Impacts of saltwater intrusion on soil nematodes community in alluvial and acid sulfate soils in paddy rice fields in the Vietnamese Mekong Delta

Van Sinh Nguyen a,b, Minh Khoi Chau b, Quang Minh Vo c, Van Khoa Le b, Thi Kim Phuong Nguyen a,b, Masaaki Araki d, Roland N. Perry e, Anh Duc Tran b, Duy Minh Dang b, Ba Linh Tran b, Gyu Lee Chol a, Koki Toyota a,∗

a Graduate School of Bio-Applications and Systems Engineering, Tokyo University of Agriculture and Technology, 2-24-16 Naka-cho, Koganei-shi, Tokyo 184-8588, Japan
b Department of Soil Science, Can Tho University, Campus II, 3/2 Street, Ninh Kieu District, Can Tho City, Viet Nam
c Department of Land Resources, Can Tho University, Campus II, Can Tho City, Viet Nam
d Environmental Bio-function Division, National Institute for Agro-Environmental Sciences, 3-1-3 Kamondai, Tsukuba 305-8604, Japan
e School of Life and Medical Sciences, University of Hertfordshire, Harfield, Hertfordshire AL10 9AB, UK

ARTICLE INFO

Keywords:
Nematode diversity
Indicator species
Paddy rice
Nematode functional guild
Salinity
Soil fertility

ABSTRACT

Saltwater intrusion is a potential risk damaging crop diversity and productivity due to degraded soil physicochemical properties. However, little is known about how salinity affects the structure and function of soil nematodes community in intensive rice cultivated area. This study aimed (1) to assess the impacts of saltwater intrusion on the nematode community in alluvial and acid sulfate soils; and (2) to evaluate its relation with soil conditions. Saltwater intrusion reduced the abundance of both free-living nematodes (FLN) and plant-parasitic nematodes (dominated by Hirschmanniella) in soils. FLN community was different among sites with different physicochemical properties. The omnivorous genera Aporcelaimellus and Thornemema were only found in non-salt-affected alluvial soil, whilst Mesodorylaimus was dominant in salt-affected acid sulfate soil, suggesting that this genus might be tolerant to higher EC and soluble Na+ , K+, Ca2+. The bacterivorous nematodes (dominant taxa Chronogaster, Rhabdolaimus) were dominant in both non-salt affected and salt-affected alluvial soils, which accounted for 48% and 40%, respectively, whilst it accounted for 21% in salt-affected acid sulfate soil. The abundance of fungivorous nematodes (Aphelenchoides, Ditylenchus, Filenchus) were greater in salt-affected alluvial soil in contrast to the other treatments, suggesting that these might be tolerant to salinity and low pH. Saltwater intrusion reduced biological diversity (Margalef, Shannon-Wiener, and Hill’s indices), maturity index (CMI, MI25), and clearly affected functional guilds of nematode community, especially c-p 5 group was reduced in both salt-affected soils. This study suggests that saltwater intrusion showed a potential risk in the degradation of soil properties, as indicated by the altered nematode community, trophic structure, functional guilds and their ecological indices in paddy fields.

1. Introduction

The Vietnamese Mekong Delta (VMD) is located in the monsoon tropical region and is surrounded by sea in the east and south. Climatic conditions of the VMD show a high temperature in the dry season, high precipitation in the wet season, and high humidity yearlong. Rice (Oryza sativa L.) is an important and dominant agricultural crop in the VMD and is planted in a total of 84% of agricultural land in this area (Tong, 2017). Rice cultivation systems are presented as single rice, double rice, and triple rice that indicates cultivating one, two, and three crops of rice in a year in the same field, respectively (Sakamoto et al., 2006).

Several soil types have been identified in the VMD (Nguyen et al., 2015). For instance, Fluvisols and Ortho-Thionic Gleysols are the biggest groups, which are the most suitable soil types for crop cultivation, including rice, upland crops, and fruit trees. Salic Gleysols soil is a followed group, which is located in surrounding coastal areas of the delta, and in this soil type, aquaculture is more widespread including permanent shrimp cultivation, and an integrated shrimp-rice system is also...
practiced. The other soil groups such as Leptosols, Arenosols, Histosols, Acrisols and Plinthosols are also present. Additionally, the occurrence of potential (predominantly as the mineral pyrite and it has not been exposed with air) and actual (sulfides react with oxygen to form sulfurous acid in the soils) acid sulfate soils is also described and it occupies >40% of the VMD. Such acid sulfate soil have developed under inundation by brackish water along the coastal line, where large amounts of organic matter and sulfuriac ion have been supplied from mangrove forests and seawater (Xuan et al., 1982; Nguyen et al., 2000). The diversity of soil types and cropping system may relate to differences in the soil physicochemical characteristics and the diversity of the soil organism community, including nematodes.

Climate changes are becoming more extreme and have severe impact on agricultural land since temperature is increasing in the dry season and precipitation is decreasing in the rainy season, resulting in increased crop damage caused by drought and salinization (Tran et al., 2019; Wassmann et al., 2019). Among these effects, saltwater intrusion is the most serious issue in the coastal areas worldwide and the VMD region particularly, because it directly affects the soil property and the physiology of crops. According to Wassmann et al. (2019), the paddy fields in Kien Giang (KG), Vinh Long (VL) and Ben Tre (BT) sites (Fig. 1) have been affected by saltwater intrusion in recent years and are now identified as fresh or slightly saline (<2 g L⁻¹), moderately saline (2 g L⁻¹–4 g L⁻¹), and highly saline (>4 g L⁻¹), respectively. Saltwater intrusion into agricultural lands has many effects, including decreased crop productivity and degraded soil property, through the inputs of water-soluble salts (Wang et al., 2019; Kruse et al., 2020; Truc et al., 2020). Sodium accumulation in soils results in the destruction of the structure of the system, which decreases plant growth and soil organisms, including microbes (Celia and Elisabeth, 2012). For example, high salinity reduces the microbial biomass (Tripathi et al., 2006), and respiration (Gennari et al., 2007) and causes increases in soil C and decreases in N mineralization, resulted in the increase of C/N ratio (Neubauer et al., 2019). Therefore, saltwater intrusion may also affect the nematode community through direct effects of a high concentration of soluble salts and indirect effects brought about by the reduction of the microbial community, which provides food resources for nematodes.

