



Taxonomic and functional diversity of nematode fauna: two sides of the same coin in the ecological quality assessment of transitional environments[☆]

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ABSTRACT

The spatiotemporal variation in taxonomic indices and functional traits of the nematode fauna was investigated along two major environmental (salinity and organic matter enrichment) gradients in the Cabras Lagoon (Sardinia, Italy). We aimed to assess, for the first time in a transitional system of western Mediterranean Sea, how taxonomic and functional components of nematode assemblage respond to such environmental factors. The taxonomic structure was the most sensitive metric to document changes in the nematode assemblage, showing significant differences in relation to all the factors and their interactions. All the taxonomic and functional nematode features, except for the maturity index (MI), proved salinity and organic enrichment to be the primary drivers of nematode distribution. The lack of significant variation in the MI is quite interesting and can be interpreted because of the marked adaptation of nematodes to highly dynamic environmental conditions of brackish and confined habitats. Temperature mainly affected faunal composition, abundance, and diversity, due to its influence on fecundity and biological cycles on almost all species. Integrating the taxonomic and functional components of nematodes as “two-sides of the same coin”, the Shannon diversity index, the percentage of coloniser–persisters and the MI were used together to assess the Ecological Quality Status (EQS) of the study sites, which ranged from poor to bad. Nematodes proved to be good indicators of the environmental variations in the investigated lagoon ecosystem. Importantly, the joint use of taxonomic and functional approaches provided integrative knowledge of nematode response to environmental gradients. We conclude that combining nematode-based taxonomic and functional indicators represent a valuable tool to assess the environmental quality of lagoonal ecosystems and may provide complementary information to macrofauna.

1. Introduction

Coastal lagoons are typical transitional ecosystems between land and sea. It has been shown that even coastal lagoons of the same typology (*sensu* Tagliapietra et al., 2009) and within a restricted climatic and geographical setting are highly heterogeneous systems depending on their origin, geomorphologic features, degree of confinement and hydrodynamics (Magni et al., 2009; Tagliapietra et al., 2012; Gravina et al., 2020). All these factors influence the lagoon's connectivity with

sea waters, including the dispersal and colonization ability of benthic animals (e.g., Magni and Gravina, 2023). Because of a relatively calm hydrodynamic environment and continuous sea-land material exchange, lagoons usually form special ecosystems that feature both sea and lakes characteristics and are valuable habitats for plants and animals requiring careful management and conservation strategies (Pérez-Ruzafa et al., 2011; Xian et al., 2022; Magni and Gravina, 2023). They further play a significant economic role and are among the most productive aquatic systems providing a variety of ecosystem services,

[☆] We heartfully dedicate this paper to the memory of our friend and colleague Davide Tagliapietra who greatly inspired our work on coastal transitional ecosystems.

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including supporting, regulating, provisioning and cultural services (Newton et al., 2018; Rodrigues-Filho et al., 2023). However, the increasing human pressure (e.g., urbanization, industrialization, agricultural, aquaculture and tourism development) is causing environmental degradation and overexploitation of the natural resources of these habitats worldwide (e.g., Daby, 2006; Arminot du Châtelet et al., 2016; Grassi et al., 2022; Magni et al., 2022). Phenomena that are amplified by the limited water exchange with the sea, shallow depths, high nutrient inputs, and climate changes that can further lead to cascading effects such as algal proliferation, excessive organic accumulation in the sediments, anoxia phenomena, trophic unbalance, and changes in the food web structure (see Magni et al., 2023 and references therein). Therefore, these systems run the risk of not meeting the criteria of good Ecological Quality Status (EQS, WFD, 2000/60/EC) and require effective surveillance and management (Semprucci et al., 2016).

Knowledge of taxonomic diversity of benthic communities is crucial for the application of biotic indices to assess the EQS of transitional waters within the European Water Framework Directive (WFD, 2000/60/EC) (Prato et al., 2009; Munari and Mistri, 2010; Reizopoulou et al., 2014). Accordingly, many studies using the benthic communities and associated diversity metrics and environmental variables have been conducted to evaluate the ecological features of lagoon ecosystems and their response to natural variability and anthropogenic pressures (see Magni et al., 2022 for review). According to the WFD, the state members must evaluate the EQS of the water bodies in a perspective of a long-term use of the environmental resources by means of selected Biological Quality Elements (BQEs), i.e., communities recognized as relevant components of ecosystems. The study of the BQEs spans from community abundance, taxonomic composition, biomass to indices, but these latter are preferred because they summarize multi-level information of community in a single output that can be easily set into EQS thresholds (Franzo et al., 2022). Within this context, many biotic indices mainly based on macrofauna have been developed (e.g., Simbora and Zenetos, 2002; Borja et al., 2000) and afterwards also applied to transitional areas (Magni et al., 2023). On the other hand, meiofauna, particularly nematodes, deserved less attention, even though they have been recognized as a useful bioindicator of the ecological status of transitional

ecosystems (Semprucci et al., 2019a; Facca, 2020). In fact, within the meiofauna, nematodes are the main component for both abundance, biodiversity and biomass from continental to marine substrates (Arminot du Châtelet et al., 2016; Semprucci et al., 2019a,b; Cocozza di Montanara et al., 2022). They are essential components of the seabed and provide important ecosystem services, including the reworking of the sediments and recycling of organic matter (Grassi et al., 2022). Their important role in the ecosystem functioning and key position at the base of food webs make them early sentinels of ecological quality changes that may allow timely conservation actions (Boufahja and Semprucci, 2015; Schratzberger et al., 2023).

Nematode biodiversity is adversely affected by many types of environmental perturbations (Moreno et al., 2011; Losi et al., 2021). However, taxonomic diversity measures cannot be the unique metrics used to assess nematode community changes since they are strongly influenced by sediment grain size variations (Semprucci et al., 2015a, 2015b). Accordingly, a concurrent use of the presence/absence of pollution-sensitive or pollution-resistant species and functional traits (i.e., trophic guilds: Wieser, 1953 and life strategies: Bongers et al., 1991) is also recommended (e.g., see Semprucci et al., 2015a; Franzo et al., 2022 for details). Furthermore, the advantage of simultaneously examining both taxonomical and functional aspects of the nematode community allow a more comprehensive understanding of the lagoonal ecosystems functioning and therefore to overcome the actual knowledge gaps that still do not allow an exhaustive approach for assessing the EQS of naturally and highly variable systems such as coastal lagoons. It is therefore obvious that for a greater appraisal of the complexity and environmental status of coastal lagoons, typically characterized by multiple and interlinked gradients (Tagliapietra et al., 2012; Foti et al., 2014), the joint analysis of taxonomic composition and functional traits of the benthic communities turns out to be of high value. This integrated approach is increasingly applied to the macrobenthic communities of Mediterranean lagoons (Marchini et al., 2008; Faulwetter et al., 2015; Nasi et al., 2018; Magni et al., 2023), while there are still fewer examples using brackish water nematodes (Semprucci et al., 2014; Jouili et al., 2017). In this context, we aimed to assess the response of taxonomic composition and functional traits of the nematodes inhabiting the

