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Ecosystem functioning along gradients of increasing hypoxia and changing soft-sediment community types

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JN, CP, JG, RR, AE, MM, MG, AN conducted the field sampling.
JG, RR, AE, SA analysed the macrofauna samples.
FL analysed the meiofauna samples.
RR, MM analysed the sediment profile image data.
CP, JG, AN, FL conducted the statistical analyses.
JN drafted the manuscript and all authors contributed.
All authors have approved the final article.

Keywords. Hypoxia; nutrient cycling; structural community changes; ecosystem functioning; macrofauna; meiofauna

Highlights

1. Hypoxia decimates macrofauna, but fauna can still contribute to nutrient cycling
2. Meiofauna is less sensitive to hypoxia compared with macrofauna
3. The link between community structure and ecosystem function is mediated by context

Abstract. Marine ecosystems world-wide are threatened by oxygen deficiency, with potential serious consequences for ecosystem functioning and the goods and services they provide. While the effects of hypoxia on benthic species diversity are well documented, the effects on ecosystem function have only rarely been assessed in real-world settings. To better understand the links between structural changes in macro- and meiofaunal communities, hypoxic stress and benthic ecosystem function (benthic nutrient fluxes, community metabolism), we sampled a total of 11 sites in Havstensfjord and Askeröfjord (Swedish west coast) in late summer, coinciding with the largest extent and severity of seasonal hypoxia in the area. The sites spanned oxic to anoxic bottom water, and a corresponding gradient in faunal diversity. Intact sediment cores were incubated to measure fluxes of oxygen and nutrients (NO_3^-, NO_2^-, NH_4^+, PO_4^{3-}, SiO_4) across the sediment-water interface. Sediment profile imaging (SPI) footage was obtained from all sites to assess structural elements and the bioturbation depth, and additional samples were collected to characterise sediment properties and macro- and meiofaunal community composition. Bottom-water O_2 concentration was the main driver of macrofauna communities, with highest abundance and biomass, as well as variability, at the sites with intermediate O_2 concentration. Meiofauna on the other hand was less sensitive to bottom-water O_2 concentration. Oxygen was the main driver of nutrient fluxes too, but macrofauna as well meiofauna were also significant predictors; DistLM analyses indicated that O_2 concentration, macrofaunal abundance or biomass, and meiofaunal abundance collectively explained 63%, 30% and 28% of the variation in sediment O_2 consumption, NH_4^+ flux and PO_4^{3-} flux, respectively. The study provides a step towards a more realistic understanding of the link between benthic fauna and ecosystem functioning, and the influence of disturbance on this relationship, which is important for management decisions aimed at protecting the dwindling biodiversity in the coastal zones around the world.

INTRODUCTION

Marine ecosystems worldwide are threatened by oxygen deficiency, with potential serious consequences for ecosystem functioning and the goods and services these ecosystems provide (Diaz and Rosenberg 2008, Rabalais et al. 2014). While eutrophication and organic enrichment are the main anthropogenic causes of hypoxia, the warming climate will further exacerbate the deoxygenation of the oceans (Breitburg et al. 2018). This highlights the urgency of better understanding how ecosystem functioning might change with increasing hypoxia, and what factors and mechanisms are driving these changes.

The deleterious effects of increasing hypoxia on soft-sediment macrofaunal communities are well documented, with a general decrease of large, deeper-dwelling animals and an increase of smaller, fast-growing species, until anoxia decimates all macrofauna (Pearson and Rosenberg 1978, Diaz and Rosenberg 1995, Gray et al. 2002, Levin et al. 2009). Through their bioturbation and bioirrigation activities, macrofauna enhance oxygen penetration into the sediments influencing all oxygen-dependent processes in the sediment, including organic matter mineralization through stimulation of microbial activity, and nutrient cycling (Levinton 1995, Aller and Aller 1998, Meysman et al. 2006, Glud 2008). These activities are reduced under hypoxic conditions due to changes in the behaviour and diversity of macrofauna. Bottom-water O_2 concentrations also influence biogeochemical processes at the sediment-water interface affecting nutrient concentrations and speciation in the water column. In particular, the release of phosphate and ammonium is enhanced under hypoxic conditions (Mortimer 1941, Ingall et al. 1993, Cowan and Boynton 1996, Slomp et al. 2002, McCarthy et al. 2008, Reed et al. 2011, Jäntti and Hietanen 2012). Nevertheless, the step from documenting structural changes in faunal composition due to hypoxia to understanding the impact on ecosystem functions (e.g., nutrient cycling) is long and we have only recently begun to assess the interacting direct (e.g. chemical release of nutrients from the

sediment) and indirect (e.g. via effects on macrofauna) effects of hypoxia on ecosystem function in natural settings (Norkko et al. 2015, Gammal et al. 2017).

The effects of hypoxia on benthic ecosystem functioning are likely to be highly context dependent, posing further challenges to building a general understanding of the effects and predicting future changes. Impacts will depend on the temporal and spatial scales of hypoxia (a function of mixing and water exchange), the type of habitat (e.g. muddy, sandy) and faunal diversity. Places with high species diversity are generally expected to tolerate stress better than low-diversity systems (insurance hypothesis, e.g., Yachi and Loreau 1999), but it is important to consider biodiversity as a much wider concept than just the number of species, including aspects such as species identity and dominance patterns. For example, dominance by one or a few species with particular functional traits may be more important than species diversity *per se* for some aspect of ecosystem functioning (Chapin III et al. 1997). A dominant species with a good hypoxia tolerance may thus maintain a vital process, e.g. bioturbation, when conditions deteriorate (Norkko et al. 2015, Rakocinski and Menke 2016)). Thus, species identity and the prevalence of functionally important traits will be important for assessing hypoxia-induced changes in ecosystem functioning.

It is difficult to directly link disturbance-induced community changes to quantifiable shifts in functioning without empirical measurements. Nevertheless, some assumptions can be made. Under anoxic conditions, when the macrofauna has been lost, there is no effect of the fauna on nutrient cycling and therefore chemical reactions, modulated by microbes, dominate solute fluxes and ecosystem function. As conditions deteriorate from normoxia, the ensuing hypoxia results in different community types, representing different successional stages (Pearson and Rosenberg 1978, Diaz and Rosenberg 1995, Nilsson and Rosenberg 1997, Rosenberg et al. 2002). Sustained hypoxia decimates big individuals, e.g., large deep-burrowing bivalves, which are particularly

76 important for nutrient cycling (Norkko et al. 2013). Thus, the macrofaunal influence on nutrient
77 cycling is likely to be reduced in a decimated community, with lower species diversity, abundance
78 and biomass. In addition, already at sub-lethal levels of hypoxic stress, behavioural and
79 physiological changes may affect the species' contribution to processes such as bioturbation, but
80 species-specific sensitivities to hypoxia vary greatly (Vaquer-Sunyer and Duarte 2008). Thus,
81 hypoxia results in a non-random species loss and the remaining community types may be adapted to
82 low-oxygen environments. It is, however, unclear how well they perform.
83
84 Much of our understanding of biodiversity-ecosystem functioning (BEF) relationships stems from
85 mechanistic, small-scale laboratory studies with only a few species and limited regard of changing
86 environmental conditions, such as increasing hypoxia (Snelgrove et al. 2014). While the potential
87 indirect effects of hypoxia via fauna on biogeochemical processes have been indicated in several
88 high-profile papers (e.g., Levin et al. 2009, Middelburg and Levin 2009, Friedrich et al. 2014),
89 actual measurements still appear to be virtually non-existent. To our knowledge, the effects of
90 hypoxia on BEF in terms of nutrient cycling have not been empirically tested in a meaningful way
91 in the laboratory or under natural field conditions. While it is challenging to assign causality in field
92 studies, relevant field measurements involving natural communities in a range of different
93 environments and geographical areas are imperative for developing a realistic understanding of the
94 effects of disturbance on BEF relationships. Using correlative field surveys and incubations of non-
95 manipulated sediment cores for measurement of benthic nutrient fluxes in a range of contrasting
96 environments, we have started to understand the importance of benthic macrofauna for nutrient
97 cycling and the concurrent effects of increasing hypoxia. This body of work includes a large-scale
98 study across the entire open Baltic Sea, spanning a salinity and corresponding diversity gradient as
99 well as areas that are more or less permanently hypoxic (Norkko et al. 2015) and a coastal study in
100 a brackish, seasonally hypoxic, low-diversity system in the northern Baltic Sea (Gammal et al.

2017). In order to pinpoint the mechanisms involved, the same research team has conducted coastal field experiments where hypoxic events of different intensity were simulated *in situ* and the responses assessed (Villnäs et al. 2012, Norkko et al. 2013, Villnäs et al. 2013). In all of these studies, macrofauna was important for explaining the variability in nutrient fluxes across the sediment-water interfaces, but this effect decreased as oxygen conditions deteriorated. It is now imperative to investigate whether the patterns in higher-diversity systems are comparable to the ones found in the low-diversity Baltic Sea.

