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Interactions between nematodes and microbial communities in a tropical soil following manipulation of the soil food web

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Abstract

The carrying capacity for microflora and nematofauna was manipulated (using a bactericide, a fungicide, manure or a growing millet plant) in a poor tropical soil, in order to identify relationships between the soil microbes and nematodes and to assess the influences of these organisms on nitrogen flux. The experiment was conducted for 4 months in containers under greenhouse conditions, with analyses of soil, nematofauna and microbial characteristics at regular intervals. Manure input and initial bactericide application led to a significant increase in bacterial-feeding and fungal-feeding nematodes of coloniser-persister classes 1 and 2, respectively, whereas high manure input stimulated omnivorous nematodes (i.e. *Microdorylaimus rapsus*) which became the dominant trophic group. Changes in abundance of the different bacterial-feeding nematode taxa between treatments seemed to be more related to changes in the structure of the microbial communities than to the total amount of micro-organisms, as suggested by the RISA fingerprint analysis of the bacterial communities. Canonical analysis of nematode feeding guilds, combined with soil microbial and mineral nitrogen parameters as well as multiple regression showed that the bacterial-feeding nematodes influenced the inorganic N content in the soil whereas microbial biomass was determined by total nematode abundance and not by any specific trophic group.

Keywords: Nematofauna; Trophic groups; Microbial biomass; Enrichment by manuring; Mineral N; Bacterial diversity

1. Introduction

Nematodes occupy several different roles in the soil food web (Hunt et al., 1987) and are essential for processes such as decomposition of organic matter and mineralisation of nutrients (Verhoef and Brussaard, 1990). In simplified experimental systems with a single microbivorous nematode species and a single species of micro-organism, the activity of bacterial-feeding or fungal-feeding nematodes can lead to an increase in soil inorganic nitrogen in the soil (Ingham et al., 1985). Also, the activity of soil mesofauna can contribute to 30% of mineralisation in field situations (Verhoef and Brussaard, 1990). However, this liberation of nutrients is not only directly related to the microbivore nematodes but to the entire nematofauna which excretes nitrogen as ammonium (Baath et al., 1981; Ekschmitt et al., 1999). Because of spatial heterogeneity, complexity of the system and changing climatic conditions, it is extremely difficult to delineate interactions between the different soil organisms under natural conditions.

In this study, the micro-organism and nematode communities that naturally occurred in a tropical soil were manipulated under controlled conditions. Actually, we manipulated the resources available for the different feeding guilds of soil nematodes in order to evaluate the consequences of changes in food availability on nematofaunal

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diversity and community structure. Nematode populations are regulated by the microflora and, through feed-back, the amount of microflora is regulated by the nematodes. However, the consequences of these biotic relationships on nutrient fluxes in the soil are exceedingly difficult to predict. We were particularly interested in this study to measure the relationship between the size and structure of both the nematofaunal and microfloral communities and nitrogen availability in the soil, using the working hypothesis that biotic interactions regulate nutrient availability.

2. Material and methods

2.1. Experimental design

The study was carried out from July to December 2000 at Dakar, Senegal. The soil used originated from the region of Dakar and had a sandy texture (sand: 75.1%, silt: 16.6%, clay: 8.3%). It was sieved at 2 mm before the experiment. Firstly, the microflora was selectively modified using bactericide and fungicide. Secondly, manure was added to directly increase resources available for bacteria, fungi and some nematodes (mostly omnivores). Finally, a growing plant was utilised to directly increase resources available for some nematodes (mostly plant feeders) and bacteria (through root exudates). The bactericide was streptomycin (added at 2 g kg^{-1} soil); the fungicide was Pelt 44 (Thiophanate methyl added at 116 mg kg^{-1} soil). The manure was locally produced and autoclaved (for 40 min at 140 °C) before being used at two different levels (see below). Chemical characteristics of the manure were: C, 18.2%; N, 0.61%; P, 0.25%; K, 0.21%; Ca, 1.88%; SiO_2, 9.6%. The plant used was millet (*Pennisetum typhoides*). The experiment was conducted under non-sterile conditions so that rapid colonisation of micro-organisms could take place.

The following six treatments were compared:

1. C: control (natural soil).
2. M1: soil enriched with manure at low dose (1.5% by dry mass (DM)).
3. M2: soil enriched with manure at high dose (5% DM).
4. M1B: soil enriched with manure at low dose (1.5% DM) plus bactericide.
5. M1F: soil enriched with manure at low dose (1.5% DM) plus fungicide.
6. M1FP: soil enriched with manure at low dose (1.5% DM), plus fungicide and growing millet plants.

The wet manure (moisture content: 100%) was mixed manually with the wet soil (moisture content: 5%) before sieving the mixture at 4 mm. Each treatment was carried out in a container (total volume 15 l: 25 cm × 40 cm × 15 cm height). Eight kilograms of wet substratum (soil or soil plus manure) were placed in each of the six containers. Containers were chosen of a sufficient size to allow for spatial heterogeneity within treatments on the scale of organisms analysed. Because sampling was destructive, samples from different dates reflected both variation in time and space, and are considered as independent replicates in the statistical sense.

