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The Antidepressants Amitriptyline and Paroxetine Induce Changes in the Structure and Functional Traits of Marine Nematodes

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Abstract: Increasing concentrations of the antidepressants amitriptyline and paroxetine were determined recently in marine habitats. However, their impact on marine biota is understudied, despite multiple undesirable effects they have on the environment. An important behavioral aspect that is increasingly measured following exposure to contaminants is the migration of fauna from contaminated areas. Hence, our aim was to better understand the migration pattern of marine meiobenthic fauna, but with a main focus on nematodes, following the exposure to both antidepressants, alone or in mixture. The experiment was carried out in microcosms, which comprised an uncontaminated upper and a lower contaminated compartment, where amitriptyline was added, alone or mixed with paroxetine, at concentrations of 0.4 and 40 µg L^{-1}. The overall abundance of meiobenthic groups decreased significantly following exposure to amitriptyline in both compartments, a pattern augmented by the mixture with paroxetine. The migration of nematodes towards the upper compartments of microcosms was triggered by the level of contamination with antidepressants. As such, the species *Terschellingia longicaudata* showed no significant change in abundance, suggesting tolerance to both antidepressants. On the other hand, the abundances of nematode taxa *Cyatholaimus prinzi*, *Calomicrolaimus* sp., *Calomicrolaimus honestus*, *Neochromadora* sp., *Chromadorina* sp. and *Chromadorina minor* decreased significantly following the exposure to both antidepressants, even at low concentrations. At the end of the experiment, the dominant migratory nematodes belonged to deposit-feeders and omnivores-carnivores trophic guilds, with tail shapes of e/f types and body-sizes longer than 2 mm. Such functional traits increase their mobility in sediments and the chance to move away from contaminated habitats. Moreover, the sex ratio was imbalanced in the favor of males in contaminated lower compartments with mixtures of the lowest and highest concentrations of amitriptyline and paroxetine, suggesting that these drugs also affect the hormone system. In conclusion, the exposure to the antidepressants amitriptyline and paroxetine triggered important changes within nematode communities, as changes in taxonomic composition were a result of migration and survival of tolerant taxa, but equally acting on the hormone system and leading to unbalanced sex-ratio among the residents.

Keywords: open microcosm; migratory behavior; marine free-living nematodes; antidepressants

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1. Introduction

The antidepressants comprise pharmaceuticals [1,2] used in the treatment of anxiety, as well as in obsessive and seizure disorders in humans [3]. A wide range of antidepressants

were determined in urban wastewater, surface and groundwater, as well as in aquatic organisms (references). Antidepressants are released into the aquatic habitats through human metabolism in trash, lavatories, wastewater from hospitals or pharmaceutical companies (reference). It is increasingly recognized that wastewaters are the main sources for spreading these antidepressants in freshwater habitats and carried further into marine ecosystems [4,5]. Following their disposal in marine habitats, the antidepressants act on local fauna and habitats, even at low concentrations, ranging from ng L^{-1} to µg L^{-1} [6]. It was reported that the antidepressants could alter the regulation of neurotransmitters, to disrupt homeostasis, induce anomalies in the embryonic development, sterility and hermaphroditism in animals, as well as to decrease bacterial biodiversity in aquatic habitats [6–8]. Furthermore, the deleterious effects of these antidepressants are equally reflected on marine fauna behavior [9], by disturbing their locomotion and feeding habits [10].

Three classes of widely prescribed antidepressants are recognized nowadays: (i) tricyclic antidepressants, (ii) selective inhibitors of serotonin reuptake and (iii) serotonin/norepinephrine reuptake inhibitors [4,5]. Among the tricyclic antidepressants, the amitriptyline was detected in domestic wastewaters following treatment in sewage treatment plants [11,12]. In France, for example, the amitriptyline was measured in concentrations ranging up to 768 ng g^{-1} in wastewaters [13]. The amitriptyline was identified, along with other selective inhibitors of serotonin reuptake antidepressants, such as the paroxetine, in sediments, fish, and seafood [14]. Salgado et al. [15] reported paroxetine concentrations of 39.73 µg L^{-1} in untreated wastewaters, leading to their further transport in marine coast habitats. The interactions between these antidepressants and non-target organisms are still poorly understood, requiring more in-depth studies, particularly in coastal habitats [9]. Few results on the toxicity of mixed selective inhibitors of serotonin reuptake antidepressants and tricyclic ones are available. One such study which focused on the water flea *Daphnia magna* adults [1–5] concluded that the effects of mixtures are much higher than when these antidepressants were tested separately, suggesting synergistic interactions. Similar findings were also observed on hydra embryos, following exposure for 14 days to these antidepressants [1–5].

Currently, the toxic impact of these types of emergent pollutants on marine meiobenthos is not well documented, particularly on sediment-dwelling invertebrates such as free-living nematodes, polychaetes, copepods, and amphipods (Figure 1). The nematodes comprise the dominant meiobenthic group and have a long history of being reliably used as ecological indicators, due their small size (1–5 mm), high abundance and diversity, as well as easy laboratory maintenance [16–19].

The (eco)toxicological effects of pollutants on nematodes are generally tested within closed experimental microcosms, which are usually filled with sediment and topped with aerated water, in containers such as bottles [20–30], jars [31], *Erlenmeyer flasks* [32], tubes [33], boxes [25–34] and cores [35]. Such containers are widely used due their low price and easy maintenance costs, but debatable in mimicking reliably the natural conditions. The classical microcosm devices do not allow the test animals to actually avoid the pollution source by moving away from it. Moreover, the migratory hebaviour of test animals in the presence of pollutants is an expected natural reaction, especially for mobile taxa such as the nematodes [36]. The amount of evidence supporting the migratory behaviour of meiobenthos following exposure to pollutants has mounted recently, due to the necessity of more realistic experimental devices where the natural behaviour, besides the standard toxicological end points, is also to be assessed [37,38]. Recently, the response of marine nematodes following exposure to the antidepressant paroxetine was tested in laboratory microcosms by Ishak et al. [19]. The results obtained showed significant changes in nematofauna taxonomic composition, as well as the dominance of species with certain functional traits (i.e., type of feeding groups, tail and amphid shapes, body size) that are best adapted following the exposure to this antidepressant. However, besides paroxetine, another antidepressant frequently measured in aquatic habitats is the amitriptyline (see above). We suspect that both antidepressants could act in synergy, with yet unknown effects on marine fauna. Hence, the

current study aimed to investigate the effects of amitriptyline, single or mixed with paroxetine, on the locomotion reaction of marine nematodes collected from the bay of Bizerte (northeast of Tunisia). An open experimental enclosure was used to study the locomotion reaction of nematodes and to assess their tolerance to these two antidepressants.