Nematodes are distributed worldwide from tropical to temperate climates, including natural agroecosystems, such as forests and grasslands, to cultivated crop systems (Yates and Bongers, 1999; Van den Hoogen et al., 2019). Moreover, they also can live in extreme climate conditions from the deserts to Antarctica. Nematodes are important biological components in soil ecological systems due to their roles in organic matter decomposition and nutrient cycling (Ferris et al., 1998; Magdoff, 2001; Buchanan et al., 2013) and they are good biological indicators for soil health assessment. For instance, some species like Caenorhabditis elegans, Dorylaimellus, Cephalobus, Aphelenchoides are very sensitive to soil disturbance, including heavy metal pollution and soil pH (Korthals et al., 1996; Yang et al., 2017; Martinez et al., 2018). Salinity, organic carbon and hydrocarbon contents of sediments are the key factors negatively influencing the density, biomass and diversity of nematodes (Mahmoudi et al., 2002). For example, Tylenchus and Aphelenchoides were tolerant to slightly saline soil conditions whilst a few species of taxon Dorylaimus and Tylencholaimus were abundant in saline soil (Ray and Das, 1980).

Okada et al. (2011) and Korobushkin et al. (2019) examined the nematode communities in paddy and upland rice fields, and among different biotope types in temperate regions to determine nematode population characteristics that relate to the water management. Both studies concluded that rice cultivation under flooding conditions increased the relative abundance of plant-parasitic nematodes (PPN) in temperate regions. However, the responses of nematode communities to saltwater intrusion in paddy fields in alluvial soil and acid sulfate soil in extremely intensive monoculture rice systems are poorly known, especially in tropical paddy fields. The hypothesis is that saltwater intrusion into paddy rice soils may break down the structures in soil physicochemical properties by supply higher soluble salts, resulting changes in soil nematode communities, diversity loss. Additionally, the temporary effect of saltwater intrusion may be a stress factor affecting specific nematode genera. Therefore, this study aimed:

- To identify the nematode assemblage composition in intensive rice cultivation systems with different soil types, alluvial and acid sulfate soils, under the effects of saltwater intrusion.
- To evaluate their relationships with the soil physicochemical and biological properties.
- To propose nematode indicators for salinity intrusion to paddy rice fields and for specific soil type.

2. Materials and methods
2.1. Location of paddy rice fields

Soil samples were collected at harvest of summer-autumn in 2019 from paddy rice fields which were located in Kien Giang (KG) (9°43'34.43" N, 105°10'55.06" E) province and in Ben Tre (BT) (9°58'22.51" N, 106°28'51.22" E) province (Fig. 1). Soil samples in Vinh Long (VL) (9°57'13.07" N, 105°55'58.01" E) province were collected at harvest of summer-autumn in 2017 and 2018. The soil type of KG was alluvial soil and that of BT acid sulfate soil and both soils were affected by saltwater intrusion in the dry season (spring-summer season), as the locations are 22 km and 16 km, respectively, from the sea. Among the locations, the distances from KG to VL and from VL to BT are 85 km and 60 km in a straight line, respectively. According to Wassmann et al. (2019), the paddy fields in VL, KG and BT sites have been affected by saltwater intrusion in recent years and identified as fresh or slightly saline (<2 g L⁻¹), moderately saline (2 g L⁻¹–4 g L⁻¹), and highly saline (>4 g L⁻¹), respectively.

Soil samples were collected at harvest of the rice crop. At each site, one field was chosen for soil collection. Several soil samples were collected at different locations in a field, and consider as replicates of each site. A total of 87 soil samples collected in the same cultivation season (summer-autumn cultivation season) were compared. Data deriving from 39 samples belonging to non-salt-affected soil in VL (27
samples in September 2017 and 12 samples in September 2018), of which 3 samples in 2017 and 3 samples in 2018 were cited from our previous paper (Nguyen et al., 2020b); 48 samples derived from salt-affected soils, including 32 samples in BT (September 2019), and 16 samples in KG (September 2019). In each soil sample, a total of five soil cores were collected randomly from the soil surface (0–10 cm depth) and evenly mixed thoroughly to obtain the homogeneous samples of independent locations. The details of the soil physicochemical properties are shown in Fig. 3.

2.2. Nematode extraction

Mist soils (100 g) were used for nematode extraction. These soils were treated with 70 °C hot 4% formaldehyde solution before extraction. The nematodes were extracted by using the decanting and sieving method (Cobb, 1918) and sucrose centrifugation (Thistletway and Riedel, 1969). A few drops of 1% rose Bengal solution were added to dye the nematodes to enhance observation and counting. The total number of nematodes were counted under an Olympus microscope (×40) and converted to the density per 100 g of dry weight (DW) soil. At least 100 individual nematodes were picked out at random for fixation and identification. Nematodes were fixed (De Grisse, 1969) and mounted in a drop of glycerin on a glass slide and sealed with a paraffin ring for nematode identification. Extracted nematodes were transferred into a staining block with the solution I (99 parts of formalin solution (4%) and 1 part of glycerin), then put into a desiccator saturated with ethanol at the bottom. The desiccator was kept in an oven at 40 °C for 24 h; the next day the staining block was taken out of the desiccator, and 1/3 partially covered by a glass piece on top to allow slow evaporation of the ethanol, and placed into the oven. Solution II (95% parts of ethanol 98% and 5 parts of glycerin) was added every 2 h for a total of 4–5 times, then solution III (50 parts of ethanol 96% and 50 parts of glycerin) was added. The day after it was checked if the nematodes were in pure glycerin (no whirling should be noticed when pure glycerin was added). Finally, the nematodes were mounted into a drop of glycerin on a glass slide and sealed with a paraffin ring. These slides were used during the identification process for nematodes.

