[Original Article]

Pratylenchus penetrans population dynamics and cabbage growth under the continuous application of sawdust cattle manure compost for year-round cabbage cropping¹

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Pratylenchus penetrans is a major nematode pest of vegetable crops in Japan. We investigated *P. penetrans* population dynamics and cabbage growth under the year-round application of sawdust cattle manure compost for continuous cabbage cropping (2002-2009) at Tsukuba, Japan. Cabbages were cultivated twice a year, during spring and autumn. Chemical fertilizer (CF), manure compost (MC) + 1/5CF, CF + 3/5MC + crop residue (CR), a triple amount of MC + 1/5CF, and 3MC + CR were applied to the field before transplanting the cabbages. Both the initial population (P_i) and final population (P_f) of *P. penetrans* were significantly high in CF, low in 3MC + 1/5CF and 3MC + CR, and average in MC + 1/5CF and CF + 3/5MC + CR in 2003-2005 and 2008-2009. Both P_f/P_i during cultivation and the decreased rate of P_i/P_f after cultivation showed no clear tendencies among compost treatments. In this study, host plant growth did not appear to be markedly restricted by the high densities of *P. penetrans*. By applying Seinhorst's formula on the P_i and P_f of *P. penetrans*, we obtained 2 curves for CF and 3MC + CR, with 16.45 and 4.54 representing "equilibrium density", respectively. Pot experiment results supported the field experiment results, whereby the number of *P. penetrans* decreased as the quantity of MC increased (from 0 to 3%w/v). On the basis of these results, we concluded that manure compost application could have a suppressive effect on *P. penetrans* population dynamics. Nematol. Res. 45(1), 1-11 (2015).

Key words: *Brassica oleracea* L. var. *capitata* L., root lesion nematode, amendment, continuous cropping

INTRODUCTION

Pratylenchus penetrans Cobb is one of the most important plant-parasitic nematodes in Japan and causes severe damage to vegetables, which reduces crop quality and hence marketability. Vegetable crops affected by this nematode include the Japanese radish (*Raphanus sativus* L. var. *longipinnatus*) (Chikaoka *et al.*, 1971), carrot (*Daucus carota* L.) (Hagiya *et al.*, 1982), and edible burdock (*Arctium lappa* L.) (Kajino, 1986). Organic soil supplements have long been utilized to control nematode damage (Nakasono, 1992). As a result, many studies have

focused on evaluating how organic soil supplements suppress the effects of parasitic nematodes, including *P. penetrans* (Nakasono, 1992; Ohbayashi, 1989; Oka, 2010; Uragami *et al.*, 2009). Although compost application has had variable effects on the populations of plant-parasitic nematodes, the population dynamics of *P. penetrans* under compost application remain unresolved (Nakasono, 1992; Oka, 2010).

In 2002, a continuous compost application experiment was initiated in a cabbage (*Brassica oleracea* L. var. *capitata* L.) field at Tsukuba Vegetable Research Station, National Agriculture and Food Research Organization (NARO) Institute of Vegetable and Tea Science, Japan. Cabbage is known to be a good host of *P. penetrans*, with high nematode population densities only minimally reducing cabbage growth (Miller, 1978; Olthof and Potter, 1973). In this study, we aimed to characterize the population dynamics of *P. penetrans* under continuous cabbage cropping conditions with the continuous application of different volumes of sawdust cattle manure compost.

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MATERIALS AND METHODS

Field experiments:

Field experiments were conducted in an experimental field located at the NARO Institute of Livestock and Grassland Science, Tsukuba, Japan (36° 02' N, 140° 11' E; 21 m above sea level). The soil type is Andosol, containing 49% sand, 46% silt, and 5% clay. Soil pH and electrical conductivity (EC) immediately prior to the experiment were 7 and 17.5 mS/m, respectively. This experimental field was naturally infested with *P. penetrans* before cabbage cultivation. *P. penetrans* was identified on the basis of morphological characteristics (Mizukubo, 2004).

In September 2002, a compost application experiment comprising 5 treatments (Table 1) was established in a randomized complete block design with 3 replications. Individual plots of 5×5 m were delineated, each with lanes of 2 m in width. Before 2002, rye (*Secale cereale* L.) or dent corn (*Zea mays* L. var. *indentata*) had been cultivated in the field for approximately 15 years, with 117 kg/ha nitrogen (N) of chemical fertilizer and 21 t/ha of compost being applied each year.

The basic amount of applied commercial sawdust cattle manure compost (MC) included approximately 250 kg/ha N, based on component analysis results (Table 2). Sawdust cattle manure compost was incorporated twice a year to 15-cm depth by using a rotary tiller, approximately 1 month before the cabbages were transplanted. Chemical fertilizer (CF; Kasei No. 8, Ryoto Hiryo Co. Ltd, Japan; N: 8; P₂O₅: 8; K₂O: 8) was applied immediately before transplanting each cabbage crop. Crop residue (CR, *i.e.*, outer leaves and roots) was incorporated into the field after harvesting the cabbage heads. In all other treatments, crop residue was removed from the field. Cabbage seedlings (cultivar “Kinkei 201” for spring cultivation and “Shutoku” or “Shutoku SP” for autumn cultivation) were transplanted to the experimental field twice a year (spring and autumn), with

continuous cultivation from September 2002 to September 2009. For the spring cultivation, cabbage seedlings were transplanted at the beginning of April and harvested in approximately late June. For the autumn cultivation, cabbages were transplanted at the beginning of September and harvested in the middle of December. After transplanting, cabbages were irrigated once by hand with a hose to ensure they survived. Cabbages were spaced at 0.4-m intervals within rows and at 0.6-m intervals between rows (7 rows per plot) with 77 plants per plot. During each cultivation period, no supplemental fertilization was applied. Pesticides, fungicides, and herbicides were applied when needed; however, chemical soil disinfectants were not applied.

