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Mediation of nitrogen by post-disturbance shelf communities experiencing organic matter enrichment

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Abstract

Microbes and benthic macro-invertebrates interact in sediments to play a major role in the biogeochemical cycling of organic matter, but the extent to which their contributions are modified following natural and anthropogenic changes has received little attention. Here, we investigate how nitrogen transformations, ascertained from changes in archaeal and bacterial N-cycling microbes and water macronutrient concentrations ($[NH_4\text{-}N]$, $[NO_2\text{-}N]$, $[NO_3\text{-}N]$), in sand and sandy mud sediments differ when macrofaunal communities that have previously experienced contrasting levels of chronic fishing disturbance are exposed to organic matter enrichment. We find that differences in macrofaunal community structure related to differences in fishing activity affect the capacity of the macrofauna to mediate microbial nitrogen cycling in sand, but not in sandy mud environments where the range of disturbance frequency was higher than that in the sandy area. Whilst we found no evidence for a change in ammonia oxidiser community structure, we did find an increase in archaeal and bacterial denitrifier (A*nirKa*, *nirS*) and anammox (*hzo*) transcripts in macrofaunal communities characterized by higher ratios of suspension to deposit feeders, and a lower density but higher biomass of sediment-reworking fauna. Our findings suggest that nitrogen transformation in shelf sandy sediments is dependent on the stimulation of specific nitrogen cycling pathways that are associated with differences in the composition and context-dependent expression of the functional traits that belong to the resident bioturbating macrofauna community.

Introduction

Marine soft-sediments cover almost 70% of the earth's surface and play a fundamental role in the remineralization of organic carbon and nutrient cycling (Olsgard et al. 2008). According to current nitrogen budgets, it is estimated that up to 80% of the nitrogen needed by primary producers in shallow shelf seas is provided by benthic remineralization processes, primarily driven by microbial organisms that occur across the oxic/anoxic interface near the sediment surface (Dale & Prego 2002; Zehr & Kudela 2011). With 23% of the global human population concentrated in coastal areas, at an average density nearly 3 times higher than the global average (Small & Nicholls 2003) and an increase in demand in food production, coastal and shelf sea benthic systems are increasingly vulnerable to anthropogenic activities such as fishing and are at higher risk of eutrophication following excessive nutrient input. Any change in the balance and distribution of reactive nitrogen (e.g. ammonium NH_4^+, nitrite NO_2^- and nitrate NO_3^-), oxygen, and organic substrates are likely to have profound consequences for nitrification, denitrification and anaerobic ammonium oxidation (anammox) that regulate coastal nitrogen budgets (Laverock et al. 2011).

Bottom fishing that uses demersal gear such as trawls and dredges to catch fish, crustaceans and bivalves living in, on or in association with the seabed, exerts a number of pressures on benthic systems that might influence sedimentary nutrient generation and budgets (Pilskaln et al. 1998; Olsgard et al. 2008). Physical processes such as sediment resuspension and sediment mixing caused by trawling alter grain size distribution, sediment sorting and porosity (Trimmer et al. 2005) that may in turn disrupt nitrification and denitrification processes (Rysgaard et al. 1994; Kitidis et al. 2017) through changes in oxygen penetration depth within the sediment (Warnken et al. 2003) and burial of organic matter to anoxic layers before aerobic remineralisation can take place (Mayer et al. 1991; Pilskaln et al. 1998). The combination of removal of surficial sediments and mixing or burial of organic matter to depth (Duplisea et al. 2001; Warnken et al. 2003) occurs on different time-

70 scales to those of alterations in community structure; changes in pore-water and bottom water

71 nutrient levels due to sediment resuspension return to pre-trawling levels within minutes to hours

72 (e.g. Falcao et al. 2003; Trimmer et al. 2005; Goldberg et al. 2014), whilst microbial assemblage

73 structure and biomass in the surficial sediment layers (upper 1 cm) return to pre-disturbance levels

74 within days (Fiordelmondo et al. 2003) to 4 - 6 months (Watling et al. 2001). In contrast,

75 macrofaunal communities can take up to 4 – 5 years to recover (Kaiser et al. 2006; Lambert et al.

76 2014). Over extended and repeated periods of fishing activity, changes in the functional

77 composition of invertebrate communities can have a disproportionate influence on nutrient cycling

78 through changes in the bioturbation potential of the benthic community (Duplisea et al. 2001;

79 Widdicombe et al. 2004). The active redistribution of particles (bioturbation) and fluids

80 (bioirrigation) by infaunal macro-invertebrates, such as bivalves, polychaetes and crustaceans,

81 directly contributes to the spatial and temporal heterogeneity of oxic and anoxic zones (Bertics &

82 Ziebis 2009), organic matter availability (Levin et al. 1997) and the distribution of metabolic

83 electron acceptors (Fanjul et al. 2007) that are important in controlling microbial process rates

84 (Gilbertson et al. 2012; Laverock et al. 2014). The close association between the macrobenthic

85 invertebrate community composition and microbial activity suggests that a reduction in bioturbation

86 potential of the benthic community ensuing from the loss or change in relative composition of

87 invertebrate species associated with bottom fishing will lead to changes in microbial-mediated

88 processes such as nitrification, denitrification and anammox. Relative to the direct physical effects

89 of fishing gear on sediment and faunal structure, however, the extent to which microbial-

90 invertebrate coupling is modified and affects nutrient budgets in post-disturbance communities has

91 received little attention.

92

93 Fluctuations in organic matter input following events such as algal blooms or run-off associated

94 with high intensity precipitation, also have a prominent influence on benthic invertebrate fauna (van

95 Oevelen et al. 2009; Zhang et al. 2015) and microbial community composition (Franco et al. 2007;

96 Mayor et al. 2012; Tait et al. 2015). Benthic communities may be affected positively as food

97 becomes available to both invertebrate grazers and bacteria and archaea, or negatively because an

98 excess of organic matter may result in deoxygenation of the sediment (Quijon et al. 2008; Mayor et

99 al. 2012). Zhang et al. (2015) observed a doubling effect on macrofaunal production and biomass

100 during a spring algal bloom relative to the end of the bloom in the Western English Channel.

101 Increases in organic matter have also been related to increases in bacterial biomass (Tait et al.

102 2015), in bacterial growth efficiency and carbon mineralization (Mayor et al. 2012), leading to

103 subsequent increases in NH_4^+ sediment flux, oxygen consumption and denitrification (Caffrey et al.

104 1993; Conley & Johnstone 1995). Whether post-disturbance benthic communities retain the

105 assimilative capacity to 'process' system-level nutrient inputs has not been adequately evaluated.

106

107 Here, we investigate how post-disturbance macro-invertebrate communities affect archaeal and

108 bacterial N-cycling community activity and composition and associated nutrient concentrations. We

109 compare communities (macro-invertebrate and microbial) from sites that have been exposed to

110 different levels of chronic fishing activity, on the *a priori* assumption that benthic macro-

111 invertebrate communities would be restructured by chronic physical disturbance and that the

112 adjusted post-disturbance community would persist long after the perturbation event (Kaiser et al.

113 2006). Further, we examine whether the effect of organic matter enrichment is sufficient to alter

114 nitrogen dynamics by activating the microbial community. Our motivation was that the addition of

115 organic matter would likely result in an increase in microbial activity, leading to increases in NH_4^+

116 sediment flux, oxygen consumption and denitrification that might be sufficient to offset any

117 negative biogeochemical effects related to faunal change associated with prior fishing activity. To

118 widen the generalizability of our conclusions, we examine the effects of chronic fishing disturbance

119 and organic matter enrichment on nitrogen cycling in different sediment types; a diffusion

120 dominated community (sandy mud) and an advection dominated community (sand). We assume

121 that the level of biogeochemical performance that is realized in either sediment type will depend at

122 least in part on the structure and composition of the post-disturbance macro-invertebrate

123 community, as the active redistribution of particles and fluids by the macrofauna disproportionately

124 influences benthic fluxes and total benthic metabolism (Mermillod-Blondin et al. 2004; Mermillod-

125 Blondin & Rosenberg 2006).

126

127 **Methods**

128

129 **Sediment collection and experimental set-up**

130 In order to investigate how the effects of organic matter enrichment (levels: Non-enriched and

131 Enriched) and previous exposure to bottom fishing (levels: Low and High) affect macro-

132 invertebrate activity and microbial transformations of nitrogen in sand (S) and in sandy mud (sM)

133 communities, we collected and maintained intact sediment cores (n = 40, 5 cores per treatment;

134 LWH, $20 \times 20 \times 12$ cm) with their associated fauna using a 0.1 m^2 NIOZ (Netherlands Institute for

135 Sea Research, Texel) corer from two fishing grounds in the Irish Sea. Sandy (S) sites were located

136 off the east coast of the Isle of Man where scallop dredging for *Pecten maximus* and some otter

137 trawling for *Aequipecten opercularis* occurs, whereas sand muddy (sM) sites were located off the

138 coast of Cumbria, England, where otter trawling for *Nephrops norvegicus* and gadoid fish occurs

139 (Table 1). Logistics such as available ship time for collecting and storage space in constant-

140 temperature rooms for housing the mesocosms posed limitations on the number of within-treatment