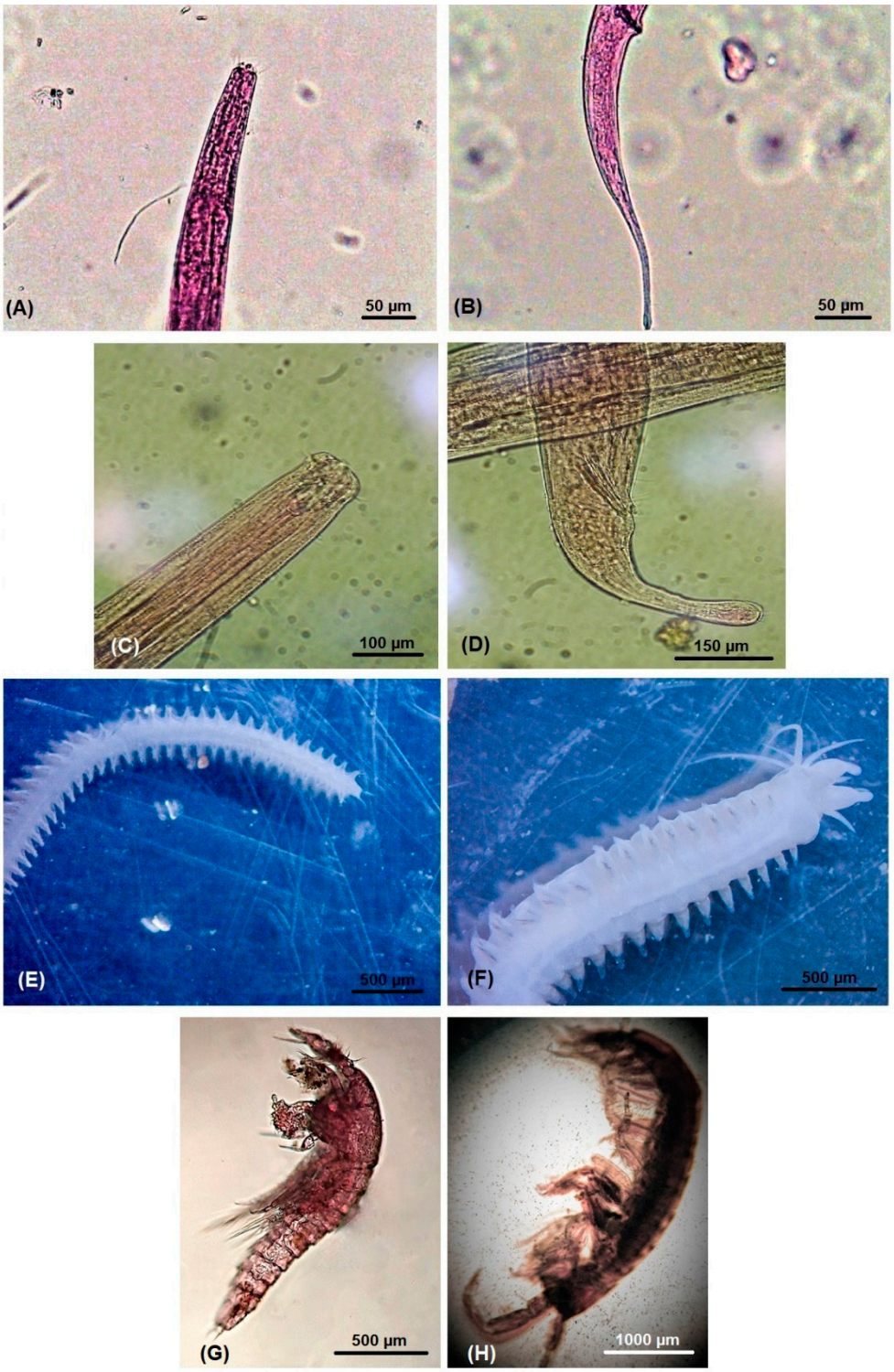

Figure 1. Photos showing the meiobenthic nematodes *Paramonohystera pilosa* (**A**,**B**) and *Oncholaimus campylocercoides* (**C**,**D**), the polychaetes (**E**,**F**), the copepods (**G**), and the amphipods (**H**).

2. Material and Methods

2.1. Sampling Site and Acclimatization

The top 5 cm sediment was sampled from the Bizerte lagoon (37°13′437″ N, 9°51′457″ E) at 50 cm water depth, on the 9 January 2021 (7 AM). This biotope of this ecosystem consists mainly of silt—clayey sediments, which is suitable to observe the natural migratory movements of nematodes over a vertical profile as opposed to sandy sediments [30]. The sand is more porous, promoting mostly the type of passive movement of nematodes within interstitial habitats [39–42]. Several hand cores (10 cm^2) were used to sample the sediment. The biotic and abiotic characteristics of the habitat were previously described by Béjaoui-Omri [43] in detail.

2.2. Experiment Set-Up

A laboratory experiment, comprising specially designed microcosms, was used (Figure 2). Each microcosm comprised 10 cm diameter polyvinyl chloride tubes, closed at both ends [19]. The main tube was separated in two equally sized compartments. The lower compartment was filled with contaminated defaunated sediments by repeated (three times) freezing (−20 °C) and thawing (12 h/48 h) [20]. The chosen antidepressants concentrations, of 0.4 and 40 µg L^{-1}, respectively, were based on the EC50/48 h of the cladoceran *Daphnia magna* [44]. The upper compartment was filled with uncontaminated natural sediments. Both compartments were isolated from each other with a thin layer of impermeable agar (Figure 2). The microcosms were kept in a controlled room, with fixed lighting (8.5 h light/15.5 h dark) and temperature (18 °C/12 °C) for three days for acclimatization, equivalent to conditions during the month before sampling (from 8 December 2020 to 8 January 2021), according to http://www.infoclimat.fr (accessed on 9 January 2021). The experiment lasted 15 days.

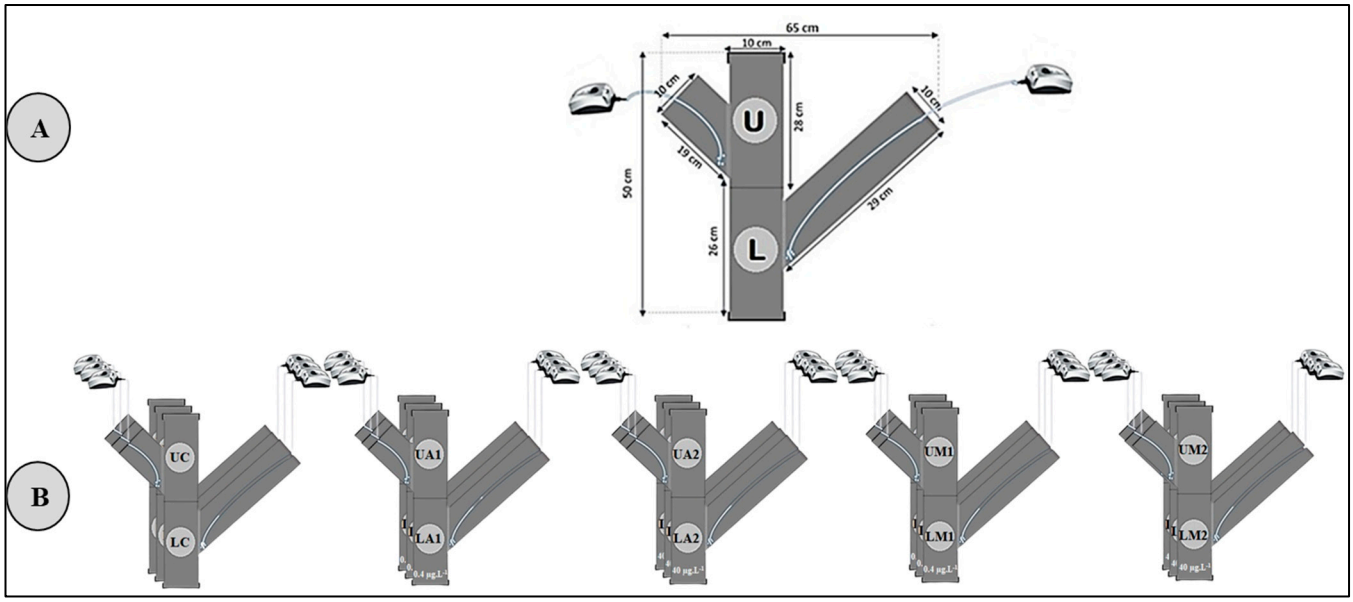

Figure 2. Experimental design (**A**) and organization (**B**) of microcosms with treatments codes: the upper compartments (U) were filled with untreated sediment, whereas the lower compartments (L) were filled with defaunated sediments contaminated with amitriptyline, alone or mixed with paroxetine. Control treatment (C); Amitriptyline (**A**: 0.4 and 40 µg L^{-1}), mixture of amitriptyline and paroxetine both at 0.4 µg L^{-1} (M1) and 40 µg L^{-1} (M2).

2.3. Sediment Contamination

The amitriptyline and paroxetine (Sigma Aldrich, St. Louis, MO, USA, 98%) were dissolved in seawater and filtered through 0.7 µm pore-size Glass Microfibre GF/F, Whatman, without using any solvent in order to prepare the stock solutions [45,46]. Each stock

solution was used separately or mixed to contaminate the defaunated sediments, and poured until the lower compartments were fully filled.

A total of 15 microcosms (i.e., three replicates per treatment) were designated. One comprised the control, with uncontaminated upper and lower compartments, noted UC and LC, respectively. Two other sets, comprising uncontaminated upper compartments, noted UA1 and UA2 and contaminated lower compartments, contaminated with the lowest and highest amitriptyline concentrations, noted LA1 and LA2, were used. Finally, two other sets, comprising uncontaminated upper compartments, noted UM1 and UM2 and contaminated lower compartments with mixture, of the lowest and highest concentrations of amitriptyline and paroxetine, noted LM1 and LM2, were also employed.

2.4. Meiofauna Study

Following the end of the experiment (i.e., 15 days), the migratory patterns of nematodes found in the lower compartments, which were azoic at the beginning, were observed. The duration of the experiment, which was 15 days, was chosen as such as to be longer than the exploratory phase, characterized by the migration of nematodes back and forth over a vertical profile in sediments, which was estimated to be 9 days by previous investigations [16]. The nematodes were extracted using the levigation-sieving technique [47] with the aid of two sieves with 1 mm and 40 µm mesh size, respectively, and stained with rose Bengal solution (0.2 $g \cdot L^{-1}$) [48]. From each compartment, 100 individuals were randomly picked under a stereomicroscope and mounted on microscopic slides [49] for taxonomic identification at species level, using the keys of Platt and Warwick [50] for genus and the Nemys database at species level [51]. In addition, the collected nematodes were subdivided according to their gender and maturity status into juveniles (hereafter J), non-gravid females (hereafter f), gravid females (hereafter fg), and males (hereafter m). The non-gravid and gravid females were merged within a single group (hereafter F) to calculate the J/F and m/F ratios. Two supplementary indices were also calculated: the relative pharyngeal lumen volume (hereafter RVPL) and the d-index according to Boufahja et al. [26].

Five additional functional and morphological traits were calculated: the amphid shape, tail shape, trophic groups, adult length, and type of life history for each genus of nematode. The amphid classification, based on the amphideal fovea shapes, was further subdivided into four groups: circular (cr), spiral (sp), pocket (pk), and indistinct (id) [52]. The shape of tails was also subdivided into four components: conical (co), clavate/conical cylindrical (cla), short/round (s/r), and elongated/filiform (e/f) [53]. The feeding types, based on oral cavity shape, were also subdivided into four groups: epiphytic (2A), deposit-selective (1A), non-selective (1B), and omnivorous/predators (2B) [54]. The body-size of adults was also ranked into three further groups: 1–2 mm, 2–4 mm, and >4 mm [55]. The life history based on the colonization success level (hereafter c-p) was ranked from a value of 1 (i.e., successful colonizers, with shortened life cycle, high reproductive rate and tolerant to various types of stress) to 5 (resilient, long-life cycle, reduced brood and sensitive to pollution), analogous to K/r strategists [56,57].

