The influence of habitat heterogeneity on Nematoda communities in *Posidonia oceanica* meadows

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Abstract Nematodes typically comprise the most abundant phylum in meiofaunal communities. We aim to characterize the specific ecological conditions that influence Nematoda communities in *Posidonia oceanica* grasslands focusing on three habitats: leaves, matte, and unvegetated adjacent sediment. We hypothesized that the constant flux of nutrients under the canopy would result in a higher concentration of metazoans compared to the unvegetated sediment outside; however, the hypothesis was not confirmed by the obtained results. The habitat heterogeneity played an important role in shaping the community, yielding a greater richness in the matte. The resource specificity of the leaves (microepiphytes) was also expected to shape the community by favoring nematodes capable of exploiting this resource (epistratum feeders with scraping-like structures), such as the family Chromadoridae that dominated this habitat. In addition, the high light exposure of the leaves explains the higher concentration of ocellated nematodes in this habitat. Organic carbon and nitrogen contents had an impact on the Nematoda community composition, meanwhile average grain size only affected the abundance of nematodes with elongated/filiform tails.

Keywords Leaves · Matte · Adjacent sediment · Epistrate feeders · Chromadoridae

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Introduction

The endemic *Posidonia oceanica* (L.) Delile, 1813 constitutes the most important meadow-forming seagrass species in the Mediterranean Sea. This marine phanerogam is actively involved in water oxygenation and has been used as bioindicator of environment well-being (Vaquer-Sunyer & Barrientos, 2021). Regarding biodiversity, *P. oceanica* meadows support a rich and plentiful community of marine organisms (Blanc & Grissac, 1984; Perès, 1984; Bourcier, 1989). According to these studies, the ever-increasing threat to these meadows in the Mediterranean Sea not only endangers their own survival, but also the conservation of vast diversity of living beings that find shelter and nourishment within this heterogeneous ecosystem. Three main habitats can be highlighted among these meadows: the leaves and the matte

(roots and rhizomes) within the bushes and the adjacent unvegetated sediment outside them.

The most well-known fauna hosted in *P. oceanica* meadows include many fishes of commercial interest as well as macroinvertebrates such as crustaceans, mollusks, and sea urchins (Cetinić et al., 1999, 2011; Albano & Stockinger, 2019; Borg et al., 2006). However, knowledge is comparatively less regarding the community of marine invertebrates of small size, namely meiofauna (García-Gómez et al., 2022). This benthic community is formed by organisms capable of passing through a 500 μm mesh and being retained in a lower 63 μm mesh size sieve (Giere, 2009). Meiofauna plays an important role in the functioning of marine ecosystems (Schratzberger & Ingels, 2018) and can be a suitable model for the study of their health because of their high abundance, fast regeneration rate, and low dispersal capacity (Woodward, 2010; Zeppilli et al., 2015). Our understanding of the diversity and ecology of meiofauna inhabiting *P. oceanica* meadows has slowly increased in recent decades (Martínez et al., 2021; García-Gómez et al., 2022). The phylum Nematoda, on the other hand, constitutes the most abundant component of the meiofauna and has been further investigated (Novak, 1982, 1989) in these *Posidonia* meadows. Previous research showed a high diversity of nematodes within *P. oceanica* and a different community composition between its habitats. For instance, Moens & Vincx (1997) highlighted that these differences are influenced by various crucial community-shaping factors, such as food availability of habitats and feeding strategy of species, while other studies attributed this to habitat complexity (Mazzella et al., 1989; Gacia & Duarte, 2001; Boström et al., 2006).

Regarding food sources, leaves within *P. oceanica* ecosystem are dominated by microepiphytic organisms, such as diatoms and dinoflagellates, whose prevalence is especially clear in the summer. Moreover, during this season, the leaves reach their maximum length, which decreases hydrodynamic forces underneath (Mabrouk et al., 2011) and favors the stability of the sediment under the canopy (Gacia & Duarte, 2001; Manca et al., 2012).

As a novel contribution with respect to previous studies on nematode communities in *P. oceanica* meadows, our research incorporates samples from outside the canopy for comparison to leaves and matte inside bushes. This work also incorporated the analyses of sediment granulometry and concentration of organic matter and nitrogen to relate it to nematode communities. Finally, we introduced the study of functional traits that were previously unexplored in the nematode communities of *P. oceanica.* These traits include presence/absence of ocelli and presence/absence of denticles on the buccal cavity and tail shape, among others previously studied.

The aim of this study is to assess dissimilarity in Nematoda communities between *P. oceanica*'s three main habitats (leaves, matte, and adjacent unvegetated sediment) due to habitat heterogeneity. These are the main hypotheses tested in this study:

- We expect to find higher density of nematodes inside the canopy than outside due to increased flux of food resources.
- The diverse availability of food and the heterogeneity of the matte lead us to expect a greater richness of genera and families in this habitat in comparison to the other, more uniform habitats.
- The resource specificity of the leaves, consisting of microepiphytes, leads to a community dominated by a group of nematodes that exploit microepiphytes as food resource, potentially resulting in a lower evenness. Conversely, the higher variety of nutrients in the matte and adjacent sediment may host more generalist feeders and foster a more even community structure.
- We anticipate that nematodes occurring in *P. oceanica* show distinct functional traits as a result of their adaptation to the specific conditions of each habitat.
- With respect to matte and adjacent sediment habitats, we expect that sediment parameters (i.e., mean grain size, nitrogen and organic matter content, and the percentages of sand, silt, and clay) will influence the genus and family composition of nematode communities.

Materials and methods

Sampling, processing, sorting and identification of meiofauna

A total of nine samples were collected by scuba diving on a *P. oceanica* meadow from Cala Cuartel (Santa Pola, Alicante, Mediterranean Spain; coordinates:

38° 12′ 34.04″ N, 0° 30′ 19.12″ W) on summer season, specifically on the 2nd of August 2016, when the phanerogam reaches its highest growth. A 1 km² area within the meadow was selected, situated at depths of 4 to 7 m (Fig. 1). Within this area, we randomly designated three sites, approximately 200 m apart, where one sample was collected from each of the three primary habitats. At each site, a 20×20 cm quadrat was used to sample leaves, matte and the nearest unvegetated area. Leaves were first cut at the ligule level and collected in a hermetic bag; then, the underlying root sediment was shoveled into another hermetic bag (Novak, 1982, 1989; Cvitkovic et al., 2017). Lastly, the nearest adjacent sediment comprised in a quadrat was as well collected in a hermetic bag.

For the extraction of the metazoans, firstly the anesthetization by magnesium chloride technique was used to isolate the soft meiofauna (Higgins & Thiel, 1988; Schmidt-Rhaesa, 2019). Once this fraction was separated, we employed the "bubble and blot" technique to extract the hard meiofauna (Higgins & Thiel, 1988). Meiofauna was collected using a 63 μm mesh size sieve and then fixed with 7% formaldehyde. Nematodes were counted from 1/5 of each sample, a measure taken in response to the notably high abundance of metazoans within the samples. Subsequently, this count was extrapolated to represent the entire sample. Approximately 100 nematode specimens were sorted and mounted on glycerine (Bianchelli et al., 2013; Rosli et al., 2018; Semprucci

