Meiofaunal communities and nematode diversity characterizing the Secca delle Fumose shallow vent area (Gulf of Naples, Italy)

Elisa Baldrighi [Corresp., 1, 2] , Daniela Zeppilli [2] , Luca Appolloni [3, 4] , Luigia Donnarumma [3, 4] , Elena Chianese [3] , Giovanni Fulvio Russo [4, 5] , Roberto Sandulli [3, 4]

[1] Istituto per le Risorse Biologiche e le Biotecnologie Marine (IRBIM), Consiglio Nazionale delle Ricerche (CNR), Lesina, Italy, Italy

[2] Laboratoire Environnement Profond, Institut Français de Recherche pour l'Exploitation de la MER (IFREMER), Plouzané, France, France

[3] Department of Science and Technology (DiST), Parthenope University of Naples, Naples, Italy, Italy

[4] Consorzio Nazionale Interuniversitario per le Scienze del Mare (CoNISMa), Rome, Italy, Italy

[5] Department of Science and Technology (DiST),, Parthenope University of Naples, Naples, Italy, Italy

Corresponding Author: Elisa Baldrighi
Email address: elisa.baldrighi@irbim.cnr.it

Hydrothermal venting is rather prevalent in many marine areas around the world, and marine shallow vents are relatively abundant in the Mediterranean Sea, especially around Italy. However, investigations focusing on the characterization of meiofaunal organisms inhabiting shallow vent sediments are still scant compared to that on macrofauna. In the present study, we investigated the meiobenthic assemblages and nematode diversity inhabiting the Secca delle Fumose (SdF), a shallow water vent area located in the Gulf of Naples (Italy). In this area, characterized by a rapid change in the environmental conditions on a relative small spatial scale (i.e., one hundred of meters), we selected four sampling sites: one diffusive emission site (H); one geyser site (G) and two inactive sites (CN, CS). Total meiofauna abundance did not vary significantly between active and inactive sites and between surface and deeper sediment layers due to a high inter-replicate variability, suggesting a pronounced spatial-scale patchiness in distribution of meiofauna. Nematofauna at site H presented the typical features of deep-sea vents with low structural and functional diversity, high biomass and dominance of few genera (i.e. *Oncholaimus*; *Daptonema*) while from site G we reported diversity values comparable to that of the inactive sites. We hypothesized that site G presented a condition of 'intermediate disturbance' that could maintain a high nematode diversity. Environmental features such as sediment temperature, pH, total organic carbon and interstitial waters ions were found to be key factors influencing patterns of meiofauna and nematofauna assemblages at SdF. Even though the general theory is that nematodes inhabiting shallow vent areas include a subset of species that live in background sediments, this was not the case for SdF vent area. Due to a marked change in nematode composition between all

sites and to the presence of many exclusive species, every single investigated site was characterized by a distinct nematofauna reflecting the high spatial heterogeneity of SdF.

2 **Meiofaunal communities and nematode diversity characterizing the Secca delle Fumose**

3 **shallow vent area (Gulf of Naples, Italy)**

5 Elisa Baldrighi[1,2], Daniela Zeppilli[2], Luca Appolloni[3,4], Luigia Donnarumma[3,4], Elena Chianese[3],

6 Giovanni Fulvio Russo[3,4] and Roberto Sandulli[3,4]

8 [1]Istituto per le Risorse Biologiche e le Biotecnologie Marine (IRBIM), Consiglio Nazionale delle

9 Ricerche (CNR), Lesina, Italy;

10 [2]Institut Français de Recherche pour l'Exploitation de la MER (IFREMER), Laboratoire Environnement

11 Profond, Plouzané, France;

12 [3]Department of Science and Technology (DiST), Parthenope University of Naples, Naples, Italy

13 [4]Consorzio Nazionale Interuniversitario per le Scienze del Mare (CoNISMa), Rome, Italy

14 **Corresponding author:**

15 Elisa Baldrighi[1,2]

16 Via Pola, 4, 71010, Lesina, Italy

17 Email address: elisa.baldrighi@irbim.cnr.it

19 **Keywords:** Secca delle Fumose, Gulf of Naples, shallow vent, meiofauna, nematode diversity

21 **Abstract**

23 Hydrothermal venting is rather prevalent in many marine areas around the world, and marine

24 shallow vents are relatively abundant in the Mediterranean Sea, especially around Italy.

25 However, investigations focusing on the characterization of meiofaunal organisms inhabiting

26 shallow vent sediments are still scant compared to that on macrofauna. In the present study, we

27 investigated the meiobenthic assemblages and nematode diversity inhabiting the Secca delle

28 Fumose (SdF), a shallow water vent area located in the Gulf of Naples (Italy). In this area,

29 characterized by a rapid change in the environmental conditions on a relative small spatial scale

30 (i.e., one hundred of meters), we selected four sampling sites: one diffusive emission site (H);

31 one geyser site (G) and two inactive sites (CN, CS). Total meiofauna abundance did not vary

32 significantly between active and inactive sites and between surface and deeper sediment layers

33   due to a high inter-replicate variability, suggesting a pronounced spatial-scale patchiness in

34   distribution of meiofauna. Nematofauna at site H presented the typical features of deep-sea vents

35   with low structural and functional diversity, high biomass and dominance of few genera (i.e.

36   *Oncholaimus*; *Daptonema*) while from site G we reported diversity values comparable to that of

37   the inactive sites. We hypothesized that site G presented a condition of 'intermediate

38   disturbance' that could maintain a high nematode diversity. Environmental features such as

39   sediment temperature, pH, total organic carbon and interstitial waters ions were found to be key

40   factors influencing patterns of meiofauna and nematofauna assemblages at SdF. Even though the

41   general theory is that nematodes inhabiting shallow vent areas include a subset of species that

42   live in background sediments, this was not the case for SdF vent area. Due to a marked change in

43   nematode composition between all sites and to the presence of many exclusive species, every

44   single investigated site was characterized by a distinct nematofauna reflecting the high spatial

45   heterogeneity of SdF.

47
48   **Introduction**
49
50   Scientific explorations have demonstrated the importance of hydrothermally influenced

51   habitats both in the deep-sea and in shallow coastal habitat (e.g., Melwani & Kim, 2008).

52   Shallow vents are often associated with active plate boundaries, and consequently volcanic or

53   seismic activities are related to sites of littoral and sub-littoral thermal venting (Tarasov et al.,

54   1999). The environmental conditions in shallow-water vents strongly differ from the surrounding

55   seafloor in terms of both increased temperature and enrichment in reduced chemical compounds

56   such as sulphide, methane, manganese, iron and arsenic (Prol-Ledesma et al., 2004). Fluids

57   formation commonly take place from relatively deep sources (1–2 km depth) and these natural

58   fluid emissions may be able to alter sea-water geochemistry (Di Bella et al., 2016). Pore-water

59   temperatures tend to significantly increase compared to ambient conditions (Pichler et al., 1999).

60   Furthermore, numerous studies have reported vent fluids with low salinity and acidic pH (i.e.,

61   Melwani & Kim, 2008 and literature therein). The high temperature, coupled with gas release

62   and variable chemical conditions due to hydrothermal activity may create biologically stressful

63   environments.

64   Differently from deep-sea hydrothermal vents based exclusively on chemosynthetic primary

65   production, shallow-water vents are characterized by the presence of light that coupled with that

66   of geothermal fluids, promotes both photo- and chemosynthetic primary production (Sorokin et

67   al., 1998; Tarasov et al., 2005). Most ecological studies investigating shallow water

68   hydrothermal vents were focused on microbial communities, which are strongly influenced by

69   the hydrothermal activity both within the water column and on the seabed (Judd & Hovland,

70   2007; Di Bella et al., 2016). Shallow-water vents can differ in terms of faunal density, diversity

71   and dominance (i.e. metazoan organisms) from the surroundings and from each other, depending

72   on the degree and effects of the venting activity (Jones, 1993; Panieri et al., 2005; Panieri, 2006a;

73   Melwani & Kim, 2008; Wildish et al., 2008).

74   At deep-sea hydrothermal vents, an increase in number of meiobenthic animals coupled with

75   a reduction in the diversity compared to non-vent areas has been reported (Copley et al., 2007

76   and literature therein; Zeppilli et al., 2018). Nematodes and copepods are the most abundant taxa

77   and studies conducted along the East Pacific Rise reported that usually copepods are the initial

78   dominant meiofaunal colonizers of 'new' vent mussel beds, with a general increase in the

79   percentage ratio of nematodes to copepods over time (Flint et al., 2006; Copley et al., 2007).

80   Deep-water hydrothermal vents in general do not show high nematode densities or biomass and

81   also their diversity is much lower than that in surrounding deep-sea sediments (Vanreusel et al.,

82   2010). Despite their ubiquitous distribution in tectonically active coastal zones, shallow-water

83   vents have been less explored than deep-sea vents in terms of biodiversity and adaptations to

84   extreme conditions (Colangelo et al., 2001; Tarasov et al., 2005). Investigations focusing on the

85   characterization of meiofaunal organisms inhabiting shallow vent sediments are still scant

86   compared to that on macrobenthic communities and they span different geographical areas, from

87   Mediterranean Aegean Sea (Dando et al., 1995; Fitzsimons et al., 1997; Thiermann et al., 1994;

88   Thiermann et al., 1997), Tyrrhenian Sea (Colangelo et al., 2001; Panieri et al., 2005; Panieri,

89   2006a; Di Bella et al., 2016), Adriatic Sea (Panieri et al., 2006b), Strait of Sicily (Sandulli et al.,

90   2015) to New Zealand (Kamenev et al., 1993), Papua New Guinea (Tarasov et al., 1999),

91   Indonesian archipelago (Zeppilli & Danovaro, 2009), the shallow sub-polar region of the Mid

92   Atlantic Ridge (Fricke et al., 1989), Azores (Cardigos et al., 2005), and including the

93   comprehensive reviews by Tarasov et al. (2005) and Zeppilli et al. (2018).

94   Shallow vents are characterized by non-endemic meiofauna that show higher diversity and

95   abundance than in background sediments (Tarasov et al. 2005). While deep-sea vents are

96   inhabited by unique nematode assemblages , at least at species level (Vanreusel et al. 1997;

97   Thiermann et al., 1997), shallow-water vent nematodes include a subset of species that can both

98   live in the background sediments and cope with extreme conditions (Zeppilli & Danovaro,

99   2009). Also for copepods, shallow vent assemblages seem to be the result of colonization from

100   adjacent areas (Colangelo et al., 2001; Zeppilli & Danovaro, 2009).

101   Hydrothermal venting in shallow water is a common phenomenon and marine shallow vents are

102   abundant in the Mediterranean, in particular in the Tyrrhenian Sea (Hall-Spencer et al., 2008).

103   The present work represents the first investigation on the meiobenthic communities inhabiting

104   Secca delle Fumose (SdF) shallow water vent area located in the Underwater Archaeological

105   Park of Baia (Gulf of Naples, Italy).

106   The peculiarity of this active area is the rapid change in the extreme environmental conditions,

107   from the diffusive vent site and geyser site with hot temperatures to inactive sites (see below), at

108   a relatively small spatial scale i.e., one hundred of meters. SdF can be considered an example of

109   how hydrothermal flux can vary at small scale in the vent habitat (Flint et al., 2006), creating a

110   gradient in the environmental conditions, from extreme to ambient conditions.

111   The present paper reports a first insight into the meiofaunal and nematode communities of SdF

112   shallow vent area and describes the distribution and diversity of meiobenthic organisms in

113   relation to seawater chemistry and sediment characteristics. Due to changes in stress regime and

114   environmental conditions, we expect different meiofauna and nematofauna assemblages

115   inhabiting the distinct sampling sites.

116

117   **Material & Methods**

118

119   **Site description**

120   The study area of SdF is located in the north-west side of the Gulf of Naples (Baia Underwater

121   Park MPA, Tyrrhenian Sea, Italy; Fig.1). The coastline consists mainly of beaches and tuff cliffs.

122   Volcanic activity is still evident both for bradyseism process, which led to the immersion of

123   many Roman structures, and for sulfurous emanations. The interaction between natural processes

124   and human activities produced a natural environment characterized by an extreme habitats'

125   variety (Fig. 1). Photophilous algae habitat (AP) is observed on docks, while strong

126   interdigitation between photophilous populations, which are setting up on the parts most exposed

127   to light, and sciaphilous populations (C) setting in the cavities (AP-C), is present on Roman

128   artifacts. Superficial muddy sands in sheltered water habitat (SVMC) dominates soft bottoms.

129   Near the submerged structures SVMC habitats are enriched with fragments come from the

130   disintegration of the artifacts and from carbonate exoskeletons of benthic organisms; here are

131   recorded species belonging to the coarse sands and fine gravels under the influence of bottom

132   currents habitat (SGCF) (as previously described in Appolloni et al., 2018). SdF is a submarine

133   relief consisting of a network of ancient Roman pillars, among which thermal vents releasing hot

134   gas-rich hydrothermal fluids occur (D'Auria et al., 2011). This area has been previously

135   investigated from a geological point of view (Todesco, 2009; D'Auria et al., 2011 and literature

136   therein), and, very recently, with the only macrobenthic and environmental published study so

137   far known (Donnarumma et al., 2019). All details regarding the sampling site are reported in

138   Donnarumma et al. (2019).

139

140

141   **Sampling methods and samples processing**

142   For our study, we selected four sampling sites, at 9-14 m water depth, over a total of ~7500 m^2

143   sampling area: one diffusive emission site (H, "White") characterized by the presence of white

144   microbial mats covering the soft bottom; one geyser site (G, "Yellow") at 65 m distance from the

145   H site, with surrounding rocky substrate covered by yellow sulphur deposits and with hot water

146   emissions reaching 80°C at the sediment surface; two inactive sites (CN and CS) located at a

147   distance of 100 m from the active sites H and G (Fig.1). Environmental data were collected as

148   previously describe in Donnarumma et al. (2019). Interstitial waters to assess ions and heavy

149   metals concentrations and sediment samples (three random replicates) were collected at the four

150   selected sites. Cylindrical corers (internal diameter 5.5 cm) were used to collect sediment for

151   grain size, total organic carbon (TOC) and meiofaunal communities by scuba diving. The

152   meiofauna sediment cores were sliced into four layers 0-1, 1-3, 3-5 and 5-10 cm and fixed in

153   buffered 4% formalin/seawater.

154

155   **Environmental variables**

156 All details on the environmental variable analyses considered in the present study i.e., interstitial
157 waters ion (Na^+, Cl^-, K^+, Mg^{2+}, Ca^{2+}, NO_3^-, SO_4^{2-} and S^{2-}) and heavy metal (Zn, Pb, Cd and Cu)
158 concentrations, sediment grain size (as percentage of gravel, sand and mud) and total organic
159 carbon (TOC) are reported in Donnarumma et al. (2019).

160

161 **Meiobenthos and nematode analyses**

162 Sediment samples were sieved through a 1000 μm mesh, and a 30 μm mesh was used to retain
163 the smallest organisms. The fraction remaining on the latter sieve was re-suspended and
164 centrifuged with Ludox HS40 according to Heip et al. (1985). All meiobenthic animals were
165 counted and classified per taxon under a stereomicroscope. From each sample, approximately
166 100-120 nematodes were picked out, transferred to anhydrous glycerin following the formalin-
167 ethanol-glycerin protocol (De Grisse, 1969), and mounted on paraffin ring glass slides for
168 microscopic identification. Nematodes were identified at genus level and diversified in putative
169 morphotypes as sp1, sp2, etc. according to the main original species descriptions (Platt &
170 Warwick, 1983, 1988; Warwick, Platt & Somerfield, 1998; Tchesunov & Schmidt-Rhaesa, 2014)
171 and pictorial keys available on the Nemys website (Bezzerra et al., 2018). Nematode biomass
172 was calculated from the biovolume using the Andrassy (1956) formula ($V = L \times W^2 \times 0.063 \times$
173 10^{-5}, in which L is the body length and W is the body width). Species richness (SR) was
174 calculated as the total number of species collected at each site; the expected number of species
175 for a theoretical sample of 51 specimens, ES(51), was selected and Pielou's (1975) evenness (J')
176 was estimated.

