



Nematode rapid adaptative response to the massive Deepwater Horizon oil spill

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ABSTRACT

The Gulf ecosystem health was of great concern after the explosion of the Deepwater Horizon oil platform (April 2010). After the spill, a large volume of oil trapped in the deep-sea plumes was transported offshore and to deep-water sediments, causing detrimental effects on the benthic fauna. In this study, the variability in nematode standing stock, structural and functional diversity has been investigated. Nematodes were collected in October 2010 from stations characterized by different levels of impact and along the sediment vertical profile. Immediately after the oil spill, nematode abundances rapidly increased moving towards impacted stations and reaching unusual abundances for the deep-sea. Despite the sediment toxicity, impacted stations showed dense populations of small-sized nematodes concentrated in the surface sediment, whereas no-impact stations displayed more even vertical distributions and more stable size spectra.

High turnover across horizontal and vertical gradients demonstrated substantial species replacement linked to contamination. Impacted stations were dominated by opportunistic and tolerant species, while sensitive taxa were restricted to no-impact stations. Functional traits provided additional resolution: impacted stations exhibited a marked dominance of juveniles, indicating rapid population turnover and resilience to hydrocarbon contamination. In contrast, no-impact stations were characterized by a higher proportion of adults and k-strategist nematodes, reflecting more stable communities. Bacterivorous/deposit-feeding nematodes dominated the impacted stations, compared to a more diversified trophic community in no-impact stations. The combined use of standing stock, taxonomic, and functional descriptors offered a powerful framework for assessing deep-sea oil spill magnitude and impacts, highlighting the nematodes as reliable bioindicators of oil pollution.

1. Introduction

The deep-sea floor, covering 65% of the Earth surface, is characterized by high spatial heterogeneity and it has been recognized as biodiversity hotspot (Ramírez-Llodra et al., 2010). Deep-sea benthos represents a source of biodiversity and covers a wide array of functions which are vital for the ecosystem functioning (Danovaro et al., 2008). The meiofauna organisms are the numerically dominant metazoan benthic component of deep-sea, and they are considered to be a good proxy of the overall deep-sea benthic biodiversity (Rex and Etter, 2010).

Among meiofaunal organisms, nematodes are often the most abundant and diversified taxon, and they can exploit at maximum the available resources into the sediments and interstitial waters due to their trophic plasticity and wide tolerance range to environmental conditions (Giere, 2009), including systems with extreme conditions (Sapir, 2021). In the deep sea, nematodes can exceed 90% of metazoan meiobenthos (Schnier et al., 2023). Recent studies have demonstrated that free-living marine nematodes can serve as valuable, cost-effective, and reliable biological indicators of various types of anthropogenic disturbance (e.g., Semprucci et al., 2022; Grassi et al., 2023). Egres et al. (2019) and

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Frontalini et al. (2025) reported how nematode communities are more sensitive to polycyclic aromatic hydrocarbons (PAHs) pollution than macrofauna, and they may have predictable trend in presence of crude or motor oil, petroleum, or PAHs (e.g., Boufahja et al., 2012; Monteiro et al., 2018; Allouche et al., 2020).

The Gulf of Mexico (GoM) encompasses a diverse array of ecosystems that provide essential services and goods (e.g. seafood, recreational activities and tourism, natural resources) in sustaining the Gulf Coast, the United States, and international economies (McKinney et al., 2021). The GoM ecosystem also faces multiple anthropogenic stressors at both local (e.g., overfishing, eutrophication, hypoxia, and oil spills) and global (e.g., ocean warming and acidification) scales, which have been exacerbated over time. Of particular concern is the oil industry, due to the rapid expansion of deep and ultra-deep wells within the exclusive economic zones of Mexico and the United States, and the increasing demand for hydrocarbon-based energy sources (Murawski et al., 2021).

The Gulf ecosystem health was of great concern after the explosion of the Deepwater Horizon offshore oil platform (April 2010). The Deepwater Horizon oil spill resulted in 134 M gal (~507 M liters) released into the ocean reported by the US District Court's Findings of Fact. The spill was extraordinarily disastrous in its volume, duration (89 days), depth of release (1525 m), and kilometers covered by the impact (Spier et al., 2013). Yan et al. (2016) reported between $1.6\text{--}2.6 \times 10^{10}$ grams of petrocarbon accumulation on the seafloor along with a sinking of spill associated substances, mediated by marine particles, for 5 months following the capping of the well. The oil-derived hydrocarbon deposition was even exacerbated by a large diatom bloom sedimentation event between August and September 2010. This event increased the sinking rates of oil spill pollutants and components of drilling mud such as barium (Hu et al., 2011).

Cleanup operations and natural mechanisms removed most of the oil. However, an estimation of 35% of the hydrocarbons were trapped and transported to the deep sediments (Montagna et al., 2013). A total of 2864 km² deep-sea floor impacted area was documented (Baguley et al., 2024).

Previous studies (e.g., Baguley et al., 2006; Wei et al., 2010) reported that the maximum benthic species diversity occurs between 1200 and 1600 m depth in the Gulf, which coincides with the depths of the Deepwater Horizon (DWH) blowout impact. Critical benthic effects of concentrations of total petroleum hydrocarbons (TPHs), PAHs and barium (Ba) on macrofauna and meiofauna communities were still persistent one year after the oil spill (Montagna et al., 2013).

Meiofauna and nematodes were the focus of some research conducted along the coastline regions, beaches and intertidal areas to evaluate the spill impact (Bik et al., 2012; Brannock et al., 2017), along the Gulf continental shelf (Landers et al., 2014; Martinec et al., 2014) and slope area (Soto et al., 2017). Other compelling studies describing the response of the deep-sea benthic communities to the oil spill (e.g., Montagna et al., 2013, 2017; Baguley et al., 2015; Reuscher et al., 2017; Schwing et al., 2020; Baguley et al., 2024; Baldrighi et al., 2024), however did not consider an analysis of the meiobenthic nematodes inhabiting the impacted sediments. Presently, there are a few works on the biogeographic patterns and community structure of meio- and nematode taxa in the Gulf of Mexico region (Sharma et al., 2011, 2012; Jones et al., 2016; Beaton et al., 2018; Cisterna-Céliz et al., 2019; Armenteros et al., 2022, 2024). In general, all mentioned studies highlighted the influence of water depth, food availability and habitat heterogeneity on the diversity and functional structure of nematode assemblages. Two recent studies focused on the spatial-temporal diversity of harpacticoid community changes after the DWH (Baguley et al., 2024; Baldrighi et al., 2024), demonstrated how a greater taxonomic resolution (i.e., focused on Harpacticoida at the family-level) can provide a more comprehensive understanding of persistent impacts or recovery trajectories of benthic communities over space and time. Immediately after the oil spill, nematode abundances 'exploded' reaching unusual densities for the deep-sea, especially at stations

located within 15 km from the wellhead, as a response to a bacterial bloom enhanced by DWH spill (Montagna et al., 2013; Romero et al., 2017).

The objective of the present study is to investigate the spatial distribution, across different impact levels and considering different sediment layers, of the nematode communities inhabiting the deep-sea ecosystem in the Gulf of Mexico after DWH oil spill. The main hypothesis tested here is that the nematode standing stock (i.e., abundance and biomass) and diversity (i.e., genus richness, composition and functional diversity as feeding groups and life strategies) are affected by different levels of impact. Functional descriptors combined with taxonomic diversity were used to test the hypothesis. Nematode functional diversity can provide complementary information on the contribution of species in the evaluation of the ecosystem quality and integrity elucidating the magnitude of the impact (Semprucci et al., 2022).

2. Methods

2.1. Sampling strategy and collection

After the DWH spill, two response cruises were carried out in the northern Gulf of Mexico in September and October 2010 aboard the *R/V Gyre* and *R/V Ocean Veritas* to collect biological and chemical deep-sea sediment samples. This large-scale effort resulted in the collection of samples from 170 stations (27–30° N, 86–91° W). Details of these sampling cruises and field collection methodology are described in prior manuscripts (Montagna et al., 2013; Baguley et al., 2015; and Montagna et al., 2017).