All nematodes were assigned to five trophic groups; bacterivorous (Ba), fungivorous (Fu), plant-parasitic nematode (PPN), omnivorous (Om), and predator (Pre) (Yeates et al., 1993). The classification of nematode colonizer-persister (cp) values were performed based on life-history strategies, which ranges from 1 to 5 and assigns the taxa of soil nematodes according to their r and K characteristics (Bongers, 1990; Bongers and Bongers, 1998). For example, nematodes of cp-1 have short generation time, high fecundity, and are mainly bacterivorous that feed on enriched media. Nematodes of cp-2, cp-3, and cp-4 have longer generation times, greater sensitivity to adverse conditions and soil disturbance, and are mainly bacterivorous, fungivorous, predator, and small omnivorous. Nematodes of cp-5 have the longest generation time, largest body, lowest fecundity, greatest sensitivity to soil disturbance, and are mainly omnivorous and predator (Ferris et al., 2001). The maturity index (MI, MI0, MI25) is an ecological measure of environmental disturbance based on nematode species composition (Bongers, 1990). The number of genera (S), density (N), Shannon-Weiner (H') diversity, species richness Margalef (d), and Hill's (N1, N2, N infinity) indices were performed by using a Primer package version 6 (Clarke and Gorley, 2006).

2.3. Soil physicochemical properties analysis

In each location, some soil samples from the total collected soils were chosen for chemical analysis, comprising six samples in VL, eight samples in KG, and eight samples in BT. Soils were air-dried after soil moisture measurement. Air-dried soils were sieved through 5 mm and 1 mm diameter screens. Soils that passed through 5 mm sieve were used to measure the maximum water holding capacity (MWHC) and set up the incubation experiment for microbial activity analysis (Moebius-Clune et al., 2017). Soils that passed through 1 mm diameter sieve were used to measure pH, electrical conductivity (EC), total nitrogen (N) and total carbon (C), and soluble cations, sodium (Na+), potassium (K+) and calcium (Ca2+). Soil moisture was measured by incubating an exact amount of moist soils at 105 °C in 48 h. The water extraction method was used for soil pH and EC based on a ratio of soil weight to water volume of 1:2.5, then measured by pH meter (744-Metrohm) and EC instrument (Twin Cond-HORIBA). A sample (0.5 g) of air-dried soil was used to analyze the total C and total N by using a CN Coder MT-700 apparatus (Yanaco Co., Japan).

Air-dried soil (10 g) was rewetted in a 30 ml glass bottle to get 60% of MWHC and incubated for 7 days under aerobic conditions at 25 °C in a Biotron (NK system). The water content in the soil was checked and maintained every day during the incubation period. The rewetted soils at 3 days incubation were used to measure the emission of CO2; the amount of CO2 emission per hour per soil weight was indicators of the microbial activity. The amount of CO2 was measured by Gas Chromatography (Shimadzu-8A) with a TDC detector. The different amounts of CO2 emission at 0 time and 3 h after the cap cover was calculated as the total amounts of CO2 emission per kg dry soil per hour.

Soil soluble cations Na+, K+, and Ca2+ were extracted by deionized (DI) water at a ratio of 1:10 of a soil weight (2.5 g) and volume of DI water (25 ml) after 1 h shaking at 120 rpm. Then, the mixtures were centrifuged at 8000 × g in 5 min. The supernatants passed through Advantec 5C filter paper were analyzed for Na+, K+ and Ca2+ by using flame photometers (BWB model BWB-XP).

2.4. Data analysis

Data were analyzed by using one-way ANOVA to determine the difference of nematode community composition and soil physicochemical properties among locations. Nematode abundance were transformed by log10 (X) to obtain the normal distribution, and homogeneity of variances assessed by Levene’s test. When the significant differences were observed from univariate results (p < 0.05), the Post hoc Tukey’s HSD test was used to compare the significant differences among treatments. All statistical analyses were performed by using the statistical
Differences at p-value < 0.05 were considered to be statistically significant.

Further, to demonstrate the distribution pattern of taxonomic nematodes communities among locations and to find out the overall of the main corresponding variables of soil physicochemical properties among locations, the principal component analysis (PCA) was performed based on taxonomic nematodes abundance, and soil physicochemical properties among locations. PCA was run on a full set (normalized data) of nematodes abundance or soil physicochemical properties to get the same metric for all variables. Non-metric multidimensional scaling (nMDS) were performed based on soil nematodes community taxonomic abundance composition to illustrate the distribution patterns of each nematode taxa among locations. nMDS was run on log (X + 1) transformed data set and a resemblance measure by S17 Bray Curtis
similarity among samples across all locations. The ‘stress’ value from nMDS should be small, at least <0.20 and ideally <0.10, showing that the reduction to two dimensions implies very little loss of information (Legendre and Legendre, 1987). The effect of soil chemical properties on the nematodes community was assessed by Mantel-test. For this test, Bray-Curtis distance was used to construct dissimilarity matrices of communities and soil characteristics (10 factors), using a vegan package of R (version 4.0.2) with 999 permutations. Significant values were considered when p < 0.05. Significant differences between groups in nematode community composition datasets were tested with PERMANOVA. Significant values were considered when p < 0.05. After the PERMANOVA tests, PERMDISP routines were performed to test for the homogeneity of multivariate dispersions. Subsequently, pairwise comparison tests were performed to identify the significant differences of entire nematodes community composition from each other (e.g., non-salt-affected and salt-affected alluvial soils, non-salt-affected alluvial soil and salt-affected acid sulfate soil, salt-affected alluvial and salt-affected acid sulfate soils). Data were square root transformed prior to analysis to downsize the effects of dominant nematodes genera (Martínez et al., 2018). The SIMPER (Similarity percentages - species contributions) analysis was used to calculate the similarity between soil samples in a location, and the dissimilarity between soils among locations based on nematodes communities. Those analyses were supported by using PRIMER version 6 with the addition of PERMANOVA+ (Anderson et al., 2008). The canonical correspondence analysis (CCA) was performed to evaluate the linkages between soil physicochemical properties and the nematode assemblage composition using the Vegan package version 2.5-6 in R version 4.0.2.