177 Furthermore, nematode genera were categorized in four feeding guilds based on their buccal
178 cavity morphology as described by Wieser (1953). Feeding guilds included "selective deposit
179 feeders" (Group 1A, small buccal cavity without teeth), "non-selective deposit feeders" (Group
180 1B, large buccal cavity without teeth), "epistrate feeders" (Group 2A, small buccal cavity with
181 teeth) and "predators/scavengers" (Group 2B, lager buccal cavity with teeth). The index of
182 trophic diversity (ITD) as $ITD = g_1 2 + g_2 2 + g_3 2 \ldots + g_n 2$, where g is the relative contribution of
183 each trophic group to the total number of individuals and n is the number of trophic groups
184 (Gambi et al., 2003). For n = 4, ITD ranges from 0.25 (highest trophic diversity, i.e. the 4 trophic
185 guilds account for 25% each) to 1.0 (lowest diversity i.e., one trophic guild accounts for 100% of
186 nematode density). To identify the life strategy of nematodes, the maturity index (MI) was

187 calculated according to the weighted mean of the individual genus scores: $MI = \Sigma v(i)f(i)$, where

188 v is the c–p value (colonizers–persisters) of genus i as given in the appendix of Bongers et al.

189 (1991) and $f(i)$ is the frequency of that genus.

190

191 **Data analysis**

192 Uni- and multivariate analyses were carried out in order to assess differences in several

193 descriptors of meiofauna (i.e. abundance, number of taxa, assemblage composition) and

194 nematode assemblage composition (i.e. abundance, biomass, SR, ES(51), J', ITD and MI)

195 between sites and layers. Faunal data were $Log_{(X+1)}$ transformed and analyzed using tests based

196 on Bray-Curtis similarity matrices (multivariate analyses) and Euclidean similarity matrices

197 (univariate analyses).

198 The sampling design included two fixed and orthogonal factors: site (4 levels: G, H, CN and CS)

199 and layer (4 levels: 0-1, 1-3, 3-5 and 5-10 cm). The distance-based permutation analysis of

200 variance (PERMANOVA; Anderson et al., 2008) in either univariate (separately for each

201 meiofauna and nematode diversity index) or multivariate data (for both meiofaunal and

202 nematode assemblages) was used for testing for differences in taxonomic composition between

203 sites and layers. Pair-wise tests were carried out to verify the significance of the differences

204 among sites and layers if any were observed in the main test.

205 Afterwards, the relative contribution of each nematode species to the average dissimilarities

206 between sites and layers was calculated using SIMPER test (using 70% as cutoff) to determine

207 the contribution of each species (Clarke & Warwick, 2001). The diversity in nematode

208 community composition is expressed as percentages of dissimilarity (Gray, 2000). A CLUSTER

209 analysis (group average) was carried out and a similarity profile test (SIMPROF) permutational

210 routine was applied to test for the significance of genuine clustering on meiofaunal assemblage

211 composition and nematode species composition characterizing the sampling sites.

212 Multivariate multiple regression analyses (DistLM forward, Anderson et al., 2008) with a

213 forward selection of the independent variables and 4999 permutation of residuals were

214 performed to test the influence of abiotic variables (sediment and interstitial water variables) on

215 meiofaunal abundance, richness of taxa and taxonomic composition, nematode biomass, species

216 composition and diversity indices, nematode trophic diversity and nematode life strategies. All

217   the analyses were carried out by means of the software PRIMER v6.0 + (Clarke & Gorley,

218   2006).

219

220

221   **Results**

222

223   **Environmental variables**

224   Environmental characteristics of sampling sites at SdF are reported and described in detail in

225   Donnarumma et al. (2019). The H site, the diffusive emission site in the southern sector, was

226   characterized by the highest sediment temperature (37.53±2.28°C) and by the lowest pH value of

227   (7.56±0.05). At this site, sediment was mainly composed by the sandy fraction enriched in TOC

228   content and the soft bottom presented a coverage of white microbial mat. At G site (geyser,

229   northern sector), located at 65 m from the H site, the sediment temperature reported was

230   29.1±2.81°C. This site was the only one characterized by the presence of sulphur ion S^{2-} into the

231   interstitial waters. CN and CS constituted the inactive sites at a distance of 100 m from G and H

232   sites. The sediment temperature (21.8°C) and pH (8.1) values were comparable to the

233   surrounding environment. All sampling sites were clearly differed due to many environmental

234   features that changed over a relatively small spatial scale (100 m) and indicating a marked spatial

235   heterogeneity (Donnarumma et al., 2019).

236

237   **Meiofauna abundance, taxonomic composition and distribution**

238   The mean meiofaunal abundances and abundance of each taxon at each sampling sites and along

239   the sediment layers are reported in Table S1. The total meiofaunal abundance ranged from

240   1142±713.8 ind./10cm² (mean ± standard error, hereafter) at CN site to 2023±1270.2 ind./10cm²

241   at G site (Fig. 2). At all sampling sites, with the only exception for the inactive site CN, an

242   increase of meiofaunal abundance from the surface sediment (0-1 cm) to the deeper sediment

243   layer (5-10 cm; Fig. 3) was recorded. Nevertheless, PERMANOVA analysis did not detect any

244   significant effect of factors 'site' and 'layer' on total meiofauna abundance (Table 1). The total

245   nematode abundances followed the total meiofaunal trends (Fig. 2 and 3). Significant lower

246   values in the total nematode abundance were reported at site H compared to sites G and CS

247   (Table 1). The abundance of nematodes increased significantly from the top 1 centimeter to the

248   deepest layer at sites H and CS (; Fig. 3 and Table 1). A total of 27 higher taxa, including

249   Foraminifera and Ciliata, were identified from the sediments of SdF (Table S1). The total

250   number of meiobenthic taxa ranged from 11±1 at site H to 20±1 at site G. PERMANOVA

251   analysis indicated that 'site' and the combined effect of SxL were the factors explaining

252   meiofaunal diversity variability in the number of major taxa (Table 1). In detail, the pair-wise

253   test showed that site H was characterized by a significant lower meiofaunal diversity compared

254   to all other sites (Table 1). Meiofaunal taxonomic composition changed significantly between

255   sites (Table 1), the pair-wise test detected major differences between site H vs. all the other sites

256   and the CLUSTER analysis clearly highlighted this separation (Figure 4).

257   With the only exception of site H, Nematoda were the most represented taxon (36-57%),

258   followed by Copepoda Harpacticoida and their *nauplii* (21-30%), Foraminifera (12-13%) and

259   Polychaeta (4-8%) (Table S1, Fig. 5). Other taxa, such as Tardigrada, Ciliata, Ostracoda,

260   Gastropoda and Halacarida typically occurred in lower numbers (2-5%) or only occasionally

261   (e.g., Kinorhyncha, Gastrotricha, Amphipoda, Tanaidacea, Cumacea, Isopoda). At site H,

262   harpacticoid copepods and their *nauplii* were the most represented groups (41%) followed by

263   Ciliata (29.5%) and Nematoda (24%). SIMPER analysis on meiofaunal taxonomic composition

264   characterizing the four sampling sites reported a dissimilarity percentage between site H and all

265   the other sites ranging from 27 to 31%. The dissimilarity was mainly due to a different

266   contribution of taxa, in term of abundances, at the different sampling sites and secondly to the

267   absence of Tardigrada and Gastrotricha at the diffusive site (H). Ostracoda, Polychaeta,

268   Gasteropoda and Halacarida, occurred frequently (2-8%) at sites G, CN and CS; other taxa, such

269   as Ciliata and Copepoda were found in high abundances at site H (Table S1, Fig. 5). Meiofauna

270   taxonomic composition did not change significantly along the vertical sediment profile. Overall,

271   nematode abundances increased with depth layer, Copepoda (with their *nauplii*), on the opposite,

272   decreased deepening into the sediment. These patterns characterized all sampling sites (Table S1,

273   Fig. 6).

274

275   **Nematode biomass, structural and functional diversity**

276   A complete list of nematodes identified and total biomass values at each sampling site and at

277   different sediment depth layers are reported in Table S2. Total biomass values ranged from

278   47.1±8.7 to 149.3±72.6 μg C at sites G and H, respectively. PERMANOVA analysis indicated

279   that nematode biomass changed significantly between sites and along the vertical profile (Table

280   1). In details, the pair-wise test reported significant higher biomass values at site H compared to

281   all the other sites and significant lower biomass values characterizing site G compared to the

282   inactive sites. Moreover, nematode biomass significantly increased going deeper into the

283   sediment and showing higher values at 3-5 cm and 5-10 cm layers compared to the top 3

284   centimeters at all sites except for CS (Table 1).

285   Nematode diversity indices (SR; $ES_{(51)}$; J), and functional diversity indices (ITD; MI) are

286   reported in Table 2. Since we did not detect any significant differences in nematode composition

287   with sediment depth layer (see below), we assessed for differences in diversity and functional

288   indices only between sites (Table 1). Site H showed significant lower values in nematode

289   diversity (SR; $ES_{(51)}$) and equitability (J') compared to all the other sites; while the inactive site

290   CS was characterized by significant lower values for J' index compared to CN and G. Overall, a

291   total of 33 families and 2 subfamilies; 156 genera and 165 species (i.e. morphotypes) were

292   identified.

293   The PERMANOVA results showed significant effect of the factor 'site' on nematode community

294   composition (Table 1). The SIMPER analysis revealed a dissimilarity of 87-94% between site H

295   and all other sites, of 55-57% between site G and inactive sites and of 50% between sites CN and

296   CS (see also CLUSTER analysis, Fig. 7).

297   Among all identified families, the most diversified in term of number of genera were

298   Desmodoridae (25 genera), Chromadoridae (21 genera), Cyatholaimidae (14 genera),

299   Comesomatidae (11 genera) and Xyalidae (10 genera). Four families, represented only by one

300   genus, were encountered just in one of the investigated sites: Ceramonematidae (site G),

301   Siphonolaimidae (site H), Rhabdolaimidae and Rhadinematidae (site CS) (Table S2). A total of

302   58 exclusive species were identified, all of them represented by few individuals (between 0.1 –

303   1.9% of the total abundances). The diffusive site H was characterized by the dominance of two

304   nematode species *Oncholaimus* sp1 and *Daptonema* sp1 (less than 1% in the other sites) and by

305   the presence of *Elzalia* sp1, *Linhystera* sp2 and *Parastomonema* sp1 as exclusive species. The

306   geyser site G was characterized by the presence of five abundant species *Chromadorita* sp1,

307   *Desmodora* sp1, *Leptolaimus* sp1, *Microlaimus* sp1and *Paradesmodora* sp1 and by 22 exclusive

308   species (Table S2). The inactive sites were characterized by the presence of 16 (at CN) and 17 (at

309   CS) unique species and by high abundances of *Desmodora* sp1, *Paradesmodora* sp1, *Perspiria*

310   sp1 and *Spirinia* sp1 at CN site and by a slight dominance of *Chromaspirina* sp1 (33.4%) and a

311   high abundance of *Spirinia* sp1 at CS (Table S2).

312   The trophic structure of nematode assemblages was dominated by predators/omnivores (2B;

313   78%) at site H and by epistrate feeders (2A) at sites G (72%) and site CN (71%); the inactive site

314   CS was characterized by the co-dominance of the groups 2A (38%) and 2B (40%) (Fig. 8).

315   Significant differences in the index of trophic diversity values between CS and all other sampling

316   sites (Table 1) was reported. Values of the maturity index were significantly higher at site H and

317   CS compared to the other sites (Table 1 and Table 2).

318

319   **Relationship between meiofauna and environmental variables**

320   The DistLM analysis was performed to assess the influence of environmental variables on faunal

321   assemblages (i.e., meiofauna abundance, taxonomic composition, number of major taxa,

322   nematode abundance and biomass, nematode composition and diversity, and nematode trophic

323   diversity). A combination of sediment (e.g., pH, T°C and TOC content) and interstitial water

324   (e.g., NO_3^{2-}, Na^+, K^+ and S^{2-}) variables considered in our investigation might explain the

325   meiofauna and nematofauna variability in different percentage and depending on the meiofauna

326   and nematode descriptors considered (Table 3). The dbRDA graph (Fig. 9) on meiofauna

327   taxonomic composition showed a separation between sites due to changes in T°C (higher at H

328   site), pH (more acid at H site) and NO_3^{2-} and Cu concentrations. All these variables could explain

329   the 73% of variability in meiofauna composition (Table 3). The dbRDA graph (Fig. 10) on

330   nematode composition showed that changes in nematode communities were significantly

331   correlated (71%) to interstitial water features (Table 3) with a separation of the sampling sites

332   into three main groups: H, G and the inactive sites CN – CS.

333

334   **Discussion**

335

336   **Small-scale spatial environmental heterogeneity induced by vent emissions**

337   Environmental conditions at hydrothermal vent systems markedly differ from background and

338   these differences include increase of temperature, decrease of pH, and enrichment in reduced

339   compounds, gases and heavy metals (Tarasov et al., 2005). Shallow and deep vents are

340   characterized by high heterogeneity and can be defined as 'patchy habitats' (Gollner et al.,

341 2010). Due to hydrothermal activity, patchiness has often been detected in physical and chemical
342 variables at small spatial scales (Dando et al., 1995; Levin et al., 2003; Melwani & Kim, 2008,
343 Gollner et al., 2010). Similarly, at the shallow vent area of SdF we detected a rapid change in the
344 environmental conditions with a consequent marked environmental heterogeneity on a relatively
345 small spatial scale (i.e., one hundred of meters).
346 SdF is characterized by three different habitats: 1) the diffusive emission site (H) in which we
347 reported the highest temperature, acidic conditions as a result of CO_2 gas bubbling (Di Napoli et
348 al., 2016) and the presence of a white microbial mat; 2) the solitary geyser site (G) which
349 differed from the other sites for the presence of sulphur ions in the interstitial waters and yellow
350 sulphurous deposits, medium-high temperature conditions but ambient pH values and 3) the
351 inactive sites (CN and CS) characterized by no extreme conditions (T and pH values within the
352 natural range), the highest percentage of gravel fraction and the lowest TOC content compared to
353 the active sites.
354
355 **Meiofauna distribution and taxonomic composition variability at vent conditions**
356 In the deep-sea, vent emissions negatively impact fauna abundance (Kamenev et al., 1993;
357 Tarasov et al., 1999; 2005), while in shallow water the amount of food material derived from
358 both chemosynthesis and photosynthesis processes (Sorokin et al., 2003) promote the meiofauna
359 abundance that can reach higher values compared to the background sediments (Kamenev et al.,
360 1993).
361 At SdF vent area active and inactive sites did not differ significantly in total meiofauna
362 abundance, contrarily to the drastic drop registered for the macrobenthic density at active sites
363 compared to the inactive sites at SdF (Donnarumma et al., 2019). Overall, total meiofauna
364 abundance reported in this study were higher than those at other shallow vent areas (Kamenev et
365 al., 1993; Thiermann et al., 1997; Tarasov et al., 1999; Colangelo et al., 2001, Zeppilli &
366 Danovaro, 2009). The lack of significant differences between sites is possibly due to a
367 pronounced inter-replicates variability that reflects the irregular and patchy distribution of
368 meiofauna. We found the same small spatial-scale patchiness in the vertical distribution of
369 meiofauna, with no significant differences in total abundances moving deeper into the sediment
370 layers but only a trend in increasing abundance values from the surface to the deeper layers.
371 Meiofauna is known to be characterized by a patchy distribution and this is particularly evident

372 at small spatial scale (i.e., microhabitats; Gallucci et al., 2009). At vents, the very small-scale

373 distribution is related to the spatial heterogeneity of biogeochemical vent processes that normally

374 occurs in these extreme environments (Tarasov et al., 2005; Di Bella et al., 2016). In this study,

375 the combination of vent small heterogeneity and meiofauna patchiness is particular evident.