141 replicates for this study. We acknowledge that we used a relatively small number of replicates and

142 caution that p-values close to a probability value of 0.05 should be interpreted with care;

143 nonetheless we adopt a conservative approach and present marginal (p<0.075) findings that indicate

144 possible trends. Within each fishing ground, sediment cores were collected from two sites of

145 contrasting exposure to chronic fishing disturbance (Table 1). We categorized fishing activity at

146 each site by calculating the number of times the site is swept by bottom fishing gear in a year (km^2

147 swept km^{-2} seabed yr^{-1}) using Vessel Monitoring System (VMS) records for UK registered vessel >

148   15 m over the 3 year period prior to our survey (further details in Sciberras et al. 2016). Since VMS

149   is only mandatory for vessels over 15 m (EC 2003), the activity of vessels smaller than 15 m,

150   particularly those between 8 and 15 m is not represented. Therefore, estimates of fishing frequency

151   may be underestimates of the actual fishing intensity, but as the spatial distribution of large and

152   small trawlers are correlated, our measure of fishing frequency is a useful indicator of the relative

153   fishing disturbance experienced by benthic communities at the sampled sites. Variation in habitat

154   characteristics (e.g. sediment grain size composition, organic matter content, water depth, bottom

155   temperature and tidal shear stress) among replicate cores collected from within each of the two

156   sediment types was minimized to ensure that any observed differences reflected differences

157   associated with changes in species composition due to fishing rather than environmental variability

158   (Table 1, Electronic Supplementary Material (ESM) 1). Sediment grain size and organic matter

159   content were determined for a separate sediment sample (ø = 5 cm, 5 cm deep) taken from each

160   NIOZ core sample collected on-site. A combination of dry sieving ($1 - 9.5$ mm at 0.25φ intervals)

161   and laser diffraction techniques (Malvern 2000 particle sizer, range: $0.21 - 1003.44$ μm) were used

162   to produce a complete particle size distribution. Organic matter content was estimated by mass loss

163   on ignition of ~ 5 g of dried sediment at 550 °C for 6 hours (Holme & McIntyre 1984).

164

165   Each intact sediment core was transferred to a Perspex aquarium, overlaid by ~20 cm (8 L) of

166   ambient seawater and incubated in the laboratory in the dark at constant temperature (13°C,

167   approximating mean sea bottom temperature during the sampling period, 22-28th June 2015) for 1

168   month. The experimental period incorporated a 15 day acclimatization period prior to the addition

169   of organic matter and a 15 day experimental period following the addition of organic matter.

170   Enriched treatments (n = 20) received 50 mL of the microalga *Isochrysis galbana* on day 16

171   (concentration of ~ 22 cells μL^{-1}; based on field observations of chlorophyll-*a* levels at ~ 10 m

172   depth for the central Celtic Sea during a typical spring algal bloom, pers. comm. Dr. Alex Poulton,

173   National Oceanography Centre, Southampton). All aquaria were aerated by bubbling with filtered

174   air for the duration of both the acclimatization and experimental periods.

175

176   **Water nutrient, microbial and macro-invertebrate community analysis**

177   A pre-filtered (0.45 μm, NALGENE) water sample was collected from approximately mid-point of

178   the overlying water column of each aquarium at the end of the experiment. Absolute concentrations

179   of ammonium ($[NH_4\text{-}N]$), nitrite ($[NO_2\text{-}N]$) and nitrate ($[NO_3\text{-}N]$) were quantified using

180   colorimetric techniques and a segmented flow nutrient autoanalyser (Bran and Luebbe, Model

181   AAIII).

182

183   To quantify abundance and activity of N-cycling associated microbes, sediment samples (1 mL)

184   were collected from the top 1 cm of the sediment from each core at the end of the experiment and

185   added to a LifeGuard Soil Preservation Solution (MoBio Laboratories, Inc., Carlsbad, California,

186   USA) and stored at -20˚C until further analysis. RNA and DNA were extracted from 0.4g sediment

187   samples using the RNA PowerSoil® Total RNA Isolation Kit with the RNA PowerSoil® DNA

188   Elution Accessory Kit (MoBio Laboratories, Inc., Carlsbad, California, USA). Changes in the

189   abundance of transcripts for key nitrogen cycling processes, nitrification (archaeal and bacterial

190   ammonia monooxygenase, *amoA* that convert NH_4^+ into NO_2^- and NO_3^-), denitrification (archaeal

191   and bacterial nitrite reductase, *nirK* and *nirS* that convert NO_3^- into N_2) and anammox (hydrazine

192   oxidoreductase, *hzo* that converts NO_2^- and NH_4^+ into N_2) were analysed *via* quantitative PCR

193   (qPCR). In addition, as proxies for bacterial and archaeal abundance and activity, archaeal and

194   bacterial 16S rRNA genes and 16S RNA were also quantified. Terminal Restriction Fragment

195   Length Polymorphism (T-RFLP) was used to compare the impact of fishing frequency and organic

196   matter addition on the composition of total and active bacterial and archaeal communities. A

197   detailed methodology for RNA, DNA and gene extraction, qPCR and associated primers and T-

198   RFLP is provided in ESM2.

199

200   All invertebrates were recovered (500 μm sieve), fixed and preserved in 4 % formaldehyde solution

201   for subsequent identification to the highest practicable taxonomic resolution (mostly species) and

202   the abundance and wet weight of each taxon was measured after blotting. Tube worms were

203   weighed excluding tubes. The values of total biomass include fragments of organisms that could not

204   be assigned to specific taxa.

205

206   **Statistical analysis**

207   Statistical analyses to examine the effects of fishing frequency and organic matter enrichment on

208   benthic communities (macrofauna, microbial) and water nutrients were kept separate for sand and

209   sandy mud, primarily because the fisheries under study at the two sediment types use different

210   fishing gears and operate in distinct habitat types with taxonomically different communities and

211   because the range of fishing frequency was not comparable between the two study locations (Table

212   1). At the sandy mud fishing ground, very low or no fishing sites were characterized by different

213   habitat conditions (sediment composition, tide and wave stress) from sites where fishing occurred.

214   Therefore, sampling from sites with fishing frequency comparable to that in sand for the low fishing

215   frequency treatment would have biased our conclusions about the effects of fishing and enrichment

216   in sandy mud. The terms 'low' and 'high' are therefore used in a relative sense.

217

218   Linear regression models (full factorial, independent nominal variables: fishing frequency F,

219   organic matter enrichment E) were fitted for the response variables for the invertebrate community

220   (total invertebrate density, biomass and species richness, the ratio of suspension to deposit feeders),

221   microbial community (abundance of bacterial and archaeal nitrifiers (AOB *amoA*, AOA *amoA*),

222   denitrifiers (A*nirKa*, *nirS*), and anammox (*hzo*)) and associated concentrations of dissolved

223   inorganic nitrogen ([NH$_4$-N], [NO$_2$-N] and [NO$_3$-N]). The ratio of suspension to deposit feeders

224   was examined as an indicator of compositional and functional change, as high levels of suspension

feeder mortality (relative to deposit feeders) in fished areas have been shown to reduce the benthic oxygen demand and result in higher rates of nitrification (Allen & Clarke 2007). Information on species feeding mode was obtained from the biological traits database generated from the BENTHIS project (Bolam et al. 2014, http://www.benthis.eu/en/benthis/Results.htm, accessed 16 July 2016). Further, to assess whether the sediment reworking potential of the macro-invertebrate community differed among treatments, species were classified as epifauna (E), surficial modifiers (SM), biodiffusors (B), upwards/downwards conveyors (C) and regenerator (R) following Solan et al. (2004) and updated by Queiros et al. (2013). Epifaunal organisms include species that occur predominantly above the sediment-water interface whose activities are limited to the near-surface sediment. Surficial modifiers are organisms whose activities are mostly restricted to the uppermost few centimetres of the sediment, rarely venturing above the sediment-water interface. Biodiffusors include organisms with activities that usually result in a constant and random local sediment biomixing over short distances (ca. 5 cm). Conveyors include burrow-building species that are vertically oriented in the sediment typically feeding head-down (upward conveyors) or head-up (downward conveyors) at depth in the sediment. Regenerators are excavators that dig and continuously maintain burrows in the sediment and by doing so they mechanically transfer sediment from depth to the surface (Solan et al. 2004; Kristensen et al. 2012). Density and biomass was summed to obtain the total of each reworking mode. A linear regression model incorporating the independent terms mode of sediment reworking (R_i), fishing frequency (F), organic matter enrichment (E), and their interactions, was fitted for total density and total biomass. A significant interaction term ($F : R_i$, $E : R_i$ or $F : E : R_i$) would indicate that changes in total density or total biomass reflect differences in response across bioturbation groups that depend on fishing frequency and/or organic matter enrichment.

