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Response of Plant Parasitic and Free Living Soil Nematodes to Composted Animal Manure Soil Amendments

M. Renčo,[1] P. Kováčik[2]

Abstract: In an outside pot experiment, dry pig manure processed on pine sawdust litter and fermented for seven days by house fly larvae (fermented manure), and pine sawdust applied alone, and in combination with a spring application of inorganic nitrogen fertilizer were used to determine their effects on plant parasitic and free-living soil nematodes on sugar beets (cv. Antek). Non amended soil was used as a control. All treatments with fermented pig manure and sawdust with nitrogen fertilizer decreased number of plant parasitic nematodes and also root-fungal feeding nematodes compared to the untreated control. Sawdust applied alone had no effect on plant parasitic and root-fungal feeding nematode suppression. Free-living nematodes which were mainly bacteriovores and fungivores were significantly more abundant in soil amended with fermented pig manure, while the sawdust had no effect on these nematodes. The effect of all tested treatments on omnivores-predators was rather random, and in general, the number of these nematodes decreased after soil amendment applications compared to the untreated control.
Key words: fermented animal manure, nematode trophic groups, nitrogen amendments, phytoparasitic nematodes, sawdust.

The European Union, the United States, and Russia are the largest sugar beet producers in the world. Today, around 35% of the sugar in the world is produced from sugar beet and the remaining 65% from sugar cane. In many beet-growing areas, plant parasitic nematodes threaten crop yields (Cooke, 1993). The beet cyst nematode *Heterodera schachtii* is one of the most damaging pests, occurring in all major beet growing areas, including the Slovak Republic (Čuri, 1964; Renčo, 2002). Besides *H. schachtii*, several other plant parasitic nematodes have been found as parasites of sugar beet including *Ditylenchus dipsaci* and *D. destructor*, the root-knot nematodes *Meloidogyne incognita, M. arenaria, M. javanica, M. hapla* and *M. Naasi,* species of the genera *Trichodorus, Paratrichodorus, Longidorus* (which vector viruses), and some species of the genera *Paratylenchus, Pratylenchus, Rotylenchulus, Tylenchorhynchus* and *Helicotylenchus* (Cooke, 1993).

According to the recent European Legislations (Reg. CE 396/2005; 1095/2007; 33 and 299/2008 and 1107/2009) which have deeply reduced and revised the use of pesticides on agricultural crops, there is increasing attention on environmental safety and human and animal health. This leads researchers to investigate new alternative control strategies of plant pests, including plant parasitic nematodes, that are environmentally sound and sustainable.

Organic amendments such as green and cattle manures, composts, and slurries, have been reported in many studies as effective control tools for important plant parasitic nematodes, and capable of stimulating beneficial and free living nematodes (Akhtar and Mahmood, 1996; D'Addabbo et al., 2003; Renčo et al., 2007, 2009, 2010, 2011; Sasanelli et al., 2002, 2006; Hu and Qi, 2010; D'Addabbo et al., 2011). Other alternative control methods or their combination such as the use of resistant cultivars, arbuscular mycorrhyzal fungi, biocontrol agents (Castillo et al., 2006; Sasanelli et al., 2007, 2008, 2009; Fourie et al., 2010), soil solarization, and steam (D'Addabbo et al., 2005), essential oils or plant exudates (Riga et al. 2004; Zouhar et al., 2009; Douda, et al. 2010; Barbosa et al., 2010; Renčo et al. 2012), and seed treatments (Cabrera et al., 2009) can reduce rates or use of synthetic nematicides (Riga, 2011) and represent sustainable methods for nematode management.

Despite in many studies on the use of organic amendments for plant parasitic nematode suppression, there are also examples where population of plant parasitic nematodes increased or were not suppressed by organic amendments (Szczech et al., 1993; McSorley and Gallaher, 1995; Sharma et al., 2000; Kimpinski et al., 2003). It seems that the nematicidal effect of composted amendments is unpredictable and may depend on the starting raw materials, the type of composting process, the maturity of the final product, the nematode species, and season of application (Rodríguez-Kábana, et al. 1987; Rivera and Aballay, 2008). Although, several mechanisms could be involved in nematode suppression, including decomposition of the compost into the soil and ammonia production (Mahran et al., 2008a,2008b), stimulation of soil microbial biomass and release of biocidal substances with nematicide activity (McSorley and Gallaher, 1996; Oka and Yermiyahu, 2002), this biocidal activity should be assessed for all composts or organic used materials.