Fig. 1 Location of Cala Cuartel in Santa Pola (Alicante). The rectangle with dotted outline delineates the 1 km² meadow area where the sample sites were designated
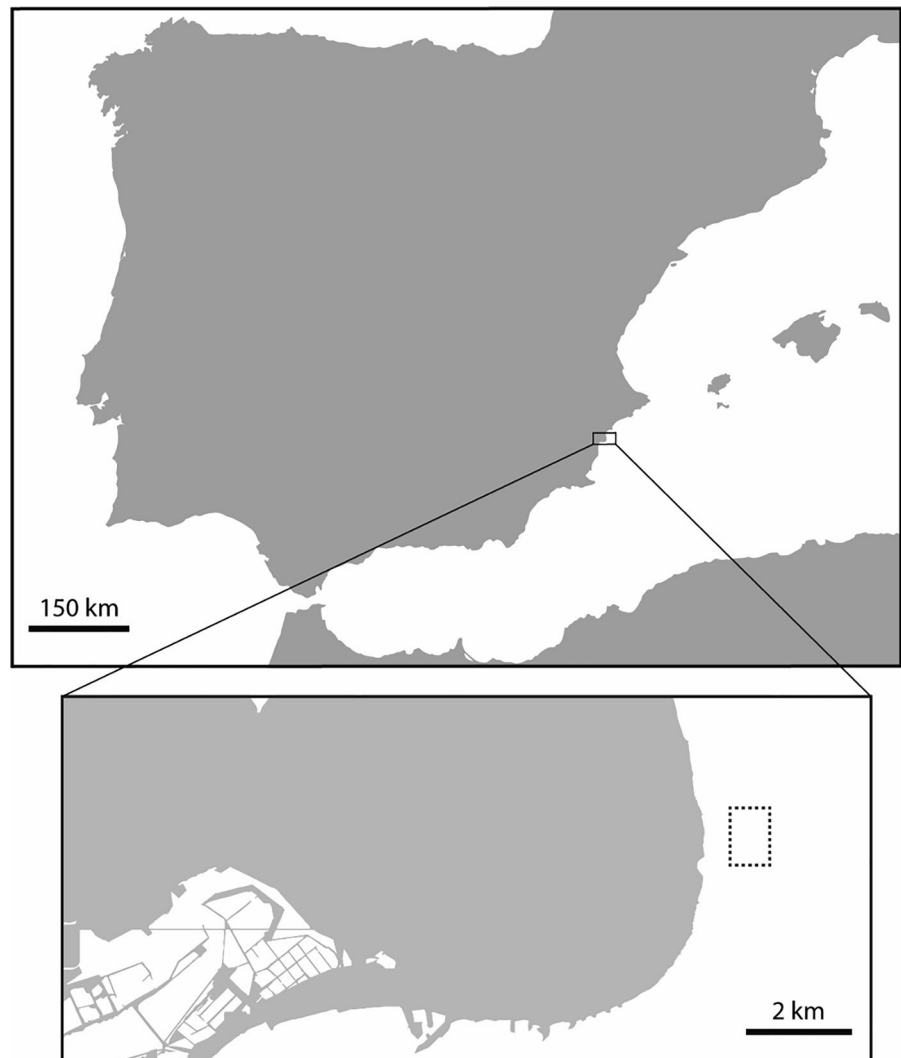
et al., 2018; Rebecchi et al., 2022), encircled by a ring of paraffin, under a stereomicroscope. Nematodes were identified to genus level using an Olympus© BX51-P microscope with differential interference contrast optics equipped with an Olympus© DP-23 camera.

Granulometry and biogenic elements analysis

Sediment samples were collected under and outside the canopy using the referred 20 × 20 cm quadrat. The first 2 cm of sediment within this quadrat was carefully shoveled into a plastic bag. After air-drying the sediment, granulometry parameters were analyzed using the methods of Guitián & Carballas (1976). Particle size classes applied in this study follow the classification adopted from Blott & Pye (2001), where the following categories are included: very coarse sand, coarse sand, medium sand, fine sand, very fine sand, very coarse silt, coarse to medium silt, fine to very fine silt, and clay. The software Gradistat v.8 (Blott & Pye, 2001) was used to obtain sediment parameters following the Folk & Ward method (1957). Nitrogen content of each sediment sample was obtained following the Kjeldahl method (Bradstreet, 1954) and organic carbon following Walkley & Black (1934) adapted to microplates reader, to subsequently calculate organic matter percentage.

Statistics

All analyses were performed on R software version 4.2.2 (R Core Team, 2022).

To test the first hypothesis, primarily, density of nematodes was calculated by dividing the total number of nematodes (abundance) of a sample by the sampling area (20 cm^2). Subsequently, habitat densities were compared using a one-way repeated measures ANOVA, employing the car package (Fox & Weisberg, 2019). The analysis was carried out considering the normality of data, which was verified through the shapiro.test function, and considering the site as within-subjects factor.

Richness corresponds to the total number of distinct genera and families present in a sample. For testing the second hypothesis, genera and family richness were calculated based on the identification of mounted specimens (approximately 100 specimens per sample) and compared between habitats using

one-way repeated measures ANOVA with the package car (Fox & Weisberg, 2019). The analysis considered data normality, verified through the shapiro.test function, and considered the site as within-subjects factor.

Regarding the third hypothesis, genera and family composition of the nematode community was studied. First, Ruziicka index with the adespatial package (Dray et al., 2023) was used to calculate beta diversity. This index considers the abundance of each taxon included in the analysis, not only the presence of the taxon. Second, these diversity indexes were compared to test for differences between habitats using a permutational analysis of variance (PERMANOVA) using the adonis2 function of the package vegan (Oksanen et al., 2022). This PERMANOVA was carried out using the beta composition as dependent variable, the habitat type as independent variable, and to address potential non-independence of habitat samples and to account for variability between sites, it was included the site as a random factor. SIMPER routine was performed to assess which genera and families contribute with the highest dissimilarities between habitats. To explore community evenness, Pielou's *J* was calculated for each sample using the function diversity of the package vegan (Oksanen et al., 2022).

To explore the fourth hypothesis, paired *t*-test through the function t.test was employed to examine differences between habitats with respect to the presence/absence of genera that typically possess ocelli based on the information extracted from the genus description, as they can easily get lost after fixation, and presence/absence of denticles on the buccal cavity. PERMANOVA analyses were conducted to test for variations in abundance of nematodes in each trophic group (selective, bacterial feeders (1A); non-selective deposit feeders (1B); epistrate or epigrowth (diatom) feeders (2A); predators/omnivores (2B)) (Wieser, 1953) and nematodes with different tail shape (short/rounded, clavate, conical, conico-cylindrical, elongated/filiform) between habitats. The tail classification was modified from Thistle et al. (1995) that considered clavate–conico-cylindrical as a single group. We divided this group into two, clavate and conico-cylindrical as in Maharning et al. (2023). For these PERMANOVA analyses, the Ruziicka index was used to compute beta diversity of the community, regarding the trophic group for one analysis and

the tail shape for the second one. The beta diversity regarding both aspects served as the dependent variable, with habitat type as independent variable. In addition, three PERMANOVA analyses were carried out with the beta composition of the trophic group as dependent variable and mean grain size, nitrogen content, and organic matter content as independent variables. To address potential non-independence between habitat samples and to account for variability between sites, it included the site as a random factor.

In terms of the fifth hypothesis, sediment parameters (mean grain size, organic matter, and nitrogen content) were compared between the matte and the adjacent sediment using Linear Mixed-Effects Models with the function lmer in the packages lme4 (Bates et al., 2015) and Matrix (Bates et al., 2022). Linear Mixed-Effects Models were also carried out to explore nematodes density, genera richness, presence of denticles, and abundance of nematodes with each tail shape in relation to sediment parameters. Furthermore, community composition with respect to genera, families, and buccal types was examined in relation to sediment parameters through PERMANOVA analyses. In these analyses, the Ruziicka index was utilized to calculate beta diversity of the community concerning its composition in families, genera, and the abundance of nematodes within each trophic group. The beta diversity related to these aspects served as the dependent variable. Each of these parameters underwent a separate PERMANOVA against mean grain size, organic matter content, and nitrogen content as explanatory variables. To account for potential non-independence among samples and variability between sites, the site was incorporated as a random factor in each analysis.

Principal Component Analysis (PCA) from the vegan package was conducted to visualize community structure of genera composition between sites: genera composition in relation to sediment parameters; community composition according to nematodes buccal type; and community composition regarding nematode tail shape and its relationship with sediment parameters. Moreover, to visualize the differential genera community composition within habitats, data were represented with a heatmap using reshape2 (Wickham, 2007) and ggplot2 (Wickham, 2016) packages.

Results

Nematoda community composition, density, genera, and family richness between the three main habitats: leaves, matte, and adjacent sediment

The study of the community composition of nematode families and genera produced similar results. Analysis based on families showed significant differences between habitats (PERMANOVA: $R^2 = 0.63141$; $P = 0.005$**), as did the analysis considering nematode genera (PERMANOVA: $R^2 = 0.51922$; $P = 0.005$**, Fig. 2). In line with Novak (1989), the family Chromadoridae dominated in the leaves (81% of all nematodes in this habitat). However, this family was poorly represented in the matte and adjacent sediment (8% and 9%, respectively, Table S1). The genus *Chromadora* accounted for most of the presence of this family, constituting 70% of the community in the leaves and only 1% and 3% in the matte and adjacent sediment, respectively. PCA clearly illustrates the association of the genus *Chromadora* with leaf samples (Fig. 3A). The family Desmodoridae also showed differential relevance among the three habitats, with the highest numbers appearing in the matte (40%), less representation outside the canopy (21%), and minimal presence in the leaves (2%). In our study, *Perspiria* and *Bolbonema* were the main representatives of the latter family. *Perspiria*, as represented in the PCA (Fig. 3A), showed different distribution between the matte (15%) and the remaining habitats (2% outside the canopy and no presence on the leaves); similarly, *Bolbonema* showed the highest abundance on the sediment habitats (11% on matte and 16% on adjacent sediment), in contrast to the leaves (0.3%, see Table S2 and Fig. 3A). The highest abundance of the family Xyalidae was found in adjacent sediment samples (16%), compared to the low representation in matte (2%) and in leaves (0.3%). Moreover, the family Oncholaimidae was best represented in the adjacent sediment habitat (20%), less abundant in the leaves (12%), and poorly present in the matte (6%).

