Global Ecology and Conservation 32 (2021) e01932

Contents lists available at ScienceDirect

Global Ecology and Conservation

journal homepage: www.elsevier.com/locate/gecco

Investigation of the soil nematode community composition in a monoculture Robusta coffee plantation in Dak Lak, Vietnam

Ha Hoang [a,b], Huong Mai Pham [a], Phuong Thi Minh Chu [a], Trang Hong Nguyen [a], Linh Huyen Tran [a], Phap Quang Trinh [c,e], Tjalf de Boer [d], Abraham Brouwer [b,d], Duong Thi Anh Nguyen [c,e], Hoang Ha Chu [a,e,*]

[a] Institute of Biotechnology (IBT), Vietnam Academy of Science & Technology (VAST), Hanoi, Viet Nam
[b] VU University, Faculty of Sciences, Amsterdam, The Netherlands
[c] Institute of Ecology and Biological Resources (IEBR), VAST, Hanoi, Viet Nam
[d] MicroLife Solutions (MLS) b.v., Amsterdam, The Netherlands
[e] Graduate University of Science and Technology, VAST, Hanoi, Viet Nam

ARTICLE INFO

Keywords:
Plant-parasitic nematodes
Community analysis
Population density
Robusta coffee
Plantation

ABSTRACT

Nematode damage to coffee poses a serious threat to coffee production all over the world, including Vietnam. Accordingly, the nematode community composition could be a reliable indicator of ecological shifts that affect coffee plants' health. Here, we analysed the nematode communities in the rhizosphere of a monoculture Robusta coffee plantation in Dak Lak of the Central Highlands, Vietnam. We found changes in the community composition of nematodes in an age gradient of replanting coffee plants and revealed distinct patterns of nematode distribution in the soils of healthy and diseased plant groups. Further analyses indicate the impact of intensive farming and inefficient nematode controls, which caused disturbances and a gradual decrease of the nematode fauna diversity in the plantation soil. These results can add to a complete prediction model for plant fitness using nematode community composition.

1. Introduction

Coffee, a native plant of tropical Africa, was introduced into Vietnam by the French from the late 19th century and was recognized by the post-war government of Vietnam to be a key agricultural product (ICC, 2019; Phan Thi et al., 2018; Vieira, 2008). Since the late 1990 s, Vietnam has become the leading producer of coffee in South East Asia and second globally (ICC, 2019). Production concentrates in the Central Highlands region, where soil, water and climate conditions are ideally suited to grow coffee, particularly the Robusta varieties (Marsh, 2007). The fast growth of the industry and the world-class yield of coffee are, however, unsustainable. The Central Highlands' coffee plantations face a reduction in soil and water resources due to excessive fertilization and irrigation, creating an unbalanced ecosystem (Wiryadiputra and Tran, 2008).

The weakened plantations create favorable conditions for diseases and pests, most notably plant-parasitic nematodes (Bigger et al., 2007; Wiryadiputra and Tran, 2008). Nematode damage to coffee is severe, causing loss throughout coffee production regions worldwide (Bell et al., 2018; Campos and Silva, 2008; Chapman, 2014; Villain, 2008; Villain et al., 2018). In Vietnam, coffee-parasitic

Abbreviations: CFF, Natural forest coffee trees; CF, Replanted coffee trees; NP Non-pat, Healthy; P Pat, Diseased.

* Correspondence to: Vietnam Academy of Science & Technology, 18 Hoang Quoc Viet, Cau Giay District, Hanoi, Viet Nam.
E-mail address: chuhoangha@ibt.ac.vn (H.H. Chu).

https://doi.org/10.1016/j.gecco.2021.e01932
Received 17 October 2021; Received in revised form 17 November 2021; Accepted 18 November 2021
Available online 22 November 2021
2351-9894/© 2021 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

nematodes were first recorded in the Central Highlands in 1995, and subsequent surveys showed that the infestation reached nearly 1000 ha in 2002 (Wiryadiputra and Tran, 2008). Another multiple-province survey recorded the abundance of 30 plant-parasitic nematode taxa associated with coffee plantations, revealing that their incidence was localized, with specific geographical distribution of several significant species (Chau and Thanh, 2001; Wiryadiputra and Tran, 2008).

The recent sampling efforts in coffee plantations nation-wide not only further highlighted the localized effect of nematode incidence, separated between North, South and the Central regions of Vietnam, but also tentatively presented the varying occurrence of nematode genera in soil of different physicochemical characteristics (Thi et al., 2018; Trinh et al., 2009). Nonetheless, these studies focused exclusively on several selected plant-parasitic genera that were considered to be important. However, the whole community of nematodes respond sensitively to environmental changes and play pivotal roles in the ecosystem (van den Hoogen et al., 2019; Yeates, 1979), which is why they are proposed bio-indicators of ecosystem health (Bongers, 1990).

Therefore, we investigated the total nematode community composition in the rhizosphere of a monoculture Robusta coffee plantation in Dak Lak Province of the Central Highlands, Vietnam. Dak Lak itself is the leading coffee cultivation area of Vietnam, with agricultural conditions being considered as a representation for the whole of the Central Highlands. However, many coffee trees in this region were older than 20 years and decrease in yield of 80% compared to younger trees (Truong, 2018). Therefore, they were completely replaced by another healthy seedlings, according to a guideline of the Vietnam Sustainable Agriculture Transformation (VnSAT) program. We applied an integrated taxonomic and functional approach to describe the nematode community functioning of soils in a different age gradient of coffee plantation. For this, we investigated wild coffee trees that grew in the natural forest, old-age coffee trees of the first seedlings in the plantation (40 years), and replanted coffee trees aged two, seven and twelve years, respectively.

