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GREENHOUSE GASES CONCENTRATIONS INFLUENCE ON VERTICAL DISTRIBUTION OF NEMATODE COMMUNITIES IN THE BA LAI RIVER, VIETNAM

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ABSTRACT

Vertical distribution of free–living nematodes from the tropical region are still poorly documented, especially in Vietnam. Field sampling was conducted at the Ba Lai river, a tributary of the Mekong river, to insight into the regularity of the vertical pattern of nematode assemblages. Furthermore, some sediment environmental characteristics such as greenhouse gases were also detected in order to understand how to influence nematode distribution. The study found that nematode composition differed significantly between the upper and deeper layers of sediment but not among the deeper layers. Nematode density showed spatial variability across layers, with higher values in the upper layer. Nematode diversity decreased with increasing depth. Non–selective deposit feeders (1B) were dominated in the surface layers, while the predator–omnivores feeders (2B) was numerous in the deeper layers. In the dry season, both methane and hydrogen sulfur were found negatively affect nematode diversity in sediment profile, particularly, methane effects negatively also to species richness and densities. However, in the rainy season, only methane has a significant correlation to the diversity, species richness, densities, and evenness of the nematode communities.

Keywords: Dam effects, indicator, Mekong, methane, monitoring, sulfur.

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INTRODUCTION

Sediments are vital for aquatic environments because of their link with eutrophication processes, chemical pollution, sedimentation, nutrient cycling, and trophic conditions of environmental ecosystems (Forsberg, 1989). These problems are becoming more widespread, especially in global warming and climate change, and they affect both natural (e.g., lakes, rivers, streams, and wetlands) and constructed systems (e.g., hydroelectric reservoirs) (Apitz, 2012; Rowe et al., 2022). The rise in global temperature and CO_2 levels in the atmosphere has serious implications for the release of greenhouse gases (GHG) (e.g., methane or other hydrocarbon gases) from aquatic sediments. GHG is normally formed in aquatic sediments at high pressures (3–5 MPa) and temperatures below 25 ^{o}C (Kvenvolden, 1993); however, when pressure reduction and/or temperature increase can trigger destabilization of GHG, consequences of a release of free methane gas into the water column and eventually to the atmosphere (Archer et al., 2009; Maslin et al., 2010). According to recent estimates, freshwater ecosystems absorb roughly 5.1 Pg C yearly from drainage basins on a worldwide scale (Drake et al., 2018); of this, a part (3.88 Pg C) returns to the atmosphere in the form of CH_4 and CO_2 (Sawakuchi et al., 2017). Several studies estimate around 0.6 Pg C is retained in sediments (Battin et al., 2009), and 0.95 Pg C returns to the environment (Regnier et al., 2013). Although these estimates can be still very imprecise since these processes are dependent on many spatial and temporal factors (e.g., quantity and quality of organic matter, electron acceptors availability, structural characteristics of sediments, trophic conditions of the system, and so on) (Cardoso et al., 2019), the constraining methane emissions from aquatic ecosystems have been increasingly recognized as substantial, yet variable, contributions to global greenhouse gas production.

The detection of identifying and estimating GHG emissions is a challenging issue since their inherently heterogeneous nature, both within and across locations (Beaulieu et al., 2016). Within a single reservoir system, for example, hotspots of methane production exist that are twice or treble the rate of methane emissions in other reservoir areas (Berberich et al., 2020). Recently, several findings showed that a large amount of methane and other GHG are emitted during reservoir creation (Ion & Ene, 2021). Song et al. (2018) have warned that the reservoir-based hydroelectric dams located in boreal and temperate regions have much lower reservoir emissions (3–70 g CO_2 eq./kW h) compared with dams located in tropical regions (8–6,647 g CO_2 eq./kW h). The author also showed that tropical reservoir-based dams could potentially have a higher emission rate than fossil-based electricity. Unlike the Arctic where methane is produced as organic matter decays within the thawing permafrost, the emission of GHG comes from the decomposition of organic matter and vegetation under anaerobic conditions in the soil or sediment layer of the reservoir (Ion & Ene, 2021). Although many studies on the evaluation of GHG emissions from hydropower reservoirs, agricultural production under an irrigation dam should also be associated with such anthropogenic emissions of major GHG (Kulshreshtha & Junkins, 2001). While studies and reviews on the effect of GHG emissions on the benthic fauna are increasingly common in the Arctic Ocean, to date, few studies have assessed the correlation in the tropical reservoir, despite the fact that a large amount of methane and other GHG are emitted in reservoirs. Nematodes, as a group of benthic fauna, exhibit direct responses to physicochemical changes in benthic habitats (Heip et al., 1985). Additionally, their high abundance and diversity often contribute to a more robust database compared to larger organisms, making them suitable for biomonitoring studies (Heip et al., 1985; Bongers & Van de Haar, 1990). Consequently, nematodes have the potential to serve as bioindicators for assessing the status of ecosystems, including disturbances in benthic ecosystems caused by natural and human-induced factors (Bongers & Ferris, 1999; Ngo et al., 2016).

Dams and reservoirs have been criticized for altering natural flow regimes, blocking fish passage, affecting sediment transport, and changing watershed characteristics, all of which contribute to water quality degradation, fish population decline, and biodiversity loss, as well as cascading social and economic issues (Song et al., 2018). With a large number of dams and reservoirs formed from the dams, Vietnam faces the risk of GHG emissions from these works. Nevertheless, to our knowledge, basic information regarding GHG emissions and its correlation to benthic fauna is still largely lacking in Vietnam. Therefore, it is crucial to understand the fate of GHG emissions in reservoir sediments in order to understand the potential impact of GHG release on aquatic ecosystems.

The objectives of this study are: (i) to evaluate the methane and hydrogen sulfide concentration in sediment deep profile; (ii) to describe the vertical distribution pattern of nematode communities with major characteristics such as diversity, densities, evenness, species composition, and trophic structure in both marine and freshwater habitats of Ba Lai river; and (iii) to detect the influence of the methane and hydrogen sulfide concentrations on the nematode communities according to sediment deep profile.

