



1 **Marine eukaryote community responses to the climate and oceanographic changes in**
2 **Storfjordrenna (southern Svalbard) over the past ~14.0 kyr BP: Insights from**
3 **sedimentary ancient DNA analysis**

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9



10 **Abstract**

11 Sedimentary ancient DNA (sedaDNA) metabarcoding is an emerging method to
12 reconstructing the response of marine organisms to past climate and oceanographic changes,
13 including rare and non-fossilized taxa. Marine *sedaDNA* records from the Arctic are scarce,
14 especially those focusing on the impact of environmental shifts on the biodiversity and
15 functional composition of marine eukaryotes communities. Here, we present a *sedaDNA*
16 eukaryotic record from the sediment core retrieved in Storfjordrenna, southern Svalbard,
17 spanning the termination of the Bølling-Allerød, the Younger Dryas, and the Holocene (13.3 -
18 1.3 kyr BP). We successfully recovered the eukaryotic communities and identified them by
19 their ecological roles. Our study showed that the eukaryotic biodiversity in Storfjordrenna
20 remained relatively stable, except during transitions between major climatic intervals. These
21 shifts were marked by changes in richness and relative abundance, driven by factors such as
22 perennial ice cover, surface water cooling, and subsurface Atlantic water influx. Cercozoans
23 and MAST emerged as dominant heterotrophs, characterized by high ecological flexibility and
24 broad tolerance. The primary productivity was primarily driven by ArW-associated
25 phytoplankton, including diatoms (*Thalassiosira* and *Chaetoceros*), green algae (*Micromonas*),
26 and autotrophic dinoflagellates (*Polarella glacialis*), as well as mixoplanktonic silicoflagellate
27 *Pseudopedinella elastica*. The ASV-based indicator analysis revealed that uncultured
28 Cercozoan lineages and MAST taxa were primarily associated with AW proxies, whereas
29 parasitic dinoflagellates (Dino-group I) and choanoflagellates were more closely aligned with
30 ArW proxies. The analysis of indicator responses shows the complex interactions within
31 eukaryotic communities, and reveals a strong association among functional ecological groups,
32 which impacts ecosystem productivity and regulation. This complexity highlights the
33 limitations of traditional single proxy approaches to accurately reconstructing
34 paleoenvironmental conditions. Our study demonstrates the potential of high-resolution marine
35 *sedaDNA* metabarcoding in elucidating responses to past climate changes and in improving
36 our understanding of the intricate interactions within eukaryotic communities in marine
37 ecosystems.

38



39 1. Introduction

40 The Arctic marine ecosystem is undergoing rapid and profound changes, primarily driven
41 by climate warming (Ipcc, 2023; Polyakov et al., 2017; Polyakov et al., 2020). A prominent
42 feature of these changes is the increasing influx of Atlantic Water (AW) into the region, a
43 phenomenon known as Atlantification. This process is associated with warming, rise of sea
44 surface temperatures (SST), reduced sea-ice cover, alterations of salinity patterns, and changes
45 in nutrient dynamics (Årthun et al., 2012; Polyakov et al., 2017).

46 These transformations in the marine environment are altering the biodiversity of Arctic
47 region and impacting the function and resilience of ecosystems (Benner et al., 2019;
48 Hallegraeff, 2010; Ribeiro et al., 2024). The Storfjordrenna region in southern Svalbard is an
49 ideal location to study these shifts, having experienced significant climate-driven changes over
50 the last ~14,000 years, driven by meltwater discharge and the interaction between cold Arctic
51 Water (ArW) and warmer AW inflows (Łacka et al., 2019; Łacka et al., 2015; Pawłowska et
52 al., 2020; Telesiński et al., 2024). The region's biodiversity has been shaped by, and remains
53 sensitive to, these fluctuating and dynamic environmental conditions (Bensi et al., 2025; Deb
54 and Bailey, 2023; Górska et al., 2022; Hop et al., 2019). Understanding how it adapts to such
55 changes is essential for reconstructing past ecological responses to climate change and for
56 predicting future trends. While the impact of climate change on Arctic marine ecosystems is
57 well documented (Deb and Bailey, 2023; Wassmann et al., 2010), relatively few studies have
58 explored marine ecosystems using sedimentary ancient DNA (sedaDNA) to assess long-term
59 biodiversity patterns (Grant et al., 2024; Pawłowska et al., 2020; Zimmermann et al., 2023).
60 Recent developments in sedaDNA techniques have increased our ability to extract and analyze
61 DNA from marine environments, providing valuable insights into eukaryotic communities and
62 their responses to environmental changes over geological time scales (Harðardóttir et al., 2024;
63 Grant et al., 2024; Zimmermann et al., 2021). Studies of marine eukaryotic sedaDNA have
64 demonstrated that even low-resolution records can provide significant data on the shifts in
65 marine communities over time, offering a window into past ecosystem dynamics (Grant et al.,
66 2024). For instance, recent studies have demonstrated the potential of the sedaDNA approach
67 in reconstructing the interactions between sea-ice cover, ocean temperatures and eukaryotic
68 community composition (Armbrecht, 2020; Grant et al., 2024; Harðardóttir et al., 2024;
69 Zimmermann et al., 2023; Zimmermann et al., 2021). However, there remains a notable lack
70 of suitable-resolution marine sedaDNA records from the Arctic, especially those focusing on
71 the impact of environmental shifts on the biodiversity and functional composition of marine
72 communities.



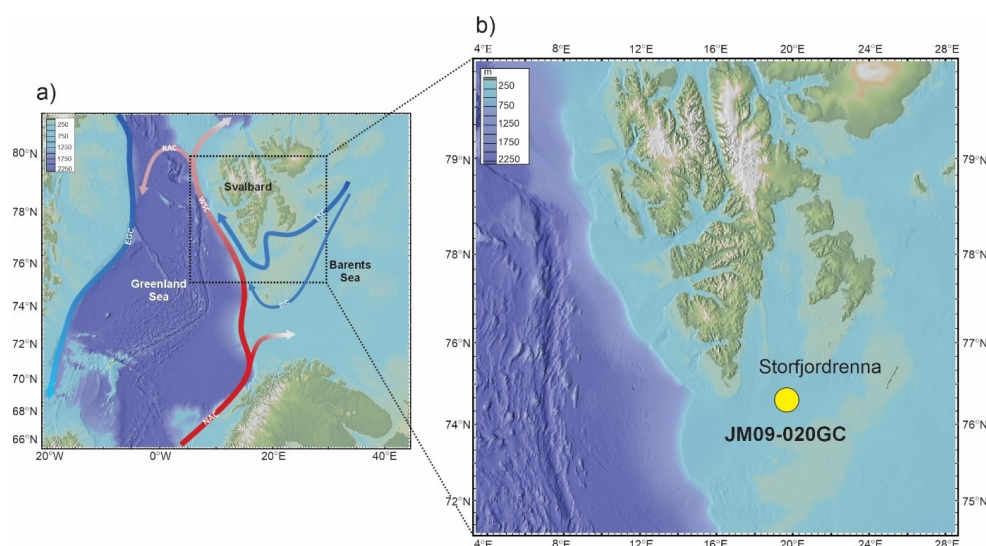
73 This study addresses this gap by reconstructing the long-term history of marine eukaryotic
74 communities using sedaDNA metabarcoding analysis from Storfjordrenna, southern Svalbard.
75 The sedaDNA record is supported by previously published sedimentological,
76 micropaleontological and geochemical records (Łącka et al., 2019; Łącka et al., 2020; Łącka
77 et al., 2015; Telesiński et al., 2024). By focusing on eukaryotic communities associated with
78 ArW and AW masses, we aim to assess their structure, ecological roles, and potential as
79 indicators of past environmental conditions. Our approach seeks to identify how marine
80 ecosystem have responded to significant climate-driven changes in this region and how these
81 responses can improve our understanding of the future trajectory of Arctic marine biodiversity
82 in the context of ongoing climate warming.

