

Article

Distribution of Root-Lesion and Stunt Nematodes, and Their Relationship with Soil Properties and Nematode Fauna in Sugarcane Fields in Okinawa, Japan

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Abstract: Sugarcane cultivation in Japan has not yet focused on suppressing plant-parasitic nematodes. For proper nematode management, it is essential to know the spatial distribution of economically important plant-parasitic nematodes and free-living nematodes that play important roles in terrestrial ecosystems. We aimed to reveal nematode fauna and soil properties in 85 sugarcane fields of three major sugarcane producing islands in Japan, and to examine their relationship by using the mixed-effect model and by visualizing the spatial distributions using the inverse distance weighting (IDW) approach. The nematode community structures were analyzed by non-metric multidimensional scaling (NMDS). Among plant-parasitic nematodes in sugarcane, the root-lesion nematodes (*Pratylenchus* sp.) and the stunt nematodes (*Tylenchorhynchus* sp.) were widely distributed in these islands, yet the abundance and the species varied geospatially. Soil pH was significantly correlated with the abundance of *Pratylenchus* and *Tylenchorhynchus* species. The abundance of *Pratylenchus* and *Tylenchorhynchus* species were significantly correlated with soil pH. The abundance of *Pratylenchus* was significantly correlated with the abundance of free-living nematodes, the number of free-living nematode species, and exchangeable cation K^+ , as were the abundance of *Tylenchorhynchus* to the clay content and that of non-*Tylenchorhynchus*. This study also revealed that the three islands had different nematode faunas, which were explained especially by soil pH, texture, and exchangeable basic cations.

Keywords: free-living nematodes; nematode diversity; plant-parasitic nematodes; *Pratylenchus*; soil physico-chemical properties; spatial distribution; *Tylenchorhynchus*

1. Introduction

More than 310 species of 48 genera of plant-parasitic nematodes have been reported in sugarcane [1], and these nematode genera include *Helicotylenchus*, *Hoplolaimus*, *Meloidogyne*, *Paratylenchus*, *Pratylenchus*, *Rotylenchulus*, *Tylenchorhynchus* and *Xiphinema*. Among these, the root-lesion nematode (*Pratylenchus* sp.), the root-knot nematode (*Meloidogyne* sp.), and the dagger nematode (*Xiphinema* sp.) are potentially

the most damaging pests associated with sugarcane [2]. Studies in Australia, Brazil, and South Africa [3–5] reported 10% to 30% sugarcane yield losses caused by plant-parasitic nematodes, and occasionally, a greater than 40% yield reduction due to plant-parasitic nematodes in Africa [1]. Previous studies [6,7] indicated about 20% yield losses in spring planting and successive ratoon crops due to plant-parasitic nematodes (principally *Pratylenchus*) in Okinawa, Japan, where our previous survey (unpublished) showed species of *Pratylenchus* and *Tylenchorhynchus* as major plant-parasitic nematodes.

Okinawa is located in the subtropical region, 1500–2000 km south-west of Tokyo. It is the major sugarcane producing area in Japan. In Okinawa Prefecture, there are 13,145 ha of sugarcane fields, and the annual sugarcane production was 742,584 t in 2019 [8]. The top three islands in the total production were Miyako island (4072 ha, 218,434 t), Okinawa island (2695 ha, 132,888 t), and Ishigaki island (1387 ha; 90,100 t). The sugarcane yield in Okinawa had been exceeding that of the world until the beginning of the 21st century. Yet, the yield has declined and was 58.8 t ha^{−1} (10-year average of 2008–2017), 5% below that of 10 years ago [8]. During the same period, the average in the world was 70.6 t ha^{−1}, 5% more than that of 10 years ago [9]. Although plant-parasitic nematodes may not be the only reason, nematodes may be one of the factors that influenced the sugarcane yield decline in Japan. Also, there has been no in-depth nematode research specific to sugarcane fields covering major production regions in Okinawa. Further, there has been no nematicide available against plant-parasitic nematodes in sugarcane in Japan until 2019, when fipronil [10] became the first agrochemical registered for suppressing *Pratylenchus zeae*, one of the major plant-parasitic nematodes in sugarcane.

In agricultural field soils, the complex microbial community plays important roles in sustainable farming [11], where nematodes are important players in soil ecosystem function, such as nutrient cycling, organic matter decomposition through regulating decomposers (bacteria and fungi), and disease suppression [12]. Nematode diversity in soil can be a good indicator of soil health [13,14]. The soil nematode communities are influenced by environmental factors including soil physical and chemical properties, moisture, temperature, and vegetation [15]. Also, the free-living nematode fauna may influence the abundance of the plant-parasitic nematodes [16]. Yet, no study has been conducted to investigate the relationship between nematode community and soil properties in sugarcane fields in Japan.

In this study, we hypothesized that root-lesion and stunt nematodes might be ubiquitously spreading over the sugarcane fields in Okinawa, but their abundance might be influenced by some of the soil physico-chemical properties and other nematode assemblages. Also, our preliminary study showed that the major species of the root-lesion nematodes might be *P. zeae* and *P. coffeae* and that those of the stunt nematodes might be *T. leviterminalis* and *T. annulatus* in Okinawa's sugarcane fields, yet the geospatial distribution of these nematode species and nematode community structures have not been studied. Therefore, the objectives of this study were (1) to examine geospatial distribution of nematodes, in particular species composition of plant-parasitic nematodes, and soil physico-chemical properties in sugarcane fields in different islands in Okinawa; (2) to determine the soil physico-chemical properties and the nematode assemblages that may influence the abundance of the root-lesion and stunt nematodes in sugarcane fields; and (3) to examine the nematode diversity and maturity indices in sugarcane fields in different islands. This is the first report of the relationship among plant-parasitic nematodes, free-living nematodes, and soil physico-chemical properties in the extended coverage of the sugarcane fields in Japan.

