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Relationship between structure of macrobenthic assemblages and

environmental variables in shallow sublittoral soft bottoms

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18 **ABSTRACT**

19 We establish baseline knowledge of abundance, diversity and multivariate structure of

20 macrobenthos from shallow sublitoral soft bottoms in the North Portuguese coast and

21 elucidate main environmental factors that shape their spatial patterns. In this area

22 distribution of soft bottoms is patchy, surrounded by boulders and rocky substrates. This

23 particular landscape and the lack of significant antropogenic disturbances are values for

24 the conservation of this habitat. Sediment and physicochemical properties of the water

25 column were studied to provide models for each studied macrobenthic variable. Our

26 models highlighted that most of variation (59%-72%) in macrobenthic spatial patterns

27 was explained by the studied environmental variables. Sedimentary variables were more

28 relevant that those of the water column. Therefore, disturbances affecting sedimentary

29 environment could cause dramatic changes in macrobenthic assemblages because of the

30 limited availability of soft bottoms in the area. In this way, results are useful to adopt

31 right management and conservation strategies.

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37   **1. Introduction**

38   Coastal ecosystems provide valuable goods and services to humans but anthropogenic

39   use has also altered the oceans through direct and indirect means (Halpern et al., 2008).

40   Particularly, in recent decades, worldwide marine ecosystems are suffering the

41   synergistic effects of multiple stressors derived from anthropogenic activities such as

42   overfishing, invasive species or pollution (Claudet and Fraschetti, 2010). These

43   stressors act as major drivers of ecosystems altering the structure and functioning of

44   their assemblages with consequences to human well-being (Worm et al., 2006). In this

45   scenario, there is an imperative need for adopting management and conservation

46   strategies in marine systems that will be crucial for the sustainable use of resources

47   (Desroy et al., 2002; Claudet and Fraschetti, 2010). However, the major constrains to

48   implement conservation strategies in marine ecosystems are the general lack of baseline

49   data prior to impacts and substantial gaps in the current knowledge of natural patterns of

50   variability of their assemblages, which are intrinsically variable (Claudet and Fraschetti,

51   2010; Schückel et al., 2015).

52   Soft bottom macrobenthos plays an important role in marine ecosystem processes such

53   as nutrient cycling, pollutant metabolism or secondary production (Snelgrove, 1998;

54   Pratt et al., 2014). Most of macrobenthic species display a sedentary lifestyle,

55   intermediate trophic level positions, relatively long life-span and varying responses to

56   changes in environmental stress that make macrobenthos an effective and useful

57   indicator for the assessment of coastal system quality (Dauvin, 2007). Over the past few

58   decades, macrobenthos has been a key element of many monitoring programmes; in this

59   way, upgrading our knowledge about its biodiversity is useful, particularly in marine

60   soft-bottoms (Ellingsen, 2002; Veiga et al., 2016). Although soft-bottoms are the largest

61   ecosystem on Earth in terms of area coverage, only a small percentage of their

62 macrobenthos has been studied and most of its species are still undescribed (Snelgrove,

63 1998). Within soft-bottom ecosystems, sublittoral macrobenthic assemblages have been

64 less studied that those from the intertidal and remaining largely unknown (Desroy et al.,

65 2002; Schückel et al., 2015). Spatial distribution of these assemblages is heterogeneous

66 (Mann and Lazier, 2006) and sediment features (e.g. grain size, organic matter content

67 and food availability) have been identified as responsible for spatial patterns of

68 macrobenthos (Ellingsen 2002; Van Hoey et al., 2004; Hily et al., 2008; Ramey and

69 Bodnar, 2008). Moreover, at greater spatial scales, physicochemical characteristics of

70 the water column and hydrodynamics, seem to control directly or indirectly abundance

71 and distribution of macrobenthos by influencing food availability, bottom-water

72 oxygenation and larval dispersion (Dauvin et al., 2004; Blanchet et al., 2005; Schückel

73 et al., 2015)

74 Spatial models such as multiple regression or canonical correspondence analyses have

75 revealed that the percentage of the variation in assemblage structure from soft-bottom

76 habitats explained by environmental factors is very variable (i.e. between 10% and 90

77 %) (e.g. Veiga et al., 2009; Olsson et al., 2013; Schückel et al., 2015). These previous

78 studies were done in subtidal areas where soft bottoms are the dominant habitat and

79 cover wide extensions. However, in some regions like north Portuguese coast, soft

80 bottoms at shallow sublittoral are restricted to patches surrounded by large extensions of

81 boulders and rocky substrates, the latter being the predominant habitat (Rodríguez et al.,

82 2011). Moreover, on the one hand, the north Portuguese coast is still an area

83 characterized by relatively low levels of anthropogenic pressure, deserving attention for

84 its conservation. Previous investigations showed that concentrations of nutrients, PAHs

85 and trace metals were near background values (e.g. Reis, 2012; Reis et al.,2014; Rubal

86 et al., 2014), indicating that North Portuguese coast is not subjected to severe

87 eutrophication or pollution by industrialization and urbanization of the surrounding

88 areas. On the other hand, benthic studies done in this area have been focused on

89 intertidal assemblages from rocky shores (e.g. Araújo et al., 2006; Rubal et al., 2011;

90 Veiga et al., 2013) and soft bottoms (Veiga et al., 2014). However, there is a gap in

91 knowledge about the structure of assemblages from subtidal soft-bottoms. As proof of

92 this, new species of macro- and meiobenthos have been recently described from shallow

93 subtidal sediments of the North Portuguese coast (Esquete et al., 2015; Rubal et al., In

94 press), indicating that this system may be also of high value for conservation.

95 The study of spatial patterns in macrobenthic assemblages in this area will let us

96 establish baseline knowledge, mandatory to detect future potential changes in species

97 distribution and helpful for monitoring and management issues(Desroy et al., 2002;

98 Claudet and Fraschetti, 2010; Dutertre et al., 2013; Schückel et al., 2015). Moreover,

99 elucidating main natural environmental factors that shape spatial patterns of

100 macrobenthic assemblages from subtidal soft-bottom will help to discriminate between

101 natural and anthropogenic changes (Glockzin and Zettler, 2008; Dutertre et al., 2013).

102 Therefore, the aims of this study were to determine the natural environmental variables

103 that shape the structure of macrobenthic assemblages in shallow sublittoral soft bottoms

104 in the North Portuguese coast and providing baseline information for assessing the

105 quality of this system in the future, which will be crucial for adopting right management

106 and conservation strategies. To achieve these aims first, spatial patterns of sediment

107 features, physicochemical properties of the water column and macrobenthic

108 assemblages were described. Then, the relationship between spatial distribution patterns

109 of macrobenthos and those of environmental factors were investigated using

110 multivariate statistical approaches. This will allow identifying useful predictor variables

111   and generating simple models to explain natural spatial variability in macrobenthic

112   assemblages.

113

114   **2. Material and methods**

115   *2.1 Study area*

116   The study was carried out on shallow subtidal soft bottoms in the North of Portugal,

117   encompassing over 22 km of coast between 41°51′10.01″N; 8°52′54.00″W and

118   41°39′39.72″N; 8°50′24.42″W (Table 1 and Figure S1). This subtidal area is

119   predominantly covered by rocky shores that constituting the 69%, whereas soft bottoms

120   are the second most abundant habitat (21%) followed by boulders (10%) (Rodríguez et

121   al., 2011). The coast in this area is north-to-south oriented, exposed to prevailing

122   northwest oceanic swell. Moreover, this coastal area is subjected to the influence of

123   river plumes, being Miño and Lima the most important rivers regarding flow and to

124   upwelling events (Lemos and Pires, 2004).

125

126   *2.2 Sampling design*

127   Sampling was conducted in May 2012 at four shallow subtidal soft bottom localities

128   (Table 1, Figure S1). A two-factor sampling design was used to assess the spatial

129   patterns of macrobenthic assemblages and their relationship with sedimentary and water

130   column environment. The largest spatial scale was that of locality, which included four

131   levels: Moledo, Âncora, Gelfa and Lima, spaced kms from one another. At each

132   locality, three sites, approximately 100s of ms apart, were randomly established within

133   each soft bottom patch. Localities and sites were selected considering the availability of

134   soft bottoms (Figure S1) based on a previous work that had characterised main habitats

135   of the study area including bathymetric and geomorphologic analyses (Rodríguez et al.,

136   2011). Sediment samples within each site were randomly collected, about 10s of ms

137   apart, using a Van Veen grab (sampling surface of 0.12 m^2) to a mean depth of 12 m

138   (between 9.5 and 15 m) (Table 1). At each site, a total of seven grabs were collected,

139   five to the study of macrobenthos and the remaining two to study the sedimentary

140   environment (i.e. grain size and organic matter). Macrobenthic samples were

141   immediately washed on board over a 0.5-mm mesh sieve. The retained macrofauna was

142   then preserved in 4% neutralised formaldehyde solution with Rose Bengal in labelled

143   plastic bags until its posterior study. Samples to sedimentary study were frozen. To

144   characterise the water column environment, three independent measures of oxygen

145   concentration, salinity and temperature were obtained at each locality by means of a

146   CTD SBE25. Moreover, three independent water column samples of 250 ml were

147   collected at each site and locality for nutrient analyses: nitrate (NO_3), phosphorus (PO_4)

148   and ammonium (NH_3) as close to the bottom as possible avoiding sediment

149   resuspension.

