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Sediment DNA metabarcoding and morphology provide complementary insight into macrofauna and meiobenthos response to environmental gradients in an Arctic glacial fjord

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ABSTRACT

Arctic fjords ecosystems are highly dynamic, with organisms exposed to various natural stressors along with productivity clines driven by advection of water masses from shelves. The benthic response to these environmental clines has been extensively studied using traditional, morphology-based approaches mostly focusing on macroinvertebrates. In this study we analyse the effects of glacially mediated disturbance on the biodiversity of benthic macrofauna and meiobenthos (meiofauna and Foraminifera) in a Svalbard fjord by comparing morphology and eDNA metabarcoding. Three genetic markers targeting metazoans (COI), meiofauna (18S V1V2) and Foraminifera (18S 37f) were analyzed. Univariate measures of alpha diversity and multivariate compositional dissimilarities were calculated and tested for similarities in response to environmental gradients using correlation analysis. Our study showed different taxonomic composition of morphological and molecular datasets for both macrofauna and meiobenthos. Some taxonomic groups while abundant in metabarcoding data were almost absent in morphology-based inventory and vice versa. In general, species richness and diversity measures in macrofauna morphological data were higher than in metabarcoding, and similar for the meiofauna. Both methodological approaches showed different patterns of response to the glacially mediated disturbance for the macrofauna and the meiobenthos. Macrofauna showed an evident distinction in taxonomic composition and a dramatic cline in alpha diversity indices between the outer and inner parts of fjord, while the meiobenthos showed a gradual change and more subtle responses to environmental changes along the fjord axis. The two methods can be seen as complementing rather than replacing each other. Morphological approach provides more accurate inventory of larger size species and more reliable quantitative data, while metabarcoding allows identification of inconspicuous taxa that are overlooked in morphology-based studies. As different taxa may show different sensitivities to environmental changes, both methods shall be used to monitor marine biodiversity in Arctic ecosystems and its response to dramatically changing environmental conditions.

1. Introduction

The Arctic ecosystems are increasingly suffering from stressors caused by climate warming and increased anthropogenic pressures (ACIA, 2005). Advection of warm Atlantic waters reshapes environmental conditions for pelagic organisms, resulting in northward range expansion of boreal species, rearrangements of food webs and changes in productivity (Wassmann et al., 2011). Increased primary production impacts both pelagic and benthic communities, due to strong pelagic-benthic coupling, and affects their functioning (Hop et al., 2006; Mazurkiewicz et al., 2021). Melting glaciers release vast amount of mineral materials that heavily impact coastal marine communities (Węsławski et al., 2011). All of these changes in Arctic ecosystems are predicted to intensify in the coming decades.

The species inventories are traditionally assessed via morphology-based analyses that are costly, time-consuming, and demanding highly specialized taxonomic expertise. Proper identification is also hampered by the incompleteness of the specimens, indistinctiveness of

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developmental stages, or the existence of cryptic species. Standard impact assessments are often limited to macrofaunal organisms that are widely used as indicators of anthropogenic impacts or to assess the effects of environmental changes e.g., (Borja et al., 2003; Cairns and Pratt, 1993; Pearson and Rosenberg, 1978). The smaller-sized meiofaunal biota may respond differently to disturbance but their ecology is much less known, therefore they are included less frequently in this type of surveys. The basic taxonomic assessments including species descriptions, diversity inventories, and patterns of variability in marine species-rich taxonomic groups of meiofauna (e.g., copepods, nematodes), are often fragmentary (Costello et al., 2006; Ridall and Ingels, 2021), making it difficult to infer the drivers of diversity in these groups using traditional, morphology-based taxonomy.

In Arctic fjords the interplay between the glacial meltwater inflows (usually located at the fjords head) and water advection from the open ocean creates steep environmental gradients operating along the fjords axis. Tidal glacier activity results in increased water turbidity, minerals sedimentation, sediment instability as well as hampered primary productivity and lower food availability to invertebrate consumers. All these effects are regarded as a source of natural chronic disturbance for macrofauna that respond in forming simple, impoverished, physically controlled communities (Włodarska-Kowalczuk et al., 2005) characterized by low taxonomic and functional trait diversity (Renaud et al., 2007; Włodarska-Kowalczuk et al., 2012, 2019), low standing stocks and productivity, fragmented size structures (Górska and Włodarska-Kowalczuk, 2017), and low temporal variability and spatial heterogeneity (Kędra et al., 2010; Węsławski et al., 2011; Włodarska-Kowalczuk and Węsławski, 2008). The patterns of declining macrobenthic diversity and standing stock along natural glacial disturbances are clear and consistent across various Arctic locations e.g., Svalbard (Renaud et al., 2007; Włodarska-Kowalczuk et al., 2005), Greenland (Sejr et al., 2010) or Canadian fjords (Dale et al., 1989) and resemble those documented for severe disturbances (Dolbeth et al., 2014; Pearson and Rosenberg, 1978).

The response of smaller sized benthic biota (meiofauna, Foraminifera) remains much less explored and understood. Most of the meiofauna studies are limited to low taxonomic resolution data (phylum/class level; e.g., (Bluhm et al., 2018; Górska and Włodarska-Kowalczuk, 2017; Grzelak and Kotwicki, 2012; Huang et al., 2021; Kotwicki et al., 2004; Pawłowska et al., 2011; Urban-Malinga et al., 2005). The reason for that is highly heterogeneous nature of meiofauna, with representatives of more than 20 phyla, detailed taxonomic analysis of which requires highly qualified specialists in each of these groups. Moreover, detailed taxonomic analysis of some taxa (e.g., Gastrotricha) cannot be carried out on fixed samples, which limits such analyses in most ecological studies (Higgins and Thiels, 1988). Regarding foraminiferal morphological studies they included mostly the hard-shelled organisms skipping the soft-walled ones, which does not preserve well in the dried sediment samples (Majewski et al., 2005; Sabbatini et al., 2007).

As macrofauna and meiobenthos are characterized by different life traits e.g. longevity, turnover rate, dispersal (Warwick and Clarke, 1984) they may respond differently to environmental settings, and several studies has already investigated this. For instance, (Patrício et al., 2012) showed that macrofauna and nematodes provided different, but complementary, response to changing biotic conditions (salinity, oxygen, sediment characteristics or nitrogen compounds) along Mondego estuary (Portugal). On the other hand, study of various size groups of benthic organisms along the environmental zonation in the Yenisei estuary (Kara Sea) showed similar patterns of distribution for macro- and meiofauna, which were not reflected by those observed for Foraminifera (Udalov et al., 2021a). In an Arctic fjord a comparison between nematodes and macrofauna in relation to glacier induced variability revealed similar patterns for both groups, although the authors acknowledge that their results might be affected by the inconsistencies in sampling methods employed across the visited stations (Somerfield et al., 2006).

All these studies were based on the morphological identification. Recent development of the environmental DNA (eDNA) metabarcoding provided an alternative to overcome the limitations of morphology-based approach, allowing a more holistic view of taxonomic diversity, encompassing a wider range of taxa, regardless of the size and developmental stage (Pawlowski et al., 2018; Thomsen and Willerslev 2015; Taberlet et al., 2012). The sediment DNA metabarcoding has been used to assess the diversity of benthic macrofauna and meiofauna (reviewed in Pawlowski et al. (2022)). However, only few of these studies focused on the Arctic marine ecosystems. These studies involved metabarcoding of sediments (Geraldi et al., 2024) benthic macrofauna (Willassen et al., 2022) and Foraminifera (Nguyen et al., 2022), as well as plankton communities (Ibarbalz et al., 2023; Lacoursière-Roussel et al., 2018; Sevellec et al., 2021). Up to our knowledge, none of these studies used metabarcoding to analyse macrofauna and meiobenthos together and compared with results of morphology-based approach.

In this study, we used morphology and metabarcoding to describe the composition and diversity patterns of macrofauna, meiofauna and Foraminifera from an Arctic glacial fjord. We focused on their patterns of response to strong natural disturbance gradients produced by glacial activity. We analyzed the results obtained using both methodological approaches and discussed their applicability for monitoring the ecological status and detecting the effects of environmental pressures effects in sensitive Arctic coastal systems.

2. Materials and methods

2.1. Sampling

Samples were collected in July 2019 from the board of r/v Oceania at six stations (Suppl. Table S1) located along the Hornsund fjord axis (Fig. 1), which is located on the southeast coast of Spitsbergen island, Svalbard archipelago. The stations were selected aiming to capture the natural gradient of environmental disturbance along the fjord (from fjord mouth towards glacier) i.e. decreasing impact of oceanic water masses (Cottier et al., 2005; Walczowski and Piechura, 2011), increasing mineral transport with freshwater coming from ablation (Weslawski et al., 1995) and increasing load and sedimentation of glacially transported minerals and sediment accumulation rate (Szczuciński et al., 2006).

At each station, three or four van Veen (0.1 m^2) grab samples for macrofauna and three box corer (0.02 m^2) samples for eDNA, meiofauna, and sediment characteristics were collected. Additionally, at each station sediment cores were collected using Nemisto gravity corer. Undisturbed sediment cores were sliced on board into 1 cm layers and samples were frozen until analysis. Macrofauna samples were sieved on board on a 500 μm mesh and preserved in 10% formalin solution with seawater. A set of replicate samples for meiofauna and sediment characteristics were collected from each box core. Upper 5 cm of sediment was collected using 10 cm^2 syringe and transferred to a zip bag (for grain-size analysis) or 50 ml plastic jars and preserved with 4% formaldehyde (for meiofauna analysis). Samples for the analysis of organic carbon content (C_{org}), total nitrogen (N_{tot}), $\delta^{13}C$, $\delta^{15}N$, and photosynthetic pigments were taken from the upper 2 cm and packed in sampling zip bags. For eDNA three surface (upper 2 cm) sediment samples were collected from each box-corer using a sterile wooden spatula and packed in separate sterile bags (Whirl-Pak Sample Bag), making a total of nine eDNA samples from each station. Samples for eDNA, grain size, and elementary analysis were frozen in −20 °C, for photosynthetic pigments in −80 °C.

2.2. Laboratory analysis

2.2.1. Environmental parameters

Photosynthetic pigments (chlorophyll *a* and pheopigments) contents in sediments were measured with the use of a fluorescent spectrometer (PerkinElmer LS55; (Evans et al., 1987; Evans and O'Reilly, 1982)).

