Contribution of soil free-living nematode communities to suppression of *Meloidogyne incognita* in organic fields

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Summary. Predatory and omnivorous nematodes are known as potential biological control agents for root-knot nematodes (RKN). However, it is still unclear whether free-living nematodes (FLN) communities, including microbial-feeding nematodes, contribute to RKN suppression. To evaluate their contribution, a pot experiment was conducted using soils containing either 1) microbes or 2) microbes and FLN. After six weeks of green pepper growth, we analysed the level of *Meloidogyne incognita* infection and the bacterial community with next-generation sequencing (NGS). The same analysis was conducted after an additional 1-month incubation. *Meloidogyne incognita* densities were significantly lower in soils with FLN than in those with microbes only. Moreover, *M. incognita* multiplication was suppressed even in the absence of predators and omnivores, highlighting the importance of microbial-feeding nematodes. NGS results showed that some bacterial genera had a significant negative correlation with *M. incognita* density, suggesting that bacterivores altered bacterial communities to promote microbial suppression of *M. incognita*.

Key words: bacterivores, microbial-feeding nematodes, nematode control, plant-parasitic nematode, root-knot nematode.

Plant-parasitic nematodes (PPN) are one of the most important pests in the world, and the crop losses have been estimated at $ 157 billion annually (Abad *et al.*, 2008). Among them, root-knot nematodes (RKN) are the most economically important group; they are distributed worldwide and parasitise nearly every species of plants (Moens *et al*., 2009). Currently, chemical control is the primary way to control RKN. However, many countries, including Japan, aim to reduce chemical pesticides due to their environmental and human health impacts (Ahmad *et al*., 2024). To reduce pesticide use, various approaches have been explored, such as crop rotation, resistant varieties and cover crops. In addition, many researchers have reported antagonistic microbes (Li *et al*., 2015; Poveda *et al*., 2020; Topalović *et al*., 2020; Vasantha-Srinivasan *et al*., 2024). Stirling (2014) advocated "Integrated soil biology management", which is a concept to raise soil ecological functions to reduce yield losses caused by plant-parasitic nematodes by increasing soil organic matter while reducing tillage to promote plant growth. A current approach is to combine traditional biological control methods in order to maximise their effectiveness (Ahmad *et al*., 2021).

Nematodes are a dominant component of the soil community and the most abundant animals on the earth (van den Hoogen *et al*., 2020), and among them, free-living nematodes (FLN) play an important role in soil ecosystem functions. Regarding the suppression of damage by PPN, predatory and omnivorous nematodes feed on PPN to decrease their population (Bilgrami & Khan, 2022). Khan and Kim (2005) reported that the inoculation of 200 *Mononchoides fortidens* Schuurmans Stekhoven, 1951 individuals per pot (500 g of soil) suppressed the density of *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 by 98% after 60 days of tomato growing. One of the barriers to applying predator nematodes as biological control agents is the

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difficulty of mass culture, but effective culture systems were established for some nematodes *e.g.*, *M. fortidens*, *Mononchoides gaugleri* Siddiqi *et al.*, 2004, *Mononchoides longicaudatus* (Khera, 1965) Andrássy, 1984, *Diplenteron colobocercus* Andrássy, 1964, *Butlerius degrissei* Grootaert and Jaques, 1979 (Bilgrami & Khan, 2022). In addition to applying predatory and omnivorous nematodes cultured in a laboratory, increasing their populations in farmlands has been attempted by improving farming practices such as conservation tillage and the introduction of cover crops (McSorley, 2003; Sánchez-Moreno & Ferris, 2007; Timper *et al*., 2021). Sánchez-Moreno and Ferris (2007) compared RKN suppression potentials between a mature oak woodland and a 20-year-old vineyard. The woodland soil had higher suppression, and a relatively higher abundance of predatory and omnivorous nematodes and structure index, indicating the complexity of the soil food web; all were positively correlated with the suppression of RKN. Furthermore, some papers reported that more disease-suppressive soils included greater abundance of predators or omnivores (Min & Toyota, 2013; Silva *et al*., 2022; Obidari *et al*., 2024).

Most of the soil nematode components are microbial-feeding nematodes (van den Hoogen *et al*., 2019), but information on their contributions to PPN suppression is limited. One known role is "amplifiable prey" (Ferris *et al*., 2012). They serve as prey for predators and omnivores, supporting their population growth and promoting predation pressure on PPN, thereby increasing the suppression. In addition, Topalović and Geisen (2023) suggested that microbial-feeding nematodes might contribute to PPN suppression indirectly by increasing antagonists by feeding competitors and by carrying antagonists close to PPN. Some previous papers reported an influence of nematodes on microbial communities (Griffiths *et al*., 1999; Jiang *et al*., 2017; Brondani *et al*., 2022). Xu *et al.* (2024) recently reported that *Protorhabditis* (Osche, 1952) Dougherty, 1953 promoted a bacterial antagonistic effect on the bacterial wilt pathogen *Ralstonia solanacearum* (Smith 1896) Yabuuchi *et al.*, 1995 by modifying the microbiome to increase the abundance of antagonistic bacteria.