Nematode density did not significantly differ between habitats (ANOVA: $F = 1.037$, $P = 0.314$, Fig. 4A). However, there were differences in genera and family richness between habitats (ANOVA for genera richness: $F = 10.303$, $P = 0.026$*; ANOVA for family richness: $F = 14$, $P = 0.016$*). The richest
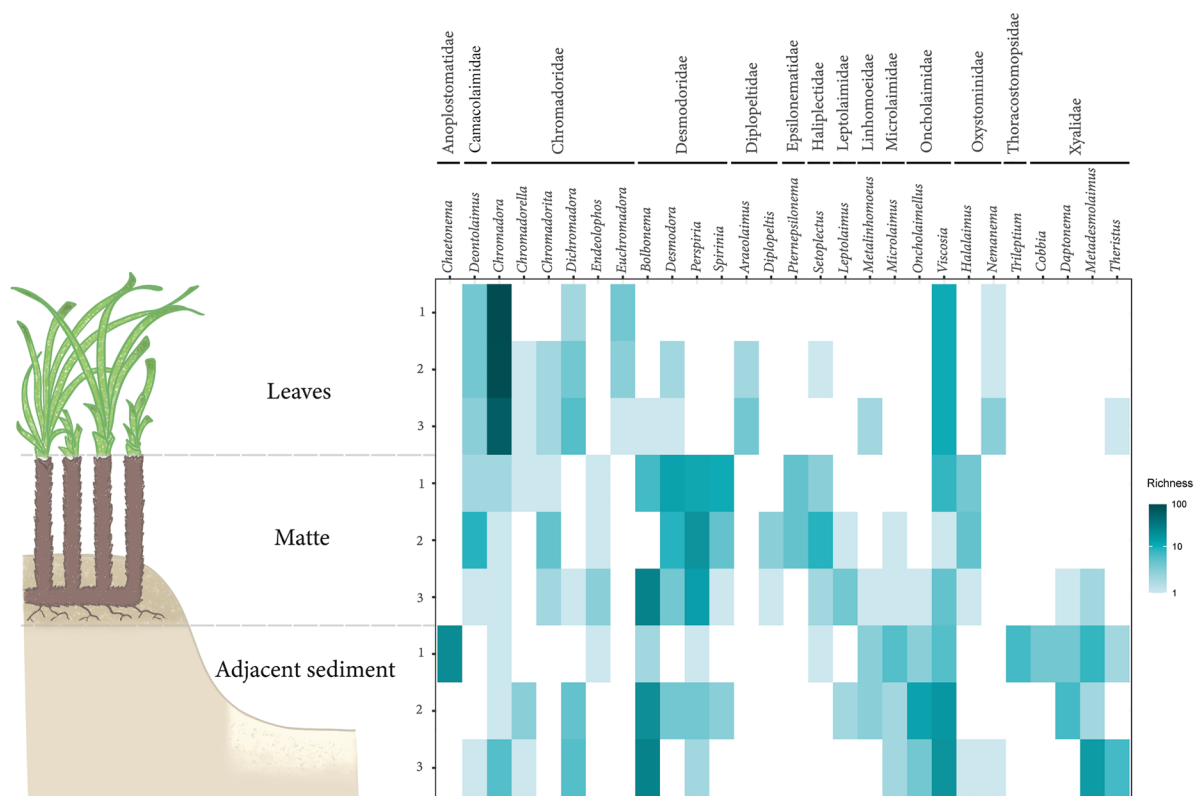
Fig. 2 Panels showing the number of specimens per genus from leaves, matte and adjacent sediment samples. Intensity of the color increases according to the number of presences. Genera that had the greatest impact on dissimilarity were included

habitat regarding genera was the matte under the canopy, although the significance was marginal when compared to the adjacent sediment (*t*-test: $P = 0.058$), and not significant when compared to the leaves (*t*-test: $P = 0.067$, Fig. 4B). Furthermore, matte family richness was significantly higher than in adjacent sediment (*t*-test: $P = 0.0067$**) and leaves (*t*-test: $P = 0.0445$*, Fig. 4C). The sediment outside the canopy showed the highest evenness of nematode genera of all the studied habitats (Pielou's *J* of 0.29, 0.30 and 0.29), followed by the matte (Pielou's *J* of 0.27, 0.28 and 0.25) and the leaves (Pielou's *J* of 0.18, 0.19 and 0.23).

Sediment parameters as community-shaping factors

Sediment nitrogen and organic matter content were compared inside and outside the canopy habitats, showing no significant differences (%nitrogen lmer: *t*-value $= 0.883$; $P = 0.43$; %organic matter lmer:

t-value $= 1.882$; $P = 0.133$). Nonetheless, our analyses demonstrated that the matte had a significantly smaller mean grain size than the adjacent sediment (lmer: *t*-value $= -4.354$; $P = 0.0489$*, Fig. 5).

The Linear Mixed-Effects Model revealed no significant differences in nematode density associated with mean grain size (lmer: *t*-value $= -0.639$; $P = 0.593$), the percentage of organic matter (lmer: *t*-value $= -0.264$; $P = 0.8049$), or nitrogen content (lmer: *t*-value $= -0.370$; $P = 0.7302$). However, genera richness significantly decreased as mean grain size increased (lmer: *t*-value $= -19.384$; $P = 0.0026$**) but remained unaffected by the other sediment parameters included in the study (lmer: organic matter: *t*-value $= -0.629$; $P = 0.5634$; nitrogen: *t*-value $= -0.661$; $P = 0.5448$).

When comparing matte and adjacent sediment, the community composition of nematode genera was significantly affected by sediment organic
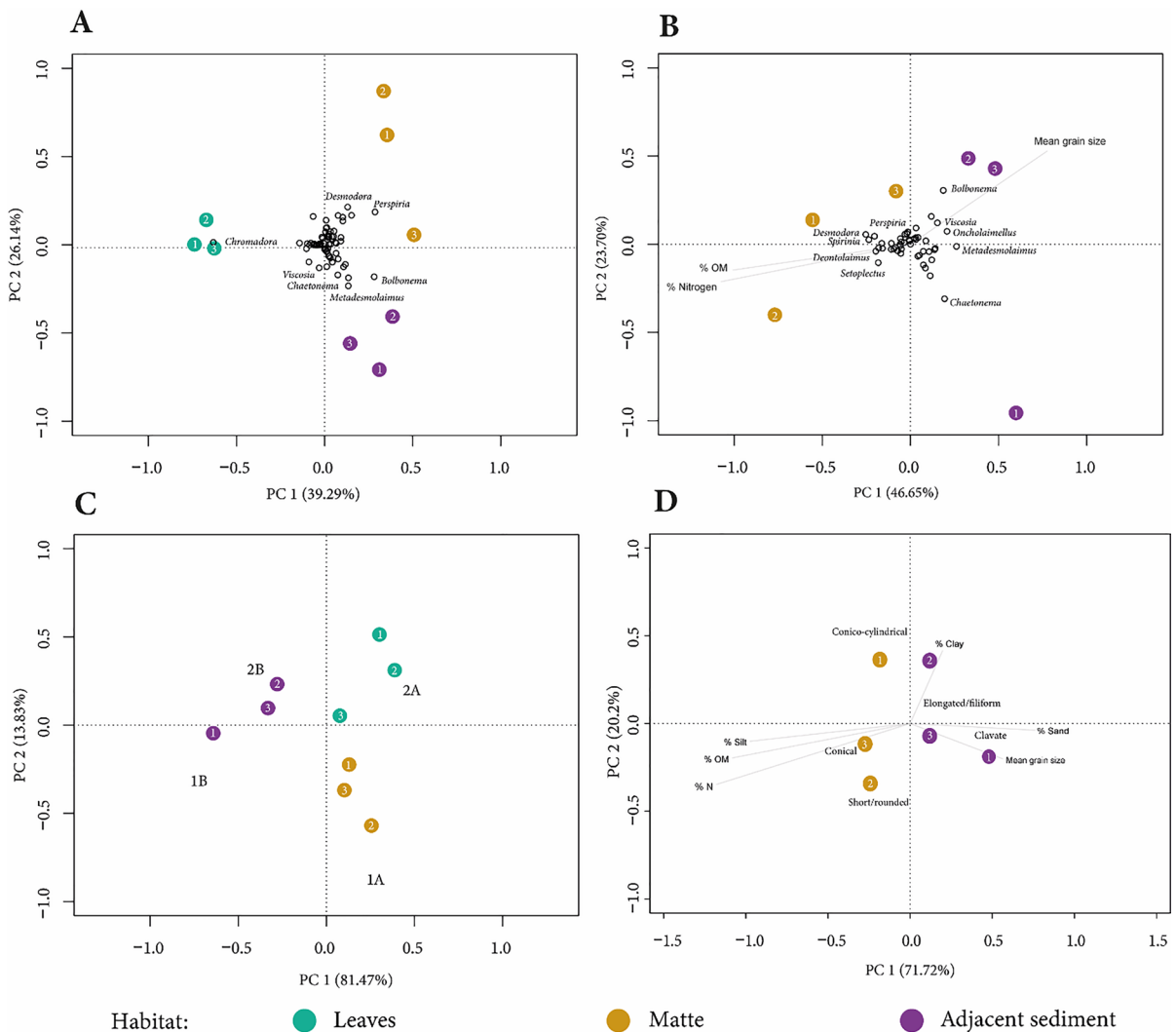
Fig. 3 Principal Component Analysis (PCA) representing **A** Nematoda community composition according to genera, **B** genera community composition in relation to sediment param- eters, **C** community composition according to buccal type, and **D** community composition according to tail shape and sedi- ment parameters; between leaves, matte, and adjacent sediment