A pot experiment was conducted on sugar beet and the effect of different rates of fermented animal manure (FM) and sawdust (SAW) on the soil nematode community was investigated. Also, the influence of these materials on chemical and physical soil parameters, the dynamics of aboveground phytomass and chlorophyll content of quantitative and qualitative parameters of sugar beet yield was assessed.

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[1]Parasitological Institute, Department of Plant Nematology, Slovak Academy of Sciences, Hlinkova 3, 040 01, Košice, Slovak Republic, e-mail: renco@saske.sk
[2]Slovak Agricultural University, Faculty of Food and Natural Sources, Tr. A Hlinku 2, 949 76, Nitra, Slovak Republic, email: peter.kovacik@uniag.sk
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TABLE 1. Experimental design.

No.	Treatment	Doses	Application time
1	Untreated control	0	-
2	FM_4	4 t/ha FM	autumn
3	FM_4 + N	4 t/ha FM + 60 kg/ha N	autumn FM + spring N
4	$FM_{8\ (4+4)}$	8 t/ha FM (4 + 4)	autumn FM (4 t/ha) + spring FH (4 t/ha)
5	FM_8	8 t/ha FM	autumn
6	FM_8 + N	8 t/ha FM + 60 kg/ha N	autumn FM + spring N
7	SAW_1	3,4 t/ha SAW	autumn
8	SAW_1 + N	3,4 t/ha SAW + 60 kg/ha N	autumn SAW + spring N
9	SAW_2	6,8 t/ha SAW	autumn
10	SAW_2 + N	6,8 t/ha SAW + 60 kg/ha N	autumn SAW + spring N

FM – fermented manure; SAW –sawdust; N – nitrogen fertilizer.

MATERIALS AND METHODS

The first experiment started in October 2009. Twenty-five kg of sandy loam soil (Haplic Luvisol) collected from an agricultural field in Dolná Malanta village were placed into 30 kg containers, and were throughly mixed with different rates of fermented manure (FM) and sawdust (SAW) (size 0.25 – 1 mm) as reported in Table 1. For each dose of amendment or treatment four replications were used. The FM is a type of organic material (compost) which comes from pig pine sawdust litter which was composted and fermented by larvae of house fly species *Musca domestica* for 7 days, during their process of development to phase of shark-moth. The flies came from the multiyear rearing at the Institute of Zoology of the Slovak Academy of Sciences. The raw pig manure was placed into fermentation halls evenly on the plane to the height of 25 cm. About 60,000 fly eggs were inoculated into every 10 kg of raw pig manure during the biodegradation process at a temperature of 35 °C.

The same clear pine sawdust used as pig litter was used in variants with SAW addition. Non amended soil was used as control. In the middle of March 2010, 4 t/ha of FM were applied as an additional treatment [$FM_{8\ (4+4)}$]. In other spring treatments an addition of 60 kg/ha of inorganic nitrogen was also considered (Table 1). Seeds of sugar beet *Beta vulgaris* L. var. saccharifera (cv Antek) were sown at the begining of April 2010, in pots filled with the amended or fertilised soil previously indicated.

To confirm the results of the first experiment, a second experiment was established the next growing season 2010-2011. For this experiment, the soil was collected from the same field as in the first experiment, the same FM and SAW was applied at the same rates and number of replications.

The chemical analysis of the FM, SAW and non amended soil used in the both experiments are reported in Table 2, whereas in Table 3 the chemical parameters of soil amended by the different treatments are shown. The doses of FM used were applied according to the maximum nitrogen doses permitted by the Slovak legislation. The applied dose of 8 t/ha of fermented pig manure was equivalent to 172 kg/ha of N.

At the end of experiments (October 2010 and 2011), to verify the effect on soil nematofauna of the different rates of FM, SW alone or in combination with N application, 200 g of soil from each pot were taken from sugar beet root zones and analysed. Each soil sample was put in a plastic bag and stored at 5°C until nematode extraction. Prior to nematode extraction, soil samples were homogenized by hand mixing and nematodes were isolated from 100 g of each soil sample by Baermann's method (1917), and then fixed in FAA and observed in permanent and provisional glycerine slides (Johansen, 1940).

