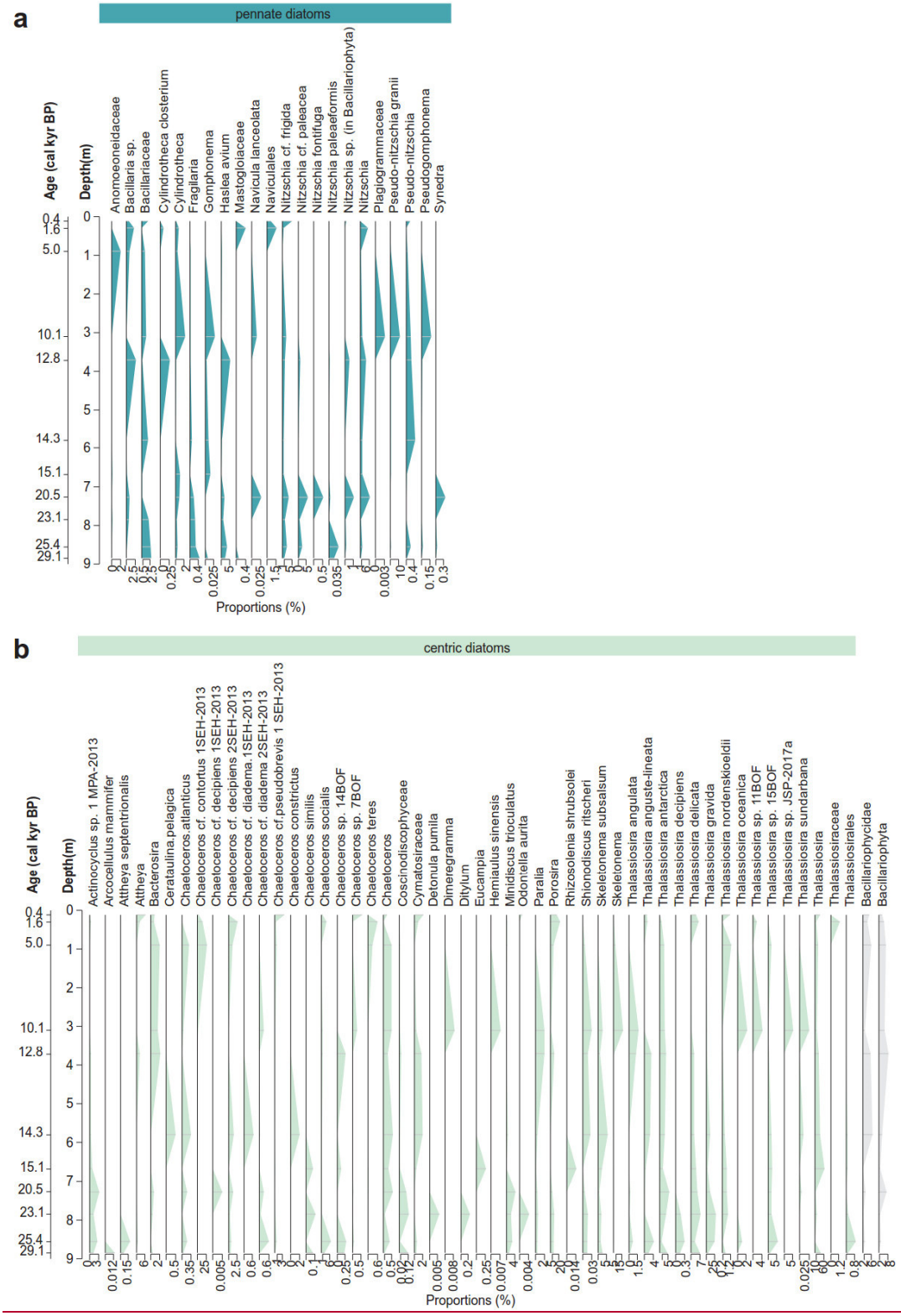


Figure 4: Barplots showing the rarefied (a) number of amplicon sequence variants (ASVs) per sample and (b) grouped ASVs assigned to the same taxonomic name ~~number of unique taxonomic names~~ for each sample with depth (m) of the sediment core MSM05/5-712-2.


a
pennate diatoms
Age (cal kyr BP)
Depth(m)
Anomoeoneidaceae
Bacillaria sp.
Bacillariaceae
Cylindrotheca closterium
Cylindrotheca
Fragilaria
Gomphonema
Haslea avium
Mastogloiaceae
Navicula lanceolata
Naviculales
Nitzschia cf. frigida
Nitzschia cf. paleacea
Nitzschia fontifuga
Nitzschia paleaeformis
Nitzschia sp. (in Bacillariophyta)
Nitzschia
Plagiogrammaceae
Pseudo-nitzschia granii
Pseudo-nitzschia
Pseudogomphonema
Synedra
Proportions (%)
b
centric diatoms
Age (cal kyr BP)
Depth(m)
Actinocyclus sp. 1 MPA-2013
Arcocellulus mammifer
Attheya septentrionalis
Attheya
Bacterosira
Cerataulina pelagica
Chaetoceros atlanticus
Chaetoceros cf. contortus 1SEH-2013
Chaetoceros cf. decipiens 1SEH-2013
Chaetoceros cf. decipiens 2SEH-2013
Chaetoceros cf. diadema 1SEH-2013
Chaetoceros cf. diadema 2SEH-2013
Chaetoceros cf. pseudobrevis 1 SEH-2013
Chaetoceros constrictus
Chaetoceros similis
Chaetoceros socialis
Chaetoceros sp. 14BOF
Chaetoceros sp. 7BOF
Chaetoceros teres
Chaetoceros
Coscinodiscophyceae
Cymatosiraceae
Detonula pumila
Dimeregramma
Ditylum
Eucampia
Hemiaulus sinensis
Minidiscus trioculatus
Odontella aurita
Paralia
Porosira
Rhizosolenia shrubsolei
Shionodiscus ritscheri
Skeletonema subsalsum
Skeletonema
Thalassiosira angulata
Thalassiosira anguste-lineata
Thalassiosira antarctica
Thalassiosira decipiens
Thalassiosira delicata
Thalassiosira gravida
Thalassiosira nordenskioeldii
Thalassiosira oceanica
Thalassiosira sp. 11BOF
Thalassiosira sp. 15BOF
Thalassiosira sp. JSP-2017a
Thalassiosira sundarbana
Thalassiosira
Thalassiosiraceae
Thalassiosirales
Bacillariophycidae
Bacillariophyta
Proportions (%)