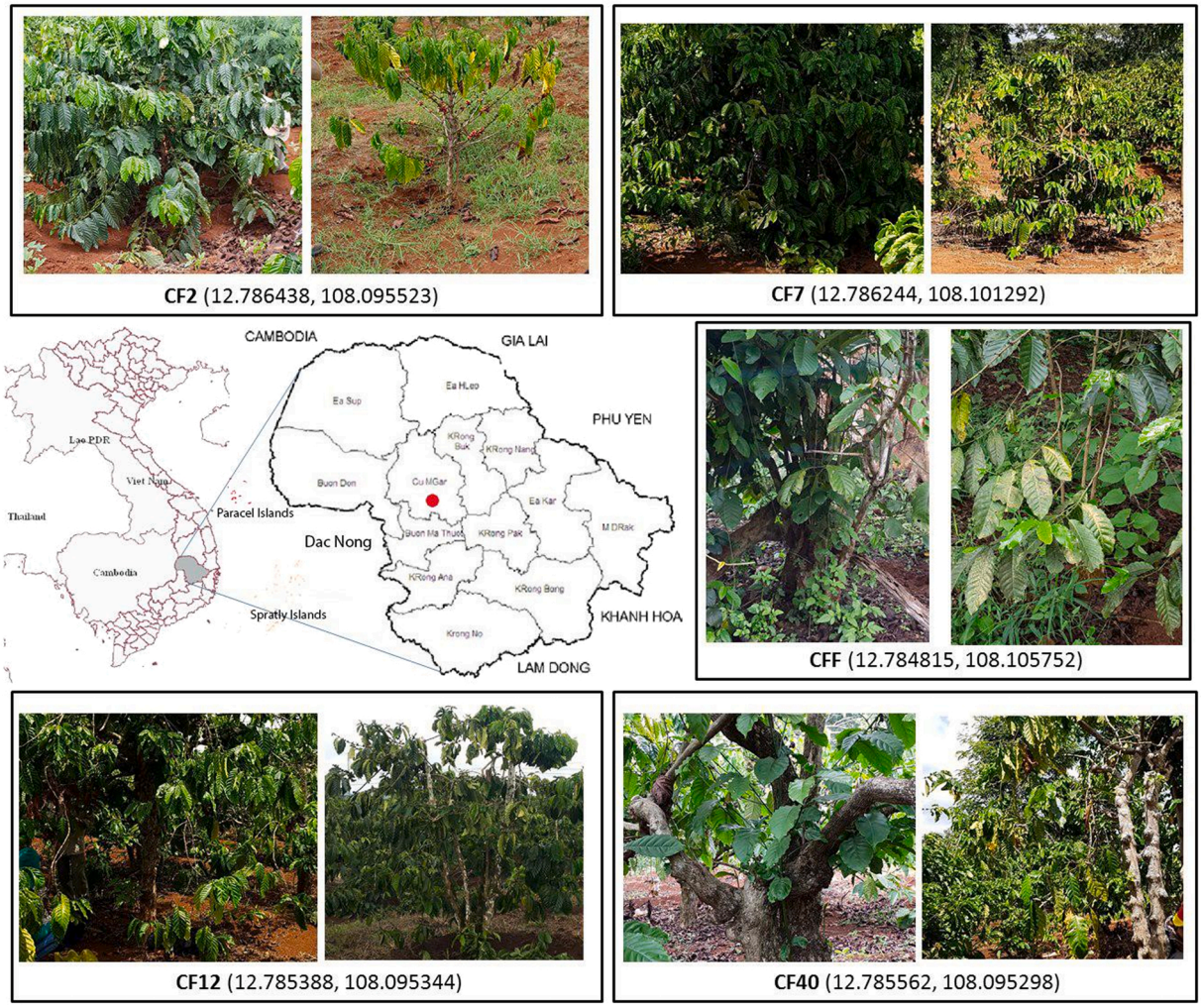


Fig. 1. The sampling site is marked with a red dot in Cu M′gar District, Dak Lak Province (modified from Nguyen et al., 2008 and Pham et al., 2011), Sample photos of the different coffee habitats (replanted coffee plants at 2 years old (CF02), 7 years old (CF07), 12 years old (CF12), coffee plants of 40 years old (CF40), and forest coffee (CFF) divided into healthy (left) and diseased (right)) are provided together with their collection location's GPS addresses. (For interpretation of the references to colour in this figure, the reader is referred to the web version of this article.)

Data analysis was implemented to assess the relationship between coffee plant ages and important plant health indices as well as nematode community composition.

2. Materials and methods

2.1. Study area

The study was conducted in the Eapok coffee plantation (371 ha area) in Cu M′gar District, Dak Lak Province (Fig. 1). The field site is situated in the Central Highlands of Vietnam, where the climate conditions correspond to a generally wetter monsoon climate with a mean annual temperature of 23.5 °C. There is a 5 °C difference between the hottest and coldest months. Dak Lak has a mean annual precipitation of approximately 1789 mm and the monthly average relative humidity of 81.9% (Pham et al., 2011; Sterling et al., 2006). The plantation was selected based on the results of an initial survey which showed that all coffee plant habitats included in this study could be found in Eapok.

2.2. Sampling

Soil nematodes samples were taken from the Eapok coffee plantation according to five different coffee plantations habitats: i) replanted coffee trees at 02 years (CF02), ii) replanted coffee trees at 07 years (CF07), iii) replanted coffee trees at 12 years (CF12), iv) coffee trees at 40 years (CF40), v) coffee trees in natural forest (CFF) (Fig. 1). The replanted coffee trees were sampled from the replanting coffee gardens, which followed the models similar to the official guideline about the process of replanting coffee trees in Vietnam with the decision number 2085/QD-BNN-TT (Ministry of Agriculture and Rural Development, 2016, in Vietnamese). This model is characterized by replanting partly or all the garden with 18–20 months old seedlings after soil treatment. Therefore, the total ages of replanted coffee trees at 02 years (CF02), 07 years (CF07), 12 years (CF12), 40 years (CF40) are around 04 years old, 09 years old, 14 years old, and 42 years old, respectively. The forest coffee trees were taken from the forest's edge, surrounded by numerous vines and small trees. The canopy shade partly covered the coffee trees. Six plants were chosen randomly for sample collection in each coffee age group: three healthy trees (Non-Pat group) and three trees showing damages from nematodes such as chlorotic and stunted growth (Pat group). The characteristic of disease syndrome among the Pat group were detailed in Supplemental Table S5. Rhizosphere soil from these plants was collected at six separated spots surrounding the tree trunk and combined to make a composite sample. A soil auger was used to collect soil from 0 to 20 cm depth at each sampling site, from which nematodes were immediately extracted from the field. At the same time, roots were collected from a standard-size excavation of 20 × 20×20 cm from the coffee tree canopy. The root gall (root knot) percentage and the necrosis percentage of dead roots (root lesion) were evaluated according to Zeck (1971) and Speijer and D. De Waele (1997), respectively; while yellow leaf symptom was assessed following Vietnam Ministry of Agriculture and Rural Development (2010).

2.3. Nematode community analysis

Nematodes were extracted from 100-g of rhizospheric soil samples by using the Baermann's funnel (Baermann, 1917) technique and Flegg (1967) sieving methods, relaxed, killed by heat, fixed in 4% formaldehyde. The nematodes were counted under a stereo-microscope (×50 magnification) and 150 individuals were picked randomly for further identification. The selected individuals were gradually processed to anhydrous glycerol following De Grisse's (1969) technique, and mounted on permanent glass slides. The nematodes were identified to genus level by using several identification keys (Abebe, 2006; Ahmad and Jairajpuri, 2010; Jairajpuri and Ahmad, 1992; Nguyen and Nguyen, 2000; Tarjan et al., 1977).

Functional diversity of nematode assemblages was assigned using individual trophic levels according to Yeates et al. (1993): Bacterivores, Fungivores, Plant Parasitic nematodes, Omnivores and Predators. Taxonomic groups were also assigned to colonizer-persister c-p values, according to (Bongers, 1990). In addition, several ecological indices were calculated: Shannon-Wiener diversity index (H′), Pielou's evenness (J′) and the total number of genera (S) in a sample. The Shannon-Wiener diversity (H′) index was calculated according to Shannon (1948) and Shannon and Weaver (1949), and was used as an indication of the diversity of the soil nematodes.