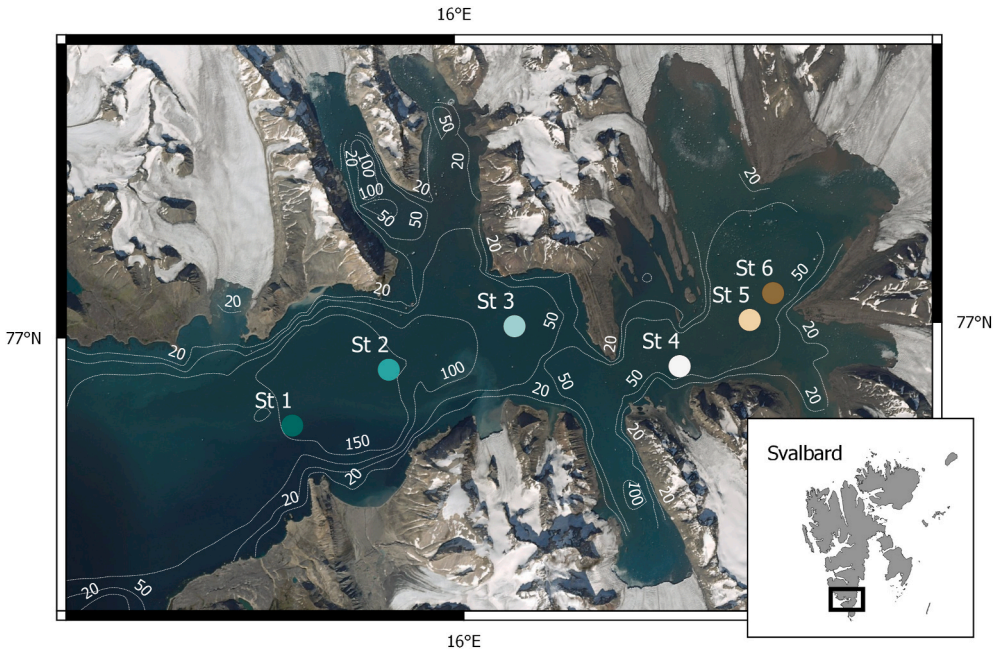

Fig. 1. Localization of sampling stations in Hornsund fjord. Stations indicated by circles; colors indicate the gradual change of environmental conditions at stations from the open ocean (dark green) towards glacier front (dark brown). Sources: satellite basemap - Esri, Maxar, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community; bathymetry – Norwegian Mapping Authority (Norwegian Mapping Authority, 2011).

Grain size composition was determined with a laser diffraction particle size analyser (Malvern Mastersizer 2000) and recalculated using GradiStat 4.0 software (Blott and Pye, 2001). Elemental analyses of sediments (C_{org} [%], $\delta^{13}C$ [‰], N_{tot} [%] and $\delta^{15}N$ [‰]) were performed with continuous flow - elemental analysis - isotope ratio mass spectrometry (CF-EA-IRMS), in a Vario Micro Cube elemental analyser.

In order to assess the sediment accumulation rates, the sediment samples were freeze-dried, and the water content was calculated. The sediment layers were dated by the ^{210}Pb method. For this purpose, the ^{210}Pb daughter radionuclide ^{210}Po was measured by alpha spectrophotometry (Zaborska et al., 2007). Chemical separation and detection efficiency was calculated for each sample using, as an internal standard, ^{209}Po. Standard reference materials (IAEA-300, IAEA-326 and IAEA-385) were measured as a control. One blank sample (not containing the sediment) was measured with every ten sediment samples. The sediment accumulation rates (mass accumulation rate - MAR and linear accumulation rate - LAR) were calculated for all individual cores using exponential profiles of excess ^{210}Pb. The supported ^{210}Pb (^{226}Ra) was obtained from gamma measurement. The CF:CS model was used to determine the maximum sediment accumulation rates and the ages of particular layers.

2.2.2. *Morphological analyses of macrofauna, meiofauna and Nematoda*

In the laboratory, macrofauna individuals were identified to the lowest possible level and enumerated. Meiofauna samples were washed in freshwater on a 32 μm sieve, organisms were extracted using centrifugation method with colloidal silica LUDOX TS50 solution (Vincx, 1996), counted and classified at higher taxonomical levels under stereomicroscope. Afterwards, a minimum of 200 individuals of the nematodes (or all nematodes if fewer were present in the sample) was randomly hand-picked from each sample and mounted in anhydrous glycerine on microscopic glass slides (Seinhorst, 1959). All nematodes on the slides were identified to the genus level under a light microscope.

2.2.3. *eDNA metabarcoding*

Environmental DNA was extracted from approximately 10 g of sediments using DNeasy PowerMax Soil Kit (Qiagen). Due to high water content, sediments were first mixed with 10 ml of PowerBead Solution in a sampling bag, then transferred to sterile falcon tubes, and centrifuged at 2500 rpm for 3 min. Next, the supernatant was carefully discarded, Powermax Beads were added to 15 ml of PowerBead Solution and treated accordingly to the manufacturer instructions. One blank sample was processed during each extraction session to control for possible contamination (11 blanks in total). Extracted DNA was precipitated by adding 200 μl 5M NaCl and 10 ml of 95% cold ethanol and leaving overnight in −20 °C. Next, samples were centrifuged at 2500 rpm for 30min, and the supernatant was discarded. The pellet was diluted in 400 μl of EDTA. The samples were kept frozen at −20 °C until PCR amplification.

Three genetic markers were used for PCR amplification: the mitochondrial cytochrome oxidase 1 gene (COI) and two hypervariable regions of the 18S rRNA gene (V1V2 and 37f). COI is regarded as a standard metazoan barcode (Folmer et al., 1994), while the ribosomal V1V2 is widely used as a universal marker for marine meiofauna (Fonseca et al., 2010). The 18S 37f region specific to Foraminifera was used to obtain foraminiferal metabarcodes (Pawlowski and Lecroq, 2010). Primer sequences and details of PCR procedures are listed in Suppl. Table S2. To allow the multiplexing of PCR products in one library, the primers were tagged with 8 unique nucleotides attached at each 5' extremity (Esling et al., 2015).

Each extracted DNA was amplified in three replicates with one negative PCR control. PCR products stained with SYBR safe were visualized by 1.5% agarose gel electrophoresis and then pooled together for further processing and quantification using high-resolution capillary electrophoresis (QIAxcel System, Qiagen). The amplicons were mixed in two equimolar pools and purified using the High Pure PCR Product Purification kit (Roche). We used the TruSeq® DNA PCR-Free Library Preparation Kit (Illumina) to prepare the libraries and the Kapa Library Quantification Kit for Illumina Platforms (Kapa Biosystems) to quantify them. The libraries were sequenced on a MiSeq instrument using 600 cycles paired-end sequencing (kit v3) for V1V2 and COI libraries and 300 cycles for foraminifera library (kit v2). A total of 53 samples (sediment replicates) were sequenced for V1V2 marker (including one blank extraction sample and two samples with the same primer tags), 48 samples for COI marker, and 53 samples for 37f marker.

2.3. Bioinformatics

Bioinformatic analyses of sequenced data were performed using SLIM pipeline (Dufresne et al., 2019). First, raw sequences were demultiplexed and assembled into full-length sequences. Next, sequences were quality filtered (Suppl. Table S3) and clustered into ASVs using DADA2 (Callahan et al., 2016). Obtained ASVs were taxonomically assigned with a vsearch with a 90% min. similarity threshold and 99% direct acceptance threshold using PR2 4.14 database (del Campo et al., 2018; Guillou et al., 2013; Vaulot et al., 2022) for V1V2 marker, 80% similarity threshold for MIDORI2 GB 243 database (Leray et al., 2022) for COI marker, and local database for Foraminifera. The unassigned ASVs for V1V2 and COI marker were additionally assigned using blast (Camacho et al., 2009) over NCBI database (in October 2022) with 99% similarity threshold for species level, 97% threshold for genus level, and 95% for family level. In the case of foraminiferal marker, we manually removed the reads not having the beginning and the end of the 37f region. To assign the sequences, we used Blast against our curated database at 95% of similarity.

For the final analysis, samples with sequencing depth >1000 reads were selected. The V1V2 samples (two samples) with the same primer tags were excluded from further analysis. Additionally, we filtered out ASVs with <10 reads across all samples and ASVs present in only one sample. One ASV was obtained in the sequenced V1V2 extraction blank sample, and it was filtered out from all the samples.

2.4. Data analysis

The statistical data analysis was performed in R 4.1.0 (R Core Team, 2022). The sum of photosynthetic pigments in the sediments – chloroplastic pigment equivalent (CPE) was calculated as a sum of chl a and peophythin concentrations. The environmental variables were inspected for collinearity using a Spearman rank correlation. In pairs with significant correlation ($p < 0.05$, rho>0.7), one of the variables was removed from the dataset used for further analysis. Principal component analysis (PCA) was applied to the final dataset of environmental variables to identify similarities in environmental conditions among stations. PCA analysis was performed using *PCA()* function from *FactoMineR* package (Le et al., 2008), with a parameter *scale.=TRUE* to obtain unit variance among analyzed variables.

Sample species richness (S) was calculated as a number of unique taxa or ASV in a sample. Shannon-Wienner index (H) was calculated using *diversity()* function form *vegan()* package (Oksanen et al., 2020). The Pearson correlation with Bonferroni correction for multiple comparisons was calculated for pairs of S calculated for morphologically identified groups (macrofauna and Nematoda) and eDNA markers, and the same was performed for H. For correlation analyses averages per stations were used for comparison with macrofauna morphological data, while for comparisons with Nematoda data averages per box corer were used, while the comparisons among metabarcoding data were calculated based on each sample data.

The taxonomic composition obtained with morphological and metabarcoding approaches was compared across all the stations on the family level for Annelida and Nematoda, two phyla dominating macrofauna and meiofauna, respectively (based on morphological analyses). Permutational analysis of variance with 9999 permutations (Permanova; *adonis2()* function from *vegan* package) was calculated based on Bray-Curtis dissimilarities calculated for proportional data to test for differences among morphological and metabarcoding data. The post hoc pairwise comparisons among each pair of data (morphological, 18S V1V2 and COI) were calculated using *pairwise.adonis2()* function from *pairwiseAdonis* package (Martinez Arbizu, 2017) based on 9999 permutations. The non-metric multidimensional scaling (nMDS) was performed with the use of *metaMDS()* function from *vegan* package for benthic abundance and presence/absence data. Morphologically identified macrofauna and Nematoda abundance data were 4th root transformed and Bray-Curtis dissimilarities were calculated. For metabarcoding abundance data, the number of reads were normalized using cumulative-sum scaling method from metagenomeSeq package (Paulson et al., 2013) and then Bray-Curtis dissimilarities were calculated. For the presence/absence data Jaccard index was calculated. To assess similarities between datasets, a Mantel test (*mantel()* function from *vegan* package) based on Spearman rank correlation with Bonferroni correction for multiple comparisons and 9999 permutations was calculated between each pair of morphologically and molecularly obtained data, separately for abundance and presence/absence data. In this case, the data were also averaged per station for the macrofauna comparisons and per box corer for the comparisons with morphologically identified Nematoda, while the comparisons among metabarcoding data were calculated based on each sample data.