These pieces of information suggest that not only predators and omnivores, but also microbial-feeding nematodes, might contribute to RKN suppression. However, previous research has studied RKN suppression by inoculating only one genus cultured on agar plates or analysing a correlation between characteristics of nematode communities and RKN suppression (McSorley, 2003; Khan & Kim, 2005; Sánchez-Moreno & Ferris, 2007; Min & Toyota, 2013; Timper *et al*., 2021; Masson *et al*., 2022; Obidari *et al*., 2024). As a result, it remains unclear whether whole soil nematode communities contribute to RKN suppression, even though research about the possibility of FLN contribution to PPN suppression has accumulated. To address this, we conducted a pot experiment with the following objectives: *i*) to reveal the contribution of soil nematode communities to suppression of *M. incognita* multiplication; *ii*) to find some characteristics of the nematode community structure related to the suppression; and *iii*) to reveal underlying mechanisms by analysing bacterial community. We hypothesised that: *i*) diverse nematode communities, including microbial feeders and predators, would have stronger suppression potential; *ii*) even though there were no predators, FLN communities could contribute to RKN suppression; and *iii*) biological suppression might occur after harvesting. To test the third hypothesis, we analysed two time points: at harvest and one month after harvest.

MATERIAL AND METHODS

Soil. Soils were collected from two different organic farming fields: Kanagawa (35°32'11.96" N, 139°18'19.75" E) and Ibaraki (35°58'35.61" N, 140°36'30.63" E). Both field soils were Andosol. Kanagawa soil had a pH (H_2O) of 5.6, a total C of 67.6 mg g^{-1}, and a total N of 4.7 mg g^{-1}. Ibaraki soil had a pH (H_2O) of 5.4, a total C of 32.3 mg g^{-1}, and a total N of 2.3 mg g^{-1}. Soil samples were collected with a shovel from ten random points at each site and were combined and mixed well, then stored at room temperature.

Pot experiment. Soil (180 g) was put into plastic pots (diam. = 9 cm) to prepare 10 pots for each sampling site. All pots were defaunated by freezing and drying (-80°C for 24 h, 80°C for 24 h, and -80°C for 24 h) to remove soil animals without affecting microbes (Bardgett *et al*., 1998). For half of the pots, 20 g of fresh soil was added to inoculate microbes and nematodes to establish an 'inoculated' treatment. For the other pots, soil suspension was added to inoculate only microbes; this treatment was named 'non-inoculated'. To prepare the soil suspension, 20 g of fresh soil was mixed with 40 ml of sterilised water, ultrasonicated for 2 min, and sieved through an 18 µm sieve to remove nematodes more reliably than in a previous study where 25 µm sieve was used (Wagg *et al.*, 2014). The water amount was adjusted to 60% of maximum water holding capacity (MWHC), and the pots were kept at 25°C for 5 days to allow inoculated soil biota to stabilise. Green

pepper (*Capsicum annuum* L. 'Kyomidori', Takii & Co., Ltd) seedlings grown for 2 weeks were transplanted to each pot as a host plant, and after 5 days, the second-stage juveniles (J2) of *M. incognita* were inoculated (450 J2 pot^{-1}). The J2 were obtained from egg masses incubated in tap water at 28°C for 2 days; the hatched J2 suspension was concentrated by centrifugation at 4,230 *g* (TOMY, low speed centrifuge LCX-100). Chemical liquid fertilisers (HYPONeX®, ×1/500, 4 ml) were applied every 2 weeks and watered daily to keep the water amount at 60% of MWHC. After 6 weeks of growth at 25°C, the pot experiment was terminated, and the plants were taken for analysis. The plants were divided into shoot and root. The shoot parts were dried at 70°C to measure the dried weight. The root parts were measured at fresh weight, cut into small pieces, and mixed well with soil in the pot, then the RKN density and FLN community structures were analysed for the first measurement (0 month) using 50 g of soil, as described below. The remained soil-root mixture was incubated for an additional month at 25°C to evaluate the biological suppression of RKN (1 month). Then, the second measurement of the RKN density and FLN community structures was conducted.

