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Relationships between environmental variables and free-living nematode communities in seasonally flooded wetlands

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ABSTRACT

The Lo Go-Xa Mat is a national park in the southeastern region of Vietnam, which has a particularly high biodiversity and it includes different wetlands which are unique diverse in species composition. It can be categorized into two types: temporarily-seasonally and permanently flooded wetlands. Ta Not grassy marsh is representative of the seasonally flooded wetland. Whilst the diversity and ecology of plants and mammals are well documented, little or no information of the benthic ecology in the seasonally flooded wetland exist. This study aims to provide a new database of the nematode's structure in the seasonally flooded wetland and its relation with environmental variables as well as detection of the ecological quality, considering nematodes as bioindicators. This work is the first investigation on nematodes communities in associate with some environmental variables in the Ta Not grassy marsh. The results showed that free-living nematodes in the Ta Not seasonally flooded grassy marsh are characterized by the high density (ranged from 235.01 to 898.43 inds.10cm^{-2}) but rather low diversity. More specifically, the genus richness (S) ranged from 8.20 to 8.60. The observed Margalef's species richness (d) was ranging from 1.07 to 1.53 and the Shannon-Wiener index (H') was measured from 2.36 to 2.52. In addition, the Pielou's evenness (J') ranged from 0.55 to 0.68 and the Hill indices indicated average values ranging between 5.46- 5.84 for N_1, between 4.32-4.60 for N_2, and between 2.64-2.86 for N_{inf}. Specifically, our results indicated that deep level, pH, and NH_4^+ showed a significant correlation with the nematode density and bio-indices. The sediment of the Ta Not grassy marsh was assessed as in good conditions in all stations based on the Maturity Index of nematodes.

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INTRODUCTION

Wetlands are sites in which water is the principal factor influencing the environment and the associated living organisms. It occurs where the water surface is at or near that of the land, or where the land is covered by water. Various wetland systems have been recognized, from freshwater to brackish or saline wetlands of coastal regions. According to the Ramsar Convention on Wetlands (Iran, 1971), wetlands can be described as "... areas of marsh, fen, peatland or water, whether natural or artificial, permanent or temporary, with water that is static or flowing, fresh, brackish or salt, including areas of marine water the depth of which at low tide does not exceed six meters." (Ramsar Handbooks, 2016). There are different classification systems of wetlands which been developed to serve various purposes. Nevertheless, based on the Ramsar Classification System for Wetland Type, wetlands have been classified into three categories, namely marine/coastal, inland, and human-made ones (Ramsar Convention Bureau, 1990). There are currently nine sites recognized as Wetlands of International Importance (Ramsar Sites), with a surface area of 120,549 hectares in Vietnam (Ramsar list, 2020). The Lo Go-Xa Mat National Park (LGXMNP), which is the largest forest cover in the region, has a total area of 30,022 hectares (including the 10,812 hectares of the Chang Riec cultural-historic forest) in the Tan Bien District, Tay Ninh Province, southeastern Vietnam. The LGXMNP has a high diversity of flora and fauna, which is being important for bio-conservation (Tordoff *et al.,* 2003). Within the national park, there are different types of wetlands (natural or artificial, temporarily-seasonally or permanently flooded with freshwater) such as rivers, streams, bogs, marshes, and canals. The LGXMNP has been applied to be recognized as one of the Ramsar sites in Vietnam. In the national park, Ta Not is the seasonally flooded grassy marshes (TNGM) in the tropical rainy season and it is the largest part of the wetland ecosystem of the LGXMNP with approximately 250 hectares. The Ta Not is assigned as "seasonal/intermittent freshwater lakes" among the listed "inland wetlands" type. Tropical wetlands, including the LGXMNP's wetland ecosystems, have been considered as the most biologically productive natural ecosystems in the world in comparison with other habitats such as tropical rain forests and coral reefs in terms of the number and variety of species (Finlayson and Moser, 1991). The tropical wetlands support the inhabiting species and provide high benefits for local people (Dugan, 1993). Unfortunately, in spite of the fact that important research progress made in recent decades, wetlands continue to be under a number of anthropogenic pressures, such as land use changes, pollution, and overexploitation of natural resources (Ramsar Handbooks, 2016). As far as TNGM is concerned, due to risks from forest fires, during the dry season in 2001, a network of roads and drainage canals were constructed in the area for firebreaks. The potential threat to biodiversity at the LGXMNP's wetland ecosystems, particularly in the TNGM, is a conversion of seasonally flooded grasslands to agriculture. The artificial canals in wetland areas make nutrient enrichment and expansion of invasive plants (Harvey *at al.,* 2011). Other threats identified are illegal timber extraction, hunting, over-exploitation of non-timber forest products, forest fires, and over-loading the wetlands with tourism activities. Among these, the conversion of wetlands to agriculture and other land use was assessed as the highest-ranked threat to biodiversity at the site. Thus, it is necessary to determine the ecological quality of wetland ecosystems in LGXMNP when they respond to a stress. In the LGXMNP's wetland ecosystems (especially TNGM), the investigation of vascular plants and mammals gets a lot of attention from ecologists. However, the studies of benthic invertebrate communities have not been fully focused yet, especially the nematode communities, which is the most abundant and diverse group (Danovaro, 2010). Nematodes are small multicellular vermiform organisms that are observed in almost all conceivable habitats (Du *et al.,* 2014). Nematode communities play crucial roles in benthic wetland sediment ecosystems processes such as: i) being the trophic link between the microfauna and larger fauna since the main food sources of nematode communities are organic detritus, bacteria, and benthic diatoms while they can provide food for a variety of predators such as juvenile fish and prawns (Majdi and Traunspurger, 2015); ii) some group of nematode communities function as decomposers, which are important for nutrient cycling and energy flow in benthic ecosystems (Semprucci *et al.,* 2013). Nevertheless, there is no study that has comprehensively investigated nematode communities and their correlation with sediment's environmental variables in the LGXMNP's wetland ecosystems. Due to the increasing interest in ecological nematodes in coastal wetlands, mainly in mangrove forests, there is more information about the content of free living