Missing from these studies is also the consideration of several size classes and trophic levels simultaneously, i.e. meiofauna and microbes. It is known that the influence of macrofauna on ecosystem function, sediment biogeochemistry and nutrient cycling, is modulated by meiofauna (Bonaglia et al. 2014, Piot et al. 2014) and by microbes (Yazdani Foshtomi et al. 2015), but studies that examine these relationships in relation to hypoxia and nutrient cycling are rare. Also, the Baltic Sea where our previous studies have been conducted is a low-diversity system (Villnäs and Norkko 2011), calling for comparative studies in fully marine systems that experience hypoxia but have higher macrofaunal diversity (and potentially greater redundancy), such as the Swedish west coast.

Hypoxic or anoxic dead zones in deeper waters (e.g. the Baltic Sea, the Gulf of Mexico) have been known for decades (e.g., Rabalais et al. 2002, Conley et al. 2009a), but the problem of near-shore, coastal hypoxia is now receiving ever more attention (Conley et al. 2011). The coastal ecosystems are heterogeneous and diverse, with enclosed inlets, and steep gradients in physical, chemical and biological properties. They are hotspots of diversity and productivity, but at the same time human impacts are very pronounced along the coasts (Levin et al. 2001, Halpern et al. 2008), highlighting the need for management actions based on sound understanding of the links between hypoxic disturbance and the functioning of these systems.

126

127 To investigate the links between benthic communities, hypoxic stress and nutrient fluxes across the
128 sediment-water interface, we conducted a field study in the seasonally hypoxic Havstensfjord and
129 Askeröfjord (Swedish west coast), at sites covering a gradient from oxic to anoxic bottom water,
130 with a corresponding gradient in diversity. The aim was to assess how macrofauna and meiofauna
131 communities change with increasing hypoxia and whether the effects of these changes on nutrient
132 cycling/fluxes could be quantified. We also investigated whether the different community types, or
133 successional stages, corresponded to different levels of sediment oxygen consumption (a proxy for
134 community metabolism). Given the higher species diversity and thus potentially higher functional
135 redundancy in Havstensfjord and Askeröfjord compared to our previous studies in the Baltic Sea
136 (Norkko et al. 2015, Gammal et al. 2017), we anticipated that the effect of hypoxia on the faunal
137 contribution to functioning would be smaller.

138

139 **MATERIAL AND METHODS**

140 **Study area, sampling and sample processing**

141 The Havstensfjord is a narrow fjord on the Swedish west coast and part of the Orust fjord system
142 (Fig. 1). The fjord extends about 25 km from north to south with its main connection to the sea
143 further south through Askeröfjord. The fjord system has been monitored since the 1950s and
144 bottom-water oxygen concentrations have steadily declined since then (Nilsson and Rosenberg
145 1997). The fjords suffer from seasonal hypoxia and particularly in the northern parts, deeper waters
146 may be anoxic for extended periods of the year (Hansson et al. 2013). The deeper parts of
147 Havstensfjord are usually ventilated once per year in late winter or early spring. The periods of
148 lower oxygen concentrations also correspond to higher concentrations of phosphate and ammonium
149 in the bottom waterers (Fig. 1).

150

During the peak of seasonal hypoxia, in early September 2011 we sampled 9 sites in the Havstensfjord and 2 outside the entrance in the Askeröfjord, covering a gradient from oxic sites outside the sill to Havstensfjord to hypoxic sites inside the sill, and then almost anoxic bottom water at the deepest site in the north (Fig. 1, Table 1). Although long-term monitoring data does not exist for all 11 sites, bottom-water oxygen concentrations in the fjord are strongly related to depth and therefore the assumption is that all sites follow a general pattern of lowest oxygen conditions at the end of summer. The choice of sampling sites was based on Nilsson and Rosenberg (1997) and the sampling was conducted on-board *R/V Skagerak*. All sites had muddy sediments, similar organic content and were 23-39 m deep.

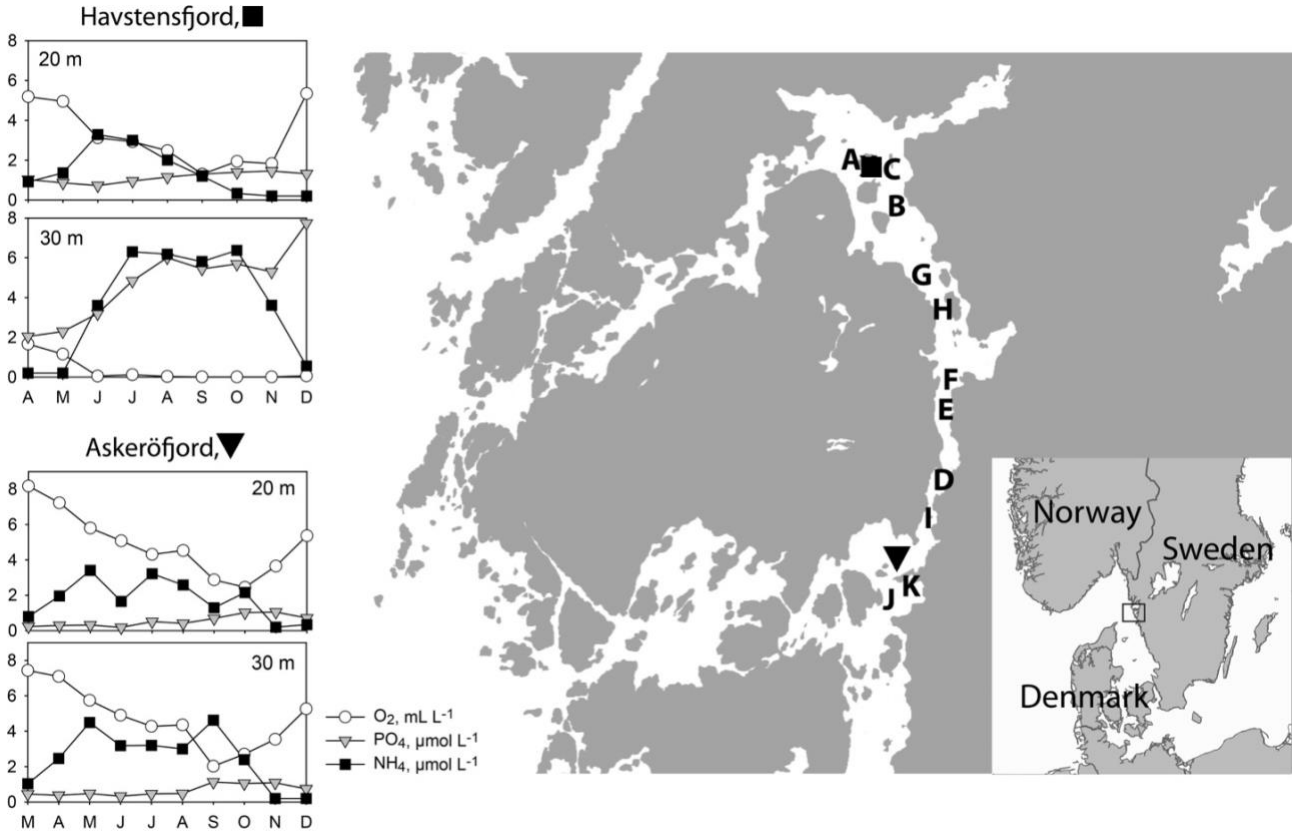


Figure 1. Map of the sampling area in the Orust fjord system on the Swedish west coast. Letters A-K indicate sites sampled during this study in September 2011. Inserted graphs present concentrations of oxygen (O_2, mL L^{-1}), phosphate (PO_4^{3-}, µmol L^{-1}), and ammonium (NH_4^+, µmol

L^{-1}) measured at 20 and 30 m depth at monitoring sites in Havstensfjord (filled square; station name Havstensfjord) and Askeröfjord (filled triangle; station name Galterö) from March or April to December 2011, as part of the national Swedish coastal monitoring program (data obtained from the SMHI database SHARKweb).

To characterise environmental conditions at each site, bottom-water salinity and temperature were determined from CTD casts (Sea-Bird). Intact sediment cores were collected with a Gemax twincorer (ID = 90 mm) and the surface sediment (0-1 cm) analysed for organic content (OC, % loss on ignition, 3 h at 500°C) and sediment silt/clay content (% <63 μm, determined by wet sieving). To illustrate differences in the sedimentary environment between anoxic, hypoxic and oxic sites, four sediment profile images (SPI) were obtained from each site and digitally analysed in PhotoShop for sediment surface/subsurface structures and mean depth of the apparent redox potential discontinuity (aRPD). Based on these variables a benthic habitat quality (BHQ) index was calculated (see details in Nilsson and Rosenberg 1997).

Oxygen and nutrient fluxes across the sediment-water interface were estimated by on-board incubation of undisturbed, intact sediment cores (n=5 per site). The upper parts of Gemax split tubes were sealed and used as flux chambers (30 cm sediment + 10 cm bottom water). The core lid contained a Teflon-coated magnetic stirring bar, which provided continuous gentle stirring by an external magnet. Core incubations (in the dark at 11ºC) started immediately after collection and water samples for O_2 and nutrient concentrations (NO_3^-, NO_2^-, NH_4^+, PO_4^{3-}, SiO_4) were obtained at the start and the end of incubation (4 h later). The differences in concentration were used to calculate solute fluxes (μmol m^{-2} d^{-1}). At the end of incubation, all cores were sieved to quantify benthic macrofaunal species richness, abundance and biomass (0.5 mm sieve, preserved in 70% ethanol, biomass estimated as blotted wwt). Dissolved oxygen was determined by Winkler titration, while the nutrient samples were filtered (GF/F) and then frozen (-20ºC) until analysed spectrophotometrically with an autoanalyser (Lachat QuickChem 8000).