3. Results

3.1. The differences of soil physicochemical properties among locations

The principal component analysis (PCA) revealed that the first two axes together explained 79.2% of the variation in soil physicochemical properties (Fig. 2). Non-salt-affected alluvial soil was characterized by greater soil moisture, total N and microbial activity, while salt-affected acid sulfate soil was characterized by greater pH, soluble cations Ca²⁺, Na⁺, K⁺, and EC and salt-affected alluvial soil by total C and C/N ratio. Soil pH was significantly greater in salt-affected acid sulfate soil than in salt-affected alluvial soil (Fig. 3b). Soil EC, soluble cations Na⁺ and K⁺ were greater (p < 0.001) in salt-affected soils than in non-salt-affected soil (Fig. 3f, g, h). Soluble Ca²⁺ was greater (p < 0.001) in salt-affected acid sulfate soil than in both salt-affected alluvial soil and non-salt-affected alluvial soil (Fig. 3i).

Total C was greater (p < 0.01) in salt-affected alluvial soil than in salt-affected acid sulfate soil (Fig. 3c). Total N was greater (p < 0.001) in non-salt-affected alluvial soil than in both salt-affected soils (Fig. 3d). C/N ratio was highest (p < 0.001) in salt-affected alluvial soil and lowest (p < 0.001) in non-salt-affected alluvial soil (Fig. 3e). Microbial activity was greater (p < 0.001) in non-salt-affected alluvial soil than in both salt-affected soils (Fig. 3k).

3.2. Impacts of salinity on nematode abundance and community composition

Total 72,302 nematode specimens were counted, and 9,238 individuals (12.8%) were identified to genera level. A total of 46 genera

Table 1
The nematode composition among different soil types in salt affected and non-salt affected areas of the Mekong Delta.

<table>
<thead>
<tr>
<th>Gradient of increasing the effect by saltwater intrusion</th>
<th>Gradient of increasing the effect by saltwater intrusion</th>
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<tbody>
<tr>
<td>Non-salt-affected alluvial soil</td>
<td>Salt-affected alluvial soil</td>
</tr>
<tr>
<td>(60.6%) Hirschmanniella (13.7%) Chronogaster (7.2%)</td>
<td>(62.0%) Aporcelaimellus (5.6%)</td>
</tr>
<tr>
<td>(64.7%) Hirschmanniella (26.5%) Mesodorylaimus (19.5%)</td>
<td>(49.4%) Mesodorylaimus (10.2%)</td>
</tr>
<tr>
<td>Salt-affected alluvial soil</td>
<td>Salt-affected acid sulfate soil</td>
</tr>
<tr>
<td>(64.7%) Hirschmanniella (25.9%) Chronogaster (12.9%)</td>
<td>(69.0%) Hirschmanniella (26.5%) Chronogaster (8.0%)</td>
</tr>
<tr>
<td>Salt-affected acid sulfate soil</td>
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Different letters indicate significant difference among locations by Tukey’s HSD comparison with p* < 0.05; p** < 0.01; p*** < 0.001; ns-non significant by one-way ANOVA test; a is more abundant than b.
were identified, belonging to 30 families, and 11 orders. The number of genera was greater in non-salt affected alluvial soil (42 genera) than in salt-affected alluvial soil (17 genera) and acid sulfate soil (16 genera) (Table 1). The abundance of PPN and FLN were greater ($p < 0.001$) in non-salt affected soil than in salt-affected soils (Fig. 4).

Permutational Multivariate Analysis of Variance (PERMANOVA) of entire nematode abundance showed significant differences among locations ($df = 2$, $F = 50.4$, $p = 0.001$), however, distant-based test for homogeneity of multivariate dispersions (PERMDISP) showed that variances were significantly heterogeneous (PERMDISP = 0.001) (Supplemental Material – SM 1); therefore, this requires careful interpretation of multivariate dispersion. Result of the pairwise test of PERMANOVA among locations showed significant differences in the nematode assemblage composition among locations, non-salt affected alluvial soil and salt-affected alluvial soil ($t = 8.67$, $p = 0.001$, PERMDISP = 0.001), non-salt affected alluvial soil and salt-affected alluvial soil ($t = 5.81$, $p = 0.001$, PERMDISP = 0.001) and salt-affected alluvial soil with salt-affected acid sulfate soil ($t = 5.26$, $p = 0.001$, PERMDISP = 0.814) (Supplemental Material – SM 1 & SM 2). Moreover, nMDS ordination analysis showed distinctly different groupings of nematode assemblage composition (Fig. 5) and the spatial distribution pattern of dominant genera like Aporcelaimellus, Mesodorylaimus and Hirschmanniella (Supplemental Material – SM 3) depending on different locations, and less fluctuations of taxonomic composition and abundance of nematode communities in the non-salt-affected alluvial soil between 2017 and 2018, with a stress of 0.14. Similarity percentages-species contributions (SIMPER) analysis showed that soils among locations had high levels of dissimilarity in nematode assemblages (Table 2). The large dissimilarity was observed between non-salt-affected and salt-affected soils, which was 62.0% between non-salt-affected alluvial soil and salt-affected alluvial soil, and 64.1% between non-affected alluvial soil and salt-affected acid sulfate soil. Nematode assemblages within the same location had high levels of similarity (60.6% – 69.0%), and Hirschmanniella (13.7% – 26.5%) was the taxon that was most similar.