2.4. Statistical analysis

Important indices for the assessment of soil nematodes, such as Maturity Index (MI), Maturity Index 2–5 (MI25), Plant Parasitic Index (PPI), Channel Index (CI), Enrichment index (EI), and Structure Index (SI), were calculated by using the toolbox from NINJA website (Nematode INdicator Joint Analysis: https://sieriebriennikov.shinyapps. io/ninja/). The diversity indices such as Total abundance, J index, Shannon index, and Species richness were analyzed in Primer 6 (Clarke and Gorley, 2006) version 6.1.13 for Windows. All indices were analyzed using one-way ANOVA to assess the differences among those ecological indices of the five different plantation types. Significant differences were subjected to post hoc tests using the Tukey HSD test. Additionally, the student *t*-test was used to find differences between two groups of healthy (Non-Pat) and diseased (Pat) coffee trees. ANOVAs, student *t*-tests, and Tukey HSD tests were performed in Python (van Rossum, 1995) version 3.7.3.

Several important nematode genera were selected by their relative abundances and grouped into their functional groups (Plant Parasites, Fungivores, Bacterivores, Predators and Omnivores) (Table 1). Two pairwise correlation analyses with Pearson were applied

to evaluate the linear relationship between specific features of samples collected on agricultural soil of Eapok plantation with the forest group (CFF) samples removed. These features include coffee plant fitness quality (Non-Pat and Pat group selection), coffee plant morphology (yellow leaf, root knot and lesion), coffee plant age (2, 7, 12, 40) and the abundance of nematode functional groups or important nematode genera. The CFF group was removed to avoid bias, due to the exclusive occurrence of multiple genera in this unique habitat. The fitness quality of each habitat was assigned as a range from 0 for unhealthy (Pat) plants to 1 for healthy (Non-Pat) plants. The results of correlation coefficient values r were then visualized in a heatmap. Besides, the relationship between nematode functional group (mean data) and plant habitat indices was examined based on a redundancy analysis (RDA). Relative abundance of nematode groups was used as species data. Among the plant habitat indices, coffee plant fitness quality, coffee plant morphology, and coffee plant age were used as environment data. The RDA result was shown in a bi-dimensional plot. All of these analyses were performed in R Studio (RStudio Team, 2020).

3. Results

3.1. Nematodes community composition

A total of 50 nematode genera were found in our study (Supplemental Table S1), while the most notable genera based on their relative abundance were summarized in Table 1. Nematode communities dwelling in the soil of nature forest plants (CFF) showed the highest number of 39 detected genera, followed by CF02, CF40, CF12, and the lowest of CF07 with 29, 28, 25, and 24 genera, respectively.

The total abundance of nematodes ranged from 1024 to 4197 individuals (ind.)/100 g soil and differed between plant age groups ($P = 0.01$) (Table 2). Plant parasites, bacterivores and omnivores (in particular *Chitwoodius*) were predominant in the plantation groups (CF02, CF07, CF12, CF40) while fungivores, omnivores and predators dominated in the forest coffee group (CFF). However, the abundance of nematodes did not differ between soils of healthy and diseased plants ($P = 0.49$) (Supplemental Table S2). We found a distinct gradient in soil nematode functions over age, and a different pattern between the healthy (Non-Pat) group and the diseased (Pat) group (Fig. 2). For the healthy group, the number of plant-parasitic nematodes can be observed to be lower in the mature plantation coffee plants (CF12-NP: 20, CF40-NP: 37) and nature forest plants (CFF-NP: 59), while the population of bacterivorous and omnivorous nematodes shifted in the opposite direction in the young, replanted plants (CF02-NP, 388–60) and mature plants (CF12-NP, 149–374). In contrast, according to functional groups, the distribution of the nematodes was more uniform in the diseased group, with plant-parasitic and omnivorous nematodes having the highest abundance.

Three omnivorous nematodes: *Aporcelaimellus, Eudorylaimus*, and *Oriverutus* were the most abundant genera in CF02 and CFF. In CF02, the nematode genus *Ceanohrabditis* was dominant (14.4%) among the bacterivores. For the fungivorous genera, *Aphelenchoides*

Table 1
Mean relative density of important nematode genera (ind./100 g soil) in different habitats. Values are mean relative abundance (n = 6). Total relative abundance of each genus and functional group are shown.

No	Nematode genera	Abbreviations	CF02 (n = 6)	CF07 (n = 6)	CF12 (n = 6)	CF40 (n = 6)	CFF (n = 6)
Plant parasites							
1	*Hemicriconemoides*	*Her*	0.0	0.0	0.0	0.0	2.7
2	*Longidorus*	*Lon*	0.9	0.2	0.9	1.6	0.4
3	*Meloidogyne*	*Mel*	9.2	15.9	2.0	3.5	1.5
4	*Pratylenchus*	*Pra*	12.6	17.2	3.7	10.4	8.8
5	*Radopholus*	*Rad*	0.0	0.0	0.0	0.0	11.0
6	*Rotylenchulus*	*Rot*	7.8	0.4	6.1	0.6	1.0
Fungivores							
8	*Aphelenchoides*	*Aph*	0.4	0.4	1.7	0.6	0.1
9	*Axonchium*	*Axo*	0.0	0.0	0.0	0.0	8.2
10	*Dorylaimoides*	*Dor*	3.1	0.0	0.0	0.5	0.2
11	*Leptonchus*	*Lep*	0.0	0.0	0.0	0.0	0.2
12	*Tylencholaimus*	*Tyl*	4.3	7.5	0.0	1.8	1.2
Bacterivores							
13	*Acrobeloides*	*Acr*	4.0	2.5	0.0	2.1	1.4
14	*Caenorhabditis*	*Cae*	14.4	7.6	5.6	9.6	3.9
15	*Eucephalobus*	*Euc*	1.6	0.6	3.9	9.9	1.7
Predators							
16	*Discolaimium*	*Dmium*	5.4	1.6	0.0	2.3	0.0
17	*Discolaimus*	*Dmus*	3.7	4.4	3.6	0.4	2.7
18	*Iotonchus*	*Iot*	0.0	0.0	0.0	0.0	7.8
Omnivores							
19	*Aporcelaimellus*	*Apo*	7.1	0.2	2.2	0.4	7.1
20	*Chitwoodius*	*Chi*	10.6	31.5	61.8	47.4	12.8
21	*Eudorylaimus*	*Eud*	4.6	0.2	0.4	0.0	1.6
22	*Opisthodorylaimus*	*Opi*	0	0	0	0	1.2
23	*Oriverutus*	*Ori*	0.0	0.7	2.7	1.4	1.6
24	*Sectonema*	*Sec*	0	0	0	0	0.1

Table 2
Nematode fauna diversity (a) and ecological indices (b) in five different coffee plantations habitats. Values are mean with ± SD (n = 6). P values of the effect of LUI are presented. (Significant of effect: p < 0.5: ' '; p < 0.1, '*'; p < 0.05 '**'; p < 0.001 '***').

