

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

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21 Abstract

Microbes and benthic macro-invertebrates interact in sediments to play a major role in the 22 23 biogeochemical cycling of organic matter, but the extent to which their contributions are modified 24 following natural and anthropogenic changes has received little attention. Here, we investigate how 25 nitrogen transformations, ascertained from changes in archaeal and bacterial N-cycling microbes 26 and water macronutrient concentrations ([NH₄-N], [NO₂-N], [NO₃-N]), in sand and sandy mud 27 sediments differ when macrofaunal communities that have previously experienced contrasting 28 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 29 30 capacity of the macrofauna to mediate microbial nitrogen cycling in sand, but not in sandy mud 31 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 32 33 an increase in archaeal and bacterial denitrifier (AnirKa, nirS) and anammox (hzo) transcripts in 34 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 35 36 transformation in shelf sandy sediments is dependent on the stimulation of specific nitrogen cycling 37 pathways that are associated with differences in the composition and context-dependent expression 38 of the functional traits that belong to the resident bioturbating macrofauna community.

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40

41 Keywords: ammonia-oxidisers, bioturbation, bottom fishing, denitrification, ecosystem

42 functioning, microbial-invertebrate interactions, nitrogen cycling.

44 Introduction

45

Marine soft-sediments cover almost 70% of the earth's surface and play a fundamental role in the 46 47 remineralization of organic carbon and nutrient cycling (Olsgard et al. 2008). According to current 48 nitrogen budgets, it is estimated that up to 80% of the nitrogen needed by primary producers in 49 shallow shelf seas is provided by benthic remineralization processes, primarily driven by microbial 50 organisms that occur across the oxic/anoxic interface near the sediment surface (Dale & Prego 51 2002; Zehr & Kudela 2011). With 23% of the global human population concentrated in coastal 52 areas, at an average density nearly 3 times higher than the global average (Small & Nicholls 2003) 53 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 54 55 eutrophication following excessive nutrient input. Any change in the balance and distribution of 56 reactive nitrogen (e.g. ammonium NH_4^+ , nitrite NO_2^- and nitrate NO_3^-), oxygen, and organic 57 substrates are likely to have profound consequences for nitrification, denitrification and anaerobic 58 ammonium oxidation (anammox) that regulate coastal nitrogen budgets (Laverock et al. 2011).

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60 Bottom fishing that uses demersal gear such as trawls and dredges to catch fish, crustaceans and 61 bivalves living in, on or in association with the seabed, exerts a number of pressures on benthic 62 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 63 64 by trawling alter grain size distribution, sediment sorting and porosity (Trimmer et al. 2005) that 65 may in turn disrupt nitrification and denitrification processes (Rysgaard et al. 1994; Kitidis et al. 66 2017) through changes in oxygen penetration depth within the sediment (Warnken et al. 2003) and 67 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 68 69 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, macrofaunal communities can take up to 4-5 years to recover (Kaiser et al. 2006; Lambert et al. 75 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 invertebrate community composition and microbial activity suggests that a reduction in bioturbation 85 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

Fluctuations in organic matter input following events such as algal blooms or run-off associated
with high intensity precipitation, also have a prominent influence on benthic invertebrate fauna (van
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 subsequent increases in NH₄⁺ sediment flux, oxygen consumption and denitrification (Caffrey et al. 103 104 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 NH₄⁺ 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

least in part on the structure and composition of the post-disturbance macro-invertebrate
community, as the active redistribution of particles and fluids by the macrofauna disproportionately
influences benthic fluxes and total benthic metabolism (Mermillod-Blondin et al. 2004; MermillodBlondin & 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) communities, we collected and maintained intact sediment cores (n = 40, 5 cores per treatment: 133 LWH, $20 \times 20 \times 12$ cm) with their associated fauna using a 0.1 m² NIOZ (Netherlands Institute for 134 135 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 each site by calculating the number of times the site is swept by bottom fishing gear in a year (km²) 146 swept km⁻² seabed yr⁻¹) using Vessel Monitoring System (VMS) records for UK registered vessel >147

