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ScienceDirect
European Journal of Soil Biology 42 (2006) S70–S78
EUROPEAN JOURNAL OF SOIL BIOLOGY
http://france.elsevier.com/direct/ejsobi

Original article

Nutrition on bacteria by bacterial-feeding nematodes and consequences on the structure of soil bacterial community

C. Blanc[a], M. Sy[b], D. Djigal[b], A. Brauman[b], P. Normand[a], C. Villenave[a,*,b]

[a] *Écologie Microbienne UMR-CNRS 5557, Université Claude-Bernard Lyon 1, 43, boulevard du 11 novembre 1918, F-69622 Villeurbanne cedex, France*
[b] *UR IBIS 083, IRD, BP 1386, Dakar, Senegal*

Available online 14 July 2006

Abstract

Bacterial-feeding nematodes are, with protozoa, the main grazers of soil bacteria. Interactions between bacteria and nematodes have important repercussions on soil functioning and particularly on nutrient availability. We assessed the influence of the bacterial strains ingested on bacterial-feeding nematodes population development and also the consequences of nematode feeding behaviour on the structure of the soil microbial community with a special attention to different soil micro-habitats for nematode and bacteria. In vivo studies conducted in the presence of single bacterial strains showed that the type of ingested bacteria conditioned the development of the different bacterial-feeding Cephalobidae nematode species tested and that the effect of bacteria differed between nematode species. The spatial distribution of soil nematodes between three soil habitats (fresh organic matter, inter-aggregates pores and aggregates) depended of the trophic behaviour of nematodes. Bacterial-feeding nematodes and fungal-feeding nematodes showed comparable distribution: being preferentially located in the fresh organic matter and in the inter-aggregate pores. Besides, the activity of inoculated bacterial-feeding nematodes modified the genetic structure of the soil microbial community. Bacterial community of the macroporosity was significantly influenced by the nematodes. On the contrary, no modification of the structure of the bacterial community linked with nematode activity was measured in the bulk soil.

Keywords: Bacteria; Soil; Nematodes; Genetic structure of bacterial community; Soil habitats

1. Introduction

Nematodes are the most abundant multi-cellular organisms in soils with densities ranging from 1 to 10 millions individuals per m^2 [17]. They present divers alimentary behaviour, some are plant-feeders, others are bacterial-feeders or fungal-feeders; the two other main types are omnivores which can use different resources and predators who consume the other nematodes [31]. The determination of the trophic behaviour is based (1) on morphological criteria, cephalic and oesophagous morphology, (2) observation of intestinal content and (3) breeding experiments in simplified environment (nutrient agar for example). However if one can prove that a nematode can multiply on a certain resource, a failure of breeding does not obligatory mean that the resource is not exploitable as the failure can be the consequence of unsatisfactory experimental conditions (humidity, temperature, resources concentration...). Moreover, breedings in laboratory have been obtained for a very limited number of species considering the huge numbers of described species [17,31]. Several bacterial-feeding nematode breeding experiments in

* Corresponding author. Fax: +33 4 72 43 12 23.
E-mail address: villenav@biomserv.univ-lyon1.fr (C. Villenave).

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doi:10.1016/j.ejsobi.2006.06.003

the presence of a single bacterial population have shown that the rates of reproduction of nematodes differed according to the ingested bacterium or that significant differences between nematodes existed in bacterial prey that they were attracted to [27] and review in it. The finding that nematodes exhibited various preferences for microbial prey is an important observation because it suggests that they are apparently not generalist feeders as has been previously assumed [8]. Selective feeding by nematodes on microbial prey may alter the outcome of inter-specific competition between microbes and may have implications on microbial community composition and distribution in soil [9,5].

Furthermore, the physical nature of soil means that movement and meeting of organisms is severely constrained. The majority of soil organisms has a non-homogeneous repartition in soil, several micro-habitats have been described and can be isolated from soil samples [12]. The location of soil organisms determined possible interactions between them, and this criterion is particularly important for interactions between nematodes and bacteria. Soil nematode activity is restricted to water films and water filled pores larger than 10 μm of diameter, consequently bacteria located in small pores of micro-aggregates are protected from grazing [17].

When populations of bacterial-feeding nematode have high density or increase in number in soil, the bacterial populations are heavily grazed by bacteriophageous nematode. As a matter of fact an adult bacterial-feeder ingests up to 10^6 bacterial cells a day. Grazing stimulates mineralisation of nutrients and is likely to affect the structures of bacterial communities as result of selecting grazing but also as indirect effect [5,22].