Indices	CF02	CF07	CF12	CF40	CFF	P
Total abundance	2852 ± 443ab	2079 ± 511ab	3563 ± 634b	2260 ± 321ab	1194 ± 170a	0.01 * *
J.index	0.8 ± 0.04b	0.73 ± 0.04ab	0.54 ± 0.06a	0.61 ± 0.1ab	0.77 ± 0.03ab	0.02 * *
Shannon (H′) index	1.96 ± 0.23ab	1.73 ± 0.09ab	1.16 ± 0.19a	1.46 ± 0.32ab	2.04 ± 0.14b	0.03 * *
Species richness	12.17 ± 2.36a	10.83 ± 0.91a	8.5 ± 1.34a	10.33 ± 1.73a	14.67 ± 1.73a	0.14
MI	3.48 ± 0.99	3.22 ± 0.94	3.39 ± 0.97	2.95 ± 1.1	3.51 ± 0.89	0.857
MI 25	4.11 ± 0.48	3.95 ± 0.51	3.69 ± 0.82	3.3 ± 0.82	4.13 ± 0.38	0.15
Plant Parasitic Index	3.19 ± 0.3	3.1 ± 0.22	3.21 ± 0.36	3.26 ± 0.28	3.27 ± 0.58	0.927
Channel Index	3.92 ± NA	5.19 ± 10.89	2.1 ± NA	26.25 ± NA	4.34 ± NA	0.481
Enrichment Index	67.24 ± 39.7	83.39 ± 20.48	52.02 ± NA	54.27 ± NA	72.67 ± 37.88	0.604
Structure Index	95.65 ± 4.8	94.35 ± 8.27	87.09 ± 16.96	78.68 ± 20.73	96.72 ± 2.82	0.107

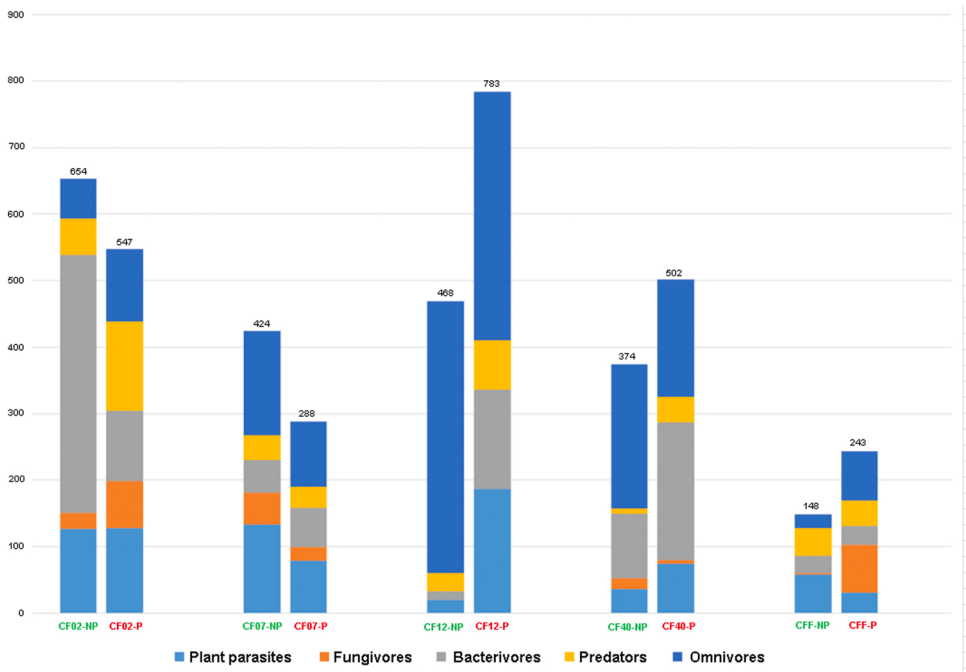


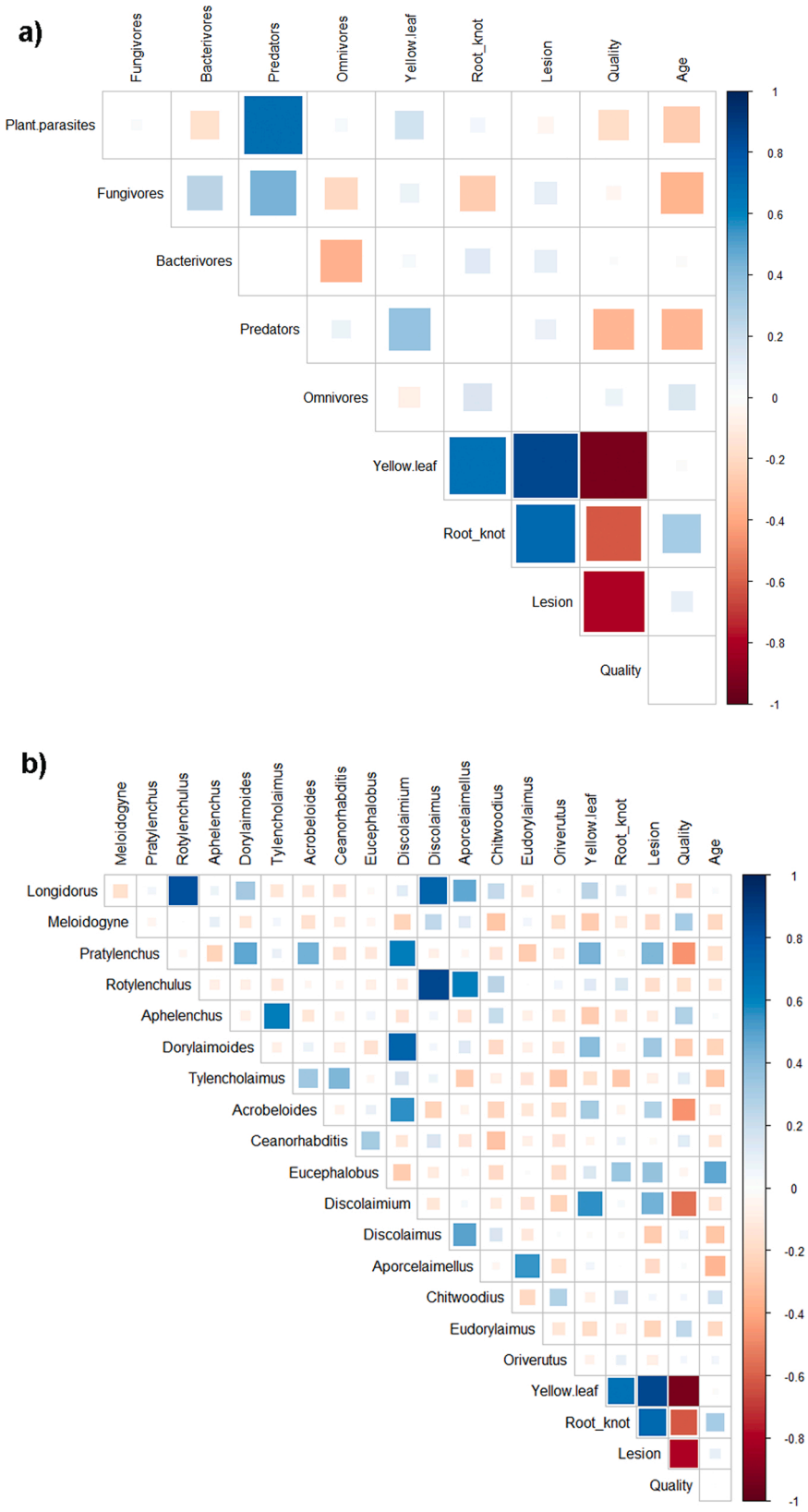
Fig. 2. Nematodes community composition. The area plots visualize the mean abundance of the nematode community in different Robusta coffee plant habitats: replanted coffee plants at 2 years (CF02), 7 years (CF07), 12 years (CF12), 40 years (CF40), and forest coffee (CFF) divided into healthy in green (Non-Pat, NP) and diseased in red (Pat, P). The number on top of the stacks show the mean value of individuals in each group.

was present in all of the habitats, while *Axonchium* and *Leptonchus* occurred only in CFF. *Pratylenchus, Meloidogyne* and *Rotylenchulus* were the most dominant plant-parasitic nematodes and possibly the main cause of diseases. They were most abundant in CF02 and CF07. Notably, the free-living *Chitwoodius* population was recorded to have an extraordinary abundance in the plantation agricultural soil. *Chitwoodius* abundance increased from the young, replanted tree group groups CF2 to CF7, peaked in the CF12 group and decreased in the old, natural groups such as CF40 and CFF (Table 1).