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 content were determined for a separate sediment sample ($\phi = 5$ cm, 5 cm deep) taken from each 159 160 NIOZ core sample collected on-site. A combination of dry sieving $(1 - 9.5 \text{ mm at } 0.25 \phi \text{ intervals})$ 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, approximating mean sea bottom temperature during the sampling period, 22-28th June 2015) for 1 167 168 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 (concentration of ~ 22 cells μL^{-1} : based on field observations of chlorophyll-*a* levels at ~ 10 m 171 172 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

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 μ m, 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 ([NH₄-N]), nitrite ([NO₂-N]) and nitrate ([NO₃-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 oxidoreductase, hzo that converts NO₂⁻ and NH₄⁺ into N₂) were analysed *via* quantitative PCR 192 193 (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.

All invertebrates were recovered (500 µm sieve), fixed and preserved in 4 % formaldehyde solution for subsequent identification to the highest practicable taxonomic resolution (mostly species) and the abundance and wet weight of each taxon was measured after blotting. Tube worms were weighed excluding tubes. The values of total biomass include fragments of organisms that could not 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

Linear regression models (full factorial, independent nominal variables: fishing frequency F, organic matter enrichment E) were fitted for the response variables for the invertebrate community (total invertebrate density, biomass and species richness, the ratio of suspension to deposit feeders), microbial community (abundance of bacterial and archaeal nitrifiers (AOB *amoA*, AOA *amoA*), denitrifiers (A*nirKa*, *nirS*), and anammox (*hzo*)) and associated concentrations of dissolved inorganic nitrogen ([NH₄-N], [NO₂-N] and [NO₃-N]). The ratio of suspension to deposit feeders was examined as an indicator of compositional and functional change, as high levels of suspension

225 feeder mortality (relative to deposit feeders) in fished areas have been shown to reduce the benthic 226 oxygen demand and result in higher rates of nitrification (Allen & Clarke 2007). Information on 227 species feeding mode was obtained from the biological traits database generated from the 228 BENTHIS project (Bolam et al. 2014, http://www.benthis.eu/en/benthis/Results.htm, accessed 16 229 July 2016). Further, to assess whether the sediment reworking potential of the macro-invertebrate 230 community differed among treatments, species were classified as epifauna (E), surficial modifiers 231 (SM), biodiffusors (B), upwards/downwards conveyors (C) and regenerator (R) following Solan et 232 al. (2004) and updated by Queiros et al. (2013). Epifaunal organisms include species that occur 233 predominantly above the sediment-water interface whose activities are limited to the near-surface 234 sediment. Surficial modifiers are organisms whose activities are mostly restricted to the uppermost 235 few centimetres of the sediment, rarely venturing above the sediment-water interface. Biodiffusors 236 include organisms with activities that usually result in a constant and random local sediment 237 biomixing over short distances (ca. 5 cm). Conveyors include burrow-building species that are 238 vertically oriented in the sediment typically feeding head-down (upward conveyors) or head-up 239 (downward conveyors) at depth in the sediment. Regenerators are excavators that dig and 240 continuously maintain burrows in the sediment and by doing so they mechanically transfer sediment 241 from depth to the surface (Solan et al. 2004; Kristensen et al. 2012). Density and biomass was 242 summed to obtain the total of each reworking mode. A linear regression model incorporating the 243 independent terms mode of sediment reworking (R_i), fishing frequency (F), organic matter 244 enrichment (E), and their interactions, was fitted for total density and total biomass. A significant 245 interaction term ($F : R_i, E : R_i$ or $F : E : R_i$) would indicate that changes in total density or total 246 biomass reflect differences in response across bioturbation groups that depend on fishing frequency 247 and/or organic matter enrichment.

248

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 \pm standard error.