The nematodes were microscopically identified to the species level using the keys by Meyl (1960), Brzeski (1998), Loof (1999), Siddiqi (2000), Andrássy (2005, 2007, 2009) and original descriptions of species. In addition, all nematode species were allocated in six trophic groups according to their feeding habits (Wasilewska, 1971; Bongers, 1990) compiled by Yeates et al. (1993), as follows: bacterivores (B), fungivores (F), root-fungal feeders (RFF), plant parasite (PP), omnivores (O) and predators (P). Due to the similar feeding behaviour of omnivorous and predators, as well as their low abundance

TABLE 2. Chemical parameters of soil before treatments, fermented manure and sawdust.

Chemical parameter	Soil	Manure	Sawdust
$N_{anorg.}$ [mg/kg]	14.3*	1030.0	187.5
$N\text{-}NH_4^+$ [mg/kg]	4.1	793.2	152.2
$N\text{-}NO_3^-$ [mg/kg]	10.2	236.9	35.3
P [mg/kg]	65.6	4908.4	9.7
K [mg/kg]	437.5	24,293	435.8
Ca [mg/kg]	1,053	5,839	871.5
Mg [mg/kg]	353	451	171
N_t [mg/kg]	1,369	19,686	806.9
C_{ox} [%]	1.34	40.4	50.23
OM [%]	2.42	80.74	93.43
C:N	9.8:1	20.5:1	632.2:1
pH_{KCl}	5.68	7.53	4.35
pH_{H2O}	6.07	7.67	4.75

OM- organic mater.
* All data are mean values of eight replication related of two experiments.

Table 3. Chemical parameters of soil amended by different treatments at the end of experiments.

No.	Treatment	$N_{anorg.}$	$N\text{-}NH_4^+$	$N\text{-}NO_3^-$	P	K	Ca	Mg	pH	pH	C_{ox}	N_t	C/N	OM
					$mg.kg^{-1}$				KCL	H_2O	%	$mg.kg^{-1}$		
1	0	19.313*	16.815	2.5	15.0	2500	820	275	6.17	7.22	1.408	1656	8.5:1	2.54
2	FM_4	16.250	13.062	3.2	42.5	1875	670	202	6.19	7.22	1.476	1968	7.5:1	2.65
3	FM_4 + N	14.438	11.312	3.1	40.0	2300	815	232.5	5.88	7.09	1.476	1751	8.4:1	2.71
4	$FM_{8\ (4+4)}$	17.313	15.375	1.9	98.8	3475	945	310	6.08	7.23	1.560	1685	9.3:1	2.80
5	FM_8	12.188	10.25	1.9	80.0	2600	745	267.5	5.90	7.27	1.640	2174	7.5:1	2.95
6	FM_8 + N	13.248	11.625	1.6.	96.3	3300	945	342.5	5.75	7.11	1.672	2024	8.3:1	2.99
7	SAW_1	10.186	9.062	1.1	45.0	3050	1060	312.5	6.27	7.37	1.460	1709	8.5:1	2.63
8	SAW_1 + N	4.408	4.312	0.1	42.5	3050	1125	355.0	6.01	7.16	1.499	1620	9.3:1	2.72
9	SAW_2	10.313	8.934	1.4	36.3	3050	1125	355.0	6.08	7.32	1.603	2002	8.0:1	2.86
10	SAW_2 + N	9.938	8.502	1.4	38.8	2725	925	312.5	5.79	7.07	1.604	2185	7.3:1	2.88

* All data are mean values of eight replication related of two experiments.

in agricultural soils during the evaluation period, those groups were combined and are presented as number of omnivorous-predators (O-P) (Fig. 5)

The chemical analysis of soil, FM and SAW before the experiments, and treated soil at the end of experiments were done as follows: $N\text{-}NH_4^+$ by Nessler's colorimetric method; $N\text{-}NO_3^-$ by colorimetric method with phenol - 2,4 disulphonic acid, $N_{anorg.}$ was calculated as a sum of $N\text{-}NH_4^+ + N\text{-}NO_3^-$; the contents of available P, K, Ca , Mg were determined by Mehlich III extraction procedure (Mehlich, 1984). Content of P was determined by colorimetric method, K and Ca by flame photometry, Mg by atomic absorption spectrophotometry, pH_{H20} and pH_{KCL} (in solution of 1.0 mol KCl dm^{-3}) potentiometrically. The total N content (N_t) was determined according to Kjeldahl method (Bremner, 1960), total carbon content (C_{ox}) spectrophotometrically after the oxidation by Ťjurin method (Orlov and Grišina 1985).