MATERIALS AND METHODS

Study area

The Ba Lai river (BLR) is a small tributary of the Mekong river system located in the Mekong Delta region, Vietnam. BLR is an average of 70 km long and 3–5 m deep, with a flow of around 50–60 m^3/s during the dry season, and approximately 250–300 m^3/s for the rainy season (Tuan et al., 2012). The flow from the My Tho river is currently the principal source of water for BLR, while another flow from the Tien river began to deteriorate since at the beginning of the 20th century more and more alluvium has accumulated in BLR's upstream.

The river serves as a source of freshwater for drinking water, agriculture, irrigation, and industry as well as a fishing spot and a major waterway commerce artery (Tran et al., 2018). An irrigation dam was erected over BLR in 2002 to prevent saltwater intrusion and conserve freshwater for 100,000 hectares of farming areas in Ben Tre Province (Mekong Delta). Besides some positive effects, Ba Lai dam has had several severe consequences on the river's ecology and hurting the livelihood of local communities (Nguyen et al., 2020, 2022; Tran et al., 2022; Ngo et al., 2017, 2022).

Sampling and sample processing

Sampling was carried out in the BLR during October (rainy season) 2020 and March (dry season) 2021. Six stations were identified from the river mouth to the upstream, in which G1 and G2 (G1–G2) were located downstream of the Ba Lai dam (DD), while G3–G6 were upstream stations (UD) (Fig. 1).

At each station, triplicate samples were sliced vertically into 10 cm layers to a sediment depth of 50 cm. The layers were labeled according to the depth in the vertical profile: layer 1 (L1, 0–10 cm), layer 2 (L2, 10–20 cm), layer 3 (L3, 20–30 cm), layer 4 (L4, 30–40 cm), and layer 5 (L5, 40–50 cm). The sediments were fixed immediately with 7% hot buffered formaldehyde solution (60 ^{o}C) and stored for further analyses.

In the laboratory, the sediment samples for nematode analyses were processed according to the centrifugation-flotation technique (Vincx, 1996) using LUDOX, with a density of 1.18 g cm^{-3}, and stained with 1% solution of Rose Bengal. All nematode organisms were counted under a stereomicroscope (50x magnification). All of nematode individuals per slides in each replicate were hand-picked out randomly and transferred to the formalin-ethanol-glycerine solution (Vincx, 1996), and mounted on permanent slides. The nematodes were identified to genus level under an

Olympus light microscope according to Platt & Warwick (1983, 1988), Warwick et al. (1988), Zullini (2010), Nguyen (2007), and the NEMYS database (Bezerra et al., 2022). The nematodes were classified into four feeding types, based on the structure of the buccal cavity according to Wieser (1953): (1A) selective deposit-feeders, (1B) non-selective deposit feeders, (2A) epistratum feeders, and (2B) predators or omnivores.

Sediment samples for environmental factors were taken from the same multiple corers used to retrieve the nematode samples. Samples were taken at 10 cm intervals to a depth of 50 cm to determine the vertical profiles in the surface sediments. Environmental variables such as pH and conductivity were measured in situ using a MILWAUKEE MW102 (pH), and a WTW ProfiLine Cond 3310 (Conductivity).

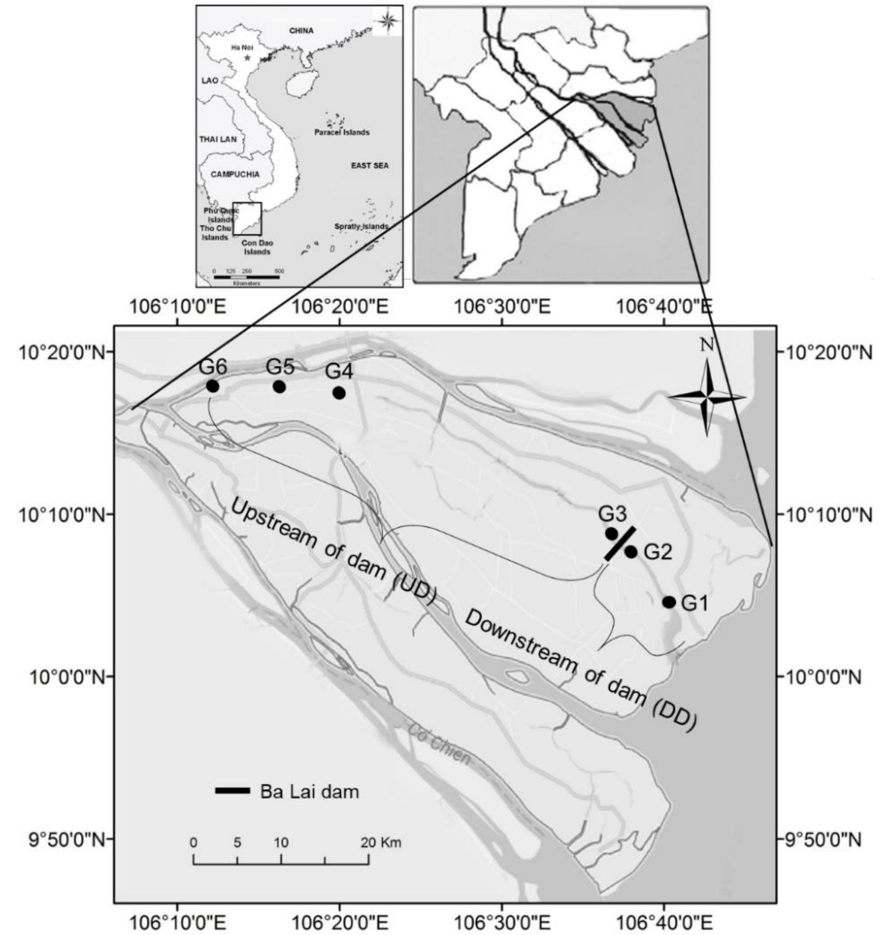

Figure 1. Overview (upper) and detailed (lower) map of the study area with location sampling stations along the Ba Lai river, Vietnam