193

194 To provide an additional and more robust estimate of benthic macrofaunal species richness,
195 abundance and biomass, than that gained from the small flux cores (0.006 m^2), we also sampled
196 with a Smith-McIntyre grab (0.1 m^2, 3 replicates per site, 1 mm sieve, preserved in 70% ethanol).

197

198 From a grab sample at each site, three subsamples (40 g wwt) of the top 2 cm sediment were taken
199 for analyses of meiofauna community composition. These samples were stored in an
200 ethanol:glycerol (95:5 %) solution and meiofauna extracted using Ludox (Burgess 2001). Sediment
201 was rinsed with tap water on a 63 μm sieve to remove ethanol, salt and organic matter. Remaining
202 sediment was transferred to a 13-ml centrifuge tube, centrifuged at 800 G for 5 minutes and the
203 water was decanted. 10 ml of Ludox AS-40 was added to the tube and the tube was then vortexed at
204 1800 rpm for 30 s and then at 1400 rpm for 4.5 min. Afterwards the sample was centrifuged at 800
205 G for 5 minutes and approximately 2 ml of the top sample was transferred to a new tube. The old
206 tube was topped up with fresh Ludox and the procedure was repeated. The retained material was
207 sieved with milliQ water through a 63-μm sieve to remove the Ludox from the extracted meiofauna
208 and preserved in ethanol:glycerol solution. The extracted meiofauna were put on petri dishes and
209 diluted with milliQ water. The petri dishes were then digitally scanned on an Epson Perfection v500
210 (6400 dpi, 16-bit grey scale in positive film mode). The meiofauna were analysed using the image
211 analysis software ZooImage (Lindgren et al. 2013) to the following major taxonomic groups:
212 Nematoda, Harpacticoida, Rotaliina, Reophax, Allogromiina, Nonionella, Tanaidacea, Polychaeta
213 and Ostracoda.

214

215 **Statistical analyses**

216 Multivariate analyses in PRIMER 6 (Clarke and Gorley 2006) based on taxon abundance was used
217 to assess inter-site variations in faunal community composition. Macrofauna community data from

grab samples were transformed (square root) to lessen the influence of dominant taxa before Bray-Curtis similarity calculations. Meiofauna data were not transformed because of the relatively coarse taxonomic resolution and even distribution of taxa. Resulting linkages were visualised in a multi-dimensional scaling (MDS) plot based on replicate data from which site clusters were identified. The statistical validity of the site clusters ($p < 0.05$) was tested using the similarity profile test (SIMPROF; Clarke 1993).

We used a correlation-based principal components analysis (PCA) to identify environmental variables (Table 1) responsible for differences among sites. The analysis was conducted on normalised environmental parameters (site average) using Euclidean distance to generate the resemblance matrix. The purpose of this analysis was to identify a reduced set of independent environmental predictors that could be used to explain variation in faunal composition and ecosystem function (here focussing on sediment oxygen consumption (SOC) and nutrient regeneration (NH_{4+} and PO_{43-} flux)).

The correlation between the site-averaged multivariate assemblage composition (grab samples) and environmental variables was examined using distance based linear models (DistLMs) in PERMANOVA+ (Anderson et al. 2008). The same approach was used for measures of ecosystem function except similarity matrices were based on Euclidean distance rather than Bray-Curtis similarity and we also included univariate measures of macrofaunal community composition (abundance, diversity and biomass from flux cores) to assess their contribution to ecosystem function. Because we had macrofaunal composition from each core, solute flux data was not site-averaged prior to analysis. Site A was omitted from the DistLM analyses because it was almost anoxic, contained no macrofauna and nutrient fluxes were much larger than the remaining hypoxic/oxic sites (see results). The only exception to this was the analysis involving the

243 relationship between meiofauna community structure and environmental variables as meiofauna
244 were present at Site A. Although DistLM is a semi-parametric, permutation-based method that does
245 not rely on normally distributed data, we checked the normality of the environmental data with
246 Shapiro-Wilks tests and no transformations were necessary. We first performed marginal tests to
247 identify strong, significant predictors, irrespective of other variables, then partial tests to assess the
248 explanatory value of a predictor variable after all other significant predictors had been accounted
249 for. Finally, DistLMs were run using the step-wise selection procedure and r^2 selection criterion to
250 identify the (linear) combinations of significant predictor variables that explained the greatest
251 proportion of variation. *P* values were obtained for predictor variables by 9999 permutations.
252

253 **Table 1.** Environmental variables at the study sites in the Havstensfjord and Askeröfjord, sampled in September 2011. Depth, bottom-water
254 temperature, salinity and oxygen concentration, surface sediment (0-1 cm) silt/clay and organic content (OC), and depth of the apparent redox
255 potential discontinuity layer (aRPD; from SPI analyses). These factors were included in the PCA analysis. In addition, the benthic habitat quality
256 (BHQ), the corresponding BHQ stage and site groupings based on macrofaunal abundance (see Table 2) are listed.
257
258

Site	Longitude	Latitude	Depth (m)	Temp (ºC)	Salinity	O_2 (ml L^{-1})	Silt/clay (%)	OC (%)	aRPD (cm)	BHQ	BHQ stage	Macrofaunal group
A	58.31482	11.77350	39.1	6.8	32.2	0.11	66.7	9.9	0.1	1.50	0	
B	58.29382	11.80400	26.3	10.6	31.0	0.89	95.0	10.4	0.3	5.00	2	1
C	58.31382	11.80182	27.0	8.5	31.5	0.99	82.4	7.9	0.2	6.75	2	1
D	58.16616	11.85200	24.2	11.3	29.4	1.02	73.7	9.8	1.7	7.50	2	2
E	58.19532	11.85000	27.5	11.0	30.0	1.20	70.7	9.1	3.7	10.75	3	3
F	58.20620	11.85116	25.8	10.4	30.1	1.23	77.7	8.8	2.7	10.25	3	3
G	58.26146	11.80730	25.6	10.2	30.6	1.28	80.7	12.0	3.2	10.25	3	3
H	58.23506	11.84782	25.2	10.4	30.4	1.34	82.4	9.4	2.3	10.75	3	2
I	58.14816	11.84116	26.9	12.4	29.8	1.36	85.1	8.6	2.3	10.50	3	4
J	58.11682	11.83050	26.7	13.8	28.6	2.11	82.9	7.7	0.7	7.00	2	4
K	58.11250	11.81950	23.2	15.1	25.1	2.91	78.6	8.4	0.7	7.25	2	4

259

RESULTS

Environmental variables

All sites had muddy sediments with a silt/clay content > 67% and OC between 8 and 12% (Table 1). Bottom-water temperature varied between 7ºC at the deepest site and 15ºC at the shallowest site. Corresponding values for salinity and O_2 concentrations were 32 and 25, and 0.1 and 2.9 ml L^{-1}, respectively. Thus, even at the shallowest site, the O_2 concentration was relatively low at the time of sampling, although all sites except the innermost sites (A, B, C) likely experience relatively good O_2 conditions during the rest of the year (Fig. 1). The 2-dimensional PCA ordination of the environmental variables (Table 1) accounted for a large fraction (72%) of the total variance and revealed sites primarily dispersed across two gradients (Fig. 2, PCA analysis). PCA1 alone accounted for 52% of the variance and was most strongly correlated ($|r| = 0.5$-0.6) with temperature, salinity and bottom-water O_2 concentration and to a lesser extent depth ($r = 0.4$). PCA2 accounted for only 20% of the inter-site variation and was driven by differences in the aRPD depth and OC ($r = 0.7$ and 0.5, respectively). Salinity and temperature were strongly correlated with oxygen concentration ($|r| > 0.9$, $p < 0.001$), consequently we used a reduced set of weakly correlated ($|r| < 0.6$) environmental variables (depth, O_2 concentration, OC, silt/clay, aRPD depth) in subsequent DistLM analyses. Variables excluded because of co-correlation explained less of the variation in response measures than the variables retained.