In the present study, data from 24 of these stations (Fig. 1) located along a suspected gradient of contaminant effects at an increasing distance from the wellhead and at a water depth range from 710 m to 1884 m are presented (see Table S1 for position, depth and impact level of the stations). The stations were divided into three main DWH effects zones as already categorized in previous studies and based on pollutant concentrations (i.e. TPHs, PAHs and Ba) into the sediment and biotic variables (sensu Montagna et al., 2013; Reuscher et al., 2020; Baguley et al., 2024): 8 high impacted (HI) and 8 moderate impacted (MI), versus 8 no-impact (NI) stations. An Ocean Scientific International Ltd. (OSIL) megacorer was used to collect 12 sediment core samples with each deployment. Sediment cores were destined, each time, to analysis of macrofauna, meiofauna, hydrocarbons, metals, pore water chemistry, and basic sediment properties (total C, total organic C, total inorganic C, total N, grain size). The core designated for meiofauna was subsampled in single replica with a 5.5 cm inner diameter sub-core and extruded into 0–1 cm and 1–3 cm sediment sections. Overlying water in the sub-core was included with the 0–1 cm fraction. Samples were narcotized in 7% MgCl₂ for body relaxation, then preserved in 4% buffered formalin and stained with Rose Bengal (Armynot du Châtelet et al., 2016).

2.2. Nematode sample processing

In the laboratory, samples were sieved over a 45-µm mesh to remove formalin and fine sediments. Meiofaunal organisms were then extracted using isopycnic centrifugation in Ludox HS-40 (Burgess, 2001). After extraction, nematodes were counted and density values expressed as ind/10cm² were calculated per each sampling site. The nematode biomass was calculated according to Baguley et al. (2004) and applying the formula $V \text{ (nL)} = \pi r^2 L / 10^6$ for biovolume estimation, where L (µm) is the nematode length. Wet mass was calculated from biovolume using a specific gravity of 1.13, and wet mass was converted to dry mass, assuming a ratio of 25%. The nematode dry mass was converted into carbon mass considering a carbon to dry mass ratios of 51.4% for nematodes.

For the identification, one hundred nematodes from each sample and sediment layer (0–1 cm and 1–3 cm; a total of 200 nematodes at each station) were randomly picked up and mounted on permanent slides in

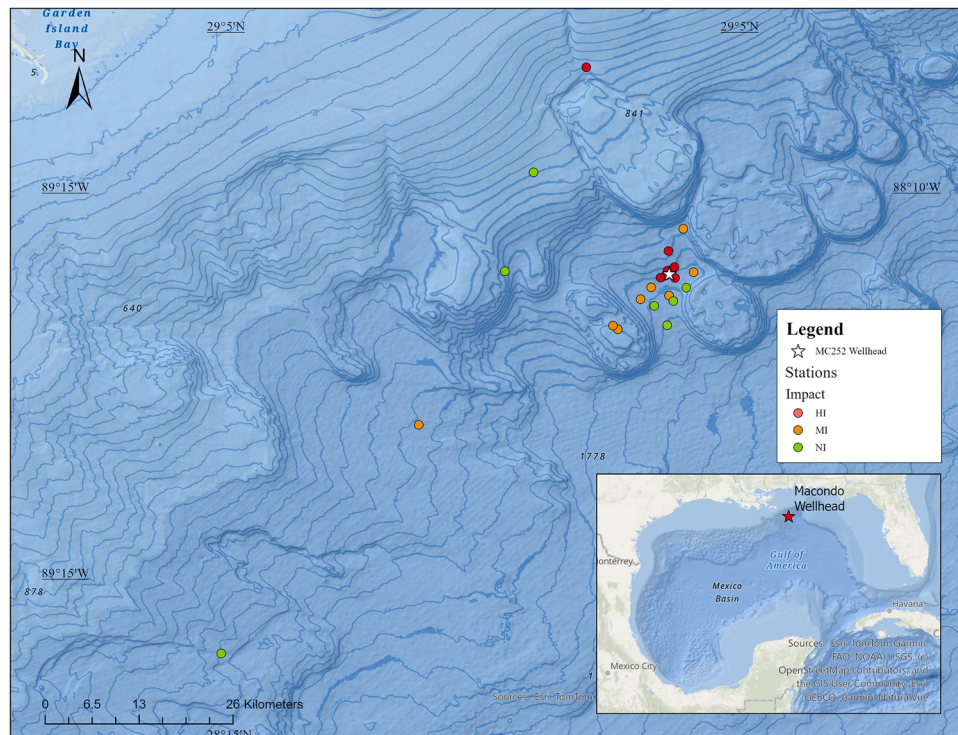


Fig. 1. Position of the Macondo wellhead in the Gulf of Mexico indicated by the star (right bottom inset) and location of the selected 24 stations sampled in 2010. Color codes: red = high impact (HI), orange = medium impact (MI) and green = no impact (NI) stations.

anhydrous glycerin according to [Seinhorst \(1959\)](#). Nematodes were identified to the lowest taxonomic level, usually genus and species level if possible. Different morphotypes were named as sp.1, sp.2, etc.; “*species affinis*” (aff.) was used for those species that resemble to known species, but it was not possible to confirm it or because differed slightly in some morphological characters. The taxonomic identification was carried out using [Platt and Warwick \(1983, 1988\)](#), [Warwick et al. \(1998\)](#) and the NeMys website (<http://nemys.ugent.be>). Additionally, the gender of the organisms was determined by the presence of spicule (male) and vulva (female, with or without eggs), while the stage of life (adult or juvenile) was determined by the presence/absence of gonads.

2.3. Nematode functional diversity

To assess the potential effects of the oil spill on nematode life traits, each nematode genus was classified into one of the five c-p (colonizers-persisters) categories according to [Bongers et al. \(1991\)](#): 1 colonizers (r-strategists, opportunistic species); 2 tolerant; 3 moderate; 4 highly sensitive to pollution and stress; 5 persisters (k-strategists, faunal components of a stable and complex food web). The weighted mean of the individual c-p values is calculated as the Maturity Index (Mi) according to the formula by [Bongers \(1990\)](#): $Mi = \sum_{i=1}^n v(i) \cdot f(i)$, where $v(i)$ is the c-p value of genus i and $f(i)$ is the frequency of that genus in the sample. Functional diversity of nematodes was also analyzed considering the feeding types following the classification by [Wieser \(1953\)](#). Nematodes were assigned to four feeding groups based on the morphology of the buccal cavity: selective feeders (1A), non-selective feeders (1B), epistrate feeders (2A) and omnivores/predators (2B). Feeding types 1A and 1B characterized by unarmed buccal cavity feed on microorganisms, while 2A-types use tooth-like structures to scrape off biofilms and 2B-types present large buccal cavities, usually with multiple ‘teeth’ to hunt and hold prey ([Wieser, 1953](#)). As an indicator of the trophic structure, the index of trophic diversity (ITD, Θ) based on [Wieser’s \(1953\)](#) classification was calculated as: $\Theta = \sum_{i=1}^n \frac{1}{q_i^2}$ where q_i is the proportion of feeding type i in the community and n is the amount of

feeding types ([Heip et al., 1985](#)).

2.4. Data analysis

Univariate nematode community-dependent variables were calculated, and they included the species richness (SR), the expected species $ES_{(50)}$ number on a standardized number of fifty individuals, and Pielou’s Evenness (J'). Species abundances were presented in k-dominance plots, in which species were ranked in decreasing order of dominance. The percentage cumulative abundance was subsequently plotted against the species rank k (see [Lambshead et al., 1983](#) for details). Multivariate characteristics of sediments were investigated using principal component analysis (PCA) to visualize data trends. PCA with Euclidean distance was performed to highlight differences in environmental variables among stations. To assess differences between impact levels and sediment layers (vertical distribution) in nematode response variables (i.e., total abundance, total biomass, SR, $ES_{(50)}$ and J'), functional diversity (i.e., ITD and Mi) and nematode community composition, a permutational multivariate analysis of variance (PERMANOVA) ([Anderson, 2001](#)) was carried out. The analysis was conducted by a multifactorial model including ‘impact’ (3 levels) and ‘layer’ (2 levels) as fixed factors, using 999 permutations under reduced model. Factors that showed significant statistical differences derived from the PERMANOVA analysis were selected to perform a pair-wise test to determine specific differences between their levels. Distance-based test for homogeneity of multivariate dispersions (PERMDISP) was performed to assess whether the dispersion between groups was significant or not. The similarity percentages (SIMPER; cut-off 80%) procedure was used to investigate the turnover diversity in nematode species composition and replacement of species among different levels of impact and sediment layers. Data were fourth root transformed to decrease the effect of numerically dominant species on the community composition and a resemblance matrix using Bray–Curtis similarity was calculated prior to multivariate analysis in Primer ([Clarke and Gorley, 2006](#)).