Analysis. At the first and second measurements, 20 g of fresh soil was taken for nematode analysis, and 30 g of soil was used for measurement of RKN density using the real-time PCR method after mixing well (Min *et al.*, 2012). Nematodes were extracted from 20 g with the Baermann funnel method at a single replicate from each pot. After extraction, nematodes were fixed with 70°C formalin liquid (4%) and stained with Rose Bengal solution (1%) (Van Sinh *et al.*, 2022). The number of total nematodes was counted under a microscope (Olympus SZX16), then approximately 100 nematodes were picked up to prepare slides for identification. Nematodes were identified at the genus level under a microscope (magnification range: ×400-1000, Olympus BW50). Feeding types and cp values were assigned to each genus according to the information on the website "NEMAPLEX" (http://nemaplex.ucdavis.edu/). Based on the number of nematodes per 20 g of fresh soil, the densities of each genus were calculated. For nematode analysis, the number of each feeding type and the number of genera of each feeding type (Richness) were calculated. In addition, the structure index (SI) and the enrichment index (EI) were calculated by following the formula: SI = 100 × [s/(s + b)], and EI = 100 × [e/(e + b)], where e, s, and b are the enrichment component, the structure component, and basal food web component, respectively (Ferris *et al.*, 2001).

Since the roots and soil were mixed well, the measured RKN density represented the number of all stages of RKN inside egg masses and in soil. Fresh soil (30 g) was dried at 60°C overnight and 20 g of dried soil was ground with a ball-mill (FastPrep®-24). Then, 0.5 g of the ground soil was used for DNA extraction by following the method of Sato *et al.* (2010). Briefly, DNA was extracted in duplicate from 0.5 g of the dried mixture of soil and root and finally dissolved in 100 µl TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). The DNA extract was diluted 10-fold and used as a template in real-time PCR. Specific primers for *M. incognita* were RKNf (5' - GCT GGT GTC TAA GTG TTG CTG ATA C - 3') and RKNr (5' - GAG CCT AGT GAT CCA CCG ATA AG - 3') (Toyota *et al.*, 2008). Real-time PCR was conducted at the following program (95°C for 10 s, (95°C for 5 s, and 60°C for 20 s) for 45 cycles in a StepOne Real-Time PCR System (Life Technologies)) (Koyama *et al*., 2015). The J2 density was calculated by using the equation ($y = -0.9179x+37.37$) between the number of J2 ($\log_2(x)$) and Ct values (y) (Watanabe *et al.*, 2013).

Because bacterivorous nematodes occupied most microbial-feeding nematodes, bacterial communities were chosen to be analysed in more detail with next-generation sequencing (NGS). Bacterial DNA was extracted from 0.5 g of fresh soil to determine the total number of copies of bacteria and analyse the community structure. The method was the same as the DNA extraction from nematodes described above, but fresh soils were used for the bacterial DNA extraction. Firstly, real-time PCR was conducted with a universal primer set to determine the copy number of bacteria. The bacterial universal primers were EUB338 (5'- ACT CCT ACG GGA GGC AGC AG - 3') and EUB518 (5' - ATT ACC GCG GCT GCT GG - 3') (Fierer *et al*., 2005). Then, the obtained Ct values were converted into the number of copies by the following formula ($y = -3.7126x + 37.861$) (y = Ct value, x = $\log_{10}$ (the number of copies)) (Sawada *et al*., 2021). From five replicates of each treatment, two replicates in which the RKN density indicated the median were selected for the NGS analysis from each measurement timing as representatives. The preparation of libraries and NGS was performed by a commercial research organisation (FASMAC, Kanagawa, Japan).

Statistical analysis. All statistical analyses were performed using the R software (version 4.3.2) except for a comparison of nematode parameters.

The effect of the FLN inoculation on the RKN density. To evaluate the effect of FLN inoculation on RKN density, a one-way ANOVA was performed, with FLN inoculation as the independent variable and

RKN density as the dependent variable. Multiple comparisons were conducted using the 'agricolae' package, and Fisher's Least Significant Difference (LSD) test was applied to assess differences between treatments. In addition, a two-way ANOVA was performed to test differences between each treatment among different field soils.

A comparison of each nematode parameter. The Excel data analysis tool was used. First, an F-test was performed to confirm whether each dispersion was equal, then Student t-test and Weltch t-test were performed for equal and unequal data, respectively, to determine if a difference between two treatments was significant.

The correlation analysis between the FLN community structure and the RKN density. A correlation analysis was conducted for each field. We removed nematode genera that were observed in only one replicate for the following analysis. The Shapiro-Wilk test was conducted to evaluate the normality of each variable. Variables that showed normality were analysed by Pearson's correlation, while those that did not show normality were analysed by Spearman's correlation.

The correlation analysis between bacterial community structure and the RKN density and nematode parameters. From five replicates of each treatment, two replicates in which the RKN density indicated the median were selected for NGS at each measurement timing as representatives, meaning four DNA extracts were used for the analysis. The bacterial OTU data were obtained from the NGS analysis conducted by FASMAC. Some OTUs were observed in only one replicate, suggesting that they might not mainly contribute to RKN suppression. Therefore, we selected OTUs that were present in all of the selected samples of each field soil. Normality and correlations were assessed using the same method as described above.

