# ~~The 16S rDNA~~ The microbiome of the Arctic planktonic foraminifera *Neogloboquadrina pachyderma* i~~s~~ comprised of fermenting and carbohydrate-degrading~~hydrocarbon-degrading~~ bacteria and a~~n~~ intracellular diatom chloroplast store.

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**Abstract.** *Neogloboquadrina pachyderma* is the only true polar species of planktonic foraminifera. As a~~It therefore~~ ~~plays a~~ key~~crucial~~ component ~~role~~ of the~~i~~ ~~n the~~ calcite flux, it ~~,~~ ~~plays a crucial role~~ ~~and~~ in the reconstruction~~s~~ and modelling of seasonality and environmental change within the high latitudes. The rapidly changing environment of the polar regions of the North Atlantic and Arctic Oceans poses challenging conditions for this (sub)polar species in terms of temperature, sea-ice ~~melt~~decline, calcite saturation, ocean pH and the progressive contraction of the polar ecosystem. To model the potential future for this important ~~high~~ high-latitude species, it is vital to investigate the modern ocean community structure throughout the annual cycle of the Arctic to understand the inter-dependencies of *N. pachyderma*. We use 16S rDNA metabarcoding and Transmission Electron Microscopy (TEM) to identify the microbial interactions of *N. pachyderma* during ~~the~~ summer ice-free conditions in Baffin Bay, and Picrust2 to predict the metabolic pathways represented by the ASVs in the foraminiferal microbiome. We demonstrate that the *N. pachyderma* diet consists of both diatoms and bacteria. The core microbiome, ~~is~~ defined as the 16S rDNA amplicon sequencing variants (ASVs) found in 80 % of individuals investigated, ~~.~~ ~~This core microbiome~~ consists of six bacterial ASVs ~~two diatom chloroplast ASVs~~ and two diatom chloroplast ASVs. On average, it ~~seven bacterial ASVs~~ ~~and~~ accounts for ~~, on average,~~ nearly ~~almost~~ 50 % of the total ASVs in any individual. The metabolic pathway predictions based on bacterial ASVs suggest that the foraminiferal microbiome is comprised of monosaccharide fermenting and polysaccharide degrading bacterial species~~bacterial ASVs represent hydrocarbon-degrading bacteria, including those~~in line with those found routinely in the diatom phycosphere. On average, the two chloroplast ASVs constitute ~~compose~~ 40 % of the core microbiome and~~.~~ ~~s~~Significantly, an average of ~~55.7~~53.3 % of all ASVs in any individual are of chloroplast origin. TEM highlights the importance of diatoms to this species by, ~~conclusively~~ revealing that intact chloroplasts remain ~~undigested~~ in the foraminiferal cytoplasm in ~~very high~~ numbers strikingly ~~,~~ comparable to th~~e~~ substantial quantities~~ose~~ observed in kleptoplastic benthic foraminifera. Diatoms are the major source of kleptoplasts in benthic foraminifera and other

kleptoplastic groups, but this adaptation has never been observed in a planktonic foraminifer. Further work is required to understand the association between *N. pachyderma*, diatoms and their chloroplasts in the pelagic Arctic realm, but such a strategy may confer an advantage to this species for survival in this extreme habitat~~., but it could also become compromised~~

35 ~~by the rapidly changing climate.~~

## 1 Introduction

The non-spinose planktonic foraminifera *Neogloboquadrina pachyderma* is found throughout the global ocean (Morard et al, 2024), ~~but occurs in greatest numbers~~ ~~in~~ in polar, ~~and~~ sub-polar and transitional upwelling waters~~oceans and also in upwelling~~ ~~water masses across the globe~~. These different biogeographies and niches are reflected by the distinct *N. pachyderma* genotypes

40 associated with ~~*N. pachyderma* genotypes found in~~ these divergent ecosystems (Darling et al., 2004; 2007; 2017). For example, whilst *N. pachyderma* is the predominant planktonic foraminiferal morphospecies of the polar oceans in both hemispheres (e.g. Bé and Tolderlund, 1971, Bé, 1977), *N. pachyderma* Type I is the only genotype found in the (sub)polar North Atlantic/Arctic Ocean (Darling et al., 2004; Darling et al, 2007) and is the major marine calcifier (Kohfeld et al., 1996) in the largest ocean carbon sink in the Northern Hemisphere (Gruber et al., 2002). ~~in the (sub)polar province of the northern North~~

45 ~~Atlantic (Kohfeld et al., 1996; Darling et al., 2004),~~ ~~the largest ocean carbon sink in the Northern Hemisphere (Gruber et al.,~~ ~~2002).~~ Present-day temperature conditions confine *N. pachyderma* Type I to the North Atlantic/Arctic Ocean ~~(sub)polar~~ water masses ~~in the high-latitude North Atlantic/Arctic Ocean~~, where summer sea surface temperature (SST) remains below 10°C (Tolderlund and Bé, 1971; Duplessy et al., 1991). Here, *N. pachyderma* exhibits strong seasonal productivity in a highly predictable pattern~~.,~~ Winter mixing re-supplies nutrients to surface waters, triggering the seasonal succession of maximal

50 phytoplankton blooms and zooplankton abundance which is followed by more nutrient depleted summer conditions (Jonkers and Kucera, 2015).

As the primary ~~major~~ component of both the modern and Quaternary fossil (sub)polar assemblage, the calcite shells of *N. pachyderma* ~~continue to~~ constitute the major contribution to ~~the~~ seasonal and environmental change reconstructions ~~and~~ ~~modelling of seasonality and environmental change~~ within the North Atlantic and Arctic Ocean (e.g. Simstich, et al., 2003;

55 Kretschmer et al, 2016; Altuna et al., 2018; Brummer et al, 2020; Livsey et al., 2020). However, the Arctic is now an unremittingly warming ecosystem, with seasonal sea ice cover constantly reducing and ice-free conditions ~~are~~ projected~~predicted~~ to appear between 2030 and 2055 (Kim et al., 2023; Jahn et al., 2024). ~~, likely to disappear within a short~~ ~~time frame (Serreze et al., 2009; Meier et al., 2021).~~ The North Atlantic/Arctic *N. pachyderma* is already considered to be particularly sensitive to the forecasted changes in seawater carbonate chemistry (Manno et al., 2012), with consequent

60 implications for the calcite flux and the biological pump. Under ocean acidification conditions, Arctic *N. pachyderma* show reduced carbonate production moderated by ocean warming, making it difficult to predict future climate change impacts as the polar habitat of *N. pachyderma* shrinks. Although the North Atlantic/Arctic *N. pachyderma* population has clearly survived the extremes of Quaternary climate cyclicity in the past (Brummer et al., 2020), it is unknown whether *N. pachyderma* will

find itself spatially displaced from its adaptive ecological range in the Arctic ecosystem (Jonkers et al., 2019; Greco et al., 2022), as we transition into the unknown territory of anthropogenically driven extreme global warming.

The warming ocean is affecting all marine organisms at multiple trophic levels (Poloczanska et al., 2016; Meredith et al., 2019; Deutsch et al., 2015). It has already been demonstrated that some planktonic protist species cannot ~~habitat~~ track their optimal temperatures as their environment changes and may undergo extinction once local thresholds are exceeded (Trubovitz, et al., 2020). Such thresholds are unknown for *N. pachyderma*, and there may soon be no true polar refugia into which to retreat. At the beginning of the 21st century within our Baffin Bay study area, the Pikialasorsuaq (the former "North Water Polynya") was considered a region of high biological productivity (Tremblay et al., 2002; 2006). However, increasing oligotrophic conditions have been reported in the last decade~~,~~ driven by meltwater from the Greenland Ice Sheet and nearby glaciers. The increas~~ing~~ed stratification and~~,~~ ~~and~~ reduc~~ed~~ mixing/upwelling ~~-~~results in a ~~causing a~~ reduction ~~specifically~~ in diatom-mediated net community production (Bergeron et al., 2014). Since diatoms are considered a major food source for *N. pachyderma* (Schiebel and Hemleben 2017; Greco et al., 2021), this reduction in diatom primary productivity poses an additional challenge to *N. pachyderma* populations. To model the impending environmental consequences for this important high latitude species, it is vital to investigate the modern ocean community structure throughout the annual cycle of the Arctic to understand the inter-dependencies of *N. pachyderma*.

Although our understanding of Arctic (sub)polar *N. pachyderma* annual/seasonal population structure and ecological behaviour is increasing (e.g. Carstens and Wefer, 1992; Kohfeld et al, 1996; Jonkers et al., 2010; Jonkers et al.,2013; Greco et al., 2019; Meilland et al., 2022), it is far from complete. The presence of only a single genotype (*N. pachyderma* Type I) ~~Small subunit ribosomal RNA (SSU rRNA) genotyping indicates that only a single *N. pachyderma* genotype (Type I) occupies the whole of the North Atlantic and Arctic Ocean water mass (Darling et al., 2004, 2007). The sub-polar North Pacific *N. pachyderma* (Type VII) is not adapted to live in Arctic polar waters (Darling et al., 2007). This~~ is good news for all the Arctic ecological investigations based on this taxon (e.g. Altuna et al., 2018; Greco et al., 2019; Meilland et al., 2022) as it simplifies analyses. ~~since different genotypes of *N. pachyderma* fill distinctly different niches within the divergent ecosystems which they inhabit (Darling et al., 2017).~~ Microbiome ~~M~~metabarcoding ~~investigations~~ of ~~other taxa within the~~ *Neogloboquadrina* ~~genus~~ species has already highlighted the diversity and complexity of the ecological community networks and symbiont/predator/prey interactions which exist between prokaryotes and protists within the water column (Bird et al., 2018). Using the 16S rDNA metabarcoding approach together with fluorescence microscopy, or TEM, Bird et al. (2018) determined the taxonomic character, trophic interactions, food source and putative symbiotic associations of *N. incompta* and *N. dutertrei* in the California Current system. Results highlight their similar feeding strategy of forming feeding cysts of particulate organic matter (POM) in the water column, but that such behaviour provides no clues to their choice of prey or potential symbiotic associations. Evidently, ecological concepts of individual planktonic foraminifera must be systematically revised, as each morphospecies and potentially each genotype has most likely evolved individually distinct interactions with the marine microbial assemblage.

A recent study used single-cell metabarcoding targeting 18S rDNA to characterise the interactions of *N. pachyderma* with the local eukaryote community (Greco et al., 2021), since the majority of data on feeding behaviour in planktonic foraminifera

100 suggests that biotic interactions are likely to be mainly with herbivorous eukaryotes (Kohfeld et al., 1996; Manno and Pavlov, 2014; Pados and Spielhagen, 2014; Schiebel and Hemleben, 2017). However, since no direct investigations have been carried out on the feeding behaviour or diet of Arctic *N. pachyderma*, this remains in question. The data shown in Greco et al. (2021) indicate that the *N. pachyderma* interactome is dominated by diatoms, with Crustacea and Syndiniales (a potential parasite) also present. Here we complement this study by examining the single cell 16S rDNA metabarcodes of the *N. pachyderma*

105 microbiome to investigate prokaryote biotic and trophic interactions and their potential symbiotic associations. In addition, we further investigate the cellular structures within *N. pachyderma* individual specimens using TEM, to examine the cellular position of the bacterial/chloroplast sources of DNA within the *N. pachyderma* cell. Our results have direct implications for understanding trophic interactions within this at-risk habitat; interpretation of the seasonal shell geochemical record; for modelling future *N. pachyderma* population dynamics and the carbonate flux under climate change; and understanding the

110 evolutionary pressures experienced by this morphospecies.

## 2 Materials and methods

### 2.1 Sampling locality and collection methods

Sampling was undertaken in Baffin Bay in July/August 2017 aboard *CCGS Amundsen* as part of ArcticNet Expedition 2017 (Leg 2b; https://arcticnet.ulaval.ca/expeditions-2017) and August/Sept. 2018 aboard the *CCGS Hudson 2018042* expedition

115 (Fig. 1). In both cases the samples were taken in open water with no sea ice cover. Details of sampling stations and collections are listed in Table 1. Sample provenance was either individual foraminifera or the water column. Samples were analysed by 16S metabarcoding of the foraminiferal microbiome or the water column bacterial assemblages from stations along the *Amundsen* cruise track.

Foraminifera were collected at seven stations by vertical net tow from 200 m depth to the surface. Foraminifera for genotyping

120 and microbiome analysis were wet picked on board, rinsed in 0.2 μm filtered surface seawater and preserved in 100 μl RNALater® (Ambion™). This reagent conserves cell integrity, inhibits nucleases at ambient temperatures, and dissolves the calcite shell. Samples were stored at 4°C for 4 hours and then transferred to a -20°C freezer until processing.

CTD data and water samples were collected from three stations (101, 115 and 323) in northern Baffin Bay (Fig. 1; Table 1) in 2017. Stations 101 and 115 are both situated within the biologically important Pikialasorsuaq between Greenland and Canada,

125 which remains sea-ice-free in winter (Eegeesiak et al., 2017). Station 101 is located close to southeast Ellesmere Island in relatively shallow water (350 m depth). Station 115 is at a similar latitude to Station 101 but deeper at 653 m and closer to Greenland. Station 323 is situated further south and outside the Pikialasorsuaq in Lancaster Sound at the entrance to the Northwest Passage. It is the deepest of the three stations at 789 m.

Water samples were collected from five depths:: surface, 50 m, 100 m, 150 m, and 200 m. 2L of seawater was filtered from each depth at each station on to 0.2 µm polycarbonate filters. Filters were then individually placed in a 1.5ml microfuge tube and covered with RNA*Later*® (Ambion™). Tubes were stored at 4°C for 4 hours and then transferred to a -20°C freezer until processing. Foraminifera for TEM analysis were collected on the CCGS Hudson cruise (2018042) in 2018 (Table 1). They were wet picked as described above and placed directly in TEM buffer (4 % glutaraldehyde, 2 % paraformaldehyde in salt-adjusted phosphate buffered saline (PBS with 24.62g/L NaCl added)), stored at room temperature for 12 hours, then kept at 4°C until further processing.
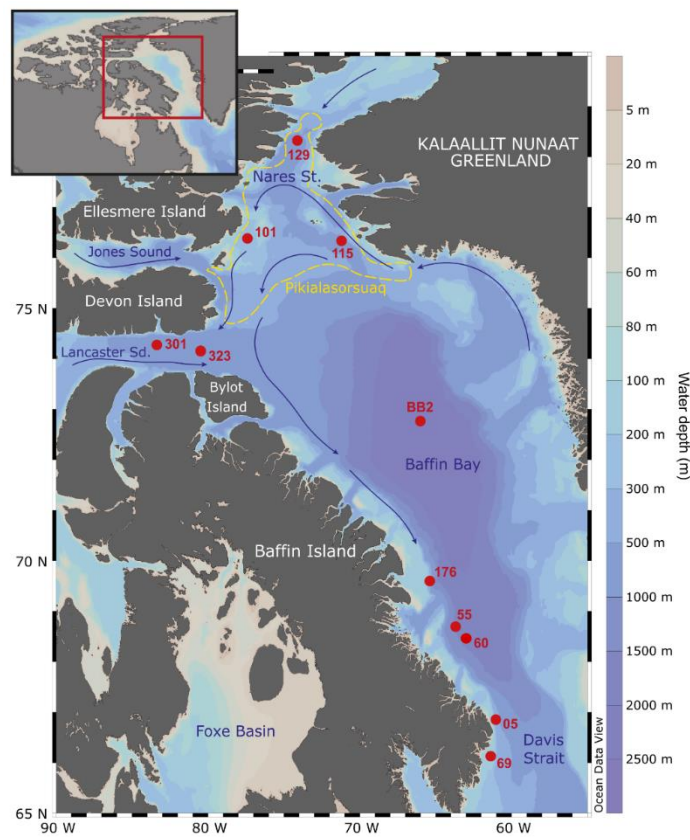
Figure 1. Map of sampling stations (numbered red spots) and the site of Pikialasorsuaq (the former 'North Water Polyna' (dashed yellow line); Eegeesiak et al., 2017). Stations BB2, 101, 115, 129, 301 and 323 were part of the *CCGS Amundsen ArcticNet* Expedition 2017 and Stations 05, 55, 60 and 69 were part of the *CCGS Hudson 2018042* Expedition 2018. Inset shows the sampling location within the wider region. The base maps were drawn in Ocean Data View v.5.6.2 (Schlitzer 2018).

Table 1. Stations and sample information including provenance, depth, and analysis type. Specimen IDs are either WC (water column) or Fm (foraminifera). This is followed by station identification (e.g. 101), and water depth (e.g. 050 = 50 metres), and replicate ID (e.g. a, b or c etc.)

| Cruise | Station | Sampling date | Specimen IDs | Latitude (°) | Longitude (°) | Water depth (m) | Sample depth (m) | Provenance | Analysis |
|---|---|---|---|---|---|---|---|---|---|
| AMD2017-2B | 101 | JUL-24-2017 | WC101_000a | 76.3844 | -77.4033 | 350 | surface | Water column | microbiome |
| | | | WC101_050a WC101_050b WC101_050c | 76.3844 | -77.4033 | 350 | 50 | Water column | microbiome |
| | | | WC101_100b | 76.3844 | -77.4033 | 350 | 100 | Water column | microbiome |
| | | | WC101_150a WC101_150b | 76.3844 | -77.4033 | 350 | 150 | Water column | microbiome |
| | | | WC101_200a WC101_200b | 76.3844 | -77.4033 | 350 | 200 | Water column | microbiome |
| | 101 | JUL-24-2017 | Fm101a Fm101b Fm101c Fm101d Fm101e Fm101f Fm101g | 76.3844 | -77.4033 | 350 | surface-200m | Foraminifera | microbiome, genotyping |
| | 115 | JUL-26-2017 | WC115_000a WC115_000b | 76.3419 | -71.2192 | 653 | surface | Water column | microbiome |
| | | | WC115_050a WC115_050b | 76.3419 | -71.2192 | 653 | 50 | Water column | microbiome |
| | | | WC115_100a WC115_100b | 76.3419 | -71.2192 | 653 | 100 | Water column | microbiome |
| | | | WC115_150a WC115_150b | 76.3419 | -71.2192 | 653 | 150 | Water column | microbiome |
| | | | WC115_200a WC115_200b | 76.3419 | -71.2192 | 653 | 200 | Water column | microbiome |
| | | | Fm115a Fm115b | 76.3419 | -71.2192 | 653 | surface-200m | Foraminifera | microbiome, genotyping |
| | 323 | JUL-31-2017 | WC323_000a | 74.1593 | -80.4753 | 789 | surface | Water column | microbiome |
| | | | WC323_050a WC323_050b | 74.1593 | -80.4753 | 789 | 50 | Water column | microbiome |
| | | | WC323_100a WC323_100b | 74.1593 | -80.4753 | 789 | 100 | Water column | microbiome |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | WC323_150a WC323_150b | 74.1593 | -80.4753 | 789 | 150 | Water column | microbiome |
| | | | WC323_200a WC323_200ab | 74.1593 | -80.4753 | 789 | 200 | Water column | microbiome |
| | 323 | JUL-31-2017 | Fm323a Fm323b | 74.1593 | -80.4753 | 789 | surface-200m | Foraminifera | microbiome, genotyping |
| | 176 | JUL-21-2017 | Fm176a Fm176b Fm176c Fm176d | 69.6032 | -65.3938 | 281 | surface-200m | Foraminifera | microbiome, genotyping |
| | BB2 | JUL-22-2017 | FmBB2a FmBB2b FmBB2c FmBB2d FmBB2e | 72.7678 | -66.0002 | 2372 | surface-200m | Foraminifera | microbiome, genotyping |
| | 129 | JUL-29-2017 | Fm129a Fm129b | 78.3254 | -74.1124 | 514 | surface-200m | Foraminifera | microbiome, genotyping |
| | 301 | AUG-03-2017 | Fm301a Fm301b Fm301c Fm301d Fm301e Fm301f | 74.2778 | -83.3641 | 716 | surface-200m | Foraminifera | microbiome, genotyping |
| Hudson 2018042 | 05 | AUG-23-2018 | BB1 | 66.8605 | -61.0668 | 337 | 100 | Foraminifera | TEM |
| | 05 | AUG-23-2018 | BB2 | 66.8605 | -61.0668 | 337 | 100 | Foraminifera | TEM |
| | 55 | AUG-31-2018 | BB8 | 68.6999 | -63.7084 | 1560 | 50 | Foraminifera | TEM |
| | 60 | SEP-02-2018 | BB9B | 68.543415 | -63.461252 | 1543 | 100 | Foraminifera | TEM |
| | 60 | SEP-02-2018 | BB9C | 68.543415 | -63.461252 | 1543 | 100 | Foraminifera | TEM |
| | 69 | SEP-04-2018 | BB11 | 66.1371 | -61.3659 | 160 | 100 | Foraminifera | TEM |
| | 69 | SEP-04-2018 | BB12 | 66.1371 | -61.3659 | 160 | 100 | Foraminifera | TEM |

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Figure 1. Map of sampling stations (numbered red spots) and the site of Pikialasorsuaq (the former 'North Water Polyna' (dashed yellow line)). Stations BB2, 101, 115, 129, 301 and 323 were part of the *CCGS Amundsen ArcticNet* Expedition 2017 and Stations 05, 55 and 69 were part of the *CCGS Hudson 2018042* Expedition 2018. Inset shows the sampling location within the wider region. The base maps were drawn in Ocean Data View v.5.6.2 (Schlitzer 2018).

## 2.2 DNA extractions, foraminiferal 18S rDNA genotyping and 16S ~~rDNA~~ rDNA metabarcoding.

Downstream washing of individual cells preserved in RNALater® for genotyping was carried out to remove the shell and shell-associated external contaminants according to Bird et al. (2017). DNA was extracted from individual foraminifera ~~using the~~in 40µl DOC buffer (Holzmann and Pawlowski, 1996) ~~extraction method~~ to identify the specific genotype~~Holzmann and Pawlowski, 1996)~~. PCR amplification of the foraminiferal 18S ~~rRNA~~ rDNA gene was performed with three rounds of PCR using a Phire Hot start DNA polymerase master mix (Thermo-Scientific), 3 % DMSO and an annealing temperature of 58°C with 25 cycles. ~~Post RNALater stored DNA, crushed into 40µl DOC extraction buffer~~DNA was diluted 1 in 20 in PCR grade water. Primer pairs were as follows: Primary PCR: C5-sB, secondary PCR: N5-N6, tertiary PCR: 14F1-N6. PCR products between rounds were diluted 1 in 100 PCR grade water and 1 µl was used in the following round of PCR. Cloning to account for intra-individual variation was carried out according to Darling et al. (2016). DNA sequencing was carried out using the BigDye® Terminator v3.1 Cycle Sequencing Kit and an ABI 3730 DNA sequencer (both Applied Biosystems). Filtrate from water samples was extracted for DNA using the DNeasy power water kit (Qiagen). Filters were removed from RNA*Later*® (Ambion™), placed in clean 1.5 ml microfuge tubes and centrifuged for 1 min at 10,000 xg. Excess RNA*Later*® (Ambion™) was removed and the filter was transferred to the bead beating tubes of the DNeasy power water kit and processed following the manufacturer's protocol. A control DNeasy power water kit extraction was carried out in parallel using a clean filter. In addition to the foraminiferal and water sample processing, four reagent controls were also processed. These were composed of two PCR controls containing no DNA template, an extraction control containing 2.5µl DOC buffer only and an extraction control containing 1µl of elute from a Qiagen DNA extraction of a clean 0.2 µm polycarbonate filter.