To visualize and compare the differences of nematode community

composition among impact levels a Canonical analysis of principal coordinates (CAP) analysis was applied on a Bray-Curtis similarity matrix of fourth root transformed data. Finally, to model the relationships between the univariate nematode variables and environmental predictors, as well as between the multivariate nematode community composition and the environmental predictors we used the distance-based linear model DistLM with dbRDA to visualize the results in PERMANOVA (Anderson et al., 2008). The environmental parameters, previously normalized, to conduct DistLM were total petroleum hydrocarbons (TPHs), total polycyclic aromatic hydrocarbons (PAHs), barium (Ba), aluminum (Al), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), (lead) Pb, magnesium (Mg), mercury (Hg), nickel (Ni), silver (Ag), titanium (Ti) and vanadium (V), and selected natural habitat characteristics (i.e., depth, sediment grain size, porosity and total organic C). *P* values were obtained with 999 permutations of the model. The Monte Carlo permutation *P* was used when the number of permutations was lower than 150. If significant differences were detected, posteriori pair-wise comparisons were performed using 999 permutations under a reduced model. The software package Primer-E v.7 was used for all the multivariate analyses and for index computation.

3. Results

3.1. Environmental variables

All environmental variables considered in the present study are reported in Table S1. PC1 included information representing the

sedimentary background of the study area encompassing variables such as Al, Cr, Fe, Ni and V. Variables that loaded strongly in the positive axis of PC2 were PAH44, TPH and Ba proxies of oil spill pollution and drilling activity (Table S2, Fig. 2A) and percentage of sand, instead mud loaded on the negative axis. The first two PCA axes accounted for 53.9% of the total variation. Higher concentration of heavy metals such as Fe, Cr, Ni and V reported at not impacted stations, drove the positions of HI and NI stations along the PC1, while MI stations were mixed with NI (Table S2, Fig. 2A). Along PC2 axis, stations were more driven by PAH44, TPH and Ba contents, higher at the HI and MI stations (Table S2). Variables most strongly loading on the positive axis PC3 were Porosity, Carbon and Al indicative of background conditions, while Cd and Ti strongly loaded on the negative axis PC3 reflecting the influence of crude oil related trace-metals (Table S2, Fig. 2B). PC1 – PC3 accounted for 64.8% of the variability explained.

3.2. Nematode assemblages

3.2.1. Nematode abundance and biomass

Nematode total abundances varied from a minimum total value of 715 ind./10cm² to a maximum total value of 8,509 ind./ 10cm² at stations 1.02 (NI) and LBNL1 (HI), respectively (Table 1). Abundances generally decreased from 0 to 1 cm to the deeper sediment layer 1-3 cm, except at two no-impact stations 1.02 and D085S (Table 1, Fig. S1A). Nematode individual average biomass ranged from 0.013 µgC/10cm² to 0.179 µgC/10cm² at D062S (NI) and D021S (NI) stations respectively, while total nematode biomass ranged from 24.1 µgC/10cm² to 316.1

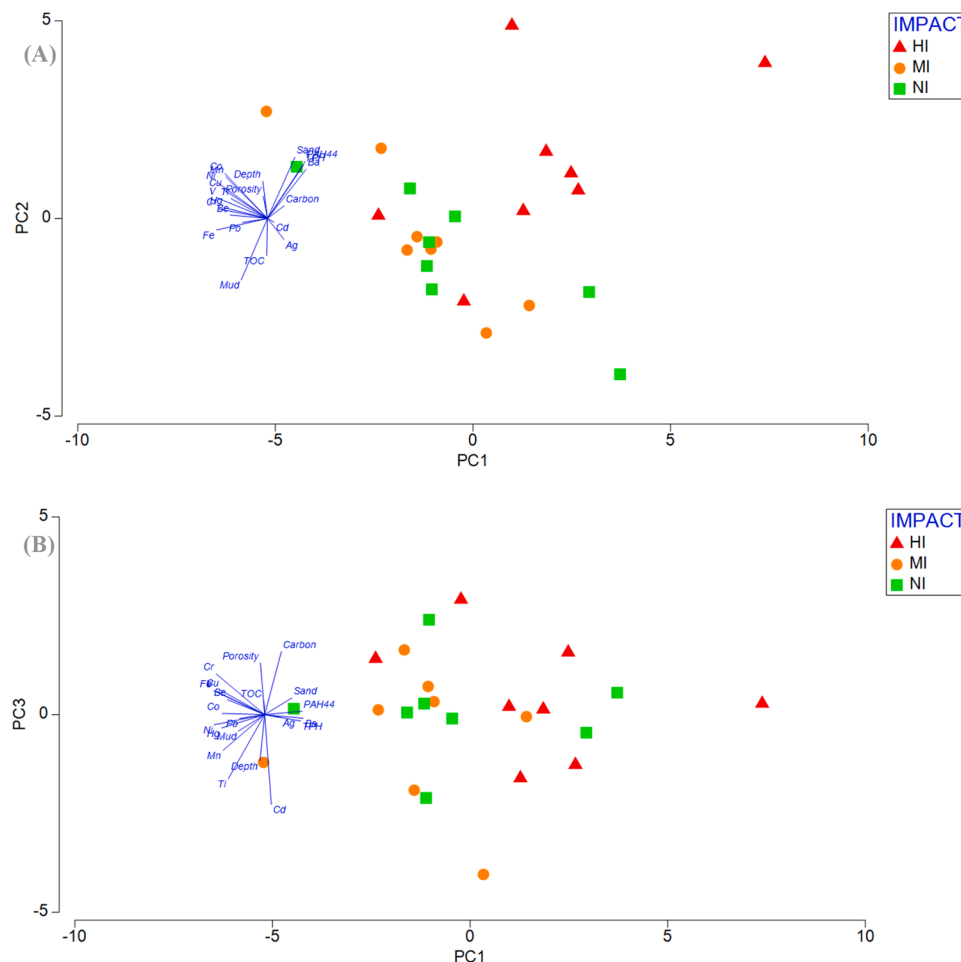


Fig. 2. A, B. Two-dimensional PCA ordination loads on PC1 – PC3 and based on sedimentary variables characterizing all investigated stations. PC1 – PC3 accounted for 64.8% of the variation. HI= high impact (red triangles), MI= medium impact (orange circles), and NI=non-impact (green squares). For clarity, overlapping labels are: Sand, PAH44, TPH, Co, Mn, Cr and Hg (2A); Ba, TPH, Fe, Cu, V, Ni and Hg (2B).

Table 1

Nematode descriptors characterizing the investigated stations and sediment layers. Abbreviations: HI =high impact; MI= medium impact; NI= no-impact; Tot ind =total individuals; NCwt = individual carbon weight; SR = species richness; ES(50) = expected species number; J' = evenness; ITD = index of trophic diversity; Mi = maturity index; 1 = 0-1 cm; 2 = 1-3 cm.

