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6 **Environmental drivers of nematode community structure in harbors: the interplay of pollution,
7 granulometry, and spatial heterogeneity**

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21 **Abstract**

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23 This meta-analysis provides a comprehensive assessment of meiofaunal nematodes in Mediterranean
24 port ecosystems, integrating taxonomic patterns, frequency of occurrence, and environmental
25 characteristics across seven ports. Only a limited set of genera occurred consistently across sites, all
26 belonging to tolerant and opportunistic taxa, confirming that nematode assemblages in ports are
27 strongly shaped by environmental filtering but do not form a community uniquely characteristic of
28 port environments.

29 Community differences among ports were driven primarily by sediment grain size, depth, and port-
30 specific hydrodynamic conditions rather than by geographical proximity. Highly impacted stations
31 were associated with a single consistent indicator genus, while moderately impacted sites hosted a
32 broader suite of transitional taxa. No genus was exclusive to non-impacted conditions, which were
33 instead characterised by more balanced and evenly structured assemblages. Contaminant levels
34 played a secondary and context-dependent role, largely modulated by sediment permeability and
35 oxygenation.

36 Overall, the findings highlight the sensitivity of nematode assemblages to both natural and
37 anthropogenic gradients and underline their value as ecological indicators when interpreted together
38 with sedimentological features, functional traits, and local port morphology.

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40 **Keywords:** nematode diversity, functional traits, port systems, environmental quality, anthropogenic
41 impact, monitoring programs.

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47 **1. Introduction**

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49 Ports are focal points of environmental stress, where industrial activities, shipping operations,
50 urban runoff, and other human interventions converge. The cumulative impact of these anthropogenic
51 activities results in the release of a wide array of pollutants, including heavy-metals, hydrocarbons,
52 nutrients, and organic contaminants into coastal ecosystems and into the sediments. Sedimentary
53 environments are critical for benthic organisms, which play a key role in nutrient cycling, sediment
54 stabilization, and food web dynamics (Frontalini et al., 2025). However, these stressors pose
55 significant risks to biodiversity, ecosystem functioning, and the overall quality of port environments
56 and of all the surrounding coastal systems (Losi et al., 2013; Franzo et al., 2022). Biomonitoring has
57 emerged as an indispensable tool for evaluating the health of marine ecosystems, particularly in
58 complex and heavily impacted environments such as ports. Assessing the ecological quality of
59 sediments is, therefore, an essential component of environmental monitoring and management in
60 ports and surrounding coastal areas (Baldrighi et al., 2019; Franzo et al. 2022).

61 The European Union has recognized the importance of using indicators in environmental
62 monitoring through legislative frameworks such as the Water Framework Directive (WFD,
63 2000/60/EC) and the Marine Strategy Framework Directive (MSFD, 2008/56/EC). These directives
64 aim to achieve "Good Environmental Status" (GES) in European waters by integrating biological,
65 chemical, and physical indicators into comprehensive assessment programs.

66 Physical disturbances such as sediment removal and replacement are common in port areas.
67 These activities, combined with reduced exposure to hydrodynamic forces (e.g., wind, waves, and
68 currents), often lead to high water residence times within ports. As a result, sediment granulometry
69 can vary markedly between the inside and outside of the port: sandy sediments typically dominate
70 external areas, while finer fractions accumulate in the inner zones. This granulometric gradient
71 contributes to higher levels of contaminants and organic enrichment within the port, as fine-grained
72 sediments tend to retain pollutants more effectively than coarser sediments (Baldrighi et al., 2019).

73 Sediment texture is a strong predictor of benthic community patterns (Steyaert et al., 1999;
74 Vanaverbeke et al., 2002). Granulometric characteristics can shape the distribution of nematode
75 communities, their taxonomic and functional diversity and modulate their responses to sediment-
76 associated pollutants (Schratzberger and Warwick, 1998; Heininger et al., 2007 and references
77 therein).

78 Benthic nematodes respond rapidly to both acute and chronic stressors reflecting the
79 cumulative effects of pollution and habitat alterations, and they provide a comprehensive
80 understanding of ecosystem status (Semprucci et al., 2019; Grassi et al., 2025). Their high abundance
81 and biodiversity in all types of environments, ease of quantitative sampling, and sensitivity to multiple
82 stressors make them cost-effective for assessing sediment quality in compliance with EU directives
83 (Cocozza di Montanara et al., 2022; Semprucci et al., 2022).

84 Many studies have explored the role of nematode-based biomonitoring in evaluating port
85 systems, where contamination gradients often pose significant challenges to ecological integrity of
86 the coasts revealing shifts in community composition, biodiversity, and functional traits (feeding
87 strategies and life-history characteristics) along disturbance gradients (Moreno et al., 2008a, b; 2009,
88 2011; Losi et al., 2013, 2021). Even if individual studies often yield useful quantitative data, they
89 give no indication of macroecological patterns and of whether the magnitude and direction of an
90 observed disturbance response differ between studies (Schratzberger et al., 2009; Fonseca and Netto,
91 2015). The combined analysis of independent studies is a useful tool for exposing general patterns in
92 assemblage responses to a gradient of pollution in different enclosed systems that permits ecological

93 questions to be examined on a much larger scale (Dernie et al., 2003). Despite the obvious limitations
94 of such analyses, including different sampling design and methodology for sample collection, time
95 of collection (interference of seasonality), study-specific differences in scale of disturbance,
96 disturbance regime and nematode genus composition, consistent patterns often emerge which
97 otherwise would not be supported by single studies. In the present study, we have adopted a meta-
98 analysis approach on a large data set of nematode data collected from Mediterranean port systems.
99 The main aim was to explore the shift of nematode community composition (i.e., taxonomic and
100 functional structure) across many ports presenting different sediment features and pollution levels.
101 Therefore, we hypothesized that nematode assemblages from Mediterranean port systems exhibits
102 discernible patterns in relation to (i) geographical location (e.g., Adriatic vs. Ligurian basins), (ii)
103 structural port differences, (iii) natural environmental variables (e.g., sediment grain size, water
104 depth), and (iv) levels of contamination (e.g., concentrations of organic pollutants).

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106 **2. Material and Methods**

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108 **2.1. Study areas and sampling**

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110 Nematode assemblages were studied at different coastal stations of the Ligurian Sea (four
111 ports: Vado Ligure, Genoa-Voltri, Marina degli Aregai and Portosole) and Adriatic Sea (three ports:
112 Koper, Trieste and Ancona) during several experimental campaigns (Fig. 1; Table 1). As shown in
113 the satellite images in Figure 1, the seven analysed ports exhibit different degrees of openness to the
114 sea, depth, and internal compartmentalization. These structural differences likely represent obstacles
115 to natural water circulation and could strongly influence the internal circulation of water, as well as
116 the movement and accumulation of sediments and associated deposits. Hereinafter, a summary of
117 each case study is reported.

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119 *Vado Ligure (Losi et al., 2021)*

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121 The study area is located in the surroundings of the port of Vado Ligure (Savona), along the
122 coast of the Western Ligurian Sea (NW Mediterranean) (Fig.1). The port of Vado is the base of a
123 commercial port that is a relevant container and terminal of petroleum products (e.g. gasoline, diesel,
124 naphtha), and is the largest industrial site of Western Liguria, where an oil-burning power plant and
125 several other industrial plants are located. The sampling was carried out at 28 stations (Table 1)
126 located at increasing distances from the port. The Northern part of the study area was characterized
127 by the port terminals, while the southernmost part is located close to the Marine Protected Area of
128 Bergeggi island. The particle size resulted to be very variable among stations, with sediments varying
129 from fine silt to very coarse sand; mud and especially gravel were the less represented components.

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131 *Genoa-Voltri (Moreno et al., 2008b, 2011)*

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133 The industrial Genoa-Voltri harbor is an important container and oil terminal located to the
134 west of Genoa (Ligurian Sea, NW Mediterranean) (Fig.1). The study area was characterized by high
135 concentrations of organic compounds (e.g. protein, carbohydrate, PAHs). Samples were collected in
136 three sampling stations, located over a distance of 1000 m (Table 1). These stations were situated at:
137 the inner part of the harbour (I), the middle (M) and the outer parts (O), and close to the open sea.
138 The sediments were dominated by a fine-sand-muddy grain size.

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Marina degli Aregai and Portosole (Moreno et al., 2008b, 2009)

Sediment samples were collected from the tourist marinas of Portosole and Marina degli Aregai, located in the Ligurian Sea (NW Mediterranean) (Fig.1). Portosole is an area of 16 ha, located close to a commercial harbour (Sanremo) experiencing both commercial and pleasure vessel traffic, in addition to the presence of the S. Francesco River plume entering the Sanremo harbor from the northwest. Marina degli Aregai is an area of 18 ha, it is located 6 miles away from Sanremo and is not influenced by river output. At both marinas, samples were collected at four stations and sediments were dominated by a fine-sand fraction (Table 1).

Koper (Baldrighi et al., 2019)

The port of Koper is located in a semi-enclosed bay in the north-eastern Adriatic (Fig. 1). The sediments consist of detrital material from the hinterland, shore erosion and riverine inflows. The samples were collected in four stations with different disturbance sources (Table1). In the first station (Kp1) container ships and touristic cruise ships anchor. The second station (Kp2) is influenced by the river Rizana, which discharges also the outflow of the main coastal wastewater treatment plant (85,000 households). In the third station (Kp3) bulk cargoes are handled, the most common of which are hard coal and iron ore. In all port stations, the sediment is stirred up by dredging operations. The sampling station Kp4 is located outside the port, next to the shipway, where there is the least traffic impact, and no dredging operation takes place. All sampling stations in Koper were mainly dominated by mud.