The relationships between the univariate measures of alpha diversity and the environmental variables were tested using multiple linear regression models. The model selection was performed through stepwise backward procedure based on Akaike Information Criterion (AIC; (Akaike, 1974)), using *ols_step_backward_aic()* function from *olsrr* package (Hebbali, 2024). Regarding beta diversity, the relationships between community composition data and environmental parameters were assessed using BIOENV analysis conducted using *bioenv()* function from *vegan* package. The analysis was based on the assessment of the best correlated set Euclidean distances calculated for environmental parameters and community dissimilarity matrix (Bray-Curtis dissimilarity for abundance data, and Jaccard similarity for presence/absence data). The vectors of variables from the best correlated set of environmental parameters were calculated using *envfit()* function from *vegan* package. Since macrofauna morphological samples could not be paired with environmental samples, we used station average values of environmental variables. Prior to the analyses, highly collinear (based on Spearman rank correlations $p < 0.05$, rho>0.7 or < -0.7) variables were excluded.

3. Results

3.1. Environmental settings

Sediment sorting was strongly correlated to mud content (rho = −0.98, Suppl. Table S4), LAR (linear accumulation rate) to CPE and Chl *a* (rho = −0.82 and rho = −0.81 respectively), Chl *a* to CPE (rho = 0.85) and C_{org} to C_{org}/N_{tot} (rho = 0.73). Therefore, sediment sorting, LAR, Chl *a* and C_{org}/N_{tot} were not considered in further analyses.

The first principal component of PCA explained 33% of the total variance among environmental data and was strongly correlated (coefficients in parenthesis) with CPE (0.87), $\delta^{15}N$ (0.74), and N_{tot} (0.67). The second principal component explained 25% of the variance and was mostly correlated with the mud content (−0.81), N (0.60), and water content (0.59). Based on the PCA biplot (Fig. 2A), station St5 strongly differed from others in being characterized by low values of mud content and CPE (and low Chl *a* and high LAR, both excluded from analyses due to high correlation to CPE, Suppl Fig. 1) and high C_{org} content in sediments (Fig. 2B). Stations St2, St3 and St6 were similar and characterized by moderate values of all the variables. In contrast, station St1 was distinct from others mostly due to high CPE, N_{tot} and $\delta^{15}N$. Station St4 characterised very high variability among samples along the second principal component (mostly due to the water content, the mud content, N_{tot} and $\delta^{15}N$, and $\delta^{13}C$ and sediment sorting; Fig. S1.).

3.2. Morphological data

3.2.1. Macrofauna

127 taxa, representing eight phyla were morphologically identified in macrofauna samples, mostly to the species level (Suppl. Table S5). The largest group were annelids (73 taxa), followed by mollusks (27 taxa) and arthropods (18 taxa). In terms of abundance, the annelids

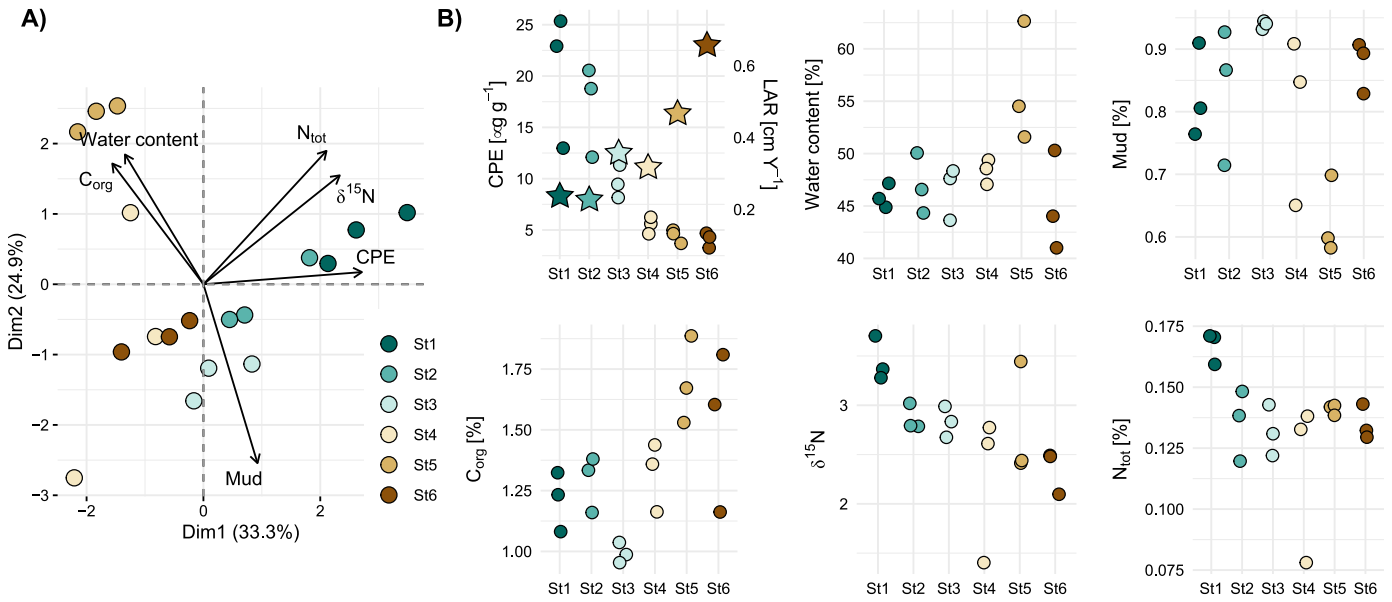

Fig. 2. A) A biplot of environmental variables distribution in Principal Component Analysis. Arrows represent loading vectors of the six variables with the highest contribution to the first and second components. B) distribution of data for individual samples at six stations. Six variables with the highest contribution to the first and second PCA components are presented as dots and a LAR (a variable highly negatively correlated with CPE) as asterisks.

accounted for an average of 89.5% of individuals in a sample, followed by mollusks (7.0%) and arthropods (2.1%). Other groups (Nemertea, Cnidaria, Sipuncula, Priapulida) constituted 1.4% of all individuals (Fig. 4). The taxonomic composition was consistent across stations, with the proportion of annelids ranging from 81 to 95% on average. Molluscs were observed in significant proportions at stations St3 – St6 (5–13%), while arthropods had a significant proportion at stations St4 and St5 (3–5%) only. The number of macrofauna specimens varied between 411 ± 180 and 464 ± 532 ind. 0.1 m^{-2} (mean ± SD) at three outer stations St1 - St3, and 157 ± 20 and 169 ± 25 ind. 0.1 m^{-2} at three inner stations (Fig. 3).

3.2.2. Meiofauna

Eight phyla were recorded morphologically in meiofauna samples (Suppl. Table S5). The assemblage was dominated by Nematoda (on average 91% of all organisms in a sample), followed by Arthropoda (4%) and Annelida (3%). Other identified phyla included Nemertea, Kinorhyncha, Mollusca, Cnidaria, and Tardigrada. The dominance of Nematoda was very consistent among stations (89–93%). The proportion of Arthropoda varied on average between 2 and 6% of meiofaunal individuals at each station. At station St6 we observed also a significant proportion of Annelida (7%). The number of meiofauna specimens identified morphologically varied from 3400 ± 300 and 3676 ± 904 ind. 10 cm^{-2} (mean ± SD) in outer stations St1 - St3 to 992 ± 242 and 1126 ± 820 ind. 10 cm^{-2} in inner stations St4 and St5 and dropped to ca. 103 ± 49 ind. 10 cm^{-2} in St6.

3.3. Molecular data

3.3.1. COI

The COI dataset comprised 2868 ASVs (Suppl. Table S6) represented by 2.48M reads across 44 samples. On average, 49% of reads in each sample were assigned to Opisthokonta (Fig. 4). Among them, 12 phyla of Metazoa were recorded. Most abundant were Annelida (75% of reads on average in a sample), followed by Nemertea (10%), Arthropoda (10%) and Cnidaria (2%). The remaining phyla (3%) included Chordata, Echinodermata, Kinorhyncha, Mollusca, Nematoda, Porifera, Priapulida and Rotifera. For further analyses of alpha and beta diversity the COI data were taxonomically filtered to include only benthic metazoan taxa: Annelida; Arthropoda: Harpacticoida, Cumacea and Balanomorpha; Bryozoa; Cnidaria: Anthoathecata; Echinodermata; Gastrotricha; Kinorhyncha; Mollusca: Nuculanida and Littorinimorpha; Nematoda; Nemertea; Placozoa; Priapulida; Rotifera and Xenacoelomorpha. Due to low number of taxa, we did not separate macrofauna and meiofauna, as it would result in zero values at some samples.

The assemblage of benthic Metazoa in COI data was dominated by

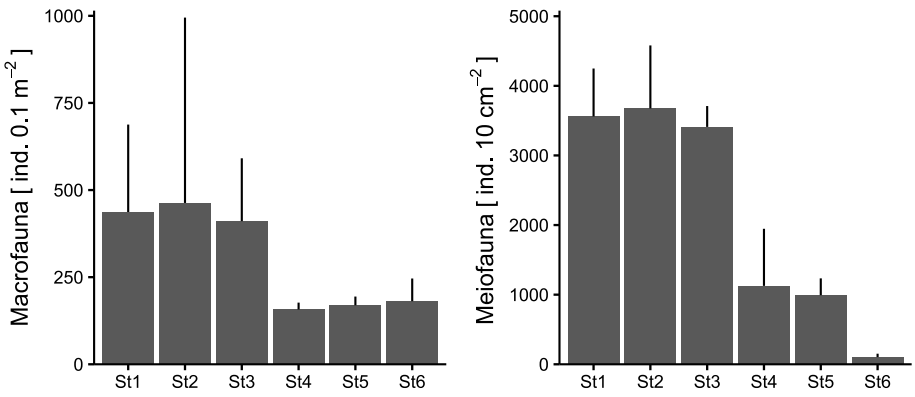

Fig. 3. Abundance of macrofauna and meiofauna assessed morphologically at sampling stations. Bars represent mean values, whiskers – mean + SD.