Where there was evidence of violation of homogeneity of variance, the data were analyzed using a generalised least squares (GLS) estimation procedure to allow the residual spread to vary with

251 individual independent variables (Zuur et al. 2009). To determine the optimal variance structure, we

252 compared the full linear regression models to the equivalent GLS models incorporating specific

253 variance structures using Akaike information criteria (AIC) and by inspection of model residual

254 patterns. The optimal fixed-effects structure was then obtained by applying a backward selection

255 using the likelihood ratio test obtained by maximum-likelihood (ML) estimation. Following Zuur et

256 al. (2009), the optimal model was estimated using REML estimation. Homogeneity of residuals was

257 established through visual examination of plotted standardized residuals versus fitted values. All

258 analyses were performed using the *nlme* package (v. 3.1, Bates et al. 2013) in the R statistical and

259 programming environment (R Development Core Team 2005). A summary of the linear regression

260 models output is presented in Table 2 and 3, coefficient tables that indicate the direction and

261 magnitude of differences among treatments are presented in supplementary material, ESM 3. Unless

262 indicated differently, univariate results are expressed as mean ± standard error.

263

264 Differences in macrofaunal and microbial community composition associated with different fishing

265 frequency and organic matter enrichment treatments were examined using PERMANOVA

266 (Permutational analysis of variance). The relative contribution of species to significant effects was

267 identified using SIMPER (Similarity percentages). All PERMANOVA and SIMPER analyses were

268 conducted in PRIMER-E (Version 7, http://www.primer-e.com/).

269

270 **Results**

271

272 **Sandy sediments**

273 Macro-invertebrate density ranged from 100 to 3700 ind. m^{-2}, total biomass from 1 to 853.25 gWW

274 m^{-2} and species richness from 3 to 22, but were not affected by fishing frequency or organic matter

275 enrichment (Models 1 − 3, Table 2a). However, the ratio of suspension to desposit feeders (Model

276 4, Table 2a, Figure 1a) and overall macro-invertebrate composition (PERMANOVA, density:

277 Pseudo-F = 6.79, p = 0.001; biomass: Pseudo-F = 2.56, p = 0.009; nMDS Figure 1b) were

278 dependent on the frequency of fishing. The nMDS ordination for density and biomass data was very

279 similar, therefore only that for density is presented in Figure 1b. Deposit feeders such as the

280 polychaete *Lagis koreni* and the echinoderms *Leptosynapta inhaerens*, *Echinocardium cordatum*

281 and *Echinocyamus pusillus* were more abundant than suspension feeders in communities that had

282 previously experienced a low frequency of bottom fishing (density: deposit feeders = 635 ± 155 ind.

283 m^{-2}, suspension feeders = 92.5 ± 20 ind. m^{-2}), whereas suspension feeders such as *Phoronis* sp.,

284 *Owenia fusiformis* and *Abra alba* were more abundant in communities that had previously

285 experienced a high frequency of bottom fishing (density: deposit feeders = 262.5 ± 70 ind. m^{-2},

286 suspension feeders = 357.5 ± 47.5 ind. m^{-2}) (Table 3a). Compositional differences were largely

287 associated with a higher density of echinoderms (in particular *L. inhaerens*, *E. cordatum*, juvenile

288 asteroids and *E. pusillus*) at lower fishing frequency, and a higher density of polychaetes (*O.*

289 *fusiformis*, *Magelona* spp., *Sthenelais limicola, Ophelina acuminata* and *Chaetozone sp.*) (SIMPER,

290 Table 3a) and larger individuals of *E. cordatum* (34g) and the bivalves *Acanthocardia echinata*

291 (6g), *Chamelea striatula* (3g) and *Thracia phaseolina* (1.4 g) at the higher fishing frequency sites

292 (SIMPER, Table 3b).

293

294 Sediment reworking group density depended on the interactive effects of sediment reworking group

295 identity and the frequency of fishing (Model 5, Table 2a); biodiffusor (e.g. *E. cordatum*, *Glycera*

296 *oxycephala*) and conveyor species (e.g. *L. koreni*, *T. phaseolina*, *Spiophanes bombyx*) were more

297 abundant after a low frequency of fishing (density: 492.5 ± 133.28 ind. m^{-2} and 430 ± 172.92 ind. m^{-}

298 ^{2}, respectively) than they were after a high frequency of fishing (density: 180 ± 80.78 ind. m^{-2} and

299 197.5 ± 42.89 ind. m^{-2}, respectively) (Figure 2a). Sediment reworking group biomass, however, was

300 dependent on the independent effects (Model 6, Table 2a) of organic matter enrichment (Figure 2b)

301 and sediment reworking group identity (Figure 2c), with a greater biomass attributed to biodiffusors

302 (e.g. *E. cordatum, Sigalion mathilde, Lumbrineris* sp.) and when sediments were enriched with

303 organic matter.

304

305 T-RFLP profiling for archaeal and bacterial 16S rRNA genes revealed significant differences in the

306 total microbial community structure between communities that experienced low and high frequency

307 of fishing activity (PERMANOVA: Archaea, Pseudo-F = 2.22, p = 0.05; Bacteria, Pseudo-F = 4.03,

308 p = 0.03), but not among enriched and non-enriched treatments (Archaea, Pseudo-F = 0.66, p =

309 0.61; Bacteria, Pseudo-F = 0.92, p = 0.42; ESM4 Figure S2). The density of the metabolically

310 active bacterial denitrifier (*nirS*) was dependent on the independent effects of fishing frequency

311 (Figure 3a) and organic matter enrichment (Figure 3b), whilst both archaeal (A*nirKa*) denitrifiers

312 (Figure 3c) and anammox (*hzo*) hydrazine oxidoreductase transcripts (Figure 3d) were dependent

313 solely on the effect of fishing frequency (Models 7 – 9, Table 2a). We found no evidence that the

314 density of bacterial (AOB *amoA*) or archaeal (AOA *amoA*) ammonia oxidisers, or ammonia

315 oxidiser community structure (AOB:AOA *amoA* ratio) were affected by the frequency of fishing or

316 the level of organic matter enrichment (Models 10 – 12, Table 2a).

317

318 For nutrients, we found that [NO_2-N] and [NO_3-N] were dependent on an independent effect of

319 fishing frequency (Models 13–14, Table 2a, Figure 4), whilst [NH_4-N] was not affected by fishing

320 frequency or organic matter enrichment (Model 15, Table 2a).

321

322 **Sandy Mud sediments**

323 Relative to sandy sediments, total density (25 to 450 ind. m^{-2}), total biomass (0.8 to 119.8 gWW m^{-}

324 ^{2}) and species richness (1 to 9) were lower in sandy mud macro-invertebrate communities. Total

325 macro-invertebrate density (Model 16, Table 2b, Figure 5a, b) and species richness (Model 17,

326 Table 2b, Figure 5c,d) were dependent on the independent effects of fishing frequency and organic

327 matter enrichment. Mean total density and species richness were highest for communities that had

328   experienced a greater frequency of fishing (density: 227.5 ± 40.5 m^{-2}; species richness: 5.00 ± 0.89

329   core^{-1}) or organic matter enrichment (density: 200.00 ± 30.51 m^{-2}, species richness: 4.70 ± 0.68

330   core^{-1}). In contrast, we found no evidence of any macro-faunal response in terms of biomass (Model

331   18, Table 2b). Similarly, the ratio of suspension to deposit feeders did not differ between any of our

332   treatments (Model 19, Table 2b). Sediment reworking group density (Model 20, Table 2b),

333   however, reflected the independent effects of fishing frequency (Figure 5e), organic matter

334   enrichment (Figure 5f) and sediment reworking group identity (Figure 5g), whilst sediment

335   reworking group biomass was influenced solely by sediment reworking group identity (Model 21,

336   Table 2b with a greater biomass attributed to biodiffusors (e.g. *Goneplax rhomboides*, *Nepthys* sp.)

337   (Figure 5h). Nevertheless, community composition did not differ between sites of low and high

338   fishing frequency (PERMANOVA, density: Pseudo-F = 1.30, p = 0.25; biomass, Pseudo-F = 1.37,

339   p = 0.22, ESM 5) or between enriched and non-enriched treatments (density: Pseudo-F = 0.82, p =

340   0.55; biomass: Pseudo-F = 0.74, p = 0.63) (Figure 6).

341

342   T-RFLP profiling for archaeal and bacterial 16S rRNA genes did not reveal any differences in total

343   microbial community structure that related to either fishing frequency (PERMANOVA, Archaea:

344   Pseudo-F = 1.07, p = 0.37; Bacteria: Pseudo-F = 1.47, p = 0.15), or organic matter enrichment

345   (Archaea: Pseudo-F = 0.23, p = 0.95; Bacteria: Pseudo-F = 1.29, p = 0.24) (ESM4 Figure S3).

346   Indeed, we were unable to find any evidence supporting the view that bacterial denitrifers (*nirS*

347   transcripts, range: 903 – 6330 copies g^{-1} sediment), anammox (*hzo* transcripts, range: 3160 –

348   165000 copies g^{-1} sediment) or bacterial (AOB *amoA* transcripts, range: 1270 – 251000 copies g^{-1}

349   sediment) or archaeal ammonia oxidisers (AOA *amoA* transcripts, range: 2700 – 83900 copies g^{-1}

350   sediment) respond to differences in fishing freqency or organic matter enrichment (Models 22, 24–

351   26, Table 2b). In contrast, however, archaeal (A*nirKa*) denitrifiers did respond (Model 23, Table

352   2b) positively to the effects of increasing fishing frequency (Figure 7a) and negatively to increasing

353   organic matter enrichment (Figure 7b), although these effects were independent of one another. We

354 also found evidence that the mean ratio of bacterial to archaeal ammonia oxidisers (AOB : AOA

355 *amoA*) increased with organic matter enrichment (Model 27, Table 2b, Figure 7c).