Trieste (Baldrighi et al., 2019)

The port of Trieste is in the Bay of Muggia, a shallow, semi-enclosed basin in the Gulf of Trieste (north-eastern end of the Adriatic Sea) (Fig.1), Trieste port is characterized by low-level hydrodynamism and by riverine inputs laden with fine sediments containing chemical fertilizers. The port has long been surrounded by industrial infrastructure and is characterized by an intense traffic of oil tankers and ferries. The main pollutants include PAHs and trace metals. The four sampling stations (Table 1) were chosen on the basis of the main activities carried out there and their consequent anthropogenic pressures: the port area (Ts1), the shipbuilding area (Ts2), the iron foundry area with a steel plant (Ts3) and the petroleum area where petroleum products are handled, stored and processed (Ts4). All sampling stations in Trieste were mainly dominated by mud.

Ancona (Baldrighi et al., 2019)

The port of Ancona is located in the Central Adriatic Sea (Fig.1) and is characterized by intense passenger and cargo traffic. The main pollutants include organic waste dumped from fishing vessels and industrial contaminants from a number of shipyards. The bottom current speed usually reported from the sampling area is 10 cm/s, with a north-eastern direction. More than one million passengers on ferries and cruise ships travel from Ancona to the eastern coasts, and both container and oil traffic have also developed in recent years. The four sampling stations (Table 1) were chosen according to the different anthropogenic activities that take place in the port subareas: Anc1 and Anc2

185 were located in the inner part of the port nearby shipping facilities such as active berths; Anc3 was
186 located in a more external position although in an area used for cargo anchorage; Anc4 was outside
187 the port where no activity takes place. All sampling stations in Ancona were mainly dominated by
188 sand.

189 **Table 1.** Geographical location, stations, replicates, and methods used at the different sampling sites. Abbreviations used: GS = grain size, PAHs = polycyclic aromatic
 190 hydrocarbons, HM = heavy metals, PRT = proteins, CHO = carbohydrates, TOM = total organic matter, Phaeo = phaeopigments and BTs = total butyltins. Nematode analyses: A =
 191 total abundance, S = genera richness, H' = Shannon index of diversity, J' = Pielou's equitability index, ITD = index of trophic diversity and Mi = maturity index. In bold
 192 environmental and nematode parameters used in the present study.

Study port	Vado Ligure	Genoa-Voltri	Marina degli Aregai	Portosole	Koper	Trieste	Ancona
Geographic area	W Ligurian Sea	W Ligurian Sea	Ligurian Sea	Ligurian Sea	NE Adriatic Sea	NE Adriatic Sea	Central Adriatic Sea
Structural features description	Close to the commercial port, but in the open sea area with sediments mainly characterized by fine silt to very coarse sand.	Large port area but highly compartmentalized, with large piers. Protected, lateral entrance, internal water masses may experience reduced renewal, especially under calm conditions. Sediments were dominated by a fine-sand-muddy grain size.	Tourist marina similar to Portosole, but even smaller and more enclosed. Single access from the southeast, with circular breakwaters. Water exchange is presumably very limited, except during strong wind conditions. Sediments were dominated by a fine-sand fraction.	Highly protected tourist marina, with narrow lateral entrances. The basin is almost entirely enclosed by piers, potentially very slow water renewal, with high susceptibility to stagnation, nutrient buildup, and elevated temperatures. Sediments were dominated by a fine-sand fraction.	Like Trieste, but with more extensive piers and compartmentalized industrial areas. Part of the port is more enclosed and protected by breakwaters, where water renewal may be slower. Good openness to the N-NE. Sediments were mainly muddy.	Open structure, directly facing the sea. Wide channels and linear docks, with few internal obstacles. Presumed good circulation, especially in the outer areas. The innermost zones (well protected) may have slow water renewal, though likely less stagnant compared to other ports. Sediments were mainly muddy.	Entrance protected by a long breakwater, with a deep internal basin. Port zones enclosed on multiple sides are characterized by a greater risk of stagnant waters, while the most exposed to the sea reveal better exchange conditions. Sediments mainly sandy.
Period of construction	The construction of the Capo Vado port began in the early 1960s, with the first docks becoming operational in 1962. The large Vado Gateway platform, a modern container terminal, was officially inaugurated on December 12, 2019.	The current commercial port of Prà (formerly Voltri) was built following extensive land reclamation and expansion works in the 1970s, with the container terminal (Voltri Terminal Europa) becoming operational in 1994.	Marina degli Aregai is a modern tourist marina, inaugurated in 1992, located on the Western Ligurian coast (Riviera dei Fiori).	The port was officially inaugurated in July 1977, quickly becoming one of the most modern tourist marinas in the Mediterranean.	The construction of the new port east of the old town began in 1957. By 1962–1963, the company Luka Koper was established, with functional terminals already in operation.	The current Old Port (then called the "New Port") was built between 1868 and 1883, in conjunction with the Austro-Hungarian railway development connecting Trieste to Central Europe.	Ancona has ancient origins: the Roman port was expanded with the northern quay built around 100 A.D. Further development with continuous expansion over the centuries.
Sampling technique	Van Veen grab	Manual corer	Manual corer	Manual corer	Box-corer	Box-corer	Box-corer
N. of stations/N. replicates	28/3	3/3	4/3	4/3	4/3	4/3	4/3
Sampling depth (m)	43.5 - 78.0	7.5 - 12.0	3.0 - 12.0	3.0 - 7.0	8.0 - 17.0	8.0 - 20.0	4.0 - 15.0
Environmental parameters	GS, PAHs, HMs PRT, CHO, TOM	OM, Chl-a, Phaeo PAHs, PRT, GS	GS, PAHs, HMs TOM, CHO, PRT	GS, PAHs, HMs TOM, CHO, PRT	TOC, PAHs, BTs GS	TOC, PAHs, BTs GS	TOC, PAHs, BTs GS
Nematode parameters	A, S, H' log, J', ITD, Mi	A, S, H', J, ITD, Mi	A, S, H', J, ITD, Mi	A, S, H', J, ITD, Mi	A, S, H', J, ITD, Mi	A, S, H', J, ITD, Mi	A, S, H', J, ITD, Mi
References	Losi et al., 2021	Moreno et al., 2008b, 2011	Moreno et al., 2008b, 2009	Moreno et al., 2008b, 2009	Baldrighi et al., 2019	Baldrighi et al., 2019	Baldrighi et al., 2019

194 **2.2. Environmental contaminant analyses and quality status of the investigated harbors**
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196 For Vado Ligure, Genoa-Voltri, Marina degli Aregai and Portosole ports, the concentrations
 197 of PAHs were measured by the PAH-GCMS/MS and GC/FID method (see details in Moreno et al.,
 198 2009 and Losi et al., 2021). In the Adriatic ports, PAH quantification was carried out by high
 199 performance liquid chromatography (HPLC, Ultimate3000) with a fluorescence detector (RF2000)
 200 and a photodiode array detector (Baldrighi et al. 2019).
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 203 **Table 2.** Impact levels of stations located in the investigated ports based on PAHs content and on MD 176/2016.
 204 Abbreviations used: NI = non-impacted; MI = moderately impacted; HI = highly impacted. I = inner port area; M = middle
 205 port area; O = outer port area.

Port	Stations	Impact level	Source
Vado Ligure	A7, B6, B7, C6,	HI	Losi et al. 2021
	B5, C5, C7, E4	HI	
	E5, F2, F3, H1, K2	HI	
	K3, H2, H3, I1, I2	HI	
	G2, G3, J1, L2, M3	MI	
	K1, L1, L3, M1, M2	NI	
Ligurian Sea	Portosole St1	HI	Moreno et al., 2008a;
	Portosole St2	HI	Moreno et al., 2008b;
	Portosole St3	HI	Moreno et al., 2009;
	Portosole St4	HI	Moreno et al., 2011
	Genoa-Voltri I	HI	
	Genoa-Voltri M	HI	
	Genoa-Voltri O	HI	
	Marina Aregai St.1	HI	
	Marina Aregai St.2	HI	
	Marina Aregai St.3	HI	
Marina Aregai St.4	HI		
Koper	Kp1, Kp2,	NI	Baldrighi et al., 2019
	Kp3, Kp4	NI	Franzo et al., 2022
Trieste	Ts1	MI	
	Ts2, Ts3	HI	
	Ts4	NI	
	ANC1, ANC2	NI	
Ancona	ANC3, ANC4	NI	

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 207 The concentration of PAHs characterizing the Ligurian and Adriatic ports were then compared
 208 with threshold values for chemicals specified in the Ministerial Decree 173/2016 (MD 173/2016), the
 209 Italian normative that rules the management of dredged sediments and sets out their quality.
 210 According to the MD 173/2016, only those values exceeding the upper thresholds values (L2) are
 211 defined as alerting values, while PAH values below the lower limits (L1) are in the normal range.

