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

Annalisa Franzo[1], Elisa Baldrighi[2]*, Eleonora Grassi[3], Mateja Grego[4], Maria Balsamo[3,5,6], Marco Basili[2], Federica Semprucci[3,5,6]

[1]Oceanography Section, Istituto Nazionale di Oceanografia e di Geofisica Sperimentale, OGS I-34151 Trieste, Italy - afranzo@inogs.it

[2]Institute for Biological Resources and Marine Biotechnologies (IRBIM), Italian National Research Council (CNR), Largo Fiera della Pesca 1, 60125, Ancona, Italy - marco.basili@irbim.cnr.it

[3]Department of Biomolecular Sciences (DiSB), University of Urbino 'Carlo Bo', loc. Crocicchia, 61029 Urbino, Italy - e.grassi5@campus.uniurb.it; federica.semprucci@uniurb.it; maria.balsamo@uniurb.it

[4]National Institute of Biology, Marine Biology Station Piran, Fornace 41, SI-6330 Piran, Slovenia - Mateja.Grego@nib.si

[5]Fano Marine Center, The Inter-Institute Center for Research on Marine Biodiversity, Resources and Biotechnologies, 61032, Fano, Italy

[6]Consorzio Nazionale Interuniversitario per le Scienze del Mare (CoNISMa) Rome, URL-Centro Direzionale—Isola C4, 80143 Napoli, Italy

*Corresponding author: elisa.baldrighi@irbim.cnr.it

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 provided some suggestions for a proper application of the 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 (Chatzinikolaou et al., 2018; Darbra et al., 2005). 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 (Briant et al., 2013; Demopoulos et al., 2016; Moreno et al., 2008; Veiga et al., 2009;). 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; Chatzinikolaou et al., 2018; Thibaut et al., 2017).

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 (Franzo et al., 2018b, 2019; Moreno et al., 2011;

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; Kandratavicius et al., 2018). 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 (Moreno et al., 2008; Trannum et al., 2004).

Free-living nematodes have been successfully used as BQEs in coastal areas (e.g. Hong et al., 2020), lagoons (Franzo et al., 2019; Jouili et al., 2017), 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, Total Organic Carbon and contaminants)? 3) which EQS is obtained according to nematode assemblages in the port subareas?

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 (Grilli et al., 2020; Mozetič et al., 2012), 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 the different anthropogenic activities that take place in the port subareas: 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 (Adami et al. 2000; Solis-Weiss et al., 2004). 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) oil 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 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 four stations. In the first station (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 station (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 station (Kp3) bulk cargoes are handled, the most common of which are hard coal and iron ore (itabirritic ore). In all port stations, the sediment is stirred up by dredging operations. The sampling station Kp4 is located outside the port, next to the shipway, where there is 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 independent deployments were performed with a box-corer (40 cm × 30 cm wide and 50 cm high). For meiofauna, 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). Similarly, the top 3 cm of sediment was collected from each box-corer for the 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. Environmental analyses

For grain size determination, aliquots of fresh sediment were sieved over a 63 μm mesh. The two fractions (> 63 μm, sand; < 63 μm, mud) 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).

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, i.e. %TOC (for details see Baldrighi et al., 2019).

The following PAHs were assessed as described in Baldrighi et al. (2019): 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). Total PAH concentrations (ΣPAH) were calculated as the sum of these congers. 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 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).

2.4. 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 in NeMys (2022).

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).

2.5. 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 a previous study conducted on the same samples, Baldrighi et al. (2019) reported that the temporal variability of meiofauna main groups (i.e. Nematoda, Copepoda, Polychaeta, etc.) was not statistically relevant. Similarly, in the present study an exploratory PERMANOVA analysis on nematode dataset revealed that there were not significant differences between campaigns (data not shown). As a consequence only the spatial variability of the nematofauna was tested. For what concerns the taxonomic composition of this assemblage, 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). 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 mud (>80%). Trieste showed the highest percentage 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). Ancona stations showed the overall lower values (55.2 - 112 ng g^{-1}), while Trieste the highest concentrations (73.4 - 14,036.1 ng g^{-1}). The port of Koper was characterized by

intermediate amounts, although closer to those measured in Ancona (167.6 – 302.8 ng g^{-1}). 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 = 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 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 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

Due to their ubiquity and sensitivity to several kinds of environmental stress, free-living marine nematodes may be a cost-effective alternative to macrofauna in biomonitoring studies (Semprucci et al., 2015b). Notwithstanding, to date these organisms are still understudied especially in areas subjected to long lasting contamination such as the commercial ports, hampering their more effective exploitation as BQEs. In the present study, free-living nematodes were studied in three Mediterranean ports in order to (1) build a wider baseline of data from these neglected environments, (2) implement the sets of sensitive/tolerant genera and (3) recalibrate the EQS thresholds based on nematode metrics for achieving an EQS assessment of commercial ports as much as possible close to reality.

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 nematode assemblages are reported at the most contaminated sites (Losi et al., 2013 and references therein; Boufahja and Semprucci, 2015; Franzo et al., 2018b). Notwithstanding, some studies on macrofauna document that an increased anthropogenic stress does not necessarily correspond to a reduction of the diversity. In an intertidal mussel bed subjected to sewage outfall (NW Atlantic), Vallarino and Elias (2006) observed that the most impacted stations were inhabited by a more diverse macrobenthic community than that at the control site. The authors explained this apparent paradox in terms of different opportunistic species that might take advantage by the organic enrichment provided by the sewage. For what concerns the chronic exposure of the organisms to chemical pollutants, findings similar to ours come directly from the port of Trieste. Even at stations close to those of the present study, Solis-Weiss et al. (2004) reported that the very high concentrations of heavy metals, and in particular of lead, contrasted with the presence of a relatively diverse and structured macrofauna. The authors provide some hypothesis that fit well with our results. Among all, the chronic contamination might have favored the adaptation of several taxa.

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 (Losi et al., 2013, 2021; Moreno et al., 2008) 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 percentage of TOC was 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 iron foundry 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 data can be considered comparable. For what concerns ΣPAH, previous investigations suggested that in the port of Trieste an accumulation of these contaminants with increasing sediment

depth could be excluded (Adami et al. 2000; Notar et al. 2001). The PAHs values measured in sediment layers of different thickness (e.g. 0-15 cm) were in fact comparable to ours (Adami et al. 2000). Furthermore, the vertical distribution of these contaminants along a sediment core of 320 cm did not reveal significant higher concentrations of PAHs below the top 3 cm of sediments (Notar et al. 2001). The severe PAH contamination of Trieste has a pyrolytic origin and has been ascribed mainly to the iron foundry plant that is in front of Ts3, as suggested by the rapid decline of the PAHs contents with increasing distance from this particular point source (Adami et al. 2000; Solis-Weiss et al. 2004). During the last decades, this factory has worked without significant interruptions and its contribution to PAHs accumulation has been maintained over time, resulting in a rather homogenous vertical distribution of these contaminants through the sediments. For what concerns ΣTB, in Europe this group of contaminants has been banned in 2003 (Carreño and Lloret, 2021). The comparability of Trieste values (0-50 cm of sediment) with those measured in the other two ports (0-3 cm of sediments), allows to exclude that below the very first centimeters of Trieste sediments there is a higher accumulation of TBs, i.e. in correspondence to the ante-ban period.

Overall, the comparison of contaminants values in the three ports revealed that the sediments of Trieste are more contaminated than those of Koper and of Ancona, at least for ΣPAH and ΣTB. In is not a coincidence, in fact, that the port of Trieste has been declared 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 ΣPAH, 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 the proliferation of their main food source (i.e. benthic microalgae, Cibic et al. 2017) and/or by other components whose

identification, however, is challenging due to the intrinsic complexity of the ecosystem. Notwithstanding, the presence in the port of Trieste of specialized consortia of bacteria able to tolerate and to degrade hydrocarbons such as n-Hexadecane (Cibic et al. 2017) suggests that biota at the different trophic levels might have adapted to the chronic and long-standing contamination of this area. 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 muddy sediment and the presence of long-standing anthropogenic activities nearby Ts2 (shipbuilding) and Ts3 (iron foundry plant), might have favored PAH accumulation in this port (Baldrighi et al., 2019; Frapiccini and Marini, 2015).