matter content (PERMANOVA: $R^2 = 0.30658$; $P = 0.0403*$) and nitrogen content (PERMANOVA: $R^2 = 0.32495$; $P = 0.0097**$), with a marginal effect from mean grain size (PERMANOVA: $R^2 = 0.27326$; $P = 0.0569$, Fig. 3B). In contrast, the community composition of nematode fami- lies was not significantly affected by any of the sediment parameters (PERMANOVA: mean grain size: $R^2 = 0.14623$; $P = 0.5514$; organic matter: $R^2 = 0.26317$; $P = 0.2736$; nitrogen: $R^2 = 0.29447$; $P = 0.1535$).

Functional traits and habitat characteristics

Buccal types

The composition of the community was explored according to this trait, revealing significant dif- ferences between habitats (PERMANOVA: $R^2 = 0.73627$; $P = 0.005**$). Epistratum feeders (2A) were the most abundant nematodes in all three habi- tats, constituting 82% of the community in the leaves, 67% in the matte, and 40% in the adjacent sediment. The most significant dissimilarity between leaf and
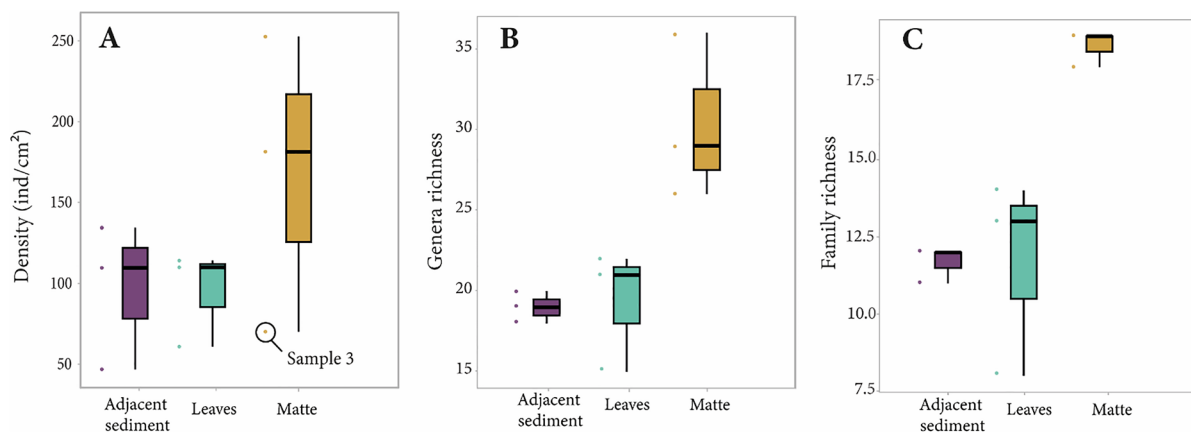
Fig. 4 Boxplots showing **A** Nematoda density (ind/cm^2), **B** genera, and **C** family richness between habitats. Sample 3 is highlighted to show its dissimilarity with the rest of samples of the matte habitat regarding nematodes density

matte habitats was observed in selective feeders (1A), whose abundance ranged from 5% in the leaves to 20% in the matte. The adjacent sediment community showed the highest evenness according to the buccal types, hosting 40% of epistratum feeders (2A), 30% of non-selective deposit feeders (1B), 27% of predators/omnivores (2B), and the least represented, 3% of selective feeders (1A) (Table S3; Fig. 6).

When analyzing nematode buccal types in relation to sediment characteristics, none of the sediment parameters, including mean grain size (PERMANOVA: $R^2 = 0.24562$; $P = 0.3431$), organic matter (PERMANOVA: $R^2 = 0.3618$; $P = 0.8$), or nitrogen content (PERMANOVA: $R^2 = 0.42513$; $P = 0.0653$), appeared to significantly affect the community (Fig. 3C).

Tail

Analyses show significant differences in the composition of nematode tail shapes between habitats (PERMANOVA: $R^2 = 0.70581$; $P = 0.005**$). The most abundant type of tail was the conical one, contributing with the highest dissimilarities between the three habitats. Conical tail constituted the 86% of the community in the leaves, 56% in matte, and 35% in adjacent sediment. The clavate tail was most prevalent outside the canopy (31%), differentiating it from the two other habitats (leaves = 16%; matte = 16%). Likewise, the elongated/filiform tail reached its highest abundance in the adjacent sediment, comprising 13% of the community, and only 3% and 7% in the leaves

and matte, respectively. Nematodes with conico-cylindrical and short/rounded tails maintained similar abundances (6, 7, and 9% for conico-cylindrical and 2, 1, and 0.3% for short/rounded) between habitats, as did nematodes with short/rounded tails (Table S4).

When analyzing the effect of sediment mean grain size, sand, silt, and clay proportions on the relative abundance of nematodes with each tail shape (Table 1), the only significant differences were found in the nematodes with elongated/filiform tails (Fig. 7). This type of tail is positively correlated to increasing mean grain size (lmer: t-value $= 5.095$; $P = 0.0276*$) as well as increasing clay percentage (lmer: t-value $= 2.830$; $P = 0.0474*$, Fig. 3D).

Ocelli

Nematodes that typically have or may present ocelli were represented in our samples by the following genera: *Araeolaimus, Axonolaimus, Bolbonema, Chaetonema, Chromadora, Chromadorella, Diplopeltis, Eurystomina, Leptosomatum, Onchium, Phanoderma,* and *Tricoma.*

The 70% of nematodes of the leaves belonged to genera mentioned above. This percentage significantly differed from the 18% of ocellated nematodes from the matte (paired t-test: $t = 5.6723$; $P = 0.0297*$) and the 30% from outside *Posidonia* bushes (paired t-test: $t = -6.776$; $P = 0.0211*$, Fig. 8A). There were no significant differences in the abundance of nematodes with ocelli between matte and adjacent sediment habitats (paired t-test: $t = 3.874$; $P = 0.0606$).
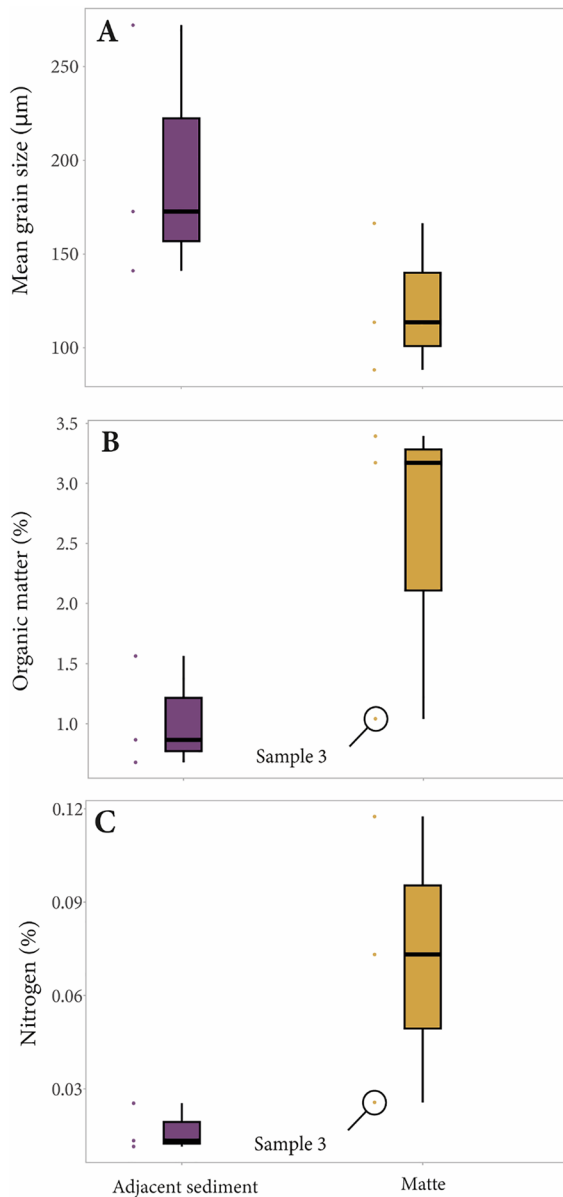
Fig. 5 Boxplots showing sediment **A** mean grain size (μm), **B** organic matter (%) and **C** nitrogen (%) of the sediment outside and inside the canopy. Sample 3 is highlighted to show its dissimilarity with the rest of samples of the matte habitat regarding organic matter and nitrogen content