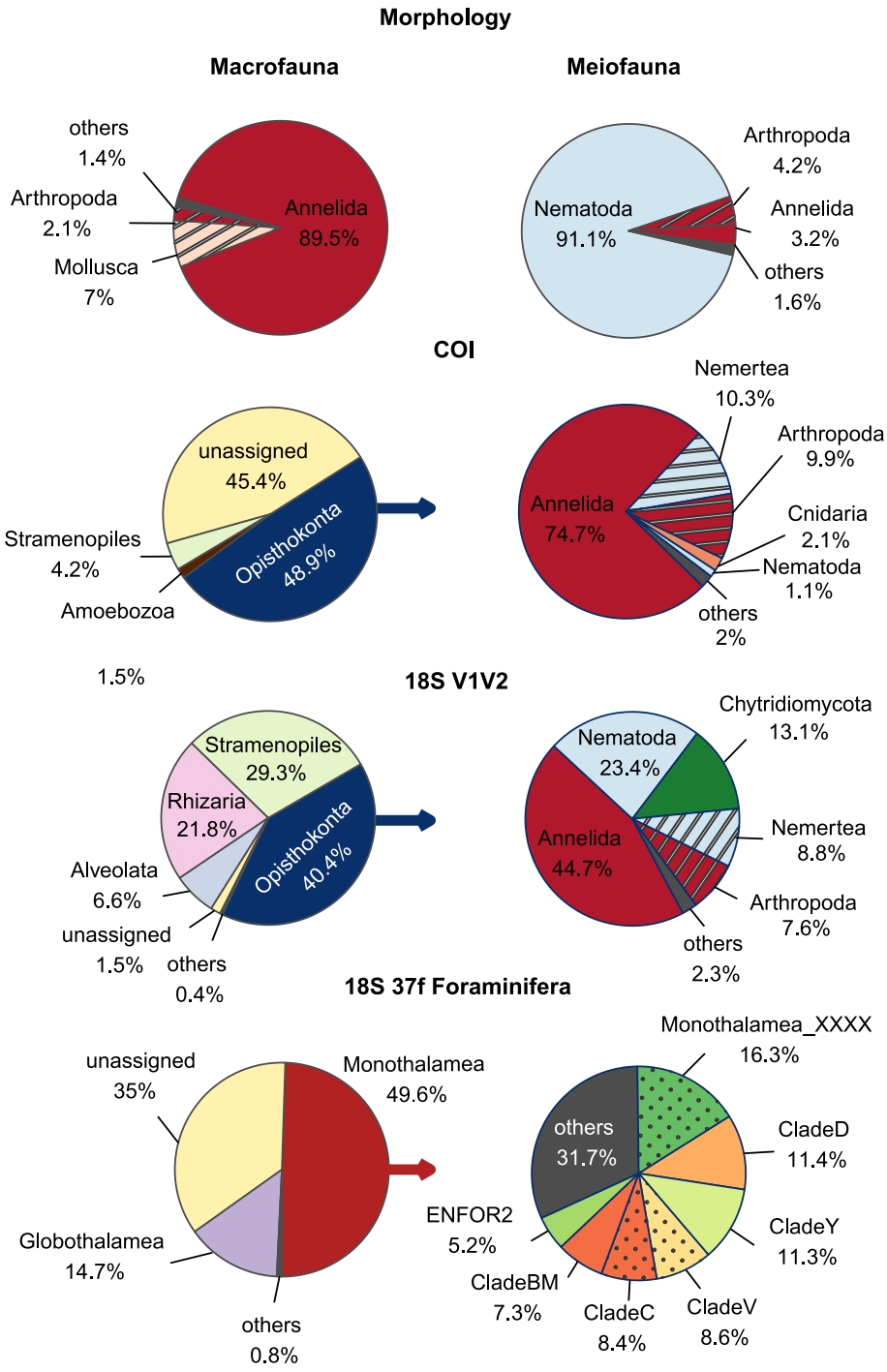

Fig. 4. Taxonomic composition in datasets obtained with morphological (meiofauna, macrofauna) and eDNA metabarcoding analyses (using COI, 18S V1V2, 18S 37f markers) based on relative abundance of individuals (macrofauna and meiofauna) or relative abundance of reads (COI, 18S V1V2 and 18S 37f data). Mean values from all the samples are presented. Others – taxa with average proportion <5 %.

Annelida across all stations (from 81 to 98%; Fig. 5). There was also a significant proportion of Nemertea (17%) in stations St3 and St5, and some Gastrotricha, Cnidaria and Placozoa and Rotifera in certain stations. Interestingly, a noticeable increase in the number of unassigned sequences was observed along the fjord axis towards the glacier from 40% at St1 to 78% at St6 (Suppl Fig. S2).

3.3.2. 18S V1V2

Metabarcoding data for 18S V1V2 comprised 942 ASVs (Suppl. Table S6) represented by 3.59M reads across 50 samples. On average 40% of the reads in each of the samples were assigned to Opisthokonta, 29% to Stramenopiles, 22% to Rhizaria, and 7% to Alveolata (Fig. 4). The Opisthokonta included 22 metazoan phyla (87% of reads) and Chytridiomycota (13%). The Metazoa were dominated by Annelida (45% of reads), followed by Nematoda (23%), Nemertea (9%), and Arthropoda (8%). The remaining metazoan phyla included Chaetognatha, Chordata, Cnidaria, Ctenophora, Gastrotricha, Hemichordata, Mollusca, Platyhelminthes, Porifera, Priapulida, Sipuncula and Xenacoelomorpha.

For the purpose of further analysis of taxonomic composition and diversity, the 18S V1V2 dataset was split into meiofauna and macrofauna. The meiofauna comprised Nematoda, Arthropoda: Harpacticoida

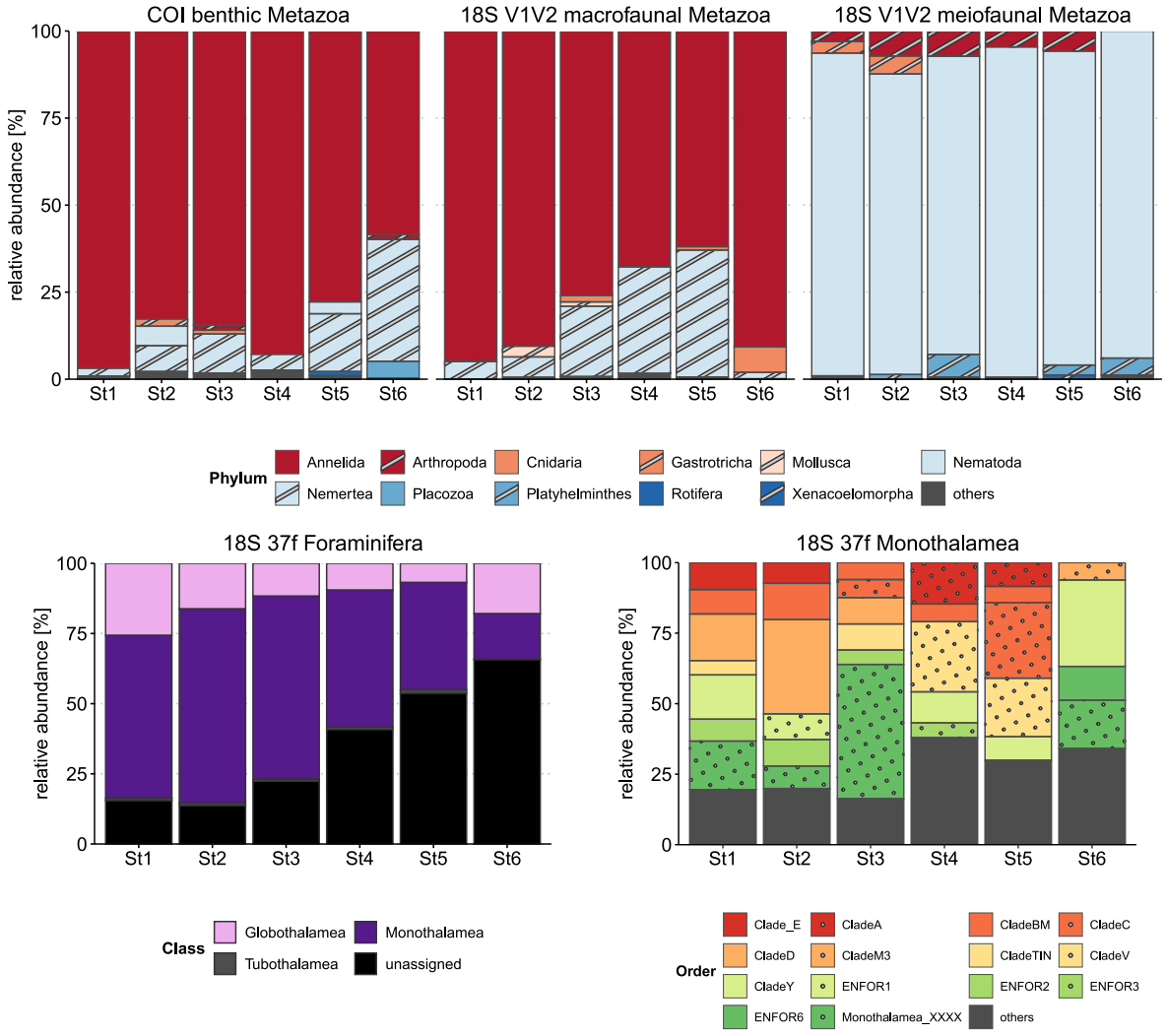

Fig. 5. Taxonomic composition of Metazoa based on COI and V1V2 markers (upper panel) and Foraminifera classes and Foraminifera Monothalamea orders at each station based on 37f marker. Others – taxa with average proportion at station <1% in the case of Metazoa and <5% in the case of Foraminifera.

and Ostracoda, Platyhelminthes, Gastrotricha and Xenacoelomorpha.

In the 18 V1V2 meiofaunal dataset Nematoda dominated in all stations (between 51 and 85% of reads at station), while Arthropoda were common (3–7%) at all stations except St6, Gastrotricha only at St1 and St2 (3 and 5%), and Platyhelminthes at stations St2, St3, St5 and St6 (1–7%).

The macrofaunal 18S V1V2 dataset consisted of Annelida, Cnidara: Anthoathecata, Hemichordata: Enteropneusta, Mollusca, Nemertea, Priapulida and Sipuncula. Annelida dominated in all stations (62–95% of reads). The proportion of Nemertea ranged from 2 to up to 36% in station St5. Cnidaria were observed in high proportion (7%) only as St6, while Mollusca were observed mainly at St2 and St3 (3% and 1%, respectively).

3.3.3. 18S 37F Foraminifera

Foraminiferal metabarcoding data comprised 1654 ASV (Suppl. Table S6) represented by 5.14M reads across 51 samples. The majority of reads were assigned to the class Monothalamea (on average 50% of reads in each sample) and Globothalamea (15%), while 35% remained unassigned (Fig. 4). The proportion of unassigned reads tended to increase along the fjord from 13.7 at St1 and 15.4% at St2 to 65.7% at St6 (Suppl. Fig. S2).