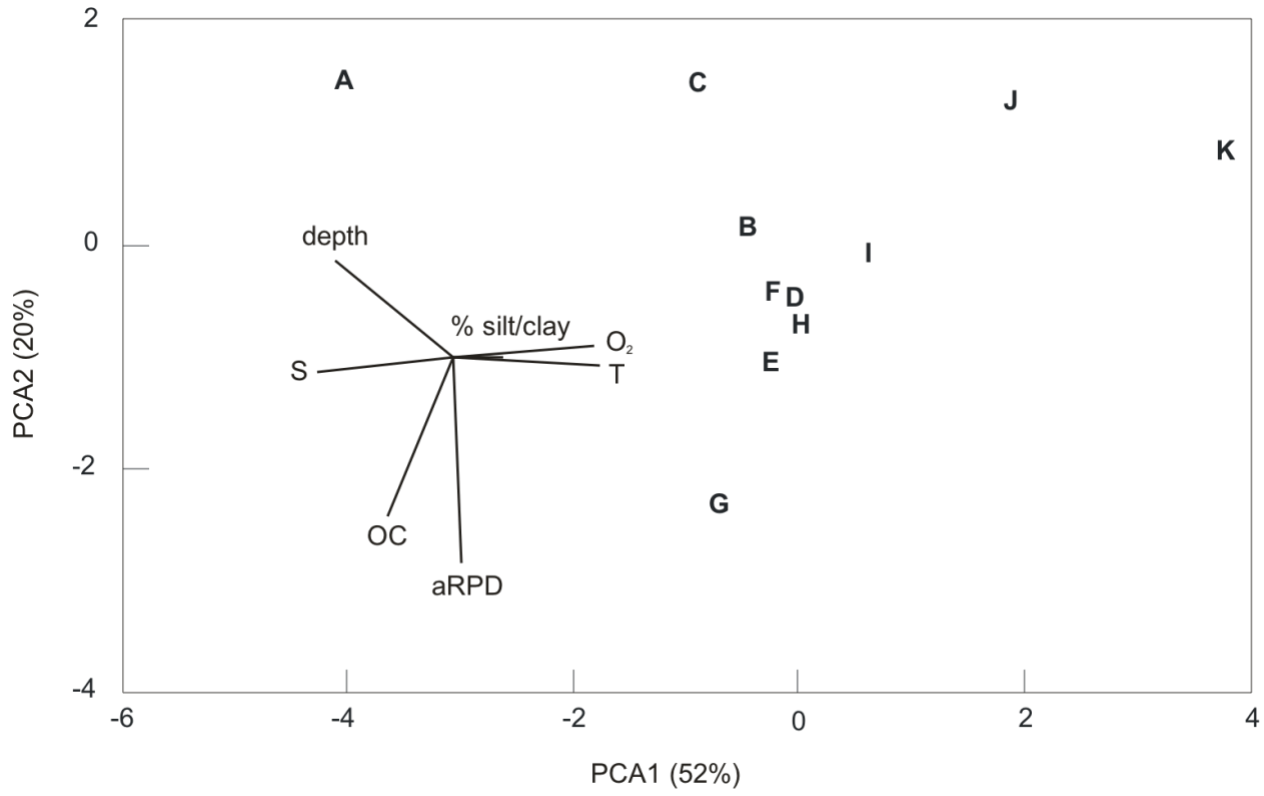
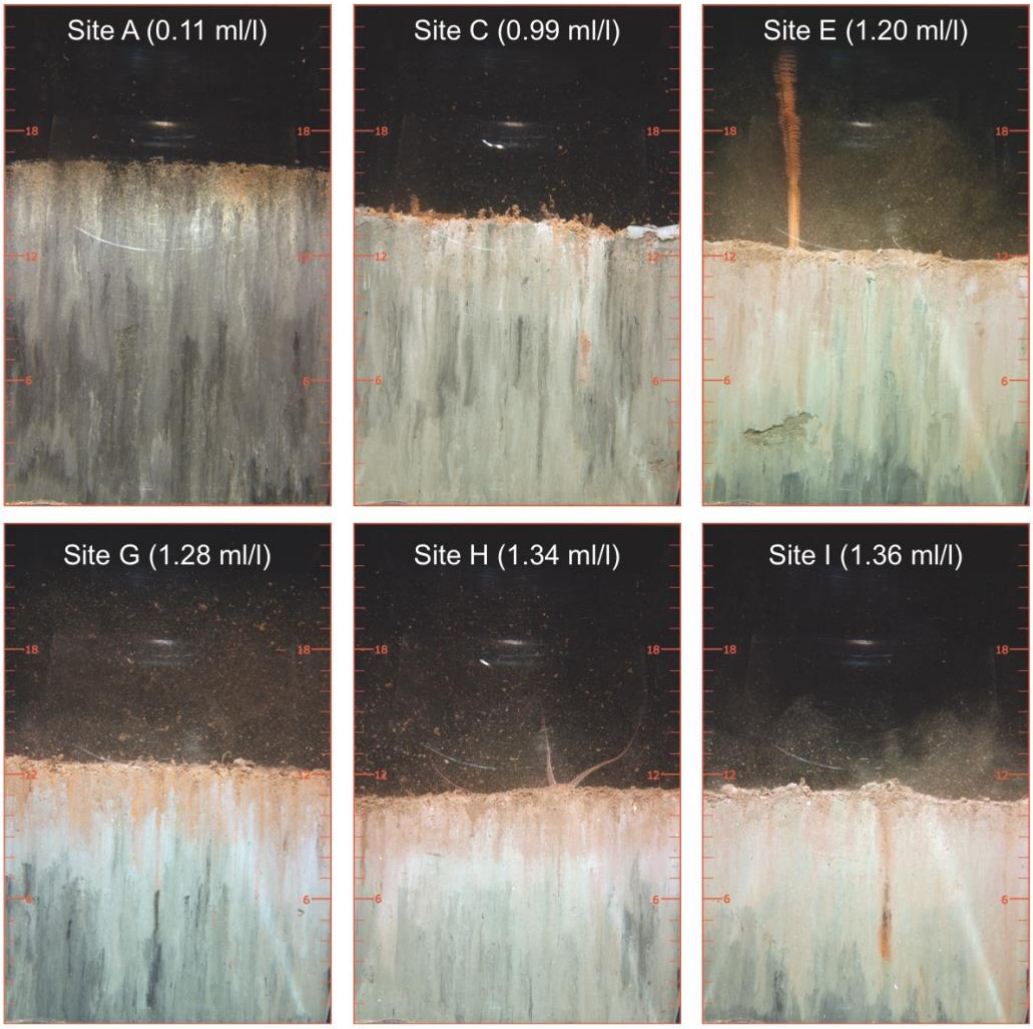


Figure 2. A two-dimensional ordination of a principal components analysis of site environmental variables, which collectively explained 72% of the variation between sites. Also shown is the correlation between the component axes and the environmental variables.

Sediment Profile Images. The O_2 gradient was also visible in the sediment profile images, with clear differences between sites with different near-bottom O_2 concentrations, here exemplified by images from almost anoxic (site A), hypoxic (sites C, E, G, H) and oxic areas (site I; Fig. 3). At the almost anoxic site A, the whole sediment was reduced and large amounts of fecal pellets were seen at the sediment surface. The image from site C, at about 1 ml L^{-1} of near-bottom O_2 concentration, showed no bioturbation activity and a reduced sediment with a marginally oxidised sediment surface, but several small tubes were at the surface. These tubes are most likely inhabited by spionids. BHQ was 5. Site E had an anthozoan, *Virgularia mirabilis*, at the well bioturbated sediment surface and several vertical burrows. A feeding void was present at a depth between 8 and 10 cm. The mean aRPD was 2.6 cm and the BHQ index was 10. Site G demonstrated great bioturbation activity at the sediment surface with some protruding tubes and several oxic burrows

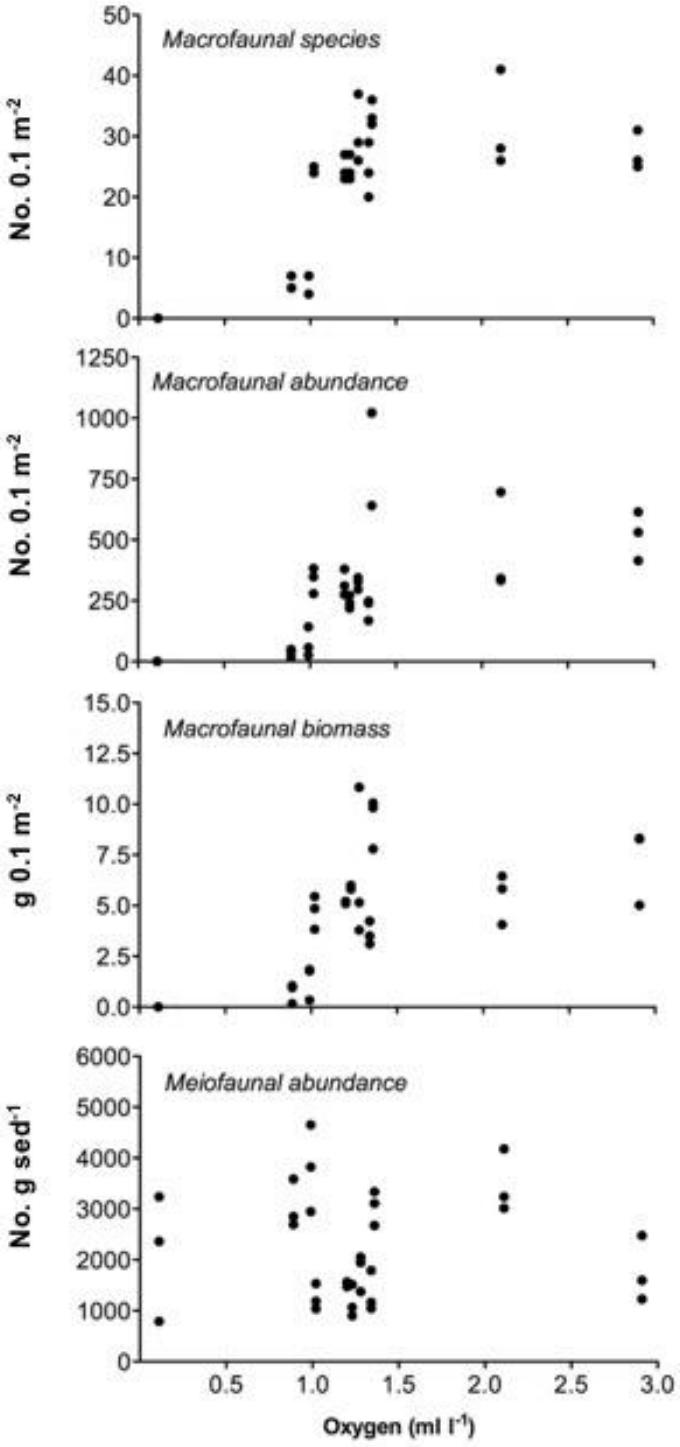
302 going down into the surrounding reduced sediment. Some vertical black patches could indicate
303 presence of dead animals. The mean aRPD was 2.0 cm and the BHQ index was 9. Similarly, site H
304 had an ophiuroid at the sediment surface and great bioturbation activity. Many vertical tubes were
305 stretching down to several centimetres in the sediment surrounded by reduced sediment. Mean
306 depth of the aRPD was 2.8 cm and the BHQ index was 11. The image from site I showed some
307 tubes at the sediment surface and some vertical oxidised burrows, where infauna is visible in one
308 burrow. The sediment surface showed signs of great bioturbation activity, and the mean depth of the
309 aRPD was 3.1 cm and the BHQ index was 12.