83

84 2. Study area

85 Storfjorden is an open fjord system located in the Svalbard Archipelago, between the islands
86 of Spitsbergen, Barentsøya, and Edgeøya (**Fig. 1a**). The cross-shelf through Storfjordrenna is
87 located south of Storfjorden's mouth. The hydrography of Storfjorden and Storfjordrenna is
88 primarily governed by the interplay of two major water masses: AW and ArW. AW is typically
89 characterized by relatively warm temperatures ($>3^{\circ}\text{C}$) and high salinity (>34.95), whereas
90 ArW exhibits lower temperatures ($<0.5^{\circ}\text{C}$) and salinity ranging from 34.3 to 34.8 (Bensi et al.,
91 2025; Skogseth et al., 2020; Sundfjord et al., 2017). AW is transported northwards by the
92 Norwegian Atlantic Current, which bifurcates upon entering the Barents Sea into the West
93 Spitsbergen Current and the North Cape Current (Blindheim and Osterhus, 2005). In contrast,
94 ArW enters the region via the East Spitsbergen Current and the Bear Island Current, bringing
95 cold, less saline waters into the Barents Sea (Hopkins, 1991). AW enters Storfjordrenna in a
96 cyclonic manner, following the bathymetry, and the Polar Front separating AW and ArW water
97 masses is located along the slope of Storfjordrenna (Bensi et al., 2025).

98



99

100 **Figure 1.** a) Map of the study area and (b) location of core JM09-020GC (yellow dot). Red
 101 arrows indicate warm currents, and blue arrows indicate cold currents. Abbreviations: NAC:
 102 North Atlantic Current, WSC: West Spitsbergen Current, RAC: Return Atlantic Current, ESC:
 103 East Spitsbergen Current, BIC: Bear Island Current, EGC: East Greenland Current.)

104 3. Materials and Methods

105 3.1 Sediment core and age model

106 Gravity core JM09-020-GC was collected in 2009 at a depth of 253 m in Storfjordrenna,
 107 northwestern Barents Sea during the expedition of R/V Jan Mayen (**Fig. 1b**). The core was
 108 stored and processed according to the methods described by (Łącka et al., 2019; Łącka et al.,
 109 2020; Łącka et al., 2015). The chronology of the core was established based on AMS¹⁴C
 110 radiocarbon dating. The dates published first by Łącka et al.(2015) were recalibrated using the
 111 Marine20 calibration curve (Heaton et al., 2020). The palaeoceanographic history of
 112 Storfjordrenna over the past ~14,000 years is well documented through detailed, multi-proxy
 113 reconstructions. These include analyses of fossil foraminifera assemblages, isotopic
 114 composition of foraminiferal tests, grainsize and elemental composition of sediments,
 115 alkenones (Łącka et al., 2019; Łącka et al., 2015), and dinoflagellate cysts (Telesiński et al.,
 116 2024).

117 3.2 SedaDNA workflow

118 3.2.1 DNA extraction, amplification, and sequencing



119 Approximately 10 g of sediment was collected from 55 sediment layers using sterile spoons
120 and transferred to sterile containers. DNA extractions were performed using the DNeasy
121 PowerMax Soil Kit (Qiagen), following the manufacturer's instructions. All DNA extracts were
122 stored at -20°C until PCR amplification.

123 The V1V2 region of the 18S rDNA (with a length of ~ 340 bp) was amplified by PCR using
124 the forward primer SSU_FO4mod (5'-GCT TGW CTC AAA GAT TAA GCC-3') and the
125 reverse primer SSU_R22 (3'-CCT GCT GCC TTC CTT RGA-5') (Lindeque et al., 2013), which
126 were tagged with a unique 8-nucleotide sequence at their 5' ends. Each sample was amplified
127 in triplicate and each PCR reaction was performed in a total volume of 25 μL , which included
128 1.5 μL of 1.5 mM MgCl_2 (Applied Biosystems, USA), 2.5 μL of 10 \times PCR buffer II (Applied
129 Biosystems), 0.5 μL of 0.2 mM deoxynucleotide triphosphates (Promega, USA), 0.5 μL of 20
130 mg/ mL bovine serum albumin (Invitrogen Ultrapure, USA), 1 μL of 10 μM of each primer,
131 0.2 μL of AmpliTaq Gold DNA polymerase (Applied Biosystems) and 2 μL of template DNA.
132 The amplification conditions consisted of a pre-denaturation step at 95°C for 5 min, followed
133 by 50 cycles of denaturation at 95°C for 30 s, annealing at 57°C for 30 s and extension at 72°C
134 for 1 min, followed by a final extension step at 72°C for 5 min. PCR products, including
135 negative control for each unique combination of tag-encoded primers, were verified by agarose
136 gel electrophoresis. PCR products were purified using the High Pure PCR Cleanup Micro Kit
137 (Roche) and quantified using a Qubit 2.0 fluorometer. Libraries were pooled in equimolar
138 quantities and the sequence library was prepared using a TruSeq library-preparation kit
139 (Illumina). Samples were then loaded into a MiSeq instrument for a paired-end run of 2×250
140 cycles. The sequencing was performed at the University of Geneva.

141 3.2.2 Data quality control and processing

142 The raw sequencing reads for each sample were processed using the SLIM web application
143 (Dufresne et al., 2019). In brief, the module *demultiplexer* was used to demultiplex the raw
144 reads according to their unique tag in the forward and reverse reads. Quality filtering, chimera
145 removal and generation of the Amplicon Sequence Variants (ASVs) table were performed using
146 DADA2 v.1.16 (Callahan et al., 2016) with pseudo-pool parameters.

147 The ASVs were curated using the LULU package v.0.1.0 (Froslev et al., 2017) with the
148 default parameters. The taxa assignment of the ASVs was performed using VSEARCH against
149 the taxonomically curated PR2 database v.4.14.1 (Guillou et al., 2013), which contains
150 functional annotations. We used a Last Common Ancestor approach, assigning to the
151 consensual taxonomic rank to up to reference sequences with at least 80% similarity. The ASVs



152 were also assigned to functional groups with at least 95% similarity; with the functional
153 attributes of Ibarbalz et al., (2019). The ASVs assigned to prokaryotes (bacteria and archaea)
154 were removed in order to analyze only eukaryotic ASVs. Additionally, fungi and gymnamoebae
155 were removed due to the high risk of contamination (Armbrecht, 2020). Unique ASVs
156 (occurring in only one sample), short sequences (<200 bp), rare ASVs (having <100 reads),
157 and low read count samples (< 1000 reads) were removed from the dataset. Additionally, the
158 unassigned sequences were blasted with NCBI to further clarify the taxonomic composition.
159 The Cumulative Sum Scaling (CSS) technique was used to transform the read counts in the
160 dataset and using 'cssNorm' function in the genomeSeq package (Paulson et al., 2013).
161 CSSlog1p (scale factor - 0.75) was used for the downstream statistical analysis in the study.

162 3.3 Statistical analysis

163 Data analysis was performed in R v.4.2.2 (Team, 2013) using several R packages. The
164 relative abundance of reads and ASVs of each eukaryote group were calculated and plotted
165 using *ggplot2* (Wickham, 2016) and Grapher 24.1.213.

166 Three alpha diversity indices were calculated for all samples using the Hill number
167 diversity (ASV richness $q = 0$, Shannon index $q = 1$, and Simpson index $q = 2$) based on the
168 functions in the *hillR* packages (Chao et al., 2014). Hill-Shannon diversity was compared
169 between the main groups using the Kruskal-Wallis's rank test in the *stats* package (Kruskal and
170 Wallis, 1952). The significance between the groups was determined using a pairwise Wilcoxon
171 rank sum test with an adjusted p value (Benjamini-Hochberg) in the *ggpubr* package
172 (Kassambara, 2023), and Permutational Multivariate Analysis of Variance (PERMANOVA)
173 with 999 permutations via *adonis2* function in the *vegan* package (Oksanen et al., 2007). A
174 Principal Coordinate Analysis (PCoA) ordination was generated using the Bray-Curtis
175 dissimilarity matrix calculated with the *ape* package (Paradis et al., 2019) and *stringer* package
176 (Wickham, 2019) to assess beta diversity and visualize dissimilarities in eukaryotic community
177 composition among the samples.