2. Materials and Methods

2.1. Study Fields

Soil was collected in the major sugarcane producing regions of Okinawa Island (Okinawa-North and South: between 26°5'41.4" N and 26°42'14.8" N and between 127°40'15.5" E and 128°1'9.7" E); in Miyako Island (Miyako: between 24°43'12.1" N and 24°53'8.5" N and between 125°15'47.0" E and 125°25'37.3" E); and in Ishigaki Island (Ishigaki: between 24°21'0.3" N and 24°28'9.2" N

and between 124°6′14.1″ E and 124°14′44.1″ E) in Japan (Figure 1; generated by leaflet package [17] in R). They were the top three sugarcane producing islands in the 2018–2019 season in Okinawa [8]. We separated Okinawa-North and Okinawa-South, because their major soil types are different. The major soil types of each region are red-yellow soil (local name in Japan: Kunigami-Mahji) in Okinawa-North and Ishigaki; dark-red soil (local name: Shimajiri-Mahji) in Miyako; and dark-red soil plus marlitic terrestrial regosols (or grey terrace soil, local name: Jahgaru) in Okinawa-South [18,19]. Soil sampling was conducted in the early fall at the end of September and in the middle of October in 2016 in Okinawa-North and South, in mid-August in 2017 in Miyako and in the early July in 2018 in Ishigaki. The 20-year averages of precipitation and day-time temperature [20] were 2152 ± 425 (standard deviation) mm annum⁻¹ and 22.9 ± 0.3 °C (the north of Okinawa); 2210 ± 439 mm annum⁻¹ and 23.4 ± 0.3 °C (the south of Okinawa); 2086 ± 317 mm annum⁻¹ and 23.9 ± 0.3 °C (Miyako); and 2129 ± 381 mm annum⁻¹ and 24.6 ± 0.3 °C (Ishigaki).

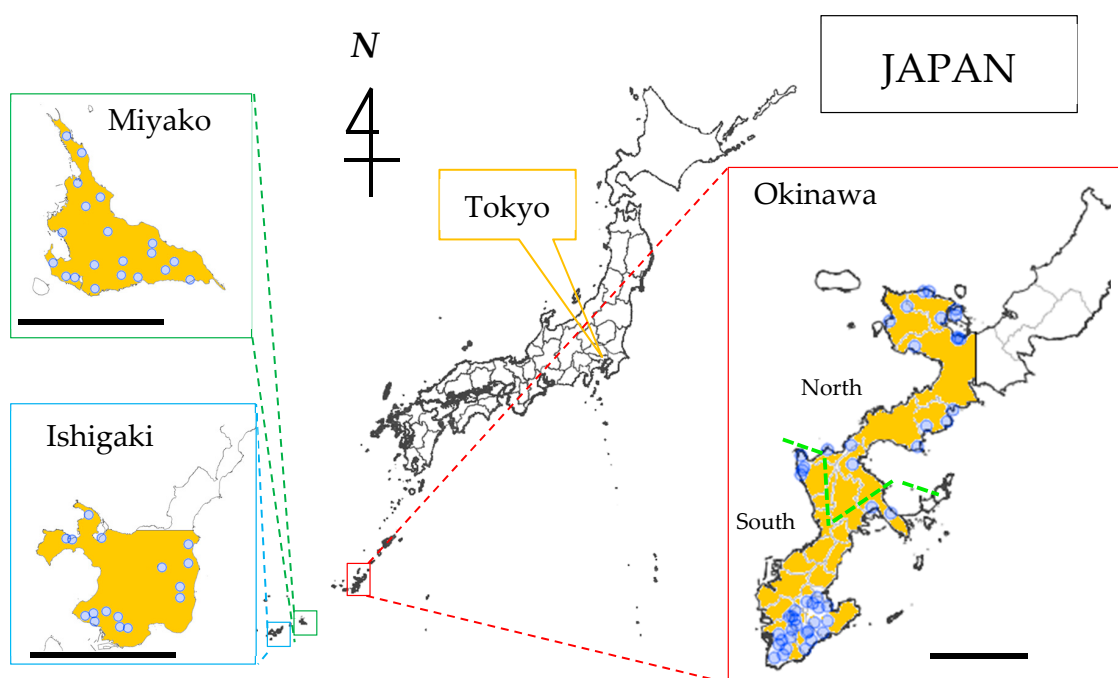


Figure 1. Soil sampling sites in each island. The green dotted line in the Okinawa map (enlarged) is to separate North and South. The scale bar at the bottom of each island is approximately 20 km. The maps are based on the blank map [21] published by Geospatial Information Authority of Japan [22] and developed using leaflet package in R.

2.2. Soil Sampling

Soil was collected for nematode and physico-chemical analyses in a total of 85 sugarcane fields (18, 31, 20, and 16 fields in Okinawa-North, Okinawa-South, Miyako, and Ishigaki, respectively). For nematode analysis, the soil was collected with an auger (3 cm diameter) at 0–30 cm depth within 10–15 cm of the base of 5 randomly selected sugarcane plants in each field. The soil was passed through a 5 mm aperture sieve to remove rocks and debris, mixed well and kept at room temperature for no more than two days before nematode extraction. The soil moisture was determined using 100.0 g fresh soil dried in an oven (60 °C $\times$ 48 h). For physico-chemical analysis, the soil was collected at a depth of 0–30 cm at 5 randomly selected spots within 20–30 cm of the sugarcane ridge in the same field as for the nematode analysis. The collected soil samples were air-dried, sieved to pass through a 2 mm mesh screen, and stored at ambient temperature until analysis.

2.3. Nematode Extraction, Identification and Enumeration

Nematodes were extracted in triplicate from 20 g subsample of each well mixed soil sample to evaluate nematode fauna using the Baermann funnel method (room temperature, 72 h [23]). The soils after the Baermann extraction were further set in the sugar-flotation nematode extraction method to extract remaining nematodes following Jenkins [24] with some modifications [25]. Nematodes were counted and identified based on their morphological characters under a stereomicroscope (BX53, Olympus, Tokyo, Japan). Up to 6 root-lesion nematodes and stunt nematodes were randomly selected from soils of each field and identified using the real-time PCR method (*P. zeae* and *T. leviterminalis* by the method in the previous study [26] and *P. coffeae* and *T. annulatus* using unpublished real-time PCR primers). Thresholds of *Pratylenchus* spp. and *Tylenchorhynchus* spp. for severe damage against sugarcane were set at 80 (and 40) and 100 (and 50) nematodes 20 g soil⁻¹, respectively, based on previous studies [25,27]. Other plant-parasitic nematodes identified in this study were *Helicotylenchus* sp., *Rotylenchulus* spp. (*R. reniformis* and *R. borealis*), *Hoplolaimus* sp., *Meloidogyne* sp., *Paratylenchus* sp., *Xiphinema* sp., and ring nematodes. Among these plant-parasitic nematodes, *Meloidogyne* sp. and *Xiphinema* sp. may be potentially harmful [1] for sugarcane, however, their presence in the investigated fields was low and therefore were not examined in this study. Free-living nematodes were counted in total and classified separately for the first 100 individuals or all, if total numbers were less than 100, based on their morphological characters [28,29] and general feeding habits [30]. The classification of free-living nematodes in the current study covered *Acrobeloides*, other Cephalobidae, *Rhabditis*, other Rhabditidae, and the other bacterial feeders. The other feeding types including fungal feeders (*Aphelenchus*, *Aphelenchoides*, *Filenchus* and *Ditylenchus*), mostly omnivorous nematodes in the Dorylaimida order and predator nematodes were also recorded. Soil DNA was extracted from 0.25 g of oven-dried (60 °C, 48 h) and homogenized soil in duplicate [31]. The DNA solutions dissolved in 100 µL Tris-EDTA buffer (10 mmol liter⁻¹ Tris-HCl, 1 mmol liter⁻¹ EDTA, pH 8.0) were diluted 10-folds with RNasefree water and used for real-time PCR assays for confirming the root-lesion and stunt nematode species existing in field samples using the same primer sets described above.