150

151   *2.3 Sampling processing*

152   Macrobenthos was sorted, identified to the lowest possible taxon (usually species level)

153   and counted. The organic matter content was calculated by measuring the loss of weight

154   on ignition in a furnace at 450ºC for 4 h. In order to study the sediment grain size,

155   samples were dried and then sieved. The following sedimentary fractions were

156   considered: coarse gravel (> 4 mm), fine gravel (2-4 mm), very coarse sand (1-2 mm),

157   coarse sand (0.5-1 mm), medium sand (0.25-0.5 mm), fine sand (0.125-0.25 mm), very

158   fine sand (0.063-0.125 mm) and silt/clay (< 0.063 mm). Then, the median particle size

159   (Md; Bale and Kenny, 2005) and sorting coefficient of the sediment (QDΦ; Yamanaka

160 et al., 2012) were calculated. Nutrient analyses were done directly in filtered seawater

161 samples by Molecular Absorption Spectrometry using a segmented flux autoanalyser

162 (San Plus System, Skalar). The concentrations of NO_3, PO_4 and NH_3 were determined

163 according to Skalar methods M461-318 (EPA 353.2), M155-008R (EPA 350.1) and

164 M503-555R (Standard Method 450-P I) (Skalar, 2004).

165

166 *2.4 Data analyses*

167 Data were analysed by means of univariate and multivariate techniques to test the

168 hypothesis that variables of the water column and sediment beside total number of

169 individuals (N), taxon richness (S), Shannon's diversity index (H′) and the multivariate

170 structure of macrobenthic assemblages will differ among localities.

171 Analyses of variance (ANOVA) were done to test for differences in the water column

172 variables (i.e. oxygen concentration, salinity and temperature) among localities. These

173 analyses were based on a one-way model, including locality as fixed factor with four

174 levels and three replicates. ANOVA was also used to test for differences in the nutrient

175 content (i.e. NO_3, PO_4 and NH_3), sediment variables (i.e. different sedimentary grain

176 sizes and organic matter content), N, S and H' of macrobenthos among localities. These

177 analyses were based on a two-way model, including Locality as fixed factor with four

178 levels and Site as random factor nested in Locality with three levels, considering five

179 replicates for macrobenthic data, three for nutrients and two for sedimentary variables.

180 Cochran's *C* tests were previously done to check for homogeneity of variances and,

181 when test was significant ($p < 0.05$), data were transformed to remove the heterogeneity

182 of variances. When this was not possible, untransformed data were analysed and results

183 were considered robust if significant at $p < 0.01$, to compensate for the increased

184 probability of type I error (Underwood, 1997). Whenever ANOVA showed significant

185 differences ($p < 0.05$), a post hoc Student-Newman-Keuls (SNK) test was done to

186 explore differences among all pairs of levels of the selected factor (i.e. locality).

187 A distance-based multivariate analysis of variance (PERMANOVA, Anderson 2001a),

188 according to the two-way aforementioned design, was used to test differences among

189 localities on the multivariate structure of macrobenthic assemblages. For each replicate,

190 values of abundance for each species were square-root transformed to downweight the

191 influence of numerically dominant taxa, which were used for the calculation of the

192 Bray–Curtis similarity matrix by permutation of residuals under a reduced model (999).

193 When PERMANOVA showed significant differences ($p < 0.05$), a pair-wise

194 comparison (999 permutations) was done to explore differences among all pairs of

195 levels of the selected factor. As the number of unique permutations for the pair-wise

196 comparison was lower than 30, Monte Carlo P-values were considered (Terlizzi et al.,

197 2005).

198 In order to test whether differences in the multivariate structure of assemblages between

199 localities were due to varying multivariate dispersion, the PERMDISP procedure was

200 done (Anderson, 2006). Multivariate patterns were illustrated by non-metric

201 multidimensional scaling (nMDS) ordination of replicates for each locality.

202 The SIMPER procedure (Clarke, 1993) was used to determine the percentage of

203 contribution ($\delta_i\%$) of each taxon to the Bray-Curtis dissimilarity between assemblages

204 sampled at each locality (δ_i). A taxon was considered important if its contribution to

205 total percentage dissimilarity was $\geq 3\%$. The ratio $\delta_i/SD(\delta_i)$ was used to quantify the

206 consistency of the contribution of a particular taxon to the average dissimilarity in all

207 pair-wise comparisons of samples among localities. Values ≥ 1 indicated a high degree

208 of consistency.

209 The relationship between the uni- and multivariate macrobenthic data and the

210 environmental variables of the water column and sedimentary environment was

211 analysed using nonparametric multivariate multiple regression (McArdle and Anderson,

212 2001). Environmental variables were subjected to a stepwise forward-selection

213 procedure to develop a model of the macrobenthic data (i.e. N, S, H´ and the

214 multivariate structure of assemblage). Analyses were based on Euclidean similarity

215 matrices for N, S and H´ and Bray-Curtis similarity matrix for multivariate data that

216 were square-root transformed. P-values were done using 9999 permutations of residuals

217 under the reduced model (Anderson, 2001b). All non-parametric multivariate multiple

218 regressions were done using the computer program DISTLM (Anderson, 2002).

219 Draftsman plots were done previously to check the skewness of environmental variables

220 and data were $\log(x+1)$ transformed. Some pairs of variables (i.e. temperature – oxygen

221 concentration; PO_4 – NO_2 and coarse sand – very coarse sand) showed strong

222 correlations ($r > 0.90$). As these pointed out that they are redundant variables,

223 temperature, PO_4 and coarse sand were removed from the analyses whereas oxygen

224 concentration, NO_2 and very coarse sand were maintained. Constrained ordination, a

225 distance-based redundancy analysis (dbRDA, Legendre and Anderson, 1999), was done

226 to explicitly investigate the relationship between environmental variables and

227 macrobenthic assemblages.

228 These analyses were also completed on sets of environmental variables that formed two

229 natural groups, those associated with the water column environment and the associated

230 with the sediment, to test the hypothesis that sedimentary variables will explain a

231 greater percentage of variability in spatial patterns of macrobenthos than that explained

232 by variables of the water column environment. Water and sediment sets were analysed

233 separately for their relationship with the macrobenthic data (ignoring the other set), and

234   both sets were then subjected to a stepwise forward-selection procedure to develop a

235   model of macrobenthic data.

236

237   **3. Results**

238   *3.1 Environmental variables*

239   Depth ranged from 9.5 m (Site 1 of Lima) to 15 m (Sites 1 of Âncora and Gelfa) (Table

240   1). Regarding water column environment (Table S1), results of ANOVA showed

241   significant differences among localities for oxygen concentration and temperature

242   (Table 2). Post hoc analyses indicated that oxygen concentration and temperature in

243   Gelfa were significantly higher than in the remaining localities (Fig. 1A and 1C).

244   However, salinity did not show significant differences among localities (Table 2, Fig

245   1B). Moreover, results of ANOVA indicated no significant differences among localities

246   for nutrient content (NO_3, NH_3 and PO_4) despite some variability was evident among

247   sites (Table 2; Figure 2A-C).

248   Sediments were predominantly composed by fine sand with the only exception of Miño

249   that showed coarse and medium sand (Table 1). Moreover, sediments were well sorted

250   and moderately well sorted, except on Site 3 of Âncora where they were very well

251   sorted (Table 1). Concerning sedimentary environment, results of ANOVA showed

252   significant differences among localities for organic matter and most of the sedimentary

253   grain sizes, except for coarse and fine gravel (Table 3). Post hoc analyses indicated that

254   very coarse, coarse and medium sand content in Miño were significantly higher than in

255   the remaining localities (Figure 3C-E). However, organic matter, fine, very fine sand

256   and silt/clay content were significantly lower in Miño (Figure 3F-I).

257

258   *3.2 Macrobenthic assemblages*

259   A total of 9669 individuals belonging to 65 taxa were identified throughout the study

260   (Table S2). Results of ANOVA showed significant differences among localities for N

261   (Table 4). Post hoc analyses indicated that N in Moledo and Âncora was significantly

262   higher than in the remaining localities (Figure 4A). However, results of ANOVA

263   indicated no significant differences among localities for S and H´ (Table 4, Figure 4B-

264   C). PERMANOVA analysis showed that the multivariate structure of macrobenthic

265   assemblages differed significantly among localities (Table 5). Pair-wise comparisons

266   indicated significant differences between Miño and the remaining localities (Table 5).

267   The documented multivariate pattern was visualized as a clear separation between

268   replicates from Miño and those of the remaining localities in the nMDS ordination

269   (Figure 5). Moreover, the PERMDISP analysis for locality (F = 1.58, p = 0.274)

270   indicated that the dispersion of replicates did not provide a significant contribution to

271   observed differences among localities.