376 Meiofauna distribution can vary more along the sediment vertical profile than along a horizontal

377 axe (Fonseca et al., 2010). Only few studies documented the vertical distribution of meiobenthic

378 organisms inhabiting shallow vent areas showing a decreasing trend with increasing sediment

379 depth (Ansari et al., 2001; El-Sehery et al., 2015; Di Bella et al., 2016). Sediment physical

380 disturbance is known to impact meiofaunal vertical distribution (Braeckman et al., 2011). In

381 some cases, nematodes can migrate from the surface disturbed layers into the less impacted

382 deeper layers (Leduc & Pilditch 2013). At SdF the highest abundances were recorded at the

383 deeper layers. The bubble streams coupled with near-bottom currents and sediment rework by

384 larger fauna might possibly have a role in meiofauna sediment penetration by inducing a

385 migratory response to the deeper layers (Colangelo 2001; Steyaert et al., 2003).

386 Meiofauna diversity was higher at SdF than in the other shallow vent meiofauna studies (Fricke

387 et al., 1989; Kamenev et al., 1993; Dando et al., 1995; Tarasov et al., 1999; Colangelo et al.,

388 2001; Zeppili & Danovaro, 2009). Nematodes represented a key taxon of meiobenthic

389 communities at shallow vents (Kamenev et al., 1993; Thiermann et al., 1997; Tarasov et al.,

390 2005). Similarly to results of Colangelo et al. (2001) and Zeppilli & Danovaro (2009), we

391 reported a dominance of copepods at one of our four sites, site H, which showed a different

392 assemblage structure in respect to the others. Previous authors (Coull, 1985; Colangelo et al.,

393 2001) explained this dominance invoking the alteration of sediment grain size and the preference

394 for coarser sediments by this taxon, coupled with the effect of intermediate disturbance created

395 by a moderate gas bubbling that seemed to promote copepod diversity (Colangelo et al., 2001).

396 At the diffusive emission site (H), we did not detect any clear alteration of sediment grain size

397 but we can hypothesize a gas bubbling effect due to the presence of CO_2 emissions. Moreover,

398 the presence of white microbial mat at site H could provide food for vent obligate copepods,

399 which have developed specific adaptation to feeding on bacterial mats (Heptner & Ivanenko,

400 2002). High abundance of copepods characterized by a high species richness was reported also

401 from deep-sea vents (Gollner et al. 2010), confirming their ability to be well adapted to the

402 extreme vent conditions. Two other taxa, usually neglected in the meiobenthic studies because

403   part of the unicellular organisms, constituted two abundant components of site H vent
404   assemblage and they probably took advantage feeding on microbial mat: foraminifers and
405   ciliates. Bernhard et al. (2000) found that the microbial mats in the anoxic, sulphidic waters of
406   the Santa Barbara Basin supported abundant communities of foraminifera, flagellates and
407   ciliates. Benthic foraminifers represent an important environmental sensitive group and their
408   composition is strictly related to venting activity (Di Bella et al., 2016). Protists (e.g., ciliates)
409   play several roles in marine ecosystems and form a trophic link between prokaryotes and higher
410   trophic levels; they impact carbon and other nutrient cycles directly and indirectly through
411   grazing on organic matter and prokaryotic prey (Anderson et al., 2012).
412   The meiofaunal taxonomic composition usually varies between surface and subsurface sediment
413   layers: copepods and *nauplii* occupy the well oxygenated surface sediment layer (Grego et al.,
414   2014), while nematodes become the dominant taxon at subsurface depths (Ingels et al., 2009;
415   Rosli et al., 2016). Meiobenthic organisms at SdF vent followed the same trends for nematodes
416   and copepods at all sites.
417

418   **Nematode community reflects Secca delle Fumose environmental heterogeneity**
419   At SdF shallow vent, nematode community composition and functional diversity varied from site
420   to site reflecting the environmental heterogeneity of the sampling area. The same marked change
421   in fauna composition was reported for the macrobenthic communities inhabiting SdF, with an
422   absence of more sensitive species at the active sites (Donnarumma et al., 2019). The high
423   variability in the nematode assemblages (i.e., percentage of dissimilarity between sites) and the
424   presence of exclusive species characterizing each sampling site accounted for the differences in
425   the biodiversity composition. This change in nematofauna was mainly due to a decrease of
426   nematode genera/species at site H and to a change in the abundances and/or replacement of
427   species among the other sites (G *vs*. CN *vs*. CS).
428   At the diffusive emission site H, where we reported the most extreme conditions, nematode
429   assemblage showed some typical traits of nematode assemblages inhabiting shallow
430   (Thiermann et al., 1994; Dando et al., 1995; Tarasov et al., 1999; Zeppilli & Danovaro, 2009)
431   and deep-sea active vent sites (Tschesunov, 2015; Zeppilli et al., 2018): biomass values from
432   twofold to threefold higher compared to that of the active site G and inactive sites due to the
433   dominance of big size *Oncholaimus* genus (i.e., very low equitability value), the lowest

434   nematode diversity (species richness) and functional diversity (trophic diversity). In this case,

435   and as reported in Donnarumma et al. (2019) for the macrofauna, the harsh hydrothermal

436   conditions affect the nematode assemblages.

437   Nematodes belonging to the family Oncholaimidae (genus *Oncholaimus*) have been reported

438   several times inhabiting sediments near to source of the emissions at shallow vents (e.g., Dando

439   et al., 1995; Thiermann et al., 1997; Zeppilli & Danovaro, 2009). This genus can tolerate high

440   sulphide concentrations by producing sulphur-containing droplets to reduce the toxic effect of

441   hydrogen sulphide (Thiermann et al., 1994, 1997). *Oncholaimus* has been originally identified as

442   predator/omnivore/scavenger (Jensen, 1987), but recently it has been shown that this nematode

443   can feed also on free-living chemoautotroph microorganisms (Zeppilli et al., 2019). This wide

444   diet makes *Oncholaimus* able to use the different food sources available at shallow vent: other

445   organisms (as alive or dead animals) and bacterial mat (Thiermann et al., 1997). Oncholaimidae

446   are also known to have symbiotic associations (Bellec et al., 2018; Bellec et al., 2019). The

447   deep-sea nematode *Oncholaimus dyvae* can harbor sulfur-oxidizing bacteria in the cuticle and in

448   its intestine (Bellec et al., 2018). *Metoncholaimus albidus* inhabiting shallow water anoxic

449   sediments hosts ectosymbiotic bacteria involved in sulfur metabolism suggesting a potential for

450   chemosynthesis in the nematode microbial community (Bellec et al., 2019). At SdF,

451   *Oncholaimus* showed endosymbiotic sulfur-oxidizing and -reducing bacteria, purple sulfur

452   bacteria and *Zetaproteobacteria* in the intestine (Bellec et al., submitted).

453   Moreover, the high abundance and biomass values characterizing *Oncholaimus* suggested that

454   this nematode could have a significant impact on the turnover of organic matter. *Daptonema sp1*

455   was the second most abundant species at site H; this genus was frequently found at vent areas

456   (Vanreusel et al., 1997; Zeppilli & Danovaro, 2009). The low trophic diversity index value was

457   the consequence of the dominance of the genera *Oncholaimus* and *Daptonema*, but deposit

458   feeders and predators/scavengers are typically reported from shallow vent systems where

459   organisms can feed on available organic resources.

460   The drastic decrease in macrobenthic biodiversity observed at geyser site due to the effect of

461   high sediment temperature and sulfide ions (Donnarumma et al., 2019), was not reported for the

462   nematofauna that showed very low diversity values only at H site.

463   The nematode diversity at geyser (site G) was comparable to that at the inactive sites, even if the

464   geyser hosted its own nematode community characterized by the highest number of exclusive

465   species (22). While the macrobenthic assemblages around site G was defined as "simplified"
466   community that represented a subset of the background biota (Donnarumma et al., 2019), the
467   presence of sulphur ion S^{2-} did not constitute a source of disturbance for nematodes structural
468   and functional diversity. According to the ecological theory of 'intermediate disturbance'
469   (Huston, 1979), across an environmental stress gradient higher diversity is expected at
470   intermediate stress levels (i.e., site G), whilst at higher levels of stress only colonizing species
471   survive (i.e., site H). Similarly, Colangelo et al. (2001) reported higher copepod diversity values
472   in areas with moderate gas seepage and sulphur deposits. At site G we did not observe a clear
473   dominance of one or few genera (i.e., high value of equitability index), and the most represented
474   genera such as *Leptolaimus, Desmodora, Paradesmodora* and *Chromadorita* were already
475   reported from other vent areas (Dando et al., 1995; Vanreusel et al., 1997; Zeppilli & Danovaro,
476   2009).
477   At inactive sites (CN and CS) the family of Desmodoridae (genera *Spirinia, Perspiria* and
478   *Chromaspirina*) was the most represented taxon. Those genera, and in particular different species
479   of *Spirinia* and *Chromaspirina*, have been reported from different environments spanning from
480   shallow (Platt, 1977; Nicholas et al., 1991; Olafsson, 1995; Vafeiadou et al., 2014) to deep-sea
481   (Da Silva et al., 2009; Leduc and Verschelde, 2015) systems and sometimes associated to
482   unstable condition and coarser sediment, as the sediment at our inactive sites from where we
483   detected the highest percentage of gravel. Another group found at inactive sites was that of
484   Stilbonematinae, reported already from shallow (Kamenev et al., 1993; Thiermann et al., 1994;
485   Dando et al., 1995) and deep-sea vent areas (Vanreusel et al., 1997; Gollner et al., 2010) and
486   inhabiting zones of volcanic activity or at the periphery of vents. Stilbonematine nematodes are
487   common in suitable tropical shallow-water carbonate sands (Ott et al., 2003, 2004), but they are
488   also adapted to life at seeps, organically enriched bottoms (Giere, 2009) and sulphidic sediments
489   where they feed on ectosymbiotic sulphur-oxidizing bacteria (Ott et al., 1991). Stilbonematine
490   nematodes have chemosynthesising ectobacteria covering their bodies, which constitute an
491   adaptation to life in silt enriched with H_2S (Powell e a., 1979; Jensen, 1986). We found
492   stilbonematine nematodes at SdF sediments with their body completely covered by ectobacteria.
493   Their presence at inactive sites, even if in low abundances, suggests the presence of localized
494   and very likely patchy reduced conditions at those sites (Tchesunov et al., 2012) caused by
495   accumulation and burial of dead organic matter.

496   The trophic structure of assemblages inhabiting the geyser and the inactive site CN was similar

497   to that reported from other shallow vent systems (Kharlamenko et al., 1995; Zeppilli &

498   Danovaro, 2009), were epistrate feeders dominated because favored by the high primary

499   biomass. The bulk of biomass does not rely solely on symbiotrophs, but on the available organic

500   resources (i.e., deposit feeders, epistrate feeders, predators/omnivores) and this was confirmed

501   also by the significant correlation between TOC content and nematode trophic diversity at SdF.

502   This is particularly true for the inactive site CS where all trophic groups were well represented

503   (i.e., the lowest value of ITD) and therefore underling the ability of nematodes to use all the

504   available food sources and /or to partition multiple food sources (Limén et al., 2007).

505   Vent nematodes belong to families and genera already known from non-vent habitats (Vanreusel

506   et al., 1997; Flint et al., 2006; Zeppilli et al., 2018), suggesting no endemicity at genera or

507   families level for this taxon. However, at a species level many of the nematode present in

508   samples from deep-sea vents appeared to be new, suggesting the presence of unique or endemic

509   species adapted to the vent conditions (Copley et al., 2007; Gollner et al., 2010). In contrast to

510   deep-sea hydrothermal vents, nematode inhabiting shallow vent areas include a subset of species

511   that live in background sediments but can survive in extreme conditions (e.g., Tarasov et al.,

512   2005; Zeppilli & Danovaro, 2009; Zeppilli et al., 2018). However, this is was not the case at SdF

513   vent area where each one of the investigated site (i.e., active H-G and inactive CN-CS sites) was

514   characterized by a distinct nematode community reflecting the high spatial heterogeneity. The

515   26% (at site CN) and 28% (at site CS) of species were unique at the inactive sites, but the highest

516   percentages of exclusive species were found characterizing the active sites H (30%) and G

517   (38%). In our study, we noticed a lack of dominance by the nematode genera usually found to be

518   abundant at shallow (e.g., *Sabatiera, Linhomoeus, Siphonolaimus*, *Pomponema, Dichromadora,*

519   *Paracanthoncus* and *Steineridora*; Dando, Hughes & Thiermann, 1995; Zeppilli & Danovaro,

520   2009) and deep-sea (*Molgolaimus*, *Monhystera* and *Thalassomonhystera*; Vanreusel et al., 1997;

521   Zekely et al., 2006) vents, except for the genus *Oncholaimus*. On the other hand, most of the

522   exclusive genera/species inhabiting the investigated sites are not frequently reported from other

523   vent areas, sustaining the presence of a typical nematofauna assemblage at SdF. Moreover, we

524   noticed a preponderance of monospecific nematode genera in all samples with the 95% of genera

525   that exhibited a species (i.e., morphotype) to genus ratio of 1 as reported from deep-sea vent

526   systems **(**Vanreusel et al., 1997; Gollner et al., 2010).

527

528 **Effects of SdF vent conditions on meiobenthic comunities**

529 In the recent study of Donnarumma et al. (2019), it has been reported how macrobenthic

530 community structure was significantly correlated with some of the environmental variables (e.g.,

531 pH, TOC, temperature and interstitial water ions), the same variables that mostly determined the

532 differences between the investigated sites. Similarly, the meiofauna and nematofauna richness

533 and community composition were correlated with the same environmental parameters (Table 3).

534 The effects of sediment temperature, TOC, pH and ions (e.g., NO_3^{2-}, Ca^{2+} and Cl^-) were

535 particularly clear in influencing patterns of meiofauna and nematofauna communities (Fig. 7 and

536 Fig. 8).

537 Separation between H, G and CN-CS sites was even more evident when we considered the

538 nematode community composition. The same separation between sites occurred if we considered

539 the macrobenthic community composition (Donnarumma et al., 2019), confirming the

540 importance of key environmental factors for benthic communities such as: sediment temperature,

541 pH value, TOC content and interstitial water characteristics. But if for the macrobenthic

542 community those key factors led to a drastic decrease in density and diversity at both active sites,

543 for the meiofauna and nematofauna assemblage this effect was reported only at H site where

544 conditions were the most extreme and were few adapted species can survive.

545
546 **Conclusions**
547
548 This study confirmed some of the trends often observed in vent-associated benthic communities,

549 i.e. a pronounced small-scale spatial variability of meiofauna and particularly nematode

550 composition that reflect the natural patchy distribution of this benthic components and the high

551 environmental heterogeneity of the study area, typically from extreme environments. We noticed

552 a migratory response to deeper layers by the meiofauna due to sediment disturbance (e.g. bubble

553 streams, near-bottom currents, sediment reworking by larger fauna), this is also a common

554 phenomenon reported from such a kind of environment. High nematode biomass values, low

555 diversity and the dominance by the single highly tolerant genus *Oncholaimus* at station with the

556 harshest conditions (H) was not surprising for vent systems. Other findings, however, appear to

557 contradict some general accepted tenets of shallow water hydrothermal vents ecology. We

558 reported higher values of meiofaunal abundance and diversity characterizing Secca delle Fumose

559 shallow vent compared to other shallow vent areas. Nematodes inhabiting sediments of SdF were

560 clearly different and each one of the investigated site was characterized by a distinct nematode

561 community, that means that nematodes at SdF shallow vent did not constitute a subset of species

562 that live in background sediments. In our study, we noticed a lack of dominance by the nematode

563 genera usually found to be abundant at shallow and deep-sea vents, except for *Oncholaimus*

564 genus, sustaining the presence of a typical nematofauna community at SdF.

