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Salinity drives meiofaunal community structure dynamics across the Baltic ecosystem

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Running title: Benthic meiofauna in the Baltic Sea

Abstract

Coastal benthic biodiversity is under increased pressure from climate change, eutrophication, hypoxia, and changes in salinity due to increase in river runoff. The Baltic Sea is a large brackish system characterized by steep environmental gradients that experiences all of the mentioned stressors. As such it provides an ideal model system for studying the impact of on-going and future climate change on biodiversity and function of benthic ecosystems. Meiofauna (animals < 1 mm) are abundant in sediment and are still largely unexplored even though they are known to regulate organic matter degradation and nutrient cycling. In this study, benthic meiofaunal community structure was analysed along a salinity gradient in the Baltic Sea proper using high-throughput sequencing. Our results demonstrate that areas with higher salinity have a higher biodiversity, and salinity is likely the main driver influencing meiofauna diversity and community composition. Furthermore, in the more diverse and saline environments a larger amount of nematode genera classified as predators prevailed, and meiofauna-macrofauna associations were more prominent. These findings show that in the Baltic Sea, a decrease in salinity resulting from accelerated climate change will likely lead to decreased benthic biodiversity, and cause profound changes in benthic communities, with potential consequences for ecosystem stability, functions and services.

35 **Introduction**

36 Biodiversity underpins essential ecosystem services for human benefits such as food
37 availability, provision of clean water, recreational areas and activities affiliated with human
38 health, and play key roles in ecosystem processes such as nutrient cycling and secondary
39 production (Pan, Marcoval, Bazzini, Vallina, & Marco, 2013). Climate change, eutrophication
40 with associated algal blooms, hypoxic bottom zones, and changes in salinity are contemporary
41 major threats for coastal biodiversity (Pan et al., 2013). Such impacts need to be understood in
42 order to predict how marine ecosystems will respond to future changes.

43 The Baltic Sea is a brackish water system that contains strong abiotic environmental
44 gradients in salinity, depth and temperature that structure its biodiversity and benthic
45 community structure (Ojaveer et al., 2010). The Baltic Sea is also affected by multiple
46 anthropogenic pressures like eutrophication (Conley, 2012) and climate change (Vuorinen et
47 al., 2015). In its deeper basins, below the halocline, hypoxic and anoxic benthic zones are
48 widespread (Conley, 2012). Low-saline areas (< 6 ppt) have expanded in the Baltic Sea since
49 the 1970s and are predicted to further increase with climate change due to increased freshwater
50 runoff and increased water column stratification (Vuorinen et al., 2015). The Baltic Sea
51 therefore presents an ideal ecosystem to study the impact of future climate change scenarios on
52 biodiversity (Ojaveer et al., 2010) and concomitant effects on benthic structure and consequent
53 benthic-pelagic coupling (Griffiths et al., 2017). Most knowledge on how benthic organisms in
54 the Baltic Sea react to these pressures are based on benthic macrofauna, while meiofauna
55 (animals < 1 mm) have been studied much less. Meiofauna is a much more abundant and
56 diverse metazoan group in sediments than macrofauna and plays an important role in a number
57 of ecosystems process (Bonaglia, Nascimento, Bartoli, Klawonn, & Brüchert, 2014;
58 Nascimento, Näslund, & Elmgren, 2012; Näslund, Nascimento, & Gunnarsson, 2010).
59 However, there are still large knowledge gaps regarding how meiofaunal diversity and structure

60 is affected by environmental changes (Bik et al., 2012). Recent DNA and RNA techniques now
61 offer new possibilities to better address such questions on larger geographical scales than
62 previously possible with traditional techniques.

63 Meiofauna have a short life span and are known to stimulate bacterial growth (reviewed
64 in B. C. Coull and Chandler (2001)). Meiofaunal diversity and community composition are
65 structured by several interacting factors; both abiotic and biotic (Giere, 2009). Oxygen is
66 important for meiofaunal survival and metabolism (Braeckman, Vanaverbeke, Vincx, van
67 Oevelen, & Soetaert, 2013), with some exceptions for facultative anaerobes with anaerobic
68 mitochondria (Tielens, Rotte, van Hellemond, & Martin, 2002). Additionally, meiofaunal
69 species richness and abundance have been found to increase with increasing salinity (Bruce C.
70 Coull, 1988). In benthic environments these organisms rework sediment particles through e.g.
71 bioturbation (Cullen, 1973), and have been found to affect porosity and increase the transport
72 of solutes in the sediment (R. C. Aller & Aller, 1992). Meiofauna utilize many sources of
73 organic substrates in the lower trophic food web, e.g. bacteria, and detritus such as settling
74 algal matter from the pelagic water (reviewed in Schratzberger and Ingels (2018)).
75 Furthermore, they have also been found to stimulate degradation of sediment organic matter
76 (OM) and bacterial denitrification (Bonaglia et al., 2014), and may therefore be key players in
77 sediment habitats influencing carbon and nitrogen cycles.

78 One of the most diverse animal groups on Earth are the roundworms, i.e. nematodes
79 (Zhang, 2013), and they are also one the most abundant meiofauna in sediments (Bruce C.
80 Coull, 1999). Nematodes have been found to enhance the oxygen production in diatom biofilms
81 (Mathieu, Leflaive, Ten-Hage, De Wit, & Buffan-Dubau, 2007), and to enhance the
82 mineralization of OM (Nascimento et al., 2012). Because of their different feeding behaviours
83 in sediments, nematodes have been widely used in functional analyses (e.g. Semprucci,
84 Cesaroni, Guidi, & Balsamo, 2018; Vanaverbeke, Merckx, Degraer, & Vincx, 2011). An

85 increased knowledge of nematode community composition in the Baltic Sea could therefore
86 further elucidate the role of trophic interactions in sediments under anthropogenic stress and
87 climate change scenarios.

88 Benthic macrofauna have been observed to control meiofauna populations (or limit in
89 some cases) through e.g. predation (Olafsson, 2003) and competition of limited resources
90 (Ingels, Dashfield, Somerfield, Widdicombe, & Austen, 2014; Nascimento, Karlson, Näslund,
91 & Elmgren, 2011; Olafsson, 2003). There has been extensive work, mainly laboratory or *in*
92 *situ* experimental approaches, conducted on meiofauna-macrofauna interactions using
93 morphological approaches (Olafsson, 2003). Such studies have yielded a variety of mixed
94 results, but also a general consensus that macrofauna bioturbation structures the meiofauna
95 community (Olafsson, 2003). These ecological interactions have been shown to have an
96 importance on biogeochemical cycles, however, studies that focus on meiofauna-macrofauna
97 interactions *in situ* and over regional and ecologically relevant scales are scarce. Macrofauna
98 diversity is generally higher in more saline regions (Gogina et al., 2016), and meiofauna-
99 macrofauna interactions might therefore be more prominent in saline regions with higher
100 diversity and species richness. Gaining such insights will help to elucidate potential trophic
101 interactions in the sediment and how these may be affected by contemporary ecological and
102 environmental pressures.

103 Studies using metabarcoding, i.e. high-throughput sequencing of taxonomically-
104 informative marker genes, to investigate meiofaunal biodiversity is a growing field (Bik et al.,
105 2012; Carugati, Corinaldesi, Dell'Anno, & Danovaro, 2015; Fonseca et al., 2010; Lallias et al.,
106 2014; Peham, Steiner, Schlick-Steiner, & Arthofer, 2017), and opportunities to facilitate such
107 insights and the investigation of 18S rRNA gene meiofauna community in the Baltic Sea are
108 now emerging (Nascimento, Lallias, Bik, & Creer, 2018). Compared to traditional
109 morphological taxonomic techniques, modern sequencing tools facilitate the study of regional

110 patterns of meiofauna diversity in less time while requiring no specific expertise in
111 morphological taxonomy (Carugati et al., 2015). However, caveats do exist, such as not being
112 able to determine absolute abundance and limitations of reference databases to assign
113 taxonomy (Carugati et al., 2015). The benthic meiofauna community of the Baltic Sea is still
114 largely unexplored although many benthic habitats in the Baltic Sea are under stress from
115 anthropogenic pressure.

116 In this study we aimed to assess Baltic Sea meiofaunal diversity and community structure
117 at the ecosystem level. An additional goal was to improve our understanding of possible future
118 trajectories of benthic coastal diversity by using the Baltic Sea as a model system. We
119 specifically tested the following hypotheses: 1) salinity is an important driver of meiofauna
120 community structure in the Baltic Sea, and 2) biotic interactions with macrofauna play a more
121 important role in structuring meiofauna communities in more saline areas coincident with
122 higher macrofaunal species richness. To test these hypotheses we sampled sediment along a
123 salinity gradient in the central Baltic Sea (Baltic Proper). In order to identify changes in
124 community composition and diversity of benthic taxa, a combination of traditional taxonomic
125 assessment for macrofauna and metabarcoding DNA analyses for meiofauna were used.
126 Meiofauna community composition was then analysed together with macrofauna community
127 composition and sediment abiotic parameters (sediment water and OM content, bottom water
128 temperature, salinity, and dissolved oxygen). Finally, because of the large relative abundance
129 and diversity of nematodes, data for the phylum Nematoda were analysed separately to
130 investigate their functional ecology (maturity index and feeding type) along the salinity
131 gradient.