272   SIMPER analysis identified 34 taxa as the main responsible for differences between

273   Miño and the remaining localities. Collectively, these taxa contributed more than 90%

274   to the total dissimilarity, although only the contribution by nine of them was ≥ 3%

275   (Table 6). The contribution to percentage of dissimilarity of *Pisione parapari* Moreira,

276   Quintas and Troncoso, 2000, *P. remota* (Southern, 1914), *Polygordius appendiculatus*

277   Fraipont, 1887, *Spio decoratus* Bobretzky, 1870, Nematodes, *Diogenes pugilator*

278   (Roux, 1829) and *Gastrosaccus spinifer* (Goës, 1864) was consistent among all the pair-

279   wise comparisons between Miño and the remaining localities (Table 6). Moreover,

280   *Eurydice* sp. and *Fabulina fabula* (Gmelin, 1791) contributed only to dissimilarity of

281   Miño with Gelfa and Lima, respectively. Noticeably, the abundance of these species

282 was larger in Miño compared to the remaining localities except for *S. decoratus* and *F.*

283 *fabula*, which reported lower abundances in Miño (Table 6).

284 *3.3 Relationship between environmental variables and macrobenthic assemblages*

285 Results of DISTLM showed that 11 environmental variables (i.e. all the studied

286 variables except fine gravel and very coarse sand) together explained 71.51% of the

287 variance in N (Table 7). The variable that explained its greatest amount of variation was

288 the fine sand content that alone explained the 56.30% followed by medium sand content

289 (3.65%) and depth (6.02%) (Table 7). However, after fitting these three variables, the p-

290 values associated with the conditional test to add silt/clay and the subsequently fitted

291 terms in the model were not statistically significant ($p > 0.05$) (Table 7). In this way,

292 based on forward selection results, the best model to explain N would be include only

293 the first three variables: fine sand, medium sand and depth that together explained the

294 65.89% of variation in N (Table 7).

295 Results of DISTLM also showed that all the studied variables, except medium sand and

296 very fine sand, together explained 60.64% of the variance in S (Table 7). The variable

297 that explained the greatest amount of variation (24.65%) was salinity, followed by

298 silt/clay content (6.65%) and depth (4.48%) (Table 7). Moreover, NO_3, NH_3 and organic

299 matter content added significantly to explain variation of S, as evidenced by the p-

300 values < 0.05 (Table 7). However, after fitting these six variables, the p-values

301 associated with the conditional test to add very coarse sand and the subsequently fitted

302 terms in the model were not statistically significant ($p > 0.05$) (Table 7). In this way,

303 based on forward selection results, the best model to explain S would be include the

304 first six variables: salinity, silt/clay, depth, NO_3, NH_3 and organic matter content that

305 together explained the 58.45% of variation in S (Table 7).

306   Results of DISTLM showed that all studied variables, except oxygen concentration,

307   salinity and coarse gravel, together explained 59% of the variability in H´ (Table 7). The

308   variable that explained the greatest amount of variation was silt/clay content that alone

309   explained the 46.46% (Table 7). However, after silt and clay content, the p-values

310   associated with the conditional test to add organic matter content and the subsequently

311   fitted terms in the model were not statistically significant ($p > 0.05$) (Table 7). In this

312   way, based on forward selection results, the best model to explain H´ would be to

313   include only silt/clay content.

314   Results of DISTLM showed that all studied variables, except fine gravel and organic

315   matter content, explained 64.41% of the variability in the multivariate structure of

316   macrobenthic assemblages (Table 7). The variable that alone explained the greatest

317   amount of variation (25.61%) was very coarse sand content followed by very fine sand

318   content (8.27%) and salinity (4.99%) (Table 7). Moreover, all the remaining variables

319   added significantly to explain variation in the multivariate structure of macrobenthic

320   assemblages, as evidenced by the p-values < 0.05 (Table 7). In this way, based on

321   forward selection results, the best model to explain the multivariate structure of

322   macrobenthic assemblages would include the 11 variables (Table 7).

323   The first two dbRDA axes explained 66% of the fitted variation, which is about 42.2%

324   of the total variation in the structure of the macrobenthic assemblages (Figure 6). All

325   dbRDA axes together explain 100% of the fitted variation and 64% of the total

326   variation.

327   The results of the multivariate analyses based on two sets of variables (sediment and

328   water column) are shown in Table 8. The set of sedimentary variables showed the

329   greatest descriptive power, which explained 70% of the variation in the macrobenthic

330   abundance and more than 54% in the diversity (S and H') and the multivariate structure

331   of macrobenthic assemblages (Table 8). Once the sedimentary variables were fitted, the

332   set of water column variables only added another 7% to the explained variation in the

333   multivariate structure of macrobenthic assemblages although this was statistically

334   significant. However, in terms of abundance and diversity, the set of water column

335   variables appeared to be redundant in the model (p > 0.05) (Table 8).

336

337   **Discussion**

338   Coastal ecosystems face strong anthropogenic pressures but are also naturally variable

339   (Crossland et al., 2005; Halpern et al., 2015). This variability makes difficult to

340   determine whether structural changes in benthic assemblages are due to natural or

341   anthropogenic disturbances, unless the latter are severe (Hardman-Mountford et al.,

342   2005; Rubal et al., 2014). In this way, studies that provide a consistent description of

343   their assemblages and associated habitats are useful in establishing a baseline for the

344   detection of ecological changes and anthropogenic impacts (Desroy et al., 2002;

345   Hardman-Mountford et al., 2005; Claudet and Fraschetti, 2010; Dutertre et al., 2013;

346   Schückel et al., 2015; Veiga et al., 2016). Particularly in the shallow subtidal, previous

347   studies have focused in analysing patterns of macrobenthic assemblages along salinity

348   or depth gradients (e.g. Bris and Glémarec, 1996; Ysebaert et al., 2003; Barros et al.,

349   2008). Moreover, many studies have focused in strongly anthropogenic areas (e.g. Lu,

350   2005; Sánchez-Moyano et al., 2010; Nishijima et al., 2015).

351   The present study explored spatial patterns of macrobenthic assemblages and main

352   features of habitat (i.e. sediment and water column) in an area characterized by

353   relatively low levels of anthropogenic pressure (Reis, 2012; Reis et al., 2014; Rubal et

354   al., 2014). Our results indicated that environmental variables varied significantly among

355   localities with the only exceptions of nutrients, salinity, temperature and sediment

356   gravel content. Moreover, the abundance and multivariate structure of macrobenthic

357   assemblages changed significantly among localities, despite these did not exhibit clear

358   differences in their depth or salinity. However, diversity measures (S and H') did not

359   differ significantly among localities. Therefore, our results partially supported the

360   hypothesis that predicted that variables of the water column and sediment beside

361   macrobenthic response variables (N and the multivariate structure of assemblages)

362   would differ among localities.

363   Identifying main environmental variables that shape spatial patterns of benthic fauna is

364   not an easy task because they differ among areas (Lu, 2005). None mechanism alone

365   has been universally capable of elucidating faunal patterns identified in different

366   environments, and at any given site, different interacting factors may be involved

367   (Snelgrove and Butman, 1994). Our results provide a good estimation of the percentage

368   of spatial variation of macrobenthos which can be explained by natural environmental

369   conditions providing models for abundance, diversity (S and H') and multivariate

370   structure of assemblages in which significant and most relevant environmental variables

371   were selected. Results of the multiple multivariate regression analyses highlighted that

372   the environmental variables here considered explained a substantial proportion of the

373   variability, between 59% for the H' and 72% for the abundance of macrobenthos.

374   Similarly, Dutertre et al. (2013) found that spatial distribution of macrobenthos along

375   the subtidal coastal fringe of South Brittany showed a relatively high correlation with

376   environmental factors, and that the combination of 16 natural abiotic variables,

377   including sediment characteristics, bathymetry and hydrodynamic conditions, explained

378   the 51% of macrobenthic distribution. This contrasts with other previous studies that

379   yielded low values of the rank correlation coefficient between macrobenthic

380 assemblages and environmental variables (Lu, 2005) or that found a lower degree of

381 variation explained by environmental variables (Olsson et al., 2013; Schückel et al.,

382 2015).

383 Our results also indicated that the identity and number of environmental variables

384 selected for each model were dependent on macrobenthic response variable. Content in

385 different sedimentary grain sizes was usually the most relevant factor, explaining the

386 highest percentage of spatial variability in N (fine sand, 56%), H' (silt/clay, 45%) and

387 the multivariate structure of macrobenthic assemblages (very coarse sand, 26%).

388 Sediment features (i.e. mud content, mean grain diameter and sorting index) were also

389 the responsible for 20% of the variation in the macrofaunal distribution along the

390 coastal fringe of South Brittany (Dutertre et al., 2013). The relationship between

391 sediment and macrobenthos has been often described in terms of the range of

392 granulometric variations tolerated by each species (Ellingsen, 2002; Van Hoey et al.,

393 2004; Hily et al., 2008). According to their lifestyle, macrobenthic species require

394 particular sediment features for instance for tube building, burrowing, or feeding (Self

395 and Jumars, 1988; Pinedo et al., 2000). Moreover, the relationship between animals and

396 sediment is quite changeable because sedimentary type can influence other variables

397 like microbial content and food supply, and trophic interactions (Snelgrove and Butman,

398 1994; Barros et al., 2008). For example, organic content tends to increase in fine

399 sediments in nearshore ecosystems, improving the food supply for many benthic species

400 (Gray and Elliot, 2009). In our case, the locality of Minho had significantly higher

401 content in very coarse, coarse and medium sand and showed as expected the lowest

402 organic matter content. Moreover, the multivariate structure of the macrobenthic

403 assemblage in Miño was significantly different to that of other localities. However, in

404 contrast to expected, Miño displayed higher abundance values. This can be explained by

405 the relatively high abundance of some typically interstitial taxa as *Pisione*, *Polygordius*

406 or Nematodes that usually live in coarse and medium sand sediments as those found in

407 Miño, which were absent or less abundant in the remaining localities. Mesh-size (0.5

408 mm) could be partially responsible for such differences, because other published works

409 rely on 1-mm mesh which usually do not retain such numbers of small-sized species as

410 those found in this paper at Miño (Lu, 2005; Glockzin and Zetter, 2008; Dutertre et al.,

411 2013). Similarly, Dutertre et al. (2013) found that homogeneous fine sediments showed

412 a relatively low value of species richness because of the absence of interstitial fauna.