3.2. Diversity and the ecological indices of soil nematode communities

Significant effects of age gradient were demonstrated by the Shannon-Wiener diversity index (H′, F = 3.06, p < 0.05), Pielou's evenness index (J′, F = 3.02, p < 0.05) (Table 2a). The value of H′ was recorded the highest number in the CFF group (2.04) and decreased significantly with increasing plantation age, from 1.97 at the replanted coffee trees at 2 years (CF02) to reach the minimum value at CF12 (1.16), and then slightly raised. Similarly, the value of J′ index showed slight variation at the group of replanted coffee (CFs). The highest and lowest mean values of these indices were observed in natural forest plants (CFF, 0.77) and 12 year-old replanted coffee trees CF12, 0.54).

In terms of ecological differences, the MI was top at CFF group (3.51), besides, it also witnessed a fluctuation in CF group and reached its minimum at the CF40 group (2.95) (Table 2b). The nature forest coffee groups (CFF) continued to display the highest mean values in the rest ecological indices compared to other plantation coffee trees. However, in general, there were no significant



(caption on next page)

Fig. 3. Heatmaps showing pairwise correlation coefficient values r with Pearson as standard correlation coefficient between a) nematode functional groups and plant habitat properties b) important nematode genera and plant habitat properties. The correlation coefficient r between 2 features is a number from − 1–1. Each feature has either positive (blue) or negative (red) correlation effects to each other. The colour intensity depicts the strength of that specific correlation.

differences found in the MI, PPI, Channel index and Enrichment index (Table 2b).

In addition, when comparing all age groups at once, only two indices were found to differ between the healthy (Non-Pat) and the diseased (Pat) coffee plants, i.e. the Shannon index and Species richness (Supplemental Table S2). Overall, the Shannon (H′) and Species richness indices were only slightly higher in the diseased plant group compared to the healthy group ($p < 0.1$ and $p < 0.05$, respectively).

3.3. Relationship between nematode communities and plant habitat properties

Regarding the result of pairwise correlation coefficient analysis, the age gradient of plantation coffee witnessed the exclusive relationship with the abundance of almost all nematode functional groups except omnivores (Fig. 3 and Supplemental Table S3). Particularly, the Pearson correlation coefficients r value between plant age and plant parasites, fungivores, bacterivores, predators, and omnivores were −0.27, −0.36, −0.02, −0.35, 0.14 correspondingly. At once, the plantation age was positively correlated with the morphological indices of replanted coffee trees, meanwhile, it displayed no correlation with plant fitness quality. Moreover, the number of yellow leaves, root knots and root lesions correlate positively with each other and negatively with coffee plant fitness. The higher the numbers of yellow leaves, root knots and root lesions were, the lower the plant fitness would be; which supported our sample selection criteria. Furthermore, the analysis showed that the abundance of omnivores, fungivores, plant parasites were negatively correlated with the number of yellow leaves, root-knot, and lesions, respectively; the abundance of bacterivores and predators, however, did positively correlate with the number of these three plant morphological features.

The ordination diagram was based on redundancy analysis (RDA) of the nematode functional groups and the coffee plant health indices for all age gradients (Fig. 4 and Supplemental Table S4). The RDA plot showed that the composition of the nematode community was clearly discriminated among the different health statuses of plantation coffee trees and showed that plant age was closely related to the distribution of nematode functional groups. The eigenvalues for the first canonical axis and all canonical axes were 0.03434 and 0.04574, respectively and the first two axes explained 17.64% of the variation. As displayed by the Axis 1, no variation was exhibited in nematode community composition among diseased coffee trees (Pat group) (apart from CFP12.1, CFP12.3, and CFP40.1) as well as the four healthy samples CFNP02.1, CFNP02.3, CFNP07.1, and CFNP07.3 (Non-pat group), but significant transformations to the positive direction of Axis 1 occurred in replanted coffee trees at 07 years old (CF07). In addition to this, nematode community composition varies remarkably in most healthy plant samples, especially mature plantation coffee trees (CF12 and CF40). The first axis was positively related to coffee plant age, indicating that the plantation age gradient drove the variation in nematode community composition. Combined with the results of Pearson's correlation analysis, it confirmed the significant responses of omnivores, fungivores and plant parasites along with plant health indices to the plant age gradient.

4. Discussion

In our study, the abundance and diversity of total nematodes differed considerably among the coffee plant age groups. Notably, the abundance of nematodes in coffee trees from the plantation was significantly higher than the forest coffee group. This can be explained by the predominance of plant-parasitic nematode populations in younger replanted coffee tree groups, particularly CF02 and CF07. The most important plant-parasitic nematode genera *Pratylenchus, Meloidogyne* and *Rotylenchulus* could be found in all samples from the plantation, similar to previous surveys by Chau and Thanh (2001) and Trinh et al. (2009). Considering that their abundance was low in the natural forests, this suggests plantation soils to be a favourable habitat. This is in agreement with the theory that plant-parasitic nematodes prefer a mature environment with few competitors (Shea et al., 2004; Zhang et al., 2015). Interestingly, many diversity indices of the young coffee group CF02 were similar to the forest group, including the species richness and diversity (Shannon index and J. index). While the forest environment brought a stable condition to the nematode community, the young coffee plants at two years might not be mature enough to carry a settled nematode population, which explains the high diversity in these groups. This result also indicates that intensive farming and inefficient nematode controls have created disturbances and gradually decreased the diversity in coffee trees of the plantation, especially in replanted groups. The natural habitats for forest coffee also included many vines and other plants, potentially affecting the composition of nematode communities, whereas these factors in replanted gardens were less pronounced. Regarding ecological indices, the nematode communities in nature forest coffee trees (CFF group) recorded higher MI, MI25, PPI, and SI than those in replanted coffee (CF group). Nevertheless, those indices did not appear to be constructive in our research of monoculture Robusta coffee as they displayed no significant differences ($p > 0.05$).

The nematode fauna diversity indices seemingly suggested that the diseased plants had higher soil nematode diversity in their rhizospheres than the healthy plants (Supplemental Table S2); however, this calculation did not take functionality into account. Many plant-parasites do not occur with healthy plants, which lowers the species richness. Hence, nematode functional groups were favoured in our further analyses to avoid the possible bias.