310

311 **Figure 3.** Selected sediment profile images with overlaying water from sites with different bottom-
312 water O_2 concentrations. The colours have been digitally enhanced to facilitate interpretation. The
313 vertical scale is in centimetres.
314
315
316 **Macrofauna.** In general, macrofauna species richness, abundance and biomass was greatly reduced
317 at the sampling sites with bottom-water O_2 concentrations < 1.3 ml L^{-1}, while the highest abundance
318 and biomass, and variability, was observed at the sites with intermediate O_2 concentration (Fig. 4).
319 Multivariate analyses of macrofaunal abundance revealed four distinctive site clusters (confirmed
320 by SIMPROF $p < 0.05$) that separated at 50-65% similarity (Fig. 5a). These groupings reflected
321 changes in macrofaunal assemblage structure associated with the gradient in O_2 concentration
322 (Table 1): severely hypoxic (0.9-1.0 ml L^{-1}, sites B, C), two hypoxic groups (1.0-1.3 ml L^{-1}, sites E,
323 F, G and sites D, H), and oxic (1.4-2.9 ml L^{-1}, sites I, J, K). The dissimilarity between the oxic and
324 the two hypoxic groups was approximately the same, likely driven by other co-varying spatial or
325 environmental factors, which cannot be determined this from this dataset. The almost anoxic site A
326 (0.1 ml L^{-1}) had no macrofauna and was excluded from this analysis. Among dominants in the
327 hypoxic as well as oxic areas were the surface deposit-feeding bivalve *Abra nitida,* the
328 chemosymbiotic bivalve *Thyasira flexuosa* (which can also suspension feed), the sub-surface
329 deposit-feeding polychaete *Scalibragma inflatum* and the facultative suspension-feeding and
330 surface deposit-feeding brittle star *Amphiura filiformis*, indicating a high level of tolerance to low
331 O_2 concentrations in these species (Table 2). *Thyasira flexuosa* was additionally dominant even in
332 the severely hypoxic areas. Conspicuous species in severe hypoxia were the tube building
333 polychaetes *Maldane sarsi* and *Polydora caulleryi,* which have minor effects on the depth of the
334 aRPD, the polychaete *Chaetozone setosa,* and the burrowing bivalve *Thyasira flexuosa.* Total
335 abundance as well as total biomass decreased from the oxic to the severely hypoxic groups (Table
336 2). Notable is that that the suspension-feeding bivalve *Arctica islandica* dominated the biomass,
337 with only a few large individuals, at sites in the two hypoxic clusters (sites D, E, F, G).
338

339 In marginal tests multivariate macrofaunal assemblage structure (abundance) was most strongly
340 correlated with aRPD depth followed by silt/clay and O_2 concentration (Table 3). The other
341 variables (depth, OC) were not significantly correlated ($p > 0.2$). After correcting for the effect of
342 the other variables (i.e. in partial tests), aRPD depth, silt/clay as well as O_2 concentration remained
343 significant predictors and in linear combination collectively accounted for 60% of the variation in
344 assemblage structure.



345

346 **Figure 4.** Macrofauna species richness, abundance and biomass (per Smith-McIntyre grab, 0.1 m2),
347 and meiofauna abundance (per gram sediment) as a function of bottom-water O2 concentration at
348 the sampling sites.
349

350

351 **Table 2.** Abundance and biomass (per 0.1 m^2) of macrofaunal dominant taxa and community
352 groupings identified as statistically distinctive site clusters using SIMPROF (based on abundance
353 data, Fig. 5). The large bivalve *Arctica islandica* was excluded from the SIMPROF analysis, but
354 dominated biomass, with only a few large individuals, at sites in the two hypoxic groups.
355

Macrofaunal groupings	**Group 1** Severely hypoxic	**Group 2** Hypoxic	**Group 3** Hypoxic	**Group 4** Oxic
Abundance of dominant taxa				
Scalibregma inflatum		88	52	233
Abra nitida		22	49	188
Nucula nitidosa		10	9	61
Thyasira flexuosa	30	63	15	46
Trochochaeta multisetosa				17
Amphiura filiformis			91	10
Amphiura chiajei		4	20	
Corbula gibba		21	9	
Anobothrus gracilis			8	
Chaetozone setosa	13	16		
Terebellides stroemi		6		
Hyala vitrea		8		
Heteromastus filiformis		4		
Maldane sarsi	7			
Polydora caulleryi	4			
Total	**54**	**242**	**253**	**555**
Biomass of dominant taxa				
Nucula nitidosa		0.1		1.8
Scalibregma inflatum		0.6	0.3	1.5
Ophiura ophiura				0.9
Abra nitida			0.1	0.8
Priapulus caudatus			0.2	0.6
Tubulanus polymorphus				0.4
Leptopentacta elongata		1.3		0.2
Amphiura sp.			1.8	
Amphiura filiformis			1.3	
Amphiura chiajei			0.3	
Thyasira flexuosa	0.6	0.2		
Corbula gibba		0.2	0.3	
Anobothrus gracilis			0.1	
Glycera alba		0.4		
Tubulanus polymorphus		0.1		
Phyllodoce groenlandica		0.1		
Maldane sarsi	0.3			
Total	**0.9**	**3**	**4.4**	**6.2**

356
357

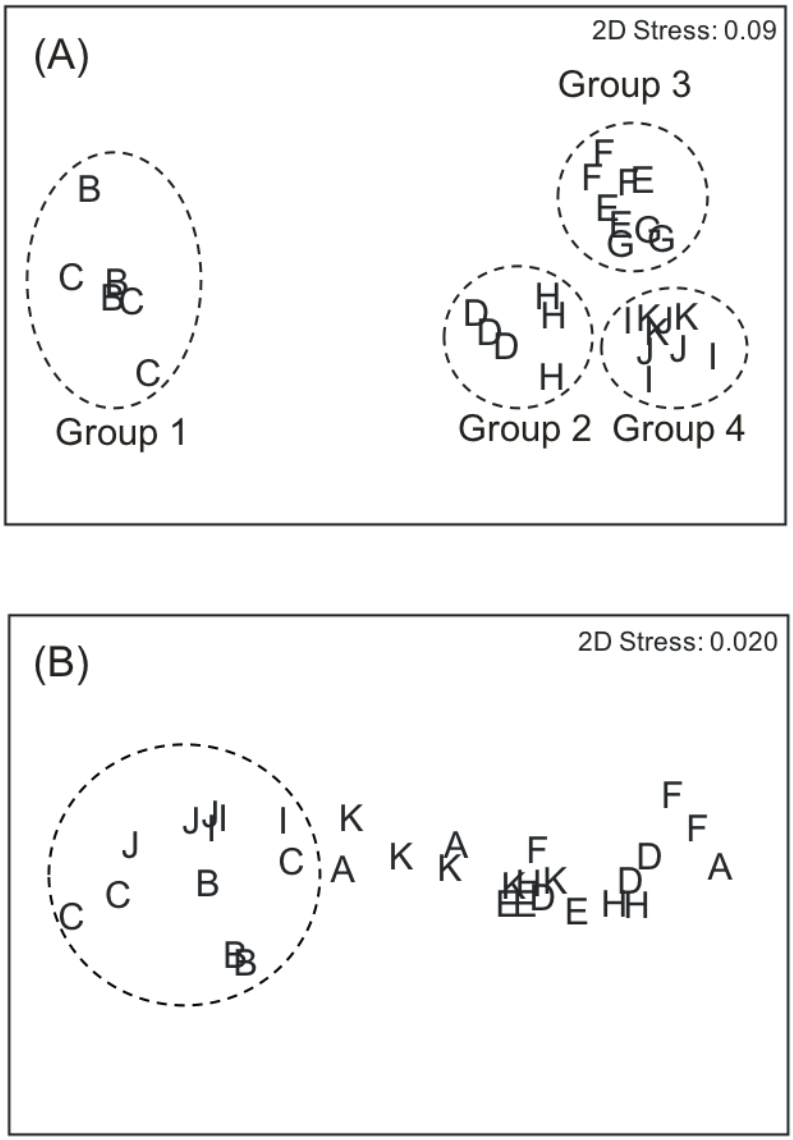


Figure 5. Two dimensional MDS ordination of (A) macrofaunal (grab samples, square root transformed) and (B) meiofaunal (raw counts) community composition based on taxa abundance. Circles denote statistically distinctive site clusters determined in SIMPROF ($p < 0.05$).