Data from the experiments were subjected to analysis of variance ANOVA and means compared by the Least Significant Difference Test (LSD) at P=(0.05). Statistical analysis were performed using the PlotIt program.

Results and Discussion

In all pots treated with different rates of FM, including those with N addition, a significantly lower number of plant parasitic nematodes were observed in comparison to the untreated control in the both experiments (Fig. 1). The two applied rates of SAW had no effect on PP reduction. These two treatments were not significantly different from the untreated control. Only SAW treatments with N addition were effective for PP reduction with no significant differences in comparison to all FM treatments (Fig. 1) (Table 4).

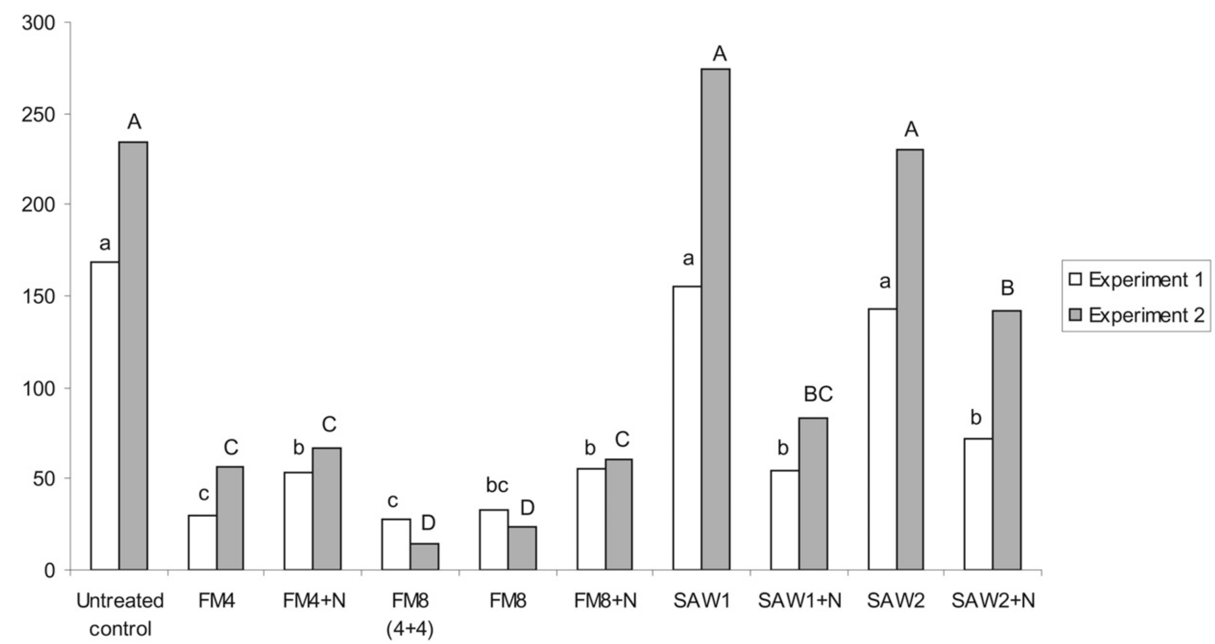

Fig. 1. Numbers of plant parasitic nematodes in soil treated by different doses of FM and SAW with or without addition of N fertilizer. Means (n=4) followed by the same letters are not significantly different according to LSD's Test (P=0.05), small letters for experiment 1, capital letters for experiment 2.

TABLE 4. Mean numbers (n=4) of individual species of plant parasitic nematodes in soil treated by different doses of fermented manure, sawdust with or without nitrogen fertilizer addition in both experiments.