nematodes in mangrove sediments around the world. Studies on the ecology of free living nematodes in mangrove wetlands have been conducted in southern Africa (Dye, 1983), southeastern coast of India (Chinnadurai and Fernando, 2007; Ansar *et al.,* 2014), southeastern Australia (Gwyther, 2000), southwestern Puerto Rico (Torres-Pratts and Schizas, 2007), and coast of China (Liu *et al.,* 2016). In addition, some information on free living nematodes in mangrove habitat has also been reported in Vietnam (Ngo *et al.,* 2007, 2008). On the contrary, the distribution, biodiversity, and the interactions between free living nematodes and environmental variables have not been well characterized in inland freshwater wetlands. To our knowledge, even fewer studies have evaluated the free living nematode structure and their responses to the environmental variables in seasonal freshwater wetlands. Knowledge about benthic habitats in general, and benthic nematodes in particular, will help us to develop strategies for maintaining and enhancing the quality and sustainability of LGXMNP's wetland ecosystems. Therefore, this research aims to: i) study relevant environmental variables in the TNGM-LGXMNP; ii) investigate free-living nematode communities by assessing their composition, abundance, distribution, and diversity; iii) investigate correlation between nematode communities and environmental variables, if any; iv) investigate the ecological quality status of sediments (EcoQ) in the TNGM based on the analyses of nematode communities in relation to their physical and biochemical environment.

MATERIALS AND METHODS

Study area and sampling design

All samples were collected in the rainy season (October) of 2017. During sampling campaigns, water and sediment samples for environmental and biological analysis were collected at three transects (the distance is approximately one kilometer, representing three habitats) of the TNGM; the transect A (Trans A, herbaceous grassy marsh), transect B (Trans B, artificial canal), and transect C (Trans C, timber/herbaceous grassy marsh) (Fig. 1). In Trans B, sampling stations were chosen in the low intertidal zone.

Sample collection and analysis of environmental variables

In order to understand the influence of environmental variables on the structure and diversity of nematode communities, a set of relevant environmental variables in water, such as water depth (cm), temperature (^{o}C), and total dissolved solids (TDS, mg/L) and in the sediment, such as temperature (^{o}C), pH, ammonia concentration (NH_4^{+}, mg/g), and organic carbon (%C), were measured together with nematode samples. Water depth, temperature, and total dissolved solids (TDS) are important variables that influence free living nematodes since they swim

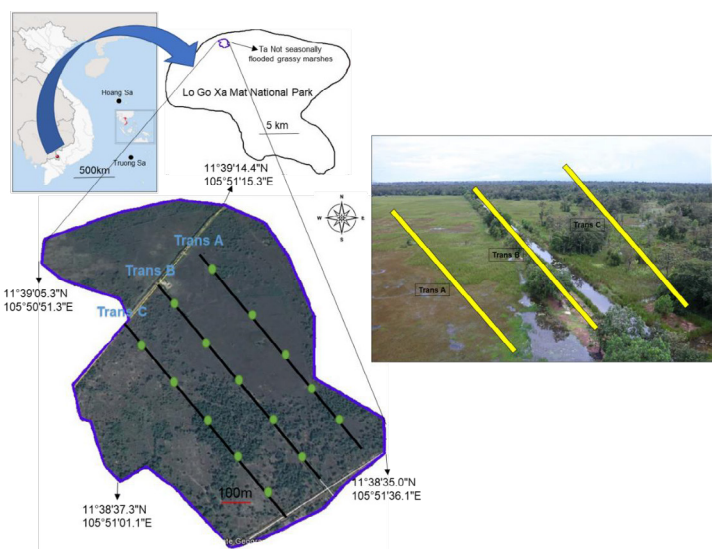

Fig. 1: Geographical location of the Ta Not grassy marsh, Lo Go-Xa Mat National Park along with the sampling stations in three transects

on surface of the sediment for food. Meanwhile, sediment temperature, pH, NH_4^+ and organic carbon can reflect the environmental condition associated with nematodes in the wetland. Water column depth (from the water surface to sediment sampling), temperature, and total dissolved solids (TDS) were measured *in situ* during the sampling campaign by using the Multiparameter Water Quality Meter Model (WQC22A). Temperature and pH of sediments were also measured *in situ*. Sediment samples were collected using a Ponar-type grab, kept in glass bottles for nutrient analysis. Samples were preserved at 4°C and transferred to the laboratory to measure ammonia concentration (NH_4^+) (VNS, 2009) and organic carbon (%C) (VNS, 2000). Each environmental variable had five replicates per transect.

Nematode sample collection and identification

Five replicates per transect were collected using cores of 3.5 cm in diameter and 30 cm in length. The cores were pushed down into the soft-bottom up to 10 cm deep (10 cm^2 surface area). The samples were fixed in a 7% formaldehyde solution at 60°C and gently stirred before transported to the laboratory of the Institute of Tropical Biology, Vietnam Academy of Science and Technology. In the laboratory, sediment samples were passed through a sieve with a mesh size of 1mm, the samples were sieved twice on a 38µm sieve. Nematode samples were extracted from the debris remaining on the 38µm sieve by flotation technique using Ludox-TM50 solution (specific gravity of 1.18) (Vincx, 1996). Samples were colored using 3–5 mL Rose Bengal solution (1%). All individual numbers of nematodes were counted under a stereomicroscope for quantitative analysis. Then, one hundred individuals were randomly picked out and processed using pure glycerin for making permanent slides (De Grisse, 1969) for supporting qualitative analysis. Nematodes were identified up to genus level using the Identification manual for Free living Marine Nematodes, Part III (Warwick *et al.,* 1998), and (Bongers, 1988), with the taxonomy literature in the NeMys database (Bezerra *et al.,* 2020).

