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Using nematode communities as an indicator of soil quality assessment for pepper (*Piper nigrum* L.) growing

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Abstract Soil nematode communities have been known as one of the most potential bio-indicators for soil quality assessment. Soil quality cultivated with black pepper (*Piper nigrum* L.) in Ba Ria-Vung Tau province, using soil nematode communities as indicator organisms was assessed. A total of 72 soil samples were elucidated at 12 sites at two soil depths of 0-10cm and 10-20cm. There were found 51 genera of nematodes which belong to 27 families in 7 orders. They are identified and classified in the 5 primary feeding groups as plant-parasites, bacterivores, fungivores, ominivores and predators. Four plant-parasitic nematode genera were found as *Meloidogyne, Ditylenchus, Discocriconemella* and *Rotylenchulus.* The total of 18 nematode genera found to be associated with black pepper cultivation. MI, PPI indices and c-p triangles showed that soil environment was stable and not appeared much stress. Correlation analysis indicated soil nematode communities had closedly interacted with environmental factors, of which, the feeding groups had better correlation than the biological index and showed potential of soil nematode communities used as bio-indicators for the monitoring and assessment of soil environmental quality.

Keywords: Ba Ria-Vung Tau, Black pepper, Soil nematodes, Soil quality assessment

Introduction

Since the 90s of the last century, the issues of sustainable land use have received more attention. In order to develop crop production in a sustainable way, it is very important to control and evaluate soil quality in order to protect agro-ecosystems (Porazinska *et al*., 1999). The relationship between land use and soil biota is perceived to be essential for a better understanding of soil

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ecosystem processes and adaptation in ecosystem management (Wu *et al.*, 2002). Agricultural ecosystems are often strongly affected by the use of chemical fertilizers and pesticides, which have degraded the environment and reduced biodiversity. Therefore, the monitoring of soil environmental quality in order to timely detect and have solutions to control soil quality is an important requirement developed and become more and more effective. Along with the advancement of technology, the methods of assessing the ecological environment in general and the quality of the soil environment in particular are increasingly rich and bring accurate results. In addition to traditional methods, biological methods using soil biomes as an indicator are increasingly proving advantageous in monitoring and assessing environmental quality. Nematode communities are considered as one of the potential indicator organisms to assess soil quality, especially in agro-ecosystems, soil nematodes respond rapidly to disturb the soil environment, as they are presented in very high abundance and diversity in soil and in different ways according to living conditions. They are suitable for using as environmental quality indicators (Bongers, 1990; Neher and Campbell, 1994; Neher, 2001; Ferris *et al.*, 2001).

In Vietnam, pepper production is one of the key agricultural commodity production industries and makes an important contribution to the economic growth of the country along with Gia Lai, Dak Lak, Dak Nong, Binh Phuoc, Dong Nai and Ba Ria-Vung Tau provinces, in which Ba Ria-Vung Tau is considered one of the six provinces for pepper cultivation and production in Vietnam. However, at present, most pepper farms mainly develop spontaneously according to traditional farming methods. Due to limitations in awareness and technology, pepper growers do not know how to control the optimal amount of agrochemical inputs, using a lot of inorganic fertilizers, and less organic fertilizers. Pests and diseases are commons, causing great impacts on all pepper growing areas. The abuse of pesticides makes the soil environment worse, affecting the yield and quality of pepper products. Therefore, the assessment of soil ecological environment quality is very necessary. The method of using nematodes in assessing the environmental quality of agricultural land is still quite new in Vietnam through the assessment and analysis of the characteristics of the nematode community structure. It is possible to identify the current quality status of the nematode community soil quality of the region and take appropriate measures to protect the soil and develop sustainable agriculture. The study of nematodes as an indicator for soil environmental quality was aimed to contribute to the diversification of monitoring method for the quality of the soil environment.

Materials and methods

Study site and collection soil samples

Soil samples were collected at 12 points in the longterm pepper growing areas which done in TanThanh (Hac Dich-HD1, Hac Dich-HD2 and Hac Dich-HD3), ChauDuc (Lang Lon-LL1, Da Bac-DB1, Kim Long-KL1, Kim Long-KL2, Quang Thanh-QT1 and Xuan Son-XS1) and XuyenMoc (Hoa Binh-HB1, Hoa Hiep-HH1 and Bao Lam-BL1) districts of Ba Ria-Vung Tau province, Vietnam (Figure 1).