The aim of our experiment was to determine (1) population development of three Cephalobidae species feed with six different bacterial strains in nutrient agar, (2) the localisation of soil nematodes in the different soil fraction and, finally, (3) the effect of two selected bacterial-feeding nematodes on the structure of the microbial community of the whole soil and of the fraction linked with soil macroporosity.

2. Material and methods

2.1. Influence of various bacteria on nematode fecundity and population growth

Four nematodes, isolated from Thysse-Kaymor, Sénégal, belonging to the family of Cephalobidae were selected for this study: *Cephalobus pseudoparvus*, *Zeldia punctata*, *Acrobeloides nanus* morphotype 1 and *A. nanus* morphotype 2. Two populations of *A. nanus*, differing by cephalic and tail morphology, were studied to estimate intra-specific differences. Six bacterial strains representative of the bacterial genera commonly found in soil were selected, based on morphology (rods, cocci or filaments), cell wall characteristics (gram-positive or gram-negative) and possible protection against predation such as mucus or spore production or motility: origin of these five following bacteria is precised in Djigal et al. [6]: *Actinomyces* sp (filamentous, 2–5 × 0.5 μm, gram+), *Bradyrhizobium* sp (rods, 1–1.5 × 0.5 μm gram– mucus producers), *Methylobacterium nodulans* (coccoides 1, 2 × 1 μm gram– mobiles), *Paenibacillus polymyxa* (incurved rods, 2 × 1 μm, gram+, produce spores) and *Pseudomonas monteilii* (rods, 1–2.5 × 0.5, gram–, mobiles). The last bacteria is *Arthrobacter* sp (young cultures: rods, 1–1.5 × 0.3, gram–; old cultures: coccoides, 1 × 0.5 μm, gram+); the strain is the ORS 3003/BOS1; it as been isolated from French soil. Bacterial strains were cultured on specific growing media as described in Djigal et al. [6]. The media for *Arthrobacter* sp was: agar: 1.5%, yeast extract: 0.35%, glucose: 0.5%.

For each bacterial isolate, 15 replicates of nutrient agar amended with cholesterol (5 μg l^{-1}) were inoculated with 10 μl of a turbid bacterial suspension (> 10^8 cells ml^{-1}) in plastic Petri dishes (60 × 15 mm). Dishes were incubated at 28 °C until a bacterial lawn was established, i.e. for 24 hours, except 72 hours for *Bradyrhizobium* (13 °C). Eggs from stock cultures of the different nematodes species were sterilised and one newly hatched juvenile was aseptically transferred to each Petri dish, considered as the founder female. Dishes were then sealed and incubated in the dark at a constant temperature (28 °C).

The total number of eggs and individuals at each life stage (juvenile, reproductive adult or non-reproductive adult) were determined under dissecting microscope daily. As offspring approached adulthood, the founder female was transferred to a new Pétri dish seeded with bacterial substrates. After the final counting, nematodes were rinsed from the surface of each Petri dish using 2 ml of sterile, deionised water. Nematodes from the 15 replicates were pooled and fixed with hot formaline solution (mixture of 4% formalin, 1% propionic acid and 1% glycerine) before mounting on slides. The length of 60 adults was measured per bacterial treatment.

2.2. Distribution of nematodes among soil fractions

Five cubes (10 × 10 × 10 cm) of undisturbed soil were extracted in a natural fallow (Thyssé-Kaymor, Sénégal). Soil fractionation was carried out after incubation of the undisturbed soil samples during 8 days at the field capacity at 28 °C. An undisturbed part of about 150 g of each cube was submerged in water to fill pores larger than 30 μm in diameter and kept at 4 °C for 24 h without agitation. Then, macropore water was emptied by gravity and the sample was then gently fractionated by hand and by wet sieving and sedimentation [3]. The different fractions were over 2000; 200–2000; 50–200 μm; 0–50 μm. The weight of each habitat was determined on an oven-dried (80 °C) basis.

Nematodes were extracted from habitats larger than 50: 50–200, 200–2000, >2000 μm by the elutriation-sieving technique and from fresh organic matter and macropore water by active passage through filter [24]. Nematodes collected from the fraction 50–200 μm are nematodes that actually resided within the inter-aggregate pores from 50 to about 300 μm in diameter [19]. Nematodes were counted and fixed with hot formalin solution. From each sample, a mean of 1098 nematodes (total of the different fractions) was identified to family or genus level under an inverted microscope. Nematode taxa were assigned to trophic groups following Yeates et al. [31].