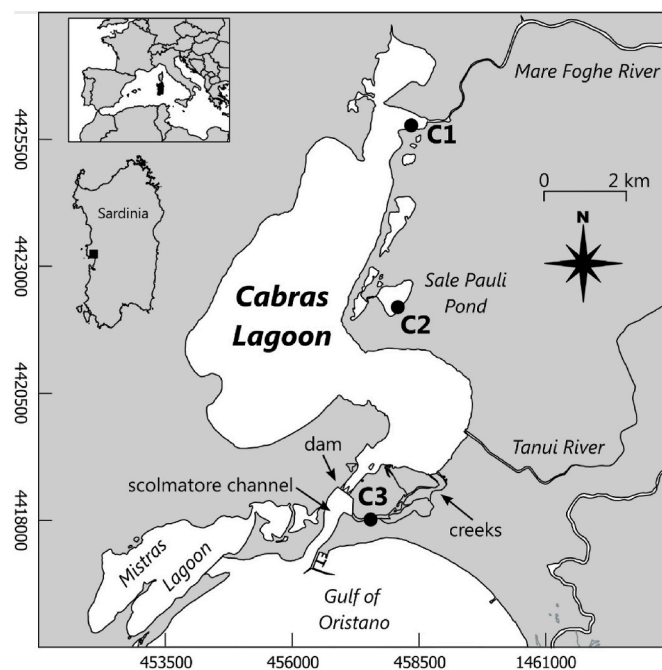


Fig. 1. Location of the sampling sites (C1, C2, C3) of the Cabras Lagoon investigated in July 2010 and February 2011.

Cabras Lagoon sediments along known environmental gradients, i.e., salinity and organic matter enrichment (e.g., Magni et al., 2022, 2023). In our opinion, the dual approach, too neglected so far, of using both assemblage composition and functional attributes of nematodes, as two sides of the same coin, to investigate the expected environmental changes along the continental-marine gradient, would be invaluable to study the lagoon ecology. To test this hypothesis, we analysed the taxonomy-based assemblage distribution patterns of nematodes and compared it with ecological information obtained through a functional trait-based approach (life and trophic strategies) in relation to the environmental gradients. Moreover, to obtain more comprehensive tools to assess the environmental quality in accordance with the European Directive and to accomplish the agenda 2030 goals, both the taxonomic and functional metrics were critically discussed for their application in the EQS assessment in transitional systems.

2. Materials and methods

2.1. Study area and sampling sites

The Cabras Lagoon (central-western Sardinia; Fig. 1) is the largest lagoon in the Sardinia Island, with a surface area of 22 km² and a watershed of ~430 km². The Rio Mare e Foghe, located in the northern sector of the lagoon, represents the main freshwater source, while southernmost narrow creeks, that flow into a larger channel, connect the lagoon with the Gulf of Oristano (Como et al., 2007; Padedda et al., 2012). An extended description of the hydrological and ecological features of this complex transitional system can be found in our previous studies (e.g., Magni et al., 2005b, 2008; Como et al., 2012; Specchiulli et al., 2018). The present investigation was conducted at three sampling sites (C1, C2 and C3, Fig. 1) located along marked salinity and organic enrichment gradients.

2.2. Field sampling

The field sampling was carried out at sites C1, C2, and C3 on July 6, 2010 and February 2, 2011. At each site and date, water temperature, salinity, and dissolved oxygen (DO) were measured using portable probes at the beginning and at the end of sampling. Sediment samples for the determination of the water content (Wc) and the analysis of organic matter (OM) and chlorophyll-*a* (Chl-*a*) were collected using a manual core (40 cm long, 5.5 cm diameter) gently pushed by hand into the sediments. Details of the hydrological measurements and chemical analysis can be found in Semprucci et al. (2019a).

For the analyses of the nematode fauna, six replicates were collected at each site and date by means of Plexiglas corers (Ø 3.6 cm) inserted 5 cm in the sediment. Details of sample treatment for the sorting and identification of the nematode fauna can also be found in Semprucci et al. (2019a). Briefly, the sediment samples were pre-filtered with magnesium chloride to allow organisms to relax before fixation and facilitate subsequent taxonomic identification (Danovaro et al., 2004). These samples were then fixed in a solution of pre-filtered seawater

containing formalin buffered with sodium tetraborate to reach a pH of ca. 8.2 (Pfannkuche and Thiel, 1988). A few drops of a Rose Bengal solution were added to the sample to facilitate the identification of organisms in the sorting phase.

2.3. Nematode community analysis

Once in laboratory, nematode analysis was carried out. The sediments were sieved through 500 µm and 42 µm meshes (Danovaro et al., 2004). The extraction of the organisms from the sediment fraction was performed by multiple centrifugation (3 times) with LUDOX HS30 (density 1.18 g cm⁻³, 3000 rpm for 10 min) as described in detail in Semprucci et al. (2019a). Meiofaunal organisms were then counted and sorted into their major taxa under stereomicroscope (Leica G26, zoom 25 × –50 ×). All the values obtained were recalculated as abundance per 10 cm². Nematodes were picked up for the taxonomical and functional identification, stored in 10% formaldehyde solution and mounted in permanent slides (Seinhorst, 1959; Danovaro et al., 2004). The identification at the genus level was completed under a light optical microscope (Nikon Optiphot-2) using the taxonomical guides of Platt and Warwick (1983, 1988), Warwick et al. (1998) and Zullini (2021), and original species descriptions available on the Nemys website (Nemys Ed., 2023).

The trophic groups were assigned to each genus according to Wieser (1953: selective, 1A and non-selective, 1B deposit feeders, epistrate feeders, 2A, and predators/omnivores, 2B) and Zullini's guide (2021: bacterivores, omnivores, endoparasites and small animals' feeders).

The Index of trophic diversity (ITD) is based on the composition of nematode trophic guilds that is reflected by the buccal cavity shape and the presence/absence of cuticularizations. 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, the lowest ITD values correspond to the highest trophic diversity, while the highest ITD to the lowest diversity.

Maturity index was initially applied in soil ecosystems and only later in marine habitats (MI, Bongers, 1990; Bongers et al., 1991). It is based on the life and reproductive strategies of free-living nematodes that are distinguished in a range from extreme r-strategist species (colonizers or c-p 1) and k-strategist species (persisters or c-p 5). R-strategists are generally bacterial-feeding, small in size and have short biological cycles and voluminous gonads that produce small, but numerous, eggs. They have a great recovery capacity, responding quickly to environmental perturbations. K-strategists are generally larger, with small gonads producing fewer but larger eggs. The index was calculated as the weighted average of the individual colonizers-persisters (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 change in composition from k-strategist genera (persisters, i.e., c-p 4 and c-p 5) to intermediate colonizers (i.e., c-p 3) and r-strategists (colonizers, i.e., c-p 1 and c-p 2) with the perturbation increase.

Finally, the Shannon-diversity index (Shannon and Weaver, 1949) and MI (Bongers et al., 1991) were utilized as Biological Quality

Table 1

Environmental data collected at each site and period in Cabras lagoon: water temperature, salinity, and dissolved oxygen (DO), water content (Wc) and the analysis of organic matter (OM) and chlorophyll-*a* (Chl-*a*). J10 = July 2010, F11=February 2011.