The fungicide and bactericide were applied the same day, in a suspension of water (100 ml kg^{-1} of soil) to allow treatment of the entire substratum. The initial humidity of the media, following application of the bactericide, fungicide or an equivalent quantity of water, was adjusted to 10% for the control, to 12% with the low doses of manure and to 15% with the high dose of manure which corresponded to the moisture content at field capacity (p*F* 4.2). Humidity was maintained during the experiment by adding water after weighing the containers three times a week. In all experiments, humidity was strictly related to manure content because humidity was kept experimentally constant at p*F* 4.2. As the bulk density of the soil was not strictly checked, it is possible that the ratio of water-filled pore space to air-filled pore space differed lightly across treatments; however, water was never limitant. The containers were partly covered to avoid water loss, except for the M1FP treatment involving plants. Three days before the start of the experiment, millet seeds were soaked in water; six germinating seeds were planted in the container of the M1FP treatment immediately following adjustment of soil water content. Water was added daily in this treatment. Containers were kept between 28 and 35 °C in a greenhouse and daily temperatures were recorded. The experiment lasted 18 weeks and nine soil samples were taken at approximate 2-week intervals, i.e. at 15, 24, 38, 54, 64, 78, 92, 106 and 121 days after the beginning of the experiment. Samples consisted of 300 g of wet soil; 200 g were used for nematological analyses and 100 g for the microbial and soil physico-chemical analyses.

2.2. Analyses

The following physico-chemical characteristics of the soil were measured at the end of the experiment: carbon (C) and nitrogen (N) content, cation exchange capacity (measured with ammonium acetate at pH 7 (Page et al., 1989)), the content of the main exchangeable cations and calcium (Ca^{+}), magnesium (Mg^{++}) and potassium (K^{+}) titrated with a flame spectrometer following exchange with ammonium acetate. Plant-available phosphorus (P) was also determined using the method of Olsen modified by Dabin (1967).

Nematodes were extracted from the soil using a modified Seinhorst method (Seinhorst, 1962) and counted at low magnification. The composition of the soil nematofauna was determined after fixation in a formalin–glycerol mixture and transfer to mass slides. On average, 170 nematodes were identified to family or genus level in every sample at 400× magnification (30 categories). We retained 17 categories for the data analysis, plus one for all rare taxa, as described in Villenave et al. (2001).

Each nematode taxon was then assigned to 1 of 5 trophic groups following Yeates et al. (1993) (bacterial feeders, fungal feeders, plant parasites, omnivores and predators) and also allocated to a coloniser–persister (c–p) class according to Bongers (1990). The coloniser–persister scale ranged from 1 to 5, respectively, and could vary within a trophic group, thus bacterial feeders with a c–p class of 1 were placed in feeding guild BF1 and fungal feeders with a c–p class of four were placed in feeding guild FF4. Nematodes which could not be assigned to one trophic group with certainty were classified in the group having the most similar morphological feeding structure.

Microbial biomass was estimated by the fumigation–extraction method, using the gain in ninhydrin-reactive N after fumigation, multiplied by 21 (Amato and Ladd, 1988). Ergosterol content of the soil was measured using 2 g fresh mass (FM) soil by quantitative high performance liquid chromatography (HPLC) analysis according to Grant and West (1986). Fungal biomass was then estimated as ergosterol content $\times 89$. Bacterial biomass was calculated as the difference between microbial biomass and fungal biomass.

Soil inorganic N was determined colorimetrically in KCl extracts (KCl 2 M) by flow injection analysis according to the method of Bremner (1965).

The structure of the bacterial community from four treatments (C, M1, M2 and M1B) was studied at day 24 and day 64 using automated ribosomal intergenic spacer analysis (ARISA). The entire ARISA procedure described below was implemented for three replicates at day 24 and two replicates at day 64. Total DNA from bacteria was extracted from 0.5 g (dry weight) soil samples using the direct extraction lysis procedure described by Porteous et al. (1997). The *rrs–rrl* intergenic spacer region was amplified by polymerase chain reaction (PCR) using the primers S-D-Bact-1522-b-S-20 (small eubacterial ribosomic subunit; 5′-TGCGGCTGGATCCCCCTCCTT-3′), which was labelled at the 5′ end with fluorochrome 6-carboxyhexafluorescein (HEX; Perkin-Elmer, Courtaboeuf, France), and L-D-Bact-132-a-A-18 (large eubacterial ribosomic subunit; 5′-CCGGGTTTCCCCATTCGG-3′; Normand et al., 1996). In addition to the intergenic spacer, the amplicons contained also 20 bp corresponding to primer S-D-Bact-1522-b-S-20 and approximately 130 bp from *rrl*. PCR amplification was carried out as described by Ranjard et al. (2001). Fragments were resolved by using the MegaBACE sequencer (Amersham-Pharmacia, Orsay, France) and electrophoresis of the DNA in a capillary tube. Samples consisted of a standardized amount (1.5–3 µl) of PCR product along with 0.25 µl of a red fluorescent internal standard (ET-ROX 900, Amersham-Pharmacia) and 1.75–3.25 µl of ultra-pure water. The samples were run under denaturing conditions at 44 °C, for 3.5 h at 6000 V. Results were analysed by the Genotype Profiler software program (Amersham-Pharmacia) which converted fluorescence data into electrophoregrams, with peaks representing fragments of different sizes and heights of the peaks representing the relative proportion of the fragments in the total product.

2.3. Data analyses

Differences in population densities of nematodes between treatments were assessed by ANOVA (StatSoft, Tulsa, USA). Treatment effects were assessed by planned comparison of selected pairs of treatments and measures of relative difference between the two paired treatments: (1) effect of low energy and nutrient input (comparison between M1 and C), (2) effect of high energy and nutrient input (comparison between M2 and M1), (3) effect of initial elimination of the bacterial community (comparison between M1B and M1), (4) effect of initial elimination of the fungal community (comparison between M1F and M1) and (5) effect of presence of live plants (comparison between M1FP and M1F).