~~The V4 region of the 16S rRNA gene was chosen for amplification using barcoded 5~~15F (Parada et al., 2016) forward and 806R (Apprill et al., 2015) reverse primer pairs modified from the original primer pair (Caporaso et al., 2011, Walters et al., 2016). PCR was used to amplify the V4 region of the 16S rDNA gene of bacteria and chloroplasts. ~~Each DNA sample and control was PCR amplified with unique dual barcoded tags that enabled demultiplexing of the samples after being pooled for sequencing.~~ PCR reactions using 5~~15F (Parada et al., 2016)~~forward (Parada et al., 2016) and 806R ~~(Apprill et al., 2015)~~ reverse ~~primer~~ (Apprill et al., 2015) primer ~~pairs~~ modified from the original primer pair (Caporaso et al., 2011, Walters et al., 2016) were performed in triplicate. Each reaction contained ~~and contained~~ 1 Unit Phusion DNA polymerase (ThermoScientific), 1 x Phusion HF buffer, 0.2 mM each dNTP, 0.4 µM of each primer, 0.4mM MgCl$_2$ and 2.5 µl (foraminifera) or 1 µl (water column) of template DNA in a 50 µl volume made up with PCR grade water (Sigma). All PCR reactions were set up in a UV sterilization cabinet (GE healthcare). Reaction tubes and PCR mixtures were treated for 15 minutes with 15 W UV light (wavelength = 254 nm) to destroy contaminating DNA, prior to addition of dNTPs, DNA polymerase primers and template DNA (Padua et al., 1999). ~~PCR products were run on a 1.2 % agarose gel and the t~~Triplicate PCR reactions were pooled before purification with the Wizard® SV Gel and PCR Clean–Up System (Promega). The purified amplicons were quantified using a Qubit® 2 fluorometer (ThermoFisher Scientific) prior to pooling at equimolar

concentrations for DNA sequencing. DNA sequencing was performed at Edinburgh Genomics using an Illumina MiSeq v3 to generate 253 base pair (bp) paired-end reads.

## 2.3 Quality filtering paired end reads, rarefaction, taxonomic assignment and sequence filtering.

190     The Quantitative Insights in Microbial Ecology 2 pipeline (QIIME2, Bolyen et al., 2019) was used for initial analyses. Sequences were trimmed and denoising was carried out using the DADA2 plugin (Callahan et al., 2016) for quality filtering, dereplication, removal of singletons, chimera identification and removal and merging paired-end reads. This method generates amplicon sequence variants (ASVs) and a set of representative sequences. Alpha rarefaction was carried out in QIIME2 (metrics: observed OTUs; Shannon; and ~~faith~~ Faith ~~pd~~PD) ~~to assess whether the sequencing depth was adequate to detect~~

195     ~~bacterial diversity~~. Samples were rarefied to the lowest sequencing depth observed across all samples (27,660) and sampling depth was adequate across all samples. A total of 60 samples including 28 foraminiferal samples, 28 water samples and 4 negative controls, produced a total of 4290 ASVs across 5,802,211 counts. ASVs with a total frequency count of 50 or less across all 60 samples were removed from the sample set, leaving 1717 ASVs. Taxonomy was assigned using an SKlearn classifier pre-trained on the database SILVA-132 99 % OTUs from the V4 515f/806R region of the sequences (Quast et al.,

200     2013). Taxonomic-based filtering was then carried out to remove ASVs assigned to mitochondria, eukaryotes, and those not assigned beyond Kingdom level, and contaminant removal (26 identified ASVs) was carried out in the R package *Decontam* (Davis et al., 2018) in R v 4.0 (R Core Team, 2017) using the prevalence with batch methods. After filtering, 1548 ASVs remained.

~~Prior to further analysis, abundance data were normalised to the total number of counts per sample as a relative abundance,~~

205     ~~and only ASVs present at > 0.1 % abundance averaged across all samples were retained (Prazeres et al., 2018). Unassigned~~ ~~taxa or those taxa assigned to mitochondria or eukaryotes (totalling 71 ASVs) were removed from the sample set leaving 59~~ ~~samples, 130 ASVs and 4,620,174 reads for analysis.~~

## 2.4 Statistical analyses

Absolute count data were transformed to centred log-ratios suitable for statistical analysis of compositional data using q2-

210     Gemelli (Martino et al., 2021). Robust Aitchison distances were calculated (Martino et al., 2019) and a PCA was performed in Vegan (Oksanen et al., 2017). This was visualised with ggplot2 (Wickham, 2009). To determine if provenance, sample depth or station significantly affected the assemblages a PERMANOVA was carried out using the *Adonis* function in QIIME2 with the default 999 iterations. ~~Statistical analyses were performed in R v 4.0 (R Core Team, 2017). Differences between the~~ ~~provenances (foraminiferal microbiome and water column assemblage) were tested using the package mvabund (Wang et al.,~~

215     ~~2012). This generalized linear model approach uses absolute count data but takes into consideration the variability in the mean-~~ ~~variance relationships across the ASVs. The negative-binomial model best fit the dataset, and ANOVA was performed to test~~ ~~for significant difference between provenances using *mvabund* and *Vegan* (Oksanen et al., 2017).~~

~~Differences in~~The taxa associated with the different provenances, ~~stations and water depths~~ were assessed using the packages Phyloseq (McMurdie and Holmes, 2013) and DESseq2 (Love at al., 2014). DESeq2 was used rather than ANCOM because ANCOM assumes that <25 % of the ASVs are changing between provenances, and here this assumption does not hold true (Mandal et al., 2015). ~~Graphical presentation of results was performed with *ggplot2* (Wickham, 2009). Bray-Curtis dissimilarity between different samples was calculated and visualised using non multidimensional scaling (NMDS) in *Phyloseq*. To determine if provenance, sample depth or station significantly affected the assemblages a multivariate analysis of variance (ANOVA) was carried out using the A*donis* function in the *Vegan* R package (Oksanen et al., 2018).~~

Compositional differences~~,~~ and specific taxa that were significantly different between provenances were identified using log2 of fold change analysis in DESeq2 by converting the phyloseq-object, containing the raw frequency counts, to a DESeq2 object. The DESeq2 analysis was run with size factor type set to "poscounts" which allows values of zero in the sample counts and accounts for the data transferal from a phyloseq-object (van den Berge et al., 2018). The significance test was set to "Wald", and a "local" fit type for fitting of dispersions.

The core microbiome, here defined as ASVs present across 80 % of the foraminifera, was identified using *Microbiome* (Lahti and Shetty et al., 2017).

Functional predictions of the foraminiferal microbiome compared to the wider water column assemblage were made using PICRUSt2 (Douglas et al., 2020). The inputs to PICRUSt2 were the representative sequences fasta file and the ASV frequency table (converted to biom format) generated in QIIME2. The default full pipeline was run with the addition of "--per_sequence_contrib" and "--coverage" to give copy number normalised, community wide pathway abundances to compare between the provenances. EPA-NG phylogenetic placement of reads (Barbera et al., 2019) was used with the default cutoff nearest sequence taxon index (NSTI) of 2.0 which removed 8 of 1548 ASVs from downstream analysis, as these could not be satisfactorily placed in the tree. ALDEx2 (Fernandez et al., 2014) was used to assess the differential abundance of functional pathway predictions between the foraminiferal microbiome and the water column assemblage. PICRUSt2 generated pathway abundance data was rounded to integers then input to aldex.clr which generates centred log-ratio transformed values (number of Monte-Carlo instances = 1000, and denominator = iqlr). The Welch's t test (aldex.ttest) and an estimate of the effect size and the within-and-between provenance values (aldex.effect) were calculated from the output of aldex.clr. The dataset was then filtered for pathways that were significantly differentially abundant between provenances (Benjamini-Hochberg corrected P value <0.05 and effect size >1).

~~The core microbiome, here defined as ASVs present across 80 % of the foraminifera, was identified using *Microbiome* (Lahti and Shetty et al., 2017).~~

**2.5 Transmission electron microscopy**

TEM was used to observe and document the structural relationships between any endobiotic micro-organisms and foraminiferal cells. After fixation in TEM fixative (see methods section 2.~~2~~1), specimens were post–fixed in 1 % Osmium

Tetroxide in 0.1 M Sodium Cacodylate for 45 minutes, followed by a further three 10-minute washes in distilled water. Specimens were then set in small cubes of 1 % low melting point agarose and decalcified in 0.1 M EDTA (pH 7.4) for 1 hour and 48 hours at 4 °C. Fixed cells were then dehydrated in 50 %, 70 % and 90 % ethanol for 2 x 15 minutes followed by 100 % ethanol 4 x 15 minutes. Two 10–minute changes in Propylene Oxide were carried out prior to being embedded in TAAB 812 resin. Sections, 1 μm thick, were cut on a Leica Ultracut ultramicrotome, stained with Toluidine Blue, and then viewed under a light microscope to select suitable specimen areas for investigation. Ultrathin sections, 60 nm thick, were cut from selected areas, stained in Uranyl Acetate and Lead Citrate and then viewed with a JEOL JEM–1400 Plus TEM. Both osmium tetroxide and uranyl acetate used here bind to unsaturated lipids such that they appear dark in TEM imaging.

## 3 Results

### 3.1 The water column

Water samples were taken from three locations: stations 101, 115 and 323 (Fig. 1, Table 1). At Station 115 to the east of the Pikialasorsuaq~~The water at Station 115~~, the water ~~to the east of the Pikialasorsuaq,~~ is derived from warm Atlantic water (Melling et al., 2001; Vincent, 2019) with ~~and the surface water temperature~~SSTs here ~~is~~ was as high as 4°C accompanied by~~, with~~ a steep thermocline to 40 m (Fig. 2). At ~~stations~~ stations 101 and 323 (~~ in the~~ west and southwest of the Pikialasorsuaq), the upper level water temperatures were colder due to ~~and to the southwest respectively,~~ Pacific-derived water enter~~ings~~ via the colder Arctic Ocean (Tremblay et al., 2002; Bergeron and Tremblay, 2014) ~~and consequently the surface waters are were colder~~. Whilst ~~S~~station 323 ha~~ds~~ a surface temperature of 3°C, it very rapidly drop~~peds~~ to -1°C by 20 m and station 101 ~~has~~ had a surface maximum temperature of only 0.75°C. The chlorophyll maximum ~~is~~ was closely associated with the temperature maximum at all stations.

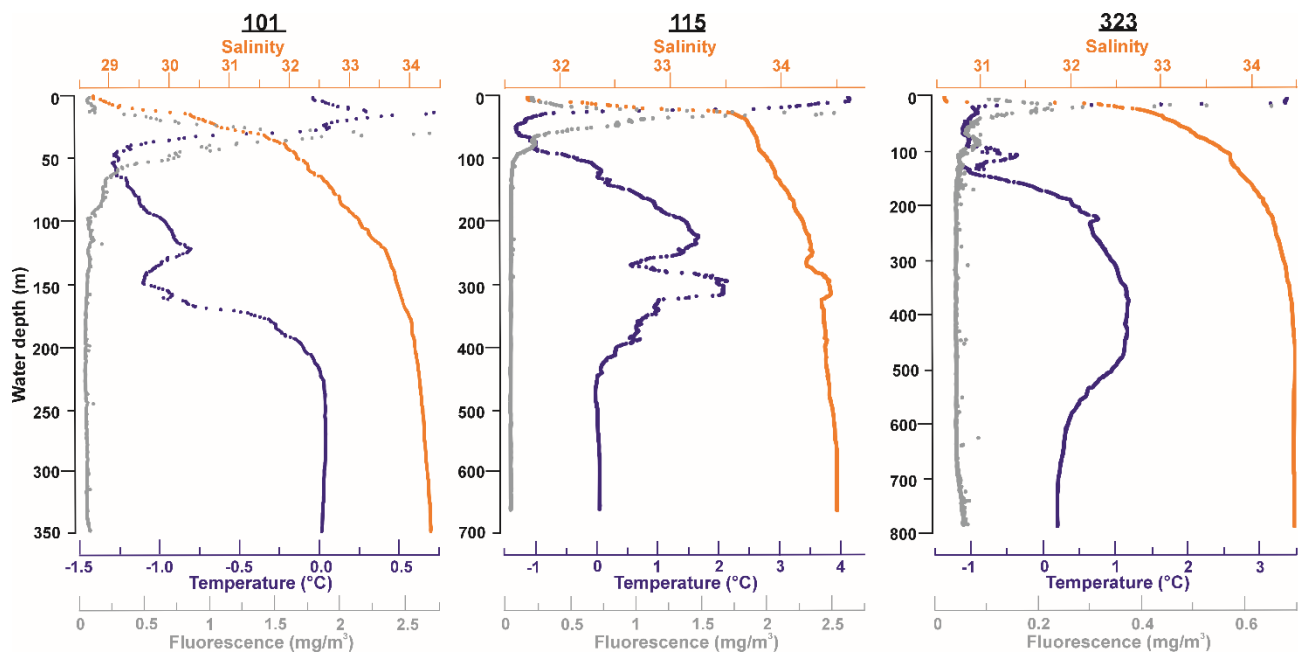



Figure 2. CTD plots for temperature, salinity, and fluorescence at stations 101 (350m), 115 (653m) and 323 (789m) where both water and foraminifera were collected.

275

The general microbial assemblages in the water column across the three stations (101, 115 and 323) displayed a similar pattern of assemblage composition with depth (Fig. 3). Surface waters contained either no, or extremely low relative abundances of chloroplast or archaeal ASVs. Chloroplast ASV relative abundance ~~increases~~ increased steeply with depth however, with the highest abundance found in the 50 m water samples, before numbers reduced again. This pattern agrees with our CTD data,

280 where the chlorophyll maximum ~~occurs~~ occurred between 20-40 m across all stations (Fig. 2). The chloroplast ASVs ~~make~~ made up an average of ~~2.97~~3.51 % of the ASVs in the water column. Archaeal ASVs (candidate Phylum Thermoplasmatota, and the Crenarchaeota~~Thaumarchaeota and Euryarchaeota~~) are most predominant below 50 m, peaking in the 100-150 m water samples. ~~Bray-Curtis~~Robust Aitchison dissimilatory (Fig. A1) and multivariate analysis (*Adonis*; ~~vegan~~QIIME2*) indicated systematic differences in composition with ~~both~~ depth ~~and~~ but not station. ~~D~~Depth ~~drives~~ drove ~~52~~55 % of the variability

285 (F.Model=7.168, Pr = 0.001) compared to ~~Station~~ station driving ~~17~~only ~~%~~2.38 % of the variability, and ~~the~~ the ASV assemblages are not significantly different between stations ~~(~~(F.Model=2.69, Pr = 0.~~001~~063)~~)~~. Results of all statistical tests can be seen in Table A1.

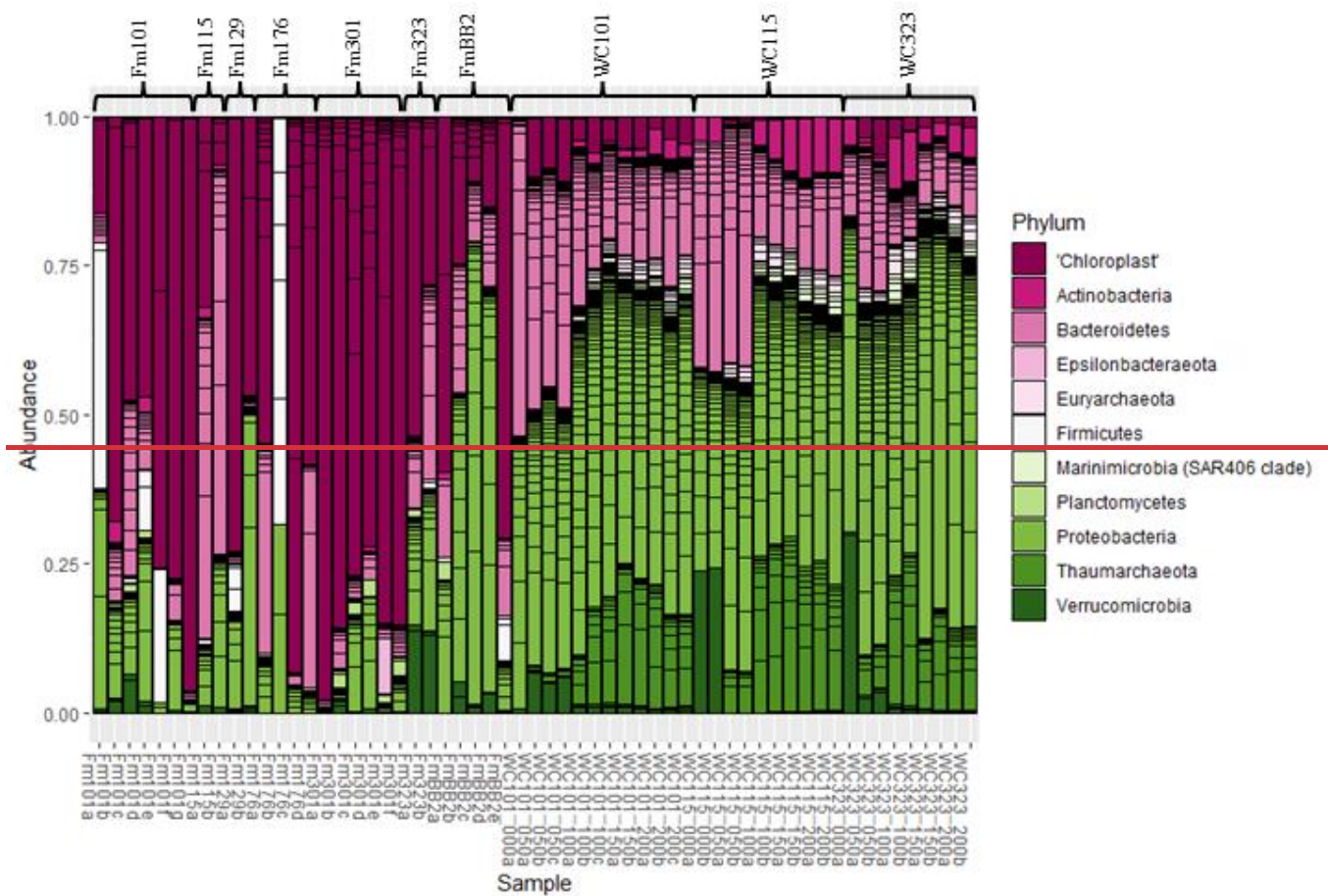
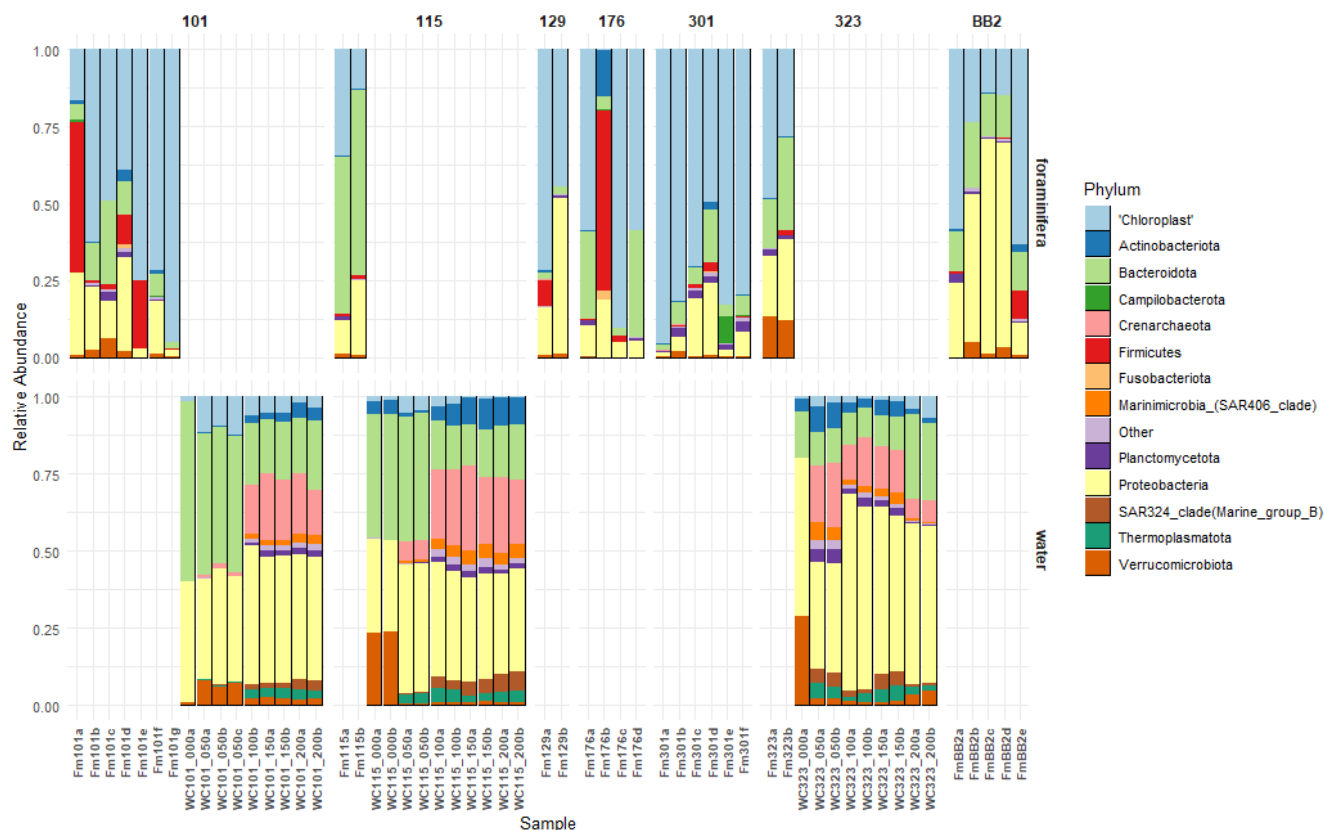



Figure 3. The relative abundance of 16S rDNA ASVs generated from the foraminiferal specimens (Fm) and the water column (WC). Note that foraminifera (top row) were successfully processed from 7 stations (see Fig. 1) and water was collected and processed from three stations (bottom row). Taxa are shown at the phylum level except for chloroplast derived 16S ASVs, which are grouped together. ~~Sample IDs (Table 1) are found on the x-axis.~~ ~~Black lines in bars indicate divisions of ASVs.~~ ~~Brackets at top indicate stations from which samples were taken, e.g. Fm101 are foraminifera samples from station 101, whereas WC101 are water samples from station 101.~~

## 3.2 Statistical comparison of water column and foraminiferal ASVs

The raw 16S metabarcoding dataset was submitted to the NCBI Sequencing Read Archive (BioProject accession PRJNA984332). The combined microbial assemblages of the ~~31~~ 28 water column samples and the 28 foraminifera specimens (see Table 1) consist~~ed~~ of ~~130~~ 1548 identified ASVs after ~~removal of very low abundance ASVs (< 0.1 % abundance averaged cross all samples)~~filtering. ~~The raw 16S metabarcoding dataset was submitted to the NCBI Sequencing Read Archive (BioProject accession PRJNA984332).~~ By far the most prevalent ASVs in the foraminifera are those from chloroplasts (averaging ~~55.7~~53.3 %), and particularly diatom chloroplasts (averaging 44.5%)~~52.7 %~~). In the water column, Proteobacteria ASVs ~~represent~~ are the most abundant ~~ASVs in the water column~~ (averaging ~~49.8~~41.2 %~~.~~). ~~(~~Fig. 3).