2.5. Statistical Analyses

Community-based indices, such as abundance, species richness (hereafter S), Margalef's species richness (hereafter d), Shannon-Weaner diversity index (hereafter H'), and evenness Pielou (hereafter J') were calculated in PRIMER 5.0 [58,59]. Normality tests (i.e., Kolmogorov–Smirnov) and the homogeneity of variance (i.e., Bartlett tests), as well as log10(x + 1) transformations, were applied to raw data [60] in STATISTICA (v5.1). One-way ANOVA and subsequent Tukey's HSD tests were used to check for overall and pairwise differences among treatments. Non-metric Multidimensional Scaling (hereafter nMDS) ordination was used, based on square root transformed species and relative functional traits abundances, based on Bray-Curtis similarity measures. The contribution of species and functional traits to the overall dissimilarity among treatments and compartments was done with SIMPER analysis (SiMilarity PERcentage analysis), see Clarke [58].

3. Results

3.1. Taxonomic Composition

At the end of the experiment, the nematofauna comprised 11 families, 17 genera, and 22 species. The most diverse families were Oncholaimidae and Xyalidae (Table 1).

Table 1. Taxonomic list of nematode species and types of functional traits in uncontaminated (i.e., UC, LC, UA1, UA2, UM1 and UM2) and contaminated (i.e., LA, LA2, LM1 and LM2) microcosms. Upper compartment (U); Lower compartment (L), Control treatment (C); amitriptyline (A: 0.4 and 40 μg L^{-1}), mixture of amitriptyline and paroxetine both at 0.4 μg L^{-1} (M1) and 40 μg L^{-1} (M2); Colonizers-Persisters scores (c-p); tail shape (Tl): conical (co), elongated/filiform (e/f), clavate (cla); amphid shape (Am): pocket-like (pk), indistinct (id), spiral (sp), circular (cr); feeding groups were classidied and ranked according to Wieser (1953) (FG) as folows: selective deposit-feeders (1A), non-selective deposit-feeders (1B), epistratum-feeders (2A), omnivores carnivores (2B); adult length (AL).

Functional Traits						Treatments									
Species	**c-p**	**Tl**	**Am**	**FG**	**AL**	**UC**	**LC**	**UA1**	**LA1**	**UA2**	**LA2**	**UM1**	**LM1**	**UM2**	**LM2**
Terschellingia sp.	2	e/f	pk	1A	2–4 mm	2.37 ± 1.17	1.68 ± 0.56	1.67 ± 0.58	4.33 ± 0.58	1.67 ± 0.58	1 ± 0.58	1 ± 0.08		1.33 ± 0.58	
Terschellingia longicaudata	3	e/f	cr	1A	1–2 mm	19.67 ± 1.72	18.18 ± 0.75	24 ± 1	22.67 ± 0.58	31.33 ± 1.15	50.33 ± 1.53	27 ± 2	41.70 ± 5.56	23.67 ± 0.58	42.33 ± 2.52
Metalinhomoeus numidicus	2	e/f	cr	1B	2–4 mm	6.44 ± 0.61	6.74 ± 0.65	5.33 ± 0.58	12.33 ± 0.58	5 ± 1.73	4.67 ± 0.58	5 ± 1.02	5 ± 0.70	4.67 ± 0.58	2.33 ± 0.58
Paracomesoma dubium	2	cla	sp	2A	1–2 mm	7.44 ± 1.43	8.43 ± 0.74	20.33 ± 1.53	17.33 ± 0.58	25.67 ± 1.15	13 ± 1	18 ± 1	14.56 ± 1.15	17.33 ± 0.58	12.66 ± 1.53
Marylynnia puncticaudata	3	e/f	sp	2A	2–4 mm	1.36 ± 0.61	1.69 ± 0.60	0.67 ± 0.58	0.33 ± 0.58		5.33 ± 1.15				
Daptonema trabeculosum	2	cla	cr	1B	1–2 mm	2.37 ± 0.55	2.35 ± 0.56	3.67 ± 0.58	2 ± 0.2	4.33 ± 2.08	3.33 ± 1.15	3 ± 1	4.66 ± 0.85	3 ± 1	3.33 ± 0.58
Paramonohystera wieseri	2	cla	cr	1B	1–2 mm	6.10 ± 1.00	6.05 ± 0.92	4.33 ± 0.58	5 ± 1.73	5 ± 1	7 ± 1	3.67 ± 0.58	8.62 ± 2.30	3.67 ± 0.58	7.33 ± 0.58
Paramonohystera pilosa	2	cla	cr	1B	1–2 mm	3.05 ± 1.04	3.03 ± 1.00	2.33 ± 0.58	1.67 ± 0.58						
Steineria sp.	2	cla	cr	1B	1–2 mm	7.46 ± 0.62	5.06 ± 1.09	4.33 ± 0.58	3.67 ± 0.58	4 ± 1	2.67 ± 0.58	4.67 ± 0.58	11.51 ± 1.05	3.33 ± 0.58	11 ± 1
Metoncholaimus pristiurus	3	cla	pk	2B	>4 mm	3.74 ± 1.23	3.36 ± 0.55	2 ± 0.2	2 ± 0.3	0.33 ± 0.58	0.33 ± 0.58		1.86 ± 0.39	1.67 ± 0.58	1.67 ± 0.58
Oncholaimelluscalvadocicus	3	cla	pk	2B	>4 mm	8.15 ± 1.14	5.41 ± 1.65	5 ± 1	0.33 ± 0.58	1 ± 1	1.33 ± 1.15	2 ± 0.38	1.97 ± 0.88	1.33 ± 0.58	1.67 ± 0.58
Viscosia cobbi	3	e/f	pk	2B	1–2 mm	4.06 ± 0.95	3.37 ± 0.65	3.33 ± 0.58	0.67 ± 0.58	2.67 ± 1.53		2 ± 0.2	1.86 ± 0.39	1	1.67 ± 0.58
Oncholaimus campylocercoides	4	cla	pk	2B	>4 mm	10.48 ± 2.39	6.41 ± 2.12	11.67 ± 0.58	5.33 ± 0.58	11.67 ± 0.58	2.67 ± 0.58	11.33 ± 0.58	12.38 ± 1.53	10.67 ± 0.58	11.67 ± 1.53
Calomicrolaimus honestus	3	co	sp	2A	1–2 mm	2.04 ± 1.04	3.03 ± 1.00	2 ± 0.19	4.67 ± 0.58						
Calomicrolaimus sp.	3	co	sp	2A	1–2 mm	4.06 ± 1.01	4.37 ± 0.55	3.67 ± 0.58	1.67 ± 0.58						
Neochromadora sp.	2	co	Id	2A	1–2 mm	2.38 ± 0.62	4.37 ± 1.52	1.67 ± 0.58	0.33 ± 0.58						
Chromadorina sp.	2	co	Id	2A	1–2 mm	1.02 ± 0.02	2.34 ± 1.51	1 ± 0.02							
Chromadorina minor	2	co	Id	2A	1–2 mm	1.70 ± 0.61	2.69 ± 0.54		0.33 ± 0.58	1.33 ± 1.15		1 ± 0.02		0.67 ± 0.58	
Anticoma acuminata	2	e/f	pk	1A	2–4 mm	1.69 ± 0.58	2.69 ± 1.14	1.33 ± 0.58	1.33 ± 0.58	1.67 ± 0.58	1 ± 0.5	1 ± 0.04		0.67 ± 0.58	
Ascolaimus sp.	2	co	cr	1B	2–4 mm	1.36 ± 0.61	3.03 ± 1.00	2 ± 0.2	1.67 ± 0.58	4.33 ± 0.58	3.33 ± 1.15	1.67 ± 0.58	2 ± 0.91	1 ± 1	1.67 ± 0.58
Cyatholaimus prinzi	3	co	sp	2A	1–2 mm	2.03 ± 1.02	2.69 ± 0.54	2 ± 0.01				0.33 ± 0.58		0.33 ± 0.58	
Synonchiella edax	4	e/f	sp	2B	2–4 mm	1.02 ± 0.02	3.03 ± 1.00	1 ± 0.02				1.67 ± 0.58	1.99 ± 0.91	0.33 ± 0.58	0.67 ± 0.58

Following the experiment completion, the most abundant species in UC compartment was *Terschellingia longicaudata* (19.6 ± 1.7%) and *Oncholaimus campylocercoides* (10.5 ± 2.4%). The remaining genera each comprised less than 10%.

In UC compartment, the genus *Chromadorina* sp. had the lowest abundance, 1.02 ± 0.02%. The other treatments registered an overall decrease in diversity and taxonomic composition compared to control. The species *O. campylocercoides*, *T. longicaudata* were detected in all compartments in high abundances. The species *Cyatholaimus prinzi*,

Calomicrolaimus sp., *C. honestus*, *Neochromadora* sp., *Chromadorina* sp., and *C. minor* disappeared from most contaminated compartments following the experiment completion.