Sampling sites	Temperature	Salinity	DO	Wc%	OM%	Chl- <i>a</i> (µg/g)
C1J-10	26.8 ± 2.7	5.6 ± 1.2	3.3 ± 1.2	27.9 ± 1.9	1.6 ± 0.2	6.6 ± 2.0
C1F-11	9.7 ± 0.7	0.8 ± 0.3	5.1 ± 0.2	32.6 ± 2.8	1.4 ± 0.3	2.5 ± 1.1
C2J-10	26.6 ± 1.1	11.7 ± 1.0	5.1 ± 0.5	69.4 ± 10	16.1 ± 4.4	63.9 ± 25.2
C2F-11	9.2 ± 0.4	4.7 ± 0.1	8.9 ± 0.2	74.8 ± 10.8	16.3 ± 5.3	29.2 ± 23.5
C3J-10	29.7 ± 3.0	26.8 ± 2.6	12 ± 2.5	50.4 ± 21.5	7.8 ± 5.3	26.5 ± 14.9
C3F-11	12.5 ± 1.3	2.5 ± 0.4	14.5 ± 0.6	42.4 ± 11.0	4.4 ± 2.3	14.6 ± 6.4

Elements (BQEs) for nematodes according to Semprucci et al. (2015a, b). These BQEs allowed the classification of the sampled sites in five EcoQ classes (i.e., “bad”, “poor”, “moderate”, “good”, and “high”) after Moreno et al. (2011), following the principles applied by Chen et al. (2018). The final classification of the EcoQ was obtained by merging the results of both MI and H': when two close EcoQ classes were found (e.g., poor and moderate), the final EcoQ assigned to the site 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 site was obtained by averaging these two scores (i.e., poor).

2.4. Data analysis

The environmental variables used in the analysis included temperature, salinity, and dissolved oxygen (DO) in the water column and water content (Wc), organic matter (OM) and chlorophyll-*a* (Chl-*a*) in the sediment. Biotic data consisted in the abundance of the nematode fauna and were used to construct a taxa-by-site and period matrix. The biotic parameters computed were the number of taxa (*S*, taxon richness), the total abundance of nematodes in 10 cm² (*Ab*, abundance), the Shannon-diversity (*H'*, diversity), Pielou-equitability (*J*, evenness), Maturity Index (MI), the Index of Trophic Diversity (ITD), as well as all the single functional traits such as the trophic guilds and c-p classes. These biotic variables were computed for the three sampling sites C1, C2, and C3, for each replicate and date.

As for multivariate analysis, the non-parametric permutational analysis of variance (two way-PERMANOVA), based on Bray-Curtis (dis) similarity measures (Anderson, 2001) was carried out to test significant differences of the structure of community among sites (three levels: C1, C2, and C3), periods (two levels: July and February), and site × period interactions as fixed factors. The data were log (*x*+1) transformed before the analysis. The PERMANOVA, based on Euclidean distance, was also used to test the significant differences of all the biotic univariate measures. A log (*x*+1) transformation of data was applied only for the total nematode fauna abundance. The significance was computed by permutation with 9999 replicates. The pairwise comparisons between all pairs of sites were computed as post-hoc test and the Bonferroni correction procedure was followed to account for multiple simultaneous correlations (Rice, 1989). The nematode fauna major taxa contributing most to (dis) similarities among the sites were identified using the similarity percentages (SIMPER) test. Principal Component Analysis (PCA) was performed using the Statistica 8.0 software. This analysis attempts to identify the underlying factors which explain the pattern of correlation within a set of observed variables. The relative nematode parameters were projected on the factor plane as additional variables (i.e., secondary variables) without contributing to the results of the analysis, while environmental variables, as such as temperature, salinity, dissolved oxygen, sediment water content, organic matter and Chl-*a* were considered as primary variables. All the data were log (*x*+1) transformed before the analysis to reduce the different magnitude order between the variables.

3. Results

3.1. Environmental variables

The main distinctions between the sites based on the measured parameters are here summarized, to shed light on the relationship between nematodes and environmental factors (Table 1). Salinity distinguished a freshwater pole at site C1 located at the northern sector of the lagoon, while at the opposite southern site C3 salinity was closer to marine conditions. The intermediate site C2 was the most confined with much reduced hydrodynamics and highest levels of OM and phytoc pigment concentrations. Dissolved oxygen progressively increased from C1 towards C3, with the widest fluctuations occurring in C2. Sediment water,

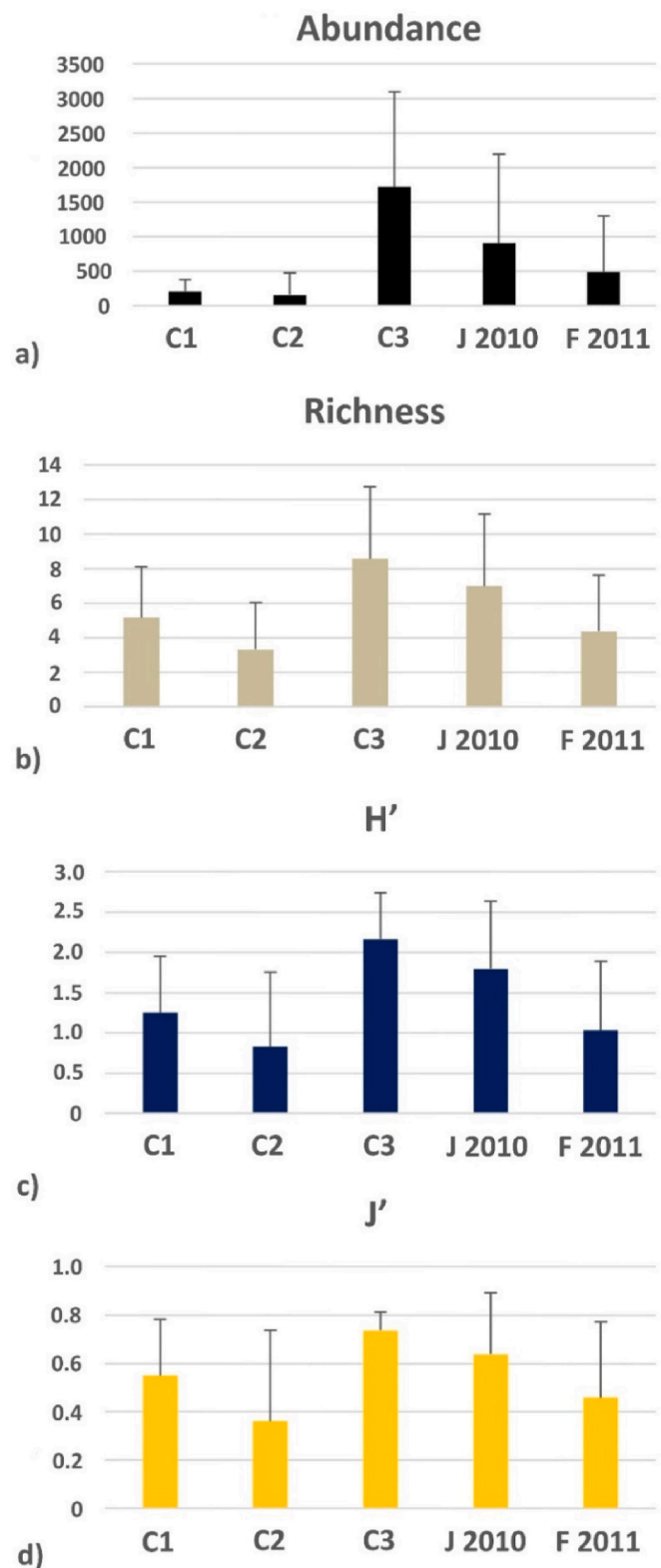


Fig. 2. Mean values ($n = 6 \pm$ standard deviation, SD) of the nematode abundance (a), richness (number of genera, b), Shannon-diversity (*H'*) (c) and Pielou-evenness (*J'*) (d) indices measured in the study area in July 2010 (J 2010) and February 2011 (F 2011).

