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CICLO XXXIV

EFFECT OF WIDESPREAD ENVIRONMENTAL DISTURBANCES ON MEIOFAUNA AND FREE-LIVING NEMATODES

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ABSTRACT

Natural and human disturbances may affect ecosystems compromising their functioning. It is, therefore, important to define prevention strategies to avoid irreparable damages, where the first action implicates the identification of possible environmental criticalities followed by the management actions.

For this purpose, it is necessary to identify reliable and easily applicable bioindicators that can give an early response to potential environmental disturbance and define the ecological quality of marine coastal systems. Meiofauna (animals from 500 µm to 45 µm in size), and in particular free-living nematodes, appear good candidates in the framework of the ecological assessment because they have all the above cited characteristics along with a wide distribution in all the types of environments and substrata. Thus, the present Ph. D. project takes into consideration six study cases set in a wide geographical span and under environmental disturbances frequently occurring in the coastal systems. The primary project aims are i) to demonstrate that meiofauna and nematodes can be efficiently used for the assessment of the health status in marine habitats; ii) to increase the number of meiobenthic indicator taxa that can be regarded as "sentinels" of human disturbance; iii) to further calibrate the tools so far available for the classification of Ecological Quality (EcoQ) to encourage the use of meiofauna in the assessment of the status of underwater life.

The first study case was carried out in the Maldivian Archipelago (Indian Ocean), where human activities such as dredging and infilling are common practices, which led to a decrease in the taxonomic diversity of many animal species. In this context, the study of nematode communities shows that their taxonomical composition and functional traits (i.e. trophic and life strategy) are mainly influenced by the reef exposure and topography (i.e., lagoon and ocean reefs) and the depth gradient. The high abundance of k-strategists in ocean reefs might indicate a higher EcoQ when compared to lagoon reefs; however, the absence of significant differences in life strategy functional diversity and functional redundancy indicates that a recovery process is underway. The results obtained suggest that nematodes could add important information to the vulnerability and resilience of the benthos and, therefore, should be included in the biomonitoring activities of the Coral Degradation Zones (CDZs) along with the reef bioconstructors.

The second case study from the Indian Ocean (Indonesia), was carried out in two seagrass meadows characterized by different sedimentological regimes and anthropogenic pressure. The lowest meiobenthic abundance and diversity found at the Barat meadow along with the major sediment modifications suggested a higher anthropogenic pressure in this site. Rare meiobenthic taxa (<1% of the total abundance in all investigated samples) revealed significant differences in the composition of the meiobenthic assemblage suggesting the key role of rare meiobenthic taxa as possible indicators of spatial variability generated by engineer species and different environmental conditions.

The third study concerned the Southern Ocean at Terra Nova Bay (Antarctica), where the effects of the human activities in the Mario Zucchelli Research Station were compared with the natural disturbance occurring in the penguin colony located at Adelie Cove. Here, the high contribution of r-strategists corroborates the hypothesis that the chronic impact of the penguin colonies may have stronger effects on the meiobenthos than the human activities at the research station and, as reported in the Indonesian study, the assemblage structure of rare meiobenthic taxa is confirmed to be more susceptible to environmental variations, rather than the whole assemblage structure.

In the fourth study, a commercial port of the Ligurian Sea, nematode communities were investigated in stations at an increasing distance from the port. The stations selected at an increasing distance from the port outlet aim to detect how ports may influence the benthic nematodes from surrounding coastal areas. The taxonomical and functional distribution of the nematode species appeared influenced by the contamination level, granulometry, and organic load of sediments. According to

these results, the nearest stations to the port outlet appeared to be the most polluted, while the coast close to Bergeggi Marine Protected Area seemed as the most pristine area. Furthermore, the study indicated a selection of the genera that may be considered as sentinels of sediment contamination and organic enrichment. The maturity index suggested that the different proportion of the nematode life-strategies is an effective tool to assess the EcoQ of sediments.

In the fifth study the sediments within three commercial ports from the Adriatic Sea (Ancona and Trieste, Italy; Koper, Slovenia) were investigated, the nematode analysis indicated that r-strategist genera were dominant in all three study areas. Despite the high contamination levels, Trieste showed high nematode biodiversity which suggests a potential adaptation of nematodes to a long-standing contamination. A co-occurrence analysis was performed to further implement the sets of bioindicator genera concerning their different opportunistic behaviors and to identify which genera cohesively respond to site-specific environmental conditions. The results encourage the use of nematodes for the EcoQ assessment of port seabeds.

Despite the good response of meiofauna and nematodes as bioindicators of anthropogenic disturbance, the taxonomic identification of these organisms remains a time-consuming activity and requires the supervision of experienced taxonomists. To overcome this problem, in the sixth study, was proposed a combination of morpho-functional traits (i.e. amphids, cuticles, buccal cavities, and tail shapes) as an alternative to the taxonomic identification of the nematode specimens. The approach was tested by employing a meta-analysis of a data set of study cases from the Mediterranean Sea. The statistical results revealed that the trait combination perfectly mirrors the changes in the taxonomic community structure at the genus level which makes it possible to suggest this approach as a rapid test of nematode fauna changes for biomonitoring purposes.

CONTENT

INTRODUCTION

ENVIRONMENTAL DISTURBANCE

Disturbance can be described as a complex phenomenon influenced by disturbance origin, intensity, duration, frequency, magnitude, timing, severity, variability, and regime (White & Pickett, 1985; Turner, 2000; Keane, 2017; Ratajczak et al., 2018).

It causes stress and perturbations that manifest themselves as temporal and spatial heterogeneity in natural assemblages (Sousa 1984; Rykiel 1985; Parker et al. 1999). Independently of its origins (i.e. natural or anthropogenic ones), the consequences are alterations, degradation and destruction of habitats with impacts from organism to community level with relevant implications for the trophic web.

After an event of stress or perturbation, the affected community responds in a complex way especially if the disturbance involves different populations.

Communities can tolerate alterations, adapt to the new environmental conditions, organize themselves into a different state, or return to the pre-disturbance state (Supp & Ernest, 2014; Arnoldi et al., 2018; Falk et al., 2019).

Recently, public opinion has increased interest and concern for the future of our planet: the environment is seen as an indispensable prerequisite for social well-being and quality of life mainly because environmental disturbance can represent a risk to human health.

Indeed, environmental pollution is responsible for ca. 9 million deaths each year due to heart, gastric, brain and cancer diseases, huge economic losses, ecosystem degradation and biodiversity loss (Landrigan et al., 2020).

DISTURBANCE: HOW TO DETECT IT?

Our planet is subject to continuous and increasing changes due to different types of disturbances.

It is important to identify and promptly manage any kind of perturbation or stressor to avoid irreparable consequences such as the loss of biodiversity, ecosystem functioning or services that would have devastating impacts on human life.

Therefore, research should develop effective and rapid methodologies/approaches that can be easily applied in the field and, at the same time, meet the legislative requirements.

In Europe, the Water Framework Directive (WFD, 2000/60/EC) and the Marine Strategy Framework Directive (MSFD, 2008/56/EC) regulate the marine environmental policy: the Ecological Quality Status (EQS) of marine environments must be assessed through Biological Quality Elements (BQEs) as fundamental parts of the ecosystem for long-term use of marine environmental resources to be possible.

Research on the disturbance effects is mainly focused on large organisms and so far, macrofauna is the most used BQE as it is characterized by organisms visible to the naked eye and, therefore, easily identifiable from a taxonomic point of view. However, many limits have been documented in the use of macrofauna as bioindicator group that makes urgent the development of alternative BQEs (Fairweather et al., 1995; Gambi et al., 2020).

Furthermore, over 90% of animal life on Earth is small in size (Naskrecki 2005).

Meiofauna and their most representative group, free-living nematodes, have been suggested as valid alternatives to macrofauna (Moreno et al., 2011; Balsamo et al., 2012; Franzo et al., 2018, 2019; Semprucci et al., 2018).

Meiofauna represents the most abundant component of the benthic domain, with densities of 10^{5}-10^{6} ind. m^2, an inhomogeneous horizontal distribution and a vertical profile limited by the oxygen penetration depth (Pusceddu et al., 2007).

Thanks to their strong sensitivity to environmental perturbations, the high number of individuals, lack of larval planktonic forms, short life cycle, and non-destructive sampling methods, meiofaunal organisms are regarded as interesting candidates for the evaluation of the disturbance processes in marine coastal systems (Giere, 2009, 2019).

In particular, nematodes, which are mainly responsible for biomineralization, bioturbation, the oxygen and nutrient cycle, can detect quickly to ongoing or recent environmental changes providing effective responses after disturbances of many origins (Schratzberger and Ingels, 2018).

Many studies have demonstrated that free-living nematodes can be effective BQEs, but they still remain neglected by policy-makers, environmental agencies and even a part of the scientific community (Semprucci et al., 2015; Chen et al., 2018; Franzo et al., 2019; Hong et al., 2020).

AIM OF THIS PH.D. THESIS

This Ph.D. project attempts to provide new evidence of the possible application of meiofauna and free-living nematodes in the Ecological Quality (EcoQ) assessment of marine ecosystems in accordance with the European Directives (WFD, 2000/60/EC and MSFD, 2008/56/EC). In detail, the aims are i) to demonstrate that meiofauna and nematodes can be efficiently used for the assessment of the health status in marine habitats; ii) to increase the number of meiobenthic indicator taxa that can be regarded as "sentinels" of human disturbance; iii) to further calibrate the tools so far available for the classification of EcoQ to encourage the use of meiofauna in the assessment of the status of underwater life.

Many coastal areas, subjected to different types of environmental disturbances (e.g., ports, sewages, natural organic enrichment, physical stress) and located in a wide range of geographical regions (from the Mediterranean Sea to the Southern Ocean) were selected. In addition, a meta-analysis approach based on already published data was carried out to test an alternative method to detect the nematode community changes in areas affected by human pressure. The approach based on nematode morpho-functional traits provided that can document nematode community structure changes can represent a rapid and low-cost method of detection.

REFERENCES

- Arnoldi, J., Bideault, A., Loreau, M., Haegeman, B. (2018). How ecosystems recover from pulse perturbations: A theory of short- to long-term responses. Journal of Theoretical Biology, 436, 79–92.
- Balsamo, M., Semprucci, F., Frontalini, F., Coccioni, R., (2012). Meiofauna as a tool for marine ecosystem biomonitoring. Marine ecosystems, 4, 77–104.
- Chen, C.A., Soo, C.L., Balsamo, M., Semprucci, F., (2018). An approach based on nematode descriptors for the classification of the ecological quality (EcoQ) of the Malaysian coasts. Marine Biodiversity, 48, 117–126.
- Fairweather, P., Ludwig, J., McIntyre, S. (1995). Use of biota to assess water quality. Australian Journal of Ecology, 20(1), 227.
-Falk, D. A., Watts, A. C., Thode, A. E. (2019). Scaling Ecological Resilience. Frontiers in Ecology and Evolution, 7, 1–16.
- Franzo, A., Asioli, A., Roscioli, C., Patrolecco, L., Bazzaro, M., Del Negro, P., Cibic, T., (2019). Influence of natural and anthropogenic disturbances on foraminifera and freeliving nematodes in four lagoons of the Po delta system. Estuarine. Coastal. And Shelf Science, 220, 99–110.
- Franzo, A., Guilini, K., Cibic, T., Del Negro, P., (2018). Structure and function of nematode assemblages in contaminated sediments: what can we learn from the mar piccolo of Taranto (Ionian Sea)? Journal of the Marine Biological Association of the United Kingdom, 98, 1845–1857.

- Gambi, C., Dell'Anno, A., Corinaldesi, C., Martire, M. L., Musco, L., Da Ros, Z., Danovaro, R. (2020). Impact of historical contamination on meiofaunal assemblages: The case study of the Bagnoli-Coroglio Bay (southern Tyrrhenian Sea). Marine environmental research, 156, 104907.
- Giere O. (2009) Meiobenthology: the microscopic motile fauna of aquatic sediments. 2nd edition. Springer, Berlin Heidelberg. pp. 527.
- Giere, O. (2019). Perspectives in meiobenthology: reviews, reflections and conclusions. Springer International Publishing. Pp. 80.
- Hong, J. H., Semprucci, F., Jeong, R., Kim, K., Lee, S., Jeon, D., Lee, W. (2020). Meiobenthic nematodes in the assessment of the relative impact of human activities on coastal marine ecosystem. Environmental monitoring and assessment, 192(2), 1-13.
-Keane, R. (2017). Disturbance Regimes and the Historical Range and Variation in Terrestrial Ecosystems. Encyclopedia of Biodiversity, Volume 2, 568–581.
-Landrigan, P. J., Stegeman, J. J., Fleming, L. E., Allemand, D., Anderson, D. M., Backer, L. C., Rampal, P. (2020). Human health and ocean pollution. Annals of global health, 86(1).
- Moreno, M., Semprucci, F., Vezzulli, L., Balsamo, M., Fabiano, M., Albertelli, G., (2011).
The use of nematodes in assessing ecological quality status in the Mediterranean
coastal ecosystems. Ecol. Indic. 11, 328–336.
- Naskrecki, N. (2005). The smaller majority: the hidden world of the animals that dominate the tropics. Cambridge, Massachusetts, USA: Belknap Press of Harvard University Press.
-Parker Jr, E. D., Forbes, V. E., Nielsen, S. L., Ritter, C., Barata, C., Baird, D. J., … Ripley, B. J. (1999). Stress in Ecological Systems. Oikos, 86(1), 179–184.
- Pusceddu, A., Gambi, C., Manini, E., & Danovaro, R. (2007). Trophic state, ecosystem efficiency and biodiversity of transitional aquatic ecosystems: analysis of environmental quality based on different benthic indicators. Chemistry and Ecology, 23(6), 505-515.
-Ratajczak, Z., Carpenter, S. R., Ives, A. R., Kucharik, C. J., Ramiadantsoa, T., Stegner, M. A., Turner, M. G. (2018). Abrupt Change in Ecological Systems: Inference and Diagnosis. Trends in Ecology and Evolution, 33(7), 513–526.
-Rykiel Jr, E. R. (1985). Toward a definition of ecological disturbance. Australian Journal of Ecology, 10, 361–365.
- Schratzberger, M., & Ingels, J. (2018). Meiofauna matters: The roles of meiofauna in benthic ecosystems. Journal of Experimental Marine Biology and Ecology, 502, 12–25.
- Semprucci, F., Balsamo, M., Appolloni, L., Sandulli, R., (2018). Assessment of ecological
quality status along the apulian coasts (Eastern Mediterranean Sea) based on
meiobenthic and nematode assemblages. Marine Biodiversity, 48, 105–115.
- Semprucci, F., Losi, V., Moreno, M., (2015). A review of italian research on free-living
marine nematodes and the future perspectives on their use as ecological indicators
(EcoInds). Mediterranean Marine Science 16 (2), 352–365.
-Sousa, W. P. (1984). The role of disturbance in natural communities. Annual review of ecology and systematics, 15, 353-391.
-Supp, S. R., & Ernest, M. S. K. (2014). Species-level and community-level responses to disturbance: a cross-community analysis. Ecology, 95(7), 1717–1723.
-Turner, A. (2000). Trace metal contamination in sediments from UK estuaries: an empirical evaluation of the role of hydrous iron and manganese oxides. Estuarine, Coastal and Shelf Science, 50, 355–371.
-White, P., & Pickett, S. (1985). Natural disturbance and patch dynamics, an introduction. In: Pickett STA, White PS (eds) The ecology of natural disturbance and patch dynamics. Academic Press, New York (pp. 3–13).

CHAPTER 1

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Taxonomic and functional nematode diversity in Maldivian coral degradation zones: patterns across reef typologies and depths

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ABSTRACT

The intensification of dredging and infilling activities in lagoons and on coral reefs are common practices in the Maldivian archipelago, and these activities alter the biodiversity of the bioconstructors and the functioning of the ecosystem.
The alteration of environmental factors can also affect inconspicuous fauna, such as free-living nematodes. The implications of a reduction in biodiversity may transcend decreased taxonomic diversity, resulting in changes in functional diversity and redundancy; however, how the environmental conditions and human pressure affects the functionality of nematodes in Maldivian coral degradation zones (CDZs) remain poorly understood. In this paper, we examined changes in the taxonomic and functional diversity and the functional redundancy in nematode communities regarding the geographic location (atolls with various levels of human pressure), the exposure and topography of the reef (lagoon and ocean), the slope of the reef, and the depth. The functional diversity and redundancy were evaluated by considering two main biological traits of nematodes: i) the trophic strategy, and ii) the life strategy. The extremely high number of nematode genera observed in the Maldives is supported by the high complexity of the carbonate sediments. The reef exposure and depth were the most relevant environmental factors that influenced the taxonomic and functional diversity. The functional diversity, according to the trophic strategies, mirrored the taxonomic diversity because the adaptive plasticity of nematode buccal cavity structures is closely associated with the high biodiversity of the phylum.
The high abundance of k-strategists in ocean reefs may indicate a higher ecological quality when compared to lagoon reefs; however, the absence of significant differences in life strategy functional diversity and functional redundancy indicates that a recovery process is underway. Analyses of nematode communities should be combined with standard investigations of reef bioconstructors during monitoring activities to assess the vulnerability of CDZ systems to future disturbances and facilitate the adoption of the most appropriate preventative actions.

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INTRODUCTION

Coral reef bioconstructions are of particular conservation and scientific interest because they support many microhabitats that promote marine biodiversity (*Ape et al., 2018*); however, they are declining worldwide due to global and local stressors (*Hoegh-Guldberg et al., 2007*; *Montefalcone, Morri & Bianchi, 2018*). In the Maldives, the lack of land and the increasing requirement for private and tourist infrastructures have necessitated the artificial expansion of the islands (*Nepote et al., 2016*). Therefore, the dredging and infilling of sediment and cement in the surrounding lagoons and coral reefs have been common practices since the 1970s and have intensified since 2005, particularly in the North and South Malé Atolls, which are the most populated areas (*Montefalcone, Morri & Bianchi, 2020*; *Pancrazi et al., 2020*). Coastal development, waste disposal and pollution are the primary local human pressures that contribute to the degradation of coral reefs in the Maldivian Archipelago and the alteration of their benthic communities (*Pancrazi et al., 2020*).

In general, the seabeds associated with reef bioconstructions are dynamic areas that are referred to as coral degradation zones (CDZs) (see *Raes et al., 2007*), where the sediments constitute the direct remains of corals, green algae (*Halimeda*), coralline red algae, foraminiferans, bryozoans, and molluscs, and are derived from the activity of bioeroders (*e.g.*, boring sponges, sea urchins, and parrotfish) (*Janßen et al., 2017*). The physical disturbance caused by anthropogenic activities (*e.g.*, coastal development, bottom trawling, and the disposal of dredged material) may impact seabeds and, consequently, result in changes in invertebrate patterns in coastal systems (*Semprucci et al., 2017*; *Schratzberger & Somerfield, 2020*).

Free-living nematodes are essential components of the seabed and provide ecosystem services, including the reworking of the sediments and recycling of organic matter (*Schratzberger, Warr & Rogers, 2007*). These small metazoans are bound to the seabed throughout their entire lifespan and, therefore, are susceptible to slight environmental alterations. The nematode density and diversity are influenced by the sediment grain size, organic enrichment, salinity, currents, and oxygen availability (*Semprucci et al., 2010*, and references therein). Natural or human physical disturbances are also important factors that control nematode assemblages in taxonomic and functional terms (*Netto, Attrill & Warwick, 1999a*; *Semprucci et al., 2011*; *Sandulli, Semprucci & Balsamo, 2014*; *Semprucci, Colantoni & Balsamo, 2016*; *Semprucci et al., 2018a*; *Semprucci et al., 2018b*; *Maria et al., 2012*; *Boufahja, Semprucci & Beyrem, 2016*). Analyses of meiofauna at higher taxonomic levels are routinely used to detect possible environmental changes (*Ape et al., 2018*; *Losi et al., 2018*; *Baldrighi et al., 2021*). However, the study of nematodes from a taxonomic and functional point of view can contribute more specific information on the ecological status and capacity of ecosystems to respond to disturbances (*Semprucci et al., 2011*; *Losi et al., 2021*; *Hong et al., 2020*; *Sahraeian et al., 2020*; *Schratzberger & Somerfield, 2020*).

A high density and biodiversity of nematodes exist in carbonate sediments in the Maldives, where there are an impressive number of microhabitats (*Semprucci et al., 2010*, *2018a*, *2018b*). As meiobenthic biodiversity has shown greater vulnerability in lagoon reefs compared to ocean reefs (*Semprucci et al., 2018b*), in the present study, the composition of nematode assemblages was analysed to detect patterns in taxonomic diversity and community structure across environmental gradients.

Biological diversity not only encompasses a variety of genes, phenotypes, species, etc. but also includes a wide variety of functions that support goods and ecosystem services. Functional groups in nematode communities, *e.g*. trophic guilds (*Wieser, 1953*) and life strategies (*Bongers, Alkemade & Yeates, 1991*), can be regarded as clusters of taxa that similarly influence ecosystem processes. According to the redundancy theory, the role of numerous species might be easily taken over by other functionally analogous species without considerably altering ecosystem functioning. Thus, a small number of keystone species may maintain the gains, recovery potential, and stability of an ecosystem after a disturbance (*e.g*., *Lawton & Brown, 1993*; *Kang et al., 2015*; *Biggs et al., 2020*). In this regard, indices that quantify functional diversity and redundancy may help to clarify the relationship between aspects of biodiversity, ecosystem functioning and stability, and environmental constraints, as well as facilitate an assessment of the actual vulnerability of ecosystems (*Loreau et al., 2001*; *Bremner, Rogers & Frid, 2003*; *Worm et al., 2006*).

Most previous studies on the effects of physical disturbances on Maldivian reefs have focused on the response of zooxanthellate scleractinian corals, which generally require long periods to recover (*Montefalcone, Morri & Bianchi, 2020*; *Pancrazi et al., 2020*); to date, no information exists regarding the taxonomic and functional changes of benthic nematodes. Hence, in this study, the geographical location of the reef (*i.e*., atolls with various levels of human pressure), the exposure and topography (*i.e*., lagoon and ocean reefs), and the slope and depth of the reef (*i.e*., the main drivers of coral reef zonation *Pancrazi et al., 2020*) were the environmental factors used to define nematode taxonomic and functional patterns and to quantify changes in functional diversity and redundancy. Four hypotheses were tested, which examined whether the nematode community structure, taxonomic diversity, and functional diversity were influenced by (1) the geographic location in distinct atolls; (2) the exposure; (3) the slope and topography of a reef, and (4) the depth.

MATERIALS AND METHODS

Study area and field activities

The Maldives islands are located in the Indian Ocean, southwest of India (Figs. 1A and 1B). The archipelago occupies a total area of 90,000 km and includes 22 atolls aligned in a north to south direction. The unconsolidated nature of atoll system sediments and seasonally opposing wind directions create an extremely dynamic coastline (*Godfrey, 1996*; *Pancrazi et al., 2020*). Atolls have a calcareous nature, and their sediments are derived from the mechanical erosion of coral reefs *via* hydrodynamism and certain bioeroders, such as the parrotfish (*Morgan & Kench, 2016*). Samples were collected in May 2013 in the atolls of North Malé, South Malé, and Felidhoo. The atolls of North and South Malé have

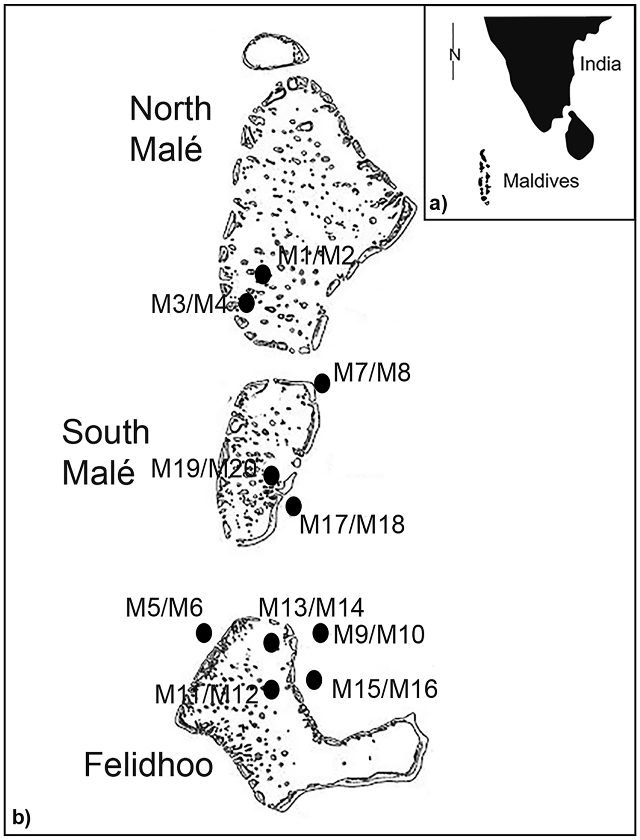

Figure 1 **Study area with the locations and codes of the sampling stations (M1 to M20).**
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experienced a constant increase in the resident population in recent decades, with a doubling of the population during the past 20 years, whilst the population of Felidhoo decreased slightly during the same period (*Montefalcone, Morri & Bianchi, 2020*). Additionally, an increase in the number of beds in the resorts (which is a proxy for touristic pressure) was one order of magnitude higher in the atolls of North and South Malé than in Felidhoo (*Montefalcone, Morri & Bianchi, 2020*).

As reported by *Semprucci et al. (2018b)*, two distinct reef typologies can be distinguished: (i) lagoon reefs, which are the lagoon-facing sides (back reef or inner reef) of the atoll rim or lagoon patch reefs; (ii) ocean reefs, which are the ocean-facing sides (fore reef or outer reef) of the atoll rims. Lagoon reefs have gentle slopes and degrade homogeneously, ending on a sandy terrace at depths no greater than a few tens of metres. Ocean reefs have steep slopes and degrade abruptly from the reef flat to depths greater than one hundred metres (*Lasagna et al., 2010*) (Figs. 1A and 1B). Erosion processes mainly affect the ocean side of the reef due to its exposure to wind and hydrodynamism.
In contrast, the sheltered and calm conditions that characterise a lagoon reef result in faster coral growth and a higher abundance of branching and tabular coral morphologies (*Stoddart & Steers, 1977*; *Morri et al., 2015*). Conversely, lagoon reefs are typically more

affected by local human pressure (*e.g.*, coastal constructions) than ocean reefs (*Montefalcone, Morri & Bianchi, 2020*; *Pancrazi et al., 2020*).

In the present study, scuba divers obtained sediment samples at five reef sites in lagoons (inner) and five reef sites on ocean-exposed reefs (outer) (Fig. 1B). At each sampling point, three stations were selected at a depth range from 19 m to 66 m (*i.e.*, <25 m, 25–50 m, >50 m) and at the three slopes (*i.e.*, <10°, 10–50°, >50°) (Table S1), except for the ocean reefs in which slopes of <10° were absent (total number of stations = 20). The sampling of sediments was conducted using a plexiglass corer (surface area 5.72 cm^2) that was manually pushed to 5 cm into the sediment. At each station, three replicates of sediments were obtained for the taxonomic analysis of nematodes (*Vincx, 1996*) and one sediment sample was obtained for sedimentological analysis. A total of sixty samples were analysed for nematodes and twenty samples were examined for granulometry. The samples for nematode analysis were treated with an aqueous solution of magnesium chloride (final concentration 7%) for the narcotisation and relaxation of the meiofaunal organisms. The samples were subsequently treated with a 10% solution of neutralised formaldehyde for fixation (see *Danovaro et al., 2004* for details).

Sedimentological analysis

The diffraction and diffusion of a monochromatic laser beam diffused on suspended sediment particles (Particle Size Analyzer Malvern Mastersizer 2,000) were utilised for the sedimentological analyses according to the protocol described by *Loizeau et al. (1994)*. The measurements ranged from 0.02 to 2,000 μm, with obscuration of 10% to 20%. The sediments were subdivided into the following granulometric classes: clay (particle size: <3.9 μm), silt (from 3.9 to 63 μm), and sand (>63 μm) and utilised for further characterisation of the faunal microhabitat.

Nematode community structure analysis and ecological indices

In the laboratory, the samples were sieved through a 500 μm mesh to remove macrobenthic organisms, while a 42 μm mesh was utilised to retain meiobenthic nematodes (*Danovaro et al., 2004*). The sediment fraction from 500 μm and 42 μm was thoroughly rinsed with a gentle jet of prefiltered water. For the extraction of the fauna from the sediment fraction, flotation and multiple decantations (10 times) were performed, followed by three Ludox gradient centrifugations (Ludox HS 30, final density 1.18 g/cm^3, 3,000 rpm for 10 min) (*Boufahja, Semprucci & Beyrem, 2016*). The extracted nematodes were preserved in a 10% formaldehyde solution that included several drops of Rose Bengal stain (0.5 $g\ L^{-1}$, see *Danovaro et al., 2004*) to facilitate subsequent counting and sorting. A total of 100 specimens were randomly chosen from each replicate under a stereomicroscope (Leica G26, zoom 25×–50×, *Semprucci et al., 2010*) and transferred into a capsule containing a glycerine solution. The specimens were preserved on permanent slides (*Seinhorst, 1959*) and identified at the genus level under a light optical microscope (Nikon Optiphoto-2) using taxonomic guides and original species descriptions available on the Nemys website (*Bezerra et al., 2021*).

The Shannon-diversity (H') and Pielou-evenness (J) (both log2) indices were computed to provide a measure of the taxonomical diversity of the nematode community. To explore the functional structure of the community, the nematode genera were classified according to two main biological traits: i) the trophic strategy, and ii) the life strategy. Four trophic guilds were identified based on the structure of nematode buccal cavities (*Wieser, 1953*): selective and non-selective deposit feeders (1A and 1B, respectively), epigrowth feeders (2A), and omnivores/carnivores (2B). Five life strategies were defined according to the five coloniser-persister (c-p) classes distinguished by *Bongers, Alkemade & Yeates (1991)*, in a spectrum from extremely tolerant species (*i.e*., extreme r-strategists or c-p 1: short generation time, high egg production, high colonisation ability, and tolerance to stress) to extremely sensitive nematodes (*i.e*., k-strategists or c-p 5: long generation time, very few offspring, low colonisation ability, and sensitivity to stress). The four trophic guilds and the five life strategies were utilised to measure the functional diversity and the functional redundancy (*Lawton & Brown, 1993*; *Petchey & Gaston, 2006*), using the Shannon-diversity (H') and the Simpson-dominance (D) indices, respectively.

Data analysis

All the nematode taxa identified were utilised to create a data matrix for the following statistical analyses. Non-metric multi-dimensional scaling (nMDS) analysis, derived from Bray–Curtis similarity matrices (fourth root transformed values), was used to visualise differences in the taxonomical diversity of nematode communities considering the following factors: (i) the geographic location (the three atolls); (ii) the exposure (lagoon and ocean reefs); (iii) the slope (*i.e*. <10°, 10–50°, >50°), and (iv) the depth (*i.e*. <25 m, 25–50 m, >50 m). The stress values of nMDS can be regarded as a measure of the goodness-of-fit of the ordination, and values lower than 0.2 underline limited probabilities of misleading interpretations (*Clarke & Warwick, 1994*). Significant differences in the four factors were further examined using analysis of similarities (ANOSIM). A similarity percentage (SIMPER) procedure (cut-off 50%) was performed on the transformed Bray–Curtis similarity matrix to assess the percentage dissimilarity in nematode composition in the four factors. The same multivariate analyses were performed on untransformed data to evaluate differences in the nematode functional traits considering the four factors (*Semprucci et al., 2018a*).

The Kruskal–Wallis test was applied to evaluate changes in the taxonomic and functional diversity values (*i.e*., Shannon-diversity, Pielou-evenness, Simpson-dominance), trophic guilds (*i.e*., 1A, 1B, 2A, 2B) and life strategies (c-p classes). The software package Primer v.6 (*Clarke & Gorley, 2006*) was utilised for all the multivariate analyses and computation of the indices (*i.e*., Shannon, Pielou, and Simpson), while the SPSS v.21 software package was used for the Kruskal–Wallis test.

RESULTS

Calcium carbonate was the dominant component in the sediments (98%). Silt and sand were the prevalent sediment fractions (sand ranged from 20% to 70%, silt from 29% to 73%), while clay was an extremely small component (less than 7.5%) (Table S1).

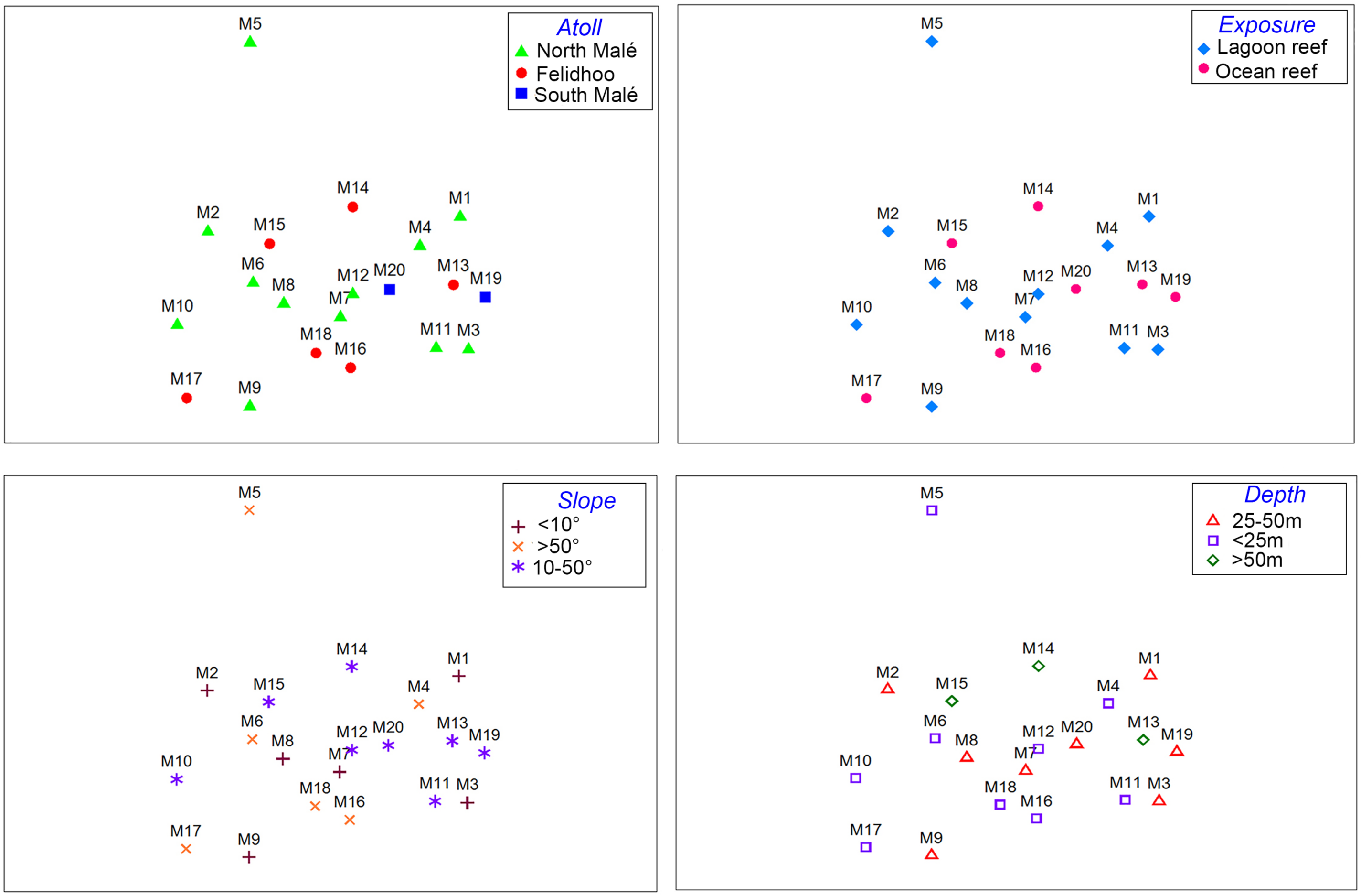

Figure 2 **Non-metric multi-dimensional scaling (nMDS, stress value = 0.17) performed on the nematode community structure (fourth root transformed values) considering the four environmental factors (*i.e.* atoll, exposure, slope, and depth).**

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A total of 173 genera and 36 families were observed in the study area. The families Desmodoridae (26 genera), Chromadoridae (19 genera), Xyalidae (13 genera), and Cyatholaimidae (12 genera) showed the highest richness of genera (Table S2). The Desmodoridae family represented 37% of the entire community, followed by Chromadoridae (11%), Xyalidae (5%), and Cyatholaimidae (5%).

The results of the nMDS (Fig. 2) and ANOSIM (Table 1) on the nematode community structure showed significant differences between the lagoon and ocean reefs ($p < 0.001$), the slope ($p < 0.01$), and the depth ($p < 0.001$), but not between the atolls ($p > 0.05$). The lagoon reefs revealed a higher number of genera compared with the ocean reefs (regarding the total number of genera and the SIMPER results) and mainly consisted of the families Desmodoridae, Microlaimidae, Cyatholaimidae, and Chromadoridae (Fig. 3A; Tables S2 and S3). The Desmodoridae were also observed in ocean reefs, but higher densities of Draconematidae, Epsilonematidae, and Desmoscolecidae were detected in these locations. Differences in the composition of the taxa with depth were observed, with the genera *Desmodorella*, *Dracograllus*, and *Epsilonema* mainly characterising depths less

Table 1 **Analysis of Similarities (ANOSIM) to detect possible differences in nematode community structure among the four environmental factors.**

Factors	R	*p*	*Pair-wise* test
Atoll		n.s.	–
Exposure	0.24	<0.001	–
Slope	0.15	<0.01	<10° *vs.* 10°–50° (R = 0.18; $p < 0.01$); <10° *vs.* >50° (R = 0.20; $p < 0.01$); >50° *vs.* 10°–50°(n.s.)
Depth	0.23	<0.001	25–50 m *vs.* >50 m (R = 0.44; $p < 0.001$); <25 m *vs.* >50 m (R = 0.25; $p < 0.04$); 25–50 m *vs.* <25 m (R = 0.13; $p < 0.05$)

Note:
n.s., not significant differences; R, ratio that compares the mean of ranked dissimilarities between groups to the mean of ranked dissimilarities within groups; p, probability level.

than 25 m; genera of the family Desmodoridae (*i.e.*, *Desmodora*, *Acanthopharynx*, *Chromaspirinia*, *Croconema*, *Spirinia*, *Zalonema*, and *Onyx*) as well as *Microlaimus*, *Paracyatholaimus*, *Sabateria*, and *Viscosia* characterising the intermediate depths (25–50 m), while *Ceramonema*, *Tricoma*, *Chromadorita*, and *Spilophorella* were dominant at the deepest stations below 50 m (Fig. 3B; Table S4). Slopes greater than 50° were observed at shallower depths and were characterised by *Desmodorella*, *Dracograllus*, and *Epsilonema*. A significant difference was observed between slopes <10° and slopes between 10° and 50° as well as between slopes of <10° and >50°, which were characterised by the genera that were more abundant at depths between 25 and 50 m (Figs. 3B and 3C; Table S5).

The taxonomic and functional diversity values, as well as the functional redundancy values, are shown in Fig. 4. The number of genera (S) and the Shannon index (H') were significantly higher in North Malé, while Felidhoo showed the lowest values ($p < 0.05$). The S, H', and J indices revealed significant differences according to the reef typology, slope, and depth (Kruskal–Wallis test, S: $p < 0.001$; H': $p < 0.001$; J: $p < 0.01$). S and H' were higher in the lagoon reefs, while J was higher in the ocean reefs. The highest values of S and H' were observed at depths of 25 to 50 m and slopes of less than 10°, while the highest values of J were observed at depths greater than 50 m and slopes between 10° and 50°. No significant changes in the functional redundancy among the factors were observed, while a significant difference was observed in the trophic functional diversity concerning the reef typology ($p < 0.05$), with lower values detected for the ocean reefs. The nematode community was mostly characterised by the trophic category 2A (grazers), which represented 54% of the total number. Selective deposivores (1A) represented 22% of the total, predators-omnivorous (2B) represented 14% of the total, and non-selective deposivores (1B) represented 11% of the total (Figs. 5A–5C). A total of 57% of the nematode community was represented by the c-p 3 life strategy, 22% were c-p 2, 20% were c-p 4, and c-p 5 and c-p 1 were only sporadically observed (0.2% and 0.1%, respectively) (Fig. 6).

The ANOSIM showed a significant change in the functional structure (*i.e.*, the trophic and life strategies) only between the lagoon and ocean reefs (R = 0.13, $p < 0.001$). The Kruskal–Wallis test also revealed significant differences among the exposure due to trophic groups such as 1A ($p < 0.05$) and 2B ($p < 0.01$), as well as the life strategy categories such as c-p 3 ($p < 0.05$) and c-p 4 ($p < 0.01$). Significant differences were only observed for

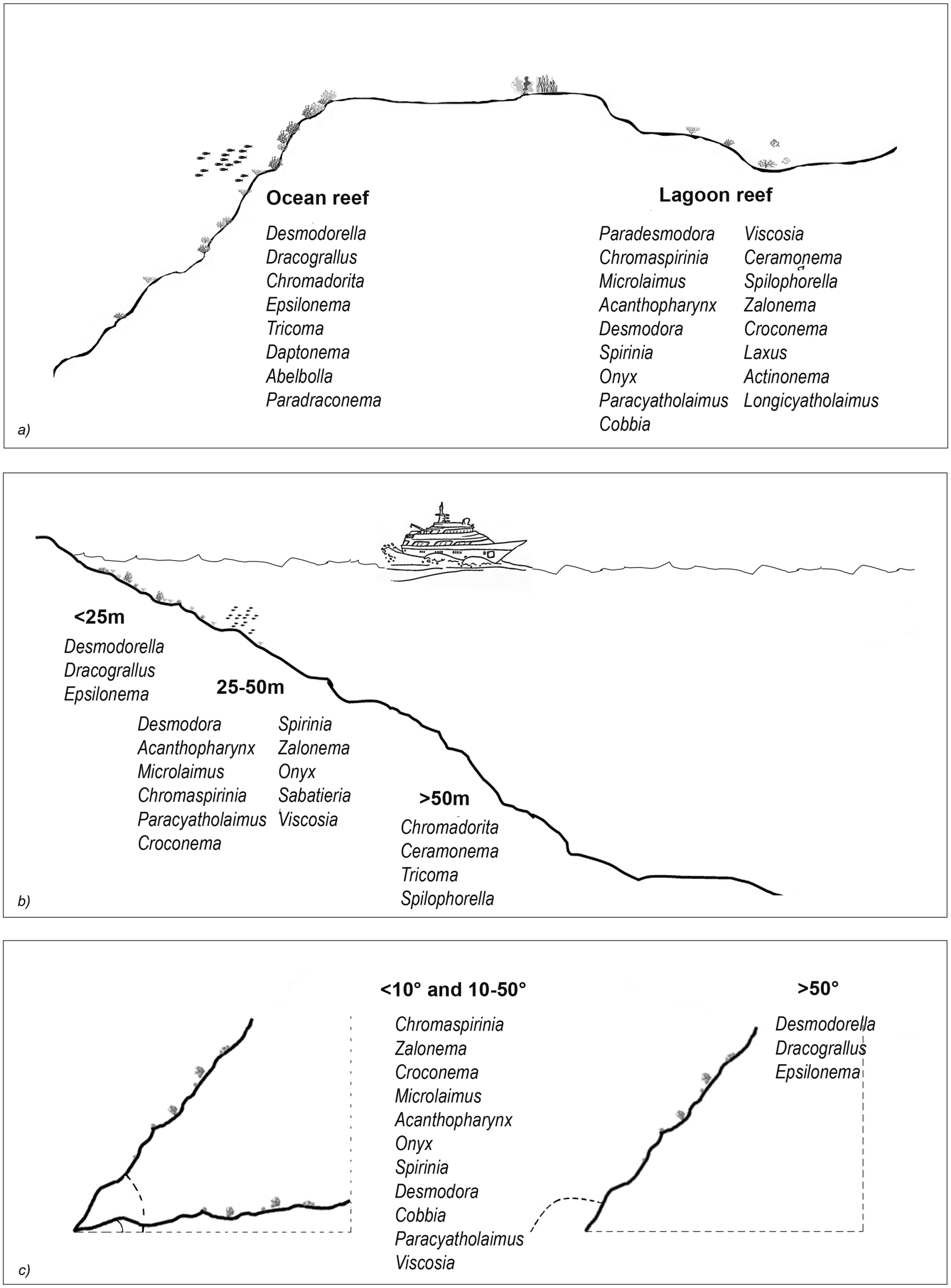

Figure 3 **The nematode genera that were important for differentiating reef typologies (A), depths (B), and slopes (C), as obtained by SIMPER analysis (cut-off 50%).** Full-size DOI: 10.7717/peerj.13644/fig-3

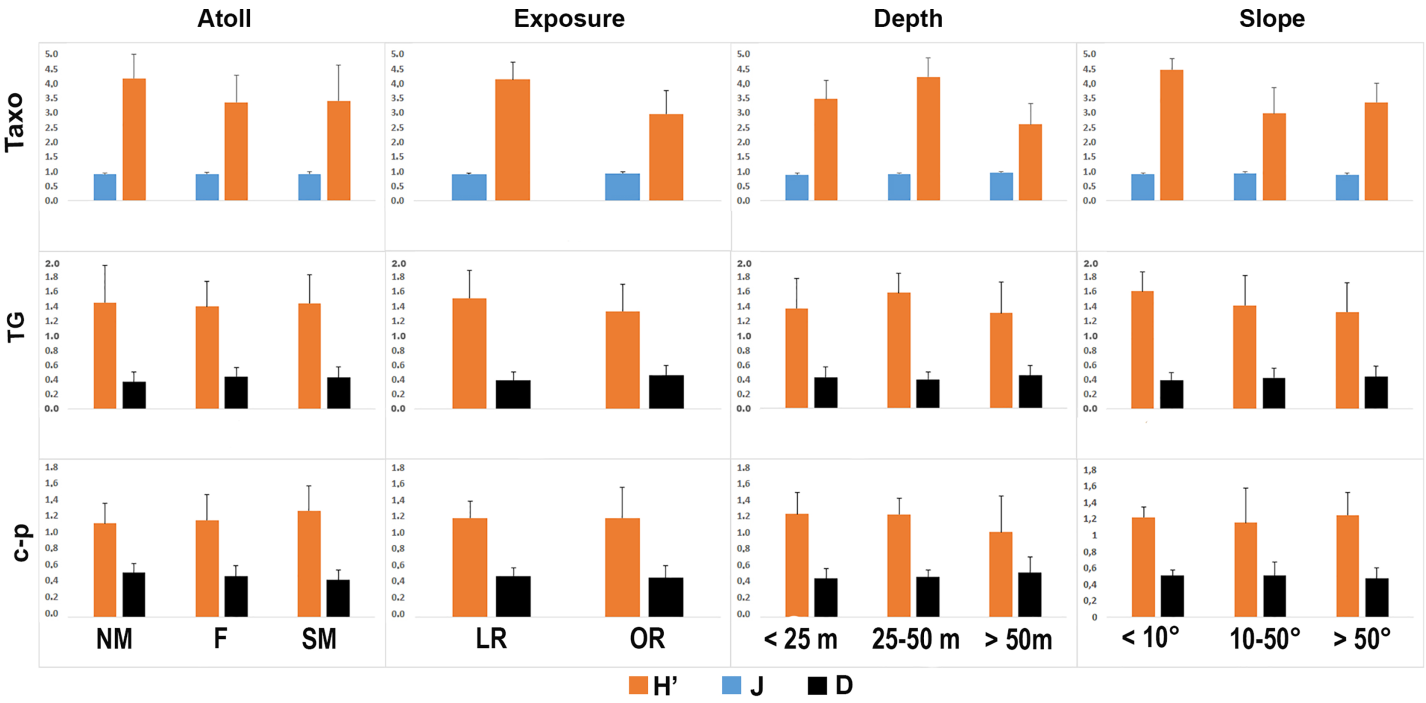

Figure 4 Values of the taxonomic (H' and J) and functional (H') diversity and the functional redundancy (D) indices in the nematode community according to the four environmental factors considered (*i.e.* atoll, exposure, depth, and slope). TAXO, taxonomic composition at the genus level; TG, trophic guilds; c-p, life strategies.

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the c-p 5 life strategy ($p < 0.001$) regarding the depth, whereas significant differences were only observed for the c-p 3 life strategy concerning the slopes ($p < 0.05$) (Figs. 5A–5C and 6).

DISCUSSION

The richness of the nematode genera recorded in the present study was notably high in comparison with data available from shallow and deep subtidal soft-bottom habitats in other Maldivian localities (*Semprucci et al., 2010*; *Semprucci et al., 2011*; *Semprucci et al., 2013*), in Kenya and Zanzibar (*Raes et al., 2007*), and the Porcupine Seabight (*Raes & Vanreusel, 2006*). The Desmodoridae, Chromadoridae, and Xyalidae were the most abundant nematode families, and species belonging to these families have been widely documented in Maldivian CDZs, from shallow to deep seabeds (*Semprucci et al., 2010*; *Semprucci et al., 2011*; *Semprucci et al., 2013*). The Desmodoridae and Chromadoridae are typical components of medium-coarse sediments and CDZs (*e.g.*, *Raes et al., 2007*; *Armenteros et al., 2019*; *Neres, Dos Santos & Esteves, 2021*). The Xyalidae, although often associated with fine-grained sediments, includes several species that are associated with coarser sediments (*Venekey et al., 2014*).

While higher values for the taxonomic richness and the Shannon index were observed at the North Malé atoll, no significant differences were observed in the nematode community structure among the atolls, *i.e.* among various levels of human pressure, even when the atolls were geomorphologically different or located far from each other. Previous

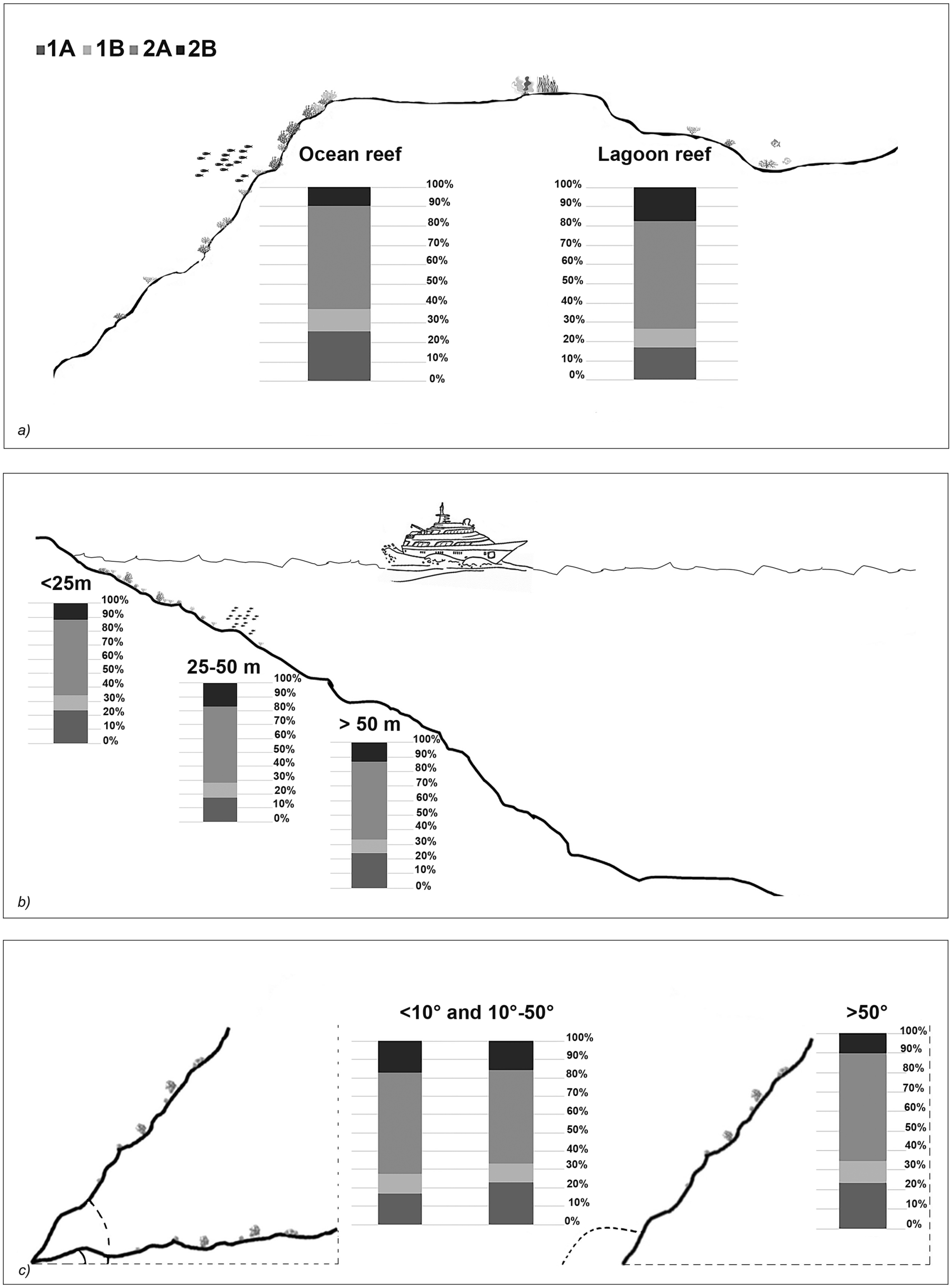

Figure 5 Nematode trophic guilds (*i.e.* 1A, 1B, 2A and 2B) that characterized each reef typology (A), depth (B), and slope (C).

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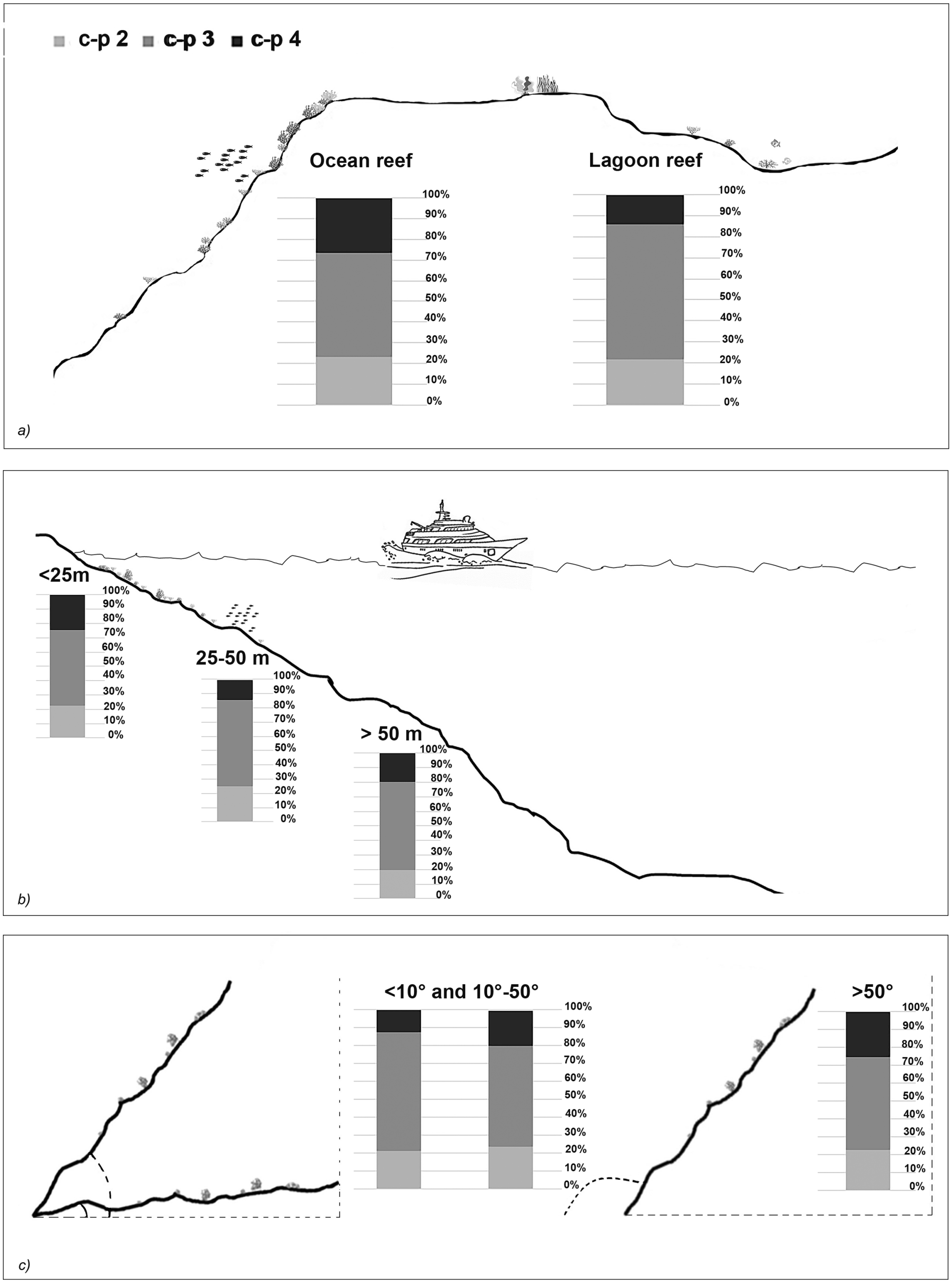

Figure 6 The nematode life strategies (*i.e.* c-p 2, c-p 3, c-p 4) that characterised each reef typology (A), depth (B), and slope (C).

Full-size DOI: 10.7717/peerj.13644/fig-6 investigations in the Felidhoo and South Malé atolls revealed similar results (*Semprucci et al., 2010*). The numerous cosmopolitan meiobenthic species ('Everything is Everywhere' paradox) and a low dissimilarity in the nematode community structure over large spatial scales are not surprising findings and are likely associated with active or passive migration and phoresis phenomena (*Baldrighi et al., 2020*; *Ingels et al., 2020*). Instead, a plethora of micro-variations in the physical-chemical environmental features (*e.g*., granulometry, biogeochemistry, food availability, and sediment oxygenation) can significantly control small-scale spatial variations of meiobenthic organisms (*Semprucci et al., 2010*).

A greater influence of exposure and depth was previously detected in meiofauna applying the surrogacy at major taxon level (*Semprucci et al., 2018b*). These natural environmental factors likely play a more predominant role in nematode communities than human influence. The dissipation of wave energy across the reef front generally results in different hydrodynamic conditions that lead to the creation of distinct environmental zonations (*Lasagna et al., 2010*). Lagoon and ocean reefs show distinct coral community composition and functioning, and coral growth, vitality and recruitment rate (*Cardini et al., 2012*; *Jimenez et al., 2012*; *Nepote et al., 2016*; *Montefalcone, Morri & Bianchi, 2018*) that support parallel shifts in the associated nematodes. The microhabitat structure, rather than hydrodynamism, seems to influence to a larger extent the nematode community composition. Living corals and coral rubble, after mechanical and bio-erosion, may supply seabeds with complex structures and provide extra surfaces for attachment and refuge, sediment or organic matter traps, and food items (*e.g*., bacteria and microalgae) for nematodes (*Ape et al., 2018*). In Indonesia, changes in the grain size distribution and composition of sedimentary facies between inner and outer reefs, due to a different proportion of coral fragments, shells, and hard parts of reef-forming and reef-associated calcifying organisms, have been documented (*Janßen et al., 2017*).

The nematode assemblages that inhabited the two reef typologies were dissimilar, with a predominance of Desmodoridae (including Stilbonematinae) and Microlaimidae in the lagoon reefs, while Desmodoridae, Draconematidae, Epsilonematidae, and Desmoscolecidae were predominant in the ocean reefs. The Desmodoridae are the most ubiquitous and abundant nematode family that inhabit carbonate sediments (*e.g*., see *De Troch et al., 2008*; *Semprucci et al., 2010* for review), therefore, itself this family cannot be considered as discriminant of a particular ecological condition. Conversely, genera of the Desmodoridae family varied among the various environmental factors. Representatives of the Stilbonematinae subfamily were consistently observed in the lagoon reefs. This taxon occurs globally in sheltered intertidal and subtidal habitats and is frequently observed in tropical carbonate sediments (*Semprucci et al., 2010*; *Armenteros, Ruiz-Abierno & Decraemer, 2014*). Species of this subfamily show an ectosymbiosis with sulphur oxidising bacteria, which cover the cuticle of the nematodes and are often genus- and species-specific consortia (*Bayer et al., 2009*; *Scharhauser et al., 2020*). As sediment-dwellers, the Stilbonematinae migrate up and down through the redox cline and, thus, provide the bacteria with electron donors and electron acceptors, such as nitrate and oxygen (*Hentschel et al., 1999*; *Bayer et al., 2009*). The exclusive occurrence of members of the

Stilbonematinae with many species in the lagoon reefs is likely associated with low hydrodynamism.

Members of the Draconematidae and Epsilonematidae families are morphologically unusual and specialised and are considered epifaunal organisms in the coral fragments of the CDZs (*Raes & Vanreusel, 2006*; *Raes et al., 2007*). They have small and sturdy bodies, cuticle annules lined with spiny ornamentation, many adhesive tubes, and a distinctive type of locomotion–looper-like crawling, as is usual in Geometridae worms (Lepidoptera). These organisms are, therefore, capable of moving across various types of substrata, from gravel to coarse sands to smooth coral surfaces or sponge fragments, where other nematode species have difficulty finding a grip (*Gad, 2009*). Such taxa that have adapted to crawl on larger surfaces and withstand hydrodynamically active environments may have a competitive advantage in ocean reefs (*Raes & Vanreusel, 2006*; *Raes, Decraemer & Vanreusel, 2008*; *Semprucci et al., 2011*). The Desmoscolecidae was an additional family that differentiated reef typologies and its members were more abundant in the ocean reefs at the greatest depths. Species of the family Desmoscolecidae have been defined as 'sediment-dwelling taxa', *i.e*., organisms that inhabit the finest coral sands trapped between larger sponge and coral fragments (*Raes & Vanreusel, 2006*; *Semprucci et al., 2013*). They show an annulated cuticle covered with desmens (*i.e*. concretion annules made of sedimentary material). Depth was the second environmental factor that accounted for significant differences in the nematode communities and their taxonomic diversity and evenness, with the overall highest values observed at depths greater than 25 m. The Desmoscolecidae were more abundant at depths greater than 50 m, as previously reported from other deep-sea habitats, such as abyssal plains and continental slopes (*e.g*., *Zeppilli et al., 2014*; *Sergeeva & Revkova, 2021*). Members of the Ceramonematidae family were also observed at the greatest depths. This family is often associated with the gravelly sediments of seamounts; the species are generally long with a coarsely annulated and extremely flexible cuticle and body, which may be highly advantageous in biogenic sands (*Vanreusel et al., 2010*).

The reef slope was less relevant for nematodes and the slight differences observed reflect the influence of the depth. Slopes of less than 10° were exclusively associated with certain depths, and it may be difficult to discriminate the effects of these two factors.

The feeding guilds differed between the lagoon and ocean reefs due to the significant contribution of selective deposivores (1A). These organisms have extremely small buccal cavities, thus, they feed selectively on small food items (*e.g*., bacteria; *Wieser, 1953*). Despite the highest hydrodynamism, bacteria are more abundant in the ocean reefs (*Ndaro & Olafsson, 1999*; *Semprucci et al., 2010*; *Semprucci et al., 2011*). The predators/omnivores (2B) were more abundant in the lagoon reefs. In the Maldives, this guild is associated with medium sands where there is a higher abundance of prey represented by other meiofaunal organisms or macrofaunal juveniles (*Netto, Warwick & Attrill, 1999b*; *Semprucci et al., 2018b*). The epigrowth feeders (2A) did not vary between the environmental factors. The high preponderance of this trophic strategy (mostly represented by the Desmodoridae) in carbonate sediments is associated with the considerable level of primary production in CDZs, where these species obtain high densities of diatoms or microalgae

(*Raes et al., 2007*). Moreover, the 2A feeders can be viewed as an opportunistic group that can also benefit from bacterial biofilms that settle in the porosities of biogenic sediments.

Furthermore, the trophic functional diversity mirrored the taxonomic diversity due to the large trophic spectrum and the high adaptive plasticity of nematode buccal cavity morphologies that are closely associated with the high biodiversity of the phylum (*De Meester et al., 2016*; *Daly et al., 2021*). Thus, a reduction in the functional diversity could, in the future, be considered an indirect measure of biodiversity loss.

The life strategy composition revealed a medium-high ecological quality in the study area. A higher percentage of c-p 4 in the ocean reefs suggests that the impact was lower here, while inside the lagoon, where a greater proportion of human activity is concentrated, the significant preponderance of intermediate colonisers (c-p 3) indicates recovery. This result, in addition to the absence of significant differences in the life strategy functional diversity and functional redundancy between the lagoon and ocean reefs, suggests higher resilience and recovery capability of the nematodes (*Semprucci, Colantoni & Balsamo, 2016*) compared to other biological components (*e.g.*, corals) regarding physical disturbances.

