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SHORT TERM EFFECT OF GREEN MANURING ON SOIL INHABITING NEMATODES AND MICROORGANISMS

by
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Summary. In a microplot experiment white mustard, tancy phacelia, oat and field pea were sown in August as aftercrops and dig into soil in October. The total number of bacteria increased rapidly after green manuring, and it was closely followed by bacteriovorous nematodes. Increase of fungi followed peak of bacteria. Fungivorous nematodes remained at a low level. The population of microorganisms and nematodes grazing on these microorganisms declined after three months. Green manuring did not affect significantly omnivorous nematodes of Dorylaimina. It was calculated that nematodes consumed 21% of C introduced with phacelia, 26% of C from oat, 29% of C from mustard and 49% of C from pea during five months following treatment. The N consumption was estimated as being between 1-3 kg N ha^{-1}.

This paper is part of a study of green manuring, a very old but not fully understood agricultural method. It has been defined as "the process of turning a crop into the soil for the purpose of effecting some agronomic improvement" (MacRae and Mehuys, 1985). As soil inhabiting nematodes are one of the main animal groups contributing to organic matter mineralization (Freckman, 1988; Persson, 1989), an investigation was undertaken on the development of microorganisms and their nematode grazers after green manuring. Although the sudden increase of soil microflora, bacteriovorous and fungivorous nematodes after organic amendments is well documented, the relation between densities of microorganisms and nematodes after green manuring has not been adequately investigated. The aim of the experiment described here was to study these relations and to ascertain the role of nematodes in mineralization of green manures.

Materials and methods

A long term microplot experiment at the Research Institute of Vegetable Crops, Skierniewice, Poland, started in 1990. The experiment consisisted of 15 plots, each 0.63 m^2. The soil fractions were - 1.0-0.1 mm - 63%, 0.1-0.02 mm - 18%, < 0.02 mm - 19%; The bulk density was 1.35 g cm^{-3}; pH in H_2O - 7.2; pH in 1 n KCl - 6.5; organic C - 1.98%; total base exchangeable cations - 38.9 me K 100 g^{-1}.

All plots were sown with onion (*Allium cepa* L.) in spring 1990, and after harvest three replicates of each of the following green manures were sown on 17 August: white mustard (*Sinapis alba* L.), tancy phacelia (*Phacelia tanacetifolia* Benth.), oat (*Avena sativa* L.), and field pea (*Pisum arvense* L.); bare fallow plots served as an unmanured control. Weeding was done by hand, and the plots were not irrigated. The green manure crops were harvested and weighed on 15 October, then immediately dug into the soil in the plots from which they were harvested. In 1991 a lettuce crop was planted in all plots on 24 April and harvested on 14 June. A low rate of nitrogen fertilizer was applied before cabbage cv Kamienna Glowa (*Brassica oleracea* L.) was planted as the main crop on 19 June; it was harvested on 22 October.

Soil samples were taken from each plot starting in August 1990 just before sowing the green manures, and then a month after their incorporation and again monthly from November 1990 until October 1991. Each sample consisted of 13 cores 2.5 cm in diameter taken to a depth of 20-25 cm. This is equivalent to 20.6 cores m^{-2}. Collected soil was thoroughly mixed and subsamples were taken for isolation of bacteria, actinomycetes, fungi and nematodes.

Two 10 g soil subsamples from each plot were added separately to 100 cm^3 of sterile water, shaken for 20 min. on a reciprocating shaker, and then serially diluted with water. Samples of 0.1 cm^3 for bacterial, actinomycete and fungal counts were spread on 5-8 petri dishes with appropriate selective media. Dishes were incubated in the dark at 28 °C (for bacteria and actinomycetes) or 25 °C (for fungi). Colonies were counted day 6 with results expressed as colony forming units (cfu) g^{-1} of soil dried at 105 °C for 24 hours. The media used were: rose bengal agar (Martin 1950) for fungi, 20% soil extract agar with K_2HPO_4 for bacteria and actinomycetes.