212 Based on selected PAH concentrations into the sediments reported from the literature (see Table 2)
213 and according to the threshold limits imposed by the Ministerial Decree 173/2016, the stations
214 investigated were classified in three main categories of impact: high impacted (HI), moderately
215 impacted (MI) and non-impacted (NI). The subdivision into three categories was based on lower (L1)
216 and upper (L2) thresholds values for PAHs (Ministerial Decree 173/2016) and as follows: for PAH
217 values < L1 stations were defined as NI (green coded), for PAH values >L1 but <L2 stations were
218 defined as MI (orange coded) and for PAH values >L2 (i.e., alerting values) stations were defined as
219 HI (red coded) (Table 2). Analyses of sediment grain size were performed in all ports as described in
220 Moreno et al., (2009) and Losi et al. (2021) for the Ligurian Sea ports and in Baldrighi et al. (2019)
221 for the Adriatic Sea ports.

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223 **2.3. Nematodes analysis**

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225 For the meiobenthic nematode analysis, the standard protocol of extraction and sample
226 treatment were applied (Heip et al., 1985). In detail, the extraction was performed in accordance with
227 the most adopted method for soft sediments, which is based on the centrifugation of the sediments
228 with Ludox-HS 40 and stained with Rose Bengal (0.5 g l⁻¹). During the counting of meiofaunal
229 organisms under a stereomicroscope (final magnification of 40–80×), 100-120 nematodes (or all the
230 specimens encountered) were randomly hand-picked using a fine pin. Collected animals were
231 transferred from formalin to glycerol through a series of ethanol-glycerol solutions and finally
232 mounted on slides in anhydrous glycerin (see Franzo et al., 2022). All nematodes on permanent slides
233 were taxonomically identified at the genus level under a 100× oil immersion objective using the
234 original species descriptions and identification keys available in NeMys (2025).

235 The trophic structure of nematode assemblage was studied by assigning each genus to one of
236 the following feeding groups (Wieser, 1953): selective (1A) and non-selective (1B) deposit feeders,
237 epistrate feeders (2A) and predators/omnivores (2B). The Index of Trophic Diversity (ITD) was
238 calculated according to Heip et al. (1985): $ITD = \sum \theta^2$, where θ is the percentage contribution of each
239 feeding type. ITD values range from 0.25 (the highest trophic diversity, i.e. each trophic group
240 accounts for 25% of the whole nematode assemblage) to 1.0 (the lowest trophic diversity, i.e. one
241 feeding type represents 100% of the assemblage). The maturity index (Mi, Bongers et al., 1991) was
242 calculated as the weighted average of the individual colonizer-persister (c-p) values: $Mi = \sum v(i) f(i)$,
243 where v is the c-p value of genus i and $f(i)$ is the frequency of that genus. This index is based on the
244 gradual distinction among r -strategist nematodes (colonizers, i.e., c-p 1 and c-p 2), intermediate
245 colonizers (i.e., c-p 3), and k -strategist genera (persisters, i.e., c-p 4 and c-p 5). It therefore reflects
246 the relative prevalence within the community of genera with varying adaptive capacities in response
247 to environmental disturbances.

248 Finally, the frequency of occurrence of nematode genera according to Arasaki et al. (2004) was
249 calculated. Genera were classified as 'constant' if they occurred in at least half of the samples ($F \geq$
250 50%), 'common' ($25\% \leq F < 50\%$), or 'rare' ($F \leq 25\%$). The rare category was further subdivided into
251 'rare class 1' ($10\% \leq F < 25\%$), 'rare class 2' ($5\% \leq F < 10\%$), and 'rare class 3' ($F < 5\%$) to allow for
252 a more detailed discrimination of low-frequency genera.

253 For nematode genera defined as 'constant' and 'common' in the entire dataset, the traditional
254 feeding group classification by Wieser (1953) was compared to the recent feeding group classification
255 proposed by Hodda (2022). Indeed, Hodda suggested a more detailed division of nematodes into
256 different trophic groups according to the most recent literature and feeding habits of the various
257 genera. Based on this new classification, seven feeding categories were defined for free-living

258 nematodes, with additional subcategories emerging within the original Wieser' microbial and
259 predator trophic groups. Among microbial feeders, the following sub-categories were defined: sucker
260 with a suspension as type of food (equivalent to 1A trophic group), sucker with particulate as type of
261 food (equivalent to 1B trophic group), processor with a suspension as type of food (1B), processor
262 with particulate as type of food (1B), scraper with particulate as type of food (equivalent to 2B trophic
263 group), crusher with particulate as type of food (1B), piercer with cellular type of food. Instead,
264 among predators, chewer and piercer feeding methods were identified from the original 2B Wieser's
265 guild.

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267 **2.4. Statistical analysis**

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269 Univariate and multivariate analyses were performed using the PRIMER v. 7 software
270 package (Clarke and Warwick, 2001) with the PERMANOVA add-on package (Anderson et al.,
271 2008). Taxonomic diversity indices (total genera GR; Pielou-evenness J' , Shannon-diversity H' \log_e
272 indices) were calculated based on the percentages of nematode genera.

273 To test the null hypothesis of no significant difference on each of the main nematode
274 descriptors (GR, H' \log_e , J , ITD and M_i) among ports and impact levels, a two-way PERMANOVA
275 analysis was applied using 'port' (7 levels) and 'impact' (3 levels) as fixed factors and based on
276 Euclidean-distance similarity matrices with 9999 permutations of residuals under a reduced model.
277 The data were square root transformed prior to the analysis. The null hypothesis was rejected when
278 the p-level was <0.05 . The Monte Carlo permutation P was used when the number of permutations
279 was lower than 150. If significant differences were detected, posteriori pair-wise comparisons were
280 performed. A Distance-based test for homogeneity of multivariate dispersions (PERMDISP) was
281 performed to assess whether the dispersion between groups was significant or not.

282 A two-way crossed Analysis of Similarity (ANOSIM) (Anderson et al. 2008) was run to test
283 the null hypothesis that nematode community composition does not change across ports and impact
284 levels. The two-way design was applied using data matrix based on the Bray-Curtis similarity on
285 previously square root transformed data. The sample statistic (R) and the significant statistic at the
286 0.1% level (p-level =0.001) were calculated.

287 Multivariate characteristics of sediments were investigated using principal component
288 analysis (PCA) to visualize data trends. PCA with Euclidean distance was performed on previously
289 normalized data to highlight differences in environmental variables (*i.e.*, sediment grain size, depth
290 and PAH concentrations) among stations located in different ports.

291 A Canonical Analysis of Principal coordinates (CAP) was run based on the Bray-Curtis
292 resemblance matrix to visualize the differences in nematode composition among different impact
293 levels (*i.e.*, HI, MI and NI) and among ports. CAP was used among all other analyses, since one of
294 the purposes of CAP is to find axes through the multivariate cloud of points that are the best at
295 discriminating among *a priori* groups. CAP visualizes the presence of real differences among *a priori*
296 groups in multivariate space that cannot be easily seen in an unconstrained ordination (such as the
297 traditional MDS plot). Genera were assigned to a specific impact category when their vector was
298 clearly directed toward the corresponding group in CAP.

299 The relationships between environmental predictor variables and nematode assemblage
300 structure were investigated using distance-based linear models (DistLM) with dbRDA to visualize
301 the percentage of variability in the original resemblance data matrix of the nematode community
302 fitted the model and explained by dbRDA axes (Anderson et al., 2008). P values were obtained with
303 9999 permutations of the model.

304

305 **3. Results**

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307 **3.1. Environmental features and sediment contamination**

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309 A complete list of depth, sediment grain size and PAHs values from all sampling stations is
 310 reported in Table S1. According to the Italian law (Ministerial Decree 173/2016), most (18 out of 28)
 311 of the stations in the port of Vado Ligure were classified as HI, while the rest of the stations were MI
 312 or NI. Porto Sole, Voltri and Marina degli Aregai ports were classified all as HI according to the
 313 threshold limits imposed by the Ministerial Decree 173/2016. In the Adriatic Sea, nine stations out of
 314 twelve resulted as NI, only two stations located in Trieste port were HI (Table 2). PAHs
 315 concentrations ranged from a maximum avg. of 3,019,545.5 $\mu\text{g kg}^{-1}$ (Portosole – Voltri – Marina) to
 316 a minimum avg. of 76 $\mu\text{g kg}^{-1}$ (Ancona) (Table S1). Grain size was different across ports and stations
 317 spanning from muddy sediments (i.e., Koper and Trieste stations and some stations located in Vado
 318 Ligure port) to sandy sediments (i.e., Portosole, Voltri, Marina degli Aregai and Ancona stations).
 319 The depth range was also wide with deeper stations in Vado Ligure (avg. 51 mt) and very shallow in
 320 all other ports (avg. 10 mt) (Table S1).

321 To visualize trends in environmental parameters, a Principal Component Analysis (PCA) was
 322 performed, including grain size composition (sand% and silt/mud%), concentrations of PAHs and
 323 water depth. The PCA revealed that the sampling stations differed primarily in terms of grain size,
 324 followed by levels of pollution and water depth (Table 3; Figure 2). The highest eigenvectors for the
 325 first Principal Component (PC1) that explain 56% of the variance are those of the grain size (Table
 326 3). Along PC1, stations were primarily separated based on the proportion of sand *versus* silt/mud.
 327 PC2, which explained 27% of the variability, was mainly associated with PAH concentrations, and
 328 to a lesser extent with water depth, both of which contributed to the grouping of stations (Fig. 2).