565 **Acknowledgments**

566 We thank the Soprintendenza of the Underwater Archeological Park of Baia (prot. 5667,
567 24/10/2016) for the authorization to sampling. We are in debt to the diving center Centro SUB
568 Pozzuoli (Guglielmo Fragale) for support during diving activities. We thank Dr. Renato Bruno,
569 Dr. Aurélie Tasiemski and Dr. Céline Boidin-Wichlacz for helping in sampling activities.
570
571
572

573 **References**
574
575 Anderson MJ, Gorley RN, Clarke KR. 2008. *PERMANOVA+ for PRIMER: guide to software*
576   *and statistical methods.* PRIMER-E Ltd., Plymouth, United Kingdom.
577 Anderson R, Winter C, Jürgens K. 2012. Protist grazing and viral lysis as prokaryotic mortality
578   factors at Baltic Sea oxic-anoxic interfaces. *Marine Ecology Progress Series* 467:1-14
579   DOI: https://doi.org/10.3354/meps10001.
580 Andrassy I. 1956. The determination of volume and weight of nematodes. *Acta Zoologica*
581   *Hungarica* 2: 1–15.
582 Ansari A, Rivonkar CU, Sangodkar UMX. 2001. Population fluctuation and vertical distribution
583   of meiofauna on a tropical mudfat at Mandovy estuary, west coast of India. *Indian Journal of*
584   *Marine Sciences* 30: 237-245 DOI: http://nopr.niscair.res.in/handle/123456789/4627.
585 Appolloni L, Sandulli R, Vetrano G, Russo GF. 2018. Assessing the effects of habitat patches
586   ensuring propagule supply and different costs inclusion in marine spatial planning through
587   multivariate analyses. *Journal of Environmental Management* 214, 45–55.
588   doi:10.1016/j.jenvman.2018.02.091.
589 Bernhard JM, Buck K R, Farmer MA, Bowser SS. 2000. The Santa Barbara Basin is a symbiosis
590   oasis. *Nature* 403(6765): 77 DOI: https://doi.org/10.1038/47476.
591 Bezzerra TN, Decraemer W, Eisendle-Flöckner U, Hodda M, Holovachov O, Leduc D, Miljutin
592   D, Mokievsky V, Peña Santiago R, Sharma J, Smol N, Tchesunov A, Venekey V, Zeng Z,
593   Vanreusel A. 2018. NeMys: World Database of Nematodes, available at:
594   http://nemys.ugent.be/ (accessed March, 2018).
595 Bongers T, Alkemade R, Yeates GW. 1991. Interpretation of disturbance-induced maturity
596   decrease in marine nematode assemblages by means of the Maturity Index. *Marine Ecology*
597   *Progress Series* 76: 135–142 DOI: https://www.jstor.org/stable/24825556.
598 Braeckman U, Provoost P, Moens T, Soetaert K, Middelburg JJ, Vincx M, Vanaverbeke J. 2011.
599   Biological vs. Physical Mixing Effects on Benthic Food Web Dynamics. *PLoS ONE* 6(3):
600   e18078. DOI:10.1371/journal.pone.0018078.

601   Cardigos F, Colaço A, Dando PR, Ávila SP, Sarradin PM, Tempera F, Conceição P, Pascoal A,
602   Serraõ SR. 2005. Shallow water hydrothermal vent field fluids and communities of the D.
603   João de Castro Seamount (Azores). *Chemical Geology* 224: 153–168 DOI:
604   https://doi.org/10.1016/j.chemgeo.2005.07.019.
605   Clarke KR, Gorley RN. 2006. *User manual/tutorial*. PRIMER-E Ltd., Plymouth, UK.
606   Clarke KR, Warwick RM. 2001. *Change in Marine Communities: An Approach to Statistical*
607   *Analysis and Interpretation*. PRIMER-E Ltd., Plymouth, UK.
608   Colangelo MA, Bertasi R, Dall'Olio P, Ceccherelli VH. 2001. *Meiofaunal biodiversity on*
609   *hydrothermal seepage off Panarea (Aeolian Island, Tyrrhenian Sea)*. In: Faranda FM,
610   Guglielmo L, Spezie G (eds) Structure and processes in Mediterranean ecosystems. Springer-
611   Verlag, Heidelberg, p 353–359.
612   Copley JTP, Flint HC, Ferrero TJ, Van Dover CL. 2007. Diversity of meiofauna and free-living
613   nematodes in hydrothermal vent mussel beds on the northern and southern East Pacific Rise.
614   *Journal of the Marine Biological Association of the United Kingdom* 87: 1141–1152 DOI:
615   https://doi.org/10.1017/S0025315407055956.
616   Coull BC. 1985. Long-term variability in estuarine meiobenthos: an 11 year study. *Marine*
617   *Ecology Progress Series* 24:205-218 DOI: https://www.jstor.org/stable/24816886.
618   D'Auria L, Giudicepietro F, Aquino I, Borriello G, Del Gaudio C, Lo Bascio D, Martini M,
619   Ricciardi G P, Ricciolino P, Ricco C. 2011. Repeated fluid-transfer episodes as a mechanism
620   for the recent dynamics of Campi Flegrei caldera (1989–2010). *Journal of Geophysical*
621   *Research-Solid Earth* 116: B04313 DOI:10.1029/2010JB007837.
622   Da Silva MC, De Castro FJV, Da Fonseca Cavalcanti M, Da Fonseca-Genevois V. 2009.
623   *Spirinia lara* sp. n. and *Spirinia sophia* sp. n. (Nematoda, Desmodoridae) from the Brazilian
624   continental margin (Campos Basin, Rio de Janeiro). Zootaxa 2081: 31–45.
625   Dando PR, Hughes JA, Leathy Y, Niven SJ, Smith C. 1995. Gas venting rates from submarine
626   hydrothermal areas around the Island of Milos, Hellenic Volcanic Arc. *Continental Shelf*
627   *Research* 15: 913–929 DOI: https://doi.org/10.1016/0278-4343(95)80002-U.
628   De Grisse A. 1969. Redescription ou modifications de quelques technique utilisés dans l'etude
629   des nématodes phytoparasitaires. *Meded. Rijksfak. LandbWet*. Gent 34 : 251–369.
630   Di Bella L, Ingrassia M, Frezza V, Chiocci FL, Martorelli E. 2016. The response of benthic
631   meiofauna to hydrothermal emissions in the Pontine Archipelago, Tyrrhenian Sea (central
632   Mediterranean Basin). *Journl of Marine Systems* 164:53–66 DOI:
633   https://doi.org/10.1016/j.jmarsys.2016.08.002.
634   Donnarumma L, Appolloni L., Chianese E, Bruno R, Baldrighi E, Guglielmo R, Russo GF,
635   Zeppilli D, Sandulli R. 2019. Environemental and benthic community patterns of the shallow
636   hydrothermal area of Secca della Fumose (Baia, Naples, Italy). *Frontiers in Marine Science*
637   6:685 DOI: 10.3389/fmars.2019.00685.
638   Duquette A., James B. McClintock, Charles D. Amsler, Alberto Pérez-Huerta, Marco Milazzo,
639   Jason M. Hall-Spencer 2017. Effects of ocean acidification on the shells of four
640   Mediterranean gastropod species near a CO2 seep. *Marine Pollution Bulletin* 124 :917-928
641   DOI: http://dx.doi.org/10.1016/j.marpolbul.2017.08.007.
642   Eleftheriou A. 2013. *Methods for the study of marine benthos. Fourth Edition*, ed. A.
643   Eleftheriou John Wiley & Sons, Ltd.
644   Fitzsimons MF, Dando PR, Hughes JA, Thiermann F, Akoumianaki I, Pratt SM. 1997.
645   Submarine hydrothermal brine seeps off Milos, Greece. Observations and

646   geochemistry. *Marine Chemstry* 57: 325-340 DOI: https://doi.org/10.1016/S0304-
647   4203(97)00021-2.
648 Flint HC, Copley JTP, Ferrero TJ, Van Dover CL. 2006. Patterns of nematode diversity at
649   hydrothermal vents on the East Pacific Rise. *Cahiers de Biologie Marine* 47:365-370 DOI:
650   https://eprints.soton.ac.uk/id/eprint/35558.
651 Fricke H, Giere O, Stetter K, Alfredson GA, Kristjansson JK, Stoffers P, Svavarsson J. 1989.
652   Hydrothermal vent communities at the shallow subpolar Mid-Atlantic Ridge. *Marine Biology*
653   102: 425–429 DOI: https://doi.org/10.1007/BF00428495.
654 Gallucci F, Moens T, Vanreusel A, Fonseca G. 2008. Active colonisation of disturbed sediments
655   by deep-sea nematodes: evidence for the patch mosaic model. *Marine Ecology Progress*
656   *Series*
657   367:173–183 DOI:10.3354/meps07537.
658 Gambi C, Vanreusel A, Danovaro R. 2003. Biodiversity of nematode assemblages from deep-sea
659   sediments of the Atacama Slope and Trench (South Pacific Ocean). *Deep Sea Research Part*
660   *I: Oceanographic Research Papers* 50:103–117 DOI: https://doi.org/10.1016/S0967-
661   0637(02)00143-7.
662 Giere O. 2009. *Meiobenthology. The microscopic motile fauna of aquatic sediments*. Berlin,
663   Heidelberg: Springer-Verlag. 527 p.
664 Gollner S, Riemer B, Martìnez Arbizu P, Le Bris N, Bright M. 2010. Diversity of meiofauna
665   from the 9°50'N East Pacific Rise across a gradient of hydrothermal fluid emissions. *PLoS*
666   *ONE* 5(8): e12321 DOI:10.1371/journal.pone.0012321.
667 Gray JS. 2000. The measurement of marine species diversity, with an application to the benthic
668   fauna of the Norwegian continental shelf. *Journal of Experimental Marine Biology and*
669   *Ecology* 250: 23–49 DOI: https://doi.org/10.1016/S0022-0981(00)00178-7.
670 Grego M, Riedel B, Stachowitsch M, De Troch M. 2014. Meiofauna winners and losers of
671   coastal hypoxia: case study harpacticoid copepods. *Biogeosciences* 11:281–292 DOI:
672   10.5194/bg-11-281-2014.
673 Hall-Spencer JM, Rodolfo-Metalpa R, Martin S, Ransome E, Fine M, Turner SM, Rowley SJ,
674   Tedesco D, Buia MC. 2008. Volcanic carbon dioxide vents show ecosystem effects of ocean
675   acidification. *Nature* 454: 96–99 DOI: 10.1038/nature07051.
676 Heip C, Vincx M, Vranken G. 1985. *The ecology of marine nematodes*. Aberdeen University
677   Press.
678 Heptner MV, Ivanenko VN. 2002. *Hydrothermal vent fauna: composition, biology and*
679   *adaptation. Copepoda*. Biology of hydrothermal systems (ed. A. Gebruk), 159-176.
680 Huston M. 1979. A general hypothesis of species diversity. *The American Naturalist* 113: 81-
681   101. DOI: https://doi.org/10.1086/283366.
682 Ingels J, Kiriakoulakis K,Wolff GA, Vanreusel A. 2009. Nematode diversity and its relation to
683   the   quantity and quality of sedimentary organic matter in the deep Nazare´ Canyon,Western
684   Iberian Margin. *Deep Sea Research Part I: Oceanographic Research Papers* 56:1521–1539
685   DOI: 10.1016/j.dsr.2009.04.010.
686 Jensen P. 1987. Feeding ecology of free-living aquatic nematodes. *Marine Ecology Progress*
687   *Series* 35:187–196 DOI: www.jstor.org/stable/24825022.
688 Jones RW. 1993. *Preliminary observations on benthonic foraminifera associated with biogenic*
689   *gas seep in the North Sea*. In: Jenkins, D.G. (Ed.), Applied Micropalaeontology. Springer
690   Science, Dordrecht, pp. 69–91.

691 Judd AG, Hovland M. 2007. *Seabed Fluid Flow: The Impact on Geology, Biology and the*
692   *Marine Environment*. first ed. Cambridge University Press, Cambridge.
693 Kamenev GM, Fadeev VI, Selin NI, Tarasov VG, Malakhov VV. 1993. Composition and
694   distribution of macro- and meiobenthos around sublittoral hydrothermal vents in the Bay of
695   Plenty, New Zealand. *New Zealand Journal of Marine and Freshwater Research* 27: 407–418
696   DOI: https://doi.org/10.1080/00288330.1993.9516582.
697 Kharlamenko VI, Zhukova NV, Khotimchenko SV, Svetashev VI, Kamenev GM. 1995. Fatty
698   acids as markers of food sources in a shallow-water hydrothermal ecosystem (Kraternaya
699   Bight, Yankich Island, Kurile Islands). *Marine Ecology Progress Series* 120:231–241
700   DOI: 10.3354/meps120231.
701 Leduc D, Pilditch CA. 2013. Effect of a physical disturbance event on deep-sea nematode
702   community structure and ecosystem function. *Journal of Experimental Marine Biology and*
703   *Ecology* 440: 35–41 DOI: http://dx.doi.org/10.1016/j.jembe.2012.11.015.
704 Leduc D, Verschelde D. 2015. New *Spirinia* and *Stygodesmodora* species (Nematoda,
705   Spiriniinae) from the Southwest Pacific, and a revision of the related genera *Spirinia*,
706   *Chromaspirina* and *Perspiria*. *European Journal of Taxonomy* 118: 1–25 DOI:
707   http://dx.doi.org/10.5852/ejt.2015.118.
708 Limén H, Levesque C, Juniper SK. 2007. POM in macro-/meiofaunal food webs associated with
709   three flow regimes at deep-sea hydrothermal vents on Axial Volcano, Juan de Fuca Ridge.
710   *Marine Biology* 153:129–139 DOI: 10.1007/s00227-007-0790-1.
711 Melwani AR, Kim SL. 2008. Benthic infaunal distributions in shallow hydrothermal vent
712   sediments. *Acta Oecologica* 33: 162–175 DOI: https://doi.org/10.1016/j.actao.2007.10.008.
713 Nicholas WL , Elek JA, Stewart AC, Marples TG. 1991 . The nematode fauna of a temperate
714   Australian mangrove mudflat; its population density, diversity and distribution .
715   *Hydrobiologia*   209: 13-27 DOI: https://doi.org/10.1007/BF00006714.