Data analysis

All data of nematodes and environment of each transect were presented as average ± standard deviation using Microsoft Excel 2007. The abundance of nematodes was standardized for a 10 cm^2 (Pfannkuche and Thiel, 1988). The diversity of nematodes was measured using indices such as the genus richness (S), the Shannon diversity index ($H'log_2$), Margalef's species richness (d), Pielou's evenness (J'), and Hill indices (N_1, N_2, N_{inf}). The Maturity Index (MI, Bongers, 1990; Bongers *et al.,* 1991; Bongers and Ferris, 1999) was calculated as the weighted average of the individual colonizers-persister (c-p) values: MI= $\sum_{i=1}^{n} v(i).f(i)$, where v(*i*) and f(*i*) are the c-p score and the frequency of genus *i* in a sample. This index has been proposed as a semi-quantitative value giving an indication of benthic ecological health according to the nematode communities and can be converted in EcoQ using the fixed scale provided by Moreno *et al.* (2011): High, MI>2.8; Good, 2.8≥MI>2.6; Moderate, 2.6≥MI>2.4; Poor, 2.4≥MI>2.2, Bad, MI≤ 2.2. The SIMPER analysis (SIMilarity PERcentages) was used for identifying (i) average similarity and dissimilarity between sampling transects (at a genus level), and (ii) the genera responsible for similarity and dissimilarity between sampling transects. All the analyses were computed with the PRIMER-E ver. 6 (Clarke and Warwick 2001). Univariates (environmental variables and nematode characteristics) significant differences between sampling transects were tested by the bootstrap method (with 1,000 replications), using the boot package in the R software (Canty *et al.,* 2016). The standardized 95% confidence intervals of the bootstrapped parameters were compared. Non-parametric Spearman rank correlation coefficients were used ($p<0.05$) to identify correlations between the environmental variables and the nematode characteristics. All the analyses were computed with the software STATISTICA ver 7.0.

RESULTS AND DISCUSSION

Environmental variables of water and sediment

The environmental variables of the overlying water (depth, temperature, and TDS) and of the sediment (temperature, pH, NH_4^+, and %C) for the three transects in the TNGM are shown in Table 1. The results indicated that water temperature fluctuated between sampling transects. In Trans A, aboveground biomass of grassland is having a high canopy cover which was reflected in the lowest temperature values (28.55±0.23°C). Contrastingly, grassland cover was little in Trans B and C, resulting in slightly elevated temperature values (33.21±0.80°C and 33.23±4.27°C, respectively). TDS values showed no consistent patterns between sampling transects, exhibiting values between 12.07±1.38 and 15.07±6.09 mg/L. The

depth was highest at Trans B-canal (45.76±15.98 cm), while showing the lowest levels (12.70±10.52 cm) in Trans C in general. The same trend as for water temperature, the lowest sediment temperature values (25.51±0.42°C) was recorded in Trans A, while showing the highest levels (30.92±3.16°C) in Trans C. Because the Trans A had high biomass of herbaceous grasses, which lead to slightly elevated carbon concentration (1.74±0.25%) in the sediment while this value was lower levels in Trans B and C (1.19±0.26%, 1.09±0.44%, respectively). Also, pH value was highest for Trans B (5.35±0.73%) and showing the lowest value (4.20±0.20%) for Trans C. Furthermore, NH_4^+ did not vary so much, exhibiting values between 0.016±0.002 mg/g and 0.024±0.008 mg/g. The result of the Bootstrap analysis showed the significant difference between transects for all variables, except for TDS (Table 1 and Fig. 2).

Characteristics of nematode communities from the seasonally flooded wetland

In total, nematode communities in the TNGM consisted of 31 genera belonging to 24 families, 9 orders, which include Chromadorida, Dorylaimida, Enoplida, Monhysterida, Mononchida, Plectida, Rhabditida, Triplonchida, and Triplonchida. They mainly belong to class Chromadorea accounting for 64.39% of total individuals compared to class Enoplea (35.61%) (Fig. 3).

Nematode communities in the Trans C were expressed as the highest density (898.43±403.01 inds/10 cm^2) followed by Trans A (498.72±313.62 inds/10 cm^2). In contrast, Trans B was showed the lowest density with ranges from 235.01±185.95 inds/10 cm^2 (Fig. 4A). The result of Bootstrap analysis showed that the nematode density in the Trans C was significantly higher than the nematode density in the Trans B (with

Table 1: Water (W), sediment (Se) environmental variables (mean±SD) measured at the Ta Not seasonally flooded grassy marsh and Bootstrap result test

Variables	Trans A	Trans B	Trans C	Bootstrap results
Temp (°C,W)	28.55±0.23	33.21±0.80	33.23±4.27	A<B (-5.27:-3.99, 95%CI mean A-mean B); A<C (-8.05:-1.38, 95%CI mean A-mean C)
TDS (mg/L,W)	15.07±6.09	12.07±1.38	13.80±5.20	No significant difference
Depth (cm,W)	38.13±16.57	45.76±15.98	12.70±10.52	B>C (-48.36:-18.96, 95%CI mean C-mean B); A>C (8.50:38.07, 95%CI mean A-mean C)
Temp (°C,Se)	25.51±0.42	30.03±0.79	30.92±3.16	A<B (-5.23:-3.82, 95%CI mean A-mean B); A<C (-7.92:-2.90, 95%CI mean A-mean C)
pH (Se)	4.34±0.16	5.35±0.73	4.20±0.20	A<B (-1.59:-0.41, 95%CI mean A-mean B; B>C (-1.78:-0.58, 95%CI mean C-mean B
NH_4^+ (mg/g, Se)	0.016±0.002	0.024±0.008	0.018±0.009	A<B (-0.013:-0.0006, 95%CI mean A-mean B)
%C (Se)	1.74±0.25	1.19±0.26	1.09±0.44	A>B (0.29:0.83, 95%CI mean A-mean B); A>C (0.24:1.00, 95%CI mean A-mean B)