Generation of the bacterial heatmap. To visualise a change in bacterial community structure caused by the FLN inoculation, a differential test was performed using the DESeq2 (pheatmap) package in R. Since the OTUs obtained from the NGS analysis showed a relative abundance, they were converted to absolute abundance using copy number data. Statistical analysis was conducted for each field separately, using nematode presence or absence as an explanatory factor. OTUs with adjusted P value < 0.05, and both increased ($\log_2$ fold change > 0) and decreased ($\log_2$ fold change < 0), were included for heatmap generation. Among those OTUs, those identified at the genus level were used to generate a heatmap. Each variable was standardised (z-score normalisation), and the heatmap was generated using the pheatmap package. Hierarchical clustering was applied only to a taxon.

RESULTS

Plant growth and RKN infection. Plant growth did not significantly differ between the two treatments in each field soil. In Kanagawa soil, dried shoot weights were 0.958 ± 0.037 and 0.880 ± 0.094, and wet root weights were 3.15 ± 0.240 and 3.19 ± 0.468 g in non-inoculated and inoculated treatments, respectively. In Ibaraki soil, dried shoot weights were 0.528 ± 0.034 and 0.484 ± 0.048, and wet root weights were 3.25 ± 0.312 and 3.09 ± 0.328 g in non-inoculated and inoculated, respectively.

The number of *M. incognita* was significantly lower in the inoculated treatment, both at 0 and 1 months ($F_{1,16}$ = 5.2 and 15.4, P < 0.05 and 0.01, respectively) (two-way ANOVA: Field; NS, Field × RKN; NS). The values in Kanagawa soil were 417,000 (0 month) and 508,000 (1 month) (J2 eq (20 g of fresh soil)$^{-1}$, non-inoculated and inoculated treatments, respectively). The values in Ibaraki soil were 314,000 (0 month) and 265,000 (1 month) (J2 eq (20 g of fresh soil)$^{-1}$, non-inoculated and inoculated treatments, respectively). Furthermore, the suppressions by the inoculated treatment were significant in both soils at 1 month ($F_{7,32}$ = 5.1 and P < 0.001) (one-way ANOVA) (Fig. 1).

Nematode community. A total of 14 genera were observed across the two fields: 8 in Kanagawa and 13 in Ibaraki (Table 1). *Acrobeloides* Cobb, 1928 and *Cephalobus* Bastian, 1865 were observed in both treatments, while many genera were observed in only the inoculated treatments. No omnivorous nematodes were observed in either soil at 0 month, but they were detected in both inoculated treatments at 1 month. In addition, opposite trends were observed between the two field soils. In Kanagawa field, the numbers of all genera increased over time in the inoculated treatment, whereas in Ibaraki, the number of 7 out of 10 genera decreased. All of the calculated indices tended to be higher in soils inoculated with nematodes, and richness was significantly higher in the inoculated treatment except for at 0 month in Kanagawa (Table 2). There was no significant negative correlation between the RKN density and any characteristic of nematode communities of each field. On the other hand, the number of bacterivores showed a significant positive correlation with the RKN density (Kanagawa: R^2 = 0.46 and P < 0.01, Ibaraki: R^2 = 0.21 and P < 0.05), especially the number of *Acrobeloides* (Kanagawa), and *Cephalobus* (Ibaraki) were positively correlated with the RKN density (R^2 = 0.47 and R^2 = 0.59, respectively. P < 0.01).

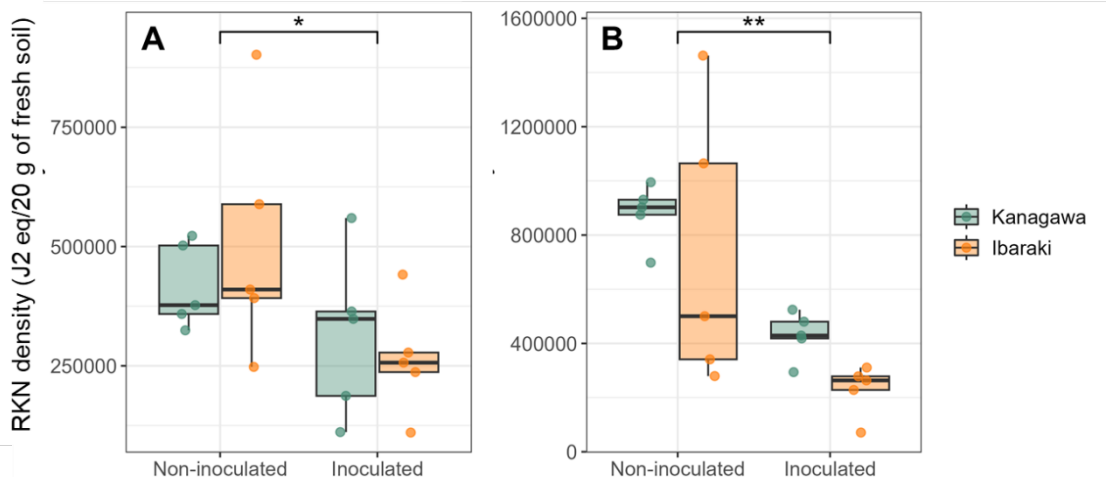

Fig. 1. The density of *Meloidogyne incognita* estimated from Ct values (Watanabe *et al*., 2013) in two soils (Kanagawa and Ibaraki prefectures) after 6 weeks of growth (0 month) (A) and after further 1 month incubation (1 month) (B) . *: $P < 0.05$, **: $P < 0.01$ (Two-way ANOVA: Field; NS, Field × RKN; NS). The values indicate the mean value ± SD (n = 5).