Focusing on the nematode fauna, the EQS obtained according to the thresholds proposed by Moreno et al. (2011) 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 (Table S4), evidence of a better environmental quality compared to the more polluted stations within the same port, suggests that H' is a metrics reliable to catch the lower contamination levels that characterized this station. Since this difference was not detected by analysing the meiofaunal main groups (Baldrighi et al., 2019; Cibic et al., 2017; Moreno et al., 2008), 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, the wide application of MI in different environmental contexts might allow the identification of its intrinsic limits as already done for dedicated macrobenthic indices (see Borja and Muxika,

2005) and favour the development of specific guidelines for its proper use. Similarly to AMBI, there are environmental conditions where there is natural increase in opportunistic species (e.g. confined environments: Armenteros et al., 2009; Jouili et al., 2017; Moreno et al., 2009) and decrease in MI values, providing contradictory results. Therefore, in order to minimise these problems, we recommend, as suggested by Moreno et al. (2011), the use of MI together with other metrics (e.g. H' and c-p%) in such environmental contexts (i.e. transitional environments and ports) that provides a more comprehensive view of the benthic community status (Table S4).

The co-occurrence analysis 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 (Franzo et al., 2018b; Gambi et al., 2020; Losi et al., 2013, 2021; Moreno et al., 2008). 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 taking in to account 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.

633 I within the Genoa-Voltri port and subsequently the authors proposed it as an indicator of
634 'Bad' EQS (Moreno et al. 2011). Based on these results we confirm *Aponema* and
635 *Paracomesoma* as resistant genera, i.e. mainly found in polluted sediments.

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

645 Both *Ptycholaimellus* and *Prochromadorella* were considered sensitive in Moreno et al.
646 (2011), while *Prochromadorella* was reported as tolerant in Losi et al. (2021). The co-
647 occurrence analysis confirmed the widespread presence of these two genera because they
648 represented the dominant members of the assemblage in the polluted sediments of Trieste
649 and, to a lesser extent, even at the least impacted station of Koper (Group 6, Fig. 2).
650 Furthermore, *Ptycholaimellus* was reported as dominant at the virtually unpolluted station
651 C1 in the Gulf of Trieste (Franzo et al., 2018a). Taking into account all these evidences, we
652 propose to consider *Ptycholaimellus* and *Prochromadorella* as opportunistic genera, i.e.
653 equally found in both polluted and unpolluted sediments.

654 In Group 10 (Fig. 2), *Parapinnanema* and *Halalaimus* co-occurred in the sediments of both
655 the least impacted station of Koper and at the contaminated site Ts1. Since these two genera
656 have been reported as sensitive to anthropogenic impact, we advise to consider them as
657 opportunistic. Furthermore, in Ts1, *Parapinnanema* and *Halalaimus* co-occurred with
658 *Paracanthonchus and Desmoscolex,* although these latter genera presented lower
659 percentages. Since Losi et al. (2021) proposed *Paracanthonchus* as a sensitive genus while
660 we observed it mainly in polluted sediments, we propose to downgrade it as opportunistic.
661 On the contrary, *Desmoscolex* seems to tolerate polluted conditions in ports (Losi et al.,
662 2021), therefore, it has been considered as resistant.

663 The genera *Terschellingia, Sabatieria* and *Parodonthophora* are known for their tolerance
664 to contaminants, organic enrichment and environmental instability (Losi et al., 2013, 2021;

665 Moreno et al., 2011). Interestingly, in the present study *Sabatieria* and *Parodonthophora* did
666 not correlate with any other genus although observed in noticeable abundances, especially
667 *Sabatieria.* Notwithstanding, both *Sabatieria* and *Terschellingia* represent the two dominant
668 genera at the port stations of Koper (Fig. S1), i.e. where the nematode assemblage was the
669 least structured and biodiverse. Taking into account all these aspects, we propose to
670 maintain these three genera in the category of those considered resistant to the impacted
671 conditions of big commercial hubs.

672

673 ***5. Conclusions***

674 The present study represents a contribution to the needed baseline of data about the free-
675 living nematodes inhabiting large commercial ports. The main findings suggest that the
676 assemblage might adapt to long-standing contamination in synergy with other ecosystem
677 components such as autotrophic and heterotrophic microbiota, resulting in a relatively fairly
678 diverse and structured assemblage even in the contaminated port of Trieste. For what
679 concerns the EQS assessment, we recommend the concomitant use of both diversity (e.g.
680 H') and functional indices (e.g. MI) for a more reliable assessment of the ecological quality
681 of commercial ports. Finally, the co-occurrence analysis has allowed the assignment or the
682 reallocation of some genera to three proposed categories - resistant, opportunistic and
683 relatively opportunistic - in order to implement the use of nematodes for the EQS
684 assessment of these heavily modified environments.

685

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694

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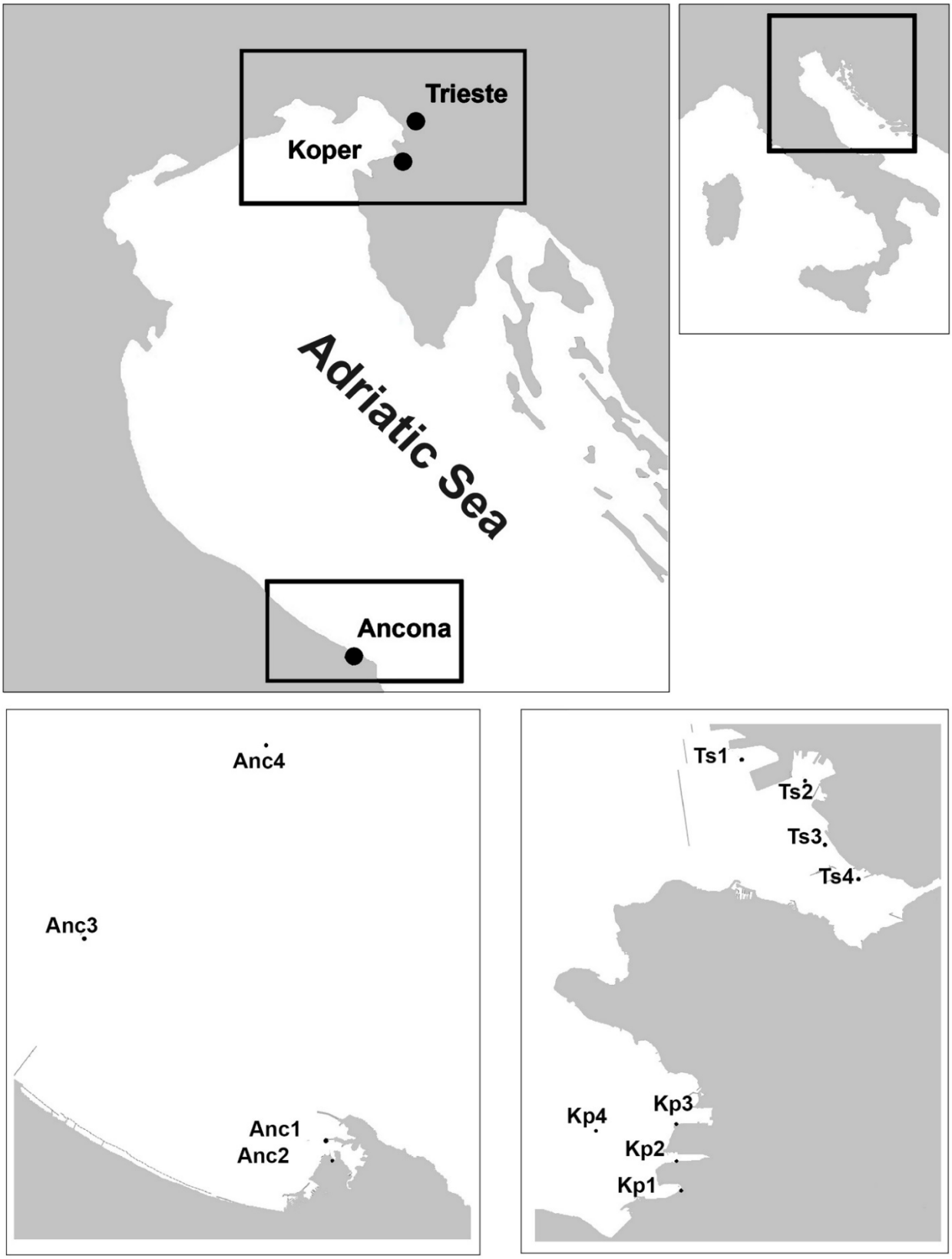

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944 **Fig. 1. Study area with the location of the ports of Ancona, Trieste and Koper and of the**
945 **sampling stations inside each port.**