755

Figure 5: Stratigraphic diagrams with sequences assigned to (~~A~~a) pennate diatoms (blue) and (~~B~~b) centric diatoms (green) and higher level sequences assigned between family and phylum level (graey) The taxonomic composition with relative proportions (%) of the 360 detected sequence variants is grouped into 75 unique taxonomic names based on identically assigned taxonomic names of sediment core MSM05/5-712-2. ~~Gray, dashed lines indicate zones recommended by CONISS. Taxonomic names are sorted by according to the weighted average with depth.~~

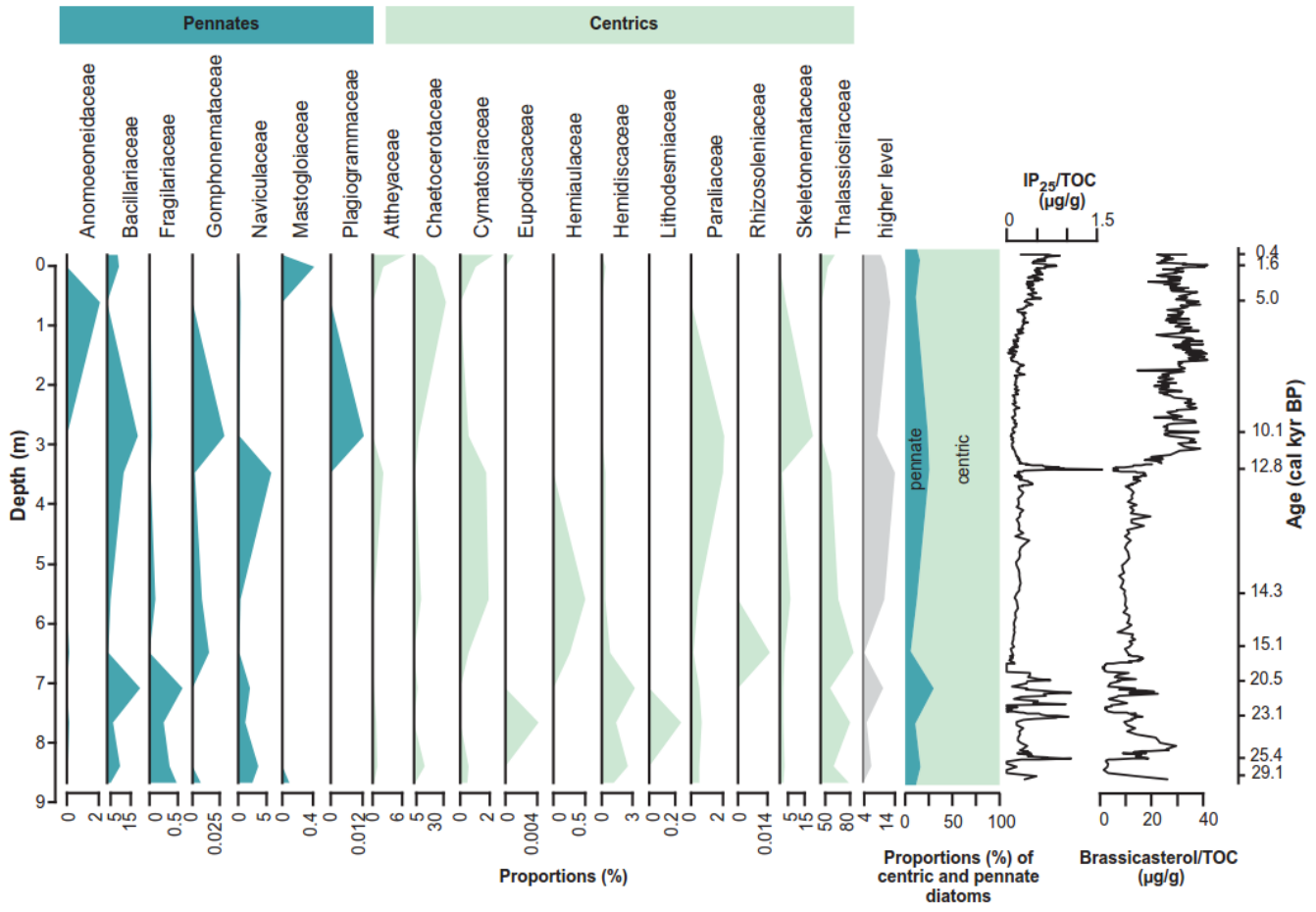


Figure 6: Proportions of sequences assigned to diatoms grouped on family level and down-core proportions of centric (blue) and pennate diatoms (green) as well as concentrations of the sea-ice biomarker IP_{25} (Müller et al., 2012; Müller and Stein, 2014) and the phytoplankton biomarker brassicasterol (Müller et al., 2012; Müller and Stein, 2014). Higher level (graey) contains sequences assigned between family and phylum level. ~~Horizontal gray, dashed lines indicate CONISS zone boundaries.~~ TOC = total organic carbon.