In order to analyze the sulfide (H_2S) analysis, the top 2 cm of each sediment layer sample was immediately isolated and stored in 50 mL capped vials (polypropylene screw cap, conical bottom tubes, ISOLAB) on dry ice (Ion-selective electrode method). Once returned to the laboratory, samples were transferred to a -18 ^{o}C freezer until further analysis. Total free H_2S concentrations were determined following the method of Brown et al. (2011). Briefly, the sediment sample in the 50 mL plastic vial was mildly defrosted at 4 ^{o}C, then centrifuged at 3,000 rpm for 5 min. After the water layer was discarded, the sediment was homogenized with a stainless steel spatula. A 10 mL portion of the sample was transferred into another graduated plastic vial containing 10 mL of SAOB and further vortexed. The mixture was measured as quickly as possible to avoid sulfide conversion.

For the methane (CH_4) concentration, about 10 mL of the top 2 cm of each sediment layer samples were immediately stored after sampling into a tared 40 mL serum vial containing 5 mL of 0.1 N NaOH to terminate further bacterial activity (GC-FID method). The vial was quickly sealed with a silicone stopper to minimize the potential loss of methane and placed on dry ice. Once returned to the laboratory, samples were transferred to a -18 ^oC freezer until further analysis (Leloup et al., 2007). Methane in the headspace was analyzed using gas chromatography equipped with an Alumina Sulfate PLOT (30 m × 0.53 mm × 10 µm, Supelco, USA), a split/splitless injector, and a flame ionization detector. The injector was operated in splitless mode at a temperature of 35 ^oC. The carrier gas, nitrogen, had a linear velocity of 90 cm/s. The detector was set at 250 ^oC, with an air flow rate of 400 mL/min and a hydrogen flow rate of 40 mL/min. Quantification of methane was performed by constructing a calibration curve using various volumes of standard methane gas (15 ppmv, Agilent). Each sample and standard were injected five times for replication.

Determination of terrestrial organic matter (TOM) by loss on ignition (LOI). The sediment samples were first fried at 90–100 ^oC in a ceramic crucible for one hour until a constant weight was obtained. While the sediment was heating, the samples can be smashed by a glass-rod until it was smooth. Cooling the sediment sample in a desiccator to room temperature. Taking the correct sediment to a ceramic crucible, it was weighed again m_1 (g) (Frangipane et al., 2009). The sediment and ceramic crucible were heated in a muffle furnace until 450–500 ^oC for at least 1 hour to obtain constant weight, and let cool to room temperature, the samples had been weight again m_2 (g). The difference between m_1 and m_2 is the amount of organic matter ignited (Byers et al., 1978).

Data analysis

Both parametric and non-parametric permutational ANOVA was performed to test for significant differences in several characteristics of nematode assemblages and environmental variables between sediment layers between stations. The data set was analyzed using two-factors mixed model design, with the following factors: “Sediment layer” (Sl: L1, L2, L3, L4, L5; fixed), and “Seasons” (Se: Dry, Rainy; fixed). The Pseudo-F and *P* values were calculated using 9999 permutations of the residuals in a reduced model. The significant level for all statistical tests was considered when $p < 0.05$. After the PERMANOVA routines, pair-wise comparisons between layers and seasons were performed. Subsequently, the PERMDISP-test was performed to check for homogeneity of variances, and data were transformed when needed.

The univariate characteristics of the nematode communities, including total abundance (N, inds.$10cm^{-2}$), species richness (S), Shannon diversity index (H′ log_2), Pielou’s evenness index (J′), were conducted by PERMANOVA. The multivariate community data on species level (log-transformed, Bray-Curtis similarity was used to calculate resemblance) was analyzed by PERMANOVA. Subsequently, the SIMPER analysis (SIMilarity PERcentages) was performed to assess the percentage dissimilarity in nematode communities’ composition between sediment layers and stations.

Environmental variables, including pH, conductance, TOM, CH_4, and H_2S, were tested by PERMANOVA tests on normalized Euclidean distance similarity resemblance matrices. Non-parametric Spearman rank correlation coefficients were computed to identify correlations between environmental variables and characteristics of the nematode assemblages. All described analyses were performed within PRIMER v6 with

PERMANOVA + add-on software and STATGRAPHICS 18 software.

RESULTS

Greenhouse gases and some sedimental characteristics in the Ba Lai river

The GHG concentrations showed high variation between the sampling layers and seasons (Table 1). Methane ranged from 9.47 ± 2.92 ppm to 14.56 ± 10.77 ppm in the dry season and from 49.01 ± 59.38 ppm to 196.58 ± 380.28 ppm in the rainy season. The two-way PERMANOVA results indicated that methane values were only significantly different between seasons ($p_{se} < 0.001$). Sulfur concentrations varied between 36.30 ± 28.48 ppm and 58.08 ± 85.31 ppm for the dry season and from 8.08 ± 2.79 ppm to 29.04 ± 44.12 ppm for the rainy season, and its values were also significantly different between seasons ($p_{se} < 0.001$) (Table 2). The pairwise comparison illustrates that there is a high variability in GHG concentrations between seasons. The rainy season mainly showed consistently high values for methane whereas the dry season consistently had high values for sulfur.