329

330 **Table 3.** The table presents the results of the Principal Component analysis (PCA). Eigenvectors for the individual
 331 environmental variables (i.e., water depth, total PAHs sediment content and grain size) for the first two axes in the
 332 ordination space of the principal component analysis. PC1 and PC2 with a measure of variability in % extracted by each
 333 axis. Abbreviations used: Depth = water depth, PAHs = polycyclic aromatic hydrocarbons, Cum.% = cumulative
 334 variation.

335

Variable	PC1	PC2
Depth	-0.396	0.432
PAHs	0.162	-0.836
Sand (%)	0.638	0.245
Silt/Mud (%)	-0.640	-0.234
Eigenvalue	2.24	1.080
%Variation	56	27
Cum.%Variation	56	82.9

336

337 **3.2 Frequency of occurrence and functional characterization of nematode fauna in the port**
 338 **systems**

339

340 Fourteen genera out of one hundred eighty occurred in 50% or more of all samples and they
 341 are considered as 'constant'. *Daptonema*, *Desmodora*, *Molgolaimus*, *Prochromadorella*, *Sabatieria*
 342 and *Terschellingia* occurred in more than 70% of samples (Table 4). Twenty-eight genera and one

343 family occurred in $\geq 25\%$ of all samples and indeed defined as 'common', while all the rest (i.e., 138
 344 genera and 5 families) occurred in $\leq 25\%$ of all samples and here defined as 'rare'.

345 Among the 'rare' category, further classes were identified based on genera frequency. In line
 346 with this subdivision, rare taxa in class 1 and class 2 were represented by 40 genera, while class 3
 347 included 63 genera (Fig. 3, Table S2).

348 Among the 'constant' genera, *Daptonema*, *Halalaimus*, *Molgolaimus*, *Sabatieria* and
 349 *Terschellingia* were also the genera with the highest abundance percentages (i.e., as relative
 350 abundance %) across ports and impact levels, followed by *Paracomeseoma*, *Desmodorella*, (both
 351 defined as 'common') and *Paralongicyatholaimus* (defined as 'rare').

352 In detail, *Daptonema* was the most widespread and represented genus (max. relative
 353 abundance: 23.7%) crossing different port conditions and pollution levels, followed by *Terschellingia*
 354 reported from six ports out of seven and from NI conditions with a maximum relative abundance of
 355 16%. *Richtersia* dominated the Vado Ligure community (relative abundance = 20%) and impacted
 356 conditions, while *Sabatieria* thrived better in less impacted conditions of the Adriatic Sea (max.
 357 relative abundance = 21.5%).

358 In Table 4, functional measures (i.e., trophic diversity, c-p values and maturity index) of
 359 nematode communities are reported. Most genera (35 out of 43) belonged to c-p classes 2 and 3,
 360 indicating that nematodes in port systems revealed varying degrees of *r*-strategist expression, while
 361 only a minority of them was classified as *k*-strategists (i.e., c-p 4). Regarding the trophic guilds, most
 362 of the genera were 2A, 1A and 1B, while only three genera were identified as 2B (Table 4). Overall,
 363 the most represented Hodda's trophic groups were the microbial scrapers, followed by microbial
 364 sucker suspension and particulate feeders (Table 4).

365

366 **Table 4.** List of 'constant' (bold numbers) and 'common' (italic numbers) nematode genera according to Arasaki et al.
 367 (2004) and their frequency (F%). Functional measures are also listed as trophic groups according to Wieser and Hodda
 368 and colonizer-persister (c-p) values. Abbreviations: mic= microbial, scrap= scraper, suck= sucker, part= particulate
 369 feeder, susp= suspension feeder, proc= processor.

370

Genus	F (%)	Trophic (Wieser 1953)	Trophic (Hodda 2022)	c-p
<i>Anticoma</i>	39.2	1A	mic-scrap	2
<i>Campylaimus</i>	31.4	1B	mic-suck-part	3
<i>Chromadorina</i>	39.2	2A	mic-scrap	3
<i>Chromadorita</i>	29.4	2A	mic-scrap	3
<i>Comesa</i>	35.3	2A	mic-scrap	2
<i>Daptonema</i>	74.5	1B	mic-suck-part	2
<i>Desmodora</i>	78.4	2A	mic-scrap	2
<i>Desmodorella</i>	35.3	2A	mic-scrap	3
Desmodoridae	29.4	n.a.	n.a.	3
<i>Desmoscolex</i>	56.9	1A	mic-scrap/mic-suck-susp	4
<i>Dorylaimopsis</i>	35.3	2A	mic-scrap/mic-proc-part	2
<i>Halalaimus</i>	68.6	1A	mic-suck-susp	4
<i>Leptolaimus</i>	66.7	1A	mic-suck-susp	2
<i>Longicyatholaimus</i>	27.5	2A	mic-scrap	3
<i>Marylynnia</i>	37.3	2A	mic-scrap	3
<i>Metadesmolaimus</i>	37.3	1B	mic-suck-part	2
<i>Metalinhomoeus</i>	58.8	1B	mic-suck-part	2
<i>Metoncholaimus</i>	25.5	2B	pred-ingest	4
<i>Microlaimus</i>	56.9	2A	mic-scrap	2

<i>Molgolaimus</i>	76.5	1A	mic-proc-part	3
<i>Neotonchus</i>	33.3	2A	mic-scrap	2
<i>Odontophora</i>	25.5	1B	mic-crush	2
<i>Oxystomina</i>	43.1	1A	mic-suck-susp	4
<i>Paracanthonchus</i>	49.0	2A	mic-scrap	2
<i>Paracomesoma</i>	29.4	2A	mic-proc-susp	2
<i>Parodontophora</i>	35.3	1B	mic-crush	2
<i>Pierrickia</i>	25.5	1B	mic-suck-part	2
<i>Prochromadorella</i>	72.5	2A	mic-scrap	2
<i>Pselionema</i>	49.0	1A	mic-suck-susp	3
<i>Pseudochromadora</i>	31.4	2A	mic-proc-part	3
<i>Ptycholaimellus</i>	49.0	2A	mic-scrap	3
<i>Quadricoma</i>	49.0	1A	mic-suck-susp	4
<i>Rhips</i>	31.4	2A	mic-scrap	3
<i>Richtersia</i>	64.7	1B	mic-suck-part	3
<i>Sabatieria</i>	78.4	1B	mic-suck-part	2
<i>Setosabatieria</i>	43.1	1B	mic-suck-susp	2
<i>Sphaerolaimus</i>	47.1	2B	pred-ingest	3
<i>Spilophorella</i>	31.4	2A	mic-scrap	2
<i>Terschellingia</i>	88.2	1A	mic-suck-susp	3
<i>Thalassoalaimus</i>	29.4	1A	mic-suck-susp	4
<i>Thalassomonhystera</i>	35.3	1B	mic-suck-susp	1
<i>Tricoma</i>	68.6	1A	mic-suck-susp	4
<i>Viscosia</i>	58.8	2B	pred-ingest	3

371 A detailed analysis of the relative abundance of trophic categories across ports and impact
372 level showed that microbial sucker particulate feeder and microbial sucker suspension feeder
373 categories were the most represented across ports and impacts, while microbial scrapers, processors
374 particulate and suspension feeders were ascribed only to some ports and to HI stations (*i.e.*, microbial
375 particulate suspension feeders) (Figure S1).

376

377 **3.2. Comparison of the nematode community structure across impact levels and port systems**

378

379 A total of 186 morphotypes were identified (Table S2). Diversity as genus richness (GR)
380 ranged from 6 to 63 at A7 and G3 stations (Vado Ligure, Ligurian Sea), respectively, while Shannon-
381 Weaver index (H') ranged from 1.08 at Kp2 station (Koper, Adriatic Sea) to 3.68 at G3 station. Pielou
382 index values (J') varied from 0.39 (Kp2 station) to 0.89 (G3 and I1 stations) (Table S3). Despite the
383 wide range of values observed in the univariate measures of nematode communities across all
384 sampling stations, the PERMANOVA analysis did not reveal any significant differences among ports
385 ($P(\text{MC}) = 0.845$) or among impact levels ($P(\text{MC}) = 0.315$).

386 A two-way crossed ANOSIM was performed to assess differences in nematode composition
387 across impact levels and ports (Table S4). Community composition significantly differed between
388 impacts (HI vs. NI, $p = 0.001$ and MI vs. NI, $p = 0.02$) and across ports (Vado Ligure vs. all ports, $p \leq$
389 0.02) (Table S4). The CAP was applied to assess patterns in nematode genus composition related to
390 impact levels (Fig. 4) and ports (Fig. 5). Taxonomic composition changed gradually from high-
391 impacted stations to non-impacted stations (Fig. 4). Moderately impacted stations were mixed among
392 HI in the upper-left part of the plot showing a transitional community of nematodes in between two
393 opposite ecological quality conditions.

394 *Richtersia* genus exhibited the strongest and most consistent association with HI stations,
395 forming the primary taxon aligned with the HI cluster. *Terschellingia* and *Sabatieria* also projected
396 toward the HI group, although their vectors occupied an intermediate position between HI and MI
397 stations. MI stations were associated with a broader set of transitional genera, including
398 *Spilophorella*, *Halalaimus*, *Leptolaimus*, *Sphaerolaimus*, *Thalassomonhystera*, *Pselionema*, *Rhips*,
399 *Desmoscolex*, *Tricoma*, *Quadricoma*, *Steinera* and *Gnomoxyala*. Due to the short vectors and low
400 frequency of occurrence, no genus displayed a distinct association with NI stations.