Data collection:

The total carbon and nitrogen contents of the soils, which were collected just after every cabbage cultivation, were estimated using a nitrogen and carbon analyzer (Sumigraph NC-22F; Sumika Chemical Analysis Service Ltd., Tokyo, Japan). Soil pH and EC were also analyzed using a pH meter (F-14; HORIBA, Kyoto, Japan).

At harvest, 4 uniformly grown healthy cabbage plants were cut from the ground of each plot and individually weighed, with the average of the 4 plants being used for data analysis.

From May 2003 to June 2005 and from September 2008 to December 2009, the population levels of *P. penetrans* and free-living nematodes in the soil were assessed immediately before transplanting the cabbages

Table 2. Compositions of the compost used in the experiment

Year	N (%)	P ₂ O ₅ (%)	K ₂ O (%)	C/N ratio
2003	2.5	-	-	17
2006	2.4	2.1	2.7	16.6
2009	3.7	2.3	3.5	11

The commercial manure compost was analyzed before each application

Table 1. Amount of organic materials and nitrogen incorporated into the plot per cabbage planting for 5 different treatments

Treatment	Treatment abbreviation	Organic material (tFW/ha)	Nitrogen (kg/ha)
Chemical fertilizer	CF	0	250
Manure compost + 1/5 of chemical fertilizer	MC+ 1/5CF	32	250 + 50
Chemical fertilizer + 3/5 of manure compost + crop residue	CF + 3/5MC + CR	20 + 33	250 + 150 + 80
Triple amount of manure compost + 1/5 of chemical fertilizer	3MC + 1/5CF	97	750 + 50
Triple amount of manure compost + crop residue	3MC + CR	97 + 33	750 + 80

(P_i : initial population) and just after harvest (P_f : final population). The first 3 years and the last 2 years in the 7 years of cabbage cultivation with compost treatment were assessed for nematode densities. For MC + 1/5CF and 3MC + 1/5CF, the assessment began from July 2003, *i.e.*, P_f in spring 2003 (2003S). In 2009, the nematode population assessments were also performed on January 19, February 19, March 19, August 14, and October 21. The population assessment for each plot in each sampling period was based on a composite sample of 9 soil subsamples collected from a depth of 5-15 cm, totaling approximately 1 kg wet weight. Soil samples were passed through a sieve with 2-mm openings to remove the cabbage roots and were gently hand-mixed. Nematodes were extracted from two 20-g subsamples, using the Baermann funnel method, for 72 hr at room temperature. The number of nematodes was counted using a biological microscope (E-600; Nikon, Tokyo, Japan), and was calculated as the average of the 2 subsamples. Seasonal multiplication rates were calculated as the P_f/P_i of a cultivation period, and the rates of decline during fallow periods were calculated as P_i/P_f by using the P_f of a cultivation period and the P_i of the following period.

Data for hourly soil temperatures at 10-cm depth were obtained from the website for the National Institute for Agro-Environmental Sciences. These measurements were made using a New Automated Weather System located approximately 1 km northwest of the experimental field (36° 01' N, 140° 07' E; 25 m above sea level). The effective accumulative temperatures (degree-days) for *P. penetrans* development during each cultivation period were calculated using developmental zero degree of 4.2°C (Mizukubo and Adachi, 1997).

Pot experiments:

Soil was collected from CF plots just after the cabbage harvest on July 20, 2010, and passed through a 2-mm sieve. Eleven kilograms of the soil was then placed into a 15-l plastic pot (surface area, 500 cm²; height, 30 cm). Zero percent, 1%, and 3% (w/v) (0, 30, and 90 t/ha, respectively) of commercial sawdust cattle manure compost used in the field experiment was incorporated into the pot. The average soil moisture content was 0.52 (g/g). Each treatment contained 10 pots, which were maintained in a glass house. Average, maximum, and minimum soil temperatures at 5-cm depth during the experiment were 28, 38, and 24 °C, respectively. The water content of the pots was kept uniform by weighing and refilling the pots with water to the original weight once a week. Nematodes were extracted at 45 and 76 days

after treatment. The population assessment for each pot was based on two 20-g soil samples. Nematodes were extracted from each sample by using the Baermann funnel method, with counts following the same technique as that used for the field experiments.

Statistical analysis and model formula analysis:

Statistical analysis was performed using Statistica 6.0 (Statsoft Japan). We conducted analysis of variance (ANOVA) and Tukey's HSD test on cabbage weight; soil properties; P_i , P_f , P_i/P_f , and P_f/P_i ; and nematode populations in pots. P_i , P_f , P_i/P_f , P_f/P_i and nematode populations in pots were analyzed after Box-Cox transformation because of the lack of normality and homoscedasticity.