178 A co-occurrence heatmap representing most of families in the study was generated. The
179 *corrplot* (Wei and Simko, 2024) and *pheatmap* packages were used based on spearman method
180 to analyze the correlation between eukaryote families (a cutoff mark in correlation as > 0.5 ,
181 and p value Benjamini-Hochberg (BH) adjusted < 0.05). Environmental parameters
182 represented by paleoceanographic proxies were used to identify the response of eukaryote
183 species through three analytical methods. Seven proxies were used including indicators of sea
184 surface temperature (SST U_{37}^{K*}) (Łacka et al., 2019), AW indicators foraminifera *Nonionellina*



185 *labradorica* and *Buccella frigida* (Łącka et al., 2015), dinocyst *Operculodinium centrocarpum*
186 (Telesiński et al., 2024), ArW/meltwater indicator (%C37:4) (Łącka et al., 2019), glaciomarine
187 condition indicator foraminifera *Elphidium excavatum* and *Cassidulina reniforme* (Łącka et
188 al., 2015), sea ice indicators dinocysts *Echinidinium karaense* from (Telesiński et al., 2024),
189 and bottom current dynamics (mean grain size 0-63 μm) (Łącka et al., 2015). Fuzzy set
190 ordination (FSO) plots were generated for each environmental variable to assess their influence
191 on eukaryote communities and identify key proxies for downstream analysis (Roberts, 2008).
192 Firstly, a heatmap of sparse partial least squares (sPLS) regression between ASVs, and proxies
193 was generated using the *spls* and *cim* function in *mixOmics* package (Froslev et al., 2017; Kim-
194 Anh Lê Cao et al., 2008). The potential ASV based indicators were selected based on a
195 correlation coefficient threshold of > 0.3 and BH adjusted p value of < 0.05 . Secondly, a
196 Spearman correlation heatmap of the top 100 most significant ASVs ($\rho > 0.3$ and p-adjust $<$
197 0.05) as generated using the *pheatmap* package. Finally, the dataset was analyzed using DEseq2
198 analysis, and the dataset was curated based on $\log_2\text{FoldC} \geq 1$ (BH-adjusted p value < 0.05),
199 and lower base mean (Love et al., 2014). Potential indicator ASVs were categorized based on
200 their correlation strength and consistent detection across at least two methods or strong
201 association with multiple paleo-proxies.

202 4. Results

203 4.1 Metabarcoding data

204 A total of 2,620,808 raw sequence reads were generated from 55 samples. After filtering, 13
205 samples which mainly spanned the period between 4.0 and 7.5 kyr BP, were removed due to
206 low number of reads. This reduced our dataset to 1,609,500 sequence reads and 273 ASVs in
207 42 samples (Table S1, S2).

208 4.2 Alpha diversity

209 Alpha diversity indices varied across time intervals, although Hill's Shannon diversity
210 showed no significant differences (Kruskal-Wallis, $p = 0.48$; Fig. S1, Table S1). Overall, the
211 number of observed ASVs ranged from 4 to 144 (Fig. S1). The highest values were observed
212 in the Younger Dryas, Early Holocene and Late Holocene, particularly at 12.3 kyr BP, 11.3 kyr
213 BP, 9.5 kyr BP, 4.0 kyr BP, and 2.8-2.3 kyr BP. In contrast, a significant decrease in richness
214 was observed around 13.3 kyr BP, 11.8 kyr BP, 2.17 kyr BP, and 1.8 kyr BP (Fig. S1, Table
215 S1). Similar trends were revealed by both the Shannon and Simpson indices, with minimal
216 diversity observed around 12.8 kyr, 11.7 kyr, 9.2 kyr, and 3.4 kyr BP. Between 11.7 kyr and

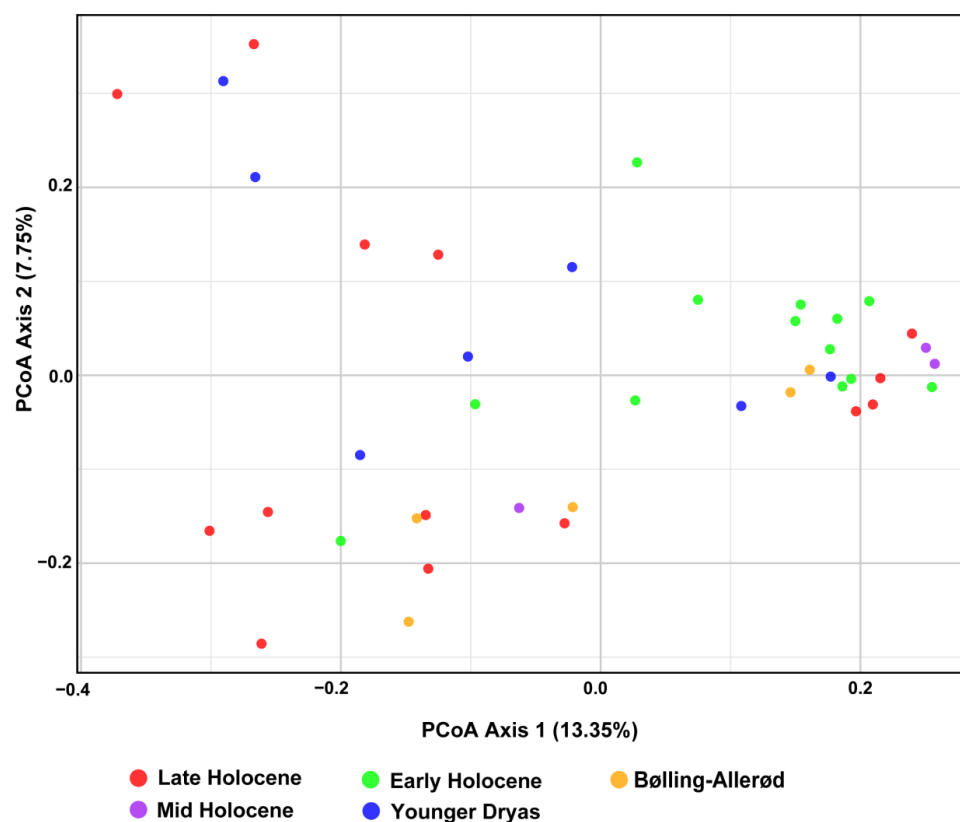


217 9.2 kyr BP. Due to limited data resolution, no clear trends in alpha diversity could be discerned
218 between 9.2kyr and 3.4 kyr BP. However, a decline was evident after 3.4 kyr BP, continuing
219 towards 1.3 kyr BP (**Fig. S1**).

220 **4.3 Beta Diversity**

221 Beta diversity analyses revealed minor changes in community composition during the
222 transitions from the Bølling-Allerød to the Younger Dryas, and from the Younger Dryas to the
223 Holocene (**Fig. 2**). The PCoA plot revealed overlap across different time intervals. Late
224 Holocene and Younger Dryas samples were widely dispersed, while Early Holocene samples
225 clustered separately along Axis 1, respectively. In contrast, Bølling-Allerød and Mid Holocene
226 samples were largely scattered along the same axis (**Fig. 2**). PERMANOVA results supported
227 significant differences in community composition ($p < 0.05$) between the Late and Early
228 Holocene, Late Holocene and Younger Dryas, Early Holocene and Younger Dryas, and
229 Younger Dryas and Bølling-Allerød (**Table S3**). FSO plots revealed a significant relationship
230 between the samples and several paleo-environmental proxies, including the dinocyst
231 *Operculodinium centrocarpum* from (Telesiński et al., 2024), ArW/meltwater indicator
232 (%C37:4) (Łacka et al., 2019), and sea surface temperature (SST U_{37}^{K*}) (Łacka et al., 2019) (**Fig.**
233 **S2**).