2.3. Effect of nematode activity on the genetic structure of the bacterial community

Soil used in this experiment originated from the 0 to 10 cm layer (17.1% clay; 22.1% loam; 60.8% sand; ph 7; CEC 64 meq kg^{-1}) of a maize field located at La Côte St André, France. The indigeneous nematode community was eliminated using five freezing and thawing cycles (7 days at −18 °C and 4 days at room temperature) of the wet soil (i.e. 15.5 g H_2O 100 g^{-1} soil). Fifty grams of wet soil (15.5%) were deposited in flask (125 ml) closed with parafilm and placed during 3 weeks in an incubator (28 °C). Four treatments, realised in three replicates (three microcosms) were compared: (T) control, soil not inoculated with nematodes, (C) soil inoculated with *Cephalobus* sp. (Cephalobidae), (P) soil inoculated with *Poikilolaimus oxycerca* (Rhabditidae), and (C + P) soil inoculated with the two nematodes species. Nematodes have been previously extracted from the same soil and bred in nutrient agar supplemented with cholesterol (4 g l^{-1} of TSB, 15 g l^{-1} of agarose, 5 mg l^{-1} of cholesterol) with the bacterial strain *Escherichia coli* DH10B. Nematodes were inoculated at a density of 23 ± 5 g^{-1} dry soil for Cephalobidae and 17 ± 3 g^{-1} dry soil for Rhabditidae. Humidity was fitted to 21 g H_2O for 100 g of dry soil, humidity close to, but bellow, the field capacity. After 3 weeks of incubation at 28 °C, nematodes were extracted from 35 g of soil of each microcosm using Cobb's decanting and sieving method (modified according to s'Jacobb and van Bezooijen [23] followed by sugar centrifugation and enumerated under binocular microscope (×40).

Ten grams of soil were treated to obtain the inner and the outer part of soil aggregates (modified from [13]). The soil was transferred from the microcosms into 50 ml of sterile 0.8% NaCl solution in a 250 ml Erlenmeyer flask. After gentle shaking on a gyratory shaker (100 rotation min^{-1}) for 1 min, soil suspensions were kept still for 1 min to allow soil particles to settle down. The supernatant was then carefully transferred to a sterile centrifuge tube. Fifteen successive washings were done. The supernatants containing microorganisms released from the outer part of soil aggregates, were pooled and centrifuged at 9800 × *g* for 20 min at 20 °C.

Three DNA extractions (MO BIO kit Ultra Clean(TM) Soil DNA extraction) were realised on the outer part of soil aggregate and the total soil on 0.25 g soil for each microcosm. The structure of the bacterial communities was studied by the automated ribosomal intergenic spacer analysis (ARISA). The *rrs-rrl* intergenic spacer region was amplified by PCR using primers S-D-Bact-1522-b-s-20 (small eubacterial ribosomic subunit 5′TGCGGCTGGATCCC CTCCTT-3′) and L-D-Bact-132-a-A-18 [18]. PCR amplification was done as described by Ranjard et al. [20]. Fragments were resolved by using the Mega-BACE sequencer (Amersham-Pharmacia, Orsay, France), the samples were run under denaturing conditions at 44 °C, for 3.5 h at 6000 V. The data were analysed by the Genotype Profiler software program (Amersham-Pharmacia). 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.

2.4. Statistical analysis of the data

Comparisons of means between bacterial treatments for generation time, nematode length, and between localisation of the nematode feeding group and fraction

were carried out using analysis of variance. Fisher PLSD tests ($P < 0.05$) were used to compare means.

The matrix describing the genetic structures of the microbial community was subjected to a principal component analysis (PCA) of covariance using ADE-4 software [25]. 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. A permutation test ($N = 1000$) was used to discriminate between treatments.