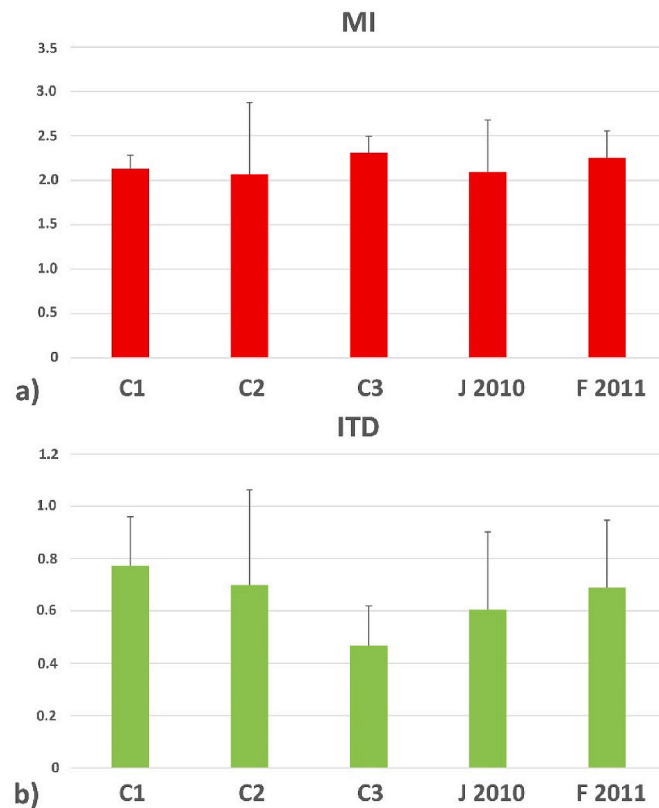


Fig. 3. Mean values ($n = 6 \pm$ standard deviation, SD) of the nematode functional indices: a) Maturity Index (MI); b) Index of Trophic Diversity (ITD) measured in the study area in July 2010 (J 2010) and February 2011 (F 2011).

as indirect measurement of the fine fraction of the sediment, was lowest at C1 and highest at C2, with C3 characterized by intermediated levels (Table 1).

3.2. Nematode community analysis

A total of 4149 nematode specimens were analysed and 39 genera were overall identified. Many of them are well recognized as typical of a wide range of environments from marine, brackish water to freshwater, while 11 genera are reported by international nematode database being exclusive of one type of environment, e.g. *Idiodorylaimus*, *Neotobrilus*, *Tridentula* are documented only in freshwater sediments and *Megadesmolaimus*, *Metadesmolaimus*, *Metalinhomoeus*, *Paracomosoma*, *Paramonohystera*, *Paradesmodora*, *Parapinnanema* and *Stylotheristus* only for marine bottoms (see Nemys Ed., 2023 Appendix 1). The most abundant genera were *Daptonema* (21%) and *Sabatieria* (16%), followed by *Ter-schellingia* (9%), *Triphyloides* (8%), *Dichromadora* (7%), *Anoplostoma*, *Leptolaimus* and *Hirschmanniella* (5% each).

Nematode abundance was 9 times higher at C3 than in C1 and C2 both in summer and winter (Fig. 2). Similar trends were observed also for the number of genera (here after named as richness), Shannon-diversity and Pielou-evenness indices even if to a less extent (Fig. 2). The abundance and taxonomic univariate variables are always higher during the warmer season (J 2010) than in winter (Fig. 2). In detail, abundance ranged from 0.0 ind. 10 cm² (C2F-2011) to 5053 ind. 10 cm²

(C3J-2010), the number of genera from 0.0 (C2J-2010 and C2F-2011) to 16 (C3J-2010), H' from 0.0 (C2J-2010, C1F-2011, C2F-2011) to 3.16 (C3J-2010) and J from 0.0 (C2J-2010, C1F-2011, C2F-2011) to 0.89 (C3J-2010).

Regarding the functional traits, it was discernible a high dominance of c-p 2 (71% of total nematode community), followed by c-p 3 (21%), c-p 4 (4%) and c-p 1 (1%) (Fig. 4a), with MI values that ranged from 0.0 (C2J-2010 and C2F-2011) to 3.0 (C2F-2011) (Fig. 3a). The trophic structure of the nematode community was prevalently characterized by non-selective deposit feeders that dominated the community (58%), followed by selective deposit feeders (15%), epistrate feeders (12%), endoparasites in roots of aquatic plants (5%) and predators-omnivorous (3%) (Fig. 4b). ITD ranged from 0.0 (C2J-2010 and C2F-2011) to 1.0 (C1J-2010, C2J-2010, C1F-2011 and C2F-2011) (Fig. 3b).

PERMANOVA test revealed that the taxonomic structure of the nematode community was the only parameter significantly affected by both single factors (i.e., sites, periods) and their interactions (Table 2). The most marked differences were between sites for both univariate taxonomic and functional parameters (abundance, richness, J , H' , trophic structure and ITD) and community structure as a whole. Contrarily, significant differences between dates were detected only for the number of genera, H' and community structure. The only exception is the MI that did not show any significant difference (Table 2).

The percentage contribution to the average dissimilarity between the sampling sites, measured by the SIMPER analysis, is reported for the top

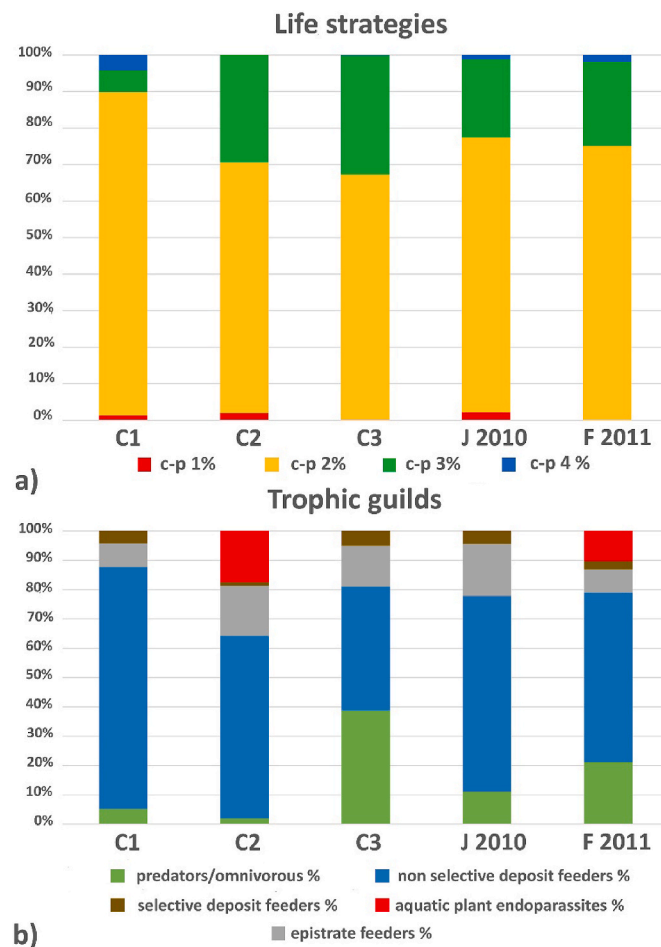


Fig. 4. Nematode functional traits: a) life strategies with colonizers-persisters classes; b) trophic guilds, measured in the study area in July 2010 (J 2010) and February 2011 (F 2011).