The nematode community was different between locations, especially in some dominant FLN genera such as non-salt-affected alluvial soil (Aporcelaimellus, Thornei, Dorylaimus, Chronogaster, Rhabdolaimus) and salt-affected alluvial soil (Aphenlenchoidea, Diylenchus, Filenchus) and salt-affected acid sulfate soil (Mesodorylaimus, Aquatid) and some PPN genera like Hirschmanniella, Helicotylenchus, Bitylenchus (Table 1). Aporcelaimellus (60 ± 43 ind. 100 g$^{-1}$ DW soil) and Thornei (76 ± 128 ind. 100 g$^{-1}$ DW soil) were identified only in non-salt-affected alluvial soil. The abundance of Mesodorylaimus was greater ($p < 0.001$) in salt-affected acid sulfate soil (90 ± 76 ind. 100 g$^{-1}$ DW soil) than in both non-salt-affected and salt-affected alluvial soils (6 ± 11 ind. 100 g$^{-1}$ DW soil). Dorylaimus was greater ($p < 0.001$) in non-salt-affected alluvial soil (67 ± 55 ind. 100 g$^{-1}$ DW soil) than in both salt-affected soils, while Aquatid showed high abundance in salt-affected acid sulfate soil. Chronogaster was counted at all locations (accounted for 3.7% to 7.0% total nematodes each location) and its abundance was greater ($p < 0.001$) in non-salt-affected alluvial soil (71 ± 58 ind. 100 g$^{-1}$ DW soil) than in salt-affected alluvial soil (31 ± 25 ind. 100 g$^{-1}$ soil) and salt-affected acid sulfate soil (12 ± 13 ind. 100 g$^{-1}$ DW soil). Rhabdolaimus was greater ($p < 0.001$) in non-salt-affected alluvial soil (60 ± 63 ind. 100 g$^{-1}$ DW soil) than in both salt-affected areas (alluvial soil, 1 ± 3 ind. 100 g$^{-1}$ DW soil, and acid sulfate soil, 13 ± 21 ind. 100 g$^{-1}$ DW soil). Hirschmanniella was the most dominant genus at all locations, accounting for >50% of the total nematode abundance. The abundance of Hirschmanniella was greater in non-salt-affected alluvial soils (725 ± 408 ind. 100 g$^{-1}$ DW soil) than in salt-affected area (337 ± 50% of the total nematode abundance. The abundance of Hirschmanniella was greater in non-salt-affected alluvial soils (725 ± 408 ind. 100 g$^{-1}$ DW soil) than in salt-affected area (337 ±
Ecological Indicators 122 (2021) 107284

3.3. Impacts of saltwater intrusion on trophic structure, functional guilds of free-living nematode community in paddy rice fields

Bacterivorous nematodes were greater (*p* < 0.001) in non-salt-affected alluvial soil (40%) and salt-affected alluvial soil (48%) than in salt-affected acid sulfate soil (21%) (Fig. 6). The relative abundance of omnivorous nematodes was greater (*p* < 0.001) in salt-affected acid sulfate soil (70%) than in salt-affected alluvial soil (4%) and non-salt-affected alluvial soil (47%). Fungivorous nematodes were significantly greater in salt-affected alluvial soil (43%) than in non-salt-affected alluvial soil (4%) and in salt-affected acid sulfate soil (6%). Predator nematodes were less abundant at all locations and its relative abundance was significantly greater in non-salt-affected alluvial soil (8%) than in salt-affected acid sulfate soil (2%).

The relative abundance of functional guilds of the FLN community varied among sites (Fig. 7). Nematodes of cp-1 were lowest at both sites (varied: 0.06% – 2.98% total FLN), and were significantly (*p* < 0.001) greater in salt-affected alluvial soil (2.9%) compared with that in non-salt-affected alluvial soil (0.46%) and salt-affected acid sulfate soil (0.06%). There were significantly (*p* < 0.001) more nematodes of cp-2 in salt-affected alluvial soil (53.6%) than in non-salt-affected alluvial soil (7.3%) and salt-affected acid sulfate soil (9.6%). Nematodes of cp-3 occurred in significantly (*p* < 0.001) greater numbers in both non-salt-affected (33.6%) and salt-affected (34.0%) alluvial soil than in salt-affected acid sulfate soil (17.4%). Numbers of nematodes of cp-4 were greatest (*p* < 0.001) in salt-affected acid sulfate soil (70.5%) and lowest in salt-affected acid sulfate soil (6.4%). Nematodes of cp-5 was significantly (*p* < 0.001) greater in non-salt-affected alluvial soil (36.4%) than in salt-affected alluvial soil (3.1%) and acid sulfate soil (2.5%).
3.4. Effects of salinity intrusion on diversity and nematodes indices

The number of genera (Fig. 8a), Margalef richness index (d), Shannon-Wiener index (H') and Hill’s indices were greater (p < 0.001) in non-salt-affected alluvial soil than in salt-affected areas (Fig. 8b & c). Both ∑MI and MI25 were greater (p < 0.001) in non-salt-affected alluvial soil than in salt-affected soils, and it was significantly the lowest in salt-affected alluvial soil (Fig. 8d). The PPI index was lower (p < 0.001) in non-salt-affected alluvial soil than in salt-affected soils (Fig. 8d).