356

357 Despite changes in the microbial and macrofaunal attributes of our sandy mud communities, [NH$_4$-

358 N], [NO$_2$-N] and [NO$_3$-N] were not affected by fishing frequency or organic matter enrichment

359 (Models 28 – 30, Table 2b).

360

361 **Discussion**

362

363 Our findings suggest that nitrogen transformation in shelf sea sediments is dependent on whether

364 specific microbial transcripts are influenced by differences in the composition of the bioturbating

365 macrofauna, environmental context (here, nutrient enrichment and sediment type), and recent

366 history of anthropogenic disturbance (here, frequency of bottom fishing), although these effects are

367 not necessarily interactive and their relative importance is context dependent (Wohlgemuth et al.

368 2017). We find that the modification of invertebrate community structure following bottom fishing

369 is particularly important for the mediation of biogeochemical processes and is not necessarily offset

370 by the effects of organic matter enrichment on microbial composition and activity. These effects

371 were observed in sand but not in sandy mud (where the range of fishing frequency from bottom-

372 towed fishing gear was higher than that in the sandy area). In sand, we found that sediment

373 characterized by higher ratios of suspension to deposit feeders and a lower density but higher

374 biomass of bioturbating fauna, was associated with increased activity of denitrifying archaea and

375 bacteria (A*nirKa*, *nirS*) and anammox (*hzo*) and higher levels of bottom water [NO$_2$-N] and [NO$_3$-

376 N]. The higher biomass of bioturbating species, in particular *Echinocardium cordatum*,

377 *Acanthocardia echinata* and *Chamelea striatula* and the higher density of bioirrigating tube-

378 building species such as *Phoronis* sp. and *Owenia fusiformis,* offers an explanation for the enhanced

379 denitrifier activity. *E. cordatum* is known to displace large volumes of sediment (20 000 cm^3 m^{-2} d^{-1}

by 40 individuals m^{-2}, Lohrer et al. 2005) and although the shallow-burying bivalves *A. echinata* and *C. striatula* and the tube-building polychaetes do not build extensive burrow systems deep within the sediment, their active mixing of the uppermost sediment layers and their dominating biomass, means that their bioturbation activities are likely to have stimulated microbial denitrification and anammox, possibly by increasing the flux of [NO_2-N] and [NO_3-N] across the water-sediment interface, which constitutes the substrate for nitrite reductase and anammox (Howe et al. 2004; Dang et al. 2010). Measured sediment particle reworking rates using fluorescent sediment profile imaging techniques at the same study sites confirm more intense particle reworking activity in communities that have previously experienced a higher frequency of bottom fishing, substantially extending the maximum depth of sediment reworking (low frequency fishing, 1.99 ± 0.19 cm; high frequency fishing, 4.64 ± 0.5 cm) and increasing the volume of sediment available for nitrification (Hale et al. 2017). It is interesting to note that, although some bioturbation groups - such as biodiffusors and upwards/downwards conveyors – were, on average, twice as abundant at sites with a history of low frequency of fishing, the biomass of biodiffusors and surficial modifiers was substantively higher (24 times and 60 times higher, respectively) at sites with a history of high frequency of fishing. It appears that biomass had an overriding effect over density; larger individuals with a greater *per capita* effect on sediment mixing have a disproportionate effect on microbial activity and composition and, in turn, nutrient concentrations (Osinga et al. 1995; Bird et al. 1999). The increased abundance of active suspension feeders (relative to deposit feeders) is also likely to have stimulated microbial denitrification and anammox, through an increase in the provision of [NO_2-N] and [NO_3-N] as water is actively moved into the sediment during feeding (Howe et al. 2004; Dang et al. 2010). Although we may conclude that macrofaunal bioturbation and bioirrigation activities had a stimulatory effect on microbial denitrification, it remains unclear why we did not detect any change in archaeal or bacterial nitrifier (*amoA* gene) abundance in either sand or sandy mud sediments.

406   Given the findings elsewhere that report higher mortality of suspension feeders at locations that are

407   subject to fishing activity (e.g. Tillin et al. 2006; van Denderen et al. 2015), it is surprising that we

408   found a higher ratio of suspension to deposit feeders in sand communities that experienced a higher

409   frequency of chronic fishing. However, a clear trend that emerges from previous studies is that the

410   degree of natural disturbance in which a community develops determines the degree to which it is

411   affected by bottom fishing (Kaiser & Spencer 1996; Hiddink et al. 2006; Sciberras et al. 2013). The

412   macro-invertebrate communities at our sandy study sites are adapted to living in physically dynamic

413   areas that are characterized by relatively high near-bed current flows (Hiddink et al. 2009) and

414   infrequent fishing activity (1.63 times yr^{-1}), so community recruitment and growth is unlikely to be

415   significantly affected by fishing. In contrast, the macro-invertebrate communities of our sandy mud

416   sites show substantive compositional changes that relate to a fishing frequency of 3.8 times yr^{-1},

417   such that further increases in fishing activity (8.4 times yr^{-1}) have proportionally less effect on

418   microbial and/or macrofaunal community composition and structure. We recognize that our study

419   would benefit from additional locations where bottom fishing is absent, but such areas were not

420   comparable as they were characterized by very different habitat conditions (sediment composition,

421   tide and wave stress). In agreement with Braeckman et al. (2014), however, we find that that

422   benthic functional diversity (expressed as community bioturbation potential, BP_C) had a strong

423   influence on biogeochemical cycling (sediment community oxygen consumption, denitrification

424   rates, alkalinity and NH_4 fluxes) in sandy sediments with high BP_C but not in muddy sediments,

425   where the BP_C was found to be significantly lower than in sand.

426

427   We hypothesized that the addition of organic matter would increase microbial activity (measured

428   here as the gene transcript abundance). Thus, the correlation between enrichment and macrofaunal

429   density and species richness in sandy mud and the relative biomass of different functional groups in

430   sand was unexpected (given the short time scale of the experiment) and most likely the result of

431   stochastic variation in the abundance of infauna and unrelated to the enrichment treatment.

432 However, in sandy sediments, organic matter enrichment correlated with a reduction in the variation

433 and mean activity of bacterial denitrifiers (*nirS*), and in sandy mud sediments with a reduction in

434 mean activity of archaeal denitrifiers (A*nirKa*), and a change in ammonia oxidiser community

435 structure, altering the ratio of ammonia oxidising bacteria to ammonia oxidising archaea (as in

436 Gilbertson et al. 2012). An effect, however, was not found for all microbes measured, for example

437 for the nitrifiers (AOB *amoA*, AOA *amoA*) and anammox (*hzo*) in either sand or sandy mud. A

438 number of studies have reported increases in bacterial biomass and activity upon addition of organic

439 material within days or even hours (e.g. Luna et al. 2002; Gihring et al. 2009). However, others

440 have reported a delayed microbial response (~ 1 - 2 weeks) (Tait et al. 2015) as instead of feeding

441 directly on sinking phytodetrital material, benthic microbes may consume the organic matter

442 released via the grazing activity of deposit and suspension feeders, thus explaining the lack of

443 response for some microbes in our study. The lesser importance of organic matter enrichment

444 relative to fishing frequency related changes in macrofaunal composition documented here, may be

445 emphasising habitat-specific differences in organic matter incorporation rate and/or differences in

446 the response time of different components of the benthic community. Our study highlights the

447 importance of understanding the response of multiple ecosystem components over the longer term if

448 we are to provide ecosystem-relevant evidence to underpin decisions that aim to secure the

449 protection of natural capital (Pittman & Armitage, 2016), and ensure the sustainable management of

450 coastal and shelf sea ecosystem services (Voss et al. 2013).

451

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681 **Table 1.** Summary of environmental characteristics and bottom fishing frequency of our study sites. The mean ± SE (n = 10) values for percentage

682 sand, percentage mud and organic matter content (mg) for areas that have experienced low and high fishing frequency in sandy Mud (sM-low and sM-

683 high) and sandy sediments (S-low and S-high) are listed.

684

Site code	Geographical location (latitude, longitude)	Fishing frequency (times fished per annum)	Depth (m)	Tide stress (Nm^{-2})	Wave stress (Nm^{-2})	Sand (%)	Mud (%)	Organic matter (mg)
sM - low	54.15 N, -3.63 W	3.8	26.00	0.17	0.69	33.48 ± 2.62	66.48 ± 2.62	70 ± 6.33
sM - high	54.26 N, -3.73 W	8.4	28.54	0.22	0.68	36.43 ± 1.98	63.55 ± 1.99	90 ± 12.65
S - low	54.20 N, -4.05 W	0.25	19.80	0.17	1.00	99.51 ± 0.38	0.02 ± 0.02	40 ± 6.33
S - high	54.26 N, -4.19 W	1.63	18.79	0.11	0.73	94.2 ± 0.68	4.7 ± 0.53	70 ± 9.49

685

686

687 **Table 2a.** Linear regression models to examine the effects of fishing frequency and enrichment (full factorial, F × E) **in sand (S)**, for macro-
688 invertebrate community (Models 1-4: invertebrate density, biomass and species richness, the ratio of suspension to deposit feeders), sediment
689 reworking groups (Ri, reworking group density and biomass, Models 5-6), microbial community (Models 7-12: abundance of bacterial and archaeal
690 denitrifiers (A*nirKa*, *nirS*), anammox (*hzo*), bacterial and archaeal nitrifiers (AOB *amoA*, AOA *amoA*) and ratio of bacterial and archaeal *amoA*
691 transcripts) and associated levels of dissolved inorganic nitrogen (Models 13 -15: [NO_2-N], [NO_3-N], [NH_4-N]). The test statistic (L-ratio or F value),
692 degrees of freedom (df) and probability value (p) are listed for marginal (p<0.075) or significant (p < 0.05) terms. Where all independent variables
693 were found insignificant, we present the intercept only model. The class of variance-covariate used to specify different variances for each level of
694 stratification within-group are also provided.