Table 1. The number of nematode genera (individuals/20 g of fresh soil) in the soil under green pepper plants infested by *Meloidogyne incognita* after 6 weeks of growth (0 month) and after further 1 month of incubation (1 month).

Nematode genera	Feeding type	cp	0 month		1 month	
			Non-inoculated	Inoculated	Non-inoculated	Inoculated
Kanagawa						
Mesorhabditis	Bacterivore	1	0	4 ± 8	0	29 ± 65
Acrobeles	Bacterivore	2	0	0	0	8 ± 19
Acrobeloides	Bacterivore	2	40 ± 39	453 ± 26	2290 ± 305	1650 ± 133
Cephalobus	Bacterivore	2	12 ± 26	0	0	0
Eucephalobus	Bacterivore	2	0	0	0	15 ± 33
Aphelenchus	Fungivore	2	0	0	0	7 ± 16
Aphelenchoides	Fungivore	2	0	4 ± 9	0	85 ± 90
Aporcelaimellus	Omnivore	5	0	0	0	7 ± 16
Ibaraki						
Mesorhabditis	Bacterivore	1	0	4 ± 8	0	3 ± 6
Panagrolaimus	Bacterivore	1	0	24 ± 29	0	0
Diploscapter	Bacterivore	1	0	0	0	7 ± 10
Fictor	Bacterivore	1	0	0	0	2 ± 5
Acrobeloides	Bacterivore	2	485 ± 189	721 ± 166	4150 ± 1030	424 ± 71
Plectus	Bacterivore	2	0	6 ± 9	0	0
Cephalobus	Bacterivore	2	73 ± 102	5 ± 7	331 ± 450	20 ±39
Eucephalobus	Bacterivore	2	0	7 ± 17	0	12 ±9
Prismatolaimus	Bacterivore	3	0	7 ± 11	0	3 ± 6
Aphelenchus	Fungivore	2	0	5 ± 8	0	0
Tylenchus	Fungivore	2	0	38 ± 55	22 ± 49	3 ± 6
Aphelenchoides	Fungivore	2	25 ± 48	195 ± 136	491 ± 536	63 ± 16
Aporcelaimellus	Omnivore	5	0	0	0	2 ± 5

The values indicate the mean value ± SD (n = 5).

Table 2. The number of nematodes of different trophic groups (individuals/20 g of fresh soil) and nematological indices in the soil under green pepper plants infested by *Meloidogyne incognita* after 6 weeks of growth (0 month) and after further 1 month of incubation (1 month).

Conditions of pot experiment	0 month		1 month	
Nematological parameters	Non-inoculated	Inoculated	Non-inoculated	Inoculated
Kanagawa				
Bacterivore	52 ± 45	457 ± 32**	2290 ± 305**	1700 ± 100
Fungivore	0 ± 0	4± 9	0 ± 0	93 ± 83
Omnivore	0 ± 0	0 ± 0	0 ± 0	7± 16
SI	0 ± 0	0 ± 0	0 ± 0	2.3 ± 5.2
EI	0 ± 0	3.5 ± 5.6	0 ± 0	9.9 ± 10.7
Shannon index	0.20 ± 0.29	0.068 ± 0.094	0 ± 0	0.31 ± 0.22 *
Richness	1.0 ± 0.71	1.4 ± 0.55	1.0 ± 0	2.6 ± 1.1 *
Bacterivore richness	1.0 ± 1.0	1.2 ± 0.45	1.0 ± 0	1.6 ± 0.89
Ibaraki				
Bacterivore	558 ± 158	774 ± 175	4480 ± 765 **	471 ± 65.7
Fungivore	25 ± 48	238 ± 120 *	512 ± 541	66 ± 20
Omnivore	0 ± 0	0 ± 0	0 ± 0	2 ± 5
SI	0 ± 0	1.6 ± 2.5	0 ± 0	3.4 ± 4.9
EI	3.9 ± 7.2	25.7 ± 8.4 **	8.8 ± 8.7	18.0 ± 7.0
Shannon index	0.38 ± 0.29	0.81 ± 0.21 *	0.48 ± 0.32	0.73 ± 0.17
Richness	2.2 ± 0.45	5.2 ± 0.83 **	2.6 ± 0.89	4.6 ± 0.89 **
Bacterivore richness	1.6 ± 0.55	3.2 ± 1.3 **	1.6 ± 0.55	3.2 ± 1.3 **

The values indicate the mean value ± SD (n = 5). *: $P < 0.05$, **: $P < 0.01$, marked with an asterisk for the significantly different values (t-test).