discriminating nematode genera accounting for up to 95% of the cumulative percentage (Table 3). The highest dissimilarity was observed between C1 and C3 (overall average dissimilarity: 94%), followed by the intermediate average dissimilarity (82%) found between C1 and C2, and the lowest average dissimilarity (79%) between C2 vs. C3. C1 was distinguished mainly by *Daptonema*, *Triphyloides*, *Theristus*, *Neotobrilus*, *Terschellingia*, *Amphimonhystrella* and *Thornia*, C2 by *Sabatieria*, *Daptonema*, *Dichromadora*, *Leptolaimus*, *Hirschmanniella*, *Anoplostoma*, *Aphanonchus* and *Thalassomonhystera*, while C3 by *Terschellingia*, *Leptolaimus*, *Sabatieria*, *Dichromadora*, *Anoplostoma*, *Theristus*, *Viscosia*, *Marylynnia*, *Sphaerolaimus* (Table 3).

The PCA results showed that 81% of the variance was explained by the first two factors: the first axis accounting for 51% of the explained variance and the second one for 30% (Fig. 5). The scatter diagram showed a clear separation of the sample-points along the first axis, with C2 sample-points located on the left side, while C3 and C1 placed centrally and on the right side, respectively. The separation appeared more marked for C1 samples. The second axis discriminated monthly replications of all stations. On the first axis the predominant environmental variables were Chl-*a* (−0.90), OM (−0.87), salinity and Wc (−0.79), while temperature (0.88) and DO (−0.57), showed a more marked role in the second axis (Fig. 5). Among the superimposed genera, *Sabatieria* (−0.58), *Anoplostoma* (−0.57), *Sphaerolaimus* (−0.41), *Paradesmodora* (−0.39), *Viscosia* (−0.35), *Aphanonchus*, *Parapinnanema* (−0.35), *Thornia* (0.37), *Amphimonhystrella*, *Neotobrilus* (0.44) and *Triphyloides* (0.61) exhibited the highest coordinates along the first axis (Fig. 5). Along the second axis, *Theristus* (0.52), *Thalassomonhystera* (0.44), *Mesodorylaimus*

(0.35), *Daptonema* (0.34) resulted more associated to the highest temperature of July 2010, while *Hirschmanniella* (−0.35) revealed the highest abundances in February 2011. Among the univariate measures, predator/omnivorous (−0.39), c-p 3 (−0.33), MI (0.20), non-selective deposit feeders (0.33), ITD (0.39), freshwater small animals' feeders (0.44), c-p 4 (0.47), c-p 2 (0.48), freshwater omnivores (0.51) were significantly distributed along the salinity and organic enrichment gradients. C-p 1 (0.44), H', number of genera (0.41), J (0.30), abundance (0.26) resulted higher in J-2010, while freshwater endoparasites (−0.39) in F-2011 (Fig. 5).

The Ecological Quality Status EQS of Cabras Lagoon detected by the taxonomical and functional data is reported in Table 4. C1 exhibited bad EQS in both the sampling periods, C2 bad EQS in July 2010 and poor in February 2011, while C3 was assessed as poor in both dates.

4. Discussion

The present study highlighted a clear relationship between the nematode fauna and the known environmental gradients occurring in the Mediterranean brackish lagoon of Cabras. About 69% of the nematodes found in Cabras Lagoon was represented by euryecious species known to occur in a wide range of habitats, from continental to marine ones, while the remaining assemblage comprised stenoeccious taxa, i.e., more properly marine or freshwater taxa (Nemys Ed., 2023). In particular, the PERMANOVA and SIMPER analyses indicated salinity and organic matter as the main drivers of nematode assemblages, as both taxonomy and functional components resulted significantly different in

Table 2
PERMANOVA main test results (two-way crossed design with Site and Season as main factors) for nematode abundance (A), number of genera (S), Pielou-evenness (J), Shannon-diversity (H'), Maturity Index (MI), Index of Trophic Diversity (ITD), nematode trophic structure and taxonomic structure (Df = degrees of freedom, SS/SM = sums and means of squares, p values < 0.05 are in bold and p values < 0.01 in bold and italic).

Source	Nematode abundance (A)			Number of genera (S)			Pielou-Evenness (J)			Shannon-Diversity (H')		
	Sum of squares	df	p	Mean square	df	p	Mean square	df	p	Mean square	df	p
Sites	2.7404	2	0.0003	1.3702	2	0.0014	1.3432	2	0.0046	1.5202	2	0.0025
Dates	0.21679	1	0.412	0.21679	1	0.412	0.22439	1	0.1739	0.46679	1	0.46679
Interaction	0.41481	2	0.4964	0.20741	2	0.4964	0.1096	2	0.41579	0.739	2	0.073763
Residual	6.8766	30	0.22922	0.22922	30	0.22922	0.22922	30	0.22922	0.22922	30	0.22922
Total	10.249	35		535.64	35		5.6312	35	6.3164	35		6.3164

Source	Maturity Index (MI)			Index of Trophic Diversity (ITD)			Nematode trophic structure			Taxonomic community structure		
	Sum of squares	df	p	Mean square	df	p	Mean square	df	p	Mean square	df	p
Sites	0.71155	2	0.037986	0.35578	2	0.0001	1.4186	2	0.7093	7.3814	2	1.1878
Dates	0.060997	1	0.027123	0.060997	1	0.060997	0.17404	1	0.17404	1.8111	1	1.0296
Interaction	0.10948	2	-0.061779	0.054739	2	0.054739	0.10837	2	0.054183	0.56387	2	0.58189
Residual	2.0055	29	0.03811	0.068849	30	0.068849	2.4984	26	0.096093	6.8369	26	0.26296
Total	2.8875	34		2.8875	35		4.1994	31	11.406	11.406	31	11.406

the distribution patterns of the three sites. Contrarily, the temporal variations were not relevant for most univariate and multivariate metrics (with the only exception of the number of genera, Shannon-diversity index, and taxonomic structure) and, thus, demonstrated the persistence of distribution patterns along the spatial scale gradient over time. A similar result was found at the same study sites of the Cabras Lagoon for the macrobenthos by Foti et al. (2014). The continental site C1, where freshwater inputs of the Mare e Foghe river flow into the lagoon and low OM levels occurred, was characterized by intermediate values of nematode abundances, richness and biodiversity. In this area, some typical freshwater/brackish water genera, such as *Amphimonhystrella*, *Neotobrilus*, *Thornia*, *Triphyloides*, along with many euryecious genera composed the most distinctive faunal composition of the lagoon. In the most confined site C2, the environmental conditions were particularly marked by muddy and organically enriched sediments with a very limited exchange with the lagoon's main body (Bartoli et al., 2009). This affected mainly the abundances and diversity of nematodes that were the lowest found in the study sites. The nematode composition at site C2 was significantly different as well, but it revealed a lower dissimilarity compared with the seaward site (C3) located in a creek connecting the lagoon to the Gulf of Oristano. Its sediment characteristics are intermediate between the sandy, organically poor C1 and the muddy, organically enriched C2, but salinity dropped in winter, nullifying temporarily the salinity gradient, and further highlighting the seasonal and interannual environmental variability of the Cabras Lagoon (Padedda et al., 2012; Semprucci et al., 2019a). The transition from a freshwater fauna to a typical estuarine assemblage and then to a more marine fauna is evident throughout the Cabras Lagoon system and this agrees with the results from other coastal lagoons and estuaries worldwide (Heip et al., 1985; Barnes et al., 2008; Ferrero et al., 2008). From a functional point of view, the assemblages were dominated by non-selective deposit feeders (1B), and general opportunistic nematodes, in accordance with the common occurrence of these functional traits in estuaries, semi-enclosed bays and coastal lagoons (Armenteros et al., 2009; Alves et al., 2014; Semprucci et al., 2014; Franzo and Del Negro, 2019).