Nematodes were extracted from 100 cm^3 subsamples by sugar centrifugation. Repeated extractions of the same soil samples until no more nematodes were obtained indi-

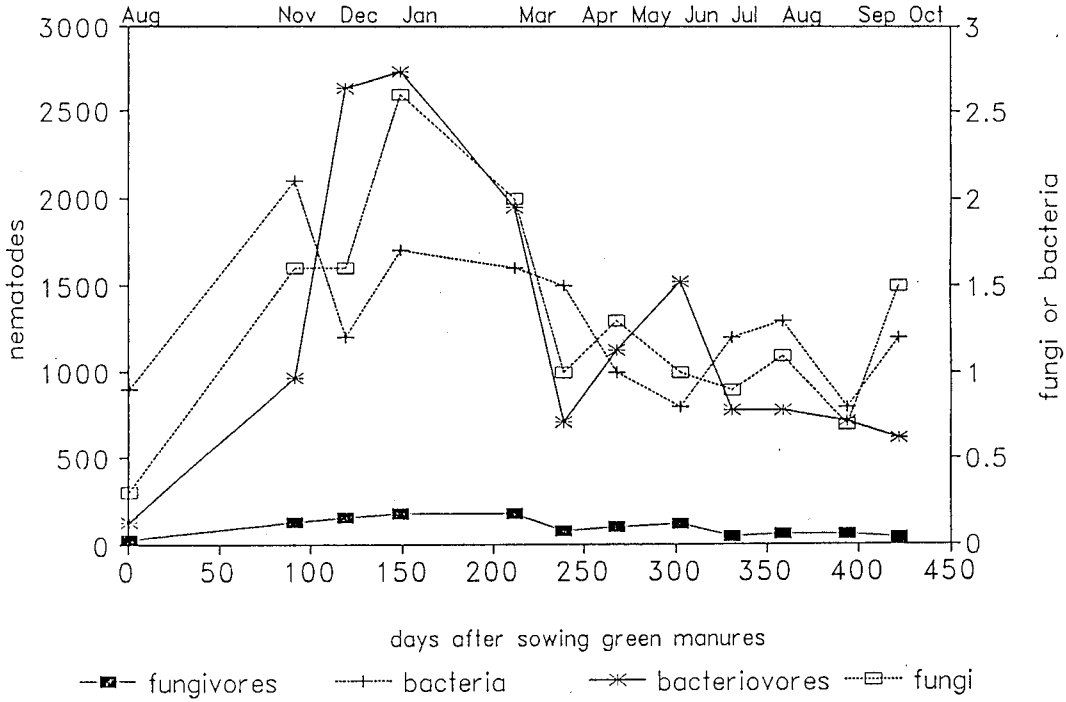

Fig. 1 - Abundance of bacteria, bacteriovorous nematodes, fungi and fungivorous nematodes in soil following green manuring with mustard. Number of nematodes expressed as specimens in 100 cm^3 of soil, number of bacteria x 10^7 and fungi x 10^5 in gram of air dried soil.