413 Salinity is often regarded among the major factors affecting the species richness and

414 composition of macrobenthic assemblages (e.g. Laine, 2003; Bonsdorff, 2006; Gorgina

415 et al., 2010). Lu (2005) found that species number and abundance was negatively

416 correlated with salinity because a lower salinity values mean higher freshwater input

417 from rivers, which is, in turn, a source of nutrients that can be used as food supply.

418 Thus, freshwater input might have affected positively to macrobenthos here. Our results

419 showed that salinity was the most important factor in explaining spatial pattern of

420 macrobenthic richness and the third most relevant for the multivariate structure of

421 macrobenthic assemblages. However, it was not selected in the models of N and H'

422 because its contribution was not significant. However, in our study area, salinity was

423 not significantly different among localities contrasting with previous studies focused on

424 macrobenthos along estuarine gradients (Bris and Glémarec, 1996; Ysebaert et al.,

425 2003; Barros et al., 2008); this may explain its minor relevance in shaping macrobenthic

426 assemblages in the north Portuguese coast. Similarly, Glockzin and Zettler (2008) found

427 that salinity did not vary strongly in their study area and thus its potential value as the

428 main predictor on the benthic assemblage was insignificant.

429   Depth, through its impact on other factors such as organic content, sorting or

430   permeability of sediment, has been usually considered mainly responsible for spatial

431   patterns of macrobenthic assemblages (Bonsdorff, 2006; Zettler et al., 2006; Glockzin

432   and Zettler, 2008; Gorgina et al., 2010; Schückel et al., 2015). In our study, however,

433   depth was only the third most relevant factor for N and S, explained a low percentage of

434   variability in the assemblage multivariate structure and it was not selected for H'. This

435   lower influence of depth could be explained because in our study area depth range was

436   small (between 9.5 and 15 m).

437   When variables were individually analysed by sets (i.e. sediment and water column),

438   results showed a significant contribution of both sets although sedimentary variables

439   explained a higher percentage of variation in all the considered macrobenthic response

440   variables. However, when sets were sequentially fitted, water variables did not add a

441   significant contribution once that sedimentary set was already included in the model.

442   This points out that, at the scale of the present study, sedimentary variables seem to be

443   more relevant that those of the water column in shaping spatial patterns of

444   macrobenthos, although there is a degree of interdependence among both. Therefore,

445   these results supported our second hypothesis (i.e. sedimentary variables would explain

446   a greater percentage of variability in spatial patterns of macrobenthos than that

447   explained by variables of the water column environment). This may be explained by the

448   greater sediment heterogeneity across localities whereas the water column environment

449   was more homogenous. Dutertre et al. (2013) indicated that physical-chemical

450   properties of the water column were important to understand the broad-scale species

451   distribution in coastal ecosystems. Schückel et al. (2015) studied macrobenthic

452   assemblages in three tidal channels of the Wadden Sea, and found that differences in

453   spatial distribution of macrofaunal assemblages and species composition were best

454 explained by the variability of tidal current velocity and depth, followed by sediment

455 characteristics. Therefore, within the tidal channels (i.e. smaller spatial scale), the

456 significance of sediment characteristics became higher, in concordance with our results

457 (Schückel et al., 2015).

458 Our models highlighted that most variation in spatial patterns of macrobenthic

459 assemblages was explained by the studied environmental variables. However, some

460 variation still remains unexplained (between 21 and 48%). This suggests that other

461 abiotic or biological variables did not considered in the present study, such as food

462 supply, source of larvae or interspecies competition might also play a significant role

463 (Wildish, 1977).

464 To develop realistic conservation and management strategies to identify and ameliorate

465 anthropogenic impacts, managers need baseline ecological measurements from

466 appropriate spatial scales. The lack of these baseline data often results in poor decision-

467 making and environmental policy (Yaffee, 1997).Therefore, analysis and measurement

468 of the relationship between species and their environment is essential to build predictive

469 models, that provide a global visualisation harmonious with ecosystem management

470 (Gorgina and Zettler, 2010; Méléder et al., 2010). In this way, our study distinguished

471 and categorised different natural environmental factors responsible for shaping

472 macrobenthic assemblages from soft-bottom shallow sublittoral in the North Portuguese

473 coast and contributes to incorporate a local ecological study in a wide-ranging

474 ecosystem background. The good correlation obtained between natural environmental

475 factors and different macrobenthic response variables advocates that our models may be

476 useful to support conservation and management strategies. Moreover, results of this

477 study showed some deviations from generality (i.e. lack of relevance for salinity or

478 depth) proving that our understanding of benthic assemblages is locally specific and

479 thus, conclusions based on studies considering different habitats, spatial scales or

480 different components of the benthos cannot be extrapolated as generalities. On the one

481 hand, we establish appropriate baseline data that will be indispensable to future

482 monitoring studies that aim detecting ecological changes as those derived from

483 anthropogenic impacts or preserving the integrity of shallow subtidal areas including

484 their associated biodiversity (Desroy et al., 2002). On the other hand, our results allow

485 focusing on certain environmental factors that have proved shaping the distribution of

486 macrobenthic abundance, diversity and multivariate structure of the assemblage

487 (Gorgina and Zettler, 2010; Méléder et al., 2010). The identification of key

488 environmental factors, mainly related with sediment, is crucial for the conservation of

489 the studied habitat. Any anthropogenic activity that potentially modifies these

490 environmental factors (i.e. dredging, modification of the hydrological regimen due to

491 coastal structures or changes in fluvial discharge) could, in turn, modify intensely the

492 structure of soft bottom assemblages. The disturbance of sedimentary habitat for any of

493 the previous activities could be especially dramatic in this particular area because of the

494 habitat fragmentation (dominated by rocks) and the limited sources of sediment to

495 buffer these changes.

496

507

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728

729 **Figure caption**

730 **Fig. 1** Mean values (+SE) of oxygen concentration (A), salinity (B) and temperature (C)

731 in the water column at each site of the four studied localities. Letters indicate significant

732 differences among localities ($p < 0.05$) as detected by SNK test. ns: not significant

733 differences among localities.

734 **Fig. 2** Mean values (+SE) of NO_3: nitrate (A), PO_4: phosphorus (B) and NH_3:

735 ammonium (C) in the water column at each site of the four studied localities. ns: not

736 significant differences among localities.

737 **Fig. 3** Mean values (+SE) of sediment grain size (A-H) and organic matter content (I) in

738 percentage, at each site of the four studied localities. Letters indicate significant

739 differences among localities ($p < 0.05$) as detected by SNK test. ns: not significant

740 differences among localities.

741 **Fig. 4** Mean values (+SE) of N: total number of individuals (A), S: taxon richness (B)

742 and H': Shannon diversity index of macrobenthos at each site of the four studied

743 localities. Letters indicate significant differences among localities ($p < 0.05$) as detected

744 by SNK test. ns: not significant differences among localities.

745 **Fig. 5** nMDS ordination of macrobenthic assemblages based on square-root transformed

746 abundances and Bray-Curtis similarities in the four studied localities. Stress: 0.18.

747 **Fig. 6** Distance-based redundancy (dbRDA) plot illustrating the DISTLM model based

748 on the macrobenthic assemblages and the fitted environmental variables as vectors

749 based on DistLM analysis in Table 7 for the assemblage.

750

Table 1. Environmental data of the studied localities. Md: median particle size of sediment; QDΦ: sorting coefficient of sediment.