Station	Impact level	Tot ind./10cm ²	NC_wt (μg)	NCwt (μg)/10cm ²	SR	ES ₍₅₀₎	J	ITD	Mi
D040S_1	HI	2095.1	0.019	73.0	27	17	0.64	0.53	1.87
D040S_2	HI	95.2	0.040	5.2	22	19	0.88	0.46	2.34
D038SW_1	HI	778.6	0.034	49.8	16	11	0.56	0.79	1.41
D038SW_2	HI	55.2	0.078	17.3	11	10	0.69	0.71	1.58
ALTNF001_1	HI	3857.0	0.027	21.4	17	13	0.67	0.64	1.49
ALTNF001_2	HI	129.7	0.049	2.7	27	22	0.83	0.46	2.15
NF006MOD_1	HI	2982.4	0.039	81.4	19	13	0.49	0.72	1.49
NF006MOD_2	HI	217.3	0.052	5.0	21	14	0.51	0.70	1.49
NF011_1	HI	3689.0	0.024	193.1	29	17	0.62	0.62	1.84
NF011_2	HI	458.2	0.046	21.6	24	16	0.79	0.59	2.10
3.33_1	HI	2813.5	na	na	28	20	0.78	0.39	2.13
3.33_2	HI	181.1	na	na	25	19	0.83	0.48	2.24
LBNL1_1	HI	8037.9	0.025	73.7	7	5	0.35	0.94	1.22
LBNL1_2	HI	471.2	0.032	6.8	12	8	0.43	0.76	1.35
D031S_1	HI	1446.1	0.019	70.6	18	13	0.74	0.59	2.07
D031S_2	HI	221.9	0.058	26.4	17	15	0.83	0.40	2.98
D050S_1	MI	5323.4	0.048	46.9	29	19	0.72	0.41	2.20
D050S_2	MI	896.6	0.032	18.8	31	22	0.83	0.50	2.07
FF010_1	MI	1598.1	0.034	182.1	20	14	0.66	0.51	1.62
FF010_2	MI	473.3	0.149	134.0	34	23	0.86	0.45	2.25
LBNL3_1	MI	2486.7	0.018	37.2	23	16	0.69	0.45	2.34
LBNL3_2	MI	759.3	0.016	23.9	25	19	0.84	0.57	2.69
LBNL5_1	MI	1812.5	0.022	35.1	21	14	0.52	0.63	2.20
LBNL5_2	MI	943.7	0.071	33.7	25	18	0.84	0.47	2.65
D057S_1	MI	2089.2	0.014	35.7	28	18	0.56	0.58	2.05
D057S_2	MI	1481.9	0.035	26.5	32	22	0.82	0.38	2.22
NF008_1	MI	4126.1	0.020	35.8	9	9	0.82	0.59	1.85
NF008_2	MI	251.8	0.025	23.2	20	15	0.76	0.63	1.96
ALTNF015_1	MI	977.8	0.016	66.2	23	15	0.67	0.57	2.18
ALTNF015_2	MI	585.8	0.057	14.4	18	17	0.96	0.35	2.44
NF013_1	MI	1522.3	0.037	57.0	26	17	0.67	0.55	2.21
NF013_2	MI	527.7	0.052	27.4	29	20	0.84	0.46	2.45
LBNL17_1	NI	1577.9	0.016	9.6	25	17	0.74	0.49	2.08
LBNL17_2	NI	437.1	0.046	25.3	20	14	0.64	0.43	2.25
D019S_1	NI	584.5	0.022	17.2	28	19	0.81	0.37	2.34
D019S_2	NI	546.6	0.042	24.1	32	22	0.78	0.46	2.38
D085S_1	NI	827.1	0.014	8.6	31	22	0.87	0.30	2.64
D085S_2	NI	1126.5	0.041	19.0	30	22	0.88	0.34	2.31
1.02_1	NI	352.1	na	na	45	31	0.91	0.30	2.57
1.02_2	NI	362.6	na	na	23	18	0.87	0.49	2.07
D010S_1	NI	754.2	na	na	39	27	0.90	0.32	2.91
D010S_2	NI	432.1	na	na	32	23	0.91	0.34	2.48
NF014_1	NI	1403.2	0.024	20.2	27	17	0.71	0.42	2.03
NF014_2	NI	759.3	0.053	59.9	33	24	0.83	0.41	2.43
D021S_1	NI	768.1	0.017	27.0	23	16	0.73	0.34	2.44
D021S_2	NI	579.5	0.179	78.3	24	17	0.69	0.51	2.32
D062S_1	NI	604.7	0.013	18.1	30	22	0.86	0.32	2.54
D062S_2	NI	462.8	0.050	38.3	34	25	0.91	0.31	2.53

μgC/10cm² at ALTNF001 (HI) and FF010 (MI) stations. Overall, individual average biomass increased from the top 1 cm to the deeper sediment layer (Table 1, Fig. S1B). PERMANOVA analysis reported significant differences in nematode abundances across impacts, between sediment layers and significant impact-layer interaction factors (Table 2). In detail, pair-wise test detected differences between NI stations and HI – MI stations with lower abundances characterizing NI stations (i.e., avg = 3442±2337 ind./10cm², avg = 3232±1517 ind./10cm² and avg = 1447±528 ind./10cm² at HI, MI and NI stations respectively). Moreover, pair-wise test reported significant difference between sediment layers at HI and MI stations but none at NI stations (Table 2). PERMDISP test did not show any significant dispersion around centroids, neither among impacts (P(per)= 0.407) nor between layers (P(per)= 0.490). Nematode individual average biomass changed significantly only between layers (PERMANOVA, Table 2 and PERMDISP test P(per)= 0.652) (Fig. S2), while a significant interaction between impact and sediment layers was detected when the total nematode biomass was considered (Table 2). In detail, significant changes in total biomass were reported between layers at HI and NI

stations, with higher values characterizing the top one centimeter at HI stations and an opposite trend at NI stations.

3.2.2. Nematode diversity and community composition

A total of 128 genera and 189 species/morphotypes were identified, belonging to 32 families (Table S3). The lowest diversity was reported at impacted station LBN1 (SR = 7 and ES₍₅₀₎ = 5), while the highest diversity characterized the NI station 1.02 (SR = 45 and ES₍₅₀₎ = 31) (Table 1, Fig. S3). Equitability ranged from 0.35 (at station LBNL1, HI) to 0.96 (at station ALTNF015, NI) (Table 1). Diversity and equitability changed significantly across all impact levels and through the two sediment layers (PERMANOVA, Table 3), reporting higher values at NI>MI>HI stations and an overall higher diversity and equitability characterizing the deeper sediment layer at HI stations and at some of the MI and NI stations (Table 1). PERMDISP test did not report any significant dispersion around centroids, meaning that the differences among impacts and layers were due to a real difference in nematode diversity indices (P(per) = 0.750 and P(per) = 0.615). The k-dominance curves (Fig. S2) graphically illustrate a distinct picture of a

Table 2

PERMANOVA results and pairwise tests for nematode abundance, individual biomass and total biomass. Symbols: * $p < 0.05$, ** $p < 0.01$, n.s. = not significant. Abbreviations: df = degree of freedom; SS = sum of squares; MS = mean of squares; P(MC) = Monte Carlo test; IM = impact; LA = layer; 1 = 0–1 cm; 2 = 1–3 cm; Res = residuals.

Nematoda total abundance						Nematode individual biomass					
Source	df	SS	MS	Pseudo-F	P(MC)	Source	df	SS	MS	Pseudo-F	P(MC)
IM	2	9.61E+06	4.80E+06	3.83	*	IM	2	0.00	0.00	0.07	n.s.
LA	1	3.34E+07	3.34E+07	26.68	**	LA	1	0.01	0.01	14.76	**
IMxLA	2	1.48E+07	7.38E+06	5.89	**	IMxLA	2	0.00	0.00	0.79	n.s.
Res	42	5.26E+07	1.25E+06			Res	36	0.03	0.00		
Total	47	1.10E+08				Total	41	0.04			

PAIR-WISE TESTS				Term 'IM'		Nematode total biomass					
Groups	P(MC)					Source	df	SS	MS	Pseudo-F	P(MC)
HI, NI	*					IM	2	3.56E+10	1.78E+10	1.22	n.s.
MI, NI	**					LA	1	5.79E+10	5.79E+10	3.96	n.s.
HI, MI	n.s					IMxLA	2	1.48E+11	7.39E+10	5.05	*
PAIR-WISE TESTS						Res	36	5.27E+11	1.46E+10		
Term 'IMxLA' for pairs of levels of factor 'IMPACT'						Total	41	7.82E+11			
Within level '1'			Within level '2'			PAIR-WISE TESTS					
Groups	P(MC)		Groups		P(MC)	Term 'IMxLA' for pairs of levels of factor 'LAYER'					
HI, NI	*		HI, MI	**		Within level 'HI'			Within level 'NI'		
MI, NI	*		HI, NI	*		Groups	P(MC)		Groups	P(MC)	
HI, MI	n.s		HI, MI	n.s		1, 2	*		1, 2	*	
PAIR-WISE TESTS						Within level 'MI'					
Term 'IMxLA' for pairs of levels of factor 'LAYER'						Groups	P(MC)				
Within level 'HI'			Within level 'MI'			1, 2	n.s.				
Groups	P(MC)		Groups	P(MC)		Term 'IMxLA' for pairs of levels of factor 'IMPACT'					
1, 2	**		1, 2	**		Within level '1'			Within level '2'		
Within level 'NI'						Groups	P(MC)		Groups	P(MC)	
Groups	P(MC)					HI, NI	*		HI, NI	*	
1, 2	n.s.					MI, NI	*		MI, NI	n.s.	
						HI, MI	n.s.		HI, MI	n.s.	

tendency to dominance and decreasing diversity with impact level. Contaminated station curve (Fig. S4, red triangles) was above all curves with 10 species accounting for >70% of the total abundance. Orange circle curve, indicating MI stations, was in between HI and NI stations with 10 species accounting for >50% of the total abundance and 25 species accounting for >70% of the total abundance. The NI station curve (Fig. S2, green squares) below all the others indicating a more diverse community of nematodes, with 10 species accounting for <45% total abundance and 40 species accounting for >70% of the total abundance.