3. Results

3.1. Population development of the nematodes in nutrient agar

All the tested nematodes did not produce reproductive offsprings with the *Actinomyces* sp. (Table 1). Similarly, the rate of nematodes producing reproductive offspring was lower with *Arthrobacter* sp. than with the other bacteria (with the exception of *A. nanus* 2). On the other hand, *P. polymyxa*, *P. monteilii*, *Bradyrhizobium* sp. and *Methylobacterium nodulens* allow for more than 75% of replicates the development of reproductive offsprings (with the exception of *Bradyrhizobium* sp., 58%). The weakest generation time was obtained when the four nematodes were fed with *P. monteilli*. On the contrary, the longest generation times were not obtained with the same bacteria for all the nematodes: it was *P. polymyxa* for *A. nanus* 1 and *A. nanus* 2 (if one omits *Arthrobacter* sp.), *M. nodulens* for *C. pseudoparvus*, and *Bradyrhizbium* sp. for *Z. punctata*. Also, average length of adults depended of the ingested bacteria. If the weakest length was always obtained with *P. polymyxa* whatever the nematode, on the contrary the greatest lengths were obtained with different bacteria for each of the nematodes. *A. nanus* 1, *A. nanus* 2 and *Z. punctata* have highest numbers of offspring when they were fed with *M. nodulens* while *C. pseudoparvus* obtained highest number of offsprings with *P. polymyxa* (Fig. 1). In the presence of *Arthrobacter* sp. and *Bradirhizobium* sp., offspring numbers were very low whatever the nematode species.

3.2. Distribution of nematodes among soil fractions

The soil fraction corresponding to inter-aggregates pores represented 15.7% of the total weight of soil. The figurative organic matter represented only 0.2%

Table 1
Fecundity and growth characteristics of four nematodes in nutrient agar ($N = 15$) with six bacterial strains as food source

	Bacterial inoculum	Percent of feeder females having reproductive offspring	Generation time (days)		Mean adult length (mm)	
A. nanus 1	Actinomyces sp.	0	na		na	
	Arthrobacter sp.	50	na		na	
	P. polymyxa	75	15.8	c[a]	0.43	a
	P. monteilii	100	7.4	a	0.54	bc
	Bradyrhizobium sp.	92	7.8	a	0.53	b
	M. nodulens	100	10.3	b	0.55	c
A. nanus 2	Actinomyces sp.	0	na		na	
	Arthrobacter sp.	100	10.6	b	na	
	P. polymyxa	80	8	a	0.5	a
	P. monteilii	100	7.4	a	0.5	a
	Bradyrhizobium sp.	100	7.6	a	0.5	a
	M. nodulens	80	7.5	a	0.6	b
C. pseudoparvus	Actinomyces sp.	0	na		na	
	Arthrobacter sp.	69	na		na	
	P. polymyxa	100	6.8	c	0.36	a
	P. monteilii	93	4.9	a	0.44	b
	Bradyrhizobium sp.	93	6.1	b	0.48	c
	M. nodulens	100	7.4	d	0.44	b
Z. punctata	Actinomyces sp.	0	na		na	
	Arthrobacter sp.	27	na		na	
	P. polymyxa	87	12.8	b	0.80	a
	P. monteilii	87	10.4	a	0.90	b
	Bradyrhizobium sp.	58	14.8	c	0.89	b
	M. nodulens	100	10.2	a	0.90	b

na: not applicable since no nematode developed.

[a] Different letters for generation time and length indicate significant differences between bacterial inoculi at $P < 0.05$.