263

Differences in macrofaunal and microbial community composition associated with different fishing frequency and organic matter enrichment treatments were examined using PERMANOVA (Permutational analysis of variance). The relative contribution of species to significant effects was identified using SIMPER (Similarity percentages). All PERMANOVA and SIMPER analyses were 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. m^{-2} , suspension feeders = 92.5 ± 20 ind. m^{-2}), whereas suspension feeders such as *Phoronis* sp., 283 284 Owenia fusiformis and Abra alba were more abundant in communities that had previously experienced a high frequency of bottom fishing (density: deposit feeders = 262.5 ± 70 ind. m⁻², 285 suspension feeders = 357.5 ± 47.5 ind. m⁻²) (Table 3a). Compositional differences were largely 286 287 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 abundant after a low frequency of fishing (density: 492.5 ± 133.28 ind. m⁻² and 430 ± 172.92 ind. m⁻ 297 ², respectively) than they were after a high frequency of fishing (density: 180 ± 80.78 ind. m⁻² and 298 197.5 ± 42.89 ind. m⁻², respectively) (Figure 2a). Sediment reworking group biomass, however, was 299 300 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, p = 0.03), but not among enriched and non-enriched treatments (Archaea, Pseudo-F = 0.66, p = 308 309 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 (AnirKa) 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

For nutrients, we found that $[NO_2-N]$ and $[NO_3-N]$ were dependent on an independent effect of fishing frequency (Models 13–14, Table 2a, Figure 4), whilst $[NH_4-N]$ was not affected by fishing frequency or organic matter enrichment (Model 15, Table 2a).

321

322 Sandy Mud sediments

Relative to sandy sediments, total density (25 to 450 ind. m⁻²), total biomass (0.8 to 119.8 gWW m⁻ and species richness (1 to 9) were lower in sandy mud macro-invertebrate communities. Total macro-invertebrate density (Model 16, Table 2b, Figure 5a, b) and species richness (Model 17, Table 2b, Figure 5c,d) were dependent on the independent effects of fishing frequency and organic matter enrichment. Mean total density and species richness were highest for communities that had

experienced a greater frequency of fishing (density: $227.5 \pm 40.5 \text{ m}^{-2}$; species richness: 5.00 ± 0.89 328 core⁻¹) or organic matter enrichment (density: $200.00 \pm 30.51 \text{ m}^{-2}$, species richness: 4.70 ± 0.68 329 core⁻¹). In contrast, we found no evidence of any macro-faunal response in terms of biomass (Model 330 331 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 enrichment (Figure 5f) and sediment reworking group identity (Figure 5g), whilst sediment 334 335 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, p = 0.22, ESM 5) or between enriched and non-enriched treatments (density: Pseudo-F = 0.82, p = 339 340 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). Indeed, we were unable to find any evidence supporting the view that bacterial denitrifers (nirS 346 transcripts, range: 903 - 6330 copies g⁻¹ sediment), anammox (hzo transcripts, range: 3160 -347 165000 copies g⁻¹ sediment) or bacterial (AOB *amoA* transcripts, range: 1270 – 251000 copies g⁻¹ 348 349 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 (AnirKa) 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

- also found evidence that the mean ratio of bacterial to archaeal ammonia oxidisers (AOB : AOA *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 (AnirKa, nirS) and anammox (hzo) and higher levels of bottom water [NO₂-N] and [NO₃-376 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 denitrifier activity. E. cordatum is known to displace large volumes of sediment (20 000 cm³ m⁻² d⁻¹ 379