The model used to assess the relationship between initial and final nematode densities, developed by Seinhorst (1966) for migratory nematodes (such as *Pratylenchus* spp.), is as follows:

$$P_f = \frac{aEP_i}{(a-1)P_i + E}$$

where a = rate of multiplication at $P_i \rightarrow 0$ and E = equilibrium density

We applied this model to the regression analysis of *P. penetrans* population data from the field experiments, to quantify how compost application inhibited an increase in nematode population size (24 sets of P_i and P_f for CF and CF + 3/5MC + CR, 21 sets for MC + 1/5CF and 3MC + CR, and 18 sets for 3MC + 1/5CF, respectively). Three samples of 3MC + CR with P_f/P_i values below 1 were removed as aberrant values. Model formula analysis was performed with DataFit 9 software (Oakdale Engineering, Oakdale, U.S.A.).

RESULTS

Cabbage growth and soil properties:

Until the 2004 spring cropping season, cabbage growth (based on the weight of the above-ground part of the plant) was greater in CF and CF + 3/5MC + CR than in MC + 1/5CF, 3MC + 1/5CF, and 3MC + CR (Table 3). After the 2004 autumn cropping season, the growth of cabbages in plots with 3MC was similar to that of cabbages in plots with CF. Cabbage growth in the CF + 3/5MC + CR plots remained consistently high until the end of the experiments in 2009. According to the factorial analysis on the cabbage above-ground weights, there were no significant differences between the spring and autumn plantings in 2003 and 2004 ($P = 0.765$) and in 2009 (0.054), respectively.

Total nitrogen and total carbon content of the soil for

all treatments, except CF, increased with time. This increase was noticeably rapid for 3MC + 1/5CF and 3MC + CR (Table 4). The soil pH of CF, CF + 3/5MC + CR, and MC + 1/5CF decreased with time, whereas that for the other compost types changed minimally. The soil EC of CF + 3/5MC + CR remained consistently high for all other plots.

Nematode population:

The number of *P. penetrans* on May 23, 2003, in uncultivated areas adjacent to the experimental plot was $2.80 \pm 0.73/\text{g}$ soil ($n = 3$).

When we performed ANOVA on *Pi* and *Pf* of *P. penetrans* in two parts (2003-2005 and 2008-2009), there were significant differences among treatments ($P < 0.001$ for all ANOVA). Both *Pi* and *Pf* of *P. penetrans* were high in CF and low in 3MC + 1/5CF and 3MC + CR in 2003-2005 and 2008-2009 (Table 5). *Pi* of *P. penetrans* in CF plots showed the highest mean value among all treatments, except in autumn 2009 (2009A) (Table 5). *Pi* of *P. penetrans* in 3MC + 1/5CF and 3MC + CR showed the lowest mean values among all treatments. The ranges of *Pi* for CF, CF + 3/5MC + CR, MC + 1/5CF, 3MC + 1/5CF, and 3MC + CR were 1.77-9.32, 0.46-3.80, 1.25-

Table 3. Above-ground weight (kg) of cabbage plants (*i.e.*, excluding the roots) in spring (S) and autumn (A) cabbage cultivation during the 5 trial years in a *Pratylenchus penetrans*-infested field

Treatment	2003S	2003A	2004S	2004A	2005S	2008A	2009S	2009A
CF	2.63 a	2.26 ab	2.73 a	1.93 ab	2.75 ab	2.24 ab	2.88 ab	2.63 b
MC + 1/5CF	1.91 b	1.55 c	1.34 b	1.69 b	1.82 c	1.53 c	2.33 b	2.00 c
CF + 3/5MC + CR	2.61 a	2.33 ab	2.87 a	1.97 ab	3.19 a	2.54 a	3.27 a	3.09 a
3MC + 1/5CF	1.94 b	2.02 b	1.48 b	2.11 a	2.47 bc	2.05 ab	2.97 ab	2.97 ab
3MC + CR	1.34 c	1.63 c	0.84 b	1.91 ab	2.50 bc	1.86 bc	2.73 ab	2.69 b

Values represent the means of 3 replicates.

Means followed by the same letter in each column were not significantly different according to Tukey's HSD test at the 5% level.

Chemical fertilizer (CF), manure compost (MC), and crop residue (CR), either separately or in combination, were applied before transplanting the cabbages.

Table 4. Soil properties of the experimental plots during continuous cabbage cultivation in plots treated with organic materials

Date	Treatment	Total-N (g/kg)	Total-C (g/kg)	pH	EC (mS/m)
2003/8/12	CF	3.2 a	35.1 a	6.5 d	25 cdef
	MC + 1/5CF	3.7 abcd	40.2 abc	7.2 gh	16 ab
	CF + 3/5MC + CR	3.6 abc	38.0 ab	6.8 e	34 gh
	3MC + 1/5CF	4.5 def	50.6 ef	7.2 gh	22 bcdef
	3MC + CR	5.0 ef	53.9 f	7.2 gh	28 fgh
2006/7/24	CF	3.3 ab	35.6 a	5.3 b	19 abcde
	MC + 1/5CF	4.1 bcde	42.8 bcd	6.9 ef	13 a
	CF + 3/5MC + CR	4.4 cdef	45.6 cde	6.1 c	25 def
	3MC + 1/5CF	6.4 g	66.5 g	7.2 gh	17 abc
	3MC + CR	6.3 g	65.0 g	7.4 h	18 abcd
2009/7/28	CF	3.4 ab	36.2 ab	4.6 a	26 efg
	MC + 1/5CF	5.2 f	50.4 ef	6.7 de	14 ab
	CF + 3/5MC + CR	4.9 ef	48.2 def	5.2 b	45 i
	3MC + 1/5CF	8.8 h	77.6 h	7.1 fg	20 abcde
	3MC + CR	9.1 h	86.0 i	7.1 fgh	35 h

Values represent the means of 3 replicates.