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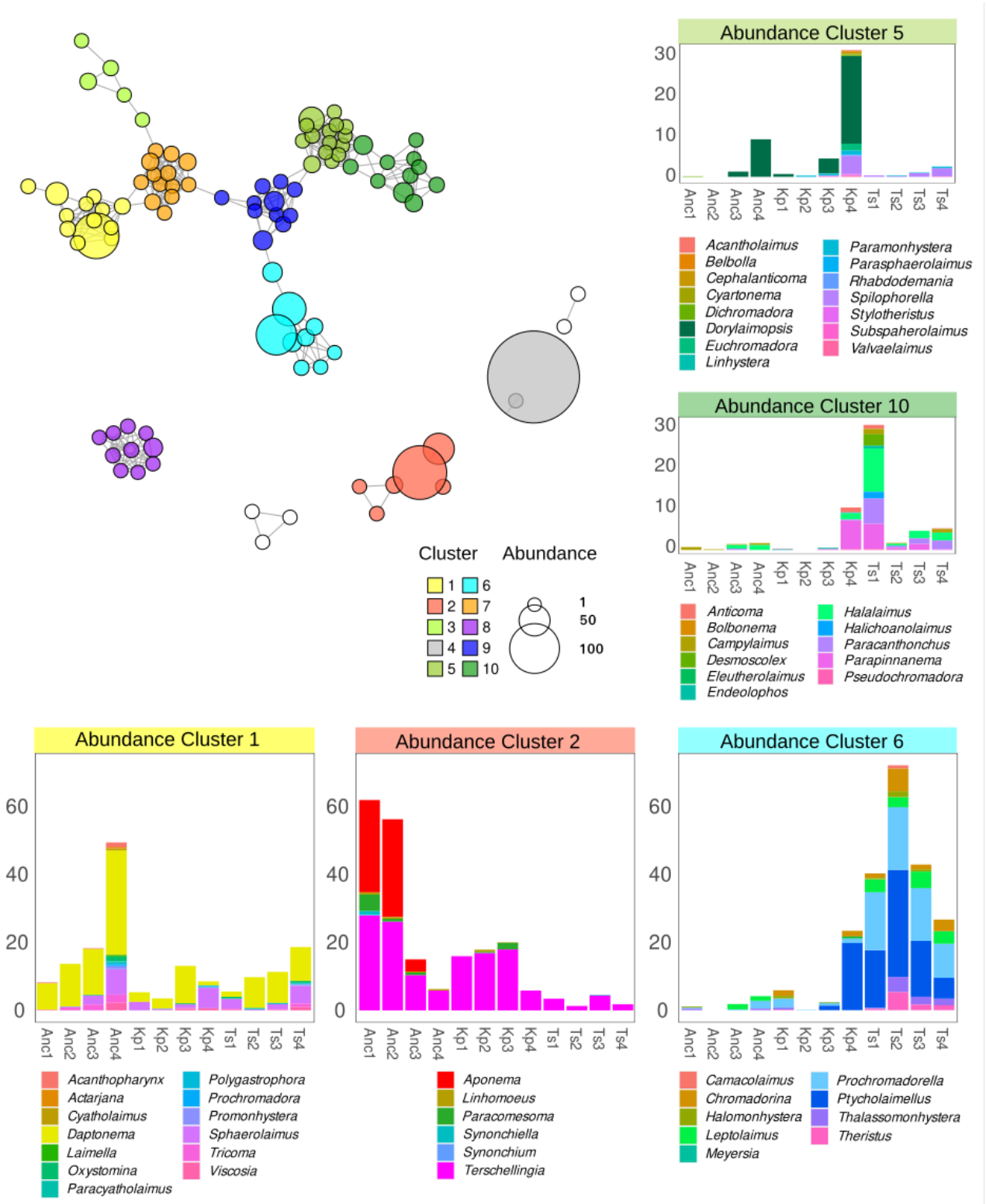
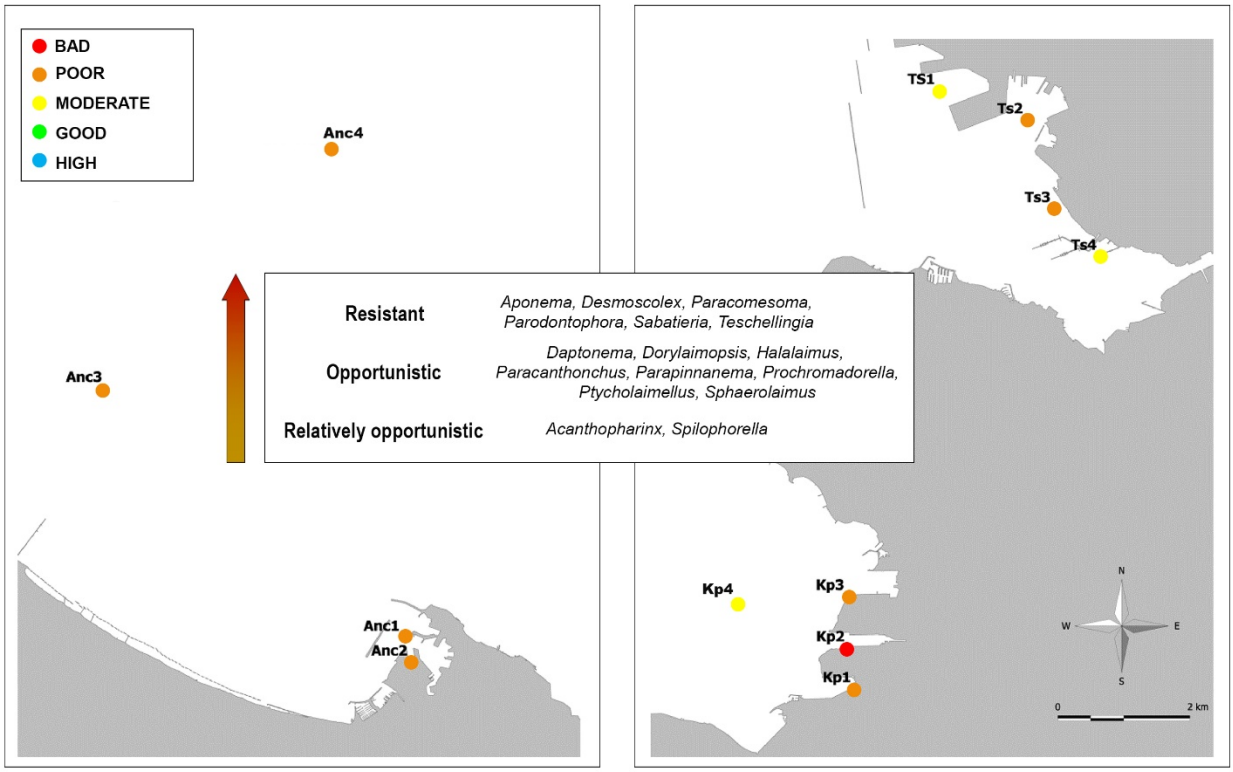


Fig. 2. Outputs of the co-occurrence analysis performed on the entire nematode dataset. Each node indicated a genus, and its size represents the sum of the relative abundances. Each edge represents a positive Spearman correlation between genera (ρ > 0.7). The clusters of nematodes with similar distribution 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.



954

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

Table 1. Environmental parameters measured within the three ports: % of 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)	% mud (>63µm)	ΣBT (ng Sn g^{-1}dw)	ΣPAH (ng g^{-1}dw)	
Anc 1	78	22	15	55.2	1.1
Anc 2	82	22	8	99.8	0.73
Anc 3	81	22	7	112.0	0.89
Anc 4	71	29	-	-	-
Ts 1	6	94	2	3,785.2	2.74
Ts 2	6	94	9	13,958.8	2.61
Ts 3	6	94	6	14,036.1	4.78
Ts 4	6	90	5	73.4	2.23
Kp 1	0	100	-	-	-
Kp 2	0	100	9	261.9	1.25
Kp 3	0	100	7	167.6	1.04
Kp 4	0	100	9	302.8	1.21

964 **Table 2. PERMANOVA outputs carried out to ascertain the differences among ports according**
965 **to nematode assemblage structure, abundance (ABU), Shannon-diversity (H'), total genera**
966 **(S), Pielou-evenness (J), Maturity index (MI), Index of Trophic Diversity (ITD). df= degree of**
967 **freedom; SS= sum of square; MS= mean square; F=F statistic; P= probability level (in bold**
968 **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**

969

970 **Table 3. DistLM outputs carried out on the nematode assemblage structure. P = significant**
971 **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			

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Supplemental online material

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

Franzo A[1], Baldrighi E[2]*, Grassi E[3], Grego M[4], Balsamo M.[3,5,6], Basili M[2], Semprucci F[3,5,6]

[1]Oceanography Section, Istituto Nazionale di Oceanografia e di Geofisica Sperimentale, OGS I-34151 Trieste, Italy

[2]Institute for Biological Resources and Marine Biotechnologies (IRBIM), Italian National Research Council (CNR), Largo Fiera della Pesca 1, 60125, Ancona, Italy

[3]Department of Biomolecular Sciences (DiSB), University of Urbino 'Carlo Bo', loc. Crocicchia, 61029 Urbino, Italy