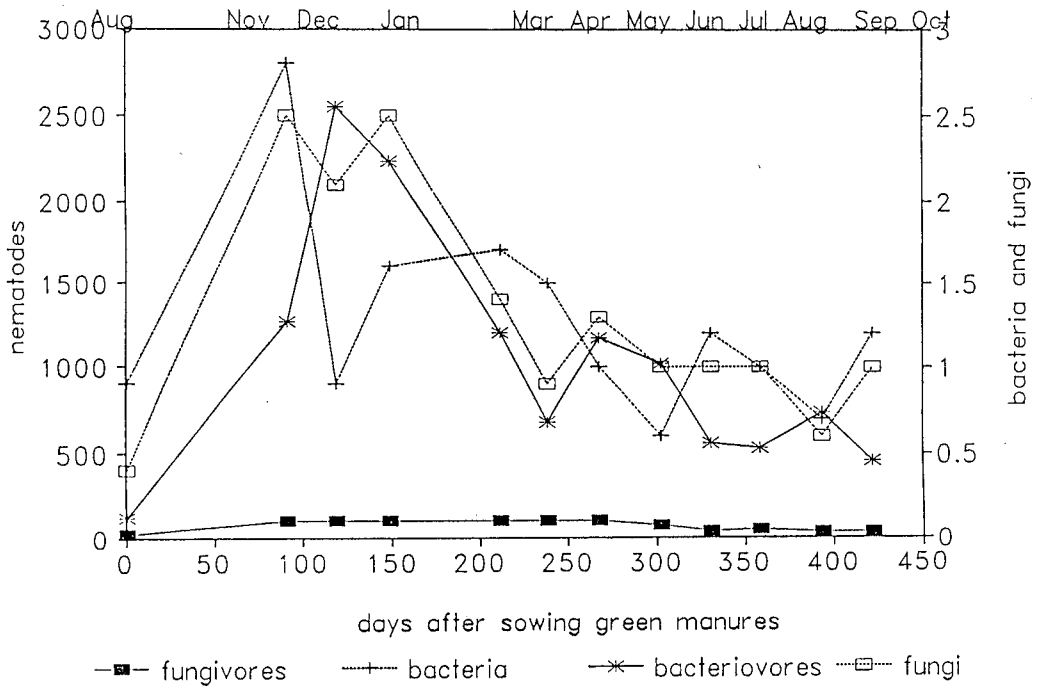

Fig. 2 - Abundance of bacteria, bacteriovorous nematodes, fungi and fungivorous nematodes in soil following green manuring with phacelia. Number of nematodes expressed as specimens in 100 cm^3 of soil, number of bateria x 10^7 and fungi x 10^5 in gram of air dried soil.

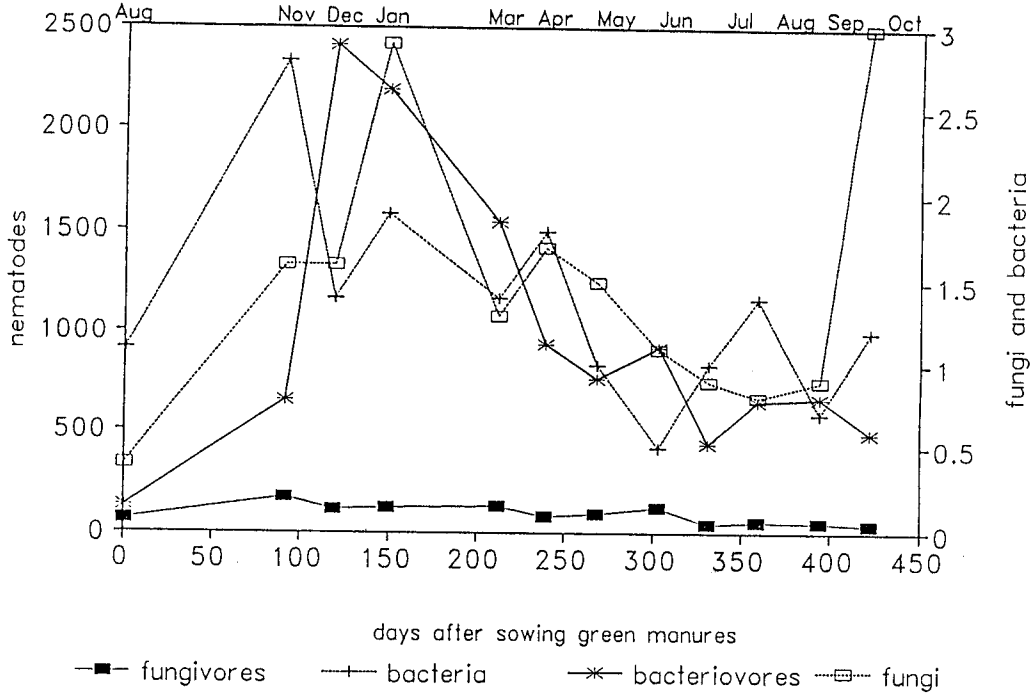

Fig. 3 - Abundance of bacteria, bacteriovorous nematodes, fungi and fungivorous nematodes in soil following green manuring with oat. Number of nematodes expressed as specimens in 100 cm^3 of soil, number of bacteria x 10^7 and fungi x 10^5 in gram of air dried soil.

cated single extraction efficiency as 80 ± 10% of nematodes present in soil. Nematode eggs were not isolated from the soil. Following extraction nematodes were concentrated in 10 ml of water, killed by slow heating and fixed with formalin. Identification was carried to trophic group level, sometimes to the genus level.