Among Monothalamea, the proportion of different clades varied between samples (Fig. 5). In total, the most abundant were Clade D and Clade Y (11% each; Pawlowski et al. (2002)). Clade D dominated in St2 (33%), while Clade Y dominated in station 6 (30%). The most abundant were also the Clade V that dominated in station 4 (25%), and the Clade C dominating in station 5 (27%). Among other clades, the most common were Clade E (10% in St1 and 7% in St2), Clade A (15% in St 4 and 8% in St 5), and ENFOR6 (12% in St.6). Clade BM was present at all stations except St6 and showed a tendency to decrease between the most outer stations (St1 - St2, 9–13% respectively) and the rest of stations (4–6%). At the genus level, Clade E was dominated by *Psammophaga*, Clade C by *Marsipella*, Clade TIN by *Tinogullmia* and Clade BM by *Micrometula*. High proportion of reads (16%) remained unassigned or assigned exclusively to class level (Monothalamea X).

The class Globothalamea was dominated by the order Rotaliida (79% of reads), followed by order Textulariida (20%), and order Robertinida (<1%; Suppl. Fig. S3). The proportions of all orders was consistent across all stations (Suppl. Fig. 3).

3.4. Comparison of morphological and metabarcoding data

3.4.1. Macrofauna/Annelida

The taxonomic composition of Annelida in morphological and metabarcoding data differed significantly (Permanova $F(2, 114) = 13.34$, $p < 0.001$). The post hoc comparison showed that differences were significant among all three groups (metabarcoding data, COI and 18S V1V2

data, pairwise Permanova $p < 0.05$). More Annelida taxa were recorded morphologically, than in COI and 18S V1V2 data (Suppl. Fig. S4). Morphologically analyzed annelids were composed mainly of Lumbrineridae (13–34%, Fig. 6), noticeably declining at stations close to the glacier, and Cirratulidae, numerous (24–55%) at all stations except St1 where they constituted only 5%. In metabarcoding data, the Lumbrineridae were also abundant (19–60% for COI marker and 23–64% for V1V2 marker) at all stations except St6. Cirratulidae were also numerous in V1V2 data (10–83%), but only at St4 – St6. The family Terebellidae was well represented in the metabarcoding datasets (29–100% for COI marker at all stations and 28–42% for V1V2 marker for stations St3 – St5), while in the morphological samples, Terebellidae were present at all stations but only in a very small proportion (max 5%). On the other hand, Sabellidae made 2–8% of all annelids in morphological samples at all stations except St4, while in metabarcoding data they were observed only in 18S V1V2 dataset at St6 (3%). Sigalionidae were found in significant proportions in metabarcoding data at stations St2 – St4 (COI: 3–12%; 18S V1V2 11–25%), while they constituted only 1% of morphological assemblage at St3 and St4.

3.4.2. *Meiofauna/Nematoda*

Similar to Annelida, the taxonomic composition of Nematoda in the morphological and metabarcoding datasets also differed significantly (Permanova $F(2, 97) = 51.92$, $p < 0.001$). The post hoc comparison showed that differences were significant among all three groups (metabarcoding data, COI and 18S V1V2 data, pairwise Permanova $p < 0.05$). The number of Nematoda taxa was higher in morphological than in metabarcoding datasets (Suppl. Fig. S5). COI marker revealed the presence of only four nematode families: Chromadoridae, Linhomoeidae, Monhysteridae and Xyalidae, which were also present in morphological dataset at only two stations: St2 (all of them) and St5 (only Chromadoridae and Xyalidae).

Five nematodes families dominated in morphological dataset: Oxystominidae (10–46% at all stations), Chromadoridae (5–11% at all stations except St6), Comesomatidae (7–48% at all stations), Linhomoeidae at stations St1 – St3 (11–23%), and Xyalidae (5–14%). Except Linhomoeidae, the same families dominated in 18S V1V2 dataset but in different proportions. Oxystominidae accounted for 31–70% (Fig. 6), Chromadoridae for 12–40%, Comesomatidae for 13 and 29% at stations St1 and St2 and 6% at St6, while Xyalidae occurred only at stations St1 – St3 (1–3%). Other families numerous in V1V2 data included Desmoscolecidae at St3 (8%) and Microlamidae at stations St5 and St6 (5–11%). Four families: Aphanolaimidae, Axonolaimidae, Teratocephalidae, Tripyloididae, present in V1V2 dataset were not recorded in the morphological analysis.

3.5. *Patterns of taxonomic richness and alpha diversity*

We noticed differences in taxonomic richness (S) and Shannon-Wiener index (H) among stations, with a tendency to decrease along fjord axis towards the glacier. These patterns were not consistent across studied groups/markers (Fig. 7). Taxonomic richness of morphologically identified macrofauna was relatively high (up to 56 taxa) and variable at three outer stations St1 – St3, while in the inner basin it drastically dropped (to about 20 taxa) and remained stable across stations St4 – St6. Such pattern was unique to macrofauna in morphological data. In nematodes identified morphologically and all molecular datasets, the richness in inner basin was more variable. It usually was lower in the inner basin, except for the nematode morphological data (St5) and 18S V1V2 macrofauna dataset. The strongest difference in diversity between

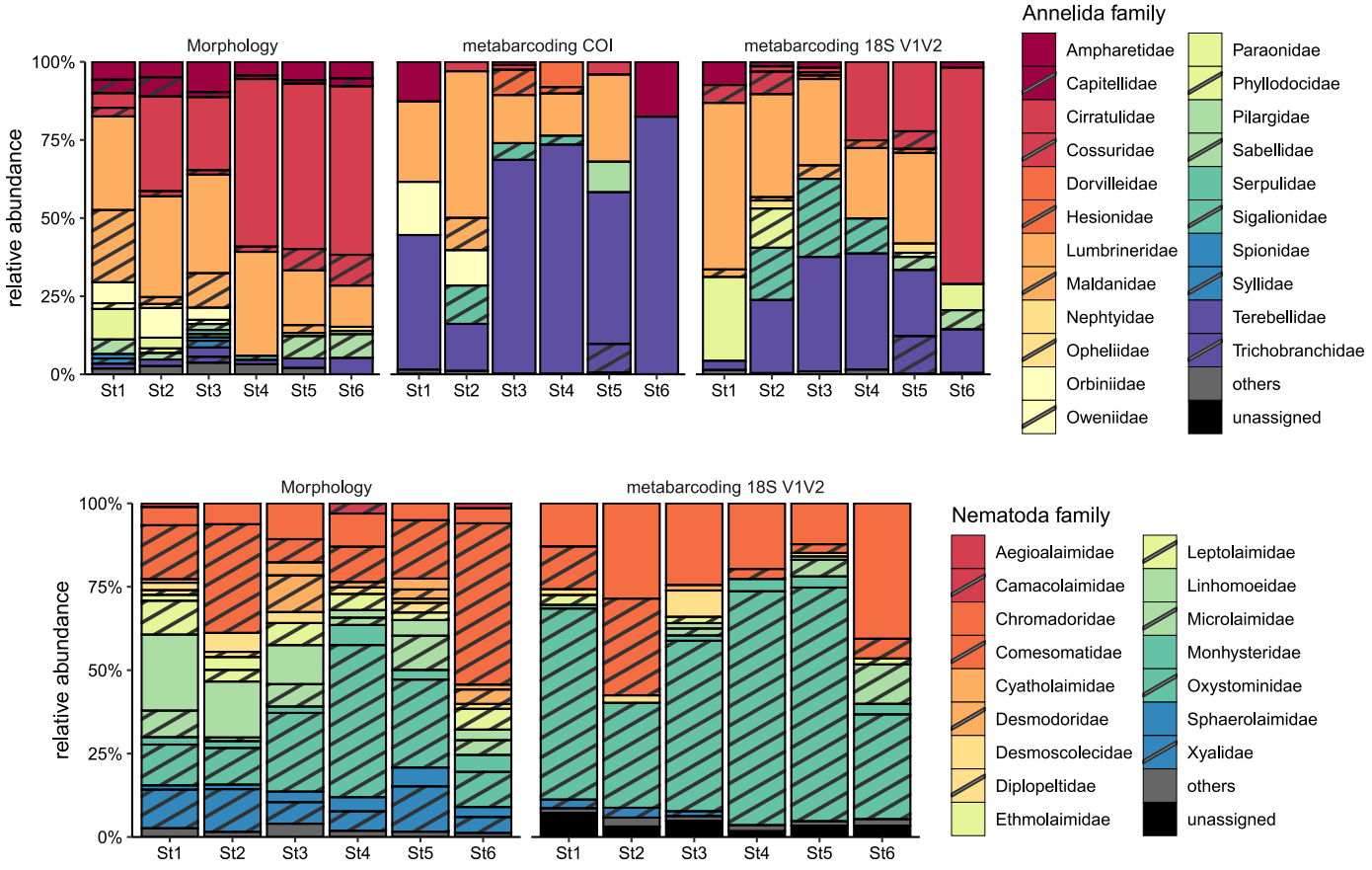

Fig. 6. Relative abundance of individuals/ASVs of Polychaeta (upper panel) and Nematoda (lower panel) families in data obtained with morphological and eDNA analysis (Polychaeta - COI and V1V2 markers, Nematoda - V1V2 marker). Mean values are presented. Others – all the families with mean relative abundance at station <5%.

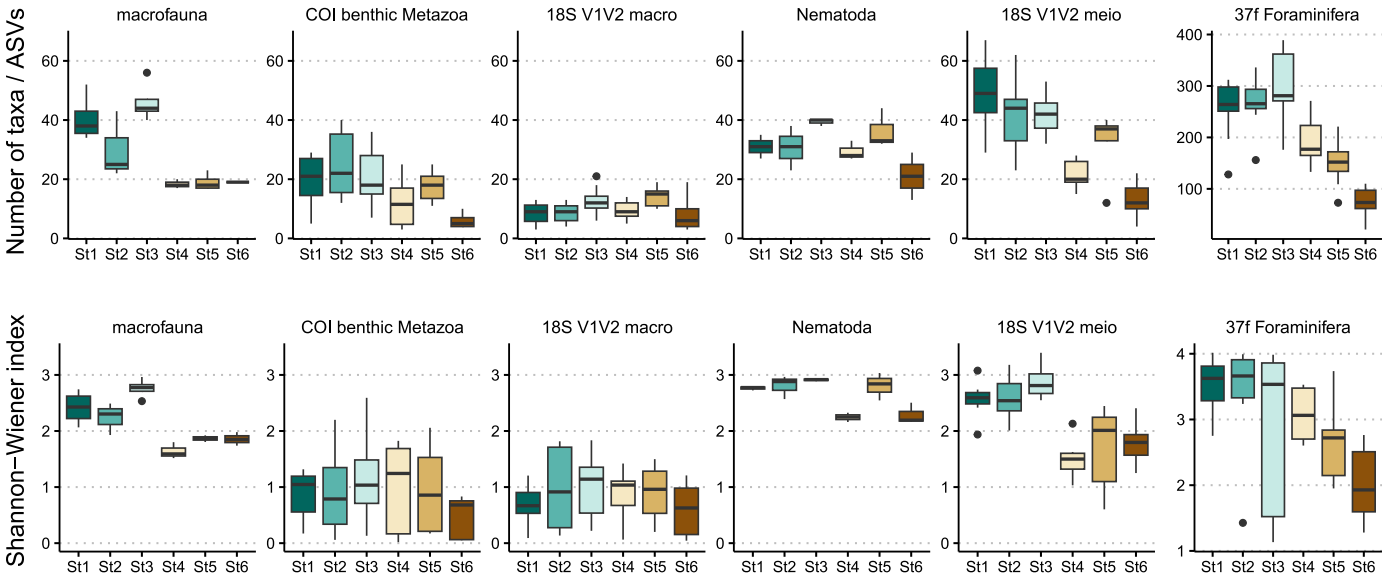

Fig. 7. Taxonomic richness (number of taxa/ASV per sample, upper panel) and diversity (Shannon-Wiener index, lower panel) at stations for morphologically identified macrofauna and meiofaunal Nematoda and metabarcoding data (using COI, 18S V1V2 and 18S 37f markers). Macrofauna – taxa identified to the lowest possible level, mostly species, Nematoda – identified to genus level, 18S V1V2 meio - ASVs obtained with 18S V1V2 marker regarded as meiofaunal Metazoa, 18S V1V2 macro - ASVs obtained with 18S V1V2 marker regarded as macrobenthic Metazoa, COI for ASVs regarded as benthic Metazoa, 18S 37f Foraminifera - all ASVs included.