[4]National Institute of Biology, Marine Biology Station Piran, Fornace 41, SI-6330 Piran, Slovenia

[5]Fano Marine Center, The Inter-Institute Center for Research on Marine Biodiversity, Resources and Biotechnologies, 61032, Fano, Italy

[6]Consorzio Nazionale Interuniversitario per le Scienze del Mare (CoNISMa) Rome, URL-Centro Direzionale—Isola C4, 80143 Napoli, Italy

*Corresponding author: elisa.baldrighi@irbim.cnr.it

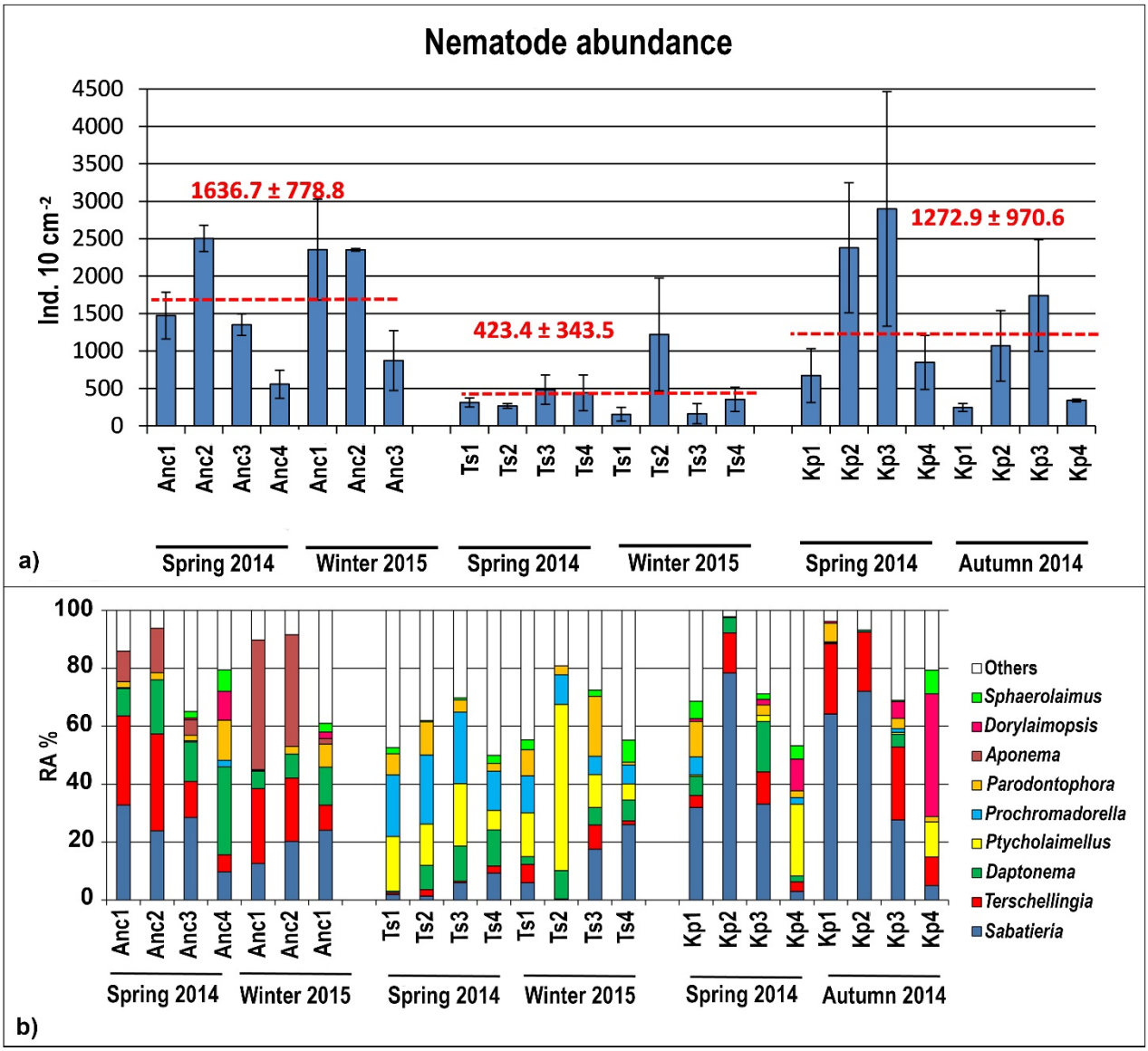

Fig. S1. Nematode total abundances (A) and Relative abundance (RA%) of the dominant genera (B) in the three ports during the two sampling periods.

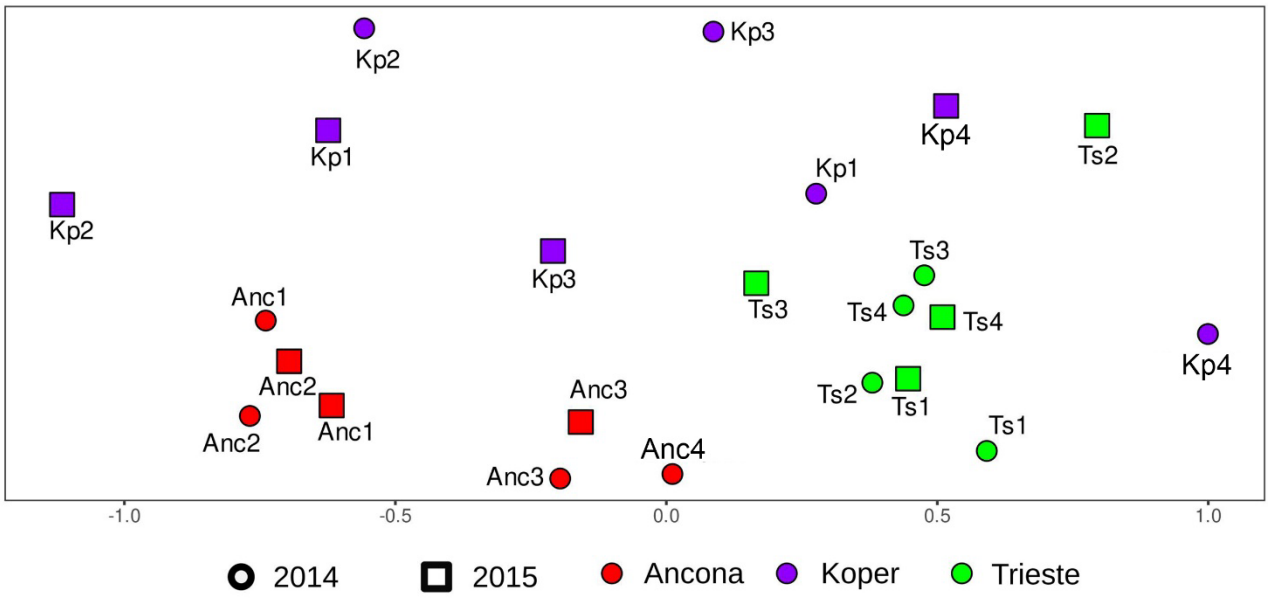

Figure S2. Non-metric multidimensional scaling (nMDS) ordination plot based on Bray-Curtis similarity obtained from replicates dataset in 2014 (circles) and 2015 (squares). Stress value = 0.17.