234



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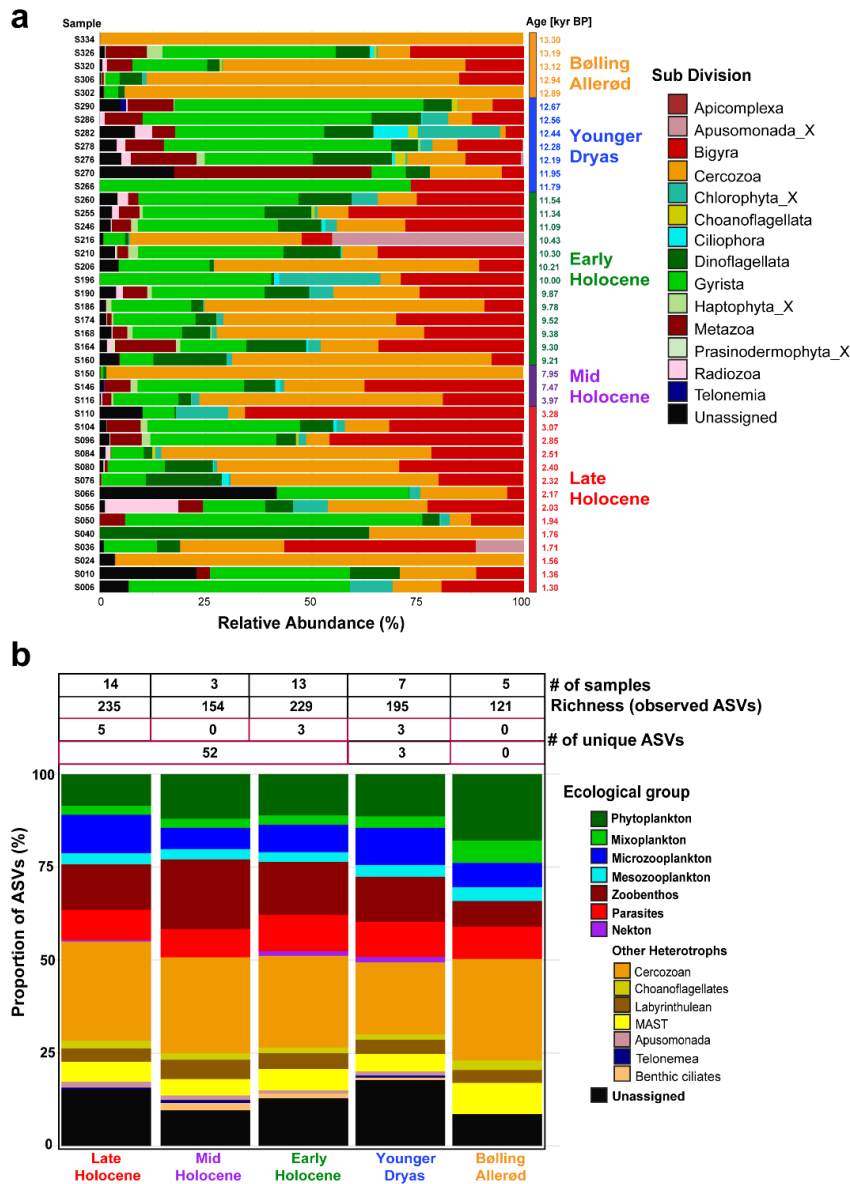
236 **Figure 2.** PcoA based on the Bray-Curtis dissimilarity matrix method with the eukaryote
237 dataset (raw data converted into CSSlog1p formation).

238 4.4 Community composition

239 Within the dataset, a total of 236 ASVs were assigned to 14 Sub-Divisions, while 37 ASVs
240 remained unassigned (**Table S2**). The Cercozoa was the most abundant sub-division,
241 comprising 67 ASVs, accounting for 24.54% of the total ASVs (**Fig. 3 & Table S2**). Overall,
242 the taxonomic structure of eukaryotes based on read abundance fluctuated significantly
243 between samples (**Fig. 3a**). In contrast, ASV richness remained stable across different time
244 periods except during the Bølling-Allerød period (**Fig.3b**). The number of unique ASVs was
245 highest during the Late Holocene, with five unique ASVs identified. The Younger Dryas and
246 Early Holocene each exhibited three unique ASVs. Conversely, no distinctive ASVs were
247 identified during the Mid Holocene and Bølling–Allerød periods. Across the entire Holocene,



248 a total of 52 unique ASVs were recorded (Fig.3b).



249
250 **Figure 3:** (a) Bar plot showing the downcore distribution of eukaryotic sub-divisions based on
251 their relative abundance. (b) Proportional richness of distinct ecological groups across selected
252 time periods (Bølling-Allerød, Younger Dryas, Early Holocene, Mid Holocene, and Late
253 Holocene), expressed as the percentage of ASVs. The accompanying table provides the number
254 of samples, the total number of observed ASVs and unique ASVs within each climate time
255 interval.



256 The ASVs were categorized based on their ecological roles, such as phytoplankton (31
257 ASVs), mixoplankton (8 ASVs), mesozooplankton (8 ASVs), microzooplankton (23 ASVs),
258 parasites (24 ASVs), zoobenthos (36 ASVs), nekton (2 ASVs), and other heterotrophs (104
259 ASVs) (**Fig. 3b, Table S2**). The latter category comprises multiple taxonomic groups
260 characterized by complex habitats and feeding behaviors, many of which have poorly
261 understood ecological roles. This group includes Cercozoa (67 ASVs), Labyrinthulea (11
262 ASVs), Choanoflagellata (5 ASVs), MAST (15 ASVs), benthic ciliophora (2 ASVs),
263 Apusomonada (3 ASVs), and one ASV from the Telonemea flagellate group. The unassigned
264 taxa (37 ASVs) also remained ecologically uncategorized. (**Fig. 3b**).

265 The phytoplankton community consisted of diatoms, green algae, haptophytes, and
266 autotrophic dinoflagellates, most of which were associated with ArW (**Table S2**). In terms of
267 read abundance, *Thalassiosira* spp. and *Chaetoceros* sp. dominated among diatoms, while
268 *Micromonas polaris* was the dominant species within the green algae. The haptophytes group
269 was primarily represented by *Phaeocystis* sp., whereas the *Gymnodinium* spp. and sea-ice-
270 associated species *Polarella glacialis* were dominant within the autotrophic dinoflagellate
271 group (**Fig. S3**). The mixoplankton community was primarily composed of mixotrophic
272 dinoflagellates and silicoflagellates. In terms of read abundance, mixotrophic dinoflagellates
273 were mainly present from the Younger Dryas to the beginning of the Early Holocene, whereas
274 mixotrophic silicoflagellates, represented by *Pseudopedinella* sp., was present throughout the
275 entire core (**Fig. S3**).

276 The zooplankton community was divided into two size-based groups: micro- and
277 mesozooplankton. The microzooplankton group included heterotrophic protists such as
278 radiolarians, pelagic ciliates, dinoflagellates, and silicoflagellates. These groups were
279 identified as being present at specific time periods, e.g. ~12.4 to ~10.2 kyr, and ~2.3 to 1.3 kyr
280 BP (**Fig. S4, Table S2**). The mesozooplankton group comprises small metazoans and was
281 dominated by arthropods (Copepoda and Malacostraca) and larvaceans (Appendicularia). The
282 copepod *Calanus* spp. represented the majority of the mesozooplankton around the study area
283 (**Fig. S4**).

284 The zoobenthos was recorded as the most diverse group, primarily representing
285 macrobenthic species. This group included annelids, ascidiacean, molluscs, cnidarians, and
286 echinoderms (**Fig. S5, Table S2**). Zoobenthos taxa were most abundant around ~9.3 kyr BP,
287 as well as between ~12.3 kyr and 12.0 kyr BP (**Fig. S5**).

288 The parasites were represented by six classes: Dinophyceae (Syndiniales),
289 Gregarinomorphea, Paragregarea, Peronosporae, Hyphochytrae, and Enoplea (**Fig. S6, Table**