CONCLUSIONS

Understanding the effects of anthropogenic impacts on tropical regions is extremely important, as these geographic areas are experiencing significant environmental changes. The nematode community of the Maldivian coral reefs investigated during the present study revealed high biodiversity and overall good ecological quality. The nematode life strategies suggested the higher ecological quality of the ocean reefs and signs of community recovery in the lagoons. However, the results suggest that the taxonomic and functional structure of the nematodes is mostly guided by natural environmental conditions and not anthropogenic pressure. Thus, this study confirms the requirement to include small invertebrates, such as nematodes, in the biomonitoring activities of CDZs because these species may contribute to an assessment of the vulnerability and resilience of the benthos. Based on the results of the present study, an assessment of the natural environmental characteristics, together with the nematode structure, is recommended for inclusion in ecological impact assessments on coral reefs.

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Competing Interests

The authors declare that they have no competing interests.

Author Contributions

- Eleonora Grassi performed the experiments, analyzed the data, prepared figures and/or tables, and approved the final draft.
- Monica Montefalcone conceived and designed the experiments, authored or reviewed drafts of the article, and approved the final draft.
- Lucia Cesaroni performed the experiments, prepared figures and/or tables, and approved the final draft.
- Loretta Guidi analyzed the data, prepared figures and/or tables, and approved the final draft.
- Maria Balsamo conceived and designed the experiments, authored or reviewed drafts of the article, and approved the final draft.
- Federica Semprucci conceived and designed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.

Data Availability

The following information was supplied regarding data availability:

The raw measurements are available in the Supplemental Files.

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.13644#supplemental-information.

REFERENCES

Ape F, Gristina M, Chemello R, Sarà G, Mirto S. 2018. Meiofauna associated with vermetid reefs: the role of macroalgae in increasing habitat size and complexity. *Coral Reefs* **37(3)**:875–889 DOI 10.1007/s00338-018-1714-x.

Armenteros M, Pérez-García JA, Marzo-Pérez D, Rodríguez-García P. 2019. The influential role of the habitat on the diversity patterns of free-living aquatic nematode assemblages in the cuban archipelago. *Diversity* **11(9)**:166 DOI 10.3390/d11090166.

Armenteros M, Ruiz-Abierno A, Decraemer W. 2014. Taxonomy of stilbonematinae (nematoda: desmodoridae): description of two new and three known species and phylogenetic relationships within the family. *Zoological Journal of the Linnean Society* **171(1)**:1–21 DOI 10.1111/zoj.12126.

Baldrighi E, Dovgal I, Zeppilli D, Abibulaeva A, Michelet C, Michaud E, Franzo A, Grassi E, Cesaroni L, Guidi L, Balsamo M, Sandulli R, Semprucci F. 2020. The cost for biodiversity: records of ciliate-nematode epibiosis with the description of three new suctorian species. *Diversity* **12(6)**:224 DOI 10.3390/d12060224.

Baldrighi E, Vasapollo C, Grassi E, Alvisi F, Cesaroni L, Balsamo M, Semprucci F. 2021. Meiobenthic assemblages as ecological indicator of natural variability induced by ecosystem engineers. *Regional Studies in Marine Science* **45(1)**:101824 DOI 10.1016/j.rsma.2021.101824.

Bayer C, Heindl NR, Rinke C, Lücker S, Ott JA, Bulgheresi S. 2009. Molecular characterization of the symbionts associated with marine nematodes of the genus Robbea. *Environmental Microbiology Reports* **1(2)**:136–144 DOI 10.1111/j.1758-2229.2009.00019.x.

Bezerra TN, Eisendle U, Hodda M, Holovachov O, Leduc D, Mokievsky V, Peña Santiago R, Sharma J, Smol N, Tchesunov A, Venekey V, Zhao Z, Vanreusel A. 2021. Nemys: world database of nematodes. *Available at http://nemys.ugent.be*.

Biggs CR, Yeager LA, Bolser DG, Bonsell C, Dichiera AM, Hou Z, Keyser SR, Khursigara AJ, Lu K, Muth AF, Negrete B, Erisman BE. 2020. Does functional redundancy affect ecological stability and resilience? A review and meta-analysis. *Ecosphere* **11(7)**:e03184 DOI 10.1002/ecs2.3184.

Bongers T, Alkemade R, Yeates GW. 1991. Interpretation of disturbance-induced maturity decrease in marine nematode assemblages by means of the maturity index. *Marine Ecology Progress Series* **76**:135–142 DOI 10.3354/meps076135.

Boufahja F, Semprucci F, Beyrem H. 2016. An experimental protocol to select nematode species from an entire community using progressive sedimentary enrichment. *Ecological Indicators* **60(10)**:292–309 DOI 10.1016/j.ecolind.2015.07.002.

Bremner J, Rogers SI, Frid CLJ. 2003. Assessing functional diversity in marine benthic ecosystems: a comparison of approaches. *Marine Ecology Progress Series* **254**:11–25 DOI 10.3354/meps254011.

Cardini U, Chiantore M, Lasagna R, Morri C, Bianchi CN. 2012. Size-structure patterns of juvenile hard corals in the Maldives. *Journal of the Marine Biological Association of the United Kingdom* **92(6)**:1335–1339 DOI 10.1017/S0025315411001561.

Clarke KR, Gorley RN. 2006. *PRIMER v6: user Manual/Tutorial*. Plymouth: PRIMER-E.

Clarke KR, Warwick RM. 1994. *Changes in marine communities: an approach to statistical analyses and interpretation*. United Kingdom: Natural Environment Research Council.

Daly AJ, De Meester N, Baetens JM, Moens T, De Baets B. 2021. Untangling the mechanisms of cryptic species coexistence in a nematode community through individual-based modelling. *Oikos* **130(4)**:587–600 DOI 10.1111/oik.07989.

Danovaro R, Gambi C, Mirto S, Sandulli R, Ceccherelli VU. 2004. Meiofauna. *Biologia Marina Mediterranea* **11**:55–97.

De Meester N, Gingold R, Rigaux A, Derycke S, Moens T. 2016. Cryptic diversity and ecosystem functioning: a complex tale of differential effects on decomposition. *Oecologia* **182(2)**:559–571 DOI 10.1007/s00442-016-3677-3.

De Troch M, Raes M, Muthumbi A, Gheerardyn H, Vanreusel A. 2008. Spatial diversity of nematode and copepod genera of the coral degradation zone along the Kenyan coast, including a test for the use of higher-taxon surrogacy. *African Journal of Marine Science* **30(1)**:25–33 DOI 10.2989/AJMS.2008.30.1.3.453.

Gad G. 2009. Colonisation and speciation on seamounts, evidence from Draconematidae (Nematoda) of the Great Meteor Seamount. *Marine Biodiversity* **39(1)**:57–69 DOI 10.1007/s12526-009-0007-6.

Godfrey T. 1996. *Dive maldives. a guide to the maldives archipelago*. Apollo Bay, Australia: Atoll Editions.

Hentschel U, Berger EC, Bright M, Felbeck H, Ott JA. 1999. Metabolism of nitrogen and sulfur in ectosymbiotic bacteria of marine nematodes (Nematoda, Stilbonematinae). *Marine Ecology Progress Series* **183**:149–158 DOI 10.3354/meps183149.

**Hoegh-Guldberg O, Mumby PJ, Hooten AJ, Steneck RS, Greenfield P, Gomez E, Harvell CD, Sale PF, Edwards AJ, Caldeira K, Knowlton N, Eakin CM, Iglesias-prieto R, Muthiga N,

Bradbury RH, Dubiand A, Hatziolos ME. 2007. Coral reefs under rapid climate change and ocean acidification. *Science* **318(5857)**:1737–1742 DOI 10.1126/science.1152509.

Hong JH, Semprucci F, Raehyuk J, Kim K, Lee S, Jeon D, Yoo H, Kim J, Kim J, Yeom J, Lee S, Lee K, Lee W. 2020. Meiobenthic nematodes in the assessment of the relative impact of human activities on coastal marine ecosystem. *Environmental Monitoring and Assessment* **192(2)**:81 DOI 10.1007/s10661-019-8055-2.

Ingels J, Valdes Y, Pontes LP, Silva AC, Neres PF, Corrêa GVV, Silver-Gorges I, Fuentes MMPB, Gillis A, Hooper L, Ware M, O'Reilly C, Bergman Q, Danyuk J, Sanchez Zarate S, Acevedo Natale LI, dos Santos GAP. 2020. Meiofauna life on loggerhead sea turtles-diversely structured abundance and biodiversity hotspots that challenge the meiofauna paradox. *Diversity* **12(5)**:203 DOI 10.3390/d12050203.

Janßen A, Wizemann A, Klicpera A, Satari DY, Westphal H, Mann T. 2017. Sediment composition and facies of coral reef Islands in the Spermonde Archipelago, Indonesia. *Frontiers in Marine Science* **4**:144 DOI 10.3389/fmars.2017.00144.

Jimenez H, Bigot L, Bourmaud C, Chabanet P, Gravier-Bonnet N, Hamel MA, Rilwan Y. 2012. Multi-taxa coral reef community structure in relation to habitats in the Baa Atoll Man and Biosphere UNESCO Reserve (Maldives), and implications for its conservation. *Journal of Sea Research* **72**:77–86 DOI 10.1016/j.seares.2012.04.011.

Kang S, Ma W, Li FY, Zhang Q, Niu J, Ding Y, Han F, Sun X. 2015. Functional redundancy instead of species redundancy determines community stability in a typical steppe of inner mongolia. *PLOS ONE* **10(12)**:e0145605 DOI 10.1371/journal.pone.0145605.

Lasagna R, Albertelli G, Colantoni P, Morri C, Bianchi CN. 2010. Ecological stages of Maldivian reefs after the coral mass mortality of 1998. *Facies* **56(1)**:1–11 DOI 10.1007/s10347-009-0193-5.

Lawton JH, Brown VK. 1993. Redundancy in ecosystems. In: Schulze ED, Mooney HA, eds. *Biodiversity and Ecosystem Function*. Berlin: Springer, 255–270.

Loizeau JL, Arbouille D, Santiago S, Vernet JP. 1994. Evaluation of a wide range laser diffraction grain size analyser for use with sediments. *Sedimentology* **41(2)**:353–361 DOI 10.1111/j.1365-3091.1994.tb01410.x.

Loreau M, Naeem S, Inchausti P, Bengtsson J, Grime JP, Hector D, Hooperm U, Hustond A, Raffaelli B, Schmid D, Tilmanan D, Wardle A. 2001. Biodiversity and ecosystem functioning: current knowledge and future challenges. *Science* **294(5543)**:804–808 DOI 10.1126/science.1064088.

Losi V, Grassi E, Balsamo M, Rocchi M, Gaozza L, Semprucci F. 2021. Changes in taxonomic structure and functional traits of nematodes as tools in the assessment of port impact. *Estuarine, Coastal and Shelf Science* **260(2)**:107524 DOI 10.1016/j.ecss.2021.107524.

Losi V, Sbrocca C, Gatti G, Semprucci F, Rocchi M, Bianchi CN, Balsamo M. 2018. Sessile macrobenthos (Ochrophyta) drives seasonal change of meiofaunal community structure on temperate rocky reefs. *Marine Environmental Research* **142**:295–305 DOI 10.1016/j.marenvres.2018.10.016.

Maria TF, Vanaverbeke J, Esteves AM, De Troch M, Vanreusel A. 2012. The importance of biological interactions for vertical distribution of nematodes in a temperate ultra-dissipative sandy beach. *Estuarine, Coastal and Shelf Science* **97**:114–126 DOI 10.1016/j.ecss.2011.11.030.

Montefalcone M, Morri C, Bianchi CN. 2018. Long term change in bioconstruction potential of Maldivian coral reefs following extreme climate anomalies. *Global Change Biology* **24(12)**:5629–5641 DOI 10.1111/gcb.14439.

Montefalcone M, Morri C, Bianchi CN. 2020. Influence of local pressures on Maldivian coral reef resilience following repeated bleaching events, and recovery perspectives. *Frontiers in Marine Science* **7**:587 DOI 10.3389/fmars.2020.00587.

Morgan KM, Kench PS. 2016. Parrotfish erosion underpins reef growth, sand talus development and island building in the Maldives. *Sedimentary Geology* **341**:50–57 DOI 10.1016/j.sedgeo.2016.05.011.

Morri C, Montefalcone M, Lasagna R, Gatti G, Rovere A, Parravicini V, Baldelli G, Colantoni P, Bianchi CN. 2015. Through bleaching and tsunami: coral reef recovery in the Maldives. *Marine Pollution Bulletin* **98(1–2)**:188–200 DOI 10.1016/j.marpolbul.2015.06.050.

Ndaro SG, Olafsson E. 1999. Soft-bottom fauna with emphasis on nematode assemblage structure in a tropical intertidal lagoon in Zanzibar, eastern Africa: I. Spatial variability. *Hydrobiologia* **405**:133–148 DOI 10.1023/A:1003874122971.

Nepote E, Bianchi CN, Chiantore M, Morri C, Montefalcone M. 2016. Pattern and intensity of human impact on coral reefs depend on depth along the reef profile and on the descriptor adopted. *Estuarine, Coastal and Shelf Science* **178(3–4)**:86–91 DOI 10.1016/j.ecss.2016.05.021.

Neres PF, Dos Santos PJP, Esteves AM. 2021. New chromadorid nematodes from Brazilian coral reefs: a taxonomic contribution to Chromadora Bastian, 1865. *Zootaxa* **5032(1)**:47–79 DOI 10.11646/zootaxa.5032.1.2.

Netto SA, Attrill MJ, Warwick RM. 1999a. The effect of a natural water-movement related disturbance on the structure of meiofauna and macrofauna communities in the intertidal sand flat of Rocas Atoll (NE, Brazil). *Journal of Sea Research* **42(4)**:291–302 DOI 10.1016/S1385-1101(99)00033-7.

Netto SA, Warwick RM, Attrill MJ. 1999b. Meiobenthic and macrobenthic community structure in carbonate sediments of Rocas Atoll (North-east, Brazil). *Estuarine, Coastal and Shelf Science* **48(1)**:39–50 DOI 10.1006/ecss.1998.0398.

Pancrazi I, Ahmed H, Cerrano C, Montefalcone M. 2020. Synergic effect of global thermal anomalies and local dredging activities on coral reefs of the Maldives. *Marine Pollution Bulletin* **160(2)**:111585 DOI 10.1016/j.marpolbul.2020.111585.

Petchey OL, Gaston KJ. 2006. Functional diversity: back to basics and looking forward. *Ecology Letters* **9**:741–758 DOI 10.1111/J.1461-0248.2006.00924.X.

Raes M, De Troch M, Ndaro SGM, Muthumbi A, Guilini K, Vanreusel A. 2007. The structuring role of microhabitat type in coral degradation zones: a case study with marine nematodes from Kenya and Zanzibar. *Coral Reefs* **26**:113–126 DOI 10.1007/s00338-006-0184-8.

Raes M, Decraemer W, Vanreusel A. 2008. Walking with worms: coral-associated epifaunal nematodes. *Journal of Biogeography* **35(12)**:2207–2222 DOI 10.1111/j.1365-2699.2008.01945.x.

Raes M, Vanreusel A. 2006. Microhabitat type determines the composition of nematode communities associated with sediment-clogged cold-water coral framework in the Porcupine Seabight (NE Atlantic). *Deep Sea Research Part I: Oceanographic Research Papers* **53(12)**:1880–1894 DOI 10.1016/j.dsr.2006.08.012.

Sahraeian N, Sahafi HH, Mosallanejad H, Ingels J, Semprucci F. 2020. Temporal and spatial variability of free-living nematodes in a beach system characterized by domestic and industrial impacts (Bandar Abbas, Persian Gulf, Iran). *Ecological Indicators* **118(6)**:106697 DOI 10.1016/j.ecolind.2020.106697.

Sandulli R, Semprucci F, Balsamo M. 2014. Taxonomic and functional biodiversity variations of meiobenthic and nematode assemblages across an extreme environment: a study case in a Blue Hole cave. *Italian Journal of Zoology* **81(4)**:508–516 DOI 10.1080/11250003.2014.952356.

Scharhauser F, Zimmermann J, Ott JA, Leisch N, Gruber-Vodicka HR. 2020. Morphology of obligate ectosymbionts reveals Paralaxus gen. nov.: A new circumtropical genus of marine stilbonematine nematodes. *Zoologica Scripta* **49(3)**:379–394 DOI 10.1111/zsc.12399.

Schratzberger M, Somerfield PJ. 2020. Effects of widespread human disturbances in the marine environment suggest a new agenda for meiofauna research is needed. *Science of the Total Environment* **728(4)**:138435 DOI 10.1016/j.scitotenv.2020.138435.

Schratzberger M, Warr K, Rogers SI. 2007. Functional diversity of nematode community in the south-western North Sea. *Marine Environmental Research* **63(4)**:368–389 DOI 10.1016/j.marenvres.2006.10.006.

Seinhorst JW. 1959. A rapid method for the transfer of nematodes from fixative to anhydrous glycerine. *Nematologica* **4(1)**:67–69 DOI 10.1163/187529259X00381.

Semprucci F, Cesaroni L, Guidi L, Balsamo M. 2018a. Do the morphological and functional traits of free-living marine nematodes mirror taxonomical diversity? *Marine Environmental Research* **135(3)**:114–122 DOI 10.1016/j.marenvres.2018.02.001.

Semprucci F, Colantoni P, Baldelli G, Rocchi M, Balsamo M. 2010. The distribution of meiofauna on back-reef sandy platforms in the Maldives (Indian Ocean). *Marine Ecology* **31(4)**:592–607 DOI 10.1111/j.1439-0485.2010.00383.x.

Semprucci F, Colantoni P, Baldelli G, Sbrocca C, Rocchi M, Balsamo M. 2013. Meiofauna associated with coral sediments in the Maldivian subtidal habitats (Indian Ocean). *Marine Biodiversity* **43**:189–198 DOI 10.1007/s12526-013-0146-7.

Semprucci F, Colantoni P, Balsamo M. 2016. Is maturity index an efficient tool to assess the effects of the physical disturbance on the marine nematode assemblages? A critical interpretation of disturbance-induced maturity successions in some study cases in Maldives. *Acta Oceanologica Sinica* **35(4)**:89–98 DOI 10.1007/s13131-016-0832-y.

Semprucci F, Colantoni P, Sbrocca C, Baldelli G, Rocchi M, Balsamo M. 2011. Meiofauna in sandy back-reef platforms differently exposed to the monsoons in the Maldives (Indian Ocean). *Journal of Marine Systems* **87(3–4)**:208–215 DOI 10.1016/j.jmarsys.2011.04.002.

Semprucci F, Frontalini F, Losi V, du Châtelet EA, Cesaroni L, Sandulli R, Balsamo M. 2018b. Biodiversity and distribution of the meiofaunal community in the reef slopes of the Maldivian archipelago (Indian Ocean). *Marine Environmental Research* **139(2)**:19–26 DOI 10.1016/j.marenvres.2018.05.006.

Semprucci F, Sbrocca C, Baldelli G, Tramontana M, Balsamo M. 2017. Is meiofauna a good bioindicator of artificial reef impact? *Marine Biodiversity* **47(2)**:511–520 DOI 10.1007/s12526-016-0484-3.

Sergeeva NG, Revkova TN. 2021. First finding of Greeffiella Cobb, 1922 (Nematoda, Desmoscolecida) in the Black Sea. *Ecologica Montenegrina* **42**:96–102 DOI 10.37828/em.2021.42.5.

Stoddart DR, Steers JA. 1977. The nature and origin of coral reef islands. In: Jones OA, Endean R, eds. *Biology and Geology of Coral Reefs IV, Geology II.* London: Academic Press, 59–105.

Vanreusel A, Fonseca G, Danovaro R, Cristina da Silva M, Esteves AM, Ferrero T, Gad G, Galtsova V, Gambi MC, Da Fonsêca V, Ingels J, Ingole B, Lampadariou N, Merckx B, Miljutin D, Miljutina M, Muthumbi A, Netto S, Portnova D, Radziejewska T, Raes M, Tchesunov A, Vanaverbeke J, Van Gaever S, Venekey V, Bezerra TN, Flint H, Copley J, Pape E, Zeppilli D, Martinez PA, Galeron L. 2010. The contribution of deep-sea macrohabitat heterogeneity to global nematode diversity. *Marine Ecology* **31(1)**:6–20 DOI 10.1111/j.1439-0485.2009.00352.x.

Venekey V, Gheller PF, Maria TF, Brustolin MC, Kandratavicius N, Vieira DC, Brito S, Souza GS, Fonseca G. 2014. The state of the art of Xyalidae (Nematoda, Monhysterida) with reference to the Brazilian records. *Marine Biodiversity* **44**:367–390 DOI 10.1007/s12526-014-0226-3.

Vincx M. 1996. Meiofauna in marine and freshwater sediments. In: Hall GS, ed. *Methods for the Examination of Organismal Diversity in Soils and Sediments*. Wallingfort UK: CAB International, 187–195.

Wieser W. 1953. Die Beziehung zwischen Mundhöhlengestalt, Ernährungsweise und Vorkommen bei freilebenden marinen Nematoden. In: *Arkiv för Zoologi.* Vol. 4. Frankfurt: Universitätsbibliothek Johann Christian Senckenberg, 439–484.

Worm B, Barbier EB, Beaumont N, Duffy JE, Folke C, Halpern BS, Jackson JBC, Heike Lotze HK, Micheli F, Palumbi SR, Sala E, Selkoe KA, Stachowicz JJ, Watson R. 2006. Impacts of biodiversity loss on ocean ecosystem services. *Science* **314(5800)**:787–790 DOI 10.1126/science.1132294.

Zeppilli D, Bongiorni L, Serrão Santos R, Vanreusel A. 2014. Changes in nematode communities in different physiographic sites of the condor seamount (north-East atlantic ocean) and adjacent sediments. *PLOS ONE* **9(12)**:e115601 DOI 10.1371/journal.pone.0115601.

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CHAPTER 2

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Meiobenthic assemblages as ecological indicator of natural variability induced by ecosystem engineers

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Abstract

Indonesia is biologically one of the most diverse areas in the world. Nevertheless, its biodiversity is underestimated, and this is particularly evident for meiobenthic assemblages. Seagrass meadows are known to be ecosystem engineers that can significantly modify the habitat heterogeneity and, thereby, increase the meiobenthic diversity. Therefore, a study on the meiobenthic distribution and biodiversity was conducted in two seagrass meadows around Siladen Island (Bunaken Marine National Park): Barat and Lagoon, characterized by different sedimentary regimes, as well as by different anthropogenic impact. An overall high level of diversity indices and richness were recorded (H': 1.19-1.69; J: 0.46-0.67; number of taxa: 9-14), with meiobenthic abundances ranging between 770 and 2529 ind. 10 cm^{-2}. The lowest meiobenthic abundance and diversity (i.e. Shannon-diversity, number of taxa) were reported at Barat meadow, probably in response to sedimentological modifications and higher anthropogenic pressure at this site. Differences in meiobenthic assemblage structures were particularly evident when only rare taxa were considered (i.e taxa found in low abundances and characterized by a sporadic distribution in the study area). This result underlines the role of rare species as possible indicators of spatial variability generated by engineer species and different environmental conditions.

Keywords

Meiobenthos, seagrasses, rare taxa, habitat complexity, Indonesia.

Introduction

Seagrass beds constitute a conspicuous feature of sheltered soft-shores worldwide, and are amongst the most productive marine communities (Mateo et al., 2006; Leduc and Probert, 2011). They are key intertidal habitats and important nursery grounds (Barnes et al., 2011). Seagrasses are considered to be ecosystem engineers or structural species and are known to influence their surroundings in complex ways (i.e. habitats differing in terms of grain size, sediment geochemistry, biotic communities, etc...) (Fonseca et al., 1982). They alter sediment grain size, stabilize sediments by trapping detritus, influence sediment chemistry both directly and indirectly, and alter the microbial and microphytic communities within sediments (e.g. Fonseca et al., 2011; Gallucci et al., 2020). Tropical seagrass beds can be made up by one or several species of seagrasses and their morphological complexities (i.e. shoot density, leaf shape, etc.) play a crucial role in favoring the colonization of associated invertebrates (De Troch et al., 2006; Liao et al., 2016). Thus, each seagrass species contributes to increasing habitat complexity in meadows and determining the community structure of co-existing species and their ability to exploit a unique niche, even on a spatial scale of 10 cm^2 (De Troch et al., 2003).

So far, research suggests that the presence of seagrasses positively influences macrofaunal abundance, biomass, and diversity (e.g. Hemminga and Duarte, 2000; Lee et al., 2001; Hirst and Atrill, 2008). Current studies indicate that meiobenthic density and diversity from seagrass sediments are overall higher compared to those from bare sediments (e.g. Orth et al., 1984; Fonseca et al., 2011; Liao et al., 2015). Since meiobenthic organisms spend their entire lifecycle on or around the same substrate, they are sensitive to changes in environmental conditions (i.e. bottom current impact, grain-size changes, presence of vegetated sea bottom) even over small spatial scales (Giere, 2009; Semprucci et al., 2015, 2019). Furthermore, meiobenthic assemblages can indicate warning environmental conditions due to anthropogenic activities (e.g. effects of nutrient enrichment) that otherwise would not be documented by the seagrass plants themselves (Daudi et al., 2012). This makes meiobenthic organisms important for planning effective management and conservation practices in these types of habitats (as suggested by Vasapollo, 2010; Vasapollo and Gambi, 2012; Vasapollo et al., 2015). Their numerical dominance, relatively high diversity and key ecological roles in energy transfer to higher trophic levels (e.g. macrofauna, shrimps, small fishes and juveniles of larger fishes) are well documented in seagrass systems, but meiobenthos of seagrass free sediments is comparatively more investigated (e.g. De Troch et al., 2006; Barnes et al., 2011; Leduc and Probert 2011; Semprucci et al., 2019; Ravaglioli et al., 2020).

Key strategies for the conservation of seagrass systems are pivotal considering that many seagrass beds have been degraded worldwide, with an estimated annual decline of 7% at a global level (Leduc and Probert, 2011). The total global loss of seagrass systems (ca. 29% from 1897; Beck, 2009) is likely to have a major impact on the structure and functioning of associated invertebrate communities (Kenworthy et al., 2006; Pusceddu et al., 2014).

Indonesia harbors some of the biologically most diverse ecosystems in the world, but its marine ecosystems remain one of the least studied environments of the planet (Barber et al., 2014). Preserving biodiversity and their benefits for mankind requires an understanding of how biodiversity is distributed over space and time, to fully evaluate ecosystem health and possible ecologic changes due to natural and anthropogenic causes (Cardinale et al., 2012; Balvanera et al., 2014; Gamfeldt et al., 2014). The distribution of meiobenthos is closely linked to small-scale processes such as changes in sediment structure, disturbance, and potential food sources (Somerfield et al., 2002; Giere, 2009; Sahraeian et al. 2020; Sbrocca et al. 2021). Here, the meiobenthic assemblages (including both metazoans and unicellular organisms, such as Ciliata and Foraminifera) inhabiting two multispecific seagrass meadows located in the Bunaken Marine National Park (North Sulawesi, Indonesia) were investigated and their roles as indicators of local environmental conditions were evaluated. We hence investigated changes in the meiobenthic abundance, diversity, assemblage structure by considering different spatial scales from few kilometers (i.e. between meadows) to few centimeters (i.e. sediment vertical profile) with a particular focus on the rare meiobenthic taxa as potential ecological indicators of changes in the seagrass meadows due to both natural and anthropogenic influence. In specific, we hypothesized that the increase of the habitat complexity, created by seagrass meadows as ecosystem engineering species, could promote a higher richness of meiobenthic taxa (both of temporary and of permanent meiobenthic components). This investigation is the first attempt to document the benthic response to different sedimentary regimes and putative anthropogenic pressures in an understudied geographical region. This will be a significant baseline information for future biomonitoring of the human disturbance in this Marine Protected Area.

Material and Methods

Study area

The Bunaken Marine National Park (1°37'N 124°45' E, North Sulawesi, Indonesia), established in 1991, is one of the most important Indonesian Marine Protected Areas, and covers about 90,000 ha of coral reefs, mangroves and seagrass beds (Fig. 1 and Supplementary Material, Figure S1). The park includes the coastal section of North Sulawesi and five islands (Bunaken, Siladen, Manado Tua, Nain and Montehage) with 22 villages that support a local population of 30,000 people (Fava et al., 2009). Each island is surrounded by fringing reefs characterized by reef flats with different offshore extents and steep walls along the outer edge. In this area several tourist resorts and about 20 dive operators result in an estimated tourist traffic of 25,000 guests per year (de Vantier and Turak, 2004). Human impacts include overexploitation of resources, destructive fishing practices, overcrowded diving activities, anchor and boat strike damages, as well as pollution from solid waste and sewage (Fava et al., 2009).

The investigation was carried out in February 2005, after the winter monsoon. The study area was Siladen Island (01°37'39.5" N and 01°38'20.2''N), which is one of the five islands of the Bunaken National Marine Park. This island covers a surface area of around 45 ha, with a reef area of 111 ha (Fig. 1 and Supplementary Material, Figure S1). Daily, two high and two low tides trigger strong tidal currents around Siladen (Runtukahu et al., 2007). The coral reef is of the fringing-reef type and a heterogeneous topography is present around the island. On the southern side, the reef crest is very close to the shore, with a reef flat less than 20 m wide, whereas on the western, eastern and northern side the reef flat area is wider and seagrass meadows are present close to the shore (Supplementary Material, Figure S1). A complete list and description of the seagrass species characterizing the sampling area are reported in Table 1. Of the twelve species reported for the Indonesian waters, only the following seagrass species are present around Siladen Island: *Enhalus acoroides, Halophila ovalis, Thalassia hemprichii, Cymodocea rotundata, Cymodocea serrulata, Halodule pinifolia* and *Syringodium isoetifolium* (Vasapollo 2005). The meadows are typically multispecific and occur in waters of less than 1 m depth to approximately ~ 3.5 – 5 m depth on the reef flat. Siladen Island is inhabited by approximately 350 people, of which 200 are locals, living in small villages in the southern and western sides, and 150 are resort staff or guests of the five scuba diving resorts operating mainly on the south-western coast of the island (Kyle et al., 2014). Barat is defined as a human impacted area (Fava et al. 2009). Here, a small harbor is present for the locals' small fishing vessels, with handcraft moorings that are placed in one of the seagrass meadows. Nevertheless, several boats anchor without using the moorings, thus creating a mechanical impact on the plants. Aside from the mechanical impact, some concerns arise from the discharges of wastewater from villages and resorts. In fact, several septic pits are located under the lavatories of each house and filtered by corals and natural fibers, producing a sludge that can penetrate the ground (Kyle et al., 2014).

However, the presence of strong tidal currents reduces the deposition of pollutants and sludge in the meadows (Vasapollo, 2005).

Sampling design

Meiobenthic assemblages were investigated in two seagrass meadows around the island: one located in the fishing boat harbor on the southwest side (Barat - B: 124°47’58.0’’E – 1°37’42.6’’N), and the second on the eastern side of the island (Lagoon - L: 124°48’22.4’’E – 1°37’46.9’’N). Despite, the two sampling sites are located only 1500 m apart, they show an overall different seagrass species composition (Vasapollo, 2005). At each site, samples were collected in a coast-perpendicular transect at 30 m and 50 m distance from the shoreline, named B30 and B50 at Barat and L30 and L50 at the Lagoon site (Fig. 1 and Supplementary Material, Figure S1). The two distances were selected according to the changes in seagrass species composition and density of the seagrass meadows. At the Lagoon site the seagrass composition is more homogeneous along the transect than at Barat. At each site, sediments were collected between two subsequent tides at depth of about 1.3 m and 1.5 m. In order to collect undisturbed portions of the sediments, scuba divers carried out the sampling using a manual corer (of 3.5 cm internal diameter) (e.g. Danovaro, 2010; Daudi et al., 2012) among the seagrass rhizomes down to the depth of 15 cm (e.g. Barnes et al., 2011). Two replicate cores were collected for meiofauna at each station of Lagoon and Barat and each core was immediately sliced into three layers to document possible changes in the vertical distribution (i.e. 0-5 cm, 5-10 cm and 10-15 cm). Likewise two replicate cores were taken at the same site for the grain size and subsampled at the same vertical intervals. All the samples for the meiobenthic analysis were immediately treated with a 4% formaldehyde solution in buffered pre-filtered seawater to fix the fauna and stained with Rose Bengal (0.5 g L^{-1}) (Heip et al., 1985), while sediment samples for the grain size were stored at room temperature until laboratory analysis.

Grain size

Grain size analysis was carried on bulk samples (15-25 g) collected at each station, oven-dried at 55°C for 48 hours. In order to remove the organic matter eventually present in sediment samples and to facilitate the release of sediment particles from the lumps, the samples were treated with hydrogen peroxide for 48 hours, (Picone et al., 2008; Giani et al., 2009), the muddy fraction was separated from sand and gravel-sized macro-remains (e.g. shells and pieces of corals) by wet sieving at 63 microns. The coarse fraction was further dry-sieved at 125, 500, 1000 and 2000 microns in order to evaluate different sand fraction (i.e. very fine, fine, medium, coarse, very coarse) and macro-remain concentrations. Each fraction is expressed as percentage of the total dry weight of their sum (Picone et al., 2008; Giani et al., 2009).

Meiobenthic assemblage

In the laboratory, all meiobenthic samples were carefully washed through two nested mesh nets (500 and 32 µm) (e.g. Danovaro et al. 2004). The first was used to exclude macrofaunal organisms, while the second to retain meiobenthos. The fraction retained on the 32 µm sieve was centrifuged 3 times in Ludox HS 30 (density 1.18 g cm^{-3}) in order to separate the lighter organisms from the heavier sediment grains (Heip et al., 1985; Danovaro, 2010). The organisms retained on the 32 µm sieve were then transferred into a ‘Delfuss’ Petri dish with a checkered bottom (200 squares, to make counting easier) and, with the aid of a Leica G26 stereomicroscope, sorted into major meiobenthic groups and counted. The density of all the individuals found (both temporary and permanent meiobenthos) was standardized as abundance in 10 cm^{-2}. The meiobenthic groups that were found in very low densities (<1% of the total abundance in all investigated samples) were defined as rare taxa and represented with the category named ‘Others’. Shannon diversity (*H’*) and Pielou evenness (*J*) indices ($\log_2$) were calculated to describe the meiobenthic assemblage structures at the major group level (Shannon and Weaver, 1949; Pielou, 1969). Larval stages (e.g. *nauplii* and nectochaetes) were incorporated into their respective taxonomic groups (i.e. Copepoda and Polychaeta, respectively) for the calculation of diversity and evenness indices as well as richness (number of taxa), while they were considered separately from their adult forms when the structure of the assemblage was analyzed.

Statistical analyses

All statistical analyses were carried out using the same sampling design, considering three factors as main sources of variance: ‘site’ (fixed, 2 levels: Barat and Lagoon meadows), ‘station’ (random and nested in ‘site’, 2 levels: 30 and 50 meters), and ‘depth layer’ (random and nested in station, 3 levels: 0-5, 5-10 and 10-15 cm).

Permutational multivariate analysis of variance (PERMANOVA) was used to assess the variability in the grain size, total meiobenthic abundance, assemblage composition and diversity indices (Anderson 2001; McArdle and Anderson 2001). In order to assess the possible differences of the assemblage structure, the data matrix with both entire meiobenthic assemblage and only taxa included in the ‘Others’ category (< 1%) were utilized. The exclusion of dominant groups, such as nematodes or copepods, may detect the small changes of the assemblage structure that otherwise would be masked (Pusceddu et al., 2011). Furthermore, the relative contribution of the numerically less represented meiobenthic taxa is often crucial because most of them are more sensitive to environmental changes (e.g. Bianchelli et al., 2016).

The analyses were carried out on Euclidean distances (for all the following univariate measures: grain size, meiobenthic abundance as total number of individuals on 10cm^{-2}, Shannon-diversity and Pielou-evenness indices) or Bray–Curtis similarity matrices (for multivariate measures: meiobenthic assemblage structure) of previously normalized (e.g. grain size) or fourth root transformed (e.g. faunal structure) data, using 999 permutations of the residuals under a reduced model. The fourth root transformation of the densities was chosen in order to give more relevance to rare taxa in the analysis (Anderson et al. 2008). P values for pairwise comparisons were obtained from Monte Carlo asymptotic distributions, because of the restricted number of unique permutations (Anderson, 2006).

Principal component analysis (PCA) was performed to visualize differences in environmental variables (grain size and seagrass communities, based on presence/absence data) among sites and/or stations and among sediment layers in the same station.

Similarity percentage (SIMPER) analyses were also performed to assess the percentage dissimilarity (*sensu* Gray, 2000) in the meiobenthic assemblage composition between sites, stations within the same site, and depth layers, for the entire meiobenthic assemblage as well as ‘Others’ taxa.

In order to assess if and to what extent the sediment characteristics (e.g. grain size) and seagrass meadow composition explained changes in meiobenthic assemblage characteristics at different sites, stations or layers, a non-parametric multivariate multiple regression analysis, based on Euclidean distances, was carried out using the routine distance-based linear modeling (DistLM) (McArdle and Anderson, 2001). The forward selection of the predictor variables was performed with tests by permutation. P values were obtained using 4999 permutations of raw data for the marginal tests (tests of individual variables), while for all of the conditional tests, the routine uses 4999 permutations of residuals under a reduced model. All the analyses were performed using the routines included in the software PRIMER 6 and PERMANOVA+ (Clarke and Gorley, 2006).

Results

Environmental variables

During the sampling period, temperature and salinity ranged from 27 °C to 28 °C and 33 psu to 35 psu, respectively. In general, sands (> 63 μm and ≤1000 μm) characterized sediments at both sites, with a mean value of 95% at the Lagoon site and 84% at Barat, followed by gravel percentage, whereas the muddy fraction (< 63 μm) was always rather negligible (Supplementary Material, Table S1). However, the distribution of the different grain size classes was not the same in the two meadows. At Barat, the composition was dominated by medium and fine sand fractions. Gravel represented another important fraction at B50 (mean value of 26%), whereas it was less than 5% at B30. On the other hand, the two Lagoon stations were characterized by a slightly finer grain size, with very similar values at both stations, and a gravel fraction always <12% (Supplementary Material, Table S1).

The PERMANOVA test results revealed significant differences between sites and between stations in Barat meadow (PERMANOVA, p=0.001 and p=0.005, respectively); more specifically the pair-wise tests documented a higher percentage of very coarse sand and gravel fractions at B50 when compared to B30 (p = 0.010) (Table 2). The multivariate characteristics of seagrass meadows (i.e. grain size and seagrass communities) were investigated using the PCA to visualize data trends (Fig. 2). Sites were clearly separated in first factor plane. In particular, PC1 accounted for 56.3% of the differences between sites, and PC1 and PC2 accounted together for 81.9% (Supplementary Material, Table S2). Along the PC1 axis, Barat and Lagoon sites were separated into two groups according to percentage of fine sand and seagrass species: the presence of pioneer species such as *Halophila ovalis* and *Halodule pinifolia* at the Lagoon site and the unique presence of *Cymodocea serrulata* in Barat meadow (Fig. 2). Along the PC2 axis, stations B30 and B50 were ordinated according to grain size, coarser at B50 station, along with the presence of the pioneer species *Syringodium isoetifolium*, not reported for B30.

Abundance and diversity of meiobenthic assemblages

The total meiobenthic abundance across all depth levels (0-15 cm) varied from a minimum mean value of 770±402 ind. 10 cm^{-2} at B50 to a maximum mean value of 2529±1305 ind. 10 cm^{-2} at L50 (Fig. 3). Significant differences occurred only between sites (PERMANOVA, p = 0.038, Table 2), with lower values detected at Barat compared to Lagoon. Despite significant differences of the abundances were not revealed along the vertical sediment profile, a general decrease of the abundances was observed in the deeper layers (Fig. 3). Fifteen animal taxa, including temporary meiobenthos, were identified: Nematoda, Copepoda (adults + *nauplii*), Polychaeta (adults + larvae), Oligochaeta, Turbellaria, Ostracoda, Tardigrada, Gastrotricha, Isopoda, Tanaidacea, Acarina, Nemertea, Ciliata, Loricifera and Gnathostomulida. The total highest mean value of richness of taxa was reported for station L50 with 14 taxa, whereas the total lowest number of taxa characterized station L30 with 9 taxa (Fig. 3). Lagoon comprised 14 meiobenthic taxa, while at Barat 12 taxa were present. Significant differences were detected between L30 and L50 stations (PERMANOVA, p = 0.014, Table 2). The H’ ranged from 1.19 (B30) to 1.69 (L50), while J from 0.46 (L30) to 0.67 (B30) (Fig. 3), but without any significant difference.

Meiobenthic assemblage structure

At all sampling stations, Nematoda was the dominant taxon (60-63%), followed by Copepoda (13%). The other most frequent taxa at Barat were Polychaeta (8%), Ciliata (8%) and Gastrotricha (5%), while Ciliata (10%) and Gastrotricha (4%) dominated at the Lagoon site. PERMANOVA, carried out on the whole meiobenthic assemblage structure, revealed significant differences between layers only at B50 station (PERMANOVA: $p = 0.038$ and $p = 0.031$ between 0-5 *versus* 5-10 cm and 0-5 *versus* 10-15 cm layers, respectively) (Table 2). The dissimilarity observed between the B50 layers (47-53%) was mainly due to the different contribution of Nematoda, Copepoda, Polychaeta, Turbellaria and Gastrotricha, which drastically decrease along the vertical sediment profile at Barat (Supplementary Material, Table S3).

The PERMANOVA tests carried out considering only the meiobenthic components included in the 'Others' category (Fig. 4a,b) showed significant differences between many factors considered (i.e. sediment layers, stations and interaction sites × layers) (Table 2). In detail, Barat and Lagoon revealed significant differences (PERMANOVA, $p = 0.045$) related to the presence/absence of meiobenthic components such as Tardigrada, *nauplii,* Loricifera, Tanaidacea, Halacarida and Isopoda. Tardigrada, Loricifera and Isopoda were detected exclusively in Lagoon meadow, while *nauplii*, Tanaidacea and Halacarida in Barat (Supplementary Material, Table S3, S4). Stations B30 and B50 did not show significant differences in terms of rarer taxa composition, even if the contribution of some meiobenthic components changed between the two stations (e.g. Oligochaeta, Tanaidacea, Acarina and Polychaeta larvae; Fig. 4a,b). Instead, pairwise results documented significant differences between L30 and L50 (PERMANOVA $p = 0.028$) that appeared primarily related to the occurrence of Tardigrada, Loricifera, Isopoda, Gnathostomulida at L50 and Halacarida and *nauplii* at L30 (Fig. 4a, Supplementary Material, Table S4). The distribution of the rarer meiobenthic components along the vertical profile showed significant changes only at L50 and B50 (all, PERMANOVA, $p < 0.05$). Along the vertical profile, the highest dissimilarity values were reported at L50, but a reduction of the number of these meiobenthic components with increasing sediment depth at both sites was generally observed. In detail, organisms such as Isopoda, Loricifera, Gnathostomulida, Tanaidacea and some temporary meiobenthic groups as Nemertea and Polychaeta larvae disappeared in the 10-15 cm layers (Fig. 4b).

Influence of environmental features on seagrass meiobenthos

The results of DistLM-forward analysis performed on the meiobenthic abundance, diversity (i.e. taxa richness, H' and J), structure of the whole as well as rarer taxa meiobenthic assemblages are reported in Table 3. The different grain size classes of the sediments explained most of the variance of the meiobenthic abundance and taxon richness (i.e. 46% and 37%, respectively) and very fine sands, in particular, appeared to be the most significant sediment fraction affecting meiobenthic abundance and richness. The structure of the whole meiobenthic assemblage is explained mainly by very fine (14%), followed by very coarse fractions and the occurrence of *T. hemprichii* meadow. Comparatively, the occurrence of this seagrass species appeared more relevant for the distribution of the rarer taxa (i.e. 6%) than for the other faunal parameters. However, very coarse and gravel sediment fractions were the environmental variables that explained the highest variance percentages (7 and 6%, respectively).

Discussion

In natural systems, environmental heterogeneity is important for the functioning of many different ecosystems, since it can enhance primary productivity, increase biodiversity and stabilize the system (Hastings et al., 2007; Eriksson et al., 2010). Moreover, it occurs at different scales in space and time thus forces species diversification (Gallucci et al., 2020). Studies from terrestrial and marine ecosystems have demonstrated that ecosystem engineers, i.e. organisms that deeply modify their physical environment (Jones et al., 1997), determine spatial habitat heterogeneity (Hastings et al., 2007; van der Heide et al., 2012). Among these actors, seagrasses can strongly influence their surrounding in a complex way (Fonseca, 1996; Somerfield et al., 2002): they can alter water flow regimes and deposition of particles, and thus influence food supply for benthic organisms (Jankowska et al., 2014).

Barat and Lagoon showed differences in environmental characteristics such as seagrass species composition and sediment features. The meadow at Lagoon showed similar plant species coverage and sediment grain size at different distances from the shore (i.e. L30 = L50), whereas Barat showed some differences with respect to Lagoon site, as well as between the two stations (B30 ≠ B50). Barat was also characterized by a higher presence of gravel-sized sediments at B50 than at the same distance from shoreline in Lagoon. These sedimentological data seem to suggest a higher energy environment at B50m probably due to the limited reef width on this side of the island that favored the transport of hard coral and encrusting sponge fragments from the reef edge. However, a physical disturbance cannot be excluded at Barat due to boat anchoring or disruption of corals during the recent building activities of the jetty (Vasapollo, 2005; Fava et al., 2009). The dominance of fine-medium sand at both seagrass meadows appeared to support the idea that plants can retain detritus and fine sediment particles inside the vegetated substrata (Marba et al., 2006; Leduc and Probert, 2011). Many studies, in fact, report higher amounts of finer sediments in seafloors covered by seagrass compared to bare sediments (van Katwijk et al., 2010; Jankowska et al., 2014), since they create a rough bottom surface and slow down the hydrodynamics (i.e. waves, currents and tides).

Meiobenthic abundance, diversity and taxonomic composition variability in seagrass meadows

Overall meiobenthic abundances reported from Siladen meadows fall within the range of 53 - 5314 ind.10 cm^{-2} of total abundances reported from seagrass systems worldwide (e.g. Danovaro, 1996; Daudi et al., 2012; Losi et al., 2012; Jankowska et al., 2014; Liao et al., 2015). Meiobenthic abundances were two to three times higher than abundances reported in Micronesia (Min et al., 2006), Malaysia (Zaleha et al., 2009; Long et al., 2014) and along the south-western cost of Taiwan (Liao et al., 2016) and even nine times higher than those reported in the only two studies performed in Indonesia (Bali: Leasi et al., 2016 and Sulawesi: Pusceddu et al., 2014). The diversity indices documented the presence of a highly diversified meiobenthic assemblage without the dominance of one or few groups (see J values) that is consistent with studies in similar types of environments (e.g. De Troch et al., 2001a,b, 2006; Zaleha et al., 2009; Barnes et al., 2011; Fonseca et al., 2011; Mascarat et al., 2013; Jankowska et al., 2014; Pusceddu et al., 2014). Seagrasses, in fact, support higher diversity values of associated meiobenthos if compared to bare sediments because they provide unique micro-habitats that enhance meiobenthic colonization (Ndaro and Ólafsson, 1999; Fisher and Sheaves, 2003; Jankowska et al., 2015; Losi et al., 2018).

The most abundant taxa (i.e. Nematoda, Copepoda, Polychaeta, Turbellaria and Gastrotricha) found in Siladen are mainly permanent components of meiobenthos (Polychaeta were the only exception). These taxa generally form the majority of the meiobenthic groups living within the sediment covered by seagrass (e.g. Danovaro et al., 2002; Min et al., 2006; Mascarat et al., 2013; Jankowska et al., 2014; Pusceddu et al., 2014; Liao et al., 2016). Moreover, we reported also a high contribution of Ciliata that are rarely considered in the investigations on meiobenthos, but they can constitute a relevant benthic component in many marine habitats (e.g. Daudi et al., 2012; Baldrighi et al., 2020).

Protists (e.g., Ciliates and Foraminifera) play many roles in marine ecosystems and form a trophic link between prokaryotes and higher trophic levels; they impact carbon and other nutrient cycles directly and indirectly through grazing on organic matter and prokaryotic prey (Anderson et al., 2012; Balsamo et al., 2012; Bianchelli et al., 2020). For all these reasons, they might be part of the complex detritus – bacteria – meiofauna system at the base of the functioning in seagrass meadows (Danovaro et al., 2002; Armenteros et al., 2008; Balsamo et al., 2010).

The role of seagrass in structuring the meiobenthic assemblages may depend on species composition or density of meadows (Jankowska et al., 2014; Liao et al., 2016). However, in the present study, sedimentological variations appeared more relevant to explain the meiobenthic spatial distribution than a particular seagrass species *per se*. In particular, very fine sands explained most of the variance for the total meiobenthic abundance, richness and whole assemblage structure, while very coarse sand fractions only for rarer taxa structure.

The presence of a dense and uniform vegetation coverage, as well as a wider reef flat area at Lagoon, seemed to provide a sheltered and stable environment that favored fine sediment deposition. Finer grain-sizes generally retain higher organic matter content and this could explain the prevalence of deposit-feeding meiobenthos and the higher meiobenthic abundance in Lagoon samples, which were about double as high as at Barat (e.g. Todaro et al., 2006; Giere, 2009; van Katwijk et al., 2010; Barnes et al., 2011; Jankowska et al., 2014). Furthermore, meiobenthos can be easily washed away in coarser sediments; therefore, their abundances are frequently low in these types of sediments (Semprucci et al., 2010, 2011).

The microhabitat complexity created by these ecosystem engineers appeared to be more discernible using the multivariate analysis of the rare taxa (see the components belonging to the 'Others' category) that can be regarded as sentinels of possible environmental changes (Pusceddu et al., 2011; Carugati et al., 2018). Indeed, the whole meiobenthic assemblage structure showed significant differences only between the sediment layers at Barat 50 station, while the rarer meiobenthic taxa revealed significant differences in relation to the stations, vertical profile, and interaction sites × sediment layers. The differences in the distribution of the rare taxa have been recently used as tool to assess for instance the biodiversity patterns at different spatial scales in deep-sea (Bianchelli et al., 2010) as well as the benthic environmental quality status in coastal areas characterized by different benthic trophic status (Pusceddu et al., 2011; Gallucci et al., 2020) and pollution levels (Carugati et al., 2018). The highest meiobenthic richness as well as increased presence of rare taxa in the Lagoon seafloor could be also related to a lower impact of humans in that part of the island. Indeed, even if there are no data on the organic enrichment or contaminant concentrations, we should consider that Barat is an urbanized area. There, the sewage sludge loads coming from the villages and resorts are poorly re-worked by currents (Kyle et al., 2014) and could be a possible disturbance for the most sensitive meiobenthic taxa, which show with the lowest richness at Barat.

A general decrease of meiobenthic abundance and richness occurred with increasing depth in the sediment, even if it was not statistically significant in most of the cases. This pattern of meiobenthic distribution was related to the complete disappearance of some rare taxa in the deeper sediment layers (e.g. Tanaidacea, Ostracoda; present study) and general decrease of the most abundant groups (e.g. De Troch et al., 2001a; Leduc and Probert, 2011; Daudi et al., 2012). Vertical distribution of meiobenthic organisms can be affected by numerous factors such as unequal distribution of food, sedimentological variations (with a consequent change of the interstitial spaces) and interactions with other benthic organisms (McLachlan et al., 1977; Daudi et al., 2012; Boufahja et al., 2016; Jouili et al., 2017). As Siladen Island data seem to suggest, seagrass coverage might enhance the sedimentary environment and deposition rate (by baffling currents and wave-action) and affect the composition of the infauna through direct or indirect pathways (Somerfield et al., 2002).

A significant change in the distribution of rare meiobenthic taxa along the vertical profile was detected only in the stations far from the coast; L50 showed four rare taxa: Oligochaeta, *nauplii*, Ostracoda and Tardigrada in the deepest sediment layers, while B50 showed only Oligochaeta and *nauplii*. This pattern of distribution seems to further confirm the greater habitat heterogeneity and potentially a higher ecological quality of the meadows in the Lagoon. De Troch et al. (2001) and De Troch and Fiers (2001) found a similar variability in the vertical distribution in shallow meadows and also in that case meiofaunal assemblages appeared largely structured by biogenic factors. Among the taxa that characterized the deeper sediments at L50, there were Tardigrada that mostly feed on bacteria, algal cells and detritus (Nelson et al., 2015). Their vertical distribution is poorly investigated but, being closely related to the food availability (Giere, 2009), it is possible to hypothesize that they found greater trophic supply in the deeper layers of L50. Similarly, Ostracoda are widely distributed in all types of aquatic environments and generally more abundant in fine-grained sediments (Dügel et al., 2008; Giere, 2009). They are generally restricted to the upper layers, but some species show wide levels of tolerance and preference for specific physical-chemical variables (Jahn et al., 1996; Frontalini et al., 2018) that could explain their unusual presence in the deeper layers of Siladen seafloors. Direct adaptation to sediments is one of the main causes for variation in the sediment-dwelling fauna (Snelgrove and Butman, 1994). Thus, the presence of some rare taxa, even in the deepest sediment layers, was in line with their ability to colonize a specific range of environmental conditions. From our analysis, rare taxa were the only taxa influenced by the presence/absence of particular seagrass species (e.g. *T. hemprichii*). In detail, we reported a close association among higher Oligochaeta abundance, *T. hemprichii* occurrence and lower percentage of very coarse sand fraction. This association would deserve an accurate study, as well as the other association emerged between rarer taxa and specific meadows and/or grain size.

Conclusions

The present study documents, for the first time, the meiobenthic composition and diversity of an area (i.e. Siladen Island) that is part of one of the most important Marine Protected Areas in Indonesia (Bunaken Marine National Park). Despite, the preliminary nature of this survey, the data collected so far seem to support the idea that rare meiobenthic taxa (i.e. the taxa with a fragmentary distribution or numerically less represented) are more sensitive to the different ecological conditions than the whole meiobenthic assemblage. The fact that they are rare may indicate their preference for specific environmental conditions that make them more suitable as sentinels of spatial variability generated by engineer species and proxies of environmental condition changes. Although the mechanism is not yet totally clear, the habitat heterogeneity characterizing the seagrass beds may have an important role in favoring the occurrence of a rich meiobenthic assemblage. Therefore, our study strengthens the idea that the conservation of engineering species habitats leads to preserve high levels of diversity of the benthos closely associated with seagrass plants and is crucial in maintaining the coastal biodiversity and functioning of the ecosystem.

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Conflict of interest

The authors declare that they have no conflicts of interest.

Availability of data and material

Not applicable

Ethical approval

No special ethics approval was needed

References

Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. Aust J Ecol 26: 32–46

Anderson MJ (2006) Distance-based tests for homogeneity of multivariate dispersions. Biometrics, 62: 245–253

Anderson M, Gorley R, Clarke K, (2008) Plymouth: primer-E; 2008. PERMANOVA+ for PRIMER: Guide to software and statistical methods: 214

Anderson R, Winter C, Jürgens K (2012) Protist grazing and viral lysis as prokaryotic mortality factors at Baltic Sea oxic-anoxic interfaces. Mar Ecol Prog Ser 467: 1-14

Armenteros M, Williams, JP, Creagh B, Capetillo N (2008) Spatial and temporal variations of meiofaunal communities from the western sector of the Gulf of Batabanó, Cuba: II. Seagrass systems Rev Biol Trop 56: 55-63

Baldrighi E, Zeppilli D, Appolloni L, Donnarumma L, Chianese E, Russo GF, Sandulli R (2020) Meiofaunal communities and nematode diversity characterizing the Secca delle Fumose shallow vent area (Gulf of Naples, Italy). PeerJ 8: 9058

Balsamo M, Albertelli G, Ceccherelli VU, Coccioni R, Colangelo MA, Curini-Galletti M, Danovaro R, D'Addabbo R, Leonardis C, Fabiano M, Frontalini F, Gallo M, Gambi C, Guidi L, Moreno M, Pusceddu A, Sandulli R, Semprucci F, Todaro MA, Tongiorgi P (2010) Meiofauna of the Adriatic Sea: current state of knowledge and future perspectives. Chem Ecol 26, 1: 45-63

Balsamo M, Semprucci F, Frontalini F, Coccioni R (2012) Meiofauna as a tool for marine ecosystem biomonitoring. In: A. Cruzado (Ed) Marine Ecosystems. InTech Publisher 4: 77-104

Balvanera P, Siddique I, Dee L, Paquette A, Isbell F, Gonzalez A, Byrnes J, O'Connor MI, Hungate BA, Griffin JN (2014) Linking biodiversity and ecosystem services: current uncertainties and the necessary next steps. BioScience 64: 49-57

Barber PH, Ablan-Lagman MCA, Berlinck RGS, Cahyani D, Crandall ED, Ravago-Gotanco R, Juinio-Menez MA, Mahardika IGN, Shanker K, Starger CJ, Toha AHA, Anggoro AW, Willette DA (2014) Advancing biodiversity research in developing countries: the need for changing paradigms. Bull Mar Sci 90: 187-210

Barnes N, Bamber RG, Bennell G, Cornelius N, Glassom D, Conroy-Dalton Henderson S, Jiddawi N, Lee CNW, Macia A, Msangameno DJ, Paula J, Yahya S, Ferrero TJ (2011) Assessment of regional and local biodiversity in tropical and subtropical coastal habitats in the East African Marine Ecoregion. Biodivers Conserv 20: 2075-2109

Beck MB (2009) Shellfish reefs at risk: a global analysis of problems and solutions. The Nature Conservancy, Arlington, VA, 52 pp.

Bianchelli S, Gambi C, Zeppilli D, Danovaro R, (2010) Metazoan meiofauna in deep-sea canyons and adjacent open slopes: a large-scale comparison with focus on the rare taxa. Deep Sea Res I 57: 420-433

Bianchelli S, Buschi E, Danovaro R, Pusceddu A, (2016) Biodiversity loss and turnover in alternative states in the Mediterranean Sea: a case study on meiofauna. Sci Rep: 6, 1: 1-12

Bianchelli S, Nizzoli D, Bartoli M, Viaroli P, Rastelli E, Pusceddu A, (2020) Sedimentary Organic Matter, Prokaryotes, and Meiofauna across a River-Lagoon-Sea Gradient. divers, 12: 189

Boufahja F, Semprucci F, Beyrem H (2016) An experimental protocol to select nematode species from an entire community using progressive sedimentary enrichment. Ecol Indic 60: 292–309

Cardinale BJ, Duffy JE, Gonzalez A, Hooper DU, Perrings C, Venail P, Narwani A, Mace GM, Tilman D, Wardle DA, Kinzig AP, Daily GC, Loreau M, Grace JB, Larigauderie A, Srivastava DS, Naeem S (2012) Biodiversity loss and its impact on humanity. Nature 486: 59–67

Carugati L, Gatto B, Rastelli E, Lo Martire M, Coral C, Greco S, Danovaro R (2018) Impact of mangrove forests degradation on biodiversity and ecosystem functioning. Sci Rep 8:13298

Clarke KR, Gorley RN (2006) In PRIMER v6: User Manual/Tutorial (PRIMER-E, Plymouth)

Danovaro R (1996) Detritus-bacteria-meiofauna interactions in a seagrass bed (*Posidonia oceanica*) of the NW Mediterranean. Mar Biol 127: 1-13

Danovaro R (2010). Methods for the Study of Deep-sea Sediments, Their Functioning and Biodiversity. CRC Press Taylor & Francis Group

Danovaro R., Gambi C., Mirto S. (2002). Meiofaunal production and energy transfer efficiency in a seagrass *Posidonia oceanica* bed in the western Mediterranean. Mar. Ecol. Prog. Series. 234: 95-104.

Danovaro R., Gambi C., Mirto S., Sandulli R., Ceccherelli V.U. (2004). Meiofauna. In: Gambi M.C., Dappiano M. (Eds.), Mediterranean marine benthos: a manual of methods for its sampling and study. Bio Mar Mediterr, 11: 55-97

Daudi NL, Lugomela C, Nduku Uku J, De Troch M (2012) Effect of nutrient enrichment on seagrass associated meiofauna in Tanzania. Mar Env Res 82:49-58

De Troch, M., Fiers, F., 2001. Alpha and beta diversity of harpacticoid copepods in a tropical seagrass bed: the relation between diversity and species' range size distribution. Mar. Ecol. Prog. Ser. 215, 225–236.

De Troch, M., Fiers, F., Vincx, M., 2003. Niche segregation and habitat specialization of harpacticoid copepods in a tropical seagrass bed. Mar. Biol. 142, 345–355.

De Troch, M., Gurdebeke, S., Fiers, F., 2001. Zonation and structuring factors of meiofauna communities in a tropical seagrass bed (gazi bay, Kenya). J. Sea Res. 45, 45–61.

De Troch, M., Van Gansbeke, D., Vincx, M., 2006. Resource availability and meiofauna in sediment of tropical seagrass beds: Local versus global trends. Mar. Environ. Res. 61, 59–73.

de Vantier L, Turak E (2004) Managing Marine Tourism in Bunaken National Park and Adjacent Waters, North Sulawesi, Indonesia. NRM III, Jakarta, Indonesia

Dügel M, Külköylüoğlu O, Kılıç M (2008) Species assemblages and habitat preferences of Ostracoda (Crustacea) in Lake Abant (Bolu, Turkey). Belg J Zool 138: 50-59

Eriksson BK, van der Heide T, van de Koppel J, Piersma T, van der Veer HW, Olff H (2010) Major changes in the ecology of the Wadden Sea: human impacts, ecosystem engineering and sediment dynamics. Ecosystems, 13, 5: 752-764

Fava F, Ponti M, Scinto A, Calcinai B, Cerrano C (2009) Possible effects of human impacts on epibenthic communities and coral rubble features in the marine Park of Bunaken (Indonesia). Estuar Coast Shelf Sci 85: 151–156

Fisher R, Sheaves MJ (2003) Community structure and spatial variability of marine nematodes in tropical Australian pioneer seagrass meadows. Hydrobiologia 495: 143–158

Fonseca MS (1996) The role of seagrasses in nearshore sedimentary processes: a review. In: Nordstrom KF, Roman CT (eds) Estuarine shores: evolution, environments and human alterations. Wiley Scientific Publishers, Chichester, p. 261–286

Fonseca MS, Fisher JS, Zieman JC, Thayer GW (1982) Influence of the seagrass *Zostera marina* on current flow. Estuar Coast Shelf Sci 15: 351–358

Fonseca G, Hutchings P, Gallucci F (2011) Meiobenthic communities of seagrass beds (Zostera capricorni) and unvegetated sediments along the coast of New South Wales, Australia. Estuar Coast Shelf Sci 91: 69-77

Frontalini F, Semprucci F, Di Bella L, Caruso A, Cosentino C, Maccotta A, Scopelliti G, Sbrocca C, Bucci C, Balsamo M, Martins V, Armynot du Châtelet E, Coccioni R (2018) The response of cultured meiofaunal and benthic foraminiferal communities to lead contamination: results from mesocosm experiments. Environmental Toxicol Chem 37: 2439–2447

Gallucci F, Christofoletti R, Fonseca G, Dias GM (2020) The effects of habitat heterogeneity at distinct spatial scales on hard-bottom-associated communities. Diversity 12, 1: 39

Gamfeldt L, Lefcheck JS, Byrnes JEK, Cardinale BJ, Duffy JE, Griffin JN (2014) Marine biodiversity and ecosystem functioning: what's known and what's next? Oikos, 124, 3: 252-265.

Giani M, Berto D, Rampazzo F, Savelli F, Alvisi F, Giordano P, Ravaioli M, Frascari F (2009) Origin of sedimentary organic matter in the north-western Adriatic Sea. Estuar Coast Shelf Sci 84: 573-583

Giere O (2009) Meiobenthology: the microscopic motile fauna of aquatic sediments, 2nd edn. Springer-Verlag, Berlin

Gray JS (2000) The measurement of marine species diversity, with an application to the benthic fauna of the Norwegian continental shelf. J Exp Mar Biol Ecol 250: 23–49

Hastings A, Byers JE, Crooks JA, Cuddington K, Jones CG, Lambrinos JG, Talley TS, Wilson WG, (2007) Ecosystem engineering in space and time. Ecol Lett 10, 2: 153-164

Heip C, Vincx M, Vranken G (1985) The ecology of marine nematodes. Oceanogr Mar Biol: An Annual Review 23: 399–489

Hemminga M, Duarte CM (2000) Seagrass Ecology. Cambridge University Press, Cambridge (United Kingdom)

Hirst JA, Atrill MJ (2008) Small is beautiful: an inverted view of habitat fragmentation in seagrass beds. Estuar Coast Shelf Sci 78: 811-818

Jahn A, Gamenick I, Theede H (1996) Physiological adaptation of *Cyprideis torosa* (Crustacea, Ostracoda) to hydrogen sulphide. Mar Ecol Prog Ser, 142: 215-223

Jankowska E, Jankowska K, Wlodarska-Kowalczuk M (2015) Seagrass vegetation and meiofauna enhance the bacterial abundance in the Baltic Sea sediments (Puck Bay). Environ Sci Pollut Res 22::14372-14378

Jankowska E, Wlodarska-Kowalczuk M, Kotwicki L, Balazy P, Kuliński K (2014) Seasonality in vegetation biometrics and its effects on sediment characteristics and meiofauna in Baltic seagrass meadows. Estuar Coast Shelf Sci 139: 159-170

Jones CG, Lawton JH, Shachak M (1997) Positive and negative effects of organisms as ecosystem engineers. Ecol 78, 1946–1957

Jouili S., Essid N., Semprucci F., Boufahja F., Nasri A., Beyrem H. 2017. Environmental quality assessment of El Bibane lagoon (Tunisia) using taxonomical and functional diversity of meiofauna and nematodes. J Mar Biol Ass UK 97: 1593-1603.