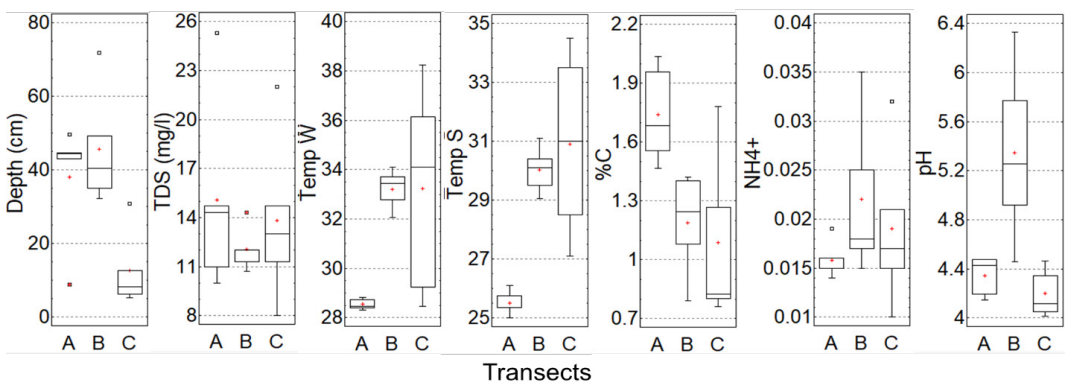

Fig. 2: Water (W) and sediment (S) environmental variables. Thick black lines, asterisk, and empty circles represent for the median, the mean, and the outlier value, respectively. Lower box indicates the first quartile and upper box indicates the third quartile. Upper line of the boxes shows the maximum value and lower line shows the minimum value

95% confidence interval and 1,000 replications). A similar result was found in comparing the nematode density in Trans A and Trans C, the results of the bootstrap analysis confirmed that the nematode density in Trans A was significantly lower than Trans C (Table 2). The average diversity indices showed no consistent patterns between sampling transects. More specifically, the genus richness (S) ranged from 8.20±1.92, 8.20±1.30 (Trans C, B) to 8.60±2.61 (Trans A) (Fig. 4B). Margalef's species richness (d), indicated average values ranging from 1.07±0.25 (Trans C) to 1.53±0.55 (Trans B), while that of Trans A was 1.24±0.36 (Fig. 4C). The Shannon-Wiener index (H') in Trans A, B, and C was 2.52±0.31, 2.48±0.43, 2.36±0.58, respectively (Fig. 4D). Also, Pielou's evenness (J') measured varied from 0.55±0.19 (Trans A) to 0.68±0.03 (Trans C) (Fig. 4E). The Hill indices indicated average values ranging between 5.46±1.96-5.84±1.34 for N_1, between 4.32±1.80-4.60±1.65 for N_2, and between 2.64±0.44-2.86±0.98 for N_{inf} (Fig. 4F-H). The result of the Bootstrap analysis showed no significant difference among the sampling transects for all indices (Table 2).

The SIMPER analysis

Results of SIMPER analysis confirmed that the average similarity percentages in nematode communities were high (37.85%) in the Trans A. Five genera that contributed the most in the communities were *Cryptonchus* (26.99%), *Achromadora* (22.68%), *Eudorylaimus* (19.36%), *Prismatolaimus* (16.77%), and *Rhabdolaimus* (8.23%). In Trans B, the average similarity percentage in nematode communities was lower (29.15%). *Eudorylaimus*, *Alaimus*, and *Achromadora* were also known as the most contributing genera (with 55.95%, 14.14%, and 12.09%, respectively). The sub-contribution genera were *Chronogaster*, *Paractionlaimus* (6.30% and 8.49%, respectively). Furthermore, the average similarity in nematode communities was lower (25.50%) in Trans C. Four genera contributed most to the communities in this transect were *Rhabdolaimus* (29.00%), *Achromadora* (16.54%), *Prismatolaimus* (13.19%), and *Eudorylaimus* (10.06%). In three transects, genus *Achromadora* and *Eudorylaimus* were among the most contributors (Table 3).

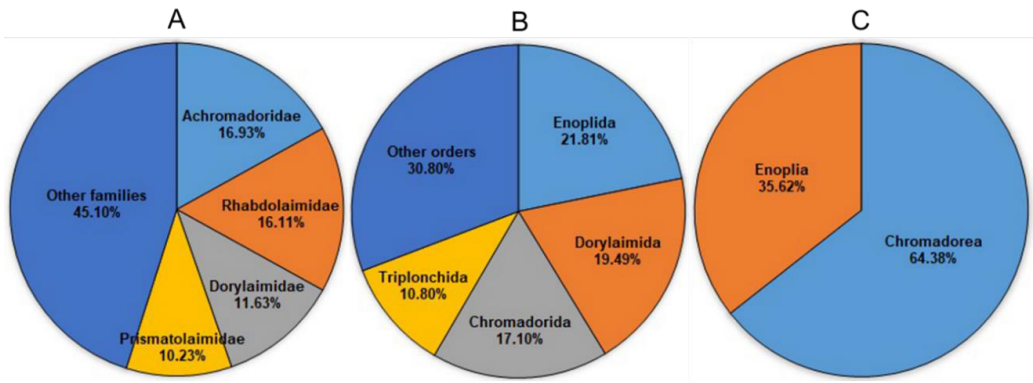

Fig. 3: Nematode families (A), orders (B), and (C) class composition

Table 2: The nematode density, bio-indices, and Bootstrap result test

Den and indices	Trans A	Trans B	Trans C	Bootstrap results
Density (inds/10 cm^2)	498.72±313.62	235.01±185.95	898.43±403.01	C>B (2.05:554.65, 95%CI mean C-mean B); A<C (-832.96:-41.79, 95%CI mean A-mean C)
S	8.60±2.61	8.20±1.30	8.20±1.92	
d	1.24±0.36	1.53±0.55	1.07±0.25	
H' (log2)	2.52±0.31	2.48±0.43	2.36±0.58	
J'	0.83±0.05	0.82±0.12	0.78±0.13	No significant difference
N_1	5.84±1.34	5.77±1.52	5.46±1.96	
N_2	4.48±0.90	4.60±1.65	4.32±1.80	
N_{inf}	2.64±0.44	2.86±0.98	2.77±1.17	

The average dissimilarities between transects A-C and B (artificial canal) were considerably high, varied from 76.53% in the case of Trans A&B to 83.48% in the case of Trans C&B. The genera responsible for those dissimilarities were *Cryptonchus*, *Prismatolaimus*, *Eudorylaimus*, *Achromadora*, *Metalinhomoeus*, *Rhabdolaimus*, *Paractionlaimus*, *Alaimus*, *Chronogaster*, *Neotobrilus*, *Monhystera*, and *Udonchus.* Pairwise comparison of transect A and C showed also relatively high dissimilarity (73.83%). Several genera, such as *Rhabdolaimus* (c-p 4), *Achromadora* (c-p 3), *Neotobrilus* (c-p 3), and *Prismatolaimus* (c-p 3), were more abundant and served as the principal genera responsible for those dissimilarities. This lead to the percentage of the groups c–p 4 and c–p 3 being the most abundant in all of the stations (Table 4).