695

Sediment type: SAND (S)							
Macro-invertebrate community (Initial linear model: Response variable ~ F × E)							
Model ID	**Model**	**Response variable**	**Fishing frequency (F)**	**Enrichment (E)**	**Interaction (F : E)**	**Intercept only**	**Variance-covariate**
1	GLS	Macro-invertebrate density				L = 2.54, df = 1, p = 0.11	E
2	GLS	Macro-invertebrate biomass				L = 3.37, df = 1, p = 0.07	E × F
3	GLS	Species richness				L = 1.32, df = 1, p = 0.25	E
4	GLS	suspension: deposit feeders ratio	L = 17.07, df = 1, p < 0.001				F

Sediment reworking groups (Initial linear model: Response variable ~ F × E × Ri)								
Model ID	**Model**	**Response variable**	**Fishing frequency (F)**	**Enrichment (E)**	**Reworking mode (Ri)**	**F : E**	**F : Ri**	**Intercept only**	**Variance-covariate**
5	GLS	Ri density					L = 15.92, df = 4, p = 0.003		Ri × F
6	GLS	Ri biomass		L = 4.09, df = 1, p = 0.04	L = 27.05, df = 4, p <				Ri × F

30

| | | | | | | | 0.0001 | | | |

Abundance of active N-cycling associated microbes (Initial linear model: Response variable ~ F × E)

Model ID	Model	Response variable	Fishing frequency (F)	Enrichment (E)	Interaction (F : E)	Intercept only	Variance-covariate
7	GLS	*nirS*	L = 3.63, df = 1, p = 0.05	L = 11.26, df = 1, p < 0.001			F
8	GLS	*AnirKa*	L = 6.41, df = 1, p = 0.01				E × F
9	GLS	*hzo*	L = 6.59, df = 1, p = 0.01				F
10	GLS	AOA *amoA*				L = 1.25, df = 1, p = 0.26	E
11	GLS	AOB *amoA*				L = 2.50, df = 1, p = 0.11	F
12	GLS	AOB : AOA *amoA* ratio				L = 3.28, df = 1, p = 0.07	E

Water nutrient concentration (Initial linear model: Response variable ~ F × E)

Model ID	Model	Response variable	Fishing frequency (F)	Enrichment (E)	Interaction (F : E)	Intercept only	Variance-covariate
13	GLS	[NO_2-N]	L = 5.99, df = 1, p = 0.01				F
14	GLS	[NO_3-N]	L = 19.47, df = 1, p < 0.0001				F × E
15	GLS	[NH_4-N]				L = 1.12, df = 1, p = 0.29	E × F

696
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31

699   **Table 2b.** Linear regression models to examine the effects of fishing frequency and enrichment (full factorial, $F \times E$) **in sandy mud (sM)**, for macro-

700   invertebrate community (Models 16-19: invertebrate density, biomass and species richness, the ratio of suspension to deposit feeders), sediment

701   reworking groups (R_i, reworking group density and biomass, Models 20-21), microbial community (Models 22-27: abundance of bacterial and archaeal

702   denitrifiers (*nirS*, A*nirKa*), anammox (*hzo*), bacterial and archaeal nitrifiers (AOB *amoA*, AOA *amoA*) and ratio of bacterial and archaeal *amoA*

703   transcripts) and associated levels of dissolved inorganic nitrogen (Models 28-30: [NO_2-N], [NO_3-N], [NH_4-N]). The test statistic (L-ratio or F value),

704   degrees of freedom (df) and probability value (p) are listed for marginal ($p<0.075$) or significant ($p < 0.05$) terms. Where all independent variables

705   were found insignificant, we present the intercept only model. The class of variance-covariate used to specify different variances for each level of

706   stratification within-group are also provided.

707

**Sediment type: sandy Mud (sM)**								
**Macro-invertebrate community (Initial linear model: Response variable ~ $F \times E$)**								
Model ID	**Model**	**Response variable**	\multicolumn{2}{l}{**Fishing frequency (F)**}	**Enrichment (E)**	**Interaction (F : E)**	**Intercept only**	**Variance-covariate**
16	GLS	Macro-invertebrate density	\multicolumn{2}{l}{L = 5.27, df = 1, p = 0.02}	L = 4.31, df = 1, p = 0.04			F
17	GLS	Species richness	\multicolumn{2}{l}{L = 3.83, df = 1, p = 0.05}	L = 7.75, df = 1, p = 0.005			F
18	GLS	Macro-invertebrate biomass	\multicolumn{2}{l}{}			L = 1.29, df = 1, p = 0.26	$F \times E$
19	LM	suspension: deposit feeders ratio	\multicolumn{2}{l}{}			F = 12.33, df = 16, p = 0.77	-
**Sediment reworking groups (Initial linear model: Response variable ~ $F \times E \times Ri$)**								
Model ID	**Model**	**Response variable**	**Fishing frequency (F)**	**Enrichment (E)**	**Reworking mode (Ri)**	**F : E**	**F : Ri**	**Intercept only** **Variance-covariate**
20	GLS	Ri density	L = 8.51, df = 1, p = 0.004	L = 5.59, df = 1, p = 0.02	L = 54.17, df = 1, p < 0.0001			$F \times E$
21	GLS	Ri biomass			L = 8.23, df = 1, p = 0.02			$F \times Ri$

Abundance of active N-cycling associated microbes (Initial linear model: Response variable ~ F × E)							
Model ID	**Model**	**Response variable**	**Fishing frequency (F)**	**Enrichment (E)**	**Interaction (F : E)**	**Intercept only**	**Variance-covariate**
22	LM	*nirS*				F = 0.005, df = 17, p = 0.94	**-**
23	LM	*AnirKa*	F = 3.86, df = 1, p = 0.07	F = 3.97, df = 1, p = 0.06			-
24	LM	*hzo*				F = 0.33 , df = 17, p = 0.57	-
25	LM	AOA *amoA*				F = 0.46 df = 17, p = 0.51	**-**
26	LM	AOB *amoA*				F = 0.17 , df = 17, p = 0.68	**-**
27	LM	AOB : AOA *amoA* ratio		F = 13.33, df = 1, p = 0.002			-
Water nutrient concentration (Initial linear model: Response variable ~ F × E)							
Model ID	**Model**	**Response variable**	**Fishing frequency (F)**	**Enrichment (E)**	**Interaction (F : E)**	**Intercept only**	**Variance-covariate**
28	GLS	[NO_2-N]				L = 1.37, df = 1, p = 0.24	F × E
29	LM	[NO_3-N]				F = 0.49 , df = 19, p 0.49	-
30	GLS	[NH_4-N]				L = 1.73, df = 1, p = 0.19	F × E

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711 **Table 3.** The similarity percentage (SIMPER) dissimilarity tables (up to 90% of cumulative
712 differences) of taxa (a) density and (b) biomass in **sandy sites** that experienced contrasting levels of
713 fishing frequency (Levels: low and high fishing frequency). Information on species feeding mode/s
714 (SDF for surface deposit feeder; SubDF for subsurface deposit feeder; ASF for active suspension
715 feeder; PSF for passive suspension feeder; Pred for predator; Scav for scavenger; Det for
716 detritivore; feeding mode was not allocated to taxon level higher than genus and are denoted by "-
717 "), sediment reworking functional type (E for epifauna; SM for surficial modifiers; UC/DC for
718 upward and downward conveyors; B for biodiffusors; and R for regenerators) and mobility (1 for
719 organisms that live in fixed tubes; 2 indicates limited movement; 3 indicates slow, free movement
720 through the sediment matrix; 4 indicates free movement via burrow system) are provided.
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a. Groups tested: Taxon density between low and high fishing activity in sandy sediment						
Species	**Feeding mode**	[3]**Sediment reworking functional type**	[3]**Mobility**	**Low fishing frequency**	**High fishing frequency**	**Contr. Diss. (%)**
Lagis koreni	[1]SubDF	UC/DC	1	3.59	1.56	8.81
Phoronis sp.	[1]PSF, ASF	SM	1	0.34	3.00	8.74
Leptosynapta inhaerens	[1]SubDF, Det	SM	3	2.88	0.00	8.39
Echinocardium cordatum	[1]SDF, SubDF	B	3	2.57	0.85	6.93
Asteroid juvenile	-	E	3	1.46	0.00	4.29
Owenia fusiformis	[1]PSF, ASF, SDF, SubDF	SM	1	0.20	1.11	3.36
Magelona sp.	[2]SDF	SM	2	0.14	0.73	2.64
Glycera oxycephala	[1]Pred, Scav	B	3	0.94	0.20	2.60
Echinocyamus pusillus	[1]SDF, SubDF	SM	3	0.84	0.00	2.43
Sthenelais limicola	[2]Pred, Scav	B	3	0.10	0.71	2.28
Ophelina acuminata	[2]SubDF	B	3	0.20	0.76	2.27
Chaetozone sp.	[2]SDF	SM	2	0.00	0.64	2.07
Magelona johnstoni	[2]SDF	SM	2	0.20	0.66	2.03
Dosinia lupinus	[2]ASF, PSF	SM	2	0.54	0.00	1.94
Poecilochaetus serpens	[1]SDF, SubDF, PSF, ASF	SM	2	0.54	0.41	1.91
Thracia phaseolina	[1]PSF, ASF, SDF,	UC/DC	2	0.47	0.34	1.79