by 40 individuals m⁻², Lohrer et al. 2005) and although the shallow-burying bivalves A. echinata 380 381 and C. striatula and the tube-building polychaetes do not build extensive burrow systems deep 382 within the sediment, their active mixing of the uppermost sediment layers and their dominating 383 biomass, means that their bioturbation activities are likely to have stimulated microbial 384 denitrification and anammox, possibly by increasing the flux of [NO₂-N] and [NO₃-N] across the 385 water-sediment interface, which constitutes the substrate for nitrite reductase and anammox (Howe 386 et al. 2004; Dang et al. 2010). Measured sediment particle reworking rates using fluorescent 387 sediment profile imaging techniques at the same study sites confirm more intense particle 388 reworking activity in communities that have previously experienced a higher frequency of bottom 389 fishing, substantially extending the maximum depth of sediment reworking (low frequency fishing, 390 1.99 ± 0.19 cm; high frequency fishing, 4.64 ± 0.5 cm) and increasing the volume of sediment 391 available for nitrification (Hale et al. 2017). It is interesting to note that, although some bioturbation 392 groups - such as biodiffusors and upwards/downwards conveyors - were, on average, twice as 393 abundant at sites with a history of low frequency of fishing, the biomass of biodiffusors and 394 surficial modifiers was substantively higher (24 times and 60 times higher, respectively) at sites 395 with a history of high frequency of fishing. It appears that biomass had an overriding effect over 396 density; larger individuals with a greater per capita effect on sediment mixing have a 397 disproportionate effect on microbial activity and composition and, in turn, nutrient concentrations 398 (Osinga et al. 1995; Bird et al. 1999). The increased abundance of active suspension feeders 399 (relative to deposit feeders) is also likely to have stimulated microbial denitrification and anammox, 400 through an increase in the provision of [NO₂-N] and [NO₃-N] as water is actively moved into the 401 sediment during feeding (Howe et al. 2004; Dang et al. 2010). Although we may conclude that 402 macrofaunal bioturbation and bioirrigation activities had a stimulatory effect on microbial 403 denitrification, it remains unclear why we did not detect any change in archaeal or bacterial nitrifier 404 (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 affected by bottom fishing (Kaiser & Spencer 1996; Hiddink et al. 2006; Sciberras et al. 2013). The 411 412 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 infrequent fishing activity (1.63 times yr^{-1}), so community recruitment and growth is unlikely to be 414 415 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} , such that further increases in fishing activity (8.4 times vr^{-1}) have proportionally less effect on 417 418 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 NH₄ 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

We hypothesized that the addition of organic matter would increase microbial activity (measured here as the gene transcript abundance). Thus, the correlation between enrichment and macrofaunal density and species richness in sandy mud and the relative biomass of different functional groups in sand was unexpected (given the short time scale of the experiment) and most likely the result of 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 mean activity of archaeal denitrifiers (AnirKa), and a change in ammonia oxidiser community 434 435 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 released via the grazing activity of deposit and suspension feeders, thus explaining the lack of 442 response for some microbes in our study. The lesser importance of organic matter enrichment 443 444 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 importance of understanding the response of multiple ecosystem components over the longer term if 447 448 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).

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Table 1. Summary of environmental characteristics and bottom fishing frequency of our study sites. The mean \pm 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-

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

Table 2a. Linear regression models to examine the effects of fishing frequency and enrichment (full factorial, $F \times E$) in sand (S), for macro-687 688 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 (AnirKa, 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: [NO₂-N], [NO₃-N], [NH₄-N]). The test statistic (L-ratio or F value), 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 692 693 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.

Sediment	type: SAI	ND (S)									
Macro-inv	vertebrate	e community (Initia	l linear model: I	Respor	nse varia	ble ~ $\mathbf{F} \times \mathbf{E}$)					
Model ID	Model	Response variable	Fishing freque (F)	ency	y Enrichment (E)		Interaction (F : E)		Intercept only		Variance- covariate
1	GLS	Macro-invertebrate density							L = 2.54, c 0.11	lf = 1, p =	Е
2	GLS	Macro-invertebrate biomass							L = 3.37, 0 0.07	lf = 1, p =	$\mathbf{E} \times \mathbf{F}$
3	GLS	Species richness							L = 1.32, 0 0.25	df = 1, p =	Е
4	GLS	suspension: deposit feeders ratio	L = 17.07, df = 1 0.001	l, p <							F
Sediment	reworkin	g groups (Initial line	ear model: Resp	oonse v	variable ~	$-\mathbf{F} \times \mathbf{E} \times \mathbf{Ri}$)					
Model ID	Model	Response variable	Fishing frequency (F)	Enri	chment (E)	Reworking mode (Ri)	F : E		F : Ri	Intercept only	Variance- covariate
5	GLS	Ri density						L = 1 4, p =	5.92, df = = 0.003		Ri×F
6	GLS	Ri biomass		L = 4. 1, p =	09, df = 0.04	L = 27.05, df = 4, p <					$Ri \times F$