Kenworthy WJ, Wyllie-Echeverria S, Coles RG, Pergent G, Pergent-Martini C (2006) Seagrass conservation biology: an interdisciplinary science for protection of the seagrass biome. In Larkum, A.W.D, Orth, R.J. and Duarte, C.M. (eds) Seagrasses: biology, ecology and conservation. Dordrecht: Springer, pp. 595–623

Kyle A, Rymer C, Sarmiento J (2014) Design of a low-impact wastewater Collection And Treatment System for Siladen Island In Indonesia. Civil Engineering Senior Theses. 18

Leasi F, Gaynus C, Mahardini A, Moore TN, Norenburg JL, Barber PH (2016) Spatial and ecologic distribution of neglected microinvertebrate communities across endangered ecosystems: meiofauna in Bali (Indonesia). Mar Ecol 37: 970–987

Leduc D, Probert PK (2011) Small-scale effect of intertidal seagrass (*Zostera muelleri*) on meiofaunal abundance, biomass, and nematode community structure. J Mar Biol Ass UK 91: 579–591

Lee SY, Fong CW,Wu RSS (2001) The effects of seagrass (*Zostera japonica*) canopy structure on associated fauna: a study using artificial seagrass units and sampling of natural beds. J Exper Mar Biol Ecol 259: 23-50

Liao JX, Yeh HM, Mok HK (2015) Meiofaunal communities in a tropical seagrass bed and adjacent unvegetated sediments with note on sufficient sample size for determining local diversity indices. Zool Stud 54: 14

Liao JX, Yeh HM, Mok HK (2016) Do the abundance, diversity, and community structure of sediment meiofauna differ among seagrass species? Marine Biological Association of the United Kingdom. J Mar Biol Assoc UK 96, 3: 725

Long SM, Abdullah AAFA, Ab Rahim SA (2014) Marine gastropod and bivalves of Sampadi Island, Lundu, Sarawak. In Monograph Aquatic Science Colloquium pp. 75-87

Losi V, Montefalcone M, Moreno M, Giovannetti E, Gaozza L, Grondona M, Albertelli G (2012) Nematodes as indicators of environmental quality in seagrass (*Posidonia oceanica*) meadows of the NW Mediterranean Sea. Adv Oceanogr Limnol 3: 69–91

Losi V, Sbrocca C, Gatti G, Semprucci F, Rocchi M, Bianchi CN, Balsamo M (2018) Sessile macrobenthos (Ochrophyta) drives seasonal change of meiofaunal community structure on temperate rocky reefs. Mar Environ Res 142: 295-305

Marba N, Holmer M, Gacia E, Barron C (2006). Seagrass beds and coastal biogeochemistry. In Larkum A.W.D, Orth R.J. and Duarte C.M. (eds) Seagrasses: biology, ecology and conservation. Dordrecht: Springer, pp. 135–157.

Mascarat T, Lepoint G, De Troch M (2013) Meiofauna and harpacticoid copepods in different habitats of a Mediterranean seagrass meadow. J Mar Biol Assoc UK 93: 1557–1566

Mateo MA, Cebrian J, Dunton KH, Mutchler T (2006) Carbon flux in seagrass ecosystems. In Larkum A.W.D, Orth R.J. and Duarte C.M. (eds) Seagrasses: biology, ecology and conservation. Dordrecht: Springer, pp. 159–192

McArdle BH, Anderson MJ (2001) Fitting multivariate models to community data: a comment on distance-based redundancy analysis. Ecol 82: 290–297

McLachlan A, Winter PED, Botha L (1977) Vertical and horizontal distribution of sublittoral meiofauna in Algoa Bay, South Africa. Mar Biol 40: 355-364

Min WG, Kim DS, Kwon MS (2006) Meiobenthic Community Structures on the Sediment of Seagrass Bed and Mangrove Forest in Chunk Lagoon, Micronesia. Ocean and Polar Res 28, 1: 13-23

Ndaro SGM, Ólafsson E (1999) Soft-bottom fauna with emphasis on nematode assemblage structure in a tropical intertidal lagoon in Zanzibar, eastern Africa: I. spatial variability. Hydrobiologia 405: 133–148

Nelson DR, Guidetti R, Rebecchi L (2015) Phylum Tardigrada. In: Thorp, J., Rogers, D.C. (Eds.), Ecology and General Biology: Thorp and Covich's Freshwater Invertebrates, Academic Press: 347–380

Orth RJ, Heck Jr KL, van Montfrans J (1984) Faunal communities in seagrass beds: a review of the influence of plant structure and prey characteristics on predator-prey relationships. Estuaries 7: 339-350

Picone S., Alvisi F., Dinelli E., Morigi C., Negri A., Ravaioli M., Vaccaro C. 2008. New insights on late Quaternary palaeogeographic setting in the Northern Adriatic Sea (Italy). J Quat Sci 23 5: 489-501

Pielou EC (1969) An Introduction to Mathematical Ecology. Wiley-Interscience, New York

Pusceddu A, Bianchelli S, Gambi C, Danovaro R (2011) Assessment of benthic trophic status of marine coastal ecosystems: significance of meiofaunal rare taxa. Estuar Coast Shelf Sci 93: 420-430

Pusceddu A, Gambi C, Corinaldesi C, Scopa M, Danovaro R (2014) Relationships between meiofaunal biodiversity and prokaryotic heterotrophic production in different tropical Habitats and oceanic regions. PLoS ONE 9, 3

Ravaglioli C, Lardicci C, Pusceddu A, Arpe E, Bianchelli S, Buschi E, Bulleri F (2020) Ocean acidification alters meiobenthic assemblage composition and organic matter degradation rates in seagrass sediments. Limnol Oceanogr 65, 1: 37-50

Runtukahu F, Pinca S, Scaps P (2007) Acropora community composition at Siladen Island, North Sulawesi, Indonesia. Galaxea, JCRS 9: 23-33

Sahraeian N., Sahafi H.H., Mosallanejad H., Ingels J., Semprucci F. (2020) Temporal and spatial variability of free-living nematodes in a beach system characterized by domestic and industrial impacts (Bandar Abbas, Persian Gulf, Iran). Ecol Ind, 118: 106697.

Sbrocca C., De Troch M., Losi V., Grassi E., Balsamo M., Semprucci F (2021) Habitat-diversity relations between sessile macrobenthos and benthic copepods in the rocky shores of a Marine Protected Area. Water, 13: 1020.

Semprucci F, Colantoni P, Baldelli G, Rocchi M, Balsamo M (2010) The distribution of meiofauna on back-reef sandy platforms in the Maldives (Indian Ocean). Mar Ecol – Evol Persp 31: 592-607

Semprucci F, Colantoni P, Sbrocca C, Baldelli G, Rocchi M, Balsamo M (2011) Meiofauna in sandy back-reef platforms differently exposed to the monsoons in the Maldives (Indian Ocean). J Mar Syst 87: 208–215

Semprucci F., Frontalini F., Sbrocca C., Armynot du Châtelet E., Bout-Roumazeilles V., Coccioni R., Balsamo M. 2015. Meiobenthos and free-living nematodes as tools for biomonitoring environments affected by riverine impact. Environ Monit Asses. 187:251.

Semprucci F, Facca C, Ferrigno F, Balsamo M, Sfriso A, Sandulli R (2019) Biotic and abiotic factors affecting seasonal and spatial distribution of meiofauna and macrophytobenthos in transitional coastal waters. Estuar Coast Shelf Sci 219: 328–340

Shannon CE, Weaver W (1949) The mathematical theory of communication. The University of Illinois. Urbana, Chicago, London pp. 3–24

Snelgrove PVR, Butman CA (1994) Animal sediment relationships revisited: cause versus effect. Oceanogr Mar Biol Annu Rev 32: 111-177

Somerfield PJ, Yodnarasri S, Aryuthaka C (2002) Relationships between seagrass biodiversity and infaunal communities: implications for studies of biodiversity effects. Mar Ecol Prog Ser 237: 97–109

Todaro MA, Leasi F, Bizzarri N, Tongiorgi P (2006) Meiofauna densities and gastrotrich community composition in a Mediterranean sea cave. Mar Biol 149: 1079-1091

van der Heide T, Eklöf JS, van Nes EH, van der Zee EM, Donadi S, Weerman EJ, Olff H, Eriksson BK, (2012). Ecosystem Engineering by Seagrasses Interacts with Grazing to Shape an Intertidal Landscape. PLoS ONE 7, 8

van Katwijk, MM, Bos AR, Hermus DCR, Suykerbuyk W (2010) Sediment modification by seagrass beds: muddification and sandification induced by plant cover and environmental conditions. Estuar Coast Shelf Sci 89: 175-181

Vasapollo C (2005) Studio preliminare della distribuzione e zonazione delle praterie di fanerogame dell’isola di Siladen (Nord Sulawesi, Indonesia). Master Thesis p: 48

Vasapollo C (2010) Spatio-temporal variability of plant features and motile invertebrates in Posidonia oceanica seagrass meadows. Ph.D. Thesis. The Open University, Mylton Kyenes, UK

Vasapollo C, Gambi MC (2012) Spatio-temporal variability in *Posidonia oceanica* seagrass meadows of the Western Mediterranean: shoot density and plant features. Aquat Biol. 16: 163–175

Vasapollo C, Villano L, Gambi MC (2015) Spatio-temporal variability of borer polychaetes in *Posidonia oceanica* beds and its relation to meadow structure. Mediterr Mar Sci. 16: 136–146

Zaleha KMFD, Amirudin A (2009) Benthic community of the Sungai Pulai seagrass bed, Malaysia. MJS 28, 2: 143-159

Table 1. Presence and absence (+/-) of seagrass species at each sampling site and some key features of each seagrass species. B30, B50 = Barat 30 and Barat 50; L30, L50 = Lagoon 30 and Lagoon 50.

Seagrass species	B30	B50	L30	L50	Seagrass features / habitat
Enhalus acoroides	+	+	-	-	Long roots, usually presents in both highly bioturbated environments subjected to high wave action or sheltered environments.
Halophila ovalis	-	-	+	+	**Pioneer species,** presents in bioturbated sediments, proxy of unstable environment, it colonizes bare sediment subjected to regular stress including emersion and physical effects of waves.
Cymodocea rotundata	+	+	+	+	It colonizes different sediment types, it stabilizes the sediment, characteristic of stable community.
Cymodocea serrulata	+	+	-	-	It colonizes mainly sheltered and calm environments, medium grain size sediments.
Halodule pinifolia	-	-	+	+	**Pioneer species,** proxy of unstable environment, it colonizes bare sediment subjected to regular stress including emersion and physical effects of waves.
Syringodium isoetifolium	-	+	+	+	**Pioneer species,** proxy of unstable environment, it colonizes bare sediment subjected to regular stress including emersion and physical effects of waves.
Thalassia hemprichii	+	-	+	+	Long and strong roots; it colonizes coarse sediments with coral rubbles, presents in both highly energy and sheltered environments .

Table 2. Results of the PERMANOVA test carried out to ascertain differences in grain size, meiobenthic assemblage (i.e. total abundance, taxa richness, Shannon H' and Pielou J indices, whole meiobenthic structure and rarer taxa structure) between sites (Si), stations (St) and sediment layers (La). Reported are df= degree of freedom; MS= mean square; F=F statistic; P= probability level. In italic and bold significant P values.

	Source	df	SS	MS	F	P		Source	df	SS	MS	F	P
Grain size	Site	1	54.441	54.441	15.406	***0.001***	**Shannon (H')**	Site	1	1.8292	1.8292	0.266	0.640
	St(Si)	2	40.855	20.428	5.781	*0.005*		St(Si)	2	43.648	21.824	3.172	0.093
	De(St(Si))	8	23.299	2.9123	0.824	0.686		De(St(Si))	8	141.43	17.678	2.570	0.076
	Res	12	42.405	3.5337				Res	11	75.672	6.8793		
	Total	23	161					Total	23	267.11			
pair-wise test	B30 vs B50					***0,010***							
Total meiofauna abundance	Site	1	1128.1	1128.1	5.630	***0.038***	**Evenness (J)**	Site	1	17.1	17.1	1.453	0.219
	St(Si)	2	739.41	369.7	1.845	0.174		St(Si)	2	15.771	7.8856	0.670	0.549
	De(St(Si))	8	2967.4	370.92	1.851	0.120		De(St(Si))	8	79.917	9.9896	0.849	0.592
	Res	12	2404.7	200.39				Res	11	129.43	11.767		
	Total	23	7239.6					Total	23	230.72			
Taxa richness	Site	1	329.73	329.73	2.086	0.175	**Meiofauna composition**	Site	1	446.68	446.68	1.325	0.269
	St(Si)	2	1829.1	914.53	5.786	***0.017***		St(Si)	2	1400.6	700.32	2.077	0.072
	De(St(Si))	8	2491.7	311.46	1.970	0.145		De(St(Si))	8	6089	761.13	2.257	***0.007***
	Res	10	1580.7	158.07				Res	10	3372.1	337.21		
	Total	23	5763.3					Total	23	11433			
pair-wise test	L30 vs L50					***0,014***	*pair-wise test*	0-5 vs 5-10 cm					***0.038***
							Barat 50	0-5 vs 10-15 cm					***0.031***
Meiofauna rare taxa	Site	1	1938.8	1938.8	1.606	***0.045***							
	St(Si)	2	6492.6	3246.3	2.015	***0.043***							
	De(St(Si))	8	28694	3586.8	2.121	***0.031***							
	Res	12	38391	3199.3									
	Total	23	75517										
pair-wise test	L30 vs L50					***0.028***							

Barat 50	0-5 vs 5-10 cm	***0.035***
	0-5 vs 10-15 cm	***0.023***
	5-10 vs 10-15 cm	***0.027***
Lagoon 50	0-5 vs 5-10 cm	***0.041***
	0-5 vs 10-15 cm	***0.038***
	5-10 vs 10-15 cm	***0.031***

Table 3. Results of DistLM-forward carried out to assess the role of different environmental variables on total meiobenthic abundance, taxon richness, structure of the whole meiobenthic assemblage and "Other" taxa, Shannon-diversity (H') and Pielou-evenness (J) indices. Results of the sequential tests. SS=mean square; F=F statistic. In italic and bold significant p values.

	Variable	SS	F	*p*	Prop %	Cum.Prop %
Total meiofaunal abundance	very fine sands (%)	11.675	10.107	***0.004***	31.5	31.5
	coarse sands (%)	3.372	3.213	***0.010***	9.1	40.6
	fine sands (%)	1.812	1.791	***0.010***	4.9	45.5
Taxa richness	very fine sands (%)	321.31	8.5795	***0.006***	28.1	28.1
	gravel (%)	55.015	1.5025	***0.035***	4.8	32.9
	coarse sands (%)	46.718	1.2938	***0.043***	4.1	36.9
Whole assemblage structure	very fine sands (%)	3289.3	3.462	***0.007***	13.6	13.6
	very coarse sands (%)	1137.1	1.2081	***0.029***	4.7	18.3
	T. hemprichii	1106.8	1.1863	***0.031***	4.6	22.9
Structure of "Others"	*T. hemprichii*	5175.7	1.307	***0.020***	5.6	5.6
	very coarse sands (%)	6404.2	1.6661	***0.037***	6.9	12.5
	gravel (%)	5559.2	1.4793	***0.041***	6.0	18.6
H'	all			ns		
J	all			ns		

Figures and figure captions

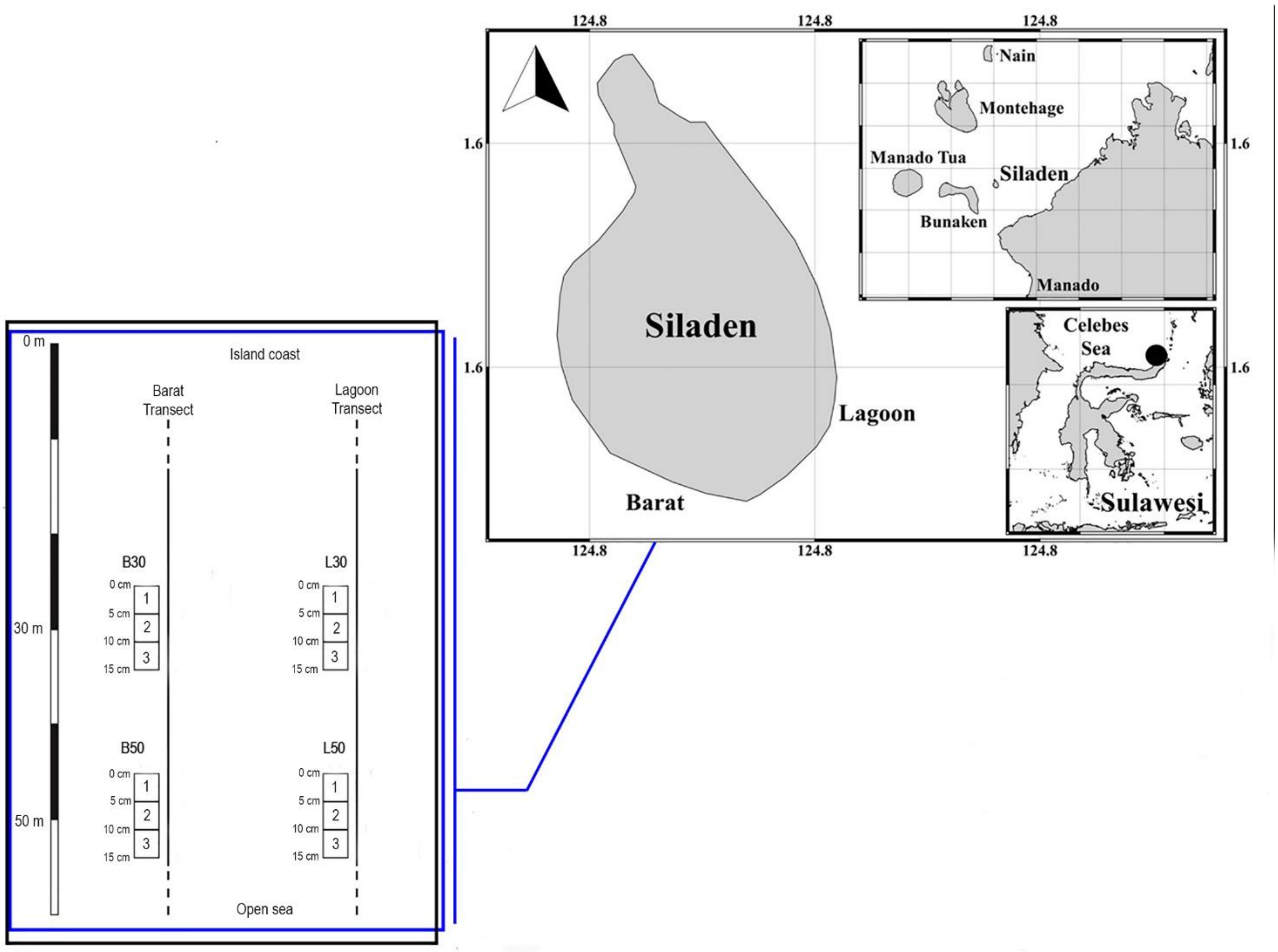

Fig. 1 Study area and location of the two investigated seagrass meadows: Barat (B) and Lagoon (L) (right side), and position of the stations within each transect (left side).

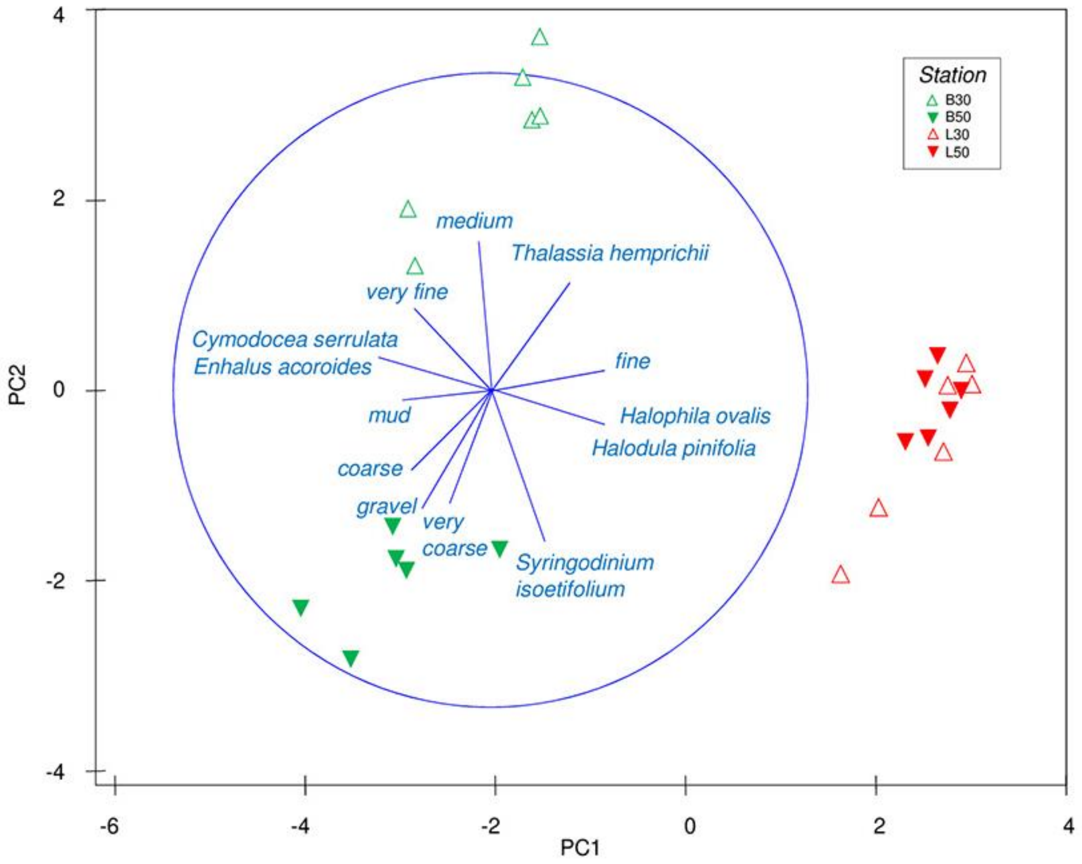

Fig. 2 PCA outputs of the environmental data at all sites and stations. Ordination of sampling stations using the first and second Principal Component. Very fine, fine, medium, coarse and very coarse labels in the graph are referred to the different sandy fractions.

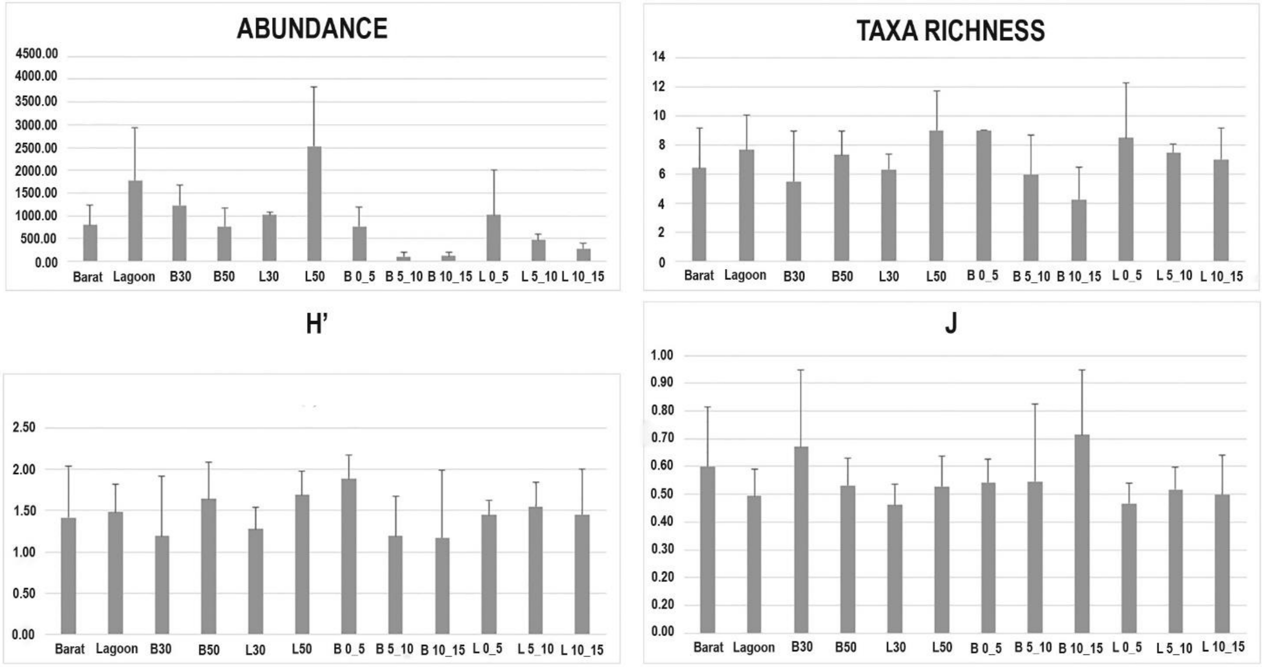

Fig. 3 Values of the meiobenthic abundance, richness of taxa, Shannon-diversity (H'), Pielou-evenness (J) in the sampling sites, stations and sediment layers.

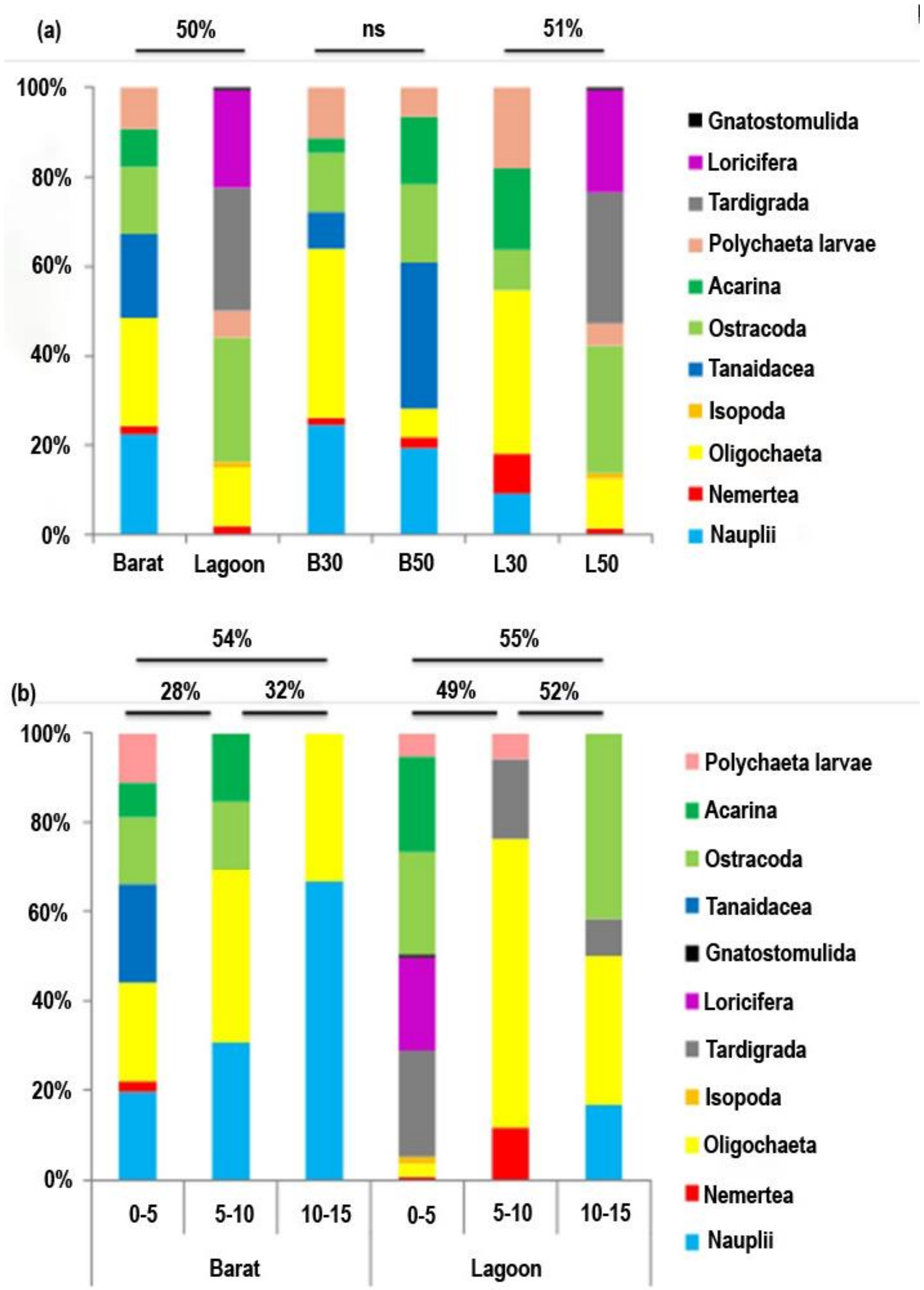

Fig. 4 Assemblage structure of the meiobenthic taxa belonging to the category Others (i.e. rare taxa): (a) at all investigated stations and (b) at all sediment layers (cm). Above each column, the percentage of dissimilarity between sites, stations and sediment layers is also reported.

Appendix A. Supplementary data available at:https://doi.org/10.1016/j.rsma.2021.101824

CHAPTER 3

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Antarctic Special Protected Area 161 as a Reference to Assess the Effects of Anthropogenic and Natural Impacts on Meiobenthic Assemblages

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Abstract: The Antarctic region is usually considered a pristine area. Nevertheless, regional warming effects and increasing human activities, including the presence of several research stations, are inducing considerable environmental changes that may affect the ecosystem's functions. Therefore, during the XXXIII Antarctic expedition, we carried out an investigation in Terra Nova bay (Ross Sea), close to the Antarctic Specially Protected Area (ASPA) n.161. In particular, we compared the effects of two different types of impacts on the meiobenthic assemblages: anthropogenic impact (AI), associated with the activity of Mario Zucchelli Research Station (MZS), and natural impact (NI) attributable to a large colony of Adélie penguins (*Pygoscelis adeliae)* in Adelie Cove. For each impacted site, a respective control site and two sampling depths (20 and 50 m) were selected. Several environmental variables (pH, dissolved oxygen, major and minor ions, heavy metals, organic load, and sediment grain size) were measured and analysed, to allow a comprehensive characterization of the sampling areas. According to the criteria defined by Unites States Environmental Protection Agency (US EPA 2009), heavy metal concentrations did not reveal critical conditions. However, both the MZS (AI20) and penguin colony (NI20) sites showed higher heavy metal concentrations, the former due to human activities related to the Italian research station, with the latter caused by the penguins excrements. Meiobenthic richness and abundance values suggested that the worst ecological condition was consistently related to the Adélie penguins colony. Furthermore, the higher contribution of r-strategists corroborates the hypothesis that the chronic impact of the penguin colonies may have stronger effects on the meiobenthos than the human activities at the MZS. Food is not limited in shallow Antarctic bottoms, and microscale differences in primary and secondary production processes can likely explain the greater spatial heterogeneity, highlighted both by the univariate and multivariate attributes of meiobenthic assemblage (i.e., richness, diversity, abundance, whole structure assemblage, and rare taxa) at the deeper stations. As reported in other geographical regions, the assemblage structure of rare meiobenthic taxa is confirmed to be more susceptible to environmental variations, rather than the whole assemblage structure.

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1. Introduction

Despite the fact that polar regions might be particularly sensitive to anthropogenic impacts (e.g., climate changes), Antarctic coasts are still amongst the least studied on Earth, due to their remoteness and harsh regional climatic conditions [1]. Available data from Antarctica show a high marine faunal biodiversity, even though this knowledge is mainly limited to mega- and macrofauna, while the meiobenthic component (body size: 45–500 µm) is overlooked or partly studied in Antarctic Peninsula (East Antarctica) (e.g., [2,3]). However, recent metabarcoding data reveal high levels of meiobenthic diversity within the same magnitude, as in temperate regions, confirming that meiobenthos plays a significant role in the global biogeochemical cycles of inorganic and organic compounds [3,4]. Many studies support the idea that meiobenthos is essential for understanding the functioning and resilience of marine ecosystems (e.g., [5]). Furthermore, they are more abundant, compared to the macrobenthic taxa, and require only a limited sediment volume to obtain representative samples [6]. Because of their widespread distribution, high turnover rate, life cycles spent entirely in the sediment, high biodiversity, and specific ecological requirements, meiobenthos responds more precociously to several types of environmental changes (e.g., [7–12]). Hence, they are considered effective bio-indicators of natural and anthropogenic disturbances (e.g., [13–15]). Nematodes and copepods generally constitute more than 80% of the total meiobenthic abundance, whereas many other taxa often represent less than 1% each. However, these latter, defined as 'rare' by Bianchelli and co-authors [16], may be useful to disentangle the differences in environmental conditions, thus providing a clearer figure of the ecosystem status, compared to the whole meiobenthic assemblage [17].

Although heavy metals can be introduced into the environment by natural causes, the major input derives from anthropogenic origin [6]. One of the main problems associated with these contaminants is their persistence; unlike organic pollutants, they do not decay but show high bioaccumulation and biomagnification rates. Over the last four decades, many field studies and laboratory experiments have documented significant changes in meiobenthic structure and diversity after exposure to heavy metals (e.g., [6,18]). The chemical form, as well as the type of trace element, is generally important in determining the toxicity effects on meiobenthic organisms (see [19] for review). However, laboratory studies have shown that the effect of trace elements depends not only on the nature of the element, but also on some environmental conditions, such as temperature, salinity, and trophic availability [19].

Since meiobenthic organisms have a crucial role in detritus decomposition, nutrient cycling, and energy flow, their change in composition and structure is assumed to be a good proxy for detecting biodeposition effects (e.g., [20–23]). The primary anthropogenic sources of organic enrichment in marine ecosystems are related to sewage discharges and aquaculture activities (see [6] and reference therein), but there are also possible natural origins of organic enrichment. Penguins constitute an important bird biomass in the Southern Hemisphere, where they breed in colonies on different sites, from hundreds to thousands of individuals [24]. In the Ross Sea region (Antarctica), large colonies of Adélie penguins (*Pygoscelis adeliae*) have been forming extensive deposits of ornithogenic sediments [25], and the biochemical characterization of the sediments revealed organic matter concentrations higher than those reported for highly productive areas [26–29]. Chemical contamination, especially through fuel spills and exhaust gases, is the most widespread environmental impact of human activities in Antarctica, especially near the land stations [30]. In particular, human presence has determined a marked increase in products discharged at sea, such as fuels, sewage waste, and the 'grey water' originating from toilets, laundry facilities, accommodation, and cooking areas [31–34]. Thus, products such as heavy metals/metalloids (typically copper, lead, zinc, cadmium, mercury, and arsenic), hydrocarbons, and desalination plant brine [35] can strongly affect Antarctica benthic assemblages, inducing possible higher abundance of resilient taxa, such as polychaetes and gastropods, when compared to uncontaminated assemblages [36]. In addition to human activities, some products, mostly heavy metals, may derive from penguin faeces; for example, [37] observed the presence of cadmium in Edmonson Point (Terranova Bay), due to the Adélie penguins' guano. Similar results were found by [38], revealing cadmium, copper, and zinc in penguins' faeces.

Among the main types of human activities in Antarctica, research has demonstrated that the limited sewage treatment of the research stations represents an impact on marine ecosystems [33,36,39,40]. In the early 1990s, the Madrid Protocol required that national programs prevent or mitigate the adverse impacts of human activity on the environment and natural resources of the Antarctic continent, but disturbances and impacts still occur [41–44].

This study investigates the response of the meiobenthic assemblages to different sources and magnitudes of disturbance. To achieve this objective, during the XXXIII Antarctic expedition, environmental parameters (i.e., pH, dissolved oxygen, major and minor ions, and heavy metals), organic load, sediment grain size, and meiobenthos were investigated close to the Mario Zucchelli Station (MZS, Terra Nova Bay, Ross Sea) and along the coast of the proximate Antarctic Specially Protected Area (ASPA) n.161. Based on previous knowledge [29], two main sampling locations (and their respective controls) were selected: the Mario Zucchelli Station (MZS, hereafter named as anthropogenic impact site, AI) and Adelie Cove (natural impacted site, NI). Previous studies highlighted that research stations and penguin colonies are characterized by an organic and chemical enrichment in sediments, due to the impact of the untreated, domestic wastewaters, related to the summer research activities, and by the penguin excreta accumulation, respectively [30,32,35,38,45,46]. The main questions were: (1) do anthropogenic and natural impact sources show different effects on the meiobenthic assemblages? (2) Can rare taxa add more information in the detection of the distinct environmental conditions of the sampling sites than those supplied by the whole meiobenthic assemblage?

2. Material and Methods

2.1. Study Area and Sampling Sites

MZS is located at 74°42′ S, 164°07′ E in the Terra Nova Bay area (Ross Sea) and accommodates about 120 people; although, during the summer, more than 250 people live in the base for short and long periods (Figure 1). Station facilities include an electrical generator; desalination plant, for drinkable water from sea water; incinerator; and a sewage primary treatment plant. The base is supplied with food and other material by ships from New Zealand. All waste is subjected to differentiated collection, locally treated, or brought back to Italy for recycling or disposal.

Adelie Cove, a small 70-m deep V-shaped bay, located at 74°46′ S and 164°1′ E in Terra Nova Bay (Ross Sea), hosts an Adelie penguin colony, composed of hundreds of individuals (Figure 1). It is separated from the open sea by a 12- to 15-m deep sill, which forms a barrier to inflow and outflow. This area is strongly affected by katabatic wind events, blowing down a glacial valley towards the open sea [47].

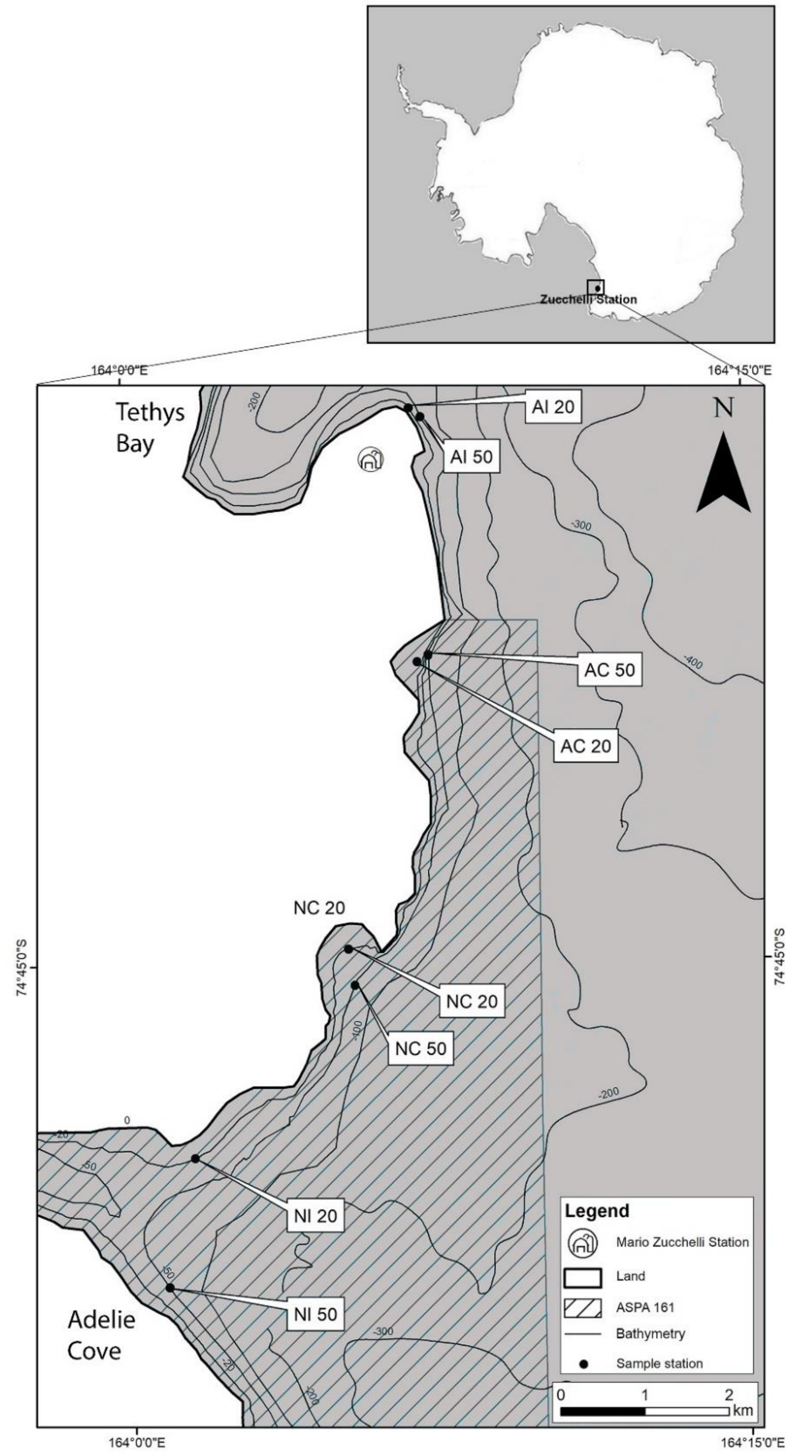

Figure 1. Study area in the Terra Nova Bay area (Ross Sea, Antarctica). Sediments were collected in four sites: anthropically impacted site (AI) and its control (AC); naturally impacted site (NI) and its control (NC). Two stations were also selected in each area, at 20 and 50 m in depth. The limits of the Antarctic Specially Protected Area (ASPA) were also reported on the map.

During the Antarctic summer season, 2017/2018, four sites were selected to assess the effects of anthropogenic and natural impacts on meiobenthic assemblages. In particular, the MZS area was considered as the anthropogenic impact source (AI), while a *P. adeliae* colony, located in Adelie Cove area, was considered as the natural impact source (NI). Two control sites, named the anthropogenic control (AC) and natural control (NC), were also chosen at about 2 Km from the AI and NI, respectively. At each area, sediments were collected from two stations, located 20 and 50 m in depth (Figure 1), by means of a modified Van Veen grab (total volume 12 l). This modified grab model permits the insertion of a Plexiglass corer into the central part of the grab, allowing for the collection of undisturbed sediments. Three independent deployments of the grab were utilized to collect three sediment replicates. Thus, one subsample for total organic content (TOC), one for grain-size, and one for meiobenthos analyses were collected for each grab sample, by means of corers (inner diameter 2.7 cm), down to a sediment depth of 5 cm. The sediment was fixed with 4% neutralized formalin in seawater and stained with Rose Bengal. Interstitial water was also collected by 20 mL syringe and immediately frozen for the further physico-chemical analyses.

2.2. Environmental Parameters Analyses

Analysis of major and minor ions, heavy metals, and chemical-physical parameters for interstitial waters was carried out at the Laboratory of Chemistry at Parthenope University of Naples.

Samples were filtered with cellulose filters (porosity: 0.20 µm); for each filtered sample, pH, salinity, dissolved oxygen (O2), and conductivity values were measured using a pHenomenal MU 6100 L (VWR) multimeter. Samples were then treated with H_2O_2 (100 µL in 10 mL of sample) for the digestion of organic content; then, samples were fractioned in two aliquots for ions and metals determination.

For the analysis of major and minor ions concentration, interstitial water samples were analyzed using a Dionex ICS1100 system, equipped with an ASRS 300-4 mm suppressor (applied current of 33 mA and an AS22 column working with a buffer solution 3.5 mM of sodium carbonate/bicarbonate, at a flow rate of 1.20 mL/min) for anions detection. This system allows for the determination of Cl^-, F^-, Br^-, $NO_2{}^-$, $NO_3{}^-$, $PO_4{}^{3-}$, and $SO_4{}^{2-}$, as the inorganic species, and $HCOO^-$, CH_3COO^-, and $C_2O_4{}^{-2}$ as the organic species.

In addition, the system was equipped with a CERS 500-4 mm suppressor, with an applied current of 15 mA and CS12A column (working with 20 mM methanesulfonic acid solution as eluent, at a flow rate of 0.25 mL/min) for cations determination (Li^+, Na^+, K^+, $NH4^+$, Ca^{2+}, and Mg^{2+}). For both anions and cations, calibration curves were defined using certified multistandard solutions.

For metals determination, voltammetric analyses were carried out with a Metrohm 797 VA Computrace and multimode working Mercury electrode. An Ag/AgCl electrode was used as reference and a Pt electrode as auxiliary electrode. For calibration, the standard addition method was applied to limit the matrix effects. All elements were quantified using linear regression, based on the height of voltammogram peaks. Anodic stripping voltammetry (ASV), with the hanging mercury drop electrode (HMDE), was used to determine all those metals that are soluble in mercury; this method allows the determination of zinc, cadmium, lead, and copper.

In order to consider the effects of soluble species, coming from ultrapure water, LODs (limit of detections) were calculated for each species [48].

For the grain size analysis, sediment was sieved over a series of sieves, with mesh sizes ranging from 1 mm to 0.25 mm, considering three main sediment fractions: coarse sand (sediment fraction ≥ 1 mm), sand (<1 mm and ≥0.25 mm), and fine sand (<0.25 mm) [49]. Fractions were dried in oven at 60 °C for 48 h and weighed; data were expressed as percentages of the total sediment dry weight, differencing it in the three size classes. Total organic carbon (TOC) was determined, according to [50], and expressed as a percentage in the sediment. In detail, a known weight of sample was placed in a ceramic crucible (or similar vessel), which was then heated to between 350 and 500 °C overnight. The sample was then cooled in a desiccator and weighed. Organic matter content was calculated as the difference between the initial and final sample weights divided by the initial sample weight times 100%. All weights are dried and put to 60 °C for 48 h, prior to organic matter combustion.

2.3. Meiobenthic Assemblage Analysis

In the laboratory, all meiobenthic samples were carefully washed through two nested mesh sieves (500 and 45 µm) (e.g., [51]). The first was used to exclude macrofaunal organisms, while the second was used to retain meiobenthos. The residual fraction obtained was centrifuged three times (10 min at 3000 rpm) with Ludox HS 30 colloidal silica (density 1.18 g cm^{-3}) for specimen extraction purposes [52]. The organisms retained on the 45 µm sieve were then transferred into a 'Delfuss' Petri dish with a checkered bottom and, with the aid of a Leica G26 stereomicroscope, sorted into major meiobenthic groups and counted. The density of all the individuals found (both temporary and permanent meiobenthos) was standardized as abundance in 10 cm^{-2}. The meiobenthic groups that were found in low densities (less than 1% of the total abundance in all investigated samples) were defined as rare taxa and named as reported in the 'Others' category [16].

In order to describe the meiobenthic assemblage structure, synecological indices were used. In particular, the number of meiobenthic taxa, the number of individuals in 10 cm^{-2} (A), diversity index, calculated by Shannon index (H'), and evenness, calculated according to Pielou index (J) (both using $\log_2$ data), were calculated for each station, at the major group level. Ecological quality (EcoQ) status was assessed using meiofaunal richness, according to Danovaro et al. (2004 and modified in agreement with the WFD classification): bad = $\leq$4 taxa, poor = 4–7 taxa, moderate = 8–11 taxa, good environmental quality = 12–16, and high environmental quality = $\geq$16 taxa.

2.4. Statistical Analyses

The obtained environmental and biological data set was used to assess possible significant differences between the different impacted areas. As applied in previous studies (e.g., [53–55]), the ACI (after-control/impact) experimental design was chosen selecting two factors: area factor (Ar, fixed and orthogonal with 4 levels: anthropically impacted area = AI, the relative anthropogenic control area = AC, naturally impacted area = NI, and the relative natural control area = NC) and depth factor (De, fixed and orthogonal with 2 levels: 20 and 50 m), with $n = 3$.

All statistical analyses were performed with PRIMER-E 6 + PERMANOVA [56].

Environmental data were normalized, and a permutational multivariate analysis of variance (PERMANOVA, [57]), based on Euclidean distance, was performed, in order to assess differences in data composition imputable to the anthropogenic or natural impacts. Each term in the analysis was tested by 4999 random permutation [58], and a post-hoc pair-wise comparison, using PERMANOVA t-statistic, was also conducted, if necessary. The p values for pairwise comparisons were obtained from Monte Carlo asymptotic distributions, because of the restricted number of unique permutations. Multivariate patters were visualized through principal component analyses (PCA) ordination plot.

To test for spatial differences in the total meiobenthic assemblage and rare taxa, data matrix based on the faunal abundances were constructed by applying the Bray–Curtis similarity (biological data were fourth-root transformed). Each term in the analysis was tested by 4999 random permutations, and a post-hoc pair-wise comparison, using PERMANOVA t-statistic, was also conducted, if necessary. The p values for pairwise comparisons were obtained from Monte Carlo asymptotic distributions, because of the restricted number of unique permutations. Multivariate patters were visualized through canonical analysis of principal coordinates ordination plot (CAP, [59]), and taxa were correlated by Pearson index (ρ), if needed. Based on the results of PERMANOVA, SIMPER analyses [60] were employed to identify taxa that mainly affected dissimilarities: within total or rare assemblages, among area factor levels, and/or between depth factor levels. Univariate PERMANOVA analyses were performed on synecological indices, based on Euclidean distances [61], in order to test for differences among assemblage structures.

Environmental data were here related to meiobenthic assemblages through distance-based linear modelling (DistLM, [62]), using stepwise as the selection procedure and adjusted r^2 (hereafter Adj. r^2) as the selection criterion, in order to assess what variable affected biological patterns. Relations among assemblages and environmental variables, selected by distLM, were visualized through distance-based redundancy analysis ordination plot (dbRDA, [63]).

3. Results

3.1. Environmental Data

Values of zinc (Zn^{2+}), cadmium (Cd^{2+}), lead (Pb^{2+}), copper (Cu^{2+}), nitrate ion (NO_3^-), phosphate ion (PO_4^{3-}), sulphate ion (SO_4^{2-}), pH, O2, coarse sand, medium sand, fine sand, and TOC in the sampling stations are reported in the Table 1.

Table 1. Environmental conditions of each station. Data were expressed as nanomoles (nM), milligrams/liter (mg/L), or as percentage of total sample (%); nd. refers to no detectable concentration. Standard deviation (SD) of each variable is also shown.

Station	**Zn^{2+} (nM)**	**Cd^{2+} (nM)**	**Pb^{2+} (nM)**	**Cu^{2+} (nM)**	**pH**	**O2 (mg/L)**	**NO_3^- (mM)**	**PO_4^{3-} (mM)**	**SO_4^{2-} (mM)**	**Coarse Sand %**	**Sand %**	**Fine Sand %**	**TOC %**
AI20	41.994	7.894	17.556	50.177	8.33	5.7	0.494	nd.	23.002	63.618	35.426	0.956	1.732
±SD	8.344	1.150	0.741	2.083			0.013		0.045	0.983	0.144	0.398	0.008
AC20	13.735	1.031	12.700	14.838	8.70	6.2	nd.	nd.	18.362	26.844	73.142	0.014	4.043
±SD	3.181	0.581	1.091	1.629					0.036	0.923	0.689	0.006	0.008
NC20	21.939	nd.	7.269	11.024	8.85	5.7	nd.	nd.	20.411	7.800	89.740	2.460	1.263
±SD	2.686		4.184	2.457					0.064	0.674	0.965	0.893	0.001
NI20	45.453	2.033	14.802	13.976	8.86	5.9	nd.	1.864	24.183	3.814	50.015	46.172	2.348
±SD	8.680	0.598	1.913	1.176				0.012	0.052	0.380	0.501	0.138	0.006
AI50	11.482	2.639	5.698	9.727	8.55	6.2	nd.	nd.	24.770	53.867	45.075	1.057	2.800
±SD	3.643	1.395	1.540	0.825					0.031	0.251	0.255	0.041	0.009
AC50	7.423	2.106	nd.	1.938	8.70	6.2	nd.	0.569	23.907	17.025	76.701	6.275	5.711
±SD	2.681	0.807		0.710				0.014	0.048	0.876	0.019	0.818	0.010
NC50	6.942	0.706	nd.	0.642	8.85	5.7	0.313	0.438	24.465	3.341	62.875	33.784	1.153
±SD	0.873	0.331		0.115			0.017	0.009	0.056	0.595	0.636	0.740	0.007
NI50	11.722	nd.	nd.	1.484	8.71	6.2	0.205	nd.	24.342	32.872	61.760	5.369	3.867
±SD	4.413			0.176			0.014		0.043	0.965	0.595	0.099	0.007

The highest concentration of PO_4^{3-} was detected in the stations NI20, although it was also found in NC50 and AC50, with very low concentrations. NO_3^- was detected in AI20, NI50, and NC50. The pH and O2 concentrations did not show high spatial variability among stations, ranging between 8.85 and 8.33 for the former and between 6.2 and 5.7 mg/L the latter. Finally, SO_4^{2-} was detected in all the sampling stations with comparable concentrations, except for AC20, where it reached the lowest value.

Metal concentrations showed a high spatial variability (Figure 2). Zn^{2+} concentration ranged between 41.99 ± 8.344 nM in AI20 and 6.942 ± 0.873 nM in NC50; Cd^{2+} between 7.894 ± 1.15 nM in AI20 and nd (not detectable concentration) in NC20 and NI 50; Pb^{2+} between 17.556 ± 0.741 nM in AI20 and nd in NC50, NI50, AC50; finally, Cu^{2+} between 50.177 ± 2.083 nM in AI20 and 0.642 ± 0.115 nM in NC50.

The sum of the detected heavy metals showed the same spatial variability, from the north to the south stations. In particular, for the 20 and 50 m sampling stations, the highest values were reached in the stations AI and NI (117.621 nM and 76.264 nM at 20 m, as well as 29.546 nM and 13.206 nM at 50 m, respectively).

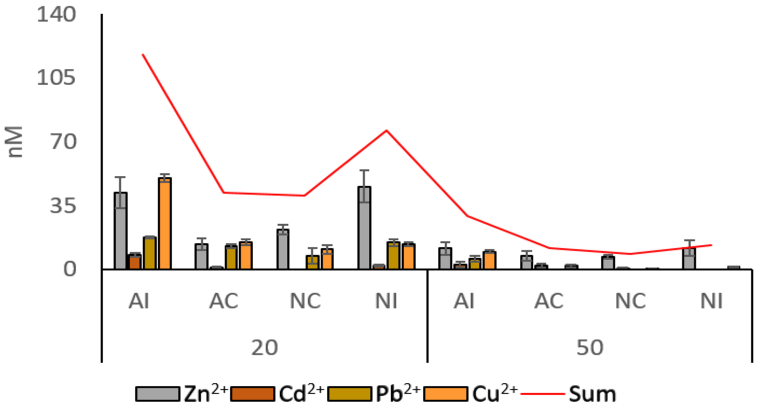

Figure 2. Heavy metals concentration (nM) among stations; "Sum" indicates the sum of all detected metals in each station.

As reported in the Figure 3, the highest percentages of coarse sand were detected both at AI20 (63.62%) and AI50 (53.87%), while sand was prevalent at AC20 and NC20 (73.14% and 89.74%, respectively) and AC50 and NC50 (76.70% and 62.87% respectively). Finally, the highest fine sand percentages were found in NI20 (46.17%) and NC50 (33.78%). TOC concentrations showed the highest values at AC20 (4.04%) and AC50 (5.71%) and the lowest at NC20 (1.26%) and NC50 (1.15%).

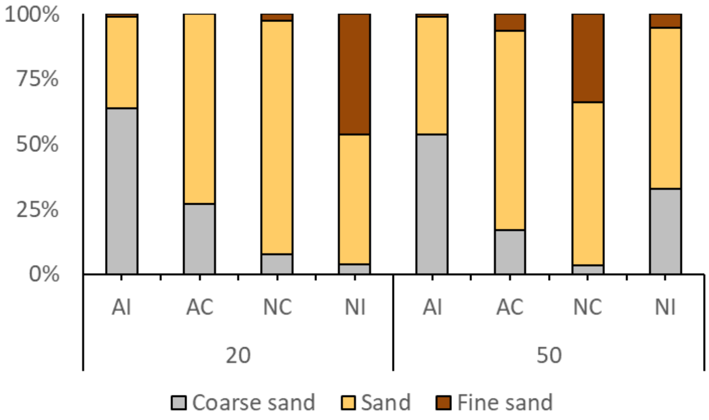

Figure 3. Cumulative percentages of three main gran size classes in the sampling stations.

PERMANOVA (Supplementary S1 Table S1) performed on environmental data showed that the interaction between areas and depths factors (Ar × De) determine significant differences (PERMANOVA, $p < 0.001$). In particular, t-statistics highlighted that, within the depth levels (i.e., 20 and 50 m), all sites showed significant differences (PERMANOVA, t-statistic $p < 0.001$) between each other. The same results were observed within the areas levels (AI, AC, NC, and NI), where environmental characteristics were strongly different for each pair of 20 and 50 m (PERMANOVA t-statistic, $p < 0.001$) samples.

The PERMANOVA results were consistent with PCA analyses (Figure 4), where the PC1 axis accounts for the 38.2% of total variance, with PC2 accounting for 25.4%. The PCA plot shows four different clusters made up of AI20 samples (Group 1), strongly correlated to coarse sand fraction and, to a lesser extent, heavy-metals and NO_3^-; NI20 samples (Group 2) mainly correlated to pH, fine sand, and PO_4^{3-}; NC20 and NC50 stations (Group 3) were associated with medium sand; and, finally, AI50, NI50 AC20, and AC50 samples (Group 4) correlated with O2 and TOC.

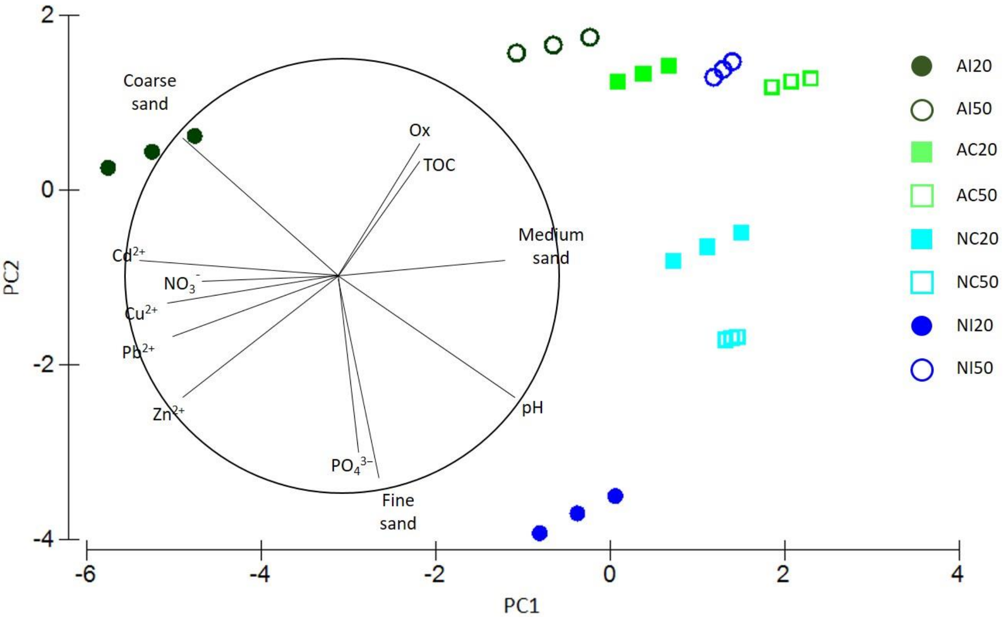

Figure 4. Ordination plot on environmental data and coming from PCA analysis. Replicates of Ar × De interaction are here shown. Strength lines are vectors that graphically show the correlation of physical and chemical correlation with different clusters.

3.2. Meiobenthic Assemblage Analysis

Meiobenthic richness (number of taxa) accounted for a total of 14 taxa, with values ranging between 6 taxa at NI20 and 11 taxa at AI50 and NC50. According to the classification proposed by Danovaro et al. [51], the sampling areas were characterized by a moderate ecological quality, with the exception of the natural impacted area that showed a poor ecological quality. Significant differences were detected among the levels of the factor area (PERMANOVA, $p = 0.0258$) and between the levels of the factor depth (PERMANOVA, $p = 0.0284$). In particular, t-statistics on the number of taxa account for differences only within the level 50 m between AI and AC (PERMANOVA t-statistic, $p = 0.018$) and AI and NI (PERMANOVA t-statistic, $p = 0.02$) (Supplementary S1 Table S2; Figure 5a).

The total abundance (A) of the meiobenthic assemblage ranged from 401 ± 347 ind./10 cm^2 at NC20 to 3753 ± 1006 ind./10 cm^2 at NC50, with generally higher values at the control stations and the lowest at NI (Supplementary S2 Table S8). Significant differences were detected only for the interaction Ar × De (PERMANOVA, $p = 0.004$), due to differences within the level 50 m. Indeed, the t-statistics highlight significant differences among all pairs of stations, except between AI and NI. In particular, significant differences were detected only within the 50 m level, between AI and AC (PERMANOVA t-statistic, $p = 0.004$), AI and NC (PERMANOVA t-statistic, $p = 0.035$), AI and NI (PERMANOVA t-statistic, $p = 0.0008$), AC and NC (PERMANOVA t-statistic, $p = 0.0054$), and NC and NI (PERMANOVA t-statistic, $p = 0.0068$) (Supplementary S1 Table S3; Figure 5b).

The dominant taxon was Nematoda (Figure 5c), with a mean density ranging from 72 ± 47 ind./10 cm^{-2} at AI50 to 3507 ± 925 ind./10 cm^2 at NC50 (on average, 53% of the total meiofauna ranging between the 4% and 93%). The next most abundant taxa were Copepoda (on average 22% of the total meiofauna from 3% to 62%), Ciliata (on average 16%, range: 0.5–69%), Gastrotricha (on average 7%, range: 0.1–32%), and Platyhelminthes (on average 1%, range: 1–4%). Nemertea, Bivalvia, Oligochaeta, Polychaeta, Ostracoda, Syncarida, Halacaridae, Insecta, and Chaetognata were all included in the rare taxa, i.e., taxa sporadically found in the study area and with less than 1% of abundance (see 'Others' category in Figure 5c).