The most diversified nematode families were Chromadoridae and Xyalidae (both 26 spp./morphotypes) and Comesomatidae (23 spp./morphotypes). The most represented species in the whole sampling area were *Halomonhystera aff. hickeyi* (26%; Monhysteridae), *Daptonema* sp.1 (14%; Xyalidae), *Pierrickia* sp.1 and *Sabatieria* sp.1 (5% and 4%; both Comesomatidae). All other nematodes accounted for <3%. Seventy-five spp./morphotypes were reported from all impact levels, while seventy-four were unique and reported only from stations belonging to the same impact level; forty spp./morphotypes were shared between two impact levels (Fig. 3). The NI stations reported the highest number of unique species (35 out of 74). However, the abundances of all unique species across impact levels were $\leq 1.2\%$, as shown in the Venn diagram reporting the three most represented species at each impact level (Fig. 3).

According to PERMANOVA analysis, the nematode community composition changed significantly between HI and NI stations (PERMANOVA, $p = 0.001$; PERMDISP test $P(\text{perm}) = 0.357$) and between the two sediment layers (PERMANOVA, $p = 0.001$; PERMDISP test $P(\text{perm}) = 0.159$) (Table 3). The SIMPER analysis reported a dissimilarity of 69.8% between HI vs. NI stations and the species *Halomonhystera aff. hickeyi*, highly represented at HI stations, as the one that mostly contributed to the dissimilarity between the impact levels (Table S3). CAP analysis showed a clear separation between HI and NI groups of stations along CAP1, with MI stations between the two groups (Fig. 4) indicating a shift in community composition across impacts. Along

CAP2, MI stations are grouped in the upper part of the graph, separately from HI and NI stations. In Fig. 4 the nematodes that mostly explained (i.e., correlation >0.5) the station distribution were superimposed. *Halomonhystera aff. hickeyi* characterized the HI stations, while many unique species reported only from the NI stations identified that group positioned on the left side of the graph (e.g., *Anoplostoma* sp., *Anticoma* sp., *Belbolla* sp., *Calligyris* sp., *Daptonema* sp.2, *Dagda* sp.). *Doliolaimus* sp. was reported only once from MI station group. A dissimilarity percentage of 72% was reported between sediment layers. *Halomonhystera aff. hickeyi* and *Linhystra* sp.1 characterized the top 1 centimeter of the sediment, while *Pierrickia* sp.1 was reported particularly abundant from the deepest layer (Tables S3 and S4).

3.2.3. Nematode functional diversity and gender

The functional diversity indices, ITD and Mi, varied significantly according to the impact level (PERMANOVA, Table 3 and PERMDISP test $P(\text{perm}) = 0.512$). The ITD values ranged from an avg. = 0.61 (HI stations) to an avg. = 0.51 (MI stations) and an avg. = 0.38 (NI stations) (Table 1). Higher ITD values indicated the dominance of one or two trophic groups in impacted stations, mainly deposit feeders (1A and 1B represented >80%), compared to the presence of more trophic groups in NI stations (i.e., epistrate feeders 2A or/and predators/omnivores 2B constituted 30–40%). The maturity index increased from an avg. = 1.86 (HI stations) to avg. = 2.39 (NI stations) (Table 1), indicating a dominance of colonizers and opportunistic nematodes (c-p values equal to 1 or 2, Table S4) at the more impacted stations versus a population of more persistent/intermediate nematodes (c-p values equal to 3 or 4, Table S4) at NI stations. Juveniles represented most of the population at HI (60%) and MI (59%), while adults represented 51% of the population at NI stations (Fig. S3). Females were more abundant than males at NI stations (27% vs. 24%, respectively), but their contribution decreased with increasing impact level (18–19%) while males' contribution remained stable (22–24%) (Fig. S5). Significant differences in nematode gender contribution were reported between NI vs. HI-MI stations (PERMANOVA, $p = 0.001$; PERMDISP test $P(\text{perm}) = 0.151$).

Table 3

PERMANOVA results and pairwise tests for nematode diversity, equitability, community composition and functional diversity (i.e., ITD and Mi). Symbols: * $p < 0.05$, ** $p < 0.01$, ns = not significant. Abbreviations: df = degree of freedom; SS = sum of squares; MS = mean of squares; P(MC) = Monte Carlo test; IM = impact; LA = layer; Res = residuals.

Nematode diversity						Nematode ITD					
Source	df	SS	MS	Pseudo-F	P(MC)	Source	df	SS	MS	Pseudo-F	P(MC)
IM	2	976.47	488.23	10.98	**	IM	2	0.41	0.21	17.40	**
LA	1	171.14	171.14	3.85	*	LA	1	0.01	0.01	0.89	n.s.
IMxLA	2	250.96	125.48	2.82	n.s.	IMxLA	2	0.04	0.02	1.80	n.s.
Res	42	1867.20	44.46			Res	42	0.50	0.01		
Total	47	3265.70				Total	47	0.96			
PAIR-WISE TESTS						PAIR-WISE TESTS					
Groups	P(MC)					Groups	P(MC)				
HI, MI	*					HI, MI	*				
HI, NI	**					HI, NI	**				
MI, NI	**					MI, NI	**				
Nematode equitability						Nematode Mi					
Source	df	SS	MS	Pseudo-F	P(MC)	Source	df	SS	MS	Pseudo-F	P(MC)
IM	2	0.18	0.09	7.22	**	IM	2	2.37	1.19	11.31	**
LA	1	0.12	0.12	9.33	**	LA	1	0.34	0.34	3.20	n.s.
IMxLA	2	0.07	0.03	2.68	n.s.	IMxLA	2	0.43	0.22	2.06	n.s.
Res	42	0.53	0.01			Res	42	4.40	0.10		
Total	47	0.90				Total	47	7.54			
PAIR-WISE TESTS						PAIR-WISE TESTS					
Groups	P(MC)					Groups	P(MC)				
HI, MI	*					HI, MI	*				
HI, NI	**					HI, NI	**				
MI, NI	*					MI, NI	*				
Nematode community composition											
Source	df	SS	MS	Pseudo-F	P(MC)						
IM	2	7969.80	3984.90	1.82	**						
LA	1	12399.00	12399.00	5.66	**						
IMxLA	2	4844.70	2422.40	1.11	n.s.						
Res	42	91989.00	2190.20								
Total	47	117200.00									
PAIR-WISE TESTS											
Groups	P(MC)										
HI, NI	**										
HI, MI	n.s.										
MI, NI	n.s.										

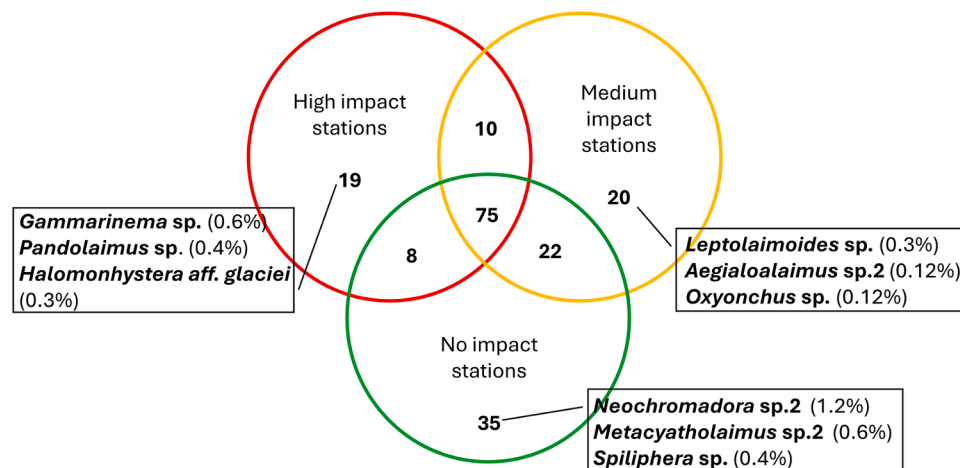


Fig. 3. Venn diagram showing the number of shared species between impact levels and the number of unique species reported from different impacts. The top three most represented nematode species from each impact level (names in the rectangles) and their percentages are reported.