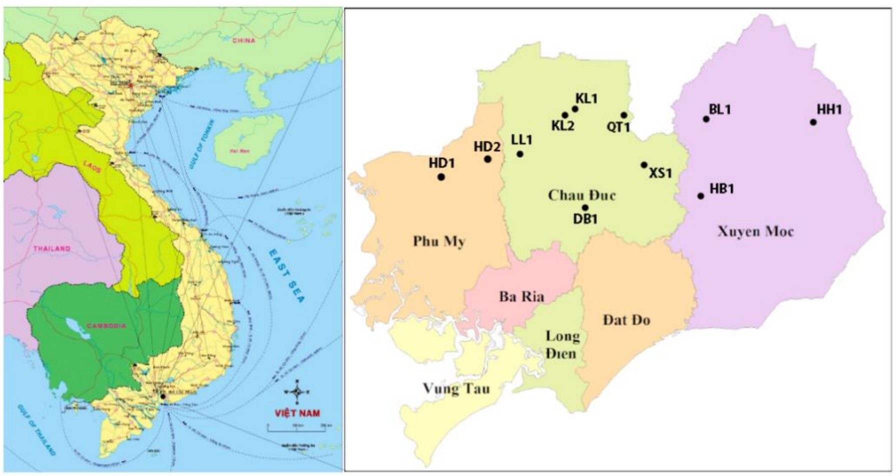

Figure 1. Location map of sampling at Ba Ria-Vung Tau province, Vietnam

Three replicates were collected at each site, each sample was collected at two depths: from 0-10cm and from 10-20cm. Soil samples were collected at a distance of 30cm from the root, weighing about 500g of soil (for biological and physicochemical analysis), then stored in plastic bags and transported within a day to the laboratory and analyzed immediately for physiochemical parameters. Soil samples were stored at 4 °C and analyzed for soil nematodes within 7 days. The temperature at the time of sampling was about 30-33^{0}C.

Analytical methods for soil physicochemical parameters

The soil physicochemical parameters were selected for analysis including pH, total nitrogen content, total phosphorus, total organic matter and were determined according to the corresponding Vietnamese standards: TCVN 5979:2007, TCVN 6498:1999, TCVN 8940:2011, TCVN 8941:2011.

Extraction and identification of soil nematodes

Nematodes were extracted from 100 gram soil each sample by decanting and centrifugation with sugar media (Smol, 2007). After counting on the square counting dish, nematodes were picked up and mounted by Seinhorst's method (1959), then 200 individuals per site were randomly selected and identified to taxonomic genus according to Fauna in Vietnam (Chau and Thanh, 2000; Thanh, 2007), Freshwater nematode, Ecology and Taxonomy (Abebe *et al.*, 2007) for Aphelenchida, Longidoriae and Trichodoridae (Hunt, 1993).

Trophic group and ecological indices

All extracted nematodes were classified into plant parasites, bacterivores, fungivores, omnivores and predators based on the classification of Yeates *et al.* (1993). Ecological indices of MI (Yeates, 1994) were calculated as follows:

$$\mathrm{MI} = \sum_1^f v_i \cdot \frac{n_i}{\sum_1^f n_i}$$

Where: MI – maturity index of the nematode community
v_i *– the c-p index (i) in the sample (defined according to Yeates et al., 1993)*
n_i *– number of nematodes in family (f) in sample*

The ecological triangle model (c-p triangle) of ecosystems was proposed by De Goede *et al.* (1993) with the sides of the triangle being % c-p values: the left side corresponds to % cp=1 value, the right edge corresponds to the value % cp=2, the bottom edge corresponds to the value % cp from 3 to 5. The cp ecological grouping resulted of any ecosystem was combined by 3 groups, whose value corresponded to the 3 sides of the triangle. From the % c-p values of each combination group defined on each side of the triangle, 3 lines were drawn parallel to the 3 sides of the triangle, created a common intersection for the 3 lines. The location of the identified intersection indicated the quality values as well as the trend of the environment: If the 3-line intersection points towards the top of the triangle (the cp=1 index group predominates), the environment is under pressure stress of organic matter. Towards the right (predominant cp=2 groups), the environment is under pressure of chemicals. Towards the left (predominant cp=3-5 groups), it is stable environment and without any stress.

Data processing

Data were processed through mean, standard deviation for repeated samples according to statistical principles using Microsoft Excel 2013 and R i386 3.1.1 software. Comparison of differences in soil physicochemical properties, distribution density, maturity index, ratio of trophic groups between depths and survey sites were used two-way ANOVA for data following the analysis normally distribution (or after converting the data to a normal distribution) and with uniform variance. The data that do not follow the normal distribution, using the non-parametric method of the Kruskal-Wallis test to check for difference between survey points and between two depths. The data for the correlation between physicochemical and biological factors do not follow a normal distribution, so the correlation coefficient used in the study was the Spearman correlation coefficient (instead of the Pearson coefficient). The significance level $\alpha=0.05$ was chosen to compare the difference.

Results

Analysis of physicochemical properties of pepper growing soils

The quick measurement results showed that the pH value at this study, at a depth of 0-10cm ranged from 5.0-6.95 and from 4.60 to 6.81 at the second depth (Figure 2A). Two-way ANOVA analysis showed that the soil pH value had a statistically significant difference between the two depths (p=0.039) and between the survey sites ($p=2.14.10^{-9}$). Thus, the pH at the depth of 0-10cm was higher than the depth of 10-20cm and varied which depending on the survey locations. Soil pH in the study area was in the range of medium to slightly acidic (5-7), the suitable pH range for pepper plants to grow was from 5.5-7.0.