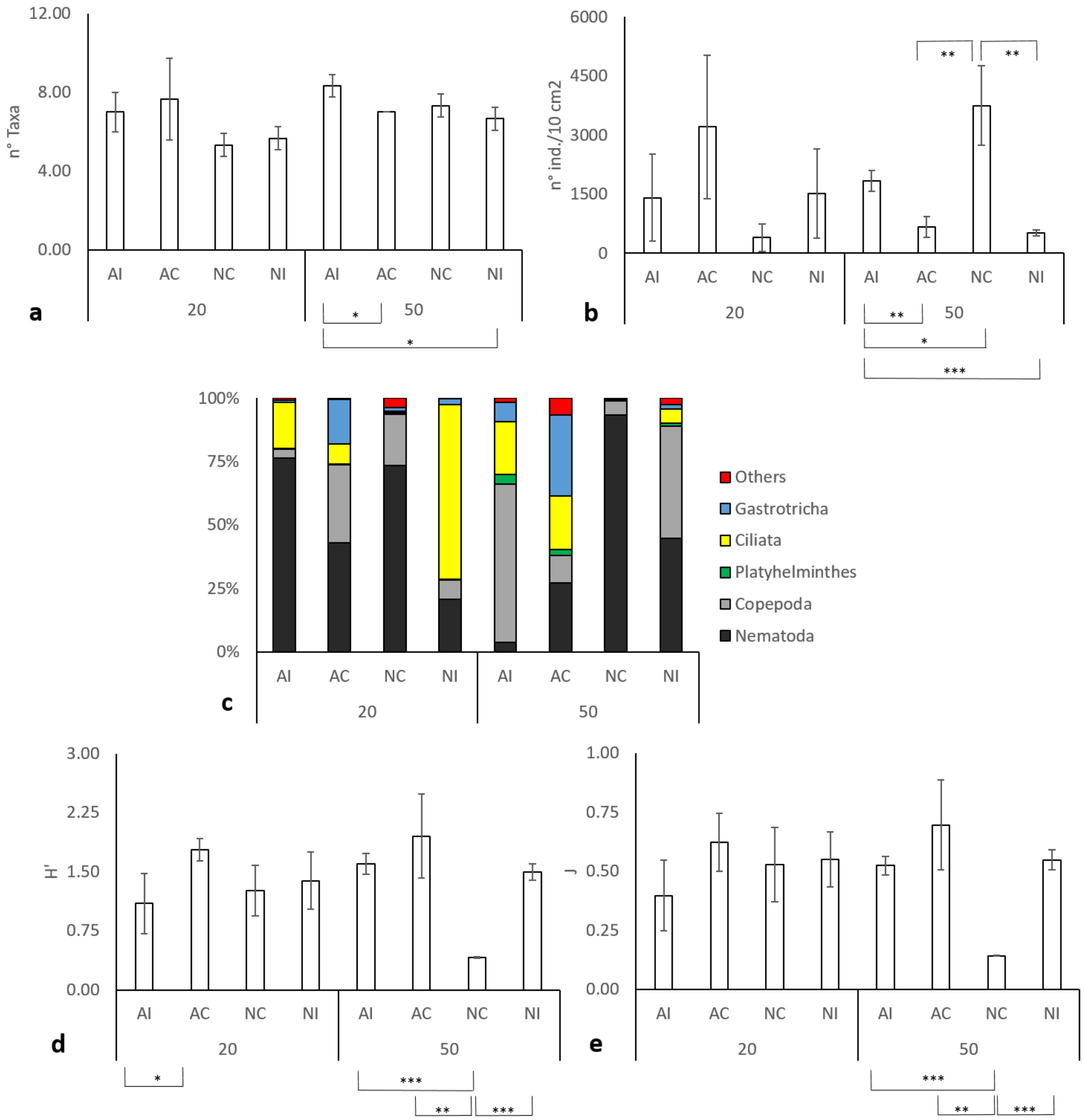

Figure 5. Trends of synecological indices and results of univariate analyses. (**a**) Number of meiobenthic taxa, (**b**) abundances, (**c**) percent composition of meiobenthic taxa (those taxa with less than 1% were grouped in the "Others" category), (**d**) diversity (H′) calculated as Shannon index, and (**e**) evenness (J) calculated as Pielou index. PERMANOVA significant differences among stations are also shown: * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

Both diversity (H′, Figure 5d) and evenness (J, Figure 5e) indices revealed the highest values at station AC50 (1.95 ± 0.53 and 0.70 ± 0.19, respectively), while the lowest values were recorded at station NC50 (0.41 ± 0.012 and 0.14 ± 0.001). Significant differences were detected for the interaction of Ar × De (PERMANOVA, $p_{H'} = 0.0076$ and $p_J = 0.007$) and for the single factor area (PERMANOVA, $p_{H'} = 0.0008$ and $p_J = 0.0032$). Within 20 m in depth, H′ showed significant differences only between AI and AC (PERMANOVA t-statistic, $p = 0.045$), while J did not show significant differences; within 50 m in depth, both H′ and J showed significant differences between the AI and NC (PERMANOVA t statistic, $p_{H'} = p_J = 0.0002$), AC and NC (PERMANOVA t-statistic, $p_{H'} = 0.007$, $p_J = 0.005$), and NC and NI comparisons (PERMANOVA t-statistic, $p_{H'} = 0.0002$, $p_J = 0.0004$) (Supplementary S1 Tables S4 and S5).

PERMANOVA analyses performed on assemblage structure (Supplementary S1 Table S6) showed that there are significant differences, both considering the single factors (PERMANOVA, areas: $p = 0.0002$; depths: $p = 0.0336$) and their interactions Ar × De (PERMANOVA, $p < 0.001$). In particular, statistical differences are detected, both among areas (PERMANOVA, $p = 0.0002$) and between depths (PERMANOVA, $p = 0.0336$). A list of taxa affecting for more than 70% of dissimilarities for each pair Ar × De interaction (SIMPER test) is reported in Supplementary S3 Table S9. In detail, the most frequent taxa here were: Gastrotricha, Nematoda, Ciliata, Copepoda, Platyhelminthes, Ostracoda, and Oligochaeta. The t-statistic highlighted that, within the 20 m level of the depth, there were significant differences between AC and NC and AC and NI (Table 2a). Within the 50 m level of the depth, all pair-wises showed significant differences with only one exception (Table 2b). Within levels of the factor area, significant differences between assemblages at 20 and 50 m depth were detected only at AC (PERMANOVA, t-statistic, $p = 0.044$) and NI (PERMANOVA, t-statistic, $p = 0.022$) stations.

Table 2. Pair-wise comparisons and t-statistics for differences in total meiobenthic assemblages among stations, within levels of the depth factor. Significant tests relevant to the hypothesis are given in bold. (**a**) Within level '20' of factor 'Depth'. (**b**) Within level '50' of factor 'Depth'.

	(a)			**(b)**	
Station Pair	**t**	***p***	**Station Pair**	**t**	***p***
AI vs. AC	1.4662	0.147	AI vs. AC	1.8175	0.0718
AI vs. NC	1.6671	0.105	AI vs. NC	3.989	**0.0032**
AI vs. NI	1.0871	0.356	AI vs. NI	4.0749	**0.0018**
AC vs. NC	2.5469	**0.0244**	AC vs. NC	3.1563	**0.012**
AC vs. NI	2.2636	**0.028**	AC vs. NI	2.4701	**0.0208**
NC vs. NI	1.9268	0.0544	NC vs. NI	2.7836	**0.0136**

In the CAP ordination plot on total assemblages (Figure 6; Supplementary S4 Figure S1), although clear clusters are not evident, it is possible to recognize that AI50 and NI20 are strongly correlated, and AI20 is weakly correlated with the Platyhelminthes and Ciliata taxa; AC20 with Copepoda and Gastrotricha; NC50 with Bivalvia and Nematoda; and NC20 was weakly correlated with Halacaridae. These stations formed four separated clusters, located at the plot edges.

PERMANOVA analyses, performed on the assemblages and composed by the only rare taxa, showed significant differences for both the factors (PERMANOVA, area $p = 0.0002$ and depth: $p = 0.0014$) but not for their interactions (Supplementary S1 Table S7). SIMPER analysis showed that Oligochaeta, Ostracoda, Polychaeta, Halacaridae, and Bivalvia are the taxa affecting more than 70% dissimilarities for each area pair-wise comparison (Supplementary S3 Table S10). The first four taxa were also those that contributed more than 70% to dissimilarity between the 20 and 50 m levels of the factor depth (Supplementary S3 Table S11). The t-statistics highlighted that no differences were detected among areas within the 20 m depth (Table 3a), while differences were detected among all pairs of station within the 50 m depths (Table 3b), except for the pairs AI and AC and AI and NC.

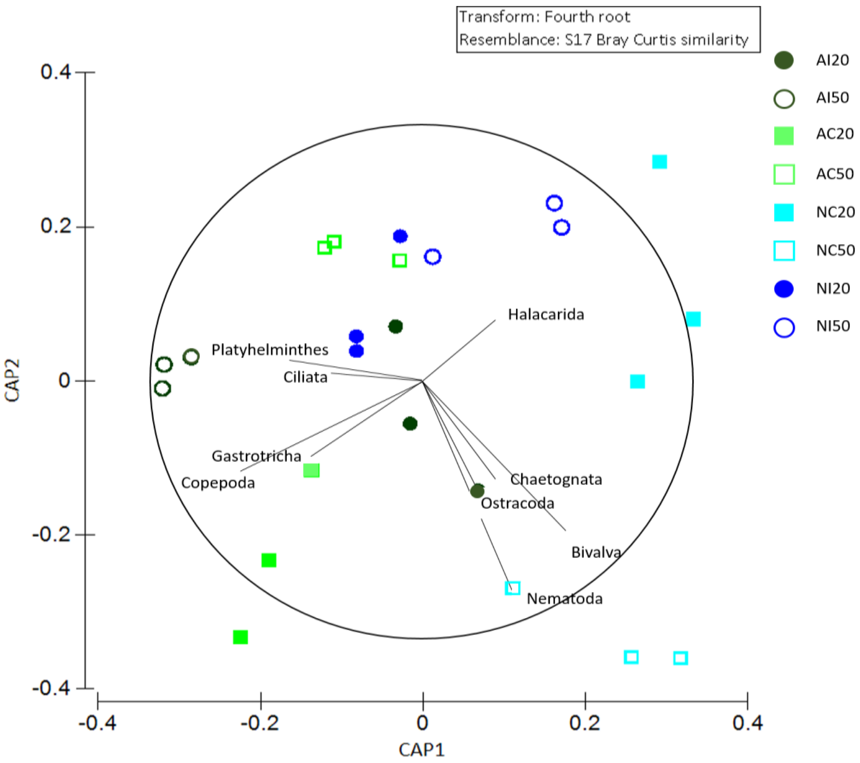

Figure 6. Canonical analysis of principal coordinates (CAP) of the interaction of Ar × De factors. Dataset on total taxa is correlated by the Pearson index (ρ > 0.35). Straight lines are vectors of taxa, whose orientation and length are proportional to the most correlated plot elements. Circle represents 95% confidence interval.

Table 3. Pair-wise comparisons and t-statistics for differences in rare meiobenthic assemblages among stations within levels of the depth factor. Significant tests relevant to the hypothesis are given in bold. (**a**) Within level '20' of factor 'Depth'. (**b**) Within level '50' of factor 'Depth'.

(a)			**(b)**		
Station Pair	**t**	***p***	**Station Pair**	**t**	***p***
AI vs. AC	0.46728	0.8484	AI vs. AC	0.72994	0.6404
AI vs. NC	1.3267	0.2092	AI vs. NC	2.0195	0.0548
AI vs. NI	1.4224	0.1976	AI vs. NI	3.1182	**0.019**
AC vs. NC	1.3719	0.1856	AC vs. NC	2.1586	**0.043**
AC vs. NI	1.5315	0.1794	AC vs. NI	2.9999	**0.0152**
NC vs. NI	2.136	0.0586	NC vs. NI	2.5334	**0.0396**

Significant differences between assemblages at 20 and 50 m depth were detected only at the NI area (PERMANOVA t-statistic, $p = 0.019$) within its levels.

In the CAP ordination plot on rare taxa assemblages (Figure 7; Supplementary S4 Figure S2), clear clusters were not evident, since a high overlapping rate existed among the area and depth factors elements. However, it is possible to observe that all the stations belonging to AI and AC are grouped together in the positive quadrant of the CAP1 and CAP2 and are weakly correlated to the Oligochaeta and Nemertea taxa.

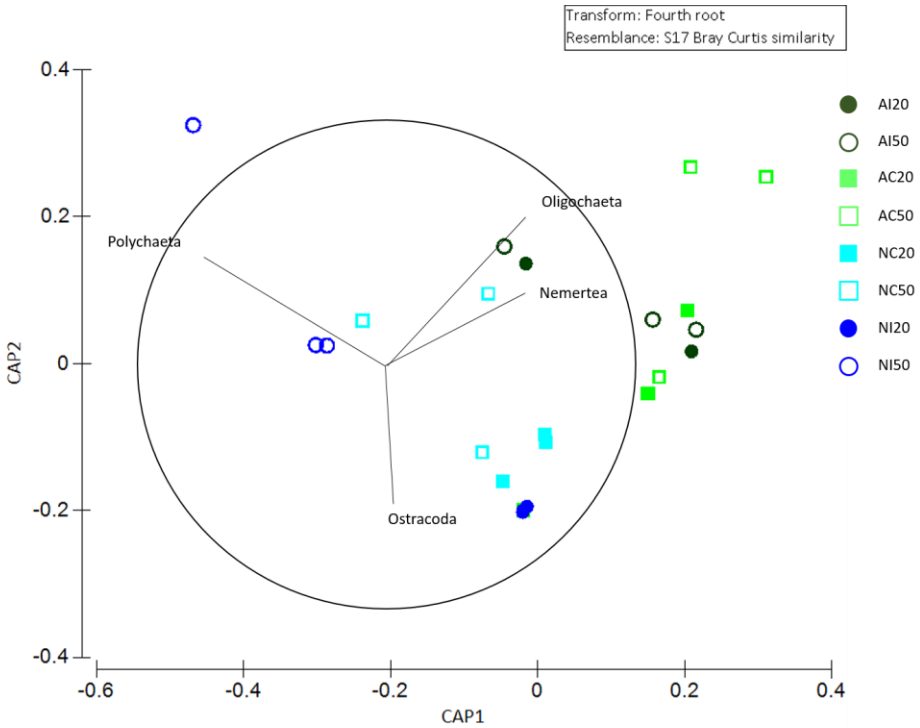

Figure 7. Canonical analysis of principal coordinates (CAP) of the interaction Ar × De factors. Dataset on rare taxa is correlated by Pearson index ($\rho > 0.35$). Straight lines are vectors of taxa whose orientation and length are proportional to the most correlated plot elements. Circle represents 95% confidence interval.

3.3. Interaction between Environmental and Biological Data

DistLM performed on total assemblages selected Cd^{2+}, NO_3^-, coarse sand percentage, pH, and PO_4^{3-} as the best combination of environmental variables affecting meiobenthic assemblages and concurring for more than 55% of total variance (Adj. $r^2 = 0.56$). However, Cd^{2+}, NO_3^-, and pH were significantly correlated with assemblage structures (Table 4).

Table 4. Variables selected by distLM as the most affecting biological data showed in their cumulative contribution to total variation Adj, r^2, and the significance (*p* in bold) of their own pseudo-F value.

Variable	Adj. r^2	Pseudo-F	*p*
+Cd^{2+}	0.27427	3.9724	**0.0018**
+NO_3^-	0.35437	3.6054	**0.0032**
+Coarse sand	0.46235	2.4745	0.051
+pH	0.52549	3.2622	**0.019**
+PO_4^{3-}	0.56052	2.2752	0.0832

dbRDA performed on total assemblages and related to environmental variables, selected by distLM (Figure 8), showed three different clusters: one composed by AC20, AC50 and AI50 replicates and positioned in the central part of the dbRDA2; one composed by AI20, NI20 and NI50 stations polarized at the plot centre; and one composed by NC20 and NC50 polarized in the negative part of dbRDA1. Of these clusters, AI20 elements were correlated with Cd^{2+} and coarse sand percentage, NI20 and NI50 with PO_4^{3-}, and, finally, NC20 and NC50 with NO_3^- and pH.

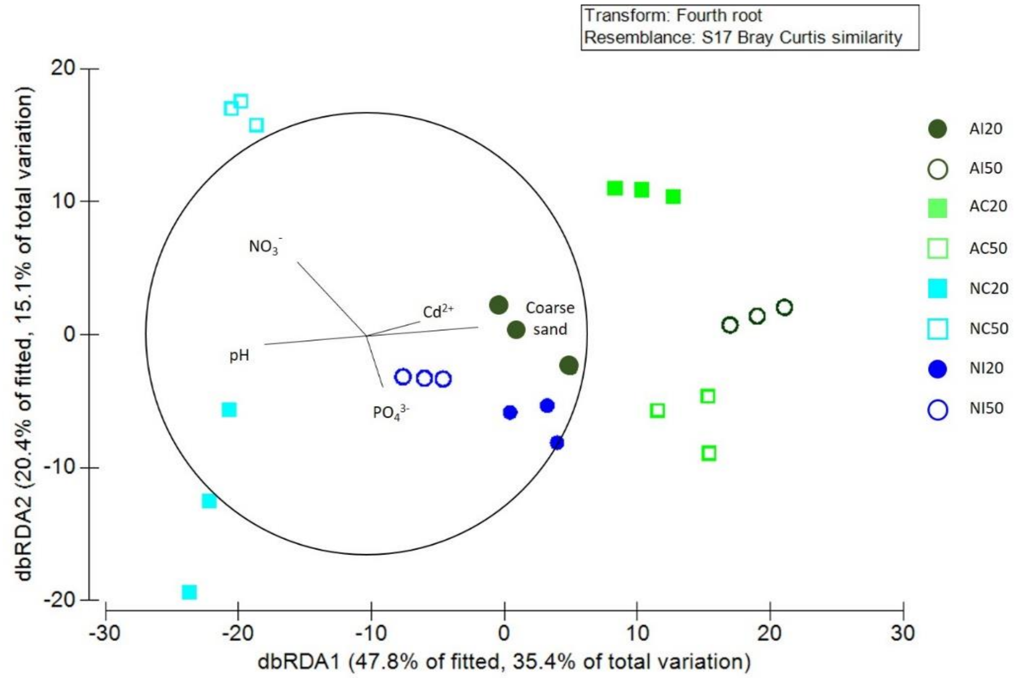

Figure 8. The dbRDA (distance-based redundancy analysis) ordination of total meiobenthic assemblages vs. the significant explanatory environmental variables selected by distLM. Vector overlays represent multiple partial correlations of the explanatory variables with the distance-based redundancy analysis (dbRDA) axes. See text for further explanation.

DistLM performed on rare assemblage structures of the selected TOC, Cu^{2+}, pH, NO_3^-, coarse sand percentage, Zn^{2+}, and Cd^{2+} as environmental variables affecting rare meiobenthic taxa and concurring for more than 50% of total variation (Adj. r^2 = 0.505). However only TOC, Cu^{2+}, pH, and coarse sand were significantly positive correlated with assemblages (Table 5).

Table 5. Variables selected by distLM as the most affecting biological data showed in their cumulative contribution to total variation Adj, r^2, and the significance (*p* in bold) of their own pseudo-F value.

Variable	Adj. r^2	Pseudo-F	*p*
+TOC	0.1047	3.6896	**0.0112**
+Cu^{2+}	0.19544	3.4813	**0.0146**
+pH	0.27149	3.1921	**0.0302**
+NO_3^-	0.29641	1.7084	0.1804
+Coarse sand	0.4142	4.7817	**0.003**
+Zn^{2+}	0.48903	2.2048	0.1056
+Cd^{2+}	0.50471	1.2803	0.3088

dbRDA performed on rare assemblages and related to environmental variables, selected by distLM (Figure 9), grouped the 20 m elements in the positive part of dbRDA2 strongly correlated with Zn^{2+}, Cu^{2+}, and pH, while 50 m depths in the negative part of dbRDA strongly correlated with coarse sand, NO_3^-, and TOC. It is noteworthy that AI20 and AI50 appeared to be more correlated with Cd^{2+}.

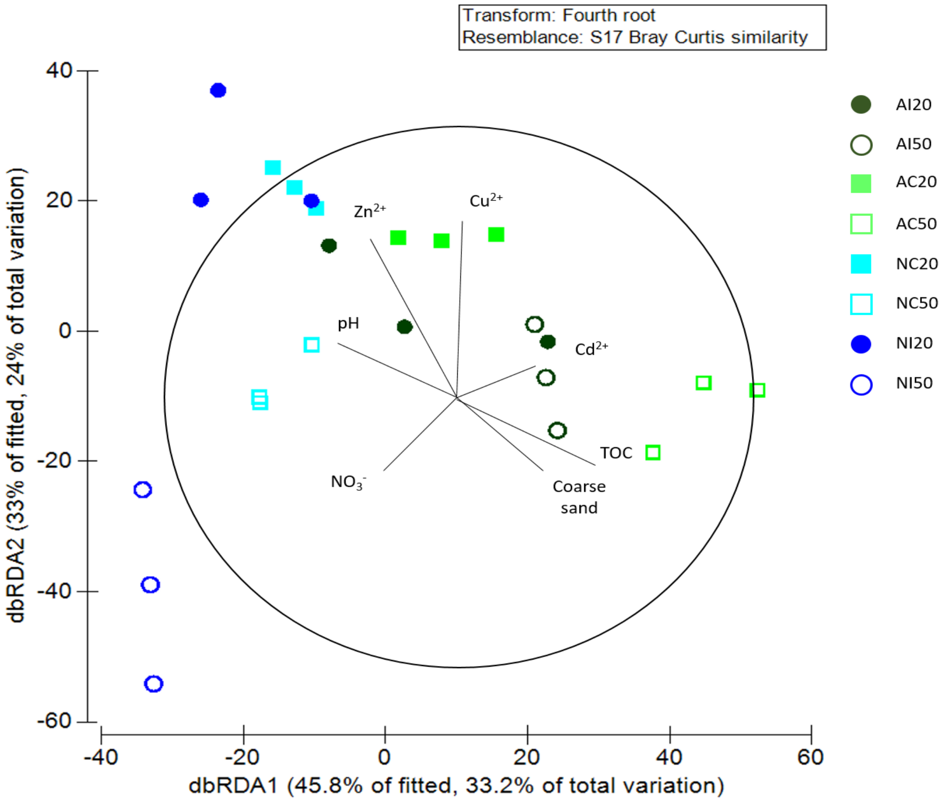

Figure 9. The dbRDA (distance-based redundancy analysis) ordination of rare meiobenthic assemblages vs. the significant explanatory environmental variables. Vector overlays represent multiple partial correlations of the explanatory variables with the distance-based redundancy analysis (dbRDA) axes. See text for further explanation.

4. Discussion

4.1. Environmental Characterization of the Study Area

High levels of lead, cadmium, copper, and zinc are generally considered indicators of anthropogenic contamination; in addition, they can result in adverse effects on several benthic components (e.g., [6]). Their concentrations were in general agreement with other published data related to the impacted sites in the Antarctic region ([64]). From Figure 2, it is possible to note that the highest metals concentrations were at AI20 (i.e., Zn^{2+}, Cd^{2+}, Pb^{2+}, and Cu^{2+}) and NI20 (only Zn^{2+} and Pb^{2+}), suggesting that, in these stations, the highest impact of pollutants (emission or accumulation) is present, with respect to the other ones. This result should not be considered surprising, due to the presence of MZS (in AI20), where heavy metals, organic matter, and hydrocarbon are produced by fuel spills, sewage waste, and the 'grey waters' that originate from station toilets, laundry facilities, accommodation, and cooking areas. In addition, the presence of the Adelie penguin colony in Adelie Cove (NI20) could contribute to the circulation of all the metals that are here considered [65,66]. In particular, Chu et al. [65,66] reported that heavy metals and other trace elements are present in Antarctica, due to both global events (global circulation of air masses and water) and local phenomena, such as human (including activities related to research stations) and natural activities (principally, penguins faeces). With reference to the latter point, [66] proposed a mechanism for the bio-transport of metals in the Antarctic sediments by penguins that can act, depending on their diet, as bioaccumulators of environmental contaminants [67].

The Unites States Environmental Protection Agency [68] set the limit concentration of the heavy metals in seawater. In particular, the "criterion maximum concentration" (CMC), as an estimate of the highest concentration of a material in surface water to which an aquatic assemblage can be briefly exposed to without a negative impact, was established;

in addition, a "criterion continuous concentration" (CCC), which stands for the highest concentration for indefinite exposure, was defined [69]. As far as our data are concerned, it is possible to state that, at present, there is no critical condition for the contamination, due to heavy metals; however, this aspect must be monitored for the future.

Phosphate and nitrate ions play the role of nutrients and are generally regarded as the most typical bioindicator for penguin input [70]. In this investigation, phosphate was detected in the stations NI20, NC50, and AC50, with the highest concentration measured in the sampling station NI20; these values were high, compared to the background level recorded in seawater and interstitial water samples [71].

The concentrations, discussed here, for nitrate ion were very low, compared to levels recorded in seawater samples, with the highest value at station AI 20. Our data are in agreement with previous measures in interstitial waters, collected in coastal sediments in Antarctica; Monien et al. [71] assumed that nitrate loss might be due to a variation in redox sediment conditions. The sulphate ion is a conservative component of seawater, and its concentration depends on physical phenomena, such as evaporation and precipitation. The values recorded in the present study are lower than typical background values for pore waters and might be attributable to microorganism activities, as reported by Monien et al. [71].

O2 represents an important measure of water quality. All the investigated stations show optimal values of this parameter, since O2 concentrations are higher than 4.8 mg/L, which is considered the background protective value for biological health, survival of juveniles and adults, growth, and larval recruitment [68].

Sediment granulometry and TOC percentage values highlight high environmental heterogeneity, mostly at deeper stations. Indeed, Ross Sea soft bottom habitats are mainly composed of: (a) basal tills sediments, which display all the characteristics of continental shelf and indicate deposition by grounded ice; (b) residual glacial marine sediments, derived from floating ice and icebergs, where the fine fraction has been removed by marine currents; and (c) compound glacial marine sediments, derived, in part, from floating ice but containing a significant current-derived fine component [72]. Moreover, during the water freezing period, plankton is trapped among the ice crystals, and its amount is variable each year, depending on specific spatio-temporal environmental parameters (i.e., the current regime and geomorphological features). During the ice melting period, the previously trapped plankton is released; it then settles on the seabed as organic matter, thus contributing to the TOC concentration and its great spatial heterogeneity [73].

4.2. Meiobenthic Assemblage Structure

Total meiobenthic abundances were lower than that documented in many studies carried out in the Antarctic Peninsula (see [74] and references therein), but they were comparable with those reported for Ross Sea [2]. However, as suggested by Pasotti et al. [74], comparisons of abundance data are often impaired by the use of different sampling methods or mesh size sieves, applied during the meiobenthic sampling routine or separation process. Nematodes and copepods (adults and *nauplii*) were the most abundant taxa in our data set; besides those two, Ciliata, Gastrotricha, and Platyhelminthes showed relevant abundances and were regularly documented among the prevalent taxa in many polar expeditions [2,74–80]. The statistical analysis did not reveal clear effects of anthropogenic or natural impacts or depth gradient on the meiobenthic richness; however, a significantly higher number of taxa at the station AI50 was detected, suggesting that human influence is minimal at the deeper stations. When the number of taxa was used to obtain a classification of the ecological quality of the sediments [51], all the sampling stations showed a moderate quality, with the exception of the sites corresponding to the penguin colonies (NI20 and NI50), which showed a poor ecological quality. The total abundance appeared to be affected more by natural and human impacts than the number of taxa, with particularly lower values in the proximity of the penguin colonies.

The general positive relation between medium sands and meiobenthic diversity and evenness [8] was not clearly discernible in the present study (see Figures 3 and 5d). However, the sites with the lowest H' and J values (i.e., NC50) were characterized by a high percentage of fine sediment fraction, which likely contributed to the reduction of the interstitial space in the sediment matrix [81]. Here, the almost total absence of the *phylum* Gastrotricha taxon, mainly showing interstitial lifestyle [82], and particularly high abundance of the more tolerant nematodes seem to corroborate this hypothesis.

There were significant differences in the structure of the whole meiobenthic assemblage, due to area, which were larger than those that were due to the depths, as well as their interactions. Almost all pair-wise comparisons showed significant differences within the 50 m depth level, revealing a high degree of environmental heterogeneity, especially at deeper sites. Despite Ciliata and nematodes resulted more abundant in the impacted sediments in the present study, the former, as well as other groups such as Chaetognata, are often ignored in investigations on meiobenthos. Indeed, Ciliata are prokaryotes, and the latter were previously considered only macrobenthic components and only an accidental taxon in meiobenthos until a few years ago. However, ciliates, as part of the complex detritus–bacteria–meiofauna system, might play an important role in the marine ecosystem functioning [4,11,83]. In addition, numerous worldwide investigations have recently documented the presence of a very small meiobenthic species, belonging to the Chaetognata genus *Spadella*, suggesting that meiobenthos may number more specialized forms than expected [84–88].

When rare taxa were taken into account, PERMANOVA revealed significant differences only for the single factors, underlining a more relevant difference between areas, rather than depths, as revealed also by the whole assemblage structure. Indeed, the positive side of the CAP1 contained all AI and AC samples, where Oligochaeta and Nemertea were more abundant.

The higher spatial heterogeneity of the meiobenthic assemblages (at both total community and rare taxa levels) at the deeper stations (i.e., 50 m) seems to confirm the previous observations by Pasotti et al. [74], who reported a high spatial heterogeneity of meiofauna, closely associated to food availability and microscale differences in primary (microphytobenthos and macroalgae) and secondary (bacteria and protozoans) production processes.

Although the analyzed environmental parameters explain only a part of the assemblage structure variations (suggesting the possible influence of additional abiotic variables), Cd^{2+}, pH, NO_3^-, and coarse sand percentage appeared to be among the most relevant variables for both whole meiobenthic assemblage and rare taxa structure. Moreover, rare taxa appeared to be more susceptible to small environmental variations, as underlined primarily by the total variation, accounting for more than 57% (Figure 9), and, secondly, by a high number of parameters affecting the assemblages (see distLM results: i.e., 5 vs. 7 total meiofauna and rare taxa, respectively; Tables 4 and 5). In particular, heavy metals, coarse sediments, and TOC (this latter only for rare taxa) seem to control the assemblages of the anthropogenically impacted sediments, while the NO_3^-, PO_4^{3-}, and pH values appear to influence the assemblages in the stations where the penguin colonies are located. However, these observations need to be confirmed by a higher number of sites and studied within each combination of factors in the future sampling campaigns in the Ross Sea. The poor ecological quality, highlighted by the taxonomic richness values, low meiobenthic abundances, and prevalence of r-strategy lifestyle taxa (e.g., ciliates and nematodes, characterized by high reproduction rate, high surviving ability, and physiological adaptations to changing environments) [9,18,51], suggests that the chronic impact of penguin colonies might have stronger effects on the meiobenthic assemblages than the human activities at the MZS.

5. Conclusions

Understanding the anthropogenic impacts on Antarctica has become a crucial issue, as the world is experiencing major environmental changes. Despite the benefits of many of the research programs carried out in Antarctica, the presence of scientific field infrastructure is causing adverse impacts on the environment, which need to be accurately monitored. Meiobenthic organisms have proven to be useful as early biological indicators and for documenting all of the ecosystem dynamics in many field and laboratory studies. Based on the present comparison between naturally and anthropogenically impacted areas, we can conclude that the large and old colony of Adélie penguins (*Pygoscelis adeliae*) at Adelie Cove has a heavier impact on the meiobenthic community than the Mario Zucchelli Station. Despite the fact that environmental and faunal data did not reveal critical conditions or the overcoming of international guideline thresholds, the presence of the Antarctic Specially Protected Area (ASPA) n.161 and vulnerability of this ecosystem require future accurate monitoring.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/d13120626/s1, Supplementary S1 Table S1: results of PERMANOVA analyses on environmental data; Supplementary S1 Table S2: results of PERMANOVA analyses on Species Richness results; Supplementary S1 Table S3: results of PERMANOVA analyses on Abundances results; Supplementary S1 Table S4: results of PERMANOVA analyses on Diversity Species index results; Supplementary S1 Table S5: results of PERMANOVA analyses on Evenness results; Supplementary S1 Table S6: results of PERMANOVA analyses on Assemblages structure; Supplementary S1 Table S7: results of PERMANOVA analyses on Assemblages composed by rare taxa; Supplementary S2 Table S8: Mean (±SD) abundances of taxa (n° ind./10 cm^2); Supplementary S3 Table S9: Mean (±SD) abundances of taxa (n° ind./10 cm^2), Taxa affecting for more than 70% dissimilarities for each pair AreaXDepth interaction detected by SIMPER analysis. Contrib% = percentage contribution of each Taxa to dissimilarities; Cum.% = percentage cumulative contribution of each Taxa to dissimilarities; Supplementary S3 Table S10: results of SIMPER analysis showing main TAXA affecting dissimilarities of area pairs meio-benthic communities compose by rare TAXA. Contrib% = percentage contribution of each TAXA to dissimilarities; Cum.% = percentage comulative contribution of each TAXA to dissimilarities; Supplementary S3 Table S11: results of SIMPER analysis showing main TAXA affecting dissimilarities between 20 and 50 meters meio-benthic communities compose by rare TAXA. Contrib% = percentage contribution of each TAXA to dissimilarities; Cum.% = percentage comulative contribution of each TAXA to dissimilarities. Supplementary S4 Figure S1: Canonical Analysis of Principal coordinates (CAP) of the factor a) Area and b) Depth on dataset of total taxa; Supplementary S4 Figure S2: Canonical Analysis of Principal coordinates (CAP) of the factor a) Area and b) Depth on dataset of rare taxa.

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References

1. Cook, W.M.; Yao, J.; Foster, B.L.; Holt, R.D.; Patrick, L.B. Secondary succession in an experimentally fragmented landscape: Community patterns across space and time. *Ecology* **2005**, *86*, 1267–1279. [CrossRef]
2. Fabiano, M.; Danovaro, R. Meiofauna distribution and mesoscale variability in two sites of the Ross Sea (Antarctica) with contrasting food supply. *Polar Biol.* **1999**, *22*, 115–123. [CrossRef]

3. Fonseca, V.G.; Sinniger, F.; Gaspar, J.M.; Quince, C.; Creer, S.; Power, D.M.; Peck, L.S.; Clark, M.S. Revealing higher than expected meiofaunal diversity in Antarctic sediments: A metabarcoding approach. *Sci. Rep.* **2017**, *7*, 1–11.
4. Bianchelli, S.; Nizzoli, D.; Bartoli, M.; Viaroli, P.; Rastelli, E.; Pusceddu, A. Sedimentary organic matter, prokaryotes, and meiofauna across a river-lagoon-sea gradient. *Diversity* **2020**, *12*, 189. [CrossRef]
5. Schratzberger, M.; Ingels, J. Meiofauna matters: The roles of meiofauna in benthic ecosystems. *J. Exp. Mar. Bio. Ecol.* **2018**, *502*, 12–25. [CrossRef]
6. Balsamo, M.; Semprucci, F.; Frontalini, F.; Coccioni, R. Meiofauna as a tool for marine ecosystem biomonitoring. *Mar. Ecosyst.* **2012**, *4*, 77–104.
7. Semprucci, F.; Balsamo, M.; Appolloni, L.; Sandulli, R. Assessment of ecological quality status along the Apulian coasts (eastern Mediterranean Sea) based on meiobenthic and nematode assemblages. *Mar. Biodivers.* **2018**, *48*, 105–115. [CrossRef]
8. Boufahja, F.; Semprucci, F.; Beyrem, H. An experimental protocol to select nematode species from an entire community using progressive sedimentary enrichment. *Ecol. Indic.* **2016**, *60*, 292–309. [CrossRef]
9. Frontalini, F.; Semprucci, F.; Di Bella, L.; Caruso, A.; Cosentino, C.; Maccotta, A.; Scopelliti, G.; Sbrocca, C.; Bucci, C.; Balsamo, M. The response of cultured meiofaunal and benthic foraminiferal communities to lead exposure: Results from mesocosm experiments. *Environ. Toxicol. Chem.* **2018**, *37*, 2439–2447. [CrossRef]
10. Appolloni, L.; Zeppilli, D.; Donnarumma, L.; Baldrighi, E.; Chianese, E.; Russo, G.F.; Sandulli, R. Seawater Acidification Affects Beta-Diversity of Benthic Communities at a Shallow Hydrothermal Vent in a Mediterranean Marine Protected Area (Underwater Archaeological Park of Baia, Naples, Italy). *Diversity* **2020**, *12*, 464. [CrossRef]
11. Baldrighi, E.; Zeppilli, D.; Appolloni, L.; Donnarumma, L.; Chianese, E.; Russo, G.F.; Sandulli, R. Meiofaunal communities and nematode diversity characterizing the Secca delle Fumose shallow vent area (Gulf of Naples, Italy). *PeerJ* **2020**, *8*, 1–28. [CrossRef] [PubMed]
12. Semprucci, F.; Cesaroni, L.; Guidi, L.; Balsamo, M. Do the morphological and functional traits of free-living marine nematodes mirror taxonomical diversity? *Mar. Environ. Res.* **2018**, *135*, 114–122. [CrossRef] [PubMed]
13. Donnarumma, L.; Appolloni, L.; Chianese, E.; Bruno, R.; Baldrighi, E.; Guglielmo, R.; Russo, G.F.; Zeppilli, D.; Sandulli, R. Environmental and Benthic Community Patterns of the Shallow Hydrothermal Area of Secca Delle Fumose (Baia, Naples, Italy). *Front. Mar. Sci.* **2019**, *6*, 1–15. [CrossRef]
14. Hong, J.-H.; Semprucci, F.; Jeong, R.; Kim, K.; Lee, S.; Jeon, D.; Yoo, H.; Kim, J.; Kim, J.; Yeom, J. Meiobenthic nematodes in the assessment of the relative impact of human activities on coastal marine ecosystem. *Environ. Monit. Assess.* **2020**, *192*, 1–13. [CrossRef] [PubMed]
15. Sahraeian, N.; Sahafi, H.H.; Mosallanejad, H.; Ingels, J.; Semprucci, F. Temporal and spatial variability of free-living nematodes in a beach system characterized by domestic and industrial impacts (Bandar Abbas, Persian Gulf, Iran). *Ecol. Indic.* **2020**, *118*, 106697. [CrossRef]
16. Bianchelli, S.; Gambi, C.; Zeppilli, D.; Danovaro, R. Metazoan meiofauna in deep-sea canyons and adjacent open slopes: A large-scale comparison with focus on the rare taxa. *Deep. Res. Part I Oceanogr. Res. Pap.* **2010**, *57*, 420–433. [CrossRef]
17. Pusceddu, A.; Bianchelli, S.; Gambi, C.; Danovaro, R. Assessment of benthic trophic status of marine coastal ecosystems: Significance of meiofaunal rare taxa. *Estuar. Coast. Shelf Sci.* **2011**, *93*, 420–430. [CrossRef]
18. Losi, V.; Grassi, E.; Balsamo, M.; Rocchi, M.; Gaozza, L.; Semprucci, F. Changes in taxonomic structure and functional traits of nematodes as tools in the assessment of port impact. *Estuar. Coast. Shelf Sci.* **2021**, *260*, 107524. [CrossRef]
19. Coull, B.C.; Chandler, G.T. Pollution and meiofauna: Field, laboratory, and mesocosm studies. *Oceanogr. Mar. Biol. Annu. Rev.* **1992**, *30*, 191–271.
20. Mirto, S.; La Rosa, T.; Danovaro, R.; Mazzola, A. Microbial and meiofaunal response to intensive mussel-farm biodeposition in coastal sediments of the Western Mediterranean. *Mar. Pollut. Bull.* **2000**, *40*, 244–252. [CrossRef]
21. Vezzulli, L.; Moreno, M.; Marin, V.; Pezzati, E.; Bartoli, M.; Fabiano, M. Organic waste impact of capture-based Atlantic bluefin tuna aquaculture at an exposed site in the Mediterranean Sea. *Estuar. Coast. Shelf Sci.* **2008**, *78*, 369–384. [CrossRef]
22. Grego, M.; De Troch, M.; Forte, J.; Malej, A. Main meiofauna taxa as an indicator for assessing the spatial and seasonal impact of fish farming. *Mar. Pollut. Bull.* **2009**, *58*, 1178–1186. [CrossRef] [PubMed]
23. Dal Zotto, M.; Santulli, A.; Simonini, R.; Todaro, M.A. Organic enrichment effects on a marine meiofauna community, with focus on Kinorhyncha. *Zool. Anzeiger-A J. Comp. Zool.* **2016**, *265*, 127–140. [CrossRef]
24. Espejo, W.; Celis, J.E.; Sandoval, M.; González-Acuña, D.; Barra, R.; Capulín, J. The impact of penguins on the content of trace elements and nutrients in coastal soils of North Western Chile and the Antarctic Peninsula area. *Water Air Soil Pollut.* **2017**, *228*, 116. [CrossRef]
25. Emslie, S.D. Ancient Adélie penguin colony revealed by snowmelt at Cape Irizar, Ross Sea, Antarctica. *Geology* **2021**, *49*, 145–149. [CrossRef]
26. Batalla Salvarrey, A.V.; Kotzian, C.B.; Spies, M.R.; Braun, B. The influence of natural and anthropic environmental variables on the structure and spatial distribution along longitudinal gradient of macroinvertebrate communities in southern Brazilian streams. *J. Insect Sci.* **2014**, *14*, 1–23. [CrossRef]
27. Bueno, C.; Brugnoli, E.; Bergamino, L.; Muniz, P.; García-Rodríguez, F.; Figueira, R. Anthropogenic and natural variability in the composition of sedimentary organic matter of the urbanised coastal zone of Montevideo (Río de la Plata). *Mar. Pollut. Bull.* **2018**, *126*, 197–203. [CrossRef]

28. Hermand, R.; Salen-Picard, C. *Responses of a Macrobenthic Mediterranean Community Submitted to Allochtonous Natural or Anthropic Sedimentary Inputs*; Universite de l a Mediterranee, Centre d'Oceanologie de Marseille, Station Marine d'Endoume: Marseille, France, 2008; Volume 33, p. 18.
29. Danovaro, R.; Chiantore, M.; Pusceddu, A.; Cattaneo Vietti, R.; Manini, E.; Fabiano, M. Sediment organic matter, bacteria and protozoans in Terra Nova bay, Ross Sea, Antarctica, during austral summer 1993/1994. In *National Programme for Antarctic Research—ROSSMIZE (Ross Sea Marginal Ice Zone Ecology)*; Faranda, F.M., Guglielmo, L., Povero, P., Eds.; G.Lang Editor: Genoa, Italy, 1997; pp. 423–430.
30. Bargagli, R. *Antarctic Ecosystems: Environmental Contamination, Climate Change, and Human Impact*; Czeschlik, D., Ed.; Springer Science & Business Media: Berlin/Heidelberg, Germany, 2006; Volume 175, ISBN 3540264655.
31. Hughes, K.A.; Thompson, A. Distribution of sewage pollution around a maritime Antarctic research station indicated by faecal coliforms, Clostridium perfringens and faecal sterol markers. *Environ. Pollut.* **2004**, *127*, 315–321. [CrossRef]
32. Stark, J.S.; Smith, J.; King, C.K.; Lindsay, M.; Stark, S.; Palmer, A.S.; Snape, I.; Bridgen, P.; Riddle, M. Physical, chemical, biological and ecotoxicological properties of wastewater discharged from Davis Station, Antarctica. *Cold Reg. Sci. Technol.* **2015**, *113*, 52–62. [CrossRef]
33. Stark, J.S.; Corbett, P.A.; Dunshea, G.; Johnstone, G.; King, C.; Mondon, J.A.; Power, M.L.; Samuel, A.; Snape, I.; Riddle, M. The environmental impact of sewage and wastewater outfalls in Antarctica: An example from Davis station, East Antarctica. *Water Res.* **2016**, *105*, 602–614. [CrossRef] [PubMed]
34. Corbett, P.A.; King, C.K.; Stark, J.S.; Mondon, J.A. Direct evidence of histopathological impacts of wastewater discharge on resident Antarctic fish (*Trematomus bernacchii*) at Davis Station, East Antarctica. *Mar. Pollut. Bull.* **2014**, *87*, 48–56. [CrossRef]
35. Tin, T.; Fleming, Z.L.; Hughes, K.A.; Ainley, D.G.; Convey, P.; Moreno, C.A.; Pfeiffer, S.; Scott, J.; Snape, I. Impacts of local human activities on the Antarctic environment. *Antarct. Sci.* **2009**, *21*, 3–33. [CrossRef]
36. Stark, J.S.; Snape, I.; Riddle, M.J. The effects of petroleum hydrocarbon and heavy metal contamination of marine sediments on recruitment of Antarctic soft-sediment assemblages: A field experimental investigation. *J. Exp. Mar. Bio. Ecol.* **2003**, *283*, 21–50. [CrossRef]
37. Ancora, S.; Volpi, V.; Olmastroni, S.; Focardi, S.; Leonzio, C. Assumption and elimination of trace elements in Adélie penguins from Antarctica: A preliminary study. *Mar. Environ. Res.* **2002**, *54*, 341–344. [CrossRef]
38. Espejo, W.; Celis, J.E.; Acuna, D.G.; Jara, S.; Barra, R. Concentration of trace metals in excrements of two species of penguins from different locations of the Antarctic Peninsula. *Polar Biol.* **2014**, *37*, 675–683. [CrossRef]
39. Stark, J.S.; Mohammad, M.; McMinn, A.; Ingels, J. Diversity, Abundance, Spatial Variation, and Human Impacts in Marine Meiobenthic Nematode and Copepod Communities at Casey Station, East Antarctica. *Front. Mar. Sci.* **2020**, *7*, 480. [CrossRef]
40. Conlan, K.E.; Kim, S.L.; Thurber, A.R.; Hendrycks, E. Benthic changes at McMurdo Station, Antarctica following local sewage treatment and regional iceberg-mediated productivity decline. *Mar. Pollut. Bull.* **2010**, *60*, 419–432. [CrossRef]
41. Lenihan, H.S.; Peterson, C.H.; Kim, S.L.; Conlan, K.E.; Fairey, R.; McDonald, C.; Grabowski, J.H.; Oliver, J.S. Variation in marine benthic community composition allows discrimination of multiple stressors. *Mar. Ecol. Prog. Ser.* **2003**, *261*, 63–73. [CrossRef]
42. Conlan, K.E.; Kim, S.L.; Lenihan, H.S.; Oliver, J.S. Benthic changes during 10 years of organic enrichment by McMurdo Station, Antarctica. *Mar. Pollut. Bull.* **2004**, *49*, 43–60. [CrossRef]
43. Hill, S.; Cavanagh, R.; Knowland, C.; Grant, S.; Downie, R. *Bridging the Krill Divide: Understanding Cross-Sector Objectives for Krill Fishing and Conservation*; WWF Living Planet Centre: Woking, UK, 2014.
44. Stark, J.S.; Mohammad, M.; McMinn, A.; Ingels, J. The effects of hydrocarbons on meiofauna in marine sediments in Antarctica. *J. Exp. Mar. Bio. Ecol.* **2017**, *496*, 56–73. [CrossRef]
45. Stark, J.S.; Bridgen, P.; Dunshea, G.; Galton-fenzi, B.; Hunter, J.; Johnstone, G.; King, C.; Leeming, R.; Palmer, A.; Smith, J.; et al. Chemosphere Dispersal and dilution of wastewater from an ocean outfall at Davis Station, Antarctica, and resulting environmental contamination. *Chemosphere* **2016**, *152*, 142–157. [CrossRef]
46. Stark, J.S.; Kim, S.L.; Oliver, J.S. Anthropogenic disturbance and biodiversity of marine benthic communities in Antarctica: A regional comparison. *PLoS ONE* **2014**, *9*, e98802. [CrossRef]
47. Povero, P.; Chiantore, M.; Misic, C.; Budillon, G.; Cattaneo-Vietti, R. Land forcing controls pelagic-benthic coupling in Adelie Cove (Terra Nova Bay, Ross Sea). *Polar Biol.* **2001**, *24*, 875–882. [CrossRef]
48. Chianese, E.; Tirimberio, G.; Riccio, A. PM2.5 and PM10 in the urban area of Naples: Chemical composition, chemical properties and influence of air masses origin. *J. Atmos. Chem.* **2019**, *76*, 151–169. [CrossRef]
49. Wentworth, C.K. A Scale of Grade and Class Terms for Clastic Sediments. Published by The University of Chicago Press. *J. Geol.* **1922**, *30*, 377–392. [CrossRef]
50. Schumacher, B.A. *Methods for the Determination of Total Organic Carbon (TOC) in Soils and Sediments, NCEA-C-1282*, 1st ed.; Schumacher, B.A., Ed.; US Environmental Protection Agency, Office of Research and Development: Washington, DC, USA, 2002.
51. Danovaro, R.; Gambi, C.; Mirto, S.; Sandulli, R.; Ceccherelli, V. Meiofauna. In *Mediterranean Marine Benthos: A Manual of Methods for Its Sampling and Study*; Gambi, M.C., Dappiano, M., Eds.; S.I.B.M-Società Italiana di Biologia Marina: Genova, Italy, 2004; pp. 55–97.
52. Heip, C.; Vincx, M.; Vranken, G. *The Ecology of Marine Nematodes*; Aberdeen University Press: Aberdeen, UK, 1985.

53. Appolloni, L.; Bevilacqua, S.; Sbrescia, L.; Sandulli, R.; Terlizzi, A.; Russo, G.F. Does full protection count for the maintenance of β-diversity patterns in marine communities? Evidence from Mediterranean fish assemblages. *Aquat. Conserv. Mar. Freshw. Ecosyst.* **2017**, *27*, 1–11. [CrossRef]
54. Azzurro, E.; Matiddi, M.; Fanelli, E.; Guidetti, P.; La Mesa, G.; Scarpato, A.; Axiak, V. Sewage pollution impact on Mediterranean rocky-reef fish assemblages. *Mar. Environ. Res.* **2010**, *69*, 390–397. [CrossRef]
55. Underwood, A.J. Beyond BACI: Experimental Designs for Detecting Human Environmental Impacts on Temporal Variations in Natural Populations. *Aust. J. Mar. Freshw. Res.* **1991**, *42*, 569–587. [CrossRef]
56. Anderson, M.; Gorley, R.N.; Clarke, K.R. *PERMANOVA + for PRIMER User Manual*; PRIMER-E Ltd.: Plymouth, UK, 2008.
57. Anderson, M.J. A new method for non parametric multivariate analysis of variance. *Austral. Ecol.* **2001**, *26*, 32–46. [CrossRef]
58. Anderson, M.J. Permutation tests for univariate or multivariate analysis of variance and regression. *Can. J. Fish. Aquat. Sci.* **2001**, *58*, 626–639. [CrossRef]
59. Anderson, M.J.; Willis, T.J. Canonical Analysis of Principal Coordinates: A Useful Method of Constrained Ordination for Ecology. *Ecology* **2003**, *84*, 511–525. [CrossRef]
60. Clarke, K.R.; Gorley, R.; Sommerfield, P.J.; Warwick, R.M. *Change in Marine Communities—An Approach to Statistical Analysis and Interpretation*, 3rd ed.; PRIMER-E: Plymouth, UK, 2014.
61. Terlizzi, A.; Anderson, M.J.; Fraschetti, S.; Benedetti-Cecchi, L. Scales of spatial variation in Mediterranean subtidal sessile assemblages at different depths. *Mar. Ecol. Prog. Ser.* **2007**, *332*, 25–39. [CrossRef]
62. Anderson, M.J. DISTLM v. 5: A FORTRAN computer program to calculate a distance-based multivariate analysis for a linear model. *Dep. Stat. Univ. Auckl. N. Z.* **2004**, *10*, 2016.
63. Legendre, P.; Anderson, M.J. Distance-based redundancy analysis: Testing multispecies responses in multifactorial ecological experiments. *Ecol. Monogr.* **1999**, *69*, 1–24. [CrossRef]
64. Larner, B.L.; Seen, A.J.; Snape, I. Evaluation of diffusive gradients in thin film (DGT) samplers for measuring contaminants in the Antarctic marine environment. *Chemosphere* **2006**, *65*, 811–820. [CrossRef]
65. Chu, W.-L.; Dang, N.-L.; Kok, Y.-Y.; Yap, K.-S.I.; Phang, S.-M.; Convey, P. Heavy metal pollution in Antarctica and its potential impacts on algae. *Polar Sci.* **2019**, *20*, 75–83. [CrossRef]
66. Chu, Z.; Yang, Z.; Wang, Y.; Sun, L.; Yang, W.; Yang, L.; Gao, Y. Assessment of heavy metal contamination from penguins and anthropogenic activities on fildes peninsula and ardley island, antarctic. *Sci. Total Environ.* **2019**, *646*, 951–957. [CrossRef]
67. Metcheva, R.; Yurukova, L.; Teodorova, S.E. Biogenic and toxic elements in feathers, eggs, and excreta of Gentoo penguin (*Pygoscelis papua* ellsworthii) in the Antarctic. *Environ. Monit. Assess.* **2011**, *182*, 571–585. [CrossRef]
68. *US EPA Ambient Aquatic Life Water Quality Criteria for Dissolved Oxygen (Saltwater): Cape Cod to Cape Hatteras*; Environmental Protection Agency, Office of Water, Office of Science and Technology: Washington, DC, USA, 2000.
69. Dimitrakakis, E.; Hahladakis, J.; Gidarakos, E. The "Sea Diamond" shipwreck: Environmental impact assessment in the water column and sediments of the wreck area. *Int. J. Environ. Sci. Technol.* **2014**, *11*, 1421–1432. [CrossRef]
70. Huang, T.; Yang, L.; Chu, Z.; Sun, L.; Yin, X. Geochemical record of high emperor penguin populations during the Little Ice Age at Amanda Bay, Antarctica. *Sci. Total Environ.* **2016**, *565*, 1185–1191. [CrossRef]
71. Monien, P.; Lettmann, K.A.; Monien, D.; Asendorf, S.; Wölfl, A.-C.; Lim, C.H.; Thal, J.; Schnetger, B.; Brumsack, H.-J. Redox conditions and trace metal cycling in coastal sediments from the maritime Antarctic. *Geochim. Cosmochim. Acta* **2014**, *141*, 26–44. [CrossRef]
72. Anderson, J.B.; Kurtz, D.D.; Domack, E.W.; Balshaw, K.M. Glacial and glacial marine sediments of the antarctic continental shelf. *J. Geol.* **1980**, *88*, 399–414. [CrossRef]
73. Pusceddu, A.; Dell'Anno, A.; Fabiano, M. Organic matter composition in coastal sediments at Terra Nova Bay (Ross Sea) during summer 1995. *Polar Biol.* **2000**, *23*, 288–293. [CrossRef]
74. Pasotti, F.; De Troch, M.; Raes, M.; Vanreusel, A. Feeding ecology of shallow water meiofauna: Insights from a stable isotope tracer experiment in Potter Cove, King George Island, Antarctica. *Polar Biol.* **2012**, *35*, 1629–1640. [CrossRef]
75. Gradinger, R. Integrated abundance and biomass of sympagic meiofauna in Arctic and Antarctic pack ice. *Polar Biol.* **1999**, *22*, 169–177. [CrossRef]
76. Vanhove, S.; Beghyn, M.; Van Gansbeke, D.; Bullough, L.W.; Vincx, M. A seasonally varying biotope at Signy Island, Antarctic: Implications for meiofaunal structure. *Mar. Ecol. Prog. Ser.* **2000**, *202*, 13–25. [CrossRef]
77. Lee, H.; Vanhove, S.; Peck, L.S.; Vincx, M. Recolonisation of meiofauna after catastrophic iceberg scouring in shallow Antarctic sediments. *Polar Biol.* **2001**, *24*, 918–925. [CrossRef]
78. Veit-Köhler, G.; Durst, S.; Schuckenbrock, J.; Hauquier, F.; Suja, L.D.; Dorschel, B.; Vanreusel, A.; Arbizu, P.M. Oceanographic and topographic conditions structure benthic meiofauna communities in the Weddell Sea, Bransfield Strait and Drake Passage (Antarctic). *Prog. Oceanogr.* **2018**, *162*, 240–256. [CrossRef]
79. Ehrlich, J.; Schaafsma, F.L.; Bluhm, B.A.; Peeken, I.; Castellani, G.; Brandt, A.; Flores, H. Sympagic fauna in and under Arctic pack ice in the annual sea-ice system of the new Arctic. *Front. Mar. Sci.* **2020**, *7*, 452. [CrossRef]
80. Leasi, F.; Sevigny, J.L.; Hassett, B.T. Meiofauna as a valuable bioindicator of climate change in the polar regions. *Ecol. Indic.* **2021**, *121*, 107133. [CrossRef]

81. Jouili, S.; Essid, N.; Semprucci, F.; Boufahja, F.; Nasri, A.; Beyrem, H.; Mahmoudi, E. Environmental quality assessment of El Bibane lagoon (Tunisia) using taxonomic and functional diversity of meiofauna and nematodes. *J. Mar. Biol. Assoc. United Kingdom* **2017**, *97*, 1593–1603. [CrossRef]
82. Balsamo, M.; Albertelli, G.; Ceccherelli, V.U.; Coccioni, R.; Colangelo, M.A.; Curini-Galletti, M.; Danovaro, R.; D'Addabbo, R.; Leonardis, C.; Fabiano, M.; et al. Meiofauna of the Adriatic Sea: Current state of knowledge and future perspectives. *Chem. Ecol.* **2010**, *26*, 45–63. [CrossRef]
83. Ferrigno, F.; Russo, G.F.; Semprucci, F.; Sandulli, R. Unveiling the state of some underexplored deep coralligenous banks in the Gulf of Naples (Mediterranean Sea, Italy). *Reg. Stud. Mar. Sci.* **2018**, *22*, 82–92. [CrossRef]
84. Kapp, H.; Giere, O. Spadella interstitialis sp. nov., a meiobenthic chaetognath from Mediterranean calcareous sands. *Meiofauna Mar.* **2005**, *14*, 109–114.
85. Casanova, J.P.; Hernández, F.; Jiménez, S. Spadella lainezi n. sp., the first cave chaetognath from the Eastern Atlantic Ocean. *Vieraea* **2006**, *34*, 17–24.
86. Curini-Galletti, M.; Artois, T.; Di Domenico, M.; Fontaneto, D.; Jondelius, U.; Jörger, K.M.; Leasi, F.; Martínez, A.; Norenburg, J.L.; Sterrer, W. Contribution of soft-bodied meiofaunal taxa to Italian marine biodiversity. *Eur. Zool. J.* **2020**, *87*, 369–384. [CrossRef]
87. Semprucci, F.; Colantoni, P.; Baldelli, G.; Rocchi, M.; Balsamo, M. The distribution of meiofauna on back-reef sandy platforms in the Maldives (Indian Ocean). *Mar. Ecol.* **2010**, *31*, 592–607. [CrossRef]
88. Semprucci, F.; Frontalini, F.; Losi, V.; du Chatelet, E.A.; Cesaroni, L.; Sandulli, R.; Coccioni, R.; Balsamo, M. Biodiversity and distribution of the meiofaunal community in the reef slopes of the Maldivian archipelago (Indian Ocean). *Mar. Environ. Res.* **2018**, *139*, 19–26. [CrossRef]

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CHAPTER 4

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Changes in taxonomic structure and functional traits of nematodes as tools in the assessment of port impact

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Abstract

Numerous anthropogenic activities take place within or nearby ports, thereby increasing environmental pressure in coastal zone that become the final destination of many environmental contaminants. Thus, port areas are crucial to understand the possible long-term effects of contaminants on metazoans that are at the base of the food webs. The port of Vado Ligure is the outlet to the sea of one of the largest industrial areas of the West Liguria (NW Mediterranean). Sediment quality was evaluated in twenty-eight stations at increasing distance from the port. The nematode fauna was characterized (i.e., assemblage composition, taxonomic diversity and functional traits) and organic matter quantity and composition and contaminant concentrations (heavy-metals, polycyclic aromatic hydrocarbons and heavy, long-chain hydrocarbons) were measured. The area resulted mainly polluted by mercury, followed by arsenic, zinc, and polycyclic aromatic hydrocarbons. The faunal distribution (both from a taxonomical and functional point of view) appeared influenced by the contamination, as well as by sediment granulometry and organic matter content and composition. According to nematode assemblages and environmental variables, two groups of stations showed the most marked differences: one located in front of the Vado Ligure port, characterised by the most contaminated stations, the highest organic matter content, and fine sediments, and the other one, close to Bergeggi Marine Protected Area, characterised by more pristine stations, low organic matter content and coarser sediments. A suite of nematode genera that may be considered as bioindicators of pollution and organic enrichment was selected. Maturity index (MI) resulted negatively affected by contamination, suggesting that the different proportion of the nematode life-strategies is useful in assessing the environmental quality of sediments.

The ecological quality status of the sampled stations obtained using abiotic and biotic descriptors (i.e., contamination level, organic matter content and nematode diversity and maturity index) was compared. Both the abiotic and nematode variables showed a general consistency for the stations of the two groups characterised by the highest and the lowest levels of contamination and organic enrichment.

Keywords: Nematoda; polycyclic aromatic hydrocarbons; organic enrichment; heavy-metals; functional traits; Maturity index.

1. Introduction

Ports and harbours constitute key economic areas, where several commercial activities take place, they provide a service to many other industrial sectors and are also important job generators. The two terms are often used interchangeably, but they are distinguished by the main purpose of the areas: a port is a commercial water facility used for ships and their cargo, while a harbour is a naturally or artificially sheltered zone for ship and boat docking. Problems of water and sediment quality are generally relevant in these environments being areas characterized by low hydrodynamism, low rate of water renewal and high sedimentation rate (Moreno et al., 2008). The commercial use of the port and the high concentration of numerous activities (e.g., shipping, sediment dredging, industrial activities in port areas, use of antifouling) that could generate environmental impacts (e.g, through leaks of petroleum derivatives, toxicants leaching from antifouling coatings, wastewater discharges, oil spill accidents, resuspension of sediments, invasive species introduction) determine a higher human impact in ports than in harbours (Darbra et al., 2005; Baldrighi et al., 2019). As a consequence, port sediments may be contaminated with a large number of pollutants, with potential effects on benthic assemblages that are the most impacted biological components (e.g., Fichet et al., 1999; Trannum et al., 2004; Moreno et al., 2008; Losi et al 2013; Baldrighi et al. 2019).

Polycyclic aromatic hydrocarbons (PAHs) are among the most ubiquitous and long-lived petroleum contaminants in port sediments (Chatzinikolaou et al., 2018). They mainly arise from the transport vessel sewage or refuelling, and loading/unloading of oil, when petroleum and other fuels transport activities take place. A release of hydrocarbons always has negative impact on the marine environment, the extent of which depends on the quantity and type of products released, as well as on the environmental characteristics of the area affected by the pollution. Heavy or high molecular weight (HMW) PAHs are more persistent (less readily bio-degraded) than light or low molecular weight (LMW) PAHs and can be incorporated by marine biota. Microorganisms have the ability of breaking down the heavier compounds present in the oil slicks deposited in bottom sediments, making the most persistent and harmful hydrocarbons available for consumers that pose important concerns due to their bioaccumulation and biomagnification potential throughout the food chain (Abdulla and Linden, 2008; Olayinka et al., 2018; Ma et al. 2020). Metal based antifouling systems are an additional cause of contamination in port sediments along with effluent from waterfront industries and industrial wastewater. In the 20th century, lead was used in paints to prevent corrosion and fouling. Then, it was replaced by arsenic, mercury, copper and tributyltin (TBT). However, many of these contaminants have been gradually forbidden in many countries due to adverse impacts on organisms. Copper is now the main inorganic biocide used in antifouling systems, while zinc oxides and other chemical compounds are commonly added to paints as booster biocides (Briant et al., 2013). The high concentrations of these chemicals represent a persistent and ongoing threat for the benthos living in ports (Veiga et al., 2009; Briant et al., 2013; Sedano et al., 2014; Demopoulos et al., 2016; Schintu et al., 2016) and might have implications also for the sediments of the surrounding areas. In ports, anoxia phenomena are frequent and mainly due to the high inputs of organic matter that may determinate relevant recruitments of r-strategist species (Moreno et al., 2008; Ingole et al., 2009; Semprucci and Balsamo, 2014; Semprucci et al., 2018b). For all these reasons, ports are considered as “Heavily Modified Water Bodies” that cannot meet the common criteria of good ecological quality status (WFD 2000/60/EC) (Chatzinikolaou et al., 2018).

Although sediment quality guidelines can give good indications of site contamination, they do not always reflect *in situ* conditions due to several limitations and therefore could generate false positive and negative predictions (Burton, 2002). Furthermore, ER-M and ER-L thresholds are based on laboratory toxicity and bioaccumulation tests made on model organisms (i.e., amphipods *Ampelisca abdita* and *Rhepoxynius abronius* and sea urchins *Arbacia punctulata* and *Strongylocentrus purpuratus*) (Long and MacDonald, 1998) easily culturable, with a high fecundity rate or progeny number (r-strategist species for definition). Such organisms can give an insight of the response to contaminants at the individual or population levels of biological organization, but they cannot reflect the complexity of a community response (e.g., Beyrem et al., 2007; Balsamo et al., 2012; Schratzberger and Somerfield, 2020). Finally, mean ER-M quotients do not take into account the interactive effects of the pollutants (e.g., potential synergistic or antagonistic effects), assuming just an additive effect.

Thus, a multi-approach considering abiotic and biotic descriptors is certainly recommended to have a complete overview of the ecological quality status of sediments. Indeed, the most recent scientific acquisitions have led the sediment quality assessment process towards a univocal path based on several lines of evidence, according to the so-called Weight Of Evidence (WOE) approach (Chapman, 2007; Chapman and Hollert, 2006; Chapman et al., 2002), which overcomes the limitations of the tabular "pass to fail" approach. In fact, in a contaminated environment, the effect on aquatic organisms is given by the "resultant" of the simultaneous action of complex mixtures of pollutants and their interaction with physico-chemical conditions. Therefore, it is not possible to derive direct cause-effect correlations between the concentration in the environment of a specific and single substance and the biological effects produced in organisms, but an integrated approach in which multiple components are assessed must be used.