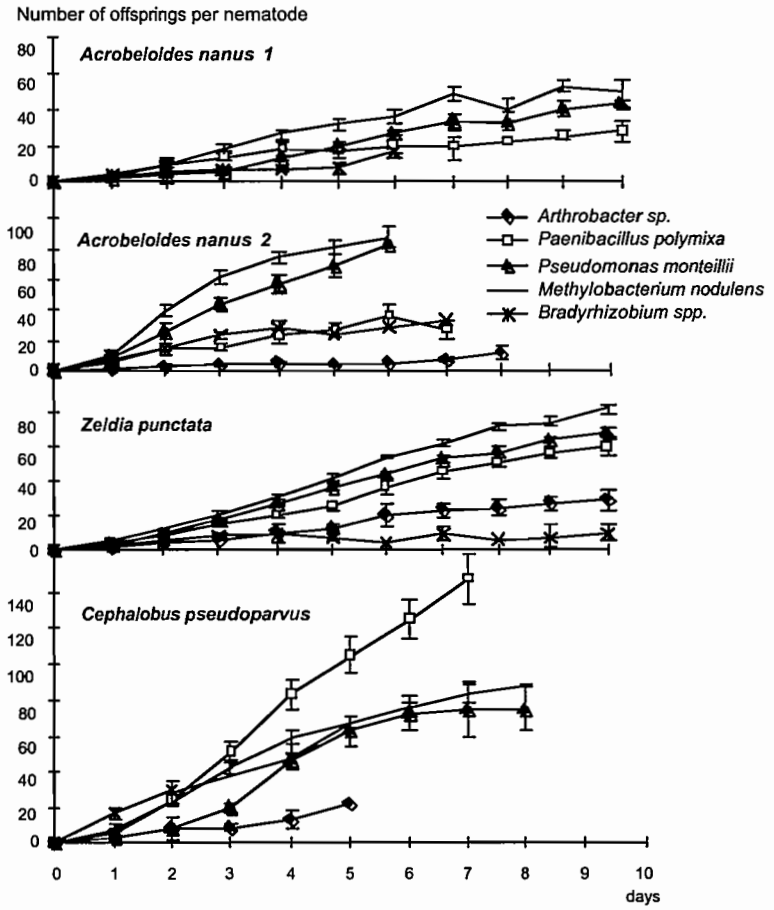

Fig. 1. Number of offsprings for the four nematodes grown with different bacterial stains as food source. T0 represents the day before the first egg hatching. Bars are standard errors.

of the sample, aggregates contributed to 84.1% of the total weight of soil. Bacterial-feeding nematodes were essentially localised in inter-aggregate pores. Indeed more than 50% of these nematodes were found outside aggregates (Table 2). An important proportion of these nematodes were localised in fresh organic matter (24%). A relatively similar distribution was observed for fungal-feeding nematodes, which were very abundant in fresh organic matter. The other trophic groups presented slightly different distributions: plant-feeders and Tylenchidae, in particular, has more than 50% of their total number in aggregates > 200 μm. Predators and omnivores have a significant lower relative abundance than fungal-feeders and bacterial-feeders in fresh organic matter and lower relative abundance than plant-feeders in aggregates, they were essentially localised in inter-aggregate pores. The density of bacterial-feeding nematodes was 17 times higher g^{-1} of soil of the outer

Table 2
Distribution of the different feeding groups of soil nematode between three soil fractions (in % of the total nematode number in the soil sample)

	Aggregates > 200 μm		Inter-aggregate pores		Fresh organic matter	
Bacterial feeders	24.0	b[a] A[b]	53.3	a B	22.6	b A
Fungal feeders	33.2	b A	40.7	a A	26.2	b A
Root-hair feeders (Tylenchidae)	59.8	c B	36.9	a B	3.3	a A
Plant-feeders	51.4	c B	38.7	a B	9.9	a A
Predators	24.9	b B	74.0	b C	1.1	a A
Omnivores	0.0	a A	93.0	b B	7.0	a A

[a] Different lower case letters indicate a significant difference in a column.
[b] Different upper case letters indicate a significant difference in a line.

part of soil aggregates (e.g. in inter-aggregate pores and in fresh organic matter) than in the inner part.

3.3. Effect of nematode activity on the genetic structure of the bacterial community

Final nematode densities were, respectively, 9.1 ± 6.2 g^{-1} dry soil (mean ± standard error) for *Cephalobus* sp., 16.4 ± 0.1 for *P. oxycerca*. When the two nematodes were inoculated simultaneously, final density were 11.5 ± 1.3 and 9.7 ± 0.1, respectively, for *Cephalobus* sp. and for *P. oxycerca*. The outer part of the soil represented $21.0\% \pm 3.3\%$ (standard error) of the total soil for treatment T, $27.3\% \pm 1.8\%$ for C, $21.0\% \pm 2.6\%$ for P and $23.7\% \pm 2.6\%$ for C + P, there was no significant differences between treatments.

Genetic profiles of bacterial communities for the whole soil and the outer part of the soil aggregate were complex, containing from 70 to 250 fragments from 250 pairs of bases to 1150 pairs of bases. For every microcosm, three independent analyses (three soil samples for DNA extraction, three PCR and three A-Risa) have been realised, the PCA of the structure of the microbial community for the total soil on one hand and for the outer part on the other hand in the different microcosms showed the independence of the intra-microcosm analyses (test of permutation not significant; data not shown). Consequently, the 72 samples (36 for the total soil 36 for the outer part of aggregates) have been considered as independent replicates in the following analysis.

The structure of the microbial community of the outer part of soil aggregates was different from that of the total soil (Fig. 2); the two clouds of points separated on the main plan of the PCA mainly on the axis 1 which explained 14% of the total variability while the axis 2 explained 9%. Differences between microbial community of the outer part of soil aggregate and the total soil are developed in Blanc et al. (in prep).