Differences in the bacterial community structure between treatments were assessed by converting electrophoregrams to a data matrix with bacterial pools as rows and peaks as columns. Presence/absence and relative intensity (height) of each peak in a given profile were taken into account. Bands below 450 bp were not included in the analysis since no differences were observed between profiles. The data matrix was subjected to a principal component analysis (PCA) of covariance using ADE-4 software (Thioulouse et al., 1997). This method provided an ordination of bacterial communities and encoded bands which were then plotted in two dimensions based on the scores for the first two principal components.

Responses of the 11 nematode feeding guilds to soil factors (mineral N content, microbial and fungal biomass, temperature and humidity) were analysed by Canonical Correlation. The Statistica for Windows package (StatSoft, Inc., Tulsa, USA) was used.

In order to assess the biotic regulation of soil N status, we performed multiple regressions using nitrate, ammonium levels as dependent variables. Independent variables were the density of bacterial feeders, fungal feeders, omnivores, plant parasites and of predators, total nematode abundance, microbial C content, soil ergosterol content, day of sampling during the experiment, soil humidity and temperature (weekly mean). In addition, multiple regressions on microbial biomass and soil ergosterol were carried out using the same variables to investigate the effect of the nematofauna on the microflora.

3. Results

3.1. Soil physico-chemical characteristics

At the end of experiment, C and N contents of the soil increased by 23 and 27%, respectively, with the low manure dose compared to the control and by 73 and 82%,

Table 1
Soil physico-chemical characteristics for the six enrichment treatments after 121 days

Parameter	Treatment						Manure effects relative difference (%)		Pesticide and plant effects relative difference (%)		
	C	M1	M2	M1B	M1F	M1FP	M1/C	M2/M1	M1B/M1	M1F/M1	M1FP/M1F
C content (mg C/g DM soil)	8.6	10.6	14.9	10.8	10.3	10.5	23	73	2	−3	2
Total N content (mg N/g DM soil)	0.60	0.76	1.09	0.76	0.70	0.73	27	82	0	−8	4
Assimilable P (ppm P)	177	260	365	275	246	224	47	106	6	−5	−9
pH water	6.9	7.1	7.3	7.1	7.1	7.3	3	6	0	0	3
Ca^{+} (mequiv./100 g DM soil)	3.95	4.08	4.12	3.97	3.86	4.35	3	4	−3	−5	13
K^{+} (mequiv./100 g DM soil)	0.34	0.41	0.62	0.40	0.39	0.15	21	82	−2	−5	−62
Mg^{+} (mequiv./100 g DM soil)	1.13	1.25	1.48	1.21	1.14	1.13	11	31	−3	−9	−1
ECC (mequiv./100 g DM soil)	5.14	5.24	5.69	5.06	5.19	5.56	2	11	−3	−1	7

C: control; M1: low manure dose; M2: high manure dose; M1B: low manure dose plus bactericide; M1F: low manure dose plus fungicide; M1FP: low manure dose plus fungicide and millet plants.

respectively, with the high dose compared to the low dose (Table 1). Manure addition also led to an increase in pH, assimilable P and exchangeable cations. The bactericide and fungicide additions did not induce any marked modifications in the soil physico-chemical characteristics. The presence of the millet plants led mainly to a decrease in exchangeable K.

3.2. Nematofauna

On average, manure addition (M1) led to an increase of 108% in nematode density compared to the control (Table 2). The high dose of manure caused an additional increase in nematode density (M2/M1: +95%). For the other treatments with the low manure dose, only the bactericide addition led to a significantly increased nematode density (M1B/M1: +68%).

Bacterial-feeding nematodes were significantly more abundant overall in M1 compared with C (+100%); there was also a trend, however non-significant, of an increase in bacterial-feeder density when the bactericide was implemented (M1B/M1: +67%; Table 2).

The bacterial-feeder densities followed the same temporal pattern in treatments M1 and M1F; densities increased gradually during the first 50 days and then stabilised at a higher level than that in C (Fig. 1). The pattern was very different for the three other treatments. Bacterial-feeding nematodes were 3-fold higher in M2 than in the other treatments (except M1B) at day 15 and day 24, then the density decreased to that of M1 and M1F at day 45. In the presence of plants (M1FP), the density of bacterial feeders increased later but was twice that in treatments M1, M2 and M1F by the end of the experiment. In M1B, the bacterial-feeder density was consistently greater than that found in M1 and M1F.

Soil enrichment with manure favoured most of the abundant bacterial feeders: in particular the Rhabditidae and the Cephalobidae including *Cephalobus* increased in numbers (Table 2). The high dose of manure (M2) did not incur significant differences in densities of the different taxa compared to the low dose (M1). When the fungicide was used, Rhabditidae showed a significantly lower density (M1F/M1). Furthermore, the presence of millet favoured the development of two taxa of bacterial-feeding nematodes; *Zeldia* and Rhabditidae (M1FP/M1F: +712 and 713%, respectively).

Fifteen days after the beginning of experiment, a very high density of Rhabditidae was measured in M2: 8-fold higher than in M1 and these nematodes represented 45% of the entire nematofauna of M2 (data not shown). This very high density was ephemeral and a density comparable to the other treatments was measured by day 38.