The high similarity of the nematode communities in the soils of diseased and healthy plants indicates only small changes in the community of nematodes that favor disease symptoms. Identifying these differences can potentially predict their trend in fitness. A

RDA analysis in the present study indicated that the nematode communities in different coffee plant ages clustered corresponding to plant fitness. As can be seen, the plant morphological indices were closely related to the distribution of nematode functional groups, plant parasites, fungivores, bacterivores, and predators. In particular, nematode communities in the samples CFNP02.1, CFNP02.3, CFNP07.1, and CFNP07.3, belonging to healthy plant group (Non-pat) as well as young replanted coffee tree groups (CF02 and CF07), showed a development toward the diseased state, which is likely if the abundance of bacterivores and fungivores decreases while the proportion of plant-parasitic nematodes increases (Fig. 4). This trend contributes to the hypothesis that lower diversity and a shift towards dominance of plant-parasitic nematodes appeared to be the indicators of diseased plants. Meanwhile, omnivores were positively correlated with the age and fitness quality of coffee plants, especially mature replanted coffee groups (CF12 and CF40), which contain diverse and well-structured microbial communities. This functional group correlated weakly to the diseased samples, as these groups prefer a more stable environment (Pothula et al., 2019; Wang and Hooks, 2011). Overall, young replanted coffee trees (CF02, CF07) are commonly weak, susceptible to pathogens, and died at young age, besides, older replanted plants (CF12, CF40) or plants in natural forest (CFF) have associated nematode communities reaching equilibrium and specified diversity. Indeed, the land affected by nematodes in Vietnam can take 2–3 years for soil treatment and plantation rotation to grow coffee again due to excessive production; and it takes a minimum 3 years for the newly planted to produce a crop (Chapman, 2014). Hence, in farms where nematodes had already been prevalent, it can take 5–6 years to complete the process. On the other hand, many replanting areas were not successful after the beginning period of 3–4 years due to damages caused by parasites, and it was still unclear whether the source of contamination was from unclean soil or seedling materials (Chapman, 2014). Plant-parasitic nematodes especially pose a major problem for coffee replanting projects. Replanting coffee in an infested area causes early and rapid death of the seedling's tap root, leaving plants loosely attached to the soil and can be easily pulled out by hand. These symptoms become apparent two to three years after the replanting (Loang, 2002). These drawbacks prevent farmers from investing in costly and potentially risky large-scale replanting. There is an inherent risk for the Vietnamese coffee industry of losing not only the current crops, but also further coffee plantations if the damages from nematode infestations cannot be controlled. Thus, the need for a biological control for nematode damage in Vietnam coffee plantations is of a significant importance and urgency.

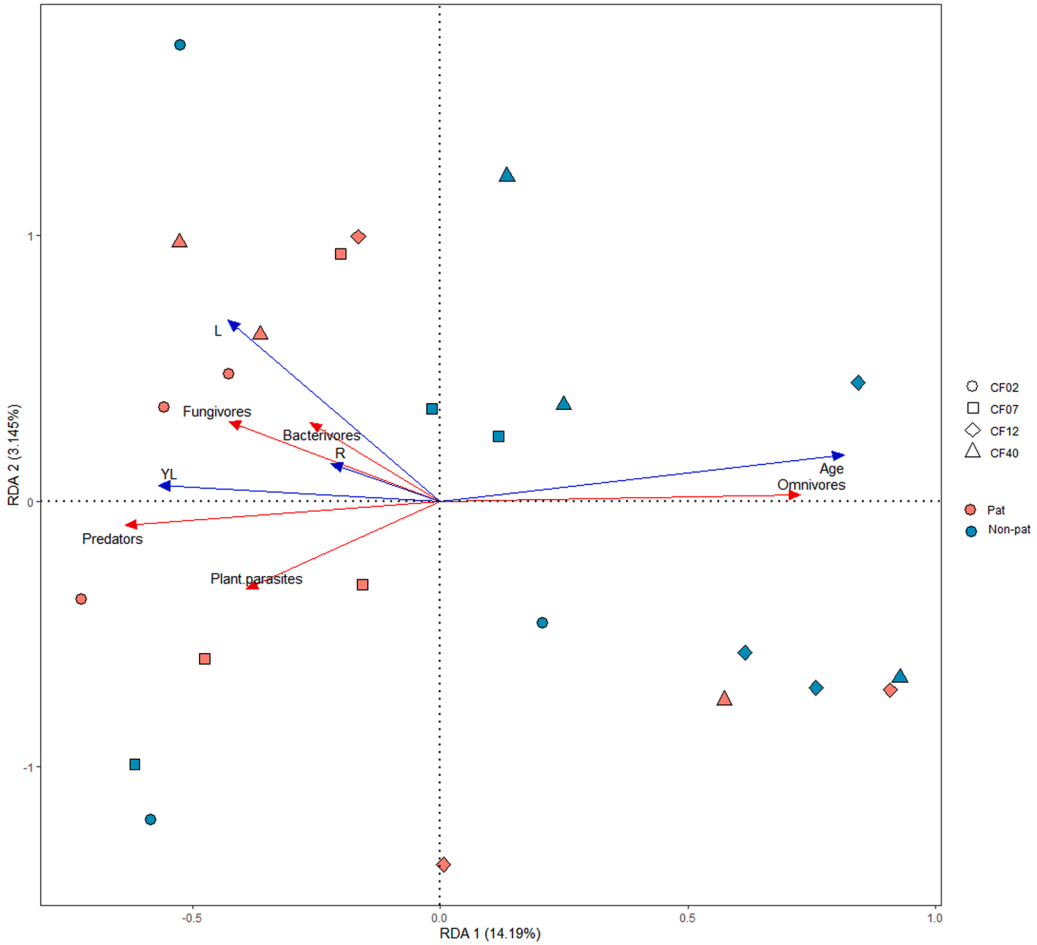


Fig. 4. Redundancy analysis (RDA) between nematodes and plant habitat indices. RDA results were summarized in a bi-dimensional plot. YL: Yellow leaf; R: Root-knot; L: Lesion; CF02–40: replanted coffee trees at 02–40 years old; Non-pat: Healthy; Pat: Diseased.