Three weeks of activity of nematodes did not lead to significant modification of the structure of the microbial community of the total soil: treatments did not separate on the main plan of the PCA; the permutation test was not significant (P H0 > 0.05). On the other hand, the microbial community of the outer part of the soil aggregates was significantly affected by nematodes. The microbial communities of four nematode treatments separated on the main factorial plan of the PCA (Fig. 3). Samples inoculated with Cephalobidae (C or C + P) are situated globally positively on the axis 1 which explains 18% of the total variability. On the contrary samples inoculated only with P take place nega-

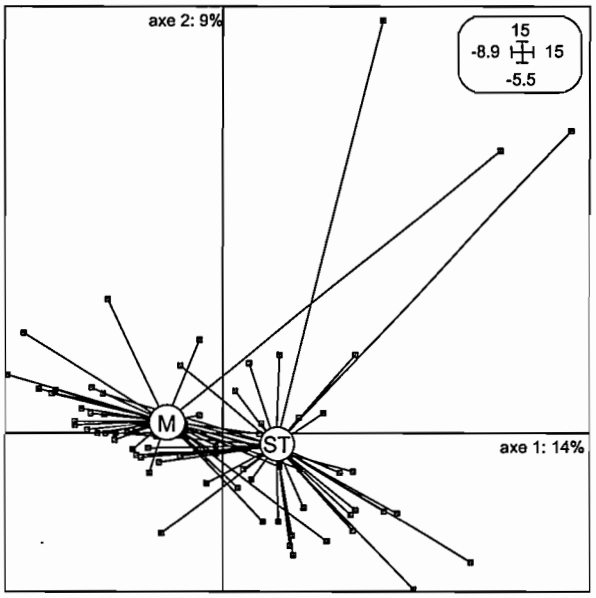

Fig. 2. Ordination of bacterial communities of the two soil fractions: the total soil and of the outer part of soil aggregates. In the plan defined by the axis 1 and axis 2 of the PCA; result of permutation test: P H0 < 0.005.
(M) outer part of soil aggregates (Macroporosity); (ST) total soil.
For each soil fraction, points are linked to the gravity centre of the clouds.

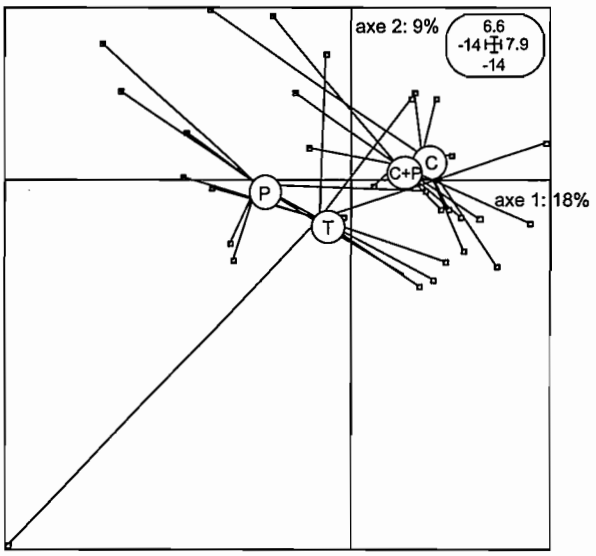

Fig. 3. Ordination of bacterial communities of the outer part of soil aggregates for the four nematode treatments in the plan defined by the axis 1 and axis 2 of the PCA; result of permutation test P H0 < 0.005.
(T) Control soil not inoculated with nematodes. (C) soil inoculated with *Cephalobus* sp. (Cephalobidae). (P) soil inoculated with *P. oxycerca* (Rhabditidae) and (C + P) soil inoculated with the two nematodes species.
For each treatment, points are linked to the gravity centre of the clouds.

tively on the axis 1. Control samples corresponding take place negatively on the axis 2, which explains 9% of the total variability.