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Species	Feeding mode	Sediment reworking functional type	Mobility	Low fishing frequency	High fishing frequency	Contr. Diss. (%)
	SubDF					
Aricidea sp.	[2]SDF, SubDF	SM	3	0.58	0.10	1.75
Spiophanes bombyx	[1]PSF, ASF, SDF, SubDF	UC/DC	1	0.61	0.10	1.70
Corystes crassivelanus	[1]Pred, Scav	R	4	0.40	0.60	1.67
Venus casina	[2]ASF, PSF	SM	2	0.44	0.30	1.62
Ophiuroid juvenile	-	SM	2	0.54	0.10	1.61
Scalibregma inflatum	[1]SDF, SubDF	B	4	0.14	0.50	1.58
Abra alba	[1]PSF, ASF, SDF, SubDF	SM	2	0.00	0.56	1.57
Nephtys caeca	[1]Pred, Scav	B	3	0.44	0.00	1.53
Goniada sp.	[1]Pred, Scav	B	3	0.34	0.24	1.51
Ensis juvenile	[1]ASF	SM	2	0.34	0.20	1.22
Sthenelais sp.	[2]Pred, Scav	B	3	0.00	0.38	1.20
Nephtys sp.	[1]Pred, Scav	B	3	0.10	0.34	1.15
Spio sp.	[1]SDF, SubDF	UC/DC	2	0.30	0.20	1.14
Terebellidae	-	UC/DC	1	0.20	0.24	1.09
Syllidae	-	B	3	0.00	0.30	1.05
Abra prismatica	[1]PSF, ASF, SDF, SubDF	SM	2	0.24	0.00	0.79
Gattyana cirrhosa	[1]Pred, Scav	B	3	0.10	0.20	0.76
Nematoda	-	SM	2	0.00	0.24	0.75
Scolelepis squamata	[2]SDF	UC/DC	2	0.24	0.00	0.70
Scoloplos armiger	[2]SubDF	B	3	0.24	0.00	0.70
Sabellidae	-	SM	1	0.10	0.20	0.64
Bathyporeia gracilis	[2]SDF, SubDF	SM	3	0.20	0.00	0.57
Orbinia sp.	[2]SubDF	B	3	0.10	0.10	0.57
Pagurus sp.	[1]SDF, Pred, ASF	E	4	0.00	0.20	0.53
Cerebratulus sp.	[2]Pred, Scav	B	3	0.20	0.00	0.52

b. Groups tested: Taxon biomass between low and high fishing activity in sandy sediment

Species	Feeding mode	[3]Sediment reworking functional type	[3]Mobility	Low fishing frequency	High fishing frequency	Contr. Diss. (%)
Echinocardium	[1]SDF,	B	3	0.28	1.06	19.76

cordatum	SubDF					
Corystes crassivelanus	[1]Pred, Scav	R	4	0.30	0.29	11.17
Thracia phaseolina	[1]PSF, ASF, SDF, SubDF	UC/DC	2	0.05	0.25	6.19
Lagis koreni	[1]SubDF	UC/DC	1	0.23	0.12	6.06
Acanthocardia echinata	[1]PSF, ASF	SM	2	0.00	0.24	4.94
Chamelea striatula	[1]PSF, ASF	SM	2	0.00	0.17	4.41
Nephtys incisa	[1]SDF, SubDF	B	3	0.09	0.03	3.06
Glycera oxycephala	[1]Pred, Scav	B	3	0.11	0.01	2.94
Phoronis sp.	[1]PSF, ASF	SM	1	0.01	0.1	2.56
Sigalion mathilde	[2]Pred, Scav	B	3	0.00	0.06	2.13
Lumbrineris sp.	[1]Pred, Scav	B	3	0.00	0.07	1.96
Ophelina acuminata	[2]SubDF	B	3	0.01	0.06	1.92
Owenia fusiformis	[1]PSF, ASF, SDF, SubDF	SM	1	0.01	0.07	1.91
Sthenelais limicola	[2]Pred, Scav	B	3	0.01	0.05	1.88
Venus casina	[2]ASF, PSF	SM	2	0.03	0.07	1.85
Scolelepis squamata	[2]SDF	UC/DC	2	0.06	0.00	1.38
Pagurus sp.	[1]SDF, Pred, ASF	E	4	0.00	0.04	1.36
Abra alba	[1]PSF, ASF, SDF, SubDF	SM	2	0.00	0.07	1.26
Abra prismatica	[1]PSF, ASF, SDF, SubDF	SM	2	0.04	0.00	1.26
Asteroid juvenile	-	E	3	0.04	0.00	1.22
Cerianthus sp.	[1]PSF, Pred	SM	1	0.00	0.05	1.18
Nucula hanleyi	[1]SubDF, Det	SM	3	0.00	0.05	1.01
Dosinia lupinus	[2]ASF, PSF	SM	2	0.03	0.00	0.95
Glycera alba	[1]Pred, Scav	B	3	0.02	0.01	0.88
Travisia forbesi	[1]SDF, SubDF	B	3	0.03	0.00	0.87
Ensis juvenile	[1]ASF	SM	2	0.00	0.03	0.82
Scalibregma inflatum	[1]SDF, SubDF	B	4	0.01	0.02	0.80
Gattyana cirrhosa	[1]Pred, Scav	B	3	0.02	0.02	0.77

| *Sthenelais* sp. | [2]Pred, Scav | B | 3 | 0.00 | 0.05 | 0.77 |

Data sources

[1] MarLIN, 2006. *BIOTIC - Biological Traits Information Catalogue.* Marine Life Information Network. Plymouth: Marine Biological Association of the United Kingdom. [03/07/2017] Available from <www.marlin.ac.uk/biotic>

[2] Biological traits database developed under the BENTHIS (Benthic Ecosystem Fisheries Impact Studies) project. [16/07/2016]. http://www.benthis.eu/en/benthis/Results.htm

[3] Queiros AM, Birchenough SNR, Bremner J, et al (2013) A bioturbation classification of European marine infaunal invertebrates. Ecol Evol 3: 3958-3985

732 **Figure 1.** The (a) independent effect of fishing frequency on the ratio of suspension:deposit feeders

733 (mean ± SE) and (b) a non-metric multidimensional scaling (nMDS) ordination of square-root

734 transformed Bray-Curtis resemblance matrix of macro-invertebrate density for communities in

735 sand. In (b) contrasting levels of bottom fishing frequency (open symbol low, closed symbol high)

736 and organic matter enrichment (circle non-enriched, square enriched) are presented, and the MDS

737 dimensionality representation stress value is indicated.

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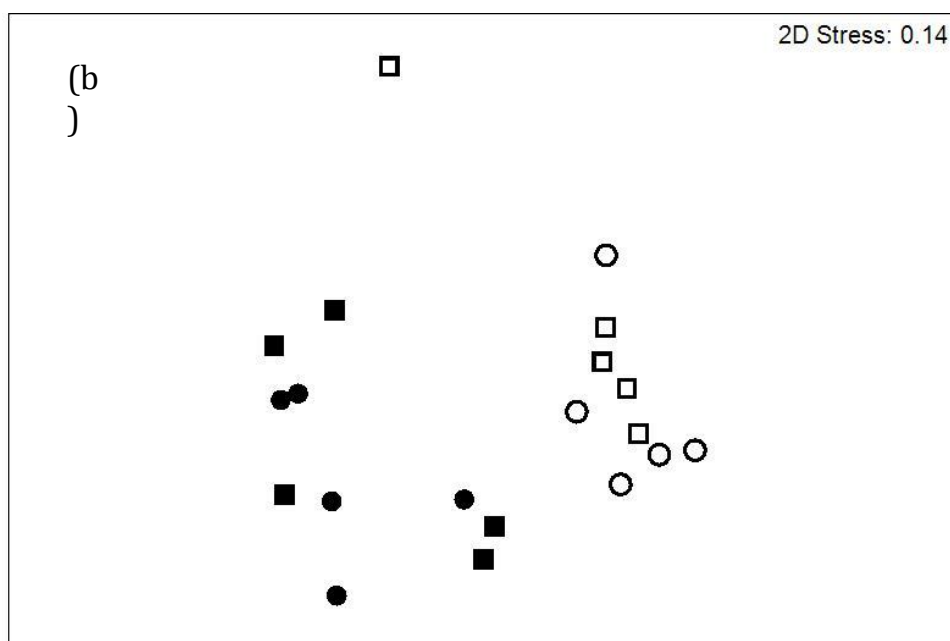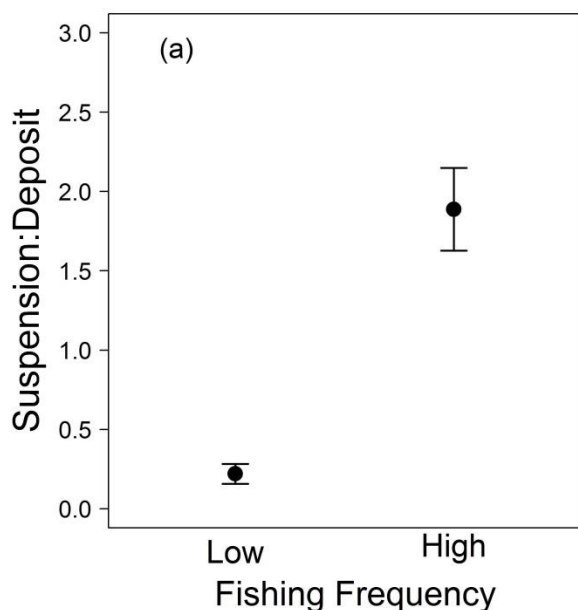
Figure 2. The (a) interactive effects of sediment reworking group identity and the frequency of fishing on sediment reworking group density, and (b-c)

