

Interactive comment on “Multiproxy evidence of the Neoglacial expansion of Atlantic Water to eastern Svalbard: Does ancient environmental DNA complement sedimentary and microfossil records?” by Joanna Pawłowska et al.

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We would like to thank the Referee for constructive review, that will help us to improve the manuscript. Written below are our responses to the Referee’s comments. The comments were reproduced and are followed by our responses. Based on the comments, we propose the changes of the manuscript. The revised version of the manuscript will be prepared based on the decision of the Editor. Anonymous Referee #1

This paper presents an interesting multiproxy dataset to document the paleoceanogra-

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phy near Svalbard and compares traditional sedimentary and microfossil proxies with a novel approach involving ancient environmental DNA. As such, the dataset certainly deserves publishing, but I have some comments/reservations about the age model and the discussion of the results. The discussion has some writing-technical issues. In several cases the own results are presented, without clear arguments supporting the interpretation (e.g. P12, L9–11 & L28–30; P15, L12–15) but rather followed by a literature review. The own results need to be better used to document the paleoceanographic/ environmental signal that is gained from this new site and data, before comparing to the literature. Figures integrating the own results with key records from previous studies is also advised.

Major comments

Referee's comment: First of all, the raw data needs to be made publicly available and/or presented with the manuscript. Needed are tables that list unique sample labels and relevant metadata such as core coordinates, sampling depths, measured data for each proxy (sedimentology, foraminifer assemblage data, stable isotopes and aDNA), etc.

Response: According to the Reviewer's suggestion, the raw data will be provided as electronic supplementary material.

Referee's comment: Age model. The ages used for the age model seem arbitrary. What is the argument to choose 1500, 2700 and 7890 yr BP? Those ages are not the average of the 2 sigma calibrated yrs BP. The most up-to-date radiocarbon calibration (Calib 7.1) was not used. Why?

Response: The calibration was refined with the use of the latest version of Calib program. However, the calibration dataset (Marine 13, Reimer et al. 2013) remained the same, thus the obtained results of calibration have not changed. The dates used in the age model marked the tops of probability curves on the probability distribution plot provided by Calib 7.1 program (see Fig. 2).

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Referee's comment: There is 9 cm sediment between 2700 and 7890 cal yr BP (43.5–52.5 cm), or a sedimentation rate of 0.0017 cm/yr assuming a constant sedimentation rate. Have you considered the possibility of a hiatus? Are there changes in the sedimentology/lithology? Additional dating could help solve this issue. Using your proxies to support the age model (P10, L23), make your environmental interpretation become circular. You need to separate the age model from the environmental proxies.

Response: We agree with the Reviewer that additional dating would improve the age model. According to the linear age model, the beginning of the Neoglacial was recorded at 46 cm sediment depth. Therefore, we decided to provide an additional radiocarbon date from this layer. The dating of foraminiferal tests revealed the age of 4.5 cal ka BP, which confirms our previous age estimation. We also agree that environmental proxies should be separated from the age model, therefore, we decided to remove the sentence considering our proxy record from the mentioned above paragraph. The low sediment accumulation rate recorded for the period from 7890 to 2700 yr BP was most likely a result of glacial retreat and consequent low delivery of sedimentary material. SAR recorded in the studied core was consistent with the results obtained by ŁÄËcka et al. (2015) in Storfjordrenna for this time period. On the other hand, Knies et al. (2017) and Rasmussen and Thomsen (2015) recorded higher accumulation rates in the inner Storfjorden. However, their studied cores were located relatively close to the shore, and, in our opinion, were more affected by sedimentary material delivery.

Referee's comment: Methods. This type of study (aDNA) is still very new in paleoceanography and more details about the aDNA method would be useful. For example, a short account of the bioinformatics (how were sequences translate to OTUs) would be advisable, rather than referring to other papers. How did you determine that the aDNA was in fact ancient?