Table 1. The average and standard deviation of the greenhouse gases (CH_4: Methane, H_2S: Hydrogen Sulfide) and other environmental factors (Con: Conductance, TOM: Total Organic Matter) among sediment layers (Sl: L1, L2, L3, L4, L5) during the dry (D) and the rainy season (R) in the Ba Lai river

Sl	CH_4 (ppm)		H_2S (ppm)		pH		Conduct (g/L)		TOM (%)	
	D	R	D	R	D	R	D	R	D	R
1	9.98 ± 2.07	49.01 ± 59.38	36.30 ± 28.48	19.64 ± 29.88	6.82 ± 0.20	7.03 ± 0.19	10.91 ± 9.70	2.68 ± 2.97	6.40 ± 0.69	6.63 ± 0.42
2	9.47 ± 2.92	79.77 ± 78.27	38.88 ± 26.00	8.08 ± 2.79	6.76 ± 0.20	6.98 ± 0.25	9.48 ± 10.15	2.85 ± 2.83	6.52 ± 0.99	6.51 ± 0.64
3	14.56 ± 10.77	83.54 ± 88.66	59.45 ± 60.02	8.81 ± 3.46	6.80 ± 0.22	6.93 ±0.35	8.29 ±9.72	3.39 ± 3.22	6.29 ±0.96	6.35 ± 0.69
4	9.70 ± 4.35	83.29 ± 103.24	57.57 ± 38.00	10.78 ± 6.91	6.85 ± 0.21	7.05 ± 0.39	7.23 ± 8.62	3.78 ± 3.79	6.11 ± 0.67	6.91 ± 0.50
5	10.53 ± 4.40	196.58 ± 380.28	58.08 ± 85.31	29.04 ± 44.12	6.94 ± 0.27	7.02 ± 0.37	6.62 ± 7.86	4.05 ± 4.47	6.36 ± 0.89	6.99 ± 0.72

Table 2. Results of PERMANOVA analysis for significant differences in environmental variables (Con: Conductivity, TOM: Total Organic Matter, CH_4: Methane, H_2S: Hydrogen Sulfide) among sediment layers (Sl), seasons (Se), and interaction terms

Source	df	pH		Con (g/L)		TOM (%)		CH_4 (ppm)		H_2S (ppm)	
		ps-F	CV	ps-F	CV	ps-F	CV	ps-F	CV	ps-F	CV
Sl	4	1.12	5.7E-2	0.24	-0.13	1.05	3.6E-2	1.67	0.12	1.23	7.4E-2
Se	1	17.28**	0.40	24.46**	0.48	9.77*	0.30	19.96**	0.43	32.56**	0.54
Sl×Se	4	0.40	-0.17	0.98	-3E-2	2.09	0.23	1.67	0.18	1.04	4.3E-2
Res	170		0.96		0.94		0.96		0.93		0.91
Total	179										

Note: Significant effects: *: P < 0.05, **: P < 0.001 are indicated next to values of PERMANOVA ‘pseudo’ F statistic (ps-F).

The pH value in the dry season was lower than 7, indicating an acidic environment, and its value in the dry season (6.76–6.94) was lower than those in the rainy season (6.93–7.05). The results indicated that especially conductivity fluctuated between both sediment layers and sampling events. Conductivity in the dry season (6.62–10.91) was lower than those in the rainy season (2.68–4.05). These values decreased from layer 1 to layer 5 during the dry season, whereas they increase from layer 1 to layer 5 during the rainy season. TOM showed no consistent patterns either between seasons or layers, exhibiting values between 6.11 and 6.52 for the dry season, and ranging from 6.35 to 6.99 for the rainy season (Table 1). The two-way PERMANOVA results indicated that pH, conductivity, and TOM were only significantly different between seasons. The rainy season showed consistently high values for pH and TOM whereas the dry season had high values for conductivity (Table 2).

Nematode communities in the Ba Lai River, focus on vertical patterns

The nematode community composition of the Ba Lai River consisted of species belonging to both classes, Enoplea and Chromadorea. In the dry season, species were distributed over 11 orders, 51 families, 114 genera, and 179 species while for the rainy season, 10 orders, 42 families, 86 genera, and 134 species were observed.

Table 3. Results of PERMANOVA tests for differences in univariate (N: Total abundance (inds.10^{-2}), S: Genera richness, H′$\log_2$: Shannon–Wiener diversity index, J′: Pielou’s evenness index)

Source	df	Community		N		S		H′$\log_2$		J′	
		ps-F	CV	ps-F	CV	ps-F	CV	ps-F	CV	ps-F	CV
Sl	4	3.43**	1.76	37.17**	11.73	9.88**	4.56	2.73*	1.20	5.81**	0.73
Se	1	13.78**	21.40	101.22**	12.35	83.01**	8.76	47.77**	3.94	9.08*	0.60
Sl×Se	4	1.78**	11.83	4.08*	4.84	6.92**	5.27	5.44*	2.71	0.55	-0.31
Res	170		56.11		11.70		9.18		5.47		2.02
Total	179										

Note: Significant effects: *: $P < 0.05$; **: $P < 0.001$ are indicated next to values of PERMANOVA ‘pseudo’ F statistic (ps-F); sediment layers (Sl); seasons (Se); components of variation (CV).

Table 4. Results from pair-wise multivariate PERMANOVA analyses for differences in nematode community structure. Bold italic values indicate significant differences at $p < 0.05$

Factors	t	p(perm)	Unique perms
Seasons			
D, R	3.71	**0.0001**	9902
Layers			
L1, L2	2.21	**0.0001**	9902
L1, L3	2.54	**0.0001**	9915
L1, L4	3.14	**0.0001**	9905
L1, L5	2.84	**0.0001**	9909
L2, L3	0.83	0.838	9912
L2, L4	1.27	**0.041**	9900
L2, L5	1.06	0.284	9907
L3, L4	0.91	0.641	9913
L3, L5	0.79	0.914	9906
L4, L5	0.75	0.934	9910

The nematode composition differed significantly among sediment layers and seasons, with seasons being the most important, as indicated by its highest component of variation (Table 3). Moreover, the nematode composition differed significantly between layers, indicating that multivariate variation in nematode communities was also affected by the vertical profile of the sediment layers. Overall, the nematode community structure differed significantly between the surface layers (L1) and the deeper layers (L2, 3, 4, and 5). However, nematode composition at the deeper layers (L2 to L5) did not differ significantly from each other (except for layers 2 and 4) (Table 4).