At a depth of 0-10cm, the total organic matter content (OM) in the soil ranged from 7.24 to 11.57% and from 7.40 to 10.56% at depth 10-20cm (Figure 2B). The two-way ANOVA results showed that OM content between the two depths did not shown a statistically significant difference (p=0.1769) but there was a difference between the survey points ($p=2.49.10^{-8}$). Thus, the influence of depth on the variation of OM content in the soil in the study area was not significantly compared to the influence of the survey site locations.

The results indicated total nitrogen (TN) content at Ba Ria-Vung Tau province is assessed at the average total nitrogen richness in the study area, ranging from 0.14-0.34% at a depth of 0-10cm and from 0.12-0.33% at a depth of 10-20cm (Figure 2C). The average total phosphorus (TP) content at the study area at the first depth ranged from 0.05-0.47% and from 0.08-0.43% at the second depth (Figure 2D). Using two-way ANOVA (after converting the data to log(x) showed that the total nitrogen (TN) and average total phosphorus (TP) values between the two survey depths in the study area were not statistically different (p=0.1872). However, there was a statistically significant difference between the survey points ($p=1.70.10^{-15}$).

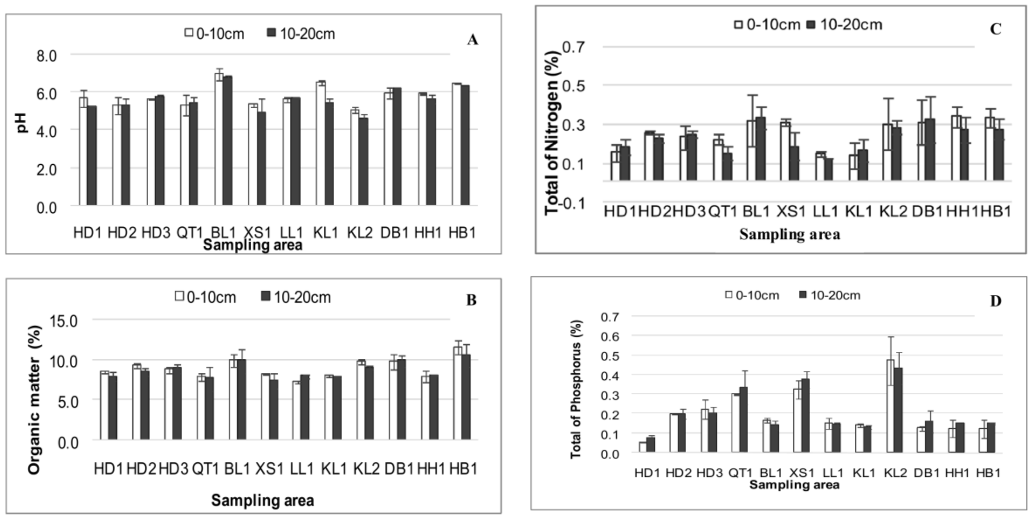

Figure 2. Calculation results of some soil physicochemical parameters at survey points by depth: (A) pH value, (B) total organic matter content, (C) total nitrogen content, (D) total phosphorus content

Structure of nematode community composition in pepper growing soils

Nematode genera and trophic groups

The number of nematode genera reflected the biodiversity of the soil habitat. Analysis results in Ba Ria-Vung Tau province were identified to be a total of 51 nematode genera belonging to 27 families, 7 orders, and are classified into 5 typical trophic groups (Table 1).

Table 1. Nematode genera and occurrence frequency of soil nematode communities in Ba Ria-Vung Tau province, Vietnam

Order	Family	Genera	Frequency of appearance (%)		Trophic group	Group cp
			0-10 cm	10-20 cm		
Aphelenchida	Aphelenchidae	*Aphelenchus*	0,67 ± 0,46	0,13 ± 0,07	Fungivores	2
	Aphelenchoididae	*Aphelenchoides*	1,40 ± 0,82	2,33 ± 1,10	Fungivores	2
Araeolaimida	Chronogasteridae	*Chronogaster*	0,11 ± 0,06	0,26 ± 0,16	Bacterivores	2
	Plectidae	*Plectus*	0,03 ± 0,03	0,01 ± 0,01	Bacterivores	2
Dorylaimida	Aporcelaimidae	*Aporcelaimellus*	0,20 ± 0,12	0,32 ± 0,12	Omnivore	5
	Belondiridae	*Axonchium*	0,17 ± 0,14	0,01 ± 0,01	Omnivore	5