746   the independent effects of organic matter enrichment and sediment reworking group identity on sediment reworking group biomass in sandy sediments

747   (mean ± SE). In (a) contrasting levels of bottom fishing frequency (open symbol low, closed symbol high) are presented. In (a) and (c) sediment

748   reworking groups include epifauna (E), surficial modifiers (SM), conveyors (C), biodiffusors (B) and regenerators (R).

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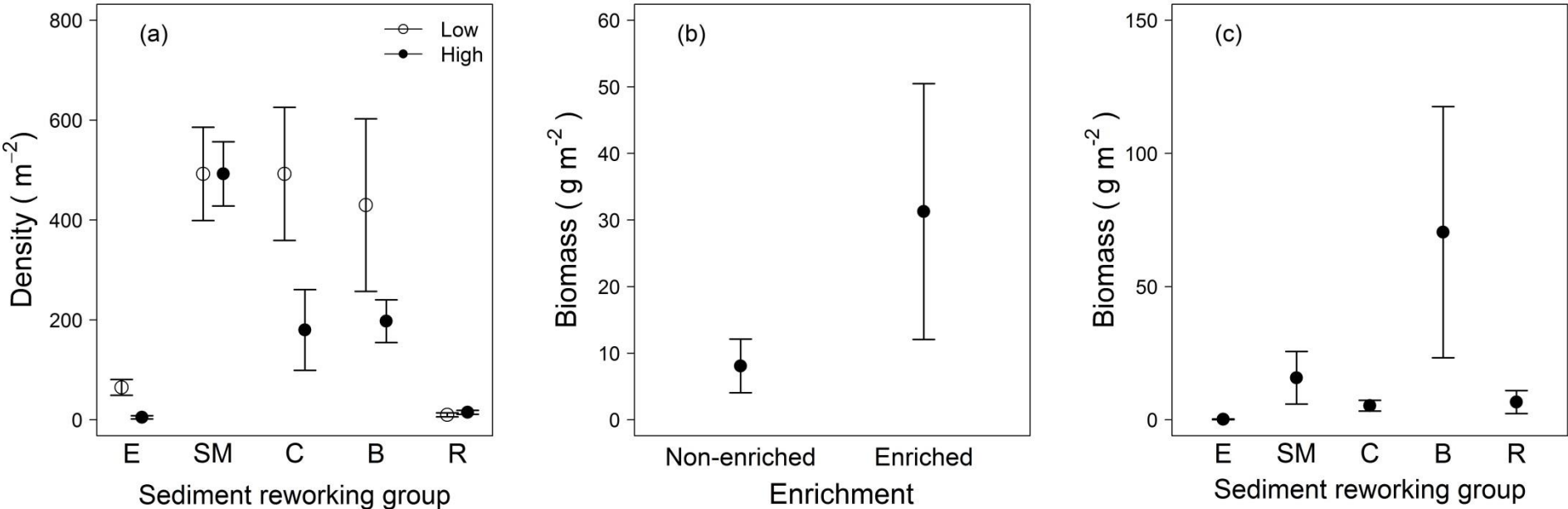
Figure 3. The independent effects of (a) fishing frequency and (b) organic matter enrichment on

abundance of the metabolically active bacterial denitrifier (*nirS*) and the independent effect of

fishing frequency on (c) archaeal (A*nirKa*) denitrifiers and (d) anammox (*hzo*) hydrazine

oxidoreductase transcripts in sandy sediments. Values plotted are mean ± SE.
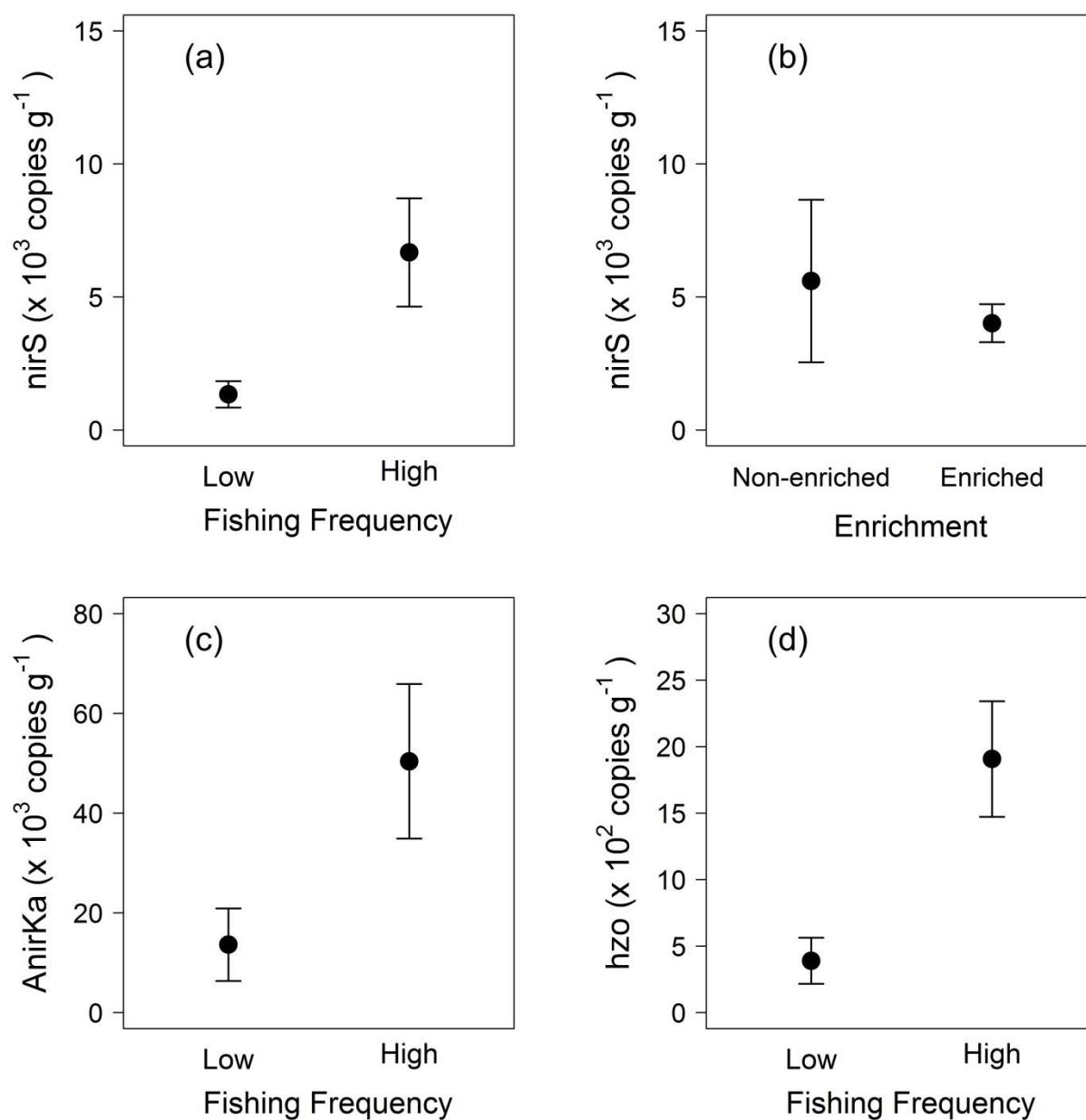
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760 **Figure 4.** The independent effect of fishing frequency on (a) [NO2-N] and (b) [NO3-N] in sandy

761 sediments. Values plotted are mean ± SE.