2.4. Physico-Chemical Properties of Soil

Soil pH and electric conductivity (EC) were determined in deionized water (H₂O) and 1 M KCl at a soil-to-solution ratio of 1:5. Soil particle size distribution was determined by the wet-sieving and pipet method [32]. Total carbon (TC) and total nitrogen (TN) were quantified by the dry combustion method with an NC analyzer (SUMIGRAPH NC TR22, Sumika Chemical Analysis Service, Ltd., Osaka, Japan). Available phosphate (Available-P) was evaluated by the Truog method [33]. A batch extraction with ammonium acetate (1 M, Ph 7.0) was performed to measure the cation-exchange capacity (CEC) and exchangeable basic cations (K⁺, Na⁺, Ca²⁺, and Mg²⁺), which were determined using atomic absorption spectroscopy (A-A-640-01; Shimadzu, Kyoto, Japan).

2.5. Free-Living Nematode Biodiversity and Maturity Indices

Several important indices were used to assess the biodiversity of free-living nematodes in sugarcane fields, i.e., the Shannon-Wiener index (*H'*), to evaluate the diversity of soil free-living nematode fauna, the Simpson's *D* to assess dominance of abundant taxa, species richness (Margalef index), and evenness *J'* to evaluate using genus and family levels as taxa classification [13]. Maturity index [34], maturity index (colonizer-persister (cp) value 2–5) [35], and maturity index (Cephalobidae adjusted) [29] were also used to gauge the condition of the soil ecosystem.

2.6. Statistical Analysis

For nematode abundance, percentages of each free-living nematode species, diversity and maturity indices, and soil physico-chemical properties, multiple comparisons (Tukey-Kramer's method) were conducted among four different sites (Okinawa-North and South, Miyako, and Ishigaki). Stepwise

regression analysis was used to narrow down the potential predictors among physico-chemical parameters, nematode biodiversity indices and non-target nematode abundance to explain the abundance of the target nematodes, *Pratylenchus* and *Tylenchorhynchus* nematodes. The variance inflation factor (VIF) was used to detect the presence of linear relationships among variables. Then, linear mixed model analysis was performed using the parameters remained (pH (H₂O), K⁺, the abundance of the free-living nematodes, and the number of free-living nematode species for *Pratylenchus*, and pH (H₂O), the abundance of non-*Tylenchorhynchus* nematodes, and the clay content for *Tylenchorhynchus*) as fixed effects with site (Okinawa-South and North, Miyako, and Ishigaki) as a random effect to control for variation in categorical explanatory variables among different locations of soil collection. The statistical analysis was conducted in R v.3.6.1 (R Core Team, Vienna, Austria) [36] using multiple comparisons (multicomp library [37]) and linear mixed-effects models (lmer library of lme4 package [38]) for all variables (square-rooted). Further, in order to understand the spatial pattern of *Pratylenchus* and *Tylenchorhynchus* distribution together with these parameters, geospatial mapping was conducted in each island by the inverse distance weighting (IDW) method using gstat package [39,40] in R. Non-metric multidimensional scaling (NMDS) using Bray-Curtis dissimilarities [41] was applied for the nematode community analysis using the nematode classification in the Section 2.3 above. The result of NMDS analysis highlighted the difference among regions and pH levels (high: ≥ 8.3 : the 1st quartile; middle: < 8.3 : the 2nd and 3rd quartiles; low: < 5.4 : the 4th quartile of all the 85 data) using vegan package [42] and shown in graph using ggplot2 package [43] in R. Then the physico-chemical variables were fitted to the NMDS as environmental parameters.

3. Results

3.1. Nematodes in Sugarcane Fields

Plant-parasitic nematodes were significantly ($p < 0.05$) more in Okinawa-North than in Miyako and Ishigaki, and more than 80% of the plant-parasitic nematodes in each region were species of *Pratylenchus*, *Tylenchorhynchus* and *Helicotylenchus* (Table 1). Species identification of individual nematodes using the real-time PCR method showed that all the root-lesion nematodes were either *P. zeae* or *P. coffeae*, and all the stunt nematodes were *T. leviterminalis* or *T. annulatus*. The abundances of *Pratylenchus* and *Helicotylenchus* nematodes were significantly ($p < 0.05$) higher in Okinawa-North than in the other 3 regions, while *Tylenchorhynchus* was more in Okinawa-South than Miyako and Ishigaki. Nematodes of the genus *Meloidogyne* were not populous in all four regions and found in one field each in Okinawa-North and South and Ishigaki, and 7 fields (35%) in Miyako. *Rotylenchulus* sp. was detected at 28% and 68% of the studied fields in Okinawa-North and South, respectively, and 100% and 94% in Miyako and Ishigaki, respectively. The abundance of *Rotylenchulus* sp. also tended to be more in Okinawa-South and Miyako than in Okinawa-North and Ishigaki. *Xiphinema* sp. was found only in 2 fields in Okinawa-South.

Table 1. Nematodes per 20 g soil.

	Okinawa-North		Okinawa-South		Miyako		Ishigaki	
Free-living nematodes	146	a	82	bc	128	ab	54	c
Plant-parasitic nematodes	372	a	273	ab	200	b	146	b
<i>Pratylenchus</i>	63	a	17	b	20	b	17	b
<i>Tylenchorhynchus</i>	148	ab	203	a	77	b	83	b
<i>Helicotylenchus</i>	153	a	21	b	64	b	23	b
<i>Hoplolaimus</i>	2	a	3	a	4	a	3	a
<i>Meloidogyne</i>	0	a	0	a	3	b	0	a
<i>Paratylenchus</i>	2	a	0	a	0	a	0	a
<i>Rotylenchulus</i>	1	a	27	ab	32	b	12	ab
<i>Xiphinema</i>	0	a	0	a	0	a	0	a
Ring nematodes	3	ab	0	a	0	a	7	b

Numbers are the mean of each sampling region (Okinawa-North: N = 18; Okinawa-South: N = 31; Miyako: N = 20; Ishigaki: N = 16). Different letters indicate significant difference (Tukey-Kramer method; $p < 0.05$) among the regions.