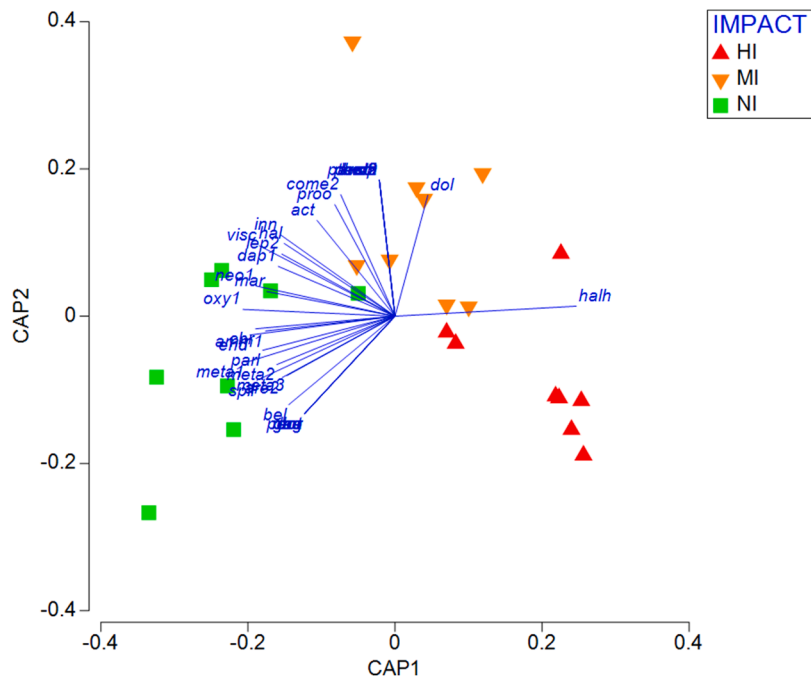


Fig. 4. CAP output with superimposition of nematodes on stations (correlation >0.5). Abbreviations: HI = high impact, MI= medium impact, NI = no impact. Abbreviations of nematode names are listed in Table S4. For clarity, overlapping labels are: parc2 and parm2 pointing towards the MI stations; chr, end, anto1, parl, meta1, meta2, meta3, spil, bel, gno, gre and pasp pointing towards the NI stations.

3.3. Influence of pollution and environmental features on nematode communities

The results of the DistLM-forward analysis and sequential test reported in Table 4, show that 16% of the variance in the nematode community composition was significantly explained by the TPH and Mn content of the sediment. In dbRDA graph (Fig. 5) environmental variables reported as *best solution* (i.e., most parsimonious model of explanatory variables that effectively predicts a variation in nematode communities) are shown. TOC content, mud, and some heavy metals (e.

g., Ni, Ag, Hg, Ti, Fe) were related to the NI and MI stations; while TPH, PAH44 and Ba are strongly related to HI stations. TOC content, PAH44 and Be explained 45% of the variability in nematode diversity. Nematode functional diversity (as trophic diversity and maturity index) was explained (ITD 49% and Mi 66%) by heavy metals and pollutant content in the sediment and secondarily by percentage sand fraction characterized HI stations (Table 4). None of the pollutants and environmental variables considered in the present study significantly explained the variability in nematode standing stock (i.e., abundance and biomass).

4. Discussion

Under the Oil Pollution Act of 1990 (OPA-90, 33 U.S.C.), federal and state trustees are in charge of assessing damages to natural resources caused by an oil spill as part of the Natural Resource Damage Assessment (NRDA) process. After a large-scale spill such as DWH, there have been consistent conclusions that adequate pre-spill baseline contamination and ecosystem monitoring studies would have made the NRDA process much more focused on the incremental impacts of the spill rather than being focused on disentangling a particular spill from background contamination from various anthropogenic inputs and natural factors (Murawski et al., 2021). While environmental/habitat sensitivity indices (E/HSIs) are used in current planning documents (e.g., BOEM, 2018), they also are primarily summarized at a coarse level of spatial resolution (e.g., for entire planning areas and comparisons among them; BOEM, 2018). Since deepwater biota highly differ from those located on continental shelves and at coastal margins, oil spills in those areas will encounter a completely different scenario and ecosystem(s), and thus resources at risk will be different (BOEM, 2018).

4.1. Nematodes standing stock and diversity after the DWH oil spill

The first short-time response registered in the deep-sea benthic compartment after the DWH spill was an impressive peak in the total meiofauna abundances around the wellhead (Montagna et al., 2013, and Baguley et al., 2015). This unusual abundances in deep-sea meiofauna were mainly due to a proliferation in the nematode communities with

Nematode community composition					
Variable	SS(trace)	Pseudo-F	p	Prop.	Cumul.
Mn	3541.70	2.05	*	0.09	0.09
TPH	2964.10	1.78	*	0.07	0.16
Nematode Diversity					
TOC	121.10	8.52	*	0.28	0.28
PAH44	53.71	7.00	*	0.12	0.40
Be	20.63	5.39	*	0.05	0.45
Index of trophic diversity (ITD)					
Cu	0.07	5.27	*	0.17	0.17
Pb	0.07	15.78	**	0.16	0.33
Sand	0.05	4.61	*	0.12	0.45
Cu	0.02	6.70	*	0.04	0.49
Maturity index (Mi)					
TPH	0.47	4.14	*	0.16	0.16
PAH44	0.47	4.86	*	0.16	0.32
Sand	0.44	5.58	*	0.15	0.47
Porosity	0.37	5.76	*	0.12	0.59
Cd	0.21	4.64	*	0.07	0.66

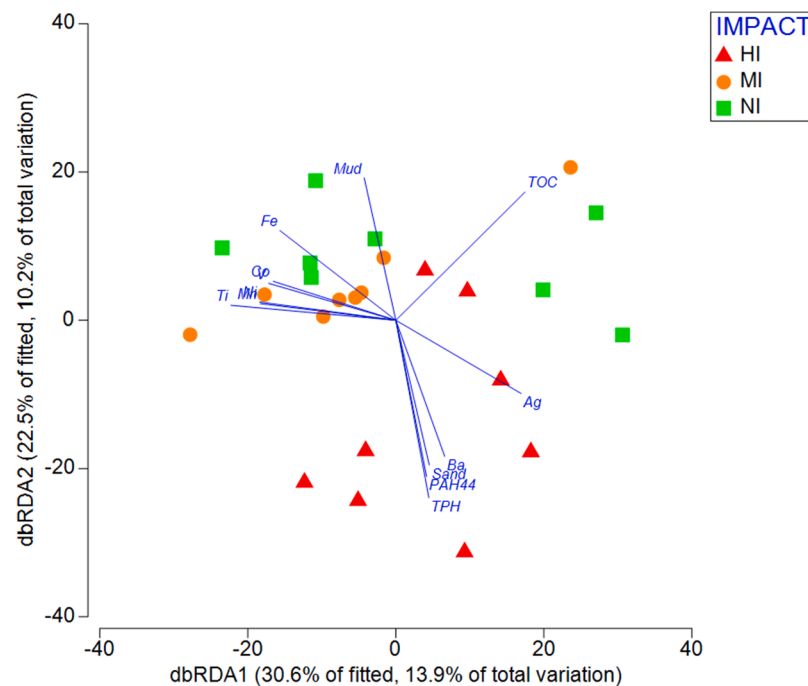


Fig. 5. dbRDA graph on nematode community composition showing the effects of the most significant environmental variables on nematode distribution across impact levels.

increasing level of impact.

In the present study, the nematode fauna inhabiting the deep-sea Gulf of Mexico sediments after the DWH oil spill was characterized for the first time. Nematode densities reported from impacted stations were two times higher compared to the background stations (NI stations) and compared to other studies conducted in the Gulf of Mexico at comparable depths (Sharma et al., 2012; Vargas-Esposito et al., 2023), in shallower sites (Beaton et al., 2018) and from sediments surrounding offshore oil platforms (Montagna and Harper, 1996). Similar abundances were reported in the Gulf of Mexico continental slope (Baguley et al., 2006) from shallower stations (212–763 m depth) characterized by high sedimentation rates and from a canyon site located at 1826 m depth. Nematode abundances reported from no-impact stations (avg. 1774 ind./10cm²) were, instead, comparable to background conditions and to abundances typical reported from deep-sea sediments (Sharma et al., 2012; Schnier et al., 2023; Armenteros et al., 2024). The rapid increase in meiofauna and particularly in nematodes was explained by the enrichment-toxicity paradox: the oil spill promotes the development of indigenous bacteria, which in turn become available food for deep-sea fauna (Montagna et al., 2013; Fisher et al., 2014; Baguley et al., 2015). This rapid reaction of the benthic communities is evidence for a community-level trophic response and an example of bottom-up control on meiofauna and nematode fauna populations to bacterial and/or other organic constituents of the DWH fallout plume (Valentine et al., 2014; Baguley et al., 2015; Ghosh and Mandal, 2021). Responses to oil contamination can be different according to the habitats and pollution intensity. For instance, Stark et al. (2017) reported that the increase in microbial abundances in sediments from shallow Antarctic Sea did not appear to have any positive effect on nematode abundance, indicating that the type of bacteria was not suitable for nematodes or that the toxic components had effects that were stronger than the effect of increased food.