For nematode biomass determination, at least 50 nematodes of each group were measured and biomass was calculated according the formula of Andràssy (1956). The calculations may be slightly overestimated, as Robinson (1984) showed that the bias of volume calculation using the Andràssy formula may vary from 0 to about 20% and depends on nematode species and the developmental stage. Thus, all further calculations must be considered as rough estimates.

Respiratory (R) metabolism of nematodes was calculated according to formula $R = 1.40W^{0.72}$ (Klekowski *et al.*, 1972). The values for respiration were used to calculate consumption (C) and production (P) assuming that R/C = 0.18 and P/C = 0.12 (Duncan *et al.*, 1974; Persson, 1989). Biomass turnover was estimated as production divided by mean monthly biomass. The amount of C introduced with green manures was estimated as 40% of dry weight. C consumed by nematodes was calculated assuming that the calorific equivalent of 1 mg CO_2 equals 10.47 J. Total N in plants was determined by the Kjeldahl method.

Results

The mean yields of green manures are shown in Table I. C input into the soil was calculated assuming that air dried matter of plant consists of 40% C.

Increase in numbers of bacteria, actinomycetes and fungi occurred after green manuring. Bacteria peaked a month after green manures were incorporated, the total number of bacteria associated with phacelia, oat, pea and mustard were 2.7, 2.7, 2.4 and 2 times higher than in control unamended soil, respectively (Figs. 1-5).

Actinomycetes increased slightly a month after soil amendment with tancy phacelia, field pea and oat, but no differences were observed between actinomycete cfu in unamended and mustard amended soils (Fig. 6).

Number of fungi, measured as cfu g^{-1} dry soil, in soil amended with phacelia and field pea peaked a month following treatment, while in soil amended with oat and mustard the highest density was observed two months following amendments. Differences in the numbers of bacteria, actinomycetes and fungi in treated and untreated soils diminished gradually following peak densities.

The number of bacterial feeding nematodes closely followed the increasing number of bacteria (Figs. 1-4). The highest density was associated with mustard and the lowest with pea green manure. The fungal feeding