The SIMPER test revealed similarity values for the surface layers (L1) and the deeper layers of 21.54% and 17.49%, respectively. As expected from its dominant occurrence at layer 1, *Theristus consobrinus* and *Terschellingia media* contributed the most to similarity within this layer (12.64 and 10.00%, respectively). For the other layers, a broader range of genera was responsible for the similarity between layers, such as *Mesodorylaimus* sp. (14.32%), *Rhabdolaimus minor* (10.75%), and *Monhystrella* sp. (10.53%). The dissimilarity between layer 1 and the other layers was significantly high (87.45%), with *Theristus consobrinus*, *Theristus rigidus*. *Terschellingia media*, *Dichromadora rigida*, and *Monhystera wangi* as the main discriminating species (Table 5).

The densities of nematodes showed a high spatial and temporal variability across layers and between seasons. Densities ranged from 59.83 ± 38.92 inds.10 cm^{-2} to 844.28 ± 758.89 inds.10 cm^{-2} in the dry season and from 12.42 ± 10.98 inds.10 cm^{-2} to 801.17 ± 1080.40 inds.10 cm^{-2} in the rainy season. However, higher densities were observed on average in the dry season when compared with the rainy season. The highest abundance of nematodes occurred in layer 1, followed by layers 2 and 3, whereas the lowest abundance was observed in layers 4 and 5 (Fig. 2). PERMANOVA results based on the total density showed significant differences between sediment layers and seasons ($p_{sl}<0.001$, $p_{se} < 0.001$) as well as a significant interaction effect ($p_{sl\&se} < 0.05$) (Table 3). Pairwise comparisons showed significantly higher densities at layer 1 compared with the other layers (Table 6).

Table 5. Results from analysis of species contributions to similarities and dissimilarity between layers SIMPER similarity (%, contribution > 5%)

Layer 1	21.54	The other layers	17.49
Theristus consobrinus	12.64	*Mesodorylaimus* sp.	14.32
Terschellingia media	10.00	*Rhabdolaimus minor*	10.75
Theristus rigidus	9.02	*Monhystrella* sp.	10.53
Dichromadora rigida	7.18	*Rhabdolaimus aquaticus*	8.61
Monhystera wangi	7.02	*Theristus consobrinus*	8.31
		Mylonchulus amurus	7.13
		Mononchulus sp.	5.14
SIMPER dissimilarity (%, contribution > 3%)			
Layer 1 vs. the other layers	87.45		
Theristus consobrinus	4.36		
Theristus rigidus	3.71		
Terschellingia media	3.70		
Dichromadora rigida	3.24		
Monhystera wangi	3.07		