Denticles

Nematodes that present denticles in their buccal armature are represented in our samples by the following genera: *Actinonema, Chromadora, Chromadorita, Cyatholaimus, Dichromadora, Euchromadora,*
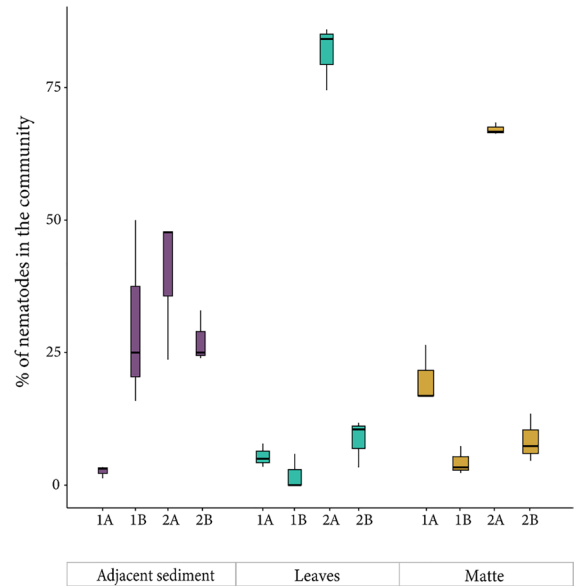
Fig. 6 Boxplots showing percentage of nematodes with different buccal types according to Wieser ([1953](#)): selective (bacterial) feeders (1A), non-selective deposit feeders (1B), epistrate or epigrowth (diatom) feeders (2A), and predators/omnivores (2B) in the community per habitat

Eurystomina, Longicyatholaimus, Metachromadora, Paramarylynnia, Pomponema, Preacanthonchus, Rhips, and *Spilophorella.*

The 72% of nematodes from the leaf community had denticles in their buccal cavity. This habitat had a significant higher relative density of nematodes provided with denticles than matte (paired *t*-test: $t = 9.3978$; $P = 0.0111^*$) and adjacent sediment (paired *t*-test: $t = -9.9015$; $P = 0.0101^*$), but no significant difference was observed between the last two (paired *t*-test: $t = -0.75056$; $P = 0.5312$, Fig. [8](#)B). This functional trait did not seem to be significantly affected by the organic matter content when comparing the two sedimentary habitats, matte, and adjacent sediment (lmer: *t*-value $= 1.992$; $P = 0.1841$).

Discussion

Nematode density is not significantly different between habitats

No significant differences in nematode density were observed between the studied habitats, although we

Table 1 lmer results relating sediment mean grain size, sand, silt, and clay proportions to the relative abundance of nematodes with each tail shape

	Mean grain size		Sand (%)		Silt (%)		Clay (%)	
	t-value	P	t-value	P	t-value	P	t-value	P
Elongated/filiform	5.095	0.0276*	− 0.056	0.958	− 0.476	0.6591	2.830	0.0474*
Clavate	0.881	0.428	1.048	0.354	− 1.480	0.213	0.521	0.630
Conical	− 2.879	0.0913	− 0.722	0.510	0.967	0.3884	− 0.376	0.7259
Conico-cylindrical	− 0.285	0.789	− 0.221	0.836	0.330	0.7579	− 0.257	0.810
Short/rounded	0.229	0.830	− 1.025	0.406	1.204	0.336	0.305	0.776

expected the matte to host a higher density than the adjacent sediment. It is worth noting that one of the matte samples (sample 3) showed a significant deviation from the other two samples in terms of biogenic element concentrations. This particular sample exhibited significantly lower levels of organic matter and nitrogen compared to the other sampled mattes (Fig. 5, see the surrounded sample). Nematode density is strongly influenced by nutrient concentration (Moens & Vincx, 1997), and, as such, the atypical parameters of this sample may be a possible reason why we did not find differences between habitats. This is in contrast to the rest of the matte samples, which were characterized by higher levels of organic carbon and nitrogen than the adjacent sediment samples.

Habitat heterogeneity and food specificity shape the community

The matte within the phanerogam canopy had the highest family richness but not the highest genera richness. This habitat was dominated by nematodes from the Desmodoridae family (40%), specifically *Perspiria* (15%), *Bolbonema* (11%), and *Desmodora* (9%). These results align with Novak (1989), where Desmodoridae was the most abundant family in the matte habitat in September. Desmodorids are frequently reported in enriched estuarine sediments with low oxygen levels, although tolerance to anoxic conditions may be partly species specific and/or context specific (Moens et al., 2013). The abundance of this family in the matte habitat, with high organic matter concentration, would support the idea of desmodorids inhabiting enriched sediments. Most desmodorids in this study samples have conical tails, except for *Spirinia*, which have a conico-cylindrical shape. This is in tune with the fact that nematodes with a conical tail constitute the highest percentage in the matte

(56%). In addition, the sediment under the canopy constitutes the habitat with the highest abundance of selective (bacterial) feeding nematodes (1A buccal type; Fig. 6). The constant organic matter flow from the canopy to the matte sediment likely promotes the growth of organic matter-degrading bacteria, providing an abundant food source for nematodes with such a feeding strategy. Furthermore, this habitat had the lowest abundance of nematodes with ocelli (18%). Since the matte is the darkest habitat under the leaves, photoreceptors seem a less useful adaptation in this environment and, therefore, could explain its low abundance.

The leaf habitat exhibited moderate genera richness and an uneven distribution of abundances among taxa. This habitat was dominated by the family Chromadoridae, composing the 80% of the community and mostly represented by the genus *Chromadora*. This habitat is also characterized by the high occurrence of specimens with denticles (72%) and conical tails (86%). Denticles are known to be used for scraping off bacteria or microalgae from substrata (Moens et al., 2013). During summertime, diatom populations and biofilms covering *Posidonia* leaves reach their maximum development (Mabrouk et al., 2011), providing a plentiful food source for epistratum feeding nematodes (buccal type 2A; Fig. 6). These nematodes use teeth to scrape diatoms from the surface. In addition, the leaf habitat hosted by far the highest percentage of nematodes with ocelli, likely an adaptation to the well-lighted conditions on *P. oceanica* leaves.