401 CAP analysis also indicated a clear association of nematode communities with geographical
402 location of the ports. The genus composition of the seven ports was distinct among three major areas:
403 Adriatic Sea (*i.e.*, Ancona, Trieste and Koper ports), south Ligurian Sea (*i.e.*, Vado Ligure port) and
404 north-west Ligurian Sea (*i.e.*, Portosole, Marina degli Aregai and Genoa-Voltri ports) (Fig. 5).

405 Depth was the variable explaining the largest independent portion of variability (22.6%;
406 marginal test), while the other granulometric variables each showed smaller independent effects
407 (Table 5). When combined in the sequential model using non-correlated variables, depth and silt/mud
408 explained 27.4% of the variability (sequential test), whereas PAHs were not significant.

409 The dbRDA graph showed 22.6% of the variability explained along the first axis by a
410 separation of stations according to different depths, with many Vado Ligure stations deeper than other
411 stations from north-west Ligurian Sea and Adriatic Sea (Fig. 6A). Along the second axis, which
412 accounted for 5% of the total variation, the grouping of stations was primarily influenced by
413 differences in sediment grain size. Koper, Trieste, and some stations in Vado Ligure were
414 characterized by very muddy sediments, in contrast to the coarser sediments observed in the other
415 ports and stations (Table S1). In Figure 6B, the relative abundances of the nematode genera were
416 projected on the factor plane as secondary variables without contributing to the results of the dbRDA.
417 Thus, graph showcased how deep-muddy stations were characterized by the presence of genera such
418 as *Molgolaimus*, *Desmodoridae* sp. 1, *Desmodorella*, *Pselionema*, *Desmoscolex*, *Richtersia*,
419 *Comesoma*, *Microlaimus* and *Quadricoma*; *Prochromadorella* and *Daptonema* characterized
420 shallow-sandy stations, while shallow-muddy stations were characterized by genera such as
421 *Terschellingia*, *Parodontophora*, *Ptycholaimellus*, *Monhystera*, *Longicyatholaimus*,
422 *Halomonhystera* and *Cyatolaimidae* sp.1 (Fig. 6B).

423 The functional diversity of nematode communities was explored through their trophic
424 diversity and their lifestyle strategy composition indices (*i.e.*, ITD and Mi, respectively). The ITD
425 values ranged from 0.31 (M3 station) to 0.70 (Kp2 station), while Mi varied from 1.9 (B5 station) to
426 3.4 (L1 station) (Table S3). PERMANOVA detected significant differences in nematode functional
427 diversity indices (*i.e.*, ITD and Mi) only between ports ($P(\text{MC}) = 0.002$), and specifically between
428 Vado Ligure vs. Marina degli Aregai ($p = 0.005$), Koper ($p = 0.002$), Trieste ($p = 0.05$) and Ancona
429 ($p = 0.013$). Vado Ligure was characterized by lower values in the ITD (avg. 0.38) compared to the
430 other ports (avg. 0.47), which means that all trophic groups were represented instead of a few of them
431 which dominated. Instead, Mi values were generally higher at Vado Ligure (Mi avg. = 3), indicating
432 a community with a relatively higher proportion of *k*-strategist species compared to the other ports
433 (Mi avg. = 2.4; Table S3). PERMDISP test did not show any significant dispersion around centroids,
434 confirming that the differences among the sampling stations were due to a real difference in nematode
435 functional diversity indices ($P(\text{perm}) = 0.886$).

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437
438

439 **Table 5.** The table presents the results of the sequential DistLM test searching for relationships between variability in
 440 nematode community composition and environmental variables (water depth, PAHs and grain size). Statistical
 441 significant variables are in bold. Abbreviations used: SS= sum of squares; Prop = proportion of the variability
 442 explained; Cumul = cumulative proportion of the variability explained.

MARGINAL TESTS					
Variable	SS(trace)	Pseudo-F	<i>p</i>	<i>Prop.</i>	<i>Cumul.</i>
Depth	29218	14.29	0.001	0.226	
PAHs	4371.6	1.7132	0.068	0.034	
Sand (%)	9614.9	3.9329	0.001	0.074	
Silt/Mud (%)	9703.5	3.9721	0.002	0.075	
SEQUENTIAL TESTS					
Variable					
Depth	29218	14.29	0.001	0.226	0.226
Silt/Mud (%)	6263	3.2007	0.001	0.048	0.274
PAHs	2753	1.4192	0.132	0.021	0.295

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4. Discussion

4.1. Is there a typical fauna in port systems?

449 The frequency of occurrence revealed that only 14 genera were ‘constant’ (e.g., *Daptonema*,
 450 *Desmodora*, *Molgolaimus*, *Prochromadorella*, *Sabatieria*, and *Terschellingia*), all known as tolerant
 451 or opportunistic taxa capable of thriving under stressful or disturbed conditions (Balsamo et al., 2012;
 452 Sandulli et al., 2014; Hedfi et al., 2022; Schratzberger et al., 2023) (Fig. 3).

453 Therefore, although nematode assemblages in port areas can be interpreted as communities
 454 shaped by intense environmental filtering, clearly reflecting the anthropic effects (Moreno et al.,
 455 2011; Losi et al., 2021; Franzo et al., 2022 and references therein), our meta-analysis did not identify
 456 a community composition that could be considered typical or exclusive to port systems. Indeed, many
 457 of the taxa recurrently found in Mediterranean ports are cosmopolitan, associated with a wide range
 458 of habitats, from semi-enclosed coastal systems to deep-sea environments, and frequently occur in
 459 sediments that are either disturbed or simply characterized by fine grain-size (Heip et al., 1985;
 460 Semprucci et al., 2014; Magni et al., 2022; Grassi et al., 2023).

461 'Rare' taxa ($F \leq 25\%$), although not driving the main community patterns, may provide useful
 462 ecological information. In the context of port environments, identifying and monitoring 'rare'
 463 nematode taxa might provide key insights since their decline or disappearance might signal the loss
 464 of potentially endemic or specialized populations, unable to tolerate cumulative stressors.
 465 Incorporating their analysis into port monitoring frameworks can enhance the resolution of impact
 466 assessments, contributing to a more nuanced understanding of ecosystem alteration, resilience, and
 467 change in these highly dynamic and human-dominated systems (Franzo et al., 2022). In this regard,
 468 the further subdivision of the 'rare' category into multiple frequency-based classes can help to
 469 distinguish between occasionally occurring genera and those on the edge of exclusion, providing a
 470 finer diagnostic tool in future to interpret subtle but ecologically meaningful community changes
 471 (Fig. 3). However, to reliably use 'rare' taxa as ecological indicators, a broader and more integrated
 472 dataset is required. Indeed, comparative data from other geographical regions of the world are still

473 scarce, and even more critically, long-term temporal datasets are essential to detect trends and
474 distinguish natural variability from anthropogenic change.

475

476 **4.2. How do impact, structural port complexity, natural features influence nematode** 477 **communities?**

478 Environmental factors such as sediment transport/deposition and the resultant grain-size
479 structure of the seafloors can affect the dispersion and dilution of pollutants, the distribution of
480 oxygen and nutrients, and consequently the taxonomic structure and functioning of living benthic
481 fauna (Zhang et al., 2014; Grassi et al., 2023). Therefore, any attempt to correlate or interpret the
482 composition of nematode communities in relation to pollutant concentrations must consider the
483 potential influence of site-specific hydrodynamic regimes, sedimentological variability, local
484 pollution sources as well as management actions (Moreno et al., 2011; Sahraean et al. 2017).

485 In this meta-analysis, PCA clearly revealed a separation among stations, primarily driven by
486 the sedimentological features of the sea bottoms, which were certainly, at least in part, related to the
487 structural complexity of each port system (Fig. 1 and 2). Stations located in closed or semi-enclosed
488 areas, as well as in shallow waters surrounding ports, were characterized by sandy sediments and,
489 based on the PAH levels detected, appeared generally less impacted. In contrast, stations with a high
490 proportion of silt and clay were typical of offshore stations near Vado Ligure or of open port structures
491 directly facing the sea, such as those of Trieste and Koper. This last observation may appear
492 counterintuitive, considering that more enclosed or semi-enclosed areas are generally expected to
493 exhibit lower hydrodynamic energy and therefore higher sedimentation rates. However, several
494 factors must be considered when interpreting these results, including the complex interactions
495 between local hydrodynamics, geomorphology, and riverine inputs. For instance, the
496 geomorphological setting of the northern Adriatic Sea, where the ports of Koper and Trieste are
497 located, is naturally predisposed to the accumulation of fine sediments, which helps explain the
498 complex depositional patterns observed. Although both ports are relatively open, the Gulf of Trieste
499 is shallow, hydrodynamically weak, and influenced by riverine inputs (*e.g.*, the Isonzo River), which
500 deliver large amounts of silt and clay into the area (Grilli et al., 2005). Furthermore, the cyclonic
501 circulation of the Adriatic Sea tends to drive fine particulate matter toward the northernmost part of
502 the basin, further promoting the accumulation of fine sediments in these regions (Colantoni et al.,
503 2004). This combination of factors may have led to greater sediment accumulation in the northern
504 Adriatic ports. In contrast, ports located along the Ligurian Sea are influenced by deeper waters,
505 stronger coastal currents, and the absence of major freshwater inputs, conditions that favour sediment
506 resuspension and the predominance of sandy substrates.