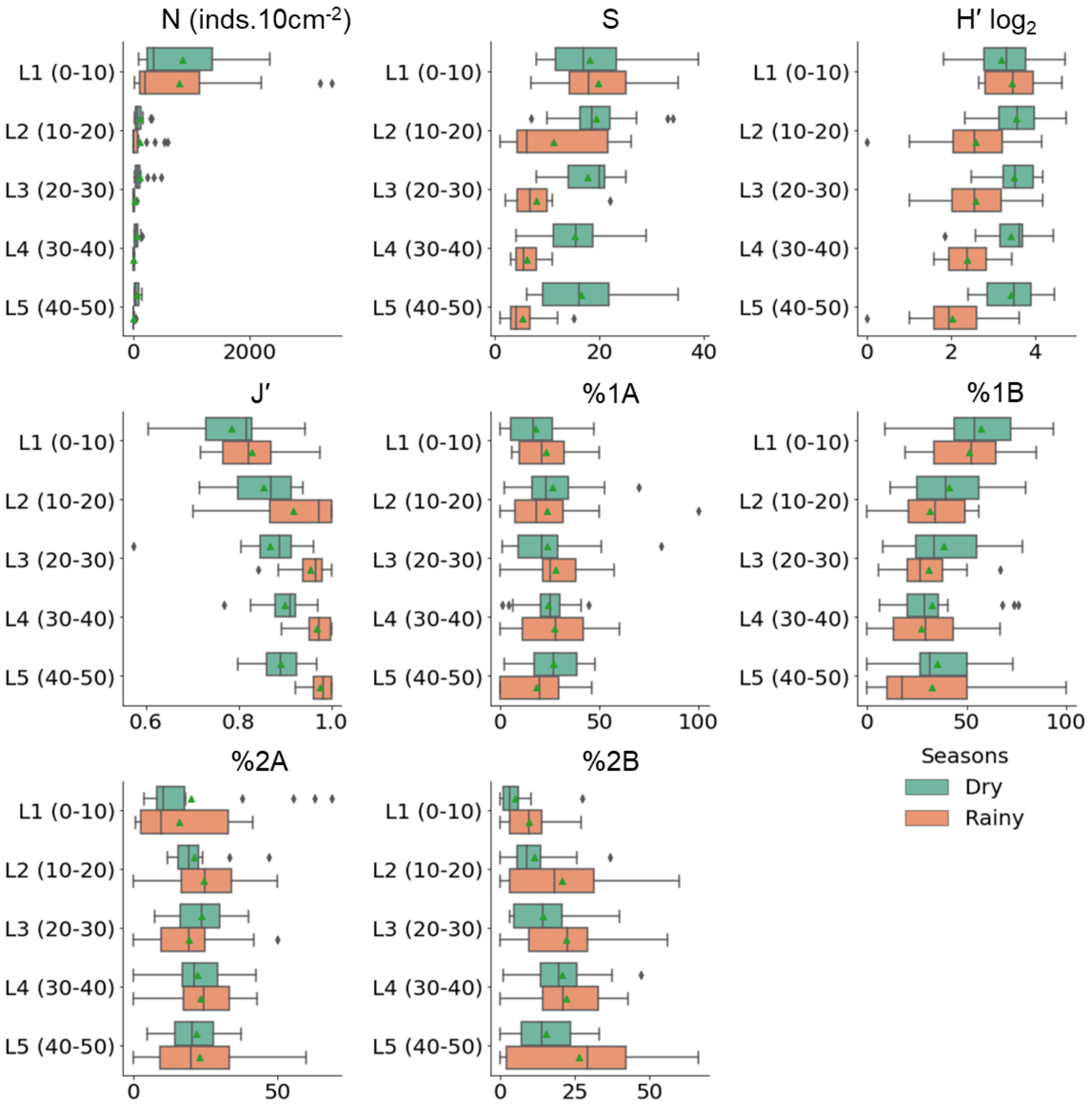

Figure 2. Box and whisker plot for the abundance (N), biodiversity indices (S: Genus richness, H′($\log_2$): Shannon–Wiener diversity index, J′: Pielou's evenness index), and feeding types (1A: Selective deposit-feeders, 1B: Non-selective deposit feeders, 2A: Epistratum feeders, 2B: Predators or omnivores) of the nematode communities along with the vertical and horizontal sediment profile

The average species number per layer ranged from 15.28 ± 5.70 to 19.33 ± 7.10 in the dry season and from 5.36 ± 3.76 to 19.72 ± 8.34 in the rainy season. Their average and standard deviation were lowest in the deepest layers (layers 4 and 5), and highest in the surface layers (layers 1 and 2) in both seasons (Fig. 2). The two-way PERMANOVA showed significant differences between seasons ($p_{se} < 0.001$) and sediment layers ($p_{sl} < 0.001$) as well as between the interaction factors (psl&se < 0.001) (Table 3). Pairwise comparisons showed a significantly higher species at the surface layer compared with the other deeper layers (Table 6).

Table 6. PERMANOVA pair-wise comparisons for nematode characteristics among sediment layers and seasons

Variables	Layers	Seasons
N	L1 > L2, 3, 4, 5; L2 > L4, 5; L3 > L5	D > R
S	L1 > L2, 3, 4, 5; L2 > L4 , 5; L3 > L5	D > R
H′	L1 > L4, 5	D > R
J′	L1 < L2, 3, 4, 5; L2 < L4; L3 < L5	D < R
%1A	n.s	n.s
%1B	L1 > L2, 3, 4, 5; L3 > L5	D > R
%2A	n.s	D < R
%2B	L1 < L2, 3, 4, 5; L2 < L4	n.s

Note: n.s: Not significant.

Shannon–Wiener diversity showed no vertical patterns in the dry season, exhibiting values between 3.20 ± 0.78 and 3.56 ± 0.59. However, the Shannon–Wiener diversity decreased from layer 1 (3.44 ± 0.64) to layer 5 (2.04 ± 0.96) in the rainy season. These values showed high temporal variability between seasons (Fig. 2). The two-way PERMANOVA analysis showed significant differences between layers ($p_{sl} < 0.05$) and seasons ($p_{se} < 0.001$) and for the interaction terms ($p_{sl\&se} < 0.05$) (Table 3).

Table 7. Results of PERMANOVA tests for the proportions of the feeding types among sediment layers (Sl), seasons (Se), and interaction terms

Source	df	%1A		%1B		%2A		%2B	
		ps-F	CV	ps-F	CV	ps-F	CV	ps-F	CV
Sl	4	0.72	-1.85	3.57*	4.70	1.02	0.57	5.42*	7.45
Se	1	2.16	2.40	8.88*	5.21	11.69*	6.69	2.35	2.61
Sl×Se	4	2.71*	6.51	1.20	1.86	0.22	-4.19	1.41	3.24
Res	170		21.16		17.61		20.21		21.27
Total	179								

Note: Significant effects: *: P < 0.05 is indicated next to values of PERMANOVA ‘pseudo’ F statistic (ps-F); components of variation (CV).

Pielou’s evenness index ranged from 0.78 ± 0.08 to 0.90 ± 0.05 in the dry season and from 0.83 ± 0.08 to 0.97 ± 0.03 in the rainy season (Fig. 2). The lowest evenness occurred in layer 1, followed by layers 2 and 3, whereas the highest abundance was observed in layers 4 and 5. PERMANOVA results indicated significant differences between sediment layers and seasons (Table 3).

The trophic structure of the nematode communities in the dry season consisted of a high percentage of non-selective deposit feeders 1B (from 32.59 ± 20.50% to 57.36 ± 23.71%). The highest proportions of this group were found at layers 1 and 2. Selective deposit feeders and epistratum feeders contributed less (1A: 17.52 ± 14.38% – 2 7.09 ± 14.37%; 2A: 20.22 ± 20.90% – 23.62 ± 9.93%). There was a low percentage of predator-omnivores feeders found in all layers, except for layers 4 and 5 (2B: 20.83 ± 11.94%, 15.44 ± 9.63%, respectively). In the wet season, the trophic structure consisted of a high percentage of non-deposit feeders 1B (from 27.30 ± 20.37% to 51.17 ± 21.47%) in all stations. All other feeding types showed a lower percentage (1A: 18.01 ± 16.02% – 27.77 ± 14.55%; 2A: 16.16 ± 15.67% – 24.59 ± 14.75%; 2B: 9.60 ± 7.50% – 26.57 ± 22.04%) (Fig. 2). The results of PERMANOVA tests for the proportions of the feeding types are presented in Table 7.