3.2. Univarites Indices

Besides the nematodes, other taxonomic meiobenthic groups were also considered in the current experiment. As such, in UC, the nematodes were the dominant group (1343 ± 109 ind.), followed by copepods (73 ± 4.58 ind.), polychaetes (13.66 ± 1.52 ind.) and amphipods (10.33 ± 0.57 ind.). The treatments recorded significant decreases in the abundance of nematodes in both upper and lower compartments, compared to control (Figure 3). Likewise, the abundance of copepods was significantly lower in both compartments in any type of treatment compared to control. The abundance of polychaetes was significantly lower in all upper compartments compared to UC, except for UA1, and in lower compartments only in LM2 compared to LC. The abundance of amphipods was significantly different in the upper compartments of UM1 and UM2 compared to UC, as well as in the lower compartments only in UM2 compared to LC.

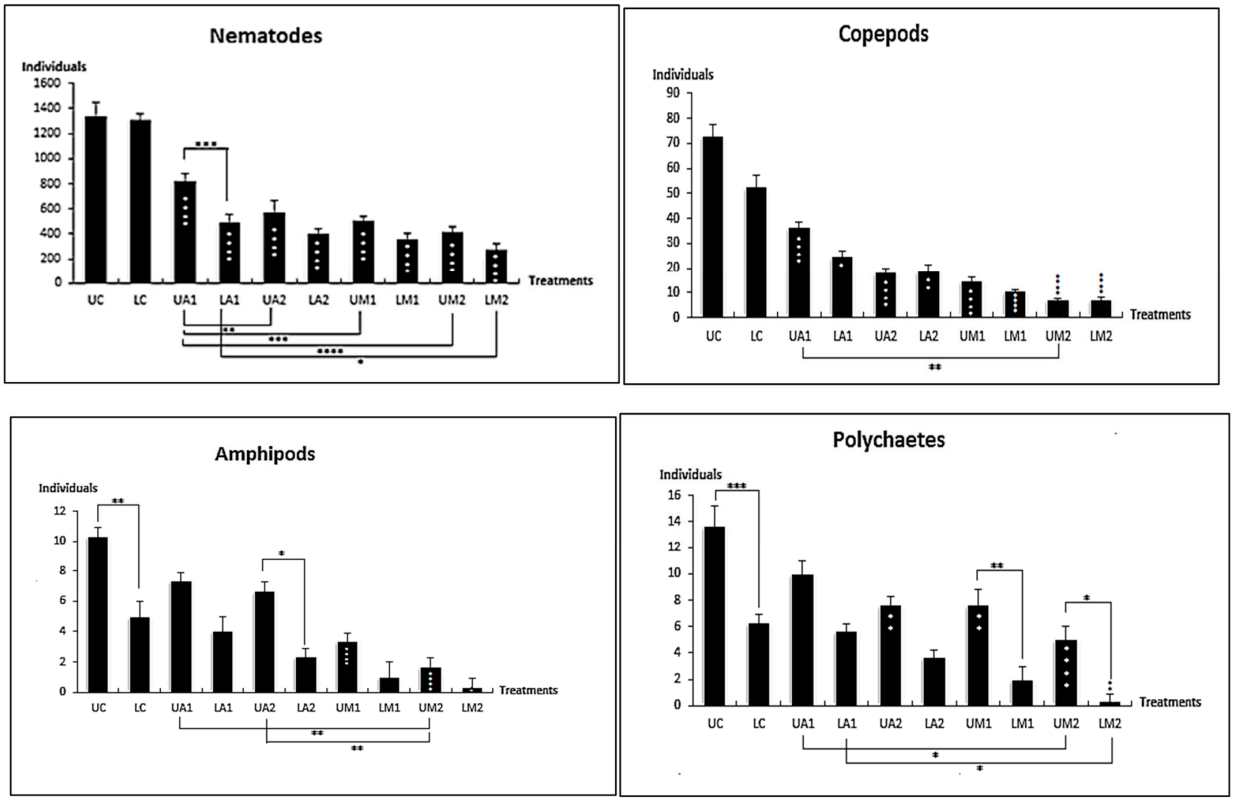

Figure 3. Abundances of meiobenthic taxa from uncontaminated (UC, LC, UA1, UA2, UM1 and UM2) and contaminated (LA1, LA2, LM1 and LM2) compartments. U = Upper compartment, L = Lower compartment, C = Control treatment, A = amitriptyline (0.4 and 40 μg L^{-1}), M = mixture of amitriptyline and paroxetine both at 0.4 μg L^{-1} (M1) and 40 μg L^{-1} (M2). * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, **** = $p < 0.0001$.

The diversity (S) of the upper compartments showed significant decrease in most compartments, except for UA1 compared to UC (Table 1). The diversity of lower compartments also showed significant differences in all compartments compared to LC. The Margalef's species richness showed a significant decrease in the upper sections of microcosms compared to UC (Figure 4) except for UA1, and differed in the lower compartments of all treatment combinations compared to LC. Pielou's evenness which differed significantly between UC and LA2 and the Shannon-Wiener index showed a significant decrease in all

upper compartments compared to UC, except for UA1 (Figure 4). The latter index in lower compartments differed significantly in all types of treatments compared to LC.

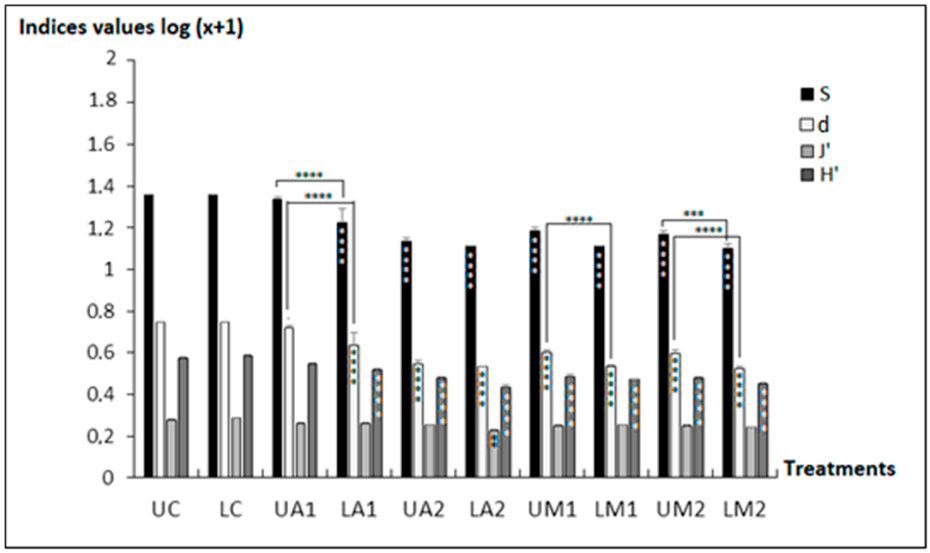

Figure 4. Distribution of univariate indices of nematofauna from uncontaminated (i.e., UC, LC, UA1, UA2, UM1 and UM2) and contaminated (i.e., LA1, LA2, LM1 and LM2) compartments. U = Upper compartment, L = Lower compartment, C = Control treatment, A = amitriptyline (0.4 and 40 µg L^{-1}), M = mixture of amitriptyline and paroxetine both at 0.4 µg L^{-1} (M1) and 40 µg L^{-1} (M2), H' is the Shannon-Weaner index, d the Margalef's species richness, J' the Pielou's evenness and S species richness. The stars indicate significant differences according to pairwise Tukey tests: $p < 0.01$ (**), $p < 0.001$ (***), and $p < 0.0001$ (****).

3.3. Multivariate Indices

The nMDS ordination (Figure 5) indicated that the taxonomic composition and abundance of nematodes differed among treatments and control (Stress = 0.11). The LA2 communities were situated the farthest from control, whereas the UA1 the closest. The dissimilarity values showed an increase in the average dissimilarity value between control and contaminated compartments. The lowest dissimilarity was observed between UC and UA1 (20.35%) and the highest between LC and LA2 (46.2%). SIMPER results reveal that the species which contributed most to the overall average dissimilarity were *Paracomesoma dubium*, *O. campylocercoides*, *Oncholaimellus calvadocicus*, *Steineria* sp. and *T. longicaudata* (Table 2).

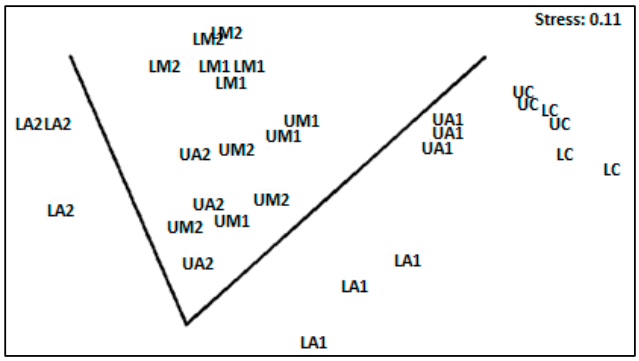

Figure 5. nMDS plot based on √-transformed nematode abundances from uncontaminated (i.e., UC, LC, UA1, UA2, UM1 and UM2) and contaminated (i.e., LA1, LA2, LM1 and LM2) compartments from microcosms treated with amitriptyline and paroxetine. U = Upper compartment, L = Lower compartment, C = Control treatment, A = amitriptyline (0.4 and 40 µg L^{-1}), M = mixture of amitriptyline and paroxetine both at 0.4 µg L^{-1} (M1) and 40 µg L^{-1} (M2).