3.5. Correlation of dominant taxa, trophic structure, and diversity to soil physicochemical properties

Mantel test was used to estimate the main factors of soil physicochemical properties that affect the distribution of nematode assemblage composition among locations. Results showed that the soil physicochemical properties affected on nematode composition, particularly soil moisture (p < 0.001) in non-salt-affected alluvial soil than in salt-affected areas (Fig. 8b & c). Both ∑MI and MI25 were greater (p < 0.001) in non-salt-affected alluvial soil than in salt-affected soils, and it was significantly the lowest in salt-affected alluvial soil (Fig. 8d). The PPI index was lower (p < 0.001) in non-salt-affected alluvial soil than in salt-affected soils (Fig. 8d).

4. Discussion

4.1. Effects of saltwater intrusion on nematode abundance and composition in rice field

The nematodes communities were identified in three different locations. The abundance of nematodes, both FLN and PPN, was greater in non-salt-affected alluvial soils than in salt-affected soils. Their distribution may be related to the soil physicochemical properties affected by locations and saltwater intrusion. The alluvial soil was most fertile due to the higher total C, total N, and microbial activity. These conditions provide more food resources and better environmental conditions for the nematode community (Okada et al., 2011; Yan et al., 2018; Chen et al., 2019; Nguyen et al., 2020a). By contrast, in salt-affected areas, the factors that differ the most were soil EC, soluble cations such as Na⁺, K⁺, Ca²⁺, and C/N ratio. In general, salinity causes, directly or indirectly, a harmful influence on soil quality, since it affects the physical, chemical and biological properties of the soil (Celia and Elisabeth, 2012).

In this study, saltwater intrusion decreased almost the abundance of nematodes composition, particularly in some dominant nematodes genera, such as Hirschmanniella, Aporcelaimella, Thorneema, Dorylaimus, Rhabdolaimus, and Rhizolaimus. We considered that saltwater intrusion reduced the abundance of the genera through direct and indirect effects. A direct effect may be osmotic pressure due to soluble salts because nematode abundances were significantly negative correlations with soil EC, soluble Na⁺, and soluble K⁺. Also, Wu et al. (2015) reported that the total abundance of nematodes was greater in non-saline soils than in saline soils due to the lower EC, and the nematode abundance never exceeded 2 nematodes g⁻¹ DW soil at a salinity level of >2 dS m⁻¹. A bacterivorous nematode species, Caenorhabditis elegans, is the most extensively studied and tolerates up to 15.5 g NaClL⁻¹ for 96 h or up to 20.5 g NaClL⁻¹ in 24 h (Khanna et al., 1997). Moreover, Moens and Vincx (2000) reported that salinity had significantly impacted the viability of the juvenile stage of bacterivorous nematode species in extreme salinity conditions. As an indirect effect on soil nematodes community, saltwater intrusion reduces microbial biomass or plant biomass, resulting in less food resources for microbial growth and production. In this study, the microbial activity declined in both salt-affected soils, which may result in less food sources for nematodes. Indeed, saltwater intrusion reduced microbial biomass and respiration (Tripathi et al., 2006; Mavi et al., 2012). Also, Neubauer et al. (2013) reported that soils irrigated with saltwater had significantly lower soil C and N contents and higher C/N ratios compared to the treatments irrigated with fresh water. These findings suggest that the total abundance of nematodes declined directly through salt stress and indirectly through reduced microbial biomass by saltwater intrusion.

Salinity is an important environmental factor driving distribution patterns of nematode assemblage composition within the estuaries. Many studies have reported the nematode community assemblage in the different estuarine environments, such as European estuaries (Soetert et al., 1995; Adao et al., 2009; Alves et al., 2014) and the Vietnamese Mekong estuaries (Nguyen, 2011; Quang et al., 2016; Yen et al., 2020). However, information is lacking on the nematode composition in the intensive paddy rice fields affected by salinity. Our results showed that the nematode community assemblage in the paddy rice soils in this study was markedly different to that in the sediment of the estuarine
environment in the VMD region. For example, the nematode community present in the paddy rice field at severely salt-affected soils in Ben Tre site in this study differed in taxonomic composition to the nematode assemblage in the estuarine sediment in the upstream of the Mekong River (Yen et al., 2020), for example, the predominant species in paddy rice in Ben Tre site were *Hirschmanniella*, *Mesodorylaimus*, *Aquatides*, *Rhabdolaimus*, *Filenchus*, *Chronogaster* and *Dorylaimus* in this study, but were *Parodontophora*, *Theristus*, *Daptonema*, *Terschellingia*, *Sphaerotheri-istus*, *Mesodorylaimus*, *Rhabdolaimus* and *Viscosia* in the estuarine sediment. Interestingly, the two dominant nematode genera *Mesodorylaimus* and *Rhabdolaimus* were found both in the sediment of the estuarine environment in the Mekong River (Yen et al., 2020) and in soils from paddy fields having the greatest EC and soluble cations in this study. In particular, *Mesodorylaimus* had a high abundance in the paddy rice field...
and soluble Na\(^+\) in Ben Tre site and showed significantly positive correlation to soil EC.

Moreover, the presence of taxa *Daptonema* was only observed in the paddy rice field in Ben Tre site in this study, which was considered to be the most severely salt-affected soil. In general, species of taxon *Daptonema* are found in the intertidal zone and are adapted to salt conditions (Soetaert et al., 1995; Adao et al., 2009). Our data agree with the results of Quang et al. (2016) and Yen et al. (2020), who reported that *Daptonema* was found in the estuarine sediment in the intertidal zone in the Mekong River. Therefore, our hypothesis that the presence of *Daptonema* in paddy rice soil in Ben Tre site may result from the intrusion of saltwater in the dry season and irrigation practices. Also, we found that the abundance of *Aquatides* was significantly greatest in the Ben Tre site where the salinity in soil showed the highest values. In general, *Aquatides* is present in freshwater environment (Eyualm-Abebe et al., 2006) and was not found in sediments from estuarine environment (Soetaert et al., 1995; Alves et al., 2014; Quang et al., 2014). However, the understanding of the behavioral ecology of this taxon in the paddy fields is lacking, particularly about the effects of salinity exposure; therefore, further tests on the survival of *Aquatides* taxon in different salinity levels may be important for understanding their adaptation to different levels of salinity. We suggest that the monitoring of the presence of nematode assemblage composition in the paddy rice soils can be applied to assess the effects of saltwater intrusion on taxonomic composition and on biological indices of their community.