In terms of the effects of nematode genera occurences on coffee's health, *Pratylenchus* spp. (root-lesion nematodes), in particular, *P. coffeae,* and *Meloidogyne* sp. (root-knot nematodes) are the most important and widespread plant-parasitic nematodes in coffee roots in the world (Bell et al., 2018; Villain et al., 2018) and Vietnam (Chapman, 2014; Wang and Hooks, 2011). Our analysis revealed that the genus *Pratylenchus* have the strongly negative correlation with coffee plant fitness quality ($r = 0.46$, $p = 0.025$) and positive with the diseased symptoms (yellow leaf, root-knot) (Fig. 3b and Supplemental Table S3), which corroborates previous studies in the Central Highlands of Vietnam (Chau and Thanh, 2001; Loang, 2002; Q.P. et al., 2001; Trinh et al., 2009). Unexpectedly, the occurrence and abundances of *Meloidogyne* did not correlate with the diseased symptoms. Notably, our study has revealed the involvement of fungivorous nematodes, highlighted by analyses showing their association with the occurrence of plant-parasitic nematodes and important plant health indices. In particular, the fungivorous *Aphelenchoides* was present in all samples with high relative abundance. *Aphelenchida* nematodes (cp-2) can respond rapidly to organic amendment of stressful environments (Ferris and Bongers, 2006; Pothula et al., 2019). Thus, it can be hypothesized that the occurrence of *Aphelenchoides* was induced by the increased supply of food due to damaged roots of the diseased coffee plants, suggesting the proliferation of pathogenic fungi and decomposers following damage to the root system. While the correlation between *Dorylaimoides* and *Pratylenchus* found in this study was possibly the characteristic fungivorous and plant-parasitic nematode relationship in the food chain (Gomes et al., 2003), *Aphelenchoides* did not correlate with any herbivorous genus (Fig. 3b and Supplemental Table S3). Thus, the occurrence of *Aphelenchoides* could be an important indicator of plant damages and infections, which are not necessarily caused by plant-parasitic nematodes. Another important free-living nematode genus is *Chitwoodius*, populating the plantation soil in a remarkable abundance. Our previous survey (Ha et al., 2018) and this study are the first records of abundant distribution of *Chitwoodius* species in tropical East Asia territories. This genus is also a matter of contention between the plant-feeding and omnivorous groups (Yeates et al., 1993). Stylet's structure of *Chitwoodius* is strong and typical for puncturing plant cell walls, suggesting a herbivorous diet (Hoang et al., 2020). However, we have yet to find pathological evidence in our surveys that *Chitwoodius* spp. feed on Robusta coffee plant roots. Furthermore, the Pearson analysis results illustrated that the occurrence of the free-living *Chitwoodius* did not significantly correlate with coffee plant's fitness or the occurrence of other nematode genera. A schematic illustration to summarise the relationships between the nematode genera and coffee plant health indices in our study was constructed (Supplemental Fig. S1). The therein shown correlations suggest these nematode genera as ecological indicators regarding coffee plant habitat and their survival potential. However, further longitudinal studies of nematode communities in different coffee plant age gradients with larger sample sizes and rigorous quantitative methods are still required to verify our observations and to construct a pre0diction model.

5. Conclusions

Our findings give an initial overview of the rhizosphere nematode communities in a monoculture Robusta coffee plantation in Dak Lak, Vietnam. We confirmed the importance of *Pratylenchus* as the major plant-parasitic nematodes in this area and reported for the first time the significant involvement of free-living soil nematodes. The plantation ecosystem's imbalance was reflected by the distribution and relative abundance of important nematode functional groups. Colonization of plant-parasitic nematodes occurs as the plant ages, and the predominance of these nematodes coincides with lower diversity of the nematode fauna. Our data add to the development of a predictionary model in the future, which will create a positive impact on the Vietnamese coffee industry.

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.

Acknowledgments

This work was supported by the project of Vietnam Ministry of Science and Technology for "Study on metagenomics of plant rhizosphere microbiomes of tumeric and coffee in order to increase yield and quality of plants", grant number: ĐTĐLCN.14/14, Vietnam; in corporated with the Dutch Ministry of Economic Affairs, grant number: BE-Basic preoject 7.3.7, Netherlands.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.gecco.2021.e01932.

References

Abebe, E., 2006. Freshwater Nematodes: Ecology and Taxonomy.
Ahmad, W., Jairajpuri, M., 2010. Mononchida: the predaceous nematodes. Nematol. Monogr. Perspect. 7.
Baermann, G., 1917. Eine einfache Methode zur Auffindung von Ankylostomum (Nematoden) Larven in Erdproben. Geneesk. Tijdschr. Ned-Indië 57, 131–137.
Bell, C.A., Atkinson, H.J., Andrade, A.C., Nguyen, H.X., Swibawa, I.G., Lilley, C.J., McCarthy, J., Urwin, P.E., 2018. A high-throughput molecular pipeline reveals the diversity in prevalence and abundance of pratylenchus and meloidogyne species in coffee plantations. Phytopathology 108, 641–650. https://doi.org/10.1094/PHYTO-10-17-0343-R.