When the relationship among the environmental and faunal variables was analysed by means of the PCA, the results confirmed the primary importance of the salinity and organic matter in structuring the nematode composition of Cabras Lagoon. The functional traits and indices were also mainly driven by these gradients, as it can be seen from the highest coordinate scores along the first axis. In accordance, the marine predators/omnivores, epistrate feeders and intermediate colonizers (c-p 3), all increased in relation to higher salinity and organic matter enrichment, occurring in the heterogeneous marine and brackish sectors of the lagoon. Otherwise, MI and the ITD indices along with non-selective deposit feeders, freshwater small animals' feeders, omnivores feeders persists c-p 4 and extreme colonizers c-p 2) resulted more associated with the lower end of salinity and organic matter gradients, where both freshwater and low organic load of sediments most affect the community, selecting the most tolerant or highly adapted species to these conditions. On the other hand, the taxonomic univariate variables (i.e., richness H', and J) along with abundance resulted more strictly associated to temperature, as proxy of the temporal fluctuations. Indeed, the values of these metrics resulted higher in the warmer season (July 2010) when the fecundity and the biological cycles of the species are favoured. Instead, the MI and ITD indices along with non-selective deposit feeders, freshwater small animals' feeders and omnivores, c-p 4, c-p 2 resulted more associated to freshwater.

The trophic diversity significantly changed at the three sites of the lagoon showing the lowest diversity in the organically enriched sediments of C2. Our finding agrees with the results documented by Jouilli et al. (2017) from El Bibane Lagoon in Tunisia, who reported the lowest trophic diversity in association with the highest organic matter content. Otherwise, the lack of significant differences in MI between sites and dates corroborates the 'Estuarine Quality Paradox' theorized by Elliott

Table 3

List of the top discriminating nematode genera with contribution more than 5% by SIMPER analysis at the three investigated sites.

Overall Average Dissimilarity C1 vs. C2 = 82%					
Taxon	Average dissimilarity	Contribution %	Cumulative %	Mean abundance C1	Mean abundance C2
<i>Daptonema</i>	20.7	25.2	25.2	41.3	30.6
<i>Sabatieria</i>	17.6	21.4	46.6	0.0	35.2
<i>Triphylloides</i>	12.6	15.3	61.9	25.2	0.0
<i>Dichromadora</i>	6.7	8.2	70.1	7.6	7.8
<i>Theristus</i>	4.7	5.7	75.8	9.2	2.5
<i>Hirschmanniella</i>	4.7	5.7	81.6	0.0	9.4
<i>Anoplostoma</i>	3.5	4.2	85.8	0.0	6.9
<i>Thalassomonhystera</i>	1.5	1.8	87.6	1.3	2.5
<i>Neotobrilus</i>	1.4	1.7	89.2	2.8	0.0
<i>Terschellingia</i>	1.3	1.6	90.8	2.6	0.0
<i>Aphanonchus</i>	1.2	1.5	92.3	0.0	2.5
<i>Amphimonhystrella</i>	1.2	1.5	93.8	2.4	0.0
<i>Thornia</i>	1.2	1.5	95.2	2.4	0.0
Overall Average Dissimilarity C1 vs. C3 = 94%					
Taxon	Average dissimilarity	Contribution %	Cumulative %	Mean abundance C1	Mean abundance C3
<i>Daptonema</i>	20.5	21.8	21.8	41.3	0.7
<i>Triphylloides</i>	12.6	13.4	35.1	25.2	0.0
<i>Sabatieria</i>	12.1	12.8	48.0	0.0	24.1
<i>Terschellingia</i>	11.3	12.0	60.0	2.6	23.5
<i>Leptolaimus</i>	7.5	7.9	67.9	1.8	14.6
<i>Dichromadora</i>	6.6	7.0	74.9	7.6	8.5
<i>Anoplostoma</i>	6.0	6.3	81.3	0.0	12.0
<i>Theristus</i>	5.0	5.3	86.6	9.2	3.1
<i>Viscosia</i>	1.4	1.5	88.1	0.0	2.8
<i>Neotobrilus</i>	1.4	1.5	89.5	2.8	0.0
<i>Amphimonhystrella</i>	1.2	1.3	90.8	2.4	0.0
<i>Thornia</i>	1.2	1.3	92.1	2.4	0.0
<i>Marylynnia</i>	1.2	1.2	93.3	0.0	2.3
<i>Sphaerolaimus</i>	1.0	1.1	94.4	0.1	2.1
<i>Microalaimus</i>	0.8	0.8	95.3	0.4	1.2
Overall Average Dissimilarity C2 vs. C3 = 79%					
Taxon	Average dissimilarity	Contribution %	Cumulative %	Mean abundance C2	Mean abundance C3
<i>Sabatieria</i>	15.6	19.9	19.9	35.2	24.1
<i>Daptonema</i>	15.1	19.2	39.1	30.6	0.7
<i>Terschellingia</i>	11.8	15.0	54.1	0.0	23.5
<i>Leptolaimus</i>	7.3	9.3	63.4	0.0	14.6
<i>Dichromadora</i>	6.6	8.3	71.8	7.8	8.5
<i>Anoplostoma</i>	5.9	7.5	79.3	6.9	12.0
<i>Hirschmanniella</i>	4.7	6.0	85.2	9.4	0.0
<i>Theristus</i>	2.1	2.7	87.9	2.5	3.1
<i>Viscosia</i>	1.4	1.8	89.7	0.3	2.8
<i>Aphanonchus</i>	1.3	1.7	91.4	2.5	0.6
<i>Thalassomonhystera</i>	1.2	1.6	92.9	2.5	0.2
<i>Marylynnia</i>	1.2	1.5	94.4	0.0	2.3
<i>Sphaerolaimus</i>	1.1	1.4	95.8	1.2	2.1

and Quintino (2007) that appeared to be extendable to other confined environments (see Franzo et al., 2022 for review). This model suggested that the faunal assemblage features under anthropogenic disturbance may coincide with those under natural stress because of the high fluctuations of the physico-chemical parameters of the transitional environments, and that species living in such environments are well adapted to these fluctuations, becoming tolerant to further changes (Adão et al., 2009; Jouili et al., 2017). Our results are in line with this hypothesis, as the meiofaunal assemblages were dominated by nematodes particularly able to withstand stressors in the various sites of the Cabras Lagoon (Semprucci et al., 2019a), and this could be the reason for the limitation of the applicability of MI in confined environments.