Most of the environmental variables showed significant differences among the three transects (except for TDS), especially for depth. The depth was highest in the artificial canal (Trans B), while showing the low level in Trans A and C in general. Furthermore, the result of SIMPER analysis showed that the average dissimilarities between transects A-C and B were considerably high. These results indicated that the environmental variables and the nematode density must have been different among the three sampling transects (especially between Trans A, C, and Trans B). Artificial canals and levees are the foundation of wetland water-management systems. Several studies have recognized ecological and hydrological impacts of canals and levees. A canal bisected the largest section of grassland in Tinh Doi wetland (An Giang Province-Mekong Delta). In addition, there was quantitative evidence that these canal constructions lead to a decline in water quality and biodiversity value of this area. The Kien Luong wetland site (Kien Giang Province-Mekong Delta) is threatened by a flood-control canal that will bisect the site, leading to further fragmentation, changes in flooding regimes, and subsequent deterioration of soil fertility through acidification. The construction of this canal was also likely to have severe impacts on endangered water birds (Buckton *et al.,* 1999). Harvey *et al.* (2011) have warned that canals in South Florida provided deep-water, nutrient-enriched habitats for the expansion of non-native invasive plants. These plants can modify water chemistry, deplete oxygen levels, shade out native species, decrease water flow, and interfere with navigation and flood control. Specific examples of non-native plants by canal had severe impacts for the native ecological ecosystem in the Tram Chim National Park (Dong Thap Province-Mekong Delta). Native plants have been replaced by invasive *Mimosa pigra* while genus *Eleocharis*, which is the favorite food of the *Sarus crane*, have been destroyed. This situation led to reduced numbers of this endangered bird species visiting the park (Triet *et al.,* 2004). In the present study, we collected 15 samples (5 samples/

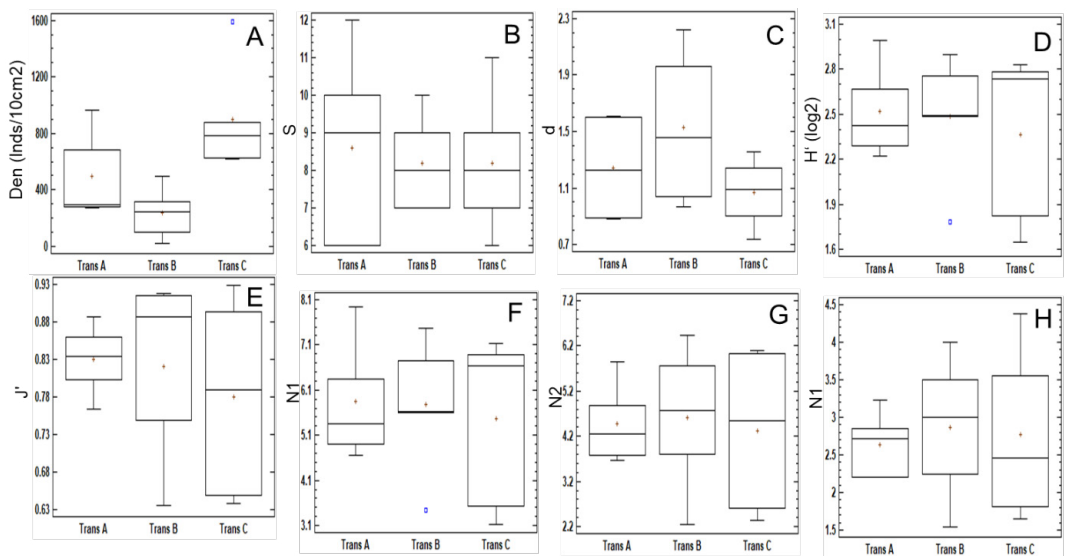

Fig. 4: Nematode boxplots data for the density (inds/10 cm^2) (A), diversity: (B) Genus richness (S), (C) Margalef's species richness (d), (D) Shannon diversity index (H'), (E) Pielou's evenness (J'), Hill indices (F-H)

Table 3: Average similarities (Av.sim) and major nematode genera were contributing to the similarity in three transects (Av.Ab: Average abundance, Con: Contribution, Cum: Cumulative, SD: Standard deviation)

Trans A (Av. Sim: 37.85%)					
Genera	Av.Ab (inds/10cm^2)	Av.Sim	SD	Con (%)	Cum (%)
Cryptonchus	70.51	10.22	1.03	26.99	26.99
Achromadora	76.56	8.58	2.37	22.68	49.67
Eudorylaimus	38.95	7.33	1.75	19.36	69.03
Prismatolaimus	108.71	6.35	0.77	16.77	85.79
Rhabdolaimus	36.33	3.11	1.00	8.23	94.02
Trans B (Av. Sim: 29.15%)					
Eudorylaimus	96.19	16.31	1.39	55.95	55.95
Alaimus	24.95	4.12	1.08	14.14	70.09
Achromadora	18.44	3.52	0.93	12.09	82.18
Chronogaster	22.46	1.84	1.54	6.30	88.48
Paractionlaimus	28.4	1.43	0.72	4.89	93.37
Trans C (Av. Sim: 25.50%)					
Rhabdolaimus	173.76	7.4	1.09	29.00	29.00
Achromadora	181.21	4.22	0.97	16.54	45.54
Prismatolaimus	48.94	3.36	1.01	13.19	58.72
Eudorylaimus	43.78	2.57	1.10	10.06	68.79
Paractionlaimus	65.18	1.74	0.99	6.84	75.63
Monhystera	66.38	1.64	0.59	6.44	82.07
Chronogaster	63.8	1.59	0.59	6.25	88.32
Udonchus	43.91	1.24	0.32	4.87	93.20