132

133 Materials and methods

134 *Field sampling, collection of macrofauna, and abiotic variables measurements*

135 Soft bottom sediment of similar clay-muddy habitats and water samples were collected in May-
136 June 2015, at 44 stations in the Baltic Sea from the Stockholm region to the southern Arkona
137 basin proper, during the yearly Swedish national and regional benthic monitoring program (Fig.
138 1). Benthic macrofauna communities were sampled with a van Veen sediment grab (0.1 m^2)
139 from each station (typically one replicate per station, except for nine stations that had three
140 replicates due to a yearly monitoring programme: 4, 5, 8, 11, 13, 14, 33, 37, and 44). All
141 macrofauna abundance and biomass data were normalized for m^2 sediment. Benthic meiofauna
142 and sediment variables were measured by collecting sediment cores from the 44 stations using
143 a Kajak gravity corer (surface area: 50 cm^2, one core per station). To investigate large spatial
144 scale variation, we sampled more stations within each region, rather than performing repeat
145 sampling within stations. The latter strategy has been demonstrated to be effective at capturing
146 both small and large spatial scale diversity of European meiofaunal communities (Fonseca et
147 al., 2014; Lallias et al., 2014). Consequently, sediment collected from stations within the same
148 region were treated as ecological replicates for further analyses. For the meiofauna and
149 sediment organic matter the top 0-2 cm layer of each sediment core was sliced and
150 homogenized into a clean and rinsed 215 ml polypropylene container (207.0215PP, Noax Lab,
151 Sweden). Sampling and slicing equipment was rinsed with de-ionized water between each
152 sample. The sliced portion was then divided into: 1) 15 ml transferred to a 90 ml polypropylene
153 container (207.0090PP, Noax Lab, Sweden) for measurement of water and OM content, and 2)
154 the remaining portion kept for meiofauna extraction. Samples were frozen at -20 °C while on
155 the boat, put on ice during transportation to the lab (~2 hours), and finally stored at -20 °C until
156 DNA extraction. Sediment collected for macrofauna was sieved through a 1 mm mesh and the
157 animals retained in the sieve were transferred to 100-1500 ml polypropylene containers (Noax
158 lab, Sweden) and conserved in 4 % buffered formaldehyde for three months (EN 16665:2014,
159 1992). Macrofauna abundance and wet weight biomass were counted visually and weighed

160 according to the European standard (EN 16665:2014, 1992). Sediment water content (%) and
161 OM content (%) were analysed according to Dybern, Ackefors, and Elmgren (1976). In more
162 detail, determination of water content was conducted by drying sediment at 80 °C to a constant
163 weight (at least for 12 hours, typically overnight). The OM content was measured by re-
164 weighing the dry sediment after loss on ignition (500 °C for two hours). Bottom water was
165 sampled at each station, approximately 20 cm above the sediment surface, with a modified
166 Niskin bottle. On deck temperature and salinity were measured in the collected bottom water
167 using a digital multimeter (WTW Cond 340i), and dissolved oxygen (O_2) was measured in
168 duplicate samples using the Winkler titration method (EN 25813:1992).
169
170 *Collection of meiofauna, DNA extraction, and sequencing*
171 The sediment collected for meiofauna analysis was thawed at the laboratory and meiofauna
172 were extracted from the sediment using the procedure described by Nascimento, Karlson, and
173 Elmgren (2008). Sediment samples were sieved through a sterilized 40 µm sieve (autoclaved,
174 rinsed with 90% ethanol and MilliQ water between samples). Meiofauna retained on the 40 µm
175 sieve were isolated by density extraction using a Levasil silica gel colloidal dispersion solution
176 (H.C. Starck) with a density of 1.3 kg m^{-3}. The isolation was performed by shaking an
177 Erlenmeyer flask with sediment and Levasil and let it stand for 5 min, while the sediment
178 particles settle and the meiofauna floats up. The top part of the solution containing the
179 meiofauna was decanted and washed with seawater (of approximately equal salinity to the
180 respective sampling site). This isolation procedure was repeated twice (a second isolation with
181 5 min of settling time, followed by a third and final isolation with 30 min of settling time). The
182 pooled content of these 3 isolations was then placed in the 40 µm sieve and washed thoroughly
183 with seawater to remove any remaining Levasil. The 40 micron sieve content was transferred
184 into a 50 ml falcon tube with a maximum final volume of 10 ml meiofauna isolate (representing

185 the total meiofauna individuals from approximately 100 g of wet sediment). The meiofauna
186 isolate was then frozen at -20 OC until DNA extraction.
187 DNA from the meiofauna isolate was extracted with the PowerMax® Soil DNA Isolation
188 Kit (MOBIO, Cat#12988). After DNA extraction, samples were frozen at -20°C in 3 mL of
189 elution buffer C6 solution (10mM Tris). Following this procedure, 100 μL of each DNA extract
190 was purified with PowerClean® Pro DNA Clean-Up Kit (MOBIO, Cat# 12997-50) and stored
191 in 100 μL of elution buffer C5 (10mM tris) solution at -20°C. All DNA extracts were
192 standardized to a concentration of 10 ng/μL before amplification. The conservative
193 metabarcoding primers TAReuk454FWD1 (5′-CCAGCA(G/C)C(C/T)GCGGTAATTCC-3′)
194 and TAReukREV3 (5′-ACTTTCGTTCTTGAT(C/T)(A/G)A-3′) (Stoeck et al., 2010) were
195 used with Q5 HS High-Fidelity Master Mix (2X) (New England Biolabs, USA) to amplify by
196 PCR the 18S rRNA gene region, targeting fragments between 365 and 410 bp excluding
197 adaptors and barcodes. Each sample was amplified in triplicates, which were then pooled, dual-
198 barcoded with Nextera XT index primers following Bista et al. (2017) and visualized by gel
199 electrophoresis. The barcoded amplicons were then purified with the Agencourt AMPure XP
200 PCR Purification kit (Beckman Coulter), quantified with Qubit (Invitrogen, USA) and pooled
201 into a library with equimolar quantities. See full details of the PCR protocol and programs in
202 Text S1. The library was sequenced with a 2×300 bp paired-end setup on the Illumina MiSeq
203 platform at the National Genomics Institute (NGI -Stockholm, Sweden).
204
205 *Bioinformatics*
206 A total of 18.4 million sequences, averaging 419 238 paired-end reads per sample (44 samples),
207 were processed following the DADA2 pipeline according to Callahan et al. (2016). DADA2
208 uses a parameterized model of substitution errors to differentiate between sequencing errors
209 and biological variation. It avoids constructing operational taxonomic units (OTUs), inferring

210 instead sequence variants. Following the DADA2 pipeline the raw sequences were trimmed to
211 remove low quality bases (the first 10 nucleotides and from position 190 and 240), filtered
212 (maximum of 2 expected errors per read), followed by merging the paired-ends. After this
213 procedure chimeras were then removed from the data set. Following quality filtering and
214 chimera removal a total of 3309 amplicon sequence variants (ASVs) and 9.2 million sequences
215 were retained, averaging 209 545 reads per sample (minimum = 45 729 reads and
216 maximum = 391 690 reads).
217 Because the taxonomic classification results from SILVA 132 could not satisfactory
218 annotate sequence variants to genus level (e.g. no Nematode sequence could be classified
219 further than Order, as well as some sequences were incorrectly classified as Arthropoda as seen
220 previously (e.g. Holovachov, Haenel, Bourlat, and Jondelius (2017)), the DADA2 sequence
221 variants were additionally aligned and annotated against the NCBI NT database using BLAST
222 2.7.1+ (Altschul, Gish, Miller, Myers, & Lipman, 1990) with a 0.001 e-value threshold and -
223 max_target_seqs 1 to only report the top hit. The NCBI NT accession numbers for each
224 sequence were imported into MEGAN 6 (with default LCA parameters (D. H. Huson & Mitra,
225 2012)) in conjunction with the “accession to taxonomy June 2018” MEGAN database
226 (nucl_acc2tax-Jun2018.abin). This made it possible to retrieve taxonomy names based on
227 NCBI accession numbers, and estimate more specific taxonomy with the use of the Lowest
228 Common Ancestor (LCA) algorithm (Daniel H. Huson, Auch, Qi, & Schuster, 2007). The
229 function “read names to taxonomy path” was used to extract all assigned DADA2 sequences
230 with their affiliated taxonomy path. These results were then combined with the DADA2
231 sequence variants counts, and the results based on the NCBI NT database were used for
232 taxonomy analyses. Sequences affiliated with Metazoa in the taxonomic description were
233 extracted from the dataset and analysed further as relative abundances (i.e. (x/sum) × 100) in
234 the software Explicet 2.10.5 (Robertson et al., 2013). In addition, Nematoda sequences were