716 Ólafsson E. 1995. Meiobenthos in mangrove areas in eastern Africa with emphasis on
717   assemblage structure of free-living marine nematodes. *Hydrobiologia* 312: 47-57. DOI:
718   https://doi.org/10.1007/BF00018886.
719 Panieri G, Gamberi F, Marani M, Barbieri R. 2005. Benthic foraminifera from a recent, shallow-
720   water hydrothermal environment in the Aeolian Arc (Tyrrhenian Sea). *Marine Geology* 218:
721   207–229 DOI: https://doi.org/10.1016/j.margeo.2005.04.002.
722 Panieri G. 2006a. The effect of shallow marine hydrothermal vent activity on benthic
723   foraminifera (Aeolian Arc, Tyrrhenian Sea). The *Journal of Foraminiferal Research* 36: 3–
724   14 DOI: https://doi.org/10.2113/36.1.3.
725 Panieri G. 2006b. Foraminiferal response to an active methane seep environment: a case study
726   from the Adriatic Sea. *Marine Micropaleontology* 61: 116–130 DOI:
727   https://doi.org/10.1016/j.marmicro.2006.05.008.
728 Pichler TP, Veizer J, Hall GEM. 1999. The chemical composition of shallow-water hydrothermal
729   fluids in Tatum Bay, Ambitle Island, Papua New Guinea and their effect on ambient water.
730   *Marine Chemstry* 64: 229–252 DOI: https://doi.org/10.1016/S0304-4203(98)00076-0.
731 Pielou EC. 1975. *Ecological diversity*. Wiley, New York.
732 Platt HM, Warwick RM. 1983. A Synopsis of the Free Living Marine Nematodes. Part I: British
733   Enoplids. Cambridge University Press, Cambridge.
734 Platt HM, Warwick RM. 1988. Free-living marine nematodes: Part II. British chromadorids. EJ
735   Brill, Dr. W. Backhuys for the Linnean Society of London and the Estuarine and Brackish-

736   water Sciences Association Synopses of the British Fauna No. 38. Cambridge University
737   Press, Cambridge.
738 Platt HM. 1977. Vertical and horizontal distribution of free-living marine nematodes from
739   Strangford Lough, Northern Ireland. *Cahiers de Biologie Marine*, Tome XVIII, pp. 261-273.
740 Prol-Ledesma RM, Canet C, Torres-Vera MA, Forrest MJ, Armienta MA. 2004. Vent fluid
741   chemistry in Bahia Concepcion coastal submarine hydrothermal system, Baja California Sur,
742   Mexico. *Journal of Volcanology and Geothermal Research* 137: 311–328. DOI:
743   https://doi.org/10.1016/j.jvolgeores.2004.06.003.
744 Riemann F, Thiermann F, Bock L. 2003. Leptonemella species (Desmodoridae,
745   Stilbonematinae), benthic marine nematodes with ectosymbiotic bacteria, from littoral sand of
746   the North Sea island of Sylt: taxonomy and ecological aspects. *Helgoland Marine Research*
747   57: 118-131 DOI: https://doi.org/10.1007/s10152-003-0149-z.
748 Rosli N , Leduc D, Rowden AA, Clark MR, Probert PK, Berkenbusch K, Neira C. 2016.
749   Differences in meiofauna communities with sediment depth are greater than habitat effects on
750   the New Zealand continental margin: implications for vulnerability to anthropogenic
751   disturbance. *PeerJ* 4:e2154 DOI: 10.7717/peerj.2154.
752 Sandulli R, Miljutin D, Angeletti L, Taviani M. 2015. Meiobenthos and nematode assemblages
753   from different deep-sea habitats of the Strait of Sicily (Central Mediterranean Sea*).*
754   *Mediterranean Marine Science* 16: 402-412 DOI: http://dx.doi.org/10.12681/mms.1145.
755 Schumacher BA. 2002. Methods for the determination of total organic carbon (TOC) in soils and
756   sediments. NCEA-C- 1282 EMASC-001.
757 Scrosati R, Heaven C. 2007. Spatial trends in community richness, diversity, and evenness across
758   rocky intertidal environmental stress gradients in eastern Canada. *Marine Ecology*
759   *Progress Series* 342: 1–14 DOI: 10.3354/meps342001.
760 Sorokin,YuI, Sorokin PYu, Zakuskina OYu. 1998. Microplankton and its functional activity in
761   zones of shallow hydrotherms in the western Pacific. *Journal of Plankton Research* 20:1015–
762   1031 DOI: https://doi.org/10.1093/plankt/20.6.1015.
763 Steyaert M, Vanaverbeke J, Vanreusel A, Barranguet C, Lucas C, Vincx M. 2003. The
764   importance of fine-scale, vertical profiles in characterising nematode community
765   structure. *Estuarine, Coastal and Shelf Science* 58: 353-366 DOI:
766   https://doi.org/10.1016/S0272-7714(03)00086-6.
767 Tarasov VG, Gebruk AV, Mironov AN, Moskalev LI. 2005. Deep-sea and shallow-water
768   hydrothermal vent communities: two different phenomena? *Chemical Geology* 224: 5–39
769   DOI: https://doi.org/10.1016/j.chemgeo.2005.07.021.
770 Tarasov VG, Gebruk AV, Mironov AN, Moskalev LI. 2005. Deep-sea and shallow-water
771   hydrothermal vent communities: two different phenomena? *Chemical Geology* 224: 5–39
772   DOI: https://doi.org/10.1016/j.chemgeo.2005.07.021.
773 Tarasov VG, Gebruk AV, Shulkin VM, Kamenev GM, Fadeev VI, Kosmynin VN, Malakhov
774   VV, Starynin DA, Obzhirov AI. 1999. Effect of shallow-water hydrothermal venting on the
775   biota of Matupi Harbour (Rabaul Caldera, New Britain Island, Papua New Guinea).
776   *Continental Shelf Research* 19: 79–116 DOI: https://doi.org/10.1016/S0278-4343(98)00073-
777   9.
778 Tchesunov AV, Schmidt-Rhaesa A. (2014). Handbook of zoology. Gastrotricha, Cycloneuralia,
779   Gnathifera. Volume 2: Nematoda.

Manuscript to be reviewed

780   Tchesunov AV. 2013. Marine free-living nematodes of the subfamily Stilbonematinae
781   (Nematoda, Desmodoridae): taxonomic review with descriptions of a few species from the
782   Nha Trang Bay, Central Vietnam. *Meiofauna Marina* 20: 71–94.
783   Tchesunov AV. 2015. Free-living nematode species (Nematoda) dwelling in hydrothermal sites
784   of the North Mid-Atlantic Ridge. *Helgoland Marine Research* 69: 343–384 DOI:
785   10.1007/s10152-015-0443-6.
786   Teixidó N, Gambi MC, Parravacini V, Kroeker K, Micheli F, Villéger S, Ballesteros E. 2019.
787   Functional biodiversity loss along natural CO2 gradients. *Nature Communications* 9:5149
788   DOI: 10.1038/s41467-018-07592-1.
789   Thiermann F, Akoumianaki I, Hugjes AJ, Giere O. 1997. Benthic fauna of a shallow-water
790   gaseohydrothermal vent area in the Aegean Sea (Milos, Greece). *Marine Biology* 128 149–
791   159 DOI: https://doi.org/10.1007/s002270050078.
792   Thiermann F, Windoffer R, Giere O. 1994. Selected meiofauna around shallow water
793   hydrothermal vents off Milos (Greece): ecological and ultrastructural aspects. *Vie Milieu* 44:
794   215–226.
795   Todesco M. 2009. Signals from the Campi Flegrei hydrothermal system: Role of a ''magmatic''
796   source of fluids. *Journal of Geophysical Research-Solid Earth* 114: B05201
797   DOI:10.1029/2008JB006134.
798   Vafeiadou AM, Materatski P, Adão H, De Troch M, Moens T. 2014. Resource utilization and
799   trophic position of nematodes and harpacticoid copepods in and adjacent to *Zostera noltii*
800   beds. *Biogeosciences* 11:4001–4014 DOI:10.5194/bg-11-4001-2014.
801   Vanreusel A, De Groote A, Gollner S, Bright M. 2010. Ecology and biogeography of free-living
802   nematodes associated with chemosynthetic environments in the deep sea: a review. *PLoS*
803   *ONE* 5(8):e12449 DOI:10.1371/journal.pone.0012449.
804   Vanreusel A, Van den Bossche I, Thiermann F. 1997. Free-living marine nematodes from
805   hydrothermal sediments: similarities with communities from diverse reduced habitats.
806   *Marine Ecology Progress Series* 157:207–219 DOI: 10.3354/meps157207.
807   Vanreusel, A, Van den Bossche I, Thiermann F. 1997. Free-living marine nematodes from
808   hydrothermal sediments: similarities with communities from diverse reduced habitats.
809   *Marine Ecology Progress Series* 157: 207–219 DOI: 10.3354/meps157207.
810   Warwick RM, Platt HM, Somerfield PJ. (1998). A synopsis of the free living marine nematodes.
811   Part III: monhysterids. Synopses of the British Fauna (New Series). Field Studies Council,
812   Shwreusbury.
813   Wieser W. 1953. Die Beziehung zwischen Mundhöhlengestalt, Ernährungsweise und
814   Vorkommen bei freilebenden marinen Nematoden. *Arkiv för Zoologi* 2/4: 439–484.
815   Wildish DJ, Akagi HM, McKeown DL, Pohle GW. 2008. Pockmarks influence benthic
816   communities in Passamaquoddy Bay, Bay of Fundy, Canada. *Marine Ecology Progress Series*
817   357: 51–66 DOI: https://doi.org/10.3354/meps07331.
818   Zeppilli D, Bellec L, Cambon-Bonavita MA, Decraemer W, Fontaneto D, Fuchs S, Gayet N,
819   Mandon P, Michel LN, Portail M, Smol N, Sørensen MV, Vanreusel A, Sarrazin J. 2019.
820   Ecology and trophic role of *Oncholaimus dyvae* sp. nov. (Nematoda: Oncholaimidae) from
821   the lucky strike hydrothermal vent field (Mid-Atlantic Ridge). *BMC Zoology* 4:6 DOI:
822   https://doi.org/10.1186/s40850-019-0044-y.
823   Zeppilli D, Leduc D, Fontanier C, Fontaneto D, Fuchs S, Gooday AJ, Goineau A, Ingels J,
824   Ivanenko VN, Kristensen RM, Neves RC, Sanchez N, Sandulli R, Sarrazin J, Sørensen MV,
825   Tasiemski A, Vanreusel A, Autret M, Bourdonnay L, Claireaux M, Coquillé V, De Wever L,

826   Rachel D, Marchant J, Toomey L, Fernandes D. 2018. Characteristics of meiofauna in
827   extreme marine ecosystems: a review. *Marine Biodiv*ersity DOI:
828   https://doi.org/10.1007/s12526-017-0815-z.
829   **Figure titles and legends**
830
831   **Figure 1. Map showing the location of the sampling area Secca delle Fumose in underwater**
832   **park of Baia MPA located in the Gulf of Naples (Tyrrhenian sea, Italy).** Selected sampling
833   sites are: the diffusive emission site (H) called "White" for the presence of white microbial mats;
834   the geyser site (G) called "Yellow" for the sulphur deposits; the inactive sites north (CN) and
835   south (CS). The different habitats are indicated as follow: AP-C = photophilous algae and
836   sciaphilous cavities; SGCF = coarse sands and fine gravels; SVMC = superficial muddy
837   sands. The base map was performed using a Side Scan Sonar (SSS) Klein 3900 - 450 kHz. The
838   SSS used 2 transducers to transmit a high-frequency acoustic signal towards the sea floor and the
839   return signal was picked up by the SSS and transmitted in real time to a graphic recorder on
840   board ship. The ship's position was continuously recorded from a positioning satellite system
841   (DGPS and Gyro GNSS Hemisphere Vector gnss VS 330). SSS and navigation data were
842   processed using SonarPro®.
843
844   **Figure 2. Total meiofaunal and nematode abundances.** Reported are mean values (±SD) at all
845   investigated sites: the diffusive emission site (H); the geyser site (G); the inactive sites north
846   (CN) and south (CS).
847
848   **Figure 3**. **Vertical distribution of total meiofaunal and nematode abundances**. Reported are
849   mean values (±SD) at four sampling sites (A-D).
850
851   **Figure 4**. **Cluster analysis with SIMPROF test performed on meiofaunal community**
852   **composition**. Red lines indicate statistically significant groupings according to the SIMPROF
853   routine. All replicates are shown and indicated as: H1, H2 and H3; G1, G2 and G3; CN1, CN2
854   and CN3; CS1, CS2 and CS3.
855
856   **Figure 5. Meiofauna taxonomic composition**. *Others*: Bivalvia, Gasteropoda, Scaphopoda,
857   Polyplacophora, Oligochaeta, Amphipoda, Isopoda, Cumacea, Tanaidacea, Cladocera,
858   Halacarida, Tardigrada, Kinorhyncha, Gastrotricha, Platyhelminthes, Nemertea, Sipuncula,
859   Porifera, Rotifera, Cnidaria and Chaetognatha.
860
861   **Figure 6. Vertical meiofauna taxonomic composition at four sampling sites (A-D).** *Others*:
862   Bivalvia, Gasteropoda, Scaphopoda, Polyplacophora, Oligochaeta, Amphipoda, Isopoda,
863   Cumacea, Tanaidacea, Cladocera, Halacarida, Tardigrada, Kinorhyncha, Gastrotricha,
864   Platyhelminthes, Nemertea, Sipuncula, Porifera, Rotifera, Cnidaria and Chaetognatha .
865
866   **Figure 7. Cluster analysis with SIMPROF test performed on nematode composition**. Red
867   lines indicate statistically significant groupings according to the SIMPROF routine. All
868   replicates are shown and indicated as: H1, H2 and H3; G1, G2 and G3; CN1, CN2 and CN3;
869   CS1, CS2 and CS3.
870

871   **Figure 8**. **Nematode trophic structure characterizing all investigated sites**. Reported are 1A
872   (selective deposit feeders), 1B (non-selective deposit feeders), 2A (epigrowth feeders) and 2B
873   (predators/omnivores) at the four sampling sites.

874

875   **Figure 9. dbRDA canonical model on meiofauna taxonomic composition**. The graph shows
876   the effect of environmental variables on meiofauna taxonomic composition based on Spearman
877   rank correlations.

878

879   **Figure 10**. **dbRDA canonical model on nematode community composition**. The graph shows
880   the effect of environmental variables on nematode community composition based on Spearman
881   rank correlations.

882
883
884
885
886
887
888
889
890
891
892
893
894

Figure 1

Figure 1. Map showing the location of the sampling area Secca delle Fumose in underwater park of Baia MPA located in the Gulf of Naples (Tyrrhenian sea, Italy).

Selected sampling sites are: the diffusive emission site (H) called "White" for the presence of white microbial mats; the geyser site (G) called "Yellow" for the sulphur deposits; the inactive sites north (CN) and south (CS). The different habitats are indicated as follow: AP-C = photophilous algae and sciaphilous cavities; SGCF = coarse sands and fine gravels; SVMC = superficial muddy sands.The base map was performed using a Side Scan Sonar (SSS) Klein 3900 - 450 kHz. The SSS used 2 transducers to transmit a high-frequency acoustic signal towards the sea floor and the return signal was picked up by the SSS and transmitted in real time to a graphic recorder on board ship. The ship's position was continuously recorded from a positioning satellite system (DGPS and Gyro GNSS Hemisphere Vector gnss VS 330). SSS and navigation data were processed using SonarPro®.

Legend

☐ Land

✕ Sampling sites

Habitats

▨ AP-C

▨ SGCF

▨ SVMC

Vents

▨ "Yellow" hydrothermal vent

◡ ◠ "White" hydrothermal vent

Gulf of Naples

14°5'20"E

CN

G

CS

40°49'22"N

40°49'22"N

H

0   30 m

14°5'20"E

N

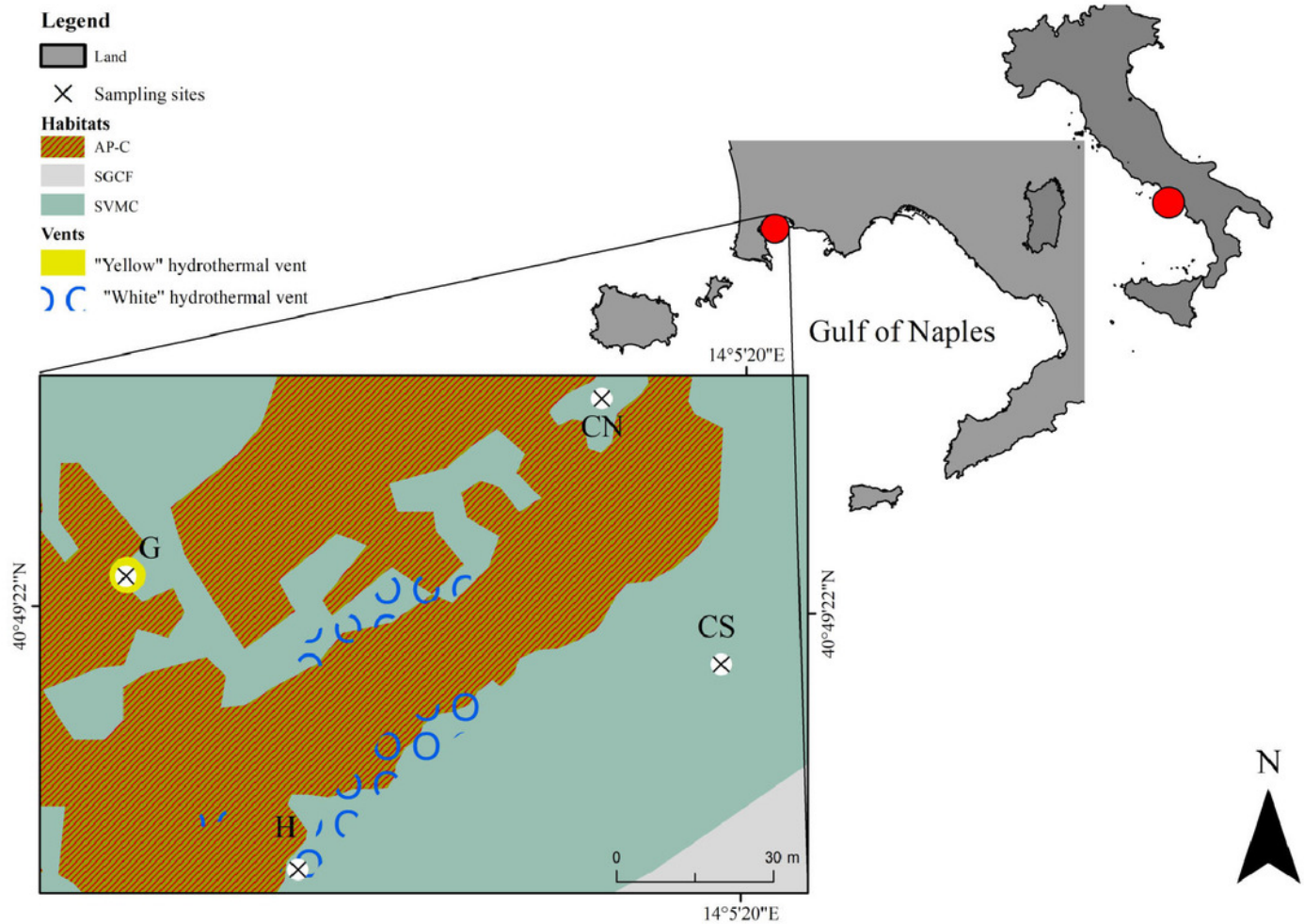



Figure 2

Total meiofaunal and nematode abundances.