transect) to assess some environmental variables and nematode structures in the TNGM. Therefore, the sample size might have been small and not a good representation of all TNGM habitats. Evidence for small sample size was data on genus accumulation. The genus accumulation curve rises faster, with a high slope, even at fifteen samples (Fig. 5). Thus, over fifteen samples, several genera will be added to nematode communities. The sample size is too small to reduce the power of the study and increases the margin of error, which can have the chance of assuming as true a false premise (Faber and Fonseca, 2014). The future study in the TNGM should collect more than fifteen samples to have more diversity in order to explain the effect of environmental variables on nematode communities in particular and the effect of artificial canals on wetland ecosystems in general.

Nematode communities in the TNGM have been first explored as a seasonally flooded wetland habitat in Vietnam. Table 5 summarizes the characteristic of nematode communities in seasonally flooded wetlands compared with other habitats. In this study, the density of nematode communities in the seasonally flooded wetland was lower than observed in brackish and marine habitats but was comparable to the density reported in freshwater habitats. The diversity was much lower than those in fresh, brackish, and marine habitats. The data gathered from the study could be used as baseline data for future research of nematodes in the seasonally flooded wetlands. Additionally, this study provides one of the first baseline surveys of nematode communities in freshwater ecosystems in Vietnam.

Associations between the environmental variables and the nematode density and bio-index

The correlation coefficients of nematode communities' variables, such as density and bio-index, with environmental variables were shown in Table 6. Specifically, the nematode density was significantly negatively correlated with pH and depth. Meanwhile, J' and biodiversity indices such as Shannon–Weiner index H' and Hill indices were having a strong positive correlation with NH_4^+.

It is well known that the density, diversity, distribution, and functional properties of nematode communities can be affected by several abiotic variables such as salinity, temperature, hydrodynamics, sediment grain size, oxygen level, and food availability (Ingels *et al.,* 2011; Cai *et al.,* 2012; Ngo *et al.,* 2013; Zeppilli *et al.,* 2013; Górska *et al.,* 2014). In this wetland habitats, nematode density was found to have a significant negative correlation with depth. A similar result was presented by Tran *et al.* (2017), which studied nematode communities in the shrimp farms in Ca Mau mangroves. Tran *et al.* (2017) indicated a decrease in densities and diversities of nematodes with increase in

Table 4: Average dissimilarities (Av. Diss) and major nematode genera were contributing to the dissimilarity between three transects

Trans A and B (Av. Diss: 76.53%)						
Genera	Trans A Av.Ab	Trans B Av.Ab	Av.Diss	SD	Con (%)	Cum (%)
Cryptonchus	70.51	1.55	12.78	1.14	16.70	16.70
Prismatolaimus	108.71	9.29	12.60	1.18	16.46	33.16
Eudorylaimus	38.95	96.19	9.80	1.13	12.81	45.97
Achromadora	76.56	18.44	7.68	1.19	10.03	56.00
Metalinhomoeus	61.83	0.00	6.96	0.48	9.10	65.10
Rhabdolaimus	36.33	8.93	4.36	1.38	5.69	70.80
Paractionlaimus	18.47	28.4	4.19	1.02	5.48	76.28
Alaimus	16.31	24.95	3.80	0.91	4.97	81.25
Chronogaster	0.00	22.46	2.68	0.78	3.50	84.75
Halalaimus	18.40	0.33	1.63	0.51	2.13	86.88
Aphelenchus	15.16	0.00	1.47	0.62	1.92	88.8
Mylonchulus	5.34	0.00	1.12	0.46	1.46	90.26
Trans A and C (Av. Diss: 73.83%)						
	Trans A Av.Ab	Trans C Av.Ab	Av.Diss	SD	Con (%)	Cum (%)
Rhabdolaimus	36.33	173.76	11.85	0.84	16.05	16.05
Achromadora	76.56	181.21	9.98	0.93	13.52	29.57
Neotobrilus	4.60	86.81	7.21	0.49	9.77	39.34
Prismatolaimus	108.71	48.94	6.43	1.37	8.71	48.06
Cryptonchus	70.51	53.22	5.92	1.32	8.02	56.08
Monhystera	0.00	66.38	4.93	0.76	6.67	62.75
Udonchus	0.00	43.91	4.15	0.72	5.61	68.37
Chronogaster	0.00	63.8	4.14	0.99	5.61	73.98
Metalinhomoeus	61.83	0.00	4.08	0.48	5.53	79.5
Paractionlaimus	18.47	65.18	3.43	0.86	4.64	84.14
Diplolaimella	0.00	28.51	2.68	0.48	3.64	87.78
Alaimus	16.31	24.89	1.95	1.02	2.64	90.42
Trans B and C (Av. Diss: 83.48%)						
	Trans B Av.Ab	Trans C Av.Ab	Av.Diss	SD	Con (%)	Cum (%)
Rhabdolaimus	8.93	173.76	15.99	0.91	19.15	19.15
Achromadora	18.44	181.21	11.9	0.86	14.26	33.41
Neotobrilus	0.00	86.81	8.75	0.48	10.48	43.89
Eudorylaimus	96.19	43.78	6.69	1.15	8.02	51.91
Monhystera	0.00	66.38	6.05	0.76	7.24	59.15
Udonchus	0.00	43.91	5.33	0.73	6.38	65.53
Chronogaster	22.46	63.80	5.03	1.17	6.02	71.55
Paractionlaimus	28.40	65.18	4.94	1.13	5.92	77.47
Cryptonchus	1.55	53.22	4.87	0.68	5.83	83.3
Prismatolaimus	9.29	48.94	4.27	1.33	5.12	88.42
Diplolaimella	0.00	28.51	3.45	0.48	4.13	92.55

depth. Furthermore, the observation of the negative correlation of nematodes with pH is also observed in Mekong estuaries (Ngo *et al.*, 2016). In the present study bio-indices, such as J', H', and Hill indices, were found to have a strong positive correlation with NH_4^+. It means ammonium ions provide a good condition for nematode communities and increase their diversity in the freshwater wetland habitat. This result is in contrast with the study of Ngo *et al.* (2016) in the brackish habitat of Mekong estuaries, which reported that NH_4^+ concentration negatively is correlated with nematode diversities ($H'log_2$). Hence, environmental variables such as depth, pH, and NH_4^+ in the TNGM influence the densities and bio-indices of nematode communities significantly.