the basins (clearly lower values in inner basin) was recorded for Foraminifera and meiofauna (except for St5). Interestingly, for all datasets except morphological analysis of macrofauna, the lowest richness was reported for the St6, located most closely to the glacier.

Diversity measured by Shannon-Wiener (H) index showed sometimes different patterns from taxonomic richness. For morphological macrofauna data the diversity decreased between the outer and inner basin, but the lowest value was found at station St4 and it slightly increased at stations St5 and St6, located closed to the glacier. A similar pattern was observed for the 18S V1V2 meiofauna. For Nematoda morphological data and Foraminifera, the patterns were very similar for taxonomic richness and H index. For 18SV1V2 macrofauna and COI benthic Metazoa we observed a high variability of H index at each station, with no clear pattern along the fjord.

No strong (rho>0.7 or rho < -0.7) and significant correlations were found in the alpha diversity indices among studied groups/markers (Suppl. Table S7). However, there was a moderate correlation in taxonomic richness (S) in two cases: between 18S V1V2 meiofauna and Foraminifera (Pearson rho = 0.49, Bonfferoni corrected $p < 0.01$) and between COI benthic Metazoa and Foraminifera (Pearson rho = 0.46, Bonfferoni corrected $p < 0.05$). For H we found significant correlation only in two cases (different than in S): between 18S V1V2 macrofauna and COI benthic Metazoa (Pearson rho = 0.62, Bonferroni corrected $p < 0.001$) as well as between morphologically assessed Macrofauna and 18S V1V2 meiofauna (Pearson rho = 0.97, Bonferroni corrected $p < 0.05$).

The multiple regression models for alpha diversity indices and environmental variables (Suppl. Table S8) revealed impact of CPE (a variable strongly, negatively correlated with LAR) and $\delta^{15}N$ on most of the indices. Other variables were also significant but it was variable among studied groups and indices. Furthermore, relatively strong relationship, as indicated by the high variance explained (adj. $R^2 > 0.7$), between alpha indices for morphological data and environmental parameters was detected. Moderate linkage (adj. R2 between 0.49 and 0.67) was observed for 37f Foraminifera species richness and both indices for 18S meiofaunal Metazoa, while the weakest relationship between alpha diversity indices and environmental variables was detected by the models for COI benthic Metazoa and 18S V1V2 macrofauna (adj. $R^2 < 0.4$).

3.6. Beta diversity

Patterns of community composition documented for different groups/markers exhibited a systematic change along the fjord axis (Fig. 8). For the macrofauna identified morphologically a distinct clustering of two groups of stations was observed, both based on transformed (4th root) abundance data and presence/absence data: i) a dense cluster of 3 stations localized closer to the glacier (St4 - St6) and ii) a more spread clustering of stations localised in the outer part of fjord (St1 - St3), with sub clusters for each station. This indicates a clear difference between glacial bay/inner basin and outer basin but also differences in the taxonomic composition among outer basin stations. The Mantel test showed that there was no significant relationship between macrofauna taxonomic composition and other groups (Bonferroni corrected $p < 0.05$, Suppl. Table 9).

Nematoda identified morphologically do not show such distinct clustering as macrofauna, but two groups of samples representing two basins were still noticeable with a clear separation of each station within the groups. The samples collected in station close to the glacier (St 6) were very different from others in abundance data but not in presence/absence data. The Mantel test showed that the Nematoda taxonomic composition was significantly correlated (Bonferroni corrected $p < 0.05$) with taxonomic compositions obtained with metabarcoding data, however a strong correlation (rho > 0.7) was observed only for abundance data concerning Foraminifera (Suppl. Table S9).

Regarding the metabarcoding data, we observed a gradual change in the taxonomic composition of the communities from St1 to St6 based on the abundance data obtained for meiofaunal and macrofauna with 18S V1V2 marker and for 18S 37f Foraminifera. The gradual change could also be noted for the presence/absence data for 18S V1V2 macrofauna. On the other hand, the presence/absence data for 18S V1V2 meiofaunal taxa and 18S 37f Foraminifera suggest a separation of stations into three groups: 1) a dense cluster of samples from stations located in the outer part of fjord (St1 and St2); 2) transition station St3, and 3) a wide cluster represented by samples from inner stations close to the glacier (St4 - St6). Moreover, these two groups (meiofauna and foraminifera) were

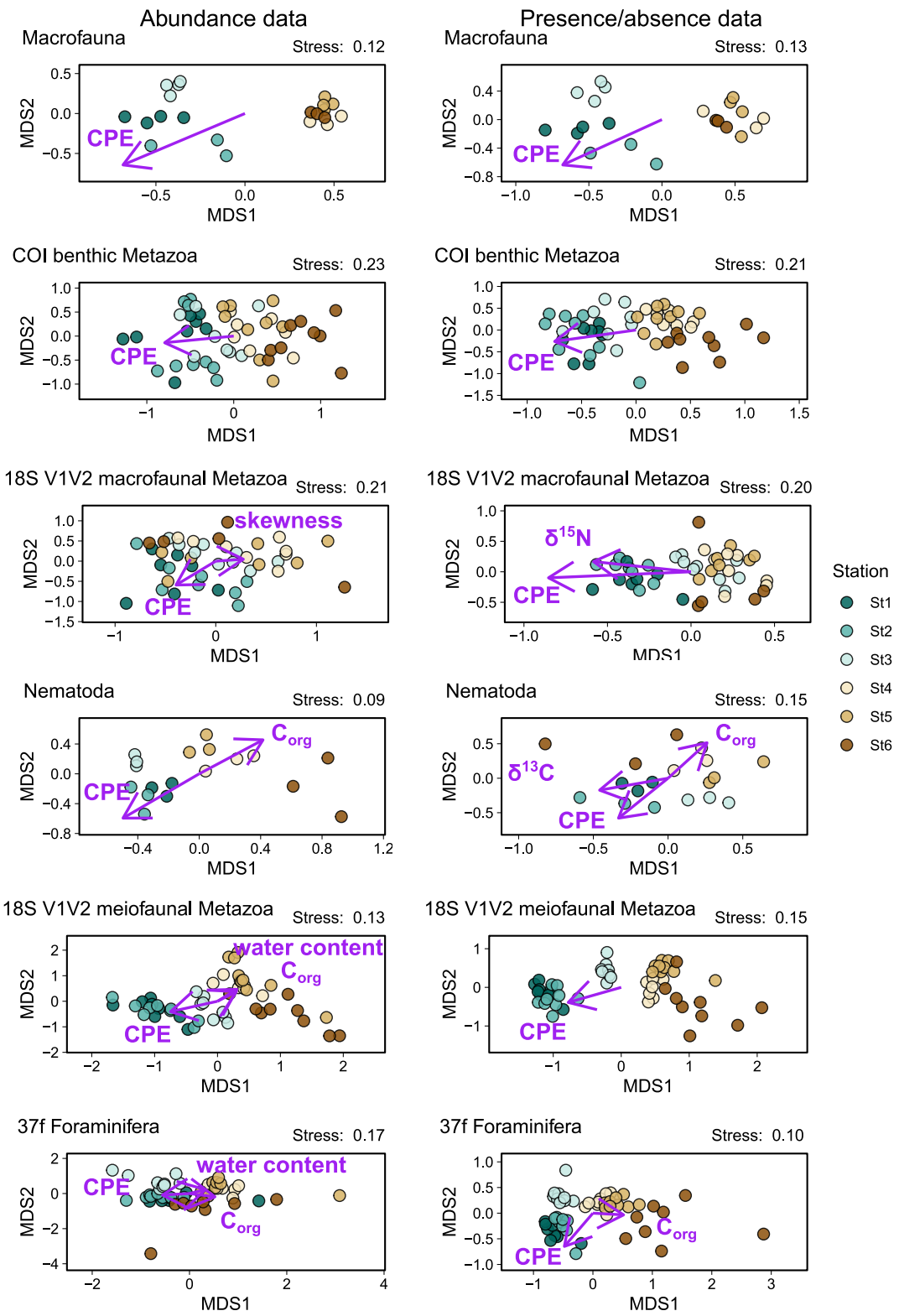

Fig. 8. nMDS plots for community data based on abundance and presence/absence data. Morphological abundance data – Bray-Curtis dissimilarities based on 4th root transformed data, metabarcoding data – Bray-Curtis dissimilarities based on cumulative-sum scaling (CSS) of reads. For presence/absence data Jaccard indexes were calculated. Purple arrows represent vectors of the environmental variables best correlated with the ordinations assessed using BIOENV analysis.

strongly correlated between each other, both in the case of presence/absence data and abundance data (rho = 0.73 and rho = 0.77, respectively, Bonferroni corrected $p < 0.05$, Suppl. Table S9). In the case of the COI benthic Metazoa (both abundance and presence/absence data) neither separation nor gradual change in the communities along the fjord could be observed.