Bacterial community. The metabarcoding data analyzed in this study were submitted to the DNA Data Bank of Japan (DDBJ) Sequence Read Archive under DRA accession number PRJDB35546 (PSUB041116). Bacterial community structures differed depending on the inoculation of FLN, especially at 0 and 1 months in Ibaraki soil, and at 0 month in Kanagawa soil (Fig. 2). The abundance of some bacterial genera was significantly different between the inoculated and non-inoculated treatments. For example, the copy number of *Conexibacter* Monciardini *et al.,* 2003 and *Candidatus* Udaeobacter Brewer *et al.,* 2016 increased by inoculating FLN, while that of *Gemmatimonas* Zhang *et al.,* 2003 and *Terrabacter* Collins *et al.,* 1989 in Ibaraki soil, and *Dyella* and *Oryzihumus* in Kanagawa soil decreased. Especially, the copy number of *Tumebacillus* Steven *et al.,* 2008 was almost twice as high in the inoculated as in the non-inoculated treatments in Ibaraki soil. The abundance of some bacteria was negatively correlated with the RKN density (Kanagawa soil: *Saccharimonadales* McLean *et al.,* 2020, Ibaraki: *Saccharimonadales, Gemmatimonas*, *Novosphingobium* Takeuchi *et al.,* 2001, *Candidatus* Udaeobacter) (Table 3).

A correlation analysis showed a significant relationship between the copy number of two bacterial genera and two nematode parameters. For example, in Ibaraki soil, the copy number of *Gemmatimonas* was positively correlated with the number of cp1 bacterivores ($R^2 = 0.50$ and $P < 0.05$), but negatively correlated with the number of *Cephalobus* ($R^2 = 0.56$ and $P < 0.05$). On the other hand, no significant correlations were observed in Kanagawa soil.

DISCUSSION

The objective of the experimental setup was to compare RKN suppression between soils with only microbes and with both microbes and nematodes, but a lot of nematodes were observed in the non-inoculated treatment, in which we expected no nematodes. Culture soils for growing green pepper seedlings were autoclaved, and sterilised water was used for all watering in this experiment. Hence, nematodes could not be contaminated through those pathways. Possible reasons were that nematode eggs survived freezing and drying, and flying insects carried nematodes. About the former reason, cp2 nematodes were more tolerant to disturbances than cp1 (Bongers & Bongers, 1998), and only cp2