507 The two complementary CAP analyses (based on impact level and by port) and the two-way
508 crossed ANOSIM revealed that spatial patterns in nematode assemblages were not driven by
509 geography *per se*, but by environmental conditions associated with different levels of disturbance and
510 by the structural properties of individual ports (Figs. 4, 5).

511 The CAP constrained by impact showed a strong and coherent clustering of NI stations
512 demonstrating that unimpacted conditions, rather than geographic proximity, were the main
513 determinant of their similarity. Indeed, if geography were the primary driver, NI stations from Vado
514 Ligure would have grouped with Ligurian HI–MI stations; instead, they consistently aligned with NI
515 sites from the Adriatic Sea.

516 When the analysis was constrained by port, stations grouped according to shared
517 environmental settings rather than geographical proximity. Adriatic ports clustered together because

518 they share a naturally silt-dominated depositional regime, whereas Ligurian ports appeared more
519 dispersed due to their heterogeneous morphologies and hydrodynamic conditions (Fig. 5).

520 Therefore, geography had no direct effect on nematode community structure. Rather, ports
521 grouped according to their structural and hydrodynamic characteristics, degree of enclosure, sediment
522 type, and water turnover, which may indirectly shape their contamination profiles and benthic
523 conditions.

524 DistLM and its dbRDA revealed that sediment type and depth were the main drivers of
525 environmental variation of the communities among stations. Northern Adriatic ports of Trieste and
526 Koper were naturally characterized by finer sediments due to basin-scale circulation and riverine
527 inputs, whereas Ligurian ports showed a wider range of sediment types due to their heterogeneous
528 geomorphology and exposure. PAHs contributed secondarily, and mostly in interaction with sediment
529 properties. Indeed, even when hydrocarbons are present, sandy and well-oxygenated sediments tend
530 to limit their bioavailability to benthic organisms, as pollutants may degrade more rapidly or be
531 dispersed within permeable substrates (Chiaia-Hernández et al., 2022). This helps explain the
532 relatively weak PAH vector observed in the dbRDA analysis: although PAHs aligned with sandy
533 stations, their presence did not translate into a strong structuring effect on nematode communities,
534 likely because coarse, oxygen-rich sediments reduce their accumulation and ecological impact (Fig.
535 6A).

536 The identification of indicator taxa was based exclusively on the CAP constrained by impact
537 level. CAP vectors revealed that *Richtersia* was the only genus strongly and consistently associated
538 with HI conditions. *Terschellingia* and *Sabatieria* also projected toward the HI cluster, although their
539 loadings were intermediate between HI and MI stations. MI stations were characterised by a broader
540 suite of transitional taxa, including *Spilophorella*, *Halalaimus*, *Leptolaimus*, *Sphaerolaimus*,
541 *Thalassomonhystera*, *Pselionema*, *Rhyps*, *Desmoscolex*, *Tricoma*, *Quadricoma*, *Steineria* and
542 *Gnomoxyala*. Many of these genera are commonly recognized as tolerant or opportunistic taxa,
543 typically thriving under high levels of organic enrichment or contamination (e.g., Heip et al., 1985;
544 Balsamo et al., 2012; Ridall and Ingels, 2021; Franzo et al., 2022). Rare taxa, characterised by short
545 vectors and low frequency of occurrence, were not considered as indicators. No genus emerged as a
546 unique indicator of NI stations, which instead exhibited a balanced and evenly structured assemblage.

547 In terms of trophic traits, it is useful to determine whether nematodes are generalist feeders
548 interacting with a wide range of organisms or functioning as specialists with a narrow dietary niche.
549 Moreover, some nematodes may switch food sources opportunistically according to what is available
550 in a particular place. Therefore, this information helps, at least to some extent, to better characterize
551 the environmental conditions in which these organisms occur (Hodda, 2022).

552 In the present study, microbial feeders were the most abundant group across all ports and
553 impact levels, consistently with findings from other studies conducted in confined and impacted
554 systems rich in organic matter (e.g., Van Colen et al., 2009; Cibic et al., 2017). Moreover, the presence
555 of pollutants and organic loads appear to stimulate rapid microbial growth as well as the bloom of
556 microphytobenthos, which can enhance dense communities of microbial feeder nematodes
557 (Montagna et al., 2013). Within the broader category of microbial feeders, multiple subgroups can be
558 identified according to both their feeding mechanisms and the specific types of food ingested (such
559 as microbes, particulate organic matter, or microphytobenthos). Sucking the food by ingesting
560 organisms small enough to be consumed whole, or molecules from solution or sucked from
561 suspension were the most common feeding modes (e.g., *Daptonema*, *Richtersia* and *Terschellingia*),
562 reflecting environmental conditions that were not food-limited and encompassing a wide range of
563 sediment types. However, some feeding groups characterized only some ports and/or impact levels

564 (Supplementary Material, Fig S1). Microbial processor particulate feeders (mic-proc-part)
565 characterized the impacted sediments of Vado Ligure (HI stations), mainly due to the presence of the
566 highly tolerant genus *Molgolaimus*, which feeds by ingesting small organisms and processing them
567 to extract their internal contents (Hodda, 2022).

568 The microbial processor suspension feeder group (i.e., organisms that ingest very small
569 particles or microbes by sucking them from suspension and processing them to extract their contents)
570 was highly represented in the Port of Voltri, primarily due to the dominance of the genus
571 *Paracomesoma*. Voltri was the most polluted port presenting a wide array of sediment grain-size and
572 *Paracomesoma* was reported by Moreno et al., (2011) to be strongly and positive correlating with
573 total organic matter and chemicals. Finally, microbial scrapers (i.e., feed by scraping microbes from
574 their attachments to substrate particles) were abundant at Vado Ligure, Portosole and Marina ports
575 due to the genera *Desmodorella* and *Paralongicyatholaimus*. These genera showed a different degree
576 of tolerance/sensitivity when compared multiple studies from different ports (e.g., Moreno et al.,
577 2011; Losi et al., 2013, 2021), along with their plasticity in inhabiting different substrate types (from
578 muddy to sandy sediments).

579 The dominance of colonizer nematodes (c-p 2 and 3) over *k*-strategist species (c-p 4) is
580 evident across the entire dataset and supports the notion that nematode assemblages in port
581 environments are strongly shaped by intense environmental filtering. This pattern clearly reflects
582 anthropogenic impacts, with a progressive disappearance of sensitive (i.e., *k*-strategist) species,
583 replaced by more opportunistic and/or resistant taxa (i.e., colonizers) capable of rapidly proliferating
584 by exploiting available food resources. The only exceptions were some stations in the shallow coastal
585 stretch from Capo Vado to Bergeggi, where coarse sand and gravel substrates create high habitat
586 heterogeneity and support a well-diversified nematode assemblage dominated by taxa belonging to
587 higher c-p classes (*k*-strategists sensu Bongers et al., 1991). These conditions contributed to a
588 significantly higher Maturity Index (Losi et al., 2021).

590 **5. Conclusions**

591
592 This meta-analysis provides an integrated assessment of meiofaunal nematodes in
593 Mediterranean port ecosystems, highlighting how their assemblages respond to both anthropogenic
594 disturbance and the natural structural heterogeneity of port environments. Frequency-based patterns
595 showed that only a small subset of genera occurred consistently across sites, all belonging to taxa
596 known for their tolerance to environmental stress. This confirms that nematode communities in ports
597 are strongly shaped by environmental filtering, yet no assemblage emerged as uniquely characteristic
598 of port systems, reflecting instead the cosmopolitan distribution of many opportunistic genera in fine-
599 grained or organically enriched sediments.

600 Multivariate analyses further demonstrated that nematode assemblages vary primarily in
601 response to sediment type and port morphology, rather than to geographic position itself. CAP
602 constrained by impact level revealed that only *Richtersia* was consistently associated with highly
603 impacted conditions, while *Terschellingia* and *Sabatieria* showed intermediate positions between HI
604 and MI sites. Moderately impacted stations were associated with a wide suite of transitional genera,
605 whereas no genus was uniquely indicative of non-impacted conditions, which were instead
606 characterised by more balanced community structures. Sediment grain size and depth were confirmed
607 as the main drivers of nematode community variations, while PAHs exhibited only a secondary and
608 context-dependent influence, largely modulated by sediment permeability and oxygenation.

609 Functional traits supported these findings: microbial feeders dominated across ports and
610 impact levels, consistent with organic enrichment and stimulated microbial production, while
611 differences in specific feeding modes reflected local port conditions. The prevalence of coloniser taxa
612 over more sensitive *k*-strategists across most stations further underscores the role of environmental
613 filtering in shaping port nematode communities, with only a few coarse-sediment stations showing
614 signs of recovery or higher ecological quality.

615 Overall, our results demonstrate that nematode assemblages offer a sensitive and informative
616 tool for assessing ecological conditions in port environments, especially when combined with
617 sedimentological and environmental information. Future efforts should prioritise expanding spatial
618 coverage, integrating long-term monitoring, and incorporating rare taxa into diagnostic frameworks
619 to improve the resolution and reliability of ecological assessments in complex, human-dominated
620 coastal systems.