As revealed by the BIOENV analysis, the patterns observed for the taxonomic composition were correlated with environmental variables with moderate strength (0.3 < rho <0.7), for most data, except macrofauna abundance data where strong correlation was found (rho = 0.7)

and Nematoda presence/absence data and COI benthic Metazoa where weak correlation was found (rho<0.3, Suppl. Table S10). The sets of environmental parameters included from one to three variables. The most frequent environmental variables selected by the BIOENV, related with nMDS ordinations, were CPE (a variable strongly correlated with LAR) that was identified in all cases, and C_{org}, but only for nematode morphological data, Foraminifera and 18S V1V2 meiofauna abundance data. CPE was the only environmental variable selected for macrofauna morphological data and 18S V1V2 macrofauna and meiofauna presence/absence data. It must be noted that in the case of macrofauna morphological data, where station average values of environmental variables were used, the CPE was also correlated with C_{org} and $\delta^{15}N$, so these variables should also be taken into account.

Vectors of the environmental variables imposed on the nMDS ordinations (Fig. 8) showed that the patterns observed among studied communities and genetic markers along the fjord, were consistent with the clines of environmental variability in the fjord. The transition of communities along the axis fjord mouth - glacier was consisted with direction of CPE vector, which was strongly and negatively correlated with LAR, and in most cases with C_{org} vector facing the opposite direction.

4. Discussion

4.1. Morphology and metabarcoding show different taxonomic composition of macrofauna, meiofauna and Foraminifera

4.1.1. Macrofauna

Morphological analysis yielded more macrofauna taxa than metabarcoding data. This is not surprising given that macrofauna was sorted from sieved grab samples (ca. 10 kilo of sediments), while metabarcoding data were obtained from about 10g sediment samples. Such small samples contain very few macroinvertebrates. They are represented in sediment DNA mainly by their traces, secretions, extracellular DNA or propagules, which greatly reduces the chances of their detection (Pawlowski et al., 2022). This is well illustrated in our study by arthropods and could be partially explained by their morphology (hard, chitin skeleton) and biology (internal fertilization), which impedes the leakage of their DNA into the environment (Klunder et al., 2022; Martins et al., 2021). More difficult to explain is the case of mollusks, which were relatively abundant in morphological dataset (7%) but very rare in our metabarcoding data. Willassen et al. (2022) also observed a very low number of mollusks, crustaceans and echinoderms in their metabarcoding study of benthic community in Svalbard fjords, despite their presence in grab samples and representation of their barcodes in reference library. The authors explained it by the competition of other organisms recognized by universal eukaryotic primers. This could also be a valid explanation in our case, but it does not seem to work equally for all macrofaunal taxa.

Interestingly, while some macrofaunal taxa are poorly represented in metabarcoding data, others yield high number of reads, although they are rarely found in morphological analysis. This is the case of ribbon worms (Nemertea), which preserve badly in formalin or ethanol samples, where their delicate bodies get fragmented and cannot be properly identified (Moore and Gibson, 2001). On the contrary, in metabarcoding data, they count for 10.3% in COI dataset and 8.8% in 18S V1V2 dataset. This can be explained by their reproductive biology, which allows their larvae to disperse by water currents over considerable distances (Leduc et al., 2019; Sevellec et al., 2021) and have been reported in high abundance in the Arctic meroplankton (Descôteaux et al., 2022). Once settled in the benthic environment, these larvae may give a strong signal in metabarcoding analysis, undetectable in morphological survey. It is also possible that the abundance of Nemertea is related to the high copy number of amplified genes as observed in other organisms (Gong and Marchetti, 2019; Krehenwinkel et al., 2017; Martin et al., 2022).

The difference between morphological analysis and metabarcoding was particularly visible in the case of annelids, the dominant phylum in macrofauna. In general, metabarcoding yielded fewer families compared to the morphological study, and the taxonomic structure was significantly different as showed by Permanova analysis. Slightly more than half of the annelid families documented by morphological analyses were not recorded in the COI data, and almost a third of them were not detected by the 18S V1V2 marker. This could be explained by the small volume of sediment DNA samples or by general primers that may hinder the detection of less abundant annelids. Nevertheless, families dominating in morphological samples, like Cirratulidae, Lumbrineridae or Sabellidae were also observed in a high abundance in molecular data. There were also three families (Hesionidae, Naididae, and Pilargidae) that were only documented in the metabarcoding datasets. Although absent from our morphological data, their presence in Svalbard region has been recorded previously. Family Hesionidae was reported in Svalbard fjords by Włodarska-Kowalczuk et al. (2007), representatives of Naididae were reported by Sirenko et al. (2022), while Pilargidae were reported in the Barents Sea close to Svalbard, but in a very low abundance (Cochrane et al., 1998).

The most striking discrepancy between the abundance of some annelid families in morphological and metabarcoding data was observed for Terebellidae and Sigalionidae. Both families were represented by a high number of reads in the metabarcoding data (up to 82% and 25% on average at the station, respectively) and less than 10 specimens per sample (mainly <5% of annelids at the station) in the morphological analysis. This discrepancy can be explained by different life-history traits, which may influence the amount of their DNA in the sediments. Terebellidae are rather medium-sized (Mazurkiewicz et al., 2020) tube-building and sessile polychaetes, with numerous tentacles used for feeding. Their unusual reproductive strategy involves larvae with two planktonic phases separated by a benthic period when the larvae settle, build a tube, and secrete mucus (Rouse and Pleijel, 2001). Each of these characteristics increase the probability of detecting this family in metabarcoding data. On the other hand, *Pholoe* (the only Sigalionidae genus detected) are rather small, surface-active and carnivorous (Cochrane et al., 2012), so it is possible that they leave substantial amounts of trace eDNA during their activity.

4.1.2. Meiofauna

Both morphological and metabarcoding data captured the same major meiofaunal taxa including Nematoda, Gastrotricha, Platyhelminthe, Kinorhyncha and Harpacticoida. Among these groups, nematodes were the only one for which we were able to provide morphological data. This was not possible for other groups due to lack of taxonomic expertise or poor preservation of organisms in our samples (i.e., Gastrotricha are best to identify alive (Higgins and Thiels, 1988)). In fact, analysis of the metabarcoding data allowed some sequences of these groups to be assigned to species level (Suppl. Table S6).

Similar to the macrofauna, metabarcoding detected fewer nematode taxa than morphological analysis. This was particularly striking for the COI marker that allowed detection of only four families, of the 26 recorded morphologically. The absence of nematodes in the COI data was also reported by Blaxter et al. (2005) or De Ley et al. (2005) and was attributed to mutations in the COI primer-binding regions (Schenk et al., 2020).

The results obtained for the 18S V1V2 marker yielded a similar list of Nematoda families as the morphological study. However, at the genus level, metabarcoding showed only a third of the genera detected morphologically. This discrepancy could be explained by the gaps in reference databases used for taxonomical assignments (Wangensteen et al., 2018). Indeed, The PR2 database contained only 34 of the 94 genera recorded in the morphological survey. Almost all of these genera (31) were identified in the metabarcoding dataset, indicating a good detectability of the 18S V1V2 marker.

The two methods also differed in the relative abundance of nematode families. In particular, the percentage of Oxystominidae reads was

significantly higher in the V1V2 dataset compared to the morphological survey. Several factors may explain these discrepancies. As in the case of macrofauna the amount of sediment from which DNA was extracted could affect the efficiency of detection by metabarcoding. In fact, the morphological study of the meiofauna was based on 0–5 cm layer of core sediments, while DNA was extracted from surface sediments (0–2 cm) only. Hence, the DNA samples were smaller in volume and may have missed taxa living in deeper layers. The lack of correlations between morphological and metabarcoding analyses could also be attributed to PCR bias (Ahmed et al., 2019), or to high intragenomic variability in the copy number of ribosomal genes, which has been shown to vary between different nematode species and limit the use of number of reads as an indicator of organism abundance (Bik et al., 2013).

4.1.3. Foraminifera

Benthic Foraminifera were not analyzed morphologically in the present study, however their composition and diversity patterns were well described in previous studies from Svalbard, including Hornsund fjord. Włodarska-Kowalczuk et al. (2013) found a consistent decrease of Foraminifera abundance and species richness in response to glacial impact along the same fjord. Hald and Korsun (1997) showed that the outer part of fjord is dominated by two calcareous rotaliid species: *Elphidum excavatum f. clavata* and *Nonionellina labradorica*, while in the inner glacial part another rotaliid *Cassidulina reniforme* constituted most of the community (up to 90%). Moreover, it was also reported that in the outer part, agglutinated textulariids were common, while they were absent in the glacial area (Zajaczkowski et al., 2010). These observations were confirmed by Szymańska et al. (2017) in Adventfjord, where the proportion of agglutinated taxa increased towards the fjord mouth, while stations located near glacier were dominated by calcareous species.

Compared to morphological analyses, our metabarcoding study showed very different taxonomic composition with single-chambered, soft-walled Monothalamea dominating benthic foraminifera communities in all stations. This is in agreement with other studies, which also showed the dominance of Monothalamea in metabarcoding datasets (Brinkmann et al., 2023; Pawlowski et al., 2014). In the Svalbard area, Pawłowska et al. (2020) studied surface sediments around the archipelago and found that most of the assigned OTUs and reads belonged to Monothalamea. Similar results were also obtained by Nguyen et al. (2022) in the various Svalbard fjords, where they recorded that Globothalamea constituted only 18% of foraminiferal ASVs. Monothalamea dominated in this study (55%) with high percentage sequences assigned to clade Y, clade V, and *Psammophaga* sp., similarly to our results. Regarding other regions dominance of Monothalamea in metabarcoding data was also reported from estuarine mudflats (Singer et al., 2023), sediments beneath fish farms (He et al., 2019) or abyssal plains (Lejzerowicz et al., 2021; Lecroq et al., 2011).

4.2. Morphology and metabarcoding provide a consistent response to glacial disturbance for meiofauna and foraminifera but not for macrofauna

The macrofauna studied morphologically showed a clearly defined pattern along the environmental gradient in the studied fjord and presented a distinct change in community between the inner, glacier-impacted, and outer parts of the fjord. The shift was reflected in a decrease of abundance, lower alpha diversity, and community distinctiveness in the two parts of the fjord. Moreover, higher variability could be observed within a community in the outer part, while the community in the inner part remained more consistent and seemed not to be influenced by local variations of other environmental factors. Our results are consistent with other studies from Hornsund (Kędra et al., 2013; Włodarska-Kowalczuk et al., 2013) and other glacial fjords, where a decrease in diversity, as well as very low spatial and temporal variability of the macrobenthic communities were observed (Kendall et al., 2003; Renaud et al., 2007; Sejr et al., 2010; Węsławski et al., 2011; Włodarska-Kowalczuk et al., 2012; Włodarska-Kowalczuk and Węsławski, 2008). The high homogeneity of macrofaunal composition and diversity in glacier-impacted regions is related to the low pool of macroinvertebrates capable of surviving chronic natural disturbances caused by strong sedimentation of glacier-transported sediments. A physically controlled, impoverished community has fewer species that can vary locally than a diverse community in the outer basin (Włodarska-Kowalczuk and Węsławski, 2008).