Meiofauna, and especially nematodes, are an abundant component of the biota of these areas where they have an important role in the biomineralization, bioturbation, oxygen and nutrient cycling (Moreno et al., 2008, 2009; Gray and Elliot, 2010). Meiofauna, living in close contact with sediment particles and interstitial water, are good indicators of sediment environmental quality, especially in soft sediments, where often represent the most abundant metazoan organisms (Gerlach, 1971). The features that make meiofauna more advantageous as bioindicators than macrofauna or other long lived organisms are: 1) the absence of planktonic larval stages that leads to a closer association with the sediment matrix and so can really document the effects of an *in situ* impact and 2) the short generation time (the entire life cycle of many meiofaunal organisms is completed in a few weeks) that can faster detects sublethal effects of toxicants on fecundity, growth rates and longevity (Kennedy and Jacoby, 1999). Furthermore, there are evidences that the different type of anthropogenic disturbance may generate a species specific response of meiofauna (e.g. Balsamo et al., 2012; Frontalini et al., 2018; Soto et al., 2017; Semprucci et al., 2018a). Marine nematodes have been successfully used as Biological Quality Elements (BQEs) to assess the ecological health status of many coasts around the world from Mediterranean basin (Semprucci et al. 2014a, 2018a; Jouili et al. 2017, Bianchelli et al., 2018), Black Sea (Ürkmez et al. 2014), Indian Ocean (Semprucci et al. 2014b), South China Sea (Chen et al. 2018) to Yellow Sea (Hong et al. 2020). At first, the percentage of some nematode indicator genera was proposed by Moreno et al. (2011) as the best method to classify the ecological status of the coastal systems, but the scant number of taxa assigned to each ecological quality class showed some limitations: not necessarily these genera are found in the area under scrutiny making impossible to assign an ecological status. Thus, the use of taxonomical and functional indices has been favored to assure the applicability of the assessment tool to any study area (Hong et al. 2020). There are many measures of the taxonomic diversity (e.g. Shannon, Margalef, Simpson indices, distinctness) or morphological nematode features thought to be related to important ecological functions (e.g. maturity and trophic diversity indices) (see Schratzberger et al., 2007 and Semprucci and Balsamo, 2012 for review). However, Shannon diversity and Maturity Indices have been identified as the ideal candidates as taxonomical and functional measures, respectively, on the basis of the extent of literature available on the indices, their spread application in field studies and their effectiveness in the detection of human disturbance (Moreno et al. 2011).

Numerous ecotoxicological laboratory experiments highlighted the negative effects of contaminants on free-living nematodes in terms of taxonomical composition, diversity and biology (see Coull and Chandler, 1992; Balsamo et al., 2012 Gallucci et at., 2015 and references therein). However, as these experiments were not designed to explain the complex ecological relationships naturally occurring in marine ecosystems, the investigation of the benthic processes in the surroundings of port areas can give interesting insights on how nematode species can respond to chronic and acute contamination events (Trannum et al., 2004; Moreno et al., 2008).

A study on the structure and diversity of the nematode assemblages associated to sediments in the surroundings of the port of Vado Ligure in the Western Ligurian Sea (NW Mediterranean) was carried out. This port is the outlet to the sea of one of the largest industrial areas of the Western Liguria as well as one of the most competitive terminals in Europe. A first survey study of the nematodes associated to sediments collected in the surrounding of Vado Ligure port was already performed by Losi et al. (2013); in that study the samples were collected in winter mainly in the vicinity of the harbour mouth. Instead, in the present study the investigated area is wider and includes both stations located in front of the port and further station (south to the port). Heavy metals, C12-C40, polycyclic aromatic hydrocarbons, organic matter and sediment grain size were analysed simultaneously with free-living nematodes at twenty-eight stations situated along an “anthropogenic gradient” from sites located near the city of Vado Ligure and its commercial port to more pristine sites close to the Marine Protected Area of Bergeggi. The main aim of this study was to assess the response of benthic nematodes to the impact of the Vado Ligure port. It is, in fact, expected a change of the nematode assemblage attributes and ecological quality along the coast of Vado in relation to different levels of contamination. In order to test this hypothesis: (1) the taxonomic structure and diversity of nematode assemblages of Vado Ligure port surroundings were analysed in relation to the chemical and organic composition of sediments, as well as to sediment type; (2) the effects of anthropogenic disturbance on nematode functional traits (i.e. life and trophic strategies) were analysed. Finally, the ecological quality of the study area has been classified separately according to nematode indices and abiotic variables (contamination and organic enrichment) and the results have been compared.

2. Materials and methods

2.1. Study area and field activities

The study area is located in the surrounding of the port of Vado Ligure (Savona), along the coast of the Western Ligurian Sea (NW Mediterranean) (Fig. 1). The port of Vado, used since Roman times as a landing place for the Po area, had an initial development after the First World War following the economic development of the Savona area. After the expansion of port infrastructures between ’70 and ’90 years, Vado Ligure is now the base of a commercial port that is a relevant container and terminal of petroleum products (e.g. gasoline, diesel, naphtha), and is the largest industrial site of the Western Liguria, where an oil-burning power plant and several other industrial plants are located. The Municipality of Vado Ligure is also interested by the presence of tourist infrastructures.

The sampling was carried out in June 2010 at 28 stations located at increasing distances from the port (Fig. 1). Sampling locations were determined with a Global Position System (GPS) receiver. The Northern part of the study area was characterized by the port terminals, while the southernmost part is located close to the Marine Protected Area of Bergeggi island. At each station, three independent replicates were collected by a modified Van Veen grab and subsampled for the analyses of grain size, organic matter concentration, biochemical composition of the sediments, contaminant concentrations (i.e. polycyclic aromatic hydrocarbons, heavy hydrocarbons and heavy metals) along with nematode assemblages. In detail, for each deployment, transparent Plexiglas tubes (inner diameter: 3.6 cm) were used to collect sediment samples at each station: only the uppermost two centimetres of sediment were collected. Sediment cores taken for nematode study were fixed with a 4% formaldehyde solution in buffered seawater to fix the fauna (see Semprucci et al., 2016 for details) and stained with a Rose Bengal aqueous solution (0.5 g L^{-1}, Semprucci et al., 2015c) to distinguish live from dead organisms. The samples for the determination of environmental variables were frozen at -20 °C.

2.2. Sedimentological analysis

Sedimentological analyses were performed by separating the sandy fraction (> 63 μm) from the muddy fraction (< 63 μm) by washing over a set of sieves. In detail, the grain size analysis of the coarser fraction was performed using a vibro-siever (Endecotts Ltd). For the dry-sieving analysis, sediment was oven dried at 105 °C for 24 h, weighed, washed on a 38 μm sieve to remove soluble salts and oven dried again for additional 24 h (see Losi et al. 2013). After the second drying, sediment was weighed, passed through a set of standard sieves and shaken by an automatic shaker for seven minutes. The sediment fraction lower than 63 μm was analysed by a sedigraph (SEDIGRAPH III 5120 Micrometrics). The results obtained were combined to classify the sediments according to the Udden and Wentworth scale (Wentworth, 1922).

2.3. Organic matter concentration and biochemical composition of the sediments

Total organic matter (TOM) was determined by loss on ignition according to Parker (1983). Protein concentration (PRT) was measured according to the technique devised by Hartree (1972) and subsequently modified by Fabiano et al. (1995). Bovine albumin solution was used as standard, and absorbance was determined at 650 nm. Carbohydrate concentration (CHO) was analyzed according to the protocol adopted by Dubois et al. (1956) and modified for sediment application by Gerchakov and Hatcher (1972). D(+) glucose solution was used as standard and absorbance was measured at 490 nm. Sediment blanks were analyzed following the same methods and sediment pre-treated at 550 °C for 4 h. Concentrations of proteins and carbohydrates were expressed as mg g^{-1} of sediment dry weight (DW). Percentages of total organic matter were compared with the thresholds defining the environmental quality status of port sediments (i.e., good/alerting/warning) according to Marin et al. (2008).

2.4. Contaminant analysis

Contaminant concentrations were analysed by the CIMA Research Foundation. The method used for heavy metals was Aqua Regia ICP/MS (Astolfi et al., 2020). Concentrations of polycyclic aromatic hydrocarbons (PAHs) and heavy, long-chain (C12−C40) hydrocarbon (C > 12) were measured by the PAH-GCMS/MS and GC/FID method (Sørensen et al., 2016; Kuppusamy et al., 2020). Then, heavy metals and PAHs concentrations were compared with the ER-L (Effect Ranged-Low) and ER-

M (Effect Ranged-Median) thresholds reported by the U.S. Environmental Protection Agency (USEPA) (Long et al., 1995).

In order to take into account for the presence of mixture of toxic chemicals, mean ER-M quotients were calculated, as suggested by Long et al. (1998). The mean ER-M quotient (ER-Mq) for a station reflects both the number and degree of ER-M guideline exceedances. For each station, mean ER-M quotients were calculated by dividing the concentrations of the individual compounds for which ER-M values exist, by their respective ER-M guidelines, summing these quotients and dividing by the total number of examined compounds (after Long et al., 1998). Mean ER-M quotients were also calculated separately for PAHs (ER-Mq PAHs), considering the concentrations of individual PAHs for which ER-M quotients exists, and heavy metals (ER-Mq Met).

This method represents a useful way to rank sites contaminated by multiple chemicals (Long and MacDonald, 1998), assuming an additive effect (even if not synergistic or antagonistic effects). The higher the values of the quotient, the higher the probabilities of toxicity. A mean ER-M quotient ≥ 1.0 implies that adverse biological effects are likely to occur (Long et al. 1998; McCready et al., 2000). Furthermore, according to the mean ER-M quotient, sites could be classified into four classes based on the probability of toxicity effects: low priority sites (≤ 0.1), medium–low priority site (0.11–0.5), high–medium priority site (0.51–1.5), and high priority site (>1.5) (Long and MacDonald, 1998).

2.5. Nematode assemblage

Samples dedicated to the meiofaunal study, after the removal of formaldehyde, were rinsed with a gentle jet of fresh water over a 500 µm sieve to exclude macrofauna, decanted over a 38 µm sieve ten times, centrifuged three times with Ludox HS40 (specific density 1.18 g cm^{-3}, according Heip et al., 1985). The supernatant obtained by extraction process was stored in 4% formaldehyde solution for further processing. Meiofaunal organisms were counted and identified at the major taxon level using a stereomicroscope (Zeiss Stemi DV4) and one-hundred nematodes from each replicate were randomly picked up and mounted on permanent slides according to Seinhorst (1959). Nematodes were identified at genus level under a Zeiss Axiolab microscope using Platt and Warwick (1983, 1988), Warwick et al. (1998), as well as NeMys database (Bezerra et al., 2018). Total nematode abundance (A), number of genera (S), Shannon-diversity index (H′, log, Shannon and Weaver, 1949), Pielou’s evenness (J′, Pielou, 1969), Index of Trophic Diversity (ITD, Heip et al., 1985) and Maturity index (MI, Bongers, 1990) were calculated. In detail, Shannon is the most common diversity index to verify the changes of the nematode structure assemblage. It is calculated as follows: $H' = -\sum(pi \ln pi)$, where: pi is the proportion of individuals belonging to the ith species in the dataset of interest (Shannon and Weaver, 1949) and it is associated to Pielou index that has the following formula: $J = H'/H'max$, where: H' is the number derived from the Shannon-diversity index and H'max is the maximum possible value of H’ (if all the species are equally represented in the sample).

Furthermore, nematodes were divided into four feeding groups (Wieser, 1953): selective (1A) and non-selective (1B) deposit feeders, epistrate feeders (2A), and predators/omnivores (2B). The composition of the trophic guilds was utilized for the calculation of the ITD: $ITD=\sum\theta^2$, where θ is the contribution of each of the four trophic groups (Heip et al., 1985). The lowest values of this index underline a highest trophic diversity, while the highest ones correspond to the lowest trophic diversity.

The Maturity index (MI) was based on the available information about the life-strategies of the different nematode species (coloniser-persister, c-p classes, Bongers, 1990). Indeed, Bongers assigned to each genus of the marine nematodes one of the five c-p classes from extreme colonizers (c-p 1) to extreme persisters (c-p 5) (Bongers et al., 1991). MI was calculated as the weighted average of the individual coloniser-persister (c-p) values, according to the following formula: $MI=\sum = (v_i \times f_i)$, where v is the c-p value of genus *i* and f(*i*) is the frequency of that genus. The obtained MI value documents the state of succession of an ecosystem and the occurrence of a disturbance may lead to a setback of succession to an earlier state that is reflected by a lower MI.

Finally, the Shannon-diversity index (Shannon and Weaver, 1949) and MI (Bongers et al., 1991) were utilized as Biological Quality Elements (BQEs) for nematodes according to Semprucci et al. (2015a,b). These BQEs allowed the classification of the sampled stations in five Ecological Quality (EcoQ) classes (i.e., “bad”, “poor”, “moderate”, “good”, and “high”) after Moreno et al. (2011), following the principles applied by Chen et al. (2018).

2.6. Statistical analyses

A principal component analysis (PCA) was performed on log (x+1) transformed environmental data (i.e., granulometry, contaminant concentrations and organic matter concentration and composition) to remove the effects of orders of magnitude difference between variables, to avoid negative numbers, and to normalize the data.

In order to explore the multivariate structure of nematode assemblages, non-metric Multi-Dimensional Scaling (nMDS) ordination was performed based on the Bray–Curtis Similarity Matrix. Nematode data were square root transformed.

To ascertain if the studied environmental variables explained changes in nematode assemblages, non-parametric multivariate multiple regression analyses, based on Euclidean distances, was carried out using the distance-based linear models (DistLM). The Akaike Information Criteria (AIC) was used to select the “best” models from all the possible combinations of predictor variables (Anderson et al., 2008). AIC selection was chosen as the method to create the most parsimonious model, as it adds a ‘penalty’ for increases in the number of predictor variables (Anderson et al., 2008). All tests were based on Bray Curtis similarities, calculated between observations for square root transformed nematode data.

A canonical correspondence analysis (CCA) was carried out to explicitly investigate the relationship between environmental variables and the nematode community assemblage. A Monte Carlo permutation was used to test statistically whether the genera were significantly related to the environmental variables and to determine the significance of first and second axes. Sensitivity or tolerance of nematode genera was estimated by the relative influence of the contaminants on nematode assemblage structure using quadrants of the CCA bi-plot, on the basis of the assumption that if a genus is tolerant or insensitive to a specific stress it occur with abundant populations, if is sensitive it decreases. Genera with a CCA score in the same quadrant as the hydrocarbons or metals were considered as insensitive to this kind of pollution, whilst genera that scored in the quadrant not influenced by contaminant vectors were regarded as sensitive to contamination; genera that fell in a quadrant influenced only by some types of contaminants (i.e., Pb and As) were assumed to have intermediate sensitivity/tolerance. Finally, in order to classify a genus sensitive or tolerant it must occur in at least two samples. A similar approach was used to rank nematode species according to their sensitivity to contamination or physical disturbance both in terrestrial and aquatic habitats (e.g., Fiscus and Neher, 2002; Höss et al. 2011; Losi et al. 2013). Spearman’s Rank Correlation Analysis was performed to test for significant relationships between abiotic variables.

All the analyses were performed using PRIMER 6 with PERMANOVA + add-on software package (Clarke and Gorley, 2006; Anderson et al., 2008) or R statistical software (R. Development Core Team, 2011).

3. Results

3.1. Environmental parameters

The particle size resulted very variable among stations, with sediments varying from fine silt to very coarse sand and from extremely poorly to moderately well sorted (Table 1). Mud and especially gravel were the less represented components (Table 1). A map of the principal sedimentary habitats is reported in Figure 1 of Supplementary Material.

Total organic matter (TOM) showed a very wide range of percentages from 0.57 % (St. K1) to 23.5 % (St. C6), as reported in Table 1. According to Marin et al. (2008), the high concentrations of TOM revealed warning conditions in 4 stations out of 28 and altered conditions in 6 stations out of 28, while all the other stations showed a low organic load in the sediments and accordingly a good EcoQ (after Marin et al., 2008), as shown in Figure 2d of Supplementary Material. PRT revealed values from 0.01 mg g^{-1} of sediment DW (St. L3) to 1.36 mg g^{-1} of sediment DW (St. C6) and CHO from 0.21 mg g^{-1} of sediment DW (St. K1) to 3.90 mg g^{-1} of sediment DW (St. C5) (Table 1).

Organic matter (TOM, PRT, CHO) resulted positively correlated with grain size (Spearman, $p < 0.001$), silt % (Spearman, $p < 0.001$) and clay % (Spearman, $p_{TOM, CHO} < 0.001$, $p_{PRT} < 0.01$) and negatively with sand % (Spearman, $p_{TOM, CHO} < 0.001$, $p_{PRT} < 0.01$) and gravel % (Spearman, $p < 0.001$).

Concentrations of the contaminants analysed in the sediments and the relative ER-M (Effect Ranged-Median) and ER-L (Effect Ranged-Low) values are shown in Fig. 2. Summary statistics of the different contaminants are reported in Table S1 of Supplementary materials.

Contaminants varied greatly across the study area. Mercury (Hg), varying from 0.05 mg kg^{-1} (St. M3) to 1.80 mg kg^{-1} (St. K3) exceeded the ER-L and ER-M limits in almost all the stations of the study area. Arsenic (As), ranging from 8 mg kg^{-1} (St. M1) to 57 mg kg^{-1} (St. G3), exceeded ER-L thresholds at all stations, and zinc (Zn), ranging from 35 mg kg^{-1} (St. L1) to 286 mg kg^{-1} (St. K3), exceeded ER-L thresholds at almost all the stations (Fig. 2). Concentration of Pb, ranging from 3 mg kg^{-1} (St. K1) to 75 mg kg^{-1} (St. K3), resulted lower the ER-L limits, with the exception of two stations (I2 and K3). Concentrations of cadmium (Cd) were lower than ER-L limit and showed the same values at all stations (0.05 mg kg^{-1}). Tin (Sn) varied from 0.05 mg kg^{-1} (St. L1) to 8.70 mg kg^{-1} (St. I2).

Total PAHs, which showed values from 0.01 mg kg^{-1} (St. M1) to 15.79 mg kg^{-1} (St. H1), exceeded ER-L limit in most of the stations, but not ER-M limit.

C > 12 values ranged from 2.0 mg kg^{-1} (St. M1) to 90.4 mg kg^{-1} (St. C5).

The classification of the stations according to the mean ER-M quotients is shown in Fig. 2 of Supplementary Material.

There were no stations in which metal concentrations may cause a high probability of adverse biological effects (ER-Mq Met < 1.0 for all stations). Mean ER-M quotients based on heavy metals indicate that 9 stations out of 28 could be classified as high–medium priority sites, about half of the stations as medium–low priority sites, and a few stations as low priority sites (after Long and MacDonald, 1998), as shown in Fig. 2 of Supplementary Material.

Considering PAHs, one station (St. H1) showed a mean ER-Mq > 1, indicating a high probability of biological adverse effects due to organic chemicals. Mean ER-M quotients based on PAHs indicate that 7 stations out of 28 could be classified high–medium priority sites, half of the stations as medium–low priority sites, and 7 stations as low priority sites (after Long and MacDonald, 1998), as shown in Fig. 2 of Supplementary Material.

All the contaminants, except for arsenic (As) and cadmium (Cd), resulted negatively correlated with grain size (Spearman, $p < 0.01$ for Pb and $p < 0.001$ for all the others), sand % (Spearman, $p_{Hg, Sn, C>12} < 0.001$, $p_{Zn, PAHs} < 0.05$) and gravel % (Spearman, $p_{Sn, C>12} < 0.001$, $p_{Hg, Zn, PAHs} < 0.01$, $p_{Pb} < 0.05$) and positively with silt % (Spearman, $p_{Sn, Hg, C>12} < 0.001$, $p_{Zn, PAHs} < 0.01$, $p_{Pb} < 0.05$) and clay %

(Spearman, $p_{Hg,\ Sn,\ C>12} < 0.001$, $p_{PAHs} < 0.01$, $p_{Pb} < 0.05$). A positive correlation with TOM was also found for C>12, PAHs (Spearman, $p < 0.001$), Hg and Sn (Spearman, $p < 0.01$).

The ordination of environmental factors determined by PCA is shown in Fig. 3. The first two axis of PCA explained the 75% of the total variance (PC1: 67.7%, PC2: 7.7%). Variance along the first axis was mainly explained by a decrease in contaminant and organic matter concentrations and an increase in granulometry, while the second axis was mainly explained by As concentrations (Fig. 3A). In particular, the environmental variables that mainly contributed to the PC1 were C>12 (-0.98), silt (-0.94), clay (-0.91), PAHs (-0.90), ER-Mq Met (-0.89), Zn (-0.88), CHO (-0.86), Sn (-0.85), Hg (-0.84), gravel (0.79), Pb (-0.78), TOM, PRT, ER-Mq PAHs (-0.77) and sand (0.69), while As (0.60) was the main variable that characterized the PC2.

3.2. Nematode assemblage

A total of 15 meiofaunal taxa was detected. The total meiofaunal abundance ranged from 76 ± 20 ind. 10 cm^{-2} (St. C7) to 3258 ± 1234 ind. 10 cm^{-2} (St. I1) with higher values in the southern part of the study area (Supplementary Material, Fig. 3). Nematodes were the most relevant taxon of the meiofauna, being on average 70% of the total assemblage (range: 39 – 93%, at St. M1 and St. J1, respectively). The following most abundant taxa were Copepoda (on average, 23%; range, 4.71 – 45.57%), Ostracoda (on average, 3 %; range,0.0 - 5.64 %), Polychaeta (on average, 2 %; range, 0.0 – 7.24 %), and Kinorhyncha (on average, 1 %; range, 0.0 – 14.31 %). The other taxa together accounted for the remaining 4% (Supplementary Material, Fig. 3).

Univariate nematode measures are reported in Table 2. Mean nematode abundance ranged between 53.51 ± 17.77 ind. 10 cm^{-2} (St. C7) and 1923.86 ± 425.03 ind. 10 cm^{-2} (St. I1) (Supplementary Material, Fig. 3). A total of 146 nematode genera were found in the present survey. The lowest number of genera (5 ± 1) and the lowest diversity (1.54 ± 0.50) were found at Station A7, the lowest evenness (0.59 ± 0.26) at Station H1. The highest number of genera (43 ± 2) and the highest diversity (4.89 ± 0.11) and evenness (0.90 ± 0.01) were recorded at Station M3.

The assemblage was mainly represented by deposit feeders (1A, 35% and 1B, 33%), followed by epistrate feeders and only marginally by predators (2A, 29% and 2B, 3%). ITD values ranged from 0.30 ± 0.0 at Station M3 to 0.57 ± 0.30 at Station B5. C-p 3 (52%) were the dominant life strategy class in the study area, followed by c-p 2 (27%), c-p 4 (18%) and only marginally by c-p 1 (3%). MI ranged from 1.91 ± 0.66 at Station B5 to 3.38 ± 0.22 at Station L1 (Table 2).

Of the 146 genera found, *Richtersia* resulted the most abundant and frequent, occurring in almost all stations, with mean relative abundances of 19% (max abundance of 60% at Station A7) (Supplementary Material, Table S2). The other most abundant and frequent genera were *Molgolaimus* (mean abundance = 6%, max = 23%), *Halalaimus* (mean abundance = 5%, max = 14%), *Desmodorella* (mean abundance = 5%, max = 33%), *Microlaimus* (mean abundance = 4%, max = 38%) (Supplementary Material, Table S2).

The nMDS ordination (Fig. 4) shows a quite good match with the PCA performed on the environmental variables (cfr. Fig. 3B), although there are some differences.

According to DistLM, almost all the predictor variables (i.e., environmental variables) result significant (Table 3). The variables that, alone, explain more variance in the nematode assemblage structure are: PRT (30%), silt (26%), CHO (25%) and C>12 (25%). The combination of Silt, Clay, As, Pb, PAHs, ER-Mq PAHs and PRT resulted as the best model, explaining more than 50% of the variance in nematode assemblage structure (Table 3).

The canonical correspondence analyses (CCA) accounted for 62% of the total variability, with the first axis (eigenvalue: 0.4098; p = 0.001) accounting for approximately 32% of the constrained variability and the second axis (eigenvalue: 0.2927; p = 0.005) accounting for 23%. The Monte Carlo test revealed a significant correlation between the environmental variables' gradient and the nematode genera (p = 0.001). CCA1 separate the stations according to a gradient mainly determined by granulometry, C>12, organic content, PAHs, and heavy metals (with the exception of As and Cd); CCA2 separate the stations according to a gradient related to Pb and As (Fig. 5a). The CCA plot showed a separation of four main groups of stations (Fig. 5b), described below.

Group 1 (Stations A7, B5, B6, B7, C5, C6, C7, E4, E5, F2 and J1): containing mostly the stations located closest to the port, characterized by the finest sediments (from fine sand to medium silt) with the highest silt content, the highest C>12 concentrations and OM content, and high level of heavy metals and PAHs. All the station of this group are classified as high–medium priority or medium–low priority sites, according to the mean ER-M quotients. *Croconema*, *Desmodorella*, Desmodoridae gen.1, *Molgolaimus*, *Pselionema*, *Pseudochromadora*, *Richtersia*, Monhysteridae gen.1 and *Pseudometachromadora* resulted the genera most strongly associated to these conditions. The group presented the lowest values of nematode densities, diversity, evenness, number of genera and MI (2.59±0.34) (Table 2), the highest abundances of c-p 3 and c-p 1 genera (on average 79% and 6%, respectively), and the lowest abundances of c-p 4 genera (6%). Non-selective deposit feeders were dominant (45%), followed by epistrate feeders (28%) and selective deposit feeders (26%), while predator/omnivores were low (1%).

Group 2 (Stations F3, G2, H1, H2, H3, I2, K2, K3, L2, and M3): containing stations located to the south-southeast of the port, with a large gradient in substrate type (with sediments classified from fine silt to coarse sand) and intermediate OM content, and characterised by highest concentrations of Pb and generally high levels of all the remaining heavy-metals and PAHs. All the stations of this group are classified as high–medium priority or medium–low priority sites, according to the mean ER-M quotients. *Crenopharynx, Hopperia, Chromaspirina, Gnomoxyala, Dorylaimopsis, Minolaimus, Sphaerolaimus, Sabatieria, Setosabatieria, Linhomoeus, Belbolla, Pierrickia, Campylaimus, Halichoanolaimus, Odontophora, Metacyatholaimus, Laimella, Neotonchus, Dichromadora* and *Steineria* resulted the genera most strongly associated to these conditions. The group showed high nematode densities, diversity, evenness and number of genera (Table 2). On average the selective deposit feeders were the most abundant (37%), but similar percentages of non-selective deposit feeders (34%) were recorded, followed by epistrate feeders (24%), while predators/omnivores represented the 5%.

Group 3 (Stations I1, L3, M2 and G3): containing some stations located to the south-southeast of the port, characterised by sandy sediments (from fine sand to very coarse sand), with low OM content, and low to intermediate contaminant concentrations. The stations of this group are classified as low priority sites or medium–low priority sites, according to the mean ER-M quotients. *Ptycholaimellus, Rhips, Paracomesoma, Longicyatholaimus, Cyartonema, Thalassomonhystera, Viscosia, Cobbia, Marylynnia, Diplopeltoides, Echinodesmodora* and *Paralongicyatholaimus* resulted the genera most strongly associated to these conditions. The group showed the highest nematode densities, diversity, evenness, number of genera and abundances of c-p 2 genera (on average 50%), and the lowest ITD (0.35±0.05) (Table 2). On average epistrate feeders and selective deposit feeders were the most abundant (39% and 37% respectively), followed by non-selective deposit feeders (24%). The percentages of epistrate feeders and predators/omnivores (6%) were the highest in comparison with the other groups.

Group 4 (Stations K1, L1 and M1): containing some of the stations furthest from the port and located to the south of it and closest to Bergeggi beach, characterized by coarse sediments (coarse sand or very coarse sand) with the lowest silt and clay content, and by the lowest contaminant concentrations and OM content. All the station of this group are classified as low priority sites, according to the mean ER-M quotients. *Onyx*, *Manunema*, *Epsilonema*, *Chromadorita*, *Eurystomina, Acanthopharynx, Innocuonema, Parapinnanema, Paracanthonchus, Draconema, Metoncholaimus, Araeolaimus, Spilophorella, Chromadorina* and *Prochromadorella* resulted the genera most strongly associated to these conditions. The group showed the highest MI and ITD (on average 3.32±0.15 and 0.50±0.03, respectively) (Table 2) and the highest abundances of c-p 4 genera (52%) and selective deposit feeders (63%) and the lowest abundances of non-selective deposit feeders (0.3%). Epistrate feeders represented the 33% and predators/omnivores the 4%. In Figure 6 the list of tolerant/sensitive genera according to CCA results is reported.

The classification of ecological quality status of the stations according to Moreno et al. (2011), obtained following the principles applied by Chen et al. (2018), is reported in Table 4. Most stations showed a good/moderate EcoQ with a general higher level of quality in the Southern area (Supplementary Material Figure 2e).

4. Discussion

4.1. Environmental characterization of Vado Ligure sediments

The sediments of study area showed heterogeneous characteristics in terms of granulometry, organic matter content and composition and concentrations of pollutants. According to the sedimentary habitats identified, it is possible to subdivide the study area in four different zones. A first zone is located in front of the port and is characterized by fine sediments (sandy silts and silts-clays); here the sediments of the Segno, Quiliano and Letimbro streams are subject, both to coastal dynamics dependent on waves and bottom currents, and to the backward dynamics of the gravitational process of Vado Ligure canyon's head (http://portovado.cimafoundation.org). A second zone, corresponding to the extension of Capo Vado to the sea, is interested by the presence of some rocky outcrops with high bioconstruction; consequently the surrounding sediments are characterised by a high content of biodetritus and therefore by an increase in the (coarser) sandy content of the sediments (http://portovado.cimafoundation.org). The third zone corresponds to the southernmost part of the Vado Ligure dam and is interested by fine sediments. The fourth zone is in front of Bergeggi beach and its coastal part is characterized by the presence of a *Posidonia oceanica* meadow, which is the continuation of that of the Bergeggi Marine Protected Area, and by some rocky outcrops at limited depths; in this area the presence of gravel reaches, in some parts, significant values and the sediment is mainly sandy (http://portovado.cimafoundation.org).

The fact that most contaminants were positively correlated with the fine fractions of sediments suggest that their distribution was influenced by sediment granulometry, since smaller grain sizes tend to adsorb more contaminants due to a higher surface area-to-volume ratio (Förstner and Wittmann, 1979; Brook and Moore, 1988; Förstner, 1989; Bubbet al., 1991; Barbanti and Bothner, 1993; Yuan et al., 2012; Jiang et al., 2014; Hadlich et al. 2018).

Twenty-seven stations exceeded ER-M or, at least, ER-L thresholds, evidencing the potential of adverse effects in sensitive species, due to the high concentrations of contaminants, especially of heavy metals. Mercury (Hg) was identified as the most critical contaminant since it exceeded the ER-M guidelines at half of the stations. The contaminant concentrations were comparable with the values found in the area by previous studies (Losi et al., 2013; Bertolotto et al., 2003a,b; Bertolotto et al., 2005).

Other than the presence of the port and its activities, the detected pollutants could be related also to many other sources. According to Bertolotto et al. (2013a, b) the concentrations of Pb found in the area could be due to the production of paints and glazing, the concentrations of As, Hg and Zn can be related to chemical and electronic processes, while the values of PAHs is likely in relation to the presence of the coal electric power plant of Vado Ligure, and to the refineries and production of bitumen and tar present in the area. Finally, the streams Segno, Quiliano and Letimbro, that pass through a densely populated and industrialized area, could be significant carriers of contaminants.

4.2. Relationships between nematodes and environmental variables

Granulometry, persistent pollutants and organic matter quantity and biochemical composition (i.e. silt, clay, As, Pb, PAHs, ER-Mq PAHs and PRT) were all important in explaining the variability in nematode assemblages that is consistent with field and laboratory studies (e.g., Montagna and Li, 1997; Schratzberger and Warwick, 1998; Mirto et al., 2002; Semprucci et al. 2020a; Balsamo et al., 2012; Frontalini et al., 2018; Franzo et al., 2019). Arsenic is the only exception due to the lack of specific microcosm experiments to evaluate its impact on nematodes.

The local contamination, sediment type and OM content shaped the composition of distinct nematode assemblages in each of the groups identified by the CCA. The stations of Group 4 are located in front of Bergeggi beach, which is a coast of high naturalistic value with the presence of a *Posidonia oceanica* meadow and adjacent to Zone C - partial reserve zone - of the Protected Marine Area of the Bergeggi island. Here, nematodes were clearly distinguished in term of assemblage structure and both higher MI values and percentage of k-strategist species. Although Gr. 4 resulted to be characterized by coarse sediments, which certainly influenced the nematode assemblages, it was not possible to explain the distinction of these three stations only based on the sediment granulometry because there were other stations with a similar grain size, but different faunal composition and lower MI (see Table 1). Therefore, the proximity to the *Posidonia oceanica* meadow and to the MPA and the distance from the port mouth and main navigation routes remain the more likely explanations of the differences observed.

The presence of contaminants in the station of Group 1 is explained by its proximity to the port and the urban centre of Vado Ligure and by the influence of the streams present in the area, other than the fine sediments.

The level of contamination of many of the stations of Group 2 could be explained with the current circulation: the Mediterranean current as well as the Ligurian circuit flow along the Ligurian coast from E to W (Drago et al., 1981) likely contributing to redistribute contaminants and organic matter due to the port, the streams outlets, and the various industrial activities towards the southern coast. Furthermore, the area interested by Gr. 2 stations is interested by the most relevant shipping traffic routes towards the port terminals (see Figure 1).

The differences in the nematode assemblage structure, MI and c-p classes among the four groups (c-p1 and c-p3 decreased, while MI and c-p 4 increased from Gr. 1 to Gr. 4) support the idea that an increasing level of sediment pollution leads a progressive disappearance of “sensitive” species replaced by more “opportunistic” and/or “resistant” taxa (e.g., Hermi et al., 2009; Beyrem et al., 2011; Boufahja et al., 2011). This was well discernible also in the previous investigation carried out in Vado Ligure, where two clear pools of nematode genera with divergent life strategies have been identified (Losi et al. 2013). As reported in Figure 6, there are various taxa that match in both studies as tolerant genera (i.e., *Comesa*, *Microlaimus*, *Molgolaimus*, *Neotonchus*, *Sabatieria*, *Terschellingia* and *Thalassoalaimus*), and just one as sensitive genus (i.e, *Chromadorita*). Instead, some of the taxa reported as tolerant or sensitive in Losi et al. (2013) were not found in the present study (i.e., *Oncholaimellus* and *Eleutherolaimus* among the tolerant, and *Enoplolaimus* and *Chaetonema* among the sensitive), while some others showed a different degree of tolerance/sensitivity (i.e. *Belbolla*, *Croconema*, *Daptonema*, *Desmodorella*, *Desmoscolex*, *Dorylaimopsis*, *Eurystomina, Innocuonema, Marylynnia Quadricoma*, *Richtersia* and *Spirinia*). The differences among the two studies could be due to spatial and temporal assemblage variabilities: Losi and co-authors carried out the sampling in different stations of Vado (closest to the port) and in another season (i.e., winter).

Among the genera that distinguished the extremes of the anthropogenic gradient analysed in the present study, there were some taxa for which a lifestyle strategy is known. For instance, *Molgolaimus, Pselionema, Richtersia*, Monhysteridae, all genera that characterised Group 1, are often reported as opportunistic taxa, often abundant under stressful conditions, while *Epsilonema, Draconema* and *Chromadorita*, all genera that characterised Group 4, are widely recognized as sensitive genera (Bongers et al., 1991; Somerfield et al., 1994; Fonseca et al., 2006, 2007; Losi et al., 2013; Neira et al., 2013; Semprucci et al., 2014 Singh and Ingole, 2016). However, in the present study we also found many other genera that were associated with contaminated or clean sediments for which Bongers et al. (1991) did not reported the colonizers-persisters class and that could be potential sentinel genera (e.g., *Acanthopharynx*, *Comesa, Draconema, Manunema, Parapinnanema, Pseudochromadora, Pseudometachromadora*).

Nematode abundance is generally closely and positively related to the fine fraction of the sediments, while its relationship with anthropogenic disturbance is not univocal (see Balsamo et al. 2012 for review). In the present study, the abundance, which resulted higher in stations of Gr. 2, was not correlated to fine sediment neither affected by contamination. Nematode biodiversity is usually sensitive to contaminants. The diversity and evenness indices resulted the lowest at the Gr. 1 and the highest at Gr. 3 and may have been positively influenced by the sand contents and the quite low level of contamination due to hydrocarbons and Hg. The presence of a high fraction of medium-coarse sands is generally associated to high levels of H’ and J indices (Vanaverbeke et al., 2002; Semprucci et al., 2010b) as well as general reduction of the number of species is detectable as levels of contamination increase (Coull & Chandler, 1992; Balsamo et al., 2012; Gambi et al., 2020a).

The lowest trophic diversity (i.e. the highest ITD) was found in the stations with the lowest human impact (i.e. Gr. 4), suggesting that ITD serves no useful purpose for the human impact detection. Hong et al. (2020) and Gambi et al. (2020b) noticed, in accordance with our data, a good response of MI and the absence of consistent results with ITD in industrially polluted bottoms in South Korea and Mediterranean Sea, respectively.

The analysis of the single trophic groups clarifies the ITD trends found in the Vado Ligure study area: non-selective deposit-feeders (1B) resulted to be advantaged in the more stressed stations in front of the port outlet likely because 1B are usually characterised by short lifecycles and a high colonisation ability (Tietjen, 1980; Boufahja and Semprucci, 2015), while predators and epistrate feeders increased in abundance in the sediments of the group Gr. 3 likely, in accordance with the coarser sediment grain size (e.g. Semprucci and Balsamo, 2012; Semprucci et al., 2010b, 2011).

4.3. Multi-approach ecological assessment of the study area

In the present study, we compared the information on sediment quality obtained by the application of guidelines on contaminants (mean ER-M quotients after Long and MacDonald, 1998) and organic enrichment (TOM, after Marin et al., 2008) with the final EcoQ classification obtained with nematode indices (i.e., diversity and MI), according to Moreno et al. (2011), following the principles applied by

Chen et al. (2018). Even if there is not a perfect match of the health status defined by each of them, both the abiotic and nematode variables showed a general consistency in identifying the worst sediment conditions at the stations of the Gr. 1 (ER-Mq tot: 4 of 11 stations resulted medium-high priority sites; TOM: alerting and warning conditions at all the stations; nematodes: from moderate to bad EcoQ) as well as a general good status at the stations of the Gr. 4 (ER-Mq tot: low priority sites; TOM: good conditions; nematodes: good EcoQ). Conversely, the various classifications showed less consistent results for the stations of the Gr. 3 and of Gr. 2. In particular, at some stations of Gr.2, notwithstanding the high concentrations of some metals, the final nematode status resulted good or high. A possible explanation could be a low availability of such contaminants for the biota. Acute and sublethal toxicity of contaminants to meiofaunal organisms may be dependent on numerous environmental factors such as pH, redox potential and salinity, and biologically mediated processes (Davies et al., 1991; Di Toro et al., 1991; Schratzberger et al., 2000; Liu et al. 2015). De Carvalho et al. (2018) stressed the importance of a trace-metal sequential extraction to make a reliable assessment of the metal bioavailability for the fauna.

5. Conclusions

The sediments of the study area resulted highly heterogeneous in terms of granulometry, contamination and organic matter content and composition. The nematode assemblage structure resulted influenced by these environmental variables. A constrained ordination performed on nematode assemblages clearly separated the assemblages of two groups of stations, one located in front of the Vado Ligure port, characterised by the most contaminated stations, the highest organic matter content, and fine sediments, and one, close to Bergeggi Marine Protected Area, characterised by more pristine stations, low organic matter content and coarser sediments. This allowed to select a suite of nematode genera according to their tolerance or sensitivity to contamination and organic enrichment. Among the univariate measures, the maturity index (MI) resulted negatively affected by contamination, suggesting that the different proportion of the nematode life-strategies is useful in assessing the environmental quality of sediments.

Since port impacts are expected to increase in the future, as a consequence of growing maritime traffic and of trade globalization, robust knowledge on the response of benthic communities can be useful for public health purposes and to support decision-makers in performing environmental quality assessments (Luna et al. 2019). The ecological quality status of the sampled stations obtained using abiotic and biotic descriptors (i.e., mean ER-M quotients, organic matter content and nematode diversity and MI) was compared. Both the abiotic and nematode variables showed a general consistency for the stations of the two groups characterised by the highest and the lowest levels of contamination and organic enrichment, while the various classifications showed less consistent results for the other group of stations. Results obtained stress the importance of a multi-indicator approach to assess the port impact in the coastal system and to plan an appropriate management strategy.

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References

Abdulla, A. and Linden, O., 2008. Maritime Traffic Effects on Biodiversity in the Mediterranean Sea: Review of Impacts, Priority Areas and Mitigation Measures. IUCN Centre for Mediterranean Cooperation, Malaga, Spain, 184. p.p.

Anderson, M.J., Gorley, R.N., Clarke, K.R., 2008. PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods. PRIMER-E, Plymouth, United Kingdom.

Astolfi, M.L., Conti, M.E., Marconi, E. Massimi, L., Canepari, S., 2020. Effectiveness of Different Sample Treatments for the Elemental Characterization of Bees and Beehive Products. Molecules 25(18), 42-63.

Baeyens, W., Mirlean, N., Bundschuh, J., Winter, N., Baisch, P., da Silva Júnior, F.M.R., Gao, Y., 2019. Arsenic enrichment in sediments and beaches of Brazilian coastal waters: a review. Science of the Total Environment, 681, 143-154.

Baldrighi, E., Semprucci, F., Franzo, A., Cvitkovic, I., Bogner, D., Despalatovic, M., Berto, D., Malgorzata Formalewicz, M., Scarpato, A., Frapiccini, E., Marini, M., Grego, M., 2019. Meiofaunal communities in four Adriatic ports: Baseline data for risk assessment in ballast water management. Marine Pollution Bulletin, 147, 171-184.

Balsamo, M., Semprucci, F., Frontalini, F., Coccioni, R., 2012. Meiofauna as a tool for marine ecosystem biomonitoring. Marine Ecosystems, 4, 77-104.

Barbanti, A., and Bothner, M.H., 1993. A procedure for partitioning bulk sediments into distinct grain-size fractions for geochemical analysis. Environmental Geology, 21, 3-13.

Bertolotto, R.M., Ghioni, F., Frignani, M., Alvarado-Aguilar, D., Bellucci, L.G., Cuneo, C., Picca, M.R., Gollo, E., 2003a. Polycyclic aromatic hydrocarbons in surficial coastal sediments of the Ligurian Sea. Marine Pollution Bulletin, 46, 903-917.

Bertolotto, R.M., Tortarolo, B., Frignani, M., Bellucci, L.G., Albanese, S., Cuneo, C., 2003b. Heavy metals in coastal sediments of the Ligurian Sea off Vado Ligure. Journal de Physique IV France, 107, 159-162.

Bertolotto, R.M., Tortarolo, B., Frignani, M., Bellucci, L.G., Albanese, S., Cuneo, C., Alvarado-Aguilar, D., Picca, M.R., Gollo, E., 2005. Heavy metals in surficial coastal sediments of the Ligurian Sea. Marine Pollution Bulletin, 50, 348-356.

Bezerra, T.N., Decraemer, W.; Eisendle-Flöckner, U., Holovachov, O., Leduc, D., Miljutin, D., Sharma, J., Smol, N., Tchesunov, A., Mokievsky, V., Venekey, V., Vanreusel, A., 2018. NeMys: World Database of Free-Living Marine Nematodes. Accessed at http://nemys.ugent.be on 2018-06-09.

Beyrem, H., Boufahja, F., Hedfi, A., Essid, N., Aïssa, P., Mahmoudi, E., 2011. Laboratory Study on Individual and Combined Effects of Cobalt- and Zinc-Spiked Sediment on Meiobenthic Nematodes. Biological Trace Elements Research, 44, 790-803.

Bianchelli, S., Buschi, E., Danovaro, R., Pusceddu, A., 2018. Nematode biodiversity and benthic trophic state are simple tools for the assessment of the environmental quality in coastal marine ecosystems. Ecological Indicators, 95, 270-287.

Bongers, T., 1990. The maturity index: an ecological measure of environmental disturbance based on nematode species composition. Oecologia, 83, 14-19.

Bongers, T., Alkemade, R., Yeates, G.W., 1991. Interpretation of disturbance-induced maturity decrease in marine nematode assemblages by means of the Maturity Index. Marine ecology progress series, 76, 135-142.

Bongers, T. and Ferris, H., 1999. Nematode community structure as a bioindicator in environmental monitoring. Trends in Ecology & Evolution, 14, 224-228.

Boufahja, F., Hedfi, A., Amorri, J., Aïssa, P., Beyrem, H., Mahmoudi E., 2011. An Assessment of the Impact of Chromium-Amended Sediment on a Marine Nematode Assemblage Using Microcosm Bioassays. Biological Trace Elements Research, 142, 242-255.

Boufahja, F. and Semprucci, F., 2015. Stress-induced selection of a single species from an entire meiobenthic nematode assemblage: is this possible using iron enrichment and does pre-exposure affect the ease of the process? Environmental Science and Pollution Research, 22, 1979-1998.

Briant, N., Bancon-Montigny, C., Elbaz-Poulichet, F., Freydier, R., Delpoux, S., Cossa, D., 2013. Trace elements in the sediments of a large Mediterranean marina (Port Camargue, France): Levels and contamination history. Marine Pollution Bulletin, 73, 78-85.

Brook, E. J., and Moore, J. N., 1988. Particle-size and chemical control of As, Cd, Cu, Fe, Mn, Ni, Pb, and Zn in bed sediment from the Clark Fork River, Montana (U.S.A.). Science of the Total Environment, 76, 247-266.

Bubb, J. M., Rudd, T., Lester, J. N., 1991. Distribution of heavy metals in the River Yare and its associated broads. III. Lead and zinc. Science of the Total Environment, 102, 189-208.

Burton Jr, G.A., 2002. Sediment quality criteria in use around the world. Limnology, 3(2), 65-76.

Chapman, P.M., 2007. Determining when contamination is pollution—weight of evidence determinations for sediments and effluents. Environment International, 33 (4), 492-501.

Chapman, P.M., and Hollert, H., 2006. Should the sediment quality triad become a tetrad, a pentad, or possibly even a hexad? Journal of Soils and Sediments, 6 (1), 4-8.

Chapman, P.M., McDonald, B.G., Lawrence, G.S., 2002. Weight-of-evidence issues and frameworks for sediment quality (and other) assessments. Human and Ecological Risk Assessment, 8 (7), 1489-1515.

Chatzinikolaou, E., Mandalakis, M., Damianidis, P., Dailianis, T., Gambineri, S., Rossano, C., Scapini, F., Carucci, A., Arvanitidis, C., 2018. Spatio-temporal benthic biodiversity patterns and pollution pressure in three Mediterranean touristic ports. Science of the Total Environment, 624, 648-660.

Chen, C.A., Soo, C.L., Balsamo, M., Semprucci, F., 2018. An approach based on nematode descriptors for the ecological quality (EcoQ) classification of the Malaysian coasts. Marine Biodiversity, 48, 117-126.

Clarke, K.R. and Gorley, R.N., 2006. PRIMER V6: User Manual/Tutorial. PRIMER-E, Plymouth, UK.

Coull, B.C., and Chandler, G.T., 1992. Pollution and meiofauna: field, laboratory, and mesocosms studies. Oceanography and Marine Biology an Annual Review, 30, 191-271.

Dalto, A.G., Grémare, A., Dinet, A., Fichet, D., 2006. Muddy-bottom meiofauna responses to metal concentrations and organic enrichment in New Caledonia South-West Lagoon. Estuarine, Coastal and Shelf Science 67, 629-644.

Darbra, R.M., Ronza, A., Stojanovic, T.A., Wooldridge, C., Casal, J., 2005. A procedure for identifying significant environmental aspects in sea ports. Marine Pollution Bulletin, 50, 866-874.

Davies, C.A., Tomlinson, K., Stephenson, T., 1991. Heavy metals in River tees estuary sediments. Environmental Technologies, 12, 961 - 972.

de Carvalho Aguiar, V.M., Abuchacra, P.F.F., Neto, J.A.B., de Oliveira, A.S., 2018. Environmental assessment concerning trace metals and ecological risks at Guanabara Bay, RJ, Brazil. Environmental monitoring and assessment, 190 (8), 1-17.

Demopoulos, A.W.J., Bourque, J.R., Cordes, E., Stamler, K.M., 2016. Impacts of the Deepwater Horizon oil spill on deep-sea coral-associated sediment communities. Marine Ecology Progress Series, 561, 51-68.

Di Toro, D.M., Zarba, C.S., Hansen, D.J., Berry, W.J., Cowan, C.E., Pavlou, S.P., Allen, H.E., Thomas, N.A., Paquin, P.R., 1991. Technical basis for establishing sediment quality criteria for nonionic organic chemicals using equilibrium partitioning. Environmental Toxicology and Chemistry, 10, 1541-1583.

Drago, M., Cosma, B., Contardi, V., 1981. Distribution of heavy metals in superficial marine sediments of Savona Harbor and Vado Ligure Bay (Ligurian Sea). Att. Soc. Toscana. Sc. Nat., 88, 117-136.

Dubois, M., Gilles, K., Mamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric method for determination of sugar and related substances. Analytical Chemistry, 28, 350-356.

Fabiano, M., Danovaro, R., Fraschetti, S., 1995. A three-year time series of elemental and biochemical composition of organic matter in subtidal sandy sediment of the Ligurian Sea (NW Mediterranean). Continental Shelf Research, 15, 1453-1469.

Fichet, D., Boucher, G., Radenac, G., Miramand, P., 1999. Concentration and mobilisation of Cd, Cu, Pb and Zn by meiofauna populations living in harbour sediment: their role in the heavy metal flux from sediment to food web. The Science of the Total Environment, 243, 263-272.

Fonseca, G., Muthumbi, A.W., Vanreusel, A., 2007. Species richness of the genus *Molgolaimus* (Nematoda) from local to ocean scale along continental slopes. Marine Ecology, 28, 446-459.

Fonseca, G., Vanreusel, A., Decraemer, W., 2006. Taxonomy and biogeography of *Molgolaimus* Ditlevsen, 1921 (Nematoda: Chromadoria) with reference to the origins of deep sea nematodes. Antarctic Science, 18, 23-50.

Förstner, U., 1989. Contaminated Sediments. Springer, Berlin.

Förstner, U., and Wittmann, G.T.W., 1979. Metal Pollution in the Aquatic Environment. Springer-Verlag, Berlin.

Franzo, A., Asioli, A., Roscioli, C., Patrolecco, L., Bazzaro, M., Del Negro, P., Cibic, T. 2019. Influence of natural and anthropogenic disturbances on foraminifera and free-living nematodes in four lagoons of the Po delta system. Estuarine, Coastal and Shelf Science, 220, 99-110.

Frontalini, F., Semprucci, F., Di Bella, L., Caruso, A., Cosentino, C., Maccotta, A., Scopelliti, G., Sbrocca, C., Bucci, C., Balsamo, M., Martins, V., Armynot du Châtelet, E., Coccioni, R., 2018. The response of cultured meiofaunal and benthic foraminiferal communities to lead contamination: results from mesocosm experiments. Environmental Toxicology and Chemistry, 37, 2439–2447.

Gallucci F., Braga de Castro I., Perina F.C., Modelo de Souza Abessa D., de Paula Teixeira A., 2015. Ecological effects of Irgarol 1051 and Diuron on a coastal meiobenthic community: A laboratory microcosm experiment. Ecological Indicators, 58, 21-31.

Fiscus, D.A., Neher, D.A., 2002. Distinguishing sensitivity of free-living soil nematode genera to physical and chemical disturbances. Ecological Applications 12, 565-575.

Gambi, G., Canals, M., Corinaldesi, C., Dell'Anno, A., Manea, E., Pusceddu, A., Sanchez-Vidal, A., Danovaro, R. 2020a. Impact of historical sulfide mine tailings discharge on meiofaunal assemblages (Portmán Bay, Mediterranean Sea). Science of The Total Environment, 736, 139-641.

Gambi, G., Dell'Anno, A., Corinaldesi, C., Lo Martire, M., Musco, L., Da Ros, Z., Armiento, G., Danovaro R., 2020b. Impact of historical contamination on meiofaunal assemblages: The case study of the Bagnoli-Coroglio Bay (southern Tyrrhenian Sea), Marine Environmental Research, 156, 104-907.

García-Rico, L., Valenzuela Rodríguez, M., Jara-Marini, M.E., 2006. Geochemistry of mercury in sediment of oyster areas in Sonora, Mexico. Marine Pollution Bulletin, 52, 453-458.

Gerchakov, S.M. and Hatcher, P.G., 1972. Improved technique for analysis of carbohydrates in sediments. Limnology and Oceanography, 17, 938-943.

Gerlach, S.A., 1971. On the importance of marine meiofauna for benthos communities. Oecologia (Berlin), 6, 176-190.

Gray, J.S. and Elliot, M., 2010. Ecology of Marine Sediments: From Science to Management. UK: Oxford University Press.

Hägerbäumer, A., Höss, S., Heininger, P., Traunspurger, W., 2015. Experimental studies with nematodes in ecotoxicology: an overview. Journal of nematology, 47, 11-27.

Hadlich, H.L., Venturini, N., Martins, C.C., Hatje, V., Tinelli, P., de Oliveira Gomes, L.E., Bernardino, A. F. 2018. Multiple biogeochemical indicators of environmental quality in tropical estuaries reveal contrasting conservation opportunities. Ecological Indicators, 95, 21-31.

Hartree, E.F., 1972. Determination of proteins: a modification of the Lowry method that give a linear photometric response. Analytical Biochemistry, 48, 422-427.

Heip, C., Vincx, M., Vranken, G., 1985. The ecology of marine nematodes. Oceanography and Marine Biology - An Annual Review, 23, 399-489.

Hermi, M., Mahmoudi, E., Beyrem, H., Aïssa, P., Essid N., 2009. Responses of a Free-Living Marine Nematode Community to Mercury Contamination: Results from Microcosm Experiments. Archives of Environmental Contamination and Toxicology, 56, 426-433.

Hong, J.H., Semprucci, F., Raehyuk, J., Kim, K., Lee, S., Jeon, D., Yoo, H., Kim, J., Yeom, J., Lee, S., Lee, K., Lee, W., 2020. Meiobenthic nematodes in the assessment of the relative impact of human activities on coastal marine ecosystem. Environmental Monitoring and Assessment, 192, 1-13.

Höss, S., Claus, E., Von der Ohe, P.C., Brinke, M., Güde, H., Heininger, P., Traunspurger, W., 2011. Nematode species at risk e a metric to assess pollution in soft sediments of freshwaters. Environment International 37, 940-949.

Ingole, B., Sivadas, S., Nanajkar, M., Sautya, S., Nag, A., 2009. A comparative study of macrobenthic community from harbours along the central west coast of India. Environmental Monitoring and Assessment, 154, 135-146.

Jiang, X., Teng, A., Xu, W., & Liu, X., 2014. Distribution and pollution assessment of heavy metals in surface sediments in the Yellow Sea. Marine Pollution Bulletin, 83, 366-375.

Jouili, S., Essid, N., Semprucci, F., Boufahja, F., Nasri, A., Beyrem, H., 2017. Environmental quality assessment of El Bibane lagoon (Tunisia) using taxonomical and functional diversity of meiofauna and nematodes. Journal of Marine Biological Association of the United Kingdom, 97, 1593-1603.

Kennedy, A.D. and Jacoby, C.A., 1999. Biological Indicators of Marine Environmental Health: Meiofauna – A Neglected Benthic Component? Environmental Monitoring and Assessment, 54, 47–68.

Kuppusamy, S., Maddela, N.R., Megharaj, M., Venkateswarlu, K., 2020. Methodologies for Analysis and Identification of Total Petroleum Hydrocarbons. Total Petroleum Hydrocarbons. Springer, 29-50.

Liu, X., Huang, D., Zhu, Y., Chang, T., Liu, Q., Huang, L., Zhao, W., Lin, K., Liu, L., 2015. Bioassessment of marine sediment quality using meiofaunal assemblages in a semi-enclosed bay. Marine Pollution Bulletin, 100, 92-101.

Long, E.R., Field, L.J., MacDonald, D.D., 1998. Predicting toxicity in marine sediments with numerical sediment quality guidelines. Environmental Toxicology and Chemistry, 17, 714-727.

Long, E.R. and MacDonald, D.D., 1998. Recommended uses of empirically derived sediment quality guidelines for marine and estuarine ecosystems. Human and Ecology Risk Assessment, 4, 1019-1039.

Long, E.R., MacDonald, D.D., Smith, S.L., Calder, F.D., 1995. Incidence of adverse biological effects within ranges of chemical concentrations in marine and estuarine sediments. Journal of environmental management, 19, 867–876.

Losi, V., Ferrero, T.J., Moreno, M., Gaozza, L., Rovere, A., Firpo, M., Marques, J.C., Albertelli, G., 2013. The use of nematodes in assessing ecological conditions in shallow waters surrounding a Mediterranean harbour facility. Estuarine, Costal and Shelf Science, 130, 209-221.

Losi, V., Montefalcone, M., Moreno, M., Giovannetti, E., Gaozza, L., Grondona, M., Albertelli, G., 2012. Nematodes as indicators of environmental quality in seagrass (P*osidonia oceanica*) meadows of the NW Mediterranean Sea. Advances in Oceanography and Limnology, 3, 69-91.

Luna, G.M., Manini, E., Turk, V., Tinta, T., D'Errico, G., Baldrighi, E., Baljak, V., Buda, D., Cabrini, M., Campanelli, A., Cenov, A., Del Negro, P., Drakulović, D., Fabbro, C., Glad, M., Grilec, D., Grilli, F., Jokanović, S., Jozić, S., Kauzlarić, V., Kraus, R., Marini, M., Mikuš, J., Milandri, S., Pećarević, M., Perini, L., Quero, G.M., Šolić, M., Vukić Lušić, D., Zoffoli, S., 2019, Status of faecal pollution in ports: A basin-wide investigation in the Adriatic Sea, Marine Pollution Bulletin, 147, 219-228.

Ma, Y, Sun, Y, Li, Y, Zheng, H, Mi, W, 2020. Polycyclic aromatic hydrocarbons in benthos of the northern Bering Sea Shelf and Chukchi Sea Shelf, Journal of Environmental Sciences, 97, 194-199.

Marin, V., Moreno, M., Vassallo, P., Vezzulli, L., Fabiano, M., 2008. Development of a multistep indicator-based approach (MIBA) for the assessment of environmental quality of harbours. – ICES Journal of Marine Science, 65, 1436-1441.

McCready, S., Slee, G.F., Birch, G.F., Taylor, S.E., 2000. The distribution of polycyclic aromatic hydrocarbons in surficial sediments of Sydney Harbour, Australia. Marine Pollution Bulletin, 40, 999-1006.

Mirto, S., La Rosa, T., Gambi, C., Danovaro, R., Mazzola, A., 2002. Nematode community response to fish-farm impact in the western Mediterranean. Environmental Pollution, 116, 203-214.

Mirto, S., Arigò, C., Genovese, L., Pusceddu, A., Gambi, C., Danovaro, R., 2014. Nematode assemblage response to fish-farm impact in vegetated (*Posidonia oceanica*) and non-vegetated habitats. Aquaculture Environment Interactions, 5(1), 17-28.

Montagna, P.A. and Li, J., 1997. Modelling contaminant effects on deposit feeding nematodes near Gulf of Mexico production platforms. Ecological Modelling, 98(2,3), 151-162.

Moreno, M., Albertelli, G., Fabiano, M., 2009. Nematode response to metal, PAHs and organic enrichment in tourist marinas of the Mediterranean Sea. Marine Pollution Bulletin, 58, 1192-1201.

Moreno, M., Ferrero, T.J., Gallizia, I., Vezzulli, L., Albertelli, G., Fabiano, M., 2008. An assessment of the spatial heterogeneity of environmental disturbance within an enclosed harbour through the analysis of meiofauna and nematode assemblages. Estuarine, Coastal and Shelf Science, 77, 565-576.

Moreno, M., Semprucci, F., Vezzulli, L., Balsamo, M., Fabiano, M., Albertelli, G., 2011. The use of nematodes in assessing ecological quality status in the Mediterranean coastal ecosystems. Ecological Indicators, 11, 328-336.

Neira, C., King, I., Mendoza, G., Sellanes, J., DeLey, P., Levin, L.A., 2013. Nematode community structure along a central Chile margin transect influenced by the oxygen minimum zone. Deep-Sea Research Part I, 78, 1-15.

Nikolaou, A., Kostopoulou, M., Petsas, A., Vagi, M., Lofrano, G., Meric, S. 2009. Levels and toxicity of polycyclic aromatic hydrocarbons in marine sediments. TrAC Trends in Analytical Chemistry, 28, 653-664.

Olayinka, O.O., Adewusi, A.A., Olarenwaju, O.O., Aladesida A.A., 2018. Concentration of Polycyclic Aromatic Hydrocarbons and Estimated Human Health Risk of Water Samples Around Atlas Cove, Lagos, Nigeria. Journal of Health and Pollution, 8(20), 181-210.

Parker, J.G., 1983. A comparison of methods used for the measurement of organic matter in sediments. Chemistry and Ecology, 1, 201-210.

Pielou, E. C., 1969. An Introduction to Mathematical Ecology. Wiley-Interscience: New York.

Platt, H.M. and Warwick, R.M., 1983. Free-living Marine Nematodes. Part I. British Enoplids. Synopses of the British Fauna (New Series). Cambridge University Press: Cambridge.

Platt, H.M. and Warwick, R.M., 1988. Free-living Marine Nematodes. Part II. British Chromadorids. Synopses of the British Fauna (New Series). Brill, Leiden.

Schintu, M., Marrucci, A., Marras, B., Galgani, F., Buosi, C., Ibba, A., Cherchi, A., 2016. Heavy metal accumulation in surface sediments at the port of Cagliari (Sardinia, western Mediterranean): Environmental assessment using sequential extractions and benthic foraminifera. Marine Pollution Bulletin, 111, 45-56.

Schratzberger, M., & Warwick, R.M., 1998. Effects of the intensity and frequency of organic enrichment on two estuarine nematode communities. Marine Ecology Progress Series, 164, 83-94.

Schratzberger, M., Rees, H.L. Boyd, S.E., 2000. Effects of the simulated deposition of dredged material on the structure of nematode assemblages-the role of burial. Marine Biology, 136, 519-530.

Schratzberger, M., Warr, K., Rogers, S.I., 2007. Functional diversity of nematode communities in the southwestern North Sea. Marine Environmental Research, 63, 368-89.

Schratzberger, M. and Somerfield, P.J., 2020. Effects of widespread human disturbances in the marine environment suggest a new agenda for meiofauna research is needed. Science of the Total Environment, 728, 138-435.

Sedano, F, Marquina, D., Espinosa, F., 2014. Usefulness of meiofauna at high taxonomic levels as a tool to assess harbour quality status. Marina del Este Harbor (Granada, Spain) as a case study. Revista Ciencias Marinas y Costeras, 6, 103-113.

Seinhorst, J.W., 1959. A rapid method for the transfer of nematodes from fixative to unhydrous glycerine. Nematologica 4, 67-69.

Semprucci, F. and Balsamo, M., 2012. Key role of free-living nematodes in the marine ecosystem. In: F. Boeri and Jordan A.C. (Eds) Nematodes: Morphology, Functions and Management Strategies. NOVA Science Publishers, Inc. Hauppauge, NY, 109-134.

Semprucci, F. and Balsamo, M., 2014. Free-living Marine Nematodes as Bioindicators: Past, Present and Future Perspectives. Trends in Environmental Science, 6, 17-36.

Semprucci, F., Balsamo, M., Appolloni, L., Sandulli, R., 2018a. Assessment of ecological quality status along the Apulian coasts (Eastern Mediterranean Sea) based on meiobenthic and nematode assemblages. Marine Biodiversity, 48, 105-115.

Semprucci, F., Balsamo, M., Frontalini, F., 2014. The nematode assemblage of a coastal lagoon (Lake Varano, Southern Italy): ecology and biodiversity patterns. Scientia Marina, 78, 579-588.

Semprucci, F., Balsamo, M., Sandulli, R., 2016. Assessment of the Ecological quality (EcoQ) of the Venice lagoon using the structure and biodiversity of the meiofaunal assemblages. Ecological Indicators, 67C, 451-457.

Semprucci, F., Boi, P., Manti, A., Covazzi-Harriague, A., Rocchi, M., Colantoni, P., Papa, S., Balsamo, M., 2010a. Benthic communities along a littoral of the Central Adriatic Sea (Italy). Helgoland Marine Research, 64, 101-115.

Semprucci, F., Colantoni, P., Baldelli, G., Rocchi, M., Balsamo, M., 2010b. The distribution of meiofauna on back-reef sandy platforms in the Maldives (Indian Ocean). Marine Ecology: An evolutionary perspective, 31, 592-607.