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632

633

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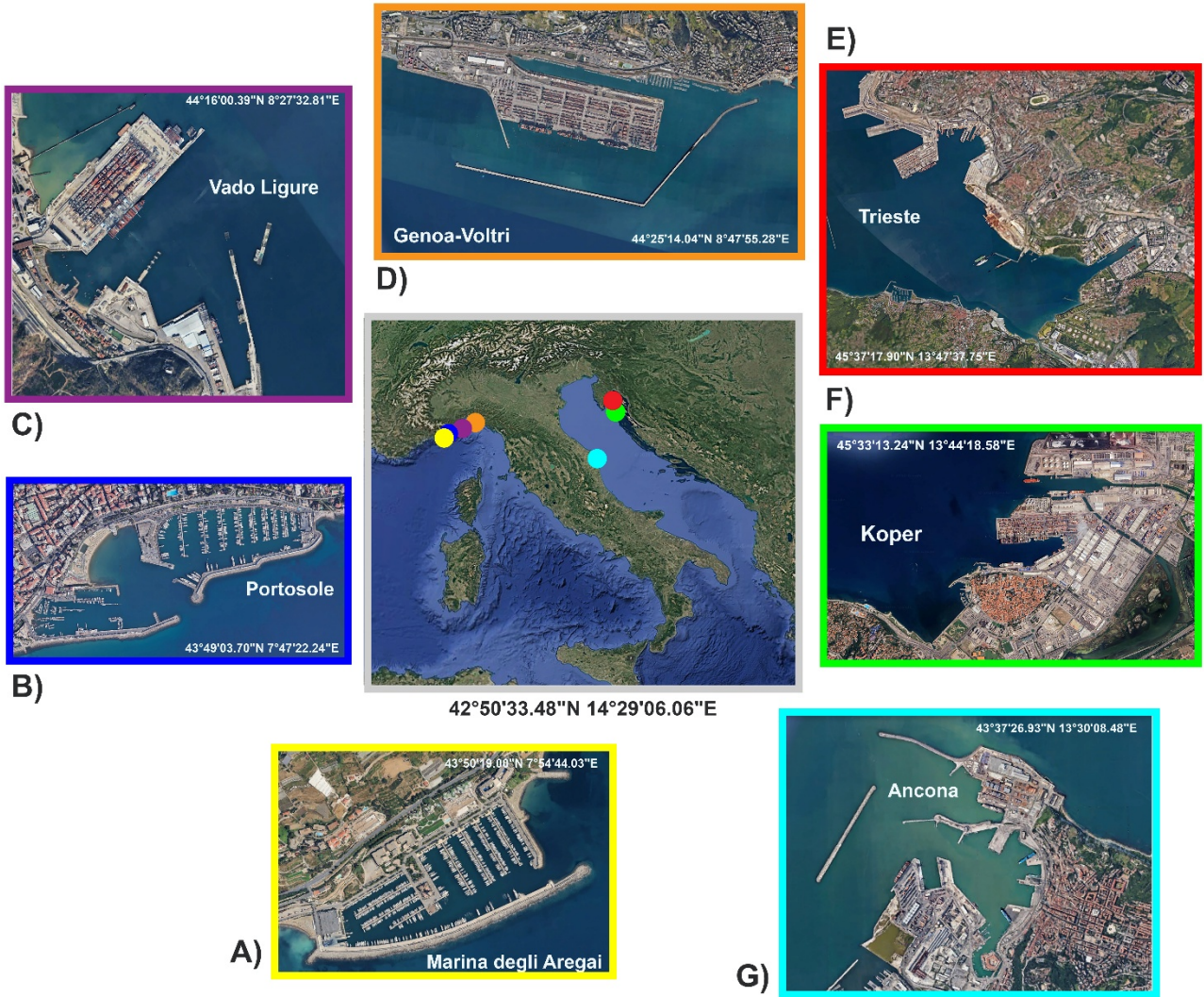
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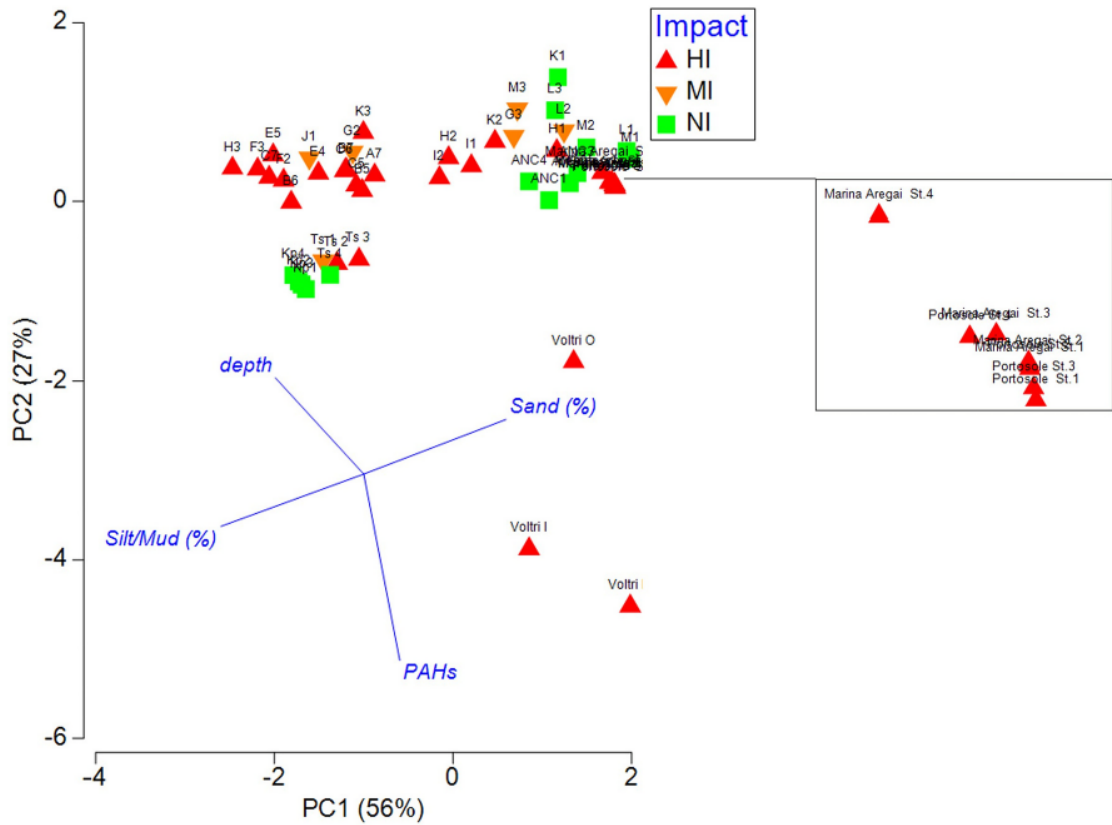
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786 **Figure 1.** Map with locations of all sampled ports in the Adriatic Sea (Koper, Trieste and Ancona)
787 and Ligurian Sea (Vado Ligure, Genoa-Voltri, Portosole and Marina degli Aregai).
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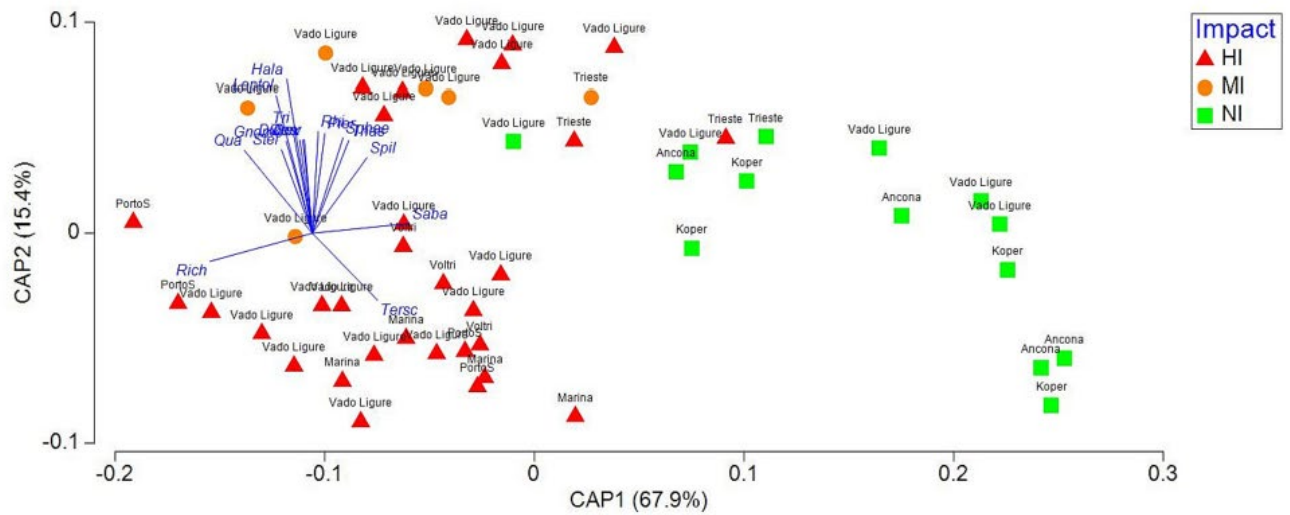