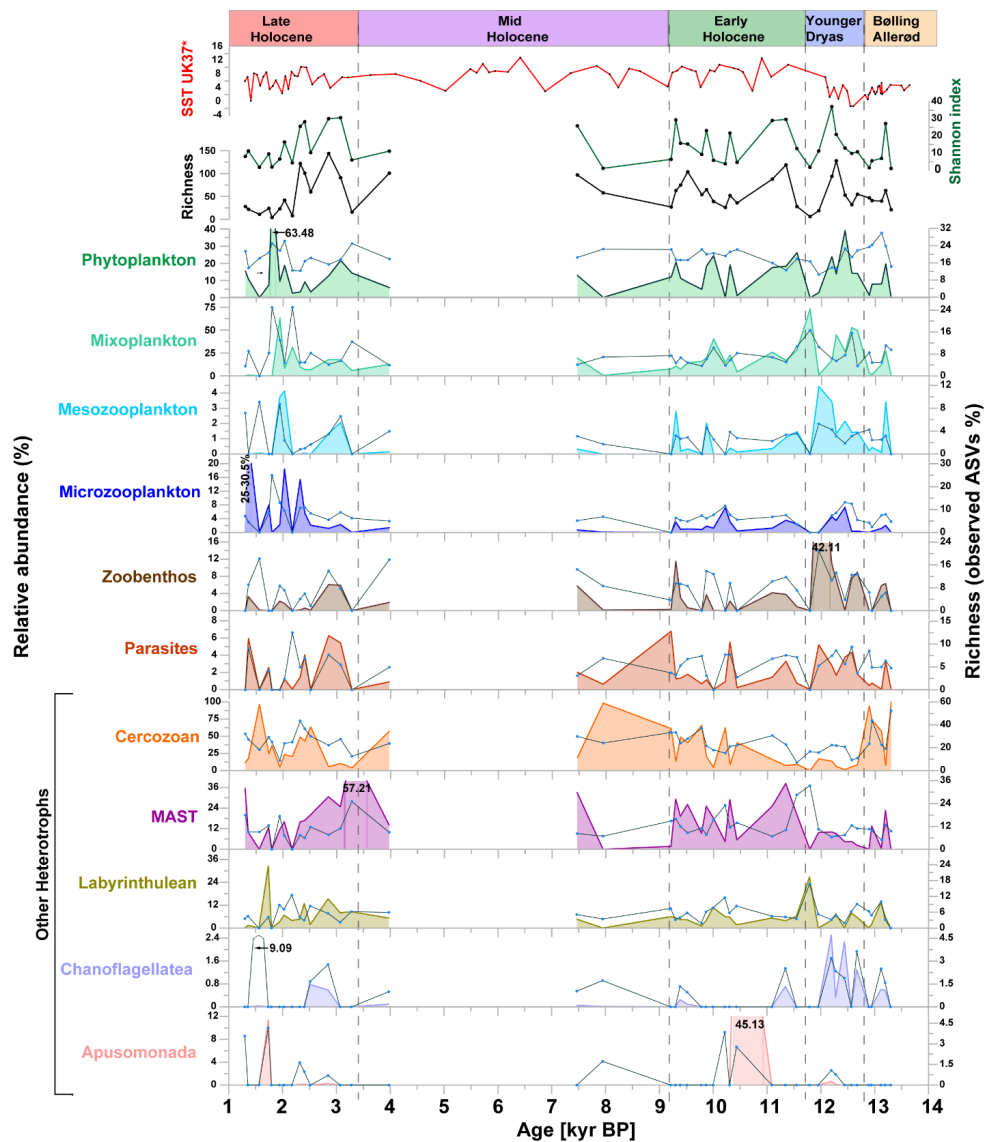


290 **S2).** Among them, Syndiniales have the highest abundance and diversity, with 18 ASVs (mainly
291 uncultured) detected throughout the studied time interval (**Fig. S6**). The nekton group included
292 two ASVs assigned to Arctic cod (order Gnathostomata), which were detected only during the
293 Younger Dryas and Early Holocene.

294 Among other heterotrophs, Cercozoa were dominant, accounting for a significant proportion
295 of reads throughout the study period (**Fig. S7, Table S2**). Five classes of Cercozoa were
296 identified: Ascetosporea, Phytomyxea, Granofilosea, Thecofilosea, and Imbricatea.
297 Thecofilosea exhibited the highest richness with 51 ASVs (**Fig. S7, Table S2**). Ecologically,
298 cercozoans can be classified as parasitic, predatory, and bacterivorous. *Cryothecomonas* spp.
299 were identified as predatory, while those in the classes Ascetosporea and Phytomyxea were
300 classified as parasites. Other cercozoan ASVs were identified only to the family level, limiting
301 precise ecological interpretations.

302 The other heterotrophs also include the MAST (Marine Stramenopiles), Labyrinthulea,
303 Choanoflagellata and Apusomonada. The MAST group included 16 ASVs, representing four
304 main sub-clades: MAST-1, MAST-3, MAST-9, and MAST-12. MAST 9 and MAST 12
305 dominated throughout the studied time period and exhibited high richness (**Fig. S6, Table S2**).

306 The Labyrinthulea included saprotrophic Thraustochytriaceae, and Aplanochytriidae,
307 revealing a dominant presence and high richness around ~11.8 kyr to ~2.2 kyr, and ~1.7 kyr
308 BP (**Fig. 4**). Most choanoflagellate ASVs belonged to environmental clades, except for
309 *Calliacantha* sp., which was dominated around ~12.7 kyr to ~12.2 kyr BP (**Fig. 4**).
310 Apusomonada, represented by the class Apusomonadea, appeared during certain time intervals,
311 especially the Early Holocene (~10.4 kyr BP), and Late Holocene (~1.7 kyr BP) (**Fig. 4, Table**
312 **S2**). (see **Supplementary document** for more details).



313

314 **Figure 4:** Relative abundance and richness (expressed as observed ASVs percentage) of major
315 ecological groups, along with Shannon index, richness, and sea surface temperature [SST
316 UK'37] from Łacka et al. [2019]. Lines represented ASVs abundance (%), and area represented
317 the read abundance (%).

318



319 **4.5 Indicator taxa for Arctic and Atlantic water conditions**

320 A total of 39 ASVs were identified as potential indicator taxa using three analytical
321 approaches (sPLS, Spearman correlation, and DESeq) (**Fig. S8, S9, Table S4**). Of these, 14
322 ASVs were identified as potential AW indicators, and belonged to the following groups:
323 phytoplankton (1), microzooplankton (1), cercozoans (6), MAST (2), zoobenthos (1),
324 labyrinthulean (1), and unassigned ASVs (2). The AW plankton indicators included a green
325 algae *Pyramimonas* sp. (ASV55) and a pelagic ciliate *Cyclotrichium* sp. (ASV265), while the
326 AW benthic indicators comprised a polychaete *Tharyx* sp. (ASV278) (**Fig. S10**).

327 In contrast, 24 ASVs were associated with ArW (**Fig. S12**), primarily parasites (5),
328 zoobenthos (2), phytoplankton (3), choanoflagellates (2), microzooplankton (2),
329 mesozooplankton (1), mixoplankton (1), benthic ciliate (1), nekton (1), and unassigned ASVs
330 (6 s) (**Table S4**). Potential ArW ASV-based indicators were identified among both planktonic
331 and benthic taxa. Planktonic ASVs comprised autotrophic dinoflagellates (*Prorocentrum* sp.,
332 ASV64 and ASV153), diatoms (*Chaetoceros gelidus*, ASV18), mixotrophic dinoflagellate
333 (*Heterocapsa* sp., ASV204), pelagic ciliate (*Strombidium* sp., ASV123), radiolarian
334 (*Heteracon* sp., ASV54), and mesozooplankton (*Oikopleura* sp., ASV500). Parasites included
335 three Syndiniales dinoflagellates (ASV213, ASV341, and ASV391) and apicomplexan
336 (*Paralecudina* sp., ASV958). Benthic indicators included benthic ciliate (*Holosticha* sp.,
337 ASV87), echinoderm (*Ctenodiscus* sp., ASV104), and bivalve (*Tridonta* sp., ASV137) (**Fig.**
338 **S11**).

339 **4.6 Ecological interactions among eukaryote families**

340 Spearman correlation analysis ($r > 0.5$, adjusted $p < 0.001$) was used to explore potential
341 ecological interactions among eukaryotic families inhabiting similar environmental niches
342 (**Fig. S12, Table S5**). Parasitic cercozoans (Ascetosporea and Phagomyxidae) strongly
343 correlated with algae families (Phaeocystaceae, Thalassiosiraceae, Pyramimonadaceae, and
344 Prasinodermataceae), and dinoflagellates (Suessiaceae, and Gymnodiniaceae). Other
345 cercozoans (CCW10-lineage, Novel-Clade-2, Cryothecomonas-lineage, and Ventricleftida)
346 revealed significant correlations with algal and dinoflagellate groups (**Fig. S12, Table S5**).
347 Among MAST groups, MAST-12 showed positive associations with algae families
348 (Prasinodermataceae, Thalassiosiraceae, and Chaetocerotaceae) and parasites (Pirsoniaceae),
349 while MAST-9 correlated with multiple phytoplankton families (**Fig. S12, Table S5**). Parasitic
350 dinoflagellates (dino-group-II) showed strong correlation with haptophyte algae
351 (Phaeocystaceae), diatom (Thalassiosiraceae), and dinoflagellates (Suessiaceae, and



Gymnodiniaceae). Parasitic alveolates of the family Lecudinidae showed a strong positive correlation with the mesozooplankton (Malacostraca), radiolarians and ophiuroids (**Fig. S12, Table S5**). Another parasitic superfamily of Stramenopiles, the Pirsoniaceae displayed strong associations with various taxa, including MAST-12, cercozoans (Thaumatomonadidae), green algae (Prasinodermataceae, and Chlamydomonadales), haptophytes (Phaeocystaceae), diatoms (Thalassiosiraceae), silicoflagellates (Actinomonadaceae), dinoflagellates (Amphidiniopsidaceae), and polychaetae (Chaetopteridae) (**Fig.S12, Table S5**).