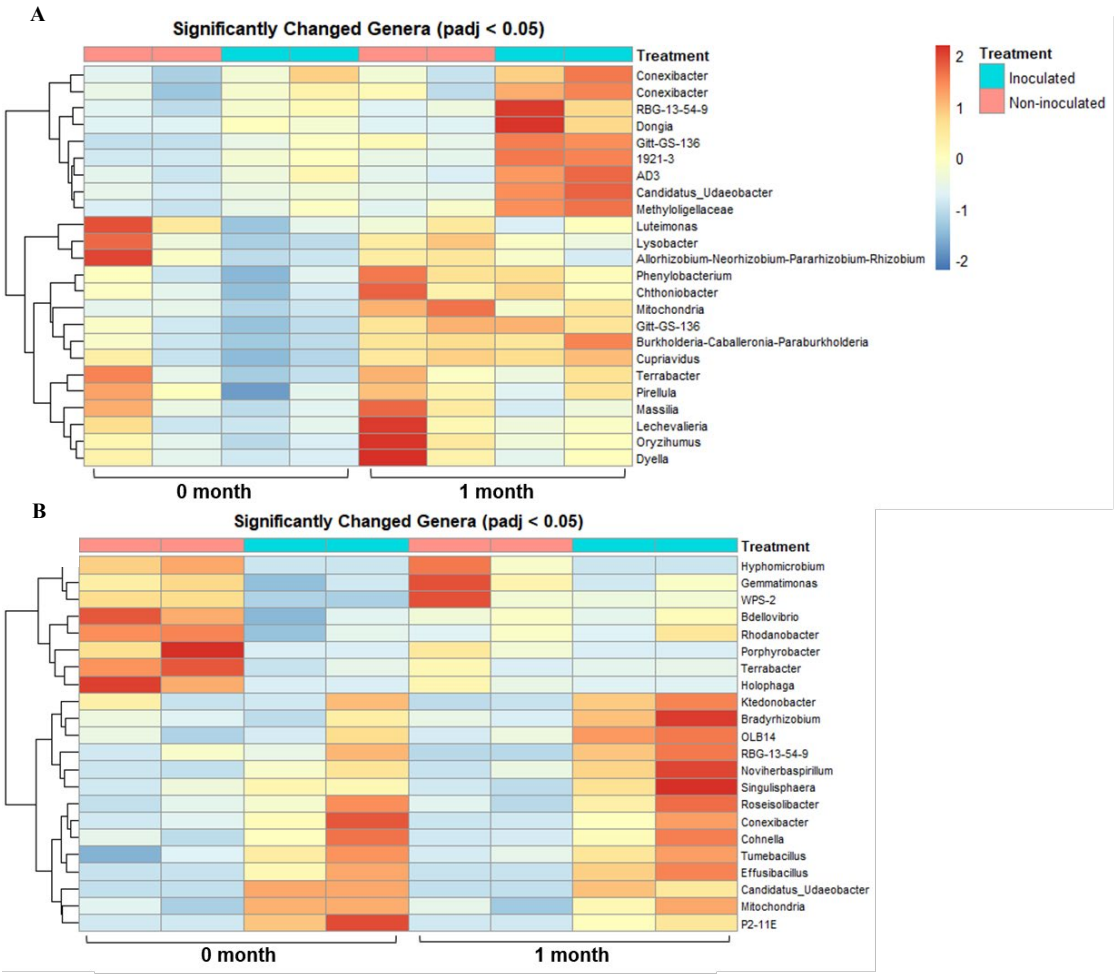

Fig. 2. Heat map of bacterial communities including bacteria whose abundance was significantly changed by the inoculation of free-living nematodes after 6 weeks of growth (0 month) and after further 1 month of incubation (1 month) in Kanagawa (A) and Ibaraki (B). Values represent z-scores calculated to reflect the relative abundance of each genus.

Table 3. Bacterial genera that showed a significant negative correlation with the number of *Meloidogyne incognita.*

Bacterial genus	R	*P* value
Kanagawa		
Saccharimonadales sp.	-0.77	< 0.05
Ibaraki		
Saccharimonadales sp.	-0.85	< 0.01
Gemmatimonas sp.	-0.74	< 0.05
Novosphingobium sp.	-0.71	< 0.05
Candidatus Udaeobacter sp.	-0.75	< 0.05

nematodes were seen in non-inoculated soils through this experiment. Therefore, this supports the possibility that some nematode eggs survived. Regarding the latter, Steel *et al.* (2013) reported that flies carried some nematodes to colonise a compost. Some flies were found in the growth chamber in this study. Carryover by flies could be the case. However, since the FLN communities were different between non-inoculated and inoculated treatments in terms of diversity, we concluded that our experimental setup worked enough to observe the contribution of nematode communities.

The RKN multiplication was suppressed by the FLN inoculation. As many papers have reported that predators and omnivores prey on plant-parasitic nematodes (Sánchez-Moreno & Ferris, 2007;

Bilgrami & Khan, 2022; Obidari *et al*., 2024), omnivorous nematodes were observed in both sites at 1 month, suggesting that they contributed to RKN suppression. In Kanagawa and Ibaraki soils, only one omnivorous genus, *Aporcelaimellus* Heyns, 1965 was seen. One species of this genus was reported to have significant biopesticidal potential on plant-parasitic nematodes (Khan *et al*., 1991). Khan and Kim (2005) reported that the application of eight *M. fortidens* per 20 g of soil seemed appropriate for *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949 management. At 1 month, two to seven individuals (20 g soil)$^{-1}$ of *Aporcelaimellus* were observed, meaning that the omnivores might prey on *M. incognita* and suppress their infection. However, in both soils, the omnivores were found in only one of five replicates, implying that they were not widely distributed. These results suggested that the contribution of omnivorous nematodes was not the main mechanism of RKN suppression in this study. Furthermore, the RKN multiplication was suppressed in the inoculated soils at the 0 month measurement, but no omnivores and predators were found. It suggested that microbial-feeding nematodes mainly contributed to the suppression at least for 0 month of analysis. Some previous papers reported that soils, containing predators, did not always show PPN suppression (Min & Toyota, 2013; Steel & Ferris, 2016; Timper *et al.*, 2021), although they did not mention the role of microbial-feeding nematodes. This is, therefore, the first paper to show a possible contribution of microbial-feeding nematodes to RKN suppression, and it supported our second hypothesis that FLN communities could contribute to RKN suppression in the absence of predators or omnivores.