Response: We have followed the Reviewers suggestion and added a broader description of post-sequencing data analysis. The added text is as follows: The post-sequencing data processing was performed with the use of SLIM web app (Dufresne

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et al., 2019) and included demultiplexing the libraries, joining the paired-end reads, chimera removal, Operational Taxonomic Units (OTUs) clustering, and taxonomic assignment. Sequences were clustered into OTUs using Swarm module (Mahe et al. 2014) and each OTU was assigned to the highest possible taxonomic level using vsearch (Rognes et al., 2016) against a local database and then reassigned using BLAST (Altschul et al., 1990). In order to ensure that obtained results represent ancient DNA, we have kept stringent precautions at each step of the analysis, from sampling to laboratory analysis. These include samples storage and processing in a sterile environment, using physically isolated work area at each step of the analysis and providing negative (blank) controls during DNA extraction, PCR amplification, and quantification. The DNA extraction was performed in the laboratory free from foraminiferal and diatom DNA in the Institute of Oceanology PAN, while PCR amplification and DNA sequencing were performed in laboratories adapted for work with ancient environmental DNA at the University of Geneva.

Referee's comment: Discussion. You write in the results section (P7, L18-20): "However, the extremely low time resolution between 9 cal ka BP and 4 cal ka BP precluded making any general conclusion about that interval. Therefore, the manuscript focuses only on the last 4 cal ka BP (the Neoglacial)." It is not clear where the 9 and 4 cal ka BP come from? The only "certain" ages are 7890 and 2700 cal yr BP (but see my comments above) measured in samples that are only 9 cm away from each other, and thus showing an extremely low time resolution. With only 2 samples analysed in this interval, this is clearly not sufficient to warrant the lengthy discussion (P10–12) on the interval prior to 2700 yr BP. While the fossil assemblages and aDNA may give valuable information about the environment, it is not possible to say something meaningful with regard to timing of events in this interval. That would require analysis of additional samples and ^{14}C dates (but preferably a record with a higher sedimentation rate).

Response: The date 9 000 results from the linear interpolation of accumulation rate based on SAR calculated for the period prior to 7890 cal ka BP. We agree that it is an

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oversimplification, therefore we have decided to keep the date 7890 cal ka BP as the oldest certain age. As mentioned above, we have decided to provide additional radiocarbon date. The obtained date was in accordance with the existing age model and confirmed that the onset of the Neoglacial was recorded at 46 cm sediment depth. We agree that the Discussion about the period prior to ~ 2.7 cal ka BP is disproportionately long compared to the low number of samples in this interval. Therefore, we decided to shorten this part of the Discussion. Now the text is as follows: During the period prior to ~ 2.7 cal ka BP, the ST_1.5 sedimentary record displayed elevated and variable IRD delivery and coarsening of the 0-63- μm sediment fraction (Fig. 4). These results are in agreement with the record from Storfjordrenna (ŁÄĚcka et al., 2015), where peaks in IRD were noted during the Neoglacial and were attributed to increased iceberg rafting due to fluctuations in the glacial fronts (e.g. Forwick et al., 2010). Coarser 0-63 μm may suggest winnowing of fine grained sediment, however, foraminiferal fauna showed no clear response for sediment removal. The ST_1.5 foraminiferal assemblage was dominated by glacier-proximal fauna (primarily *C. reniforme*) and indicators of frontal zones (primarily *M. barleeanum*; Fig. 5). The presence of *C. reniforme* and *M. barleeanus* is linked to cooled and salty AW (e.g., Hald and Steinsund, 1996; Jernas et al., 2013). Moreover, these species are also associated with the presence of phytodetritus, which may be related to the delivery of fresh organic matter observed in frontal zones and/or near the sea-ice edge (Jennings et al., 2004). Relatively light $\delta^{13}\text{C}$ (Fig. 4), followed by the maximum percentage of sea-ice species *Thalassiosira antarctica* (cf Ikävalko, 2004; Fig. 8) may indicate primary production associated with the presence of sea-ice and/or periodic inflow of ArW The typical response of a foraminiferal community to high trophic resources is an increase in diversity and standing stock (Wollenburg and Kuhnt, 2000). According to our data, the foraminiferal community showed no clear signs of increased productivity, as the abundance and flux of foraminifera were low prior to ~ 2.7 cal ka BP (Fig. 4). Similarly, Rasmussen and Thomsen (2015) noted a decrease in concentration of benthic foraminifera in Storfjorden at that time, which was attributed to the more extensive seasonal sea-ice cover. Also, Knies et al. (2017) suggested a vari-