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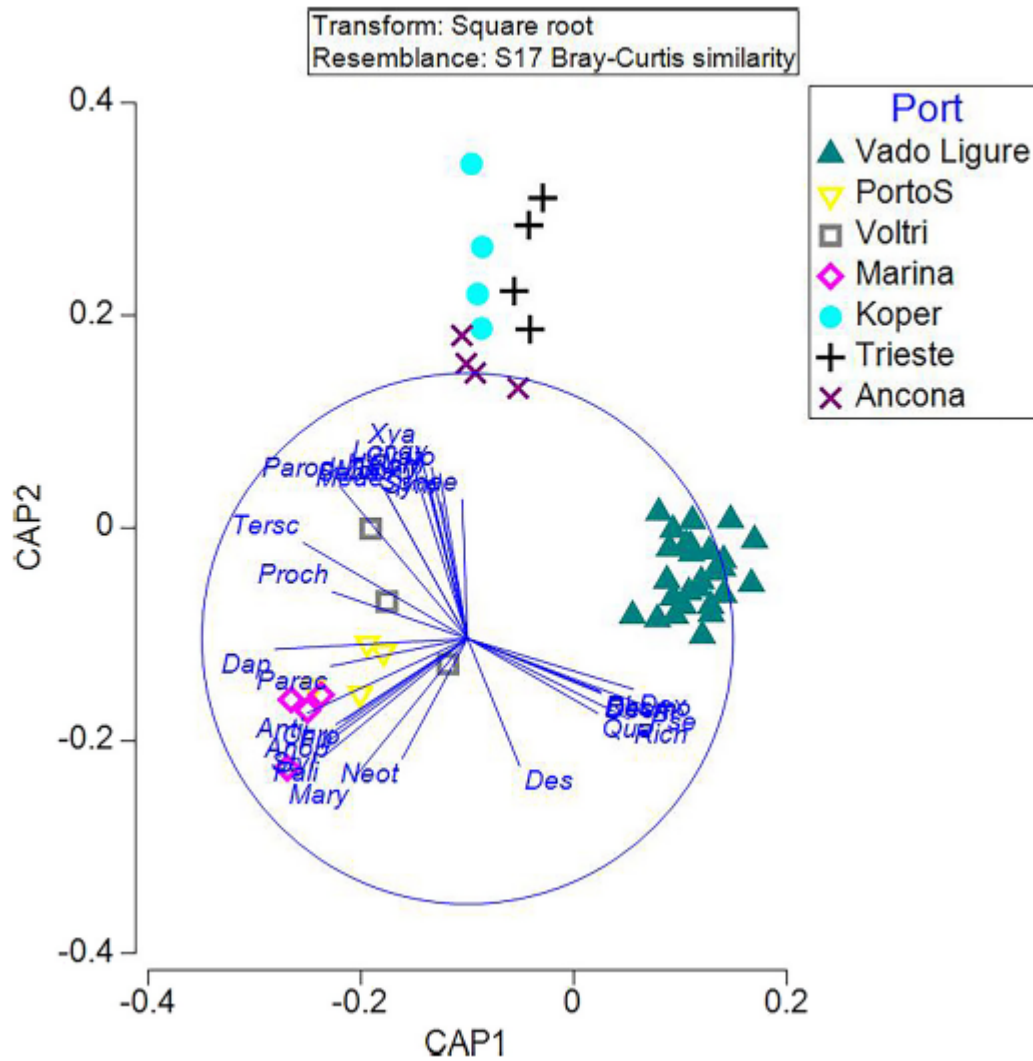
Figure 2. PCA output of environmental variables of all sampling stations. Ordination of sampling stations using the first and second principal components. The lines indicate the superimposed vectors. The square is the zoom-in on the names of HI stations located on the upper right side of the graph, for clarity. Numbers in brackets are the percentages of explained variation on the axes. Abbreviations used: NI = non-impacted; MI = moderately impacted; HI = highly impacted.



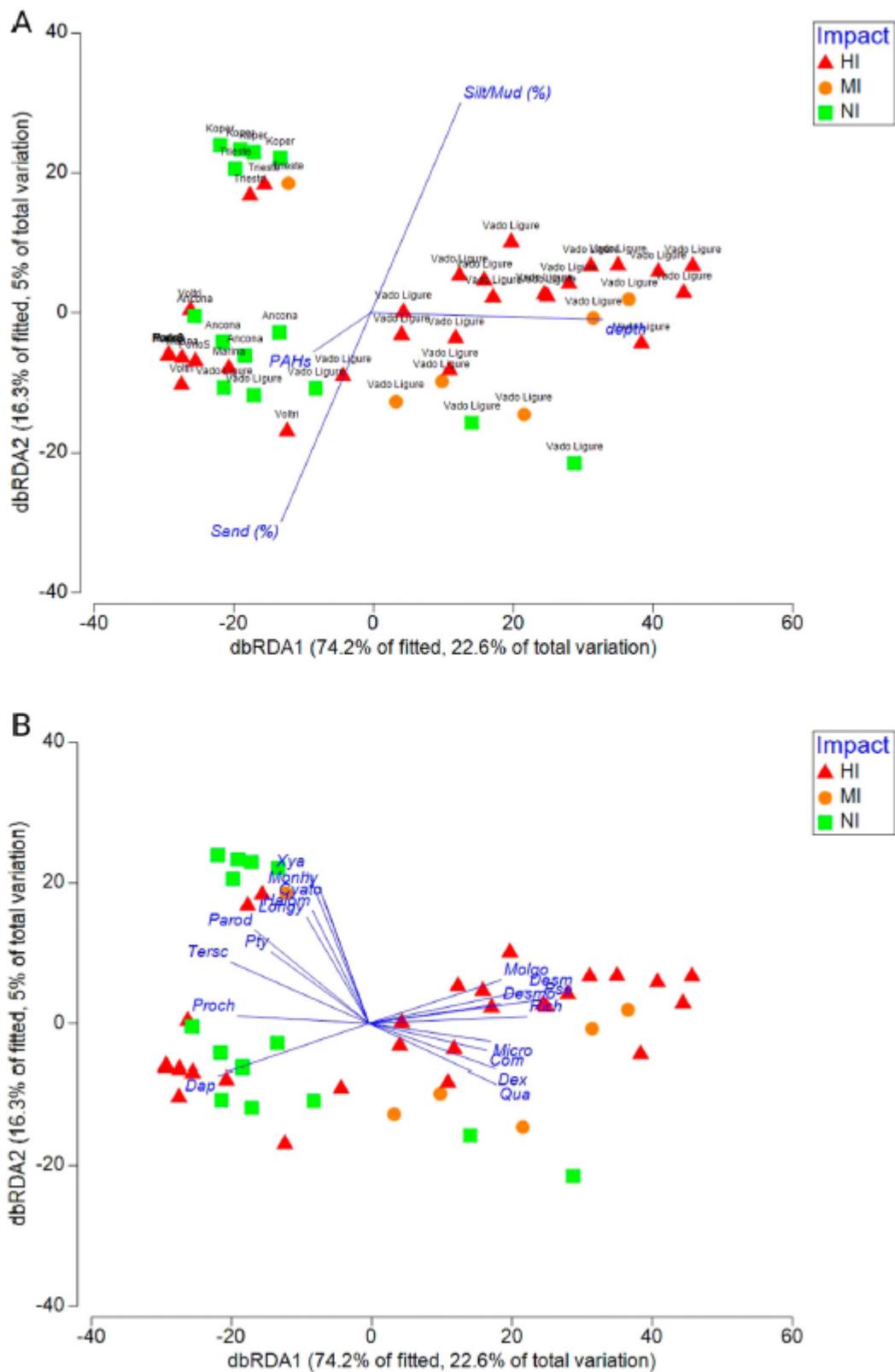
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 798 **Figure 3.** Genera classified as “constant” ($F \geq 50\%$), “common” ($25\% \leq F < 50\%$), “rare class 1”
 799 ($10\% \leq F < 25\%$), “rare class 2” ($5\% \leq F < 10\%$), and “rare class 3” ($F < 5\%$) on the base of the
 800 occurrence frequency in the study area.
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 803 **Figure 4.** Scatter plot of the first two axes of the canonical analysis of principal coordinates (CAP)
 804 which best explained the structure of the nematode assemblages at three levels of impact. A vector
 805 overlay on the CAP-chart was performed. Only genera with correlations $r > 0.5$ with at least one
 806 CAP-axis were used. HI = high impacted (red triangles), MI = moderately impacted (orange circles)
 807 and NI = non-impacted (green squares). Abbreviations of nematode genera are reported in Table
 808 S2. Data were square root transformed and a Bray-Curtis similarity was applied.
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811 **Figure 5.** Scatter plots of the first three axes of the canonical analysis of principal coordinates (CAP)
812 which best explained the structure of the nematode assemblages at different ports showing the first
813 two PCs. A vector overlay on the CAP-chart was performed. Only genera with correlations $r > 0.5$
814 with at least one CAP-axis were used. Abbreviations of nematode genera are reported in Table S2.
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Figure 6. dbRDA graph showing (A) superimposition of environmental variables on station distribution according to different impact levels and (B) superposition of nematode genera on dbRDA graph. Only nematode genera with a Correlation >0.5 with the two axes are shown. Abbreviations used: NI = non-impacted; MI = moderately impacted; HI = highly impacted. Data were square root transformed and a Bray-Curtis similarity was applied.