				0.0001			
Abundan	ce of activ	e N-cycling asso	ociated microbes (Initial lin	ear model: Response	variable ~ $\mathbf{F} \times \mathbf{E}$)		
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				$\mathbf{E} \times \mathbf{F}$
9	GLS	hzo	L = 6.59, df = 1, p = 0.01				F
10	GLS	AOA amoA				L = 1.25, df = 1, p = 0.26	Е
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	Е
Water nu	trient con	centration (Init	ial linear model: Response	variable ~ $\mathbf{F} \times \mathbf{E}$)			
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				$F \times E$
15	GLS	[NH ₄ -N]				$L = \overline{1.12}, df = 1, p$ = 0.29	$E \times F$

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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, AnirKa), 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: [NO₂-N], [NO₃-N], [NH₄-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.

Sedimen	t type: sa	ndy Mud (sM)									
Macro-in	vertebrat	e community (Initia	al linear model:]	Response	varia	ble ~ $\mathbf{F} \times \mathbf{E}$)					
Model ID	Model	Response variable	Fishing freque	ency (F)	En	richment (E)	Interaction E)	(F :	Interce	pt only	Variance- covariate
16	GLS	Macro-invertebrate density	L = 5.27, df = 1,	p = 0.02	$= 0.02 \qquad \begin{array}{c} L = 4.31, df = 1, p = \\ 0.04 \end{array}$						F
17	GLS	Species richness	L = 3.83, df = 1,	p = 0.05	L = 7 0.005	.75, df = 1, p =					F
18	GLS	Macro-invertebrate biomass							L = 1.29 0.26	, df = 1, p =	$\mathbf{F} \times \mathbf{E}$
19	LM	suspension: deposit feeders ratio							F = 12.3 = 0.77	3, df = 16, p	-
Sediment	reworkin	g groups (Initial lin	near model: Resp	ponse var	iable -	$-\mathbf{F} \times \mathbf{E} \times \mathbf{Ri}$)			·		
Model ID	Model	Response variable	Fishing frequency (F)	Enricht (E)	ment	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 1, p = 0.0	, df = 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 × Ri

Abundan	ce of activ	e N-cycling asso	ociated microbes (Initial line	ear model: Response va	ariable ~ $\mathbf{F} \times \mathbf{E}$)		
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 nut	trient con	centration (Initi	al linear model: Response v	variable ~ $\mathbf{F} \times \mathbf{E}$)			
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	$\mathbf{F} \times \mathbf{E}$
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	$F \times E$

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 fishing frequency (Levels: low and high fishing frequency). Information on species feeding mode/s 713 714 (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.

a. Groups tested:	Taxon densit	y between low	and high fi	shing activity	in sandy sedin	nent
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	В	3	2.57	0.85	6.93
Asteroid juvenile	-	Е	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	В	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	В	3	0.10	0.71	2.28
Ophelina acuminata	² SubDF	В	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