Species/treatment	Untreated Control	FM4	FM4+N	FM8 (4+4)	FM8	FM8+N	SAW1	SAW1+N	SAW2	SAW2+N
Geocenamus brevidens	35 (45)	5 (8)	12 (6)	8 (3)	6 (2)	5 (20)	32 (67)	11 (8)	15 (38)	9 (18)
Bitylenchus dubius	11 (5)	1 (3)	9 (15)	(2)	2	3	6	(26)	10 (46)	5
Helicotylenchus digonicus	15 (39)	3	10 (12)	5	14 (5)	18	22 (45)	2 (5)	15 (30)	10 (34)
Helicotylenchus dihystera	3 (17)	1	(5)	3	4 (6)	5 (15)	17 (54)	5 (8)	(4)	3
Heterodera schachtii (J2+♂)	15 (37)	2 (5)	7 (7)	8	2 (5)	8	18 (15)	5 (7)	21 (17)	2
Paratylenchus bukowinensis	59 (42)	16 (9)	6 (19)	2		5 (5)	15 (39)	11	48 (39)	8 (30)
Paratylenchus microdorus	2	1 (8)		(2)	3		(15)	2 (15)	6 (25)	14 (27)
Pratylenchus penetrans	26 (39)	1 (7)	4 (3)		2 (2)	11 (21)	37 (24)	12 (14)	9 (21)	3 (15)
Pratylenchus pratensis	3 (10)	(16)	5	2 (7)	(4)		8 (15)	6	19 (10)	18 (18)

( ) – mean number of nematodes of individual species recorded in experiment 2.

Results from our experiment clearly demonstrate that soil FM treatments with higher N-NH4$^+$ content significantly suppressed PP nematode populations in comparison to SAW treatments with a lower N-NH4$^+$ content. Similar results were also obtained for root-fungal feeding nematodes (Fig. 2). Other authors have reported similar decreasing PP populations in soils amended by different organic materials (different types of composts or sewage sludge) with narrow C/N ratio (Rodríguez-Kábana et al., 1987; Castagnone-Sereno and Kermarrec, 1991; Nico et al., 2004; Renčo et al., 2009, 2010).

Kirmani et al. (1975) observed that when more nitrogen sources were available in organic treatments with an appropriate C/N ratio, PP nematode control was enhanced. Ismail et al. (2006) recorded the highest reduction of *Rotylenchulus reniformis* in soil amended with composts with lower C/N ratio (6.7:1). On the contrary, yard waste compost (leaves, branches, grass clippings) with C/N ratio from 35:1 to 46:1 had no effect on densities of the plant parasitic nematodes *Pratylenchus* spp. and *Meloidogyne incognita* (McSorley and Gallaher, 1995). Similarly in our experiment, sawdust with a C/N ratio 623:1 had no effect on PP nematodes, but when inorganic nitrogen fertilizer was added, the suppression of PP and RFF nematodes was apparent. Similarly, ĎAddabbo et al. (2003), and Renčo et al. (2007), reported that compost with addition of urea had more suppressive effect on *M. incognita* and *Globodera rostochiensis* reproduction than other tested composts without the urea addition.

Contrary to our results, Sohlenius and Boström (1986), Akthar and Mahmood (1996) and Vestergard (2004) reported that N-fertilization had an adverse