Table 2. Percentages of dissimilarity between treatment microcosms (in bold) and SIMPER output, based on square-root transformed data. Only the nematode species and their functional groups that accounted for more then 70% of overall dissimilarity were considered. More or less abundant are represented by + and −, and elimination as Φ.

	UC vs. UA1 (20.35%)	**UC vs. UA2 (37.83%)**	**UC vs. UM1 (32.25%)**	**UC vs. UM2 (32.84%)**
Species	*Paracomesoma dubium* (31.66%) + *Terschellingia longicaudata* (11.34%) + *Oncholaimellus calvadocicus* (7.34%) − *Steineria* sp. (7.33%) − *Oncholaimus campylocercoides* (5.43%) + *Metoncholaimus pristiurus* (4.07%) − *Chromadorina minor* (4.07%) −	*Paracomesoma dubium* (24.45%) + *Terschellingia longicaudata* (15.99%) + *Oncholaimellus calvadocicus* (9.34%) − *Calomicrolaimus* sp. (5.33%) − *Metoncholaimus pristiurus* (4.45%) − *Steineria* sp. (4.44%) − *Paramonohystera pilosa* (4.00%) −	*Paracomesoma dubium* (18.23%) + *Terschellingia longicaudata* (13.08%) + *Oncholaimellus calvadocicus* (10.25%) − *Calomicrolaimus* sp. (6.83%) − *Metoncholaimus pristiurus* (6.27%) − *Paramonohystera pilosa* (5.13%) − *Steineria* sp. (4.55%) − *Paramonohystera wieseri* (3.99%) −	*Paracomesoma dubium* (17.61%) + *Oncholaimellus calvadocicus* (11.75%) − *Terschellingia longicaudata* (7.62%) + *Steineria* sp. (7.05%) − *Calomicrolaimus* sp. (7.04%) − *Paramonohystera pilosa* (5.29%) − *Viscosia cobbi* (5.27%) − *Paramonohystera wieseri* (4.11%) − *Neochromadora* sp. (4.11%) −
Feeding groups	10.66% 2A + 1B −	15.90% 2B −	12.27% 1A +	13.17% 1A + 2B −
Tail shape	3.32% cla +	9.03% co −	11.02% co −	11.94% co −
Amphid shape	11.03% sp +	8.16% sp + pk −	18.56% cr + pk −	12.36% cr + pk −
Adult length	7.81% 1–2 mm +	12.63% 1–2 mm +	9.80% 1–2 mm +	9.40% 1–2 mm +
c-p score	4.43% c-p3 −	10.58% c-p3 −	7.49% c-p3 −	7.69% c-p3 −
	LC vs. LA1 (32.26%)	**LC vs. LA2 (46.22%)**	**LC vs. LM1 (39.08%)**	**LC vs. LM2 (43.46%)**
Species	*Paracomesoma dubium* (14.93%) + *Metalinhomoeus numidicus* (9.40%) + *Oncholaimellus calvadocicus* (8.31%) − *Terschellingia longicaudata* (7.75%) + *Neochromadora* sp. (6.63%) − *Synonchiella edax* (4.98%) − *Calomicrolaimus* sp. (4.44%) − *Cyatholaimus* sp. (4.42%) − *Viscosia cobbi* (4.42%) − *Terschellingia* sp. (4.42%) +	*Terschellingia longicaudata* (35.88%) + *Paracomesoma dubium* (5.19%) + *Calomicrolaimus* sp. (4.81%) − *Neochromadora* sp. (4.81%) − *Oncholaimellus calvadocicus* (4.47%) − *Oncholaimus campylocercoides* (4.07%) − *Marylynnia puncticaudata* (4.05%) + *Viscosia cobbi* (3.70%) −	*Terschellingia longicaudata* (24.32%) + *Steineria* sp. (6.62%) + *Calomicrolaimus* sp. (5.75%) − *Neochromadora* sp. (5.75%) − *Oncholaimus campylocercoides* (5.74%) + *Paracomesoma dubium* (5.73%) + *Oncholaimellus calvadocicus* (4.88%) − *Calomicrolaimus honestus* (3.99%) − *Paramonohystera pilosa* (3.99%) −	*Terschellingia longicaudata* (28.40%) + *Steineria* sp. (6.99%) + *Oncholaimus campylocercoides* (6.19%) + *Neochromadora* sp. (5.06%) − *Calomicrolaimus* sp. (5.06%) − *Metalinhomoeus numidicus* (5.05%) − *Paracomesoma dubium* (5.02%) + *Oncholaimellus calvadocicus* (4.29%) − *Calomicrolaimus honestus* (3.51%) −
Feeding groups	13.81% 2B −	32.04% 1A +	19.36% 1A +	21.78% 1A +
Tail shape	13.38% co −	27.58% e/f −	20.73% co −	11.94% co −
Amphid shape	15.55% cr + id −	29.83% cr +	23.55% cr + id Φ	16.93% cr + sp −
Adult length	7.56% >4 mm −	15.85% 1–2 mm +	13.65% 1–2 mm +	17.10% 1–2 mm +
c-p score	8.50% c-p2 +	8.50% c-p3 +	5.88% c-p2 −	9.30% c-p2 −
	UC vs. LC (15.19%)	**UA1 vs. LA1 (22.95%)**	**UA2 vs. LA2 (30.71%)**	**UM1 vs. LM1 (18.41%)**	**UM2 vs. LM2 (26.24%)**
Species	*Oncholaimus campylocercoides* (13.29%) − *Oncholaimellus calvadocicus* (8.89%) − *Steineria* sp. (7.77%) − *Synonchiella edax* (6.67%) + *Neochromadora* sp. (6.66%) + *Ascolaimus* sp. (5.55%) + *Terschellingia longicaudata* (5.21%) − *Paracomesoma dubium* (4.84%) + *Chromadorina* sp. (4.43%) + *Calomicrolaimus honestus* (4.07%) +	*Metalinhomoeus numidicus* (15.96%) + *Oncholaimus campylocercoides* (14.45%) − *Oncholaimellus calvadocicus* (10.63%) − *Paracomesoma dubium* (6.82%) − *Calomicrolaimus honestus* (6.08%) + *Terschellingia* sp. (6.07%) + *Viscosia cobbi* (6.06%) −	*Terschellingia longicaudata* (31.54%) + *Paracomesoma dubium* (21.03%) − *Oncholaimus campylocercoides* (14.95%) −	*Terschellingia longicaudata* (28.57%) + *Paracomesoma dubium* (16.44%) − *Steineria* sp. (16.32%) +	*Terschellingia longicaudata* (41.13%) + *Steineria* sp. (16.90%) + *Paracomesoma dubium* (10.38%) −
Feeding groups	7.97% 2A +	14.87% 2B −	20.99% 1A +	11.60% 2A −	14.29% 2A −
Tail shape	8.90% cla −	11.42% e/f +	22.63% e/f +	2.11% e/f +	21.93% e/f +
Amphid shape	9.85% pk − sp +	11.35% cr +	20.28% cr +	14.01% cr +	16.85% cr +
Adult length	7.93% >4 mm −	11.31% 2–4 mm +	8.84% >4 mm −	5.36% 1–2 mm +	9.17% 1–2 mm +
c-p score	5.53% c-p2 +	10.50% c-p2 +	24.40% c-p3 +	6.48% c-p3 +	10.86% c-p3 +

3.4. Diversity of Functional Traits

The functional categories of the initial community and different compartments communities were composed as follows (Figure 6):

- The UC community feeding groups were dominated by non-selective deposit feeders (1B) and omnivorous ones (2B), comprising 26.7 ± 0.7% and 27.4 ± 1.35% of the nematofauna, respectively. The experiment showed a significant increase in groups

1A in all compartments, except for UA1. The group 2A in LA2 and the group 1B in UA1, LA2, UM1 and UM2 decreased significantly compared to control. The nMDS results indicate that LA2 treatment was the furthest from LC, followed by LM2 and LM1, whereas UA1 and UA2 treatments were the closest to UC.

- The amphid shapes of the control community (UC) were dominated by circular (cr) and pocket-shaped (pk) amphides, representing 46.4 ± 2.5% and 30.5 ± 2.7% of the nematofauna. A significant decrease was observed in the cr amphid shape in all compartments, except for UA1. In addition, the id amphid shape significantly decreased in LA1, LA2, LM1 and LM2 compared to control. The pk amphid shape percentage decreased in all compartments compared to control. The nMDS results indicate that LA2 was the furthest from LC, whereas UA1 and UA2 treatments were the closest to UC.
- Tail shapes were dominated by elongated/filiform (e/f) and clavate (cla) tails, representing 36.6 ± 0.5% and 48.8 ± 1.9% of the control nematofauna (UC), respectively. The contamination induced a significant increase in the shape of the e/f tails in LA2 and LM2. The shape of the cla tail showed significant differences between UM2 vs. LM2 and the e/f shape between UA2 vs. LA2 and UM2 vs. LM2. The nMDS indicated that the LM2 community was the furthest from LC, whereas all types of treatments were close to UC.
- The control nematofauna (UC) life history was dominated by cp3 and cp2 types, representing 45.1 ± 2.7%, and 43.3 ± 1.3. A significant decrease was observed in cp3 in the LA2 and LM2 compartments. Conversely, the results show a significant decrease in cp3 in the uncontaminated compartments UA2, UM1 and UM2. The cp2 showed significant differences between UC and LC; UA1 and LA1 and UA2 and LA2, the cp3 between UA2 and LA2 as well as UM1 and LM1, and UM2 and LM2, and the cp4 between LC and LA2. The nMDS ordination showed that LA2 and LM2 comprised a clear different cluster compared with UC, whereas UA1 treatment was the closest.
- The body-length intervals were dominated by 1–2 mm and >4 mm size-classes, comprising 61.1 ± 2.4% and 23.8 ± 0.8% of the control nematofauna (UC), respectively. The contamination induced a significant increase in the body-size class 1–2 mm in all compartments compared to control and a significant decrease in the 2–4 mm size-class between UC vs. UM2 and LC vs. LM2, as well as of the >4 mm size-class in all compartments compared to control, except for LM1 and LM2. The body-size interval 1–2 mm significantly differed between the control (UC and LC) and all other analyzed compartments, as well as between UA2 vs. LA2 and UM1 vs. LM1. The 2–4 mm size-class showed significant differences between UC and UM2 and between LC and LM2 and the >4 mm class between UC and LC. The results of the nMDS indicated that LA2 and LM2 were the furthest from LC, whereas the UA1 community the closest to UC.