Order	Family	Genera	Frequency of appearance (%)		Trophic group	Group cp
			0-10 cm	10-20 cm		
Enoplida	Dorylaimidae	*Belondira*	0,03 ± 0,02	-	Omnivore	5
		Crocodorylaimus	0,21 ± 0,12	-	Omnivore	4
		Discolaimus	-	0,07 ± 0,06	Carnivore	5
		Dorylaimus	0,02 ± 0,02	0,01 ± 0,01	Omnivore	4
		Eudorylaimus	0,85 ± 0,45	0,72 ± 0,30	Omnivore	4
		Labronema	0,72 ± 0,66	0,35 ± 0,27	Omnivore	4
		Laimydorus	0,04 ± 0,04	0,35 ± 0,35	Omnivore	4
		Mesodorylaimus	0,32 ± 0,13	0,13 ± 0,07	Omnivore	4
		Prodorylaimus	0,10 ± 0,05	0,05 ± 0,04	Omnivore	4
	Dorylaimoididae	*Dorylaimoides*	0,20 ± 0,09	0,20 ± 0,09	Omnivore	4
	Longidoridae	*Xiphinema*	0,59 ± 0,56	2,21 ± 2,14	Herbivores	5
	Alaimidae	*Alaimus*	0,03 ± 0,02	-	Bacterivores	4
		Amphidelus	0,08 ± 0,05	0,09 ± 0,06	Bacterivores	4
	Cryptonchidae	*Cryptonchus*	0,04 ± 0,04	-	Bacterivores	4
	Ironidae	*Ironus*	0,01 ± 0,01	0,01 ± 0,01	Predators	4
	Prismatolaimidae	*Prismatolaimus*	0,52 ± 0,34	0,67 ± 0,38	Bacterivores	3
Monochida	Mononchidae	*Prionchulus*	-	0,01 ± 0,01	Predators	4
	Mylonchulidae	*Mylonchulus*	0,07 ± 0,06	0,10 ± 0,06	Predators	4
	Anatonchidae	*Iotonchus*	0,12 ± 0,06	0,21 ± 0,16	Predators	4
Rhabditida	Cephalobidae	*Acrobeles*	0,04 ± 0,03	0,03 ± 0,02	Bacterivores	2
		Acrobeloides	-	0,10 ± 0,07	Bacterivores	2
		Cephalobus	0,24 ± 0,11	0,58 ± 0,23	Bacterivores	2
		Eucephalobus	0,36 ± 0,15	0,70 ± 0,33	Bacterivores	2
		Heterocephalobus	0,05 ± 0,04	0,25 ± 0,21	Bacterivores	2
	Panagrolaimidae	*Panagrolaimus*	0,04 ± 0,04	0,06 ± 0,04	Bacterivores	1

Order	Family	Genera	Frequency of appearance (%)		Trophic group	Group cp
			0-10 cm	10-20 cm		
Tylenchida	Rhabditidae	*Cuticularia*	0,01 ± 0,01	0,02 ± 0,02	Bacterivores	1
		Diploscapter	0,04 ± 0,04	-	Bacterivores	1
		Rhabditis	7,84 ± 4,66	4,87 ± 1,58	Bacterivores	1
	Anguinidae	*Ditylenchus*	15,87 ± 6,26	16,19 ± 6,06	Herbivores	2
	Belonolaimidae	*Tylenchorhynchus*	0,48 ± 0,37	0,13 ± 0,10	Herbivores	3
	Criconematidae	*Criconemella*	4,74 ± 3,93	2,17 ± 1,51	Herbivores	3
		Discocriconemella	12,33 ± 5,91	13,80 ± 6,08	Herbivores	3
		Hemicriconemoides	0,05 ± 0,05	0,01 ± 0,01	Herbivores	3
	Heteroderidae	*Meloidogyne*	27,16 ± 6,63	32,12 ± 7,72	Herbivores	3
	Hoplolaimidae	*Helicotylenchus*	2,55 ± 1,40	1,22 ± 0,72	Herbivores	3
		Rotylenchulus	15,94 ± 4,35	14,58 ± 4,87	Herbivores	3
		Scutellonema	0,01 ± 0,01	0,14 ± 0,14	Herbivores	3
	Pratylenchidae	*Pratylenchus*	0,69 ± 0,66	0,28 ± 0,28	Herbivores	3
	Tylenchidae	*Aglenchus*	0,01 ± 0,01	-	Herbivores	2
		Discotylenchus	-	0,02 ± 0,02	Herbivores	2
		Eutylenchus	-	0,02 ± 0,02	Herbivores	2
		Filenchus	0,14 ± 0,09	0,25 ± 0,15	Herbivores	2
		Psilenchus	0,12 ± 0,09	-	Herbivores	2
		Tylenchus	4,35 ± 2,02	3,65 ± 1,97	Herbivores	2
	Tylenchulidae	*Tylenchulus*	0,40 ± 0,21	0,56 ± 0,22	Herbivores	2

Of these, 46 genera were recorded at a depth of 0-10cm, and 44 genera were recorded at a depth of 10-20cm. Some genera are only recorded at a depth such as *Aglenchus, Alaimus, Belondira, Crocodorylaimus, Cryptonchus, Diploscapter, Psilenchus*only found at depths of 0-10cm and *Discolaimus, Prionchulus, Acrobeloides, Discotylenchus, Eutylenchus* only found at 10-20cm deep. The 5 genera with the highest frequency were *Meloidogyne* (the average rate at two depths was 29.64%), *Ditylenchus* (16.03%), *Rotylenchulus* (15.26%), *Discoriconemella* (13.07%) and *Rhabditis* (6.35%), of which only the genus *Rhabditis* belongs to the group of free nematodes, the remaining 4 genera belong to the group of plant parasitic nematodes and ther cultivars had a negligible frequency (<5%). Two-way ANOVA analysis showed that there was no statistically significant difference in the number of nematode genera recorded at the sampling points as well as at the two survey depths (p=0.2893 and p=0.5314).