Reported are mean values (±SD) at all investigated sites: the diffusive emission site (H); the geyser site (G); the inactive sites north (CN) and south (CS).

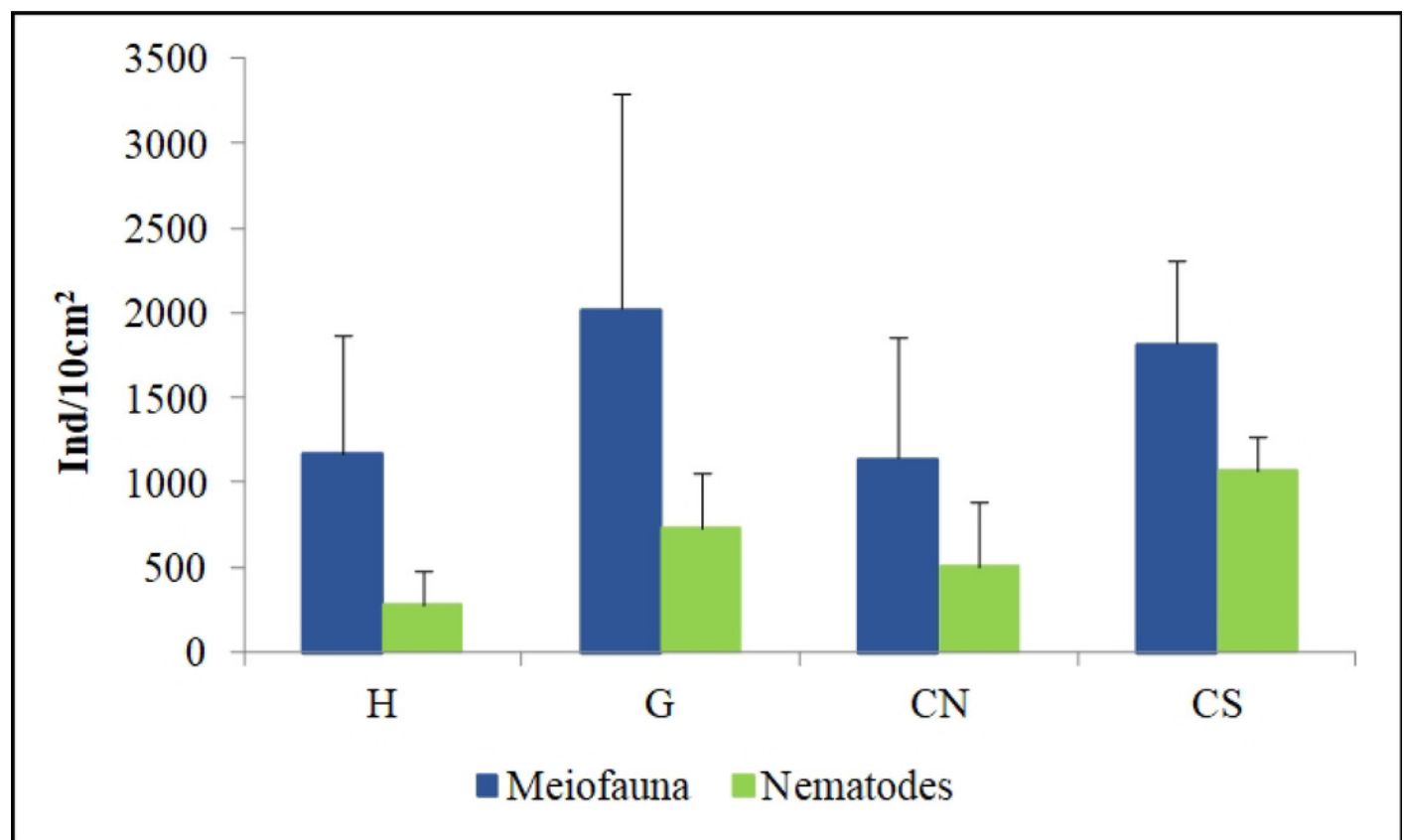



Figure 3

Vertical distribution of total meiofaunal and nematode abundances.

Reported are mean values (±SD) at four sampling sites (A-D).

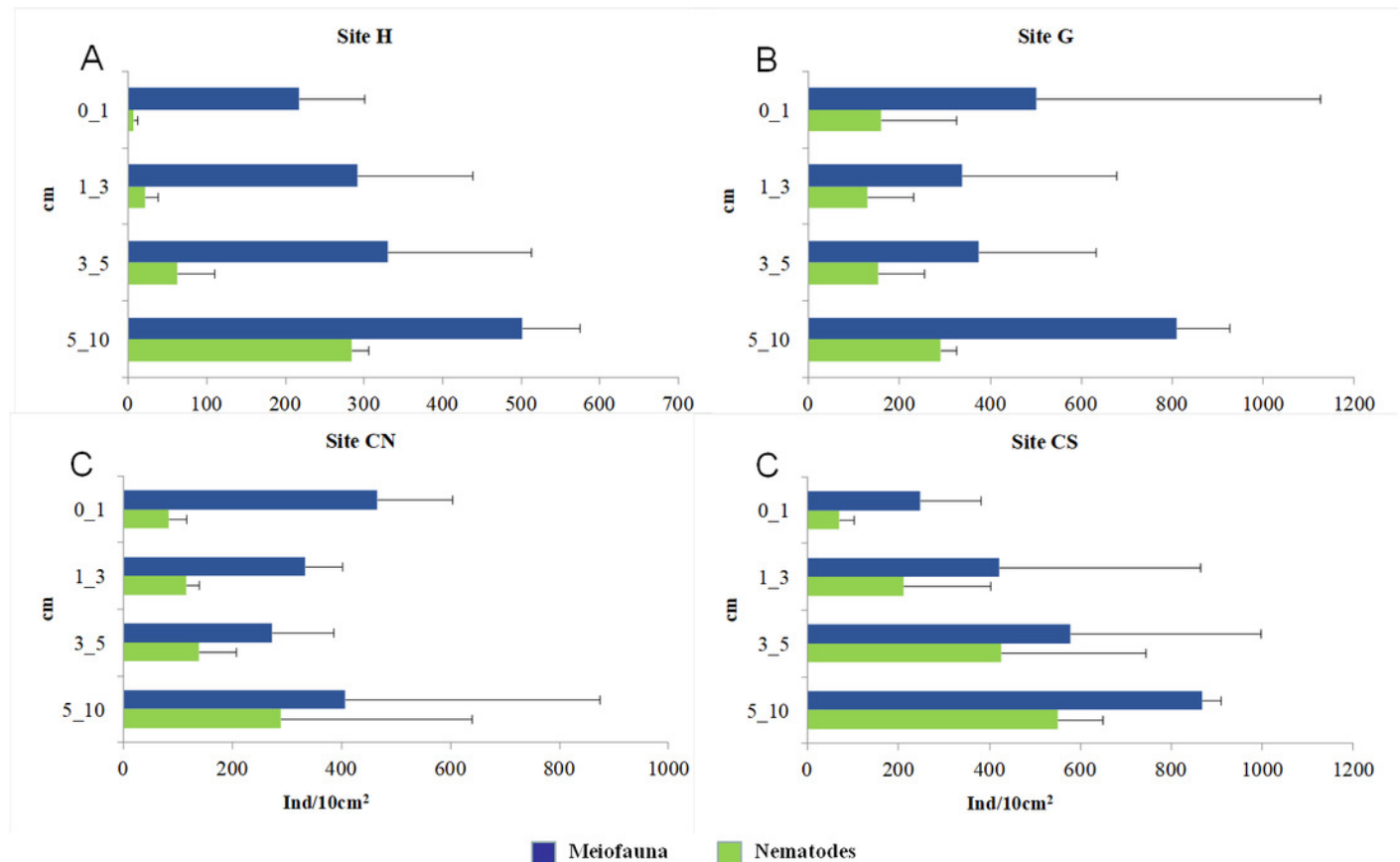



Figure 4

Figure 4. Cluster analysis with SIMPROF test performed on meiofaunal community composition.

Red lines indicate statistically significant groupings according to the SIMPROF routine. All replicates are shown and indicated as: H1, H2 and H3; G1, G2 and G3; CN1, CN2 and CN3; CS1, CS2 and CS3.

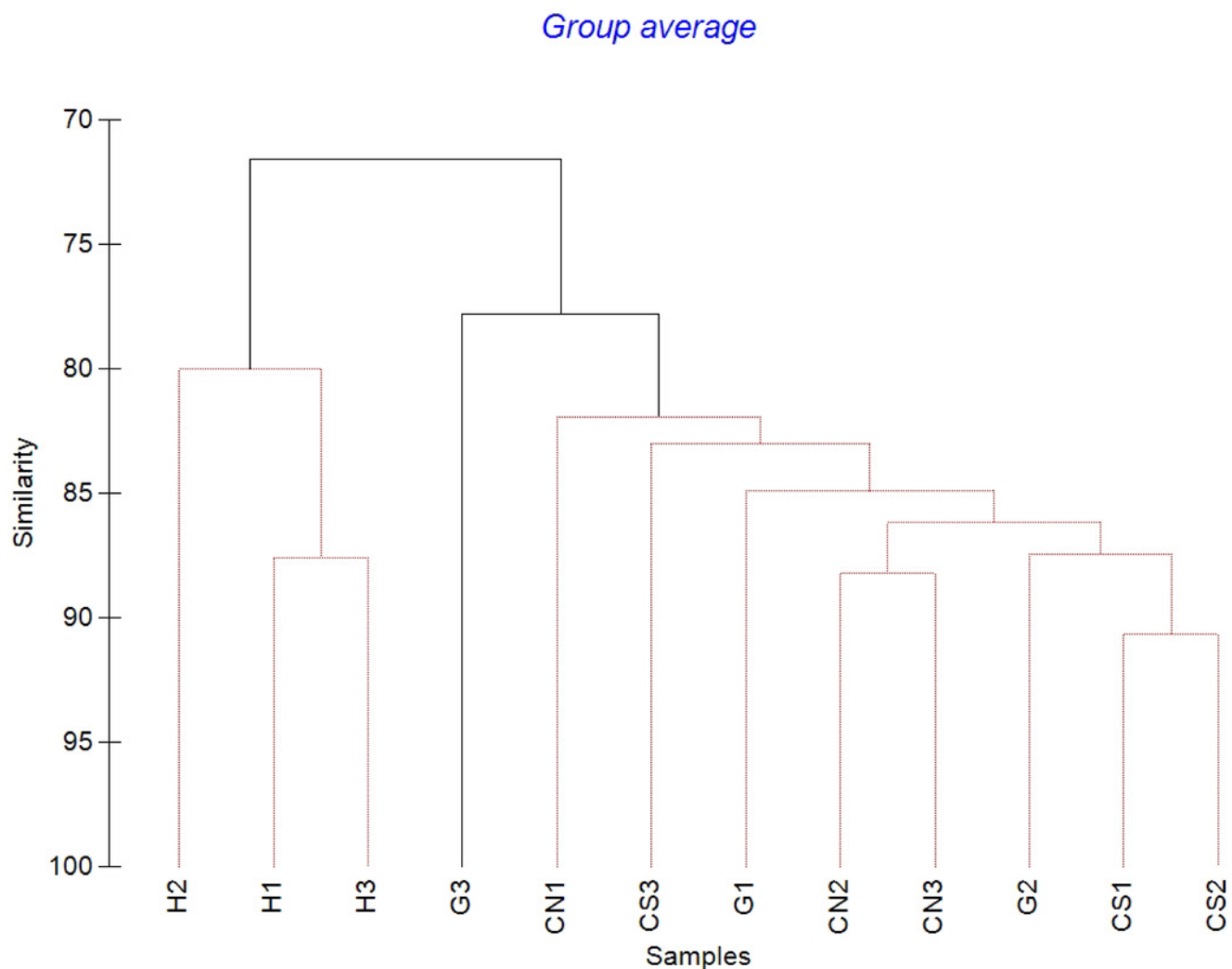



Figure 5

Meiofauna taxonomic composition

Others: Bivalvia, Gasteropoda, Scaphopoda, Polyplacophora, Oligochaeta, Amphipoda, Isopoda, Cumacea, Tanaidacea, Cladocera, Halacarida, Tardigrada, Kinorhyncha, Gastrotricha, Platyhelminthes, Nemertea, Sipuncula, Porifera, Rotifera, Cnidaria and Chaetognatha.