The abundance of free-living nematodes was significantly ($p < 0.05$) more in Okinawa-North than in Okinawa-South and Ishigaki (Table 1). Nematodes of the genus *Acrobeloides* accounted for around 50% of all free-living nematodes in Okinawa-North and South, and around 15% in Miyako and Ishigaki, while other Cephalobidae did for 15% to 19% with no significant difference among the regions (Table 2). The proportion of *Rhabditis* sp. was significantly ($p < 0.05$) more in Okinawa-North and South than in Miyako and Ishigaki, yet other Rhabditidae had no significant difference among the regions. The proportion of other bacterial feeders was significantly ($p < 0.05$) more in Miyako and Ishigaki than in Okinawa-North and South. The proportion of *Filenchus* sp. was significantly ($p < 0.05$) higher in Ishigaki than in the other regions. The proportions of Dorylaimida and predator nematodes were significantly ($p < 0.05$) more in Miyako and Ishigaki than in Okinawa-North and South (Table 2).

Table 2. Composition of free-living nematodes

	Okinawa-North		Okinawa-South		Miyako		Ishigaki	
<i>Acrobeloides</i>	48%	a	51%	a	15%	b	13%	b
Other Cephalobidae	16%	a	15%	a	15%	a	19%	a
<i>Rhabditis</i>	8%	a	7%	a	0%	b	3%	b
Other Rhabditidae	9%	a	10%	a	12%	a	11%	a
Other bacterivores	10%	a	10%	a	31%	b	20%	c
<i>Aphelenchus</i>	3%	a	2%	a	3%	a	1%	a
<i>Aphelenchoides</i>	0%	ac	0%	a	3%	b	2%	bc
<i>Filenchus</i>	3%	a	3%	a	4%	a	13%	b
<i>Ditylenchus</i>	0%	a	0%	a	0%	a	0%	a
<i>Dorylaimida</i>	3%	a	2%	a	16%	b	16%	b
Predators	0%	a	0%	a	1%	b	2%	b

Numbers are the mean of each sampling region (Okinawa-North: N = 18; Okinawa-South: N = 31; Miyako: N = 20; Ishigaki: N = 16). Different letters indicate significant difference (Tukey-Kramer method; $p < 0.05$) among the regions.

3.2. Spatial Distribution of *Pratylenchus* and *Tylenchorhynchus*

The root-lesion nematodes (*Pratylenchus*) were detected in all the sugarcane fields except 3 fields in Okinawa-North and South. The dominant root-lesion nematode species was *P. zeae* in Okinawa-North and Ishigaki, while it was *P. coffeae* in Okinawa-South and a mixture of *P. zeae* and *P. coffeae* in Miyako. There were more than 80 root-lesion nematodes 20 g soil⁻¹ observed in 28%, 5%, and 6% of the fields in Okinawa-North, Miyako and Ishigaki, respectively, and more than 40 root-lesion nematodes 20 g soil⁻¹ in 72%, 13%, 20%, and 6% of the fields in Okinawa-North, Okinawa-South, Miyako, and Ishigaki, respectively (Figure 2). The stunt nematodes (*Tylenchorhynchus*) were detected in all the sugarcane fields except 2 fields in Okinawa and Ishigaki. The dominant stunt nematode species was *T. leviterminalis* throughout most of the four regions, while *T. annulatus* were detected in Okinawa-North, in the most of Miyako and occasionally in Ishigaki. There were more than 100 stunt nematodes 20 g soil⁻¹ observed in 54% of the fields in the four regions, and more than 50 stunt nematodes 20 g soil⁻¹ in 74% of the fields (Figure 3).

3.3. Soil Physico-Chemical Properties

Values of pH (in both H₂O and KCl) and CEC were significantly ($p < 0.05$) lower in Okinawa-North and Miyako than in Okinawa-South and Ishigaki (Table 3). The clay content was significantly ($p < 0.05$) lower in Okinawa-North and Miyako than in Okinawa-South and Ishigaki, while the sand content was significantly ($p < 0.05$) higher in Okinawa-North and Miyako than in Okinawa-South and Ishigaki. Soil moisture, exchangeable K⁺, and Na⁺ were significantly ($p < 0.05$) higher in Miyako than the other three regions, and total C and total N were higher in Miyako than Okinawa-South and Ishigaki. Exchangeable Ca²⁺ was significantly ($p < 0.05$) higher in Okinawa-South than the other three regions (Table 3).

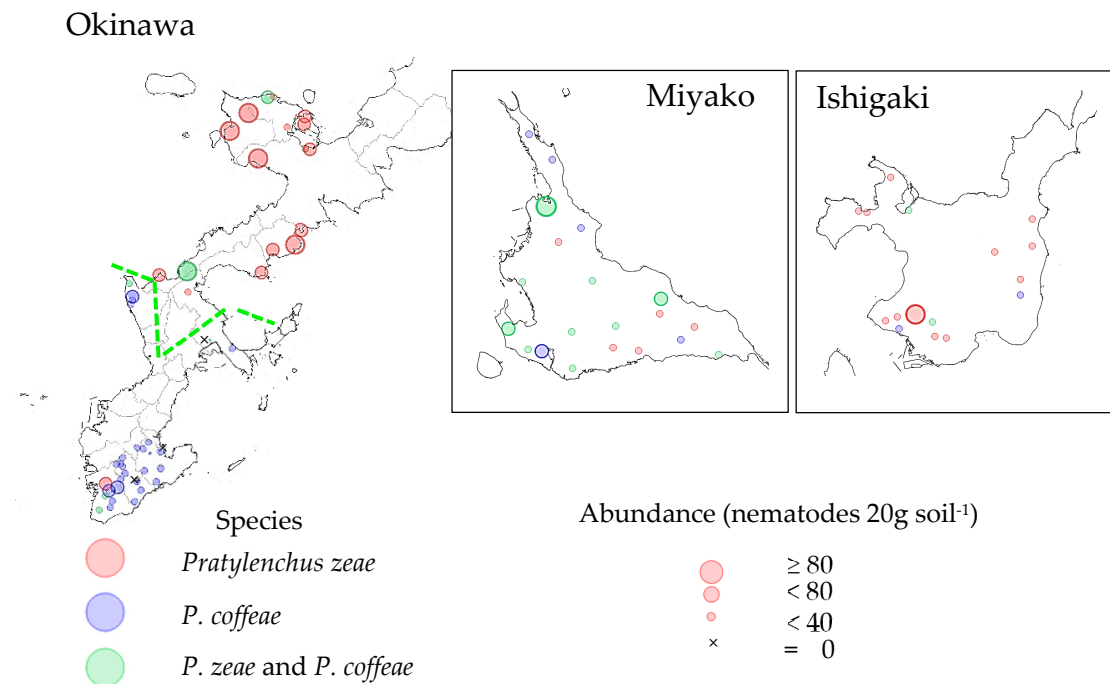


Figure 2. Distribution and abundance of *Pratylenchus* spp. in Okinawa, Miyako, and Ishigaki. The green dotted line in Okinawa map is to separate North and South. The maps are based on the blank map [21] published by Geospatial Information Authority of Japan [22].