The relationship between nematode concentrations with environmental communities and greenhouse gas variables

Table 8. The r and p values of the Spearman correlation between nematode communities' univariates and environmental parameters in the dry season (n = 90). Significant results ($p < 0.05$) in **bold**

Variables used	Correlation coeff.	pH	Conductivity	TOM	H_2S	CH_4
N	r	0.159	0.572	-0.135	-0.019	-0.215
	p	0.133	< **0.001**	0.202	0.860	**0.043**
S	r	-0.006	0.363	-0.034	-0.162	-0.453
	p	0.957	**0.001**	0.747	0.126	< **0.001**
H′ ($\log_2$)	r	-0.085	0.220	-0.051	-0.273	-0.418
	p	0.423	**0.038**	0.631	**0.010**	< **0.001**
J′	r	-0.272	-0.375	0.107	-0.148	-0.007
	p	**0.01**	< **0.001**	0.311	0.162	0.948
%1A	r	0.052	0.158	0.089	0.026	-0.397
	p	0.624	0.136	0.401	0.803	< **0.001**
%1B	r	-0.113	-0.106	-0.187	-0.147	0.208
	p	0.285	0.318	0.077	0.167	0.050
%2A	r	0.108	0.131	0.084	0.026	0.011
	p	0.309	0.217	0.430	0.804	0.916
%2B	r	0.008	-0.259	0.035	0.063	0.143
	p	0.941	**0.015**	0.744	0.550	0.176

Table 9. The r and p values of the Spearman correlation between nematode communities' univariates and environmental parameters in the rainy season (n = 90). Significant results ($p < 0.05$) in **bold**

Variables used	Correlation coeff.	pH	Conductivity	TOM	H_2S	CH_4
N	r	0.371	0.100	-0.016	-0.028	-0.325
	p	**0.001**	0.348	0.880	0.790	**0.002**
S	r	0.419	0.177	-0.061	-0.004	-0.297
	p	< **0.001**	0.096	0.566	0.973	**0.005**
H′ ($\log_2$)	r	0.439	0.208	-0.075	0.033	-0.251
	p	< **0.001**	0.050	0.482	0.756	**0.018**
J′	r	-0.243	-0.047	0.012	-0.016	0.284
	p	**0.022**	0.657	0.908	0.880	**0.007**
%1A	r	0.117	0.053	0.134	0.135	-0.118
	p	0.272	0.615	0.207	0.204	0.266
%1B	r	-0.069	-0.223	-0.106	-0.173	-0.029
	p	0.516	**0.035**	0.317	0.103	0.788
%2A	r	0.066	0.142	0.081	0.141	-0.066
	p	0.532	0.181	0.443	0.185	0.535
%2B	r	-0.056	0.188	-0.079	-0.107	0.141
	p	0.597	0.076	0.458	0.312	0.183

Correlations between nematode univariate data (total densities, species richness, Shannon–Wiener diversity, evenness index, and percentage of the four trophic groups) and environmental variables (pH, conductivity, TOM, H_2S, and CH_4) are shown in Tables 8 and 9 for the dry and wet season, respectively. In which, densities, species richness, Shannon–Wiener diversity were significantly negative correlated with CH_4 in both seasons. Shannon–Wiener diversity had a negative correlation with H_2S in the dry season, whereas the evenness index showed a positive correlation with CH_4 in the rainy season.

DISCUSSION

Greenhouse gases concentrations in the Ba Lai river in comparison to adjacent rivers

Many quantitative research on GHG concentrations are now conducted across the world, although the majority of these studies are conducted in reservoirs from hydroelectric dams, with very few studies for irrigation dams. Our results found that methane and hydrogen sulfur concentrations from Ba Lai River sediment are relatively high at a deeper lever. These values of methane concentrations ranged from 9.47 ± 2.922 to 196.58 ± 380.28 ppm. This range is high compared to the ranges for methane emission in several Brazilian hydroelectrical reservoirs (e.g., Funil, Santo Antônio, and Três Marias) reported by Hällqvist (2012) which measured from 1.21 ppm to 34.77 ppm. But they were lower than the methane emission of several boreal lakes in Finland reported by Huttunen et al. (2006), which measured from 17 ppm to 51,000 ppm.

Vertical fluctuations of nematode communities in the river sediment

Nematode communities structure differed significantly between the surface layers (L1) and the deeper layers (L2, 3, 4, and 5) in terms of density, diversity, and trophic structure. The density and diversity decreased with increasing depth. In the trophic structure, non-selective deposit feeders 1B dominated in the surface layers, while the predator-omnivores feeders 2B was numerous in the deeper layers. Similar results have been reported by Pinto et al. (2013), nematodes often exhibit an aggregated spatial distribution within the sedimentary habitat, both horizontally and vertically. Generally, nematode densities usually decrease with increasing depth in both muddy and sandy substrates (Long & Ross, 1999). The majority of the total population of nematodes can be found in the upper 2 cm of sediment (Shimanaga et al., 2000). Nematode characteristics are vertically in the sediment, in terms of total abundance (Steyaert et al., 1999; Górska et al., 2014), biomass (Ingels et al., 2011; Leduc & Rowden, 2018), diversity (Steyaert et al., 1999; Ingels et al., 2011; Leduc & Rowden, 2018), community structure (Steyaert et al., 1999; Leduc & Rowden, 2018), trophic structure (Long & Ross, 1999; Leduc & Rowden, 2018; Maria et al., 2012), and body morphometrics (Maria et al., 2012; Brustolin et al., 2013).

Nematodes were mainly concentrated on the sediment surface, with about 70% present in the top 5 cm (Kotwicki et al., 2005). Brown & McLachlan (1990) found that sandy beach meiofauna can be distributed to the depth of 50 cm or deeper on well-oxygenated beaches. Particularly, Munro et al. (1978) recorded nematodes down to 105 cm at such beaches. However, most studies supposed that nematodes appear unable to survive to 15 cm below sediments (Hauquier et al., 2011; Urban–Malinga et al., 2014). Our results showed that (i) nematodes were present up to 50 cm depth, and (ii) sometimes, the diversity (e.g., Shannon–Weiner index) was higher in the deeper layers than in the upper layers. The rationale could be given about:

a) Sediment trapping by Ba Lai dam: Dams interrupt the continuity of sediment transport through river systems, causing sediment to accumulate in the reservoirs (impairing reservoir operation and decreasing storage) (Kondolf et al., 2014). The bottom of Ba Lai river has accreted from 1.5 m to 2 m, with an average of 1.75 m during the period from 2002 to 2009 (Tran et al., 2021). Moreover, after its construction, the Ba Lai dam produced a high silt deposition throughout the Ba Lai estuary downstream, since the water current could no longer remove the substrate and was transported inland by the tidal regime (Veettil & Ngo, 2018).