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able sea-ice cover extent and a fluctuating sea-ice margin in Storfjorden prior to ~ 2.8 cal ka BP. In contrast, our data may suggest the presence of high-energy environment during the interval prior to ~ 2.7 cal ka BP, what may be the major factor limiting the development of the foraminiferal community. However, low sampling resolution during that period precluded making any general conclusion and the latter assumption should be confirmed by further studies.

Referee's comment: Higher current speeds (i.e. P.11, L5) can strongly influence paleoceanographic records. What is the effect of bottom water currents on the microfossil and aDNA records here? Could this bias your interpretation?

Response: The change in the grain size in the 0-63 μm fraction may suggest selective removal of sediment due to the winnowing of fine sediments. However, there was no clear response in fossil foraminifera. Foraminiferal flux and abundance were extremely low at that time and the assemblage was strongly dominated by *C. reniforme* and *M. barleeanum*, taxa that are associated with the delivery of fresh phytodetritus. Relatively light $\delta^{13}\text{C}$, followed by increased % of aDNA sequences of *Thalassiosira antarctica* may suggest that primary production was associated with the presence of sea ice at that time. Despite potentially high food supply, foraminiferal standing stock remained low, which may result from higher bottom currents speed and winnowing that limited foraminiferal community development. On the other hand, the flux and abundance of *C. lobatulus*, which is considered a bottom currents indicator, remained relatively low and stable during the Neoglacial. The major peak in abundance was recorded at ~ 0.4 cal ka BP, followed by minor peaks at ~ 2.3 and ~ 1.5 cal ka BP. Our observations are consistent with the record of [LÄckö et al. \(2015\)](#) from Storfjordrenna. They observed an increase in the mean grain size ($> 63 \mu\text{m}$) during the late Holocene (i.e., after 3.6 cal ka BP), what may indicate more vigorous bottom currents and winnowing of fine-grained sediment. However, it was not followed by the increase in *C. lobatulus* abundance. In the case of monothalamous foraminifera, no bottom currents indicators were identified so far. The knowledge about monothalamids' ecology and environmental tolerance is

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incomplete, and using them as a proxy is still limited. Therefore, no clear information about bottom currents activity can be inferred from aDNA record.

Referee's comment: Do the foram assemblages, and diatom and foram DNA assemblage data show a change supporting the interpreted shift from polynya conditions to densely packed sea ice environment at 2700 cal yr BP?

Response: As explained in the Discussion, our record contradicts other interpretations suggesting that Storfjorden was covered by densely packed sea-ice after ~ 2.7 ka BP (cf. Knies et al. 2017). We proposed an alternative scenario that assumed pulsed inflows of AW after ~ 2.7 cal ka BP, which caused a periodic breakup of sea ice cover and allowed primary productivity. These pulses were recorded in the abundance and taxonomic composition of fossil foraminifera assemblages as well as in shifts in monothalamous foraminifera inferred from aDNA. Moreover, the presence of diatom aDNA during the entire Neoglacial suggested continuous primary production (see P13, L9 – P14, L34). Referee's comment: The AW pulses at 2.3 and 1.7 cal kyr BP show an opposite pattern in foraminifer flux and abundance (Fig. 3, lower two panels): low at 1.7, while high at 2.3 cal kyr BP. Why are these such different patterns to AW pulses? How does this compare to the aDNA records? Response: Indeed, the response of the foraminiferal community showed differences between ~ 2.3 cal ka BP and ~ 1.7 cal ka BP. The dominant components of foraminiferal assemblage at ~ 2.3 cal ka BP were *M. barleeanum* and *E. excavatum*, while at ~ 1.7 cal ka BP, *N. labradorica* and *C. reniforme* reached higher percentages. The major difference in environmental conditions between these two "AW episodes" was noticeably coarser 0-63 μm sediment fraction noted ~ 2.3 cal ka BP, what may indicate more intensive winnowing and consequent sediment sorting, what creates favorable conditions for development of highly opportunistic species, such as *E. excavatum*, which reached its' maximum flux and percentage at that time. Moreover, slightly lighter $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ at ~ 1.7 cal ka BP suggested a slight difference in AW characteristics. The difference may be supported by the presence of more diverse monothalamous assemblage and the occurrence of

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sequences of diatom *T. hispida* at ~ 1.7 cal ka BP. The relevant information has been added to the Discussion.