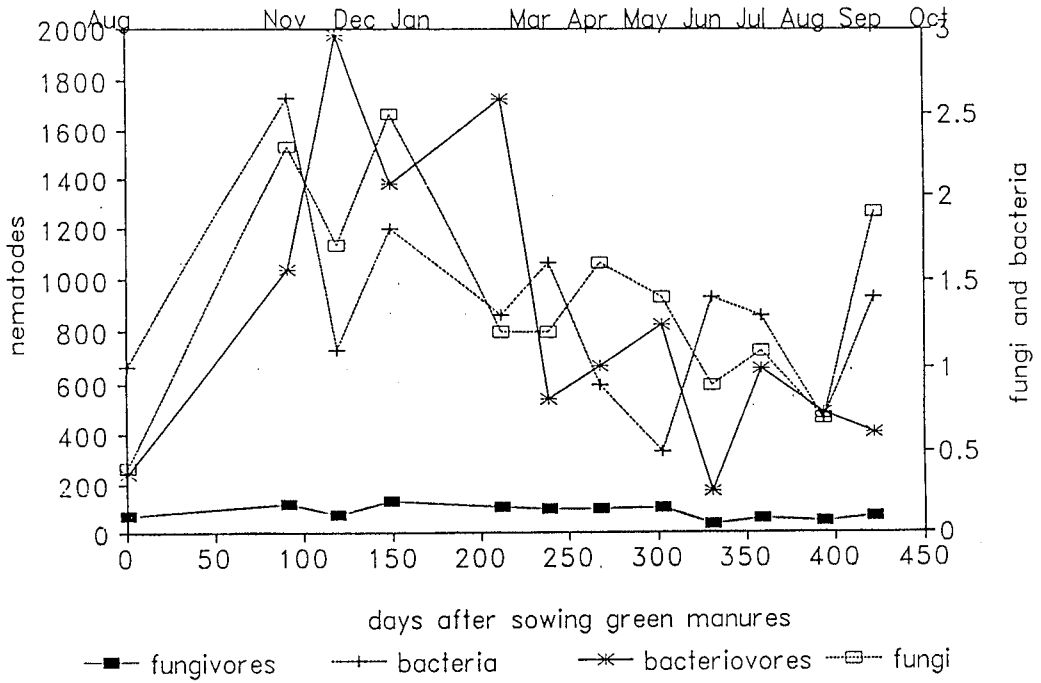

Fig. 4 - Abundance of bacteria, bacteriovorous nematodes, fungi and fungivorous nematodes in soil following green manuring with field pea. Number of nematodes expressed as specimens in 100 cm^3 of soil, number of bacteria x 10^7 and fungi x 10^5 in gram of air dried soil.

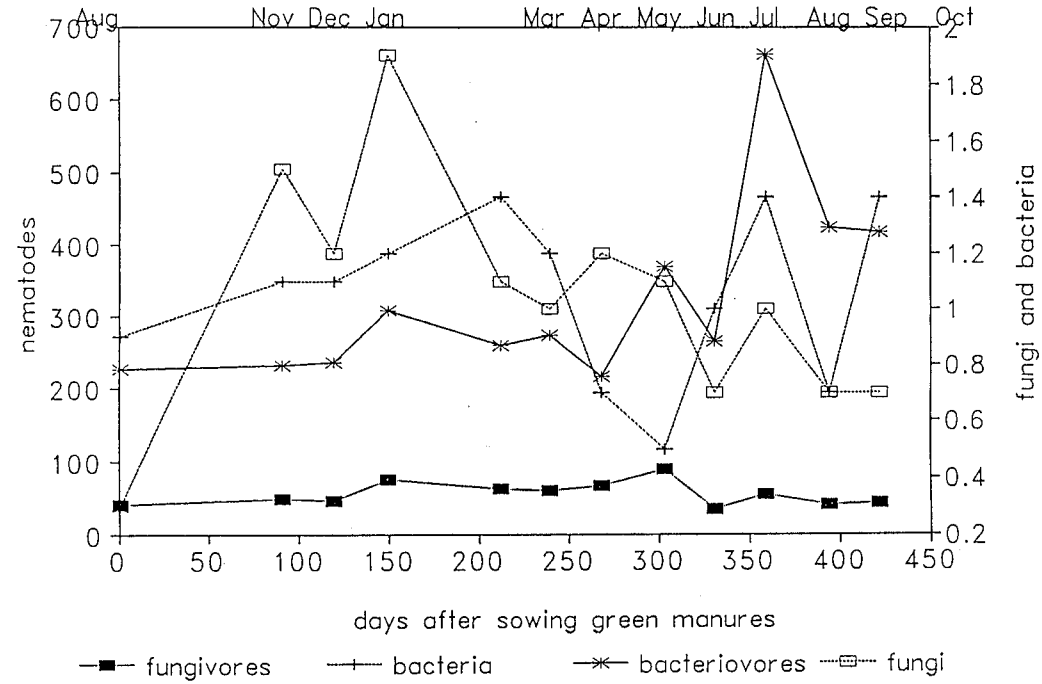

Fig. 5 - Abundance of bacteria, bacteriovorous nematodes, fungi and fungivorous nematodes in unmanured soil of check plots. Number of nematodes expressed as specimens in 100 cm^3 of soil, number of bacteria x 10^7 and fungi x 10^5 in gram of air dried soil.