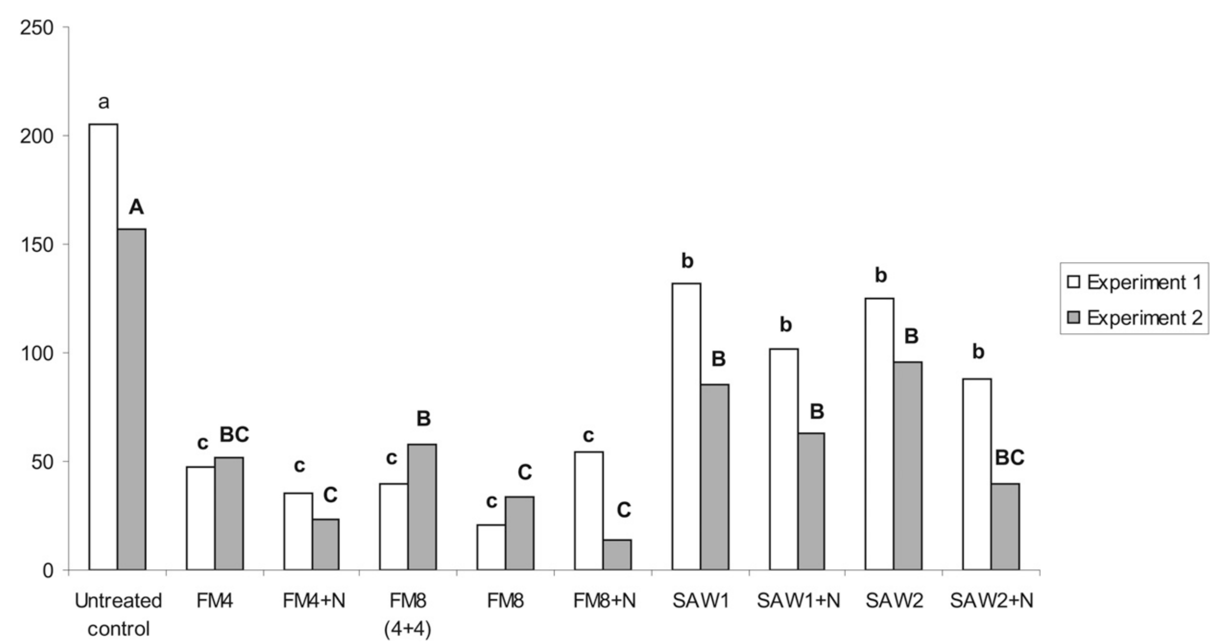

FIG. 2. Numbers of root-fungal nematodes in soil treated by different doses of FM and SAW with or without addition of N fertilizer. Means (n=4) followed by the same letters are not significantly different according to LSD's Test (P=0.05), small letters for experiment 1, capital letters for experiment 2.

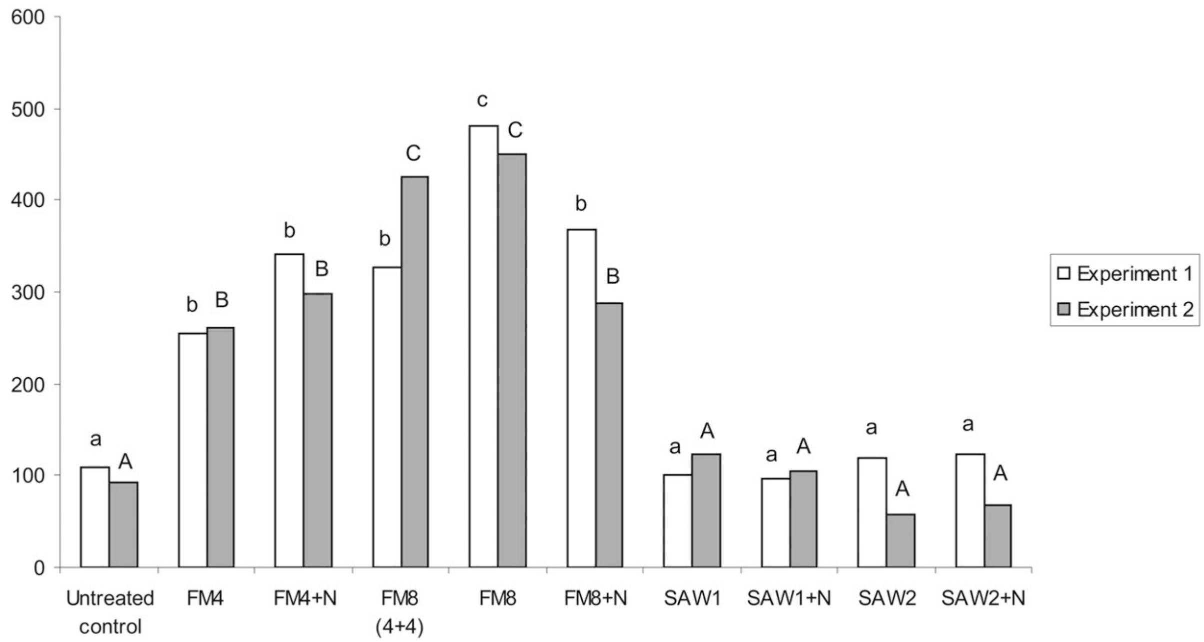

FIG. 3. Numbers of bacteriovorous nematodes in soil treated by different doses of FM and SAW with or without addition of N fertilizer. Means (n=4) followed by the same letters are not significantly different according to LSD's Test (P=0.05), small letters for experiment 1, capital letters for experiment 2.

effect, and it stimulated the increase of PP and RFF nematodes. This was ascribed to the positive effect of N-fertilization on an increase of plant production and nutrition quality of plant roots (Sohlenius and Boström, 1986).