Meiofauna. In contrast to the macrofauna, meiofaunal total abundance changed less along the oxygen gradient (Fig. 4). Furthermore, multivariate analysis of meiofauna abundance data also did not identify distinct clusters of sites related to bottom-water O_2 concentration (Fig. 5b). For example, cluster analysis identified a group containing sites B, C, I and J (confirmed by SIMPROF), which contained in equal measures sites from the most oxic and severely hypoxic sites. Significant differences in community structure among sites was in most cases (97%) due to density

differences of nematodes (which was the most abundant group in all samples) and harpacticoids, however the foraminiferan group Rotaliina was also responsible in a few cases.

Multivariate analyses revealed that aRPD depth and silt/clay, but not O_2 concentration, were significant predictors also of meiofaunal assemblage structure (Table 3). The only other significant predictor in marginal tests was OC. Collectively, aRPD depth, silt/clay content and OC explained 48% of the variation in meiofaunal assemblage structure. High fractions of silt/clay were found at sites B, C, H, I and J. This coincided with the highest densities of nematodes, with the exception of site H. Low levels of OC were found at site C and J, which corresponded with the highest abundances of harpacticoids. Low aRPD values were found at sites A, B and C, which matched high abundances of nematodes, with the exception of site A.

Table 3. Proportion of variation in infaunal multivariate assemblage composition (based on replicate grab sample abundance) explained by significant correlations with (site averaged) environmental variables derived from DistLMs. Marginal tests examine a single predictor separately, while partial tests take into account the effect of the remaining predictors. There was no macrofauna at site A so it was excluded from the analysis.

	Macrofauna		Meiofauna	
	Marginal	Partial	Marginal	Partial
Oxygen	0.26***	0.18***		
OC			0.11**	0.05*
Silt/clay	0.19***	0.05***	0.30***	0.22***
aRPD	0.28***	0.24***	0.22**	0.21***

*$p < 0.1$, **$p < 0.05$, ***$p < 0.01$

Ecosystem function

Sediment oxygen consumption and nutrient fluxes varied strongly between sites (Table 4). For further analyses, we focussed on SOC, NH_4^+ and PO_4^{3-}. SOC increased while the PO_4^{3-} flux showed a general decrease with increasing bottom-water O_2 concentration, also when averaged between the sites corresponding to the groups identified based on the macrofaunal communities (Fig. 6). The

393 NH_4^+ flux was highly variable with no clear relationship with O_2 concentration, but similar to PO_4^{3-},
394 there was a large efflux at the almost anoxic site A.

395

396 **Table 4.** Sediment O_2 consumption (ml m^{-2} d^{-1}) and nutrient fluxes (µmol m^{-2} d^{-1}) across the
397 sediment-water interface at the study sites in the Havstensfjord and Askeröfjord, sampled in
398 September (average ± SD, five replicate flux cores per site). Negative values denote an influx into
399 the sediment. Unfortunately, the NH_4^+ samples from sites B, E and J went missing during analysis.
400

Site	O_2	NO_3^-	NO_2^-	NH_4^+	PO_4^{3-}	SiO_4
A	73 ± 94	8 ± 65	1 ± 20	2473 ± 809	3554 ± 1756	3128 ± 2544
B	-48 ± 66	-1138 ± 128	-50 ± 65	-	298 ± 162	2092 ± 908
C	-79 ± 117	752 ± 2219	57 ± 116	473 ± 409	442 ± 490	6323 ± 6034
D	-109 ± 37	-737 ± 110	-98 ± 19	910 ± 425	268 ± 108	3173 ± 688
E	-124 ± 21	-440 ± 241	-130 ± 17	-	199 ± 89	2495 ± 514
F	-61 ± 66	-571 ± 228	-110 ± 14	132 ± 304	91 ± 52	1789 ± 769
G	-149 ±34	-1179 ± 208	-176 ± 31	244 ± 164	112 ± 100	3130 ± 254
H	-100 ± 128	-911 ± 2521	-86 ± 154	208 ± 278	25 ± 314	1022 ± 6574
I	-227 ± 76	-1323 ± 235	-178 ± 8	1274 ± 401	130 ± 82	5188 ± 1143
J	-124 ± 45	-116 ± 63	-76 ± 28	-	97 ± 51	2273 ± 899
K	-431 ± 74	80 ± 178	-13 ± 14	494 ± 315	43 ± 35	3103 ± 1842

401

402 In the DistLM we used the same four independent site environmental variables as above for the
403 community analyses (depth, O_2 concentration (core specific, i.e. concentration at start of
404 incubation), aRPD, OC and silt/clay), and the following faunal variables: core specific macrofauna
405 abundance, biomass (less *Arctica*) and Shannon diversity and the site-averaged meiofaunal
406 abundance and Shannon diversity. We did not include the number of taxa as a predictor variable
407 because for macrofauna it was highly correlated with abundance (Pearson's $r > 0.8$) and the number
408 of meiofauna taxa did not vary among sites. Site A was omitted from analyses because of the
409 absence of macrofauna and predominantly chemically driven changes to measured fluxes,
410 particularly for NH_4^+ and PO_4^{3-}. Unfortunately, the NH_4^+ samples from sites B, E and J went
411 missing during analysis and so the DistLM analysis were conducted on a reduced set of sites.
412 Fortunately, the missing sites were scattered across the oxygen gradient.

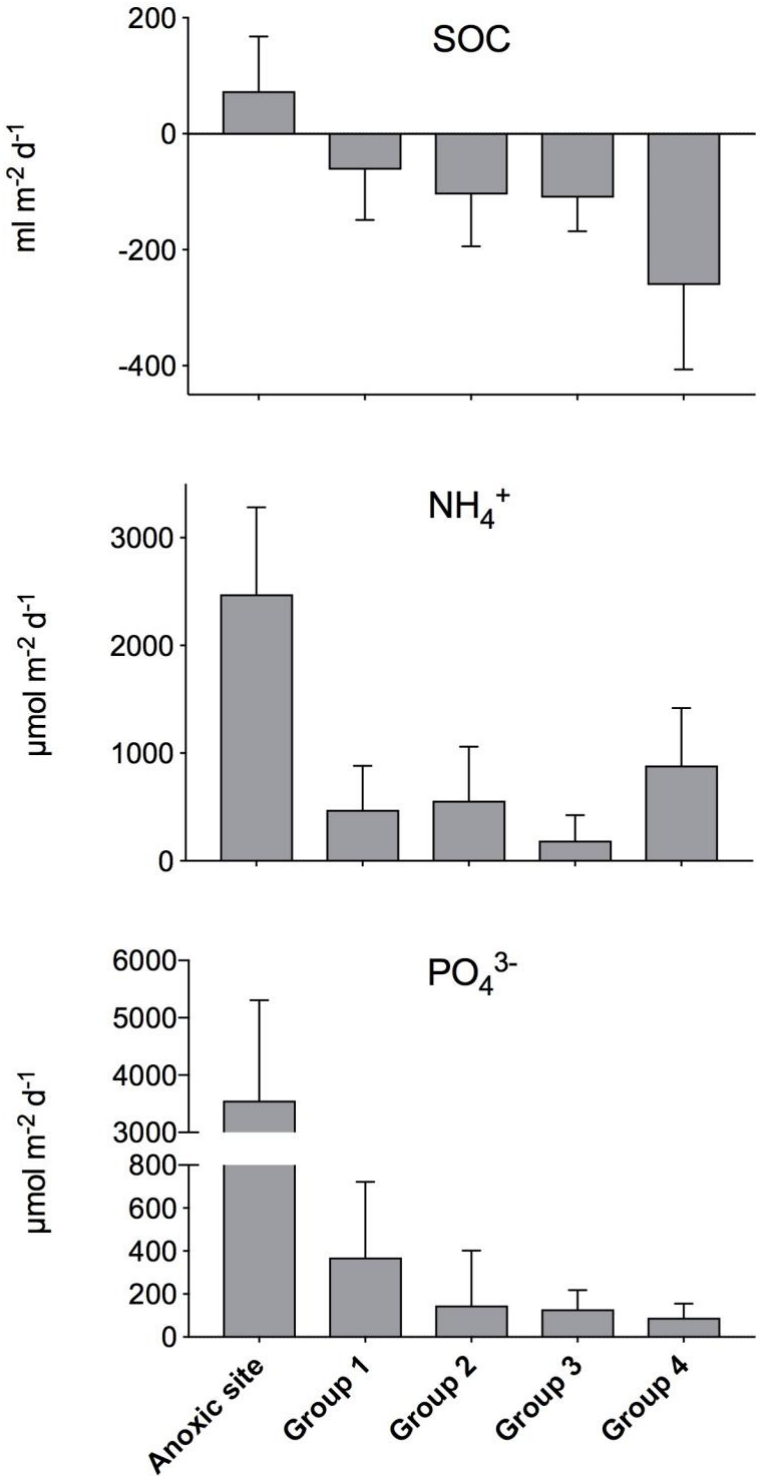


Figure 6. Sediment oxygen consumption (SOC) and effluxes of NH_4^+ and PO_4^{3-} (average ±SD) in the site groupings identified based on multivariate analyses of macrofaunal abundances (Fig. 5), where Group 1 = severely hypoxic, Group 4 = Oxic. In addition, data from the almost anoxic site (site A) is included.