Manure addition (M1/C) also led to a significant increase in overall fungal-feeder density: +163% (Table 2). The high level of manure dose, however, did not lead to a significant increase in fungal-feeder density overall (M2/M1). Among the other treatments that received the low manure dose, only the bactericide addition led to an increase in fungal-feeder density (M1B/M1: +233%). Furthermore, the temporal pattern of fungal-feeder density was very similar between treatments M1, M2, M1F and M1FP (Fig. 1). The densities for these four treatments were, with the exception of the last day, greater than those of C. On the other hand, the density of fungal feeders was greater in M1B than that of the other treatments and this difference increased continuously with time. Among the most abundant fungal feeders, *Ditylenchus* had a significantly

Table 2
Mean soil parameters, abundance of nematode c–p feeding classes and selected nematode taxa from day 15 to day 121 for six enrichment treatments and relative differences between treatments

Parameter	c–p	Treatment mean over time						Manure effects relative difference (%)		Pesticide and plant effects relative difference (%)			ANOVA, *p*-value
		C	M1	M2	M1B	M1F	M1FP	M1/C	M2/M1	M1B/M1	M1F/M1	M1FP/M1F	
Total nematode abundance (Ind./100 g DM soil)		405	844	1648	1423	709	1002	+**108**	+**95**	+**68**	−16	+41	**0.0000**
Bacterial feeders (Ind./100 g DM soil)		215	430	460	718	351	464	+**100**	+7	+67	−18	+32	**0.0000**
Cephalobus+Acrobeloides	2	120.8	217.2	274.6	349.7	257.7	316.9	+**80**	+26	+61	+19	+23	**0.0000**
Chiloplacus	2	48.6	51.6	24.1	66.4	43.8	52.2	+6	−53	+29	−15	+19	**0.0069**
Stegelleta	2	17.5	40.3	40.3	88.0	21.6	24.5	+130	+0	+119	−46	+14	**0.0041**
Zeldia	2	4.4	7.3	8.8	10.9	9.5	76.7	+67	+20	+49	+29	+**712**	**0.0106**
Rhabditidae	1	2.8	68.4	48.9	122.5	2.4	19.6	+**2308**	−29	+79	−96	+**713**	**0.0000**
Acrobeles	2	2.5	17.6	20.7	25.6	4.9	10.0	+604	+18	+46	−72	+102	0.1328
Amphidelus	4	1.1	15.7	27.1	19.9	7.6	3.6	+1285	+73	+27	−51	−53	0.2114
Fungal feeders (Ind./100 g DM soil)		26	67	113	224	59	77	+**163**	+68	+**233**	−12	+29	**0.0000**
Aphelenchina	2	15.4	37.3	37.8	151.8	21.3	31.8	+142	+1	+**307**	−43	+49	**0.0000**
Ditylenchus	2	9.4	33.7	53.9	66.5	29.3	37.9	+**259**	+60	+97	−13	+30	**0.0000**
Tylencholaimoidea	4	2.2	0.6	21.4	6.9	8.9	7.1	−74	+**3590**	+1097	+**1435**	−20	**0.0300**
**Plant parasites (Ind./100 g DM soil)*		66	64	20	66	41	152	−4	−**69**	+4	−36	+**273**	**0.0000**
Scutellonema cavenessi	3	26.3	25.3	8.0	17.9	16.1	12.3	−4	−**68**	−29	−36	−23	**0.0337**
Helicotylenchus dihystera	3	21.6	12.4	6.8	23.3	13.2	37.1	−43	−45	+88	+7	+181	**0.0170**
Tylenchorhynchus	3	12.3	23.3	3.3	12.9	3.1	21.5	+89	−86	−45	−87	+591	**0.0346**
Telotylenchus	3	1.7	1.3	0.0	6.8	5.9	81.3	−24	−100	+435	+368	+**1275**	**0.0000**
Omnivores (Ind./100 g DM soil)		89	235	1011	364	249	296	+163	+**330**	+55	+6	+19	**0.0000**
Dorylaimidae	5	86.9	221.9	956.5	343.8	247.2	295.0	+155	+**331**	+55	+11	+19	**0.0000**
Predators (Ind./100 g DM soil)		9	48	44	50	8	13	+438	−9	+3	−84	+71	**0.004**
Dorylaimoidae predateurs	5	4.8	19.6	11.7	35.8	2.4	11.3	+308	−40	+83	−88	+363	**0.0016**
Discolaimus	5	4.2	28.8	32.1	13.9	5.2	1.9	+589	+11	−52	−82	−64	**0.0005**
c–p class (Ind./100 g DM soil)													
c–p class 1		3	60	49	127	2	18	+**2007**	−18	+**112**	−96	+**640**	**0.0000**
c–p class 2		224	412	464	780	391	510	+**84**	+13	+**89**	−5	+30	**0.0000**
c–p class 3		76	75	32	76	42	155	−2	−58	+1	−44	+**268**	**0.0000**
c–p class 4		6	27	103	46	19	11	+368	+277	+70	−32	−40	0.255
c–p class 5		96	270	1000	394	255	308	+180	+**270**	+46	−6	+21	**0.0000**
Soil parameters													
Ammonium (μg N/g DM soil)		3.58	5.17	13.01	7.35	4.86	6.51	+45	+152	+42	−6	+34	**0.034**
Nitrate (μg N/g DM soil)		104.17	92.51	42.68	142.07	83.39	24.05	−11	−**54**	+54	−10	−**71**	**0.0000**
Microbial biomass (μg C/g DM soil)		72.88	104.38	152.63	95.38	104.88	99.50	+43	+46	−9	+0	−5	0.239
Ergosterol (μg/g DM soil)		0.06	0.23	0.76	0.31	0.29	0.32	+315	+231	+36	+25	+13	0.0000

Differences were tested by means of ANOVA and Spjotvoll and Stoline's post hoc tests. Significant effects are emphasised in bold. C: control; M1: low manure dose; M2: high manure dose; M1B: low manure dose plus bactericide; M1F: low manure dose plus fungicide; M1FP: low manure dose plus fungicide and millet plants. Other nematodes found: *Cervidellus*, *Rhabdolaimus*, Prismatolaimidae, *Wilsonema*, *Pratylenchus*, Tylenchidae, Geomonhystera, *Pseudacrobeles*, *Macrolaimellus*, *Drilocephalobus*, *Dorylaimellus*, Ecphyadophoridae, plus other morphotypes of Dorylaimidae.