The functional traits dissimilarities were less than 30%, excepting the trophic group in LA2 (Table 2). SIMPER results show significant decreases in feeding group 2A and cp3 life history percentages, as well as in feeding group 2B and size-class 1–2 mm in most treatments compared to control. Second-stage nMDS ordination (Figure 7) indicated that the nematodes feedback to contaminants was mainly driven by their tail shape (84.7%), amphid shape (84.4%) and adult body-size (83.1%), followed by life history (71.4%).

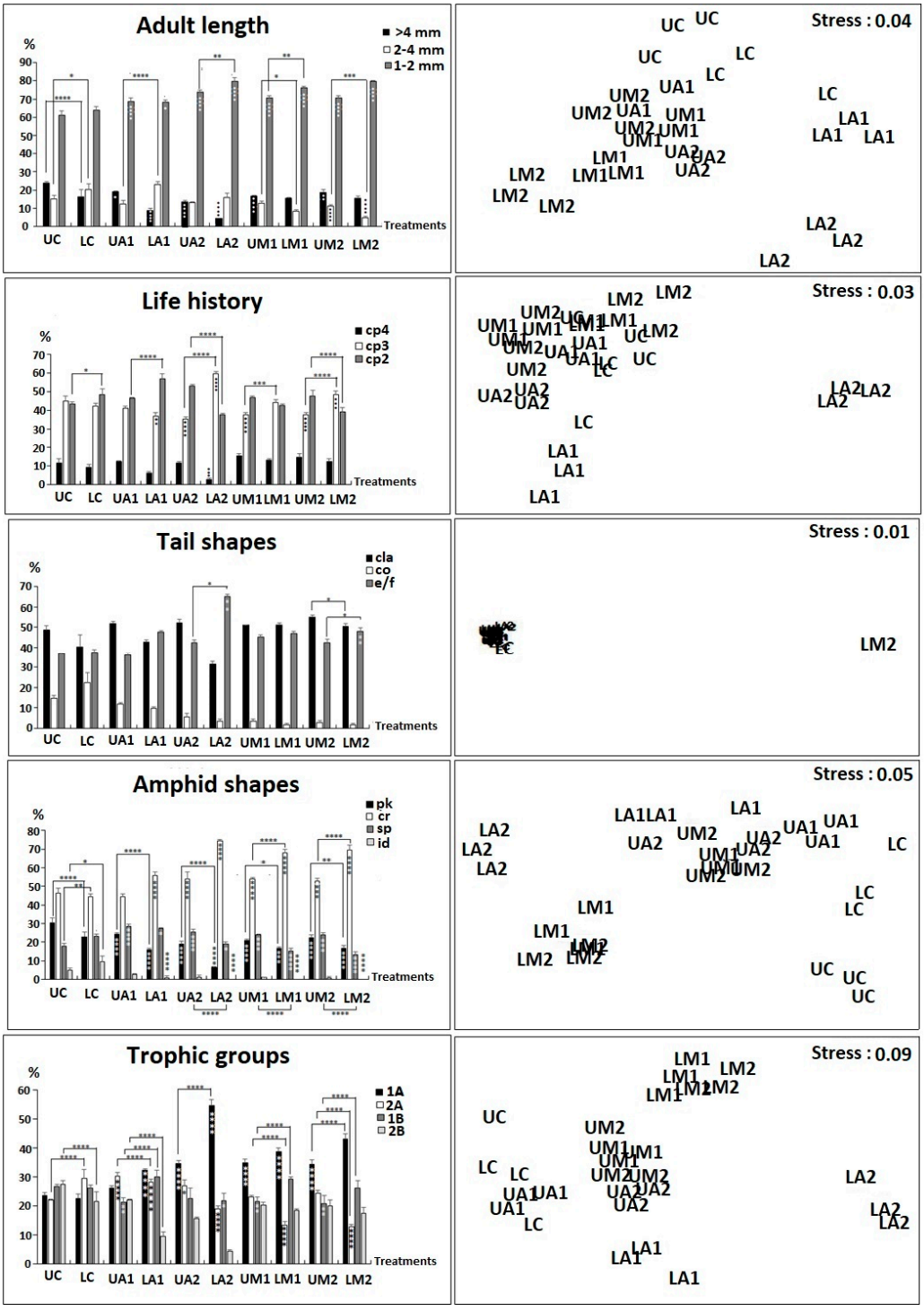

Figure 6. nMDS plot based on √-transformed nematode functional groups from uncontaminated (i.e., UC, LC, UA1, UA2, UM1 and UM2) and contaminated (i.e., LA1, LA2, LM1 and LM2) compartments from microcosms treated with amitriptyline and paroxetine. U = Upper compartment, L = Lower compartment, C = Control treatment, A = amitriptyline (0.4 and 40 μg L^{-1}), M = mixture of amitriptyline and paroxetine both at 0.4 μg L^{-1} (M1) and 40 μg L^{-1} (M2), 1A = Selective deposit feeders, 1B = non-selective deposit feeders, 2A = epigrowth feeders, 2B = omnivores-carnivores, s/r = short/round, e/f = elongated/filiform, co = conical, cla = clavate/conical-cylindrical, sp = spiral, pk = pocket-like, id = indistinct, cr = circular. Stars above bars indicate significant differences with corresponding controls after Chi-square test (√-transformed data): $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.01$ (***) and $p < 0.0001$ (****).

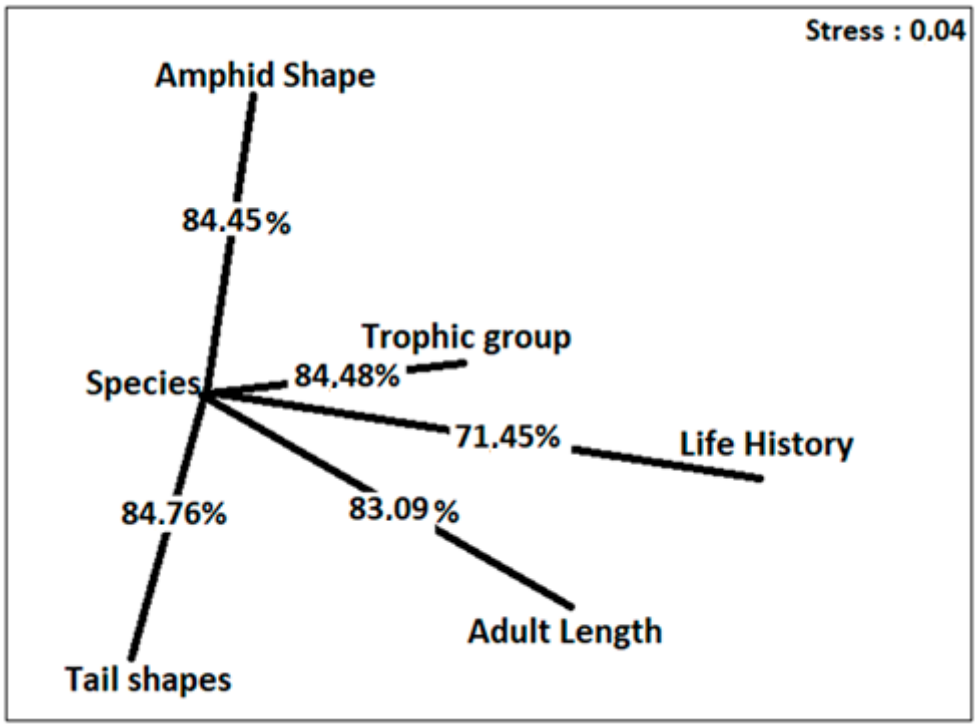

Figure 7. Second-stage nMDS ordination of the inter-matrix rank correlations. For species see Figure 4 and for functional traits Figure 5. The values comprise the mean similarity percentages between nMDS of species and functional traits.