Based on the results of classifying nematode communities for the study area according to 5 main trophic groups based on morphological features, the study showed that plant parasites nematode group was the group that accounted for a high percentage of dominant species in the whole sample and at both survey depth of 0-10cm, this group accounted for 85.5% and 87.4% at a depth of 10-20cm. The second dominant group was the bacterivores group with an average rate of 9.4% at the first depth and 7.6% at the second depth. The remaining groups accounted for a very low proportion, the lowest was the predators group with an average rate of only about 0.2% at a depth of 0-10cm and 0.4% at a depth of 10-20cm (Figure 3E).

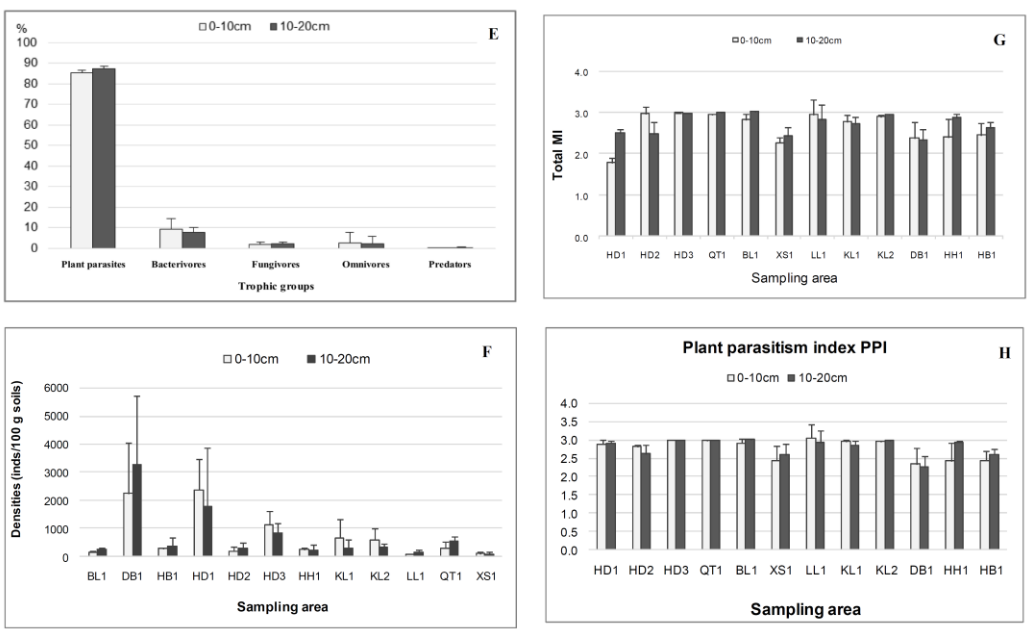

Figure 3. Trophic groups (E), distribution density (F), maturity index of the nematode community (G) and the plant parasitism index of soil nematodes at survey sites by depth (H)

Distribution density

The density of nematodes obtained at the survey sites ranged from 84-2370 individuals/100g of soil at a depth of 0-10cm, and from 88 to 3255 individuals/100g of soil at a depth of 10-20cm (Figure 3F). The results of testing the difference in the distribution density of nematode communities using two-way ANOVA after converting the data to log(x) form showed that the density of nematodes between the sampling points had a significant difference statistically significant ($p=1.53.10^{-7}$). However, the density of nematodes between the two survey depths was not statistically different ($p=0.9993$).

Evaluation of soil quality based on bio-indicators of nematode communities

The MI indices of soil nematode communities

The total of maturity index (MI) was calculated in the basis of the frequency of occurrence of nematode genera presented in the samples and the corresponding cp index for each genus, the results showed that MI of the study area fluctuated in range from 1.78 (HD1 area) to 3.00 (HD3) at 0-10cm depth and from 2.34 (DB1) to 3.03 (BL1) at 10-20cm depth (Figure 3G).

Plant Parasitism Index

The plant parasitism index (PPI) for the study area was calculated on the basis of the frequency of occurrence of parasitic nematodes in the samples and the corresponding c-p index for each variety. The PPI was ranged mean from 2.35 ± 0.43 (DB1) to 3.05 ± 0.36 (LL1). Average 2.77 ± 0.34 at depth of 0-10cm and from 2.26 ± 0.31 (DB1) to 3.01 ± 0.01 (BL1) (mean 2.81 ± 0.28) at a depth of 10-20cm (Figure 3H).