Means followed by the same letter in each column were not significantly different according to Tukey's HSD test at the 5% level.

Chemical fertilizer (CF), manure compost (MC), and crop residue (CR), either separately or in combination, were applied before transplanting the cabbages.

Table 5. The initial population (P_i), the final population (P_f), and the multiplication rate (P_f/P_i) of *Pratylenchus penetrans* in plots treated with organic materials during spring (S) and autumn (A) cabbage cultivation in each year

	2003S			2003A			2004S		
	P_i^1	P_f^1	P_f/P_i	P_i	P_f	P_f/P_i	P_i	P_f	P_f/P_i
CF	6.68 a	11.08 a	2.02 a	3.20 a	10.11 a	3.08 a	4.47 a	28.24 a	7.50 ab
MC + 1/5CF	-	4.63 ab	-	2.10 ab	5.26 ab	2.65 a	3.47 a	11.98 b	6.50 ab
CF + 3/5MC + CR	3.18 ab	1.69 bc	0.79 a	0.73 bc	2.81 ab	4.69 a	0.68 a	7.0 b	12.98 a
3MC + 1/5CF	-	1.05 c	-	0.39 c	3.40 ab	7.97 a	1.10 a	2.40 c	2.54 ab
3MC + CR	0.21 b	0.93 c	6.26 a	0.52 c	1.81 b	3.75 a	2.11 a	1.08 c	0.58 b

	2004A			2005S		
	P_i	P_f	P_f/P_i	P_i	P_f	P_f/P_i
CF	1.77 a	12.16 a	6.96 a	9.32 a	18.24 a	2.01 a
MC + 1/5CF	1.25 ab	9.98 a	8.62 a	3.55 b	8.34 ab	2.34 a
CF + 3/5MC + CR	0.46 ab	3.73 b	10.37 a	2.63 b	13.58 a	5.05 a
3MC + 1/5CF	0.52 ab	2.14 b	6.93 a	1.53 c	2.23 bc	1.40 a
3MC + CR	0.14 b	0.48 c	8.10 a	1.00 c	1.32 c	1.35 a

	2008A			2009S			2009A		
	P_i	P_f	P_f/P_i	P_i	P_f	P_f/P_i	P_i	P_f	P_f/P_i
CF	3.74 a	7.89 a	2.15 a	7.12 a	15.77 a	2.44 a	2.28 ab	4.89 a	2.15 b
MC + 1/5CF	1.63 bc	4.28 ab	2.57 a	4.26 a	7.88 ab	1.66 a	3.38 a	6.49 a	1.35 b
CF + 3/5MC + CR	2.37 ab	4.23 ab	1.85 a	3.80 a	12.69 a	3.58 a	0.89 b	4.67 a	7.44 a
3MC + 1/5CF	0.75 c	1.81 bc	2.65 a	1.98 a	3.81 b	1.96 a	0.82 b	2.08 b	2.82 b
3MC + CR	0.53 c	1.60 c	3.35 a	2.48 a	3.48 b	1.56 a	0.77 b	2.33 b	3.33 b

¹ P_i = initial population and P_f = final population of *P. penetrans* in each cultivation period per g soil extracted from 20 g soil (Baermann funnel method).

Cabbage plants were cultivated twice a year in spring and autumn.

Values represent the means of 3 replicates.

P_i , P_f and P_f/P_i were analyzed after Box-Cox transformation to reduce non-normality.

Means followed by the same letter in each column were not significantly different according to Tukey's HSD test at the 5% level.

Chemical fertilizer (CF), manure compost (MC), and crop residue (CR), either separately or in combination, were applied before transplanting the cabbages.

4.26, 0.39-1.98, and 0.14-2.48, respectively. All the mean values of P_f/P_i , except CF + 3/5MC + CR in 2003S and 3MC + CR in 2004S, were > 1, showing that the *P. penetrans* population increased during cabbage cultivation. P_f/P_i during the 8 cultivation seasons ranged from 0.58 to 12.98, with no statistical trend among treatments (ANOVA, $P > 0.05$, df: 4, MS: 0.709, F -value: 1.088).

Comparison of the mean P_f values among treatments showed that the P_f values of CF remained the highest until the spring cultivation in 2009 (Table 5). The ranges of P_f for CF, MC + 1/5CF, CF + 3/5MC + CR, 3MC + 1/5CF, and 3MC + CR were 4.89-28.24, 4.28-11.98, 1.69-13.58, 1.05-3.81, and 0.48-3.48, respectively. All mean P_i/P_f values, except 3MC + CR in 2003A and MC + 1/5CF in 2008A, were < 1, indicating that the *P. penetrans* population size decreased after cabbage cultivation (Table 6). P_i/P_f ranged from 0.07 to 1.59, with no statistical trend among treatments (ANOVA, $P > 0.05$, df:

4, MS: 0.760, F -value: 2.082).