Locality	Site	Latitude	Longitude	Depth (m)	Md (mm)	Sediment type	QDΦ	Sorting
Miño	1	41°51′10.01″N	8°52′54.00″W	13	0.548 ± 0.068	coarse sand	0.485 ± 0.057	well sorted
	2	41°51′2.82″N	8°52′40.80″W	10	0.435 ± 0.020	medium sand	0.510 ± 0.022	moderately well sorted
	3	41°50′44.87″N	8°52′44.76″W	13.5	0.332 ± 0.018	medium sand	0.410 ± 0.058	well sorted
Âncora	1	41°48′54.72″N	8°52′54.48″W	15	0.162 ±0.001	fine sand	0.378 ± 0.009	well sorted
	2	41°48′37.80″N	8°52′48.78″W	12.5	0.164 ± 0.001	fine sand	0.371 ± 0.004	well sorted
	3	41°48′17.47″N	8°53′4.86″W	13	0.178 ± 0.001	fine sand	0.325 ± 0.002	very well sorted
Gelfa	1	41°47′8.88″N	8°53′14.28″W	15	0.239 ± 0.079	fine sand	0.45 ± 0.010	well sorted
	2	41°46′52.38″N	8°53′6.84″W	12.5	0.232 ± 0.070	fine sand	0.426 ± 0.051	well sorted
	3	41°46′34.26″N	8°53′4.20″W	11	0.169 ± 0.002	fine sand	0.375 ± 0.008	well sorted
Lima	1	41°40′13.86″N	8°50′22.74″W	9.5	0.152 ± 0.002	fine sand	0.602 ± 0.015	moderately well sorted
	2	41°39′57.73″N	8°50′22.50″W	10.5	0.140 ± 0.008	fine sand	0.599 ± 0.011	moderately well sorted
	3	41°39′39.72″N	8°50′24.43″W	11	$0.137 \pm 5.561\ 10^{-6}$	fine sand	0.578 ± 0.002	moderately well sorted

Table 2. Results of ANOVAs testing differences in coastal water variables among localities; [**]: $p < 0.01$; [***]: $p < 0.001$; [s]: significant; [ns]: not significant. Relevant significant differences indicated in bold.

Source of variation	df	Oxygen concentration		Salinity		Temperature	
		MS	F	MS	F	MS	F
Locality	3	0.0009	**9.34**[**]	0.0012	4.11	0.0645	**8.82**[**]
Residual	8	0.0001		0.0003		0.0073	
Total	11						
Cochran's test		0.8525[s]		0.9697[s]		0.8702[s]	
Transformation		none		none		none	

	df	NO$_3$		NH$_3$		PO$_4$	
		MS	F	MS	F	MS	F
Locality	3	2.1746	1.34	83.500	2.83	0.0247	1.79
Site (Locality)	8	1.6239	45.05[***]	29.531	0.55	0.0138	8.04[***]
Residual	24	0.0360				0.0017	
Total	35						
Cochran's test		0.2818[ns]		0.1699[ns]		0.3314[ns]	
Transformation		Sqrt(X+1)		none		Sqrt(X+1)	

Table 3. Results of ANOVAs testing differences in sedimentary grain sizes and organic matter content (in percentage) among localities. *: p < 0.05; **: p < 0.01; ***: p < 0.001; ^{s}: significant; ^{ns}: not significant. Relevant significant differences indicated in bold.

Source of variation	df	Coarse gravel		Fine gravel		Very coarse sand		Coarse sand		Medium sand	
		MS	F	MS	F	MS	F	MS	F	MS	F
Locality	3	0.0002	1.00	0.0208	3.43	0.6724	**11.79****	1591.7965	**6.34***	2716.0566	**12.96****
Site (Locality)	8	0.0002	1.00	0.0061	2.55	0.057	12.88***	251.2306	9.48***	209.5186	0.74
Residual	12	0.0002		0.0024		0.0044		26.4955		284.7926	
Total	23										
Cochran's test		1.00^s		0.4024^{ns}		0.4022^{ns}		0.7425^s		0.5302^{ns}	
Transformation		none		none		Sqrt(X+1)		none		none	

	df	Fine sand		Very fine sand		Silt/clay		Organic matter	
		MS	F	MS	F	MS	F	MS	F
Locality	3	3927.4412	**22.25*****	868.6802	**21.78*****	115.6667	**41.87*****	0.5483	**16.07****
Site (Locality)	8	176.5464	1.07	39.8859	1.53	2.7623	1.64	0.0341	0.57
Residual	12	164.8805		26.1224		1.6820		0.0596	
Total	23								
Cochran's test		0.5289^{ns}		0.5119^{ns}		0.5007^{ns}		0.5182^{ns}	
Transformation		none		none		none		none	

Table 4. Results of ANOVAs testing for differences in the total number of individuals (N), taxon richness (S) and Shannon's diversity index (H′) of macrobenthos among localities; [*]: $p < 0.05$; [***]: $p < 0.001$; [ns]: not significant; [s]: significant. Relevant significant differences indicated in bold.

Source of variation	df	N		S		H'	
		MS	F	MS	F	MS	F
Locality	3	11.57	**6.56[*]**	27.66	0.58	2.21	5.46[*]
Site (Locality)	8	1.76	7.62[***]	47.40	7.558[***]	0.41	2.84[*]
Residual	48	0.23		6.25		0.14	
Total	59						
Cochran's test		0.19[ns]		0.26[ns]		0.32[s]	
Transformation		Ln (X+1)		none		none	

Table 5. Results of PERMANOVAs testing differences in the structure of macrobenthic assemblage among localities. Analyses based on Bray–Curtis dissimilarity matrix from square-root transformed data. All tests used 999 random permutations. Monte Carlo p-values were considered for pair-wise test because the number of unique permutations was lower than 30. [*]: $p < 0.05$; [**]: $p < 0.01$. Relevant significant differences indicated in bold.

Source of variation	df	MS	Pseudo-F	Unique perms
Lo	3	15402	**2.68**[*]	984
Si (Lo)	8	5752.8	5.42[**]	996
Residual	48	1061.6		
Total	59			
Pair-wise test			t	
Miño vs Âncora			**1.74**[*]	
Miño vs Gelfa			**1.92**[*]	
Miño vs Lima			**2.27**[**]	
Âncora vs Gelfa			0.89	
Âncora vs Lima			1.32	
Gelfa vs Lima			0.10	

Table 6. Contribution (δi) of individual taxa to the average Bray-Curtis dissimilarity among localities that showed significant differences in the structure of their assemblages.

Species	Average Abundance				Miño-Âncora			Miño-Gelfa			Miño-Lima		
	Miño	Âncora	Gelfa	Lima	δi	$\delta i\%$	$\delta i/\mathrm{SD}(\delta i)$	δi	$\delta i\%$	$\delta i/\mathrm{SD}(\delta i)$	δi	$\delta i\%$	$\delta i/\mathrm{SD}(\delta i)$
Pisione parapari	8.9	0	0	0	10.23	13.83	1.25	11.59	14.34	1.23	10.72	12.76	1.24
Nematoda spp.	6.41	0.13	0.23	0.23	7.65	10.34	2.08	8.55	10.58	2.08	7.93	9.44	2.02
Gastrosaccus spinifer	5.75	4.03	1.01	0.78	7.48	10.12	1.15	6.66	8.24	1.14	6.52	7.76	1.18
Polygordius appendiculatus	6.05	0	0	0	6.78	9.17	1.1	7.67	9.48	1.1	7.1	8.45	1.1
Diogenes pugilator	4.24	3.09	1.54	1.18	5.38	7.27	1.05	6.89	8.53	0.9	6.52	7.76	0.92
Spio decoratus	1.24	4.62	3.41	3.46	4.5	6.08	1.04	3.85	4.77	1.54	3.16	3.77	1.69
Pisione remota	3.01	0	0	0	3.3	4.46	1.01	3.71	4.59	1.01	3.45	4.1	1.01
Bodotria arenosa	0.48	1.86	1.27	1.05	2.14	2.89	1.36	1.72	2.12	1.26	1.58	1.89	1.09
Bathyporeia tenuipes	0.07	1.58	0.74	0.57	2.11	2.85	1.26	1.1	1.37	0.88	0.93	1.1	0.69
Eurydice sp.	1.75	1.38	0.34	0.33	1.92	2.6	1.14	2.81	3.47	1.01	2.47	2.94	1.07
Scolelepis squamata	0.36	1.42	0.09	0.16	1.66	2.24	1.4	0.73	0.9	0.68	0.68	0.81	0.73
Nototropis falcatus	1.05	1.39	0.99	0.87	1.59	2.14	0.88	1.74	2.15	0.79	1.4	1.66	0.83
Pontocrates altamarinus	0.28	1.16	1.4	1.89	1.5	2.02	1.14	2.01	2.49	1.06	2.42	2.88	1.66
Calanoida spp.	1.27	0.2	0.42	0	1.47	1.99	0.81	2.07	2.56	0.87	1.59	1.89	0.8
Nephtys cirrosa	0.95	1.58	1.03	0.63	1.32	1.78	1.28	1.41	1.75	1.21	1.27	1.51	1.24
Micronephtys stammeri	0.56	1.01	0.88	1.57	1.28	1.73	1.18	1.23	1.52	1.1	1.62	1.93	1.47
Magelona johnstoni	0.07	0.97	0.99	1.26	1.25	1.69	1.02	1.45	1.79	0.99	1.73	2.05	1.19
Bathyporeia nana	0	0.77	--	--	1.06	1.43	0.81	--	--	--	--	--	--
Urothoe brevicornis	0.19	0.57	0.33	--	0.84	1.14	0.82	0.77	0.96	0.73	--	--	--
Liocarcinus marmoreus	0.64	0.33	0.07	0	0.84	1.14	1.01	1.06	1.31	0.97	0.97	1.15	0.98
Saccocirrus papillocercus	0.65	0	0	0	0.69	0.94	0.63	0.78	0.96	0.64	0.72	0.86	0.63
Diastylis rathkei	0	0.48	0.56	1.06	0.65	0.88	0.72	0.77	0.95	0.54	1.46	1.73	1.37
Spiophanes bombyx	0	0.51	0.74	0.67	0.65	0.88	0.71	1.04	1.29	0.86	0.9	1.07	0.84
Fabulina fabula	0	--	--	1.85	--	--	--	--	--	--	2.55	3.04	1.27
Mediomastus fragilis	0	--	--	1.33	--	--	--	--	--	--	1.76	2.1	1.1
Glycera tridactyla	0.4	--	0.36	0.77	--	--	--	0.84	1.04	0.83	1.17	1.39	1.05
Nephtys assimilis	0	--	--	0.69	--	--	--	--	--	--	0.97	1.16	0.95
Iphinoe trispinosa	0	--	--	0.7	--	--	--	--	--	--	0.92	1.1	0.63
Hippomedon denticulatus	0	--	--	0.56	--	--	--	--	--	--	0.77	0.91	0.69
Owenia fusiformis	0	--	--	0.5	--	--	--	--	--	--	0.68	0.81	0.75
Spisula solida	0.45	--	0	0	--	--	--	0.75	0.93	0.77	0.68	0.8	0.78
Magelona mirabilis	0	--	--	0.49	--	--	--				0.67	0.8	0.66
Eocuma dollfusi	0.07	--	0.65		--	--	--	1.06	1.32	0.83	--	--	--
Tanaidacea spp.	0.07	--	0.4		--	--	--	0.59	0.73	0.59	--	--	--