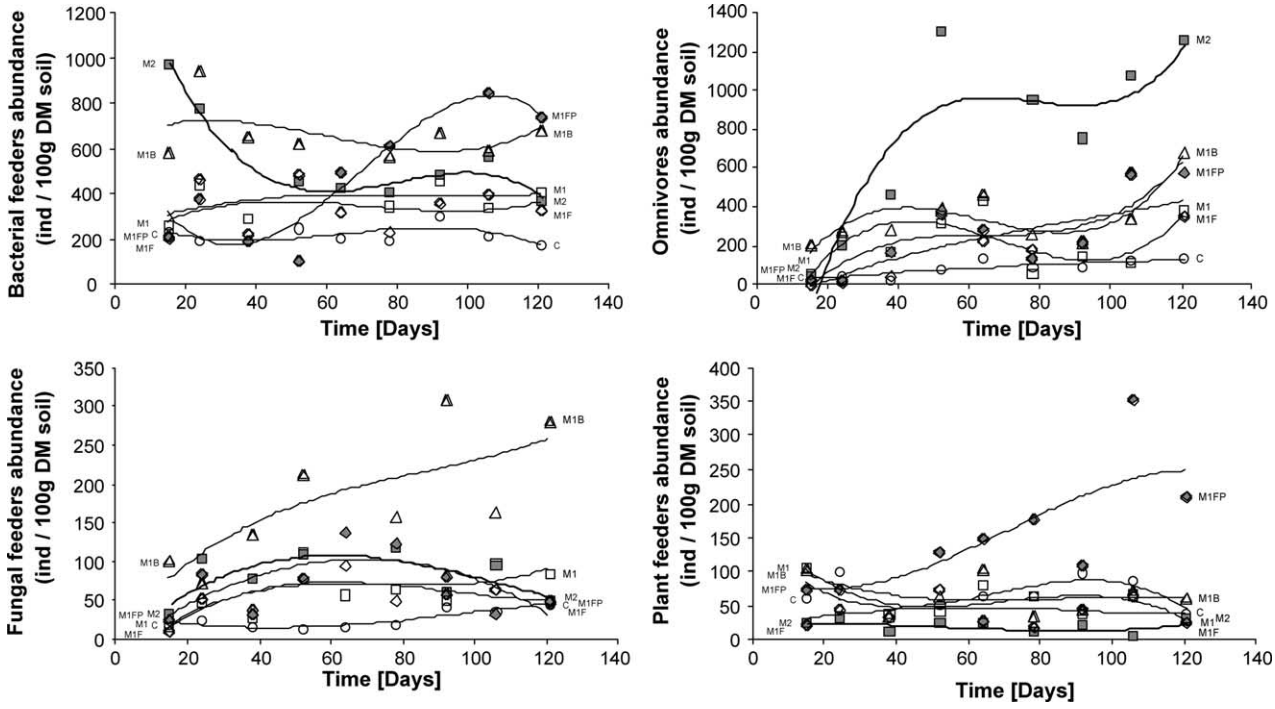

Fig. 1. Population dynamics of four different trophic groups of soil nematodes in six enrichment treatments: bacterial feeders, fungal feeders, omnivores and plant feeders. The bold line refers to the high manure dose treatment.

greater density in M1 compared with C. Tylencholaimoidea increased markedly in M2 compared to M1. The Aphelenchina density was significantly greater in M1B than in M1. Only Tylencholaimoidea increased in density in the presence of the fungicide while the density of the two other fungal feeders decreased but not significantly.

Overall plant parasite density was significantly lower with the high manure dose compared to that with the low dose (M2/M1; Table 2, $p<0.05$). No differences were observed between C and the treatments with the low manure dose, except for the treatment with millet where plant parasite density increased significantly by 273%. For the treatments without millet, the plant parasite density changed little over time (Fig. 1). In contrast, the plant parasite density increased regularly in the treatment with plants (M1FP) from day 54.

In the millet treatment, although the densities of *Helicotylenchus* and *Tylenchorhynchus* seemed to increase, only the density of *Telotylenchus* was significantly greater than without plants (M1FP/M1F; Table 2). The other treatments had no effect on the different plant parasite taxa, with the exception of M2 which led to a significant decrease in the density of *Scutellonema* compared with M1.

In overall density of omnivorous nematodes was significantly higher in M2 compared with M1 (+330%; Table 2). The difference in densities was not significant for the comparison M1/C, despite an apparent increase of 163%. On all days, the omnivorous nematode density was lower in C than in the four M1 treatments, which were also lower (from day 38) than that in M2 (Fig. 1).

The overall effect of the treatments on the predator density was significant, however no significant difference between the planned comparisons was obtained, even though predator density in M1 appeared to increase by 438% compared to C (Table 2).

The densities of all c–p classes (except c–p 4) were significantly influenced by the treatments (Table 2). Enrichment opportunists (c–p 1) were much more abundant in M1 than in C, in M1B than in M1 and in M1FP than in M1F. Also, nematodes belonging to c–p 2 were more abundant in M1 than in C and in M1B than in M1. Nematodes of class c–p 3 were more abundant in M1FP than in M1F and nematodes in class c–p 5 increased with the high manure dose.