Referee's comment: You claim an increase in fresh phytodetritus and/or phytoplankton blooms (e.g. P16, L4), but do you actually document this? It seems this is being inferred from the foram assemblages. More cautious wording is advised here.

Response: We agree with the Reviewer's comment. The sentence has been modified to "Warming was associated with pulsed inflows of AW and sea-ice melting, which may stimulate phytoplankton blooms and organic matter supply to the bottom".

Referee's comment: How does the aDNA signal reflect sea ice cover? You refer to the genera *Navicula* and *Thalassiosira* as occurring in sea ice, but these genera also occur elsewhere. For example, *Thalassiosira* is very diverse in temperate regions (Hoppenrath et al. 2007, Eur. J. Phycol.). Did you identify *Thalassiosira* species that occur in sea ice, or does the aDNA data not allow to classify to species level.

Response: We have manually checked the sequence assignment. The majority of diatom sequences were assigned to *Thalassiosira* sp., and it was not possible to assign them to species level. However, we identified the sequences belonging to *Thalassiosira antarctica*, which is a sea-ice species. We have modified the paragraph of the Discussion considering the sea-ice diatoms. Now the text is as follows: The record of diatom aDNA supports the latter assumption, as the percentage of sea-ice species *Thalassiosira antarctica* (cf. Ikävalko, 2004) reached its maximum during this period.

Referee's comment: Several studies in the region are mentioned in the discussion (e.g. Sarnthein et al. 2003, Rasmussen and Thomsen 2014, Knies et al. 2017), some of which apparently show comparable signals. This should be discussed in more detail (i.e. what is comparable), and preferably supported by a clear figure showing the key-proxies from those studies that show similarities with the own records. Response: The data showing temperature and isotopic records from GISP2 core (Cuffey and Clow, 1997; Alley, 2000) and Storfjordrenna (ŁÄËcka et al., 2015), as well as temperature

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records of Sarnthein et al., (2003), have been added to the Figure 3. Moreover, more detailed information about comparable signals has been added to the Discussion.

Referee's comment: Minor comments P5 – sampling. The core was sampled every y cm and at 5 cm for aDNA. Were all other proxies also analysed at 5 cm or at 1 cm? A list/table with raw data would help answer this question. P5, L8&11. aDNA sampling interval at 5 cm – repetition. It would be more informative to have a list of the sample depths. P6, L16. Please list these 27 levels. And provide raw data.

Response: The repetition has been removed from the text. The raw data including sampling resolution will be added to the manuscript as an electronic supplement.

Referee's comment: P6, L22. What is the primer length?

Response: The length of primers is approximately 20 base pairs (bp): the diatom-specific primers are 22 bp long, while foraminifera-specific primers are 19 bp-long. The full sequences of primers are provided in the Material and methods section in the manuscript.

Referee's comment: P8, L23. Specify “certain species”.

Response: Herein, by “certain species” we mean dominant species. To avoid confusion, the phrase “certain species” have been removed.

Referee's comment: P9, L23. Please specify the being and end of the time intervals.

Response: The mentioned above time intervals spanned the period from ~ 4 cal ka BP to 2.4 cal ka BP and ~ 1.7 cal ka BP. The relevant information has been added to the text.

Referee's comment: P10, L21 (and throughout). Please remove ST_1.5. You analyzed only one core in this study, so that does not have to be repeated.

Response: The repetitions have been removed from the text.

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Referee's comment: P11, L17. Codominant – be careful with this term, as it means that the species/groups are equally dominant. Is that always the case?