The distribution between the two sediment layers in the nematode fauna abundances reflected the environmental conditions: in the impacted stations most of the nematodes were concentrated in the top 1 centimeter, where food was abundant (e.g., POC and microbes), while in the non-impact station nematodes were equally distributed across the

sediment layers indicating more stable conditions and a certain level of sediment homogeneity along the vertical profile (Rosli et al., 2018). Another factor that could have promoted the presence of nematodes at the sediment surface of impact stations was a reduced pressure of predation by bigger size animals (e.g. macrofauna), due to their high mortality reported after the spill (Montagna et al., 2013, 2017; Reuscher et al., 2020).

Biomass is a functional descriptor, and it can be an indicator of functional changes in the system due to human and/or natural impacts. Changes in benthic biomass spectra have been reported in stressed ecosystems (e.g., Duplisea, 2000; Vanaverbeke et al., 2003). In the present study, nematode biomass has been used as a proxy of impact derived from the DWH oil spill. None of the previous studies focused on the DWH impact assessment used the benthic biomass as impact proxy (e.g. Montagna et al., 2017; Brannock et al., 2017; Baguley et al., 2024; Baldrighi et al., 2024). Even if no significant changes in individual and total nematode biomass were detected across impact levels, the distribution between sediment layers across impacts presented interesting trends related to the effects of the oil spill. Soetaert et al. (2002) reported that main differences in nematode body size may occur through the sediment vertical profile over a scale of few centimeters rather than over larger spatial scales. Small-size nematodes populated the top (0–1 cm) of the sediments at all stations, while bigger size nematode (up to three times bigger) were found inhabiting the 1–3 cm sediment layers. The exceptionally high deposition rates of organic and inorganic particles mixed with spill-related substances to the sea floor (Yan et al., 2016) and the consequent stimulation of microbial growth made the deep-sea sediments of impacted stations extraordinary food-rich compared to background stations thus, with some expectation of finding bigger size nematodes at the sediment surface. However, the presence of smaller nematodes can be explained by a dominance of juveniles instead of adults, indication of a population in a reproduction phase with an investment of energy on rapid colonization process instead of biomass storage and body growth. Smaller nematodes can often be designated as ‘colonizers’ since they show rapid growth, faster metabolic rates and early reproduction, characteristics often associated with frequently disturbed habitats and presence of pollutants (e.g., Monteiro et al.,

2018). Vanaverbeke et al. (2003) reported an increase in nematode reproduction rates during periods of enhanced deposition to the sea floor, followed by deaths of adults and the emergence of a next generation, explaining lower values in biomass when many juveniles were present. Other factors such as sediment characteristics and oxygen conditions are known to have a significant influence on the distribution of nematode standing stock and diversity into the sediments (Giere, 2009). Reduced individual biomass values coupled with high densities in the surface sediment layers and low structural and functional diversity characterized nematode communities inhabiting oxygen-depleted, organic-enriched sediments from central Chile margin (Neira et al., 2013). These findings are similar to our results from impacted stations in the GoM.

The preference of bigger nematodes for populating deeper sediment layer, reported from all impact level stations, can be explained by different reasons. Bigger nematodes inhabiting muddy substrates burrow faster through the sediment (Soetaert et al., 2002) and in this specific case study, this capability made them able to avoid stress conditions present at the surface sediments in impacted stations. Thus, it has been reported that longer nematodes may be more sensitive to environmental disturbances (Vanaverbeke et al., 2007). In background stations (NI stations), other factors such as difference in sediment characteristics across the vertical profile (e.g., organic loading, historical pollution, microscale bioturbation, avoidance of competition and/or predation) might have played an important role in determining nematode biomass trends with a 'migration' of bigger nematodes into the deeper layer (Sharma et al., 2011; De Jesús-Navarrete et al., 2022; Armenteros et al., 2024). Total biomass values reported here were overall higher than values reported from other deep-sea studies (e.g., Soetaert et al., 2009; Sharma et al., 2011; de Jesús-Navarrete et al., 2022; Armenteros et al., 2024), mainly due to high nematode abundances reported in most of the stations.

The sole analysis of nematode standing stock patterns, across impact levels and between sediment layers after the DWH oil spill, suggested the presence of an acute impact at stations located close to wellhead and defined as high and medium- impact stations compared to non-impact stations. Nematode abundance and biomass are, indeed, valuable proxies for assessing oil spill impact and consequently indicators of functional changes in nematode communities (Naidoo and Naidoo, 2024).

The total number of genera and species/morphotypes identified in the present studies were higher than values reported from other studies in the Gulf of Mexico (Sharma et al., 2012; Beaton et al., 2018; de Jesús-Navarrete et al., 2022; Armenteros et al., 2024). The diversity indices and the index of equitability well represented the increasing level of contamination across stations: the diversity was higher at no-impact stations combined with a low dominance, while opposite trends were reported from impacted stations. Low diversity-high dominance is frequently reported as a sign of environmental stress in marine ecosystem (Schratzberger et al., 2007).

4.2. Evident shift in nematode community composition across impact levels

The nematode genera reported in the present study were previously reported populating the deep Gulf of Mexico sediments from the shelf to the abyssal plain (Soto et al., 2017; Armenteros et al., 2022; Vargas-Espositos et al., 2023). Seventy-five species were shared between all impact levels and four of them were dominant (>4%) across all stations: *Halomonhystera aff. hickeyi*, *Daptonema* sp.1, *Pierrickia* sp.1 and *Sabatieria* sp.1. Most of the other shared species significantly differed across impact levels in their total abundances, indicating a different level of tolerance/sensitivity of some nematodes (e.g., *Desmoscolex* sp1, *Daptonema aff. longessimecaudata*, *Elzalia* spp., *Laimella aff. longicauda*, *Viscosia* sp1) but without being eliminated (i.e., intolerant species).

All four dominant genera are widespread and some species belonging

to these genera can easily thrive in stress conditions such as low oxygen, high organic matter loads, pollutants such as hydrocarbons and heavy metals (Armenteros et al., 2009; Allouche et al., 2020; Ghosh and Mandal, 2021; Schratzberger et al., 2023). Nematodes can also detoxify absorbed or ingested metals by using metal-binding proteins and forming subcellular inclusions (Monserat et al., 2003).

Halomonhystera aff. hickeyi was the dominant species at high and medium impact level. Species belonging to this genus, such as *H. disjuncta* (inhabiting shallow waters) and *H. hermesi* (found in deep-sea sediments), are well known to be able to thrive in unfavorable and extreme conditions. *H. hermesi* was reported inhabiting the sulphide rich bacterial mats of the Nyegga pockmark (Nordic Norwegian margin), and the Mosby mud volcano (HMMV, Barent Sea slope), situated at depths of 730 m and 1280 m, respectively (Van Campenhout et al., 2014). *H. disjuncta* showed an ability to an ovoviviparous reproduction strategy when environmental conditions become harsh (Grzelak and Kotwicki, 2016). Ovoviviparity allows higher chances of survival and early development of offspring, an adaptation strategy in toxic environmental conditions (Boffe', 1985).

Even if the contribution of *H. aff. hickeyi* remained particularly high across the study stations, it is interesting to notice how it sharply decreased from HI to MI and NI stations: 50%, 27% and to 6.5%, respectively. When considering the many shared species, gradual changes in nematode community composition were revealed between impact and no-impact stations and at different sediment depths. These changes were reflected in high values of turnover diversity (i.e., 70% among impacts and 71% along the sediment vertical profile) showing different levels of tolerance-sensitivity to PAHs pollution and presence of heavy metals. Similar results were reported in Ghosh and Mandal (2021), reporting a spectrum of responses and tolerance levels among nematode communities under high temperature and diesel contamination. Species within a community vary broadly in their response to contamination and their response is linked to many factors such as level of contamination, type of contamination, toxicity, bioavailability of contaminants into the sediments (Mahmoudi et al., 2005; Louati et al., 2015). And the abilities of each nematode genus (and species) to cope with toxic compounds of hydrocarbons would also differ (Stark et al., 2017).