3.3. Inorganic N and the microbial community

NO_3^- and NH_4^+ contents were significantly different between treatments (Table 2). Nitrate contents were significantly lower for M2 than M1 and for M1FP than M1F. Although manure addition seemed to lead to an increase in the ammonium content, adding manure appeared to decrease the nitrate content. Also, higher NH_4^+ and NO_3^- contents seemed to occur in M1B than in M1.

There were no significant differences in microbial biomass between treatments (Table 2). However, microbial biomass appeared to be low for C and high for M2 (+100% M2/C) and less than 10% relative difference was measured between treatments involving low manure dose. Overall ergosterol content was significantly different between

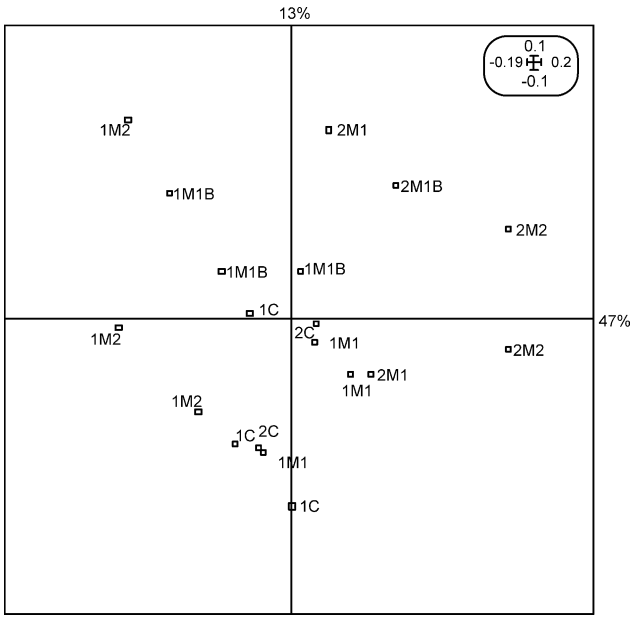

Fig. 2. PCA of the bacterial communities at day 24 (1; three replicates) and day 64 (2; two replicates) of four enrichment treatments; C, M1, M2 and M1B (C: control; M1: low manure dose; M2: high manure dose; M1B: low manure dose plus bactericide; M1F: low manure dose plus fungicide; M1FP: low manure dose plus fungicide and millet plants.).

treatments but no significant differences between planned comparisons were measured. Again, ergosterol content seemed to be low in C and high in M2 (+550% M2/C) and no differences were measured between treatments with low manure dose. The fungicide application did not decrease significantly during the experiment the ergosterol content of the soil.

In the fingerprint analysis of the bacterial communities, only one replicate for M1B (low manure dose plus bactericide) at day 64 was successfully processed until gel analysis. The ordination plot generated using the first two axes (PC1 and PC2) of the PCA of the bacterial communities on day 24 and day 64 is shown in Fig. 2. PC1 and PC2 accounted for 47 and 13% of the variability, respectively. Bacterial communities of C were very similar between the two days of analysis as were bacterial communities of M1 (except one replicate of M1 at 64 days). Changes in the bacterial communities exposed to the high manure dose (M2) and the low manure dose plus bactericide (M1B) treatments were evident on day 24 by the ordination of M2 samples on the first axis and M1B samples on the second axis compared to the control samples. Changes also occurred between the two days due to the high manure dose addition (M2) as shown by the ordination of the 1M2 samples on the left of the first axis and the 2M2 samples clustered on the right of the first axis. Due to the lack of repetition for the M1B treatment at day 64, no conclusion can be drawn regarding its longer term impact.

3.4. Relationships between soil nematofauna, inorganic N, microbial biomass and fungal biomass

Soil conditions were analysed versus the nematode c–p feeding guilds by canonical analysis (Fig. 3). The analysis included 48 sets of data, corresponding to measurements of

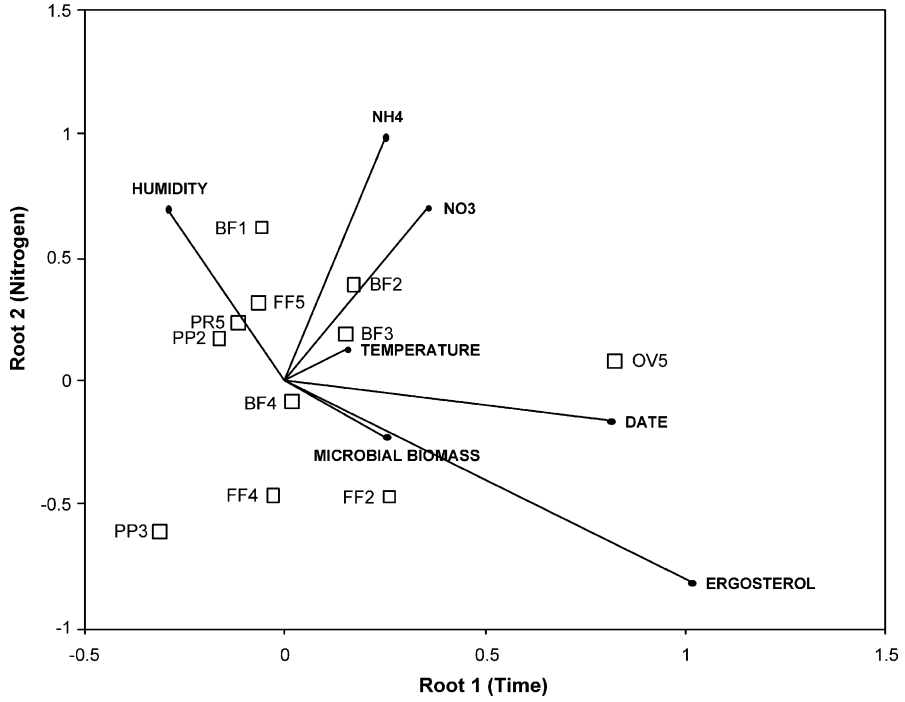

Fig. 3. Canonical analysis for soil factors versus 11 nematode feeding guilds from day 24 to day 121. BF: bacterial feeder; FF: fungal feeder; PP: plant parasite; OV: omnivore; and PR: predator. Numerals refer to coloniser–persister classes.