5. Discussion

This study expands our knowledge of eukaryotic communities' dynamics in the Storfjordrenna over the past 13,300 years by providing high-resolution sedaDNA records of both fossilized and non-fossilized groups. We demonstrate how these communities responded to major climatic shifts since the Bølling-Allerød and highlight key ecological interactions among major taxonomic groups. These findings enhance our understanding of how environmental changes have shaped eukaryotic biodiversity in southern Svalbard.

5.1.Impacts of climate change on the eukaryotic community in Storfjordrenna

5.1.1 Bølling-Allerød (13.30 kyr BP to 12.80 kyr BP)

The eukaryotic community in our record during the Bølling–Allerød reflected oceanographic conditions resembling those observed today in glacier-proximal areas of Arctic fjords characterized by high turbidity due to meltwater discharge and the presence of colder, fresher waters (Zajaczkowski, 2008; Łącka et al., 2019). Previous studies support this interpretation: the grounding line of the Svalbard Barents Ice Sheet (SBIS) retreated from Storfjordrenna before 13.95 kyr BP (Łącka et al., 2015), coinciding with SST reaching modern-like values (Łącka et al., 2019). However, despite elevated SST, primary productivity remained low—likely due to the suppressive effect of turbid meltwater input from the retreating ice sheet (Łącka et al., 2015). Biomarker data further indicated a dominance of fresher ArW over AW, which has been linked to reduced primary productivity (Łącka et al., 2019). These conditions favored the development of a eukaryotic community dominated by heterotrophs, capable of thriving in such extreme environments. The most abundant taxa, in terms of both sequence reads and ASV richness, were bacterivorous cercozoans (**Fig. 3**). The dominant cercozoan was *Limnofila* sp., a genus primarily found in fresh and brackish waters (Mylnikov et al., 2015; Nikolaev et al., 2003). Other important bacterivorous heterotrophs were MAST, particularly



383 MAST-9D and MAST-12A, known for their adaptation to extreme environmental conditions
384 (Labarre et al., 2021; Lin et al., 2022; Obiol et al., 2024).

385 Despite lower read abundance, phytoplankton, mixoplankton, and microzooplankton
386 showed high ASV richness during this period (**Fig. 3**). The phytoplankton community was
387 dominated by autotrophic, sea-ice associated taxa, such as dinoflagellates *Polarella glacialis*
388 (Harðardóttir et al., 2024) and *Gymnodinium* spp. (Kubiszyn and Wiktor, 2015), and diatom
389 *Thalassiosira* spp. (Luddington et al., 2016). The mixoplankton was represented by the
390 silicoflagellate, *Pseudopedinella elastica* (**Fig. S3**), which has been described as bacterivorous
391 under conditions of limited light and nutrients (Gerea et al., 2016). The mesozooplankton
392 community was primarily composed of herbivorous *Calanus* spp. and the omnivorous *Metridia*
393 *longa*. These species have been observed in the Svalbard region in the present environment,
394 with *Calanus* spp. dominating the ArW-AW in terms of biomass (Daase et al., 2008). Despite
395 unfavorable conditions caused by meltwater influx and low nutrient availability, both primary
396 and secondary productivity persisted, likely concentrated in ice-proximal and frontal zones
397 where the stratification enhanced nutrient retention and water column stability. This suggests
398 that environments near retreating ice sheets can act as biological hotspots, supporting
399 productivity through ice-associated blooms and complex microbial food web interactions.

400 **5.1.2 Younger Dryas (12.80 kyr BP to 11.70 kyr BP)**

401 The eukaryotic community during the Younger Dryas reflected the dramatic environmental
402 changes that occurred at that time. The most notable change was the rapid decrease in
403 biodiversity during the Bølling-Allerød and Younger Dryas transition, when alpha diversity
404 indices reached near-zero values (**Fig. 4**). It is most likely that the reorganization of
405 oceanographic conditions caused a temporary slowdown of Atlantic meridional overturning
406 circulation (AMOC) and a reduction in AW inflow leading to strong stratification, formation
407 of perennial ice cover, and anoxic conditions at the bottom (Łącka et al., 2020). The presence
408 of perennial ice cover led to a significant reduction in primary productivity in Storfjordrenna
409 (Łącka et al., 2019). In contrast, the sedaDNA record also revealed the presence of phyto- and
410 mixoplankton during this period, especially presence of phytoplankton *Thalassiosira* spp. and
411 *Gymnodinium* sp., and silicoflagellate *P. elastica*, suggesting that primary productivity, yet
412 limited, still occurred under the ice. The detection of herbivorous mesozooplankton *Calanus*
413 spp., and predatory *Cryothecomonas* spp., also coincided with the presence of the
414 phytoplankton community. Notably, the early Younger Dryas also revealed a short-term



415 increase in the relative abundance and diversity of zoobenthos, primarily polychaetae
416 (*Barantolla* sp.) and molluscs (*Tridonta* sp. and *Talochlamys* sp.) (**Fig. S4**).

417 During the latter part of the Younger Dryas (after ~12.4 kyr BP), increasing advection of
418 AW and SST warming led to the replacement of perennial sea ice by seasonal ice cover (Łącka
419 et al., 2019; Łącka et al., 2020). This shift was followed by the establishment of a more diverse
420 benthic foraminifera community (Łącka et al., 2015). Similarly, the sedaDNA record displayed
421 the increase in the richness and abundance of zoobenthic taxa, mainly annelids, molluscs, and
422 echinoderms (**Fig. S5**). However, the alkenone record suggested that the warming was
423 associated with low primary productivity, probably due to the continuous input of turbid
424 meltwater from the decaying SBIS (Łącka et al., 2015).

425 In contrast, the sedaDNA record indicated a sudden phytoplankton bloom in the latter
426 Younger Dryas, dominated by *M. polaris*, *Thalassiosira* spp., *Chaetoceros gelidus*, and
427 *Gymnodinium* spp. *Micromonas polaris* is typically associated with Arctic sea-ice
428 environments, and favorably adapted to increasing Arctic temperatures and water column
429 stratification (Grant et al., 2024). *Chaetoceros gelidus*, known for its high tolerance under
430 variable light and ocean acidification conditions (Biswas, 2022; Ribeiro et al., 2024), may play
431 a key role in plankton blooms and primary productivity, particularly during the Younger Dryas
432 (**Fig. S3**). Phytoplankton blooms stimulated the development of secondary producers, mainly
433 pelagic ciliates, and radiolarians, as well as mesozooplankton copepods (*Calanus* spp.) (**Fig.**
434 **S4**). Altogether, these findings indicate that the latter part of the Younger Dryas (after ~12.4
435 kyr BP) was associated with periods of accelerated AW inflow and an increase in SST
436 (Risebrobakken et al., 2010; Wollenburg et al., 2004), promoting phytoplankton and
437 zooplankton growth, and enhancing benthic community development..

438 **5.1.3 Early Holocene (11.70 kyr BP to 9.20 kyr BP)**

439 The transition from the Younger Dryas to the Early Holocene was characterized by a
440 significant drop in biodiversity and the dominance of mixoplankton, primarily *P. elastica*, as
441 well as mixotrophic dinoflagellates such as *Biecheleria* sp. and *Gotoius* sp. According to
442 (Łącka et al., 2020), the onset of the Early Holocene was associated with a short-term decrease
443 in SST and a decrease in foraminiferal fauna abundance. The low biodiversity and dominance
444 of mixotrophic plankton observed in the sedaDNA record might be a consequence of this short-
445 term deterioration in environmental conditions.

446 However, the further development of the Early Holocene was driven by an increasing
447 influence of AW in the area, which was followed by an increase in SST and productivity



(Devendra et al., 2023; Telesiński et al., 2017). Moreover, Arctic Front was located close to the Spitsbergen coast, leading to the formation of a highly productive frontal zone (Łacka et al., 2019). The amelioration of environmental conditions during the Early Holocene (Łacka et al., 2015) was reflected in a sudden peak of alpha diversity of overall eukaryotic community, accompanied by a notable increase in the richness and abundance of key ecological groups, including phytoplankton, zoobenthos, parasites, and other heterotrophs such as cercozoans and MAST. (**Fig. 4**). However, taxa associated with sea-ice were an important component of the assemblage, suggesting that sea-ice formation still occurred in Storfjordrenna. Despite AW dominance, the presence of a cercozoan *Limnofila* sp., green algae *M. polaris* and *Pyramimonas* sp., as well as sea-ice-indicator *P. glacialis* may suggest episodic presence of sea-ice, and transition of Arctic Front (**Fig. S3, S7**). Overall high eukaryotic biodiversity in the Early Holocene, particularly the diversity of phytoplankton, mixoplankton, mesozooplankton, and the gradual increase in MAST-9 species related to warm water further support the establishment of warm-water conditions with high nutrient availability (Łacka et al., 2019) (**Fig S3, S4, S6, and Table S4**).