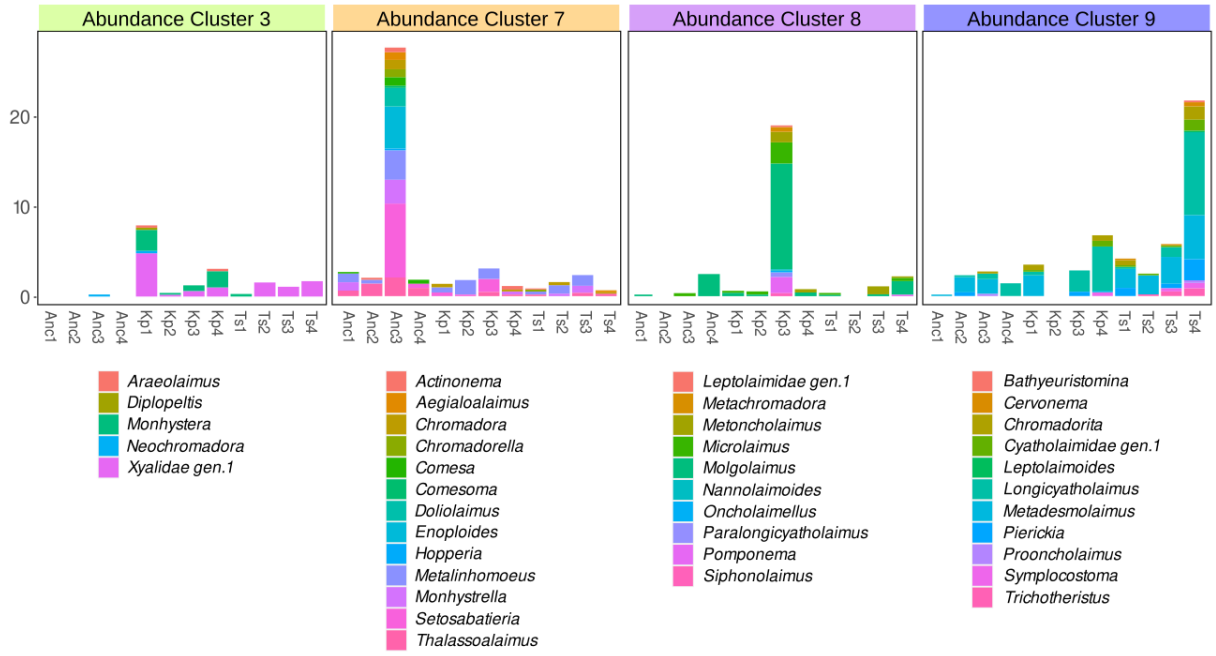

Figure S3. Bar plots of the quantitatively less important nematode groups identified by the co-occurrence analysis (< 30% of total abundance). Group 4 is not reported because lacks a statistical meaning since composed by only two genera.

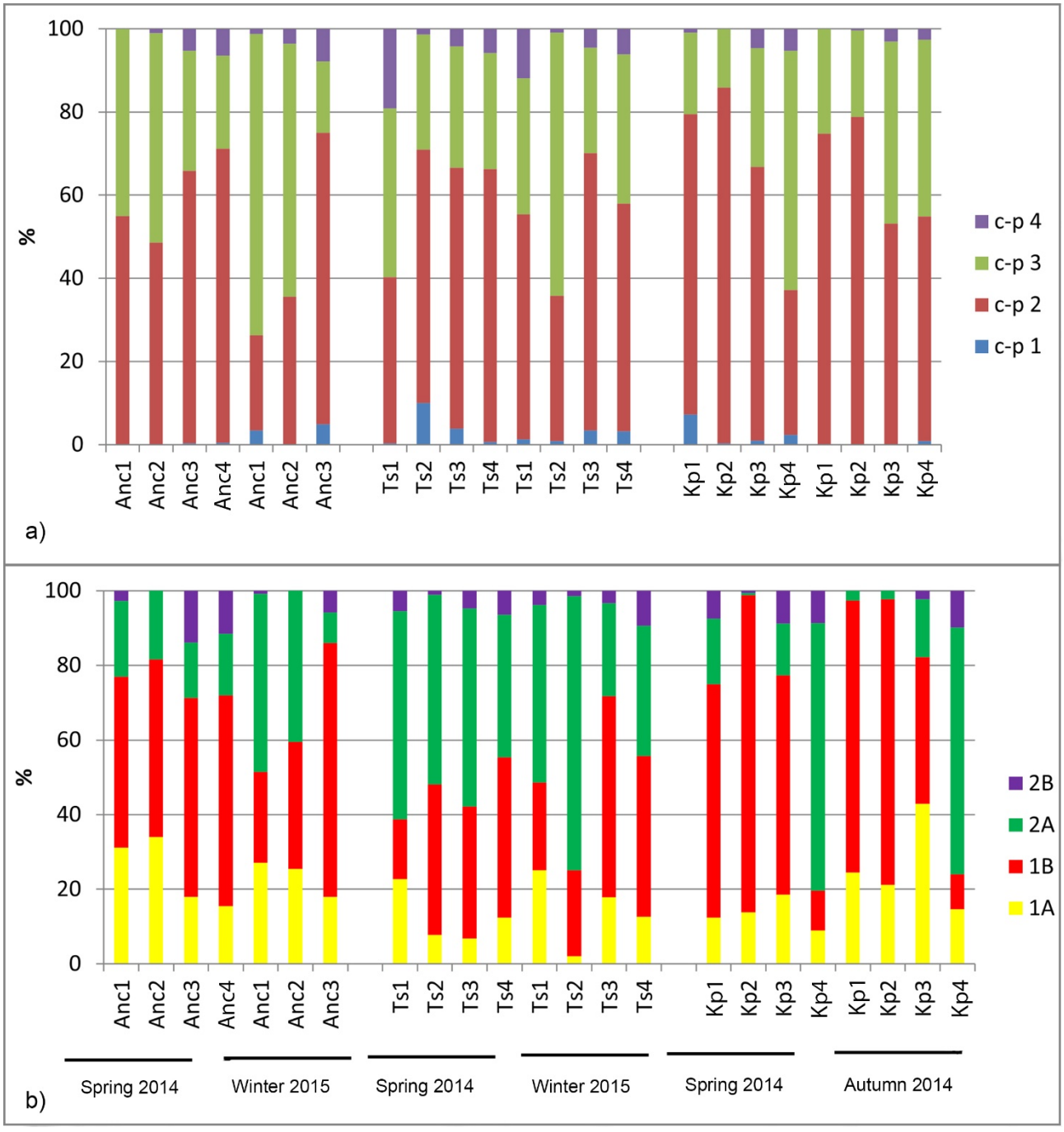

Figure S4. Percentages of c-p groups (A) and of the trophic groups (B) at the stations within the three ports.

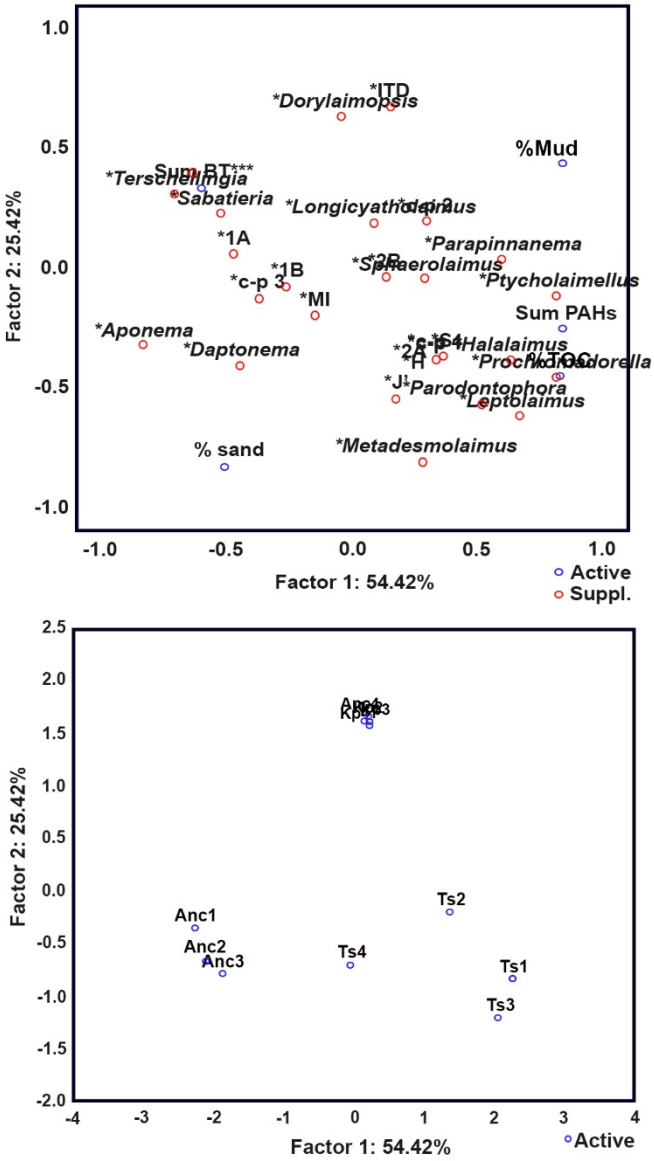

Figure S5. A) Principal Component Analysis (PCA) carried out on the environmental parameters (active variables) measured in the three ports. Nematode genera and the univariate measures were projected on the factor planes (PC1 and PC2) as supplementary variables without contributing to the analysis results; B) Scatter diagram plotting the sampling stations on the two first factors. The position of the samples highlights the relative influence of the environmental variables.

Table S1. Sampling periods, coordinates and main characteristics of the sampling stations.