### 3.2.1 Multivariate analyses of foraminiferal versus water column ASV composition.

~~Multivariate analysis determined that the composition of ASVs between the two provenances were significantly different (Bray Curtis, *mvabund*: LRT=574.3, P<0.001; A*donis*: F.model=24.703, Pr(>F)=0.001).~~ Multivariate analysis to compare provenances was carried out on two samples sets. Firstly, sample set "FW" comprised of all water column samples and foraminifera from those stations where water was collected (28 water samples and 11 foraminiferal samples). Secondly, sample set "101" comprised of water and foraminifera from station 101 only (seven foraminiferal samples and nine water samples). Analysing sample set 101 removed "station" as a variable whist providing sufficient numbers (nine water samples and seven foraminifera) to statistically verify whether the ASV compositional differences between provenances were significant within a single water column.

Multivariate analysis and ordination of the FW sample set (Fig. A2) indicated that there was a marginally significant difference between the ASV composition of the foraminifera versus the water column, with provenance driving 9.7% of the variability (F.model=3.99, Pr=0.024, Table A1). Testing the effect of station on the FW sample set indicated no significant differences (F. Model 0.93, Pr=0.375, *Adonis*, Table ~~A1and A2~~A1). For the 101 sample set (Fig. ~~A2~~A3), there was a significant difference between provenances, with 41.7 % of the differences in ASV composition driven by provenance (Pr=0.001, *Adonis*, Table A1).

### 3.2.2 Differential abundance analysis of ASVs in foraminifera versus the water column

Deseq2 fold change analysis of the FW sample set identified that 572 of 1207 ASVs are driving the significant compositional differences between provenances (P<0.05). All but 13 of those 572 ASVs are significantly more abundant in the water column. These include three Firmicutes (ASVs 1402, 609, 391), three Gammaproteobacteria (ASVs 927, 420, 116), two Bacteroidota (ASVs 743, 46), two Actinobacteriota (ASVs 1403, 509), one chloroplast ASV of unknown origin (ASV 1538) and two chloroplast ASVs from Class Bacillariophyceae (ASV355, *Fragilariopsis cylindricus*, and ASV 956, *Chaetoceros gelidus*) (Table A2, ~~Figure~~ Fig. 4~~X~~4).

Deseq2 fold change analysis of ~~sa~~the 101 sample set identified 393 of 1013 ASVs that are driving the significant compositional differences between provenances (P<0.05). All but 14 of those are significantly more abundant in the water column. These include three Firmicutes (ASVs 1402, 609, 391) five Gammaproteobacteria (ASVs 927, 194, 149, 133, 116), two Bacteroidota (ASVs 743, 46) one Actinobacteriota (ASV509), one chloroplast ASV of unknown origin (ASV472), and two chloroplast ASVs from Class Bacillariophyceae (ASV355, *Fragilariopsis cylindricus*, and ASV 956, *Chaetoceros gelidus;* Table A2, ~~Figure~~ Fig. A3A4). Importantly, ten of the ASVs that are significantly more abundant in the foraminifera are supported~~o~~ in both datasets.

~~Table 3.significantly based on DESeq2 analysis Eight of 13 ASVs remained significant across these groupings, collapsing into Genera, and Families that remained significant. These include ASVs from bacterial Families Streptococcaceae, Dietziaceae, Vibrionaceae, and ASVs from the diatom families Bacillariaceae and Chaetocerotaceae3).~~

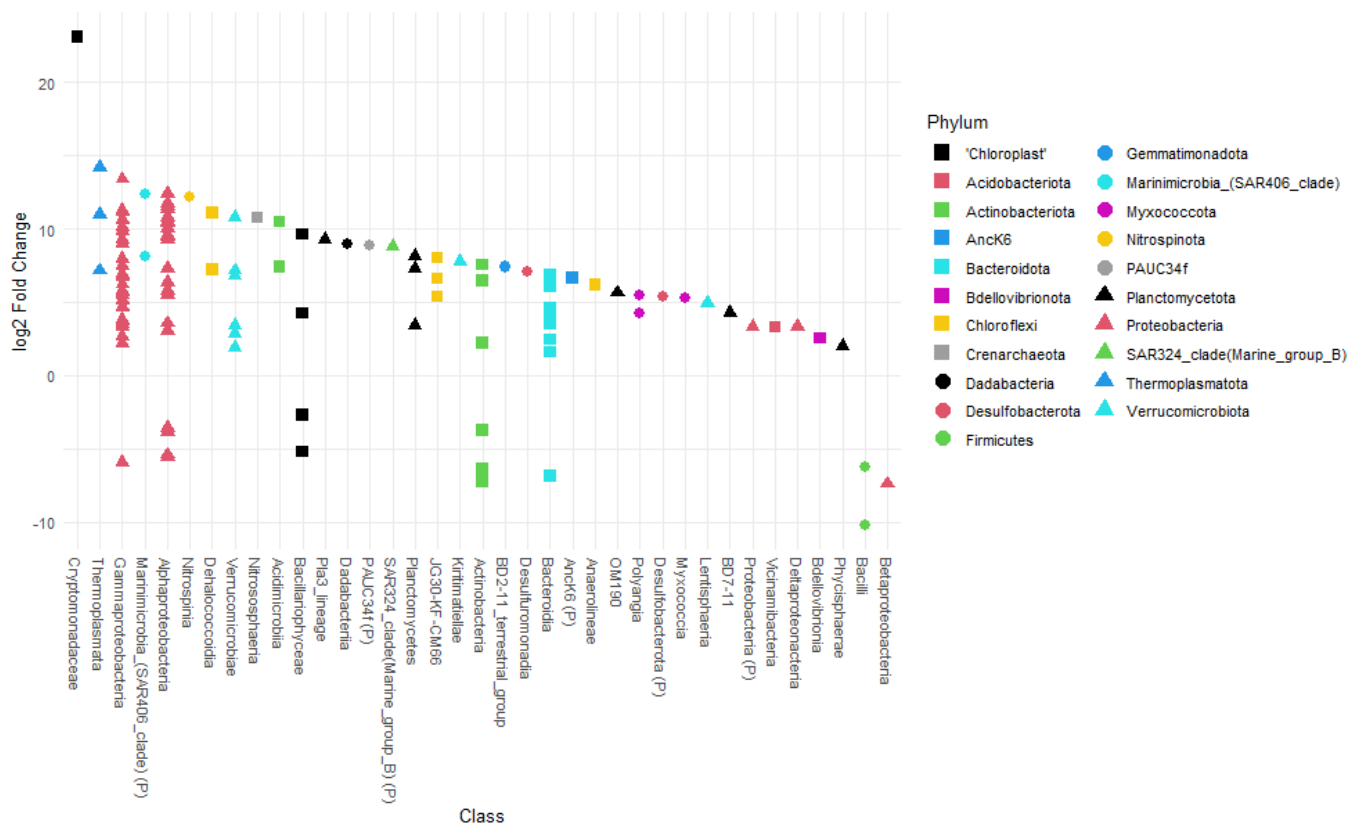



Figure 4. Differential abundance testing of FW dataset ASVs between provenances using *DESeq2*. The Log2 fold change in ASVs is the log-ratio of the ASV means in the water column and foraminifera. ASVs with positive Log2 fold change are significantly more abundant in the water column assemblage and ASVs with negative values indicate ASVs which are significantly more abundant in the foraminiferal assemblages. The Class, or the highest level of taxonomic assignment available for each ASV is given on the x-axis. (P) =Phylum

~~One ASV (ASV 927; *Acinetobacter* sp.) remained significant at both ASV and Genus level. A further ASV (ASV46, Family Chitinophagaceae) was not significant at the Genus level but appeared again to drive significant difference at the Family level. Three ASVs were not significant at any other taxonomic level (ASV1538, unknown Chloroplast, ASV420 *Oleispira* sp; ASV743 *Fluviicola* sp.)~~

~~Several additional Genera were more abundant in the foraminifera than the water column and continued to be so at the Family level including *Undibacterium* sp. (ASV317); *Lactobacillus* sp. (ASV937),~~

350

### 3.2.3 Differential abundance analysis of predicted functional pathways in the foraminiferal microbiome versus the water column assemblage.

PICRUST2 was used to identify possible functional pathways (using the MetaCyc database, Caspi et al., 2014) and to calculate functional pathway abundances based on estimated abundances of gene families that can be linked to reactions within those

360  pathways. 415 pathways were identified, and their differential abundances were compared between the microbiome of *N. pachyderma* and the water column assemblage using ALDEx2. Ninety-two pathways were identified as significantly differentially abundant between the two provenances (effect size >1 and Benjamini-Hochberg adjusted P<0.05). These 92 pathways were grouped according to metabolic types to identify broader metabolic processes that were significantly different within the two provenances (Fig. ~~4~~5). Of the 92 pathways, 38 were significantly more abundant in the foraminiferal

365  microbiome.  They include L-lysine biosynthesis (PWY-2941), peptidoglycan synthesis and recycling (four pathways, 897

ASVs), carbohydrate degradation (nine pathways, 624 ASVs), fermentation (four pathways, 496 ASVs) and the production of the secondary metabolite palmitate (PWY-1479, 4 ASVs) and butanediol production (two pathways, 49 ASVs, Fig. 5). Table A2 identifies those pathways identified in the significantly differentially abundant ASVs. The remaining 54 pathways were significantly more abundant in the water column assemblage, including 14 pathways involved in aromatic compound degradation, pathways for co-factor carrier and vitamin biosynthesis, inorganic nutrient metabolism, nucleoside and nucleotide biosynthesis, fatty acid and lipid biosynthesis, and C1 compound utilisation.

370

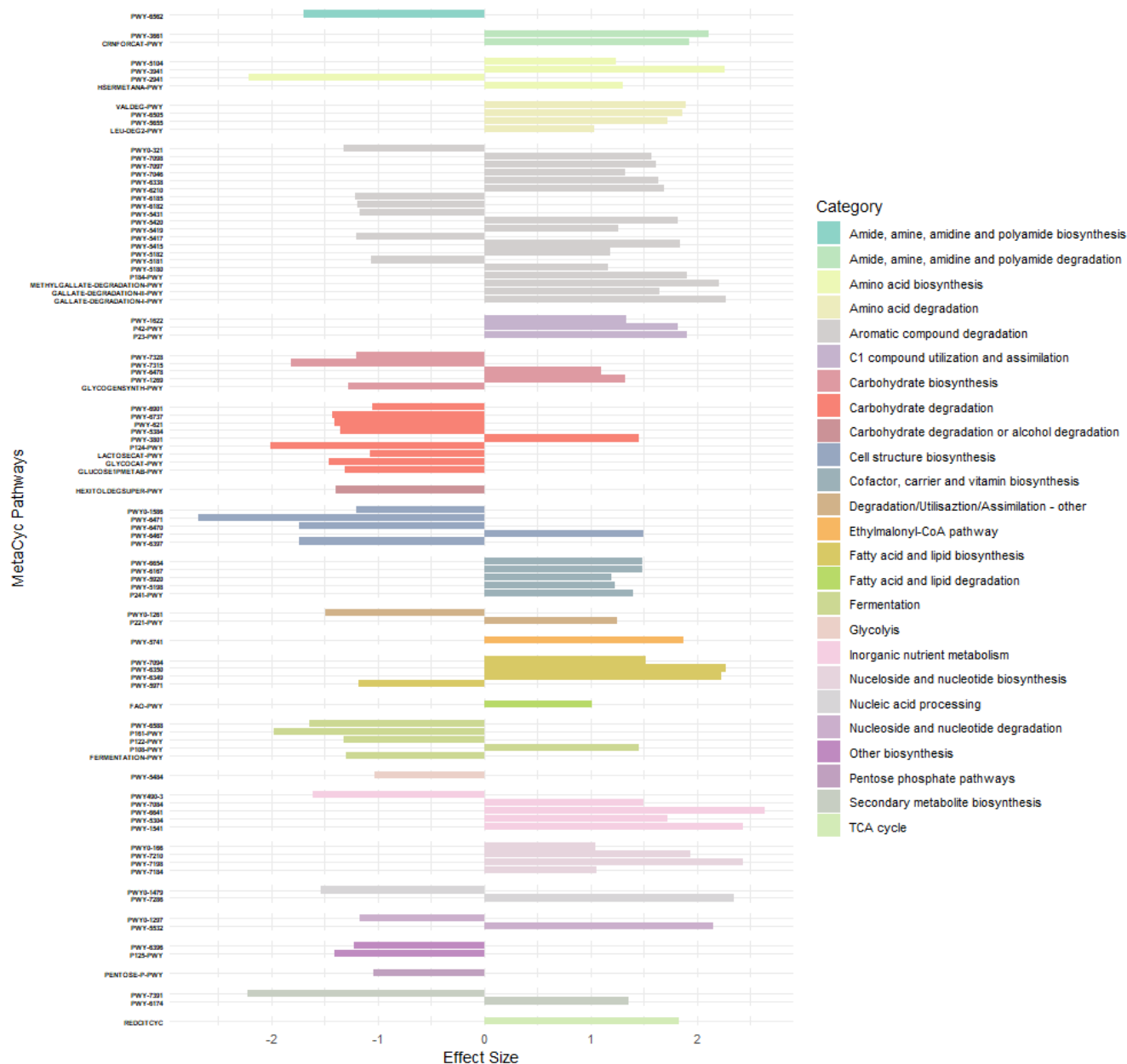



Figure 5. MetaCyc Pathways identified as being significantly differentially abundant between the two provenances are shown (y-axis) with their 'Effect Size' indicated by the x-axis. Negative values indicate the pathway is more abundant in the foraminiferal microbiome and positive values indicate the pathway is more abundant in the water column assemblage. Pathways are grouped by broader metabolic categories identified in the key.

## 3.3 Foraminiferal ASV profiles

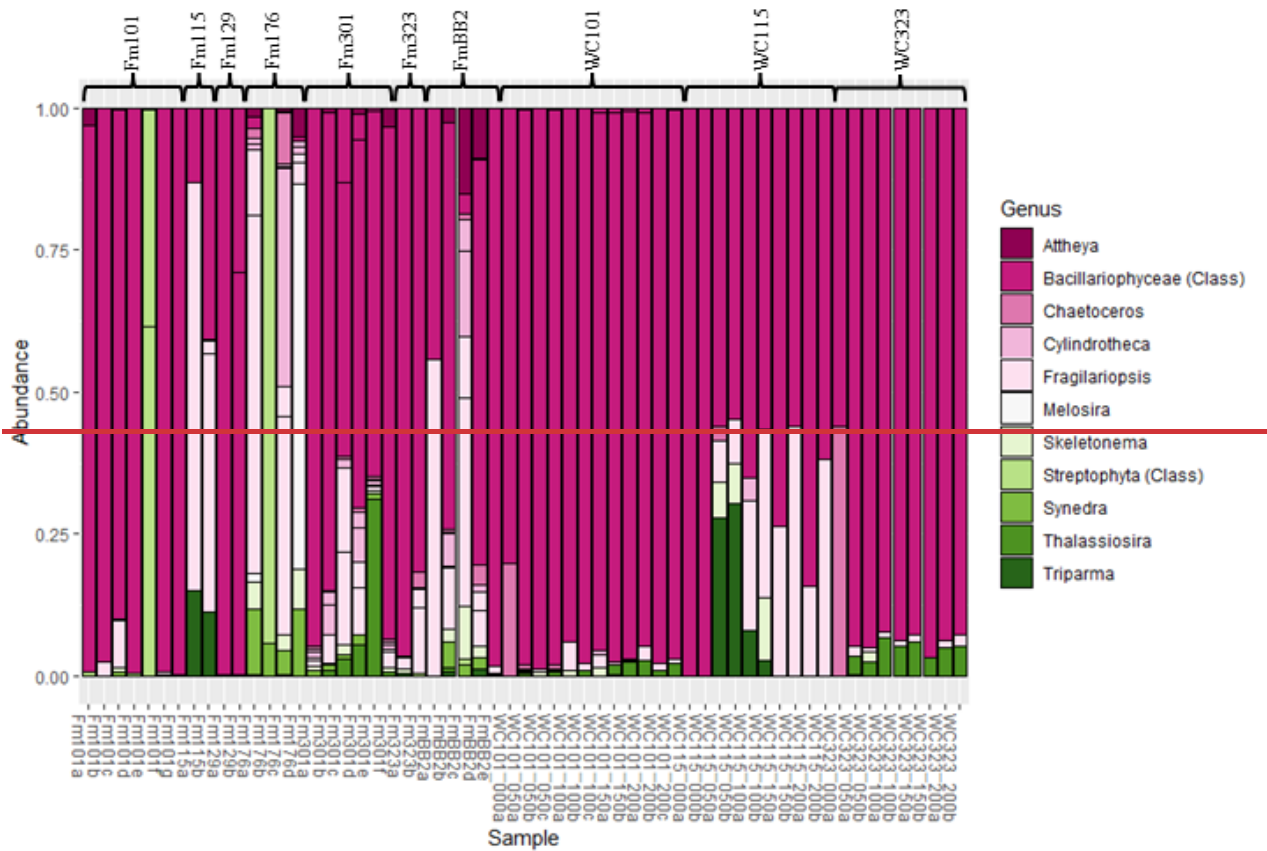
All the foraminifera were genotyped as *N. pachyderma* Type I (NCBI GenBank Accession numbers OR137988-OR138014), consistent with it being the only genotype found in the Arctic region to date (Darling et al., 2004; 2007). Since foraminifera were sampled by vertical net tow from 200 m depth to the surface, no depth correlation data are available to explore the role of depth on foraminiferal microbiome composition. However, foraminifera were sampled from seven stations and a robust Aitchison dissimilarity PCA plot showed clustering of foraminifera by Station (*Adonis*: F. model= 3.263, Pr(>F) = 0.004), with 48.3% of the variation in the foraminifera driven by station (Table A1, Fig. A5). ~~However, However, since station accounts for 17 % of the variation across water samples, we might expect to see some variation in specimen ASV profiles between foraminifera from different stations too. A Bray Curtis dissimilarity NMDS plot (Fig. A4) shows a degree of clustering of foraminifera by station (*Adonis*: F. model= 3.079, Pr(>F) = 0.001), with 49 % of the variation in the foraminifera driven by station. Of note are two specimens, Fm176b and Fm101e, which contained only 11 and nine distinct ASV respectively. All other foraminifera contain 22 or more ASVs. The phyla with some ASVs showing higher abundance counts in the foraminifera compared to the water column are Planctomycetes, Firmicutes, Bacteriodetes, and the group "Chloroplast" (Fig. A5).~~

### 3.3.1 Bacterial ASV profiles in *N. pachyderma*

ASVs were assigned to 1367 distinct prokaryote taxa across all water and foraminifera samples within 29 Phyla and 60 Classes. The major groups were Class Gammaproteobacteria which contributed on average 18.8 % of ASVs, Phylum Bacteroidota 14.6 %, and Class Bacilli (Phylum Firmicutes) 5.1 %. The only other groups that contributed >1 % of ASVs are Phylum Actinobacteria, Class Alphaproteobacteria and Class Verrucomicrobiae. The other 545 classes all contribute < 1 % each to the ASV total. This distribution reflected the ASVs driving significant compositional differences between the provenances (ASVs from Class Gammaproteobacteria, Phylum Bacteroidota, Phylum Firmicutes and Phylum Actinobacteria. Section 3.2, Table A2).

### 3.3.2~~1~~ Chloroplasts ASV profiles in *N. pachyderma*

ASVs are assigned to ~~17~~ 181 distinct chloroplast ASVs corresponding to 14 unique chloroplast-containing ~~taxa~~ Classes across all water and foraminifera samples, contributing, on average, ~~55.7~~53.3 % of all ASVs in the foraminifera and only ~~2.97~~3.51 % in the water column (Fig. 3). More specifically, those ASVs assigned to diatom~~-~~ chloroplasts (Class Bacillariophyceae) contributed on average to ~~52.7~~44.5 % of all ASVs in the foraminifera and only 2.~~92~~36 % in the water column, highlighting the major importance of diatoms ~~(Class Bacillariophyceae)~~ in the diet of the foraminifera compared to other phytoplankton taxa. ~~Of the 17 chloroplast ASVs identified, 13~~Three chloroplast ~~-~~ASVs drive the significant difference between the

foraminifera and the water column, due to greater abundance in the foraminifera. These are ~~chloroplasts from~~ one ASV (ASV1538) identified only as "Chloroplast", and two diatom ASVs identified as *Fragilariopsis cylindicus.* (ASV355) and *Chaetoceros gelidus* (ASV956) ~~three *Cylindrotheca* sp. (ASV18, ASV31 and ASV23), three *Fragilariopsis* sp. (ASV47, ASV84 and ASV59), *Synedra hyperborea* (ASV35), a *Melosira* sp. (ASV111), an *Attheya* sp. (ASV16), a single ASV from the Skeletonema family (ASV99), two ASVs identified only to the Bacillariophyceae class of diatoms (ASV14 and ASV15) and a *Chaetoceros* sp. (ASV102). The most statistically significant (in driving the differences between the foraminifera and the water column) are the two ASVs belonging to a *Cylindrotheca* sp. chloroplasts (ASV18) and a *Fragilariopsis* sp. chloroplast (ASV47; (~~Table ~~A1~~A3). The relative abundance of chloroplast ASVs in each sample is shown in ~~Figure ~~Fig. 6~~5~~.