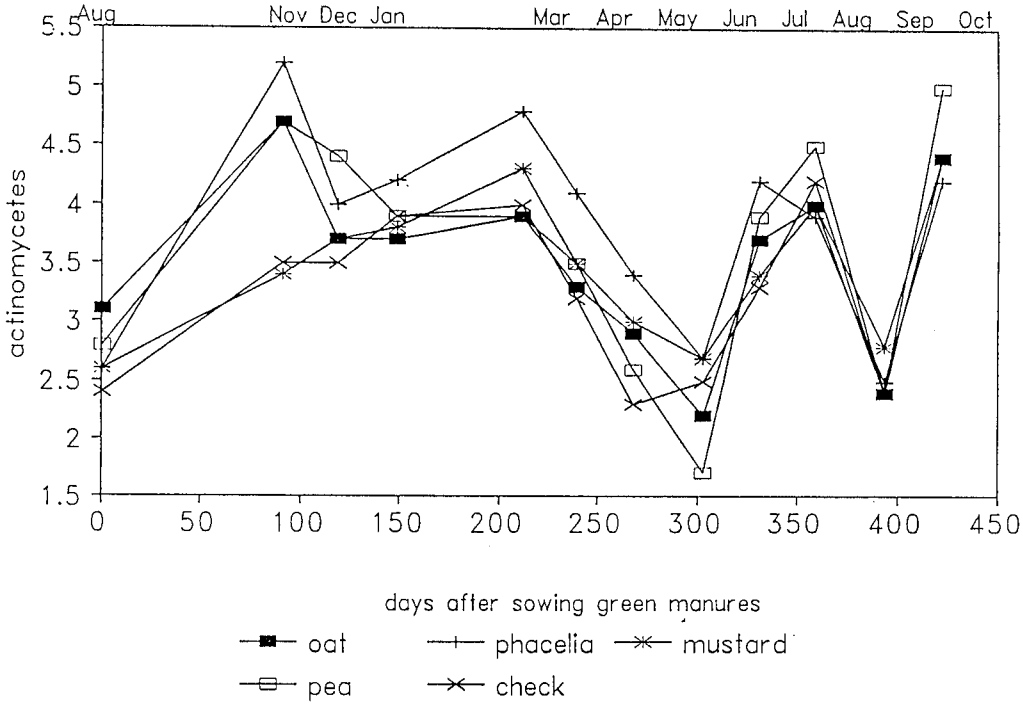

Fig. 6 - Abundance of actinomycetes x 10^6 in gram of air dry soil following green manuring and in unmanured (control) soil.

Aphelenchus avenae Bastian remained at a low level throughout the experiment and an increase of fungal cfu in amended soils was not associated with the density of fungivores (Fig. 1-4). Other fungal feeding nematodes, mainly of the genera *Dtylenchus* and *Aphelenchoides*, were very rare. Densities of dorylaimids also did not respond to green manuring (Fig. 7).

Monthly respiration, consumption and production of the most abundant trophic group of nematodes, i.e. bacteriovores, are presented in Table II. The biomass turnover was estimated to be 9.1%. Calculation of C input consumed by bacterial feeders during five months following amendment showed that nematodes consumed 21% of C from tancy phacelia, 26% from oat, 29% from mustard and 49% from field pea.

Discussion

The increase was a very rapid in the number of bacteria following green manuring, and this was closely followed by an increase in bacteriovorous nematodes. There was then a sudden decrease in bacteria, presumably due to consumption by the nematodes. This supports the observations of Clarholm *et al.* (1981) that the numbers of bacterial feeding nematodes is a possible measure of previous bacterial production. On the contrary, Freckman (1988) considers that bacteriovorous nematodes usually stimulate bacterial growth. The decrease in numbers of bacteria could also be due to accumulaton of toxic substances from decomposing plants (Weyman-Kaczmarkowa and Wójcik-Wojtkowiak, 1991). The effect of amendments on bacteriovores was of short duration and populations had declined considerably by April, six months after green manuring.

The increase of bacteria and bacteriovores in May (Figs. 1 - 5), which was more pronounced on amended plots than on control plots, could have been due to environmental conditions. Possibly favourable conditions stimulated decomposition of more persistent materials from the green manures (e.g. lignin), corresponding to the second stage of organic amendment decomposition as observed by Eitminaviciute *et al.* (1976).