235 extracted into a sub-dataset (on average 27 385 sequence counts per sample) and
236 phylogenetically placed on a reference tree as suggested by Holovachov et al. (2017). In more
237 detail, reference sequences from Holovachov et al. (2017) were downloaded from NCBI
238 GenBank, and aligned in MEGA 7 (Kumar, Stecher, & Tamura, 2016) using MUSCLE (Edgar,
239 2004) (with settings: gap open: -400, gap extend: 0, max iterations: 8, cluster method: UPGMA,
240 min diag length: 24). The alignment was used to construct a phylogenetic maximum likelihood
241 tree with 100 bootstraps (settings: Tamura-Nei model, nucleotide substitution type, rates
242 among sites: uniform rates, gaps/missing data: complete deletion, ML Heuristic model:
243 Nearest-Neighbor-Interchange). The Nematoda sequences were phylogenetically aligned using
244 PaPaRa 2.5 (Berger & Stamatakis, 2011) with the constructed reference alignments and
245 maximum likelihood tree. The output alignments were used with RAxML 8.2.12 (Stamatakis,
246 2014) to predict the taxonomy of the aligned Nematoda sequences (with the following
247 commands: -f v -m GTRCAT), that adds the input sequences on a reference tree using thorough
248 read insertion with a nucleotide General Time Reversible model. The final tree was visualized
249 in the software FigTree v1.4.3.
250
251 *Statistics*
252 To detect differences in community composition between sites Non-metric multidimensional
253 scaling (NMDS) ordination was performed by loading Metazoa sequence variants data into the
254 R package phyloseq 1.24.2 (McMurdie & Holmes, 2013) using R 3.5.1 (R Core Team, 2013).
255 In more detail, NMDS plots of Bray-Curtis dissimilarity, based on the sequence variants
256 relative abundances and presence/absence (Sørensen), were constructed using the 'ordination'
257 and 'plot.ordination' functions in phyloseq. To test for statistical differences in community
258 composition, this was followed by statistical testing with pairwise PERMANOVA tests (9999
259 permutations) using the adonis function in the vegan package (Oksanen et al., 2018). In

260 addition, the 'betadisper' function in the vegan package was used to find differences in
261 multivariate homogeneity of beta diversity variance between regions (Anderson, Ellingsen, &
262 McArdle, 2006). This was followed by PERMANOVA tests of the homogeneity variance
263 between regions, and plotted using ggplot2 package as the average distance to the centroid.
264 Alpha diversity indexes (ACE, Chao1, and Shannon's H) were based on all Metazoa sequence
265 variations counts and were calculated in the software Explicet. Before alpha diversity analysis,
266 counts were sub-sampled to 2200 counts for each station (lowest sample size; Station 14),
267 except for one station (Station 33 Gotland) that was excluded due to having fewer counts than
268 the amount of metazoan sequence variants in the dataset (station 33: 291 counts). Afterwards
269 the dataset was bootstrapped 100 times, alpha diversity was calculated, and the mean of each
270 alpha diversity index reported. In addition, ACE alpha diversity was calculated by using non-
271 subsampled counts using the fossil 0.3.7 package (Vavrek, 2011) in R.

272 Based on classified nematode genera that could be annotated according to functional traits,
273 1) the maturity index described by Bongers (1990) was calculated to identify habitat colonizers
274 or persisters (based on a 1-5 scale per genera; values closer to 1 indicate colonizers), and 2)
275 feeding type was determined according to Wieser (1953) for each genera based on available
276 literature outlining their buccal cavity morphology. Statistics on alpha diversity, taxonomic
277 groups, and nematode feeding types were conducted in the software IBM SPSS Statistics 25.
278 The normality distribution of the data was tested with Shapiro Wilk tests, and non-parametric
279 Kruskal-Wallis tests were used on data not following a normal distribution.

280 The function 'bioenv' in the R package vegan was used to test which, or combination of,
281 abiotic variables (based on euclidean distances) had the highest rank correlation explaining the
282 Bray-Curtis dissimilarity distribution of sequence variants among the sampling stations (with
283 the following parameters: method="spearman", index="bray", partial=NULL,
284 metric=c("euclidean")). This was followed by Mantel tests (Mantel, 1967) of Bray-Curtis

285 dissimilarity distances and abiotic variables (salinity and spatial distance) in R using the ade4
286 package and 9999 permutations (Dray & Dufour, 2007).
287 To find potential biotic interactions between meiofauna and macrofauna, co-occurrences
288 among meiofauna, and possible community niches based on abiotic variables we conducted
289 correlation network analysis (Röttjers & Faust, 2018). Correlation network analysis was
290 conducted by importing Metazoa genera sequence counts as primary data, and the measured
291 values for abiotic variables and macrofauna abundances per sediment m^{-2} as metadata using
292 CoNet 1.1.1 (Faust & Raes, 2016) and visualized in Cytoscape 3.6.1 (Shannon et al., 2003).
293 The setup in CoNet consisted of normalizing sequence counts as proportions per sample;
294 setting spearman correlations with rho thresholds $\leqslant$ -0.7 or $\geqslant$ 0.7, and Fisher's z *P*-value
295 threshold < 0.05 with Bonferroni adjustment for multiple-test correction. We are aware that
296 our dataset included a complicated setup, less commonly used in network software (Röttjers &
297 Faust, 2018), with 18S rRNA gene sequencing data combined with both abiotic and
298 macrofauna data. However, we applied a number of recommendations outlined in Röttjers &
299 Faust (2018) to minimize potential limitations of such an approach, namely: 1) data from
300 meiofauna were physically isolated from sediments; 2; we employed the DADA2 methodology
301 that incorporates denoising algorithms; 3) we grouped metazoan sequence variants into 125
302 groups (120 genera and 5 unclassified groups); and 4) differences in meiofaunal community
303 composition between north and south sample regions were based on the NMDS Bray-Curtis.
304 In combination with the bioenv analysis that identified salinity as a major factor of diversity
305 and community structure, we divided the data into two clusters (north and south Baltic proper)
306 remove influences of heterogeneous local environmental factors. Such precautions strengthen
307 the correlation network analysis, and emphasises ecological relevance (as reviewed in Röttjers
308 & Faust, 2018).
309

310 **Results**

311 The DADA2 analysis of the raw sequence data resulted in 3309 18S rRNA gene sequence
312 variants of which 770 belonged to the Metazoa kingdom distributed over 120 genera. On
313 average 23 % of the sequences per sample were unassigned with BLAST, and could not be
314 classified to a phyla in the SILVA database, and were therefore not included in further analyses.
315 See Table S1 for a list of all DADA2 sequence variants, the taxonomic classifications and
316 sequence counts, and Table S2 for a full list of metazoan genera.

317

318 *Meiofauna beta and alpha diversity*

319 The NMDS analysis of all meiofauna Metazoa sequence variants (based on relative
320 abundances) showed that the majority of the sampling sites formed two significantly different
321 clusters; one for sites located in the north Baltic Proper (from here on abbreviated as NBP, n =
322 33) and a second cluster for the south Baltic Proper (abbreviated as SBP, n = 11; Fig. 2a; adonis,
323 PERMANOVA tested for the two clusters, $R^2 = 0.35197$, F = 22.812, $P < 0.01$). Data based on
324 presence/absence showed similar results with the two NBP and SBP clusters being significantly
325 different (Fig. S1). PERMANOVA tests also showed a difference between the sampling
326 regions when tested with relative abundance and presence/absence for the whole model (R^2 =
327 0.54185, F = 6.0825 and R^2 = 0.46939, F = 4.5495, respectively; $P < 0.01$ for both). Looking
328 more closely at the homogeneity of beta diversity variance between the regions in the Baltic
329 Proper, Sörmland was significantly lower from all regions except Östergötland and Bornholm
330 (betadisper, PERMANOVA, $P < 0.01$; Fig. 2b, see Table S3 for a full list of P-values for the
331 geographic regions). In addition, the two regions in the SBP were significantly different from
332 each other (i.e. Bornholm being lower compared to Arkona; betadisper, PERMANOVA, P <
333 0.01; Fig. 2b). There was a relatively large abundance of pelagic Arthropoda in the 18S rRNA
334 gene dataset, and therefore, NMDS analysis was also performed without these sequence

335 variants (mainly pelagic Copepod genera *Eurytemora* and *Temora*; see Table S3 for a full list
336 of excluded genera). This analysis also showed two distinct clusters between the NBP and SBP
337 (Fig. S2a; station 33 Gotland excluded to keep statistical power, as it only contained pelagic
338 Arthropoda; adonis, PERMANOVA, $R^2 = 0.23126$, $F = 11.732$, $P < 0.01$). After removing the
339 pelagic Arthropoda there were more significant differences in homogeneity of beta diversity
340 variance between regions. For example Sörmland and Östergötland were significantly different
341 compared to all regions except Stockholm and Arkona, respectively. The deeper (64-124 m)
342 regions Sörmland offshore and Bornholm were lower compared to all other regions.
343 Furthermore, similar to the results from the whole dataset the southern region Bornholm was
344 significantly lower compared to the other southern region Arkona (betadisper, PERMANOVA,
345 $P < 0.05$ for all tests; Fig. S2b and Table S3). As such, the differences in meiofaunal
346 homogeneity variance between regions were larger after the pelagic Arthropoda had been
347 excluded from the dataset.

348 A higher alpha diversity, based on all Metazoa sequence variants, was observed in the SBP
349 stations compared to the NBP ($P < 0.01$ for all indexes (ACE, Chao1, and Shannon's H); One-
350 way ANOVA; Fig. 3). When alpha-diversity was tested on the Nematoda sequence variants
351 alone, there was also a significant difference ($P < 0.01$ for ACE and Chao1, $F = 4.1$ for both;
352 Shannon's H not significant; Fig. 3). Similar results for the nematodes were also observed when
353 ACE was tested on non-subsampled data ($P < 0.01$), although not when all metazoa sequence
354 variants were tested ($P = 0.08$). These results show that a higher diversity of Metazoa sequence
355 variants were obtained in SBP sediments. A full list of alpha-diversity indexes for each station
356 for all meiofauna and Nematoda sequence variants is available in Table S4.
357

358 *Meiofauna community composition*

359 Similar to the NMDS and alpha diversity analysis, there was a difference in relative abundance
360 in phyla between the NBP and SBP, with Arthropoda having a higher relative abundance in the
361 NBP compared to the SBP ($P < 0.01$, Mann-Whitney U test). In contrast, the phylum Nematoda
362 had a lower relative abundance in the NBP ($P < 0.01$, Mann-Whitney U test; Fig. 4a). Looking
363 closer at the genera belonging to Arthropoda, the genus *Eurytemora* was dominant in the NBP
364 compared to the SBP where *Temora* had the highest relative abundance ($P < 0.01$ for both,
365 Mann-Whitney U tests; Fig. 4b).