The populations of *P. penetrans* increased during cabbage cultivation and decreased after the harvest, as 95% of the P_f/P_i values were more than 1 and 90% of the P_i/P_f values were less than 1 (Table 5). These tendencies were not clear in free-living nematodes, especially during autumn cultivation when 60% of the P_f/P_i values of 20 treatments were less than 1 (Table 7).

We also performed ANOVA on the P_i and P_f of free-living nematodes (*i.e.*, nematodes other than plant-feeding species) in two parts (2003-2005 and 2008-2009) and found that there were clear differences among treatments ($P < 0.001$ for all ANOVA, data not shown). Both the P_i and P_f of free-living nematodes were high in 3MC + 1/5CF and 3MC + CR and low in CF in 2003-2005 and in 2008-2009 (Table 7). In CF + 3/5MC + CR and MC + 1/5CF, the mean P_i and P_f values were higher than those of CF. The mean P_i and P_f values of free-living nematodes in the treatments tended to reflect the

Table 6. The rate of population decline (Pi/Pf) of *Pratylenchus penetrans* in plots treated with organic materials after spring (S) and autumn (A) cabbage cultivation in each year.

Treatment	2003S	2003A	2004S	2004A
CF	0.30 a	0.51 ab	0.63 a	0.83 ab
MC + 1/5CF	0.45 a	0.68 ab	0.12 a	0.37 b
CF + 3/5MC + CR	0.47 a	0.23 b	0.61 a	0.29 b
3MC + 1/5CF	0.56 a	0.43 ab	0.24 a	0.80 ab
3MC + CR	0.67 a	1.59 a	0.13 a	2.47 a

Treatment	2008A	2009S
CF	0.85 a	0.15 bc
MC + 1/5CF	1.22 a	0.52 a
CF + 3/5MC + CR	0.79 a	0.07 c
3MC + 1/5CF	0.64 a	0.20 abc
3MC + CR	0.51 a	0.23 ab

Pi = initial population of the following cultivation and Pf = final population of *P. penetrans* in each cultivation period per g soil extracted from 20 g soil (Baermann funnel method).

Cabbage plants were cultivated twice a year in spring and autumn.

Values represent the means of 3 replicates.

Pi/Pf were analyzed after Box-Cox transformation to reduce non-normality.

Means followed by the same letter in each column were not significantly different according to Tukey's HSD test at the 5% level.

Chemical fertilizer (CF), manure compost (MC), and crop residue (CR), either separately or in combination, were treated before transplanting the cabbages.

amount of compost applied. Pf/Pi of free-living nematodes ranged from 0.37 to 6.32, with no statistical trend among treatments (ANOVA, $P > 0.05$ df: 4, MS: 0.387, F -value: 2.027).

When we performed ANOVA on the Pi and Pf of *P. penetrans* and free-living nematodes and analyzed the effects of cultivated seasons, there were significant differences between spring and autumn cultivations ($P < 0.001$ in the Pi of *P. penetrans*, Pf of *P. penetrans* in 2008-2009, and Pf of free-living nematodes; $P < 0.05$ in the Pi of free-living nematodes in 2003-2005), except for the Pf of *P. penetrans* in 2003-2005 (ANOVA, $P > 0.05$, df: 1, MS: 0.041, F -value: 0.093) and Pi of free-living nematodes in 2008-2009 (ANOVA, $P > 0.05$, df: 1, MS: 0.020, F -value: 0.001, Table 8). The Pi and Pf of *P. penetrans* and Pf of free-living nematodes were higher during spring cultivation than during autumn cultivation (Table 8). The mean Pi/Pf of spring cultivation was 0.357 ($n = 15$) and that of autumn cultivation was 0.729 ($n = 13$), with statistical difference (ANOVA, $P < 0.05$, df: 1, MS: 0.967, F -value: 11.367).

Table 7. The initial population (Pi), the final population (Pf), and the multiplication rate (Pf/Pi) of free-living nematodes in plots treated with organic materials for spring (S) and autumn (A) cabbage cultivation in each year

	2003S			2003A			2004S		
	Pi^1	Pf^1	Pf/Pi	Pi	Pf	Pf/Pi	Pi	Pf	Pf/Pi
CF	4.74 a	10.88 a	2.37 a	5.40 a	2.76 a	0.59 a	2.55 a	8.12 a	3.77 a
MC + 1/5CF		37.45 ab		52.33 b	35.76 ab	0.67 a	25.17 b	135.00 b	5.40 a
CF + 3/5MC + CR	45.33 b	148.37 c	3.75 a	53.58 b	46.69 ab	0.95 a	33.70 bc	132.67 b	3.95 a
3MC + 1/5CF		54.37 ab		69.23 bc	75.48 b	1.13 a	39.93 c	253.02 c	6.32 a
3MC + CR	64.54 b	80.97 b	1.29 a	100.72 c	75.29 b	0.76 a	65.18 d	164.03 bc	2.54 a