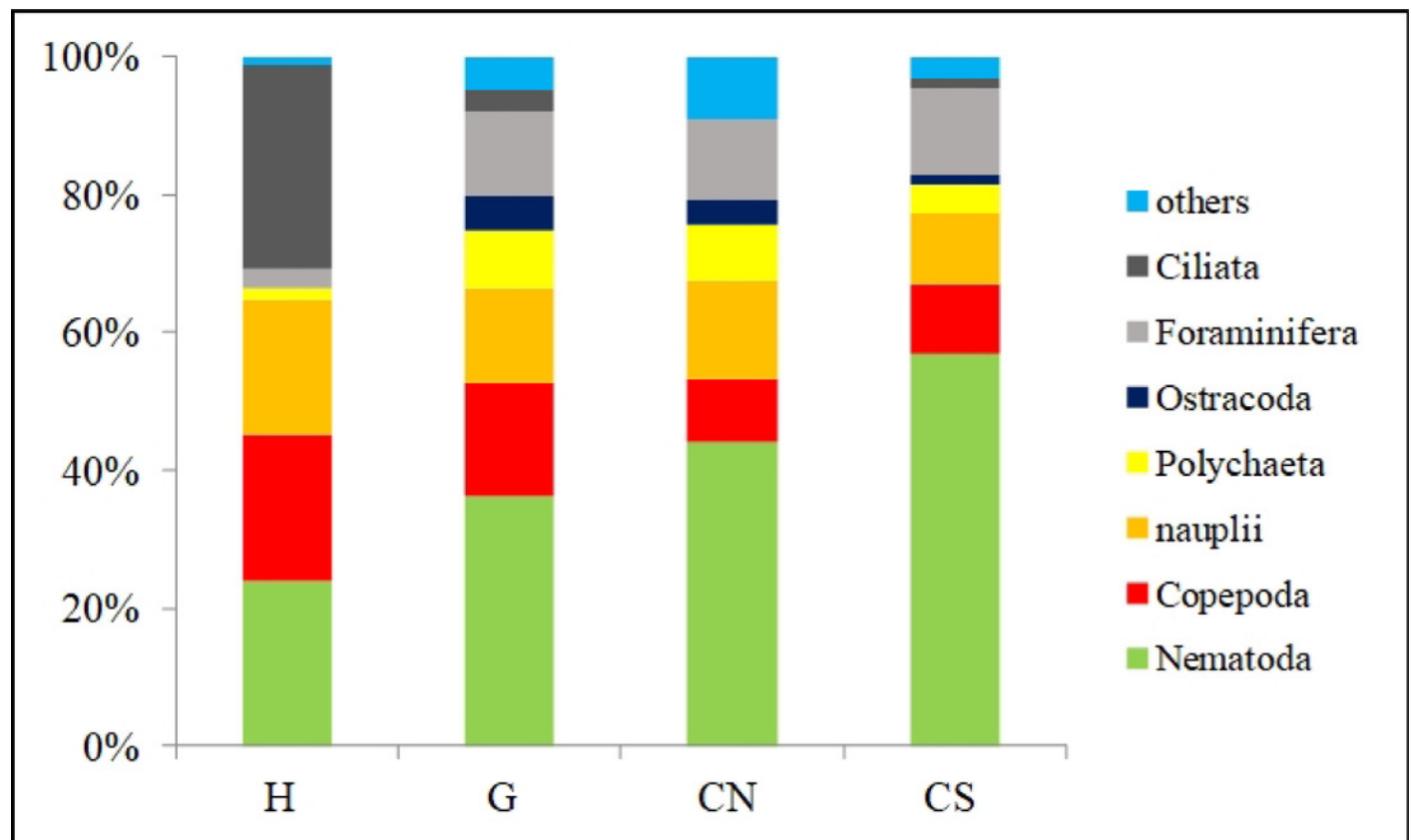



Figure 6

Vertical meiofauna taxonomic composition four sampling sites (A-D).

Others: Bivalvia, Gasteropoda, Scaphopoda, Polyplacophora, Oligochaeta, Amphipoda, Isopoda, Cumacea, Tanaidacea, Cladocera, Halacarida, Tardigrada, Kinorhyncha, Gastrotricha, Platyhelminthes, Nemertea, Sipuncula, Porifera, Rotifera, Cnidaria and Chaetognatha.

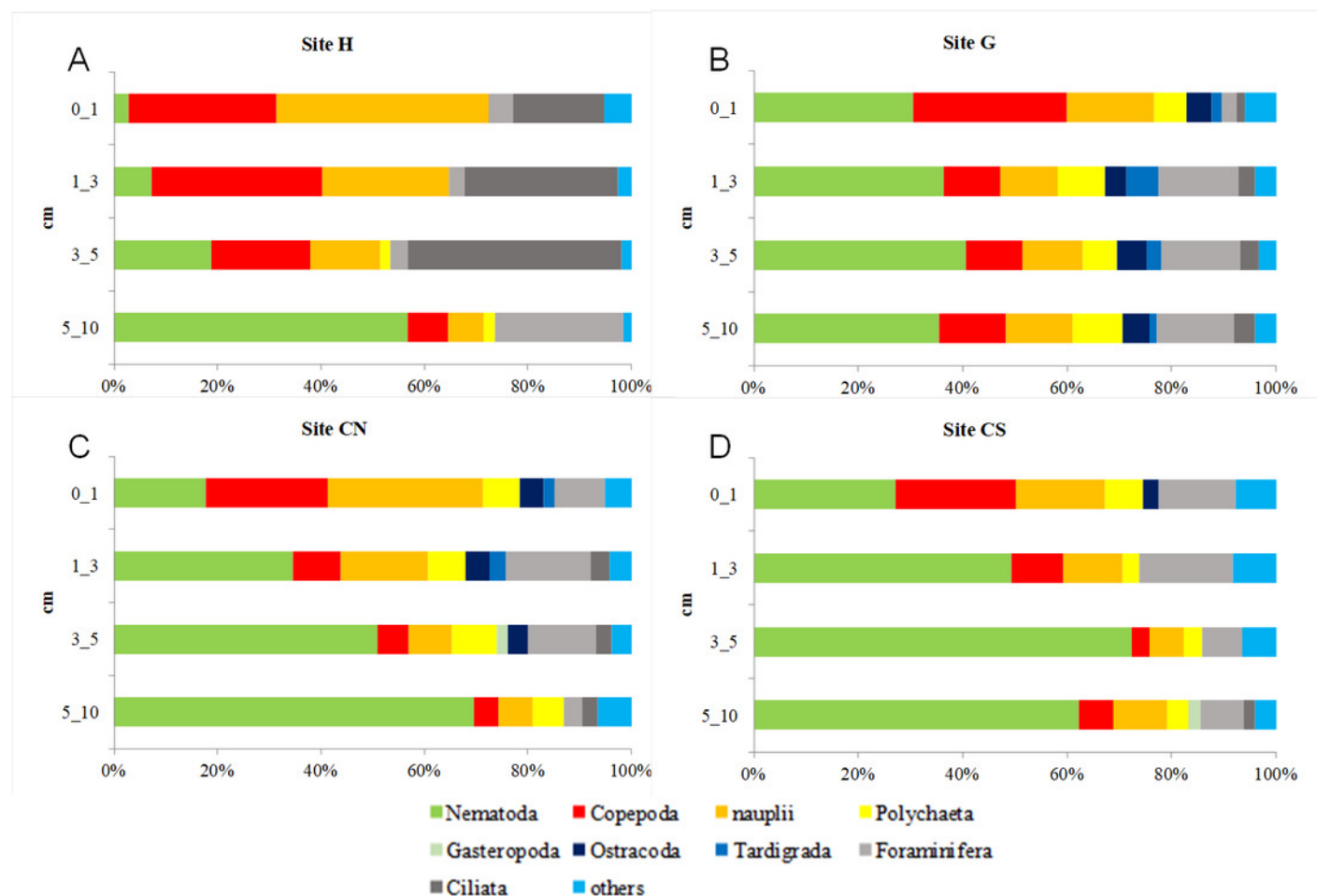



Figure 7

Cluster analysis with SIMPROF test performed on nematode composition.

Red lines indicate statistically significant groupings according to the SIMPROF routine. All replicates are shown and indicated as: H1, H2 and H3; G1, G2 and G3; CN1, CN2 and CN3; CS1, CS2 and CS3.

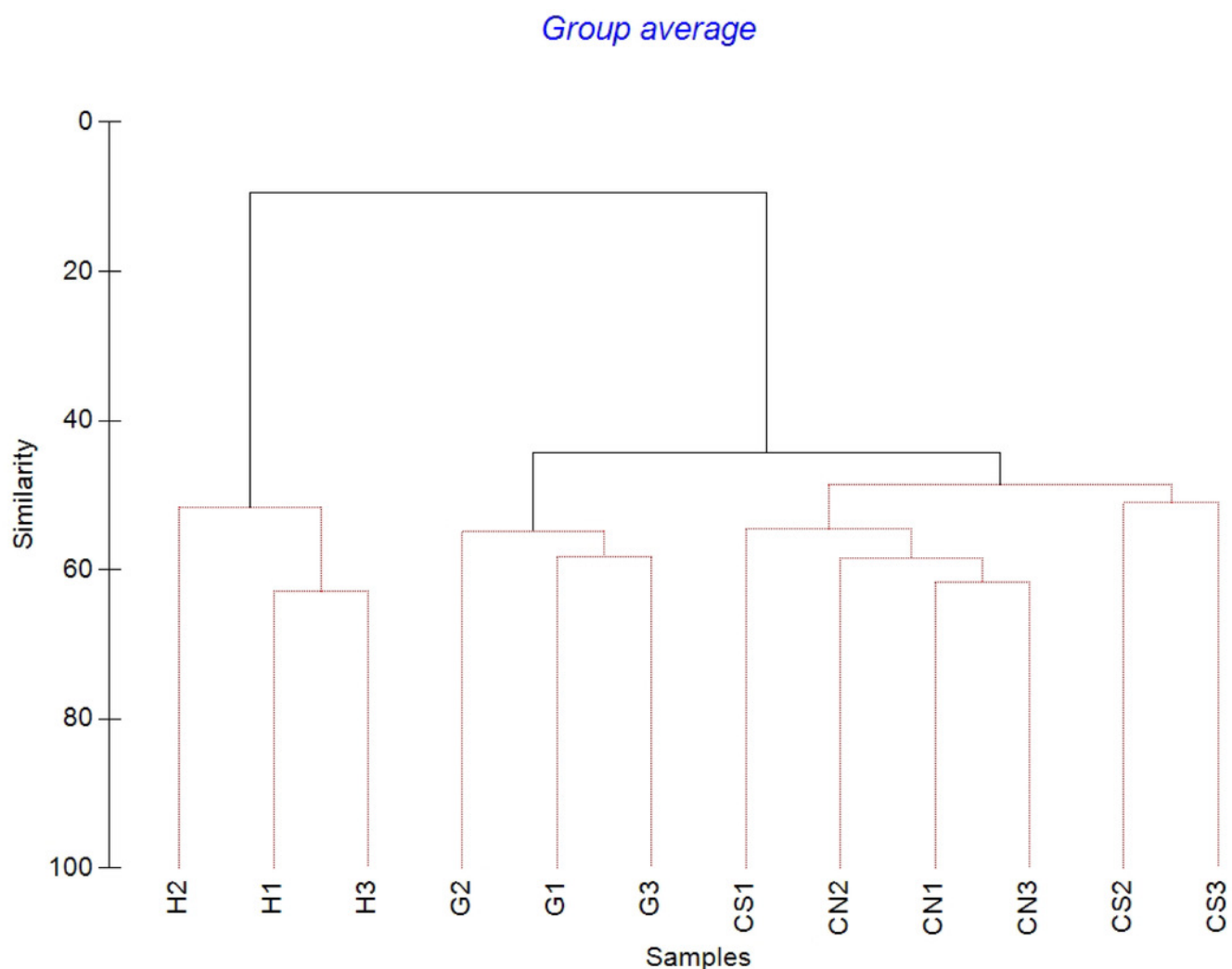



Figure 8

Nematode trophic structure characterizing all investigated sites.

Reported are 1A (selective deposit feeders), 1B (non-selective deposit feeders), 2A (epigrowth feeders) and 2B (predators/omnivores) at the four sampling sites.

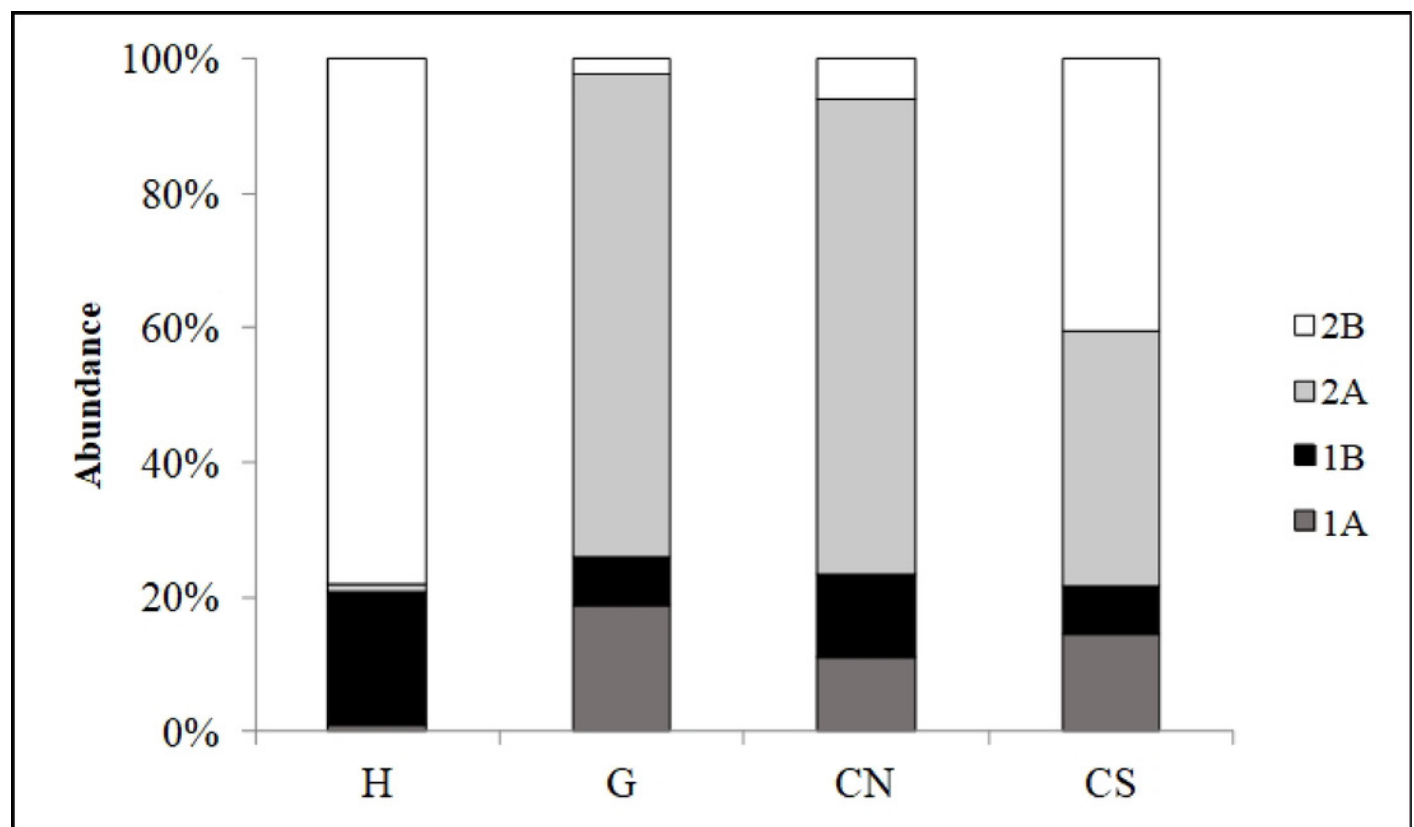



Figure 9

dbRDA canonical model on meiofauna taxonomic composition.

The graph shows the effect of environmental variables on meiofauna taxonomic composition based on Spearman rank correlations.