Port	Station	Period	Lat (N)	Long (E)	Depth (m)	Port area details
Ancona	Anc1	spring 2014	43°37'30.91"	13°29'40.00"	6.3	
	Anc2	spring 2014	43°37'12.97"	13°29'48.20"	10.8	Commercial shipping facilities - active berths (Anc1 and Anc2)
	Anc3	spring 2014	43°40'29.72"	13°24'34.37"	14.2	
	Anc4	spring 2014	43°43'27.96"	13°28'16.50"	19	Adjacent area - anchorages (Anc3)
	Anc1	winter 2015	43°37'30.91"	13°29'40.00"	6.3	Area outside the port - no activities (Anc4)
	Anc2	winter 2015	43°37'12.97"	13°29'48.20"	10.8	
	Anc3	winter 2015	43°40'29.72"	13°24'34.37"	14.2	
Trieste	Ts1	spring 2014	45° 38'0.96"	13°45'9.36"	18.5	
	Ts2	spring 2014	45°37'47.28"	13°46'10.92"	15	Commercial shipping facilities (Ts1)
	Ts3	spring 2014	45°37'4.08"	13°46'31.08"	13	Shipbuilding area (Ts2)
	Ts4	spring 2014	45°36'41.04"	13°47'3.84"	10.5	Iron foundry area (Ts3)
	Ts1	winter 2015	45° 38'0.96"	13°45'9.36"	18.5	Petroleum storage and processing area
	Ts2	winter 2015	45°37'47.28"	13°46'10.92"	15	(Ts4)
	Ts3	winter 2015	45°37'4.08"	13°46'31.08"	13	
	Ts4	winter 2015	45°36'41.04"	13°47'3.84"	10.5	
Koper	Kp1	spring 2014	45°33'7.56"	13°44'17.28"	8	
	Kp2	spring 2014	45°33'26.79"	13°44'12.37"	13	
	Kp3	spring 2014	45°33'51.74"	13°44'12.01"	11	Commercial shipping facilities - active berths (Kp1, Kp2 and Kp3)
	Kp4	spring 2014	45°33'47.64"	13°42'53.71"	17	
	Kp1	autumn 2014	45°33'7.56"	13°44'17.28"	8	Least traffic zone (Kp4)
	Kp2	autumn 2014	45°33'26.79"	13°44'12.37"	13	
	Kp3	autumn 2014	45°33'51.74"	13°44'12.01"	11	
	Kp4	autumn 2014	45°33'47.64"	13°42'53.71"	17	

Table S2: List of nematode genera (average abundance as ind. 10cm^{-2}) in all sampling stations and periods.

Genera	Spring 2014				Winter 2015		
	Anc1	Anc2	Anc3	Anc4	Anc1	Anc2	Anc3
Acantholaimus	0	0	0	0.00	0	0	0
Acanthopharynx	0	0	0	7.23	13.72	0	1.62
Actarjana	0	0	0	1.76	0	0	0
Actinonema	0	13.31	7.91	0	0	0	1.62
Aegialoalaimus	0	0	7.91	0	0	0	11.81
Amphymonhystrella	0	0	0	0	0	0	0
Anticoma	0	0	0	0	0	0	0
Aponema	162.30	398.25	74.43	0	1091.52	1028.46	13.49
Araeolaimus	0	0	0	0	0	0	0
Bathyeuristomina	0	0	0	0	0	0	0
Belbolla	0	0	0	0	0	0	0
Bolbonema	0	0	0	0	0	0	0
Camacolaimus	0	0	0	0	0	0	0
Campylaimus	3.98	0	5.43	1.76	26.02	11.46	0.00
Cephalanticoma	0	0	0	0	0	0	0
Cervonema	0	0	0	0	0	0	0
Chromadora	0	0	24.21	0	0	0	4.31
Chromadorella	0	0	18.77	0	0	0	1.62
Chromadorina	0	0	0	0	0	0	0
Chromadorita	0	0	5.77	0	0	0	0
Cobbia	0	13.31	0	0	0	0	0
Comesa	3.98	0	15.81	1.76	0.00	0.00	7.50
Comesoma	0	0	5.77	0	0	0	0
Croconema	0	13.31	0	0	0	0	0
Cyartonema	0	0	0	0	0	0	0
Cyatholaimidae sp.1	0	0	0	0	0	0	0
Cyatholaimus	0	0	0	1.76	0	0	0
Daptonema	139.73	465.35	189.54	143.38	120.82	87.52	112.85
Desmodora	4.71	0	0	0	0	0	0
Desmoscolex	0	0	0	0	0	0	0
Dichromadora	6.22	0	0	0	0	0	0
Diplopeltis	0	0	0	0	0	0	0
Doliolaimus	0	0	45.45	0	0	0	3.24
Dorylaimopsis	0	0	7.91	42.55	0	0	20.92
Eleutherolaimus	0	0	0	0	0	0	0
Endeolophos	0	0	0	0	0	0	0
Enoploides	0	0	104.58	0	0	0	17.24
Euchromadora	0	0	0	0	0	0	0
Halalaimus	0	0	7.91	5.27	0	0	15.55
Halichoanolaimus	0	0	0	0	0	0	0
Halomonhystera	0	0	0	0	14.79	0	0
Hopperia	0	0	5.43	0	0	0	0

Laimella	0	0	0	1.76	0	0	0
Leptolaimoides	0	0	0	0	0	0	0
Leptolaimus	0	0	18.77	5.27	0	0	19.86
Leptolaimidae sp.1	0	0	0	0	0	0	0
Linhomoeus	15.64	13.26	7.91	1.76	7.40	0	0
Linhystera	0	0	0	0	0	0	0
Longicyatholaimus	0	13.26	5.77	5.27	0	0	5.37
Metachromadora	0	0	0	0	0	0	0
Megadesmolaimus	0	0	0	0	0	0	0
Metadesmolaimus	0	26.57	33.59	0	7.40	53.13	5.37
Metalinhomoeus	17.38	13.31	25.20	0	14.79	11.46	45.22
Metoncholaimus	0	0	0	0	0	0	0
Meyersia	0	0	0	0	0	0	0
Microlaimus	0	0	7.91	0	0	0	0
Molgolaimus	3.98	0	0	10.74	0	0	0
Monhystera	0	0	0	0	0	0	0
Monhystrella	0	0	5.43	0	44.37	0	48.22
Nannolaimoides	0	0	0	0	0	0	0
Neochromadora	0	0	0	0	0	0	3.75
Oncholaimellus	0	0	0	0	0	0	0
Oncholaimus	0	0	0	0	0	0	0
Oxystomina	0	0	0	7.23	0	0	4.31
Paracanthonchus	0	0	0	0	0	0	7.50
Paracomesoma	111.42	0	16.30	0	63.18	11.46	1.62
Paracyatholaimus	0	0	0	1.76	0	0	0
Paralongicyatholaimus	0	0	0	0	0	0	0
Paramonhystera	0	0	0	0	0	0	0
Parapinnanema	0	0	0	0	0	0	0
Parasphaerolaimus	0	0	0	0	0	0	0
Parodontophora	29.76	66.39	24.87	62.49	7.40	57.32	60.26
Paroxystomina	0	0	0	0	0	0	0
Pierickia	0	13.26	0	0	0	11.46	0
Polygastrophora	0	0	0	1.76	0	0	0
Pomponema	0	0	0	0	0	0	0
Prochromadorella	4.71	0	5.77	10.74	7.40	0	0
Prochromadora	0	0	0	1.76	0	0	0
Promonhystera	0	13.26	0	3.51	0	0	0
Prooncholaimus	0	0	5.77	0	0	0	0
Pseudochromadora	0	0	0	0	0	0	0
Ptycholaimellus	0	0	0	0	0	0	0
Rhabdodemania	0	0	0	0	0	0	0
Sabatieria	495.55	611.01	382.74	46.70	277.20	582.46	183.02
Setosabatieria	0	0	50.56	1.76	0	0	126.16
Siphonolaimus	0	0	0	0	0	0	0
Sphaerolaimus	0	0	30.30	35.95	0	0	25.29
Spilophorella	0	0	0	0	0	0	0
Stylotheristus	0	0	0	0	0	0	0

Subspaherolaimus	0	0	0	0	0	0	0
Symplocostoma	0	0	0	0	0	0	0
Synonchiella	26.07	0	0	0	7.40	0	0
Synonchium	3.98	0	0	0	0	0	0
Terschellingia	437.11	863.16	163.22	24.79	588.19	407.41	80.19
Thalassoalaimus	0	13.26	10.87	3.51	34.84	34.39	32.29
Thalassomonhystera	0	0	0	1.76	27.45	0	0
Theristus	0	0	0	0	0	0	0
Trichotheristus	0	0	0	0	0	0	0
Tricoma	0	0	29.15	8.78	0	53.13	10.24
Valvaelaimus	0	0	0	0	0	0	0
Vasostoma	0	13.26	0	0	0	0	0
Viscosia	6.22	0	0	10.74	0	0	0
Xyalidae sp.1	0	0	0	0	0	0	0