420

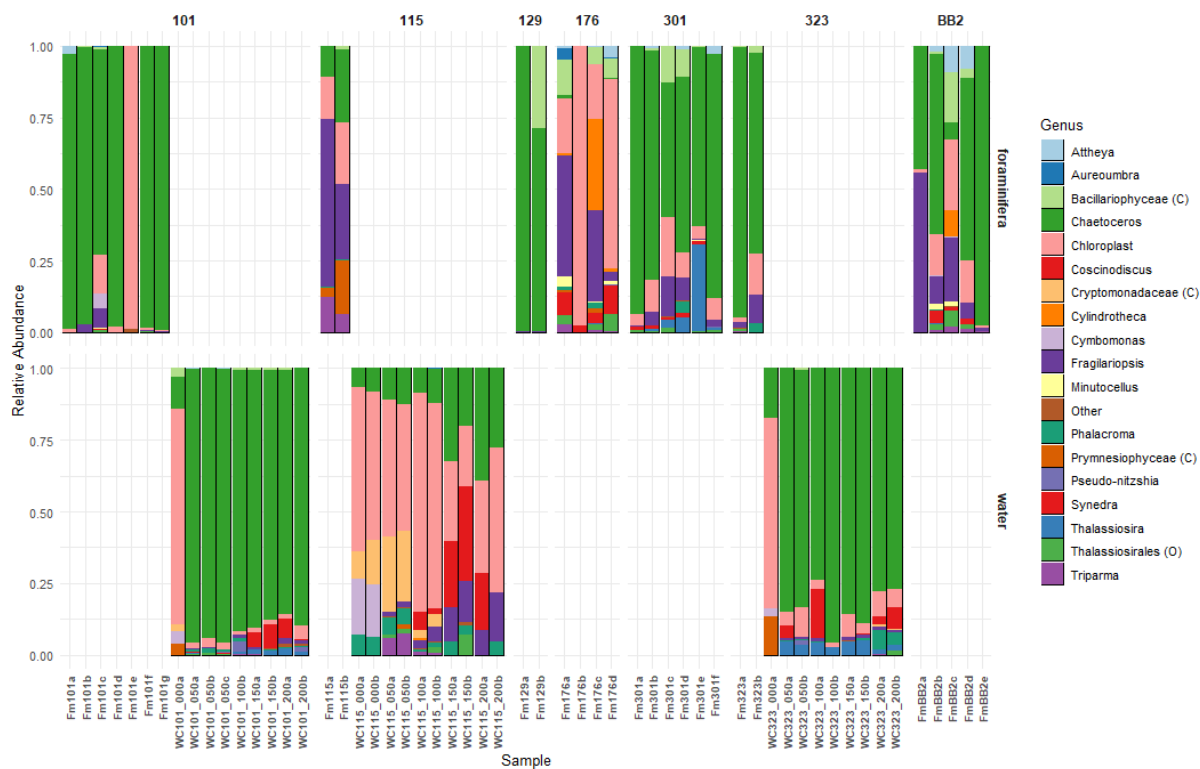



Figure 6. The relative abundance of the 13 chloroplast genera identified with a relative abundance above 2%, along with three classes, and one order identified only to these taxonomic ranks. ASVs with a relative abundance across all samples of less than 2% were grouped in the "Other" category, and ASVs that could not be taxonomically assigned beyond "Chloroplast" are grouped as such. Bars represent individual samples (foraminiferal specimens =Fm and the water column =WC. Numbers above indicate stations separated by columns and provenance is separated by row~~Figure 56. The relative abundance of the 17 chloroplast 16S rDNA ASVs generated from the foraminiferal specimens (Fm) and the water column (WC).. Taxa are shown at the genus level, except where noted in the key Black lines in bars indicate divisions of ASVs. Brackets at top indicate stations from which samples were taken, e.g. Fm101 are foraminifera samples from station 101, whereas WC101 are water samples from station 101.~~

ASV956 was ~~T~~the most abundant chloroplast ASV ~~(with a mean relative abundance across all samples of 57.6% when analysing chloroplast ASVs only. comprising > 90 % in some samples) is ASV15,~~This was identified ~~only to the level of~~ to the level of ~~c~~Class Bacillariophyceae (Fig. ~~5~~6) by the SILVA database, but with 100 % identity and coverage to the 16S rDNA of the diatom *Chaetoceros gelidus* (accession NC_063631.1) via BLASTn search. ASV 956~~It~~ ha~~ds~~ a significant Log2 fold change of ~~-4.934~~2.348 (padj = ~~7.35E-17~~0.003443). This ~~is~~ was not as significant as ~~for some other diatom~~the other two chloroplast ASVs (Table ~~A1~~A3), probably due to its higher relative abundance in the water column (Fig. ~~5~~6). ~~In a Blastn~~

search, this ASV corresponds 100 % identity and coverage to the chloroplast 16S rRNA gene of the diatom *Chaetoceros gelidus* (accession NC_063631.1). ~~ASV15~~ ASV956 ~~is~~ was common within Baffin Bay ~~as it shows~~ with an average relative

440  abundance of 57.2 % ~~>80 % relative abundance~~ (when analysing chloroplasts only) in the water column samples ~~from all but station 115, which comprises > 50 % ASV15~~ (Fig. ~~5~~6). ~~ASV15~~ ASV956 ~~is~~ was therefore relatively common in both the water column and the foraminifera and ~~is~~ was clearly a major food source for *N. pachyderma* in Baffin Bay during the summer months.

At station 101 (west of the Pikialasorsuaq polynya; Fig. 1), ~~six~~ five out of the seven *N. pachyderma* specimens contained > ~~45~~

445  50 % chloroplast ASVs, and only one specimen contained < 20 % chloroplast ASVs (Fm101a; Fig. 3). When analysing only chloroplast ASVs, six of the seven foraminifera specimens contained >~~80~~ 70 % ~~ASV15~~ ASV956 (*Chaetoceros gelidus*). Specimen Fm101e, however, contained over ~~99~~ 94 % chloroplast ASVs (472, 548) belonging to two uncharacterised chloroplasts. ~~streptophyta~~ (Fig. ~~5~~6). ~~Streptophyta ASVs were only found in one other foraminifera, Fm176b at station 176, at the most southerly cruise station for which we have no water column 16S data. In fact, despite containing a streptophyta ASV,~~

450  ~~specimen Fm176b contained a very low relative abundance of chloroplast ASVs (Fig. 3).~~ Except for specimen Fm101e, the diatom ASVs at the cooler station 101 can be said to mirror the diatom population profile in the sub-surface water column where > ~~90~~ 85 % of water column chloroplast ASVs were ~~ASV15~~ ASV956 (*Chaetoceros gelidus*) (Fig. ~~5~~5).

The two foraminifera processed at station 115 (where Atlantic-derived warmer water is found) contained <~~30~~ 35 % and <~~10~~

13 % chloroplast ASVs (Fig. 3). Again, this lower chloroplast relative abundance ~~reflects~~ reflected the water column where

455  we ~~find~~ found the lowest relative proportion of chloroplast ASVs across the three stations (Fig. 3). *Fragilariopsis* sp. contributed the greatest proportion of chloroplast ASVs (ASV355) in station 115 specimens (Fig. ~~5~~6). The proportion of *Fragilariopsis* ASVs ~~are~~ were higher in the water column at this station relative to other stations, although ~~ASV15~~ ASV956 (*Chaetoceros gelidus*) ~~remains~~ remained the major diatom ASV present. However, the highest proportion of ASVs were not identified beyond "Chloroplast" at this station. In addition, a non-diatom chloroplast (*Triparma laevis* ASV471) ~~is~~ was also

460  present in both the water column and the foraminiferal specimens. This is a relative of the diatoms, and, like most diatom species, forms external siliceous plates. *Triparma laevis* ASVs ~~are~~ were only detected in the upper water column at 50-100 m.~~, and therefore it is highly likely that the two foraminiferal specimens were also collected from this depth.~~

Finally, ~~>50~~ 48 % and 28 % of the ASVs in the two foraminifera from station 323 ~~are~~ were chloroplast ASVs (Fig. 3), and of those, ~~>90~~ 94.5 % and 69.8 % ~~are~~ were ~~ASV15~~ ASV956 (*Chaetoceros gelidus*; Fig. ~~5~~6). This ~~reflects~~ reflected the high

465  proportion of ASV956 in the water column at this station (>73 % across sub-surface samples).

Of the other foraminiferal specimens taken from stations with no comparative water column data, the foraminifera from station 176 show~~ed~~ the greatest diversity in chloroplast ASVs. Except for Fm176b which had only 3 chloroplast ASVs, Fm176a, c, and d ~~They~~ contained a much higher relative proportion of *Fragilariopsis*~~,~~ *Synedra* ~~*Melisosira,*~~ and *Cylindrotheca* diatoms and other chloroplast ASVs~~, and Streptophyta across the different specimens~~. This may indicate higher comparative diatom

470  and algal ~~(Streptophyta)~~ diversity at this more southerly station.

### 3.3.3 The *N. pachyderma* core microbiome

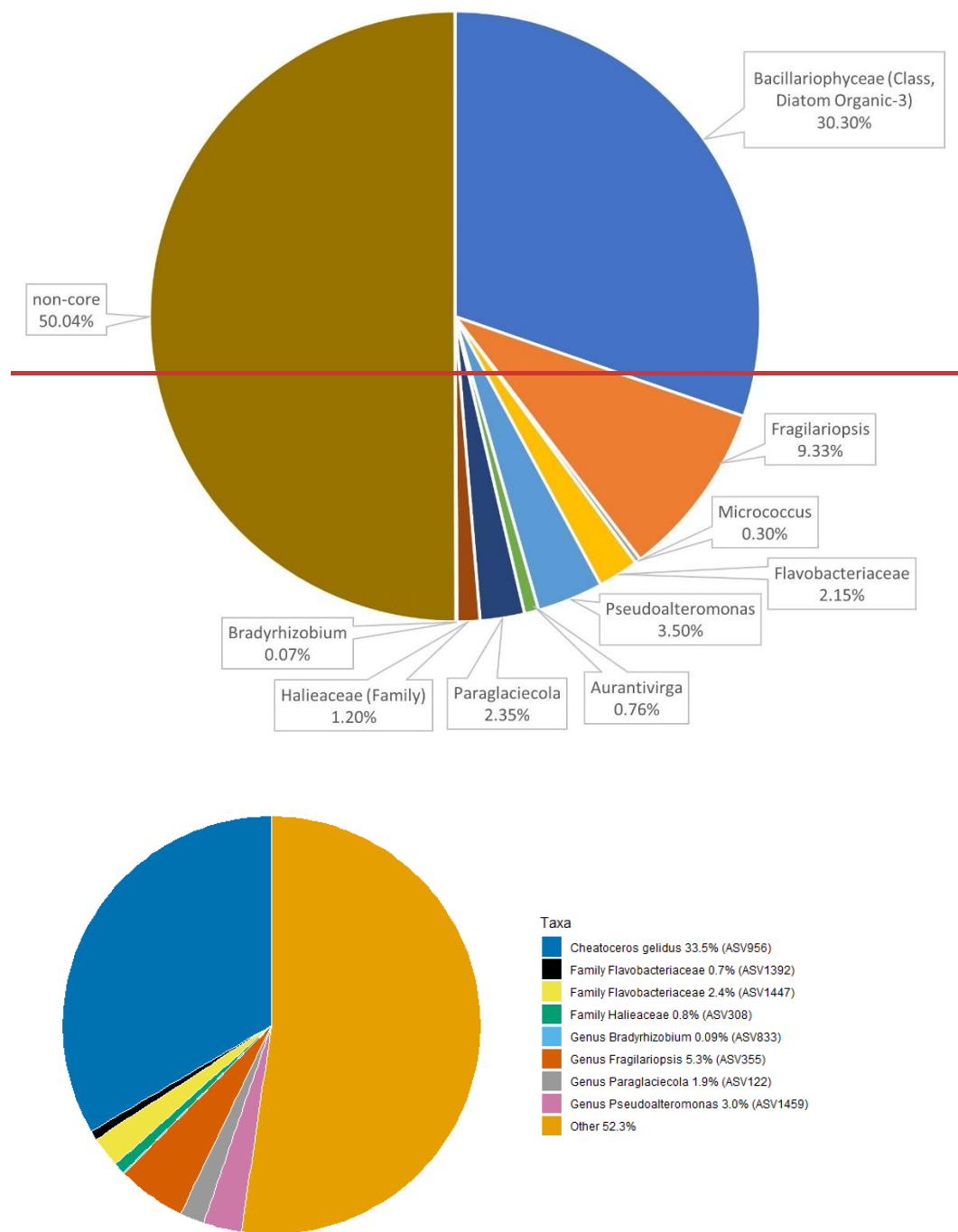
The core microbiome of *N. pachyderma* is defined here as ASVs found in 80 % of the foraminiferal specimens across all
480 stations. ~~This core microbiome could be made up of organisms which (i) the foraminifera specifically target for food, or (ii) are routinely passively ingested due to close association with specific food sources, or (iii) are endo(sym)bionts.~~ 16S metabarcoding indicate~~s~~d that there ~~are~~ were ~~nine~~ eight core ASVs. Two ~~are~~ represented by diatoms: ~~ASV15~~ASV956, *Chaetoceros gelidus* (27/28 specimens) and ~~ASV59~~ASV355, identified in BLASTn as *Fragilariopsis cylindricus* (100 % match to accession NC_045244.1, 24/28 specimens). Then ~~seven~~ six bacterial ASVs, two from the Flavobacteriaceae family
485 (ASV1392, 24/28 and 1447, ~~ASV19,~~ 23/28), the genus *Pseudoalterom*o*nas* (ASV 1459~~ASV26~~, 25/28), ~~genus *Aurantivirga* (ASV27, 24/28),~~ the genus *Paraglaciecola* (ASV 122~~ASV34~~, 25/28), the family Halieaceae (ASV308~~ASV74~~, 26/28), ~~the genus *Micrococcus* (ASV10, 25/28)~~ and genus *Bradyrhizobium* (ASV833~~ASV116~~, 23/28; Fig. ~~6~~7). Of these ~~nine~~ eight ASVs, only the two diatom ASVs (355 and 956) ~~six are~~ were also significantly more abundant in the foraminifera than the water column, driving the significant differences between the provenances. ~~These are, in order of significance, *Micrococcus* ASV10,~~
490 ~~*Fragilariopsis cylindrica* ASV59, *Bradyrhizobium* ASV116, *Chaetoceros gelidus* ASV15, Flavobacteriaceae family ASV19, and Halieaceae ASV74.~~ This foraminiferal core microbiome ~~makes~~ made up, on average ~~49.6~~47.7 % of the ASVs in the *N. pachyderma* of Baffin Bay, whereas it ~~makes~~ made up only ~~10.89~~9.42 % of ASVs in the water column. Details on the core microbiome including relative abundances and ASV frequencies in the two provenances can be seen in Table A3.

495

Figure 67. The average relative abundances of the 16S rDNA rDNA ASVs found across *N. pachyderma* Type I specimens in Baffin Bay during summer 2017. The average relative abundance of the nine eight core ASVs (found in ≥ 80 % of specimens)

are taxonomically labelled and designated the "core microbiome". These make up ~~49~~47.~~6~~7 % of the ASVs in the microbiome. 5~~2~~0.~~4~~3 % are ASVs found in fewer than 80 % of specimens, designated as non-core and ~~and are designated~~ labelled "~~non-core~~Other" above. Colour key starts at 12 O'clock and runs anticlockwise.

### 3.4 TEM analysis

~~16S metabarcoding data indicate that diatoms are the majority diet for *N. pachyderma*.~~ TEM imaging was carried out on samples collected during the 2018 cruise (Fig. 1; Table 1) to further investigate the diet/endobionts in this genotype. Whole diatoms, including frustules, were observed within the foraminiferal cell (Fig. ~~8a~~8a)~~, although the pyrenoid dissecting lamellae characteristic of diatom chloroplasts were not visible in our fixed samples~~. Empty frustules were also observed both inside and outside the foraminiferal cell. Those outside may have been ejected after the diatom organic material was digested/removed (Fig. ~~7b~~8b) or were part of the diatom-derived POM feeding cy~~ast a~~nd were likely caught in the external cytoplasm and rhizopodial network at the time of sampling and fixation, as has been reported previously (Spindler et al., 1984). These observations support the previous literature indicating that *N. pachyderma* consumes ~~diatoms~~diatoms and given the intact nature of the diatoms observed (Fig. 8a), it is not only detrital (dead) diatoms that are consumed, as reported by Greco et al. (20~~21~~).

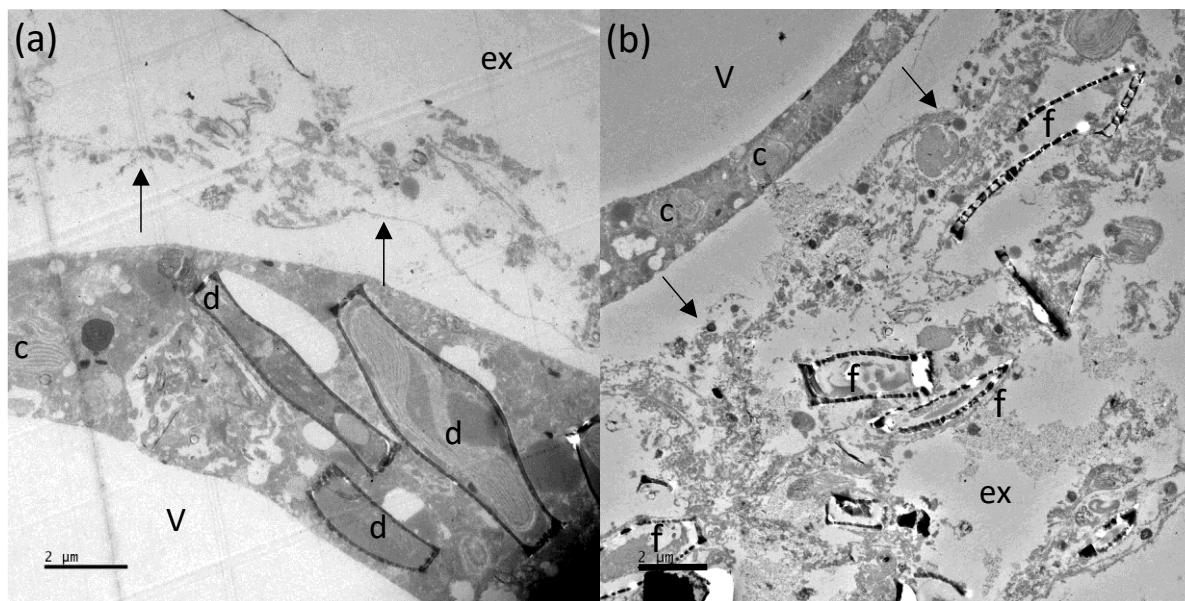



Figure ~~7~~8. TEM images of ~~three individual~~ *N. pachyderma* ~~specimens~~(specimen BB2, Table 1) showing internal and external cell and diatom structures~~relation to diatoms~~. Image **(a)**: Intact diatoms (d) observed inside the specimen cell ~~BB2 (Table 1)~~. ~~The outer membrane is identified by the black arrows, where the pore shape can be observed~~Black arrows indicate the cross section of pore plugs in the inner organic lining~~..~~. ~~v~~v = internal cell vacuole~~.~~, ex = external to the foraminiferal cell. Image **(b)**: External (ex) to specimen ~~BB2 (Table 1)~~ where debris, including empty diatom frustules (f), is apparent. The ~~outer membrane~~organic lining is identified by black arrows, c = chloroplasts inside BB2, v = internal vacuole. Large vacuoles were observed in several specimens which may be a result of the fixation process. Scale bars at bottom left ~~bar~~are ~~of 8a and 8b~~2µm.

~~In addition, the~~ TEM images also show that *N. pachyderma* contains unexpectedly high numbers of chloroplasts throughout the cell from the cell periphery to the cell centre (Fig. ~~7c~~9a-b~~, 7d & 7e~~, Fig. ~~A6~~A6). The level of preservation does not allow us to observe the number of membranes surrounding the chloroplasts or~~,~~ the pyrenoid-dissecting lamellae. ~~Due to this limited level of preservation of the foraminiferal specimens, unfortunately we cannot unequivocally determine the degradation state of all the chloroplasts present, although many appear to be in a state of degradation.~~ ~~or the degradation state of each chloroplast.~~ Nevertheless, although we cannot unequivocally determine the degradation state of all the chloroplasts present, lenticular pyrenoids, and horseshoe-shaped arrays of thylakoid membranes are visible in many chloroplasts (Fig. ~~8~~9a), as found in *Chaetoceros* spp. (Bedoshvili et al., 2009). ~~in several of the chloroplasts, obvious lenticular pyrenoids, and horseshoe-shaped arrays of thylakoid membranes are visible (Fig. 7d 9~~8a~~& 7e) as found in *Chaetoceros* spp. (Bedoshvili et al., 2009).~~ There are also abundant lipid droplets located amongst, and immediately adjacent to the chloroplasts at the cell periphery potentially indicating lipid production by the chloroplasts (Jauffrais et al., 2019a, Fig. ~~7d~~9~~9~~a).

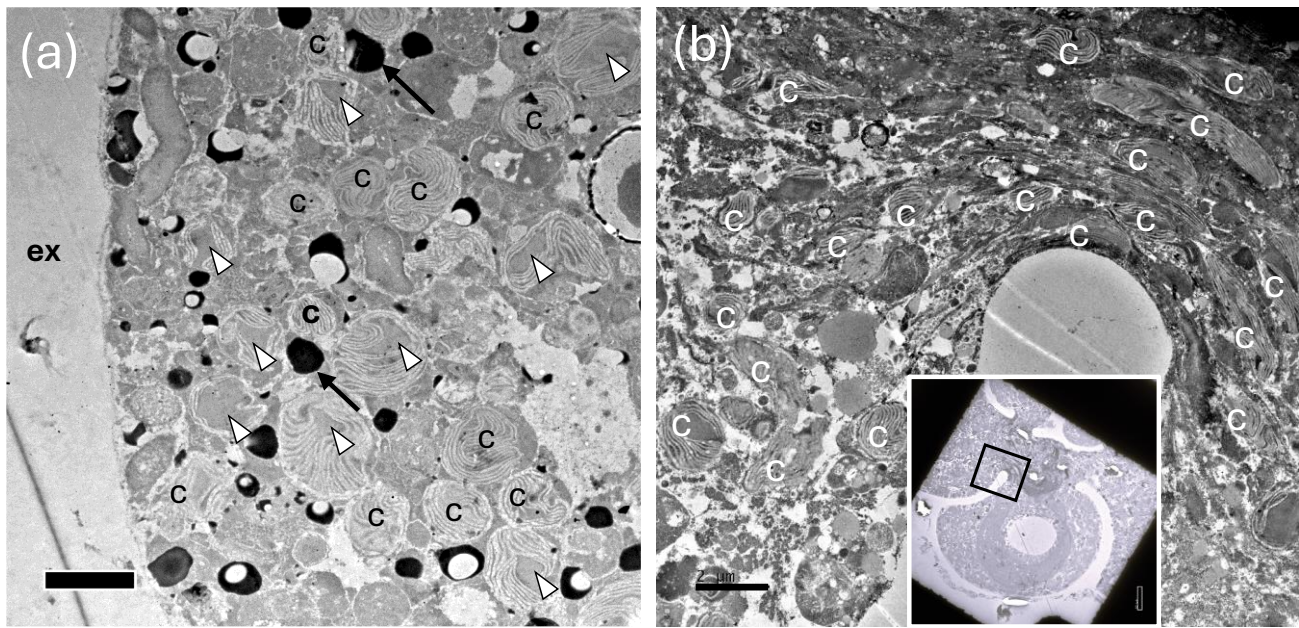



Figure 9. TEM images showing abundant chloroplasts throughout the *N. pachyderma* cell. ~~(e) Overview of thin section of BB11 (Table 1) showing chambers and identifying region of cell shown in (e). Scale bar 10μm.~~ (**~~d~~a**) Clusters of~~Many~~ chloroplasts (c) within the~~inside~~ specimen BB1 cell (Table 1) close to the cell periphery. Those~~Additional chloroplasts~~ with obvious pyrenoids are marked~~indicated~~ with ~~a~~ white arrowheads. Black spots are lipid droplets (black arrows highlight examples ~~points to an example~~). ex = external to the foraminiferal cell. Scale bar ~~1μm~~2μm. (**Inset**) Overview of thin section of specimen BB11 (Table 1) showing chambers and a black square identifying the region of the cell shown in (**b**). ~~right hand side) 10μm.~~(**~~e~~bb**) Chloroplasts ~~are~~ clustered~~found~~ in numbers ~~The~~at the ~~at~~ centre of specimen BB11 ~~(Table 1)~~also~~.~~ ~~Image location within the foraminifera is identified by black box in (c). Chloroplasts (c) are present, and~~appear stretched~~,~~ at the core ~~of the cell~~ where the chambers coalesce. Scale bar 2μm. ~~Additional chloroplasts with obvious pyrenoids are identified by black arrowheads. (c) Overview of thin section of BB11 (Table 1) showing chambers and black square identifying region of cell shown in (b). Scale bar (right hand side) 10μm.~~

## 4 Discussion

In this study, our aim ~~has been~~was to investigate the microbiome within the polar planktonic foraminifera *N. pachyderma* Type I from the Arctic Baffin Bay region. We define~~d~~ the microbiome as the combined taxa identified by taxonomic assignment of 16S ASVs generated by metabarcoding. This ~~will~~ include~~d~~ food, any endo(sym)bionts, and chloroplast-containing eukaryotes identified by their chloroplast 16S ASVs. Shedding light on their feeding preferences as well as any microbial associations that form part of the "interactome" in the context of the changing climate may afford some clues as to the ability of *N.*

*pachyderma* to withstand/adapt to its rapidly changing environment, and its contribution to the carbonate cycle and ocean alkalinity. For example, eco-physiological and trait-based models indicate that symbiont-~~-~~barren foraminifera, which *N. pachyderma* is understood to be, are predicted to experience reduced numbers and habitat decline (Roy et al., 2015), and the non-spinose species biomass is likely to be reduced by up to 11 % by 2050 (Grigoratou et al., 2022). Sound knowledge of the

560 eco-physiology and traits of foraminifera is required for model accuracy, and to that end the genotype and ~~16S~~ microbiome of the Arctic polar *N. pachyderma* ~~has~~ wasbeen investigated.