the different parameters for the six treatments on 8 days from day 24 to day 121. The overall model was significant, meaning that significant relationships between certain soil parameters and density of c–p feeding guilds can be concluded. The first two roots explained 29% of the total variance. Root 1 was largely related to time (day), whereas root 2 was related to N and soil water content (cross-correlated with organic matter content). Active fungal biomass (as measured by ergosterol) developed over time, independently of N levels. Microbial biomass increased less than did fungal biomass. The abundance of bacterial feeders was not correlated with microbial or fungal biomass, but was positively correlated with NH_4^+ and NO_3^- levels for several c–p classes. Bacterial feeders showed a gradient from BF1 to BF4 with BF1 being related to inorganic N content of the soil, while BF4 remained uncorrelated. Fungal feeders (FF2 and FF4) and omnivores (OV5) were associated with microbial biomass and ergosterol. Omnivores increased with time. Predators and plant parasites were largely indifferent to soil factors.

3.5. Nematode effects on inorganic N content of the soil and on microflora

The results of the multiple regressions with backward stepwise elimination of non-significant parameters are presented in Table 3. These analyses were performed to determine which particular nematode trophic groups influenced soil N dynamics and/or soil microflora. Other variables than those presented in Table 2 were tested but were removed when not significant (see methods).

NO_3^- content was negatively correlated with fungal biomass and plant parasite abundance, whereas it was positively correlated with bacterial feeder abundance. NH_4^+ decreased with time but was positively correlated to bacterial feeder abundance and fungal biomass and negatively correlated with fungal feeder abundance and soil temperature. Effects of nematodes on microbial biomass did not correspond to any particular nematode trophic group but to total nematode abundance. Microbial biomass was also negatively correlated to soil temperature and decreased with time. Fungal biomass was positively correlated with soil humidity, fungal feeder abundance and omnivore abundance and negatively correlated with predator abundance.

The positive correlations of microbial and fungal biomass with soil humidity were due to cross-correlations with organic matter content of the soil; as organic matter increases, water content at field capacity also increases.

4. Discussion

The aim of this study was to analyse modifications in the different nematode functional groups in response to changes in nutrient, energy and micro-organism availability in a soudano-sahelien soil, and also to measure the consequences on soil inorganic N content.

The fungicidal treatment had little effect on the parameters measured; most of the parameters for M1F overlapped those of M1. The fungicide was not applied at a sufficient dose to affect the soil fungi in a significant and durable manner. Addition of organic matter led to an increase in the microbial and fungal biomass, as expected (Leita et al., 1999; Villenave et al., 2002) and the total nematode density increased as a consequence (Aescht and Foissner, 1992; Ettema and Bongers, 1993). Three trophic groups were concerned: bacterial feeders, fungal feeders and omnivores. Density of omnivores increased most (+330%), essentially due to one species (*Microdorylaimus*

Table 3
Nematode effects on soil N pools and microflora: results of multiple regressions with backward stepwise elimination of non-significant parameters

Dependent parameter		NO_3^- (mg N/100 mg DM soil)	NH_4^+ (mg N/100 mg DM soil)	Microbial biomass (μg C/g DM soil)	Ergosterol (μg N/100 g DM soil)
Total model	*R*	0.49	0.80	0.74	0.82
	p	0.0000	0.0000	0.0000	0.0000
Independent parameters					
Intercept		103.40	67.29	1454.02	−7.20
Day (d)		n.s.	−0.08	−0.83	−4.97
Bacterial feeders (100 g^{-1} DM soil)		0.12	0.01	n.s.	n.s.
Plant parasites (100 g^{-1} DM soil)		−0.55	n.s.	n.s.	n.s.
Fungal feeders (100 g^{-1} DM soil)		n.s.	−0.02	n.s.	0.58
Omnivores (100 g^{-1} DM soil)		n.s.	n.s.	n.s.	0.34
Predators (100 g^{-1} DM soil)		n.s.	n.s.	n.s.	−1.48
Total nematode abundance (100 g^{-1} DM soil)		–	–	0.02	–
Soil moisture content (% DM soil)		n.s.	n.s.	6.62	44.91
Soil temperature (°C)		n.s.	−2.20	−50.62	n.s.
Microbial biomass (μg C g^{-1} DM soil)		n.s.	n.s.	–	–
Ergosterol content (μg N 100 g^{-1} DM soil)		−1.60	0.12	–	–

n.s.: not significant.

rapsus) which represented more than 90% of the total omnivore density. *Microdorylaimus* is closely related to *Eudorylaimus* which is considered a predator or omnivore (Yeates et al., 1993). However, given the small size of *M. rapsus* and its population explosion following input of the high manure dose, one can assume that these nematodes are omnivores.