3.5. Sex Ratio and Maturity Status

The UC nematodes community comprised 16.7 ± 4% males 65.9 ± 1.1% non-gravid females, 6.6 ± 0.5% gravid females and 7.5 ± 1.4% juveniles (Table 3). There were registered significant increases in the percentage of males in LA2, LM1 and LM2 and significant decreases in non-gravid females percentages in all compartments compared to upper and lower control. Significant differences in males' percentages were also recorded between UA2 and LA2, UM1 and LM1, and UM2 and LM2, and for non-gravid females between UA1 and LA1. The percentage of juveniles increased significantly in UA2 compared to control, but decreased between UA2 and LA2. On the other hand, the ratio j/gf increased significantly at UM2 compared to UC (Table 4).

Table 3. Relative abundances (±SD) of males (m), non-gravid females (f), gravid females (gf) and juveniles (J) in uncontaminated (UC, LC, UA1, UA2, UM1 and UM2) and contaminated (LA1, LA2, LM1 and LM2) compartments. U = Upper compartment, L = Lower compartment, C = Control treatment, A = amitriptyline (0.4 and 40 μg L^{-1}), M = mixture of amitriptyline and paroxetine both at 0.4 μg L^{-1} (M1) and 40 μg L^{-1} (M2), f = non-gravid females; gf = gravid females, J = juveniles (J), males (m). The uncontaminated compartments were filled with sediment collected at the beginning of the experiment (i.e., Upper: U), whereas the compartments where azoic sediment was used were either contaminated or not with amitriptyline and amitriptyline and paroxetine mixture (i.e., Lower: L). Bold values indicate significant differences compared to "UC" ($p < 0.05$, Tukey-HSD test). Stars ** and **** indicate significant differences between underlined treatments at $p < 0.01$ and $p < 0.0001$ (Tukey-HSD test), respectively.

%	UC	LC	UA1	LA1	UA2	LA2	UM1	LM1	UM2	LM2
m	16.76 ± 4	21.41 ± 3.63	20.71 ± 2.77	26.34 ± 2.77	23 ± 5	**32.37 ± 3.13** **	18.09 ± 2.35	**31.59 ± 3.44** ****	15.45 ± 1.33	**37.61 ± 3.63** ****
f	65.88 ± 1.16	62.09 ± 5.43	**75.13 ± 4.70**	**37.48 ± 4.32** ****	**51.33 ± 4.73**	**52.32 ± 7.11**	**40.56 ± 1.94**	**44.13 ± 5.43**	**31.88 ± 2.20**	**49.51 ± 7.42**
gf	6.55 ± 0.48	4.95 ± 0.09	5.51 ± 0.61	5.26 ± 0.91	4	5.43 ± 0.49	5.28 ± 0.55	1.87 ± 0.09	2.24 ± 0.07	2.94 ± 0.18
J	7.52 ± 1.39	9.59 ± 1.66	5.5 ± 0.44	7.86 ± 0.58	**21.66 ± 1.53**	2.22 ± 0.42 ****	5.56 ± 0.67	10.27 ± 0.44	6.22 ± 0.49	6.21 ± 0.80

Table 4. Demographic ratios (±SD) in uncontaminated (UC, LC, UA1, UA2, UM1 and UM2) and contaminated (LA1, LA2, LM1 and LM2) microcosms. U = Upper compartment, L = Lower compartment, C = Control treatment, A = amitriptyline (0.4 and 40 µg L^{-1}), M = mixture of amitriptyline and paroxetine both at 0.4 µg L^{-1} (M1) and 40 µg L^{-1} (M2). Non-gravid females (f); gravid females (gf); juveniles (J); non-gravid females + gravid females (F); males (m). Bold values indicate significant differences compared to "UC" ($p < 0.05$, Tukey-HSD test). Stars ** and **** indicate significant differences between underlined treatments at $p < 0.01$ and $p < 0.0001$ (Tukey-HSD test), respectively.

%	UC	LC	UA1	LA1	UA2	LA2	UM1	LM1	UM2	LM2
gf/F	0.1 ± 0.01	0.1 ± 0.01	0.07 ± 0.01	0.14 ± 0.02	0.08 ± 0.01	0.11 ± 0.02	0.13 ± 0.01	0.04 ± 0.01	0.07 ± 0.01	0.05 ± 0.01
J/gf	1.14 ± 0.14	1.57 ± 0.36 ****	1.01 ± 0.18	1.54 ± 0.39 **	**5.42 ± 0.38**	**0.41 ± 0.08** ****	1.1 ± 0.09	**5.5 ± 0.5** ****	**2.79 ± 0.32**	2.11 ± 0.2 ****
m/F	0.23 ± 0.06	0.22 ± 0.05	0.26 ± 0.02	0.62 ± 0.05	0.42 ± 0.13	0.56 ± 0.02	0.39 ± 0.03	0.69 ± 0.03	0.46 ± 0.07	0.72 ± 0.05
J/F	0.1 ± 0.02	0.13 ± 0.02	0.07 ± 0.01	0.19 ± 0.03	0.39 ± 0.02	0.04 ± 0.01	0.12 ± 0.01	0.23 ± 0.04	0.18 ± 0.03	0.12 ± 0.01

3.6. Taxon-Functional Traits

In the current study the nematodes *O. campylocercoides* and *T. longicaudata* were chosen for further demographic and morphometric indices investigations (Figure 8). Overall, the RVPL values increased significantly in compartments LA1, UA2, LM1, and LM2 for *T. longicaudata* and in all compartments, except UM1, for *O. campylocercoides* compared to control. The Boufahja's index increased significantly in all compartments, except for *T. longicaudata* in UM1 and for *O. campylocercoides* in UA1 and UA2, respectively, compared to control.

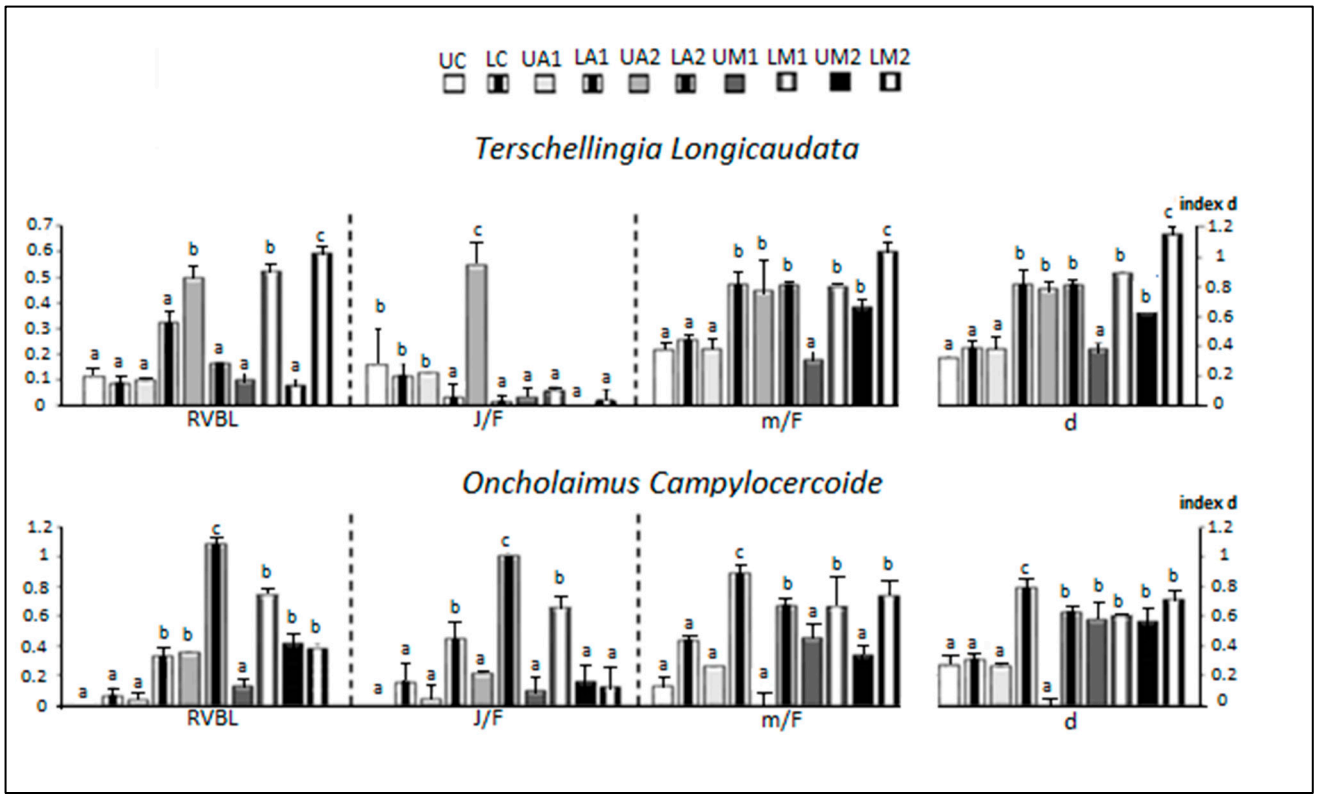

Figure 8. Mean distribution of morphometric (i.e., RVPL% and *d*) and population- (i.e., J/F and m/F) based indices of the nematodes *Terschellingia longicaudata* and *Oncholaimus campylocercoides* at uncontaminated (UC, LC, UA1, UA2, UM1 and UM2) and contaminated (LA1, LA2, LM1 and LM2) compartments with paroxetine and amitriptyline. U = Upper compartment, L = Lower compartment, C = Control treatment, A = amitriptyline (0.4 and 40 µg L^{-1}), M = mixture of amitriptyline and paroxetine both at 0.4 µg L^{-1} (M1) and 40 µg L^{-1} (M2), RVPL = Relative Volume of the Pharyngeal Light, J = Juveniles, F = non-gravid females + gravid females (F), m = Males, *d* = Boufahja's *d* index (=Total body lenght/Intermediate Piece lenght). Different letters above bars represent significant differences ($p < 0.05$) values according to Chi-square test ($\sqrt{}$-transformed data in %) and Spjotvoll-Stoline test results.