## 4.1 Divergent Feeding strategies in the Neogloboquadrinids

Our findings support previous literature stating that *N. pachyderma* feeds on diatoms (Spindler and Dieckmann, 1986; Schiebel and Hemleben, 2017, Greco et al., 2021). This study and that of Greco et al., (2021) indicate that *N. pachyderma* feeds

565 predominantly on diatoms, (~~c~~Class Bacillariophyceae), and occasionally other algae (e.g., ~~streptophyta~~ *Triparmia laevis* - this study). However, our 16S study further ~~reveals~~ revealed that *N. pachyderma* also consume~~d~~ bacteria, ~~since~~ and their ~~bacterial 16S~~ ASV composition ~~is~~ was significantly different from the water column profile (Table A1). This is most likely driven by ~~selectivity in~~the ~~fee~~ feeding behaviour, where particulate organic matter (POM) is gathered around the shell to form a feeding cyst. Once formed, the foraminifer sits within the POM microhabitat, becoming isolated from the water column. This behaviour

570 has already been observed in the Neogloboquadrinids *N. dutertrei* and *N. incompta* (Bird et al., 2018; Fehrenbacher et al., 2018), and in *Globigerinita glutinata* (Spindler et al., 1984).

Although the Neogloboquadrinids all feed within the POM microhabitat, we suggest that they are feeding on very different components within the cyst. Work carried out by Bird et al. (2018) ~~demonstrates~~ demonstrated that of the three Neogloboquadrinids, *N. incompta* ~~contains~~ contained the highest proportion of bacterial ASVs (>99.8 %), indicating that it

575 targets the bacteria within the POM microhabitat. The small proportion of chloroplast ASVs (<0.2 %) in the *N. incompta* study indicate~~d~~ that POM ~~is~~ was not being passively phagocytosed, but that the bacteria, rather than algae ~~are~~ were being specifically selected as food. In contrast, *N. dutertrei* contained only 2~~ %~~-4 % bacterial ASVs and instead ~~maintains~~ maintained a pelagophyte algal endosymbiont population and selectively fe~~eds~~ on other protists within the POM. The small proportion of intracellular bacteria in *N. dutertrei* indicate~~s~~d that it too ~~does~~ did not specifically phagocytose POM itself. However, in the

580 case of *N. pachyderma*, we ~~find~~ found a higher proportion of bacterial ASVs than was identified in *N. dutertrei* and a higher proportion of chloroplast ASVs than found in *N. incompta*, suggesting that *N. pachyderma* may feed on the POM directly for food. This ~~has been~~was suggested by Greco et al. (2021)~~,~~ who also demonstrated that the *N. pachyderma* 18S ASV assemblage revealed little difference in intracellular diatom ASVs between the surface dwelling and the deeper dwelling specimens living in diatom-free waters. This finding led them to suggest that *N. pachyderma* feeds on ~~detrital~~ dead diatoms contributing to the

585 sinking detritus, which is supportive of a POM-cyst mode of feeding. However, the ~~16S data and~~ TEM images (Fig. ~~7~~8A6) in our study indicate~~d~~ that they also feed on living diatoms, as intact diatoms were observed in their cells. Further, our evidence ~~indicates~~ indicated that a significant component~~one aspect~~ of the *N. pachyderma* diet is -~~they either~~active~~ly~~ or passive~~ly~~

consumptione of the bacteria living in the diatom "phycosphere" and diatom-derived POM (Bell & Mitchell, 1972; see Section 4.2).

590 **4.2** ~~The~~ *N. pachyderma* ~~core microbiome~~bacterial ASVs

**4.2.1 The core microbiome**

The core microbiome (defined as ASVs present across 80 % of the foraminifera) could be made up of organisms which (i) the foraminifera specifically target for food, or (ii) are routinely passively ingested due to close association with specific food sources, or (iii) are endo(sym)bionts. However, the bacteria identified in the *N. pachyderma* core microbiome point to a diatom

595 source and therefore are highly likely to be passively ingested. The core microbiome was composed of just 8 ASVs ~~and~~which ~~All specimens were genotyped as *N. pachyderma* Type I, which is unsurprising given that all specimens genotyped to date from the polar waters of the Norwegian Sea and the Fram Strait have been identified as Type I (Darling et al., 2004; 2007). Two of the specimens, Fm176b and Fm101e (Fig. 3) had a much reduced microbiome compared to all other specimens (11 and nine ASVs respectively, compared to at least 22 in all other specimens). One possible explanation for this is that these~~

600 ~~specimens were close to the end of their life span and had stopped feeding prior to producing and releasing gametes (Fehrenbacher et al., 2018). In the core microbiome analysis, Fm176b was missing seven of eight core ASVs and Fm101e was missing six of eight, highlighting their unusually sparse microbiomes compared to all other specimens (Table 2).~~ accounted for, on average, 47.7 % of ~~its~~the total microbiome. Six ~~This core microbiome could be made up of organisms which (i) the foraminifera specifically target for food, or (ii) are routinely passively ingested due to close association with specific~~

605 ~~food sources, or (iii) are endo(sym)bionts.~~bacterial ASVs contributed small percentages between 0.09 %- 2.96 %, averaging just 8.93% of the core microbiome.

~~Whilst the core microbiome accounts for almost 50 % of the total microbiome in *N. pachyderma,* the relative contributions of the nine core ASVs is highly variable, from 0.09 % 30.3 % (Fig. 6). This variability may reflect the foraminiferal food~~

610 ~~preferences if they are part of the diet, or if endo(sym)bionts, may reflect potential differences in their contribution to cellular activity, their role/function in the *N. pachyderma* cell, or that their involvement could be a function of ontogenetic stage. Of the nine core ASVs, two (ASV15 and ASV59) are taxonomically assigned to diatom chloroplasts. Both contribute to the significant difference in assemblage composition between the foraminifera and the water column since there are twelve times (ASV15) and 236 times (ASV59) more ASV counts in the foraminifera than the water column (Table A3). Chloroplasts were~~

615 ~~exceptionally abundant in our TEM images (Fig. 7 and Fig. A4), which appear very similar to observations made in kleptoplastic benthic species (Jauffrais et al., 2018; Jesus et al., 2022). This raises important questions about the nature of the relationship between the chloroplasts and *N. pachyderma* Type I, which is discussed below. Empty diatom frustules were also observed in the TEM images (Fig. 7), which is highly consistent with previous reports that diatoms are a significant part of the *N. pachyderma* diet (Hemleben et al., 1989; Scheibel & Hemleben, 2017; Greco et al., 2021).~~

The remaining core microbiome consists of diverse bacterial genera (in DESeq2 analysis) (Table A3)The bacterial ASV with the highest average relative abundance in the core microbiome (3%) was *Pseudoalteromonas* (ASV1459, Fig. 87, Table A2A3). *Pseudoalteromonas* are known hydrocarbon degraders (e.g. Calderon et al., 2018). They are the first bacteria to colonise degrading diatom aggregates (Arandia-Gorostidi et al., 2022; Costanzo et al., 2023), and in fact theyse bacteria are known to be algicidal releasing diffusible factors (Costanzo et al., 2023) in the diatom phycosphere. There were also two ASVs from the Flavobacteriaceae family (ASV1447 and ASV1392). This isFlavobacteriaceae are a large family of bacteria that are widely distributed in the marine environment and are often found associated with detritus (as well as algae, fish and invertebrates; Gavriilidou et al., 2020). Tisserand et al. (2020) isolated ten species from Baffin Bay, and all were shown to grow on exudates (dissolved organic matter) from two Arctic diatoms (*Fragilariopsis cylindricus* and *Chaetoceros neogracilis*). Since members of the Flavobacteriaceae and *Pseudoalteromonas* are shown to co-occur with diatoms (Amin et al, 2012) it is likely that *N. pachyderma* passively consumes these bacteria as it feeds on diatom detritus (Greco et al., 2021) and on living diatoms. A BLASTn search (NCBI) identified ASV1392 as 99.6 % identical to a Flavobacteriaceae of the genus *Tenacibaculum* including *T. insulae, T. haliotis and Tenacibaculum* sp. This genus contains many opportunistic fish pathogens, some of which are found to target fish teeth, a high source of calcium shown to promote the bacteria's growth (Hikida et al., 1979; Frisch et al., 2018). Growth promotion by calcium may be a common feature of the *Tenacibaculum* genus and may be another reason why this ASV is identified with *N. pachyderma,* and the calcite tests of foraminifera may provide a suitable niche for this genus. Another core ASV was 308, attributed to the OM(NOR) genus of the Family Halieaceae, order Cellvibrionales, (Spring et al., 2015). The order Cellvibrionales are gram-positive aerobes that are mesophilic and neutrophilic chemoorganotrophs. However, some members of the Family Halieaceae may additionally be capable of aerobic photoheterotrophic growth using bacteriochlorophyll a, and carotenoids for the harvesting of light. Several strains may also be able to use proteorhodopsin to utilise light as an energy source (Spring et al., 2015).

*Paraglacieocola* (ASV122) was another core ASV (Fig. 7). Numbers of ASVs and the relative abundances were substantial and similar between the provenances (Table A4A3). *Paraglacieocola* are a genus of the family Alteromonadaceae. In a BLASTn search this ASV shows 100 % identity with *Paraglaciecola psychrophila, P. arctica* and several other *Paraglaciecola* sp. sequences. *Paraglaciecola psychrophila* is a gram-negative, psychrophilic, motile rod-shaped bacteria. Identified from the sea ice of the Canadian Basin and the Greenland Sea, it is aerobic, and optimum growth is at 12°C. (Zhang et al., 2006). Unable to reduce nitrate, it may be associated with POM as an N-source and so be ingested by *N. pachyderma* as it feeds on the detritus. The final core ASV *Bradyrhizobium* ASV833 constituted on average only 0.09 % of foraminiferal and 0.004 % of water column ASVs. *Bradyrhizobium* contains mainly nitrogen fixing species that are part of phylogenetic subcluster IK of *nifH* (Chien and Zinder, 1994; Gaby et al., 2014; Fernández-Méndez et al., 2016), which encodes the nitrogen fixing enzyme nitrogenase. Sequences from subcluster IK can make up >50 % of the *nifH* sequence abundance in the open waters of the Central Arctic Ocean (Fernández-Méndez et al., 2016), supporting our identification of this ASV in the polar waters of Baffin Bay.

Of particular significance is **Micrococcus** (ASV10, 25/28 foraminifera), since its ASV counts total 7766 in the 28 foraminifera but zero in water samples (Table 2). They make up only 0.3 % of the total ASV assemblage in the foraminifera, and 0 % in the water column. The absence of ASV10 from the water column might suggest that this ASV could belong to a bacterial endo(sym)biont of **N. pachyderma**, albeit it occurs in low numbers within the cell. The mode of endobiont transfer could potentially be from an agamont mother cell to the gamont daughter cell, since asexual reproduction has been reported for this species (Kimoto and Tsuchiya., 2006; Davis et al., 2020; Meilland et al, 2022) and endobiont transfer has recently been observed during asexual reproduction in the foraminifer **Globigerinita uvula** (Takagi et al., 2020). Prior to these observations of asexual reproduction and symbiont transfer, all known symbionts of planktonic foraminifera were thought to be acquired directly from the water column by the gamonts due to the large symbiont size relative to the gametes (Hemleben et al., 1989; Bijma et al., 1990), water column prevalence, and lack of genetic drift between water column specimens and endobionts (Bird et al., 2017). **Micrococcus** is a hydrocarbon degrading genus (Atlas et al., 1995) that is also capable of chitin degradation (Annamalai et al., 2010) and is used in the production of the bioplastic polyhydroxybutyrate (Mohanrasu et al., 2021). There may be a role for Micrococcus within the foraminiferal host in helping to breakdown copepod derived chitin phagocytosed in POM (Greco et al., 2021). In addition, since diatoms are a major producer of hydrocarbons (Stonik & Stonik, 2015), and are clearly eaten in large numbers, there may be a role for **Micrococcus** to degrade such hydrocarbons within the foraminiferal cell. Indeed, several other significant hydrocarbon degrading genera are found in **N. pachyderma.** For example, of the four **Colwellia** ASVs (48, 98, 119 and 125), two ASVs (48 and 119) drive the significant differences between the foraminiferal microbiome and the water column. Combining all four **Colwellia** ASVs, **Colwellia** are present in all but the two potentially gametogenic specimens, Fm176b and Fm101e. Interestingly, **Colwellia** have been shown to break down hydrocarbons most efficiently at temperatures of around 4°C (Redmond et al., 2011). A further ASV from a hydrocarbon degrading species is ASV40 which is taxonomically assigned to the genus **Moritella**. This genus of gammaproteobacteria are psychrophilic gram-negative facultative anaerobes and were found to carry out hydrocarbon degradation in the waters of the Northwest Passage in the Canadian Arctic Archipelago (Garneau et al., 2016). This ASV was identified in 15 of 28 foraminifera. **Pseudoalteromonas** are also known hydrocarbon degraders (e.g. Calderon et al., 2018) and a single **Pseudoalteromonas** ASV was identified as a core member of the microbiome. Whether the abundance of hydrocarbon degraders within the foraminifera is a function of their presence in the hydrocarbon producing diatom's "phycosphere" (Bell & Mitchell, 1972), or whether there is a more specific foraminiferal endobiont interaction with any of these genera is yet to be determined.

### 4.2.2 The differentially abundant ASVs and PICRUSt2 pathways

Metabolic pathway abundances were predicted using PICRUST2. It is important to note that these are predictions indicating potential functional capacity and are not indicative of active processes. Nevertheless, many of the predicted pathways are consistent with the hypothesis that the bacteria in the foraminiferal microbiome are derived from foraminiferal feeding on diatoms, and diatom-derived POM.

A POM feeding cyst will contain an oxygen gradient with oxygen concentrations in the centre significantly below ambient seawater in the centre (Alldredge and Cohen 1987). Interestingly, facultatively anaerobic fermentation pathways are present in higher abundance in the foraminiferal microbiome, which may be a result of preferential ingestion of fermenting bacteria due to their presence in the centre of a low oxygen POM feeding cyst. There are two pathways categorised as "Other biosynthesis" pathways (P125-PWY and PWY-7391, Fig. 65) that are involved in the biosynthesis of the antifreeze butanediol, via the fermentation of pyruvate (Caspi et al., 2014). These pathways are attributed to 49 ASVs predominantly from Class Gamma- and Alphaproteobacteria, Phylum Firmicutes, and Phylum Actinobacteriota, and include two of the differentially abundant ASVs in the foraminiferal microbiome (Fig. 4; Table A2). Four other identified fermentation pathways utilise monosaccharides to produce ATP and reducing power (NADH). These monosaccharides which are abundant in the diatom exopolysaccharide (EPS) exudates in the phycosphere (Daly et al., 2023) and therefore, again, the phycosphere or diatom derived POM is likely to be the foraminiferal microbiome source of these fermenting bacteria. 496 ASVs contribute to this pathway, with three of the differentially abundant ASVs doing so (Table A2).

Peptidoglycan synthesis pathways are also more abundant in the foraminiferal microbiome, this is driven by 897 ASVs, of which four are also significantly differentially abundant in the foraminiferal microbiome (Table A2). A pathway for synthesis of a single amino acid L-lysine (PWY-2941) is more prevalent here, compared to higher abundance of three different amino acid biosynthesis pathways in the water column assemblage. L-lysine is essential for cell wall biosynthesis (Gillner et al., 2013) and this pathway also produces meso-diaminopimelate which is another component of the peptidoglycan cell wall (Weinberger and Gilmar 1970). Supporting this, there are four further peptidoglycan synthesis and recycling pathways (grouped in the category "Cell structure biosynthesis", Fig. 56) identified as more abundant in the foraminiferal microbiome versus the water column. Pathway PWY-6471 is specific to gram-positive bacteria, and the greater abundance of these cell wall synthesis pathways in general might indicate a greater relative abundance of gram-positive bacteria in the foraminiferal microbiome compared to the water column, although at present it is unclear why. This is again supported by the differentially abundant gram-negative Firmicutes.

The degradation of certain carbohydrates was also key in the foraminiferal microbiome with 624 ASVs associated with these pathways. For example, pathways for the degradation of the polysaccharides glycogen and starch, and the sugars sucrose, glucose and xylose were differentially abundant, and given these are abundant sugars in the diatom phycosphere (Daly et al., 2023), this is further supporting evidence that the majority of bacteria in the foraminiferal microbiome are derived from the phycosphere or diatom-derived POM. Many of the bacteria phagocytosed by *N. pachyderma* were likely to have been part of the diatom phycosphere, and bacteria with the ability to breakdown starch, and basic sugars is not surprising given that glucose and xylose for example, are abundant sugars in the diatom phycosphere (Daly et al., 2023).

Of interest were three two additional pathways. The first was norspermidine biosynthesis (PWY-6562) which plays a central role in biofilm formation in *Vibrio spp.* (Wotanis et al., 2017), whilst inhibiting biofilm formation by other species, and in particular other gram-negative bacteria (Qu et al., 2016). Norspermidine biosynthesis was identified across seven

720  Gammaproteobacterial ASVs, including three Alteromonadales and four Vibrionales, one of which (ASV116, genus *Vibrio*) was differentially abundant in the foraminiferal microbiome ~~and three Alteromonadales~~.

Finally, interestingly the palmitate biosynthesis II pathway (PWY-5971) is more abundant in foraminiferal microbiome. Palmitate is a long-chain saturated fatty acid. It is produced both by algae such as diatoms and by bacteria (Allan et al., 2023), but in our data set the ASVs responsible for this pathway are one ASV of the Genus *Alteromonas*, and three ASVs from the

725  Genus *Cellvibrio*. Palmitate, or palmitic acid, is a very abundant saturated fatty acid and key precursor for phospholipids and lipopolysaccharides essential for the bacterial plasma membrane (Cronan & Thomas, 2014). Given the universal nature of this requirement, it is surprising that genes encoding enzymes in this pathway are more abundant in the foraminiferal microbiome. Interestingly however, palmitic acid is used by pathogenic bacteria to modify their proteins and glycoproteins to avoid detection by host immune system~~s~~ TLR4 receptors (Toll-Like-Receptor family) (Subocińska et al., 2018) ~~and~~ thereby

730  increasing infectivity and the production of biofilms. However, TLR4 evolved 500 million years ago near the beginning of the vertebrate evolution (Beutler & Rehli, 2002) and is not known to be present in protists. Therefore, the reason for increased abundance of the palmitate biosynthesis II pathway in the foraminiferal microbiome compared to the water column is currently unknown.~~.~~

735  ~~There are three further core ASVs assigned to bacterial taxa: ASV19 from family Flavobacteriaceae; ASV74 from genus OM(NOR) of the family Halieaceae; and ASV116, a *Bradyrhizobium sp.* (Fig. 6). All three of these ASVs also contribute to the significant differences between foraminifera and water column samples (Fig. 4). Flavobacteriaceae are a large family of bacteria that are widely distributed in the marine environment. Tisserand et al. (2020) isolated ten species from Baffin Bay, and all were shown to grow on exudates (dissolved organic matter) from two Arctic diatoms (*Fragilariopsis cylindricus* and~~

740  ~~*Chaetoceros neogracilis*). This infers that Flavobacteriaceae may be part of the diatom phycosphere, consumed alongside diatoms by feeding *N. pachyderma*, leading to the higher proportion of Flavobacteriaceae ASV19 in the foraminifera compared to the water column. Additionally, Flavobacteriaceae are often found associated with detritus (as well as algae, fish and invertebrates; Gavriilidou et al., 2020). Therefore, *N. pachyderma*, which feeds from a detrital feeding cyst on diatoms and diatom detritus (Greco et al., 2021), could passively consume Flavobacteriaceae present in the detritus.~~

745  ~~ASV74 is attributed to the OM(NOR) genus of the Family Halieaceae, (order Cellvibrionales, Spring et al., 2015). Total ASV74 counts for the foraminifera are 31,125 (mean relative abundance = 1.2 %) and 6,224 for the water column (mean relative abundance = 0.31 %). In total, 4 % of the ASVs are assigned to the order Cellvibrionales in the water column, but only ASV74 is identified in the foraminifera as well as the water column. The order Cellvibrionales are gram positive aerobes that are mesophilic and neutrophilic chemoorganotrophs. However, some members of the Family Halieaceae may additionally be~~

750  ~~capable of aerobic photoheterotrophic growth using bacteriochlorophyll a, and carotenoids for the harvesting of light. Several strains may also be able to use proteorhodopsin to utilise light as an energy source (Spring et al., 2015).~~

~~The final bacterial ASV that contributed to both the significant difference between the water column and the foraminiferal ASV assemblage (Fig. 4) and the core microbiome (Fig. 6) is ASV116, *Bradyrhizobium*. It constitutes on average 0.09 % of~~

foraminiferal and 0.004 % of water column ASVs, indicating that the relative abundance is an order of magnitude greater in the foraminifera, although actual counts across the foraminifera (1791) versus the water column (86) are extremely low. This genus contains mainly nitrogen fixing species that are part of phylogenetic cluster I of *nifH* (Chien and Zinder, 1994; Gaby et al., 2014), which encodes the nitrogen fixing enzyme nitrogenase. *nifH* sequences that cluster with *Bradyrhizobium* in Cluster I have been isolated from the Central Arctic Ocean water column. In fact, sequences from subcluster IK which includes *Bradyrhizobium,* made up >50 % of the *nifH* subcluster sequence abundance in the open waters of the Central Arctic Ocean (Fernández Méndez et al., 2016), supporting our findings in the polar waters of Baffin Bay.