Semprucci, F., Colantoni, P., Sbrocca, C., Baldelli, G., Rocchi, M., Balsamo, M., 2011. Meiofauna in sandy back-reef platforms differently exposed to the monsoons in the Maldives (Indian Ocean). Journal of Marine Systems, 87, 208–215.

Semprucci, F., Cesaroni, L., Guidi, L., Balsamo, M., 2018b. Do the morphological and functional traits of free-living marine nematodes mirror taxonomical diversity? Marine Environmental Research, 135, 114-122.

Semprucci, F., Frontalini, F., Sbrocca, C., Armynot du Châtelet, E., Bout-Roumazeilles, V., Coccioni, R., Balsamo, M., 2015a. Meiobenthos and free-living nematodes as tools for biomonitoring environments affected by riverine impact. Environmental Monitoring and Assessment, 187, 251.

Semprucci, F., Losi, V., Moreno, M, 2015b. A review of Italian research on free-living marine nematodes and the future perspectives on their use as Ecological Indicators (EcoInds). Mediterranean Marine Science, 16, 352-365.

Semprucci, F., Sbrocca, C., Rocchi, M., Balsamo, M., 2015c. Temporal changes of the meiofaunal assemblage as a tool for the assessment of the ecological quality status. Journal of Marine Biological Association of the United Kingdom, 95, 247-254.

Shannon, C. and Weaver, W., 1949. The Mathematical Theory of Communication. University of Illinois Press, Urbana, 117.

Schintu, M., Marrucci, A., Marras, B., Galgani, F., Buosi, C., Ibba, A., Cherchi, A., 2016. Heavy metal accumulation in surface sediments at the port of Cagliari (Sardinia, western Mediterranean): Environmental assessment using sequential extractions and benthic

foraminifera. Marine Pollution Bulletin, 111, 45-56.

Singh, R. and Ingole, B.S., 2016. Structure and function of nematode communities across the Indian western continental margin and its oxygen minimum zone. Biogeosciences, 13, 191-209.

Somerfield, P.J., Gee, J.M., Warwick, R.M. 1994. Soft sediment meiofaunal community structure in relation to a long-term heavy metal gradient in the Fal estuary system. Marine Ecology Progress Series, 105, 79-88.

Soko, M.I. and Gyedu-Ababio, T.K., 2019. Free-Living Nematodes as Pollution Indicator in Incomati River Estuary, Mozambique. Open Journal of Ecology, 9, 117-133.

Sørensen, L., Meier, S., Mjøs, S.A., 2016. Application of gas chromatography/tandem mass spectrometry to determine a wide range of petrogenic alkylated polycyclic aromatic hydrocarbons in biotic samples. Rapid Communications in mass spectrometry, 30, 2052-2058.

Soto, L.A., Salcedo, D.L., Arvizu, K., Botello, A.V., 2017. Interannual patterns of the large free-living nematode assemblages in the Mexican Exclusive Economic Zone, NW Gulf of Mexico after the Deepwater Horizon oil spill. Ecological Indicators, 79, 371-381.

Team, R.C., 2011. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org

Tietjen, J.H,. 1980. Microbial meiofaunal interrelationships: a review. In Microbiology 1980. VIII Conference of the American Society of Microbiology on aquatic microbial ecology, 7-10 February 1979, Clearwater Beach, Florida. (pp. 335-338). American Society for Microbiology.

Trannum, H.C., Olsgard, F., Skei, J.M., Indrehus, J., Øverås, S., Eriksen, J., 2004. Effects of copper, cadmium and contaminated harbour sediments on recolonisation of soft-bottom communities. Journal of Experimental Marine Biology and Ecology, 310, 87-114.

Ürkmez, D., Sezgin, M., Bat, L., 2014. Use of nematode maturity index for the determination of ecological quality status: a case study from the Black Sea. Journal of the Black Sea/Mediterranean Environment, 20 (2), 96.

Vanaverbeke, J., Soetaert, K., Vincx, M., 2004. Changes in morphometric characteristics of nematode communities during a spring phytoplankton bloom deposition. Marine Ecology Progress Series, 273, 139-146.

Vanreusel, A., Fonseca, G., Danovaro, R., da Silva, M. C., Esteves, A. M., Ferrero, T., Gad, G., Galtsova, V., Gambi, C., et al., 2010. The contribution of deep-sea macrohabitat heterogeneity to global nematode diversity. Marine Ecology-An Evolutionary Perspective, 31, 6-20.

Veiga, P., Rubal, M., Besteiro, C., 2009. Shallow sublittoral meiofauna communities and sediment polycyclic aromatic hydrocarbons (PAHs) content on the Galician coast (NW Spain), six months after the Prestige oil spill. Marine Pollution Bulletin, 58, 581-588.

Vezzulli, L., Moreno, M., Marin, V., Pezzati, E., Bartoli, M., Fabiano, M., 2008. Organic waste impact of capture- based Atlantic bluefin tuna aquaculture at an exposed site in the Mediterranean Sea. Estuarine Coastal Shelf Science, 78, 369-384.

Warwick, R.M., Platt, H.M., Somerfield, P.J., 1998. Free-living Marine Nematodes. Part III. British Monhysterids. Synopses of the British Fauna (New Series). Field Studies Council, Shrewsbury: UK.

Wentworth, C.K., 1922. A scale of grade and class terms for clastic sediments. Journal of Geology, 30, 377-392.

Wieser, W., 1953. Die Beziehung zwischen Mundhöhlengestalt, Ernährungsweise und Vorkommen bei freilebenden marinen nematoden. Eine okologisch-morphologische studie. Arkiv for Zoologi, 4, 439-484.

Yuan, H., Song, J., Li, X., Li, N., Duan, L., 2012. Distribution and contamination of heavy metals in surface sediments of the South Yellow Sea. Marine Pollution Bulletin, 64, 2151-2159.

Sitography

Monitoraggio naturalistico, published in Porto Vado, http://portovado.cimafoundation.org/?page_id=192, 15/04/2021

Table 1. Coordinates, depth, sedimentological characteristics and organic matter concentration and biochemical composition at each sampling station.

	A7	B5	B6	B7	C5	C6	C7	E4	E5	F2	F3	G2	G3	H1	H2	H3	I1	I2	J1	K1	K2	K3	L1	L2	L3	M1	M2	M3
Long.	8.47	8.46	8.46	8.47	8.46	8.46	8.47	8.46	8.47	8.46	8.47	8.47	8.46	8.46	8.46	8.47	8.46	8.46	8.46	8.46	8.46	8.46	8.45	8.46	8.46	8.45	8.46	8.46
Lat.	44.28	44.27	44.27	44.27	44.27	44.27	44.27	44.27	44.27	44.26	44.26	44.26	44.26	44.26	44.26	44.26	44.26	44.26	44.25	44.25	44.25	44.25	44.25	44.25	44.25	44.25	44.25	44.25
Depth (m)	50.00	44.80	52.00	58.00	48.50	57.50	68.00	61.00	78.00	64.00	74.00	65.00	43.50	29.00	45.00	79.00	37.00	37.00	70.00	64.00	44.50	72.50	16.00	37.00	48.50	11.50	25.00	56.00
Gravel (%)	0.37	0.56	0.20	13.14	0.10	0.00	0.00	16.44	0.05	0.26	2.77	0.63	8.12	1.26	3.41	0.24	2.63	0.49	1.41	30.41	4.66	0.50	49.44	8.72	23.96	9.34	24.71	5.98
Sand (%)	38.17	32.04	14.26	20.23	32.28	32.84	14.68	9.94	20.03	17.06	11.04	37.65	69.11	82.60	55.00	8.19	59.20	51.85	26.10	68.55	67.34	44.14	49.81	80.71	67.68	89.30	66.27	77.52
Silt (%)	52.36	57.00	73.53	54.63	57.32	56.86	72.32	57.82	64.53	66.53	69.38	44.32	20.48	13.64	33.69	70.17	30.67	38.06	59.39	1.03	22.60	43.85	0.76	8.78	6.65	1.36	9.02	14.11
Clay (%)	9.10	10.40	12.00	12.00	10.30	10.30	13.00	15.80	15.40	16.15	16.81	17.40	2.30	2.50	7.90	21.40	7.50	9.60	13.10	0.00	5.40	11.50	0.00	1.80	1.70	0.00	0.00	2.40
Grain size (mm)	0.04	0.04	0.02	0.24	0.04	0.04	0.02	0.94	0.03	0.03	0.02	0.09	0.60	0.09	0.27	0.01	0.15	0.06	0.04	1.47	0.30	0.07	1.64	0.61	1.12	1.04	1.63	0.33
Grade	Coarse silt	Coarse silt	Med silt	Fine sand	Coarse silt	Coarse silt	Med silt	Coarse sand	Med silt	Med silt	Med silt	Very fine sand	Coarse sand	Very fine sand	Fine sand	Fine silt	Fine sand	Very fine sand	Coarse silt	Very coarse sand	Med sand	Very fine sand	Very coarse sand	Coarse sand	Very coarse sand	Coarse sand	Very coarse sand	Med sand
Sorting	2.40	2.53	2.05	4.19	2.25	2.32	2.17	4.88	2.45	2.37	2.45	3.38	2.65	1.15	3.33	2.37	3.01	2.65	2.45	0.63	3.01	2.85	0.95	2.13	1.98	0.55	2.41	2.29
TOM (%)	5.36	4.47	12.70	12.01	9.89	23.50	6.67	3.73	5.93	8.10	5.04	10.45	4.08	3.52	4.20	8.10	3.37	3.67	2.35	0.57	3.34	3.29	1.22	3.75	2.28	0.72	3.24	2.29
PRT (mg g DW^{-1})	1.35	1.25	1.04	1.21	1.31	1.36	1.35	0.89	1.05	0.98	1.06	0.22	0.54	0.93	0.27	0.26	0.19	0.21	0.11	0.02	0.23	0.61	0.02	0.05	0.01	0.05	0.22	0.22
CHO (mg g DW^{-1})	2.76	2.53	2.97	2.48	3.90	3.86	3.04	1.89	2.24	1.92	2.79	1.47	1.08	0.59	1.20	1.78	1.46	1.62	0.78	0.21	1.65	1.12	0.32	1.44	0.36	0.39	1.14	0.79

Table 2. Taxonomical and functional indices of the nematode assemblage revealed at each sampling station.

Station	Number of genera	Pielou index (J')	Shannon index (H')	Index of Trohic Diversity (ITD)	Maturity Index (MI)
A7	5±1	0.7±0.2	1.5±0.5	0.48±0.12	2.85±0.01
B5	11±7	0.6±0.3	2.1±1.2	0.57±0.30	1.91±0.66
B6	10±1	0.8±0.1	2.5±0.2	0.35±0.01	2.66±0.24
B7	9±1	0.8±0.1	2.4±0.3	0.36±0.03	2.71±0.07
C5	7±3	0.7±0.2	1.9±0.1	0.42±0.07	2.79±0.11
C6	12±4	0.7±0.2	2.3±0.3	0.50±0.19	2.24±0.22
C7	10±1	0.7±0.2	2.4±0.4	0.39±0.08	2.75±0.53
E4	12±1	0.8±0.1	2.7±0.6	0.34±0.01	2.61±0.10
E5	10±1	0.9±0.0	2.8±0.1	0.31±0.03	2.60±0.14
F2	11±2	0.8±0.1	2.6±0.5	0.34±0.02	2.64±0.12
F3	20±11	0.9±0.0	3.6±0.9	0.36±0.01	2.87±0.24
G2	30±11	0.9±0.0	4.2±0.3	0.32±0.08	2.86±0.24
G3	40±6	0.9±0.0	4.8±0.4	0.31±0.02	2.80±0.06
H1	11±10	0.6±0.3	2.0±1.7	0.41±0.23	2.75±0.40
H2	31±4	0.9±0.0	4.4±0.0	0.33±0.00	2.88±0.01
H3	26±6	0.8±0.0	3.8±0.5	0.33±0.06	2.68±0.02
I1	37±0	0.9±0.0	4.7±0.2	0.32±0.05	2.98±0.12
I2	33±4	0.9±0.0	4.4±0.2	0.32±0.04	2.69±0.20
J1	16±4	0.6±0.3	2.5±1.3	0.38±0.16	2.77±0.10
K1	19±1	0.7±0.1	3.0±0.3	0.54±0.00	3.24±0.15
K2	39±1	0.9±0.0	4.8±0.1	0.32±0.01	2.82±0.01
K3	30±3	0.9±0.0	4.2±0.3	0.36±0.03	2.82±0.06
L1	21±4	0.8±0.0	3.3±0.0	0.48±0.03	3.38±0.22
L2	37±4	0.9±0.0	4.4±0.2	0.32±0.04	2.64±0.19
L3	20±5	0.8±0.1	3.3±0.1	0.39±0.06	2.37±0.06
M1	19±1	0.8±0.0	3.3±0.0	0.49±0.01	3.35±0.16
M2	37±14	0.9±0.1	4.5±0.8	0.31±0.04	2.82±0.09
M3	43±2	0.9±0.0	4.9±0.1	0.30±0.00	2.88±0.07

Table 3. Results of the distance-based linear model (DistLM) of nematode assemblage composition on environmental variables: (a) marginal tests results; (b) results from the BEST model using the AIC criterion. (P, significance value; Prop., amount of explained variation).

	Variable	Pseudo-F	*P*	*Prop.*
(a) Marginal tests	Gravel	4.8498	0.001	0.15721
	Sand	5.7389	0.001	0.18082
	Silt	9.2235	0.001	0.26186
	Clay	7.5998	0.001	0.22619
	As	1.7672	0.118	6.3644e^{-2}
	Cd	-4.8397e^{-15}	1	-1.8614e^{-16}
	Hg	6.4533	0.001	0.19885
	Pb	3.6581	0.009	0.12334
	Sn	4.7031	0 .001	0.15318
	Zn	5.5117	0.002	0.17491
	PAHs	6.8442	0.001	0.20838
	C>12	8.6834	0.001	0.25036
	ER-Mq Met	5.6301	0.001	0.178
	ER-Mq PAHs	4.9477	0.002	0.15987
	TOM	6.7452	0.001	0.20599
	PRT	11.274	0.001	0.30247
	CHO	8.8784	0.001	0.25455

	Model	AIC	R^2	RSS
(b) Best solutions	Silt+Clay+As+Pb+PAHs+ER-Mq PAHs+PRT	202,63	0.61899	21976

Table 4. Ecological Quality (EcoQ) based on nematodes at each sampling station. The final classification was obtained following the principles applied by Chen et al. (2018).

Station	H' EcoQ	MI EcoQ	Final EcoQ Status using nematodes
A7	poor	high	moderate
B5	poor	bad	bad
B6	poor	good	moderate
B7	poor	good	moderate
C5	poor	good	moderate
C6	poor	poor	poor
C7	poor	good	moderate
E4	moderate	good	moderate
E5	moderate	moderate	moderate
F2	moderate	good	moderate
F3	good	high	good
G2	good	high	good
G3	high	good	good
H1	poor	good	moderate
H2	good	high	good
H3	good	good	good
I1	high	high	high
I2	good	good	good
J1	poor	good	moderate
K1	moderate	high	good
K2	high	high	high
K3	good	high	good
L1	moderate	high	good
L2	good	good	good
L3	moderate	poor	poor
M1	moderate	high	good
M2	good	high	good
M3	high	high	high

Figure 1. Map of the study area: Vado Ligure, Western Ligurian Sea (NW Mediterranean).

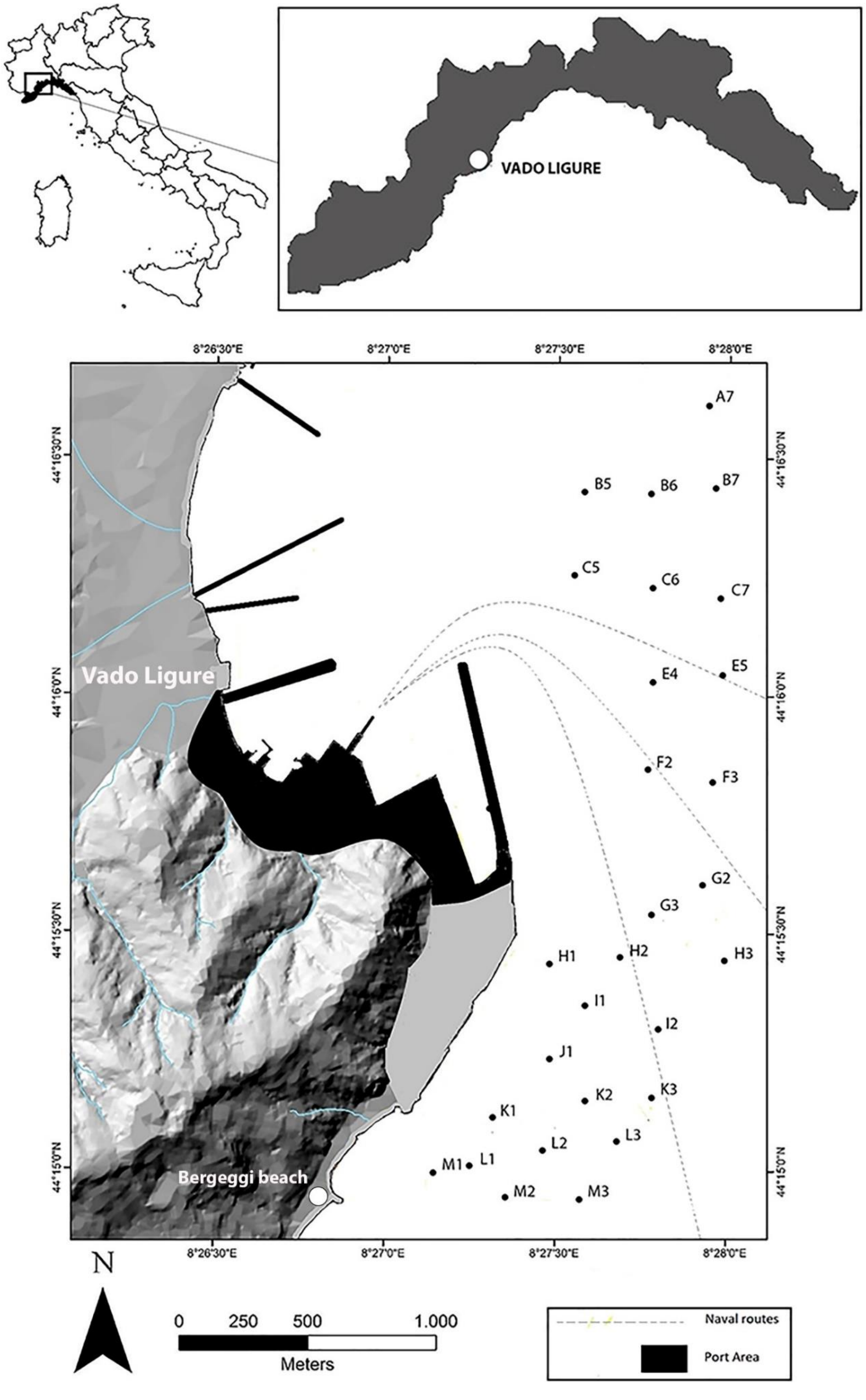

Figure 2. Heavy metals, C>12 and PAHs concentrations at the various sampling stations, with indication of ER-M and ER-L threshold (horizontal lines).

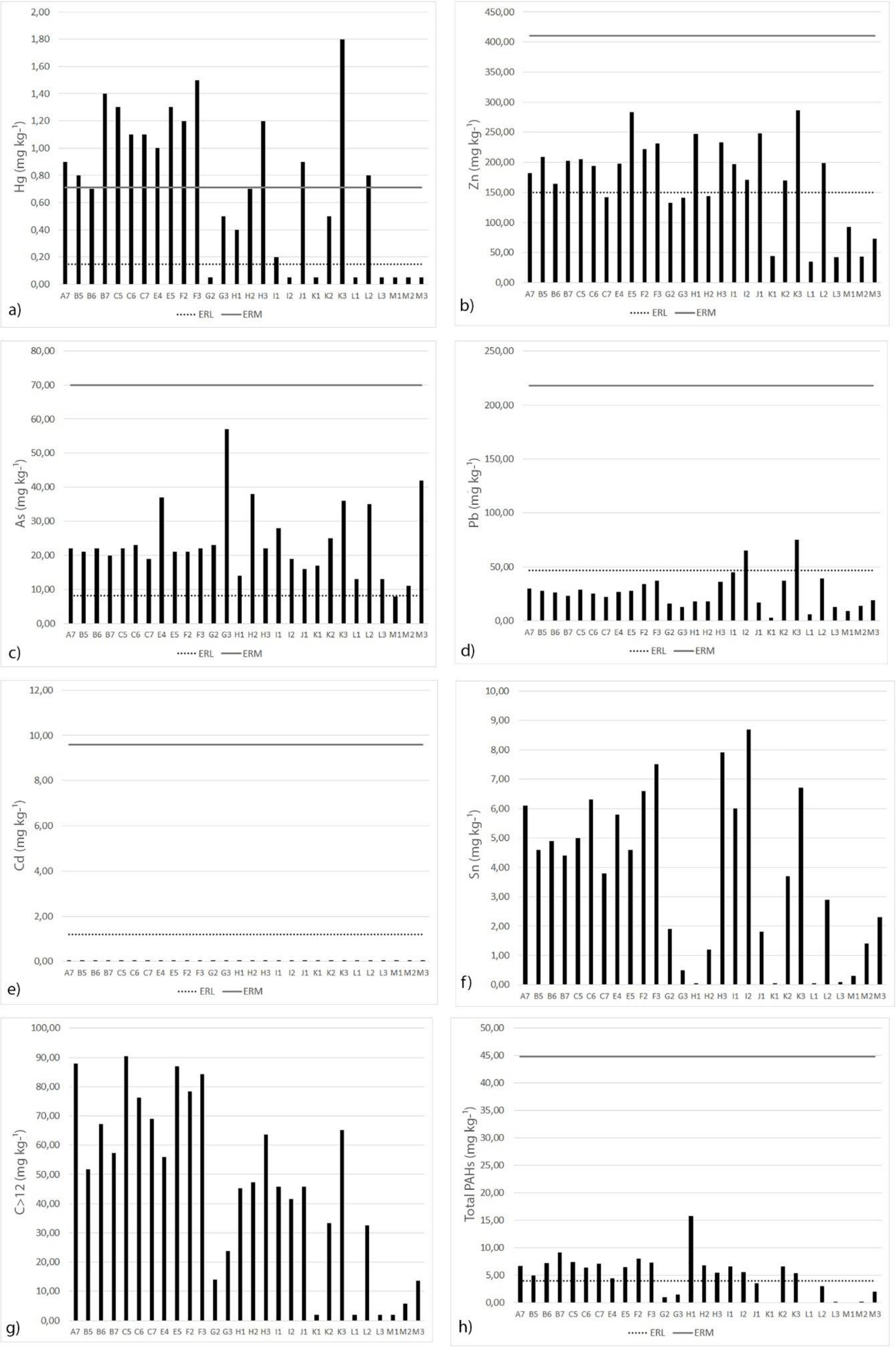

Figure 3. Principal Component Analysis (PCA) on the environmental variables: a) ordination diagram based on the environmental variables; b) Scatter diagram plotting factors 1 and 2 of sampling stations.

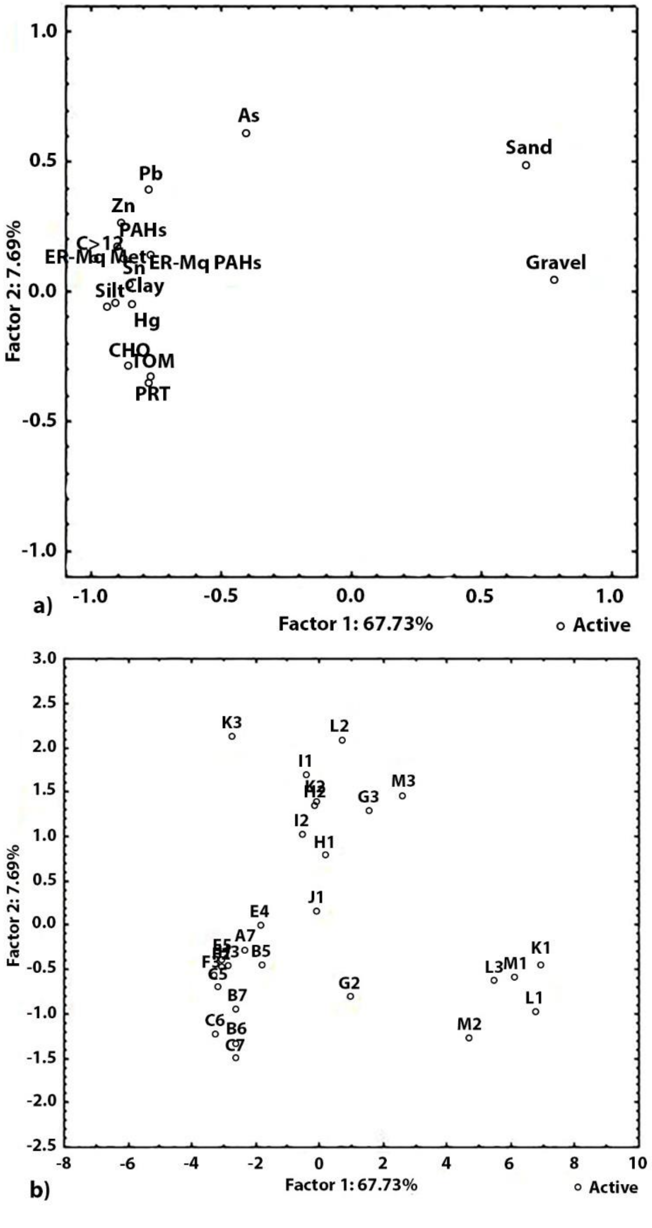

Figure 4. non-Multi-Dimensional Scaling ordination of nematode abundances (genus level)

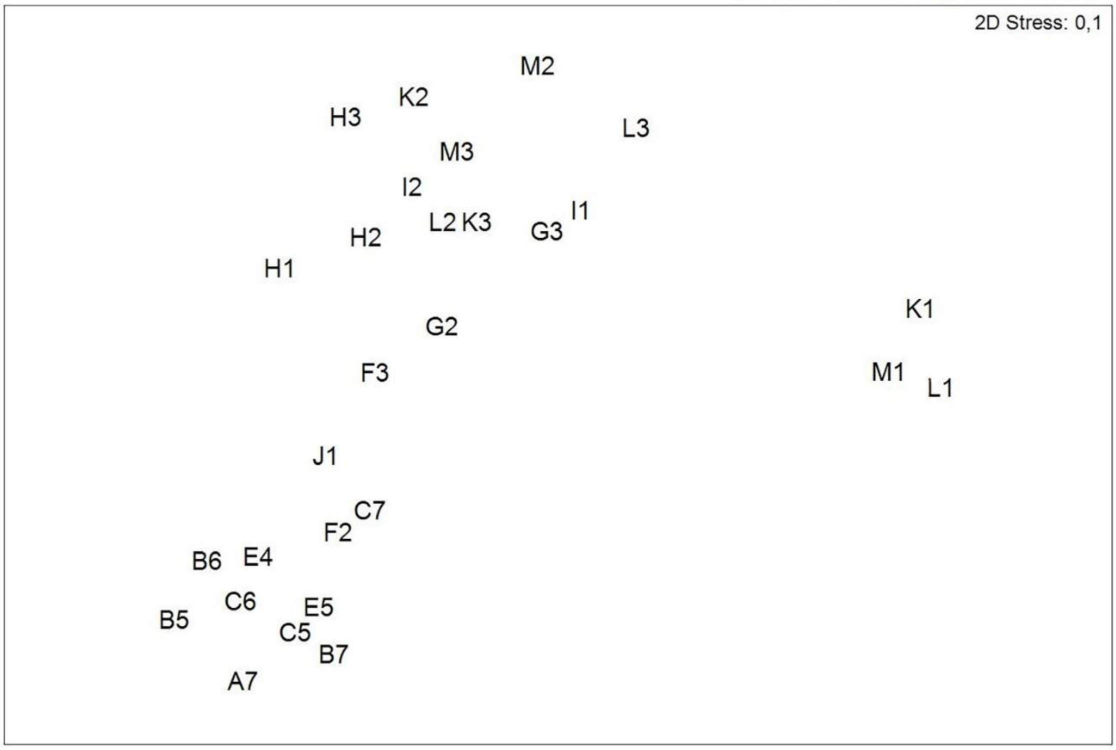

Figure 5. Canonical correspondence analysis (CCA) bi-plot showing the relationship between environmental variables and nematode genus abundance: (a) bi-plot with genera and environmental variables; (b) bi-plot with sampling stations. For clarity, only genera with frequency >0.1% are displayed; genera abbreviations as in Supplementary Material, Table S2.

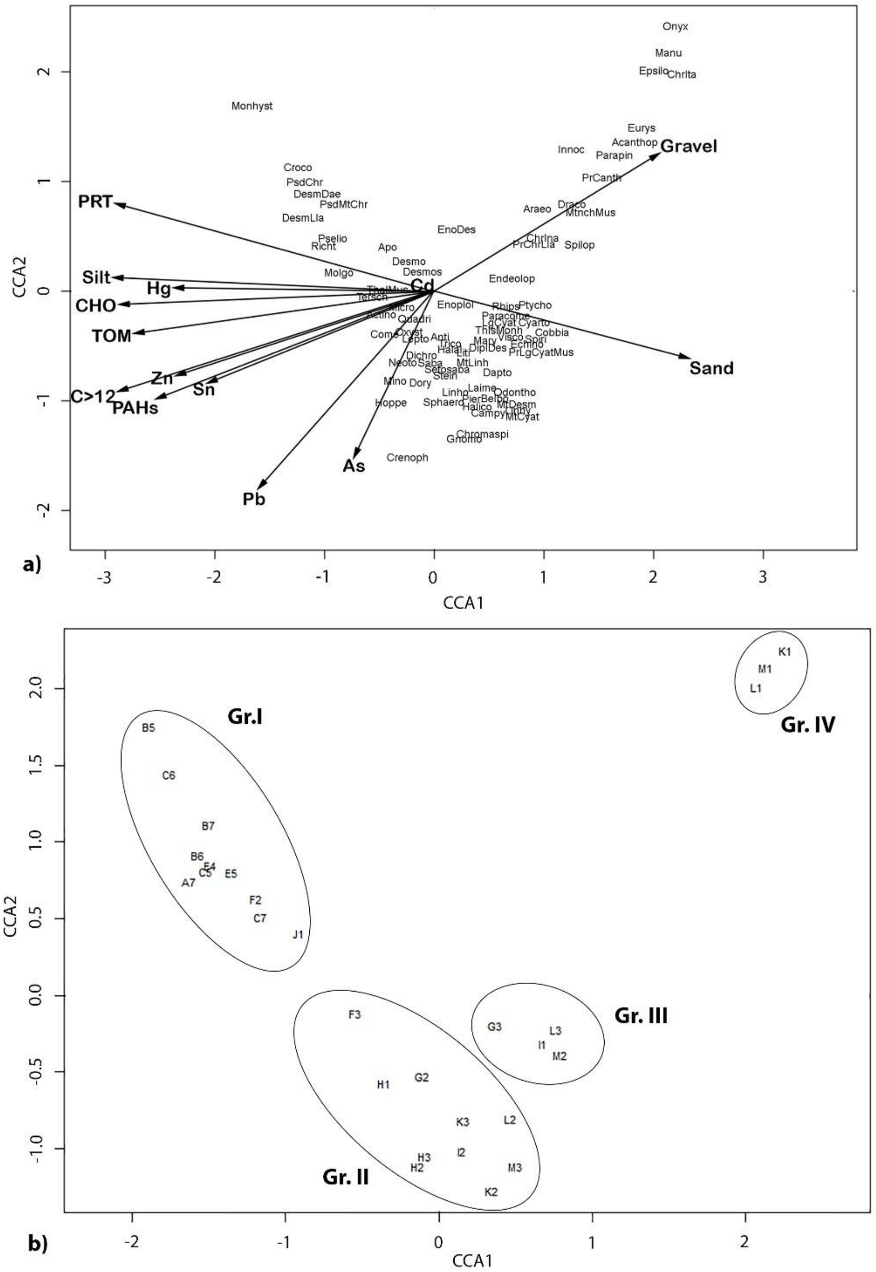

Figure 6. Nematode genera selected according to their tolerance or sensitivity to contamination and organic enrichment. Genera in bold were selected also in Losi et al. (2013). Genera that in Losi et al. (2013) resulted with intermediate tolerance/sensitivity are indicated with an asterisk.

Tolerant

Actinonema, *Aponema*, ***Comesa***, *Crenopharynx*, *Croconema**, *Desmodora*, *Desmodorella**, Desmodoridae gen. 1, *Desmoscolex**, *Dichromadora*, *Dorylaimopsis**, *Hopperia*, *Leptolaimus*, ***Microlaimus***, *Minolaimus*, ***Molgolaimus***, Monhysteridae gen. 1, ***Neotonchus***, *Oxystomina*, *Pselionema*, *Pseudochromadora*, *Pseudometachromadora*, *Quadricoma**, *Richtersia**, ***Sabatieria***, ***Terschellingia***, ***Thalassoalaimus***

Sensitive

Acantopharynx, *Araeolaimus*, *Chromadorina*, ***Chromadorita***, *Draconema*, *Endeolophos*, *Enoploides*, *Epsilonema*, *Eurystomina**, *Innocuonema**, *Manunema*, *Metoncholaimus*, *Onyx*, *Paracanthonchus*, *Parapinnanema*, *Prochromadorella*, *Spilophorella*

Appendix A. Supplementary data available at:https://doi.org/10.1016/j.ecss.2021.107524

CHAPTER 5

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Free-living nematodes of Mediterranean ports: a mandatory contribution for their use in ecological quality assessment

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Abstract

Free-living nematodes were investigated in three Mediterranean commercial ports (Ancona and Trieste, Italy; Koper, Slovenia) in terms of abundance, diversity and functionality. Results indicated that r-strategist genera were dominant in all ports and that a more diverse assemblage characterised Trieste, despite the high contamination levels, suggesting a potential adaptation to long-standing contamination. The main environmental factor that shaped the assemblage in all ports were Total Polycyclic Aromatic Hydrocarbons, while Total Organic Carbon and the grain-size were less relevant. A co-occurrence analysis was applied for identifying which genera cohesively respond to site-specific environmental conditions in order to recalibrate and implement the sets of bioindicator genera in relation to their different opportunistic behaviour. Finally, we defined some simple guidelines for a proper application of nematode indices (Maturity Index, Index of Trophic Diversity, Shannon diversity) in order to encourage the use of free-living nematodes for the environmental quality assessment of commercial ports.

Keywords: Marine nematodes; commercial ports; Mediterranean Sea; long-standing contamination; co-occurrence analysis; Environmental Quality Assessment

1. Introduction

Being sites of several productive and commercial activities, ports are crucial areas for local and national economic development. However, they are also both the recipient and the source of considerable anthropogenic disturbance for the surrounding coasts due to the chemical contamination from industrial installations, wastewater discharges, oil spill accidents, leaks of petroleum derivatives and antifouling coatings, storage and spillage of hazardous materials, transfer of invasive species with ballast waters and biofouling (Darbra et al., 2005; Chatzinikolaou et al., 2018). The high concentrations of contaminants and the relevant inputs of organic matter represent a persistent and ongoing threat, especially for the biota living in the sediments (Moreno et al., 2008; Veiga et al., 2009; Briant et al., 2013; Demopoulos et al., 2016). Furthermore, ports are considered as “Heavily Modified Water Bodies”, which cannot meet the common criteria of good ecological quality status (WFD 2000/60/EC), therefore, their effective management is crucial for the sustainable use of these maritime spaces and for the protection of the adjacent coastal habitats (Boudouresque et al., 2015; Thibaut et al., 2017; Chatzinikolaou et al., 2018).

In last decades, the concerns about the environmental degradation and the depletion of resources led to a strong demand for developing suitable bio-indicator methods, capable of quantitatively assessing the quality of marine habitats and the biotic response to various types of anthropogenic impact. In Europe the two most important EU decisions of marine environmental policy are the Water Framework Directive (WFD, 2000/60/EC) and the Marine Strategy Framework Directive (MSFD, 2008/56/EC). According to them, the state members have to assess the Ecological Quality Status (EQS) of their marine water bodies in a perspective of long-term utilisation of the environmental resources by means of selected Biological Quality Elements (BQEs) that are biological communities recognised as fundamental constituents of the ecosystem. The study of a particular BQE spans from community abundance, taxonomic composition and biomass to indices. These latter have the advantage to summarize a lot of information (e.g. the taxonomic composition or the functional traits) in a single output that can be easily inserted along a scale of thresholds that delimit the field of EQS quality judgements (i.e. from Bad to High).

Although the benthic macro-invertebrates are by far the most popular among the BQEs, in severely contaminated systems such as the ports these organisms can be too scarce and difficult to sample in a representative way, thereby providing little information that can be used effectively (Gambi et al., 2020). Furthermore, a cost-effective assessment (i.e. minimized sampling effort), less destructive as possible and fast in producing results is needed. In order to meet these requirements, biological communities alternative to macrobenthic invertebrates have been proposed as BQEs for EQS assessment. Recently, emphasis has been placed on the suitability of the most representative group of the meiofauna, i.e. the free-living nematodes (Moreno et al., 2011; Franzo et al., 2018b, 2019; Semprucci et al., 2018). Being the numerically dominant phylum of meiofauna (see Balsamo et al., 2012 and references therein), these organisms are the main responsible of the ecosystem services provided by this benthic community such as biomineralization, bioturbation, oxygen and nutrient cycling (Schratzberger and Ingels, 2018). Apart from this strict link with the benthic ecosystem functioning, the nematodes present numerous biological and practical advantages that make them potentially effective bioindicators (e.g. Balsamo et al., 2012; Franzo et al., 2019; Sahraeian et al., 2020). Due to their rapid growth and short life cycles, for examples, these organisms can respond rapidly to ongoing or recent environmental changes providing, therefore, early warnings more efficiently than macrofauna. Furthermore, the adverse effects of pollutants at the different levels of nematode biological organization (i.e. from cells to individuals, populations and communities) have been demonstrated in numerous laboratory experiments (see Coull and Chandler, 1992; Balsamo et al., 2012; Boufahja et al., 2016 and references therein). However, microcosm experiments cannot reflect the complex ecological relationships naturally occurring in marine ecosystems and field investigations are needed to assess the risks for the benthic processes and marine ecosystem functioning (Trannum et al., 2004; Moreno et al., 2008).

Free-living nematodes have been successfully used as BQEs in coastal areas (e.g. Hong et al., 2020), lagoons (Jouili et al., 2017; Franzo et al., 2019), tropical habitats (Chen et al., 2018) and both in protected and anthropogenically affected environments (for a review, see Semprucci et al., 2015b). For what concerns the ports, these organisms were investigated in Vado-Ligure (Losi et al., 2013, 2021) and Genoa-Voltri commercial hubs (Moreno et al., 2008) but their study remains still insufficient for building a robust baseline of data that can allow a reliable use of nematodes as BQEs in these environments. For example, although the analysis of nematode assemblage in terms of sensitive/tolerant genera was reported to provide the most reliable EQS scores (Moreno et al., 2011; Semprucci et al., 2015a,b), some studies detected an overall ‘good’ EQS also in severely contaminated areas (Franzo et al., 2018b; Gambi et al., 2020). These discrepancies are anything but surprising. Every ecosystem is intrinsically complex, even if heavily modified, and locally the resulting nematode assemblage is the result of the different contamination histories of each port and of several environmental factors that inevitably influence the community such as the sediment grain-size, the organic matter, the local hydrology and others.

Further studies focused on free-living nematodes in ports are therefore needed for building a wider baseline of data. This is a mandatory step for implementing the sets of the genera considered sensitive/tolerant, for calibrating the EQS thresholds based on nematode metrics such as diversity and functional indices, and finally for achieving an EQS assessment of ports as much as possible close to reality. A fruitful collaboration amongst marine scientists was promoted in order to filling this knowledge gap and the present study represents a contribution to the needed baseline of nematode data in ports. A simultaneous, multi-site investigation was carried out in three commercial hubs of the Adriatic Sea (Mediterranean Sea) - Ancona, Trieste (Italy) and Koper (Slovenia) - and the following questions were addressed: 1) do free-living nematodes differ taxonomically and functionally in port systems? 2) how are the nematofauna related to the main environmental variables (grain-size, TOC and contaminants)? 3) which EQS is obtained according to nematode assemblages in the port subareas? Aside from a proper study of the community composition at each site, a co-occurrence analysis was applied as a novel approach for the study of nematofauna in order to identify the genera that cohesively respond in the same way to site-specific environmental conditions. The outputs were integrated with previous literature for implementing the list of genera indicators for the environmental assessment of commercial ports.

2. Materials and methods

2.1 Study area

The Adriatic Sea is an elongated, semi-enclosed and shallow basin of the central Mediterranean Sea, characterized by the most extensive development of continental shelf in the Mediterranean. It is enriched by the input of nutrients, organic matter and clay from the Po River and a number of smaller Apennine rivers (Balsamo et al., 2010). Although in last decades the basin, especially its northern part, has experienced a constant PO_4^{3-} deficiency (Mozetič et al., 2012; Grilli et al., 2020), a significant positive trend of NO_3^- concentrations has been observed (Grilli et al., 2020). It is a major seaway for goods that are transported to and from Europe and also hosts an intense local traffic (David and Gollasch, 2008). Besides being surrounded by urbanized areas, the Adriatic ports are hubs of a wide range of human activities, such as industrial plants, shipbuilding activities, cargo traffic and routine sediment dredging to ensure the port access (Baldrighi et al., 2019; Luna et al., 2019).

2.1.1. Ancona

The port of Ancona (water depth, 4-15 m) is located in the Central Adriatic Sea (Fig. 1) and is characterized by intense passenger and cargo traffic (Table S1). The main pollutants include organic waste dumped from fishing vessels and industrial contaminants from a number of shipyards (Mirto and Danovaro, 2004; Spagnolo et al., 2011). Bottom water temperature ranges from 10 to 16 °C and salinity from 31 to 38 PSU in winter and spring, respectively. The main bottom current speed usually reported from the sampling area is 10 cm/s, with a north-eastern direction.

Nowadays the port of Ancona is classified as an international port by the European Union. 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. In the shipyards, ships of all kinds are designed and built and shipbuilding is the largest entrepreneurial reality in the port.

The four sampling stations were chosen according to a different degree of anthropogenic impact: Anc1 and Anc2 were located in the inner part of the port nearby shipping facilities such as active berths; Anc3 was located in a more external position although in an area used for cargo anchorage; Anc4 was outside the port where no activity takes place.

2.1.2. Trieste

The port of Trieste is located in the Bay of Muggia, a shallow, semi-enclosed basin in the Gulf of Trieste (north-eastern end of the Adriatic Sea; Fig. 1). Its depth ranges from 8 to 20 m and the sediment deposition rate is characterized by low-level hydrodynamism (Solis-Weiss et al., 2004) and by riverine inputs laden with fine sediments containing chemical fertilizers (www.porto.trieste.it) (Table S1). The port has long been surrounded by industrial infrastructures and is characterized by an intense traffic of oil tankers and ferries. The main pollutants include PAHs and trace metals. During the study, bottom water temperature ranged from 10 to 15 °C (winter and spring, respectively) and salinity from 37 to 38 PSU (spring and winter, respectively).

The development of the port dates back to the early 1900s with the construction of three external dams (1904-1909), the creation of large industrial structures in the Gaslini area, and the establishment of an iron and steel manufacturing industrial complex. In subsequent decades, other industrial structures were built, such as the industrial channel (completed in the 50s), the navigation channel (1966) as well as the construction of the Trieste - Monaco of Bavaria (SIOT) (1967) pipeline terminal (Solis-Weiss et al., 2004 and references therein), the most important pipeline that serves central Europe (ca. 36 x 10^6 tons of crude oil discharged in 2001) (www.porto.trieste.it) and finally, the expansion of the commercial docks.

The four sampling stations were chosen within the macrosites suggested by Cibic et al. (2017), i.e. established 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).

2.1.3. Koper

The port of Koper is located in a semi-enclosed bay in the northern-eastern Adriatic (Fig. 1). The sediments consist of detrital material from the hinterland, shore erosion and riverine inflows

(Ogorelec et al., 1987, 1991). The sediment deposition rate is high in the bay (from 3 to 5 mm yr^{-1}) and lower in the central area of the Gulf of Trieste (1 mm yr^{-1}; Faganeli et al., 1991; Ogorelec et al., 1991). The depth of the three sampling stations ranged from 8 to 17 m (Table S1). At the time of sampling (spring and autumn), mean water temperature and salinity were 18 °C and 37 PSU, respectively.

The samples were collected in each of the three port basins. In the first basin (Kp1) container ships and touristic cruise ships anchor. The centre of the basin is been deepened at the time of sampling, but the samples were retrieved at the end of the basin. The second basin (Kp2) is influenced by the river Rižana, which discharges also the outflow of the main coastal wastewater treatment plant (85,000 households). In the third basin (Kp3) bulk cargoes are handled, the most common of which are hard coal and iron ore (itabirritic ore). In all port basins, the sediment is stirred up by dredging operations. The sampling station Kp4 is located outside the port, next to the shipway, where there is least traffic impact and no dredging operation takes place (Fig. 1).

2.2. Sampling

Sampling was carried out in spring, autumn and winter during 2014 and 2015 (Table S1). In winter 2015, Anc4 was not sampled. At each station, three meiofaunal sediment samples were collected with a box-corer (40 cm × 30 cm wide and 50 cm high). The content of each box-corer was sub-sampled with PVC corers (inner diameter, 4.5 cm) and the top 3 cm of sediments were immediately preserved in 4% buffered formaldehyde (Danovaro, 2009).

Besides the meiofauna, the following environmental variables were collected at each station: sediment grain-size, Total Organic Carbon (TOC), Polycyclic Aromatic Hydrocarbons (PAHs) and Butyltin Compounds (BTs) (Baldrighi et al., 2019). The only exception is represented by BTs and PAHs data of the port of Trieste. These environmental parameters had been collected by the Port Authority in 2009 and 2013, in the framework of a monitoring program aimed at the environmental characterization of the area, and were measured in the top 50 cm of sediment.

2.3. Grain size

For grain size determination, aliquots of fresh sediment were sieved over a 63 μm mesh. The two fractions (> 63 μm, sand; < 63 μm, silt and clay) were dried in an oven at 60 °C and weighed. Data were expressed as a percentage of total sediment dry weight (Pusceddu et al., 2010).

2.4. Total organic carbon

TOC content was determined using a CHN Elemental Analyzer Flash 2000 apparatus calibrated with acetanilide as a standard, after removing carbonates with concentrated HCl 1 N. Concentrations were expressed as percentage of dry sediment weight. The limit of quantification (LOQ) and intermediate precision of the method were respectively 0.6% and 3% (for details see Baldrighi et al., 2019).

2.5. Polycyclic aromatic hydrocarbons

The following PAHs were assessed in the present study: napththalene (NA), acenaphthylene (Ace), acenaphthene (Apl), fluorene (Fl), phenanthrene (Phe), anthracene (An), fluoranthene (Flt), pyrene (P), benz[a]anthracene (BaA), chrysene (Chry), benz[b]fluoranthene (BbF), benz[k]fluoranthene (BkF), benz[a]pyrene (BaP), dibenz[a,h]anthracene (DahA), indeno[1,2,3-cd]pyrene (IP), and benzo[ghi]perylene (BghiP). Sediment samples were air-dried and homogenized before analysis. PAHs were extracted with a dichloromethane/acetone mixture (50:50) by ultrasonic bath (BRANSONIC 1510E-MT) for 15 min. All concentrations were expressed as a percentage of dry weight without correction for the recovery rates. Total PAH concentrations (ΣPAH) were the sum of each priority PAH. PAH concentrations in sediment were considered to indicate low (0–100 ng g^{-1}), moderate (100–1000 ng g^{-1}), high (1000–5000 ng g−1), and very high (> 5000 ng g^{-1}) contamination according to Baumard et al. (1998) and Mostafa et al. (2009). The contribution of each compound to the total PAH load was calculated as average molecular distribution of PAHs in sediments and the ratio of low molecular weight (LMW) to high molecular weight (HMW) PAHs were calculated to establish the petrogenic or pyrolytic origin of PAHs (for details see Baldrighi et al., 2019).

2.6 Butyltin compounds

The concentrations of tributyltin (TBT), dibutyltin (DBT), and monobutyltin (MBT) were determined in sediment samples (1 g) according to Binato et al. (1998), Morabito (2001) and Caricchia et al. (1993). Total BT concentrations (ΣBT) were the sum of these three compounds. For the port of Trieste, ΣBT data were collected in 2009 and provided by the Port Authority (Baldrighi et al., 2019).

2.7 Nematodes analyses

Meiofaunal organisms (body size 32-1000 μm) were extracted as described in Baldrighi et al. (2019) in accordance with the most adopted method for soft sediments, which is based on the centrifugation of the sediments with Ludox-HS 40 (Heip et al., 1985).

During the counting of meiofaunal organisms under a stereomicroscope (final magnification of 40-80×), 120 nematodes (or all the specimens encountered; Danovaro, 2009) were randomly hand-picked using a fine pin. Collected animals were transferred from formalin to glycerol through a series of ethanol-glycerol solutions and finally mounted on slides in anhydrous glycerin (Seinhorst*,* 1959). All nematodes on permanent slides were identified at the genus level under a 100× oil immersion objective using the pictorial keys of Platt and Warwick (1983, 1988) and Warwick et al. (1998), as well as the original species descriptions and identification keys available through NeMys (Bezerra et al., 2021).

The trophic structure of nematode assemblage was studied by assigning each genus to one of the following feeding groups (Wieser, 1953): selective (1A) and non-selective (1B) deposit feeders, epistrate feeders (2A) and predators/omnivores (2B). The Index of Trophic Diversity (ITD) was calculated according to Heip et al. (1985): $ITD = \sum \theta^2$, where θ is the percentage contribution of each feeding type. ITD values range from 0.25 (the highest trophic diversity, i.e. each trophic group accounts for 25% of the whole nematode assemblage) to 1.0 (the lowest trophic diversity; i.e. one feeding type represents 100% of the assemblage).

The maturity index (MI, Bongers, 1990; Bongers et al., 1991) was calculated as the weighted average of the individual colonizer-persister (c-p) values: $MI = \sum v(i) f(i)$, 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 gradual discrimination among r-strategist nematodes (colonizers, i.e. c-p 1 and c-p 2), intermediate colonizers (i.e. c-p 3) and k-strategist genera (persisters; i.e. c-p 4 and c-p 5).

Finally, the Shannon-diversity index (H’, Shannon and Weaver, 1949) and MI (Bongers et al., 1991) were utilized as Biological Quality Elements (BQEs) for nematodes according to Semprucci et al. (2015a,b). These BQEs allowed the classification of the sampled stations in five Ecological Quality

(EcoQ) classes (i.e., “bad”, “poor”, “moderate”, “good”, and “high”) after Moreno et al. (2011), following the principles applied by Chen et al. (2018). In details, the final classification was obtained by merging the EcoQ results of both MI and H': when two close EcoQ classes were found (e.g. poor and moderate), the final EcoQ assigned to the station corresponded to the worse class (i.e. poor). When the two classes were not immediately adjacent along the EcoQ gradient (e.g. bad and moderate), the final EcoQ assigned to the station was obtained by averaging these two scores (i.e. poor).

2.8. Statistical analysis

Univariate and multivariate analyses were performed using the PRIMER v. 7 software package (Clarke and Warwick, 2001) with the PERMANOVA add-on package (Anderson et al., 2008). Taxonomic diversity indices (total genera S; J’, Pielou, 1966; H’ log2, Shannon and Weaver, 1949) were calculated based on the percentages of nematode genera. In detail, to test for spatial differences in the composition of nematodes, a data matrix based on the percentages of genera at each station was constructed by applying the Bray-Curtis similarity. A one-way PERMANOVA test was conducted on this matrix using “port” as a fixed factor with 3 levels (Ancona, Trieste and Koper) and the unrestricted permutation of raw data was performed (9,999 permutations). The null hypothesis (i.e. no significant difference between nematode assemblages in the three ports) was rejected when the significance level P was < 0.05. The Monte Carlo permutation P was used when the number of permutations was lower than 150. If significant differences were detected, posteriori pair-wise comparisons were performed using 9,999 permutations under a reduced model.

To test the null hypothesis on the main nematode fauna descriptors (nematode abundance, S, H′ log2, J, ITD and MI), a one-way PERMANOVA analysis was applied using the same design described for nematode genera but based on Euclidean-distance similarity matrices with 9,999 permutations of residuals under a reduced model.

A non-metric multidimensional scaling (nMDS) was performed using a Bray-Curtis dissimilarity matrix on replicates dataset. The co-occurrence analyses were calculated as a pairwise distribution of each genus across the entire dataset using the Spearman’s correlation with coefficient (ρ) > 0.7. The network was plotted using the igraph package in the R software (Csardi and Nepusz, 2006).0 After this cut off, only the nodes with at least an edge were plotted in the network. A modularity analysis using a cluster algorithm (Clauset et al., 2004) built in the R package igraph was performed (random walks and “fast greedy” algorithms) in order to identified group of nematodes with similar distribution.

Principal component analysis (PCA) was used to investigate the effects of the environmental variable changes on the nematode taxonomical composition, diversity (H’ and J indices), functional indices and traits (MI, ITD, c-p classes and trophic guilds). The relative abundances of the nematode species as well as univariate nematode variables were projected on the factor plane as secondary variables without contributing to the results of the PCA. This routine can provide an insight into the possible influence of the environmental variables upon nematode fauna (STATISTICA v. 8 computer program).

Relationships between environmental predictor variables and nematode assemblage structure were investigated using distance-based linear models (DistLM) in PERMANOVA (Anderson et al., 2008). The environmental parameters chosen to conduct DistLM were %sand, %TOC, ∑PAH and ∑BT. For %TOC, ∑PAH, ∑BT only one replicate was analyzed and for %sand the average value of two sampling campaigns (conducted in different seasons) was calculated. As the environmental parameters were obtained as single replicate, in contrast to three replicated samples of nematode assemblage variables, the DistLM routine was done on centroids of the resemblance matrix. P values were obtained with 9,999 permutations of the model.

3. Results

3.1. Environmental variables

The three ports were characterized by different sediment grain size (Table 1). All sampling stations in Ancona were mainly dominated by sand (71.4–83.9%), while in Trieste and Koper by silt and mud (>80%). Trieste showed the highest amount of TOC especially in Ts3 (4.78%), the station nearby the iron foundry area, while comparable values were reported in Ancona and Koper and ranged from 0.73% to 1.26% (Table 1).

In all ports, ΣPAH represented the highest percentage among the pollutants considered (Table 1) and the values ranged from a minimum of 55.2 ng g^{-1} (Anc1) to a maximum of 14,036.1 ng g^{-1} (Ts3). Based on the classification of Baumard et al. (1998) and Mostafa et al. (2009), the samples from Ancona reflected a low PAH contamination, with little variation among stations (range: 55.2–112.0 ng g^{-1}; mean: 89.0 ± 29.9 ng g^{-1}), whereas those from Koper reflected a low-moderate contamination (range: 167.6–302.8 ng g^{-1}; mean: 244.1 ± 69.3 ng g^{-1}). The ΣPAH measured in Trieste showed a marked variability (range: 73.4–14,036.1 ng g^{-1}) with the highest amount in Ts2 (shipbuilding area) and Ts3 (iron foundry area).
In Trieste, ΣBT concentrations ranged from < 2 to 9 ng Sn g^{-1} while slightly higher ΣBT values characterized the other two ports since varying from 7 to 9 ng Sn g^{-1} and from 7 to 15 ng Sn g^{-1} in Koper and Ancona, respectively (Table 1).

3.2. Nematode abundance, structural and functional diversity

Nematode abundance was significantly lower in Trieste than in Koper and Ancona (PERMANOVA, p = 0.0042, Table 2). Mean values ranged from 423.4 ± 343.5 ind. 10 cm^{-2} in Trieste to 1,636.7 ± 778.8 ind. 10 cm^{-2} in Ancona, while Koper was characterised by a mean abundance of 1,272.9 ± 970.6 ind. 10 cm^{-2} (Fig. S1A).

A total of 103 nematode genera were found (Table S2) and the composition was statistically different among ports as indicated by PERMANOVA outputs (p = 0.0001, Table 2). The dominant genera (mean relative abundance or RA > 2% considering all stations) were *Sabatieria, Terschellingia, Daptonema, Ptycholaimellus, Parodontophora, Prochromadorella, Aponema, Dorylaimospis* and *Sphaerolaimus*. These genera showed variable abundances according to the ports and the stations. Ancona was characterized mainly by *Aponema*, *Terschellingia*, *Daptonema* and *Sabatieria*, Trieste by *Ptycholaimellus, Prochromadorella* and *Daptonema*, Koper by *Sabatieria* and *Terschellingia*. *Dorylaimopsis* and *Ptycholaimellus* were more abundant at Kp4 (Fig. S1B).

Koper samples were widely spread over the nMDS plot while Ancona and Trieste samples resulted well separated on the left and the right side, respectively (Fig. S2). The network output of the co-occurrence analysis, based on the genera abundances across the dataset, allows the identification of clusters of nematodes that share similar spatial patterns of abundance and that cohesively respond in the same way to the site-specific environmental conditions. Using this approach, we identified nine major groups of co-occurring genera. For each group, we subsequently highlighted the spatial distribution and the genera composition (Fig. 2, S3). The quantitatively most important (> 40% of total abundance) were group 1, group 2 and group 6 (Fig. 2). Group 1 indicates the co-presence of *Daptonema*, *Sphaerolaimus* and *Tricoma* mainly at Anc4. Group 2 suggests that Anc1 and Anc2 were characterized by the highest abundance of *Terschellingia* that co-occurred with *Aponema*. On the contrary, Group 6 separated Trieste stations and, to a lesser extent also Kp4, from the other sites. The separation of the Group 6 was mainly due to the dominance of chromadorids such as *Ptycholaimellus* and *Prochromadorella*. Although the other groups identified were less important according to a quantitative point of view (< 30% of total abundance), groups 5 and 10 deserve to be mentioned (Fig. 2). In particular, the former indicates that Kp4 was characterized by the co-occurrence of *Dorylaimopsis* and *Spilophorella,* while group 10 highlights that Ts1 was characterized by the co-occurrence of several genera such as *Parapinnanma, Parachanthonchus* and *Halalaimus*. The outputs of diversity and functional indices are reported in Table S3. In Ancona, the number of genera (S) ranged from 8 to 18, from 5 to 22 in Koper and finally from 12 to 22 in Trieste (Table S3). PERMANOVA results indicated that S was significantly higher in Trieste (on average 19 ± 5 genera) than in Ancona (13 ± 5 genera) and in Koper (13 ± 7 genera) ($p = 0.003$, Table 2). In Ancona, Shannon-diversity H’ ranged from 1.97 to 3.43, while from 0.92 to 3.67 in Koper (Table S3). Trieste showed significantly higher H’ values (range: 2.16 - 3.78) than those measured in the other two ports (PERMANOVA $p = 0.004$, Table 2 and Table S3). Mean H' values were 3.38 ± 0.50 in Trieste, 2.72 ± 0.67 in Ancona and 2.30 ± 1.08 in Koper. In Ancona, the evenness (J’) varied from 0.63 to 0.82, in Trieste from 0.61 and 0.87 and in Koper from 0.42 and 0.83. PERMANOVA highlighted significant differences of J’ ($p = 0.037$) among ports, and the pair-wise test revealed that Trieste was characterized by overall significantly higher J’ values (on average 0.81 ± 0.08) than Ancona (0.75 ± 0.09) and Koper (0.64 ± 0.19), suggesting the presence of an assemblage characterized by genera that are more equally distributed in Trieste than in the other two ports.

Considering the life history traits, in all ports the assemblage was mainly composed by c-p 2 and c-p 3 specimens (overall 58% and 36% of the whole nematode assemblage), followed by c-p 4 (4%) and c-p 1 nematodes (2%) (Fig. S4A). The resulting MI values ranged between 2.14 and 2.79 (Table S3). Ancona showed the highest MI mean value (2.49 ± 0.20), followed by Trieste (2.45 ± 0.20) and Koper (2.35 ± 0.23), but significant differences were not detected (Table 2).

Overall, non-selective deposit feeders dominated the nematode assemblages (1B: 45%) followed by epistrate feeders (2A: 31%), selective deposit feeders (1A: 19%) and predators (2B: 5%) (Fig. S4B). ITD values ranged from 0.35 to 0.77 and the average values were 0.57 ± 0.17 in Koper, 0.42 ± 0.09 in Trieste and 0.41 ± 0.09 in Ancona (Table S3). PERMANOVA outputs indicated that Koper was characterised by ITD values significantly higher than those measured in the other two ports (PERMANOVA, $p = 0.001$) (Table 2).

The Principal Component Analysis (PCA) was used to visualise any trend of nematode assemblages in relation to the environmental conditions in the three ports (Fig. S5). The 80% of the variance was explained by the first two factor planes. In detail, the first factor (PC1) explained the 54.42% of the total variance (eigenvalue: 2.72) and silt/mud% (0.84), TOC (0.83), ΣPAH (0.84) and ΣBT (-0.60) were the main primary (i.e. active) variables that characterized it. The second component explained the 25.42% of the total variance (eigenvalue: 1.27) and was discriminated by %sand (-0.84) (Fig. S5A). The factor coordinates showed that the stations of Trieste and Ancona were located mainly along the PC1 (with the only exception of Ts4), while Koper sites along the PC2 (Fig. S5B). According to the environmental variables, Koper resulted characterized by the finest sediment fraction along with intermediate levels of ΣBT and ΣPAH. On the contrary, Ancona differed from the other ports for the highest values of %sand and ΣBT, while Trieste for the highest values of TOC and ΣPAH.

Aponema, *Terschellingia*, *Sabatieria* and *Daptonema* resulted mainly associated to the sandy sediments of Ancona and to the greater amount of ΣBT. In contrast, *Ptycholaimellus*, *Prochromadorella*, *Halalaimus*, *Leptolaimus*, *Parapinnanema* (largely representing the category of 'Others', Fig. S1B) resulted more abundant in Trieste and, therefore, associated to higher values of silt/mud, TOC and ΣPAH. *Dorylaimopsis* and to a lesser extent *Terschellingia* and *Sabatieria* were mainly associated to Koper sediments (Fig. S5A). Among the nematode univariate measures, c-p 3 and the deposit feeder groups (both 1A and 1B) were clearly associated to Ancona sediments, while c-p 1, c-p 4 and the epistrate feeders (2A) to Trieste. All the other faunal parameters, i.e. MI, ITD as well as all the diversity measures, were closely related to the PC2. In particular, Koper stations showed higher ITD values in concomitance with an overall lower diversity (Fig. S5A).

DistLM marginal tests indicate that the nematode assemblage structure was significantly correlated with ΣPAH (p = 0.025, prop. = 30%) and %TOC (p = 0.021, prop. = 28%); sequential tests using a forward selection procedure highlight the significant importance of ΣPAH (p = 0.022). Four predictor variables (∑PAH, %sand, ∑BT, %TOC) explain 57% of the nematode assemblage structure in the three ports. Trieste samples were characterized by the highest ∑PAH and %TOC concentrations, while Ancona and Koper by the highest and the lowest %sand, respectively (Table 3).

4. Discussion

In a previous study conducted by Baldrighi et al. (2019) on the same samples, the potential temporal variability of main meiofauna groups was investigated. The authors reported significant differences only between stations while the temporal variability was not statistically relevant. Similarly, in the present study the PERMANOVA outputs did not indicate significant differences between campaigns (data not shown), therefore the spatial variability resulted as the sole source of variability.

1) Do free-living nematodes differ taxonomically and functionally in different port systems?

Overall, the dominant genera observed in the three ports are considered widespread in the Adriatic basin (Balsamo et al., 2010) and have been already reported in the sediments of other Mediterranean commercial ports such as Genoa-Voltri (Ligurian Sea; Moreno et al., 2008) and Vado Ligure (Ligurian Sea; Losi et al., 2013). In particular, genera such as *Terschellingia, Sabatieria, Paracomesoma, Daptonema* and *Ptycholaimellus* are commonly found in the sediments subjected to anthropogenic activities such as electricity generating plants, refineries and maritime traffic. Notwithstanding, significant differences in the taxonomic compositions were observed among Ancona, Koper and Trieste. Spatial variability was already reported also in different areas of the same port (Vado Ligure, Losi et al., 2013; 2021) and can be ascribed to the dissimilar past and ongoing anthropogenic activities that characterize each area, and to the local environmental conditions (e.g. water circulation and sediment grain-size).