The presence of unique species characterizing each impact level (see Fig. 3) was the evidence of a nematode fauna characterized by a population of intolerant species present only at NI stations (e.g., *Neochromadora* sp.2, *Metacyatholaimus* sp.2) vs a population of opportunistic and tolerant species reported only at HI (e.g., *Gammarinema* sp., *Halomonhystera aff. glaciei*) or at MI (e.g., *Leptolaimoides* sp., *Oxyonchus* sp.) stations. Species shared between HI-MI and HI-NI can be defined as resistant to different levels of pollution. The medium impact stations shared the highest number of species, 32, with high impact and no-impact stations and resulting in a 'transitional' community between two opposite conditions. The unique species reported from NI stations are well known deep-sea genera/species inhabiting pristine sediments (Sharma et al., 2011; Jones et al., 2016; Beaton et al., 2018), while unique species reported from MI and HI have been commonly reported from impacted sediments (Armenteros et al., 2009, 2024; Elarbaoui et al., 2015). Moreover, the presence of many unique genera in the NI stations (35) underlined the fact that many genera/species were sensitive to pollution and they simply disappeared from the impacted sediments (e.g., *Belbolla*, *Calligyrrus*, *Dagda*, *Rhps*, *Spiliphora*, *Spilophorella*, *Wieseria*), while more tolerant/opportunistic genera proliferate in HI stations (Soto et al., 2017; Lv et al., 2011).

In summary, the high turnover diversity in nematode community composition across impacts was supported by the presence of unique nematode species characterizing different level impact. These unique species (or morphotypes) even if rare (i.e., most of them were present as singleton or doubleton) can have a role as sentinels of different magnitude of oil spill impact affecting the deep-sea sediments in the Gulf of Mexico.

The biomass changes between sediment layers were reflected in a typical small-size nematode community (e.g., *Halomonhystera*, *Linhyss-tera*, *Elzalia*) inhabiting the top one centimeter compared to a community of bigger nematodes living the deeper layer. Large body size nematodes such as *Pierrickia*, *Sabatieria* and *Terschellingia* are known for being well adapted to anoxic conditions, for having burrowing abilities and a preference for deeper sediments where they can find free niches to occupy (Armenteros et al., 2009 and literature therein; Hong et al., 2020). Many factors can influence the vertical distribution of nematodes: oxygen depletion, organic matter availability, sediment texture, presence of pollutants (Schratzberger et al., 2006; Steyaert et al., 2007). In the present study, one of the main affecting factors was certainly the presence of high pollutant loads. Even if sediment oxygen data were not collected, we can speculate on the presence of an oxygen depletion at the sediment surface due to the high deposition rate and microbial activity (Carman et al., 2000) at the impacted stations. Low O₂ concentrations might also have been effective in influencing the nematode distribution across the two sediment layers (Neira et al., 2013). The DistLM-forward analysis reported a significant effect of TPH and PAH44 on nematode diversity and composition. At no-impact stations other factors, not tested in the present study, such as biotic interaction (e.g., competition for food resources, predation) might have intervened in the distribution of the nematode communities between layers (Steyaert et al., 2007; Rosli et al., 2018). Nematode assemblage shifts due to oil spill impact suggest that nematodes can be effective ecological indicators of this disturbance. Moreover, altered species composition could significantly influence interactions between nematodes and interactions among major benthic taxa (Carman et al., 1997; Stark et al., 2017).

4.3. Importance of using functional indices of nematode community in oil spill impact assessment

Life strategy and trophic composition of nematodes can provide useful evidence for environmental impact(s), they are good proxies in the assessment of ecological quality status (Semprucci et al., 2018; Baishnab et al., 2023) and indicators of potential changes in the ecosystem functioning (Danovaro et al., 2008).

In the present study, the trophic diversity (as ITD) and the life strategy (as Mi) of nematodes were investigated to assess the impact of DWH oil spill on the functionality of nematode fauna populations. In addition, the gender (males vs females) and the stage of life (juveniles vs adults) were investigated as important functional information of turnover dynamics of a population. The nematode functional diversity changed across impact levels. Overall, a low trophic diversity dominated by deposit feeders and colonizer/opportunistic nematodes were indicators of impacted sediments. The dominance of deposit feeders is commonly reported as feeding strategy in the deep-sea where the main sources of food are organic matter and microbes (Sharma et al., 2011; Landers et al., 2014; Joens et al., 2016; Soto et al., 2017). In the case of the impacted sediments in the Gulf of Mexico, the dominance of deposit/microbial feeders confirmed the proliferation of the microbial communities after the oil spill (Montagna et al., 2013, 2017). A feeding strategy based on rapid ingestion of microbes and organic detritus was combined with a r-strategy based on rapid colonization of the substrate. Most of the nematodes inhabiting the impacted sediments were nematodes identified as colonizers and opportunistic (c-p between 1 and 2). A similar shift towards deposit feeding – r-strategist nematodes was reported in Ghosh and Mandal (2021) under high temperature and diesel pollution conditions. Bik et al. (2012) reported a shift in nematode community post DWH spill towards a high abundance of juvenile nematodes characterizing some beach sites along the Alabama coast. The authors interpreted the abundance of juvenile life stages as a first sign of recovery. In the present study, the high abundance of juveniles was considered as an indication of a population in constant reproductive phase, fast turnover rates and high tolerance to hydrocarbon contamination (Naidoo and Naidoo, 2024).

In no-impact stations, other trophic groups such as epistrate feeders and predators/omnivore were also represented even if with lower abundances, indicating a more diversified trophic structure, an efficient partition of trophic niches (Schratzberger et al., 2019) and a better ecological quality (Moreno et al., 2011). Moreover, the life strategy of nematodes inhabiting NI sediments indicated a community of more persistent nematodes with presence of sensitive species (c-p between 3 and 4). The higher contribution of adults was an indication of a more stable community, which minimizes energy expenditure in reproduction (Vargas-Espositos et al., 2023). Very few information is available on sex and life strategy of nematodes in the deep-sea. However, in the case of the DWH oil spill these functional traits appeared to be effective indicators of impact and presence of environmental stress.

It has been reported that bacterivorous nematodes are effective in promoting oil degradation in contaminated soil and improving soil environment and quality (Zhou et al., 2022). Their dominance in the sediments of the GoM was a sign of impact by the oil spill, however it is possible to speculate a positive final effect on sediment reworking and bacterial stimulation (Schratzberger et al., 2019) by the nematode community inhabiting the deep-sea sediments.

5. Conclusions

This study provides the first comprehensive assessment of nematode standing stock, diversity, and functional traits across deep-sea sediments affected by the Deepwater Horizon (DWH) oil spill. The extraordinary increase in nematode abundances at impacted stations, two times higher than background levels, reflects a strong community-level trophic response to the massive deposition of organic- and hydrocarbon-rich material following the spill. This enrichment-toxicity dynamic resulted in dense populations of small nematodes concentrated in the surface sediment, whereas no-impact stations showed a more even distribution between the two sediment layers examined in this study.

Taxonomic and functional diversity analyses revealed distinct ecological signatures across impact levels. Impacted stations were dominated by opportunistic and tolerant species, while sensitive and rare taxa were restricted to background sediments. High turnover across stations and between the two sediment layers demonstrated substantial species replacement linked to contamination, highlighting variable tolerance thresholds among species. Functional traits provided additional resolution: impacted stations exhibited a marked dominance of juveniles, indicating rapid population turnover, high reproductive investment, and fast response to hydrocarbon contamination. In contrast, no-impact stations were characterized by a higher proportion of adults and k-strategist nematodes, reflecting more stable communities with reduced reproductive stress.

Importantly, the dominance of bacterivorous and deposit-feeding nematodes at impacted stations suggests that nematodes may play an indirect role in bioremediation processes. By stimulating microbial communities through grazing, bioturbation, and sediment reworking, nematodes can enhance microbial degradation pathways and accelerate natural attenuation following oil contamination. While they are not primary degraders of hydrocarbons, their functional responses indicate a potential contribution to the initial phases of benthic ecosystem recovery.

Overall, the combined use of taxonomic diversity and functional descriptors offers a powerful framework for assessing deep-sea oil spill impacts. The clear shifts in abundance, community composition, and functional structure underscore the sensitivity of nematodes to disturbance and their value as ecological indicators. Moreover, the interplay between juvenile-driven colonization dynamics and adult persistence provides essential insight into recovery trajectories of deep-sea benthic ecosystems. Further efforts are necessary to integrate nematodes as reliable sentinels of impact into national and international environmental policies and monitoring plans.

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CRediT authorship contribution statement

Elisa Baldrighi: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Federica Semprucci:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology. **Adélaïde Tymen:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation. **Emma Michaud:** Writing – review & editing, Writing – original draft. **Adriana Spedicato:** Writing – review & editing, Writing – original draft. **Nate Conrad-Forest:** Writing – review & editing, Writing – original draft, Formal analysis. **Yazmin Gonzalez Elizarraraz:** Writing – review & editing, Writing – original draft, Visualization. **Jeffrey G Baguley:** Writing – review & editing, Writing – original draft, Resources, Project administration, Investigation, Funding acquisition, Data curation.

Declaration of competing interest

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

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Supplementary materials

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Data availability

Data will be made available on request.

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