Three final core ASVs are *Pseudoalteromonas* (ASV26), *Aurantivirga* (ASV27) and *Paraglacieocola* (ASV34) (Fig. 6). None of these ASVs drive the differences between the foraminifera and water column; numbers of ASVs and the relative abundances are substantial and similar between these provenances, and it is therefore highly likely that these species are passively ingested during *N. pachyderma* feeding on POM. Finally, *Paraglacieocola* are a genus of the family Alteromonadaceae. In a BLASTn search this ASV shows 100 % identity with *Paraglaciecola psychrophila, P. arctica* and several other *Paraglaciecola* sp. sequences. *Paraglaciecola psychrophila* is a gram-negative, psychrophilic, motile rod-shaped bacteria. Identified from the sea ice of the Canadian Basin and the Greenland Sea, it is aerobic and optimum growth is at 12°C. (Zhang et al., 2006). Unable to reduce nitrate, it may be associated with POM as an N source, and so be ingested by *N. pachyderma* as it feeds on the detritus. As described above, *Pseudoalteromonas* is a hydrocarbon degrading genus, which may explain its presence. *Aurantivirga* is a gram-negative, aerobic, proteorhodopsin containing, rod-shaped genus of Flavobacteriaceae, described above as feeding on diatom exudates. However, a BLASTn search (NCBI) identifies ASV27 not as *Aurantivirga*, but as 99.6 % identical to an alternative Flavobacteriaceae of the genus *Tenacibaculum* including *T. insulae, T. haliotis and Tenacibaculum sp*. This genus contains many opportunistic fish pathogens, some of which are found to target fish teeth, a high source of calcium shown to promote the bacteria's growth (Hikida et al., 1979; Frisch et al., 2018). Growth promotion by calcium may be a common feature of the *Tenacibaculum* genus and may be another reason why this ASV is identified with *N. pachyderma,* and the population of foraminifera in polar waters may provide a suitable supply of accessible calcium for this genus. Finally, *Paraglacieocola* are a genus of the family Alteromonadaceae. In a BLASTn search this ASV shows 100 % identity with *Paraglaciecola psychrophila, P. arctica* and several other *Paraglaciecola* sp. sequences. *Paraglaciecola psychrophila* is a gram-negative, psychrophilic, motile rod-shaped bacteria. Identified from the sea ice of the Canadian Basin and the Greenland Sea, it is aerobic and optimum growth is at 12°C. (Zhang et al., 2006). Unable to reduce nitrate, it may be associated with POM as an N source, and so be ingested by *N. pachyderma* as it feeds on the detritus.

Lastly, of note, although not identified as a core microbiome member, members of the phylum Planctomycetes were often found in greater numbers in their foraminiferal hosts, than in the water column (Fig. A5) and one ASV (101; *Rubripirellula* sp.) contributed to driving the differences between provenances, being more abundant in the foraminifera (Fig. 4). Planctomycetes are widespread in the environment, and are essentially associated with particles including plastics, laminaria seaweeds and POM in the open water column (DeLong et al., 1993; Bondoso et al., 2015; Kallscheuer et al., 2020), again indicating the strong association of *N. pachyderma* with a POM feeding cyst.

### 4.3 *N. pachyderma* chloroplast ASVs

Two diatom chloroplasts contributed 5.3 % (ASV355, *Fragilariopsis cylindricus*) and 33.46 % (ASV956 *Chaetoceros gelidus*) of the core microbiome (Fig. 7). Both also contributed to the significant difference in assemblage composition between the foraminifera and the water column (Fig. Table A2). Chloroplasts were exceptionally abundant in our TEM images (Fig. 9 and Fig. A6), which appear very similar to observations made in kleptoplastic benthic species (Jauffrais et al., 2018; Jesus et al., 2022). This raises important questions about the nature of the relationship between the chloroplasts and *N. pachyderma* Type I and is discussed below. Empty diatom frustules were also observed in the TEM images, which is highly consistent with previous reports that diatoms are a significant part of the *N. pachyderma* diet (Hemleben et al., 1989; Scheibel & Hemleben, 2017; Greco et al., 2021).

On average 53.3 % ~~55.7 %~~ of all 16S ~~rDNA~~ rDNA ASVs in the foraminifera belong to chloroplast-containing taxa (Fig 2; Sect. 3.3.~~1~~2). This contrasts with the 3.51 % ~~2.97 %~~ average proportion found in the water column. Most of the foraminiferal intracellular chloroplast ASVs, are dominated by ~~ASV15~~ASV956, *Chaetoceros gelidus* (BLASTn) from the diatom class Bacillariophyceae. The compositional dominance of ~~ASV15~~ ASV956 in the foraminifera reflects the chloroplast ASV composition of the water column (Fig. ~~5~~6), although found in much higher proportions in the foraminifera (Fig. 3). ~~ASV15 is only missing from specimen Fm176b, one of two specimens thought to potentially have stopped feeding and be in the gametogenic stage of ontogeny.~~ The presence of ~~ASV15~~ASV956, *Chaetoceros gelidus,* in all other specimens indicates its importance to *N. pachyderma* in this location and season. It is characteristic of northern temperate and polar waters (Chamnansinp et al., 2013), and it is a known important biomass fraction in Baffin Bay (Crawford et al., 2018). In fact, eight strains were isolated from Baffin Bay only during bloom development or bloom peak (Ribeiro et al., 2020) and *Chaetoceros's* reputation for bloom forming (Booth et al., 2002) is reflected here in its high abundances compared to other species.

The diatom chloroplast 16S ASVs identified in this study (Fig. ~~5~~6) are also consistent with the diatoms found by Greco et al. (2021), who identified *Chaetoceros* and *Fragilariopsis* as major components of the *N. pachyderma* 18S ASVs from Baffin Bay. Both *Chaetoceros* (~~ASV102~~ASV1413, ~~ASV15~~ASV956) and *Fragilariopsis* 16S ASVs (~~47, 59~~ ASV355~~and 84~~) were amongst those ASVs driving the significant difference between the foraminifera and the water column, and both are major constituents of the core microbiome. Intact *Fragilariopsis* ~~was~~ were also identified in the foraminiferal TEM images (Fig. ~~7a~~8a) hinting that, like *Ammonia* sp. and the miliolid *Hauerina diversa N. pachyderma* may phagocytose the entire diatom before digesting the cell and then extruding the ~~perform intracellular ingestion of the diatom~~silicate frustules (Jauffrais et al., 2018; Pinko et al., 2023).

### 4.4 Observation of abundant chloroplasts throughout the cytoplasm of *N. pachyderma* Type I

To our knowledge this is the first report of large numbers of chloroplasts observed by TEM imaging and recorded via metabarcoding in any planktonic foraminiferal species. These observations cover two summers in different regions of Baffin Bay. The high numbers observed, and the relative abundance of diatom chloroplasts recorded, could indicate a kleptoplastic

behaviour in *N. pachyderma* Type I, a strategy ~~which~~ that is well known in several protist lineages such as benthic foraminifera (Jesus et al., 2021), dinoflagellates (Takano et al., 2014; Yamada et al., 2023), and ciliates (Johnson et al., 2007). Kleptoplasty refers to the phenomenon where an organism sequesters chloroplasts from its microalgal prey. The original definition ~~of kleptoplasty~~ does not include the requirement for temporary photosynthesis to continue in the host (Clark et al., 1990; Jauffrais et al., 2018) and is appropriate since ~~(REF) because~~chloroplasts are known to perform many functions in addition to~~as well as~~ photosynthesis. These include amino acid, nucleotide, and fatty acid synthesis as well as N and S assimilation (Cedhagen, 1991; Bobik and Burch-Smith, 2015). Further, the benthic foraminiferal species *Nonionellina labradorica* retains chloroplasts despite living in sediments below the photic zone. The photosynthetic pathway of their retained chloroplasts is therefore not functional (Cedhagen, 1991; Jauffrais et al., 2019b) and the reason for chloroplast retention in this species is unknown. However, its importance is reflected by the discovery that the kleptoplast genome in *Nonionella stella*, another benthic species that lives below the photic zone, is transcribed in the host (Gomaa et al., 2021; Powers et al., 2022). However, where kleptoplasts do continue to photosynthesize in the new host, ~~E~~evidence suggests that this has been important in supporting major evolutionary innovations crucial to the current ecological roles of such protists in the marine environment (Stoecker et al., 2009). Therefore, the role of the retained chloroplasts remains a fascinating question in many species of foraminifera, including *N. pachyderma* Type I, and it is important to assess and further investigate potential kleptoplast roles to understand any contribution they make to *N. pachyderma* evolution, successful ability to inhabit the true polar habitat, and evaluate its potential resilience to future climate change.

### 4.4.1 Evidence for potential kleptoplasty in *N. pachyderma* Type I

Foraminifera such as *N. pachyderma* that eat diatoms (and other algae) ~~such as *N. pachyderma*~~ would be expected to contain some chloroplasts in their cytoplasm as a b~~i~~yproduct of their grazing. For example, 18S metabarcoding demonstrates that the non-kleptoplastic benthic foraminifer *Ammonia* sp. (Jauffrais et al., 2016), grazes on diatoms in a comparable way to the kleptoplastic *Elphidium* sp. and *Haynesina germanica*, (Chronopoulou et al., 2019), and chloroplasts are indeed observed within the cytoplasm of *Ammonia* sp. Yet the relative plastid abundance in *Ammonia* sp., is reported as "rare" compared to "abundant" in *Elphidium* sp. and *Haynesina* sp. (Goldstein et al., 2004; Cesbron et al., 2017; Jauffrais et al., 2018), with a high proportion of chloroplasts in *Ammonia* sp. undergoing degradation (Jauffrais et al., 2018; Lekieffre et al., 2018a). In contrast, our TEM images show high numbers of chloroplasts in *N. pachyderma*, congruent with or greater than the abundance observed in the TEM images of the kleptoplastic foraminifera such as *Elphidium* sp. and *H. germanica* (Jauffrais et al., 2018; Fig. ~~7~~9, Fig. ~~A6~~A6).

Kleptoplasty is common amongst benthic foraminifera (Lopez et al., 1979; Lee et al., 1988; Cedhagen 1991; Tsuchiya et al., 2018; Jauffrais et al., 2018; Pinko et al., 2023). The molecular studies identifying the source of kleptoplasts in benthic foraminifera to date would suggest a diatom source from the family Thalassiosiraceae, but potentially, kleptoplasts from more than one ~~closely related~~ diatom species can be present (Pillet et al., 2011; Lechliter 2014; Jauffrais et al., 2019a; Tsuchiya et al., 2020; Pinko et al., 2023). More than 20 diatom species have been identified in benthic foraminifera that host intact diatom

symbionts, (Lee 1995; Schmidt et al., 2018), with potentially up to three different symbionts within a single foraminifer at the same time (Lee, 2011). In addition, diatom symbiont shuffling appears to be an adaptation to changing environmental conditions such as heat stress (Schmidt et al., 2018). These studies indicate that host-symbiont or host-kleptoplast relationships are not strictly species-specific, supporting our findings of multiple diatom ASVs.

The chloroplasts in *N. pachyderma* are distributed throughout the foraminiferal cytoplasm (Fig. ~~7d~~ 9~~& 7e~~). In the benthic kleptoplastic species, chloroplast location is specific to the foraminiferal host species, where some kleptoplasts may be associated with the cellular periphery, while others, as observed here, may be distributed throughout the cell cytosol~~.~~, (Jauffrais et al., 2018; Pinko et al., 2023). Chloroplast placement therefore cannot provide a clear-cut indicator of kleptoplasty. ~~(Jauffrais et al., 2018; Pinko et al., 2023). The presence of abundant chloroplasts in the cytoplasm of *N. pachyderma* must result from gorging on blooms of diatoms, and in particular *Chaetoceros* spp. (Booth et al., 2002).~~

~~Ordinarily chloroplasts perform many functions other than photosynthesis. These include amino acid, nucleotide, and fatty acid synthesis as well as N and S assimilation (; Bobik and Burch Smith, 2015). Further, the benthic foraminiferal species *Nonionellina labradorica* retains chloroplasts despite living in sediments below the photic zone. The photosynthetic pathway of their retained chloroplasts is therefore not functional (Jauffrais et al., 2019b) and the reason for chloroplast retention in this species is unknown. However, its importance is reflected by the discovery that the kleptoplast genome in *Nonionella stella*, another benthic species that lives below the photic zone, is transcribed in the host (Gomaa et al., 2021). The role of the retained chloroplasts remains a fascinating question in many species of foraminifera, including *N. pachyderma* and the biological advantages for the host and the impact on their shell geochemistry necessitates further investigation.~~

The degradation state of the *N. pachyderma* chloroplasts in this study is uncertain due to poor fixation of the samples, and therefore, unfortunately cannot provide information on how intact they are. However, in benthic foraminifera, actively photosynthesising kleptoplasts can remain active from just a few days to a few months before being digested (Grzymski et al., 2002; Jauffrais et al., 2018 and references therein). Given that turnover rates are extremely variable, the number of degrading versus intact kleptoplasts must also be highly variable ~~form~~from species to species.

It is ~~very~~ important to note that no photosynthetic potential was found in ~~The presence of abundant chloroplasts in the cytoplasm of *N. pachyderma* must result from gorging on blooms of diatoms, and in particular *Chaetoceros* spp. (Booth et al., 2002).~~

~~Yet it is also quite possible that *N. pachyderma* Type I may adopt a hybrid kleptoplastic lifestyle during the limited summer months, to utilise the carbon fixed by kleptoplasts alongside heterotrophic feeding on diatoms (Mitra et al., 2016), which may also have restricted availability due to plankton patchiness. A wide range of protists exhibit similar mixotrophy, including many planktonic foraminifera which house photosynthesising pelagophyte (Gastrich, 1987; Bird et al., 2018) or dinoflagellate~~

~~However, a~~six *N. pachyderma* Type VII individuals from the North Pacific using fast repetition rate (FRR) Fluorometry.

890 ~~Indeed, there was also no~~There was also no evidence of non-functional chlorophyll using this technique (Takagi et al., 2019). This is extremely surprising given the herbivorous nature of *N. pachyderma* (Spindler and Dieckmann, 1986; Schiebel and Hemleben, 2017; Greco et al., 2021; this study), and may reflect different feeding strategies in the two genotypes, whereby Type I retains chloroplasts but Type VII does not, or Type VII has a broader ranging diet and so there is no resultant build-up of chloroplasts in the cytoplasm.~~.~~

895

*N. pachyderma* Type I should now be tested using FRR fluorometry to identify whether retained chloroplasts have photosynthetic potential and therefore behave as traditional kleptoplasts or not. This potential difference could represent a divergent evolutionary adaptation in *N. pachyderma* Type I to survive and flourish in the extreme Arctic environment. *N. pachyderma* has genetically diversified to inhabit a wide range of extreme environments from the Arctic and Antarctic polar

900 waters to the frontal and upwelling systems of the transitional to tropical zones (e.g. Darling et al., 2008). Type I *N. pachyderma* diverged from its Southern Ocean counterparts during the early Quaternary (Darling et al., 2004), allowing substantial time for distinct adaptations to develop in its North Atlantic and Arctic habitat.

### 4.~~5~~ 4.2 Potential chloroplast storage to facilitate overwintering and reproduction

905 ~~Chloroplasts also represent a rich source of amino acids, fatty acids, lipids, vitamin E, pro-vitamin A, lutein, Cu, Fe, Zn and Mn (Gedi et al., 2017).~~

~~Actively photosynthesising kleptoplasts in benthic foraminifera can remain active from a few days to a few months before being digested (Grzymski et al., 2002; Jauffrais et al., 2018 and references therein). Chloroplasts also represent a rich source

910 of amino acids, fatty acids, lipids, vitamin E, pro-vitamin A, lutein, Cu, Fe, Zn and Mn (Gedi et al., 2017). be~~The ~~presence of~~ cytoplasmic ~~abundant~~ chloroplasts observed in ~~the cytoplasm of~~ *N. pachyderma* Type I (Fig. ~~8~~9) are retained ~~must result~~ from their ~~gorging on blooms of~~ diatom food sources, and in particular from *Chaetoceros* spp. (Booth et al., 2002; this study). These chloroplasts represent a rich source of amino acids, fatty acids, lipids, vitamin E, pro-vitamin A, lutein, Cu, Fe, Zn and Mn (Gedi et al., 2017). Therefore, either F~~f~~unctioning photosynthetic kleptoplasts and/or ~~the~~ chloroplasts themselves could

915 potentially provide *N. pachyderma* Type I with a substantial additional energy resource in the challenging Arctic environment. ~~Further, if~~If chloroplasts can be retained in the cytoplasm over many months before consumption, they could provide a valuable source of nutrition for the overwintering population. A similar overwintering survival strategy citing the high nutrient levels

of stored chloroplasts has been proposed for the benthic foraminifera *N. labradorica* (Salonen et al., 2021), as it is known that no photosynthesis occurs in these kleptoplasts in the winter months (Ceghagen 1991).

920 Ecological processes in the Arctic are largely governed by sea ice and light dynamics. There is a general perception of minimal biological activity in the Arctic marine surface layers during the Arctic winter, due to the low light intensity producing minimal photosynthetic activity. However, studies around Svalbard in January 2012-2015 revealed unexpectedly high biological activity in the Arctic winter, with high respiration rates per unit of biomass in the upper 100 m water column (Berge et al. 2015a, b; Falk-Petersen et al. 2015), and an earlier winter *Calanus* copepod (Arthropoda) presence than previously thought

925 (Espinasse et al., 2022). In Baffin Bay, low but significant phytoplankton growth was also observed during winter under the sea ice at extremely low light levels (Randelhoff et al., 2020). Since *N. pachyderma* Type I are thought to feed on both POM (including Arthropoda) and live diatoms (Greco et al., 2021 and this study), such wintertime POM-producing biological activity combined with stored chloroplasts (whether photosynthesising or not) could provide significant nutritional resources for an overwintering population of foraminifera.

930 These factors potentially combine to provide *N. pachyderma* with a significant nutritional resource to survive over the winter months, but questions remain about its behaviour in the water column and the form in which it may overwinter. Sediment traps in the Irminger Sea indicate a very low-level population of overwintering *N. pachyderma* and their isotopic signature profiles imply that a dormant noncalcifying population of *N. pachyderma* may remain in the water column during winter (Jonkers et al., 2010). However, it is possible that the *N. pachyderma* population they detected may not fully represent the true winter

935 population size, since sieve sizes of 150 µm would not retain smaller mature/immature *N. pachyderma* specimens. Potentially, *N. pachyderma* could also remain buoyant in the water column as non-reproducing immature cells, slowing down their cellular metabolism as largely quiescent cells during the most challenging winter months. In culture, several specimens of *N. pachyderma* Type I exhibited extended periods of dormancy or inactivity, followed by recovery (Westgard et al, 2023).

### 4.6 5 Paleoenvironments, and geochemical signatures

940 The biological adaptations and interactions of calcifying foraminifera have varying influences on the geochemistry of their shell, as photosymbionts are known to influence shell geochemistry (Spero et al., 1991; Bemis et al., 1998; 2002; Anand et al, 2003; Russell et al., 2004), and symbiont-host respiration and potentially respiration of endobiont bacteria may increase the use of metabolic (respired) Ccarbon in their shells (Rink et al., 1998; Wolf-Gladrow et al., 1999; Hönisch et al., 2003; Eggins et al., 2004; Bird et al., 2017). To fully understand variations in the geochemical signatures of Arctic *N. pachyderma* shells

945 through time in the sediment assemblagefossil record, we need to improve our understanding of the ecology and interactions between *N. pachyderma* and the intracellular microorganisms which it hosts. Interactions may exhibit ontogenetic or strong seasonal differences and may be facultative or obligate. Recent geochemical studies have used high-resolution single-specimen and even single chamber analyses to investigate both the biological and seasonal influences on shell geochemistry throughout the lifetime of calcareous foraminifera (Spindler and Dieckmann, 1986; Takagi et al., 2015, 2016; Lougheed et al.,

950 2018; Pracht et al., 2019; Metcalfe et al., 2019). Single shell analysis of $\delta^{18}O$ isotope values has identified two distinct

populations of morphologically identical *N. pachyderma* populations in the ~~north~~ North Atlantic during the last deglacial period. Isotope values indicate a temperature difference of about 4°C, potentially due to a bi-modal seasonal population with peak abundances separated temporally in late spring/early summer and late summer (Brummer et al., 2020). Spatial difference in the assemblage water depth, driven by low salinity meltwater (Brummer et al., 2020) may also contribute towards these seasonal differences. Since potential kleptoplasty (this study) could occur seasonally, obligately or facultatively, in *N. pachyderma* Type I, it is imperative to understand ~~the role of these stored chloroplasts, since~~if the~~e~~ stored chloroplasts photosynthesise or not ~~photosynthesis~~ because photosynthesis is known to influence δ$^{18}$O values (Spero & Lea 1993; Bemis et al., 1998).

~~Improving our understanding of the biology and ecology, including seasonal microbial interactions, of Arctic *N. pachyderma* is required to disentangle the palaeoproxies for this species which is so important in our understanding of the rapidly contracting Arctic biome.~~

## 5 Conclusions

The *N.~~eogloboquadrina~~ pachyderma* Type I microbiome consisted of a range of bacterial ASVs that are significantly different from those of the water column. The genera profile and the putative metabolic pathways identified imply ~~highlighted~~ that the likely source of the bacterial ASVs in the foraminiferal microbiome derive ~~come~~ from the phycosphere of their diatom food source, or from the diatom-derived POM. Although ~~Hence it can be concluded that~~ *N. pachyderma* Type I clearly utilises diatom-associated bacteria as a food source, it~~an~~ is most likely~~d~~ ~~these bacteria are likely not targeted but~~ passive~~ly~~ly ~~consum~~consumed~~ed~~ during~~as the foraminifera~~ ingestion~~s~~ and digestion of the diatom prey~~,~~ and diatom-derived POM.