The environmental complexity in which several ecosystem components variably act together might determine site-specific responses of the nematode taxonomic composition. For instance, in the port of Trieste, the dominance of chromadorids (i.e. *Ptycholaimellus* and *Prochromadorella*) can be ascribable to the presence of an active and abundant diatom assemblage even at the most polluted Ts2 and Ts3, as previously documented by Cibic et al. (2017). These nematode genera are known for feeding on diatoms (Moens and Vincx, 1997; Moens et al., 2005) by puncturing and emptying microalgae (Moens and Vincx, 1997). In a 2-year study, Franzo et al. (2018a) observed that peaks of these genera were concomitant with the highest numbers of benthic diatoms at the long-term reference station in the Gulf of Trieste. For what concerns nematodes diversity, the obtained results were comparable or slightly lower than those previously reported in Vado Ligure (Losi et al., 2013, 2021). Despite the higher contamination levels, the assemblage in the port of Trieste was significantly more diverse than those inhabiting the sediments of the other two ports, especially Koper. This result is in contrast with previous studies carried out in impacted coastal areas since generally less diverse assemblages are reported at the most contaminated sites (Losi et al., 2013 and references therein; Franzo et al., 2018b; Boufahja and Semprucci, 2015).

In all the three investigated ports, colonizer nematodes (c-p 2) were dominant. This result is in accordance with the c-p composition observed in other commercial hubs (Moreno et al., 2008; Losi et al., 2013, 2021) and in severely contaminated coastal areas (Gambi et al., 2020) confirming that in such modified environments the assemblage is dominated by r-strategists that are advantaged by peculiarities such as the rapid colonization, the short generation times and the high number of eggs. This kind of c-p composition, however, led to MI values that were not particularly low because the codominant c-p group was represented by intermediate colonizers such as c-p 3. In Bagnoli-Coroglio Bay, a coastal area characterized by a long history of high concentrations of PAHs and heavy metals, Gambi et al. (2020) ascribed the MI values >2.5 at all stations to c-p3 and c-p4 taxa. The authors argued that this result indicates a capacity of nematodes to cope unfavourable conditions as those determined by high contamination levels.

With regard to the trophic composition of nematofauna, the overall dominance of deposit feeders (1A and 1B) is in accordance with the findings of Losi et al. (2013) and Gambi et al. (2020). In Kp4 and in the port of Trieste the assemblage showed an overall higher contribution of epistrate feeders that was ascribable mainly to *Ptycholaimellus* and *Dorylaimopsis* in Koper and to *Ptycholaimellus* and *Prochromadorella* in Trieste. Although PERMANOVA outputs clearly confirm that the port of Koper was characterized by a significantly lower trophic diversity (high ITD values correspond to a lower trophic diversity because one feeding type dominates over the others) due to the dominance of non-selective deposit feeders, a critical remark here is worthy because well explains why ITD is generally considered less informative than MI or H' (Losi et al., 2021). Taken alone, ITD results in Ts2 (0.61 in winter 2015) and in Kp1 and Kp2 (0.68 and 0.64 in autumn 2014) do not allow to discriminate if such similar trophic diversities were ascribable to different trophic groups, i.e. epistrate feeders and non-selective deposit feeders, respectively. In the present study, this is meaningful because the dominance of 2A in Ts2 is ascribable mainly to c-p3 nematodes while in Koper the dominance of 1B is mainly represented by c-p 2 specimens, confirming the need of combining the analysis of the trophic diversity with that of the life strategies.

2) How are the nematofauna related to the main environmental variables (grain-size, TOC and contaminants)?

The content of TOC was sensibly higher in the sediments of Trieste than in the other two ports. The values obtained were comparable to those reported by Cibic et al. (2017) at the same stations during 2013. In Ancona, the lower values may be related to the coarser sediments. It is well known, in fact, that coarser sediments tend to retain lower amounts of organic matter and contaminants. In Koper, on the contrary, the muddy sediments should retain amounts of TOC comparable to those of Trieste. Since this is not observed, the higher values of TOC in Trieste are likely related to the anthropogenic activities settled there, such as the coal-fired steel plant (Cibic et al., 2017).

Even if contaminant concentrations were measured in different sediment layers, (i.e. surface sediments for Koper and Ancona, 0-50 cm for Trieste; Baldrighi et al., 2019), the high concentrations of PAHs in the sediments of Trieste revealed a strong and long standing contamination especially at Ts2 and Ts3, which can be related to the past and the ongoing anthropic activities. Such contamination is in fact one of the main reasons that led to the declaration of the area as a Site of National Interest (SNI) in 2003. These sites are defined by the Italian State as heavily contaminated in need of soil, surface water and groundwater remediation. The SNI are identified in relation to the characteristics of the site, the quantity and hazardous nature of pollutants, the importance of the impact on the surrounding environment in terms of health and ecology, as well as damage to cultural and environmental heritage. Although even Ancona is a port subjected to the Italian legislation, it is not a SNI, therefore to some extent this area can be considered less affected by contamination than Trieste.

DistLM outputs indicated that ΣPAH significantly explained the variability of the nematode assemblages in the study areas, followed by TOC% and by the different grain size. Although the port of Trieste was characterized by higher concentrations of these contaminants, it was inhabited by a surprisingly diverse nematode assemblage in which genera considered sensitive to pollution were observed also in non-negligible abundances. As discussed above for *Ptycholaimellus* (c-p 3), some genera can be favoured by other ecosystem components such as benthic microalgae. Their proliferation can support higher numbers of the genera that feed on them masking partially the detrimental effects of the contaminants. Furthermore, Cibic et al. (2017) reported the presence of a heterotrophic microbial community that accounts also specialized consortia of bacteria able to use and tolerate toxic compounds such as petroleum and n-Hexadecane. This suggests that in the port of Trieste the biota at the different trophic levels might have adapted to the chronic and long-standing contamination. The fact that in Koper and Ancona ports this kind of adaptation does not seem to have been achieved, calls for further investigations.

3) Which EQS is obtained according to nematodes in the port subareas?

According to the thresholds suggested by Marin et al. (2008) for abiotic variables, the three investigated ports were characterized by EQS ranging from ‘alerting’ to ‘good’. In detail, in all ports TOC% were below the threshold levels indicating a ‘good’ EQS, while ΣPAH values indicated an ‘alerting’ status only in Trieste (Baldrighi et al., 2019). The silty/muddy sediment coupled with higher TOC contents and the presence of long-standing anthropogenic activities nearby Ts2 (shipbuilding) and Ts3 (iron foundry plan), might have favored PAH accumulation in this port (Frapiccini and Marini, 2015; Baldrighi et al., 2019).

Focusing on the nematode fauna, the EQS obtained according to the thresholds proposed by Moreno et al. (2011) for H’ and MI are reported in Table S4. The scores based on H’ confirmed the suitability of this metric in detecting the differences among stations. Apart from the overall higher scores obtained in Trieste likely due to an adaptation to chronic pollution, in all ports higher EQS characterized the least impacted stations (Anc4, Kp4 and Ts4) or those located further away from the main anthropogenic activities (Anc3). The higher EQS score of Ts4 suggests that H’ was sensitive enough to catch the lower contamination levels that characterized this sampling site. Since this difference was not detected by analysing the meiofaunal main groups (Moreno et al., 2008; Cibic et al., 2017; Baldrighi et al., 2019), this result confirms the need of a more detailed taxonomic identification for detecting meiofaunal responses to the environmental changes.

The EQS obtained according to MI were rather variable and did not clearly reflect the contamination levels nor the vicinity to the anthropogenic activities. The EQS according to MI was not consistent with those obtained with H’ with the exception of Koper, where better scores characterized Kp4 and Kp3 according to both these metrics. However, previous studies underlined that when we define EQS in confined environments (e.g. ports and lagoons: Moreno et al., 2009; Armenteros et al., 2009; Jouili et al., 2017), MI scores give contradictory results in comparison to the same metric applied in open sea (e.g. Semprucci et al., 2015b; Hong et al., 2020). These observations contribute to a more effective use of MI as already done for macrobenthic biotic indices. The wide application of BENTIX and AMBI for the ecological quality assessment in different environmental contexts allowed the identification of their intrinsic limits and the consequent debate favoured the development of specific guidelines for a proper use of these indices (e.g. what are the specific environmental conditions in which a particular index should be avoided) (e.g. Simboura, 2004; Borja and Muxika, 2005). Our results support the overall accepted approach that consists in the concomitant application of different metrics in order to compensate their respective limits.

The co-occurrence analysis (to the best of our knowledge a novel approach for the study of free-living nematodes) was used for identifying nematode genera that can be regarded as indicators of human impact in commercial ports and, therefore, for integrating the previous knowledge (Moreno et al., 2008; Losi et al., 2013, 2021; Franzo et al., 2018b; Gambi et al., 2020). As shown in Figure 3, the genera were subdivided in resistant (nematodes mainly found in polluted port sediments), opportunistic (equally found in both polluted and unpolluted port sediments) and relatively opportunistic (mainly occurring in unpolluted port stations). Only the most relevant outputs of the co-occurrence analysis were considered (RA > 30%, i.e. Groups 1, 2, 5, 6, and 10) and each genus was assign to one of the three categories considering also the literature available.

According to Moreno et al. (2011), *Daptonema* indicates a Poor EQS, therefore, it is considered a tolerant genus by the authors. However, in group 1, *Daptonema* represented the dominant genus at the least impacted station of Ancona and, at the same time, it was observed also at almost all the other sites, both nearby and away from the main anthropogenic activities (e.g. the sampling sites of Trieste and Anc3, respectively) (Fig. 2). Based on these results, we propose to consider *Daptonema* as an opportunistic genus. Similarly, *Sphaerolaimus* has been assigned to the same category because co-occurred with *Daptonema* at all stations, even though it presented higher abundances at the least impacted stations Anc4, Kp4 and Ts4. On the contrary, *Acanthopharinx* co-occurred with *Daptonema* and *Sphaerolaimus* only in Anc4. Since this genus was indicated as sensitive by Losi et al. (2021) in the area nearby the Vado-Ligure port, we propose to consider it as a relatively opportunistic genus.

In group 2, *Aponema* and to a lesser extent *Paracomesoma* characterized the innermost stations of the Ancona port (Anc1 and Anc2) (Fig. 2). The former genus was reported as tolerant by Losi et al. (2021) for the area nearby the Vado Ligure. Similarly, Moreno et al. (2008) found *Paracomesoma* as the dominant genus in the contaminated sediments of St. I within the Genoa-Voltri port and subsequently the authors proposed it as an indicator of ‘Bad’ EQS (Moreno et al. 2011). Based on these results we confirm *Aponema* and *Paracomesoma* as resistant genera, i.e. mainly found in polluted sediments.

Group 5 identified *Dorylaimopsis* and *Spilophorella* as the two dominant genera that co-occurred in the sediments of Kp4 and of Anc4 (Fig. 2). These results are in accordance with Franzo et al. (2018a) who found *Dorylaimopsis* as the second dominant genus after *Ptycholaimellus* at the virtually pristine station C1 that is located nearby a Marine Protected Area of the Gulf of Trieste. Conversely, Losi et al. (2021) suggested *Dorylaimopsis* as a tolerant genus in the sediments of the Vado Ligure port. Due to these discrepancies, we proposed to consider *Dorylaimopsis* as an opportunistic genus. Since *Spilophorella* has been reported as sensitive and in the present study characterized the sediments of the least impacted station of Koper (Kp4), it is included in the group of relatively opportunistic genera.

Both *Ptycholaimellus* and *Prochromadorella* were considered sensitive in Moreno et al. (2011), while *Prochromadorella* was reported as tolerant in Losi et al. (2021). The co-occurrence analysis confirmed the widespread presence of these two genera because they represented the dominant members of the assemblage in the polluted sediments of Trieste and, to a lesser extent, even at the least impacted station of Koper (Group 6, Fig. 2). Furthermore, *Ptycholaimellus* was reported as dominant at the virtually unpolluted station C1 in the Gulf of Trieste (Franzo et al., 2018a). Taking into account all these evidences, we propose to consider *Ptycholaimellus* and *Prochromadorella* as opportunistic genera, i.e. equally found in both polluted and unpolluted sediments.

In Group 10 (Fig. 2), *Parapinnanema* and *Halalaimus* co-occurred in the sediments of both the least impacted station of Koper and at the contaminated site Ts1. Since these two genera have been reported as sensitive to anthropogenic impact, we advise to consider them as opportunistic. Furthermore, in Ts1, *Parapinnanema* and *Halalaimus* co-occurred with *Paracanthonchus and Desmoscolex,* although these latter genera presented lower percentages. Since Losi et al. (2021) proposed *Paracanthonchus* as a sensitive genus while we observed it mainly in polluted sediments, we propose to downgrade it as opportunistic. On the contrary, *Desmoscolex* seems to tolerate polluted conditions in ports (Losi et al., 2021), therefore, it has been considered as resistant.

The genera *Terschellingia, Sabatieria* and *Parodonthophora* are known for their tolerance to contaminants, organic enrichment and environmental instability (Moreno et al., 2011; Losi et al., 2013, 2021). Interestingly, in the present study *Sabatieria* and *Parodonthophora* did not correlate with any other genus although observed in noticeable abundances, especially *Sabatieria.* Notwithstanding, both *Sabatieria* and *Terschellingia* represent the two dominant genera at the port stations of Koper (Fig. S1), i.e. where the nematode assemblage was the least structured and biodiverse. Taking into account all these aspects, we propose to maintain these three genera in the category of those considered resistant to the impacted conditions of big commercial hubs.

5. Conclusions

The present study represents an important contribution to the knowledge of free-living nematodes inhabiting the sediments of large commercial ports and to define possible guidelines for the use of many nematode indices and indicators. The results suggest that the nematode assemblages might adapt to long-standing contamination in synergy with other ecosystem components such as autotrophic and heterotrophic microbiota. Indeed, Trieste commercial port is a clear example of how the benthic diatom proliferation can favor several nematode genera resulting in a fairly diverse and structured faunal assemblage even in contaminated sediments. For what concerns the EQS assessment, we recommend the use of the H' index or a combination of both the functional indices (i.e. MI and ITD) rather than solely MI or ITD to assess the ecological quality of the port sites. Finally, the co-occurrence analysis has allowed the assignment or the reallocation of some genera to three proposed categories - resistant, opportunistic and relatively opportunistic - in order to implement the use of nematodes for the EQS assessment of commercial ports.

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References

Anderson, M.J., Gorley, R.N., Clarke, K.R. PERMANOVA A+ for PRIMER: _Guide to Software and Statistical Methods. PRIMER-E, Plymouth, UK, 2008; 214 pp.

Armenteros, M., Ruiz-Abierno, A., Fernández-Garcés, R., Pérez-García, J.A., Díaz-Asencio, L., Vincx, M., Decraemer, W., 2009. Biodiversity patterns of free-living marine nematodes in a tropical bay: Cienfuegos, Caribbean Sea. Estuar. Coast. Shelf Sci. 85, 179-189. https://doi.org/10.1016/j.ecss.2009.08.002.

Baldrighi, E., Semprucci, F., Franzo, A., Cvitkovic, I., Bogner, D., Despalatovic, M., Berto, D., Malgozata Formalewicz, M., Scarpato, A., Frapiccini, E., Marini, M., Grego, M., 2019. Meiofaunal communities in four Adriatic ports: Baseline data for risk assessment in ballast water management. Mar. Pollut. Bull. 147, 171-184. https://doi.org/10.1016/j.marpolbul.2018.06.056

Balsamo, M., Albertelli, G., Ceccherelli, V.U., Coccioni, R., Colangelo, M.A., Curini- Galletti, M., et al., 2010. Meiofauna of the Adriatic Sea: current state of knowledge and future perspectives. Chem. Ecol. 26, 45–63.

Balsamo, M., Semprucci, F., Frontalini, F., Coccioni, R., 2012. Meiofauna as a tool for marine ecosystem biomonitoring. Mar. Ecos. 4, 77-104.

Baumard, P., Budzinski, H., Mehin, Q., Garrigues, P., Burgeot, T., Bellocq, J., 1998. Origin and bioavailability of PAHs in the Mediterranean Sea from mussel and sediment records. Estuar. Coast. Shelf Sci. 47, 77–90. https://doi.org/10.1006/ecss.1998.0337.

Bezerra, T.N., Eisendle, U., Hodda, M,; Holovachov, O., Leduc, D., Mokievsky, V., Peña Santiago, R., Sharma, J., Smol, N., Tchesunov, A., Venekey, V., Zhao, Z., Vanreusel, A., 2021.Nemys: World Database of Nematodes. Accessed at http://nemys.ugent.be on 2021-05-05. doi:10.14284/366

Binato, G., Biancotto, G., Piro, R., Angeletti, R., 1998. Atomic adsorption spectrometric screening and gas chromatographic-mass spectrometric determination of organotin compounds in marine mussels: an application in samples from the Venetian Lagoon. Fresenius J. Anal. Chem. 361, 333–337. https://doi.org/10.1007/s002160050898.

Bongers, T., 1990. The maturity index: an ecological measure of environmental disturbance based on nematode species composition. Oecologia 83, 14–19. https://doi.org/10.1007/BF00324627.

Bongers, T., Alkemade, R., Yeates, G.W., 1991. Interpretation of disturbance-induced maturity decrease in marine nematode assemblages by means of the Maturity Index. Mar. Ecol. Prog. Ser. 76, 135–142. https://www.jstor.org/stable/24825556.

Boudouresque, C.F., Personnic, S., Astruch, P., Ballesteros, E., Bellan-Santini, D., Bonhomme, P., et al., 2015. Ecosystem-based versus species-based approach for assessment of the human impact on the Mediterranean seagrass *Posidonia oceanica*. Marine productivity: perturbations and resilience of socio-ecosystems. Ceccaldi H., Hénocque Y., Koike Y., Komatsu T., Stora G., Tusseau-Vuillemin M.H. (eds), Springer International Publishing Switzerland: 235-241.

Boufahja, F., Semprucci, F., 2015. Stress-induced selection of a single species from an entire meiobenthic nematode assemblage: is this possible using iron enrichment and does pre-exposure affect the ease of the process? Environ. Sci. Poll. Res. 22, 1979–1998. https://doi.org/10.1007/s11356-014-3479-2.

Boufahja, F., Semprucci, F., Beyrem, H., 2016. An experimental protocol to select nematode species from an entire community using progressive sedimentary enrichment. Ecol. Ind. 60, 292–309. https://doi.org/10.1016/j.ecolind.2015.07.002.

Borja, A., Muxika, I., 2005. Guidelines for the use of AMBI (AZTI's Marine Biotic Index) in the assessment of the benthic ecological quality. Mar. Pollut. Bull. 50, 787-9.

Briant, N., Bancon-Montigny, C., Elbaz-Poulichet, F., Freydier, R., Delpoux, S., Cossa, D., 2013. Trace elements in the sediments of a large Mediterranean marina (Port Camargue, France): Levels and contamination history. Mar. Poll. Bull. 73, 78-85. https://doi.org/10.1016/j.marpolbul.2013.05.038.

Caricchia, A.M., Chiavarini, S., Cremisini, C., Morabito, R., Ubaldi, C., 1993. Analytical methods for the determination of organotins in the marine-environment. Int. J. Environ. Anal. Chem. 53, 37–52. https://doi.org/10.1080/03067319308045981.

Chatzinikolaou, E., Mandalakis, M., Damianidis, P., Dailianis, T., Gambineri, S., Rossano, C., Scapini, F., Carucci, A., Arvanitidis, C., 2018. Spatio-temporal benthic biodiversity patterns and pollution pressure in three Mediterranean touristic ports. Sci. Total Environ. 624, 648-660. https://doi.org/10.1016/j.scitotenv.2017.12.111.

Chen, C.A., Soo, C.L., Balsamo, M., Semprucci, F., 2018. An approach based on nematode descriptors for the classification of the ecological quality (EcoQ) of the Malaysian coasts. Mar. Biodiv. 48,117-126. https://doi.org/10.1007/s12526-017-0813-1.

Cibic, T., Franzo, A., Nasi, F., Auriemma, R., Del Negro, P., 2017. The port of Trieste (northern Adriatic Sea)— a case study of the “ecosystem approach to management”. Front. Mar. Sci. 4, 336. https://doi.org/10.3389/fmars.2017.00336.

Clarke, K.R., Warwick, R.M. Changes in Marine Communities: An Approach to Statistical Analysis and Interpretation. Second ed. Primer-E, Plymouth, UK, 2001; 172 pp.

Clauset, A., Mark, N., Moore, C., 2004.Finding community structure in very large networks. Phys. Rev. E. 70(6). https://doi.org/10.1103/PhysRevE.70.066111.

Coull, B.C., Chandler, G.T., 1992. Pollution and meiofauna: field, laboratory, and mesocosms studies. Oceanogr. Mar. Biol. 30, 191-271.

Csardi, G., Nepusz, T., 2006. The igraph software package for complex network research. InterJournal, complex systems, 1695(5), pp.1-9.

Danovaro, R. Methods for the study of deep-sea sediments, their functioning and biodiversity. CRC press: Boca Raton, USA, 2009; 458 pp.

Darbra, R.M., Ronza, A., Stojanovic, T.A.,Wooldridge, C., Casal, J., 2005. A procedure for identifying significant environmental aspects in sea ports. Mar. Poll. Bull. 50, 866–874. https://doi.org/10.1016/j.marpolbul.2005.04.037.

David, M., Gollasch, S., 2008. EU shipping in the dawn of managing the ballast water issue. Mar. Poll. Bull. 56, 1966–1972. https://doi.org/10.1016/j.marpolbul.2008.09.027.

Demopoulos, A.W.J., Bourque, J.R., Cordes, E., Stamler, K.M., 2016. Impacts of the Deepwater Horizon oil spill on deep-sea coral-associated sediment communities. Mar. Ecol. Progr. Ser. 561, 51-68. https://doi.org/10.3354/meps11905.

Faganeli, J., Planinc, R., Pezdič, Smodiš, B., Stegnar, P., Ogorelec, B., 1991. Marine geology of the Gulf of Trieste (northern Adriatic): geochemical aspects. Mar. Geol. 99, 93–108. https://doi.org/10.1016/0025-3227(91)90085-I.

Franzo, A., Asioli, A., Roscioli, C., Patrolecco, L., Bazzaro, M., Del Negro, P., Cibic, T., 2019. Influence of natural and anthropogenic disturbances on foraminifera and free-living nematodes in four lagoons of the Po delta system. Estuar. Coast. Shelf Sci. 220, 99-110. https://doi.org/10.1016/j.ecss.2019.02.039.

Franzo, A., Guilini, K., Cibic, T., Del Negro, P., 2018a. Interactions between free-living nematodes and benthic diatoms: insights from the Gulf of Trieste (northern Adriatic Sea). Mediterr. Mar. Sci. https://doi.org/10.12681/mms.15549.

Franzo, A., Guilini, K., Cibic, T., Del Negro, P., 2018b. Structure and function of nematode assemblages in contaminated sediments: what can we learn from the Mar Piccolo of Taranto (Ionian Sea)? J. Mar. Biol. Ass. UK. 98, 1845-1857. doi:10.1017/S0025315418000553.

Frapiccini, E., Marini, M., 2015. Polycyclic aromatic hydrocarbon degradation and sorption parameters in coastal and open-sea sediment. Water Air Soil Poll. 226, 246. https://doi.org/10.1007/s11270-015-2510-7.

Gambi, C., Dell'Anno, A., Corinaldesi, C., Lo Martire, M., Musco, L., Da Ros, Z., Armiento, G., Danovaro, R., 2020. Impact of historical contamination on meiofaunal assemblages: The case study of Bagnoli-Coroglio Bay (southern Tyrrhenian Sea). Mar. Environ. Res. 156, 104907 https://doi.org/10.1016/j.marenvres.2020.104907

Grilli, F., Accoroni, S., Acri, F., Bernardi Aubry, F., Bergami, C., Cabrini, C., Campanelli, A., Giani, M., Guicciardi, S., Marini, M., Neri, F., Penna, A., Pugnetti, A., Ravaioli, M., Riminucci, F., Ricci, F., Totti, C., Viaroli, P., Cozzi, S., 2020. Seasonal and Interannual Trends of Oceanographic Parameters over 40 Years in the Northern Adriatic Sea in Relation to Nutrient Loadings Using the EMODnet Chemistry Data Portal. Water, 12(8), 2280. https://doi.org/10.3390/w12082280

Heip, C., Vincx, M., Vranken, G., 1985. The ecology of marine nematodes. Oceanogr. Mar. Biol.: An Ann. Rev. 23, 399–489.

Hong, J.H., Semprucci, F., Jeong, R., Kim, K., Lee, S., Jeon, D., Yoo, H., Kim, Ju., Kim, Ja., Yeom, J., Lee, S., Lee, K., Lee, W., 2020. Meiobenthic nematodes in the assessment of the relative impact of human activities on coastal marine ecosystem. Environ. Monit. Assess. 192, 81. https://doi.org/10.1007/s10661-019-8055-2

Jouili, S., Essid, N., Semprucci, F., Boufahja, F., Nasri, A., Beyrem, H., 2017. Environmental quality assessment of El Bibane lagoon (Tunisia) using taxonomical and functional diversity of meiofauna and nematodes. J. Mar. Biol. Assoc. U. K. 97, 1593–1603. doi:10.1017/S0025315416000990.

Losi, V., Ferrero, T.J., Moreno, M., Gaozza, L., Rovere, A., Firpo, M., Marques, J.C., Albertelli, G., 2013. The use of nematodes in assessing ecological conditions in shallow waters surrounding a Mediterranean harbour facility. Estuar. Coast. Shelf. Sci., 130, 209–221. https://doi.org/10.1016/j.ecss.2013.02.017.

Losi, V., Grassi, E., Balsamo, M., Rocchi, M., Gaozza, L., Semprucci, F., 2021. Changes in taxonomic structure and functional traits of nematodes as tools in the assessment of port impact. Estuar. Coast. Shelf. Sci. 260, 107524. https://doi.org/10.1016/j.ecss.2021.107524.

Luna, G.M., Manini, E., Turk, V., Tinta, T., D'Errico, G., Baldrighi, E., et al., 2019. Status of faecal pollution in ports: A basin-wide investigation in the Adriatic Sea. Mar. Poll. Bull. 147, 219-228. https://doi.org/10.1016/j.marpolbul.2018.03.050.

Marin, V., Moreno, M., Vassallo, P., Vezzulli, L., Fabiano, M., 2008. Development of a multistep indicator-based approach (MIBA) for the assessment of environmental quality of harbours. – ICES. J. Mar. Sci. 65, 1436–1441.

Mirto, S., Danovaro, R., 2004. Meiofaunal colonisation on artificial substrates: a tool for biomonitoring the environmental quality on coastal marine systems. Mar. Poll. Bull. 48, 919-926. https://doi.org/10.1016/j.marpolbul.2003.11.016

Moens, T., Vincx, M., 1997. Observations on the feeding ecology of estuarine nematodes. J. Mar. Biolog. Assoc. U.K. 77(1), 211-227. doi:10.1017/S0025315400033889.

Moens, T., Bouillon, S., Gallucci, F., 2005. Dual stable isotope abundances unravel trophic position of estuarine nematodes. J. Mar. Biolog. Assoc. U.K. 85(6), 1401-1407. doi:10.1017/S0025315405012580.

Morabito, R., 2001. Metodo per la determinazione di composti organostannici in sedimenti e matrici biologiche tramite GC–MS e GC-FPD. Metodologie analitiche di riferimento del programma di monitoraggio per il controllo dell’ambiente marino costiero (triennio 2001-2003). Ministero dell’Ambiente e della Tutela del Territorio, ICRAM, Appendice I.

Moreno, M., Ferrero, T.J., Gallizia, I., Vezzulli, L., Albertelli, G., Fabiano, M., 2008. An assessment of the spatial heterogeneity of environmental disturbance within an enclosed harbour through the analysis of meiofauna and nematode assemblages. Estuar. Coast. Shelf. Sci. 77, 565-576. https://doi.org/10.1016/j.ecss.2007.10.016.

Moreno, M., Albertelli, G., Fabiano, M., 2009. Nematode response to metal, PAHs and organic enrichment in tourist marinas of the Mediterranean Sea. Mar. Poll. Bull. 58, 1192-1201. https://doi.org/10.1016/j.marpolbul.2009.03.016.

Moreno, M., Semprucci, F., Vezzulli, L., Balsamo, M., Fabiano, M., Albertelli, G., 2011. The use of nematodes in assessing ecological quality status in the Mediterranean coastal ecosystems. Ecol. Indic. 11, 328-336. https://doi.org/10.1016/j.ecolind.2010.05.011.

Mostafa, A.R., Wade, T.L., Sweet, S.T., Al-Alimi, A.K.A., 2009. Distribution and characterization of polycyclic aromatic hydrocarbons (PAHs) in sediments of Hadhramout coastal area, Gulf of Aden, Yemen. J. Mar. Syst. 78, 1–8. https://doi.org/10.1016/j.jmarsys.2009.02.002.

Mozetič P, Francé J, Kogovsek T, Talaber I, Malej A, 2012. Plankton trends and community changes in a coastal sea (northern Adriatic): Bottom-up *vs.* top-down control in relation to environmental drivers. Estuarine, Coastal Shelf Sci. 115, 138-148. https://doi.org/10.1016/j.ecss.2012.02.009

Ogorelec, B., Mišič, M., Faganeli, J., Stegnar, P., Vrišer, B., Vukovič, A., 1987. The recent sediment of the Bay of Koper (Northern Adriatic). Geologija. 30, 87–121.

Ogorelec, B., Mišič, M., Faganeli, J., 1991. Marine geology of the Gulf of Trieste (northern Adriatic): sedimentological aspects. Mar. Geol. 99, 79–92. https://doi.org/10.1016/0025-3227(91)90084-H.

Pielou, E., 1966. Shannon's formula as a measure of specific diversity: its use and misuse. Am. Nat. 118, 463–465. doi: 10.1086/282439.

Platt, H.M., Warwick, R.M. Free-living Marine Nematodes. Part I. British Enoplids. In Synopses of the British Fauna (New Series). Cambridge University Press: Cambridge, UK, 1983; vol. 28, 307 pp.

Platt, H.M., Warwick, R.M. Free-living Marine Nematodes. Part II. British Chromadorids. In Synopses of the British Fauna (New Series). Brill, Leiden, 1988; vol. 38. 502 pp.

Pusceddu, A., Bianchelli, S., Sanchez, Vidal A., Canals, M., Durrieu De Madron, X., et al., 2010. Organic matter in sediments of canyons and open slopes of the Portuguese, Catalan, Southern Adriatic and Cretan Sea margins. Deep Sea Res., Part I 57, 441–457. https://doi.org/10.1016/j.dsr.2009.11.008.

Sahraeian, N., Sahafi, H.H., Mosallanejad, H., Ingels, J., Semprucci F., 2020. Temporal and spatial variability of free-living nematodes in a beach system characterized by domestic and industrial impacts (Bandar Abbas, Persian Gulf, Iran). Ecol. Indic. 118, 106697. https://doi.org/10.1016/j.ecolind.2020.106697.

Schratzberger, M., Ingels, J., 2018. Meiofauna matters: The roles of meiofauna in benthic ecosystems. J. Exp. Mar. Biol. Ecol. 502, 12-25. https://doi.org/10.1016/j.jembe.2017.01.007.

Seinhorst, J.W.,1959. A rapid method for the transfer of nematodes from fixative to anhydrous glycerine. Nematologica. 4, 67–69.

Semprucci, F., Balsamo, M., Appolloni, L., Sandulli, R., 2018. Assessment of ecological quality status along the Apulian coasts (Eastern Mediterranean Sea) based on meiobenthic and nematode assemblages. Mar. Biodiv. 48, 105–115. https://doi.org/10.1007/s12526-017-0745-9.

Semprucci, F., Frontalini, F., Sbrocca, C., Armynot du Châtelet, E., Bout-Roumazeilles, V., Coccioni, R., Balsamo, M., 2015a. Meiobenthos and free-living nematodes as tools for biomonitoring environments affected by riverine impact. Environ. Monit. Assess. 187, 251. https://doi.org/10.1007/s10661-015-4493-7.

Semprucci, F., Losi V., Moreno, M., 2015b. A review of Italian research on free-living marine nematodes and the future perspectives on their use as Ecological Indicators (EcoInds). Mediterr. Mar. Sci. 16(2), 352-365. https://doi.org/10.12681/mms.1072.

Shannon, C., Weaver, W. The Mathematical Theory of Communication. University of Illinois Press, Urbana, 1949; 117 pp.

Simboura, N., 2004. Bentix index vs. biotic index in monitoring: an answer to Borja et al., 2003. Mar. Pollut. Bull. 48, 404– 405.

Solis-Weiss, V., Aleffi, F., Bettoso, N., Rossin, P., Orel, G., Fonda-Umani, S., 2004. Effects of industrial and urban pollution on the benthic macrofauna in the Bay of Muggia (industrial port of Trieste, Italy). Sci. Total Environ. 328, 247–263. doi: 10.1016/j.scitotenv.2004.01.027

Spagnolo A, Scarcella G, Sarappa A (2011) Benthic community response to sediment features in Ancona Harbour (Northern Adriatic Sea, Italy). Vie Milieu 61: 119-128.

Thibaut, T., Blanfuné, A., Boudouresque, C.F., Personnic, S., Ruitton, R., Ballesteros, E., et al., 2017. An ecosystem-based approach to assess the status of Mediterranean algae-dominated shallow rocky reefs. Mar. Pollut. Bull. 117: 311-329. https://doi.org/10.1016/j.marpolbul.2017.01.029.

Trannum, H.C., Olsgard, F., Skei, J.M., Indrehus, J., Øverås, S., Eriksen, J., 2004. Effects of copper, cadmium and contaminated harbour sediments on recolonisation of soft-bottom communities. J. Exp. Mar. Biol. Ecol. 310, 87-114. https://doi.org/10.1016/j.jembe.2004.04.003.

Veiga, P., Rubal, M., Besteiro, C., 2009. Shallow sublittoral meiofauna communities and sediment polycyclic aromatic hydrocarbons (PAHs) content on the Galician coast (NW Spain), six months after the Prestige oil spill. Mar. Poll. Bull. 58(4), 581-588. https://doi.org/10.1016/j.marpolbul.2008.11.002.

Warwick, R.M., Platt, H.M., Somerfield, P.J. Free-living Marine Nematodes. Part III. British Monhysterids. In Synopses of the British Fauna (New Series). Field Studies Council, Shrewsbury, UK, 1998; vol. 53, 296 pp.

Wieser, W., 1953. Die Beziehung zwischen Mundh¨ohlengestalt, Ern¨ahrungsweise und Vorkommen bei freilebenden marinen nematoden. Eine okologisch-morphologische studie. Ark. Zool. 4, 439–484.

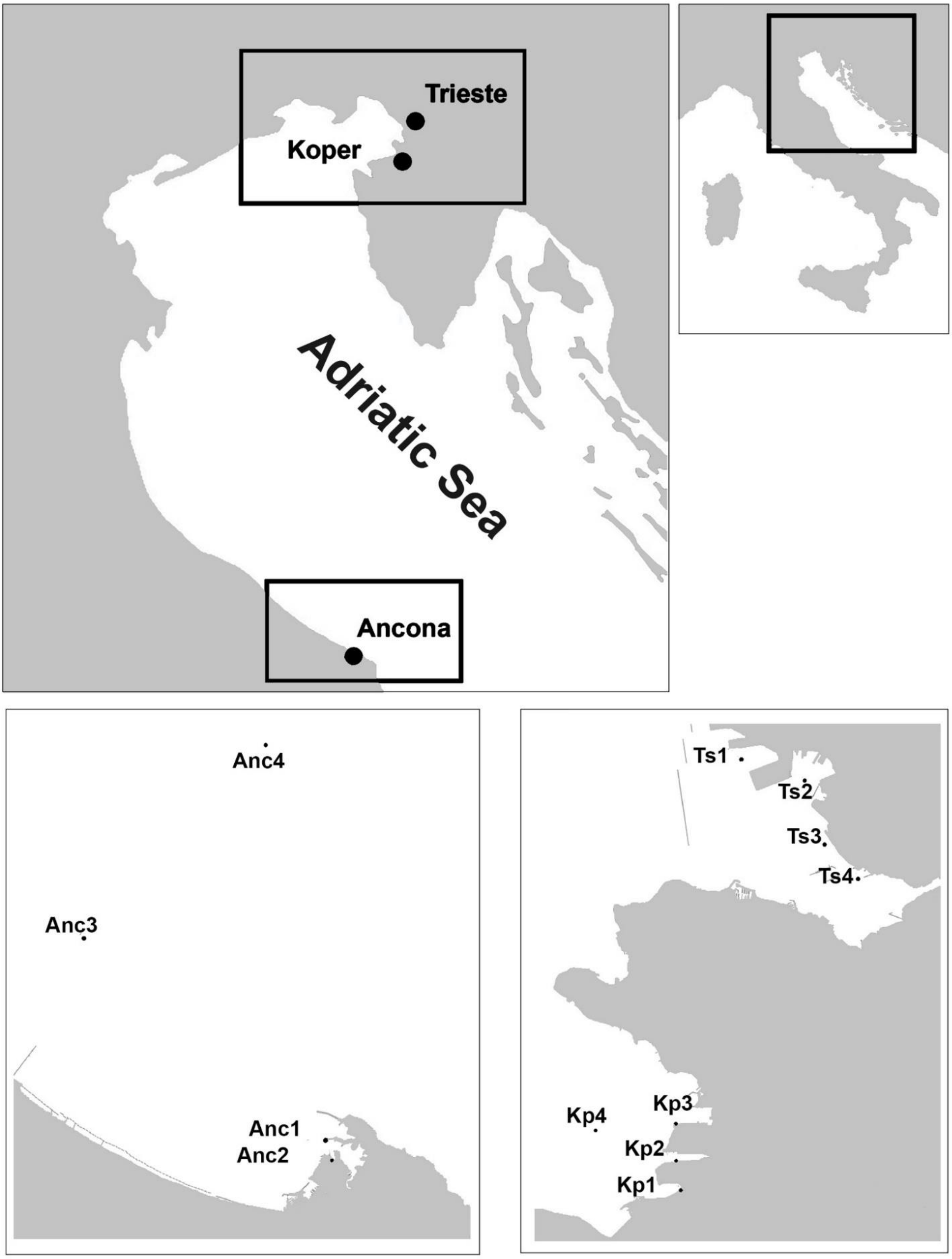


Fig. 1. Study area with the location of the ports of Ancona, Trieste and Koper and of the sampling stations inside each port.

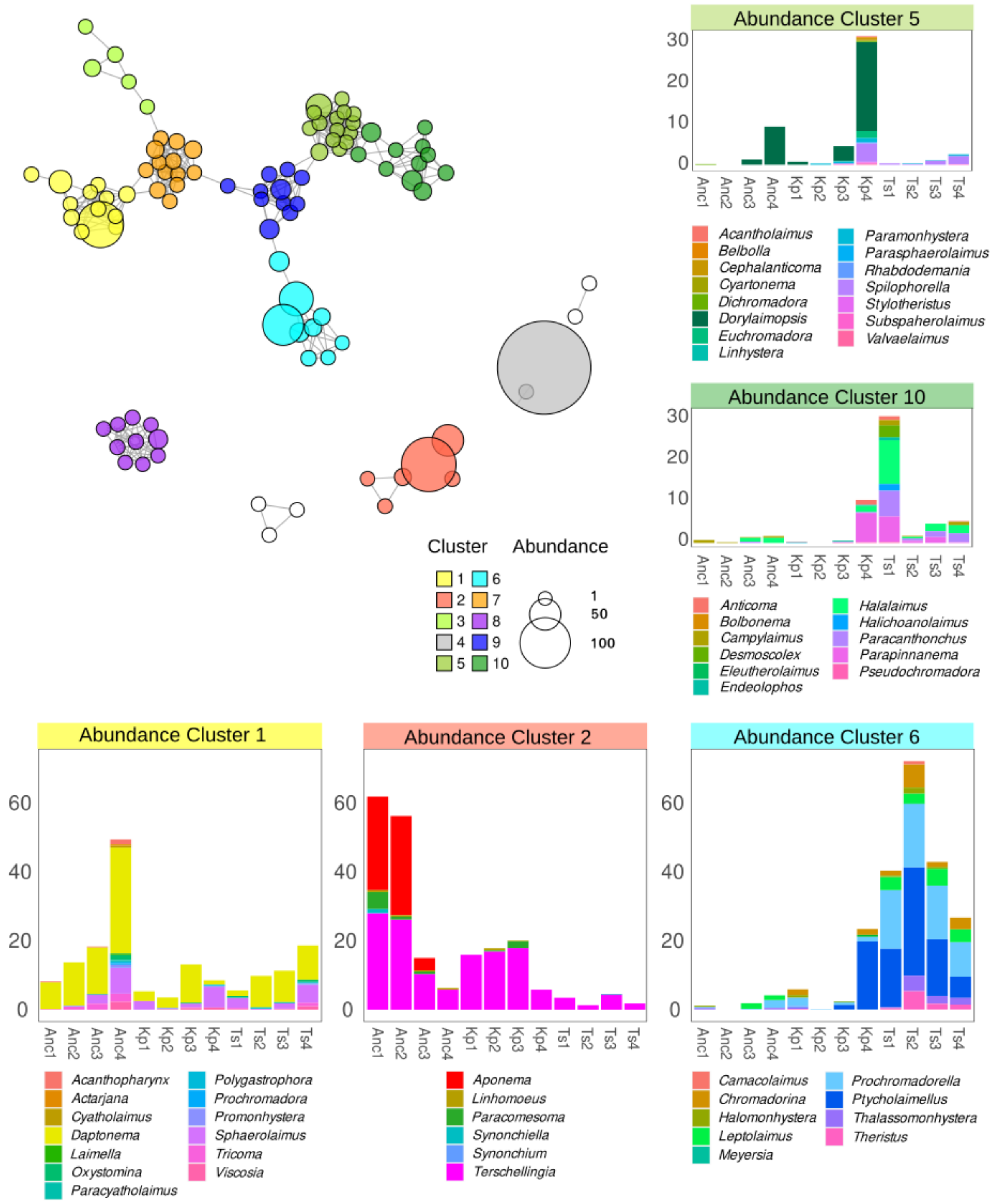
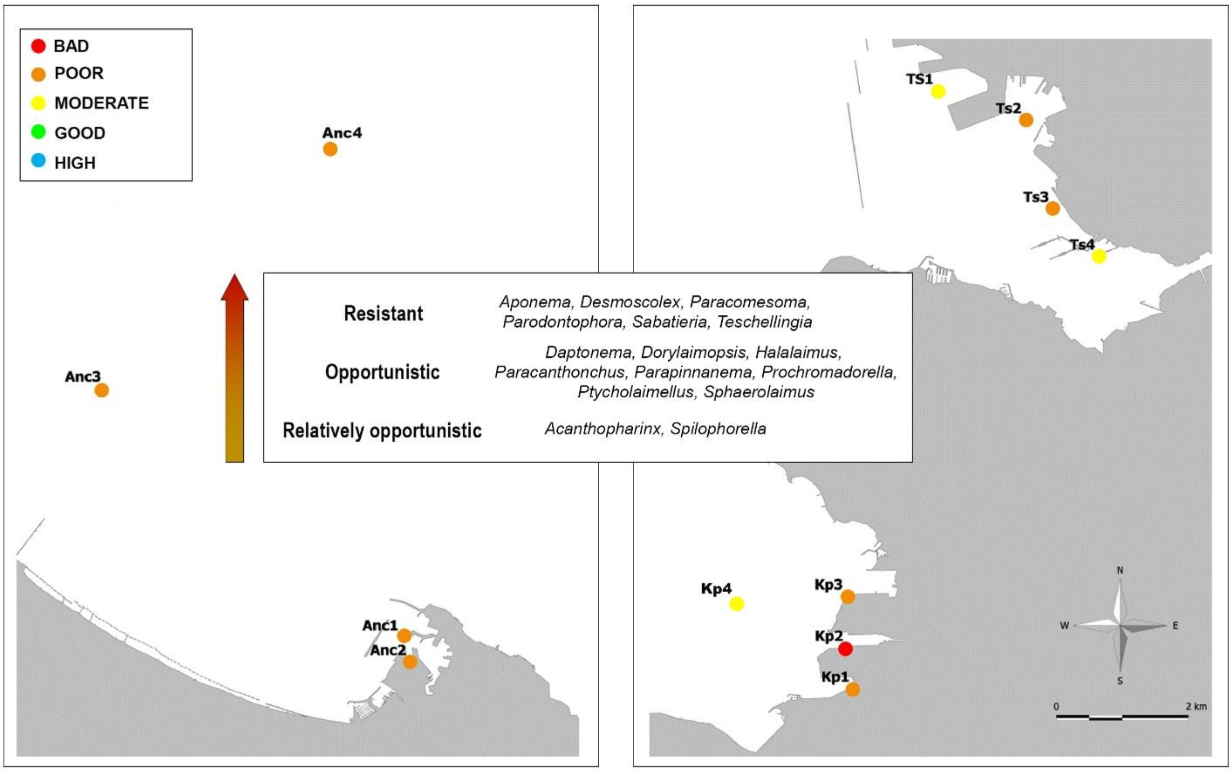


Fig. 2. Outputs of the co-occurrence analysis performed on the entire nematode dataset. Each node indicated a genus, and its size represents the sum of the relative abundances. Each edge represents a positive Spearman correlation between genera (ρ > 0.7). The clusters of nematodes with similar distribution are indicated with different colors. The quantitatively most important groups of co-occuring genera (>30% of total abundance) are reported in the bar plots at the bottom and the right.



Resistant	*Aponema, Desmoscolex, Paracomesoma, Parodontophora, Sabatieria, Teschellingia*
Opportunistic	*Daptonema, Dorylaimopsis, Halalaimus, Paracanthonchus, Parapinnanema, Prochromadorella, Ptycholaimellus, Sphaerolaimus*
Relatively opportunistic	*Acanthopharinx, Spilophorella*

Fig. 3. Subdivision of nematode genera as resistant (nematodes mainly found in polluted port sediments), opportunistic (equally found in both polluted and unpolluted port sediments) and relatively opportunistic (mainly occurring in unpolluted port stations) and correspondence to the EQS of each station.

Table 1. Environmental parameters measured within the three ports: % of silt/mud and sand fractions, Total Butyltin (ΣBT), Total Polycyclic Aromatic Hydrocarbons (ΣPAH) and % of Total Organic Carbon (TOC).

Station	Grain-size		Contaminants		% TOC
	% sand (>63µm)	% silt/mud (>63µm)	ΣBT (ng Sn g^{-1}dw)	ΣPAH (ng g^{-1}dw)	
Anc 1	78	22	15	55.22	1.1
Anc 2	82	22	8	99.85	0.73
Anc 3	81	22	7	111.99	0.89
Anc 4	71	29	-	-	-
Ts 1	6	94	2	3,785.2	2.74
Ts 2	6	94	9.0	13,958.81	2.61
Ts 3	6	94	6.0	14,036.14	4.78
Ts 4	6	90	5.0	73.41	2.23
Kp 1	0	100	-	-	-
Kp 2	0	100	9	261.91	1.25
Kp 3	0	100	7	167.56	1.04
Kp 4	0	100	9	302.77	1.21

Table 2. PERMANOVA outputs carried out to ascertain the differences among ports according to nematode assemblage structure, abundance (ABU), Shannon-diversity (H'), total genera (S), Pielou-evenness (J), Maturity index (MI), Index of Trophic Diversity (ITD). df= degree of freedom; SS= sum of square; MS= mean square; F=F statistic; P= probability level (in bold significant P values); A = Ancona; K = Koper; T= Trieste.

	Source of variation	df	SS	MS	Pseudo-F	P(perm)
Assemblage structure	Port	2	41085	20543	12.239	**0.0001**
	Residual	58	97347	1678.4		
	Total	63	1.59E+05			
	pair-wise comparisons					T≠K***
						T≠A***
ABU	Port	2	11794	5897	6.119	**0.004**
	Residual	17	16385	963.8		
	Total	22	29269			
	pair-wise comparisons					T≠K*
						T≠A**
H'	Port	2	7.250	3.625	6.089	**0.004**
	Residual	58	34.529	0.595		
	Total	63	51.421			
	pair-wise comparisons					T≠K**
						T≠A***
S	Port	2	405.73	202.86	6.658	**0.003**
	Residual	58	1767.2	30.47		
	Total	63	2506.9			
	pair-wise comparisons					T≠K*
						T≠A***
J	Port	2	0.113	0.057	3.381	**0.037**
	Residual	58	0.972	0.017		
	Total	63	1.37			
	pair-wise comparisons					T≠K*
						T≠A*
MI	Port	2	0.068	0.034	0.733	0.484
	Residual	58	2.688	0.046		
	Total					
ITD	Port	2	0.256	0.128	8.668	**0.001**
	Residual	58	0.855	0.015		

	Total	63	1.239	
	pair-wise comparisons			K≠T** K≠A**

Table 3. DistLM outputs carried out on the nematode assemblage structure. P = significant value; Prop. = amount of explained variation.

MARGINAL TESTS

Variable	SS(trace)	Pseudo-F	P	Prop.
log(ΣPAH)	4,386.3	35.063	**0.025**	0.305
TOC %	4,079.6	31.642	**0.022**	0.283
Sqr(%sand)	2,768.9	19.055	0.121	0.192
ΣBT	2,932.3	20.467	0.095	0.204

SEQUENTIAL TESTS

Variable	R^2	SS(trace)	Pseudo-F	P	Prop.	Cumul.	res.df
+log(ΣPAH)	0.305	4,386.3	35,063	**0.022**	0.305	0.305	8
+Sqr(%sand)	0.446	2,034.2	17,858	0.099	0.141	0.446	7
+ΣBT	0.536	1,289.5	11,575	0.302	0.090	0.536	6
+TOC %	0.565	423.79	0.339	0.890	0.029	0.565	5

BEST SOLUTION

	R^2	RSS	No.Vars	Selections
	0.565	6260.4	4	1;5;6;23

Appendix A. Supplementary data available at:
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CHAPTER 6

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Article

Simple Is the Best: An Alternative Method for the Analysis of Free-Living Nematode Assemblage Structure

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Abstract: An alternative approach to notice changes of the nematode community structure was evaluated in five study cases in the Mediterranean Sea. In detail, we suggested a combination of morpho-functional traits (i.e., amphid, cuticle, buccal cavity, and tail shape) as an alternative to the taxonomic identification of nematodes. The results clearly demonstrated that the matrixes made using the trait code numbers perfectly mirror the changes of the nematode community structure at the genus level. The combination of the morpho-functional traits more frequently observed in areas under human pressure were ascribable to genera belonging to Xyalidae, Linhomoeidae and Chromadoridae families. This approach might greatly speed the analyses of nematode fauna in biomonitoring programs and might also be adopted for other meiobenthic organisms that may be categorized in functional groups opening new perspectives in the ecological assessment of meiofauna.

Keywords: Nematoda; biological indicators; biomonitoring; morpho-functional traits; prevention actions

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1. Introduction

In aquatic habitats, sediments characterized by large amounts of silt, clay, and organic load adsorb persistent pollutants (e.g., metals, PCBs) and polycyclic aromatic hydrocarbons (i.e., PAHs), becoming the main sink of contaminants brought into the marine system [1–3]. These sediment-bound pollutants can alter the state of the seabed, benthic processes, and functioning of ecosystems [4–6]. One of the most consistent effects of contamination in sea bottoms is a change in the structure of benthic biological assemblages, as pollution-sensitive species are lost and replaced by more tolerant ones, e.g., [7–9]. This makes the composition/structure changes of the benthic assemblages in themselves robust signals of alterations in the ecological quality status of the sediments. Therefore, noticing early alterations and warning conditions represent worldwide aims, as highlighted by all governmental regulations and sediment quality guidelines. However, routine monitoring still focuses mainly on surface waters, while sediment quality assessment remains a major concern; see [3,10] and references therein.

Numerous indices exist that consider diversity, abundance, and autoecology of benthic taxa for the quality assessment of sediments, but they mainly focus on macrofauna due to a long tradition and an extensive taxonomic and ecological knowledge of macrobenthos, see [11–13] for review. Notwithstanding macroinvertebrates showing consistent responses to anthropogenic impact [14,15], they are only one face of the benthic coin [16]. Indeed, benthic nematodes are the dominant and most diverse group in marine ecosystems [17–19], that suggest a high degree of specificity in their choice of natural environment. Nematodes living between sand particles are strongly influenced in their structure by environmental matrix variations in physicochemical properties and contaminant concentrations [20].

This issue, along with a generally high population stability, short generation times, low mobility, and direct benthic development, results in a wide range of specificity and rapid responses of nematodes to in situ disturbances [2]. The entire life cycle of numerous nematode species is completed in a few weeks, resulting in a much higher production-to-biomass ratio of meiofauna compared with macrofauna [21]. Moreover, nematodes play a crucial role as a trophic link between bacteria and larger fauna, enhancing the rate of: (a) carbon mineralization by stimulating microbial activity through predation; and/or (b) consumption of detritus by larger deposit-feeders [22]. Given their high abundance and wide distribution in all types of habitats, they provide a highly reliable dataset for statistical analysis, even when only limited sediment volumes are collected [23]. Therefore, despite nematodes being invisible to the human eye, their changes in assemblage structures might provide remarkable advantages for preventing actions in the management of coastal systems [2,8,24,25].

A general limitation of ecological investigations based on nematodes is related to the difficult and time-consuming taxonomic identification of species that requires the aid of expert taxonomists [26]. The implementation of molecular techniques and particularly environmental DNA (eDNA) metabarcoding has certainly created new perspectives, making nematode identification easier and faster and increasing the taxonomic resolution of the investigations [3,27]. Studies of pollution response have found metabarcoding to be more sensitive in revealing assemblage changes than the traditional morphological approach. However, drawbacks (possible biodiversity assessment bias by capturing signals from dead organisms, extracellular DNA; false readings due to taxonomic selectivity and restricted sensitivity of primers; unavoidability of primers and amplification bias; lack of comprehensive genetic databases) are still present and so they require caution in the interpretation of the quantitative results [28]. Thus, although molecular methods provide numerous benefits, the promise of barcoding as a routine and cheaper alternative to current biomonitoring practices is not yet realized and more user-friendly methods of data exploration are needed.

A way to overcome some of these limits might be the use of functional traits. They capture characteristics of organisms (i.e., morphological, physiological, phenological, and/or behavioral) that are linked to life-history and ecological functioning, and, at the same time, detect responses to anthropogenic disturbances and effects on ecological processes and services [29]. Species in functional groups share morphological traits that are thought to be linked to significant ecological functions [30]. Functional groups of nematodes can be regarded as groups of species that have similar influences on ecosystem processes [31]. Morphological structure of the buccal cavity, for example, was recognized by Wieser as related to specific trophic roles of the nematodes [32,33]; tail shape has an important part in locomotion, feeding, and reproduction [34,35]; body size may be related to species life history, physiology, and energy requirements [36–38]; amphids, as the main chemosensory organs of the nematode cephalic region, [39] take a role in the search for food and partners [40]; and body cuticles (i.e., morphology and thickness) as anti-predator, pollution, and hydrodynamic energy defense [41–43].

Thus, a combining approach of a few functional traits (i.e., buccal cavities, amphids, cuticles, and the shapes of tails) that can be easily identified in a short time by inexperienced people might also reflect a possible loss of ecosystem functionality after pollution.

The aim of the present study is to demonstrate that an approach of combining functional traits may efficiently mirror the nematode taxonomic structure and document any variation of the nematode community. To deal with this aim, we have considered a number of study cases focused on various typologies of human disturbances (i.e., ports, marinas, fish farms, urban riverine discharges) carried out in the Mediterranean Sea. We compared the results of the changes in the structure of the nematode assemblages identified at the genus level and the combination of the four morpho-functional traits in five study cases. Thus, we first assigned the four morpho-functional traits to each taxon found in the single dataset, then we obtained a new data matrix based on the traits' number codes and compared the trends observed using the nematode taxonomic composition. This approach, if recognized as able to capture nematode variations, might be a very low-cost solution (both in terms of time and money) to overcome the problem of nematode taxonomic identification and an alternative method easily applicable in biomonitoring by inexperienced people (e.g., students, environmental agency staff, voluntary citizens). In this paper, three main questions are addressed: Does the combination of morpho-functional traits reflect the taxonomic composition of nematode fauna? Does the combination of morpho-functional traits detect the effects of a different human pressure? Are there recurrent combinations of morpho-functional traits in nematode communities in polluted sediments?

2. Materials and Methods

2.1. Study Areas

In the present study, the data sets of five study cases from the Mediterranean Sea were considered to demonstrate the efficiency of the combination of morpho-functional traits of nematodes. The coastal areas are exposed to different types of human activities/pressures: ports, marinas, fish farms, and discharge of urban rivers (Figure 1 and Table S1). Sediment samples (three replicates for each station) were collected by SCUBA divers or using modified Van Veen grabs. In this last case, the undisturbed aliquots of sediments were subsampled using plexiglass corers. The main information on sites, sampling routines, experimental designs, and sample processing techniques is hereafter summarized (Table S1).

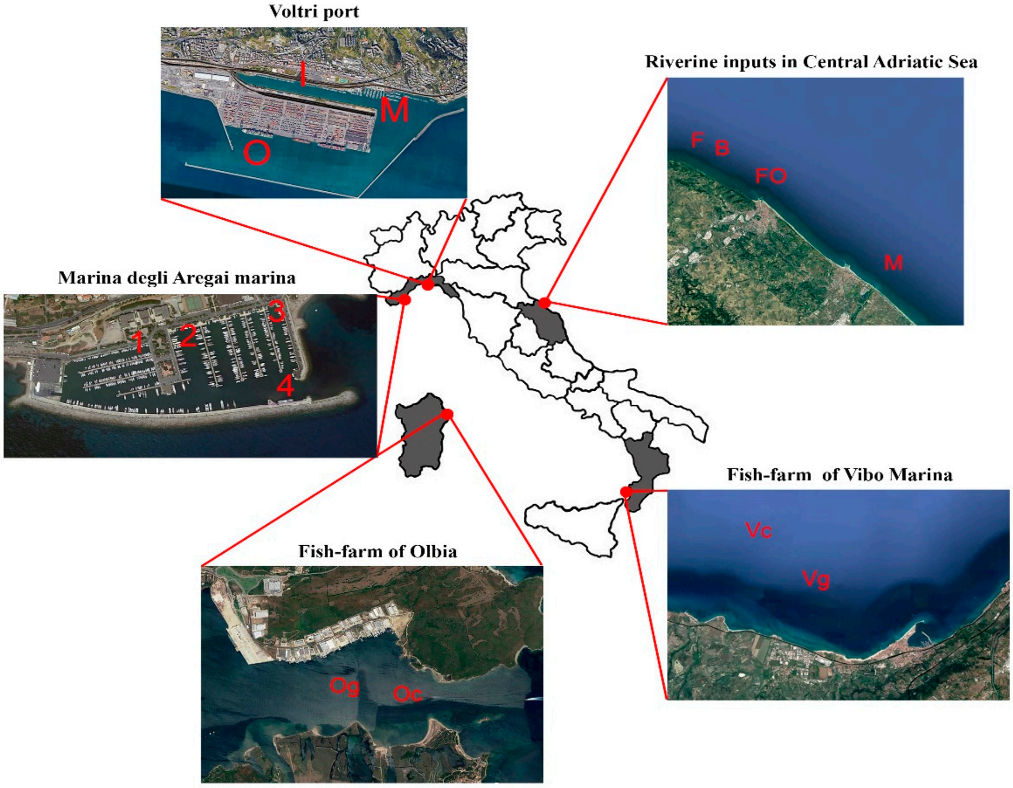


Figure 1. Localities of the study cases considered in the present study. Port contaminated stations: Voltri (St. I: inner port area, St. M: central port area, and St. O: outer port area) and Marina degli Aregai (four stations from the inner, St. 1, to outer port part, St. 4); coastal sediments influenced by riverine inputs (St. B and St. FI: Natural Regional Park; St. M and St. FO: Foglia and Metauro rivers); fish farm of Olbia (Og: under cages, Oc: controls) and tuna farming of Vibo Marina (Vg: under cages, Vc: controls).

2.1.1. Port-Contaminated Sediments from Ligurian Sea (NW Mediterranean)

The industrial port of Genoa-Voltri is an important container and oil terminal (total area ~34.5 ha). Samples were collected in four periods (i.e., June, July, and November 2002;

and February 2003) at a mean depth of 9.5 m in three sampling stations selected on the basis of their position within the port: St. I (inner port area), St. M (central port area), and St. O (outer port area, close to the open sea). Substrata were mainly characterized by fine sands and muddy sediments. St. M resulted the most contaminated area (the highest concentrations of organic matter, lowest organic matter quality i.e., low PRT:CHO ratio, highest bacterial densities, and PAHs), followed by St. I [44].

Marina degli Aregai is a tourist marina where sediments (fine sands) were collected at a range from 3 to 12 m of depth. Sampling was carried out in two periods (i.e., July 2005 and January 2006) and at four stations from the inner (St. 1) to outer port part, close to the port mouth (St. 4). Fine sands dominated the substrata and contaminants showed higher concentrations at St. 4 (i.e., highest organic load, Pb, Cr, and PAH concentrations) [45].

2.1.2. Coastal Sediments Influenced by Riverine Inputs in Central Adriatic Sea

The study area was in the Central Adriatic Sea (Central Mediterranean), close to Pesaro. Most relevant pollution sources are the runoffs of the local rivers (i.e., Foglia, Metauro, and Tavollo) and, after severe rains, Po River plumes. A total of four positions were selected for the sampling: two within the Natural Regional Park of Monte San Bartolo (i.e., St. B, and St. FI) and two others in front of the mouths of Foglia and Metauro rivers (i.e., St. M and St. FO). Samples were collected ~5 m in depth from each station in two different periods (November 2011 and June 2012). Sediments of the study area are fine sands. Both the rivers receive wastewater from urban and industrial areas and livestock farms, and the Foglia River especially causes frequent eutrophication phenomena [46,47]. Furthermore, the occurrence of low-salinity waters and high levels of Chl-a in the FI station suggested the influence of the Po River plume also confirmed by clay mineralogy data [48].

2.1.3. Fish Farm Impact in the Tyrrhenian Sea

Two areas were under scrutiny for fish farm disturbance: the first one is in the sheltered Gulf of Olbia (Northern Sardinia, Tyrrhenian Sea) [7] and the second one is an open sea area at Vibo Marina (Calabria), SW Italy (W Mediterranean Sea) [49]. Many anthropogenic activities are present in the Gulf of Olbia: tourist and commercial harbors, mussel farms, fish farming, and river outflows contributed to affect the benthic communities [7]. Sampling was carried out in two periods (i.e., April 2006 and September 2006) in two stations (~6 m of depth): one under cages (Og) and the other, regarded as control, far from the farming (Oc). Sediments were mainly characterized by fine sand fraction, especially under the cages (i.e., at St. Og) where a high level of organic matter, heavy metals, and PAHs were found.

The bluefin tuna farming of Vibo Marina was located in open sea at ~46 m in depth. Samplings were carried out in October 2005 and May 2006 at four stations: two close to the floating cages (Vg) and two others (controls) far from the farming (Vc). Sediments were mainly characterized by sand with a fine fraction accounting for ~40–50% [49]. Despite the presence of the floating cages, a moderate amount of organic matter was recorded and was slightly higher at the control station. Globally, the parameters measured in this study did not point to a detectable impact of organic waste due to the fattening of Atlantic bluefin tuna (*Thunnus thynnus*). This was likely due to the ecological context (e.g., oligotrophy) and the exposed nature of the Vibo Marine area characterized by high water depth and a strong hydrodynamic regime [49].

2.2. Nematodes

For nematode analyses, details of sample treatment and laboratory analysis are given in the relative papers reported in Table S1. Once in the laboratory, sediment samples were firstly sieved through 500–38 µm mesh sieves, then the retained fraction was centrifuged three times in a gradient of Ludox HS 30 (density 1.18 g cm^{-3}) as described by Danovaro et al. [50]. One hundred nematodes of those so obtained were randomly picked up and mounted on permanent slides according to Seinhorst [51]. Specimens were identified at the genus level with the aid of the pictorial keys of Platt and Warwick [52,53] and Warwick et al. [54], as well as the use of the NeMys website [19].

Furthermore, nematodes were characterized according to their morpho-functional traits. Amphid and cuticle structure were categorized according to the classification by Semprucci et al. [42]. Nematodes were assigned to eight categories based on amphid structure: indistinct, slit-like, pocket-like, spiral, rounded or elongate loop, circular-oval, blister-like, and longitudinal slit (Figure 2); and five based on cuticle structure: smooth, with desmens, with bacteria covering, punctuated or annulated with or without lateral differentiation, and with wide body annules and longitudinal ridges (Figure 3).