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Genera	Spring 2014				Winter 2015			
	Ts1	Ts2	Ts3	Ts4	Ts1	Ts2	Ts3	Ts4
Acantholaimus	0	0	0	0	0	0	0	0
Acanthopharynx	0	0	0	0	0	0	0	0
Actarjana	0	0	0	0	0	0	0	0
Actinonema	0.94	0	0	0	0	0	0	0
Aegialoalaimus	0	0	0	0	0	0	0	0.72
Amphymonhystrella	0	0	1.02	0	0	0	0	0
Anticoma	3.02	0	0	1.62	1.94	0	0	0
Aponema	0.00	0	0	0	0	0	0	0
Araeolaimus	0	0	0	0	0	0	0	0
Bathyeuristomina	0	0	0	0	0	0	0	1.66
Belbolla	0	0	0	0	0	0	0	0
Bolbonema	1	0	0	0	0	0	0	0
Camacolaimus	0	3.89	0	0	0	0	0	0
Campylaimus	5.86	0.88	0	3.24	0.78	0	0	3.65
Cephalanticoma	0	0	0	0.00	0	0	0	0
Cervonema	1.02	0	1.51	4.86	0	0	0	0
Chromadora	0	0.88	0	1.62	0	7.31	0	0
Chromadorella	0	0	0	0	0.78	0	0	0
Chromadorina	5.84	21.21	15.26	4.86	1.74	74.86	0	18.98
Chromadorita	2.05	0	0	3.47	0.78	0	0	5.31
Cobbia	0	0	0	0	0	0	0	0
Comesa	0	0	0	0	0	0	0	0
Comesoma	0	0	0	0	0	0	0	0
Croconema	0	0	0	0	0	0	0	0
Cyartonema	0	0	0	0	0	0	0	0
Cyatholaimidae sp.1	1.02	0	0	3.56	0	3.02	1.12	2.88
Cyatholaimus	0	0	0	0	0	0	0	0
Daptonema	1.88	21.86	53.85	42.11	4.85	129.55	7.76	23.51
Desmodora	1.02	1.94	0	0	0	0	0	0.72
Desmoscolex	16.58	0	0	0	0.97	0	0	1.66

Dichromadora	0	0	0	0	0	0	0	0
Diplopeltis	0	0	0	0	0	0	0	0
Doliolaimus	0	0	0	0	0	0	0	0
Dorylaimopsis	0	0	0	0	0	0	0	0
Eleutherolaimus	0.97	0	0	0	0	0	0	0
Endeolophos	2.82	0	0	0	0.78	0	0	0
Enoploides	0	0	0	0	0	0	0	0
Euchromadora	0	0	0	0	0	0	0	0
Halalaimus	38.14	1.76	5.99	14.59	14.76	7.31	3.84	5.86
Halichoanolaimus	9.82	0	0	0	0.97	0	0	0
Halomonhystera	0.97	8.11	4.53	0	0.97	0	1.12	0
Hopperia	0	0	0	0	0	0	0	0
Laimella	0	0	0	0	0	0	0	0
Leptolaimoides	0	0	0	0	0	0	0	1.27
Leptolaimus	5.67	9.87	15.86	17.72	4.30	20.66	9.31	13.26
Leptolaimidae sp.1	0	0	0	0	0	0	0	0
Linhomoeus	0	0	0	0	0	0	0	0
Linhystera	0	0	0	0	0	0	0	0
Longicyatholaimus	0	0	2.53	52.57	0.78	0	1.40	29.31
Metachromadora	0	0	0	0	0	0	0	0
Megadesmolaimus	0	0	0	0	0	0	0.89	0
Metadesmolaimus	2.97	8.91	21.42	45.18	6.20	0	0.70	2.54
Metalinhomoeus	0.94	4.03	1.02	0.00	0.78	0	3.14	0
Metoncholaimus	0	0	3.46	1.62	0.00	0	3.37	0
Meyersia	0	0	0	0	0	3.02	0	0
Microlaimus	0	0	0	1.94	0.97	0	0	1.66
Molgolaimus	0.94	0	1.02	1.62	0.00	0	0	11.33
Monhystera	0	0	0	0	0.78	0	0	0
Monhystrella	0	1.06	1.51	0	0	0	0.89	0
Nannolaimoides	0	0	0	0	0	0	0	0
Neochromadora	0	0	0	0	0	0	0	0
Oncholaimellus	0	0	0	0	0	0	0	0
Oncholaimus	0	0	2.04	0	0	0	0	0
Oxystomina	3.76	0.88	0	0	0	0	0	5.08
Paracanthonchus	3.84	2.83	3.55	1.94	21	0	5.62	13.28
Paracomesoma	0	0	0	0	0	0	0	0
Paracyatholaimus	1	0	0	0	0	0	0	1.27
Paralongicyatholaimus	0	0	0	0	0	0	0	0
Paramonhystera	0	0	1.51	0.92	0	3.02	0	1.27
Parapinnanema	28.47	2.12	6.57	0	4.96	0	2.02	1.66
Parasphaerolaimus	0	0	0	0	0	0	0	0
Parodontophora	22.43	29.34	21.25	13.55	17.45	21.15	29.02	4.20
Paroxystomina	0	0	0	0	0	0	0	0
Pierickia	4.88	0	4.48	5.53	0	0	0	4.04
Polygastrophora	0	1.03	2.97	1.62	0	0	0	0.72
Pomponema	0	0	0	0	0	0	0	1.27
Prochromadorella	62.63	62.88	135.34	68.16	14.49	136.86	11.98	26.64

Prochromadora	0	0	0	0	0	0	0	0
Promonhystera	0	0	0	0.92	0	0	0	0
Prooncholaimus	0	0	0	0.92	0	0	0	0
Pseudochromadora	1.88	0	0	0	0	0	0	0
Ptycholaimellus	59.09	39.46	99.35	39.19	28.79	679.40	24.62	22.05
Rhabdodemania	0	0	0	0	0	0	0	0
Sabatieria	5.92	3.82	29.18	31.56	4.49	7.31	33.00	79.06
Setosabatieria	0	0	0	0	0	0	0	0
Siphonolaimus	0	0	0	0	0	0	0	0
Sphaerolaimus	5.99	1.06	4.53	16.19	3.02	0	3.64	30.48
Spilophorella	2.82	1.03	3.46	18.00	0	0	1.40	0
Stylotheristus	0	0	0	0	0	0	0	0
Subspaherolaimus	0	0	0	0	0	0	0	1.27
Symplocostoma	0	0	2.53	4.16	0	0	0	0.00
Synonchiella	0	0	1.02	0	0	0	0	0
Synonchium	0	0	0	0	0	0	0	0
Terschellingia	1.91	5.91	3.90	11.93	12.99	0	7.49	3.10
Thalassoalaimus	1.02	0	0	0.92	0	0	0.70	0
Thalassomonhystera	0	17.79	13.05	3.56	0.78	10.33	2.52	14.55
Theristus	0	9.17	6.93	5.78	1.55	104.59	3.64	3.98
Trichotheristus	0	0.88	4.92	2.77	0	0	0	0
Tricoma	0.94	0	1.95	1.62	0.78	0	0	6.58
Valvaelaimus	0	0	0	0	0	0	0	0
Vasostoma	0	0	0	0	0	0	0	0
Viscosia	1.02	0	1.51	6.11	0	0	0	0.72
Xyalidae sp.1	0	2.97	4.04	4.49	0	13.35	0.70	6.35

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Genera	**Spring 2014**				**Autumn 2014**			
	Kp1	**Kp2**	**Kp3**	**Kp4**	**Kp1**	**Kp2**	**Kp3**	**Kp4**
Acantholaimus	0	0	0	4.17	0	0	0	0
Acanthopharynx	0	0	0	0	0	0	0	0
Actarjana	0	0	0	0	0	0	0	0
Actinonema	0	0	0	6.61	0	0	0	0
Aegialoalaimus	0	0	0	0	0	0	0	0
Amphymonhystrella	0	0	0	0	0	0	0	0
Anticoma	4.45	0	0	14.29	0	0	0	4.58
Aponema	0	0	0	0	0	0	0	0
Araeolaimus	8.14	0	0	0	0	0	0	1.62
Bathyeuristomina	0	0	0	0	0	0	0	0
Belbolla	0	0	0	2.43	0	0	0	0
Bolbonema	0	0	0	0	0	0	0	0
Camacolaimus	0	0	0	0	0	0	0	0
Campylaimus	0	0	0	0	0	0	0	0
Cephalanticoma	0	0	0	7.30	0	0	0	0
Cervonema	0	0	0	0	0	0	0	0
Chromadora	12.59	0	0	4.17	0	0	0	0
Chromadorella	0	0	0	0	0	0	0	0