5.1.4 Mid Holocene (9.20 kyr BP to 3.40 kyr BP)

Due to the lack of data from the period between 7.5 kyr BP and 4 kyr BP (see the Results section), it is possible to interpret only the early part of the Mid Holocene and the transition to the late Holocene. Therefore, this interpretation should be treated with caution due to the limited number of samples analyzed in this interval. The beginning of the Mid Holocene (9.2 kyr BP) in Storfjordrenna was marked by a significant drop in biodiversity, followed by an increase after 8.0 kyr BP. The species composition was predominantly composed of cercozoans, mainly *Limnofila* sp. and *Cryothecomonas* spp. (**Fig. S7**). Another important component of the eukaryotic assemblage was MAST species, including MAST-9D and MAST-12B, which had previously been recorded in the north Atlantic region (Lopez-Garcia et al., 2007; Newbold et al., 2012)(**Fig. S6**). The community composition resembled the one from Bølling Allerød, dominated by heterotrophic taxa adapted to extreme environmental conditions. This aligns with evidence of a minor cooling event between 9.0 kyr and 8.0 kyr BP, as proposed in prior studies (Łacka et al., 2015).

In contrast, despite their relatively low abundance, phytoplankton, mesozooplankton, and microzooplankton communities displayed relatively high diversity in the early Mid-Holocene. This period in Storfjordrenna was characterized by limited ice rafting, variable SST, and interplay between the AW and ArW water masses rather than a continuous impact of AW



481 (Łącka et al., 2019; Łącka et al., 2015). Furthermore, the low alkenone flux suggested low
482 primary productivity throughout the mid-Holocene (Łącka et al., 2019), consistent with the low
483 abundance of both phyto- and zooplankton taxa in the sedaDNA record. Low productivity was
484 also observed at that time in the Norwegian and Svalbard shelves, potentially due to the limited
485 nutrient availability. According to (Łącka et al., 2019), the reduction in primary productivity
486 resulted from enhanced vertical stratification, which reduced vertical mixing in the water
487 column, and thus, limited the nutrient resuspension to the surface. An alternative explanation
488 is the early spring bloom, that occurs in the ice-free waters, and the subsequent development
489 of mesozooplankton that graze on phytoplankton, thereby reducing the flux of organic matter
490 to the bottom. However, the low abundance of sequences assigned to both phyto- and
491 microzooplankton, as well as the increase in bacterivorous taxa, likely supports the first
492 scenario. Overall, the lack of sea ice, and the variability in water masses and SST observed at
493 the beginning of the Mid Holocene, created an unstable environment, which favored tolerant
494 heterotrophic eukaryotes such as Cercozoans or MAST.

495 **5.1.5 Late Holocene (3.40 kyr BP to 1.30 kyr BP)**

496 The onset of the Late Holocene was marked by an increase in eukaryotic biodiversity,
497 followed by a sharp decrease around 2.0 kyr BP. At this time, eukaryotic communities were
498 predominantly composed of cercozoan and MAST (**Fig. 4**). Cercozoan abundance and richness
499 exhibited an increasing, yet variable trend throughout the Late Holocene, whereas MAST
500 decreased progressively over time.

501 Both phyto- and microzooplankton exhibited high richness, but variable abundance
502 throughout the Late Holocene (**Fig. 4**). Furthermore, the presence of parasitic species,
503 including the Syndiniales dinoflagellate (dino-group-I and dino-group-II) as well as the diatom-
504 associated parasitic *Pirsonia* sp., co-occurred with the phytoplankton suggesting that parasitic
505 interactions may have influenced phytoplankton dynamics during the Late Holocene. (**Fig.**
506 **S12, Table S5**). The Late Holocene coincided with the so-called Neoglacial cooling, spanning
507 the last 4.0 kyr BP. This period was characterized by a decline in SST (Risebrobakken et al.,
508 2010), limited AW inflow and strengthening of ArW flow, which led to the formation of
509 extensive ice cover (Martrat et al., 2003; Berben et al., 2014; Devendra et al., 2023). Records
510 from Storfjordrenna also showed a cooling in the area, associated with enhanced ice rafting
511 (Łącka et al., 2019; Łącka et al., 2015). Thus, the increased abundance of phytoplankton in
512 general, and ice-associated *P. glacialis* in particular, is probably an effect of the cooling of
513 surface waters and the formation of sea-ice, which launched convective water mixing and



514 nutrient resuspension to the surface. In consequence, primary productivity increased,
515 stimulating the development of the microzooplankton community (**Fig. S4**).

516 **5.2. SedaDNA environmental indicators**

517 This study identified 39 potential eukaryotic indicator taxa associated with AW and ArW
518 conditions. Several taxa exhibited consistent temporal patterns that aligned with
519 paleoenvironmental proxies, supporting their potential for long-term reconstructions. In
520 contrast, others appeared only sporadically, reducing their reliability as potential indicators.
521 AW-associated taxa were primarily represented by cercozoans and MAST, while ArW-
522 associated taxa included diatoms, dinoflagellates, choanoflagellates, Arctic zoobenthos, and
523 zooplankton.

524 Bacterivorous cercozoans, including the Ventricleftida (ASV46), the Protaspa-lineage
525 (ASV83, ASV623, and ASV257), and the CCW10-lineage (ASV20), were identified as
526 potential indicators for the AW (**Table S4**). However, their identification is currently based
527 exclusively on molecular data, limiting ecological and biogeographical context and weakening
528 their use in environmental reconstructions (Labarre et al., 2021; Obiol et al., 2024). Similarly,
529 members of the bacterivorous MAST-9 group, notably MAST-9A and MAST-9D, were
530 exclusively detected in AW conditions (**Fig. S10, Table S4**), consistent with their known
531 tropical-to-temperate distribution. Within the phytoplankton communities, *Pyramimonas*
532 *parkeae* (a green alga that prefers higher temperature regions; (Bock et al., 2021)), and the
533 microzooplankton pelagic ciliate *Cyclotrichium* sp. (commonly found in warmer waters;
534 (Dirmenci et al., 2010; Xu et al., 2005)) were also identified as potential AW indicators.
535 However, they only occurred in a brief temporal window near the end of the Early Holocene,
536 so their reliability as indicators needs to be verified by further studies (**Fig. S10**).

537 The relatively high number of cold-water species recorded was probably due to favorable
538 overall conditions in the study area. The autotrophic *Prorocentrum* spp. are known as toxin-
539 producing, bloom-forming species with broad global distributions, including polar regions
540 (Cen et al., 2019; Goncharenko et al., 2021; Stoecker and Lavrentyev, 2018; Tillmann et al.,
541 2022). In the present study, both taxa were primarily detected at the onset of the Younger Dryas
542 (**Table S5**). Their limited distribution suggests that they may be constrained as long-term
543 environmental indicators. Similarly, *Heterocapsa* sp., a mixotrophic dinoflagellate commonly
544 associated with harmful algal blooms in Arctic and North Atlantic waters (Wu et al., 2022;
545 Rintala et al., 2010), was identified as a potential ArW indicator. The genus *Holosticha* is a
546 widespread benthic ciliate, associated with sea ice (Berger, 2003; Wilbert and Song, 2008; Petz



et al., 1995), and known to feed on diatoms and flagellates (Lei et al., 2005), which was also identified as a potential ArW indicator. However, their presence was confined to the Bølling-Allerød and Younger Dryas or the Younger Dryas and Early Holocene intervals, respectively, limiting their reliability as a long-term proxy for ArW conditions (**Fig. S11**).