4. Discussion

Tricyclic antidepressants are commonly used as sedatives, anxiolytics, antidepressants and antischizophrenics [1–61]. The high demand for these antidepressants from pacients increased significantly over the past decade, which could be easily linked to their increasing concentrations in the aquatic habitats [62–64]. The chosen antidepressants for the current experiments, namely the amitriptyline and paroxetine, were selected because their presence in terrestrial and aquatic ecosystems alike has been detected at increasing concentrations recently [65,66]. Despite previous efforts which had a main focus on assessing the toxicity of these antidepressants on the biological processes and the behaviour of non-targeted animals [67–71] the number of studies on benthic invertebrates, including nematodes, is limited [16]. Therefore, the scope of the curent experiment was to assess the effect of both antidepressants on the migratory behaviour of coast-dwelling marine nemtodes.

4.1. What Is the Effect of Contamination with Amitriptyline on Nematodes?

According to experimental results, the exposure to amitriptyline led to a significant decrease in the abundance of the benthic community in both compartments compared to control microcosms (Figure 3).

The meiobenthic communities dwelling in the upper compartments UA1 and UA2 were significantly more abundant and diverse compared to the contaminated lower compartments LA1 and LA2 (Figure 4). Previous investigations demonstrated the capacity of nematodes to avoid pollution and their subsequent reactions [36,72,73]. Among the generic taxonomic groups evaluated in the current experiment, the diversity of marine free-living nematodes also decreased compared to controls. The dominant species from control microcosms was *T. longicaudata*, which is characterized by small body-size (i.e., 1–2 mm) and is an epistrate feeder (guild 2A). However, larger (>4 mm) and omnivore-carnivores (guild 2B) species such as *O. campylocercoides* was also widespread in microcosms at the end of the experiment.

Comparing the results obtained from the current study with those of Ishak et al., [19], the differences spotted by the SIMPER analysis are not straightforward. In this work, the SIMPER analysis indicated a clearer effect of amitriptyline on the community of nematodes. The variable responses of the average dissimilarity emphasize a higher contribution of functional traits between compartments compared to Ishak et al., [19] and showed that the distribution of species was mainly driven by the contamination with amitriptyline.

The migratory behavior of certain nematodes was also influenced by contamination with amitriptyline. The vertical distribution pattern of *T. longicaudata* was particularly affected following the exposure to this antidepressant by increasing its abundance in the lower compared with the upper compartments. This nematode has a rounded amphid (cr type), known to increase the detection and hence the avoidance rate of pollutants compared to other nematodes that posess different amphid shapes [30]. The slender body of this species, associated with a filiform tail (e/f type), facilitated its vertical migration in a previous experiment, despite contamination with pollutants [39]. The results of the current experiment partially confirm the potential migration response of nematodes from polluted areas [74]. Moreover, the nématode *O. campylocercoides* was able to tolerate high amitriptyline concentrations, but its abundance was higher in uncontaminated compartments. On the other hand, species such as *Cyatholaimus prinzi*, *Calomicrolaimus* sp., *C. honestus*, *Neochromadora* sp., *Chromadorina* sp., and *C. minor* showed high sensitivity to low concentrations of amitriptyline, reflected in their elimination from most treatments (Table 1). Most of these highly sensitive species belong to indistinct (Id), spiral (sp) amphids' shape, which indicate lower efficiency of these types of amphid to detect the amitriptyline [73], leading to the conclusion that they could be considered negative bioindicators of contamination with amitriptyline.

The functional traits that contributed most to the dissimilarity between UA1 vs. LA1 and UA2 vs. LA2 were the increase in percentages of 1A feeding groups, e/f tail shapes and 2–4 mm size-class (Table 2). It can be concluded that these functional traits greatly favored

the vertical migration. Previous studies reported that undulation, body wave lengths and frequencies [75], body-size and shape [20–76], the presence of caudal [77], as well as feeding strategies [78,79] favored the migration of nematodes within marine sediments, resulting in different distribution patterns over a vertical profile.

The nematodes *T. longicaudata* and *O. campylocercoides,* with higher RVPL values, remained in the contaminated and to a lesser extent in the uncontaminated compartments. Such individuals possess larger pharyngeal light and higher pumping potential, allowing them to successfully cope with the low availability of food within spiked sediments with amitriptyline or mixtures of antidepressants. The efficacy of RVPL in biomonitoring programs was validated by Boufahja et al. [26] from field and laboratory experiments. However, the usage of this index was paused until 2011, because of the inherent difficulties and time-consuming measurements of the pharyngeal light dimensions, given their small size and diversity in shapes that is approximated afterwards to a known geometric figure that allows the calculation of the volume. The data obtained from the current work are in accordance with those observed after exposure of the species *Bathylaimus capacosus*, *Daptonema normandicum*, *Desmodora longiseta*, *Oncholaimus campilocercoides*, *Leptonemella aphanothecae*, and *Oncholaimellus mediterraneus* to metals (i.e., copper and chromium) and diesel [26].

The sex ratios highlighted significant increase in the percentage of males in LA2 and subsequent decrease in females in LA1 and LA2 (Table 3). These results could suggest a hormonal effect potentially induced by amitriptyline on females. Moreover, the abundance of juveniles was significantly higher in UA2 than in LA2, leading to the speculative conclusion that the amitriptyline may have had an impact on juveniles, by increasing their mortality. This difference in the abundance of juveniles led to a significant increase in the J/gf ratio in UA2 and decrease in LA2. A previous study showed that the toxic effects of certain glucocorticoids, including paroxetine, were also reflected in changes in the sex ratios of *Ceriodaphnia dubia* [7].

4.2. How Does the Mixture of Amitriptyline and Paroxetine Affect Nematodes?

Grosse et al. [80] showed that pharmaceutical substances can be mixed with several types of drugs, with similar or different modus operandi in water. These mixtures can cause synergistic or, less likely, antagonist effects on aquatic biota [81,82]. More recently, Benchouala [45] assessed the toxic effect of paroxetine and a tricyclic antidepressant on *Hydra attenuate* biological cycle.

The paroxetine, a selective serotonin inhibitor and one of the most prescribed antidepressants in the world, is usually found together with amitriptyline in marine habitats [63]. The abundance of all meiobenthic generic groups decreased significantly in the mixture exposure compared to the control and also lower compared to just amitriptyline exposure, suggesting potential synergistic interactions between both pharmaceuticals. The uncontaminated upper compartments UM1 and UM2 comprised a nematofauna that was more abundant and with higher values for species richness compared to the lower contaminated compartments, LM1 and LM2. The contamination with the mixture confirmed the tolerance of *T. longicaudata* to amitriptyline and added on the list of negative bioindicators the genus *Steineria* sp. The SIMPER analysis showed a high increase in Steineria sp. abundance in mixtures compared to the microcosms' contamination with amitriptyline alone. Besides, the high sensitivity of *Chromadorina* sp., and *C. prinzi* was also confirmed. Overall, the functional traits SIMPER analysis highlighted an overall response of nematofauna for mixtures as with amitriptyline alone, namely the dominance of adults with body-sizes between 1 and 2 mm, belonging to trophic groups 2A, with amphid cr, life history cp3, and tail shape e/f (Table 1). The sex ratios highlighted a significant increase in the percentage of males in LM1 and LM2, and subsequently, the decrease in that of females (Table 3). This similar output as registered for amitriptyline contamination alone supports that it could as well be neutral combination with paroxetine or less probably a slight synergic effect of both antidepressants.

5. Conclusions

The use of free-living marine nematodes as laboratory model organisms and ecological indicators for pollution has a long tradition. Until recently, ecotoxicological studies were based on rather close microcosms in the attempt to mimic natural conditions. However, the use of open microcosms design should be also considered because it allows the free movement of nematodes between compartments, facilitating their assessment as tolerant or sensitive species. The current study explored the multiple effects of two antidepressants and their interactions with meiobenthic communities, but with a main focus on marine nematodes.

The results of the current experimental reveal that the abundance of meiobenthic generic groups decreased significantly after exposure to amitriptyline alone, in both compartments, the pattern augmented by the addition of paroxetine. The migratory behavior of free nematodes was clearly influenced by contamination, mostly reflected in the behavior of the species *T. longicaudata*, which could be considered tolerant to contamination with both antidepressants. The nematodes *Cyatholaimus prinzi*, *Calomicrolaimus* sp., *C. honestus*, *Neochromadora* sp., *Chromadorina* sp. and *C. minor* could be considered susceptible to contamination, even at low concentrations. The contribution of functional traits to mean dissimilarity was mainly driven by the feeding groups 1A and 2B, e/f tail shape, and longer than 2 mm size-class adults. The sex ratios highlighted significant increases in the percentage of males, and subsequently, the decrease in females. Overall, the potential synergistic effect between the two considered antidepressants was visible for the overall decrease in traits of the meiobenthic communities.

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