The adjacent sediment outside *Posidonia's* bushes had the lowest nematode richness and the most uniform community due to the lower dominance of specific genera compared to the leaves and matte. Similar to the matte habitat, the most abundant family in the adjacent sediment was Desmodoridae (21%), mainly represented by the genus *Bolbonema* (16%). In contrast to the other two habitats, the adjacent sediment
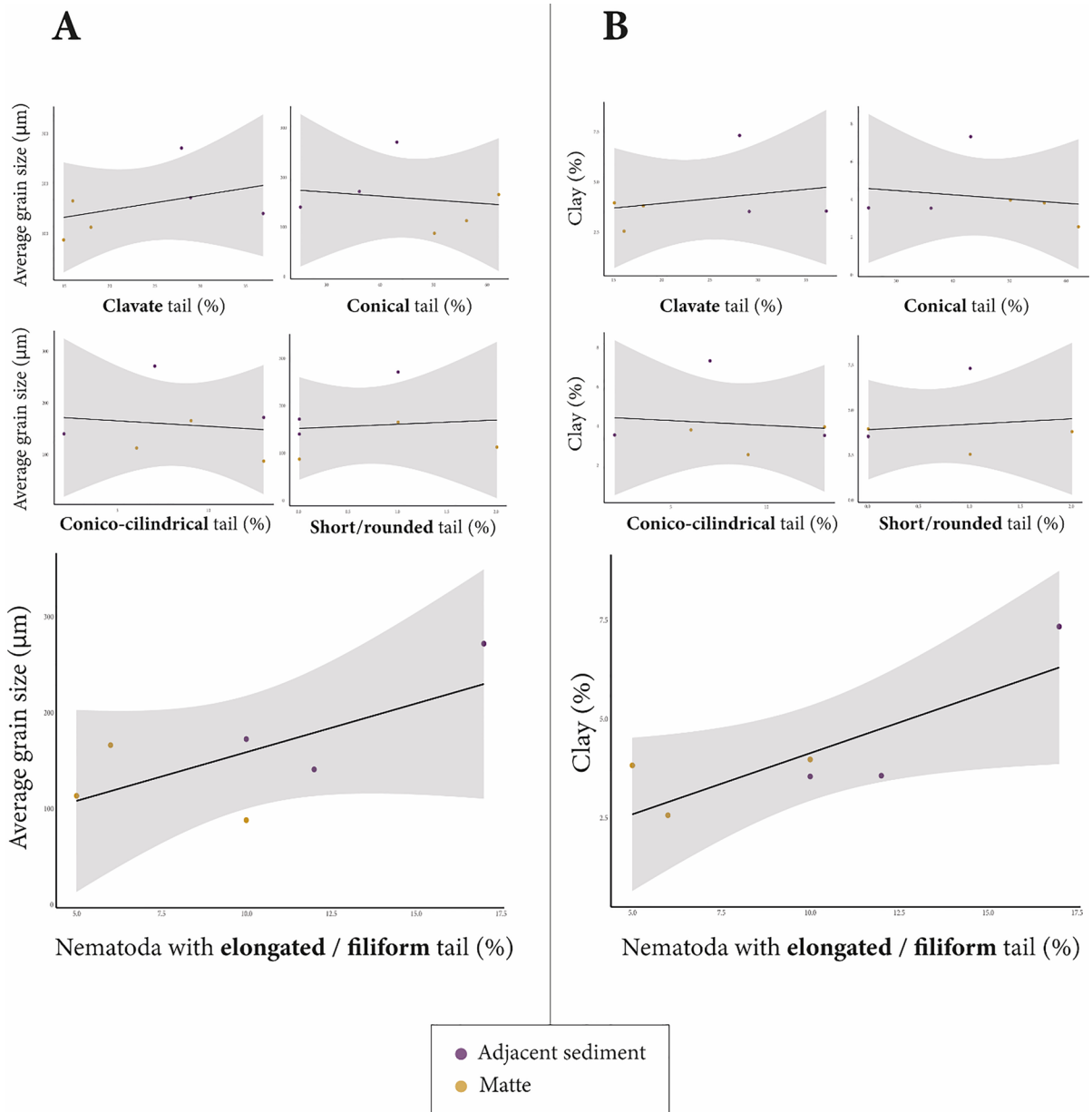
Fig. 7 Linear correlation between the five types of tail shape (clavate, conical, conico-cylindrical, short/rounded, and elongated/filiform) and the sediment **A** mean grain size and **B** percentage of clay

had a relatively high presence of the Oncholaimidae (20%) and Xyalidae (16%) families. According to Moens et al. (2013), the Xyalidae family may have developed adaptations to cope with physical disturbances (wave actions) and/or low resource availability. This fact would explain their relatively higher abundance outside the canopy where the lack of vegetation exposes the sediment to greater hydrodynamics and lower organic matter content (at least in two of our samples). On the other hand, the Oncholaimidae family is composed by nematodes that are omnivores or facultative predators (Moens et al., 2013) that could leverage whatever food resources are available and survive the unfavorable conditions

Fig. 8 Boxplots showing the proportion of nematodes with **A** ocelli and **B** denticles per habitat
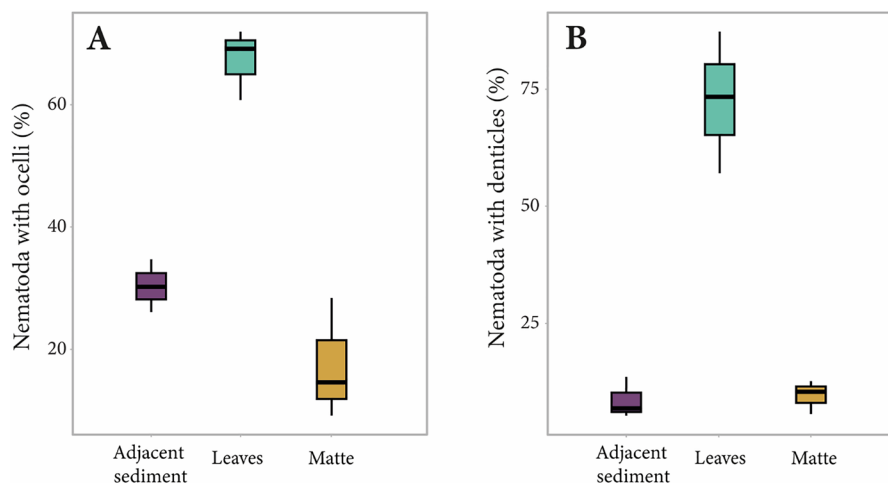
of the adjacent sediment habitat. Moreover, the nematodes within this family have a good dispersal capacity (Lorenzen et al., 1987; Prein, 1988), which may also explain their prevalence on this exposed substrates (Moens et al., 2013). The abundance of nematodes with elongated/filiform tails appears to be linked to hydrodynamic and sedimentary habitats, as they are most abundant in the adjacent sediment, which combines both characteristics. Lastly, a greater number of nematodes from outside the canopy had ocelli (30%) compared to the matte. Although both are sedimentary habitats, the adjacent sediment is more exposed to direct daylight, where photoreceptors may be more useful.

Sediment granulometry and biogenic elements concentration affect the community

Comparing the two sedimentary habitats, our results show a decrease in genera richness as mean grain size increases. This contradicts the pattern highlighted in numerous investigations (see Heip and Decraemer, 1974; Tietjen, 1977; Heip et al., 1985, 1992; Steyaert et al., 1999; Vanaverbeke et al., 2002, 2011) which relate increased diversity to coarser, more permeable sediments (Vanaverbeke et al., 2011). The composition of the nematode community in our samples is influenced by nitrogen and organic matter content and tends to change with mean grain size, consistent with previous studies (Vincx et al., 1990; Schratzberger et al., 2006, 2008a, 2008b). According to our results, genera such as *Bolbonema*, *Viscosia* and *Oncholaimellus* seem to prefer coarser sediment

grain size, while *Deontolaimus* and *Setoplectus* may be associated with high nitrogen and organic matter content (Fig. 3B). Nematodes with elongated/filiform tails are positively correlated with increasing clay percentage in our samples. On the other hand, Schratzberger et al. (2007) directly correlated nematodes with elongated/filiform tail abundance with finest sediments (but not differing between silt and clay categories). However, our samples also demonstrate the preference of nematodes with this type of tail toward coarser grain sizes. This suggests that the higher abundance of nematodes with elongated/filiform tails is not solely associated with fine sediments but may be specifically linked to the clay fraction. Riemann (1974) proposed that this tail type could be an adaptation to sand and muddy sediments where only an incomplete interstitial system exists. These elongated/filiform tails would allow the animals to retract from dead-end interstitial passageways that are too narrow to turn around and escape.

Conclusions

The habitat heterogeneity within *P. oceanica* meadows appears to influence the Nematoda community regarding richness and community composition, albeit not impacting its density. The richest habitat in terms of nematode genera and families was the matte, suggesting that habitat heterogeneity leads to a more diverse community. In line with the third hypothesis, the family Chromadoridae dominating the leaves are epistratum feeders (2A) that exploit

the abundant food resources provided by diatoms and biofilms covering the leaves of this phanerogam during the summer season. Noteworthy differences in nematode functional traits were observed among habitats. Specifically, epistratum feeders (2A) were the most abundant type of nematodes in all habitats, but particularly in the leaves. In addition, nematodes with denticles in their buccal cavity were also more prevalent on the leaves, where this structure could be employed to scrap diatoms and biofilms attached to leaf surface. Nematodes with ocelli were notably more represented on the leaves, given the greater sun exposure in this habitat. Concerning the fifth hypothesis, our results indicate that the composition of the nematode community in our samples is influenced by nitrogen and organic matter contents and tends to vary with mean grain size. More precisely, coarser grain size negatively affects genera richness but positively influences the presence of nematodes with elongated/filiform tails. Nematodes with elongated/filiform tails were also associated with the hydrodynamic adjacent sediment habitat and a higher proportion of sediment clay. Future studies could explore seasonal variations in *P. oceanica* nematode communities and investigate how the life cycle of the phanerogam, and beach hydrodynamics impact the community.