4.2. Effects of saltwater intrusion on trophic structure, functional guilds of free-living nematodes community in rice field

Among locations, trophic structures of nematodes communities showed a clear distribution pattern. Bacterivorous nematodes showed higher dominance in both salt-affected and non-affected alluvial soils than in salt-affected acid sulfate soil. This can be explained due to the higher soil fertility expressed as the higher total C, total N, and microbial activity of the alluvial soils since Pan et al. (2010) already reported that the abundance of bacterivorous nematodes was significantly correlated with soil organic carbon. The greater microbial activity in non-salt-affected soil may increase bacterivorous nematodes in this site, as Trap et al. (2016) reported that the presence of bacterivorous significantly increased soil microbial basal respiration. Jiang et al. (2017) also reported the positive correlation between bacterivorous nematodes and microbial biomass.

The abundance of fungivorous nematodes was greater in salt-affected alluvial soil (p < 0.001) than in non-salt-affected alluvial soil and salt-affected acid sulfate soil, possibly due to the higher C/N ratio and the total C in the soil. Deng et al. (2016) reported that soils with a higher C/N ratio can stimulate fungal biomass and activity that could provide food resources for fungivorous nematodes in the soil habitat (Ito et al., 2015). The greater fungivorous in salt-affected alluvial soil may also relate to the low soil pH. In general, the fungal community shows greater dominance in lower pH soils (Rousk et al., 2010). Indeed, a lower soil pH resulted in a greater abundance of fungivorous nematodes (Hyvonen and Persson, 1990), and increased soil pH reduced the abundance of fungivorous nematodes (Korthals et al., 1996). Our results showed that the genera *Aphelenchoïdes*, *Ditylenchus* and *Filenchus* were greater in salt-affected alluvial soil with the lowest pH. These fungivorous nematodes have been found to be tolerant to a low pH, like pH 3.9, and their abundance was significantly decreased by lime application (Zhao et al., 2015). In addition, *Aphelenchoïdes* and *Ditylenchus* were...
reported as the most tolerant taxa to higher zinc concentration (1000 mg kg\(^{-1}\)–3200 mg kg\(^{-1}\)) (Smit et al., 2002), which is negatively correlated with soil pH (Rutkowska et al., 2015). Also, Ocaña (1993) reported that the nematode genera in the order Tylenchida and Aphelenchida generally are tolerant to acidic pH ranges from 4.9 to 5.9.

Omnivorous nematodes are highly sensitive to changes in environmental conditions (Bongers, 1990). However, the distribution in the paddy rice ecosystem of many genera of omnivorous nematodes has not been understood. An interesting finding is the different distribution of the dominant omnivorous taxa, *Aporcelaimellus, Thornehena, Mesodorylaimus* and *Dorylaimus*, between locations in this study. *Dorylaimus* was observed at all locations and dominant in non-salt-affected alluvial soil, while *Mesodorylaimus* was dominant in salt-affected soil. Ray and Das (1980) reported that *Dorylaimus* can be tolerant in saline soil. Interestingly, the occurrence of two dominant genera *Aporcelaimellus* and *Thornehena* were only observed in non-salt-affected alluvial soil. This result may suggest that the greater soil EC and soluble salts such as Na\(^+\), K\(^+\) in salt-affected soils may suppress *Aporcelaimellus* and *Thornehena*. In general, these taxa are widespread and typical inhabitants of terrestrial habitats (Eyualem-Abebe et al., 2006). The lowest abundance of omnivorous nematodes in salt-affected alluvial soil may be caused by the lowest soil pH. Hyvonen and Persson (1990) concluded that the strong acidity markedly reduced the abundance of omnivorous nematodes in general. In addition, both *Aporcelaimellus* and *Mesodorylaimus* abundance were limited by soil pH lower than 4.0 (McSorley, 2012). In contrast to *Aporcelaimellus* and *Thornehena, Mesodorylaimus* showed a greater abundance in salt-affected acid sulfate soil and a few in non-salt-affected alluvial soil, and it was absent in salt-affected alluvial soil. In this study, salt-affected acid sulfate soil contained the highest concentration of soluble Ca\(^{2+}\) and had the highest soil pH value, and these two factors could be related to the greater abundance of *Mesodorylaimus* in this location. The application of lime increases soil pH, which resulted in a greater abundance of omnivorous and dorylaimine nematodes (Hyvonen and Persson, 1990). Also, *Mesodorylaimus* was one of the few taxa observed in high salinity conditions (Tudoranacea and Zullini, 1989).

The abundance of predator nematodes tended to decrease with the increase of EC, Na\(^+\), K\(^+\) in salt-affected soils in this study. *Nyigalaimus* and *Mononchus* were the most dominant genera in non-salt-affected alluvial soil. This result could be explained by the greater total N and microbial activity in the soil. A previous study found that the high numbers of predatory *Mononchus* nematodes was probably the most important factor explaining the increased N mineralization (Setala et al., 1990). Also, the greater microbial activity in non-salt-affected alluvial soil may stimulate the abundance of bacterivorous nematodes in this study, which might result in the increment of predator nematodes. Trap et al. (2016) reported that bacterivorous nematodes significantly increased soil microbial basal respiration and microbial turnover. Also, nematode predation has an important role in determining the composition and dynamics of the bacterial community (Jiang et al., 2017). Predator nematodes have been known to feed on a variety of soil microorganisms including nematodes such as bacterivorous and PPN (Bilgrami et al., 1986; Bongers and Ferris, 1999). Moreover, the greater soil EC and soluble cations Na\(^+\), K\(^+\) in salt-affected soils may also suppress these taxa since the species are commonly distributed in terrestrial habitats and freshwater (Eyualem-Abebe et al., 2006). Predator nematodes have the lowest fecundity and are most sensitive to environmental disturbances (Ferris et al., 2001). This suggests that saltwater intrusion in the dry season may be a factor involved in the reduction of predator nematodes in paddy rice fields.