When the high manure dose was applied, NO_3^- was immobilised by the increased microbial biomass. A decrease of the soil C:N ratio in response to high manure levels was likely responsible for the phenomenon of N immobilisation in the soil. In addition, the ratio of fungal feeders:bacterial feeders increased with the amount of manure added (0.10 for C, 0.16 for M1 and 0.24 for M2). Fungal biomass, as measured by ergosterol content, was very low in the natural soil (C). Manure addition led to a marked increase in the fungal biomass in M2 (M2/C: +1300%) likely due to an increase in suitable substrate for fungal development which was limited in the natural soil. Bacterial biomass, evaluated as the difference between microbial biomass and fungal biomass, was 93% in C, between 70 and 80% in the treatments with low manure dose and 55% in M2.

The main effect of the presence of millet was the predictable increase in the plant-feeding nematodes. However, increases in densities of some bacterial feeders were also measured, i.e. *Zeldia* and, to a lower extent, Rhabditidae. This demonstrated the attraction of these nematodes to rhizospheres where abundant microflora occurred, and bacterial feeders developed by exploiting rhizodepositions and root exudates (Griffiths, 1990; Griffiths et al., 1992; Djigal et al., 2004b). For all treatments without plants, plant-feeding nematodes survived for 4 months in the absence of any vegetation. No decrease in plant parasite density was observed, with the exception of the M2 treatment. A decrease in plant-feeding nematodes following high manure input has previously been observed and was mainly related to the presence of deleterious organic compounds (Freckman and Ettema, 1993; Villenave et al., 1998; Griffiths et al., 1994).

The high manure dose led to a high increase in bacterial-feeding nematodes at the start of the experiment, principally because of an explosive increase of the Rhabditidae. Indeed, the density of Rhabditidae in M2 on day 15 was 402 Ind 100 g^{-1} soil, then decreased to 50 Ind 100 g^{-1} soil until the end of the experiment (results not shown). Ettema and Bongers (1993) also showed a great increase in these opportunist Rhabditids following manure addition that lasted 18 weeks. They represented still 29% of total nematode numbers 18 weeks after organic input. In another study in Senegal, Villenave et al. (2002) found that organic matter input to millet fields led to a significant increase in the density of Rhabditidae for 3 months but that these nematodes remained a minority taxon (less than 4%). It seems that under the pedo-climatic conditions of Senegal, the flush of opportunist nematodes following organic matter input appeared to be extremely rapid (14 days) and did not last.

A very high density of Rhabditidae was also measured when bactericide was added: 225 Ind 100 g^{-1} soil after 24 days and, contrary to M2, this density remained until day 64. Bactericide addition did not lead to any significant effect on omnivores, plant feeders and predators but caused an increase in abundance of the opportunistic nematodes (bacterial as well as fungal feeders of mainly c–p 2 but also c–p 1). The increase in fungal-feeder density following bactericide application was predictable, however, we did not anticipate that it would be coupled with an increase in bacterial feeders.

The efficacy of the bactericide should be discussed since the initial application did not significantly reduce the microbial biomass during the experiment. However, soil recolonisation by the bacterial community was expected since the durability of the bactericide was supposed to be very short under non-sterile conditions. While no significant difference in microbial biomass between the low manure dose and the low manure dose plus bactericide treatments was measured, the M1B treatment had complex consequences on the soil nematofauna which lasted at least 4 months and led to a different nematofauna than that of the M1 treatment. It is likely that the increase in density of bacterial feeders was linked to a modification of the microbial community structure following initial partial destruction of microflora. Indeed bacterial-feeding nematodes show distinct food preferences and select the bacteria that they ingest (Schiemer, 1983; Venette and Ferris, 1998; Djigal et al., 2004a). The ARISA analysis tended to show that the structure of the soil microbial community was rapidly modified following both the high manure dose input and the bactericide application. Differences between bacterial communities between low manure dose and low manure dose plus bactericide treatments were clear 24 days after the beginning of the experiment. We assume that qualitative modification of the microbial communities could explain the differential development of nematode populations and that such processes should be investigated in further studies.

Microbial biomass was not directly determined by the nematode trophic groups nor feeding guilds, given that the only significant parameter found was the abundance of the entire nematofauna. These results confirm the concept that nematode excretion contributes to the stimulation of soil micro-organisms independently of type of nematode functional group (Ekschmitt et al., 1999; Ruess et al., 2001).

In contrast, inorganic N content of the soil was related to the particular nematode feeding guild. Canonical analysis showed correlations between bacterial feeders of classes c–p 1 and c–p 2 and soil inorganic N content. Also, there was a kind of gradient with regard to the correlation between N mineral content and the different c–p categories of bacterial feeders (BF1, BF2, BF3 and BF4) with BF4 not being correlated to N levels. Moreover, bacterial-feeding

nematode abundance (all c–p classes combined) was positively related to NO_3^- and NH_4^+ contents as measured by multiple regression. Abundance of fungal feeders was negatively correlated with NH_4^+ but not correlated with NO_3^-. Plant-feeder density was also correlated with NO_3^-, but this negative correlation seemed to be due to cross-correlation with plant growth; as the plants grew, they removed N and plant parasite density increased.

Our results showed that under tropical conditions in a poor sandy soil, microbivorous nematodes and particularly opportunistic bacterial feeders were involved in the N cycling. By studying the spatio-temporal distribution of bacterial-feeding nematodes in a wetland, Ettema et al. (1998) found that the highest densities of *Acrobeloides* (c–p 2) and Rhabditinae (c–p 1) corresponded to high concentrations in nitrate. However, they were not able to determine whether nitrate content was the consequence or the cause of these high densities of nematodes. This was also the case in our study. Thus, the relationship between availability of mineral N in the soil and densities of bacterial feeders of classes c–p 1 and 2 should be further evaluated under field situations.

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