These effects of glacial disturbance on macrofauna were not observed in metabarcoding data. In both COI and V1V2 datasets no explicit pattern related to the distance to the glacier could be detected either in alpha diversity measures or composition. This could be due to small number of sequences assigned to macrofaunal taxa. Alternatively, this could be explained by the dispersal of eDNA in aquatic environment. The benthic samples analyzed morphologically represent usually a collection of species that lived within the sampling area. Metabarcoding data encompass much wider range of taxa, including organisms that live far from the sampling area. As shown by Leduc et al. (2019) eDNA can be widely dispersed over considerable distances, leading to a more homogenized eDNA-based taxonomic composition in a study area. This would also explain the fact that when we analyzed beta diversity, different stations appeared to be more distinct from each other based on morphological macrofauna data, whereas metabarcoding data showed a rather smooth transition of the assemblages from one station to another, despite being influenced by the same environmental drivers. This is also linked with the fact that the DNA of benthic organisms can also be found in the water and may be highly seasonally variable due to spawning or larvae release (Sevellec et al., 2021). It is reasonable to assume that the seasonal variability observed in metabarcoding of water samples could also be detected in sediments. On the contrary, seasonal variation due to larval settlement or juvenile recruitment may not be easily detected in morphological analysis of benthos.

In contrast to the macrofauna, the meiofauna (both morphologically and molecularly analyzed) does not show such a dramatic decline in alpha and beta diversity in the inner basin. Instead, we observed a gradual change of meiofauna diversity along the fjord and a more subtle and complex response to environmental conditions. For example, in the inner basin meiofaunal taxonomic richness was higher at station St5 than at the other two stations, probably due to the more heterogeneous habitat with a higher proportion of coarser sediments and water content in the sediments. Noteworthy, these subtle patterns of change within the glacial bay were similar in both morphological and metabarcoding (V1V2 meiofauna) datasets, demonstrating that both methods are comparably sensitive in elucidating meiofaunal responses to environmental variability. Our study confirms the gradual changes in nematode taxonomic structure observed along the glacier impact gradient in Kongsfjorden (Somerfield et al., 2006). Compared to macrofauna, meiofaunal species are less sensitive to mechanical disturbance and sediment instability (Giere, 2009) and more resilient to pollution (Kennedy and Jacoby, 1999). Thus, they can take advantage of conditions unfavorable to macrofauna and contribute more to the structure and functioning of the benthic community (Górska and Włodarska-Kowalczuk, 2017).

Foraminifera showed very similar patterns of response to glacial disturbance as the meiofauna: relatively constant and high alpha diversity at the three outer stations and then a gradual decrease in diversity towards the glacier. It may be deduced that foraminifera, like meiofauna, are less sensitive (compared to macrofauna) to natural glacial disturbance. This finding contrasts with previous report of very similar patterns of response to glacial disturbance by macrofauna and Foraminifera in Hornsund (Włodarska-Kowalczuk et al., 2013). However, Włodarska-Kowalczuk et al. (2013) study was based only on morphological analyses, meaning the Foraminifera consisted only of hard-shelled taxa. In our study, the foraminiferal metabarcoding data were dominated by soft-walled Monothalamea. Limited knowledge of

their ecology makes it difficult to explain their response to environmental conditions (Pawłowski et al., 2014). For example, an increasing proportion of unassigned monothalamids towards the glacier suggests that many taxa adapted to the specific conditions of the inner fjord are unknown to science. The environmental response is also not very clear even for some morphologically and genetically well-defined taxa, such as the genus *Psammophaga* (clade E). In our study, this genus occurred in high numbers in the outer stations and in low numbers in the inner stations. In another metabarcoding study of Svalbard Foraminifera, Nguyen et al. (2022) reported that *Psammophaga* was more abundant in the inner basin (glacially impacted) of Isfjorden and in outer basins, away from the glacier impact in Wijdefjorden and Rijpfjorden. It is likely that glacial sedimentation is not a key factor shaping its distribution, or that the genus comprises genetically different species adapted to different ecological conditions. Obviously, our knowledge of monothalamids is too limited to link specific taxa to the gradient of glacial disturbance in Arctic fjords.

Interestingly, the patterns in the taxonomic composition showed differences between presence/absence and abundance metabarcoding data, while the patterns for these two types of data were more consistent in morphological data. In particular, there was a high variability among samples from the same site in abundance data (especially for 18S V1V2 macrofauna and 37f Foraminifera), while the presence/absence data showed lower variability and more prominent separation of particular stations. This may arise from the fact that eDNA metabarcoding is prone to some technical biases that alter abundance data (Fonseca, 2018), such as taxon-specific PCR amplification bias (Elbrecht and Leese, 2015; Krehenwinkel et al., 2017). This can induce higher dissimilarities among samples, even despite data normalization (Paulson et al., 2013), while the presence/absence data will be less affected and depend mostly on the detection of taxa.

Despite the differences discussed above in the response to environmental clines of different taxa and the methods used to analyse them, we observed a consistency between a compositional transition across the fjord and major biotic gradients. The most important environmental factor, with the strongest and most prominent change along the fjord, explaining patterns of variability in faunal composition was CPE (and/or its correlate LAR). As indicated by the CPE, availability of fresh, primary organic matter (Soetaert et al., 1991) decreased from the fjord mouth toward the glacier and was accompanied with increasing sedimentation rate (indicated by LAR). Patterns of beta diversity accompanied well these changes of environmental conditions and confirmed well described zonation patterns of Arctic benthic fauna impacted by glacier activity and food supply (Dale et al., 1989; Renaud et al., 2007; Włodarska-Kowalczuk et al., 2005). Other environmental variables like $C_{org,}$ skewness of the grain size distribution or water content in the sediment were indicated as influential too, but were more group/genetic marker specific and concerned smaller sized biota. This indicates additional biological constrains (i.e., turnover rate, sensitivity to disturbance, food requirements or resource partitioning (Warwick and Clarke, 1984) and suggests different vulnerability to smaller scale habitat variability (Patrício et al., 2012) of macrofauna and meiobenthos.

4.3. Morphology vs metabarcoding: advantages and limitations

As shown by our data, each of the methods used in this study has its advantages and limitations. Morphological analysis works better for macrofauna. It allows the detection of more taxa and it provides absolute quantitative data. It also shows clear patterns of response to glacial disturbance, which we do not observe in macrofauna metabarcoding data. As mentioned above, the main limitation of benthic macrofauna eDNA studies is the small volume of processed sediment samples. This could be overcome by analysing DNA from bulk macrofauna samples, extracted either from a mixture of organisms or from a preservative solution (Blackman et al., 2019; He et al., 2019). Bulk samples provide a higher detection rate of macrofauna taxa, but the accuracy of abundance data remains a major issue and the community structure obtained with morphological analysis and metabarcoding may still be different (Vivien et al., 2019). Among other factors limiting the efficiency of DNA-based survey of macrofauna are the gaps in barcoding database (Weigand et al., 2019) and the lack of resolution of commonly used genetic markers (Casey et al., 2021). However, we observed that some groups (e.g., Nemertea) were better represented in metabarcoding data than in morphological inventories suggesting that these limitations are not applicable to all macrofaunal taxa.

In contrast to macrofauna, the advantages of using metabarcoding to study meiofauna and Foraminifera are much more obvious. As shown by the analysis of the nematode community, most taxa can be detected in metabarcoding data, provided that a comprehensive reference database is available (Macheriotou et al., 2019). The discrepancy in taxonomic composition inferred by both methods is due to the gaps in the reference database as well as to some differences in species definition in morphological and molecular taxonomy. From a practical point of view, the main advantage of metabarcoding is the automation of taxonomic identification, without the need for personal taxonomic expertise. Metabarcoding also allows the detection of genetic variation within morphospecies, which may provide a more accurate response to environmental conditions. As shown by our presence/absence data the response to glacial disturbance is more pronounced in meiofaunal metabarcoding data than in morphological data. The accuracy of relative abundances inferred from metabarcoding data remains to be tested. However, biomass variation in meiofauna are not as important as in macrofauna, suggesting that the abundance issue is easier to address in metabarcoding studies.

Regarding the comparison of morphology and metabarcoding, the foraminifera represent a special case. Both methods target different taxonomic groups, which makes them complementary rather than substituting one to the other. The advantage of morphological analysis is that it deals with hard-shelled foraminiferal taxa, whose taxonomy and ecology is relatively well known. On the contrary, the metabarcoding data are dominated by soft-walled monothalamous taxa that normally are not included in morphological analyses (Nguyen et al., 2022; Pawlowski et al., 2022). Although the taxonomy and ecology of this group is poorly known, there is a growing evidence that they can live in the conditions where other foraminifera are absent, as illustrated by glacier-impacted zone in our study. By including all foraminiferal taxa metabarcoding provides more holistic view and can be regarded as a valuable and complementary information about the analyzed community.

5. Conclusions and perspectives

To conclude, our study confirms that metabarcoding offers a valuable tool to complement traditional morphological surveys of Arctic benthic fauna. Although macrofauna and meiofauna taxonomic pools identified in Arctic sediments by metabarcoding are lower than those assessed using morphological analyses, the metabarcoding allows detection and identification to a low taxonomic level of many taxa that are routinely not included in morphological surveys due to methodological issues or lack of highly qualified taxonomists. This is especially valid for Foraminifera, where the entire group dominating the community, the monothalamids, is absent in the morphological datasets. Therefore, the two methods can be treated as complementary rather than replaceable. This also refers to their efficiency in elucidating the environmental effects. Even the small-scale subtle differences in environmental settings, such as grain size variability within the glacial bay, were reflected in both meiobenthic datasets. On the other hand, metabarcording methodology for some groups still needs to be tested and developed. In our case evidently the macrofaunal metabarcoding data obtained based on the analyses of the small amount of surface sediments did not representatively picture the local diversity, and consequently the spatial patterns of variability. More research exploring the

complementarity gained by involving both morphological and molecular approaches on different size groups and in various environmental regimes seems to be needed to better understand the impact of environmental conditions on entire benthic communities, from small unicellular eukaryotes to macroinvertebrates, in the rapidly changing Arctic ecosystems.

CRediT authorship contribution statement

Mikołaj Mazurkiewicz: Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **Joanna Pawłowska:** Writing – review & editing, Methodology, Conceptualization. **Inés Barrenechea Angeles:** Writing – review & editing, Methodology, Investigation. **Katarzyna Grzelak:** Writing – review & editing, Investigation. **Kajetan Deja:** Writing – review & editing, Investigation. **Agata Zaborska:** Writing – review & editing, Investigation. **Jan Pawłowski:** Writing – review & editing, Validation, Supervision, Resources, Conceptualization. **Maria Włodarska-Kowalczuk:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Maria Wlodarska-Kowalczuk reports financial support was provided by National Science Centre Poland. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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