Analysis of the ecological triangle model

Based on the ecological triangle model, the study showed that the environmental quality at most of the survey sites was in a stable range. There was not much environmental pressure because the intersections of the 3 scale lines are mostly located at the same time to the left corner of the triangle. However, at some points there was a difference in environmental quality between the two depths (HD1 area) at a depth of 0-10cm, the environment was under pressure from organic matter (intersection of 3 lines of the cp percentage towards the top of the triangle), but at a depth of 10-20cm, the survey results shows that the environmental quality is stable. At the XS1 area, the results showed that the environment was relatively stable on the first depth, but at the second depth was under pressure from inorganic substances (intersection of 3 lines of the cp percentage towards the right corner of the triangle). It was a vice versa the environment at the first depth under pressure by inorganic substances, while it was relatively stable at the second depth (HB1 area). Both of depths at the DB1 place, the environments pressurized by inorganic substances. The other points of the environments were quite stable and the both depths. Especially, the intersection points is strongly shown towards the left corner of the triangle at the HD3, QT1, BL1 and KL2 places (Figure 4).

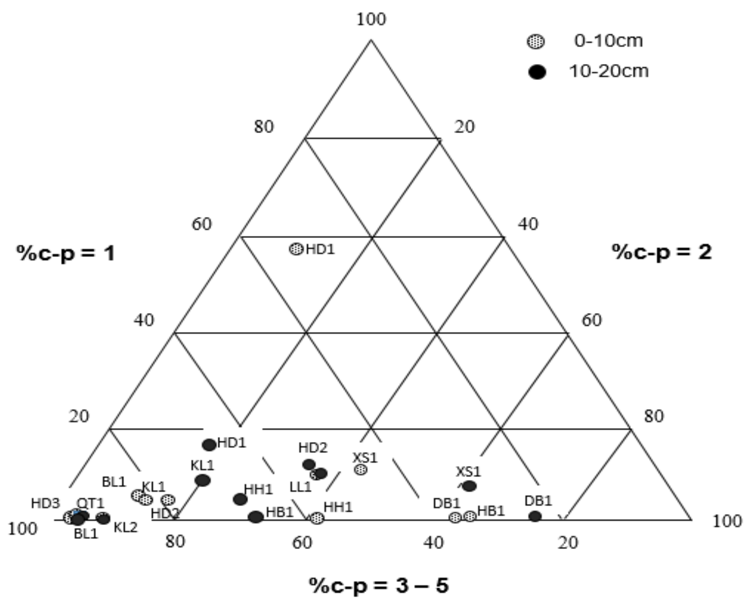

Figure 4. Ecological triangle model built for the study areas.

Evaluation of the relationship between physicochemical and biological factors

To evaluate the influence of physicochemical factors on the structure of nematode communities in the soil, the Spearman correlation coefficient for non-normally distributed data was determined as shown in Table 2.

Table 2. Values of spearman correlation coefficient between physicochemical parameters and soil nematodes communities

Variables	Soil nematode communities						
	%H	%B	%F	%O	%P	Density	MI total
TN (%)	**0.3953**	**-0.4310**	-0.0931	-0.0292	-0.0217	0.0796	0.0671
TP (%)	**0.2722**	-0.1391	0.0110	-0.0509	-0.0255	**-0.3007**	**0.3764**
pH	**0.2753**	**-0.4422**	-0.1871	0.1428	0.0208	0.1415	0.0004
OM (%)	**0.4009**	**-0.4561**	**-0.4154**	0.0850	-0.0769	**0.2822**	0.0492

Values in bold: correlation is statistically significant ($p < 0.05$).
Trophic groups:plant parasites/herbivores(H), bacterivores(B), fungivores(F), omnivores(O), predators(P).
Physical and chemical properties of soil: total nitrogen (TN), total phosphorus (TP), total organic matter (OM).

Statistical results showed that the group of plant parasitic nematodes and the group of bacterivores nematodes were the two trophic groups that showed a statistically significant correlation with the physicochemical parameters of the soil ($p<0.05$). The correlation among the groups of nematodes that fungivores, omnivores and carnivores in the physicochemical parameters were not statistically significant ($p>0.05$). The MI index did not significantly correlated with the physicochemical parameters ($p>0.05$).

Discussion

The soil pH in the study area looks was still satisfactory in the growth capacity of pepper cultivars. According to the initial survey, most pepper growing households in the study area used manure (cow, goat) to fertilize crops, the use of manure also contributed to stable soil pH. The variation in TN and TP between the survey sites was generally quite large, but the variation between the two depths was not significant, thus, the use of fertilizers, pesticides and the cultivation mode differences leaded to significant changes in the TN and TP functions in the soil at the survey sites. The percentage of natural resources in the soil was moderate to high, while the TP value in the soil was rich.