366 Nematodes showed a much higher diversity compared to the other major phyla, with 60
367 Nematoda genera compared to 28 and 19 genera belonging to Arthropoda and Platyhelminthes,
368 respectively (Fig. 4c, a full list of all genera is available in Table S2). The phylogenetic
369 placement of Nematoda sequences on a reference tree showed that the most dominant
370 Nematoda sequences (Table S1) aligned closely to NCBI reference sequences from
371 Holovachov et al. (2017) (Fig. S3). The Nematoda results also indicates that NMDS ordination
372 of Bray-Curtis dissimilarities and homogeneity of variance between geographic regions show
373 near-identical results as the meiofauna dataset without pelagic Arthropoda (Fig. S2a and S2b)
374 (Nematoda results are available in Fig. S4 and Table S3), suggesting that Nematoda were key
375 organisms affecting meiofaunal community composition. Looking closer at the Nematoda
376 genera there was a significant higher relative abundance for *Aphanolaimus*, *Cyatholaimus*, and
377 *Daptonema* in the NBP compared to the SBP (all $P < 0.01$, Kruskal-Wallis test; Fig. 4c). In
378 contrast, the genera *Axonolaimus* and *Enoplolaimus* had a higher relative abundance in the SBP
379 ($P < 0.05$ and $P < 0.01$, respectively; Kruskal-Wallis test; Fig. 4c). In addition, the relative
380 abundance of unclassified sequence variants belonging to the Nematoda phylum was higher in
381 the NBP ($P < 0.01$, Kruskal-Wallis test). The relative abundance of Nematoda unclassified
382 sequence variants was especially high in the Sörmland regions (Fig. 4c). The phylogenetic

383 placement analysis indicated that the most relatively high abundant unclassified Nematoda
384 sequences belonged to the genus *Chromadorita* (Table S1 and Fig. S3).
385 Maturity index calculations, used to estimate nematode genera as habitat colonizers or
386 persisters, showed that all observed nematode genera in the current study are classified closer
387 to colonizers rather than persisters (maturity index < 2.7; Table S5). In more detail, values
388 closer to one indicate colonizers with high reproduction able to more easily colonize new
389 habitats, while values closer to five indicate persisters with slow reproduction (Bongers, 1990).
390 Nematode genera were also classified into feeding type (according to Wieser (1953)), and
391 showed that the most southern region Arkona had more predators/omnivores compared to all
392 other regions (One-Way ANOVA Tukey HSD post hoc test, $P < 0.01$; Fig. 5d). Looking at the
393 feeding types of nematode genera with a high relative abundance in the NBP the *Cyatholaimus*
394 and unclassified sequence variants (potentially *Chromadorita*) were classified as epistrate
395 feeders (feeding type 2A) (Table S5; unclassified sequence variants not included). In the SBP
396 the genera *Enoplolaimus* was classified as predatory possessing large teeth (2B), while
397 *Microlaimus* was classified as 2A (Table S5). Other genera with a high relative abundance in
398 the Nematoda dataset such as *Aphanolaimus*, *Daptonema*, and *Axonolaimus* were classified as
399 type 1A or 1B, being either selective or non-selective deposit feeders, respectively. A full list
400 of maturity indexes and feeding type classifications is available in Table S5.
401 Looking at the Platyhelminthes the genus *Odontorhynchus* showed a significant difference
402 with a higher relative abundance in the SBP, although with high variation, ($P < 0.05$, Mann-
403 Whitney U test; Fig. 4d). In the two SBP regions the genus *Placorhynchus* was dominant in
404 the Bornholm region while *Odontorhynchus* was more prevalent in the Arkona region ($P <$
405 0.05, Mann-Whitney U test).
406
407 *Macrofauna in the sediment*

408 The Macrofauna data showed a higher species richness in the SBP than in NBP (on average 8
409 species per station compared to 4 in the NBP; Fig. 6). There were also more species belonging
410 to the Annelida phylum in the SBP, e.g. *Bylgides sarsi*, *Nepthys caeca*, *Pygospio elegans*, and
411 *Scoloplos armiger* (Fig. 6). The Bornholm region had the lowest macrofauna richness, with an
412 average of three macrofauna species per station, including the Mollusca *Arctica islandica*, and
413 two Annelida species *Bylgides sarsi* and *Capitella capitata* (Fig. 6). In contrast, other species
414 were only present in the NBP e.g. the Amphipod *Monoporeia affinis* and Isopod *Saduria*
415 *entomon* (Fig. 6). Macrofauna were found at almost all stations, except in three regions
416 (Sörmland offshore, Västervik, and Gotland; Fig. 6). A full list of measured values, i.e. not
417 relative proportions, of abundance m^{-2} sediment and gram wet weight biomass m^{-2} sediment
418 are presented in Table S6.
419
420 *Abiotic variables*
421 Bottom water salinity increased as expected in the Baltic Sea (Ojaveer et al., 2010), from the
422 NBP to SBP from 5.3 to 18.9 ppt salinity (Table 1). Bottom water temperature was generally
423 low for most stations (average of ~6 °C) except a few stations in the Östergötland region that
424 had temperatures > 10 °C (average of ~11 °C, stations 16-20; Table 1). Dissolved oxygen was
425 lower in the stations located in the SBP (~6 mg/L; stations 34-44) compared to the NBP (~9
426 mg/L). However, only the deepest stations in the dataset had oxygen concentrations that could
427 be considered hypoxic/anoxic (stations 12, 14, 32, and 33 at 79, 124, 79, and 112 m water
428 column depth; Table 1). Sediment OM was on average ~12.6 % for all stations, but especially
429 higher in the Östergötland regions that had ~16 % (stations 15-28; Table 1).
430
431 *Correlations of meiofauna with abiotic variables and macrofauna data*

432 Abiotic data from all stations were tested with Bray-Curtis dissimilarity of sequence variants,
433 and the best explainable abiotic variables were longitude, latitude and salinity (rho = 0.73).
434 Mantel tests also confirmed that these abiotic variables were significantly correlated with the
435 beta diversity measures (R^2= 0.67 and $P < 0.01$, for both salinity and spatial location tested).
436 The combination of abiotic variables latitude, sediment water content, and oxygen had the best
437 rank correlation explaining the beta diversity among the stations in the SBP (rho = 0.57;
438 'bioenv' test in R package vegan). This was in contrast to the NBP where longitude, water
439 depth, and oxygen were the best explainable variables (although with a low rank correlation,
440 rho = 0.32; in accordance to the lack of correlations with abiotic factors in the correlation
441 network; Fig. 7a).

442 Correlation network analysis was conducted on the NBP and SBP separately because the
443 NDMS Bray-Curtis indicated differences in meiofaunal community structure between the two
444 areas. In addition, the bioenv analysis showed salinity to be a strong driver influencing
445 meiofaunal community structure and diversity in the Baltic Proper. This precaution of
446 removing sample heterogeneity in a larger ecosystem-wide dataset is in accordance to Röttjers
447 and Faust (2018) to lower the risk of unwanted effects on correlation network analysis. Because
448 the macrofauna abundance m^{-2} values correlated strongly with their biomass m^{-2} data (rho =
449 0.74, $P < 0.01$; all abundance and biomass values tested together, $n = 220$), for conciseness
450 only the abundance m^{-2} data were used in the correlation network analysis.

451 The NBP did not show any major significant correlations with the dominant Metazoa
452 genera observed in Fig. 4, i.e. Arthropoda, Nematoda, and Platyhelminthes (Fig. 7a). The
453 Nematoda phyla *Axonolaimus* were correlated with Nematoda *Odontophoroides*, and two
454 Mollusca and the macrofauna species *Mya arenaria* formed a cluster of correlations with low
455 abundant nematodes and arthropods (Fig. 7a), while a few other macrofauna species correlated
456 negatively with water depth (e.g. Chironomidae, *Macoma balthica*, and *Hydrobia*; Fig. 7a;

457 correlation networks with all labels shown are available in Fig. S5 and S6 for NPB and SBP,
458 respectively). In contrast, the SBP showed a complex web of significant correlations between
459 chemistry, macrofauna and especially Nematoda genera (Fig. 7b). This difference between the
460 NBP and SBP was also confirmed when all Metazoa sequence variants were tested for
461 correlations (i.e. not tested on taxonomical genera level; Fig. S7 and Fig. S8, respectively). In
462 the SBP abundant Nematoda genera *Microlaimus* correlated positively with several other
463 nematode genera and the macrofauna crustacean species *Diastylis rathkei* (Fig. 7b). The
464 predator *Enoplolaimus*, a nematode with one of the highest relative abundance in the SBP,
465 correlated positively with the low abundant Nematoda genera *Pselionema* (Fig. 7b). The
466 predator *Bylgides sarsi* that was one of the few macrofauna species in the Bornholm region
467 correlated negatively with the Nematoda genus *Campylaimus* (Fig. S7). Other correlations
468 included e.g. Nematoda genera with other Nematoda, and the Arthropoda genus *Temora* with
469 macrofauna and Nematoda (Fig. 7b). In addition, Crustacean genera were correlated with
470 Nematoda and oxygen (Fig. 7b), and the Platyhelminthes genera *Odontorhynchus* was
471 associated with several Nematoda genera and the macrofauna *Nephtys caeca*. Mollusca species
472 such as *Arctica islandica*, *Mya truncata*, and *Hydrobia* were found in a few clusters involving
473 various meiofauna genera. Finally, a few Annelida macrofauna species such as *Pygospio*
474 *elegans*, *Polydora quadrilobata, and Heteromastus filiformis* formed the beginning or were
475 part of correlation clusters associated with low abundant meiofauna genera (Fig. 7b).
476
477 **Discussion**
478 *Abiotic explanatory variables of meiofaunal diversity*
479 Salinity was the major explanatory variable of benthic meiofauna community composition in
480 the Baltic Proper. In addition to our findings, salinity has been observed to influence
481 macrofauna in the Baltic Sea (Gogina et al., 2016) and meiofauna community structure studied