Since most microbial-feeding nematodes belonged to bacterivores, we discuss mainly bacterivorous nematodes here. The number of bacterivores positively correlated with the RKN density in both sites, although no nematode parameters showed a negative correlation with the RKN density, contrary to our expectation. Regarding the mechanisms by which bacterivores could contribute to PPN suppression, it was suggested that microbial-feeding nematodes could contribute to the suppression of plant-parasitic nematodes indirectly, by altering the microbial community to increase the abundance of antagonists, and/or by transporting antagonistic microbes closer to PPN or plant-beneficial rhizobiota closer to the roots (Topalović & Geisen, 2023). Bacterivores have been reported to affect microbial communities or activities that improve soil function, such as phosphate solubilisation, IAA production, and disease suppression (Jiang *et al*., 2017, 2023b; Zheng *et al.*, 2022; Xu *et al*., 2024). Our results showed that bacterial communities changed clearly by inoculation of FLN, supporting the idea that bacterivores contributed to the RKN suppression by changing bacterial communities. Some bacterial genera showed a significantly negative correlation with the RKN density, and among them, *Candidatus* Udaeobacter were abundant in the inoculated treatment. Although no previous reports found their antagonistic properties on RKN, those bacteria could contribute to the RKN suppression. In addition, *Tumebacillus*, which was found twice as frequently in the inoculated compared with non-inoculated treatments in Ibaraki soil, was reported to produce antibacterial compounds (Kikuchi *et al*., 2023). This genus was expected to be a beneficial microbe that could produce natural products, like *Streptomyces hydrogenans* Lindner *et al.*, 1958 which had a strong nematicidal potential (Kaur *et al.*, 2016). *Tumebacillus* could contribute to the RKN suppression through the same mechanism.

Among bacterivores, especially *Acrobeloides* (in Kanagawa soil) and *Cephalobus* (in Ibaraki soil) had a significant positive correlation with the RKN density. These nematodes were observed in both non-inoculated and inoculated treatments, and their abundance was greater in the inoculated treatment, especially at 1 month. On the other hand, other cp2 bacterivores were found only in inoculated soils, implying that even among cp2 group, they played different roles depending on the genus. Furthermore, no cp1 bacterivores were found in the non-inoculated treatment. This might be because cp1 nematodes are less tolerant to stresses, such as resource limitation and dry conditions, than cp2 (Bongers & Bongers, 1998; Ferris *et al*., 2001), and they could not survive defaunation or be transported from out of the pots. In addition, the number of cp1 bacterivores was positively correlated with the copy number of *Gemmatimonas* sp., which had a negative correlation with the RKN density, suggesting that among bacterivores, cp1 nematodes might positively contribute to RKN suppression. Previous papers reported that cp1 bacterivores had more impact on plant growth and nutrition (Brondani *et al*., 2022; Jiang *et al*., 2023a). The reason for this remains unclear, but this might be because nematodes have different life strategies, such as food preference (Liu *et al.*, 2017). Recent research suggested that nematode morpho-anatomical characteristics could better explain the nematode contribution to soil functions (N and P supplying capacity to plants) than cp classes and species identity (Brondani *et al*., 2022). Therefore, it is necessary to focus on which nematode genera play an important role and the

mechanisms behind this by focusing on their biology.

CONCLUSIONS

This study revealed that soil FLN communities contributed to the suppression of *M. incognita* multiplication. The multiplication was suppressed by an inoculation of FLN, and omnivorous and predatory nematodes were rarely observed in pots, suggesting that these nematodes were not always essential for the suppression, while microbial-feeding nematodes played an important role. Among microbial-feeding nematodes, cp1 bacterivores were likely to contribute to the RKN suppression by influencing the bacterial community. Some bacterial genera increased in abundance in soils inoculated with FLN, and one of them was estimated to have an antagonistic potential. However, it remains unclear which nematode genera contributed mainly to the RKN suppression and the mechanisms behind it. Previous research suggested that nematode morphological traits were key to explaining nematode roles, hence, further study is needed to ascertain the biological reason for the contribution of bacterivores to the suppression *via* microbial mediation

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R. Kato, S. Van Nguyen, R. N. Perry and K. Toyota. Вклад сообществ свободноживущих почвенных нематод в подавление *Meloidogyne incognita* на органических полях.

Резюме. Хищные и всеядные нематоды известны как потенциальные агенты биологического контроля корневых галловых нематод. Однако остаётся неясным, вносят ли сообщества свободноживущих нематод, включая нематод, питающихся микробами, вклад в подавление популяций корневых галловых нематод. Для оценки их роли был проведён вегетационный эксперимент в горшках с использованием двух вариантов почвы, содержащей 1) только микробные сообщества или 2) микробные сообщества и свободноживущих нематод. После шести недель выращивания растений перца (*Capsicum annuum* L.) был проведён анализ уровня заражения нематодой *Meloidogyne incognita* и структуры бактериальных сообществ с применением секвенирования нового поколения (СНП). Аналогичный анализ был проведен повторно после дополнительной месячной инкубации почвы. Результаты показали, что плотность популяции *M. incognita* была значимо ниже в почвах с присутствием свободноживущих нематод по сравнению с вариантом, содержащим только микроорганизмов. Кроме того, подавление размножения *M. incognita* наблюдалось даже в отсутствии хищных и всеядных нематод, что подчёркивает важную роль нематод, питающихся микробами, в этом процессе. Анализ данных СНП выявил, что некоторые роды бактерий демонстрировали значительную отрицательную корреляцию с плотностью популяции *M. incognita*, что позволяет предположить, что бактериотрофы модифицировали структуру бактериальных сообществ, способствуя микробному подавлению *M. incognita*.