Based on Moreno et al. (2011) approach, the final EQS of the study sites of Cabras Lagoon ranged from poor to bad, with a slight increase seaward. A similar spatial trend in the EQS was assessed by M-AMBI and M-bAMBI using the macrofauna yet giving a good EQS at site C3 (Magni et al., 2023), thus higher than the EQS obtained using free-living nematodes. However, these differences are not surprising since macrofauna and meiofauna have different ecological roles in the ecosystems and respond to disturbances at different spatial and temporal scales

(Semprucci et al., 2013). Macrofauna could be indicative of effects over larger spatial and temporal scales as related to their planktonic larval dispersal (but see Magni and Gravina, 2023). On the other hand, meiofauna, particularly nematodes, may indicate effects over shorter spatial and temporal scales due their direct benthic development and generation times as short as a few weeks (Frontalini et al., 2011; Vanaverbeke et al., 2011). These considerations suggest that the analysis of nematodes and macrofauna can reveal different, yet complementary aspects concerning the structuring of lagoonal ecosystems (see also Magni et al., 2022). Furthermore, the results of the present study on nematodes corroborate what Magni et al. (2023) underlined using the macrofauna. In particular, we believe that a thorough knowledge of benthic communities based on both taxonomic and functional aspects is indispensable for properly assessing the ecological quality of highly heterogeneous and confined systems, such as the Cabras Lagoon, where many biotic indices have been shown less effective than in coastal areas (Magni et al., 2005a; Dauvin, 2007; Ruellet and Dauvin, 2007; Semprucci et al., 2014, 2016; Hong et al., 2020; Sahraeian et al., 2020; Losi et al., 2021). Accordingly, our results underline that a combined use of taxonomic and functional indicators (i.e., MI, H' and c-p%) is necessary

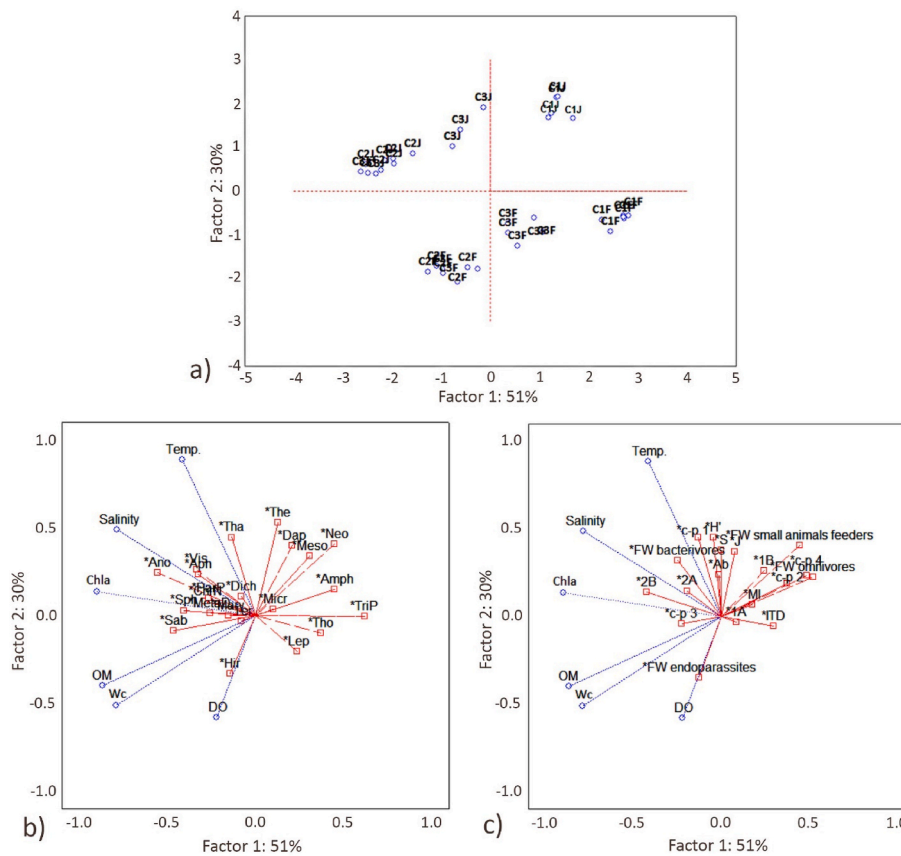


Fig. 5. a) Scatter diagram plotting factor 1 and factor 2 of sampling stations in the two sampling periods (J = July 2010 and F = February 2011) obtained by the Principal Component Analysis (PCA) carried out using environmental data as active variables (Temp. = temperature, Salinity = salinity, DO = dissolved oxygen, Chla = chlorophyll-*a*, OM = organic matter, Wc = sediment water content); b) Projection of the active variables (environmental data) and nematode genera data used as secondary variables on the first two factor-planes. c) Similar projection using indices and functional traits as secondary variables. For all the abbreviations referred to the nematode genera, indices and functional traits see [Appendix 2](#).

Table 4

Categorization of the Ecological Quality Status (EQS) of the Cabras sites in the two sampling periods according to [Moreno et al. \(2011\)](#) (MI: Maturity index, H': Shannon-diversity index, c-p 2%: percentage of coloniser-persister class 2, c-p 4%: percentage of coloniser-persister class 4). The ecological quality status (EQS) is color coded (red: bad; orange: poor; yellow: moderate).

Study sites	MI	H'	c-p 2 %	c-p 4 %	Final Ecological Quality
C1J-10	2.13	1.25	88.60	4.15	bad
C1F-11	2.17	0.94	88.01	5.37	bad
C2J-10	1.89	0.83	57.26	0.00	bad
C2F-11	1.97	0.32	53.41	0.00	poor
C3J-10	2.31	2.17	67.12	0.09	poor
C3F-11	2.25	1.85	71.19	0.00	poor

to minimize the problems related to the ‘Estuarine Quality Paradox’ ([Elliott and Quintino, 2007](#)). The present study may open the way to the development of a new nematode-based index that can combine both taxonomic and functional components of the community as a more efficient tool for assessing the EQS of the transitional water ecosystems.

5. Conclusions

The present study is one of the few in-depth investigations on the

nematode community structure, diversity and functional traits conducted in a Mediterranean transitional ecosystem. The taxonomic structure was the most sensitive metric to document changes in the nematode assemblages. However, both taxonomic and functional features of nematodes reflected the high habitat heterogeneity of Cabras Lagoon along the main gradients of salinity and organic matter enrichment of sediment. The Maturity index (based on nematode life strategies) was the only metric revealing no significant differences along the spatial gradient, suggesting a high adaptation of nematodes to highly dynamic and extreme conditions typical of the lagoon ecosystems. The EQS of the study sites ranged from poor to bad, the taxonomic and functional indices complementing and integrating well one another towards this assessment. Our results underline that the taxonomic and functional approaches, for both nematode fauna and macrofauna ([Magni et al., 2023](#)), are two sides of the same coin and that their combined use represents a robust tool to assess the environmental quality of lagoon ecosystems, also coping with issues related to the ‘Estuarine Quality Paradox’ ([Elliott and Quintino, 2007](#)).