Table 7. Results of DistLM carried out to ascertain the role of different environmental variables, considering forward-selection of variables, where amount explained by each variable added to model is conditional on variables already in the model (i.e. those variables listed above it)on the total number of individuals (N), taxon richness (S), Shannon's diversity index (H′) and the structure of macrobenthic assemblages. %Var: percentage of variance in species data explained by that variable; Cum. %: cumulative percentage of variance explained. Variables significantly related to macrobenthic assemblages indicated in bold.

N				S			
Variable	%Var	Pseudo-F	Cum (%)	Variable	%Var	Pseudo-F	Cum (%)
Fine sand	56.30	**74.73**[**]	56.30	Salinity	24.65	**18.97**[**]	24.65
Medium sand	3.56	**5.06**[*]	59.87	Silt/clay	6.65	**5.52**[*]	31.30
Depth	6.02	**9.89**[**]	65.89	Depth	4.48	**3.91**[*]	35.77
Silt/Clay	1.23	2.10	67.12	NO_3	10.68	**10.95**[**]	46.44
NO_3	1.89	3.29	69.01	NH_3	6.46	**7.41**[**]	52.90
Very fine sand	1.52	2.74	70.53	Organic matter	5.59	**7.14**[*]	58.49
NH_3	0.27	0.47	70.79	Very coarse sand	0.46	0.58	58.95
Organic matter	0.44	0.77	71.28	Fine sand	0.85	1.08	59.80
Coarse gravel	0.04	$6.29\ 10^{-2}$	71.26	Oxygen	0.12	0.15	59.92
Oxygen	0.03	$5.47\ 10^{-2}$	71.30	Fine gravel	0.34	0.41	60.25
Salinity	0.21	0.36	71.51	Coarse gravel	0.04	0.47	60.64

H'				Assemblage			
Variable	%Var	Pseudo-F	Cum (%)	Variable	%Var	Pseudo-F	Cum (%)
Silt/clay	46.46	**50.33**[**]	46.46	Very coarse sand	25.61	**20.00**[**]	25.61
Organic matter	2.96	3.33	49.42	Very fine sand	8.27	**7.13**[**]	33.88
NH_3	1.92	2.21	51.33	Salinity	4.99	**4.58**[**]	38.87
Depth	1.70	1.99	53.03	Fine sand	4.23	**4.09**[**]	43.11
NO_3	2.87	3.52	55.90	Medium sand	4.22	**4.33**[**]	47.33
Fine sand	0.96	1.17	56.86	Silt/clay	3.17	**3.39**[**]	50.50
Very coarse sand	0.46	0.56	57.31	Oxygen	4.12	**4.72**[**]	54.62
Medium sand	0.11	0.13	57.42	Coarse gravel	2.85	**3.42**[**]	57.47
Very fine sand	1.56	1.91	58.99	Depth	3.15	**4.01**[**]	60.62
Fine gravel	0.05	$5.92\ 10^{-2}$	59.04	NH_3	2.27	**3.00**[**]	62.89
				NO_3	1.52	**2.05**[*]	64.41

Table 8. Results of DistLM carried out to ascertain the role of on sets of environmental variables (coastal water and sediment), considering each set of variables taken individually (ignoring other sets) and forward-selection of sets of variables, where amount explained by each set added to model is conditional onset already in the model (i.e. that listed above it) on the total number of individuals (N), taxon richness (S), Shannon diversity index (H′) and the structure of macrobenthic assemblages. %Var: percentage of variance in species data explained by that set of variables; Cum. %: cumulative percentage of variance explained. Sets of variables significantly related to macrobenthic assemblages indicated in bold.

|	**N**				**S**		
Set	%Var	Pseudo-F	Cum (%)	Set	%Var	Pseudo-F	Cum (%)
Sets individually				**Sets individually**			
Sediment	69.67	**14.65**[**]		Sediment	54.78	**7.72**[**]	
Water	63.50	**12.93**[**]		Water	48.81	**7.08**[**]	
Sets fitted sequentially				**Sets fitted sequentially**			
Sediment	69.67	**14.65**[**]	69.67	Sediment	54.78	**7.72**[**]	54.78
Water	1.84	1.03	71.51	Water	5.85	2.38	60.64
	H'				**Assemblage**		
Set	%Var	Pseudo-F	Cum (%)	Set	%Var	Pseudo-F	Cum (%)
Sets individually				**Sets individually**			
Sediment	56.93	**8.43**[**]		Sediment	57.17	**8.51**[**]	
Water	37.62	**4.48**[**]		Water	51.26	**7.81**[**]	
Sets fitted sequentially				**Sets fitted sequentially**			
Sediment	56.93	**8.43**[**]	56.93	Sediment	57.17	**8.51**[**]	57.18
Water	2.11	0.83	59.04	Water	7.25	**3.26**[**]	64.41