In this study, the identified 18 genera of plant parasitic nematodes related to pepper, of which 12 out of 17 common parasitic nematodes in the world related to pepper cultivars were identified by Koshy *et al.* (2005). The problem of harmful parasitic nematodes on pepper cultivars in Vietnam was also recognized in many previous studies by Thuy *et al.* (2012) who studied the nematode species composition in pepper soil samples in 5 central provinces and the central highlands, and identified 19 genera of plant parasitic nematodes related to pepper. Some parasitic nematodes related to pepper were found in Quang Tri province with the study of Chau (1995) including 19 genera, of which 4 most important ones are *Meloidogyne, Rotylenchulus, Xiphinema* and *Paratrichodorus*. 14 genera were found by Khuong (1983) who stated that in the Southern provinces of Vietnam. Among 10 nematode genera discovered in the Southeast by Bien (1989) which *Meloidogyne* is the most common genus. It can be seen that the number of parasitic nematode genera fluctuates depended on the research area and research scope. Compared with the above studies, the number and composition of plant parasitic nematodes in Ba Ria-Vung Tau province were evaluated at a diverse level and presented the most typical parasitic genera related to pepper in Vietnam, especially *Meloidogyne.* The ratio of trophic groups in the study reflected the typical characteristics of agro-ecosystems, with a high percentage of plant parasites and bacterivores, especially plant parasites (herbivores), and low prevalence of the predatorous group. This conclusion was also shown in earlier studies by Wasilewska (1979) and Hendrix *et al*. (1986).

The fluctuations in nematode density are mainly influenced by different sampling locations rather than by different depths, or in other words, the horizontal distribution of nematode communities (according to sampling points) had a clearer difference than the vertical (according to the depth), which showed that the cultivation mode as well as the soil environment characteristics in different gardens affected the distribution of the community nematodes rather than the influence of soil depth. The diversity and abundance of soil nematodes depended on changes in environmental conditions (Wasilewska, 1994). Therefore, changes in physicochemical parameters such as pH, TN, TP or function the amount of total organic matter between survey sites may contribute to the variation in nematode density between these sites. Similar results were also recorded in the study of Popovici and Ciobanu (2000), which suggested that fluctuations in the soil environment in terms of pH, nitrogen content, and humus content could explain the changes in the nematode biomass.

In the study area, MI values ranged from 1.78 to 3.03 depending on each survey site. The maturity index (MI) of the study area was generally lower than that of some previous studies by Phuong *et al.* (2011) in the specialized farming area of Binh Duong province pepper (MI ranged from 2.78-2.99), studied by Hieu *et al.* (2012) in Loc Hung, Binh Phuoc province cultivated pepper (MI ranged from 2.12-3.06). The Kruskal-Wallis test showed that there was no difference in the total MI value between the two survey depths (p=0.3133), however, the total MI value between the survey points had a significant difference ($p=3.67.10^{-6}$). Thus, the difference in the maturity index of the study area is caused by soil environmental conditions and different farming practices among sampling sites rather than by depth.

In general, MI index of the study area at most of the sites was in the average range and there was no big fluctuation between the two survey depths. It showed that the environment and soil was quite stable. There had not much pressure stress. The higher MI value reflected in increasing closely to the nematode group with high cp value, except for some areas such as HD1, XS1 and DB1 where MI value was lower than the rest indicating the environment at these points less stable.

The difference in PPI values between the survey sites showed that the different distribution of nutrients as well as the different farming regimes leaded to the difference in the plant parasitism index. The results of physicochemical analysis had also shown that there was almost no difference in the physicochemical parameters by depth but there were different fluctuations according to each survey site, which showed that the soil environment characteristics were also very different. Although the group of parasites in the soil will also be affected differently from different environments, this can also be considered as a reason contributing to the variation in PPI index between survey sites. The Kruskal-Wallis ANOVA analysis showed no difference in PPI values between the two survey depths (p=0.3123). However, the PPI values between the sampling points were statistically significant different ($p=6,45.10^{-6}$).

The analysis results showed that the PPI and MI values were both low at the points XS1 and DB1. The MI and the PPI showed a tendency for the environment to be less stable at the above sampling points. However, as argued by Bongers (1990), environmental disturbance is the cause for leading to increase PPI, so the author did not include the plant parasite nematodes group in the MI growth index. Yeates (1994) has argued that the incorporation of groups of plant parasitic nematodes and free-living nematodes into the MI growth index enhanced the biological validity of the indices due to their comprehensive nature. Its value for the MI value including the phytopathogenic group decreased with environmental disturbances (Yeates *et al.*, 1993). Another study by Neher and Campbell (1994) also showed a similar trend, contributing to confirm the above argument when the PPI index decreased in disturbed environment. The results in this study also contributed to confirm the argument of Yeates (1994) and Neher and Campbell (1994) stated on the effectiveness of using the MI index in the assessment of soil environmental quality.