Maturity Index based on nematode life strategies as a potential tool to evaluate the ecological quality status of sediment

Regarding the c-p values of the transects, c-p 5 was absent in the studied samples. Group c-p 3 and c-p 4 were dominant in three transects as seen in Table 6.

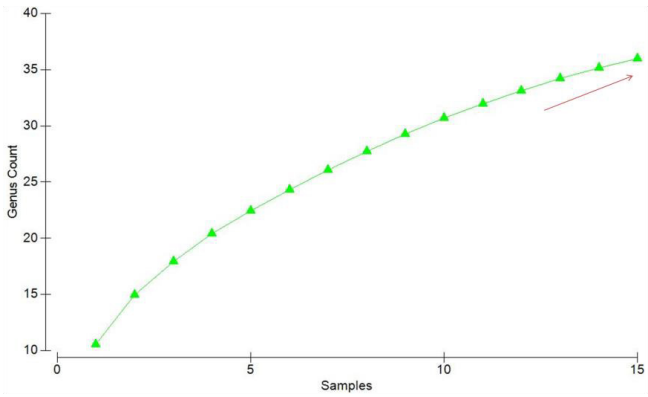

Fig. 5: Genus accumulations plot indicating cumulative genus estimation over samples (with permutations: 999)

Table 5: A summary of the characteristic of nematode communities in seasonally flooded wetland compared with fresh, brackish, and marine habitats

Habitats	Composition	Density (inds/10 cm^2)	Diversity	References
Aquatic (Fresh)	88 genera, 42 families, 10 orders in dry and 102 genera, 45 families, 10 orders in the wet season.	13.3-1,649.7	H'=0.62-3.43	Sai Gon river,Vietnam (Ngo *et al.,* 2018)
Aquatic (Brackish)	118 genera, 45 families, 12 orders, 2 class. The common taxa were *Parodontophora, Ptycholaimellus, Daptonema, Theristus, Desmodora, Terschellingia, Halalaimus,* and *Hopperia*.	83-4,580	S=13-32, H'=2.79-4.03, d=2.86-5.86	Ba Lai river, Vietnam (Tran *et al.,* 2018a)
Aquatic (Mangrove forests)	80 genera, 24 families, 7 orders. The dominant genera were *Paracomesoma, Hopperia, Halalaimus, Theristus, Neochromadora, Daptonema, Metachromadora, Parodontophor*	968.3±151.7-1758.7±436.7	H'=3.6-4.2	Can Gio Mangrove Biosphere Reserve, Vietnam (*Ngo et al.,* 2007)
Aquatic (Marine and Estuaries)	135 genera, 35 families. The dominant genera were *Desmodora, Leptolaimus, Halalaimus, Thalassomonhystera, Theristus, Daptonema,* and *Rhynchonema*	454±289.9-3,137.7±337.1	d=2.64±0.64-5.99± 1.06, H'=1.83±0.59-4 ±0.17	Mekong estuarine system (*Ngo et al.,* 2010)
Seasonally flooded wetland	36 genera, 27 families, 8 orders, 2 class. The common taxa were *Rhabdolaimus*, *Achromadora*, *Prismatolaimus*	861.80-1,699.00	S=10-11, H'=1.87-2.28, d=1.25-1.73, J'=0.55-0.68, N_1= 5.46- 5.84, N_2= 4.32-4.60, N_{inf}= 2.64- 2.86	**Present study**

Trans A revealed high abundances of c-p 3 and c-p 4 (42.31% and 48.69%, respectively). Group c-p4 and c-p 3 were found to be most abundant at Trans B and Trans C with 79.22% and 57.88%, respectively. The MI index value ranged from 2.92±0.41 (in the Trans C) to 3.74±0.10 (Trans B). Estimating the TNGM's EcoQ based on MI values showed that all of the stations could be classified as high EcoQ conditions (Table 7).

Several methods were applied for evaluating the EcoQ by using nematode communities: Diversity indices, index of trophic diversity-ITD (Wieser, 1953), abundance biomass curves-ABC (Warwick, 1986), and maturity index-MI (Bongers, 1990; Bongers *et al.,* 1991). Firstly, diversity indices, among diversity

descriptors, H' is undoubtedly one of the best candidates for the EcoQ class definition, thus its extensive use in nematode ecology (Semprucci *et al.,* 2015). In general, high values of the H' is related to healthy benthic ecosystems (Moreno *et al.,* 2011). However, an intermediate level of disturbance may favor community biodiversity (Salas *et al.,* 2006). Furthermore, links between disturbance and diversity are often very complex (Semprucci *et al.,* 2015). Also, the effect of natural variables such as sediment grain size may significantly influence the biodiversity of nematode communities (Ngo *et al.,* 2016). Consequently, there are relevant limitations in the use of diversity indices in EcoQ assessments. Secondly, ITD method, the classification of feeding types (Wieser, 1953) may not represent the real complexity of the feeding behaviors of nematodes (Moens and Vincx, 1997). Additionally, several studies have reported an ambiguous influence on the ITD of various stressors, indicating that these types of stresses do not have a real impact on the trophic structure of the nematodes (Semprucci *et al.,* 2015). Consequently, ITD cannot be considered as a good index for the detection of EcoQ (Semprucci *et al.,* 2013). Thirdly, in Vietnam, the use of the ABC method for monitoring environmental quality has been emphasized in recent papers (Tran *et al.,* 2018b). Nevertheless, ABC method did not show the abilities to discriminate the main variables affected biomass between natural stress and pollution stress (Shepherd *et al.,* 1992). Finally, the MI based on nematode life strategies, has been proposed in 1990 to assess the EcoQ in various environments (terrestrial, freshwater, marine, and brackish ecosystems (Bongers *et al.,* 1991). The MI is a single measuring stick able to give an overview of the lifestyles of nematode communities, which is easily communicated to possible environmental managers (Semprucci *et al.,* 2015). Furthermore, the MI and c-p classes proved to be potential tools as quality status indicator influenced by chemicals in several studies (OM enrichment-derived from sewage discharge or aquaculture; eutrophication-by riverine outfalls) (Semprucci *et al.,* 2015). In agreement with those results, Moreno *et al.* (2011) evaluate several nematode indices for EcoQ assessment, suggesting that MI was among the best tools to estimate the EcoQ.