On the other hand, B and F nematode populations significantly increased in soil treated by FM and FM with N fertlilizer in comparison to untreated soil (P=0.05) and those amended with SAW alone or in combination with N fertilization (Fig 3, 4) in both experiments. The highest number of B and F nematodes were found at the highest dose (8 t/ha or 8 t/ha (4+4) resp.) of applied FM. The increase of B nematodes suggested that the N-fertlilization greatly influenced the bacterial production which is caused by the increase of root production and root exudates as stated by

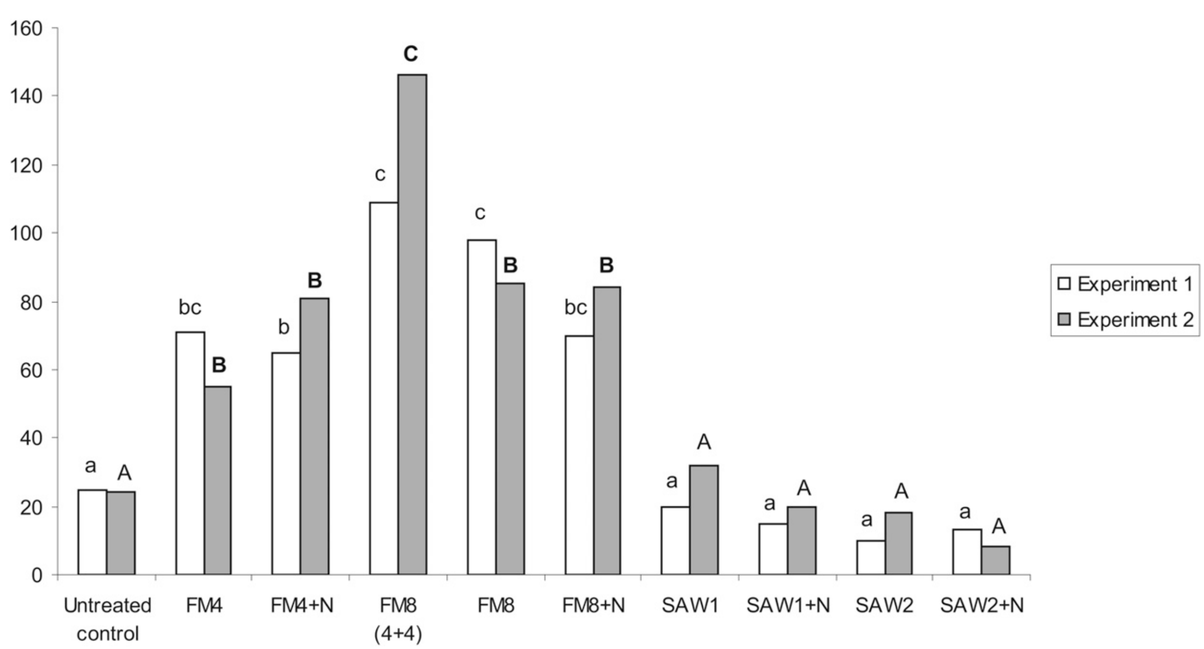

FIG. 4. Numbers of fungivorous nematodes in soil treated by different doses of FM and SAW with or without addition of N fertilizer. Means (n=4) followed by the same letters are not significantly different according to LSD's Test (P=0.05), small letters for experiment 1, capital letters for experiment 2.