419 O_2 concentration was the single best predictor of SOC closely followed by macrofaunal abundance,
420 both being positively correlated to SOC (Table 5). Depth, macrofaunal biomass and meiofaunal
421 diversity were also correlated with SOC in marginal tests however in partial tests, after fitting other
422 significant predictors first, they did not explain a significant amount of the residual variation. The
423 single best linear combination of variables included O_2 concentration and macrofaunal abundance
424 which collectively explained 63% of the variation in SOC. Inclusion of the remaining predictors
425 only explained an additional 3% of the variability.
426
427 The reduced number of sites included in the analysis of NH_{4+} flux restricted the environmental
428 gradient and as a consequence no water or sediment environmental variables were significant
429 predictors. Macrofaunal biomass was the single best predictor of NH_{4+} flux followed by
430 macrofaunal abundance then meiofaunal abundance, all being positively correlated to the NH_{4+} flux
431 (Table 5). In partial tests both macrofaunal biomass and meiofaunal abundance were still
432 significant, but macrofaunal abundance was not due it being correlated (albeit weakly) with
433 biomass. Macrofaunal biomass and meiofaunal abundance collectively explained 30% of the
434 variation in NH_{4+} flux.
435
436 In marginal tests the PO_{43-} flux was best correlated with O_2 concentration with higher fluxes
437 associated with lower O_2 concentrations (Table 5). Phosphate flux was weakly correlated with most
438 faunal predictors. Interestingly, while the flux was negatively correlated with measures of
439 macrofaunal community composition it was positively correlated with meiofaunal indices. This
440 relationship is explained in part by decline in macrofaunal abundance/biomass with decreasing O_2
441 concentration. In partial tests only O_2 concentration, macrofaunal biomass and meiofaunal
442 abundance were significant predictors of phosphate flux and combined explained 28% of the
443 variability in the data.

444 **Table 5.** Proportion of variation in sediment oxygen consumption (SOC), $NH_4^+$and PO_4^{3-} flux
445 explained by significant correlations with faunal/environmental variables (the direction is given in
446 brackets) derived from DistLMs. Marginal tests examine a single predictor separately, while partial
447 tests take into account the effect of the remaining predictors. There was no macrofauna at site A so
448 it was excluded from the analysis.
449

	SOC		NH_4^+		PO_4^{3-}	
	Marginal	Partial	Marginal	Partial	Marginal	Partial
Depth	0.23***(-)	0.02				
Oxygen	0.57***(+)	0.14***			0.13**(-)	0.07**
Macro-d	0.41***(+)	0.05**	0.13**(+)	0.04	0.10**(-)	<0.01
Macro-b	0.07*(+)	0.02	0.23***(+)	0.06*	0.06* (-)	0.05*
Macro-H’					0.11**(-)	<0.01
Meio-d			0.11*(+)	0.09*	0.11**(+)	0.07**
Meio-H’	0.13**(+)	0.01			0.13**(+)	0.02

450 Macro-d = abundance of macrofauna core^{-1}, Macro-b = g ww core^{-1}, Macro-H’ the Shannon diversity, Meio-d =
451 meiofaunal abundance g sediment^{-1}
452 *p < 0.1, **p < 0.05, ***p < 0.01
453
454

455 **DISCUSSION**

456 The deleterious effects of hypoxia on macrofaunal communities are well known and our results
457 from the seasonally hypoxic Havstensfjord and Askeröfjord corroborate this pattern, with a general
458 decrease in macrofaunal species richness, abundance and biomass with decreasing bottom-water O_2
459 concentration. Meiofaunal communities on the other hand did not appear to be similarly affected by
460 hypoxia (Table 3). Bottom-water O_2 concentration was the most important factor explaining
461 variation in sediment oxygen consumption (SOC) and fluxes of $NH_4^+$and PO_4^{3-}. Nevertheless, after
462 the key gradient in oxygen had been accounted for, macrofauna and meiofauna explained a small
463 but significant fraction of the variation in ecosystem function. This implies that faunal burrowing
464 and feeding indeed affect nutrient cycling, also when bottom-water concentrations are low, i.e. this
465 is not a purely geochemically driven process. For example, the burrowing enhances the oxygenation
466 of the sediment, thereby reducing the PO_4^{3-} efflux. The nearly anoxic site A was markedly different
467 to the other sites, with no macrofauna and with massive effluxes of PO_4^{3-} and NH_4^+ from the

468 sediment (up to an order of magnitude higher fluxes than at other sites), indicating a complete shift
469 to geochemically-driven functioning.
470
471 The patterns of structural changes in macrofaunal communities following organic enrichment and
472 ensuing hypoxia have been well known for several decades (Pearson and Rosenberg 1978), but the
473 link to changes in ecosystem functioning remains elusive. The SPI analysis showed that the depth
474 of the aRPD was shallowest at the almost anoxic site and generally deeper at more oxic sites, but
475 not consistently (Table 1). The aRPD can indeed be used as a proxy for the transition between
476 redox states (Rosenberg et al. 2001, Simone and Grant 2017), but while it was a significant
477 predictor of both macrofauna and meiofauna (Table 3; although macrofauna activity is likely also
478 affecting the depth of the aRPD, so the interaction works both ways), it was not an important
479 predictor for SOC or fluxes of NH_4^+or PO_4^{3-} (Table 5). Neither did the BHQ index correspond to the
480 nutrient fluxes, as it was a too coarse measure, with all our sites (except A) classified to the two
481 highest successional stages (BHQ 5-10 and >10). The multivariate analysis of macrofauna
482 community abundance, on the other hand, split these sites into four groups (in addition to site A).
483 There were indications that the four site clusters identified had a relationship with SOC and PO_4^{3-}
484 fluxes (Fig. 6), with increasing SOC and decreasing PO_4^{3-} flux towards the more oxic groups. The
485 four most abundant species were common in three of the four groups identified, indicating a high
486 level of tolerance to hypoxia. These species live buried in the sediment and have a generation time
487 of one year or more, and none of them are among the first colonizers in a succession pattern like
488 *Capitella* and *Polydora*. It is possible that such tolerant species might uphold bioturbation
489 throughout the year in seasonally hypoxic areas, although seasonal patterns in species-specific
490 bioturbation activity are not known. The number of sites (11 in total, 2-3 sites per group) did not,
491 however, allow analysing in detail any between-group differences in the different factors explaining
492 the variability of the fluxes. Future studies are needed to address this.

493

494 The Havstensfjord has suffered from seasonal hypoxia since the 1950s and the most likely cause is
495 eutrophication (Nilsson and Rosenberg 1997). Given the long history of hypoxic stress, the benthic
496 communities are probably adapted to this disturbance, but also likely permanently somewhat
497 degraded compared with communities that have not been exposed to hypoxia. Undisturbed
498 communities would likely be characterised by higher species richness, and large-bodied and long-
499 lived species (Pearson and Rosenberg 1978, Diaz and Rosenberg 1995, Gray et al. 2002), with a
500 potential for a stronger, more direct impact on nutrient cycling. The bottom-water O_2 concentration
501 was indeed relatively low at all sites at the time of sampling. The benthic habitat quality in relation
502 to hypoxia has previously (in 1994) been studied in the fjord using SPI (Nilsson and Rosenberg
503 1997), and no major changes in aRPD or further degradation of the BHQ index were observed in
504 the current study. Thus, the Havstensfjord appears to have been in a stable state of seasonal hypoxia
505 for decades already, with periods of good conditions every year. The big individuals of the bivalve
506 *Arctica islandica* found in the two hypoxic groups indicate that there had not been long periods of
507 complete anoxia at these sites in the last few years before the sampling, although these bivalves can
508 reduce metabolism and survive several months in hypoxic conditions. Given the proportionately
509 larger influence on nutrient cycling of such large individuals (Norkko et al. 2013), these few
510 bivalves may have a significant effect on ecosystem functioning in-between the hypoxic periods.
511
512 Fjords and other enclosed inlets and seas are prone to hypoxia because of limited water exchange.
513 Once a system has passed the threshold to hypoxia, the reversal might be difficult (Conley et al.
514 2009b), and there is evidence that once an ecosystem experiences hypoxia, it might be more
515 susceptible to hypoxia in the future (Conley et al. 2007, Diaz and Rosenberg 2008). This poses
516 challenges for the management of these systems. Another methodological challenge is often
517 introduced as part of the monitoring of hypoxia. We used the O_2 concentration measured 2-3 cm

above the sediment surface in flux chambers immediately upon core retrieval for the statistical analyses. It is important to consider the difference between these concentrations and the ones measured 1 m (or even higher with big CTD rosettes) above the sediment surface in most monitoring programmes (Rosenberg 1977). Due to the benthic boundary layer processes, where decreasing flow closer to the sediment surface usually corresponds to decreasing O_2 concentrations (e.g., Jørgensen and Des Marais 1990), the monitoring data does not necessarily reflect the real near-bottom O_2 concentrations, which hampers modelling efforts to predict species distribution patterns in relation to O_2 conditions (Virtanen et al. 2019).