Declarations

References

Albano, P. G. & M. Stockinger, 2019. The rhizome layer of *Posidonia oceanica*: an important habitat for Mediterranean brachiopods. Marine Biodiversity 49(5): 2467–2472. https://doi.org/10.1007/s12526-019-00968-6.

Bates, D., M. Maechler, B. Bolker & S. Walker, 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67(1): 1–48.

Bates, D., M. Maechler & M. Jagan, 2022. Matrix: Sparse and Dense Matrix Classes and Methods. R Package Version 1.5-1 [available on internet at https://CRAN.R-project.org/package=Matrix]. Accessed 20 April 2023.

Bianchelli, S., C. Gambi, M. Mea, A. Pusceddu & R. Danovaro, 2013. Nematode diversity patterns at different spatial scales in bathyal sediments of the Mediterranean Sea. Biogeosciences 10(8): 5465–5479. https://doi.org/10.5194/bg-10-5465-2013.

Blanc, J. J. & A. J. D. Grissac, 1984. Erosions sous-marines des herbiers à *Posidonia oceanica* (Méditerranee). In International Workshop on *Posidonia oceanica* Beds, 1984. GIS Posidonie, Marseille: 23–28.

Blott, S. J. & K. Pye, 2001. GRADISTAT: a grain size distribution and statistics package for the analysis of unconsolidated sediments. Earth Surface Processes and Landforms 26(11): 1237–1248. https://doi.org/10.1002/esp.261.

Borg, J. A., A. A. Rowden, M. J. Attrill, P. J. Schembri & M. B. Jones, 2006. Wanted dead or alive: high diversity of macroinvertebrates associated with living and 'dead'

Posidonia oceanica matte. Marine Biology 149: 667–677. https://doi.org/10.1007/s00227-006-0250-3.

Boström, C., M. Benson & C. A. Simenstad, 2006. Seagrass landscapes and their effects on associated fauna: a review. Estuarine, Coastal and Shelf Science 68(3–4): 383–403. https://doi.org/10.1016/j.ecss.2006.01.026.

Bourcier, M., 1989. Regression des herbiers à *Posidonia oceanica* (L.) Delile, a l'Est de Marseille, sous l'action conjuguée des activités humaines et des modifications climatiques. In International Workshop on *Posidonia* Beds, Vol. 2. GIS Posidonie: 287–292.

Bradstreet, R. B., 1954. Kjeldahl method for organic nitrogen. Analytical Chemistry 26(1): 185–187. https://doi.org/10.1021/ac60085a028.

Cetinić, P., I. Jardas, J. Dulčić, A. Pallaoro, M. Kraljević & A. Soldo, 1999. Effects of the "migavica" beach seine on coastal fish communities. Folia Universitatis Agriculturae Stetinensis 192: 25–35 [available on internet at https://bib.irb.hr/prikazi-rad?&rad=44703].

Cetinić, P., F. Škeljo & J. Ferri, 2011. Discards of the commercial boat seine fisheries on *Posidonia oceanica* beds in the eastern Adriatic Sea. Scientia Marina 75: 289–300. https://doi.org/10.3989/scimar.2011.75n2289.

Cvitkovic, I., M. Despalatovic, A. Žuljevic, S. Matijevic, D. Bogner, J. Lušić & A. Travizi, 2017. Structure of epibiontic and sediment meiofauna in the area invaded by invasive alga *Caulerpa taxifolia*. Marine Biology 164(1): 1–15. https://doi.org/10.1007/s00227-016-3034-4.

Dray, S., D. Bauman, G. Blanchet, D. Borcard, S. Clappe, G. Guénard, T. Jombart, G. Larocque, P. Legendre, N. Madi & H.H. Wagner, 2023. adespatial: Multivariate Multiscale Spatial Analysis. R Package Version 0.3-21 [available on internet at https://CRAN.R-project.org/package=adespatial]

Folk, R. L. & W. G. Ward, 1957. Brazos River bar [Texas]; a study in the significance of grain size parameters. Journal of Sedimentary Research 27(1): 3–26. https://doi.org/10.1306/74d70646-2b21-11d7-8648000102c1865d.

Fox, J. & S. Weisberg, 2019. An R Companion to Applied Regression, 3rd edn. SAGE, Thousand Oaks [available on internet at https://socialsciences.mcmaster.ca/jfox/Books/Companion/].

Gacia, E. & C. M. Duarte, 2001. Sediment retention by a Mediterranean *Posidonia oceanica* meadow: the balance between deposition and resuspension. Estuarine, Coastal and Shelf Science 52(4): 505–514. https://doi.org/10.1006/ecss.2000.0753.

García-Gómez, G., A. García-Herrero, N. Sánchez, F. Pardos, A. Izquierdo-Muñoz, D. Fontaneto & A. Martínez, 2022. Meiofauna is an important, yet often overlooked, component of biodiversity in the ecosystem formed by *Posidonia oceanica*. Invertebrate Biology 141(2): e12377. https://doi.org/10.1111/ivb.12377.

Giere, O., 2009. Meiobenthology. In The Microscopic Motile Fauna of Aquatic Sediments, 2nd edn. Springer, Berlin. https://doi.org/10.5860/choice.46-5018.

Guitián, F., & T. Carballas, 1976. Técnicas de análisis de suelos. Pico Sacro. ISBN 84-85170-09-1.

Heip, C. & W. Decraemer, 1974. The diversity of nematode communities in the Southern North Sea. Journal of the Marine Biological Association of the United Kingdom 54(1): 251–255. https://doi.org/10.1017/s0025315400022189.

Heip, C., M. Vincx & G. Vranken, 1985. The ecology of marine nematodes. Oceanography and Marine Biology 23: 399–489 [available on internet at https://www.marinespecies.org/imis.php?module=ref&refid=3205].

Heip, C., D. Basford, J. A. Craeymeersch, J. M. Dewarumez, J. Dörjes, P. de Wilde, G. Duineveld, A. Eleftheriou, P. M. J. Herman, U. Niermann, P. Kingston, A. Künitzer, E. Rachor, H. Rumohr, K. Soetaert & T. Soltwedel, 1992. Trends in biomass, density and diversity of North Sea macrofauna. ICES Journal of Marine Science 49: 13–22. https://doi.org/10.1093/icesjms/49.1.13.

Higgins, R. P. & H. Thiel, 1988. Introduction to the Study of Meiofauna, Smithsonian Institution Press, Washington, DC: https://doi.org/10.2307/3226350.

Lorenzen, S., M. Prein & C. Valentin, 1987. Mass aggregations of the free-living marine nematode *Pontonema vulgare* (Oncholaimidae) in organically polluted fjords. Marine Ecology Progress Series 37: 27–34. https://doi.org/10.3354/meps037027.

Mabrouk, L., A. Hamza, M. B. Brahim & M. Bradai, 2011. Temporal and depth distribution of microepiphytes on *Posidonia oceanica* (L.) Delile leaves in a meadow off Tunisia. Marine Ecology 32(2): 148–161. https://doi.org/10.1111/j.1439-0485.2011.00432.x.

Maharning, A. R., E. R. Ardli & R. E. Prabowo, 2023. Nematode Community as a tool to monitor ecosystem Health of Kembangkuning Mangrove Forest, Indonesia. IOP Conference Series 1155(1): 012009. https://doi.org/10.1088/1755-1315/1155/1/012009.

Manca, E., I. Cáceres, J. Alsina, V. Stratigaki, I. Townend & C. L. Amos, 2012. Wave energy and wave-induced flow reduction by full-scale model *Posidonia oceanica* seagrass. Continental Shelf Research 50–51: 100–116. https://doi.org/10.1016/j.csr.2012.10.008.

Martínez, A., G. García-Gómez, Á. García-Herrero, N. Sánchez, F. Pardos, A. Izquierdo-Muñoz, D. Fontaneto & S. Mammola, 2021. Habitat differences filter functional diversity of low dispersive microscopic animals (Acari, Halacaridae). Hydrobiologia 848: 2681–2698. https://doi.org/10.1007/s10750-021-04586-x.