482 elsewhere (Bruce C. Coull, 1988; Lallias et al., 2014). Interestingly, similar findings were also
483 recently discovered for sediment bacteria community composition along a salinity transect in
484 the Baltic Sea (Klier, Dellwig, Leipe, Jürgens, & Herlemann, 2018). Bottom water oxygen also
485 correlated with the difference in meiofaunal community composition, especially in the SBP.
486 The role of oxygen is not surprising considering that oxygen is essential for the majority of
487 meiofaunal organisms (Braeckman et al., 2013), and oxygen availability is known to cause
488 shifts in the community composition of e.g. nematodes (Nguyen et al., 2018). The local regions
489 as defined in this study (Fig. 1 and Table 1), also harboured significantly different communities
490 of meiofauna (Fig. 2 and 4). This difference could be attributable to specific salinity
491 preferences, but also due to the sediment substrate and available food resources (Lee, Tietjen,
492 Mastropaolo, & Rubin, 1977), and adult dispersal through water currents (Hagerman & Rieger,
493 1981). Marine meiofaunal communities have previously been shown to be heterogeneous both
494 at large (Fonseca et al., 2014) and small spatial scales (Findlay, 1981). Our results indicate
495 salinity to be a major barrier to dispersion of meiofauna species in the Baltic soft sediment, by
496 limiting the dispersion of marine species to the north and of freshwater species to the south.
497 Limitation to dispersion is an important factor driving community assembly in ecological
498 systems (Vellend, 2010). Therefore the salinity gradient in the Baltic Sea influences sediment
499 habitats with different kinds of food and predators that will in turn influence the meiofauna
500 community composition and diversity.
501
502 *Geographical differences in community composition*
503 Meiofaunal diversity was dominated by a large variety of Nematoda genera. This was not
504 surprising considering that nematodes are highly diverse (Zhang, 2013), and typically the most
505 abundant meiofauna found in the sediment surface (Bruce C. Coull, 1999). The SBP had a
506 different Nematoda community composition, likely due to the higher salinity conditions that

507 have previously been found to influence diversity and community structure in the Baltic Sea
508 (Ojaveer et al., 2010). In the NBP there was a large proportion of unclassified Nematoda
509 sequences (Fig. 4c) and could possibly be due to the lack of freshwater-brackish species being
510 classified in the reference databases (Holovachov et al., 2017). These unclassified sequences
511 were indicated in the phylogenetic placement analysis to be affiliated with the genus
512 *Chromadorita* (Fig. S3). This genus has previously been found in the Baltic Sea (Preben
513 Jensen, 1979) and contains species living on macrophytes (Preben Jensen, 1979), free-living
514 and feeding on diatoms (P Jensen, 1984), and living inside cyanobacterial biofilms (Gaudes,
515 Sabater, Vilalta, & Muñoz, 2006). The most southern region Arkona had not only a higher
516 diversity but also a higher proportion of nematode predators/omnivores (Fig. 5d), which could
517 explain why there were more ecological correlations in the SBP (Fig. 7b). Even though the
518 PCR primers used might have selected for certain eukaryotic species, and primer bias are likely
519 to pervade all metabarcoding studies, we used the same biodiversity discovery method (i.e.
520 metabarcoding primers) throughout. Despite the imperfect nature of metabarcoding (and other
521 ecological sampling approaches), the difference among regions and areas in the Baltic Proper
522 were statistically significant and showed stark dissimilarities in community composition.
523 In addition to nematodes, there was also a large relative abundance of Arthropods in the
524 18S rRNA gene dataset, especially in the NBP. The majority of the Arthropoda belonged to
525 the pelagic copepod genera *Eurytemora* and *Temora* in the NBP and SBP, respectively (Fig.
526 4a and 4b). The hatching rate and development time of e.g. *Eurytemora affinis* is negatively
527 affected by low salinity (Karlsson, Puiac, & Winder, 2018) which can explain the difference
528 between the north and south regions. Possible additional explanations for copepods being in
529 the sediment could be due to sinking marine snow containing carcasses, resting stages such as
530 buried eggs or dormancy (Dahms, 1995). The high relative abundance of Arthropoda could
531 therefore be derived from DNA being extracted from a large amounts of copepod eggs or

532 resting stages buried in the sediment surface. Considering that similar results have also been
533 observed by Nascimento et al. (2018) from sediments collected in the Stockholm region, the
534 large proportion of copepods is likely a trait for low saline waters (< 10 ppt) in the Baltic Sea.
535 Compared to the SBP where salinity is higher, the availability of copepod eggs in the low-
536 saline NBP can be a larger source of energy for benthic macrofauna populations (Karlson &
537 Viitasalo-Frösen, 2009). In addition, because the hatching rate is slower in low salinity
538 (Karlsson et al., 2018) the accumulation of a seed bank followed by subsequent hatching could
539 enhance the benthic-pelagic coupling. Our results highlight important geographic differences
540 in meiofaunal communities that are only possible to uncover with modern molecular tools
541 (Fonseca et al., 2010).
542
543 *Biotic interactions*
544 Macrofauna species richness and meiofauna diversity were both higher in the SBP (Fig. 6 and
545 Fig. 3, respectively). Nascimento et al. (2011) found that a higher species richness of
546 macrofauna increased interference competition among meiofauna and/or limited food
547 availability in a laboratory study. Potentially, this could partly explain why there were more
548 ecological connections between macro- and meiofauna in the SBP as indicated by the
549 correlations network data (Fig. 7b). On the other hand, macrofaunal bioturbation can create
550 more habitable niches and higher variety of food types allowing for a higher meiofauna
551 diversity (Meysman, Middelburg, & Heip, 2006). The significant correlations included mainly
552 Annelida as well as crustacean macrofauna, which are well-known bioturbators (Krantzberg,
553 1985). In addition, bottom water oxygen was one of the central nodes in the correlation network
554 with connections to meio- and macrofauna (Fig. 7b). It is therefore possible that oxygen rich
555 burrows made by annelids (R. Aller, 1988) or other modes of bioturbation by macrofaunal
556 organisms (Krantzberg, 1985) stimulate bacterial growth and make specific niches and habitats

557 favourable for meiofauna (reviewed in Olafsson, 2003). However, negative macro-meiofauna
558 interactions have also been previously reported (reviewed in Olafsson, 2003). High macrofauna
559 diversity can increase sediment oxygen consumption (Bolam, Fernandes, & Huxham, 2002),
560 and interference competition with meiofauna by limiting its access to freshly deposited detritus
561 (Nascimento et al., 2011). Such mechanism could explain some of the negative correlations
562 between macro- and meiofauna taxa found in our study. For example, we observed several
563 Mollusca macrofauna in correlation clusters with meiofauna genera in the SBP (Fig. 7b).
564 However, this kind of interaction was not as prominent in the NBP. This is in accordance with
565 previous experimental studies with sediments from the Sörmland region amended with bivalve
566 *Macoma balthica* that showed no significant difference on the majority of meiofauna, including
567 nematodes (Olafsson, Elmgren, & Papakosta, 1993). Considering that correlation network
568 analysis can be a major strength to visualize and detect specific habitat niches (Röttjers &
569 Faust, 2018), the meiofauna-macrofauna associations observed here could be indirect effects
570 of shared niche preference. In addition, predation is an important mechanism structuring
571 diversity in more stable and tropically complex communities (Menge & Sutherland, 1976). The
572 NBP had lower diversity and has a history of being more affected by eutrophication compared
573 to the southern region Arkona (Andersen et al., 2015). The higher relative abundance of
574 nematode predators in the SBP (Fig. 5d) could indicate a relatively more stable environment
575 where predation can maintain a higher diversity helped by more macrofauna-mediated niches,
576 biodiversity and interactions. Although, network correlations based on metabarcoding data
577 need to be treated with caution (see Röttjers & Faust, 2018), our results clearly indicate that
578 there are fewer, direct or indirect associations between meiofauna and macrofauna in low-
579 saline areas in the Baltic Sea.
580
581 *Effects of climate change and future scenarios*

582 The area of low saline regions in the Baltic Sea (surface water salinity < 6 ppt) has increased
583 since the 1970s and are predicted to further increase with climate change due to elevated levels
584 of runoff (Vuorinen et al., 2015). As indicated here a decrease in salinity might be accompanied
585 by a decrease in meiofaunal biodiversity and biotic interactions in the Baltic Sea. Salinity
586 strongly influences the community composition and diversity in other coastal systems (Lallias
587 et al., 2014; Van Diggelen & Montagna, 2016) where similar effects can happen if salinity is
588 reduced as a consequence of climate change. Additionally, it is clear from our results that a
589 continued expansion of hypoxic bottom zones will significantly alter benthic community
590 structure. This may influence important ecosystem functions regulated by meiofauna, like OM
591 degradation and nutrient cycling. Here, we show that multiple anthropogenic pressures like
592 eutrophication (Finni, Kononen, Olsonen, & Wallström, 2001), expansion of hypoxic bottom
593 zones (Meier et al., 2011), and of low-salinity areas (Vuorinen et al., 2015), will likely have
594 profound impacts on benthic communities of anthropogenically stressed coastal systems.
595 Ongoing environmental change will lead to lower benthic biodiversity and fewer biotic
596 interactions. Such structural changes to benthic community composition will likely influence
597 ecosystem functions and services, and decrease ecosystem stability (McCann, 2000).