	2004A			2005S		
	Pi	Pf	Pf/Pi	Pi	Pf	Pf/Pi
CF	5.52 a	3.41 a	0.43 a	1.97 a	9.98 a	5.09 c
MC + 1/5CF	51.67 bc	46.97 abc	0.53 a	29.60 b	47.22 ab	1.40 a
CF + 3/5MC + CR	49.49 b	30.30 abc	0.37 a	40.21 b	72.31 abc	2.90 abc
3MC + 1/5CF	79.33 c	71.48 bc	0.51 a	41.43 b	140.53 c	3.55 bc
3MC + CR	71.67 bc	86.52 c	0.70 a	60.86 c	114.86 bc	1.80 ab

	2008A			2009S			2009A		
	Pi	Pf	Pf/Pi	Pi	Pf	Pf/Pi	Pi	Pf	Pf/Pi
CF	6.03 a	10.14 a	1.67 ab	7.37 a	16.11 a	2.43 ab	14.45 a	10.10 a	0.50 a
MC + 1/5CF	31.34 ab	36.30 ab	1.53 ab	37.00 ab	56.79 ab	1.69 ab	40.46 ab	39.50 b	0.54 a
CF + 3/5MC + CR	36.59 b	44.11 b	1.00 a	25.93 ab	101.44 ab	3.20 b	45.50 b	40.50 b	0.49 a
3MC + 1/5CF	49.80 b	99.91 c	2.06 ab	70.83 bc	108.83 b	1.33 a	49.96 b	80.39 c	1.52 a
3MC + CR	37.85 b	87.85 c	2.47 b	85.74 c	137.19 b	1.90 ab	63.06 b	114.92 d	1.71 a

¹ Pi = initial population and Pf = final population of free-living nematodes in each cultivation period per g soil extracted from 20 g soil (Baermann funnel method).

Cabbage plants were cultivated twice a year in spring and autumn.

Values represent the means of 3 replicates.

Pi , Pf and Pf/Pi were analyzed after Box-Cox transformation to reduce non-normality.

Means of Pi , Pf and Pf/Pi in each column followed by the same letter were not significantly different according to Tukey's HSD test at the 5% level.

Chemical fertilizer (CF), manure compost (MC), and crop residue (CR), either separately or in combination, were applied before transplanting the cabbages.

Table 8. The initial population (P_i) and the final population (P_f) of *Pratylenchus penetrans* and free-living nematodes during spring and autumn cabbage cultivation in 2003–2005 and in 2008–2009

Cultivated season	Cultivar	<i>P. penetrans</i>				Free-living nematodes			
		2003–2005		2008–2009		2003–2005		2008–2009	
		P_i^1	P_f^1	P_i	P_f	P_i	P_f	P_i	P_f
Spring	Kinkei 201	3.40	7.59	3.93	8.72	40.21	93.98	45.38	84.07
Autumn	Shutoku or Shutoku SP	1.11	5.19	1.71	4.03	53.89	47.47	37.50	56.37
ANOVA ²		**	ns	**	**	**	**	ns	**

¹ P_i = initial population and P_f = final population of *P. penetrans* in each cultivation period per g soil extracted from 20 g soil (Baermann funnel method). Cabbage plants were cultivated twice a year in spring and autumn.

P_i and P_f were analyzed after Box-Cox transformation to reduce non-normality.

²Three-factor analysis of variance (ANOVA) results of Table 5. **: $P < 0.01$

Table 9. Number of *Pratylenchus penetrans* and free-living nematodes per g soil amended with compost in the pot experiment

		Manure compost % (w/v)	7/20/2010 (0 day)	9/3/2010 (45 days)	10/4/2010 (76 days)
<i>P. penetrans</i>	control			7.25±0.87 a	4.25±0.41 a
	1%		10.87±1.75	6.10±0.71 a	3.27±0.38 ab
	3%			5.53±0.71 a	2.46±0.31 b
Free-living nematodes	control			27.08±1.69 a	10.54±1.37 a
	1%		18.35±0.96	23.88±1.95 a	15.43±1.54 b
	3%			27.33±2.55 a	36.00±4.18 c

Means ± SE ($n = 10$).

Nematode populations were analyzed after Box-Cox transformation to reduce non-normality.

For the treatments, means followed by the same letter in rows were not significantly different according to Tukey's HSD test at the 5% level.

Fig. 1. Regression curves for the *Pratylenchus penetrans* population in a cabbage field treated with CF (●) and 3MC + CR (△); reference data.

In this study, the cabbage cultivation periods ranged from 66 to 91 days in spring and 89 to 106 days in autumn. The effective accumulative soil temperatures (degree-days) for *P. penetrans* calculated according to Mizukubo and Adachi (1997) for spring and autumn cultivations in each year ranged from 791 to 1713 and 1114 to 1261 degree-days, respectively. The fitted curves for spring and autumn were $y = 36.689x - 1611.3$ with $r^2 = 0.9967$ ($P < 0.05$, y : effective accumulative soil temperatures, x : cabbage growing-days) and $y = 7.4222x + 467.2$ with $r^2 = 0.8797$ ($P > 0.05$, y : effective accumulative soil temperatures, x : cabbage growing-days), respectively. There was no significant relationship between the effective accumulative soil temperatures and P_f of *P. penetrans* in either spring or autumn cultivations.