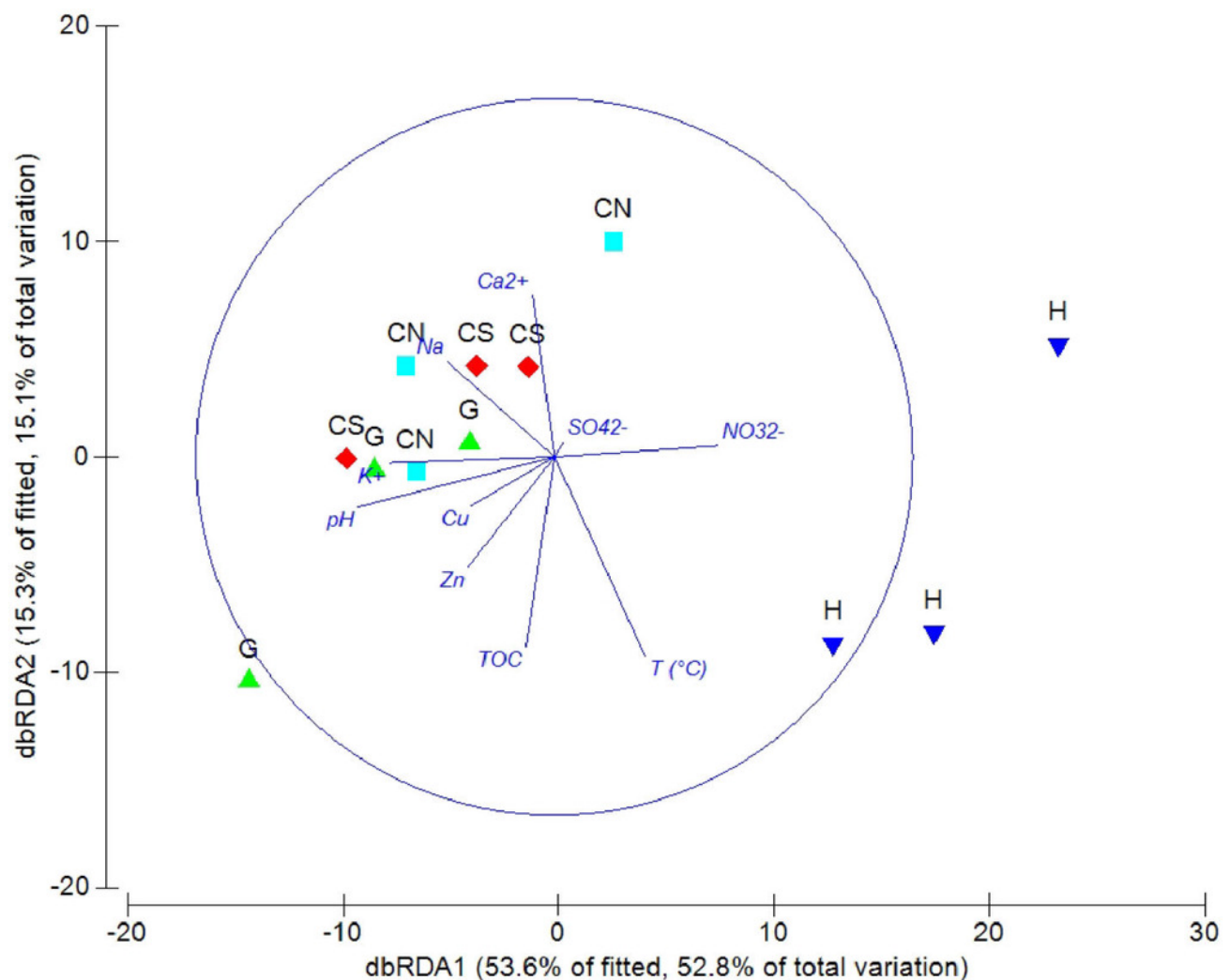
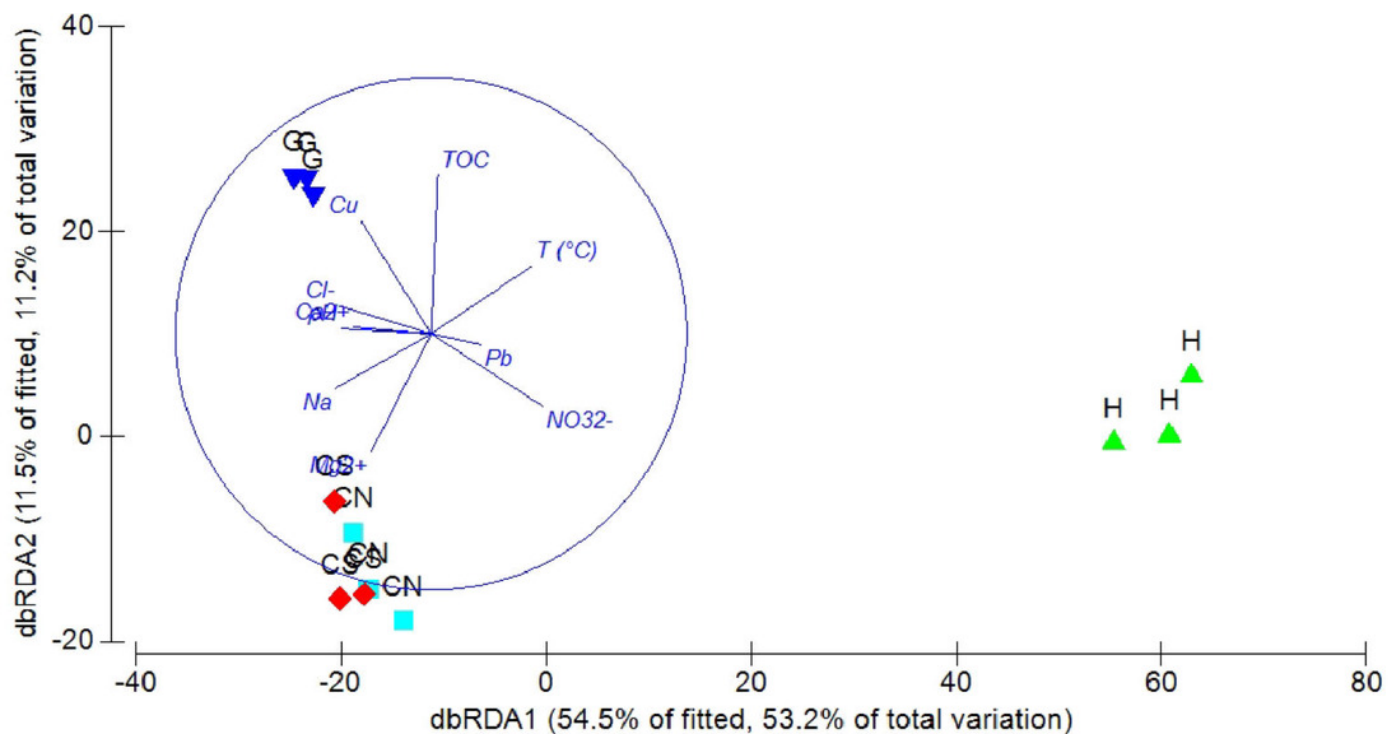



Figure 10

dbRDA canonical model on nematode community composition.

The graph shows the effect of environmental variables on nematode community composition based on Spearman rank correlations.

Results of PERMANOVA and PAIR-WISE tests for differences in meiofaunal abundance, number of taxa, meiofaunal taxonomic composition, nematode biomass and nematode species composition among sites (S) and layers (L).

Reported are (A) results of PERMANOVA Main tests and (B) Pair-wise tests. Df, degree of freedom; F, F-statistic; ns, not significant.

1 **Table 1: Results of PERMANOVA and PAIR-WISE tests for differences in meiofaunal abundance, number of taxa, meiofaunal**
2 **taxonomic composition, nematode biomass and nematode species composition among sites (S) and layers (L).** Reported are (A) results of
3 PERMANOVA Main tests and (B) Pair-wise tests. Df, degree of freedom; F, F-statistic; ns, not significant.

A)	Source	df	MS	F	P	B)	Source		P
Total meiofauna abundance	Site	3	1.194	1.244	ns	**Meiofaunal n° taxa**	Site	H vs G	0.001
	Layer	3	2.243	2.337	ns			H vs CN	0.001
	SxL	9	0.591	0.616	ns			H vs CS	0.001
	Residual	29	0.960			**Meiofaunal composition**	Site	H vs G	0.009
	Total	44						H vs CN	0.002
Meiofaunal n° taxa	Site	3	6.079	14.632	**0.001**			H vs CS	0.006
	Layer	3	1.049	2.524	ns	**Total nematode abundance**	Site	H vs G	0.024
	SxL	9	1.053	2.535	**0.023**			H vs CS	0.006
	Residual	29	0.415				Layer (within levels	0-1 vs 5-10	0.001
	Total	44					H and CS)	1-3 vs 5-10	0.002
Meiofaunal composition	Site	3	69.482	2.5462	**0.002**				
	Layer	3	30.437	1.1154	ns	**Total nematode biomass**	Site	H vs G	0.001
	SxL	9	24.074	0.8822	ns			H vs CN	0.005
	Residual	29	27.289					H vs CS	0.026
	Total	44						G vs CN	0.002
Total nematode abundance	Site	3	2.953	5.067	**0.007**			G vs CS	0.001
	Layer	3	4.622	7.931	**0.001**		Layer (within levels	0-1 vs 3-5	0.012
	SxL	9	0.556	0.954	ns		H, G and CN)	0-1 vs 5-10	0.001
	Residual	29	0.583					1-3 vs 3-5	0.014
	Total	44						1-3 vs 5-10	0.001
Total nematode biomass	Site	3	12.539	4.180	**0.001**	**Nematode composition**	Site	H vs G	0.001
	Layer	3	9.366	3.122	**0.001**			H vs CN	0.001
	SxL	9	15.528	1.725	**0.001**			H vs CS	0.001
	Residual	29	9.012	0.311			Site	G vs CN	0.001
	Total	44	44.000					G vs CS	0.001
Nematode	Site	3	64495	21498	**0.001**			CN vs CS	0.001

composition	Layer	3	5725.7	1908.6	ns	**ES(51); SR**	Site	H vs G	0.005
	SxL	9	15263	1695.9	ns			H vs CN	0.003
	Residual	29	49023	1690.4				H vs CS	0.001
	Total	44	135440			**J**	Site	H vs G	0.001
ES(51); SR	Site	3	758.17	39.535	**0.001**			H vs CN	0.001
	Res	8	19.177					H vs CS	0.001
	Total	11						CS vs G	0.02
J	Site	3	1025.2	163.61	**0.001**			CS vs CN	0.007
	Res	8	6.2663			**ITD**	Site	H vs CS	0.005
	Total	11						CS vs G	0.014
ITD	Site	3	357.23	14.045	**0.002**			CS vs CN	0.007
	Res	8	25.435			**MI**	Site	H vs G	0.002
	Total	11						H vs CN	0.001
MI	Site	3	36.44	47.043	**0.001**			H vs CS	0.03
	Res	8	0.77461					CS vs G	0.003
	Total	11						CS vs CN	0.004

Indices of diversity at all sampling sites.

Nematode species richness (SR); the expected species number (ES[51]); Pielou index (J); the index of trophic diversity (ITD) and the maturity index (MI). All replicates are reported.

Table 2:

Indices of diversity at all sampling sites. Nematode species richness (SR); the expected species number (ES[51]); Pielou index (J); the index of trophic diversity (ITD) and the maturity index (MI). All replicates are reported.

	G1	G2	G3	H1	H2	H3	CN1	CN2	CN3	CS1	CS2	CS3
SR	51	63	61	12	11	7	69	72	45	52	74	55
ES(51)	22	24	22	4	6	3	27	25	23	20	24	19
J	0.80	0.80	0.77	0.29	0.28	0.34	0.85	0.81	0.86	0.73	0.71	0.67
ITD	0.63	0.59	0.45	0.63	0.76	0.63	0.49	0.58	0.52	0.40	0.33	0.34
MI	2.9	2.8	2.7	3.5	3.8	3.6	2.8	2.9	2.7	3.2	3.3	3.4

Results of DistLM procedure (Sequential test) for fitting sediment and interstitial water variables to the meiofauna and nematofauna variables.

% Var, percentage of explained variance; % Cum, cumulative percentage explained by the added variable; ns, not significant.

2 **Table 3:**

3 **Results of DistLM procedure (Sequential test) for fitting sediment and interstitial water variables to the meiofauna and nematofauna**
4 **variables**.
5 % Var, percentage of explained variance; % Cum, cumulative percentage explained by the added variable; ns, not significant.

Meiofauna abundance

Variable	R^2	P	Var%	Cum%
Cl⁻	0.229	ns	0.23	0.23
Zn	0.280	ns	0.05	0.28
T (°C)	0.654	**0.02**	0.37	0.65
pH	0.672	ns	0.02	0.67
Ca²⁺	0.678	ns	0.01	0.68
Pb	0.820	ns	0.14	0.82
Mud	0.928	ns	0.11	0.93
Cu	0.946	ns	0.02	0.95
Na	0.994	ns	0.05	0.99
S²⁻	0.999	ns	0.01	1.00

Meiofauna n° taxa

Variable	R^2	P	Var%	Cum%
NO₃²⁻	0.849	**0.001**	0.849	0.849
Mg²⁺	0.941	**0.01**	0.093	0.941
pH	0.951	ns	0.010	0.951
Zn	0.961	ns	0.009	0.961
T (°C)	0.964	ns	0.003	0.964
S²⁻	0.971	ns	0.007	0.971
TOC	0.979	ns	0.008	0.979
Ca²⁺	0.985	ns	0.007	0.985
K⁺	0.992	ns	0.007	0.992
Pb	0.999	ns	0.007	0.999

Meiofauna taxonomic composition

Variable	R^2	P	Var%	Cum%
NO₃²⁻	0.475	**0.001**	0.475	0.475
T (°C)	0.582	**0.03**	0.107	0.582
pH	0.664	**0.03**	0.082	0.664
Cu	0.733	**0.03**	0.069	0.733
K⁺	0.785	ns	0.052	0.785
Na	0.817	ns	0.031	0.817
Zn	0.853	ns	0.036	0.853
TOC	0.921	ns	0.068	0.921
SO₄²⁻	0.964	ns	0.044	0.964
Ca²⁺	0.985	ns	0.021	0.985

Nematode abundance

Variable	R^2	P	Var%	Cum%
NO₃²⁻	0.459	**0.01**	0.459	0.459
K⁺	0.566	ns	0.107	0.566
T (°C)	0.602	ns	0.036	0.602
Na	0.716	ns	0.114	0.716
Zn	0.732	ns	0.016	0.732
Ca²⁺	0.801	ns	0.070	0.801
TOC	0.901	ns	0.100	0.901
Cl⁻	0.993	**0.004**	0.092	0.993
Mg²⁺	0.995	ns	0.002	0.995
Cd	0.998	ns	0.003	0.998

Nematode biomass

Variable	R^2	P	Var%	Cum%
K⁺	0.630	**0.001**	0.630	0.630
Cu	0.699	ns	0.069	0.699
NO₃²⁻	0.731	ns	0.032	0.731
Mud	0.742	ns	0.012	0.742
Zn	0.858	ns	0.116	0.858
TOC	0.938	ns	0.080	0.938
pH	0.982	**0.01**	0.044	0.982
Ca²⁺	0.987	ns	0.005	0.987
Cl⁻	0.991	ns	0.004	0.991

Nematode composition

Variable	R^2	P	Var%	Cum%
NO₃²⁻	0.521	**0.001**	0.521	0.521
Na	0.633	**0.001**	0.112	0.633
Ca²⁺	0.709	**0.003**	0.076	0.709
TOC	0.757	ns	0.048	0.757
T (°C)	0.798	ns	0.042	0.798
Pb	0.837	ns	0.039	0.837
Cu	0.872	ns	0.035	0.872
Mg²⁺	0.906	ns	0.035	0.906
Cl⁻	0.944	ns	0.038	0.944

Nematode diversity indices

Variable	R^2	P	Var%	Cum%
NO₃²⁻	0.888	**0.01**	0.888	0.888
T (°C)	0.939	**0.02**	0.050	0.939
S²⁻	0.976	**0.003**	0.038	0.976
Mg²⁺	0.980	ns	0.004	0.980
Zn	0.983	ns	0.002	0.983
Na	0.985	ns	0.003	0.985
K⁺	0.994	**0.03**	0.008	0.994
Ca²⁺	0.997	ns	0.003	0.997
Cu	0.998	ns	0.001	0.998

Nematode trophic diversity

Variable	R^2	P	Var%	Cum%
Cl⁻	0.627	**0.01**	0.627	0.627
TOC	0.856	**0.01**	0.229	0.856
T (°C)	0.882	ns	0.026	0.882
Cu	0.920	ns	0.039	0.920
NO₃²⁻	0.964	**0.03**	0.043	0.964
Cd	0.973	ns	0.010	0.973
Na	0.985	ns	0.012	0.985
Mg²⁺	0.989	ns	0.004	0.989
K⁺	0.994	ns	0.005	0.994

| Mg^{2+} | 0.998 | ns | 0.007 | 0.998 | pH | 0.977 | ns | 0.033 | 0.977 | Pb | 0.999 | ns | 0.001 | 0.999 | SO_4^{2-} | 0.997 | ns | 0.003 | 0.997 |