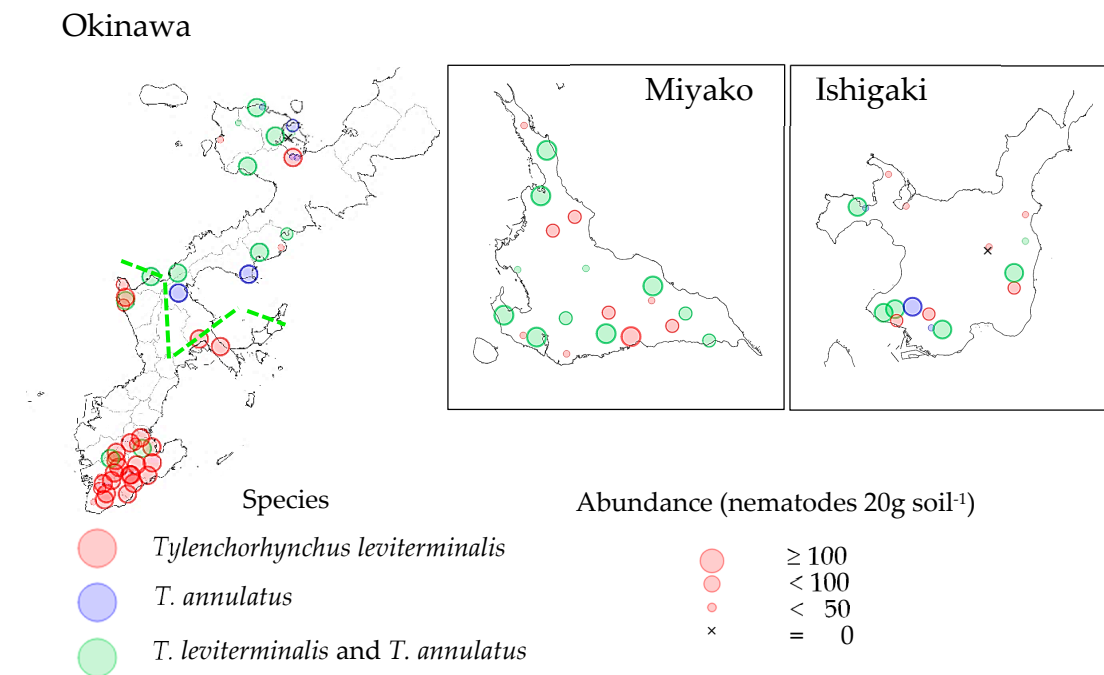


Figure 3. Distribution and abundance of *Tylenchorhynchus* spp. in Okinawa, Miyako, and Ishigaki. The green dotted line in Okinawa map is to separate North and South. The maps are based on the blank map [21] published by Geospatial Information Authority of Japan [22].

Table 3. Soil physico-chemical properties

	Okinawa-North		Okinawa-South		Miyako		Ishigaki	
pH (H ₂ O)	5.4	a	8.1	b	6.9	c	5.8	a
pH (KCl)	4.3	a	7.0	b	5.9	c	4.9	a
Soil moisture (%)	14.1	a	14.8	a	18.7	b	13.8	a
EC(mS m ⁻¹)	13.0	a	14.3	a	13.3	a	13.5	a
Clay (%)	32.9	a	41.4	b	61.6	c	29.6	a
Silt (%)	38.4	a	49.6	b	29.0	c	30.2	c
Sand (%)	28.7	a	9.1	b	9.3	b	40.1	c
Total C (g C kg ⁻¹)	0.93	ab	0.85	a	1.17	b	0.77	a
Total N (g N kg ⁻¹)	0.10	ab	0.10	a	0.14	b	0.09	a
Available P (mg P kg ⁻¹)	214.0	a	103.0	a	87.2	a	113.8	a
Exch. K ⁺ (cmol kg ⁻¹)	0.3	a	0.5	a	1.0	b	0.6	a
Exch. Na ⁺ (cmol kg ⁻¹)	0.1	a	0.2	a	0.3	b	0.1	a
Exch. Ca ²⁺ (cmol kg ⁻¹)	11.2	a	35.7	b	15.9	a	7.2	a
Exch. Mg ²⁺ (cmol kg ⁻¹)	1.6	ac	2.7	b	2.5	ab	1.1	c
CEC (cmol kg ⁻¹)	14.1	a	17.9	b	17.9	b	11.2	a

Numbers are the mean of each sampling region (Okinawa-North: N = 18; Okinawa-South: N = 31; Miyako: N = 20; Ishigaki: N = 16). Different letters indicate significant difference (Tukey-Kramer method; $p < 0.05$) among the regions.

3.4. The Relationship between Soil Properties and Abundance of *Pratylenchus* and *Tylenchorhynchus*

The mixed-effect model among the four regions to test selected variables to explain the abundance of *Pratylenchus* showed that pH (H₂O), K⁺, the abundance of free-living nematodes, and the number of free-living nematode species were significant ($p < 0.05$). All the parameters except the abundance of free-living nematodes negatively affected the *Pratylenchus* abundance. The mixed-effect model for *Tylenchorhynchus* showed that pH, the abundance of non-*Tylenchorhynchus* nematodes, and clay content were significant ($p < 0.05$). These parameters except clay content positively affected the *Tylenchorhynchus* abundance. The inverse distance weighting (IDW) prediction figures confirmed the clearer relationship for the abundance of *Pratylenchus* and *Tylenchorhynchus* against pH (H₂O) in the different directions (Figures 4 and 5). The other parameters, exchangeable cation K⁺, the abundance of free-living nematodes, and the number of free-living nematode species also showed the relationship with the abundance of *Pratylenchus* to a certain degree, as did clay content and the abundance of non-*Tylenchorhynchus* nematodes with that of *Tylenchorhynchus*.