The nematodes with a higher cp-value, e.g. nematodes of cp-5, which are mainly omnivorous and predator nematodes, have the largest body, lowest fecundity, and greatest sensitivity to soil disturbance (Ferris et al., 2001). In this study, one of the typical nematodes that were counted only in non-salt-affected alluvial soil was a cp-5 genus, *Aporcelaimellus*. Due to saltwater intrusion, which generally induces greater soil EC and soluble Na\(^+\), K\(^+\), Ca\(^{2+}\) in soil like this study, or greater Mg\(^{2+}\) (Celia and Elisabeth, 2012; Li et al., 2019), it may directly affect the structure of the nematode community, especially with the most sensitive taxa like omnivorous and predator nematodes. The greater abundance of nematodes with the higher cp-values in soil indicated a structured and matured soil ecosystem and it decreases in case of soil disturbance or degradation. Interestingly, cp-4 nematodes (most dominant genus *Mesodyrallaimus*) had the greatest abundance in salt-affected acid sulfate soil where soil EC, and soluble Na\(^+\), K\(^+\), Ca\(^{2+}\), and pH were higher compared to other sites. It is known that *Mesodorylaimus* seems the only genus common in intermitent lakes subject to high salinity and showed remarkable adaptation to extreme environments (Tudoranacea and Zullini, 1989; Eualem-Abebe et al., 2006; McSorley, 2012). Moreover, *Mesodorylaimus* was less affected by the toxicity of heavy metal, as Smit et al. (2002) reported that it can reach optimal abundance in a concentration range of zinc of 180 mg kg\(^{-1}\)–560 mg kg\(^{-1}\), and can live even in the concentration of 3200 mg kg\(^{-1}\) of zinc. Most cp-3 nematodes were bacterivorous feeders in this study and had relatively greater abundance in alluvial soils, reflecting the higher soil fertility, as described above. The cp-2 nematodes had the greatest relative abundance in salt-affected alluvial soil, which was represented almost entirely by fungivorous nematodes (dominated by *Flenchus*). The result may relate to the high soil total C, C/N ratio, and low pH, as explained above. Cp-1 nematodes mostly are bacterivorous feeder that had a high relative abundance in salt-affected alluvial soil (dominant *Protorhabditis*) in this study. This may be related to total soil C. In general, bacterivorous of the cp-1 guild, enrichment opportunists, were most responsive to increase in their food source (Ferris et al., 2004), and it was often found in disturbed environments (Ferris et al., 2001), which may be caused by saltwater intrusion.

### 4.3. Effects of saltwater intrusion on diversity and nematodes indices

Non-salt-affected alluvial soil showed a better soil quality than salt-affected soils, as indicated by the greater total N, total C and microbial activity, and lower soluble cations Na\(^+\), K\(^+\), Ca\(^{2+}\), and EC. These could be the main factors driving the greater diversity of nematodes community in non-salt-affected soil, like a study by Wu et al. (2015). The maturity index (\(\sum MI, MI_{25}\)) indicates an environmental disturbance of soils (Bongers, 1990). A higher \(\sum MI, MI_{25}\) value indicates a mature and stable food web structure in the soil habitats, which is considered better for crop productivity and ecosystem, whilst a lower \(\sum MI, MI_{25}\) indicates a higher disturbance in soil. The greater \(\sum MI, MI_{25}\) values were positively associated with soil fertility (Shaw et al., 2019). These results suggest that saltwater intrusion causes a potential reduction in diversity and food web structure of soil nematodes in paddy fields.

### 5. Conclusion

A total of 46 nematodes taxa belonging to 30 families and 11 orders have been identified in this study. Saltwater intrusion decreased the abundance of both FLN and PPN communities. *Mesodorylaimus* was the most dominant taxa in salt-affected acid sulfate soil, whilst it was absent in salt-affected alluvial soil. Two genera, *Thornehena* and *Aporcelaimellus* were only identified in non-salt-affected alluvial soil. *Aphe clenchoïdes, Dictylenchus* and *Filenchus* were capable of tolerating soils with low pH and moderate salinity in the soils. Non-salt-affected alluvial soil showed the greatest number of genera, diversity (Margalef, Shannon-Wiener, and Hill’s indices), and \(\sum MI, MI_{25}\) index. These results indicate the greater soil fertility and stability in terms of the nematode diversity and structure in non-salt-affected alluvial soil than in salt-affected soils. Saltwater intrusion reduced both the \(\sum MI, MI_{25}\) index, which indicates the loss of the soil nematodes’ component and ecological functions in salt-affected soils. Both trophic groups, Shannon-Wiener, and Margalef diversity indices had a positive correlation to MBA and total N, whilst they had a negative correlation with soil EC, Na\(^+\), and
K*. This study concluded that saltwater intrusion is a potential risk causing declining nematodes community in paddy fields as well as adversely affecting the soil physicochemical properties and microbial activity. We suggest that the use of nematode assemblage composition as an indicator is capable of assessing soil health conditions, particularly in paddy rice soils that are most vulnerable to saltwater intrusion due to climate changes.

CRediT authorship contribution statement


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.

Acknowledgement

This study was funded in part by the Can Tho University Improvement Project VN14-P6, supported by a Japanese ODA loan. We thank SUSRICE PROJECT-IJR2 financed by VLIR-UOS, Belgium for allowing us to take alluvial soil samples.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2020.107284.

References