598

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612

613 **Competing interests**

614 We have no competing interests

615

616 **References**

617

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845

846 **Data Accessibility**

847 The raw sequence data have been uploaded and are available on the NCBI database with the
848 following BioProject number: PRJNA497177.

849

850 **Author contributions**

851 E.B. analysed data and drafted the manuscript, C.R. sampled in the field and together with C.S.
852 conducted laboratory work. J.G. helped with field sampling and gave feedback on the
853 manuscript, S.C. helped designed the study and gave feedback on the manuscript. F.N.
854 designed the study, conducted laboratory work, analysed data and contributed to the manuscript
855 writing. All authors gave final approval for publication.

856

857

858 **Table 1.** List of the station numbers, region, date of sampling during 2015, latitude, longitude, and water column depth. Abiotic parameters
859 measured include bottom water salinity, temperature, dissolved oxygen (mean of two technical measurements), percentage of sediment water
860 content (WC), and sediment organic matter (OM) content. Missing data is denoted by an empty cell.

861

Station	Region	Date	Lat. (dd)	Long. (dd)	Depth (m)	Salinity (ppt)	°C	O_2 (mg/L)	WC (%)	OM (%)
1	Stockholm	May 27	59.5243	18.8533	23.5	5.3	9.0	10.7	86.1	14.0
2	Stockholm	May 27	59.5081	19.0044	58.5	6.6	5.0	8.8	62.7	4.6
3	Stockholm	May 27	59.4788	18.9215	40.3	5.8	6.0	10.9	68.1	6.0
4	Sörmland	May 17	58.8408	17.5518	22	6.3	7.7	11.1	77.0	11.7
5	Sörmland	May 19	58.8261	17.5761	39	6.6	5.0	10.7	81.5	12.2
6	Sörmland	May 17	58.8109	17.6069	37.5	6.7	4.7	10.9	82.2	12.5
7	Sörmland	May 16	58.7902	17.7284	38	6.7	4.4	10.5	80.3	9.9
8	Sörmland	May 17	58.7740	17.6914	44	6.7	4.5	11.0	68.1	6.2
9	Sörmland	May 16	58.7669	17.8313	53	6.9	4.1	11.0	73.8	7.3
10	Sörmland	May 16	58.7440	17.8140	47	6.9	4.2	10.4	79.7	9.9
11	Sörmland	May 16	58.7189	17.8423	59	7.0	4.3	10.4	68.8	6.2
12	Sörmland offshore	May 07	58.5674	17.9085	79	9.1	5.4	0.3	86.2	12.2
13	Sörmland offshore	May 07	58.5489	18.0253	78	6.5	4.9		79.8	7.7
14	Sörmland offshore	May 07	58.4941	18.1167	124	9.9	5.4	0.0	93.5	18.7
15	Östergötland	June 01	58.3961	16.8854	14	6.3	8.7	9.3	86.8	15.0
16	Östergötland	June 01	58.3791	16.9711	12.5	6.4	11.1	10.3	85.5	14.9
17	Östergötland	June 01	58.3763	16.9808	13.5	6.5	10.9	10.4	87.3	16.8
18	Östergötland	June 01	58.3739	16.9444	10	6.4	12.3	10.1	87.8	17.0
19	Östergötland	June 01	58.3697	16.9604	16	6.4	11.1	10.3	85.5	15.4
20	Östergötland	June 01	58.3621	16.9433	19.5	6.4	11.4	10.1	92.1	19.1
21	Östergötland	June 01	58.3234	16.9364	15.6	6.6	7.5	10.5	85.9	14.8
22	Östergötland	June 02	58.3220	16.9715	20.5	6.6	6.7	10.7	89.0	18.6
23	Östergötland	June 02	58.2543	16.7866	39	6.8	6.1	9.8	87.8	15.7
24	Östergötland	June 02	58.2249	16.8153	25	6.7	6.6	10.5	84.7	14.3

25	Östergötland	June 02	58.2169	16.8432	30	6.7	5.6	10.9	85.7	13.9
26	Östergötland	June 02	58.2095	16.9378	33	6.7	5.8	10.9	87.0	18.8
27	Östergötland	June 02	58.2027	16.9152	9.6	6.6	8.6	10.6	87.9	16.4
28	Östergötland	June 02	58.1980	16.8501	29.1	6.7	6.1	10.8	81.3	12.1
29	Västervik	May 08	57.7334	17.0916	72	8.5	4.8	1.5	90.0	16.9
30	Västervik	May 08	57.6019	17.0010	67	7.6	4.5	6.5	71.9	6.3
31	Västervik	May 08	57.5252	16.9691	66	7.7	4.5	6.9	90.1	18.4
32	Västervik	May 08	57.4763	17.0633	79	8.8	5.1	0.0	76.4	7.3
33	Gotland	May 14	57.4000	19.3498	112	11.0	6.2	0.1	94.3	24.7
34	Bornholm	May 09	55.7502	15.9332	64	16.0	7.6	4.7	84.6	12.7
35	Bornholm	May 12	55.6668	16.0658	71	17.2	7.5	2.7	82.0	10.8
36	Bornholm	May 09	55.6177	14.8630	80	18.3	7.2	3.7	85.4	12.7
37	Bornholm	May 12	55.2507	15.9888	91	18.9	7.1	2.8	86.2	13.6
38	Arkona	May 10	55.2334	13.3334	41	14.0	5.5	6.9	69.2	6.6
39	Arkona	May 10	55.2246	13.4182	42	13.9	5.5	7.2	69.5	10.0
40	Arkona	May 10	55.2250	13.6335	43	13.4	5.6	8.6	83.7	13.0
41	Arkona	May 10	55.2248	13.2667	40	14.0	5.6	6.2	76.3	10.1
42	Arkona	May 10	55.1333	13.6666	45	14.3	5.6	8.3	84.2	13.6
43	Arkona	May 10	55.1239	13.2615	40	12.4	5.9	8.8	56.0	4.5
44	Arkona	May 12	55.0090	14.0738	48	14.9	5.5	7.2	83.9	13.3

863 **Figure captions**
864

865 **Figure 1.** The figure shows a map of the Baltic Sea and each sampling station and geographical
866 regions (different coloured circles). Full names and details of the sampling stations are
867 presented in Table 1. The Baltic Proper was divided into two areas for this study: the north
868 Baltic Proper (NBP; stations 1-33) and the south Baltic Proper (SBP; stations 34-44). The
869 colours of the circles denote the different regions in the study, with: yellow as Stockholm; light
870 blue Sörmland; brown Sörmland offshore; purple Östergötland; green Västervik; red Gotland
871 (one station only); grey Bornholm; and orange as Arkona. The map layer is © OpenStreetMap
872 contributors.
873

874 **Figure 2.** Multivariate NMDS based on the relative abundance Bray-Curtis dissimilarities were
875 constructed based on all sequence variants classified as meiofauna (i.e. metazoan 0.40-1000
876 µm) in the 0-2 cm sediment surface layer (a), and boxplots showing the homogeneity of beta
877 diversity variance for each region (b). The colours of the symbols in the NMDS plots denote
878 the specific regions (as shown in Fig. 1), while the numbers denote each specific station.
879 Stations belonging to the north Baltic Proper are presented as circles while stations in the south
880 as triangles.
881

882 **Figure 3.** ACE, Chao1, and Shannon's H alpha diversity indexes of all meiofauna sequence
883 variants (black lines) and only the Nematoda data (orange lines). The x-axis shows the station
884 numbers (Fig. 1). The line type denotes: dashed lines, ACE; dotted lines, Chao1; and filled
885 lines, Shannon's H.
886

887 **Figure 4.** The figure shows stacked bars of the 18S rRNA gene meiofauna dataset in the north
888 and south Baltic Proper 0-2 cm sediment layer, as well as their specific geographic regions.
889 The y-axis shows the station number, and (a) shows relative abundance (%; x-axis) of Metazoa
890 phyla; (b) genera in the Arthropoda; (c) Nematoda; and (d) Platyhelminthes phyla. Bolded text
891 denotes major phyla or genera for each respective graph.
892

893 **Figure 5.** The figure shows the four Wieser (1953) nematode feeding types of the Nematoda
894 genera for each region (classification ID in parentheses). Because unclassified data could not
895 be included in the analysis the relative proportion were based on annotated genera. Each region
896 consist of replicates (i.e. stations) according to the Nematoda data shown in Fig. 4. Note the
897 different scale on the y-axes. The error bars shows the standard error.
898

899 **Figure 6.** The heatmap shows collected macrofauna from the sieved sediment. The stations are
900 numbered and region coloured on the top x-axis. Species level are shown for most macrofauna,
901 except for the class Oligochaeta and family Chrinonomidate. The grey-red gradient shows the
902 relative proportion per species (%) of abundance m^{-2} sediment, while the grey-green gradient
903 shows relative proportion per species of g wet weight biomass m^{-2} sediment. The species
904 richness are shown on the bottom x-axis.
905