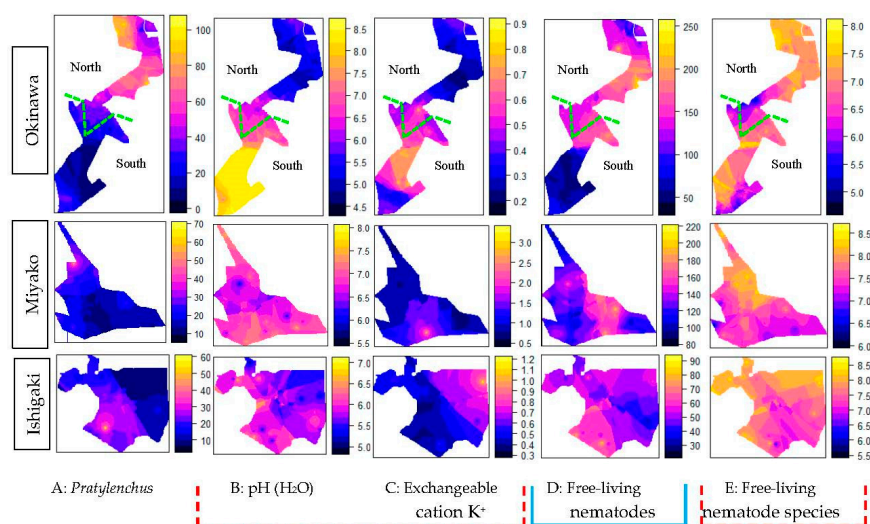


Figure 4. Spatial distribution of A: the abundance of *Pratylenchus* nematodes 20 g soil⁻¹, B: pH (H₂O), C: exchangeable cation K⁺ (cmol kg⁻¹), D: the abundance of free-living nematodes 20 g soil⁻¹, and E: the number of free-living nematode species in Okinawa, Miyako, and Ishigaki, predicted by the inverse distance weighting (IDW) approach. The green dotted line in Okinawa's figure was to separate North and South regions. To the abundance of *Pratylenchus* in the mixed-effect model, the blue solid line at the bottom has a positive relationship, and the dotted red line has a negative relationship.

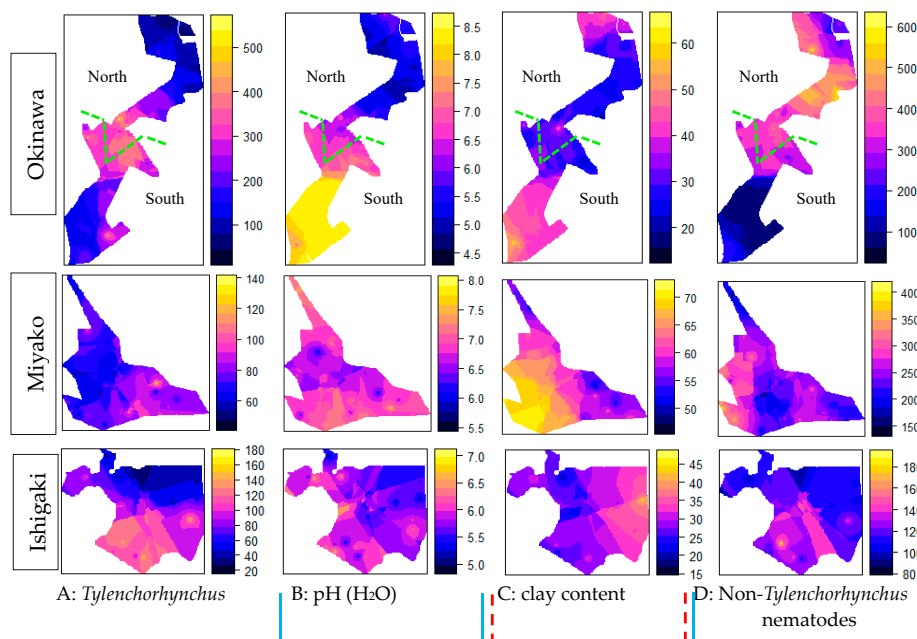


Figure 5. Spatial distribution of A: the abundance of *Tylenchorhynchus* nematodes 20 g soil^{−1}, B: pH (H₂O), C: clay content (%) and D: the abundance of non-*Tylenchorhynchus* 20g soil^{−1} in Okinawa, Miyako, and Ishigaki predicted by the inverse distance weighting (IDW) approach. The green dotted line in Okinawa's figure was to separate North and South regions. To the abundance of *Tylenchorhynchus* in the mixed-effect model, the blue solid line at the bottom has a positive relationship, and the dotted red line has a negative relationship.

3.5. Diversity of Free-Living Nematodes

The values of Shannon-Weiner index (H'), evenness J' , the number of free-living nematode species, and species richness tended to be higher in Miyako and Ishigaki than in Okinawa-North and South, while Simpson's D did in the opposite direction (Table 4). All the three maturity indices showed significantly ($p < 0.05$) lower values in Okinawa-North and South than in Miyako and Ishigaki (Table 4).

Table 4. Nematode diversity index

	Okinawa-North		Okinawa-South		Miyako		Ishigaki	
Shannon-Wiener H'	2.07	ab	1.93	a	2.43	bc	2.60	c
Evenness J'	0.90	ab	0.84	a	1.05	bc	1.13	c
Number of free-living nematode species	6.94	ab	6.58	a	7.50	ab	7.81	b
Species richness (Margalef index)	1.50	a	1.56	a	1.61	ab	2.05	b
Simpson's D	0.34	ab	0.38	a	0.23	bc	0.19	c
Maturity Index (MI)	1.90	a	1.87	a	2.20	b	2.17	b
MI (cp2–5)	1.73	a	1.69	a	2.08	b	2.02	b
MI (Cephalobidae adjusted)	1.26	a	1.21	a	1.90	b	1.84	b

Numbers are the mean of each sampling region (Okinawa-North: N = 18; Okinawa-South: N = 31; Miyako: N = 20; Ishigaki: N = 16). Different letters indicate significant difference (Tukey-Kramer method; $p < 0.05$) among the regions.