Pot experiments:

In the pot experiment, *P. penetrans* numbers significantly decreased at 45 days after the incorporation

Table 10. Results of the model curve regression variables for the *P. penetrans* population based on Seinhorst model, $Pf = aE\pi/((a-1)\pi + E)$ in the cabbage field affected by soil treatments

Treatment	Data sets	a^1	E	r^2	P value (a)	P value (E)
CF	24	6.18	16.45	0.257	0.0172	<0.001
MC + 1/5CF	21	1971	7.32	0.000	>0.05	<0.001
CF + 3/5MC + CR	24	29.18	8.34	0.051	>0.05	<0.001
3MC + 1/5CF	18	31.87	2.99	0.049	>0.05	<0.001
3MC + CR	21	4.63	4.54	0.657	0.0020	<0.001

¹ a = rate of multiplication at $Pi \rightarrow 0$; E = equilibrium density.

Chemical fertilizer (CF), manure compost (MC), and crop residue (CR), either separately or in combination, were applied before transplanting the cabbages.

of compost (Table 9). A clear trend was found in this experiment, whereby the incorporation of more compost in pots resulted in lower numbers of *P. penetrans*. The population of free-living nematodes in 90 t/ha compost plots increased significantly at 76 days after the incorporation of compost.

Application of the model formula:

When we assessed the significance of the factors (year, cultivation season, and block) of Pi and Pf of *P. penetrans*, some factors showed statistically significant differences and needed to be applied separately to the formula. However, because of the presence of 2 independent variables (a and E) in the formula, we believed that the divided sample number according to the significance of the factors would be too small to analyze. Therefore, we performed curve fitting with the entire data set of each treatment (Table 10) and used this as reference data.

Using DataFit software, we obtained 2 curves each for CF and 3MC + CR, with 6.18 and 4.63 representing a (the “rate of multiplication”) and 16.45 and 4.54 representing E (the “equilibrium density”), respectively (Fig. 1, Table 10). We were unable to obtain curves for MC + 1/5CF, CF + 3/5MC + CR, and 3MC + 1/5CF, since the P values for a were higher than 0.05. However, all the P values for E of these treatments were less than 0.001, with 8.34, 7.32, and 2.99 representing E , respectively. Hence, twice-yearly applications of 97 t/ha sawdust cattle manure compost with 33 t/ha of cabbage crop residue lowered the equilibrium density of *P. penetrans* from 16.45 to 4.54 individuals/g soil.

DISCUSSION

While cabbage is a good host for *P. penetrans*, it is also, to a certain extent, tolerant to this pest. According to

Olthof and Potter (1973), an initial *P. penetrans* density of 6,000 individuals/kg soil is required to cause a decline in the top weights of cabbage plants. In the current study, in 2005S, for example, there was no significant difference in the above-ground weights of cabbage plants between the CF treatment and CF + 3/5MC + CR treatment, which had Pi values of 9.32 and 2.63 individuals/g soil, respectively. Hence, in this study, host plant growth did not appear to be markedly restricted by the increases in *P. penetrans* population size. The soil pH in CF plots was very low during the latter half of the experiment (from 2006 to 2009; Table 4), probably because of $(\text{NH}_4)_2\text{SO}_4$ mixed in CF. However, the presence of severe soil-borne diseases (such as clubroot disease, the severity of which increases under low soil pH conditions; Murakami *et al.*, 2002) was not observed throughout the experiment.

The nematode population sizes in the experimental plots were not assessed just before cabbage was first grown; however, the distributions of *P. penetrans* and free-living nematodes in the plots did not vary greatly because the host plants of *P. penetrans* had been uniformly cultivated for approximately 15 years in the plots and the experimental plots were arranged in a randomized complete block design (3 blocks). In May 2003, following the planting of the first cabbage crops in the previous autumn, the population sizes of *P. penetrans* in the 3MC + CR plot were smaller than those in the CF plot (Table 5). The *P. penetrans* population in the 3MC + CR plots seemed to have been suppressed by the treatment. The population size of *P. penetrans* in the 3MC + CR plots was kept consistently small and those in the CF plot kept consistently large until the end of the 15th planting in 2009, although the Pf/Pi and Pi/Pf values after the 2nd planting did not differ much among treatments. It is not clear whether there was a suppressive effect of the treatment on *P. penetrans* multiplication

during cultivation after the 2nd planting, as it is well known that P_i values affect P_f/P_i values (McSorley and Gallaher, 1995) and a direct comparison of P_f/P_i values with different P_i values was not possible.

In the case of *P. penetrans*, P_f/P_i of spring and autumn cultivations did not differ much or show any tendency among treatments (Table 5). The range of effective accumulative temperatures for *P. penetrans* growth for autumn cultivations in experimental years completely overlapped with that of spring cultivations, while the growth of host plants (cabbage) for each treatment was greater in spring than in autumn and the P_i of *P. penetrans* was relatively higher in spring cultivation than in autumn cultivation (Table 8). On the basis of these results, the host plant growth for all the treatments did not seem to limit the multiplication of *P. penetrans* in both cultivation periods.