The calculated amount of organic material consumed by bacteriovores during the five months following green manuring is high, but corresponds to results of plant material decomposition published by others when the bag method was used (Eitminaviciute *et al.*, 1976; Wasilewska *et al.*, 1981; Kiss and Jager, 1987). The bacterial feeding nematodes contribute to the process of mineralization (Anderson *et al.*, 1981; Hunt *et al.*, 1984; Ingham *et al.*, 1985; Sohlenius *et al.*, 1988; Freckman, 1988; Persson,

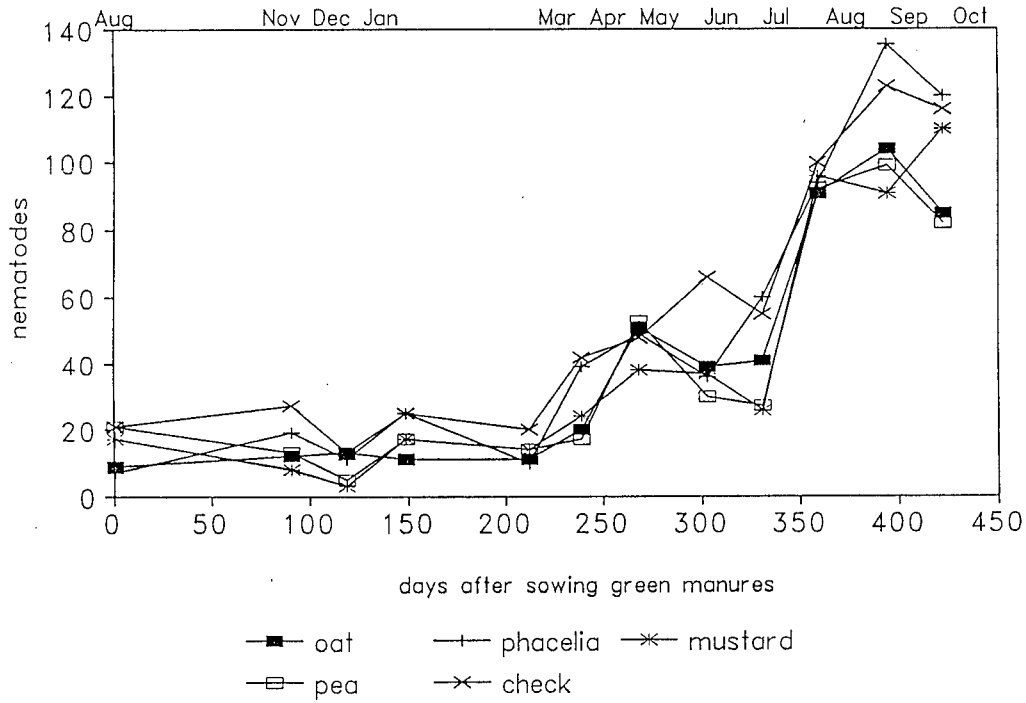

Fig. 7 - Abundance of Dorylaimina (K-strategists) nematodes in 100 cm^3 of soil following green manuring and in unmanured (control) soil.

TABLE I - *Yields of green manures.*

Green manure	Fresh weight kg m^{-2}	% dry matter	Air dry weight g m^{-2}	C input g m^{-2}
mustard	2.23	10.4	232	92.6
phacelia	3.04	8.0	243	97.2
oat	2.16	10.9	236	94.3
pea	1.05	7.7	81	32.2

1989) and this may be at least one of the reasons why green manuring only seldom increases organic C in the soil (MacRae and Mehuys, 1985). In our experiment organic C in the soil was determined in June 1991, that is 8 months after green manuring and no significant effects were observed.