Bongers, T., 1990. The maturity index: an ecological measure of environmental disturbance based on nematode species composition. Oecologia 83, 14–19. https://doi.org/10.1007/BF00324627.
Campos, V., Silva, J., 2008. Management of Meloidogyne spp. in Coffee Plantations. : Plant-Parasit. Nematodes Coffee 149–164. https://doi.org/10.1007/978-1-4020-8720-2_8.
Chapman, K.R., 2014. Final Report: Collaborative Study of Coffee Rejuvenation Strategies in Vietnam – Technical Aspects. FAO, Bangkok, Thailand.
Chau, N.N., Thanh, V.N., 2001. Plant parasitic nematodes associated with coffee in some North and Taynguyen highland provinces of Vietnam. Selected works on ecological and bio-resource studies 1996–2000. Agric. Publ. House Hanoi.
Clarke, K., Gorley, R.N., 2006. PRIMER v6: user manual/tutorial. PRIMER-E. Plymouth 29, 1060–1065.
Ferris, H., Bongers, T., 2006. Nematode indicators of organic enrichment. J. Nematol. 38, 3–12.
Flegg, J., 1967. Extraction of Xiphinema and Longidorus specks from soil by a modification of Cobb's decanting and sieving technique. Ann. Appl. Biol. 60, 429–437. https://doi.org/10.1111/j.1744-7348.1967.tb04497.x.
Gomes, S., Huang, P., Cares, J., 2003. Nematode community, trophic structure and population fluctuation in soybean fields. Fitopatol. Bras. 28 https://doi.org/10.1590/S0100-41582003000300006.
Ha, H., Ha, C., Nguyen, D., Trinh, P., Abolafia, J., Peña-Santiago, R., 2018. Morphological and molecular characterisation of Chitwoodius coffeae sp. n. (Dorylaimida: Tylencholaimidae) from Vietnam, with a revised taxonomy of the genus. Nematology 21, 1–13. https://doi.org/10.1163/15685411-00003231.
Hoang, H., Tran, H.L., Nguyen, T., Nguyen, D., Nguyen, H., Pham, N., Trinh, P., de Boer, T., Brouwer, A., Chu, H.H., 2020. Occurrence of endophytic bacteria in Vietnamese Robusta coffee roots and their effects on plant parasitic nematodes. Symbiosis 80, 75–84. https://doi.org/10.1007/s13199-019-00649-9.
van den Hoogen, J., Geisen, S., Routh, D., Ferris, H., Traunspurger, W., Wardle, D.A., de Goede, R.G.M., Adams, B.J., Ahmad, W., Andriuzzi, W.S., Bardgett, R.D., Bonkowski, M., Campos-Herrera, R., Cares, J.E., Caruso, T., de Brito Caixeta, L., Chen, X., Costa, S.R., Creamer, R., Mauro da Cunha Castro, J., Dam, M., Djigal, D., Escuer, M., Griffiths, B.S., Gutiérrez, C., Hohberg, K., Kalinkina, D., Kardol, P., Kergunteuil, A., Korthals, G., Krashevska, V., Kudrin, A.A., Li, Q., Liang, W., Magilton, M., Marais, M., Martín, J.A.R., Matveeva, E., Mayad, E.H., Mulder, C., Mullin, P., Neilson, R., Nguyen, T.A.D., Nielsen, U.N., Okada, H., Rius, J.E.P., Pan, K., Peneva, V., Pellissier, L., Carlos Pereira da Silva, J., Pitteloud, C., Powers, T.O., Powers, K., Quist, C.W., Rasmann, S., Moreno, S.S., Scheu, S., Setälä, H., Sushchuk, A., Tiunov, A.V., Trap, J., van der Putten, W., Vestergård, M., Villenave, C., Waeyenberge, L., Wall, D.H., Wilschut, R., Wright, D.G., Yang, J., Crowther, T.W., 2019. Soil nematode abundance and functional group composition at a global scale. Nature 572, 194–198. https://doi.org/10.1038/s41586-019-1418-6.
ICC, 2019. Country Coffee Profile: Vietnam. International Coffee Council 124th Session, Nairobi, Kenya.
Bigger, M., Waller, J.M., Hillocks, R.J., 2007. Coffee Pests, Diseases and Their Management. CABI, pp. 1–434.
Jairajpuri, Ahmad, W., 1992. Dorylaimida: Free-Living, Predaceous and Plant-Parasitic Nematodes. Brill, Leiden, The Netherlands.
Loang, K.T., 2002. Research on the Causal Agents of Robusta Coffee Yellow and Rot Roots Symptoms in Daklak Province And Assessment Of The Control Potential. Hanoi Agriculture University.
Marsh, A., 2007. Diversification by Smallholder Farmers: VietNam Robusta Coffee. FAO, Rome, Italy.
Nguyen N.C., Nguyen V.T., Plant parasitic nematodes in Vietnam. The Fauna of Vietnam Sci. Technol. pub. 400 2000.
Pham, H., Doan, H., Phan, T., Minh, N., 2011. Ecological factors associated with dengue fever in a Central Highlands Province, Vietnam. BMC Infect. Dis. 11, 172. https://doi.org/10.1186/1471-2334-11-172.
Phan Thi, T., Duc Niem, L., Minh, T., Ho, H., Burny, P., Lebailly, P., 2018. Economic analysis of perennial crop systems in Dak Lak Province, Vietnam. Sustainability 11. https://doi.org/10.3390/su11010081.
Pothula, S.K., Grewal, P., Auge, R., Saxton, A., Bernard, E., 2019. Agricultural intensification and urbanization negatively impact soil nematode richness and abundance: a meta-analysis. J. Nematol. 51, 1–17. https://doi.org/10.21307/jofnem-2019-011.
S. Q.P T. M.H T. T.H E. Al, 2001. Research on coffee yellow syndrome and control measures Minist. Sci. Technol. Environ. 2001.
van Rossum, G., 1995. Python tutorial.
RStudio Team, 2020. RStudio: Integrated Development for R. RStudio, Inc., Boston, MA. ⟨http://www.rstudio.com/⟩.
Shannon, C.E., 1948. A mathematical theory of communication. Bell Syst. Tech. J. 27, 379–423. https://doi.org/10.1002/j.1538-7305.1948.tb01338.x.
Shannon, C.E., Weaver, W., 1949. The Mathematical Theory of Communication. Urbana Univ. Ill. Press.
Shea, K., Roxburgh, S.H., Rauschert, E.S.J., 2004. Moving from pattern to process: coexistence mechanisms under intermediate disturbance regimes. Ecol. Lett. 7, 491–508. https://doi.org/10.1111/j.1461-0248.2004.00600.x.
Speijer, P.R., D. De Waele, D., 1997. Screening of Musa germplasm for resistance and tolerance to nematodes. INIBAP Tech. Guidel. 1, 48.
Sterling, E., Hurley, M., Le, M., 2006. Vietnam: A Natural History. Yale University Press.
Tarjan, A.C., Esser, R.P., Chang, S.L., 1977. Interactive diagnostic key to plant parasitic, free living and predaceous nematodes. J. Water Pollut. Control Fed. 49, 2318–2337.
Thi, P., Huyen, T., Giang, P., Toan, N., Trinh, P., 2018. Correlation between the distribution of nematodes and soil physicochemical characteristics in coffee rejuvenation areas. EnvironmentAsia 11, 141–156. https://doi.org/10.14456/ea.2018.11.
Trinh, P., De, E., de la Peña, E., Nguyen, C., Nguyen, H., Moens, M., 2009. Plant-parasitic nematodes associated with coffee in Vietnam. Russ. J. Nematol. 17, 73–82.
Truong H., 2018. Areas of old coffee plants are increasing in Vietnam. The Western Highlands agriculture & forestry science institute (WASI) ⟨http://wasi.org.vn/thach-thuc-cua-san-xuat-ca-phe-o-vietnam/⟩.
Vieira, H.D., 2008. In: Souza, R.M. (Ed.), Coffee: The Plant and its Cultivation BT - Plant-Parasitic Nematodes of Coffee. Springer, Netherlands, Dordrecht, pp. 3–18. https://doi.org/10.1007/978-1-4020-8720-2_1.
Vietnam Ministry of Agriculture and Rural Development, 2010. National technical regulation on Surveillance method of plant pests, QCVN 01–38-2010, Circular 71/2010/TT-BNNPTNT (20/12/2010).
Villain, L., 2008. Economic importance, epidemiology and management of pratylenchus sp. in coffee plantations. Plant-Parasitic Nematodes of Coffee. CIRAD, pp. 65–84. https://doi.org/10.1007/978-1-4020-8720-2_5.
Villain, L., Salgada, L., Sônia, M., Trinh, P.Q., 2018. Nematode parasites of coffee and cocoa. Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. CABI,, Wallingford.
Wang, K.H., Hooks, C.R.R., 2011. Chapter 4: managing soil health and soil health bioindicators through the use of cover crops and other sustainable practices. In: Brust, G.E. (Ed.), MD Organic Vegetable Growers. University of Maryland, pp. 1–18.
Wiryadiputra, S., Tran, L.K., 2008. Indonesia and Vietnam. In: Souza, R. (Ed.), Plant-Parasitic Nematodes of Coffee. Springer, Netherlands, pp. 277–292.
Yeates, G.W., 1979. Soil nematodes in terrestrial ecosystems. J. Nematol. 11, 213–229.
Yeates, G.W., Bongers, T., De Goede, R.G., Freckman, D.W., Georgieva, S.S., 1993. Feeding habits in soil nematode families and genera-an outline for soil ecologists. J. Nematol. 25, 315–331.
Zhang, Y.-Y., Ling, J., Yang, Q.-S., Wang, Y.-S., Sun, C.-C., Sun, H.-Y., Feng, J.-B., Jiang, Y.-F., Zhang, Y.-Z., Wu, M.-L., Dong, J.-D., 2015. The diversity of coral associated bacteria and the environmental factors affect their community variation. Ecotoxicology 24, 1467–1477. https://doi.org/10.1007/s10646-015-1454-4.