Seinhorst (1967) demonstrated that the maximum rate of multiplication and equilibrium density of nematode populations depend on external conditions. In other words, unfavorable conditions may reduce both the maximum rate of multiplication and equilibrium density of a nematode species compared to other populations of the same species under optimal external conditions. To clarify the effect of compost application on the *P. penetrans* population, we applied the model formula of Seinhorst (1966) and compared the relationship between the initial and final densities of migratory nematodes; 3MC + CR and CF fitted the model formula. The other three treatments did not get significant a (rate of multiplication), since the P_f values corresponding to lower P_i varied greatly. However, the P_f values of higher P_i each converged to significant E (equilibrium density). By comparing the equilibrium density values of *P. penetrans* derived from the model formula for treatments, we observed that the application of higher amounts of sawdust cattle manure compost reduced the equilibrium density to a greater degree (Table 10). Because food availability for plant-feeding nematodes did not seem to limit nematode multiplication, the unfavorable external conditions of continuous organic material application seem to cause the low multiplication rate and low equilibrium density observed in the 3MC + CR and 3MC + 1/5CF treatments in the field experiment. The results of our pot experiment support this observation.

Regarding the observed population fluctuation of *P. penetrans* in the 3MC + CR and CF + 3/5MC + CR or 3MC + 1/5CF treatments and the amount of organic materials that were applied in each treatment, we could

not confirm that CR application affected the population density of *P. penetrans* to a greater extent than the same amount of MC. Hence, the influence of CR on *P. penetrans* survival might be similar to that of MC, although cabbage is known to contain isothiocyanate-liberating glucosinolates (Kirkegaard and Sarwar, 1998) and is a potential biofumigation candidate.

The population drops of *P. penetrans* during fallow periods may be caused by the host plants' absence, antagonistic biological activities (Stirling, 2014; Stirling, *et al.*, 2005), and environmental disturbance from the tillage after harvest (Rahman, *et al.*, 2007). The population of *P. penetrans* was reduced to a greater extent after spring cultivation than autumn cultivation, although the cultivation interval was shorter after spring cultivations. This may be because of higher chemical and biological activities under high temperature during the cultivation interval after spring cultivation. The absolute P_i and P_f values of *P. penetrans* and free-living nematodes for each treatment did not differ substantially throughout the experimental period; *i.e.*, after the 2nd cultivation until the 15th cultivation. This means that cattle manure compost application twice a year to the soil affected the population levels of *P. penetrans* and free-living nematodes before and after crop cultivation; however, the rate of change did not increase progressively.

Possible factors that cause the suppression of population growth include accumulated nitrogenous compounds derived from organic materials (Oka and Yermiyahu, 2002; Renčo *et al.*, 2010) and/or biological mechanisms that cause the number of antagonistic and non-antagonistic microorganisms to increase in the soil (Jaffee *et al.*, 1998; Stirling *et al.*, 2005). The results of our pot experiment show that 1% (w/v) manure compost application reduced the population of *P. penetrans* effectively. Oka and Yermiyahu (2002) showed that the application of 200 to 1000 m³/ha cattle manure compost is effective in lowering the *Meloidogyne javanica* galling index in tomato plants, even though the compost-amended soil in pots was washed with excess water. The EC values of the field soil in our study were much lower than those reported by Oka and Yermiyahu (2002), possibly because of the differences in compost composition and preparation. Considering that the concentration of the compost used in this study was approximately 300 m³/ha (100 t/ha), the amount of nitrogenous compounds in the soil might be similar to those in the washed soil in the study of Oka and Yermiyahu (2002), and this might be a reason for the

suppression of the *P. penetrans* population.

The results of this study show that the population of free-living nematodes increased through the incorporation of manure compost. Several studies have reported the biological suppression of plant-parasitic nematodes by compost treatments accompanied with population increases of free-living nematodes. For instance, Jaffee *et al.* (1998) demonstrated that, although the difference was not great, more species of nematode-trapping fungi were found in compost-treated organic plots than in conventional inorganic plots. In a sugarcane field experiment conducted by Stirling *et al.* (2005), the populations of *Pratylenchus zae* and *Tylenchorhynchus annulatus* in soil amended with sugarcane trash were reduced by 85% and 71%, respectively. They found that amendments increased microbial activity and the number of free-living nematodes, but had no effect on known predators such as dorylaimid and mononchid nematodes or nematode-trapping fungi (although an unidentified predatory fungus was found in only amended soil). Stirling (2014) has explained the role of free-living nematodes: "bacteria and fungi that decompose organic matter are consumed by bacterivorous and fungivorous nematodes, and since they then become a food source for generalist predators, continuous organic inputs are the key to maintaining a diverse range of natural enemies". The abiotic and biotic effects of organic material application were hypothesized to cause the decline in *P. penetrans* population under conditions of continuous cabbage cultivation in the field.

On the basis of our results, we concluded that the application of 32 to 53 tFW/ha of sawdust cattle manure compost was insufficient to suppress the population of *P. penetrans*. Rather, 97 to 130 tFW/ha of sawdust cattle manure compost appeared to be sufficient for the stable suppression of *P. penetrans* population levels. Application of organic material at 97 to 130 tFW/ha per cultivation period greatly exceeded the normal application level in Japan. Near the area of Tsukuba, which is where the experimental field was located, the CF + 3/5MC + CR treatment is the standard amount of organic material applied for cabbage cultivation, and heavy organic material application would not be considered as a mainstream procedure for *P. penetrans* suppression. Further studies for providing quantitative information about the limits for the practical application of composts to crops will be needed. The suppression mechanisms of *P. penetrans* in the current system are expected to clarify the effects of organic materials on plant parasitic nematode population dynamics.

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