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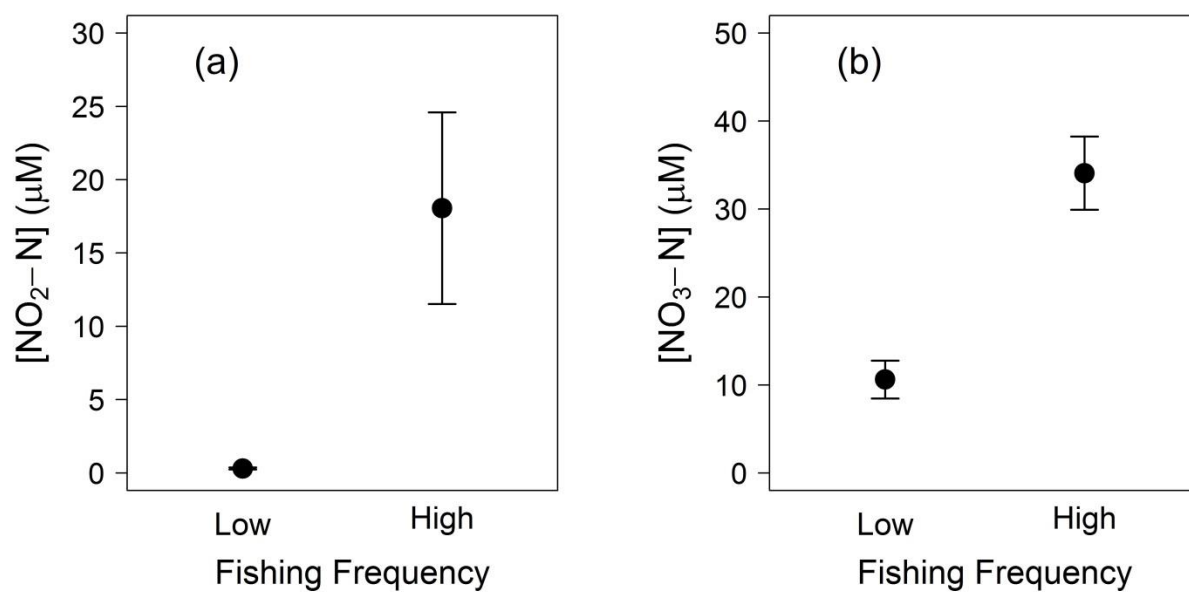
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765 **Figure 5.** The independent effects of fishing frequency and organic matter enrichment on (a,b) total macrofaunal density, (c,d) species richness and

766 (e,f) sediment reworking group density. Sediment reworking group density (g) and biomass (h) were dependent on sediment reworking group identity

767 (SM: surficial modifiers, C: conveyors, B: biodiffusors). There were no species for sediment reworking groups E (epifauna) and R (regenerators) in

768 sandy mud. Values plotted are mean ± SE.

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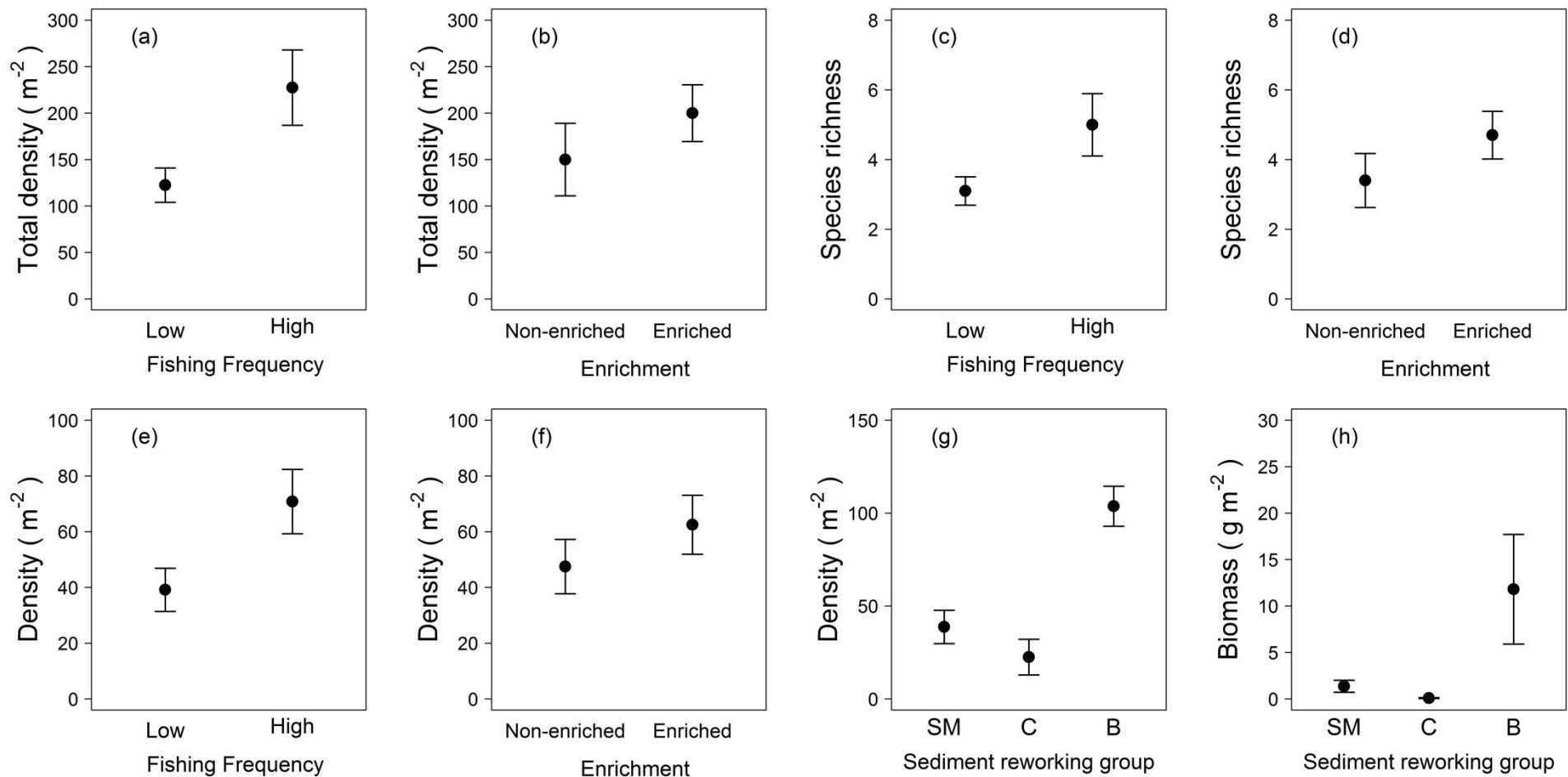
771 **Figure 6.** Non-metric multidimensional scaling (nMDS) ordination of square-root transformed
772 Bray-Curtis resemblance matrix of macro-invertebrate density for communities in sandy mud that
773 experienced contrasting levels of bottom fishing frequency (open symbol low, closed symbol high)
774 and of organic enrichment (circle non-enriched, square enriched). MDS dimensionality
775 representation stress value = 0.1.

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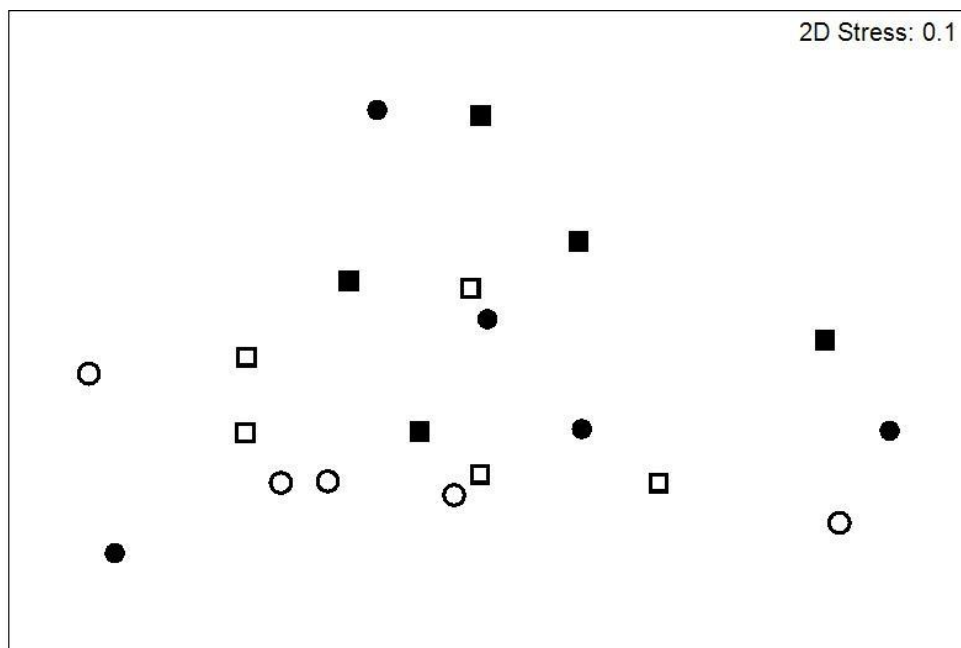
Figure 7. The independent effects of (a) fishing frequency and (b) organic matter enrichment on archaeal (*AnirKa*) denitrifiers, and of (c) organic

matter enrichment on the ratio of bacterial to archaeal ammonia oxidisers (AOB : AOA *amoA*). Values plotted are mean ± SE.

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