*N. pachyderma* Type I also ~~predominantly feeds on diatoms and~~ retains large numbers of intact diatom chloroplasts in its cytoplasm. Whilst our TEM images cannot account for the degradation state of the chloroplasts, they appear ~~do highlight the many chloroplasts retained in the cell cytoplasm,~~ commensurate with TEM images derived from kleptoplastic benthic foraminifera. 16S metabarcoding data suggests that most of the chloroplasts inside *N. pachyderma* Type I across Baffin Bay during the summer of 2017 derived from the diatom *Chaetoceros gelidus*, and that these comprised the majority component of the core microbiome at this time. Work still remains ~~still needs to be done~~ to understand the relationship between the chloroplasts and the foraminiferal "host" to determine the chloroplast role in the nutrition of *N. pachyderma* Type I, be it photosynthesis, a facultative nutrient rich overwintering store, or simply a build-up of chloroplasts due to gorging on diatoms. Improving our understanding of the biology, ecology and seasonal microbial interactions of Arctic *N. pachyderma* is essential to disentangle the palaeoproxies for this species and develop an understanding of its susceptibility/adaptability to climate change in the rapidly contracting Arctic biome.

## 6 Appendix

985

990

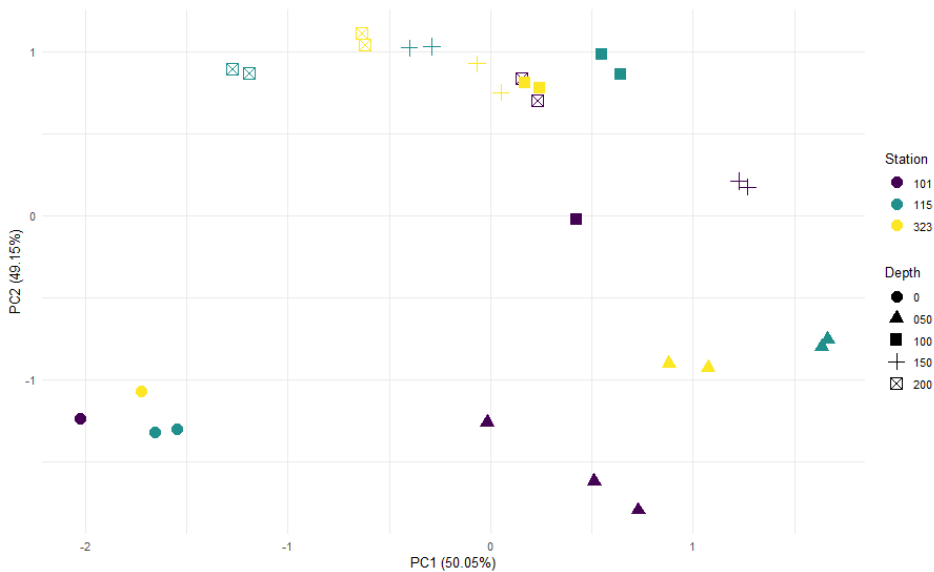



Figure ~~A1~~A1. ~~Bray Curtis~~Aitchison dissimilarity ~~NMDS~~ PCA plot of water samples from different depths at three stations. Colours represent ~~depths~~ Station and shapes represent ~~stations~~Depth. Depth drives ~~52~~55 % of the variability (Pr = 0.001) compared to Station driving ~~17~~2.38% of the variability (Pr = 0.063, *Adonis*; QIIME2*).* ~~In support, GLM analysis using~~

995   ~~*manyglm* in mvabund also identified a significant difference in water samples with depth (LRT = 3386, P< 0.001) but also a~~ ~~marginally significant result for station (LRT=1319, P<0.035).~~

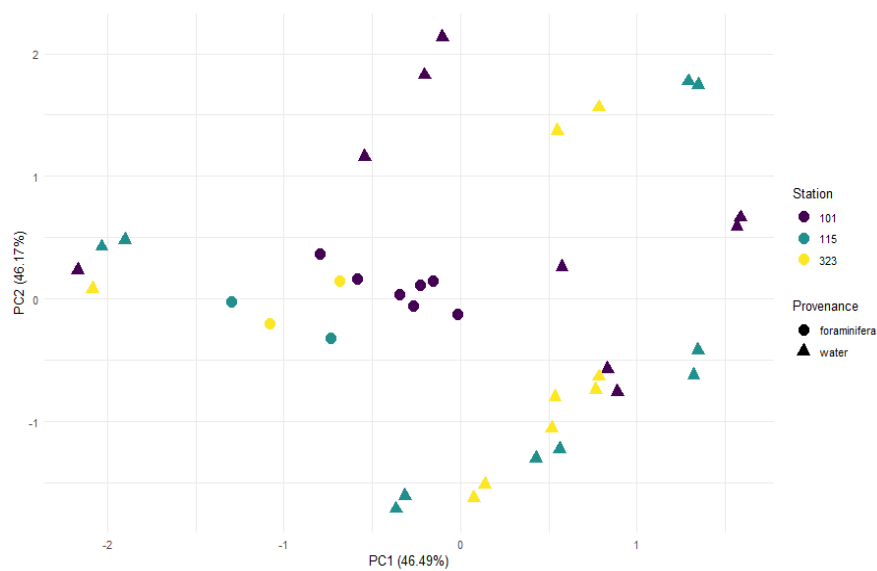



Figure A2. Aitchison dissimilarity PCA ordination of the foraminiferal and water column samples (sample set FW). There is a degree of clustering of the foraminiferal samples reflecting the significant difference identified by PERMANOVA (*Adonis; QIIME2*).

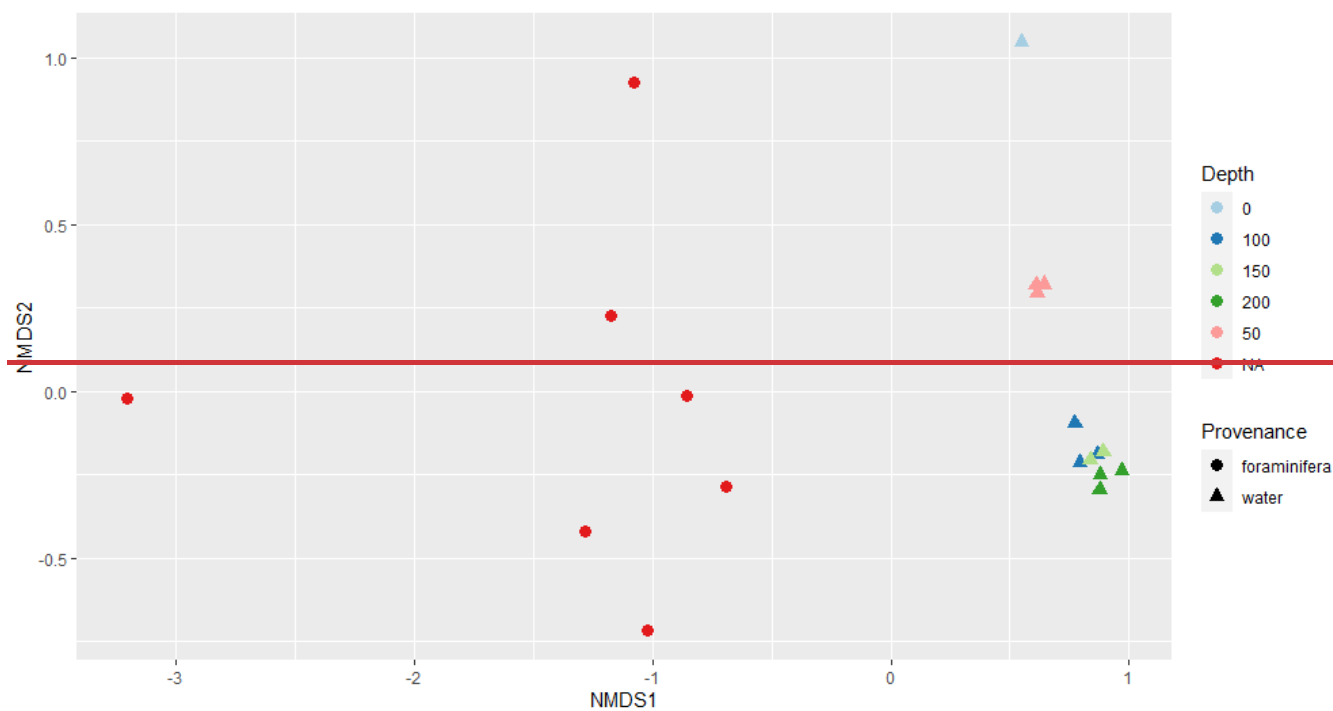
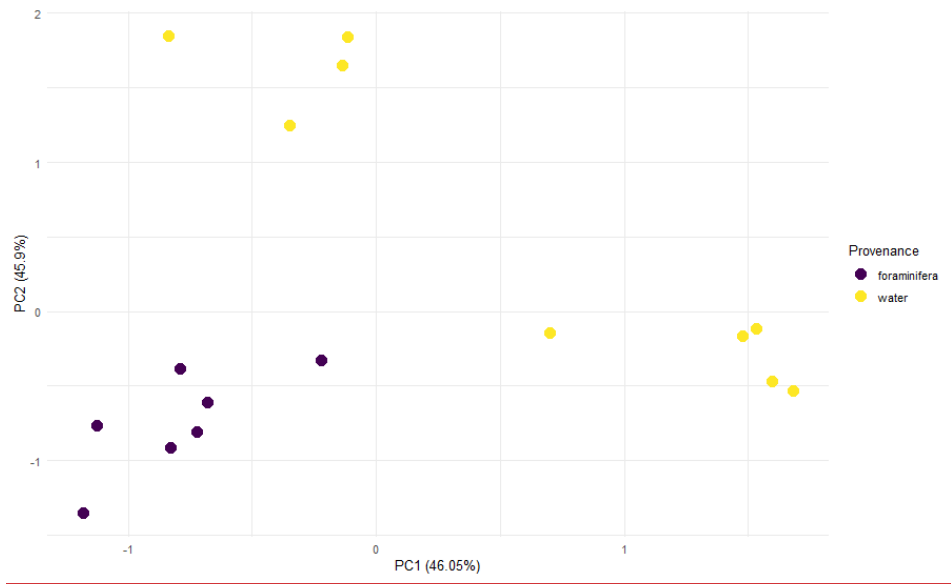



Figure A2A3. Aitchison dissimilarity PCA plot of water column (yellow) and foraminiferal samples (purple) from station 101.

1010    41.7 % of the differences in ASV composition are driven by provenance (P<0.001, (*Adonis¸* QIIME2).

1015

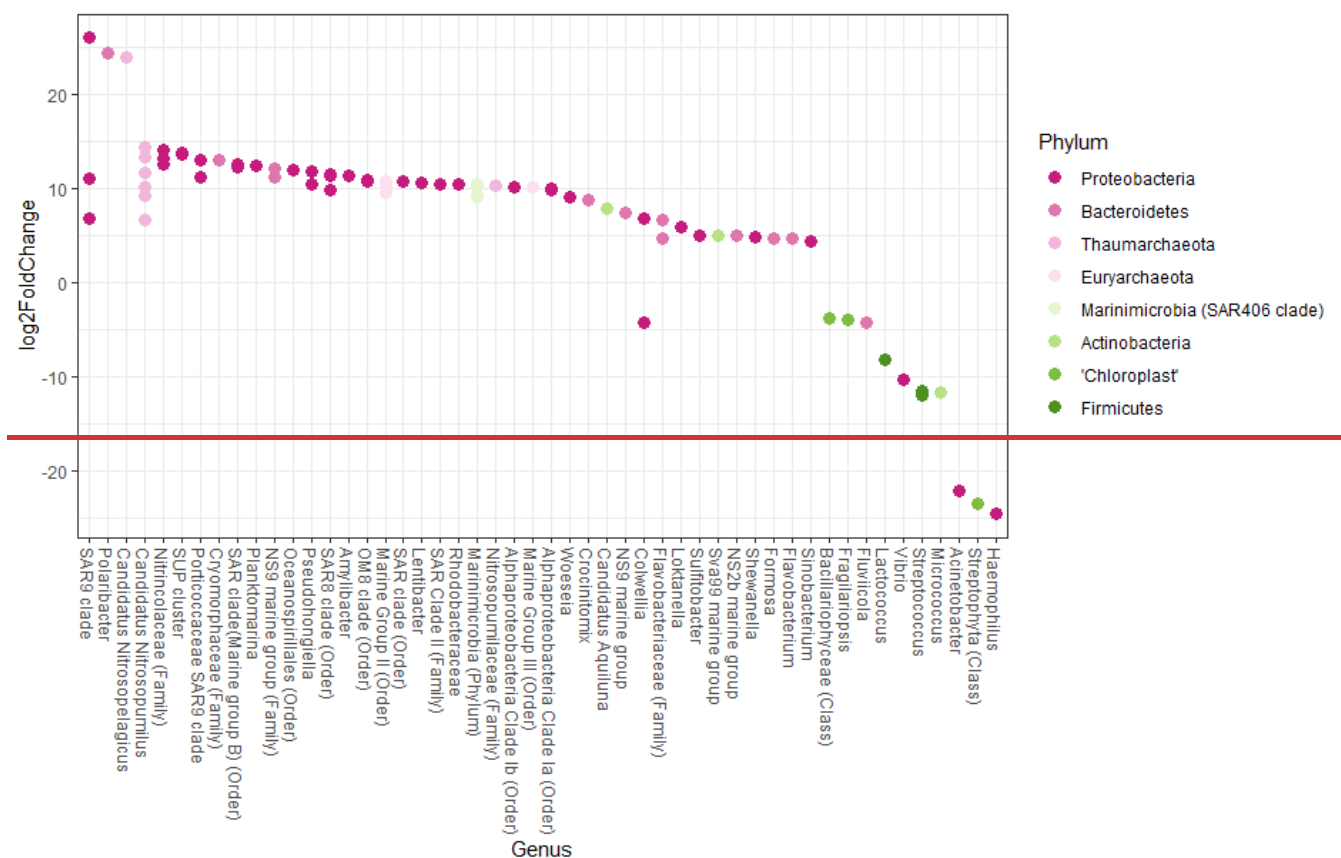
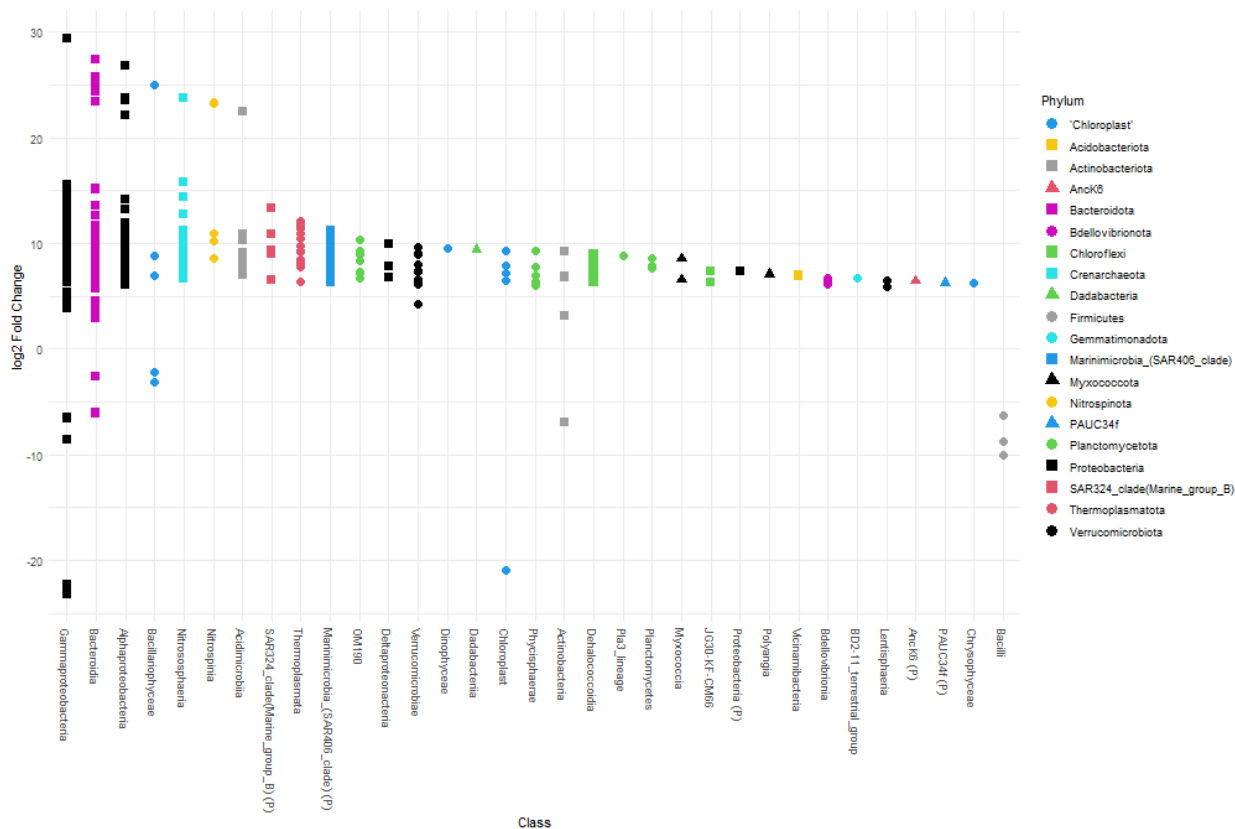
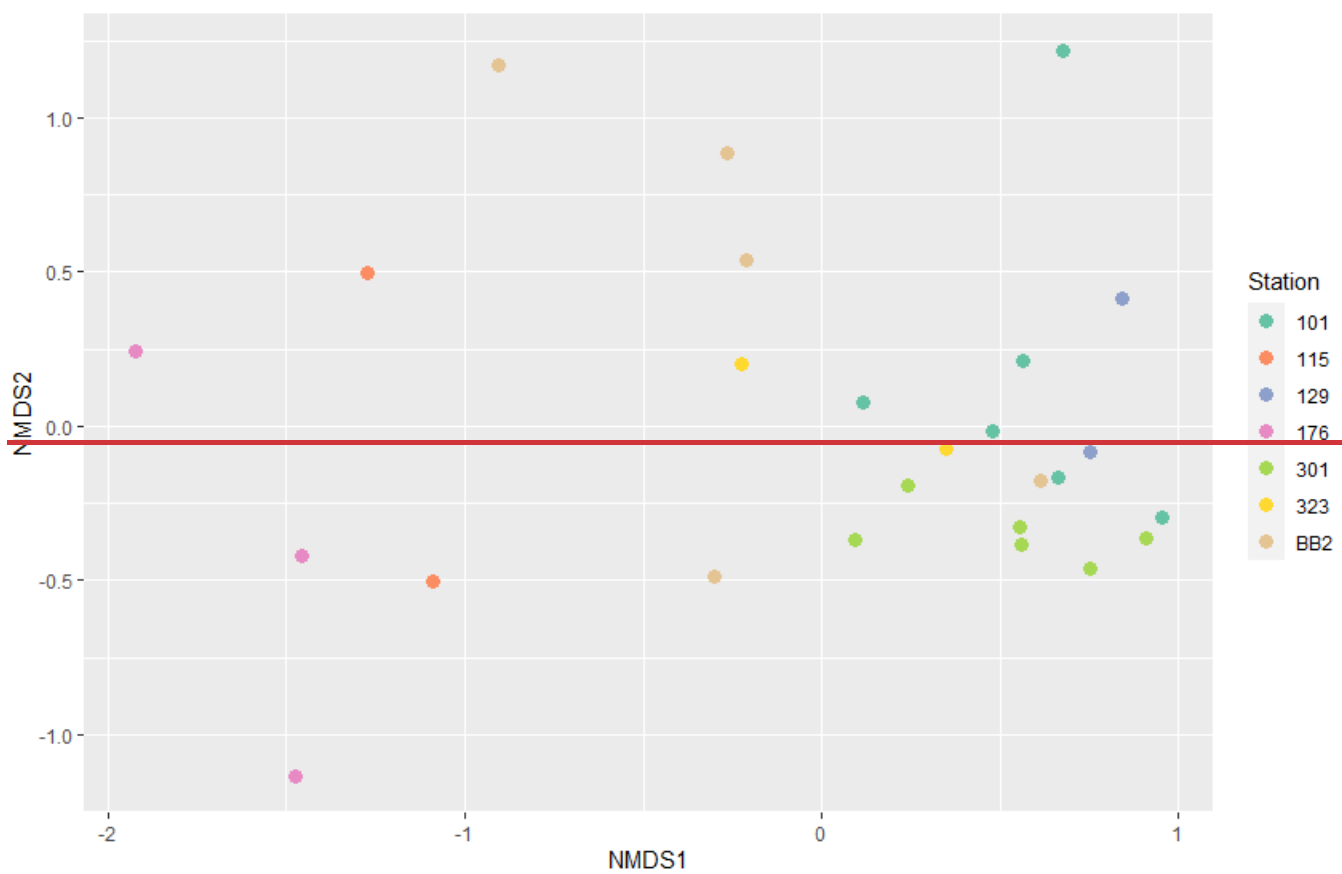
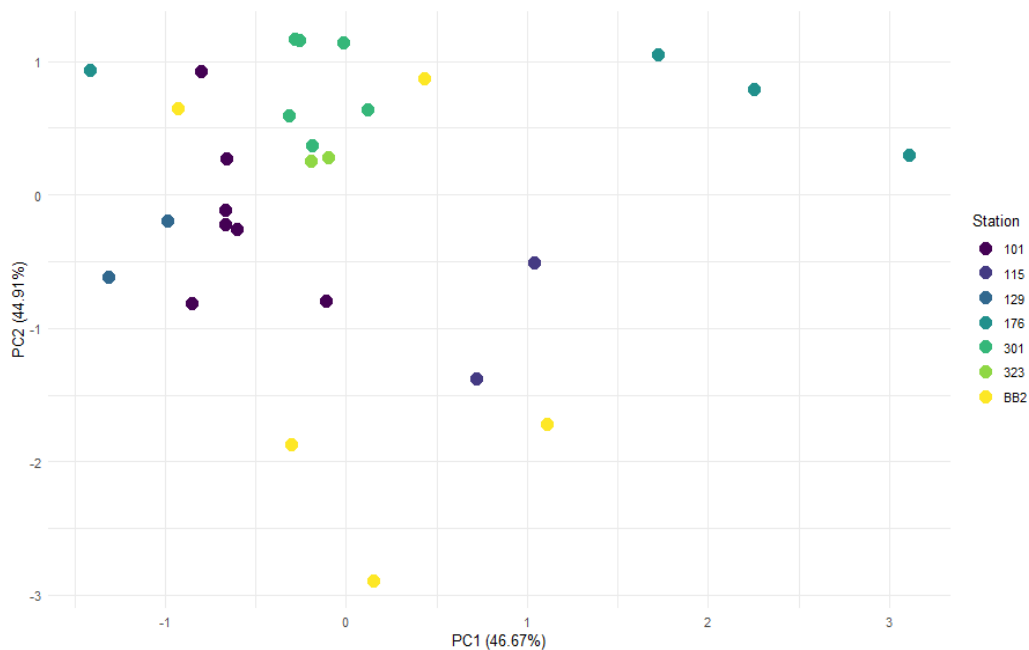
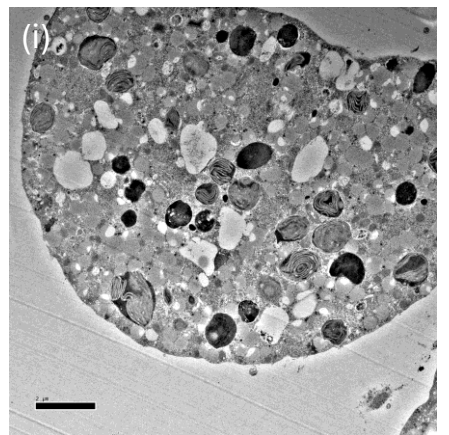
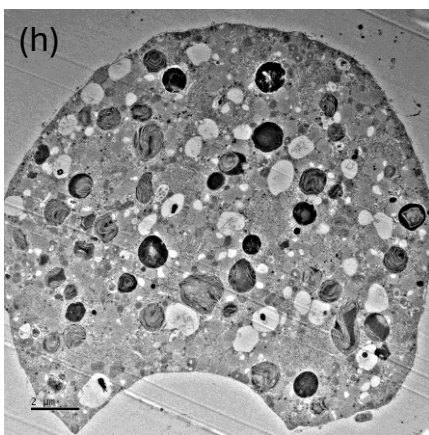
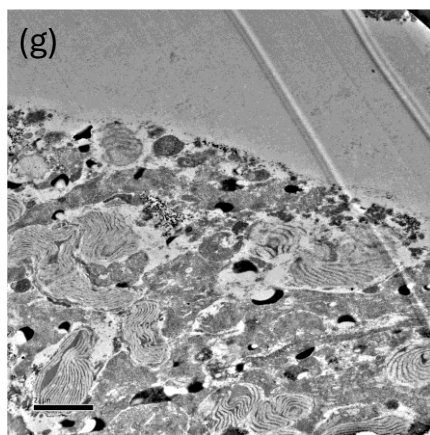
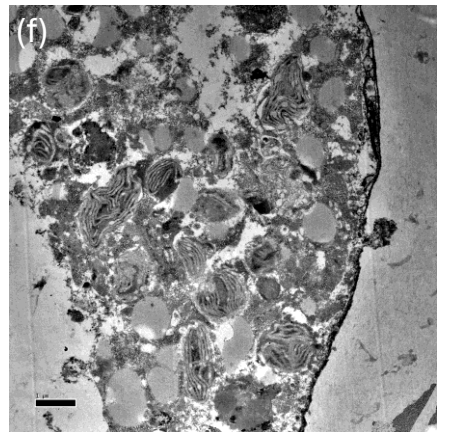
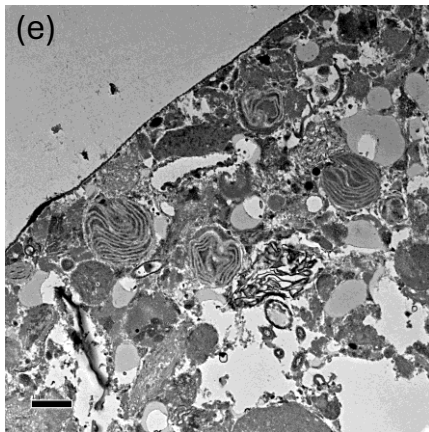
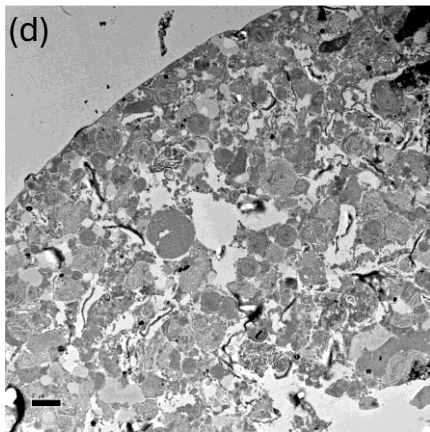
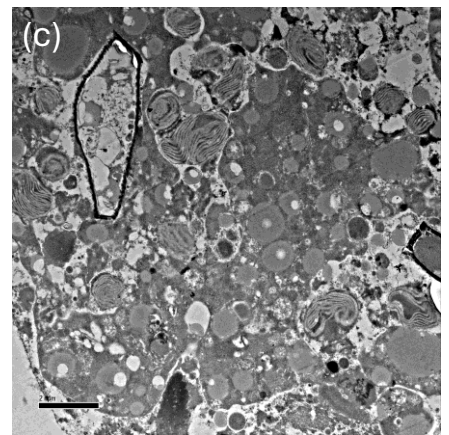
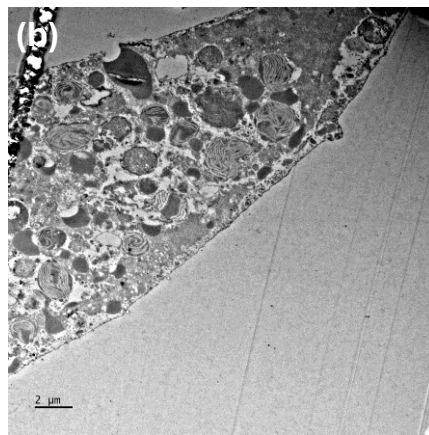
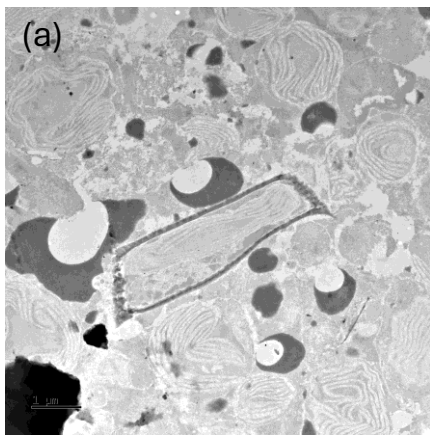
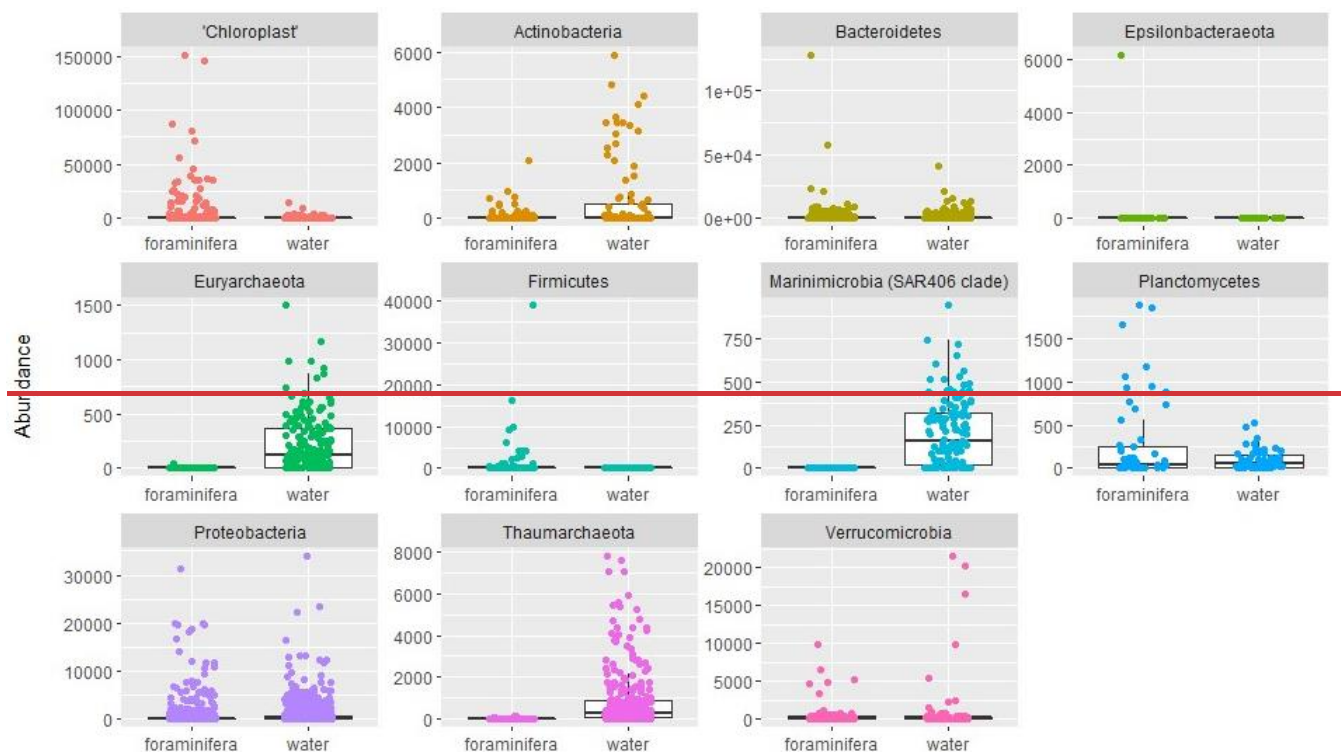