Response: Each of the mentioned above foraminifera indicators groups made up to 40% of foraminiferal abundance. However, we have decided to change the word “codominated” to “dominated”.

Referee's comment: P12, L9–11. What does this mean in terms of environment/paleocenaography?

Response: Our record displayed an almost 10-fold increase in sediment accumulation rate, accompanied with a decrease in IRD delivery and coarsening of <63 μm fraction. The increase in SAR resulted most likely from glacial advance observed in Storfjorden at that time (cf. Rasmussen and Thomsen, 2015) and consequent settling of sedimentary material. Sediment accumulation may be also enhanced by the slow-down of bottom currents, as indicated by the decrease in <63 μm fraction. Moreover, glacial advance is typically followed by more intensive IRD delivery (cf. Rasmussen and Thomsen 2015). However, Storfjorden was covered by densely packed sea ice at that time (Knies et al., 2017) and the majority of icebergs may be trapped in the innermost part of Storfjorden. The relevant explanations have been added to the text.

Referee's comment: P12, L28–30. As above. It would help to put P13, LL4–8 first in the paragraph.

Response: Indeed, placing the information about benthic foraminifera abundance and change in diatom community at the beginning of the paragraph will make our interpretation more clear and easy-to-follow. Therefore, we have modified the paragraph according to the Reviewer's suggestion.

Referee's comment: P13, L12–14. What data that you present do you base this interpretation?

Response: The proposed scenario is based on the alkenone record from Storfjor-

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drenna provided by ŁÄËcka et al. (article after review)

Referee's comment: P13, L15. Which diatom aDNA sequences? Could these be transported (currents) rather being than reflection of local production?

Response: Herein, we mean diatom sequences in general. Our aim was to pay attention to the continuity of the diatom aDNA record over the Neoglacial. The changes in taxonomic composition were discussed in the other parts of the discussion. We agree that diatoms may be transported by sea currents. However, the record was dominated by one genus (*Thalassiosira*) and taxonomic composition was relatively stable in the entire record, therefore there are no clear signs of the presence of extraneous taxa.

Referee's comment: P14, L2. . . . are not [a] coherent . . .

Response: The sentence has been corrected.

Referee's comment: P14, L9. This is speculation.

Response: Indeed, Clade Y is still poorly studied, therefore most information about its ecology are assumptions. Therefore, we have decided to remove the latter part of the sentence.

Referee's comment: P14, L24–34. It is not clear what the conclusion is from this list of examples.

Response: The aim of this paragraph was to shortly describe the monothalamous taxa recorded in the studied core and to highlight the relation of listed taxa to the presence of phytodetritus. The general conclusions about the changes in monothalamous assemblages are presented in the following paragraph (P15, L1-11).

Referee's comment: P15, L12–16. it is not clear what are own results and what comes from literature.

Response: There was a mistake in the sentence, the word “and” is unnecessary. Now the text is as follows: The decrease in the percentage of foraminiferal sea-ice indicators

that started after ~ 1.7 cal ka BP suggests a gradually diminishing sea-ice coverage in Storfjorden (Fig. 4). Modern-like conditions were established in Storfjorden ~ 0.5 cal ka BP, with seasonally variable sea-ice cover resulting in intensified but variable polynyal activity (Rasmussen and Thomsen, 2014; Knies et al., 2017).

Referee's comment: P15, L16. The IP . . . (capital)

Response: The sentence has been corrected.

Referee's comment: P15, L25. Can you identify the LIA in your record?

Response: Yes, it is possible to identify LIA in our record, however, it spanned only one sample (at 4 cm sediment depth), therefore we avoided making any general conclusion about the LIA.

Referee's comment: P16, L4. Did you actually prove phytoplankton blooms occurred or rather that benthic forams responded to changes in environment and productivity?

Response: We have based our conclusion both on microfossil and molecular records of benthic foraminifera and on molecular record of diatoms. Indeed, microfossil and aDNA record of benthic forams shows response of foraminiferal community to environmental changes, however, the aDNA record of diatoms may be an indicator of the primary production.

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