Temperature, salinity, and near-bottom O_2 concentrations were strongly correlated and therefore only O_2 was included in the statistical analysis. Since all three variables can affect community structure as well as ecosystem function, we cannot rule out the potential impact of co-variables, but we cannot disentangle these effects from our field sampling because the hypoxic water was deep, beneath the thermocline and therefore salty and/or cold. However, the lack of oxygen will most likely override the effects of the other two on nutrient cycling, as it stresses the fauna as well as alters biogeochemical pathways. While oxygen dropped below detrimental levels in this study, neither the range in salinity (25-32) nor temperature (7-15) is likely to be a major driver of the differences in function observed, given that this is an estuarine environment with organisms adapted to fluctuations. It should also be noted, that in this relatively cool fjord system organisms are more likely to oxyregulate and the effects of hypoxia may be therefore be less stressful compared to warmer systems, where organisms are more likely to experience metabolic depression, i.e. oxyconformation (Pörtner et al. 2005). Temperature does, however, affect nutrient fluxes, with higher reaction rates at warmer temperatures. In our study, the bottom-water temperature varied from 7 to 15 degrees, and the incubations were done at a standard 11 degrees, so it is possible that

542 the fluxes at the shallowest and the deepest site were slightly underestimated and overestimated,
543 respectively. This would, however, not have affected the main conclusions.
544
545 The importance of macrofauna for explaining variability in SOC and nutrient fluxes in the current
546 study was similar to that found across gradients of increasing hypoxia by Gammal et al. (2017) and
547 (Norkko et al. 2015), in the coastal and open Baltic Sea, respectively. This indicates that the number
548 of species is not crucial for functioning; the Baltic is very species poor (open sea often only 5-7
549 species) compared with the Swedish west coast (up to 40 species recorded per grab in the current
550 study). This is contrary to our prediction that the effect of hypoxia on the faunal contribution to
551 functioning would be smaller in a system with a higher background diversity. The number of
552 macrofaunal species was highly correlated with the macrofaunal abundance, further indicating that
553 the number of species is not the sole driving factor for functioning. Indeed, in previous studies
554 under normoxic conditions it has been suggested that species-specific traits of some particularly
555 important individual species, for example, the sediment reworking by large and abundant burrowing
556 urchins or bivalves, may override both species richness and functional diversity in terms of
557 influencing benthic nutrient cycling (Lohrer et al. 2004, Norling et al. 2007, Norkko et al. 2013).
558
559 Of particular interest is then whether the fauna can still contribute to ecosystem functioning when
560 conditions deteriorate, i.e. before the fauna is decimated, but when physiological and behavioural
561 changes are already likely to have been initiated. Hypoxia tolerance is highly species specific
562 (Vaquer-Sunyer and Duarte 2008), with potential for changes in behaviour already at relatively high
563 O_2 concentrations. For example, urchin grazing rates dropped already at 5.5 mg/L (3.85 ml L^{-1}),
564 with potential consequences on kelp recruitment (Low and Micheli 2018). Macrofauna can still
565 influence nutrient cycling in low O_2 conditions, although not as much as under good O_2 conditions
566 (Norkko et al. 2015). This points to the increasing importance of tolerant species when conditions

567 deteriorate, e.g. the invasive polychaete *Marenzelleria* spp. in the Baltic Sea (Norkko et al. 2015).
568 Such deep-burrowing species may also affect the propensity for rapid oxygen consumption by
569 burying organic matter deeper into the sediment, which slows down the oxidation of OM (Josefson
570 et al. 2012). Other studies have also found that larger-bodied, tolerant species may mediate
571 ecosystem functioning during periods of low-oxygen conditions (Rakocinski and Menke 2016). In
572 the present study the large bivalve *Arctica islandica* may have performed this role. Nevertheless,
573 the hypoxia-induced changes in species behaviour that is influencing nutrient cycling are not
574 sufficiently known and this requires further mechanistic studies.
575
576 **Meiofauna, microbes and altered energy pathways**
577 The differences in meiofaunal communities were due to the silt/clay fraction, OC and aRPD, not
578 differences in O_2 concentrations, which was the major factor explaining differences in the
579 macrofauna. Various taxa of meiofauna can use aerobic metabolism at O_2 concentrations as low as
580 0.1 µmol/l (Giere 2009). In addition, the non-correlation to O_2 concentration has been noted before,
581 by e.g. Josefson and Widbom (1988), who recorded no major decrease of any meiofaunal taxa
582 during a several month long period of anoxia in the Gullmar Fjord, Sweden. In the Baltic Sea,
583 Elmgren (1975) noted that meiofauna did decrease with decreasning oxygen conditions, but that the
584 meiofauna extended deeper into the hypoxic zone compared with the macrofauna. In the current
585 study the aRPD, which approximates the depth of the oxygenated sediment layer, also significantly
586 affected the differences in meiofaunal community structure, and thus O_2 is probably still involved in
587 the vertical distribution of the meiofauna, possibly through indirect effects on macrofaunal
588 bioturbation. Sediments with high silt/clay fractions have a lower permeability compared to coarser
589 sediments, which directly influences the amount of oxygenated water that can penetrate into the
590 sediment. Large abundances of nematodes were found at the sites with a high silt/clay fraction (B,
591 C, H, I, J), which is a common representation. The nematode community is then often made up of

592 non-selective deposit-feeding nematodes grazing on bacteria and detritus (Heip et al. 1985,
593 Boeckner et al. 2009, Delgado et al. 2009). Larger grain size tends to lead to more harpacticoids;
594 however they can thrive in various conditions (Giere 2009).
595
596 At site B and J high levels of NH_4^+ were registered. It is also at these sites the highest abundances of
597 meiofauna were found. This relationship can be stressed further using site H, which had low NH_4^+
598 while at the same time holding one of the lowest total meiofaunal abundances of the analyzed sites.
599 The high ammonium at site B and C can furthermore be explained by a reduced bioturbation under
600 the anoxic conditions at those sites. This can in its turn affect the nitrification and denitrification;
601 instead of nitrogen being removed as N_2 in denitrification processes, ammonia, ammonium and
602 phosphate are the main fluxes out of the sediment (Diaz and Rosenberg 2008). In addition,
603 meiofauna is known not to control the abundance of the microbial community with their grazing,
604 but to enhance the growth rate (Piot et al. 2014). In general, high OC leads to higher abundances of
605 meiofauna but at the sites in this survey the relationship was reversed. Nevertheless, no site had
606 very low OC (range 7.7 – 12%) and OC was likely not a limiting factor for the meiofauna.
607
608 Prolonged hypoxia will have larger-scale functional consequences, with miniaturization of benthic
609 food webs and altered energy pathways (the last successional stages 1 and 0) (Diaz and Rosenberg
610 2008). For example, deep-burrowing species which are important for organic matter processing,
611 priming it for further processing by the microbes, will be lost (Table 2), translating also into a loss
612 of functional spaces and a reduction of surface area. While our study design did not allow for
613 quantifying this, it is possible that meiofaunal and microbial communities will have a
614 proportionately larger influence on ecosystem function in stressed systems compared with
615 macrofauna. The resistance of sediment microbial communities to hypoxia is however poorly
616 known, but field experiments with *in situ* -induced hypoxia indicated that microbial diversity

617 decreased with increasing duration of hypoxic stress, concurrently with the deteriorating
618 macrofaunal community, likely due to the reduced macrofaunal bioturbation activity (Sinkko et al.
619 submitted).
620
621 **Conclusions and management implications**
622 Many studies on BEF have failed to demonstrate real-world relevance, highlighting the need to
623 combine insights derived from theory with detailed experiments and broad-scale monitoring and
624 well-designed field surveys (Snelgrove et al. 2014). While causality cannot be assigned in
625 correlative field studies, they are imperative for understanding the generality of the BEF
626 relationships. They need to be conducted in a range of different environments and geographical
627 areas, using the same methodology and focussing on the relative similarities or differences. There
628 are however inherent logistic constraints to field work, as the number of sites that it is possible to
629 include in a field study is almost always limited.
630
631 Ecosystem management decisions are based on model predictions of key ecosystem processes.
632 While our understanding of the links between biodiversity and ecosystem functioning, also under
633 increasing hypoxia, is growing, there are still large gaps in our knowledge about these processes
634 and how they can be included in the models, with significant effects on model performance
635 (Carstensen et al. 2014). For example, while there are sophisticated models used for nutrient
636 management around the entire Baltic Sea (BALTSEM; Savchuk et al. 2012), macrofauna data is
637 currently lacking altogether from these models, which is a serious drawback for being able to
638 predict, for example, the time required for recovery of the sea from eutrophication. Indeed our
639 failure to account for biodiversity in models of biogeochemical cycling is severely impeding our
640 ability to understand, quantify and predict, the consequences of changes in eutrophication status and
641 climate as expressed through altered carbon pathways (Snelgrove et al. 2018). This work thus needs

642 to continue with field studies targeting macrofauna, meiofauna as well as microbes linked with
643 controlled laboratory studies where specific mechanisms can be quantified, conducted in parallel
644 with model development. This will become increasingly important as we grapple with trying to
645 protect the dwindling biodiversity and trying to predict future changes in ecosystem functioning.

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