Mazzella, L., M. B. Scipione & M.-C. Buia, 1989. Spatiotemporal distribution of algal and animal communities in a *Posidonia oceanica* meadow. Marine Ecology 10(2): 107–129. https://doi.org/10.1111/j.1439-0485.1989.tb00069.x.

Moens, T. & M. Vincx, 1997. Observations on the feeding ecology of estuarine nematodes. Journal of the Marine Biological Association of the United Kingdom 77(1): 211–227. https://doi.org/10.1017/s0025315400033889.

Moens, T., U. Braeckman, S. Derycke, G. Fonseca, F. Gallucci, R. Gingold, K. Guilini, J. Ingels, D. Leduc, J. Vanaverbeke, C. Van Colen, A. Vanreusel & M. Vincx, 2013. 3. Ecology of free-living marine nematodes. En De Gruyter eBooks: 109–152. https://doi.org/10.1515/9783110274257.109.

Novak, R., 1982. Spatial and seasonal distribution of the meiofauna in the seagrass *Posidonia oceanica*. Netherlands Journal of Sea Research 16: 380–388. https://doi.org/10.1016/0077-7579(82)90044-8.

Novak, R., 1989. Ecology of nematodes in the Mediterranean seagrass *Posidonia oceanica* (L.) Delile 1. General part and faunistics of the nematode community. Marine Ecology 10(4): 335–363. https://doi.org/10.1111/j.1439-0485.1989.tb00077.x.

Oksanen, J., G. Simpson, F. Blanchet, R. Kindt, P. Legendre, P. Minchin, R. O'Hara, P. Solymos, M. Stevens, E. Szoecs, H. Wagner, M. Barbour, M. Bedward, B. Bolker, D. Borcard, G. Carvalho, M. Chirico, M. De Caceres, S. Durand, H. Evangelista, R. FitzJohn, M. Friendly, B. Furneaux, G. Hannigan, M. Hill, L. Lahti, D. McGlinn, M. Ouellette, E. Ribeiro Cunha, T. Smith, A. Stier, C. Ter Braak & J. Weedon, 2022. vegan: Community Ecology Package. R Package Version 2.6-4 [available on internet at https://CRAN.R-project.org/package=vegan]. Accessed 20 April 2023.

Perès, J., 1984. La regression des herbiers à *Posidonia oceánica*. In International Workshop *Posidonia oceanica* Beds, Vol. 1. GIS Posidonie, Marseille: 445–454.

Prein, M., 1988. Evidence for a scavenging life-style in the free-living nematode *Pontonema vulgare* (Enoplida: Oncholaimidae). Kieler Meeresforschung Soderheft 6: 389–394.

R Core Team, 2022. R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna:

Rebecchi, F., D. Zeppilli, E. Baldrighi, A. Di Cosmo, G. Polese, A. Pisaniello & J. Grall, 2022. First insights into the meiofauna community of a Maerl bed in the Bay of Brest (Brittany). Scientia Marina 86(1): e024. https://doi.org/10.3989/scimar.05230.024.

Riemann, F., 1974. On hemisessile nematodes with flagilliform tails living in marine soft bottoms and on microtubes found in deep sea sediments. Mikrofauna Des Meeresbodens 40: 1–15.

Rosli, N. M., D. Leduc, A. A. Rowden, P. K. Probert & M. R. Clark, 2018. Regional and sediment depth differences in nematode community structure greater than between habitats on the New Zealand margin: implications for vulnerability to anthropogenic disturbance. Progress in Oceanography 160: 26–52. https://doi.org/10.1016/j.pocean.2017.11.006.

Schmidt-Rhaesa, A., 2019. Introduction. In Schidt-Rhaesa, A. (ed), Guide to the Identification of Marine Meiofauna. Verlag Dr. Friederuch Pfeil, Munchen.

Schratzberger, M. & J. Ingels, 2018. Meiofauna Matters: the roles of meiofauna in benthic ecosystems. Journal of Experimental Marine Biology and Ecology 502: 12–25. https://doi.org/10.1016/j.jembe.2017.01.007.

Schratzberger, M., K. Warr & S. I. Rogers, 2006. Patterns of nematode populations in the southwestern North Sea and their link to other components of the benthic fauna. Journal of Sea Research 55: 113–127. https://doi.org/10.1016/j.seares.2005.07.002.

Schratzberger, M., K. J. Warr & S. N. Rogers, 2007. Functional diversity of nematode communities in the southwestern North Sea. Marine Environmental Research 63(4): 368–389. https://doi.org/10.1016/j.marenvres.2006.10.006.

Schratzberger, M., R. M. Forster, F. Goodsir & S. Jennings, 2008a. Nematode community dynamics over an annual production cycle in the central North Sea. Marine Environmental Research 66: 508–519. https://doi.org/10.1016/j.marenvres.2008.08.004.

Schratzberger, M., T. A. D. Maxwell, K. J. Warr, J. R. Ellis & S. I. Rogers, 2008b. Spatial variability of infaunal nematode and polychaete assemblages in two muddy subtidal habitats. Marine Biology 153(4): 621–642. https://doi.org/10.1007/s00227-007-0836-4.

Semprucci, F., L. Cesaroni, L. Guidi & M. Balsamo, 2018. Do the morphological and functional traits of free-living marine nematodes mirror taxonomical diversity? Marine Environmental Research 135: 114–122. https://doi.org/10.1016/j.marenvres.2018.02.001.

Steyaert, M., N. Garner, D. van Gansbeke & M. Vincx, 1999. Nematode communities from the North Sea: environmental controls on species diversity and vertical distribution within the sediment. Journal of the Marine Biological Association of the United Kingdom 79: 253–264. https://doi.org/10.1017/s0025315498000289.

Thistle, D., P. J. D. Lambshead & K. M. Sherman, 1995. Nematode Tail-Shape Groups Respond to Environmental Differences in the Deep Sea. HAL (Le Centre pour la Communication Scientifique Directe) [available on internet at https://hal.sorbonne-universite.fr/hal-03051534].

Tietjen, J. H., 1977. Population distribution and structure of the free-living nematodes of Long-Island Sound. Marine Biology 43: 123–136. https://doi.org/10.1007/bf00391260.

Vanaverbeke, J., T. Gheskiere, M. Steyaert & M. Vincx, 2002. Nematode assemblages from subtidal sandbanks in the Southern Bight of the North Sea: effect of small sedimentological differences. Journal of Sea Research 48: 197–207. https://doi.org/10.1016/s1385-1101(02)00165-x.

Vanaverbeke, J., B. Merckx, S. Degraer & M. Vincx, 2011. Sediment-related distribution patterns of nematodes and macrofauna: two sides of the benthic coin? Marine Environmental Research 71: 31–40. https://doi.org/10.1016/j.marenvres.2010.09.006.

Vaquer-Sunyer, R. & N. Barrientos, 2021. En Vaquer-Sunyer, R. & N. Barrientos (eds), Conselleria de Medi Ambient del Govern de les Illes Balears, & N. Marbà. Área de distribución de *Posidonia oceanica*. Informe Mar Balear [available on internet at https://informemarbalear.org/es/habitats-protegidos/imb-posidonia-oceanica-esp.pdf].

Vincx, M., P. Meire & C. Heip, 1990. The distribution of nematodes communities in the Southern Bight of the North Sea. Cahiers de Biologie Marine 31: 107–129 [available on internet at https://purews.inbo.be/ws/files/19200894/221136.pdf].

Walkley, A. & I. A. Black, 1934. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. Soil Science 37(1): 29–38. https://doi.org/10.1097/00010694-193401000-00003.

Wickham, H., 2007. Reshaping data with the reshape package. Journal of Statistical Software 21(12).

Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis, Springer, New York:

Wieser, W., 1953. Die Beziehung zwischen Mundhöhlengestalt, Ernährungsweise und Vorkommen bei freilebenden marinen Nematoden. Arkiv För Zoologie 4: 439–484.

Woodward, G., 2010. Integrative Ecology: From Molecules to Ecosystems, Academic, New York:

Zeppilli, D., J. Sarrazin, D. Leduc, P. M. Arbizu, D. Fontaneto, C. Fontanier, A. J. Gooday, R. M. Kristensen, V. N.

Ivanenko, M. T. Sørensen, A. Vanreusel, J. Thébault, M. Mea, N. Allio, T. Andro, A. L. Arvigo, J. Castrec, M. Danielo, V. Foulon, et al., 2015. Is the meiofauna a good indicator for climate change and anthropogenic impacts? Marine Biodiversity 45(3): 505–535. https://doi.org/10.1007/s12526-015-0359-z.