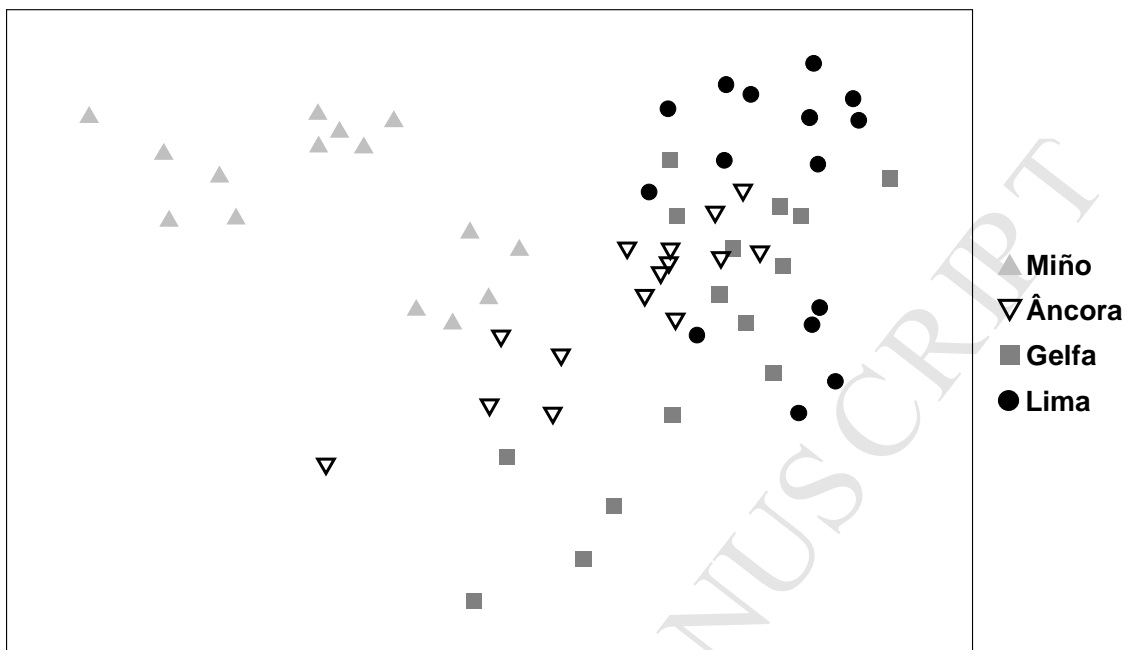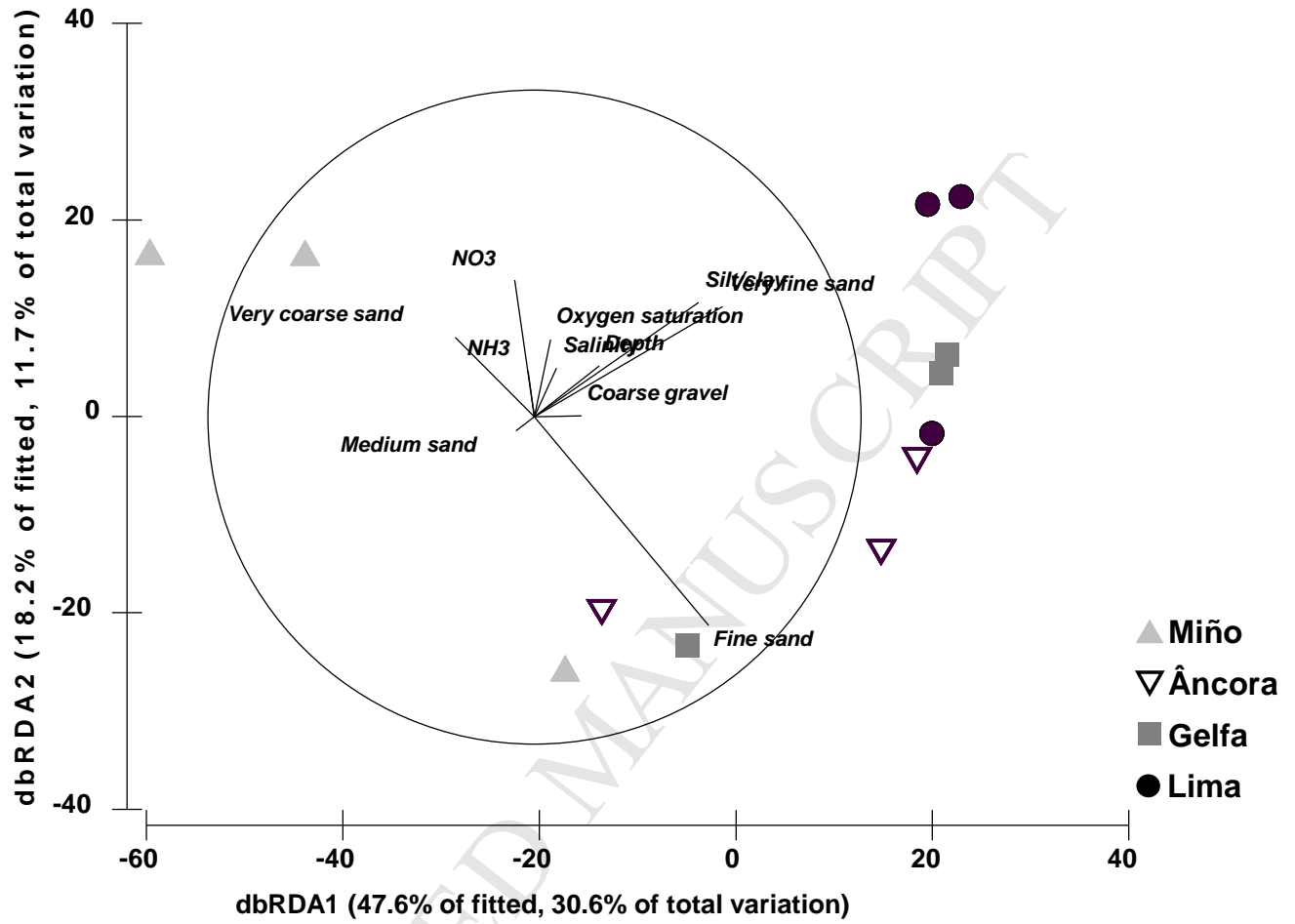**Fig. 1**
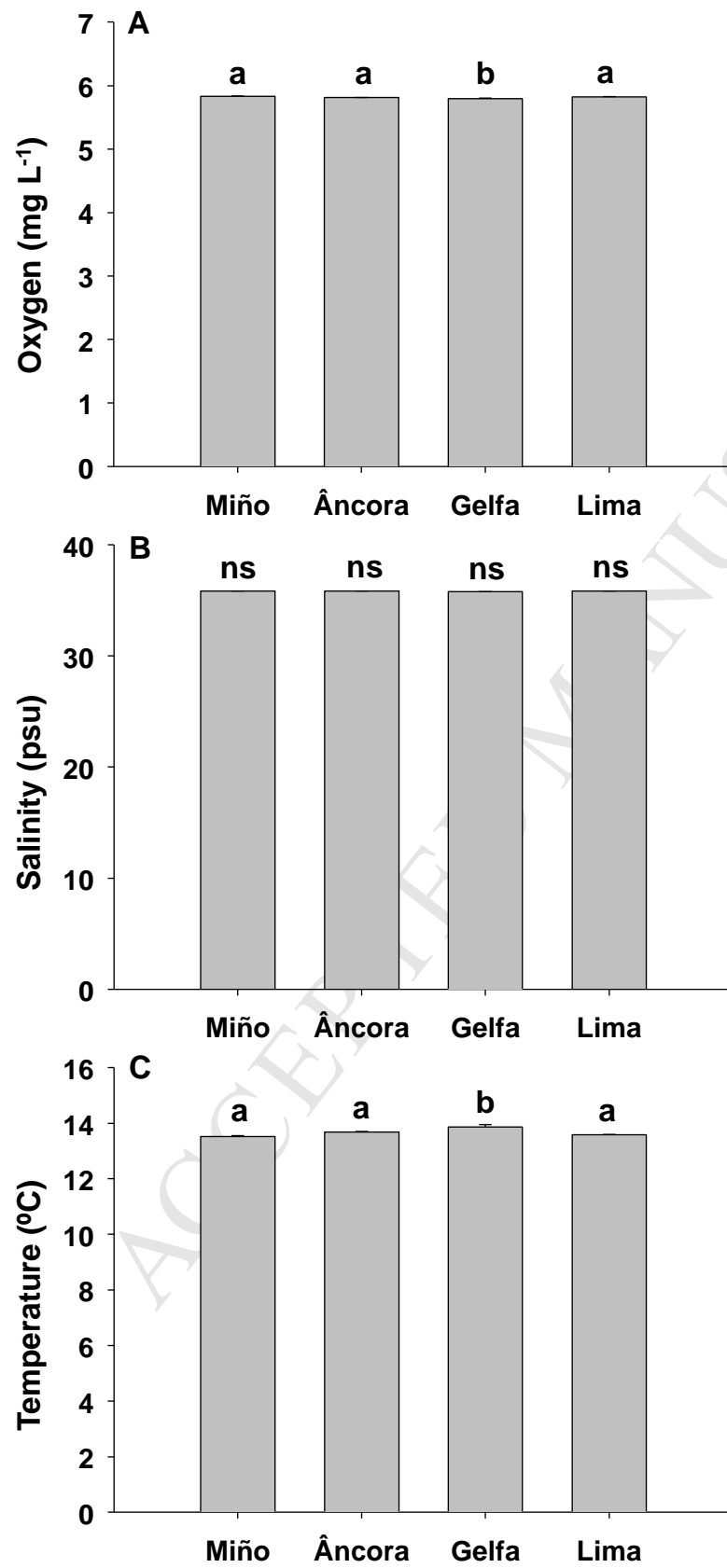
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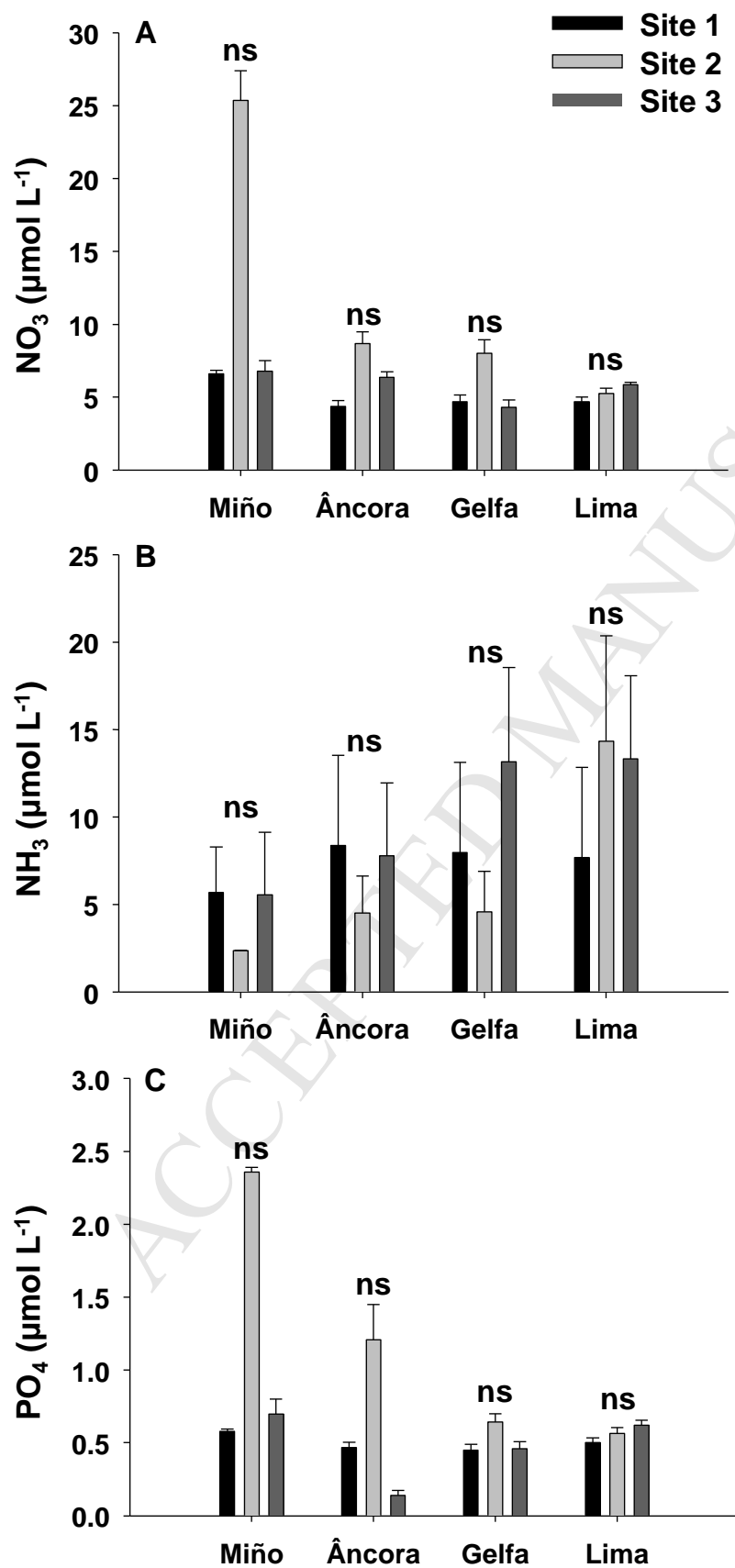
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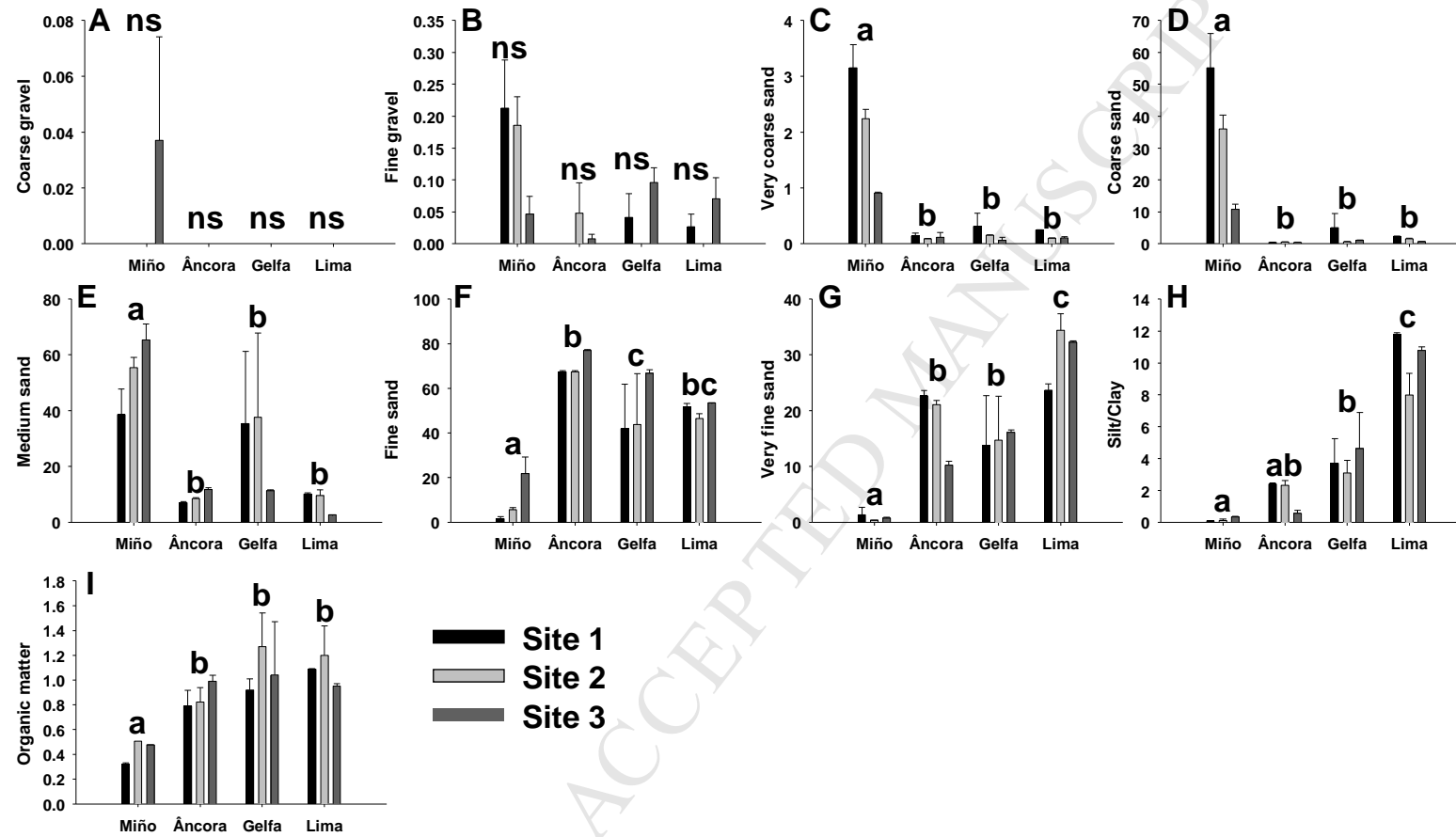
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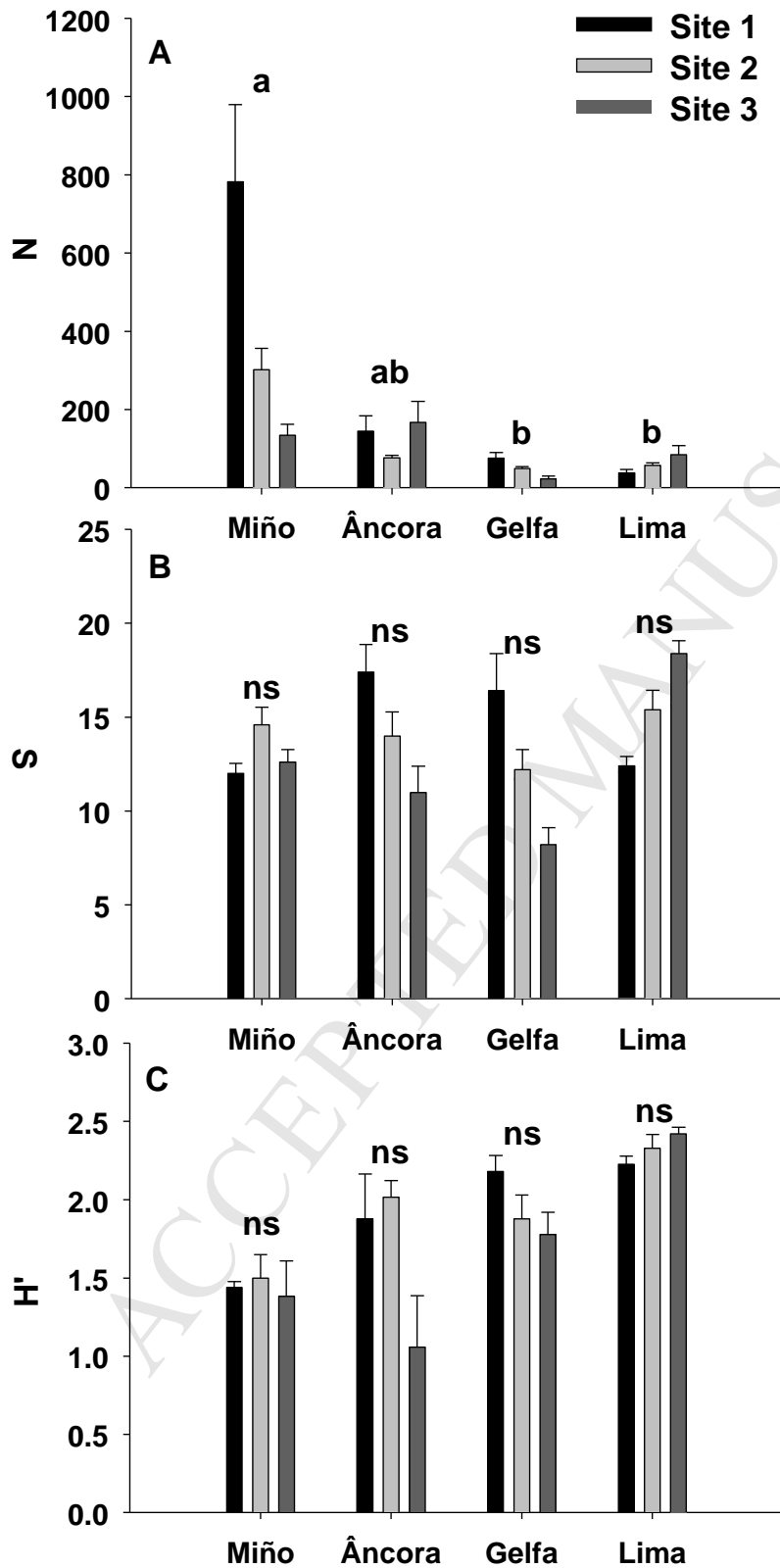
Fig. 5

Fig. 6

Spatial patterns of macrobenthos in shallow sublittoral sediments.

Abundance, diversity and multivariate structure of macrobenthos.

Models selecting main environmental variables shaping macrobenthos.

Most of macrobenthic variability was explained by environmental variables.

Sedimentary variables were more relevant that those of the water column.