Chromadorina	43.78	0	8.11	14.14	0	0	0	1.53
Chromadorita	20.73	0	0	7.30	0	0	0	0
Cobbia	0	0	0	0	0	0	0	0
Comesa	0	0	0	0	0	0	0	0
Comesoma	0	0	0	0	0	0	0	0
Croconema	0	0	0	0	0	0	0	0
Cyartonema	0	0	0	2.43	0	0	0	0
Cyatholaimidae sp.1	0	0	0	0	0.53	0	0	4.86
Cyatholaimus	0	0	0	0	0	0	0	0
Daptonema	80.79	160.22	547.99	18.08	0.53	8.36	88.11	0
Desmodora	0	0	0	2.43	0	0	13.16	0
Desmoscolex	0	0	0	0	0	0	0	0
Dichromadora	0	0	0	1.77	0	0	0	1.53
Diplopeltis	4.45	0	0	0	0	0	0	0
Doliolaimus	0	0	0	0	0	0	0	0
Dorylaimopsis	8.91	0	60.85	99.35	1.72	0	140.64	142.10
Eleutherolaimus	0	0	4.99	0	0	0	0	0
Endeolophos	0	0	0	2.43	0	0	0	0
Enoploides	0	0	0	0	0	0	0	0
Euchromadora	0	0	0	16.58	0	0	0	0
Halalaimus	4.45	0	0	13.01	0	0	3.58	4.58
Halichoanolaimus	0	0	0	0	0	0	0	0
Halomonhystera	17.82	0	0	0	0	0	0	0
Hopperia	0	0	0	0	0	0	0	0
Laimella	0	0	0	0	0	0	0	0
Leptolaimoides	0	0	0	0	0	0	0	0
Leptolaimus	0	0	0	1.77	0	0	3.58	3.05
Leptolaimidae sp.1	0	0	0	0	0	0	8.66	0
Linhomoeus	0	0	0	0	0	8.63	4.39	0
Linhystera	0	0	4.99	2.43	0	0	0.00	0
Longicyatholaimus	12.59	0	107.66	51.33	0	0	25.29	9.72
Metachromadora	0.00	0	30.59	0	0	0	8.66	0
Megadesmolaimus	0.00	0	0.00	0	0	0	0	0
Metadesmolaimus	44.55	0	0.00	0	1.65	0	0	1.53
Metalinhomoeus	0	0	0.00	0	2.19	27.94	29.48	0
Metoncholaimus	0	0	56.74	0	0	0	0	3.05
Meyersia	0	0	0	0	0	0	0	0
Microlaimus	8.14	8.79	84.79	0	0	5.30	7.17	0
Molgolaimus	4.45	0	153.37	3.54	0.53	2.16	220.67	0
Monhystera	73.26	8.79	45.89	29.22	0	0	0	0
Monhystrella	0	0	0	0	0	0	0	3.05
Nannolaimoides	0	0	8.11	0	0	0	0	0
Neochromadora	0	0	0	0	0.86	0	0	0
Oncholaimellus	0	0	4.99	0	0	0	0	0
Oncholaimus	4.45	0	0	4.87	0	0	0	0
Oxystomina	0	0	0	0.00	0	0	7.17	0
Paracanthonchus	0	0	4.99	1.77	0	0	0	0

Paracomesoma	0	0	0	0	0	4.32	53.81	0
Paracyatholaimus	0	0	0	0	0	0	0	0
Paralongicyatholaimus	0	0	9.97	0	0	0	3.58	0
Paramonhystera	0	20.59	30.59	8.35	0	0	8.66	4.58
Parapinnanema	0	0	0	60.39	0	0	0	18.97
Parasphaerolaimus	0	0	0	1.77	0	0	0	0.00
Parodontophora	148.99	0	86.04	25.74	15.81	0	68.01	6.20
Paroxystomina	0	0	0	0	0	3.07	0	0
Pierickia	0	0	8.11	0	0	0	7.97	0
Polygastrophora	0	0	0	3.54	0	0	0	0
Pomponema	0	0	64.85	0	0	0	11.56	0
Prochromadorella	83.71	3.91	0	18.52	0	0	34.64	0
Prochromadora	0	0	0	1.77	0	0	0	0
Promonhystera	0	0	0	0	0	10.59	0	0
Prooncholaimus	0	0	0	0	0	0	0	0
Pseudochromadora	0	0	0	0	0	0	3.58	1.53
Ptycholaimellus	8.14	0	56.74	213.05	1.12	0	7.17	41.76
Rhabdodemania	0	0	0	2.43	0	0	0	0
Sabatieria	452.48	1780.12	931.22	23.39	165.01	788.93	531.39	16.19
Setosabatieria	8.14	8.79	62.68	0	0.86	0	4.39	0
Siphonolaimus	0	0	16.21	0	0	0	0	0
Sphaerolaimus	72.65	0	48.63	37.30	0	0	8.66	26.97
Spilophorella	0	0	4.99	73.99	0	0	0	0
Stylotheristus	0	0	0	2.43	0	0	0	0
Subspaherolaimus	0	0	8.11	0	0	0	0	3
Symplocostoma	0	0	0	8.35	0	0	0	0
Synonchiella	0	0	0	0	0	0	0	0
Synonchium	0	0	0	0	0	0	0	0
Terschellingia	43.78	379.28	419.16	34.11	51.05	208.47	404.09	33.95
Thalassoalaimus	0	0	0	0	0	0	12.36	1.53
Thalassomonhystera	8.91	0	0	0	0	0	0	0
Theristus	4.45	0	0	0	0	0	0	0
Trichotheristus	0	0	0	0	0	0	0	0
Tricoma	0	0	0	0	0	0	4	0
Valvaelaimus	0	0	0	2.43	0	0	0	0
Vasostoma	0	0	0	0	0	0	0	0
Viscosia	0	3.91	8.11	7.30	0	0	13.04	0
Xyalidae sp.1	119.18	3.91	16.21	10.12	1.93	0	3.58	1.53

Table S3. Mean values of the nematode univariate measures: number of genera (S), Shannon-diversity (H') and Pielou-evenness (J), Maturity index (MI), Index of Trophic Diversity (ITD).

Periods	Stations	S	H'	J	MI	ITD
Spring 2014	Anc1	10.33	2.41	0.72	2.45	0.37
Spring 2014	Anc2	11.00	2.39	0.69	2.52	0.39
Spring 2014	Anc3	18.00	3.38	0.81	2.39	0.38
Spring 2014	Anc4	18.00	3.30	0.81	2.35	0.41
Winter 2015	Anc1	9.33	1.97	0.63	2.72	0.45
Winter 2015	Anc2	8.33	2.26	0.75	2.68	0.35
Winter 2015	Anc3	18.00	3.43	0.82	2.28	0.52
Spring 2014	Ts1	21.00	3.48	0.80	2.79	0.41
Spring 2014	Ts2	18.67	3.48	0.83	2.20	0.44
Spring 2014	Ts3	21.67	3.37	0.76	2.34	0.43
Spring 2014	Ts 4	21.67	3.78	0.86	2.39	0.41
Winter 2015	Ts1	16.00	3.35	0.87	2.55	0.36
Winter 2015	Ts2	11.50	2.16	0.61	2.64	0.61
Winter 2015	Ts3	15.00	3.32	0.85	2.31	0.41
Winter 2015	Ts 4	22.33	3.72	0.83	2.45	0.34
Spring 2014	Kp1	18.50	3.25	0.77	2.14	0.53
Spring 2014	Kp2	5.00	0.92	0.42	2.14	0.77
Spring 2014	Kp3	14.00	2.86	0.76	2.37	0.44
Spring 2014	Kp4	21.67	3.67	0.83	2.66	0.60
Autumn 2014	Kp1	7.33	1.32	0.46	2.25	0.68
Autumn 2014	Kp2	5.33	1.18	0.49	2.22	0.64
Autumn 2014	Kp3	16.33	2.87	0.72	2.50	0.39
Autumn 2014	Kp4	14.50	2.77	0.73	2.47	0.49

Table S4. Ecological Quality Status (EQS) based on the Shannon-diversity (H'), Maturity index (MI) and colonizer-persister groups according to Moreno et al. (2011).

Stations	H'	MI	c-p 2 & 4%	Final Ecological Quality status
Anc1	poor	moderate	poor	poor
Anc2	poor	moderate	bad	poor
Anc3	moderate	poor	moderate	moderate
Anc4	moderate	poor	good	moderate
Ts1	moderate	moderate	good	moderate
Ts2	poor	poor	moderate	poor
Ts3	moderate	poor	moderate	moderate
Ts 4	good	poor	moderate	moderate
Kp1	poor	bad	moderate	poor
Kp2	bad	bad	moderate	bad
Kp3	moderate	poor	moderate	moderate
Kp4	moderate	moderate	moderate	moderate