Thus, in the study area, the results showed that organic pollution tended to appear in the surface layer from 0-10cm, while pollution by inorganic substances are occured at both survey depths. The results of environmental quality assessment by the ecological triangle were quite similar to the results of maturity index and PPI analysis. In the five trophic groups, the total nitrogen content in the soil showed a positive correlation with the group of plant parasites nematode ($p<0.01$), this result is also shown in many previous studies in the world. The study of Ou *et al*. (2005) on the distribution characteristics of nematode communities under different land use conditions also showed a very positive correlation trend between the group of plant parasitic nematodes with nitrogen content in all four types different land use patterns are maize fields, fallow land and forest land, respectively; however, contrary to the research results of this study, the group that bacterivores had a positive correlation with the total nitrogen content ($p<0.01$). Meng *et al*. (2006) also showed a similar trend: the group of plant parasites nematode was positively correlated with total nitrogen in all three ecosystems of cultivated land, fallow land and forest land ($p<0.01$). The positive correlation between total nitrogen and the plant parasites nematode group was also shown in the study of Liang *et al.* (2007) on the response of nematode communities in grassland soils in Northeast China ($p<0.01$). The positive effect of nitrogen on the plant parasitic nematode group may be due to the stimulating effect of plant growth (the host of the plant parasitic nematodes) of nitrogen leading to increase the parasitic nematode group.

The results of the study did not show a relationship between the nitrogen content and the density of the nematode community ($p<0.05$). The previous studies of Cheng *et al.* (2008) and Sarathchandra *et al.* (2001) also showed similar results with the addition of nitrogen to the soils. Zhao *et al*. (2014) stated that the reason for not finding a relationship between nitrogen and nematode communities was due to the distinct response of different nematode genera in a trophic group or a cp group to increase nitrogen lead to the composition of the trophic groups as well as the total number of nematodes which were not affected by the nitrogen content in the soils. However, contrary to the research results of the topic, the group bacterivores in the studies showed a trend of positive correlation with total nitrogen content ($p<0.05$). Within the research area of the topic, at the survey sites, there was a high frequency of encountering groups of bacterivores of the family Rhabditidae, through morphological analysis. It can be seen that most of the larvae of this group in the "long lived larva" stage (dauerlarva), this was the stage that appeared in the nematode life cycle when environmental conditions which unfavorable for their growth and development. The appearance of the "larvae" and their "long lived" environments signalled that the environment was previously very nutrient rich but only for a short time, despite the high frequency of encounters, they do not represent nutrient rich environmental conditions but rather the current period of nutritional poverty (Ronn *et al*., 2012). Possibly for this reason the microbial feeding group was inversely correlated with the nitrogen content of the soils. The omnivorous and predatorous groups did not clearly correlated with the nitrogen content of the soil, possibly because the frequency of encounters was small that it was difficult to observe a relationship with changes in soil properties.

Similar to nitrogen, phosphorus content had a positive effect on plant parasites ($p<0.05$) but had a negative effect on nematode population density ($p<0.05$). The positive effect of phosphorus on plant parasites nematode was also noted in the study results of Ou *et al.* (2005). The results of their study suggested that in all four different land use types including maize fields, fallow land and forest land. Compared with nitrogen, studies focusing on the effect of increased phosphorus minerals in soil were reported to be are rare. The effect of phosphorus on nematode community composition and structure is often unclear information (Todd, 1996 and Zhao *et al*., 2014). The nematode densification effect of phosphorus can be explained by the phosphorus cycle in the soil, when the ecosystem is poor in phosphorus, plants allocate essential resources (including extracellular phosphatase enzyme) to soil organisms break down waste and release phosphorus into the soil that plants can use. Under increased phosphorus conditions, plants can obtain sufficient phosphorus from the soil directly thus, plants would reduce the input source for soil organisms. Therefore, the decrease in nematode density in the soil may be due to the restriction of food sources after the increase of phosphorus, the increased phosphorus also leads to salt toxins harmful to the nematodes (Zhao *et al*., 2014).

The positive correlation between organic matter content and nematode density as well as between total organic matter content and plant parasites nematode group were also shown in the study of Liang *et al.* (2007) on the response of nematode communities in grassland soils in Northeast China ($p<0.01$). The study by authors Bongers and Ferris (2006) also showed a similar trend among plant parasites nematode and organic matter. The total organic matter content exhibited a negative correlation with the bacterivores group, which is contrary to the studies of Liang *et al.* (2009). Normally, the use of organic fertilizers will increase the bacteria in the soil leading to increase the group of bacterivores nematodes. However, the explanation of the correlation between this group and total nitrogen content in the soil in the bacterivores group was mainly detected in the long lived larval stage in the nutrient poor stage. On the other hand, it is analyzed the total organic matter content become an important factor to determine the total nitrogen content in the soil because of its ability to hold nutrients, especially nitrogen. These two parameters are positively correlated poles. Therefore, these two parameters tended to be negatively correlated with the bacterivores group.

The value pH was a soil physicochemical parameter that had a positive correlation with the organic matter content of the soil because of its ability to stabilize the physical structure of the soil and to hold water and soil nutrients, stabilizing the soil pH because organic matter content is positively correlated with the percentage of plant parasitic nematodes leading to soil pH become a factor to be positively correlated with this nematodes group.

The results of the evaluation of the correlation between the nematode community and the physicochemical parameters showed that the trophic groups showed a better correlation with the soil physicochemical parameters than the biological indices. The analysis of disturbances in soil ecology through trophic groups proved more effective than biological indices. In general, the soil nematode community has shown a very clear interaction with the soil environment through statistically significant correlations, reflecting the impact of the environment on the change of the population community.

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