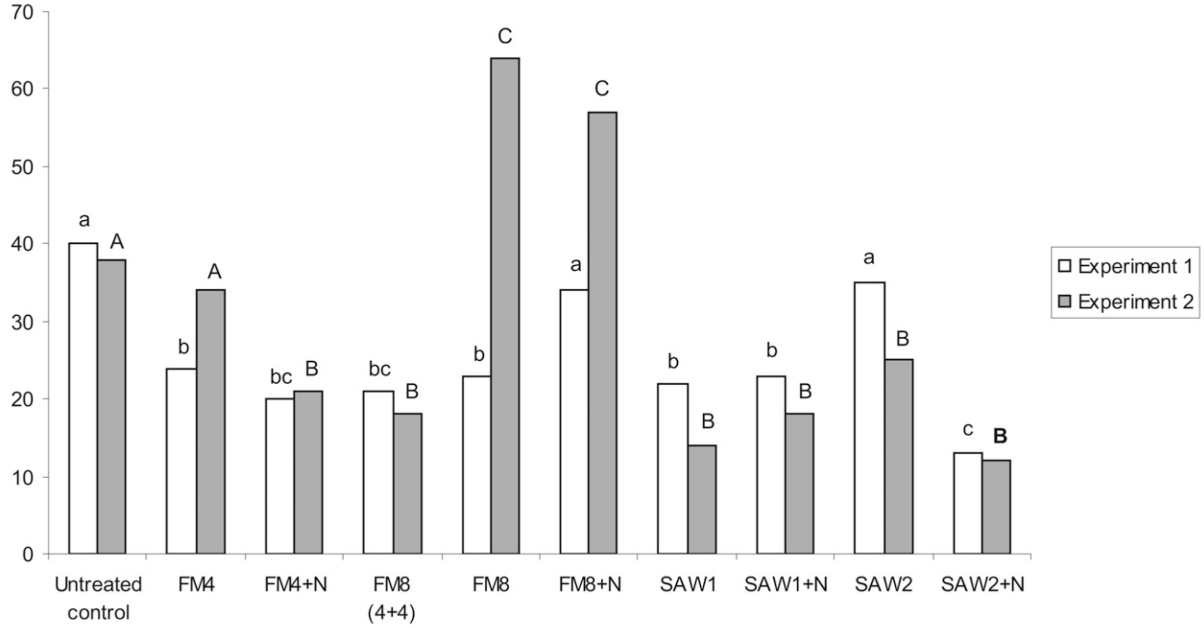

FIG. 5. Numbers of omnivores-predators in soil treated by different doses of FM and SAW with or without addition of N fertilizer. Means (n=8) followed by the same letters are not significantly different according to LSD's Test (P=0.05), small letters for experiment 1, capital letters for experiment 2.

Sohlenius and Boström (1986). An increase of abundance of F nematodes after N-fertilization has also been reported by Kozlowska and Domurat (1977). They noticed an increase of mycophages in a Polish potato field after N addition. Instead Sohlenius and Boström (1986) reported that F nematodes were lower in fertilized than in unfertilized soil plots and they stated that nitrogen addition had no effect on soil for F and RFF nematode development.

The increase in B and F nematodes can be attributed to increased food availability for these nematodes (Griffiths et al., 1994). After addition of organic matter to the soil, organic residues must be decomposed to release nutrients for plant uptake. This decomposition can be divided in two pathways, the faster-bacterial decomposition and the slower fungal-based decomposition. Soil ecosystem type and nutrient status (C:N ratio) determine the predominant decomposition mechanism (Ingham et al., 1985). As an extension of these decomposition pathways, when the bacterivorous and fungivorous nematodes graze on these microbes, they produce CO_2 and $NH4^+$ and other nitrogenous compounds, affecting C and N mineralization directly (Ingham et al., 1985). However, Wasilewska (1995) stated that the addition of manure to the soil increased the proportion of B, F and PP nematodes and decreased the number of O-P. In our study, the addition of FM in the first experiment decreased numbers of O-P (P=0.05), but in the second experiment significantly increased with FM and FM+N addition (Fig. 5).

In conclussion, it is clear, that organic soil amendments can stimulate the development of populations of soil microorganisms and reduce plant parasitic nematode populations, and/or plant diseases, as well as improve soil fertility, physical properties of soil, water retention, water infiltration, permeability, aeration and plant growth. The majority of studies have focused on the different types of organic amendments as suppressants of plant parasitic nematodes, especially root-knot nematodes, because of their large host range and reproduction rate.

Farmers are constantly under pressure to reduce the use of pesticides, including nematicides, and fertilizers. However, they must maintain economic profitability and crop quality. For an effective control of plant parasitic nematodes, it is necessary to choose an appropriate combination of several control methods. Despite the fact that each control method has a limited use because of different developmental nematode cycles and a variable range of host plants, an appropriate combination of control methods can lead to a considerable reduction of the number of phytoparasitic nematodes in soil and in plants.

Because the price of pesticides and fertilizers is high, it is desirable to use environmentally friendly alternative control methods to chemicals. The application of these methods can allow the reduction of crop production costs. In addition, pesticides are usually selective and they destroy only the targeted organisms without a positive effect on plant growth and soil quality. For this reason, the use of organic fertilizer or amendments as a tool for the control of PP nematodes and other soil pathogens is beneficial because these materials are abundant and often low cost, and nutrients and nematicidal substances are often gradually released throughout the entire vegetation period.

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