Nitrogen input into the soil resulting from green manuring is shown in Table III. Assuming that bacterial C:N is near 10 (Hunt *et al.*, 1984) and that bacteriovores consumed only bacteria, the C:N ratio of their food remained the same. Thus nematodes consumed only about 1-3 kg N ha^{-1}. That is a negligible part of the total N present in the soil. Considering that the analyses cover about half a year and bacteriovores only, this figure is more or less between the data of Wasilewska (1974) and those of Sohlenius *et al.* (1988). The ratio C:N in the plants was calculated as being very near 10. These calculations are based on the assumption that 40% of plant dry matter is C (Table I) and total N contents (Table III). Thus, the bacteria and their nematode grazers utilised little N from the soil pool. However, if bacterial C:N is accepted as being near 5 (Persson, 1989), then the estimates of nematode N consumption (Table III) would be doubled, but are still very low from agronomic point of view.

TABLE II - *Monthly means of biomass, consumption, respiration and production of nematodes.*

Green manure	Nov--Dec	Dec--Jan	Jan--Feb	Feb--March	March--April	April--May	May--June	June--July	July--Aug	Aug--Sept	Sept--Oct
					Biomass, gm^{-2}						
Mustard	1.54	2.29	2.11	1.88	1.13	0.79	1.13	0.99	0.67	0.65	0.58
Phacelia	1.62	2.04	1.62	1.32	0.81	0.79	0.94	0.68	0.49	0.56	0.51
Oat	1.31	1.96	1.88	1.50	1.07	0.74	0.73	0.59	0.47	0.56	0.49
Field pea	1.28	1.43	1.28	1.38	0.97	0.52	0.64	0.43	0.36	0.49	0.39
Unmanured	0.21	0.24	0.26	0.24	0.24	0.19	0.27	0.29	0.41	0.48	0.37
					Consumption, kcal. m^{-2}						
Mustard	48.7	77.8	78.9	66.2	34.7	26.0	35.9	31.3	21.3	25.7	18.2
Phacelia	51.6	69.3	60.5	46.5	24.7	26.1	29.9	21.6	15.6	22.3	16.2
Oat	41.3	66.6	53.4	52.7	32.9	24.2	23.2	18.7	14.8	22.3	15.6
Field pea	40.8	48.6	47.7	48.3	29.7	17.2	20.4	13.6	11.4	19.6	12.3
Unmanured	6.6	8.2	9.7	8.6	7.2	6.3	8.7	9.2	12.9	18.9	11.7
					Respiration, kcal. m^{-2}						
Mustard	8.8	14.0	14.2	11.9	6.2	4.7	6.5	5.6	3.8	4.6	3.3
Phacelia	9.3	12.5	10.9	8.4	4.4	4.7	5.4	3.9	2.8	4.0	2.9
Oat	7.4	12.0	9.6	9.5	5.9	4.3	4.2	3.4	2.7	4.0	2.8
Field pea	7.3	8.7	8.6	8.7	5.3	3.1	3.7	2.4	2.1	3.5	2.2
Unmanured	1.2	1.5	1.7	1.5	1.3	1.1	1.6	1.6	2.3	3.4	2.1
					Production, kcal. m^{-2}						
Mustard	5.8	9.3	9.5	7.9	4.2	3.1	4.3	3.8	2.6	3.1	2.2
Phacelia	6.2	8.3	7.3	5.6	3.0	3.1	3.6	2.6	1.9	2.7	1.9
Oat	5.0	8.0	7.6	6.3	3.9	2.9	2.8	2.2	1.8	2.7	1.9
Field pea	4.9	5.8	5.7	5.8	3.6	2.1	2.4	1.6	1.4	2.3	1.5
Unmanured	0.8	1.0	1.2	1.0	0.9	0.8	1.0	1.1	1.6	2.3	1.4

TABLE III - *Total nitrogen (N_t) content in soil and plants and estimated nematode consumption of N.*

Green manure	N_t in soil g kg^{-1} dry matter		N_t in plant g kg^{-1} dry matter	N_t input with green manures g m^{-2}	N_t consumption by nematodes g m^{-2}
	Aug '90	June '91			
Mustard	2.86	1.61	35.7	8.3	2.7
Phacelia	3.08	1.89	34.7	8.4	1.0
Oat	3.00	1.81	39.7	9.4	2.4
Field pea	3.34.	2.03	46.5	3.8	2.0

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