Echinocardium	¹ SDF,	B	3	0.28	1.06	19.76
Species	Feeding mode	³ Sediment reworking functional type	³ Mobility	Low fishing frequency	High fishing frequency	Contr. Diss. (%)
b. Groups tested:	Taxon bioma	ass between low	v and high f	ishing activity	y in sandy sedin	ment
Cerebratulus sp.	² Pred, Scav	В	3	0.20	0.00	0.52
Pagurus sp.	¹ SDF, Pred, ASF	Е	4	0.00	0.20	0.53
<i>Orbinia</i> sp.	² SubDF	В	3	0.10	0.10	0.57
Bathyporeia gracilis	² SDF, SubDF	SM	3	0.20	0.00	0.57
Sabellidae	-	SM	1	0.10	0.20	0.64
Scoloplos armiger	² SubDF	В	3	0.24	0.00	0.70
Scolelepis squamata	² SDF	UC/DC	2	0.24	0.00	0.70
Nematoda	-	SM	2	0.00	0.24	0.75
Gattyana cirrhosa	¹ Pred, Scav	В	3	0.10	0.20	0.76
Abra prismatica	¹ PSF, ASF, SDF, SubDF	SM	2	0.24	0.00	0.79
Syllidae	-	В	3	0.00	0.30	1.05
Terebellidae	-	UC/DC	1	0.20	0.24	1.09
<i>Spio</i> sp.	¹ SDF, SubDF	UC/DC	2	0.30	0.20	1.14
Nephtys sp.	¹ Pred, Scav	В	3	0.10	0.34	1.15
<i>Sthenelais</i> sp.	² Pred, Scav	В	3	0.00	0.38	1.20
<i>Ensis</i> juvenile	¹ ASF	SM	2	0.34	0.20	1.22
Goniada sp.	¹ Pred, Scav	В	3	0.34	0.24	1.51
Nephtys caeca	¹ Pred, Scav	В	3	0.44	0.00	1.53
Abra alba	¹ PSF, ASF, SDF,	SM	2	0.00	0.56	1.57
Scalibregma	¹ SDF, SubDE	В	4	0.14	0.50	1.58
Ophiuroid juvenile	-	SM	2	0.54	0.10	1.61
Venus casina	² ASF, PSF	SM	2	0.44	0.30	1.62
Corystes crassivelanus	¹ Pred, Scav	R	4	0.40	0.60	1.67
Spiophanes bombyx	¹ PSF, ASF, SDF, SubDF	UC/DC	1	0.61	0.10	1.70
Aricidea sp.	² SDF,	SM	3	0.58	0.10	1.75
	SubDF					

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	В	3	0.09	0.03	3.06
Glycera oxycephala	¹ Pred, Scav	В	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	В	3	0.00	0.06	2.13
Lumbrineris sp.	¹ Pred, Scav	В	3	0.00	0.07	1.96
Ophelina acuminata	² SubDF	В	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	В	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	Е	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	-	Е	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	В	3	0.02	0.01	0.88
Travisia forbesi	¹ SDF, SubDF	В	3	0.03	0.00	0.87
Ensis juvenile	¹ ASF	SM	2	0.00	0.03	0.82
Scalibregma inflatum	¹ SDF, SubDF	В	4	0.01	0.02	0.80
Gattyana cirrhosa	¹ Pred, Scav	В	3	0.02	0.02	0.77

Sthenelais sp. 2 Pred, ScavB30.000.050.7'
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- 723 Data sources
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- ^{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
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Figure 1. The (a) independent effect of fishing frequency on the ratio of suspension:deposit feeders (mean \pm SE) and (b) a non-metric multidimensional scaling (nMDS) ordination of square-root transformed Bray-Curtis resemblance matrix of macro-invertebrate density for communities in sand. In (b) contrasting levels of bottom fishing frequency (open symbol low, closed symbol high) and organic matter enrichment (circle non-enriched, square enriched) are presented, and the MDS 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) the independent effects of organic matter enrichment and sediment reworking group identity on sediment reworking group biomass in sandy sediments (mean \pm SE). In (a) contrasting levels of bottom fishing frequency (open symbol low, closed symbol high) are presented. In (a) and (c) sediment 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 (AnirKa) denitrifiers and (d) anammox (hzo) hydrazine
- oxidoreductase transcripts in sandy sediments. Values plotted are mean \pm SE.



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



Figure 5. The independent effects of fishing frequency and organic matter enrichment on (a,b) total macrofaunal density, (c,d) species richness and (e,f) sediment reworking group density. Sediment reworking group density (g) and biomass (h) were dependent on sediment reworking group identity (SM: surficial modifiers, C: conveyors, B: biodiffusors). There were no species for sediment reworking groups E (epifauna) and R (regenerators) in sandy mud. Values plotted are mean \pm SE.

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