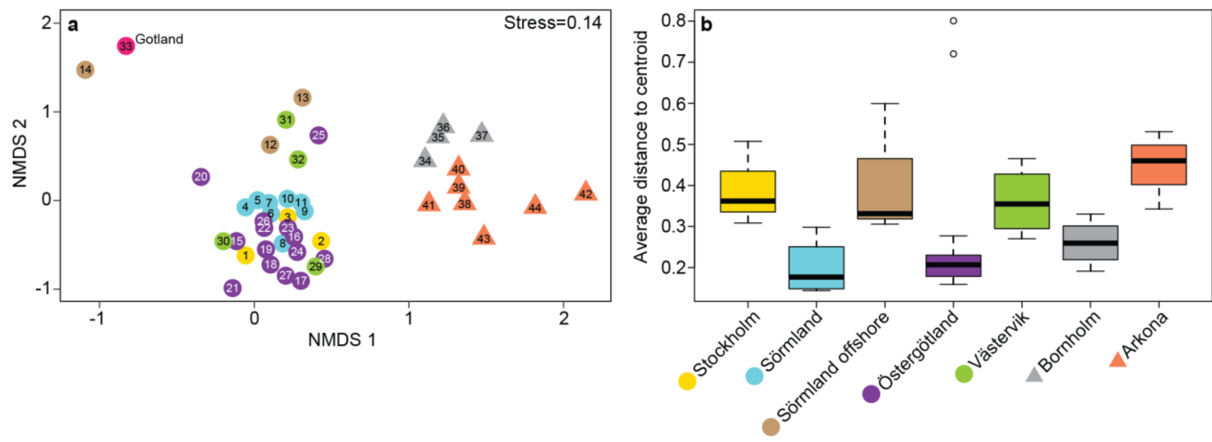
906 **Figure 7.** Correlation networks of spearman correlations based on data from north (a) and south
907 Baltic Proper (b). The correlations included meiofauna 18S rRNA gene data (each node
908 represents one Metazoa genus), abiotic variables, and macrofauna abundance data. The mean
909 was used for the two oxygen technical replicates. The colour of the lines denote rho ≥ 0.7 (red)
910 or ≤ -0.7 (blue). All correlations are statically significant ($P < 0.05$). All abiotic nodes have
911 been labelled as well as a few genera/macrofauna nodes according to the results presented in

912 the text. Nodes with black borders denote unclassified sequences belonging to a certain
913 phylum.
914