Table 6: Correlation coefficients of the nematode density and diversity with the environmental variable (n=15). Significant results ($p < 0.05$) in **bold**

Den/Diver indices	Co. coeff.	Depth	TempW	TDS	TempS	NH_4^+	%C	pH
Density	r	-0.76	0.12	0.19	0.32	-0.30	-0.27	-0.54
	p	**0.001**	0.67	0.50	0.25	0.28	0.32	**0.04**
S	r	-0.31	0.03	0.47	-0.09	0.10	-0.04	0.06
	p	0.26	0.92	0.08	0.74	0.72	0.88	0.84
d	r	0.33	0.06	0.20	-0.06	0.30	-0.15	0.31
	p	0.23	0.84	0.48	0.83	0.28	0.59	0.26
J'	r	0.14	-0.09	0.27	-0.37	0.61	0.45	-0.09
	p	0.62	0.76	0.34	0.17	**0.01**	0.09	0.75
H'	r	-0.09	-0.01	0.46	-0.29	0.55	0.28	-0.02
	p	0.75	0.98	0.09	0.29	**0.04**	0.31	0.93
N_1	r	-0.14	0.04	0.48	-0.25	0.55	0.24	-0.04
	p	0.61	0.90	0.07	0.36	**0.03**	0.39	0.89
N_2	r	-0.07	0.05	0.42	-0.27	0.68	0.29	-0.08
	p	0.82	0.86	0.12	0.34	**0.01**	0.29	0.77
N_{inf}	r	-0.07	0.22	0.27	-0.16	0.68	0.32	-0.06
	p	0.81	0.44	0.33	0.56	**0.005**	0.25	0.84

Table 7: The MI values, percentages of each c-p and the ecological quality status of sediment at the Ta Not seasonally flooded grassy marsh

Transects	% c-p group				MI	EcoQ
	cp 4	cp 3	cp 2	cp 1		
Trans A	48.69	42.31	9.00	0.00	3.36±0.43	High
Trans B	79.22	20.48	0.30	0.00	3.74±0.10	High
Trans C	29.55	57.88	11.50	1.07	2.92±0.41	High

CONCLUSION

The present study is one of the first records of nematode communities in the seasonally flooded wetland, particularly in Vietnam and tropical regions in general. The observed relationships between environmental variables and free-living nematode communities in seasonally flooded wetlands in southeastern Vietnam can be summarized as follows:

i) The nematode communities are remarkably attributed by the slightly high density and low diversity in comparison to other habitats, such as fresh and brackish. Several genera, such as *Cryptonchus*, *Prismatolaimus*, *Eudorylaimus*, *Achromadora*, *Metalinhomoeus*, *Rhabdolaimus*, *Paractionlaimus*, *Alaimus*, *Chronogaster*, *Neotobrilus*, *Monhystera*, and *Udonchus*, were served as the principal genera and could play a crucial role in the communities. ii) In addition, nematode communities were sampled and environmental variables were measured at three different transects. While there were significant differences among the three transects for density, bio-indices did not differ. As far as the environmental variables are concerned, there were significant differences among the three transects for all variables, apart from TDS. This can be due to smaller sample size and hence the future study should collect at least more than fifteen samples. iii) The nematode density was significantly correlated with pH and depth negatively. Meanwhile, J' and biodiversity indices, such as Shannon–Weiner index H' and Hill indices, have shown a strong positive correlation with NH_4^+. In fact, nematode communities in seasonally flooded wetlands are governed by several environmental variables (e.g. depth, pH, and NH_4^+). iv) The TNGM's EcoQ based on MI values can be easily classified as high EcoQ conditions. Furthermore, MI is found to be a good method to assess the EcoQ due to its relation to a wide range of anthropogenic environmental drivers. In a nutshell, the results presented in this study can be used as the baseline data for future research of nematodes in the seasonally flooded wetlands and wetland ecosystems in general.

AUTHOR CONTRIBUTIONS

The study idea, field survey, data analysis and the writing manuscript have been performed by T.T. Tran. Y.M.T. Nguyen, Q.X. Ngo have supported data analyses and manuscript preparation. L.T. Pham; S.N. Hoang and B.K. Veettil have performed making revisions to the manuscript, and also writing partly the discussion section.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy have been completely observed by the authors.

ABBREVIATIONS

ABC	Abundance Biomass Curves
NH_4^+	Ammonia concentration
Av.Ab	Average abundance
Av.diss	Average dissimilarities
Av.sim	Average similarities
cm^2	Cubic centimeter
CI	Confidence Intervals
Con	Contribution
Cum	Cumulative
d	Margalef's species richness
EcoQ	Ecological quality status of sediments
$H' log_2$	Shannon diversity index
ITD	Index of trophic diversity
J'	Pielou's evenness
LGXMNP	Lo Go-Xa Mat National Park
MI	Maturity Index
N_1	Hill indices
N_2	Hill indices
N_{inf}	Hill indices
%C	The percentage of organic carbon
S	Genus richness
Se	Sediment
SD	Standard deviation

SIMPER	SIMilarity PERcentages
TDS	Total Dissolved olids
Tempt	Temperature
TNGM	Ta Not seasonally flooded grassy marsh
W	Water

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