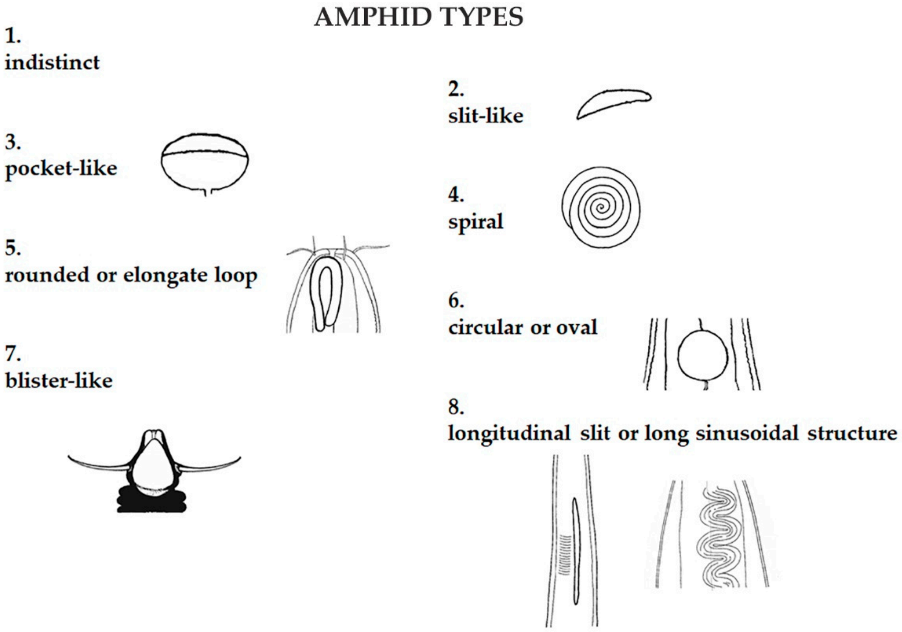


Figure 2. The amphids, chemosensory organs located in the head region of nematodes, play a role in the search for food and partners. Eight categories were identified on the base of the amphidal fovea shape.

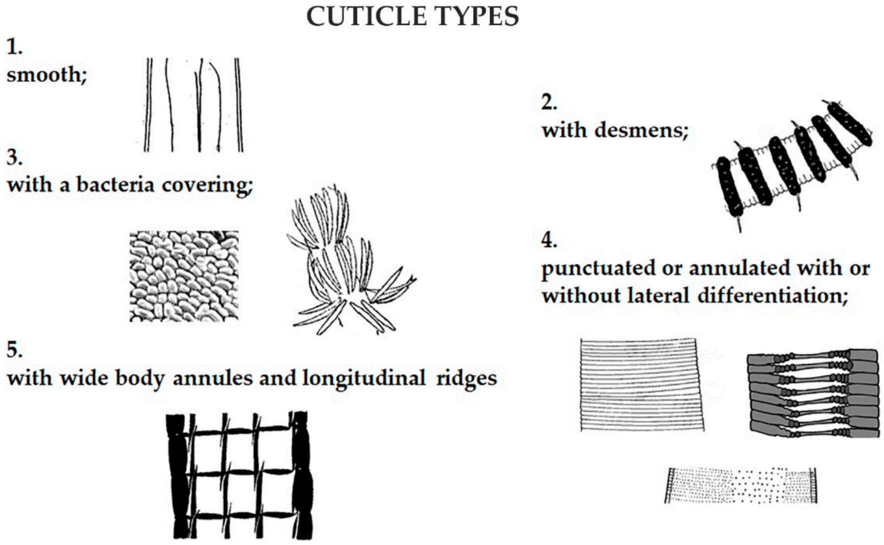


Figure 3. Cuticle has a role in defense against predators, pollution, and hydrodynamic energy and in nematode locomotion. Five categories of cuticles were recognized on the base of their morphology and thickness.

Specimens were then assigned to four trophic groups, according to Wieser [32], based on buccal morphology: selective (1A) and non-selective (1B) deposit feeders, epistratum feeders (2A), and predators/omnivores (2B) (Figure 4). As for the tail shape, specimens were assigned to one of four categories: short/round; elongated/filiform; conical and clavate/conical-cylindrical [35] (Figure 5). The four functional traits (buccal cavity, amphid, cuticle, tail) were assigned to each specimen found obtaining a four-digit number that identified each nematode (see Figure 6).

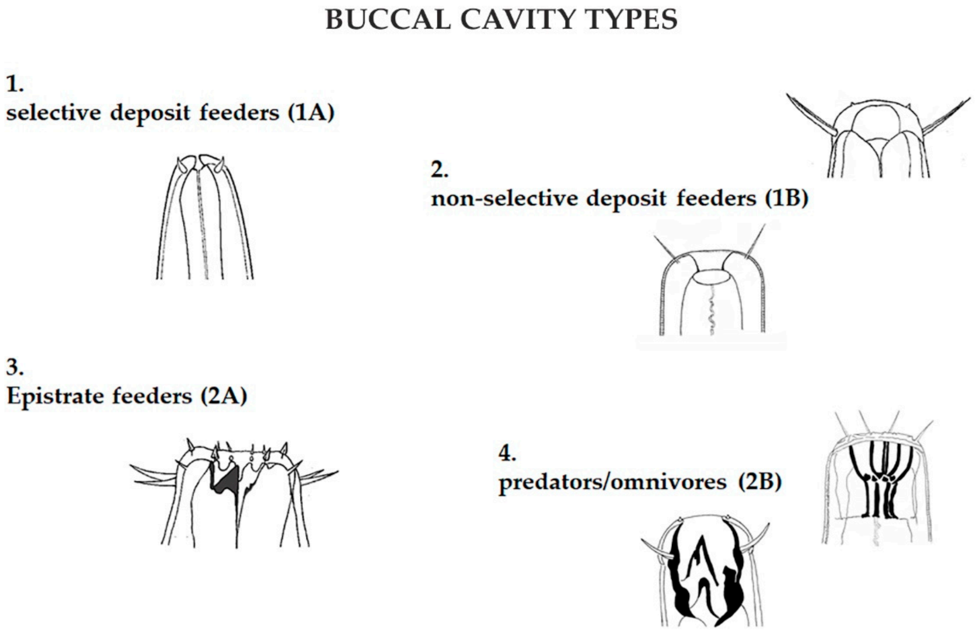


Figure 4. Buccal cavity morphologies reflect the trophic role of the species in the ecosystem: selective (1A) and non-selective (1B) deposit feeders, epistratum feeders (2A), and predators/omnivores (2B).

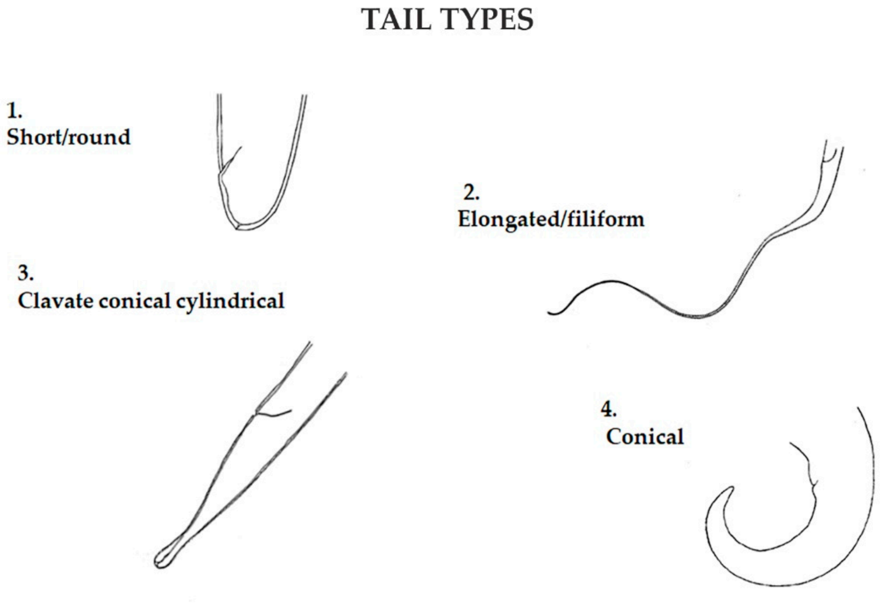
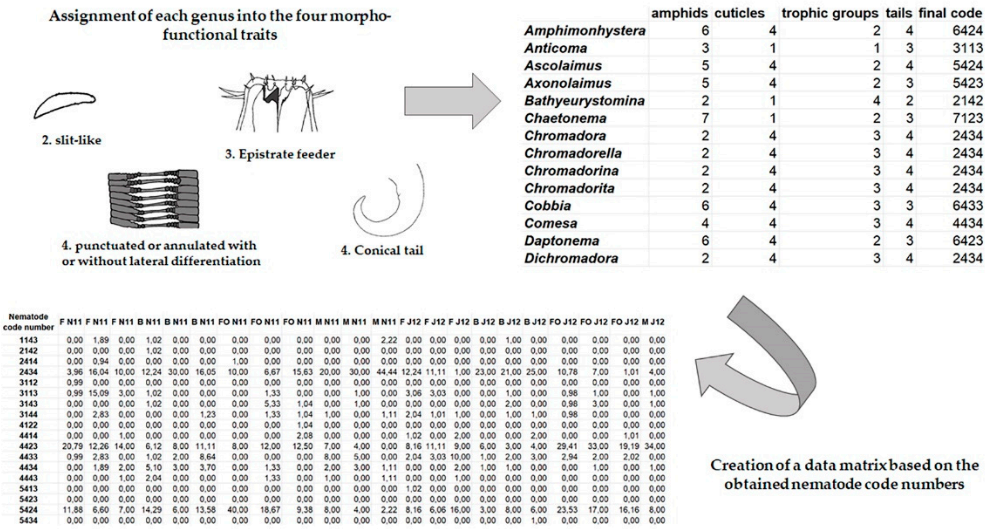


Figure 5. Tail shape has an important role in nematode locomotion, feeding, and reproduction. Four categories were designed: short/round; elongated/filiform; conical; and clavate/conical-cylindrical.



	amphids	cuticles	trophic groups	tails	final code
Amphimonhystera	6	4	2	4	6424
Anticoma	3	1	1	3	3113
Ascolaimus	5	4	2	4	5424
Axonolaimus	5	4	2	3	5423
Bathyeurystomina	2	1	4	2	2142
Chaetonema	7	1	2	3	7123
Chromadora	2	4	3	4	2434
Chromadorella	2	4	3	4	2434
Chromadorina	2	4	3	4	2434
Chromadorita	2	4	3	4	2434
Cobbia	6	4	3	3	6433
Comesa	4	4	3	4	4434
Daptonema	6	4	2	3	6423
Dichromadora	2	4	3	4	2434

Nematode code number	F N11	F N11	F N11	B N11	B N11	B N11	FO N11	FO N11	FO N11	M N11	M N11	M N11	F J12	F J12	F J12	B J12	B J12	B J12	FO J12	FO J12	FO J12	M J12
1143	0,00	1,89	0,00	1,02	0,00	0,00	0,00	0,00	0,00	0,00	0,00	2,22	0,00	0,00	0,00	0,00	1,00	0,00	0,00	0,00	0,00	0,00
2142	0,00	0,00	0,00	1,02	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
2414	0,00	0,94	0,00	0,00	0,00	0,00	1,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
2434	3,96	16,04	10,00	12,24	30,00	16,05	10,00	6,67	15,63	20,00	30,00	44,44	12,24	11,11	1,00	23,00	21,00	25,00	10,78	7,00	1,01	4,00
3112	0,99	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
3113	0,99	15,09	3,00	1,02	0,00	0,00	0,00	1,33	0,00	0,00	1,00	0,00	3,06	3,03	0,00	0,00	1,00	0,00	0,98	1,00	0,00	1,00
3143	0,00	0,00	0,00	1,02	0,00	0,00	0,00	5,33	1,04	0,00	1,00	0,00	0,00	0,00	0,00	0,00	2,00	0,00	0,98	3,00	0,00	1,00
3144	0,00	2,83	0,00	0,00	0,00	1,23	0,00	1,33	1,04	1,00	0,00	1,11	2,04	1,01	1,00	0,00	1,00	1,00	0,98	0,00	0,00	0,00
4122	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	1,04	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
4414	0,00	0,00	1,00	0,00	0,00	0,00	0,00	0,00	2,08	0,00	0,00	0,00	1,02	0,00	2,00	0,00	0,00	2,00	0,00	0,00	1,01	0,00
4423	20,79	12,26	14,00	6,12	8,00	11,11	8,00	12,00	12,50	7,00	4,00	0,00	8,16	11,11	9,00	6,00	3,00	4,00	29,41	33,00	19,19	34,00
4433	0,99	2,83	0,00	1,02	2,00	8,64	0,00	0,00	0,00	8,00	5,00	0,00	2,04	3,03	10,00	1,00	2,00	3,00	2,94	2,00	2,02	0,00
4434	0,00	1,89	2,00	5,10	3,00	3,70	0,00	1,33	0,00	2,00	3,00	1,11	0,00	0,00	2,00	1,00	1,00	0,00	0,00	1,00	0,00	1,00
4443	0,00	0,00	1,00	2,04	0,00	0,00	0,00	1,33	0,00	1,00	0,00	1,11	0,00	0,00	1,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
5413	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	1,02	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
5423	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
5424	11,88	6,60	7,00	14,29	6,00	13,58	40,00	18,67	9,38	8,00	4,00	2,22	8,16	6,06	16,00	3,00	8,00	6,00	23,53	17,00	16,16	8,00
5434	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	1,00	0,00	0,00	0,00	0,00

Figure 6. Assignment of each genus in the four morpho-functional traits and creation of the data matrix based on the combination of morpho-functional traits.

2.3. Data Analysis

All nematodes with the same code number were grouped together and utilized to produce a data matrix for each study case. Data matrices for the multivariate analyses were based on percentages of nematode genera and combinations of morpho-functional characteristics to compare changes in nematode community structure among sampling stations at each study area.

To visualize and compare the differences of the community structure (genus composition *versus* the combination of morpho-functional traits), non-Multidimensional scaling (nMDS) was applied to genera and trait codes after the Bray–Curtis similarity index computation. According to Schratzberger et al. [31], the matrix based on the combination of morpho-functional traits was not transformed and, to make comparison possible, neither were those on the genera. Spatial and temporal trends were further checked by means of Analysis of Similarities (ANOSIM) to assess the presence of significant differences between nematode communities at the stations. SIMPER test (cut-off, 90%, Bray–Curtis similarities) was applied to the genera and combination of functional traits to find the taxa or traits that mainly distinguished the disturbed stations. Shannon diversity (H′, log2) and Pielou evenness (J) indices were calculated for the functional and taxonomic structure of the nematode communities, respectively, in order to check whether the indices show the same result/trend in each case. The software package Primer v.6 [55] was utilized for all the multivariate analyses as well as index computation (i.e., H′ and J).

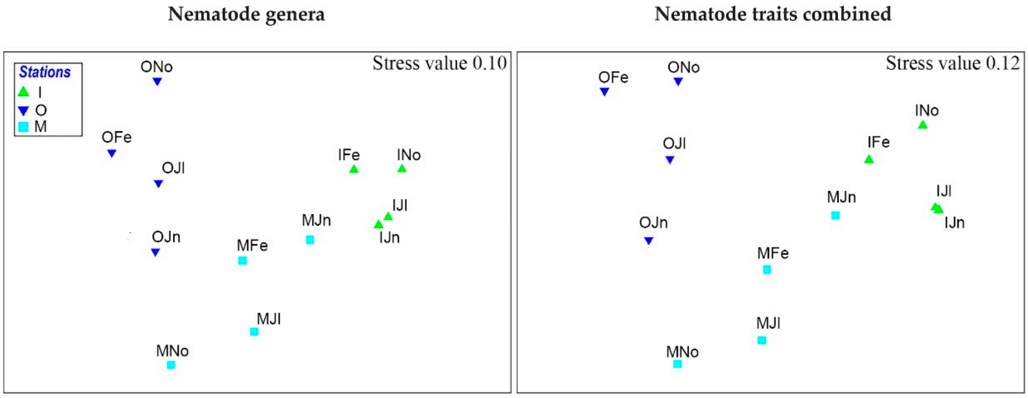
3. Results and Discussion

A total of 12,000 nematodes were analyzed, which could be assigned to 121 genera from 35 families and 47 different morpho-functional trait combinations (Table S2). As reported above, we posed three questions that are addressed in light of the outcomes from the analysis of genera composition and combination of the morpho-functional traits collected at five study case stations in the Mediterranean Sea.

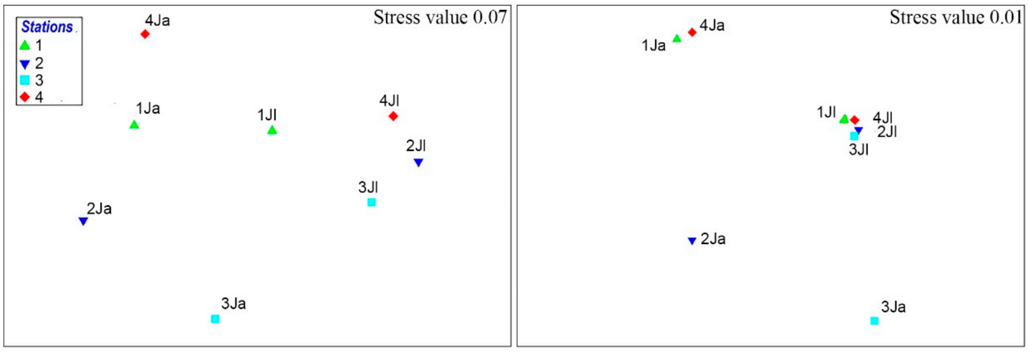
3.1. Does the Combination of Morpho-Functional Traits Reflect the Taxonomic Composition of Nematode Fauna?

Many surveys on nematodes have highlighted in the past decades that the nematode assemblage structure is the first and most efficient tool to detect environmental changes and pollution effects [7]. The need to develop alternative indices (i.e., index of trophic diversity, maturity index, biological trait analysis) [56] or tools for ecological assessment is driven by the necessity to speed the analysis process and overcome the problems related to nematode taxonomic identification. Semprucci et al. [42] and Zullini and Semprucci [40] suggested that there are detectable morphological differences between free-living aquatic (both marine and freshwater) and soil nematodes strictly associated to the environmental variations and that might be used to document the changes in the structure composition.

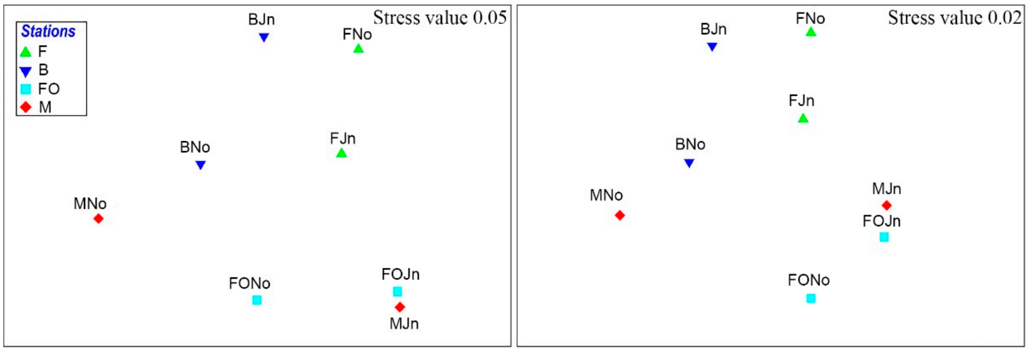
Multivariate statistical analysis such as nMDS revealed that the two data matrixes i.e., genera and combination of morpho-functional traits showed similar sub-divisions of the sampling stations in all the study cases considered (see Figures 7 and 8). This was further evidenced by the analysis of similarities that detected approximately the same R ratio as well as level of significance (when occurred), especially in the comparisons between stations (see Table 1).



Industrial port of Genoa-Voltri

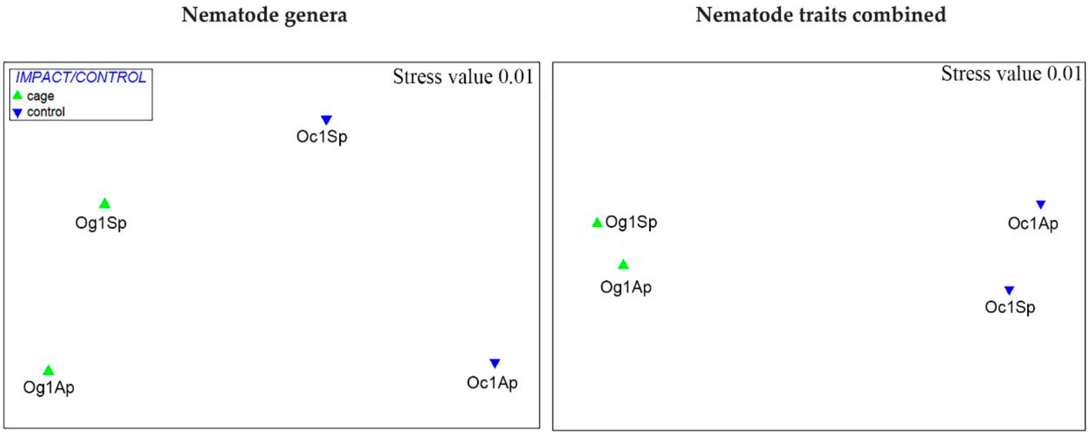


Marina degli Aregai tourist marina

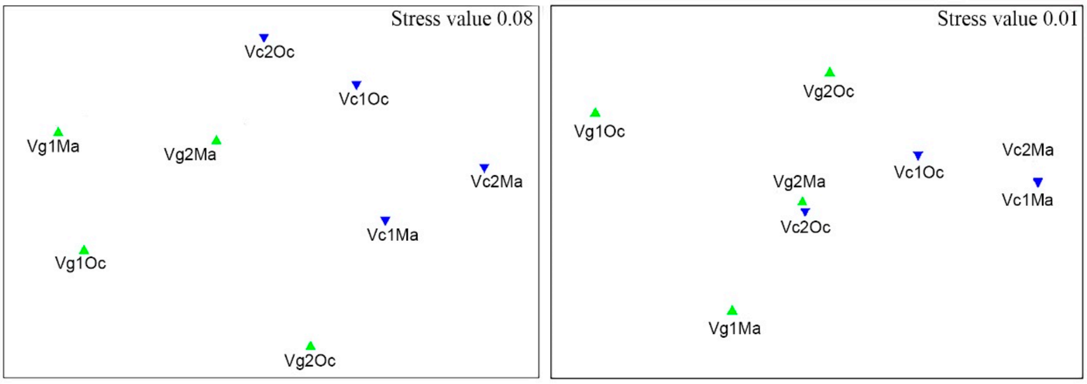


Riverine outfalls in Central Adriatic

Figure 7. nMDS carried out on untransformed data matrixes of both nematode genera and functional trait codes (Bray–Curtis similarity) from port seabeds (Ligurian Sea) and sediments under riverine outfall pressures (Adriatic Sea) (Ja = January; Fe = February; Jn = June; Jl = July; No = November).



Fish farm of Gulf of Olbia



Farming of Vibo Marina

Figure 8. nMDS carried out on untransformed data matrixes of both nematode genera and functional trait codes (Bray–Curtis similarity) from fish farm sediments (Tyrrhenian Sea) (Ap = April; Ma = May; Sp = September; Oc = October).

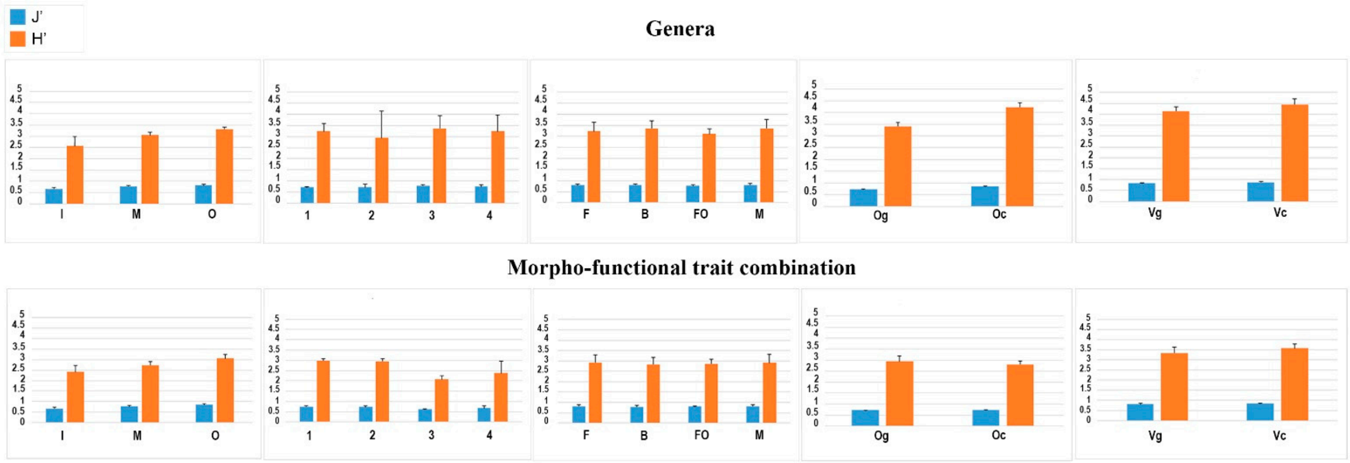
Thus, the finding that the functional trait code reflects the taxonomic structure of the nematode communities at the genus level in all cases studied suggests that this simple method could be a way to routinely use nematodes in biomonitoring programs at low financial cost and in a more time-efficient way.

The potentialities of this approach are further demonstrated by Armenteros et al. [57] that analyzed functional changes in the nematode assemblages across the wide span of habitats investigated in the Cuban Archipelago. They, in particular, reported an unclear pattern of amphidal fovea and cuticle types across the habitats analyzed and argued that different types of amphidal fovea or cuticle did not play an adaptive role in the behavior of nematodes, but likely reflect phylogenetic signatures from families/genera living in different habitat conditions. However, this statement is *per se* a crucial point that corroborates our idea. Most of the characteristics considered diagnostic in nematodes are functionally determined [58]. Therefore, the combination of these morpho-functional traits can take a snapshot of the taxonomic composition and identify possible assemblage changes.

The diversity measures (i.e., H′ and J) calculated for morpho-functional and taxonomic nematode community structure revealed consistent results in the ports from the Ligurian Sea and in the Vibo fish farm, but not in the Olbia farm or Adriatic Sea (Figure 9). Therefore, more than the univariate diversity measures, the multivariate approach to trait combination is recommended.

Table 1. Results of Analysis of Similarities (ANOSIM) performed to detect the presence of significant differences among the stations and sampling periods of each study case (n.s. indicates when significant differences were not found).

Study Case	Structure at Genus Level		Structure at Morpho-Functional Level	
	Sample Statistic	Pairwise Tests	Sample Statistic	Pairwise Tests
Genoa Voltri port	Stations R = 0.80; p = 0.001	St. I vs. St. O: R = 0.99; p = 0.03 St. I vs. St. M: R = 0.65; p = 0.03 St. O vs. St. M: R = 0.75; p = 0.03	Stations R = 0.78; p = 0.001	St. I vs. St. O: R = 0,99; p = 0.03 St. I vs. St. M: R = 0.60; p = 0.03 St. O vs. St. M: R = 0.70; p = 0.03
	Periods p = n.s.		Periods p = n.s.	
Aregai marina	Stations: p = n.s. Periods R = 0.81; p = 0.029		Stations p = n.s. Periods p = n.s.	
Riverine outfall areas	Stations R = 0.38; p = 0.001	F vs. B: R = 0.26; p = 0.05 F vs. FO: R = 0.60; p = 0.002 F vs. M: R = 0.48; p = 0.002 B vs. FO: R = 0.67; p = 0.002 B vs. M: R = 0.30; p = 0.017 FO vs. M: n.s.	Stations R = 0.35; p = 0.001	F vs. B: R = 0.25; p = 0.05 F vs. FO: R = 0.59; p = 0.004 F vs. M: R = 0.34; p = 0.004 B vs. FO: R = 0.70; p = 0.002 B vs. M: R = 0.28; p = 0.017 FO vs. M: n.s.
	Periods R = 0.24; p = 0.001		Periods R = 0.17; p = 0.016	
Olbia Fish-farm	Stations p = n.s. Periods p = n.s.		Stations p = n.s. Periods p = n.s.	
Vibo Fish-farm	Stations p = n.s. Periods p = n.s.		Stations p = n.s. Periods p = n.s.	


Genera

Morpho-functional trait combination

Figure 9. Trends of the Shannon diversity (H′) and Pielou evenness (J′) computed on the matrixes based on genus and combination of morpho-functional traits for each study case.

3.2. Does the Combination of Morpho-Functional Traits Detect the Effect of a Different Human Pressure?

Spatial and temporal trends were checked by ANOSIM. The trait combination perfectly distinguished the pollution gradient of the port of Voltri from the inner station to the open sea station. St. M resulted a sampling point with all pollutants showing higher concentrations [44] along with a distinct morpho-functional trait combination represented mainly by 6423 (combination of circular or oval amphids, punctuated or annulated cuticle with or without lateral differentiation, 1B guild, clavate conical cylindrical tail) and 3143 (i.e., pocket-like amphid, smooth cuticle, 2B group, clavate conical cylindrical body end) (Table S3), which partially fits with the abundant taxa found by Moreno and co-authors in the original paper (i.e., *Daptonema*, *Terschellingia*, *Paracomesoma* and *Metoncholaimus*) [44] (Table S4).

In the Aregai tourist marina, more marked temporal rather than spatial differences were observed in the genus assemblage structure [45] as well as in the trait combination (Table 1) (Figure 7). However, a noticeably greater spatial heterogeneity of the communities (genus and functional trait combined) as well as the lowest diversity values were detected in January, likely in relation with the highest contaminant concentrations in the winter period [45].

The SIMPER analysis revealed that the most abundant functional trait combination in the most polluted station (i.e., St. 4) was again 6423 (circular or oval amphids, punctuated or annulated with or without lateral differentiation cuticle, 1B guild, clavate conical cylindrical tail) together with 2434 (slit-like amphid, punctuated or annulated cuticle with or without lateral differentiation, 2A guild, and conical tail) likely related to the higher abundance of Xyalidae, Linhomoeidae (e.g., 6423), and Chromadoridae (2434), respectively (Tables S3 and S4) [45].

The nMDS plots of the Adriatic Sea showed very comparable station clusters with both the approaches. This area is known for eutrophication phenomena mainly related to local river runoffs. Accordingly, the nematode assemblages of the Foglia and Metauro rivers (FO and M) showed the most marked differences from the other two stations (i.e., B and FI) as confirmed by ANOSIM. Indeed, FO and M stations did not show significant differences between them but proved to be significantly different from all other localities. Another station that revealed a high level of dissimilarity from the others was Fiorenzuola (St. F), but these differences were very much related to the effects of the Po River plume rather than to the influence of the local rivers [46–48] (Table 1). As in the Voltri port and Aregai tourist marina, 6423 was one of the combinations of traits more frequently found in the human disturbed stations, along with 4423 (i.e., spiral amphid, punctuated or annulated with or without lateral differentiation cuticles, 1B group, clavate conical cylindrical tail) and 5424 (rounded or elongate loop, punctuated or annulated cuticle with or without lateral differentiation, 1B, conical tail) (Table S3). This finding matches with the genera that mainly distinguished the stations mostly influenced by riverine discharges (i.e., *Paramonohystera*, *Stylotheristus*, *Theristus, Sabatieria* and *Odontophora*) [46].

The differences between the assemblages of fish farm cages and of controls are evident in both areas studied in the Tyrrhenian Sea even if they were more marked in Olbia (Sardinia). Indeed, SIMPER routine revealed a higher level of dissimilarity between Olbia control and cage (57%) than between Vibo control and cage (43%) (Table S3). Trait combination 6423 again characterized the stations under the cages in both fish farms (mainly due to the high abundance of *Daptonema*) together with trait combination 6413 (i.e., round or oval amphids, punctate or annulate cuticle with or without lateral differentiation, 1A trophic group, conical-cylindrical tail) in Olbia (represented by the high abundance of *Terschellingia*) and feature combination 2434 in Vibo (e.g., *Ptycholaimellus*) (Tables S3 and S4).

Instead, the diversity computed from the functional trait matrix was inconsistent and less helpful than the diversity based on genera in identifying areas with greater anthropogenic disturbance, so we suggest caution in its use (Figure 9).

3.3. Are There Recurrent Combinations of Morpho-Functional Traits in Nematode Communities in Polluted Sediments?

The high frequency of 6423 and 2434 combinations in the most impacted areas allows us to conclude that there are recurring trait associations in stressful conditions and are mainly related to the high abundance of genera belonging to Xyalidae, Linhomoeidae, and Chromadoridae genera. As reported in Figure 10, circular-oval and slit-like amphids, punctuated or annulated cuticles with or without lateral differentiation, 1B and 2A trophic guilds, and clavate conical cylindrical/conical tails are the prevalent traits.

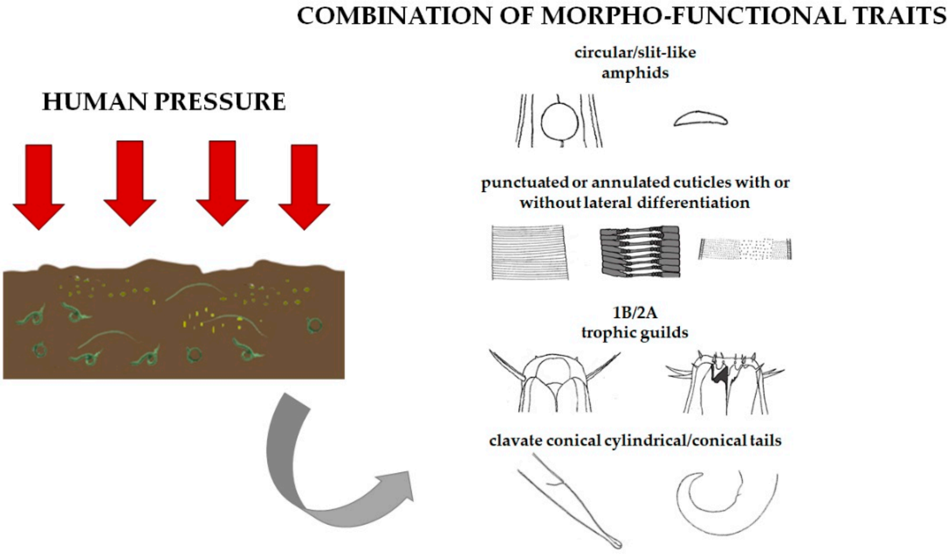


Figure 10. Combination of morpho-functional traits in nematodes inhabiting sediments under human pressure.

The most anthropogenically disturbed stations favor opportunistic trophic guilds such as 1B and 2A that are able to take advantage of a wide range of food items from organic deposits and bacteria biofilm to microphytobenthos [59–61]. All of them are abundant in sediments under anthropogenic conditions [44,62]. In addition, 2A feeding mode (i.e., scraping food off surfaces, piercing it, and sucking out the content) allows nematodes to reduce the probability of ingesting xenobiotics, which is an advantage in contaminated sediments [60]. The occurrence of 2B group, e.g., in the commercial port of Voltri, is mainly restricted to the scavenging Oncholaimids. Scavengers still follow a tactical trophic strategy through which nematodes take advantage of the consumption of dead organisms (including plant material). They play a key role in the ecosystems and many species belonging to Oncholaimidae are successful extremophiles [61,63–65]: *Oncholaimus campylocercoides* shows a sulphide detoxification via formation of inclusions that allow it to tolerate sulphidic conditions and to benefit from the 'sulphide niche'; *Oncholaimus dyvae* and *Metoncholaimus albidus* appear able to adapt to and be very abundant in extreme conditions due to their association with chemosynthetic micro-organisms.

Free-living nematodes evaluate the quality of the environment that they inhabit and food resource diversity mainly through the complex chemosensory system of the amphid [66,67]. In polluted sediments, nematodes exhibit a high frequency of circular amphids, which confirms the role of such a type and size of amphidal fovea in the detection and avoidance of xenobiotics [68]. Instead, small slit-like amphids belong to Chromadorids that, due to their trophic strategy (i.e., 2A), are already able to reduce their ingestion of xenobiotics. Furthermore, the occurrence of such a high percentage of small amphids is related to the fact that food is not a limiting factor in some of the disturbed sediments, especially for nematode species able to profit from a wide spectrum of trophic resources (microbes, organic material, microalgae).

Nematode cuticle is an extremely flexible and resistant exoskeleton essential for maintenance of body morphology and integrity and has a critical role in protection and locomotion via attachment to body-wall muscles [69]. Punctuated or annulated cuticle with or without lateral differentiation is a type of trait shared by numerous marine nematode families. For instance, nematodes such as *Terschellingia longicaudata* (Linhomoeidae) and *Ptycholaimellus ponticus* (Chromadoridae) belong to this category, and it might be related to oxygen consumption rate and locomotion [58,70]. Instead, clavate conical cylindrical/conical tails are probably strictly associated with the silt sediment fraction that characterized the sediments of the main part of the study areas [31,71].

4. Conclusions

In this paper, we suggested an approach based on a combination of morpho-functional traits (i.e., amphid, cuticle, buccal cavity, and tail shape) that might greatly speed the analyses of nematodes and might be used by unexperienced people in biomonitoring programs. We demonstrated that the simple combination of the four traits in a single code number may perfectly mirror the taxonomic structure of the nematode assemblage at the genus level and we, therefore, predict that similar results can be also obtained directly encoding nematode specimens with the four selected traits. This type of approach, if corroborated by additional studies, might also be adopted for other meiobenthic organisms that can be categorized in functional groups such as foraminifera or copepods, opening new perspectives in the assessment of many benthic taxa.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w14071114/s1, Table S1: Overview of the study cases considered in the present analysis; Table S2. List of families and genera from each sampling area and relative combination of traits. Table S3. Results of the SIMPER test carried out on the functional trait codes. Table S4. Results of the SIMPER test carried out on the nematode genera.

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Conflicts of Interest: The authors declare no conflict of interest.

References

1. Matsuyama, A.; Yano, S.; Taninaka, T.; Kindaichi, M.; Sonoda, I.; Tada, A.; Akagi, H. Chemical characteristics of dissolved mercury in the pore water of Minamata Bay sediments. *Mar. Pollut. Bull.* **2018**, *129*, 503–511. [CrossRef] [PubMed]
2. Losi, V.; Grassi, E.; Balsamo, M.; Rocchi, M.; Gaozza, L.; Semprucci, F. Changes in taxonomic structure and functional traits of nematodes as tools in the assessment of port impact. *Estuar. Coast. Shelf Sci.* **2021**, *260*, 107524. [CrossRef]
3. Pawlowski, J.; Bruce, K.; Panksep, K.; Aguirre, F.I.; Amalfitano, S.; Apothéloz-Perret-Gentil, L.; Baussant, T.; Bouchez, A.; Carugati, L.; Cermakova, K.; et al. Environmental DNA metabarcoding for benthic monitoring: A review of sediment sampling and DNA extraction methods. *Sci. Total Environ.* **2021**, *19*, 151783. [CrossRef]
4. Armynot du Châtelet, E.; Bout-Roumazeilles, V.; Coccioni, R.; Frontalini, F.; Francescangeli, F.; Margaritelli, G.; Rettori, R.; Spagnoli, F.; Semprucci, F.; Trentesaux, A.; et al. Environmental control on a land-sea transitional setting–Integrated microfaunal, sedimentological, and geochemical approaches. *Environ. Earth Sci.* **2016**, *75*, 123. [CrossRef]
5. Gambi, C.; Dell'Anno, A.; Corinaldesi, C.; Lo Martire, M.; Musco, L.; Da Ros, Z.; Armiento, G.; Danovaro, R. Impact of historical contamination on meiofaunal assemblages: The case study of Bagnoli-Coroglio Bay (southern Tyrrhenian Sea). *Mar. Environ. Res.* **2020**, *156*, 104907. [CrossRef] [PubMed]
6. Schratzberger, M.; Somerfield, P.J. Effects of widespread human disturbances in the marine environment suggest a new agenda for meiofauna research is needed. *Sci. Total Environ.* **2020**, *728*, 138435. [CrossRef] [PubMed]
7. Moreno, M.; Semprucci, F.; Vezzulli, L.; Balsamo, M.; Fabiano, M.; Albertelli, G. The use of nematodes in assessing ecological quality status in the Mediterranean coastal ecosystems. *Ecol. Indic.* **2011**, *11*, 328–336. [CrossRef]
8. Hong, J.H.; Semprucci, F.; Raehyuk, J.; Kim, K.; Lee, S.; Jeon, D.; Yoo, H.; Kim, J.; Kim, J.; Yeom, J.; et al. Meiobenthic nematodes in the assessment of the relative impact of human activities on coastal marine ecosystem. *Environ. Monit. Assess.* **2020**, *192*, 81. [CrossRef]
9. Sahraeian, N.; Sahafi, H.H.; Mosallanejad, H.; Ingels, J.; Semprucci, F. Temporal and spatial variability of free-living nematodes in a beach system characterized by domestic and industrial impacts (Bandar Abbas, Persian Gulf, Iran). *Ecol. Indic.* **2020**, *118*, 106697. [CrossRef]
10. Baldrighi, E.; Semprucci, F.; Franzo, A.; Cvitkovic, I.; Bogner, D.; Despalatovic, M.; Berto, D.; Malgorzata Formalewicz, M.; Scarpato, A.; Frapiccini, E.; et al. Meiofaunal communities in four Adriatic ports: Baseline data for risk assessment in ballast water management. *Mar. Pollut. Bull.* **2019**, *147*, 171–184. [CrossRef]

11. Nelson, W.G. A quantitative assessment of organic carbon content as a regional sediment-condition indicator. *Ecol. Indic.* **2020**, *114*, 106318. [CrossRef] [PubMed]
12. Kiddon, J.; Sullivan, H.; Nelson, W.; Pelletier, M.; Harwell, L.; Nord, M.; Paulsen, S. Lessons learned from 30 years of assessing U.S. coastal water. In *Water Quality–Science, Assessments and Policy*; Summers, K., Ed.; InTechOpen: Rijeka, Croatia, 2020; pp. 1–22.
13. Guerra-García, J.M.; Navarro-Barranco, C.; Ros, M.; Sedano, F.; Espinar, R.; Fernández-Romero, A.; Martínez-Laiz, G.; Cuesta, J.A.; Giráldez, I.; Morales, E.; et al. Ecological quality assessement of marinas: An integrative approach combining, biological and environmental data. *J. Environ. Manag.* **2021**, *15*, 286–112237. [CrossRef]
14. Diaz, R.J.; Solan, M.; Valente, R.M. A review of approaches for classifying benthic habitats and evaluating habitat quality. *J. Environ. Manag.* **2004**, *73*, 165–181. [CrossRef] [PubMed]
15. Borja, A.; Barbone, E.; Basset, A.; Borgersen, G.; Brkljacic, M.; Elliott, M.; Garmendia, J.M.; Marques, J.C.; Mazik, K.; Muxika, I.; et al. Response of single benthic metrics and multi-metric methods to anthropogenic pressure gradients, in five distinct European coastal and transitional ecosystems. *Mar. Pollut. Bull.* **2011**, *62*, 499–513. [CrossRef]
16. Vanaverbeke, J.; Merckx, B.; Degraer, S.; Vincx, M. Sediment-related distribution patterns of nematodes and macrofauna: Two sides of the benthic coin? *Mar. Environ. Res.* **2011**, *71*, 31–40. [CrossRef]
17. Appeltans, W.; Ahyong, S.T.; Anderson, G.; Angel, M.V.; Artois, T.; Bailly, N.; Costello, M.J. The Magnitude of Global Marine Species Diversity. *Curr. Biol.* **2012**, *22*, 2189–2202. [CrossRef]
18. Jouili, S.; Essid, N.; Semprucci, F.; Boufahja, F.; Nasri, A.; Beyrem, H. Environmental quality assessment of El Bibane lagoon (Tunisia) using taxonomical and functional diversity of meiofauna and nematodes. *J. Mar. Biol. Assoc. UK* **2017**, *97*, 1593–1603. [CrossRef]
19. Bezerra, T.N.; Decraemer, W.; Eisendle-Flöckner, U.; Hodda, M.; Holovachov, O.; Leduc, D.; Miljutin, D.; Mokievsky, V.; Peña Santiago, R.; Sharma, J.; et al. Nemys: World Database of Nematodes. Available online: http://nemys.ugent.be/ (accessed on 19 December 2021).
20. Coull, B.C.; Chandler, G.T. Pollution and meiofauna: Field, laboratory and mesocosm studies. *Oceanogr. Mar. Biol.* **1992**, *30*, 191–271.
21. Giere, O. *Meiobenthology: The Microscopic Motile Fauna of Aquatic Sediments*, 2nd ed.; Springer: Berlin/Heidelberg, Germany, 2009; p. 527.
22. Schmid-Araya, J.M.; Schmid, P.E. Trophic relationships: Integrating meiofauna into a realistic benthic food web. *Freshw. Biol.* **2000**, *44*, 149–163. [CrossRef]
23. Balsamo, M.; Semprucci, F.; Frontalini, F.; Coccioni, R. Meiofauna as a tool for marine ecosystem biomonitoring. *Mar. Ecosyst.* **2012**, *4*, 77–104.
24. Chen, C.A.; Soo, C.L.; Balsamo, M.; Semprucci, F. An approach based on nematode descriptors for the ecological quality (EcoQ) classification of the Malaysian coasts. *Mar. Biodivers.* **2018**, *48*, 117–126. [CrossRef]
25. Semprucci, F.; Balsamo, M.; Appolloni, L.; Sandulli, R. Assessment of ecological quality status along the Apulian coasts (Eastern Mediterranean Sea) based on meiobenthic and nematode assemblages. *Mar. Biodivers.* **2018**, *48*, 105–115. [CrossRef]
26. Boufahja, F.; Semprucci, F.; Beyrem, H.; Bhadury, P. Marine nematodes taxonomy in Africa: Promising prospects against scarcity of information. *J. Nematic.* **2015**, *47*, 198–206.
27. Derycke, S.; Maes, S.; Van den Bulcke, L.; Vanhollebeke, J.; Wittoeck, J.; Hillewaert, H.; Ampe, B.; Haegeman, A.; Hostens, K.; De Backer, A. Optimisation of metabarcoding for monitoring marine macrobenthos: Primer choice and morphological traits determine species detection in bulkDNA and eDNA from the ethanol preservative. *Front. Mar. Sci.* **2021**, *8*, 675.
28. Ruppert, K.M.; Kline, R.J.; Rahman, M.S. Past, present, and future perspectives of environmental DNA (eDNA) metabarcoding: A systematic review in methods, monitoring, and applications of global eDNA. *Glob. Ecol. Conserv.* **2019**, *17*, e00547. [CrossRef]
29. Sechi, V.; De Goede, R.G.M.; Rutgers, M.; Brussaard, L.; Mulder, C. Functional diversity in nematode communities across terrestrial ecosystems. *Basic Appl. Ecol.* **2018**, *30*, 76–86. [CrossRef]
30. Chalcraft, D.R.; Resetarits, W.J. Mapping functional similarity of predators on the basis of trait similarities. *Am. Nat.* **2003**, *162*, 390–402. [CrossRef]
31. Schratzberger, M.; Warr, K.; Rogers, S.I. Functional diversity of nematode community in the south-western North Sea. *Mar. Environ. Res.* **2007**, *63*, 368–389. [CrossRef]
32. Wieser, W. Die beziehung zwischen mundhöhlengestalt, ernährungsweise und vorkommen bei freilebenden marinen nematoden. Eine ökologisch-morphologische studie. *Ark. Zool.* **1953**, *4*, 439–484.
33. Wieser, W. Free-living Marine Nematodes IV. General Part. *Acta Univ. Lund.* **1959**, *55*, 1–111.
34. Thistle, D.; Sherman, K.M. The nematode fauna of a deep-sea site exposed to strong near-bottom currents. *Deep Sea Res.* **1985**, *32*, 1077–1088. [CrossRef]
35. Thistle, D.; Lambshead, P.J.D.; Sherman, K.M. Nematode tail-shape groups respond to environmental differences in the deep sea. *Life Environ.* **1995**, *45*, 107–115.
36. Vanaverbeke, J.; Steyaert, M.; Vanreusel, A.; Vincx, M. Nematode biomass spectra as descriptors of functional changes due to human and natural impact. *Mar. Ecol. Prog. Ser.* **2003**, *249*, 157–170. [CrossRef]
37. Losi, V.; Moreno, M.; Gaozza, L.; Vezzulli, L.; Fabiano, M.; Albertelli, G. Nematode biomass and allometric attributes as indicators of environmental quality in a Mediterranean harbour (Ligurian Sea, Italy). *Ecol. Indic.* **2013**, *30*, 80–89. [CrossRef]

38. Franzo, A.; Del Negro, P. Functional diversity of free-living nematodes in river lagoons: Can biological traits analysis (BTA) integrate traditional taxonomic-based approaches as a monitoring tool? *Mar. Environ. Res.* **2019**, *145*, 164–176. [CrossRef] [PubMed]
39. Thiele, T.R.; Faumont, S.; Lockery, S.R. The neural network for chemotaxis to tastants in Caenorhabditis elegans is specialized for temporal differentiation. *J. Neurosci.* **2009**, *29*, 11904–11911. [CrossRef]
40. Zullini, A.; Semprucci, F. Morphological differences between free-living soil and freshwater nematodes in relation to their environments. *Nematology* **2020**, *22*, 125–132. [CrossRef]
41. Raes, M.; De Troch, M.; Ndaro, S.G.M.; Muthumbi, A.; Guilini, K.; Vanreusel, A. The structuring role of microhabitat type in coral degradation zones: A case study with marine nematodes from Kenya and Zanzibar. *Coral Reefs.* **2007**, *26*, 113–126. [CrossRef]
42. Semprucci, F.; Cesaroni, L.; Guidi, L.; Balsamo, M. Do the morphological and functional traits of free-living marine nematodes mirror taxonomical diversity? *Mar. Environ. Res.* **2018**, *135*, 114–122. [CrossRef]
43. Ichiishi, K.; Ekino, T.; Kanzaki, N.; Shinya, R. Thick cuticles as an anti-predator defense in nematodes. *Nematology* **2021**, *24*, 11–20. [CrossRef]
44. Moreno, M.; Ferrero, T.J.; Gallizia, I.; Vezzulli, L.; Albertelli, G.; Fabiano, M. An assessment of the spatial heterogeneity of environmental disturbance within an enclosed harbour through the analysis of meiofauna and nematode assemblages. *Estuar. Coast. Shelf. Sci.* **2008**, *77*, 565–576. [CrossRef]
45. Moreno, M.; Albertelli, G.; Fabiano, M. Nematode response to metal, PAHs and organic enrichment in tourist marinas of the Mediterranean Sea. *Mar. Poll. Bull.* **2009**, *58*, 1192–1201. [CrossRef] [PubMed]
46. Semprucci, F.; Boi, P.; Manti, A.; Covazzi Harriague, A.; Rocchi, M.; Colantoni, P.; Papa, S.; Balsamo, M. Benthic communities along a littoral of the Central Adriatic Sea (Italy). *Helgol. Mar. Res.* **2010**, *64*, 101–115. [CrossRef]
47. Semprucci, F.; Sbrocca, C.; Rocchi, M.; Balsamo, M. Temporal changes of the meiofaunal assemblage as a tool for the assessment of the ecological quality status. *J. Mar. Biolog. Assoc. UK* **2015**, *95*, 247–254. [CrossRef]
48. Semprucci, F.; Frontalini, F.; Sbrocca, C.; Armynot du Châtelet, E.; Bout-Roumazeilles, V.; Coccioni, R.; Balsamo, M. Meiobenthos and free-living nematodes as tools for biomonitoring environments affected by riverine impact. *Environ. Monit. Assess.* **2015**, *187*, 251. [CrossRef] [PubMed]
49. Vezzulli, L.; Moreno, M.; Marin, V.; Pezzati, E.; Bartoli, M.; Fabiano, M. Organic waste impact of capture- based Atlantic bluefin tuna aquaculture at an exposed site in the Mediterranean Sea. *Estuar. Coast. Shelf. Sci.* **2008**, *78*, 369–384. [CrossRef]
50. Danovaro, R.; Gambi, C.; Mirto, S.; Sandulli, R.; Ceccherelli, V.M. *Mediterranean Marine Benthos: A Manual of Methods for Its Sampling and Study*; Gambi, M.C., Dappiano, M., Eds.; S.I.B.M-Società Italiana di Biologia Marina: Genova, Italy, 2004; pp. 55–97.
51. Seinhorst, J.W. A rapid method for the transfer of nematodes from fixative to unhydrous glycerine. *Nematologica* **1959**, *4*, 67–69. [CrossRef]
52. Platt, H.M.; Warwick, R.M. Free-living Marine Nematodes. Part, I. British Enoplids. In *Synopses of the British Fauna (New Series)*; Cambridge University Press: Cambridge, UK, 1983; Volume 28, p. 307.
53. Platt, H.M.; Warwick, R.M. Free-living Marine Nematodes. Part II. British Chromadorids. In *Synopses of the British Fauna (New Series)*; Brill: Leiden, The Netherlands, 1988; Volume 38, p. 502.
54. Warwick, R.M.; Platt, H.M.; Somerfield, P.J. Free-living Marine Nematodes. Part III. British Monhysterids. In *Synopses of the British Fauna (New Series)*; Field Studies Council: Shrewsbury, UK, 1998; Volume 53, p. 296.
55. Clarke, K.R.; Gorley, R.N. *PRIMER v6: User Manual/Tutorial*; PRIMER-E: Plymouth, MA, USA, 2006; p. 192.
56. Semprucci, F.; Losi, V.; Moreno, M. A review of Italian research on free-living marine nematodes and the future perspectives on their use as Ecological Indicators (EcoInds). *Mediterr. Mar. Sci.* **2015**, *16*, 352–365. [CrossRef]
57. Armenteros, M.; Pérez-García, J.A.; Marzo-Pérez, D.; Rodríguez-García, P. The Influential Role of the Habitat on the Diversity Patterns of Free-Living Aquatic Nematode Assemblages in the Cuban Archipelago. *Diversity* **2019**, *11*, 166. [CrossRef]
58. Inglis, W.G. The structure of the nematode cuticle. *Proc. Zool. Soc. Lond.* **1964**, *143*, 465–502. [CrossRef]
59. Mirto, S.; La Rosa, T.; Gambi, C.; Danovaro, R.; Mazzola, A. Nematode community response to fish-farm impact in the western Mediterranean. *Environ. Pollut.* **2002**, *116*, 203–214. [CrossRef]
60. Schratzberger, M.; Wall, C.M.; Reynolds, W.J.; Reed, J.; Waldock, M.J. Effects of paint-derived tributyltin on structure of estuarine nematode assemblages in experimental microcosms. *J. Exp. Mar. Biol. Ecol.* **2002**, *272*, 217–235. [CrossRef]
61. Boufahja, F.; Semprucci, F. Stress-induced selection of a single species from an entire meiobenthic nematode assemblage: Is this possible using iron enrichment and does pre-exposure affect the ease of the process? *Environ. Sci. Pollut. Res.* **2015**, *22*, 1979–1998. [CrossRef]
62. Cibic, T.; Franzo, A.; Nasi, F.; Auriemma, R.; Del Negro, P. The port of Trieste (northern Adriatic Sea)—A case study of the "ecosystem approach to management". *Front. Mar. Sci.* **2017**, *4*, 336. [CrossRef]
63. Bellec, L.; Cambon-Bonavita, M.-A.; Durand, L.; Aube, J.; Gayet, N.; Sandulli, R.; Brandily, C.; Zeppilli, D. Microbial communities of the shallow-water hydrothermal vent near Naples, Italy, and chemosynthetic symbionts associated With a free-living marine nematode. *Front. Microbiol.* **2020**, *11*, 2023. [CrossRef] [PubMed]
64. Lorenzen, S.; Prein, M.; Valentin, C. Mass aggregations of the free-living marine nematode *Pontonema vulgare* (Oncholaimidae) in organically polluted fjords. *Mar. Ecol. Prog. Ser.* **1987**, *37*, 27–34. [CrossRef]
65. Thiermann, F.; Vismann, B.; Giere, O. Sulphide tolerance of the marine nematode *Oncholaimus campylocercoides*-a result of internal sulphur formation? *Mar. Ecol. Prog. Ser.* **2000**, *193*, 251–259. [CrossRef]

66. Bargmann, C.I. *Chemosensation in C. elegans*; WormBook, 2006; pp. 1–29. Available online: https://www.ncbi.nlm.nih.gov/books/NBK19746/ (accessed on 13 February 2022).
67. Guden, R.M.; Derycke, S.; Moens, T. A Multi-Faceted Approach to Understand How Resource Diversity Can Mediate the Coexistence of Cryptic Marine Nematode Species. *Front. Mar. Sci.* **2021**, *8*, 777425. [CrossRef]
68. Wakkaf, T.; Allouche, M.; Harrath, A.H.; Mansour, L.; Alwasel, S.; Mohamed Thameemul Ansari, K.G.; Beyrem, H.; Sellami, B.; Boufahja, F. The individual and combined effects of cadmium, polyvinyl chloride (PVC) microplastics and their polyalkylamines modified forms on meiobenthic features in a microcosm. *Environ. Pollut.* **2020**, *266*, 115263. [CrossRef]
69. Page, A.P.; Johnstone, I.L. The cuticle. WormBook. 2007. Available online: http://wormbook.org/chapters/www_cuticle/cuticle.pdf (accessed on 13 February 2022).
70. Boufahja, F.; Semprucci, F.; Beyrem, H. An experimental protocol to select nematode species from an entire community using progressive sedimentary enrichment. *Ecol. Indic.* **2016**, *60*, 292–309. [CrossRef]
71. Armenteros, M.; Ruiz-Abierno, A.; Fernández-Garcés, R.; Pérez-García, J.A.; Díaz-Asencio, L.; Vincx, M.; Decraemer, W. Biodiversity patterns of free-living marine nematodes in a tropical bay: Cienfuegos, Caribbean Sea. *Estuar. Coast. Shelf Sci.* **2009**, *85*, 179–189. [CrossRef]

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CONCLUSIONS

The assessment of human activity impact on underwater life is one of the primary challenges of marine biology. The early detection of an environmental disturbance in a coastal system may contribute to the prevention of more serious damage to the ecosystem and could avoid many human diseases.

Meiofauna, and especially free-living nematodes, are recognized as good bioindicators in the marine systems with many advantages over other benthic components. Thus, the main focus of this thesis was to analyze the meiobenthic response to different types of disturbances (i.e. anthropogenic and natural sources such as ports, sewages, natural organic enrichment and physical stress) to assess their effects on meiobenthic and nematodes parameters and further select the best descriptor of the health status. The case studies considered (from the Mediterranean Sea to the Southern Ocean) underlined the overall good detection power of the “rare taxa” when it has been applied the concept of “taxonomic sufficiency” in the meiobenthic identification. Whereas when the nematode genera were identified, the number of opportunistic or resistant taxa to contamination or organic enrichment was updated increasing the nematode indicator lists so far available for the classification of the Ecological Quality (EcoQ). Effects of severe, acute anthropogenic disturbances are relatively straightforward to establish, while it is less clear how moderate, more prolonged anthropogenic disturbances affect benthic populations. In this respect ports further appear to be the ideal systems to investigate to what extent the meiofaunal response is related to the history of disturbance events. A general limitation of the use of nematodes in the EcoQ assessment is related to the difficult and time-consuming taxonomic identification. Therefore, it has been developed an alternative approach using a combination of nematode morpho-functional traits to notice changes in the nematode community structure in coastal areas under human pressure. The trait combination was tested on a wide data set from the Mediterranean Sea, and it clearly demonstrated that trait code numbers perfectly mirror the changes of the nematode community structure at the genus level. This approach might greatly speed the analyses of nematode fauna in biomonitoring programs and might also be adopted for other meiobenthic organisms that may be categorized in functional groups opening new perspectives in the ecological assessment of meiofauna as bioindicator.

OTHER PUBLICATIONS

ADDITIONAL JOURNAL PAPERS

1. Baldrighi, E., Dovgal, I., Zeppilli, D., Abibulaeva, A., Michelet, C., Michaud, E., Franzo, A., **Grassi, E.**, Cesaroni, L., Guidi, L., Balsamo, M., Sandulli, R., Semprucci, F. (2020). The cost for biodiversity: records of ciliate–nematode epibiosis with the description of three new suctorian species. *Diversity*, *12*(6), 224.
2. Sbrocca, C., De Troch, M., Losi, V., **Grassi, E.,** Balsamo, M., Semprucci, F. (2021). Habitat-diversity relations between sessile macrobenthos and benthic copepods in the rocky shores of a Marine Protected Area. *Water*, *13*(8), 1020.
3. Cocozza di Montanara, A., Baldrighi, E., Franzo, A., Catani, L., **Grassi, E.,** Sandulli, R., Semprucci, f. Nematodes research: state of the art, prospects, and future directions. A network analysis approach. Ecological Informatics, *submitted*

CONGRESS CONTRIBUTIONS

1. **Grassi E.,** Cesaroni L., Guidi L., Balsamo M. Semprucci F. 2019. Meiofaunal and nematode biodiversity trends in tropical subtidal habitats. XXIX Congresso S.It.E SOCIETà Italiana di Ecologia. Ferrara 10-12 settembre 2019.
2. Semprucci F., **Grassi E.,** Sbrocca C., Noli N., Cesaroni L., Balsamo M., 2018. Contribution to the knowledge of meiobenthic copepoda (crustacean) from the sardinian coast (Italy). UZI, UNIONE ZOOLOGICA ITALIANA, Lecce, 25-27 Settembre 2018, (POSTER)
3. Semprucci F., Cesaroni L., **Grassi E.,** Losi V., Guidi L., Balsamo M., 2019. Taxonomic composition and morpho-functional traits of nematodes in maldivian subtidal habitats. Unione Zoologica Italiana, Roma, 23-26 settembre 2019, (POSTER)
4. Guidi L., Semprucci F., Balsamo M., Fraternale D., Colomba M., Cecchini S., **Grassi E.**, Cesaroni L., Burattini S., Albertini M.C. Phenotypic cell plasticity of Trichoplax adhaerens Schulze, 1883 (Placozoa) after treatment with ethanol and Prunus spinosa L. ethanol extract, MCM2019 14th multinational congress on microscopy september 15–20, 2019 in Belgrade, Serbia (POSTER).
5. **Grassi E.**, Cesaroni L., Appolloni L., Donnarumma L., Russo G., Di Donato P., Balsamo M., Semprucci F., Sandulli R., First data on the meiofauna from the enigma lake (Antarctic). Journées annuelles 2019 de la Société zoologique de France, Périgueux (Dordogne), 2-8 Ottobre 2019, (POSTER)
6. Appolloni L., Donnarumma L., Semprucci F., Cesaroni L., **Grassi E.**, Balsamo M., Russo G.F., Sandulli R. preliminary assessment of anthropogenic and natural impacts on meiobenthic assemblages in the terranova bay (Antarctica), 51° Congresso della Società Italiana di Biologia Marina. Trieste, 8-12 giugno 2020 (POSTER).
7. Catani L., **Grassi E.**, Cocozza di Montanara A., Fraternale D., Guidi L., Sandulli R., Manachini B., Semprucci. F. Oli essenziali e loro applicazione in agricoltura: un'analisi della letteratura at-traverso il software VOSviewer. XIV Convegno Nazionale - Società Italiana di Nematologia. Napoli, 5-7 ottobre 2022. (POSTER)
8. Catani L. **Grassi E.**, Montefalcone M., Guidi L., Cocozza di Montanara A., Baldrighi E., Sandulli R., Balsamo M., Semprucci F. Valutazione dello stato ecologico in ambiente subtidale tropicale mediante l'utilizzo della diversità tassonomica e funzionale dei nematodi. XIV Convegno Nazionale - Società Italiana di Nematologia. Napoli, 5-7 ottobre 2022.
9. Cocozza di Montanara A., Baldrighi E., Franzo A., Catani L., **Grassi E.**, Sandulli R., Semprucci F. Analisi della letteratura scientifica sui nematodi di vita libera in ambienti terrestri, marini e di acqua dolce. XIV Convegno Nazionale - Società Italiana di Nematologia. Napoli, 5-7 ottobre 2022.

10. Semprucci F., **Grassi E.**, Catani L., Balsamo M. 2022. Combinazione di tratti morfo-funzionali come metodo alternativo per l'analisi della struttura della comunità dei nematodi a vita libera. XIV Convegno Nazionale - Società Italiana di Nematologia. Napoli, 5-7 ottobre 2022.
11. Baldrighi E., López Correa M., Donnarumma L., Gambi M.C., Ferrigno F., Simoncini N., Appolloni L., Sandulli R., Franzo A., **Grassi E.**, Cocozza Di Montanara A., Catani L., Semprucci F. First insight into meiofauna inhabiting the shallow CO2-seeps around Castello Aragonese (Ischia, Italy). UNIONE ZOOLOGICA ITALIANA, Trieste, 20-23 settembre 2022.
12. Franzo A., Baldrighi E., **Grassi E.**, Grego M., Balsamo M., Basili M., Catani L., Cocozza Di Montanara A., Sandulli R., Semprucci F. How can different taxonomic structure and functional traits of nematodes reflect different human pressures in “Heavily Modified Water Bodies”? UNIONE ZOOLOGICA ITALIANA, Trieste, 20-23 settembre 2022.