Then, these organic materials can be buried in the sediment following fluctuations in run-off and flow regimes, thereby providing substantial resources for deep-sediment fauna (Tank et al., 2010). This suggests that buried organic material still harbours a diverse community of microbial decomposers that might serve as a food resource for a variety of nematode species. Moreover, nematode length, diameter and biovolume increased with sediment depth (Fleeger et al., 2011).

Larger nematodes seem to dwell deeper in sediment, while populations of smaller species are concentrated in the upper sediment layer (Traunspurger & Drews, 1996). For example, the large species *Mesodorylaimus* sp., *Mylonchulus amurus* dominated in deeper sediment. *Laimus* sp. in this study. The higher proportion of fungal feeders and omnivores (e.g., *Mesodorylaimus* sp., *Mylonchulus amurus*, and *Mononchulus* sp.) in the deeper sediment is in line with this suggestion.

b) Surface sediments disturbance: Since the dam’s operation, numerous changes to water quality and sedimentary conditions have been observed in the area. In upstream areas, sediment quality has declined and become enriched with organic materials (Tran et al., 2018; Nguyen et al., 2020). The high occurrence of typical opportunistic species such as *Theristus consobrinus*, *Terschellingia media*, *Theristus rigidus*, *Dichromadora rigida*, and *Monhystera wangi* in the surface sediment layer is in agreement with the evaluations. Adapting to the situation, the colonisation of deeper sediments might provide a safe refuge and thus a guarantee of longer-term stability for nematode populations (Palmer et al., 1992).

The effect of GHG concentrations on the current nematode communities

In the Ba Lai River, nematode communities’s characteristics such as diversity, densities, species richness, and evenness were found to have a significantly negative influence from methane and hydrogen sulfur in the sediment profile. This is supported by Olu et al. (1997) and Van Gaever et al. (2006) found that the abundance of nematode communities increases in methane deep-seep habitats. A possible alternative explanation for the observed results is that at depths under 200 m photosynthetic organic matter is more available for benthic consumers due to stronger bentho-pelagic coupling (Levin, 2005). However, at greater depth, the amount of photosynthetic organic matter decreases, and chemosynthesis starts to play a significant role in local organic matter production. As a result, despite the presence of methane and sulfides (unfavourable for most organisms due to toxicity), unique and diverse ecosystems can develop at deep-sea cold seeps (e.g., the Arctic Ocean) (Dando et al., 1993). Unlike abundance, the diversity exhibit substantially reduced species diversity in seep environments, with a few species dominating (e.g., *Halomonhystera* sp., *Sabatieria mortenseni*, and *Desmodora* sp.) (Van Gaever et al., 2006).

Furthermore, the vertical distribution of nematodes in the sediment layers is highly correlated with many factors, such as the redox potential discontinuity (Long & Ross, 1999; Steyaert et al., 2003), biological interactions (Defeo & McLachlan, 2005; Maria et al., 2012), sediment characteristics (Teiwes et al., 2007), oxygen conditions (Teiwes et al., 2007), food availability (Steyaert et al., 1999).

On the other hand, the effects of GHG, which are emitted from reservoirs, cause a significant reduction in the density and biodiversity of nematode communities. The TOM can undergo transformations along the water column of the aquatic environments, and once in the sediments, this TOM can present three main destinations: (1) be mineralized by microorganisms in the sediments and then release to the water column as CO_2 and CH_4; (2) undergo resuspension of the sediments and to be mineralized in the water column; or (3) be permanently sedimented, functioning as a carbon stock in the sediments (Cardoso et al., 2013). At the first destination, the TOM is degraded by several redox reactions. These reactions release decreasing amounts of energy, ranging from more oxidative (greater release of energy) to less oxidative (less release of energy), and take into account the availability of the electron acceptors in the system (Fenchel et al., 2012).

In the superficial layer, the oxygen reduction reactions (aerobic respiration) prevail, until the oxygen is exhausted. Subsequently, reactions of nitrate reduction (nitrification), manganese reduction, iron reduction, sulfate reduction, and methanogenesis occur. After the reactions, the final products of anaerobic mineralization (CH_4, H_2S, Fe^{2+}, Mn^{2+}) can dilute and diffuse in the water column or diffuse into the upper layer of the sediment, which cause toxic for most aquatic organisms, especially sedimental nematodes. Our study showed that nematode densities, species richness, and Shannon–Wiener diversity were significantly negative correlated with CH_4 in both seasons. Furthermore, Shannon–Wiener diversity had a negative correlation with H_2S in a dry season. Most studies have warned that methane and sulfide are toxic to all organisms (Hentschel, 1999; Vanaverbeke et al., 2011), the toxicity could be an explanation of its reversible inhibition of the enzyme cytochrome *c* oxidase at even nanomolar concentrations and the latter through the formation of oxygen radicals, such as the hydroxyl radical (OH^-) and hydrogen peroxide (H_2O_2) (Hentschel, 1999).

CONCLUSION

Therefore, vertical distribution nematode communities such as composition, densities, diversity, species richness, and trophic structures were found up to 50 cm deep in the Ba Lai River. In which, their composition differed significantly between the upper and deeper layers of sediment but not among the deeper layers. Nematode densities showed spatial variability across layers, with higher values in the upper layer. Furthermore, their diversity decreased with increasing depth. In the communities, the non-selective deposit feeders 1B dominated in the surface layers, while the predator-omnivores feeders 2B were numerous in the deeper layers. These communities' characteristics were influenced by methane, hydro sulfur, and other sediment environmental variables due to both methane and hydrogen sulfur showing significant correlation to nematode communities in terms of diversity, densities, species richness, and evenness in the sediment profile.

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