915

916 Figure 1

917

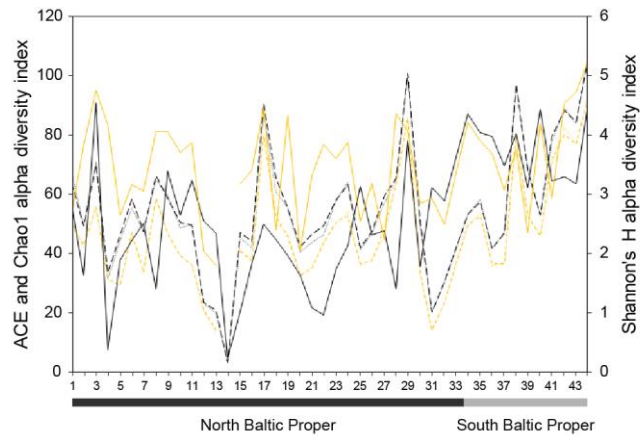


918

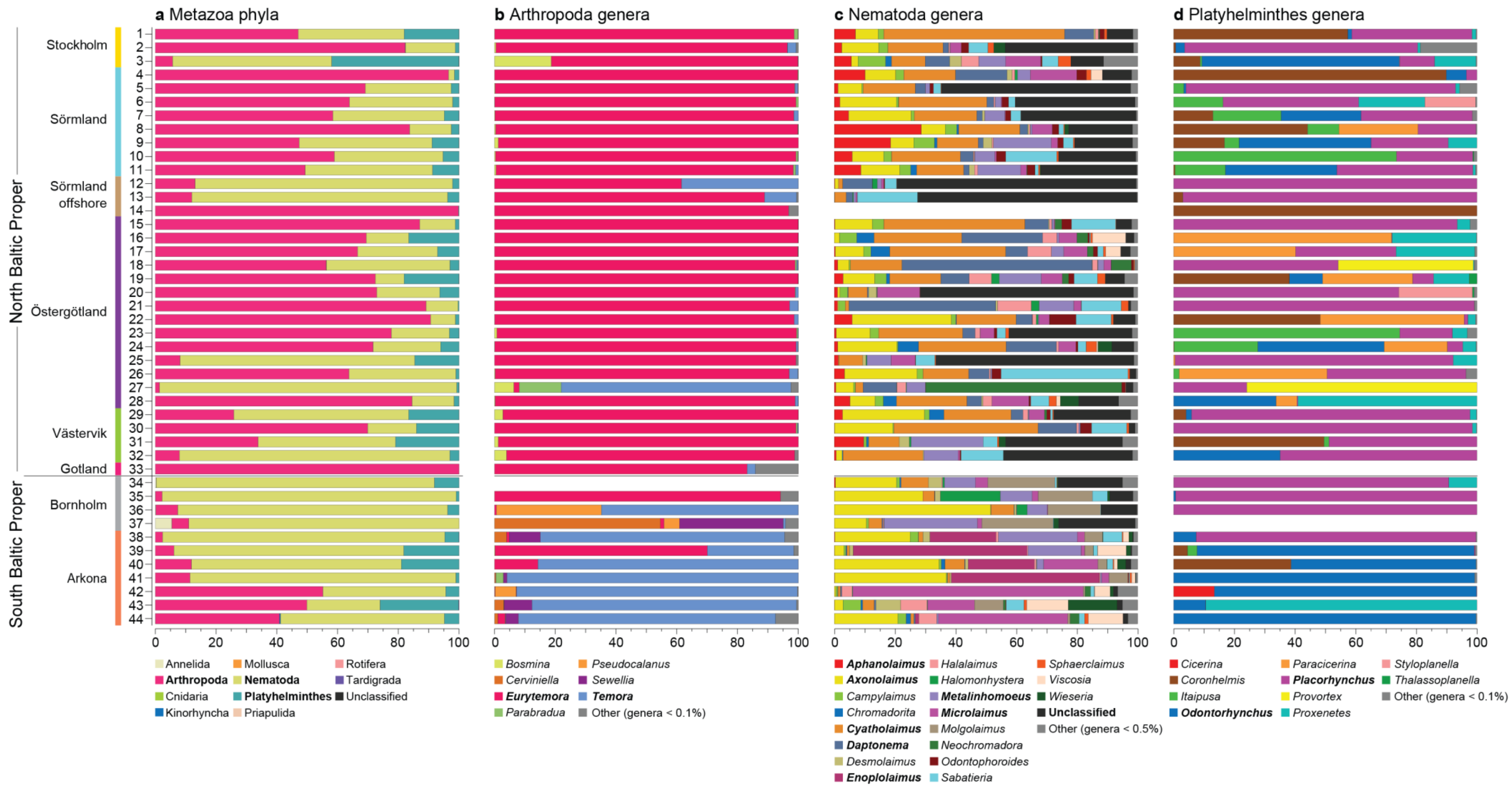
919 Figure 2

920

921



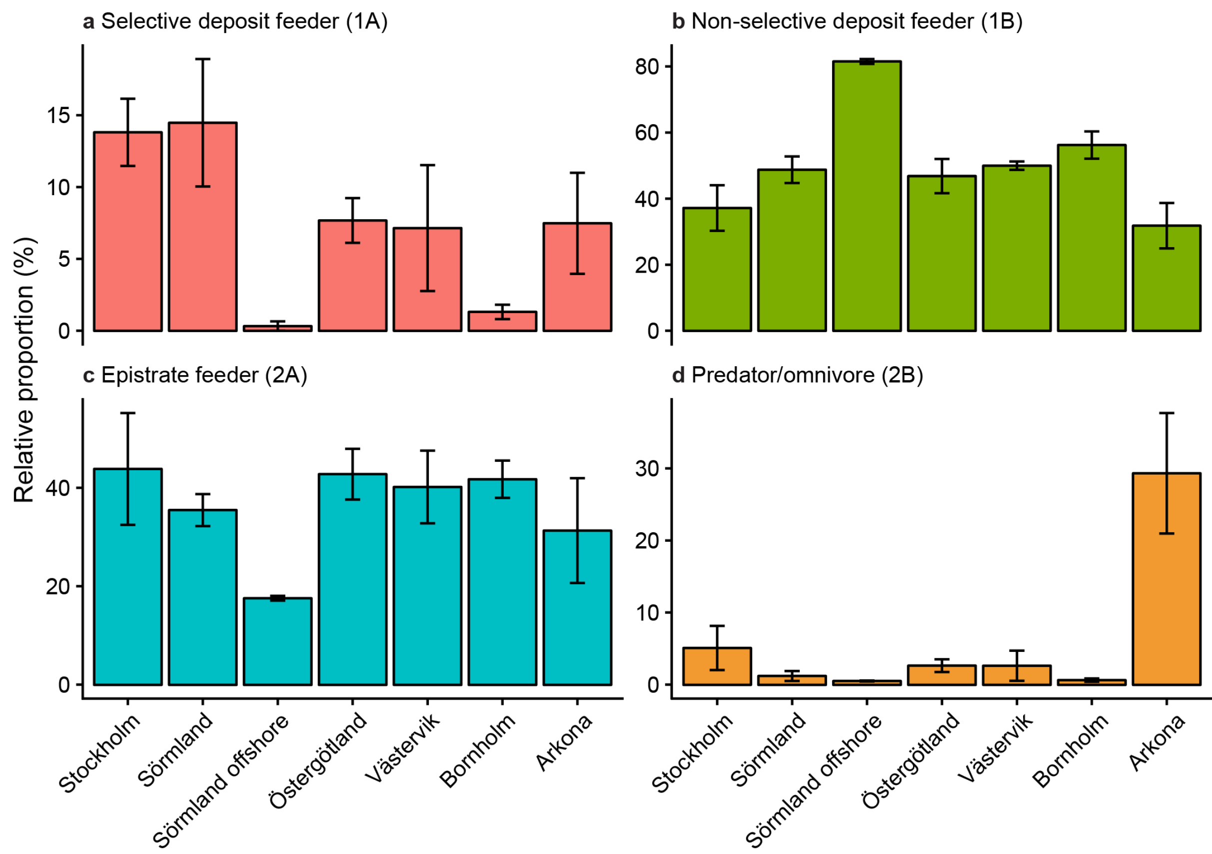
922 Figure 3



923

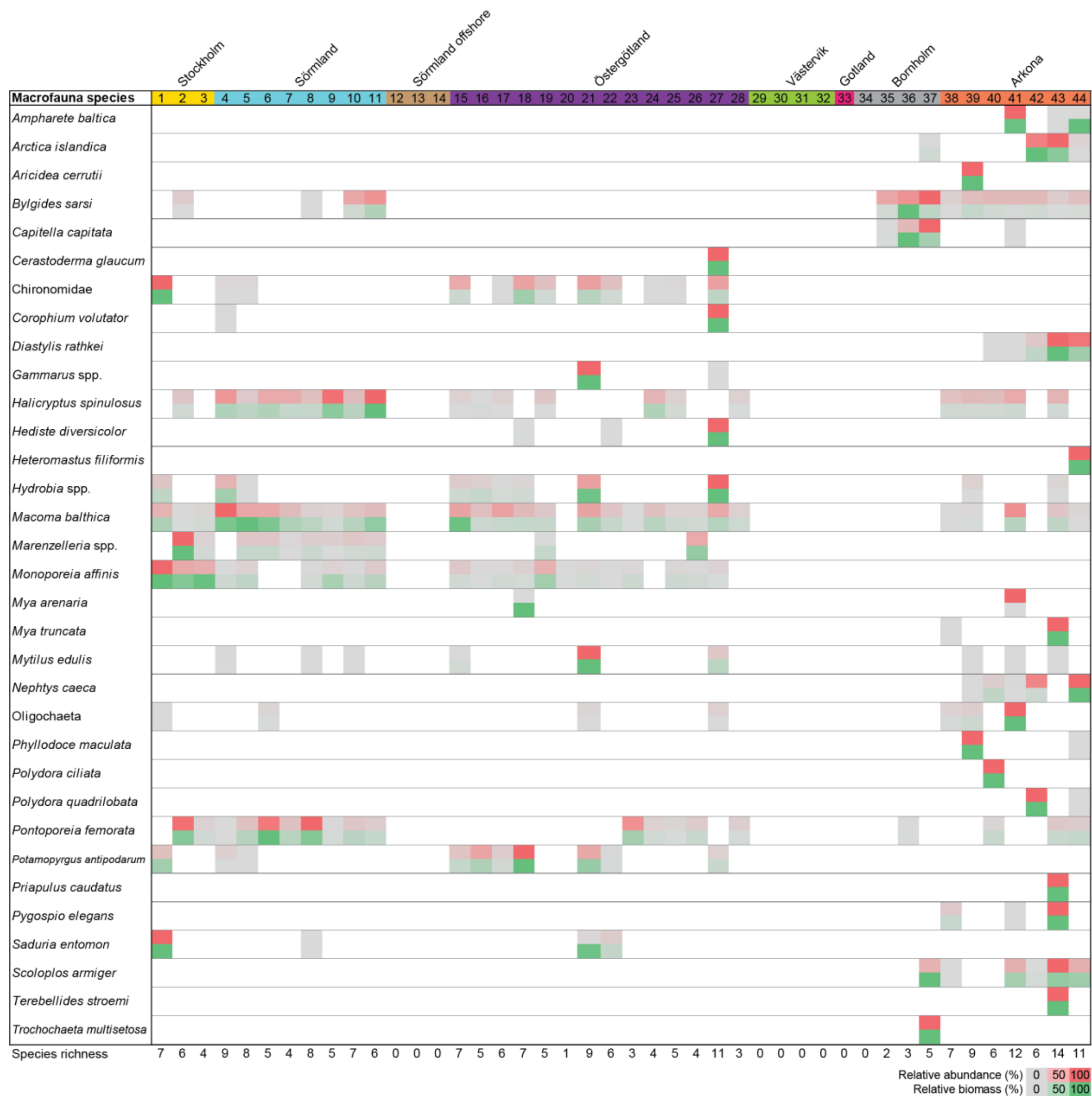
924 Figure 4

925



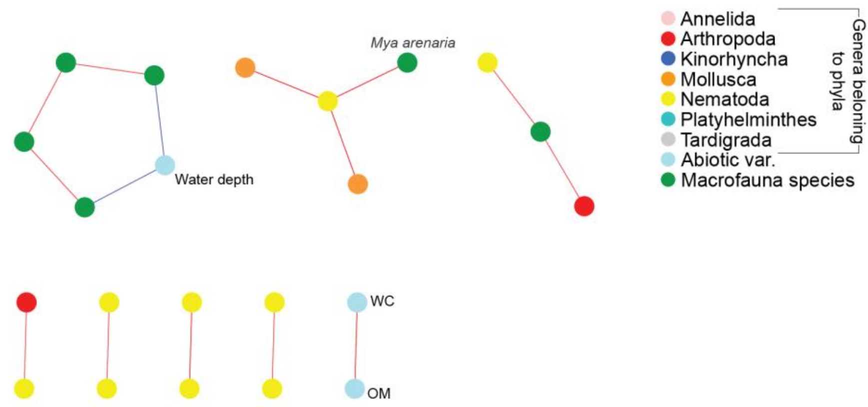
926 Figure 5

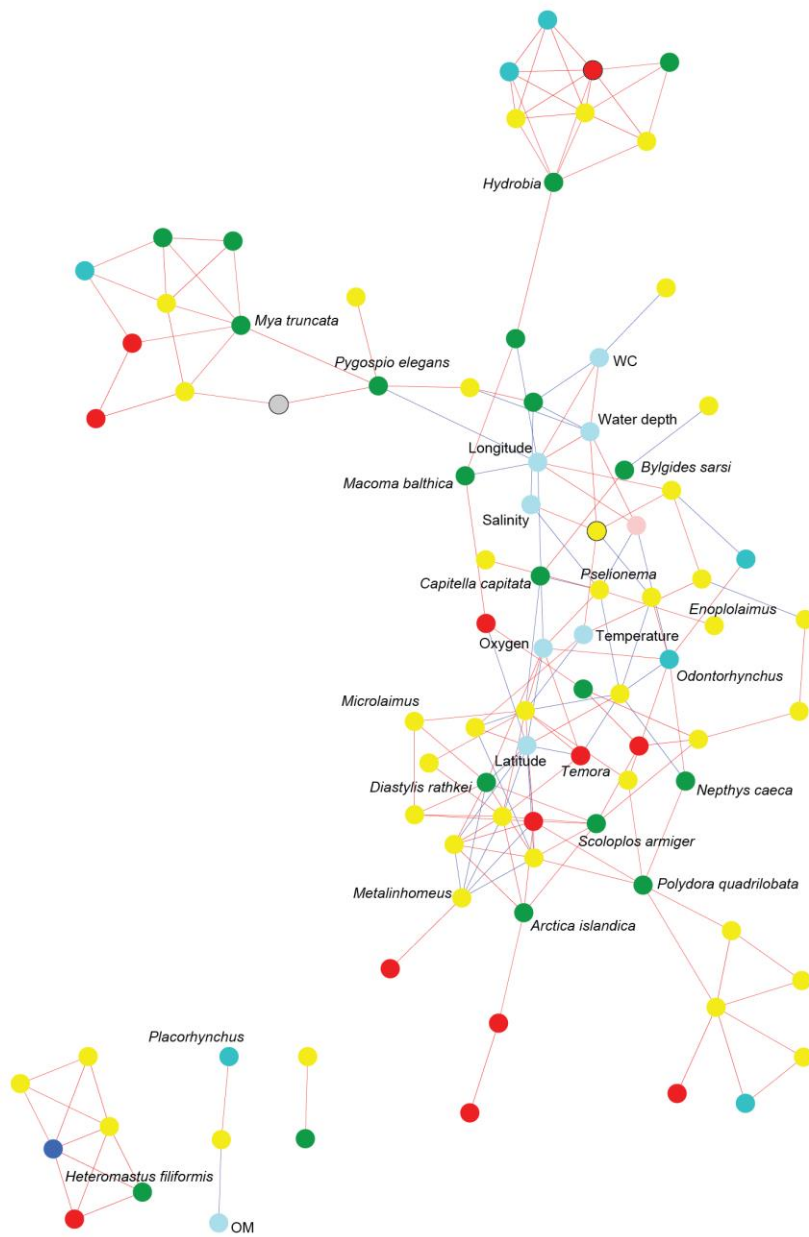
927



928

929 Figure 6





930

931 Figure 7