3.6. Nematode Fauna in Sugarcane Fields

NMDS generated the 3-dimensional graphs (Figure 6), which was suitable for this analysis with the stress value of 0.157 (the 2-dimensional NMDS showed unclear results with the stress value over 0.2). The result showed clear difference between Okinawa (North and South in the blue oval) and the other islands (Miyako and Ishigaki) but not among the levels of pH (Figure 6A). The graph also presented the environmental factors, pH, clay, silt, K⁺, Na⁺, and Ca²⁺, which showed significant ($p < 0.01$) difference. The second graph (Figure 6B) showed differences among Okinawa (both North and South: in the blue oval), Miyako (in the yellow oval), and Ishigaki (in the red oval). The graph included

the significant ($p < 0.01$) environmental factors, pH, clay, silt, sand, K^+ , Na^+ , and Ca^{2+} . Further, the third graph (Figure 6C) showed that Miyako and Ishigaki tended to be separated in which Okinawa-North and Okinawa-South were leaning toward Ishigaki and Miyako, respectively. The environmental factors, pH, clay, silt, sand, total C, Ca^{2+} , Mg^{2+} , and CEC were significant ($p < 0.01$) in this graph. Among the environmental parameters in all the dimensions together, soil pH and exchangeable basic cations (K^+ , Na^+ , and Ca^{2+}) showed significant difference ($p < 0.001$) as the soil chemical properties, so did ($p < 0.01$) the clay, silt and sand contents as the soil physical properties. Moisture, total C, total N, Mg^{2+} , and CEC were also significantly ($p < 0.05$) different in the NMDS analysis.

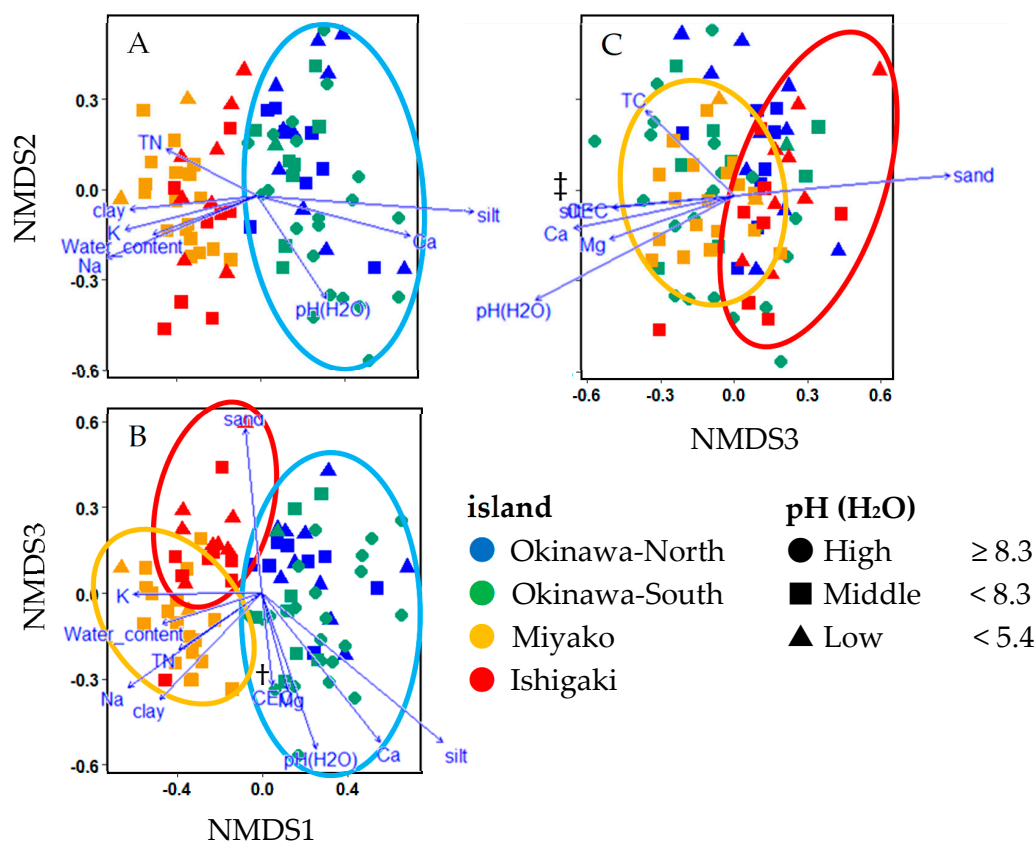


Figure 6. Non-metric multidimensional scaling (NMDS) plots for nematode community structures in four regions with environmental factors. A: dimension 1 and 2; B: dimensions 1 and 3; and C: dimensions 3 and 2. The graphs include significant ($p < 0.05$) environmental factors, pH (H₂O); clay; silt; sand; TC (total C); K (K^+); Na (Na^+); Ca (Ca^{2+}); Mg (Mg^{2+}) and CEC. The blue oval is in relation to Okinawa-North and South. The yellow oval is in relation to Miyako. The red oval is in relation to Ishigaki. †: Mg^{2+} and CEC; and ‡: silt and CEC.

4. Discussion

Species of *Pratylenchus* and *Tylenchorhynchus* were ubiquitously detected in all the four regions in Okinawa. The result was consistent with the previous study [25], which reported that *Pratylenchus* and *Tylenchorhynchus* were found in all 15 studied fields in Kitadaito island, another major sugarcane producing region in Okinawa. The survey in the current study found that *Pratylenchus* nematodes were more than threshold or half of that in most of the fields in Okinawa-North, so were *Tylenchorhynchus* in Okinawa-North and South. The abundance of *Pratylenchus* sp. was influenced by pH, K^+ , the abundance of free-living nematodes, and the number of free-living nematodes species. *Pratylenchus* sp. abundance was negatively correlated with the pH among these parameters, suggesting that *Pratylenchus* may be more populous in lower pH areas (e.g., Okinawa-North) than in higher pH areas (e.g., Okinawa-South). The result was consistent with the previous study [44] reporting inverse relationship between

the abundance of *Pratylenchus* and pH. With regard to K^+ , the result was consistent with the previous study [45], which reported a positive correlation of K^+ and plant-parasitic nematodes. Also diverse free-living nematodes can suppress plant-parasitic nematodes possibly through the increase of predator nematodes shown in Miyako and Ishigaki. Yet, another parameter, the abundance of free-living nematodes, was positively correlated with the abundance of *Pratylenchus*, though a negative relationship between total free-living nematodes and *P. crenatus* was reported [16]. The exact reason on the different results was not known.

The abundance of *Tylenchorhynchus* was affected by pH, clay contents and the abundance of non-*Tylenchorhynchus* nematodes. Unlike *Pratylenchus*, the abundance of *Tylenchorhynchus* was positively correlated with pH. This relationship was consistent with the previous study [46] reporting that *Tylenchorhynchus maximus* increased with positive relationship with pH. The current study showed inverse relationship between the abundance of *Tylenchorhynchus* and the soil clay content. On this point, the previous study [47] reported differently on *Tylenchorhynchus clarus* in soils with 4 to 32% clay, possibly due to the excessive clay contents in the fields of the current study and the difference of species, yet the exact reason was unknown. The nematode abundance other than *Tylenchorhynchus* had a positive relationship with the abundance of *Tylenchorhynchus*. This can indicate that the more nematodes were found, the more *Tylenchorhynchus*. CEC did not show significant influence on *Tylenchorhynchus* sp. (and *Pratylenchus* described above) in this study. Norton et al. [48] also reported that none of the plant-parasitic nematodes were significantly correlated with CEC.