Figure ~~A3~~A4. Differential abundance testing of ASVs between ~~P~~provenances at station 101 using *DESeq2*. The Log2 fold change in ASVs is the log-ratio of the ASV means in the water column and foraminifera. ASVs with positive Log2 fold change are significantly more abundant in the water column assemblage and ASVs with negative values indicate ASVs that are significantly more abundant in the foraminiferal assemblages. The ~~Genus~~Class, or the highest level of taxonomic assignment available for each ASV is given on the ~~X~~x-axis. (P) =Phylum

1030    Figure ~~A4~~A5. ~~Bray Curtis~~Aitchison dissimilarity ~~NMDS~~ PCA ordination of foraminiferal samples across all stations. There is a degree of clustering of foraminifera by station (*Adonis*: F. model= 3.~~079~~263, Pr(>F) = 0.~~001~~004), with ~~49~~48.3% of the variation in the foraminifera driven by station.

1040

~~Figure A5. Box plots showing individual ASV abundance counts. Dots represent single ASV counts within a single sample, grouped according to phyla, and provenance. The median, and upper and lower quatiles are shown. Note the different scales on the y axis~~Figure ~~A6~~A6. TEM images of *N. pachyderma* specimens (a) BB1 (b) BB9B, (c) BB11, (d)-(f) BB8, (g) BB9C

1045    and (h)-(i) BB12. Scale bars are 1 µm ((a), (e), and (f) all others are 2µm. TEM imaging shows that chloroplasts were observed in all fixed specimens.

Table A1. Results of PEMRANOVA (*Adonis,* QIIME2) tests on robust Aitchison (centred log) transformed ASV compositions of foraminifera and water column samples. Sample set "FW" is all foraminifera and all water samples from stations 101, 115

1050    and 323. Sample set "101" is all water column and foraminiferal samples from station 101.

| Test on | Res.Df | Df | Sum of Squares | Mean Sqs | F. Model | R2 | Pr(>F) |
|---|---|---|---|---|---|---|---|
| Water only: Depth | 23 | 4 | 31.72 | 7.93 | 7.17 | 0.55 | 0.001 |
| Water only: Station | 18 | 1 | 1.36 | 1.36 | 0.63 | 0.0238 | 0.54 |
| Sample set FW: Provenance | 37 | 1 | 8.26 | 8.26 | 3.99 | 0.097 | 0.024 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Sample set FW: Station | 38 | 1 | 2.085 | 2.09 | 0.93 | 0.025 | 0.375 |
| Sample set 101: Provenance | 14 | 1 | 14.7 | 14.7 | 9.997 | 0.417 | 0.001 |
| Forams only: Station | 21 | 6 | 30.83 | 5.14 | 3.263 | 0.483 | 0.004 |

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Table A2. The ASVs demonstrating significantly higher differential abundance in the foraminiferal samples compared to the water column samples based on DESeq2 analysis of the FW and 101 sample sets (See section 3.2.1). The contribution of the bacterial ASVs to differentially abundant pathways identified in PICRUSt2 is also shown.

| Phylum | Family | Genus | Species | ASV # | log2 fold change | p-adjust | PICRUSt2 pathways contribution |
|---|---|---|---|---|---|---|---|
| **ASVs differentially abundant in FW sample set only** | | | | | | | |
| Proteobacteria (Gamma) | Saccharospirillaceae | *Oleispira* | NA | 420 | -5.75999 | 4.0E-02 | Carbohydrate degradation |
| Chloroplast | Chloroplast | Chloroplast | Chloroplast | 1538 | -23.36711 | 2.7E-14 | |
| Actinobacteriota | Nocardiaceae | *Gordonia* | NA | 1403 | -5.54588 | 3.5E-02 | Peptidoglycan synthesis, antifreeze production, carbohydrate degradation |
| **ASVs differentially abundant in FW and 101 sample set** | | | | | | | |
| Firmicutes | Streptococcaceae | *Streptococcus* | NA | 1402 | -24.62896 | 1.2E-13 | Peptidoglycan synthesis, and carbohydrate degradation |
| Firmicutes | Streptococcaceae | *Streptococcus* | *S. salivarius* | 609 | -9.35070 | 3.6E-14 | Peptidoglycan synthesis, fermentation and antifreeze production |
| Firmicutes | Streptococcaceae | *Lactococcus* | *L. lactis* | 391 | -6.10004 | 4.2E-04 | Peptidoglycan synthesis and carbohydrate degradation |
| Proteobacteria (Gamma) | Moraxellaceae | *Acinetobacter* | NA | 927 | -6.86770 | 2.7E-03 | None identified |
| Proteobacteria (Gamma) | Vibrionaceae | *Vibrio* | NA | 116 | -6.69359 | 4.0E-04 | Norspermidine biosynthesis, fermentation and carbohydrate degradation |
| Bacteroidota | Crocinitomicaceae | *Fluviicola* | NA | 743 | -2.36485 | 4.5E-02 | None identified |
| Bacteroidota | Chitinophagaceae | Chitino-phagaceae (F) | NA | 46 | -5.84016 | 3.7E-02 | Fermentation and carbohydrate degradation |
| Actinobacteriota | Dietziaceae | *Dietzia* | NA | 509 | -6.64266 | 2.2E-03 | Peptidoglycan synthesis |
| Stramenopiles | Bacillariaceae | *Fragilariopsis* | *F. cylindricus* | 355 | -4.88348 | 8.6E-08 | |
| Stramenopiles | Chaetocerotaceae | Chaetoceros | *C. gelidus* | 956 | -2.34838 | 3.4E-03 | |
| **ASVs differentially abundant in 101 sample set only** | | | | | | | |
| Proteobacteria (Gamma) | Pseudomonadaceae | Pseudomonas | NA | 194 | -22.7872 | 2.41E-13 | Fermentation, carbohydrate degradation |
| Proteobacteria (Gamma) | Pseudo-alteromonadaceae | Pseudo-alteromonas | NA | 149 | -22.2687 | 8.37E-13 | Fermentation, carbohydrate degradation |
| Proteobacteria (Gamma) | Alteromonadaceae | Altero-monadaceae (F) | NA | 133 | -6.52626 | 0.046644 | Fermentation, carbohydrate degradation |
| Chloroplast | Chloroplast | Chloroplast | Chloroplast | 472 | -20.9888 | 1.72E-11 | |

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Table A3. Table A3. The taxonomic assignment, Log2 fold change and abundance characteristics of the eight ASVs that make

 up the foraminiferal core microbiome.

| ASV | Log2 fold change | Taxonomy | ASV Relative abundance in foraminifera | Total ASV counts in foraminifera | ASV Relative abundance in water | Total ASV counts in water | Forams ASV present in | Samples that ASV is missing from |
|---|---|---|---|---|---|---|---|---|
| ASV956 | -2.348 | Bacillariophyceae (*Chaetoceros gelidus*) | 33.46 % | 783,473 | 2.55% | 54,821 | 27/28 | Fm176b |
| ASV355 | -4.883 | Fragilariopsis (*Fragilariopsis cylindricus*) | 5.30 % | 240,554 | 0.04% | 672 | 24/28 | Fm176b, Fm101a, Fm101b, Fm101e, |
| ASV1447 | NA | Flavobacteriaceae (Family) | 2.40 % | 55,735 | 0.45 % | 9,135 | 23/28 | Fm176b, Fm101d, Fm101e, Fm101g, Fm301a |
| ASV1459 | 2.551 | Pseudoalteromonas | 2.96 % | 91.180 | 3.76 % | 85,466 | 25/28 | Fm176b, Fm101e, Fm301b |
| ASV1392 | 2.277 | Flavobacteriaceae (Family) | 0.72 % | 19,781 | 1.46 % | 29,237 | 24/28 | Fm176b, FmBB2a, FmBB2c, Fm101e, |
| ASV122 | NA | Paraglaciecola | 1.88 % | 61,050 | 0.88% | 21,455 | 25/28 | Fm176b, FM301a, Fm301f, |
| ASV308 | NA | Halieaceae (Family) | 0.88 % | 31,168 | 0.27 % | 5,374 | 26/28 | Fm176b, Fm101e |
| ASV833 | -3.7123 (sig at G and F level) | Bradyrhizobium | 0.09 % | 1799 | 0.004 % | 83 | 23/28 | Fm176b, Fm101b, Fm101c, Fm115a, Fm301d |

Table A1. Major ASVs responsible for the significant compositional differences (log2 fold change > +/- 10). between the provenances (water column and the foraminiferal microbiome). Negative values indicate a reduction in the water column mean relative abundance of the ASV compared to the foraminiferal mean relative abundance of the ASV and positive values indicate an increase in ASV in the water column compared to the foraminifera.

| ASV | Log2fold | padj | Significance in: | Taxonomy |
|------|-----------|--------|-------------------|-----------|
| ASV130 | -10.64059789 | 6.57E-29 | foraminifera | *Streptococcus* sp. |
| ASV42 | -10.6621809 | 4.32E-18 | foraminifera | *Vibrio* sp. |
| ASV10 | -10.84377001 | 2.79E-38 | foraminifera | *Micrococcus* sp. |
| ASV107 | -23.69049864 | 1.22E-15 | foraminifera | *Haemophilus* sp. |
| ASV39 | -25.23598127 | 1.37E-21 | foraminifera | *Haemophilus* sp. |
| ASV11 | -25.70969167 | 1.22E-41 | foraminifera | *Acinetobacter* sp. |
| ASV84 | -26.68731833 | 1.14E-25 | foraminifera | Bacillariophyceae (diatom class) |
| ASV47 | -27.43574907 | 1.77E-20 | foraminifera | *Fragilariopsis* sp. |
| ASV18 | -28.15660108 | 2.61E-25 | foraminifera | *Cylindrotheca* sp. |
| ASV6 | -28.90488689 | 2.17E-48 | foraminifera | *Streptococcus* sp. |
| ASV45 | 11.46484725 | 2.82E-48 | water column | SAR clade (Marine group B) (Order) |
| ASV95 | 10.69026983 | 1.26E-29 | water column | Pseudohongiella |
| ASV71 | 10.46031032 | 7.44E-32 | water column | Marinimicrobia (Phylum) |
| ASV17 | 10.42038191 | 4.02E-33 | water column | Alphaproteobacteria Clade Ib (Order) |
| ASV28 | 10.34374957 | 1.44E-38 | water column | Candidatus Aquiluna |
| ASV20 | 10.32459242 | 1.77E-31 | water column | Marine Group III (Order) |
| ASV122 | 10.29927908 | 4.06E-43 | water column | Marinimicrobia (Phylum) |
| ASV2 | 10.28800033 | 3.73E-35 | water column | Marine Group II (Order) |
| ASV52 | 10.18517168 | 3.36E-38 | water column | Candidatus Nitrosopumilus |
| ASV24 | 10.09864971 | 5.62E-19 | water column | Candidatus Nitrosopelagicus |
| ASV90 | 10.00382159 | 8.50E-25 | water column | Marine Group II (Order) |

Table A2. Taxonomic designations of those ASVs that drive the significant compositional differences between provenances, with a higher relative abundance in the foraminiferal microbiome.

| Prokaryote Phylum | ASV ID | Taxonomy |
|---|---|---|
| | | |
| Prokaryote Phylum | ASV11 | Acinetobacter |
| | ASV107 | Haemophilius sp. |
| Proteobacteria | ASV39 | Haemophilius sp. |
| | ASV107 | Vibrio spp. |
| | ASV40 | Vibrio spp. |
| | ASV116 | Bradyrhizobium sp. |
| | ASV119 | Colwellia sp. |
| | ASV48 | Colwellia sp. |
| | ASV91 | Alteromonas sp. |
| | ASV38 | Moritella sp. |
| | ASV71 | Family Halieaceae |
| | ASV106 | Flaviicola sp. |
| | ASV3 | Crocinitomix sp. |
| Bacteriodetes | ASV79 | Family Cryomorphaceae |
| | ASV71 | Family Cryomorphaceae |
| | ASV67 | Family Flavobacteriaceae |
| | ASV15 | Family Flavobacteriaceae |
| | ASV29 | Family Saprospiraceae |
| | ASV19 | Family Cyclobacteriaceae |
| Firmicutes | ASV8 | Streptococcus sp. |
| | ASV6 | Streptococcus sp. |
| | ASV130 | Streptococcus sp. |
| | ASV110 | Lactococcus sp. |
| Planctomycetes | ASV101 | Rubripirellula sp. |
| Actinobacter | ASV10 | Micrococcus sp. |

## 7 Data availability

All sequence data have been submitted to NCBI where they are freely available. *N. pachyderma* 18S sequences can be found at NCBI, GenBank Accession numbers OR137988-OR138014. The metabarcoding dataset can be found in the NCBI Sequencing Read Archive under BioProject accession PRJNA984332. No code was written for this analysis. All code used was standard scripts for each package utilised.

## 8 Author Contribution

CB contributed to the conception and design of the work, gained funding to carry out the molecular work and wrote the manuscript. KD contributed significantly to the writing of the manuscript and conception of the work. AP made a substantial contribution to the conception of the work, the acquisition of funding for sample collection, collected samples in 2017, and contributed to the editing of the manuscript. RT collected samples in 2018 and contributed to the editing of the manuscript.

## 9 Competing interests

The authors declare that they have no conflict of interest.

## 10 Acknowledgements

The authors wish to acknowledge the work of Steve Mitchell at the University of Edinburgh TEM facility for substantial contributions to sample processing and TEM analysis.

Sample collection was funded by the Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grant (RGPIN-2016-05457) awarded to AJP. Some of the data presented herein were collected by the Canadian research icebreaker CCGS Amundsen and made available by the Amundsen Science program, which was supported by the Canada Foundation for Innovation and Natural Sciences and Engineering Research Council of Canada. The views expressed in this publication do not necessarily represent the views of Amundsen Science or that of its partners.
Transmission Electron Microscopy costs were covered by the Marine Alliance for Science and Technology for Scotland (MASTS) small grant scheme awarded to CB, and molecular lab work was support by a Carnegie Research Incentive Grant awarded to CB.

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