CRediT authorship contribution statement

Eleonora Grassi: Writing – original draft, Visualization, Investigation, Formal analysis, Data curation, Conceptualization, Methodology, Writing – review & editing. **Linda Catani:** Writing – original draft, Visualization, Investigation, Formal analysis, Data curation, Conceptualization, Methodology, Writing – review & editing. **Paolo Magni:** Writing – original draft, Supervision, Investigation, Funding acquisition, Conceptualization, Visualization, Writing – review & editing. **Maria**

Flavia Gravina: Writing – original draft, Investigation, Conceptualization, Visualization, Writing – review & editing. **Federica Semprucci:** Writing – original draft, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

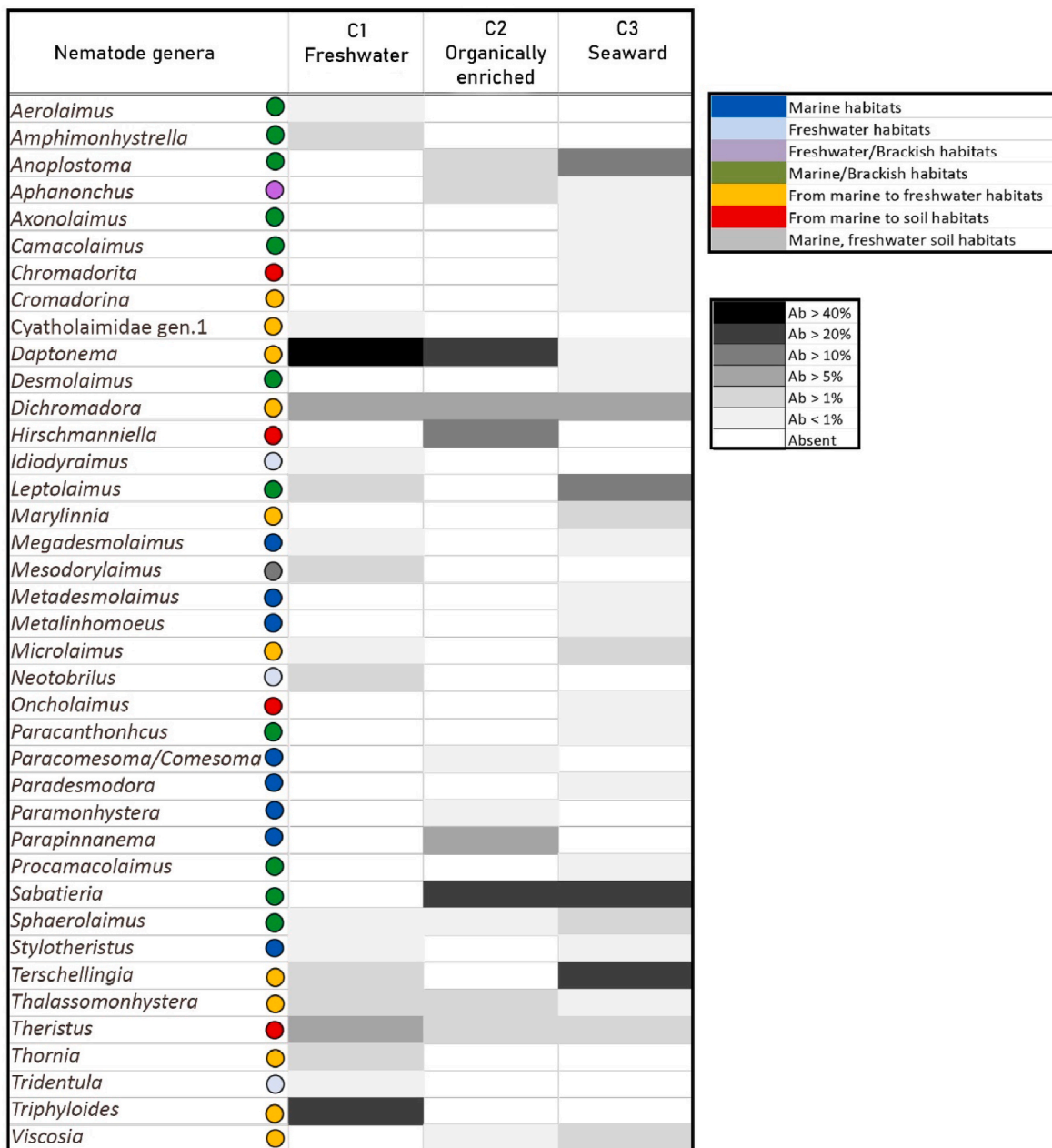
Data availability

Data will be made available on request.

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Appendix 1. Diagram showing the distribution and abundance percentage of the 39 nematode genera found along the Cabras Lagoon. The relationship between genera and type of habitat reported in the figure have been obtained by [Nemys Ed. \(2023\)](#)



Appendix 2

List of nematode genera, indices and functional traits and their relative abbreviations applied in the paper.

Genera, indices and functional traits	Abbreviations
<i>Aerolaimus</i>	Aer
<i>Amphimonhystrella</i>	Amph
<i>Anoplostoma</i>	Ano
<i>Aphanonchus</i>	Aph
<i>Axonolaimus</i>	Axo
<i>Camacolaimus</i>	Cam
<i>Chromadorita</i>	ChrT
<i>Cromadorina</i>	ChrN
<i>Cyatholaimidae</i> gen.1	Cyge1
<i>Daptonema</i>	Dap
<i>Desmolaimus</i>	Des
<i>Dichromadora</i>	Dich
<i>Hirschmanniella</i>	Hir
<i>Idiodyraimus</i>	Idi
<i>Leptolaimus</i>	Lep
<i>Marylinnia</i>	Mary
<i>Megadesmolaimus</i>	Mega
<i>Mesodorylaimus</i>	Meso
<i>Metadesmolaimus</i>	MetaD
<i>Metalinhomoeus</i>	MetaL
<i>Microlaimus</i>	Micr
<i>Neotobrilus</i>	Neo
<i>Oncholaimus</i>	Onch
<i>Paracanthocheilus</i>	ParS
<i>Paracomesoma/Comesoma</i>	ParM
<i>Paradesmodora</i>	ParD
<i>Paramonhystera</i>	ParH
<i>Parapinnanema</i>	ParP
<i>Procamacolaimus</i>	Pro
<i>Sabatieria</i>	Sab
<i>Sphaerolaimus</i>	Sph
<i>Stylotheristus</i>	Sty
<i>Terschellingia</i>	Ter
<i>Thalassomonhystera</i>	Tha
<i>Theristus</i>	The
<i>Thornia</i>	Tho
<i>Tridentula</i>	TriD
<i>Triphyloides</i>	TriP
<i>Viscosia</i>	Vis
Maturity index	MI
Index of Trophic diversity	ITD
Pielou-evenness	J
Shannon-diversity	H'
selective deposit feeders%	1A%
non-selective deposit feeders%	1B%
epistrate feeders%	2A%
predators/omnivores%	2B%
freshwater bacterivores%	FW bacterivores%
freshwater omnivores %	FW omnivores %
freshwater endoparasites%	FW endoparasites%
freshwater small animals' feeders %	FW small animals' feeders%
coloniser-persister class 1%	c-p 1%
coloniser-persister class 2%	c-p 2%
coloniser-persister class 3%	c-p 3%
coloniser-persister class 4%	c-p 4%

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