Species of *Helicotylenchus* were the third most populous plant-parasitic nematodes found in this study. Though Berry et al. [49] discussed that *H. dihystra* should provide sustainable means of reducing the effect of more pathogenic species, no clear relationship was found between *H. dihystra* and the target plant-parasitic nematodes (*Pratylenchus* and *Tylenchorhynchus*) in this study. *Meloidogyne* species were rarely found at the studied sites and this was possibly due to the clayey soil texture, which may not be suitable for *Meloidogyne* inhabitation [1]. The previous study [50] reported *R. reniformis* was found from more than 50% of fields (various crops) in Okinawa, though the current study showed *Rotylenchulus* detection of over 90% of the fields in Miyako and in Ishigaki, respectively, and around 30% and 70% in Okinawa-North and South, respectively. The result suggested that difference in crops and studied fields might cause the inconsistent results.

This study also revealed that the species of *Pratylenchus* and *Tylenchorhynchus* were different among the regions. The combination in Okinawa-North was primarily *P. zae* and both *T. leviterminalis* and *T. annulatus*, while that in Okinawa-South was *P. coffeae* and *T. leviterminalis*. In Miyako, the combination was the mixture of *P. zae* and *P. coffeae*, and that of *T. leviterminalis* and *T. annulatus*. Cadet and Spaul [1] reported that the abundance of *P. zae* was greater in clay soils with some exception in Australia and South Africa. *Pratylenchus zae* was most numerous in Okinawa-North, however, the soil in this region was not necessarily most clayey compared to the other regions. Though Miyako's soil was most clayey among the regions, the abundance of *Pratylenchus* was less than in Okinawa-North (if we simply compared these two regions without considering other differences) and the genus was a combination of *P. zae* and *P. coffeae*. The data reported in the other study [3] for Australian sugarcane fields showed some tendency of inverse relationship between the ratio of clay in soil and the abundance of *P. zae*. This may suggest that the clay contents may influence *Pratylenchus* species suitable for particular sugarcane field with appropriate level of clay contents for the specific species. The level of pH may also influence the species of *Pratylenchus* as the previous study [51] reported that raising the soil pH from 5.0 to 6.9 in which alfalfa was grown increased the abundance of *P. penetrans* and greatly reduced the abundance of *P. crenatus*. The same study commented that temperature also affected the reproduction rates of these species. Since both *P. zae* and *P. coffeae* were increased in the 25–30 °C range [52], in which the studied fields in the current study were, the difference of pH can influence more on the reproduction of both species than temperature. Stirling et al. [53] discussed that total C and total N from crop residues and roots may increase the nematode trapping fungi, which may have been one of the suppressive factors of plant-parasitic nematodes in sugarcane soils. Our study,

however, did not clearly show the relationship of total C and total N to the abundance of the root-lesion and stunt nematodes. Since the current study did not cover the nematode trapping fungi, the exact reason was unclear on this point.

The free-living nematodes tended to be separated between the group of Okinawa (North and South) and that of Miyako and Ishigaki. This tendency led to the similar combination in diversity and maturity indices. The result suggested that the free-living nematode faunas were similar within each group. Though the exact mechanism was not understood, geographical and climatic differences may play the major role in this grouping. Okinawa-North and South are in the same island, while Miyako and Ishigaki are the islands next to each other (just 50 km away). Therefore, the temperature and precipitation are very similar within each group. Overall, the trends of the three maturity indices were consistent among the regions. The result was not in line with the previous study [29] conducted in northern climate zones in Japan. *Rhabditis* and other Rhabditidae affected the value of MI (cp2–5) and Cephalobidae does that of MI (Cephalobidae adjusted). Since the compositions of these nematodes to the total free-living nematodes were consistent across the regions, the adjustments made no apparent difference on the indices of each region. The maturity index values of Okinawa-North and South were lower than those of Miyako and Ishigaki primarily because of the different composition of the high c-p value Dorylaimida to the total free-living nematodes.

Further, many of the soil physico-chemical properties (i.e., pH, clay and sand contents, Ca^{2+} , Mg^{2+} , and CEC) tended to be separated between the group of Okinawa-North and Ishigaki and that of Okinawa-South and Miyako, while the other parameters did not. Such difference may be due to the different soil materials, causing the different soil types of those two groups. Though no detailed mechanism was identified, such geographical separation of the physico-chemical properties may result in the difference in dominant *Pratylenchus* species in each group. Such separation coupled with that of the free-living nematodes mentioned above may be involved in the clear differences of nematode communities (1) between the group of Okinawa-North and South and that of Miyako and Ishigaki; (2) among Okinawa-North and South, Miyako, and Ishigaki; and (3) between the group of Okinawa-South and Miyako and that of Okinawa-North and Ishigaki, shown in the NMDS analysis. This analysis reinforced that pH and soil texture may impact not only the plant-parasitic but also the free-living nematodes. The result was basically consistent with the previous study [15], which reported that the nematode community may be a well reflected indicator of the soil properties such as soil moisture, pH, and organic soil layer. Yet, further study is needed for more comprehensive understanding of below-ground microbial ecosystem.

5. Conclusions

This study revealed both *Pratylenchus* and *Tylenchorhynchus* species were widely distributed in sugarcane fields in Okinawa, Japan. *Pratylenchus* sp. was highly abundant and may be a yield limiting factor especially in Okinawa-North, so was *Tylenchorhynchus* sp. in all the regions examined. It was confirmed that the major species of the root-lesion nematodes were *P. zeae* and *P. coffeae* and that those of the stunt nematodes were *T. leviterminalis* and *T. annulatus*. It was also found that soil pH influenced the abundance of both *Pratylenchus* and *Tylenchorhynchus* nematodes in different directions. Then, the abundance of free-living nematodes, the number of free-living nematode species, and exchangeable cation K^+ were shown to be important parameters to the abundance of *Pratylenchus* species, as were the clay content and non-*Tylenchorhynchus* nematode abundance to that of *Tylenchorhynchus*. Further, this study revealed that the three islands (the four regions) had different characteristics of the nematode fauna, and the difference was explained especially by pH, physical properties (soil texture) and exchangeable basic cations. This study provided basic data for proper management of the plant-parasitic nematodes and free-living nematodes in sugarcane fields in Okinawa to improve their productivity.

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