In contrast, the ArW-associated diatom species *Chaetoceros gelidus* was consistently abundant, (**Fig. S12**), contributing to bloom formation and demonstrating adaptability to low light conditions (Hoppe et al., 2018; Biswas, 2022). Among the zooplankton, two taxa were identified as potential indicators: the microzooplankton radiolarian species *Heteracon* sp. and the mesozooplankton filter feeder appendicularian *Oikopleura vanhoeffeni* (Deibel, 1986, 1988) (**Fig. S12**). Likewise, the cold-water bivalve species *Tridonta* sp. which is commonly found in the North Atlantic and Arctic region (Marincovich et al., 2002; Petersen, 2001), demonstrated strong potential as an indicator species. Within parasitic dinoflagellates, two potential indicators belonging to dino-group-I, mostly associated with sea-ice conditions (Clarke et al., 2019), were identified. Choanoflagellate recorded in the study can be identified as a sea-ice associated group due to the presence of two potential ArW indicator taxa (Buck and Garrison, 1988; Thomsen and Ostergaard, 2017). These taxa were all consistently present throughout the study period, suggesting a stable association with cold marine conditions.

Chaetoceros gelidus, *Oikopleura vanhoeffeni*, *Calliacantha* sp. and *Tridonta* sp. demonstrated the greatest indicative potential, with consistent presence and alignment with multiple paleo proxies (**Table S4**). However, their Spearman correlation coefficients between environmental variables ranged from 0.3 to 0.5 ($p < 0.05$), indicating a weak to moderate association. This may be due to a combination of interspecific competition and the influence of multiple external environmental variables in the study area. These interacting factors contribute to the complexity of the ecosystem and limit the effectiveness of using single-proxy approaches when interpreting the responses of indicator species in paleoenvironmental reconstructions. Further studies of eukaryotic communities in other Arctic regions are therefore needed to validate these taxa as robust indicator species.

5.3. Interactions within eukaryotic community structure in Storfjordrenna

The biodiversity of eukaryotic communities in Storfjordrenna was previously influenced by the interplay between ArW and AW masses, as well as sea-ice coverage over the past 13.30 kyr BP. Throughout the study period, eukaryotic biodiversity remained relatively stable, with a notable exception during the transitions between major climatic intervals (**Fig. 3**). Peaks of



579 biodiversity coincided with the presence of sea-ice margins and frontal zones, environments
580 known to promote phytoplankton growth and primary productivity (**Fig. 4**).

581 Analysis of phytoplankton diversity revealed a consistent presence of green algae
582 throughout the study period, except during the Bølling Allerød interstadial, when diatoms
583 dominated. Taxonomic abundance showed dynamic fluctuations, with gradual declines
584 observed during the transitions between major climatic intervals. This suggests that
585 environmental instability may have influenced the structure of the phytoplankton community.

586 Key contributors to primary productivity in the Storfjordrenna included diatoms
587 (*Thalassiosira* spp., and *Chaetoceros* spp.), green algae (*M. polaris*), and autotrophic
588 dinoflagellates (*P. glacialis*, and *Gymnodinium* spp.). Additionally, Spearman rank correlation
589 analysis showed that the family Actinomonadaceae, mainly represented by *Pseudopedinella*
590 sp., was positively associated with diatoms. Spearman rank correlation analysis also revealed
591 a strong positive association between parasitic cercozoans and phytoplankton communities
592 (**Table S5**), indicating that the presence of parasitic cercozoans may play a significant role in
593 shaping ecological interactions within phytoplankton assemblages (Bass et al., 2009; Cavalier-
594 Smith and Chao, 2003; Hartikainen et al., 2014). Conversely, the parasitic apicomplexan family
595 Lecudinidae exhibited a robust association with zoobenthos (e.g., Heteroconchia and
596 Ophiurida) and mesozooplankton (e.g., Malacostraca), highlighting their parasitic relationships
597 with marine invertebrates (Rueckert et al., 2015). Parasitic dinoflagellates (dino-group-II)
598 maintained a positive association with haptophytes and diatoms. The parasitic nanoflagellate
599 Pirsoniaceae: *Pirsonia* sp., demonstrated a strong correlation with autotrophic microbes,
600 including, dinoflagellates, diatoms, and silicoflagellates. (Kuhn et al., 2004; Schweikert and
601 Schnepf, 1997). This raises questions about the nature of their ecological interactions, and
602 whether they are strictly parasitic or co-occur under similar environmental conditions. These
603 findings emphasize the need for further investigation to understand the mechanisms driving
604 these interactions and their broader implications for microbial community dynamics.

605 Cercozoans have emerged as the most dominant group within the eukaryotic community, in
606 terms of both abundance and species richness. Cercozoan community included taxa previously
607 recorded in various habitats including fresh and marine environments (Chantangsi and Leander,
608 2010; Irwin et al., 2019). Their occurrence across a wide range of environmental conditions
609 highlights their ecological flexibility and broad tolerance. Although cercozoans as a group
610 exhibit high richness, only a few lineages, such as the Imbricata-novel clade 2, *Protaspa* spp.,
611 *Cryothecomonas* spp., and *Ascetosporea*, persisted consistently throughout the study period,



612 while most of the others were restricted to specific time intervals (**Fig. S7**). This suggests that,
613 although cercozoans as a whole group may not showed sensitivity to environmental shifts,
614 individual lineages are likely to be more responsive.

615 MAST species also constituted a major microbial group within the eukaryotic community,
616 with MAST-9 dominant overall and MAST-12 particularly prevalent during the late Holocene.
617 These two MAST subgroups are commonly associated with temperate regions or extreme
618 environments such as cold seeps (Lin et al., 2022; Obiol et al., 2024). The statistical analysis
619 identified potential warm-water indicator species within these groups (**Fig. S10, Table S4**).
620 Based on the co-occurrence relation, the MAST-12, and MAST-9 subgroup revealed a strong
621 correlation (> 0.6) with the parasitic family of Pirsoniaceae and the phytoplankton families
622 (**Table S5**), providing insight into their ecological activities within the eukaryotic community.
623 Further analysis of the ecological traits and distribution of micro eukaryotic taxa, mainly
624 cercozoans and MAST, could provide deeper insights into their ecological responses within the
625 Storfjordrenna ecosystem.

626 **6. Conclusions**

627 Using sedaDNA metabarcoding, we reconstructed the paleoecology of eukaryotic
628 communities in Storfjordrenna over the last 13.30 kyr BP, elucidating their sensitivity and
629 adaptability to environmental variables. Overall, the eukaryotic biodiversity in Storfjordrenna
630 remained relatively stable, with a notable exception during the transitions between major
631 climatic intervals. Peaks of biodiversity coincided with the presence of sea-ice margins and
632 frontal zones, environments known to foster favorable conditions for phytoplankton
633 development. Cercozoans and MAST emerged as dominant groups, highlighting their
634 ecological flexibility and broad tolerance. This study revealed that primary productivity in the
635 Storfjordrenna region over the investigated time period was mainly driven by phytoplankton,
636 including diatoms (*Thalassiosira* spp., *Chaetoceros* spp.), green algae (*Micromonas* spp.), and
637 autotrophs dinoflagellates (*P. glacialis*.) as well as mixoplankton species such as
638 *Pseudopedinella elastica*. Several potential ASV-indicators were identified through multi-
639 method analyses, including taxa associated with specific water masses. Our findings also
640 underscore the complex interplay of environmental drivers shaping community composition,
641 revealing both positive and negative associations among key microbial taxa. Our findings
642 highlight the potential of sedaDNA for reconstructing past eukaryotic communities and
643 detecting environmental change. However, improving taxonomic resolution and validating



644 indicator taxa remain essential for establishing robust palaeoecological indicators in
645 Storfjordrenna and the broader Svalbard region.

646

647 **Data availability.** Raw reads of 18S-V1V2 rDNA sequencing generated in this study were
648 deposited in the National Center for Biotechnology Information (NCBI) under Bio Project
649 PRJNA1299363, and the remaining data and additional details used for this study can be found
650 in the Supplement tables (Table S1-S5), Supplementary figures (S1-S12), and Supplementary
651 document.

652 **Supplement.** The supplement related to this article is available online at XXX.

653 **Author contributions.** HN and Joanna P, designed the study. Joanna P extract the DNA.
654 HN analyzed the DNA data, and performed bioinformatic, statistical analyses, and interpret the
655 results. Joanna P, Jan P and N-LN helped with the bioinformatic analysis, and interpret the
656 results. ML and DD help to clarify the age depth model, and the paleoenvironmental data. HN
657 drafted the paper, and prepared the figures, and tables. All authors contributed to data
658 interpretation and writing of the manuscript.

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