4. Discussion

Population developments of each nematode species were different according to the prey bacteria. The tested nematodes did not multiply in the presence of the filamentous bacteria *Actinomyces* sp. and of the gram-positive bacteria *Arthrobacter* sp. (with the exception of the strain 2 of *A. nanus*). These results are in agreement with other works which showed that, large and filamentous bacterial cells can escape uptake by some nematodes has a result of their small buccal cavity [26, 27]. As regards aquatic and soil protozoa, size-selective predation has often been demonstrated, protozoa prefer middle class bacteria [11,22]. Moreover, gram-positive bacteria may be less suitable protozoan and nematode food than gram-negative bacteria. The lower edibility of gram-positive bacteria may be related to a lower rate of digestion of the gram-positive cell wall, which may enable survival during passage through microbial grazers [22,27]. However the other gram-positive bacteria used in our experience *P. polymyxa* allowed a good multiplication of all the nematodes and was even the bacteria that allowed the highest population development for *C. pseudoparvus*. In our study, all the bacteria attracted the nematodes which remained localised in the bacterial lawn, with the exception of *P. polymyxa* in the presence of whom nematodes moved indifferently on the Petri dishes. If *Bacillus* sp. did not attract *A. nanus* in the experiment of Anderson and Coleman [1], it did not allow either a good development of the population. On the other hand, in this study, *Arthro bacter* sp. led to a good reproduction of the nematodes.

The development of nematodes depends on ingested bacteria, and, additionally, experimental data suggest that some nematode species can distinguish between and preferentially consume different strains of bacteria, the preference differing between even closely related nematode species [14,16]. However, the same confrontations of monospecific populations of a nematodes and a bacterial strain did not lead to the same results in the soil that in nutrient agar. In an additional experiment, we showed that the bacteria that produced mucus, *Bradyrhizobium* sp. did not allow a good development of *Z. punctuata* in nutrient agar media but allowed it in the soil where the mucus production must be lower because of the limitation of resource availability for the bacteria [6].

The soil is a structured and heterogeneous environment, the localisation of nematodes and bacteria condition their meeting possibilities. A large proportion of bacterial-feeding nematodes as well as fungal-feeding nematodes were found in fresh organic matter. The soil used in this study originated from a natural fallow rich in organic matter compared with most of the cultivated soils. If one omits this fresh organic fraction, we found more than 75% of bacterial-feeding nematodes localised in inter-aggregates pores and, less than 25% localised in aggregates higher than 200 μm in diameter. This work confirms results obtained by Quénéhervé and Chotte [19] by the same method and specifies the distribution among main fractions for the different nematode trophic groups. Other studies have shown that the abundance of nematodes is correlated with soil porosity [12,15]. Indeed, nematodes have not entry to pores smaller than their own diameter 10–50 μm. Consequently, soil nematodes can not feed directly on microbes in, or protected by, pores narrower than 10 μm. Yeates et al.'s [32] work show that it is not the volume of pore of diameter higher than 20 μm filled with water which explains distribution and activity of nematodes but a parameter more difficult to measure which is the surface of particles of soil, in porosity larger than 10 μm, covered with a film of water. As a consequence, nematode activity is possible in a wide range of humidity of the soil even when the pore < 20 μm are not filled with water. A thin film of water on soil particle is sufficient to allow nematode activity. Furthermore, nematode movement and feeding on bacteria may even be more efficient in thin rather that thick water films.

Considering these results, we estimate that the largest proportion of the bacterial-feeding nematodes inoculated in the soil concentrated in the outer part of the soil aggregates as we collected it.

Nematode activity (at a density of about 10 individual g^{-1} of dry soil during 21 days) led to modifications of the structure of the microbial community of a particular soil fraction: the outer part of the soil whereas changes were not significant at the scale of the total soil. Nematodes mainly and directly affected bacteria present in their influence area and these bacteria do not represent the majority of total soil bacterial community [21].

The effects of bacterial predator on microbial community are the resultant of several mechanisms which can or not apply at the same time (1) selective feeding by the predator, (2) difference in sensibility of bacteria to predation and (3) indirect effects linked with the conditions of growth of bacterial populations (e.g. nutrient

and substrate availability). As a matter of fact even in absence of selective feeding, grazing may favour bacteria with high growth rates because they will be able to replace cells lost by predation [10]. Grazing may also affect microbial community structure indirectly as bacterial number decrease and nutrients are mineralised; hence the competition for substrates and limiting nutrients is reduced.

Changes of the structure of the microbial community by microbial grazers has been shown by Djigal et al. and Griffiths et al. [5,9] but these studies did not identified the bacteria taxa which were affected by the grazers. Rønn et al. [22] found that the effect of protozoa on soil bacterial communities was different according to the feeding ecology of the flagellates. And, furthermore, they showed that high-G + C gram-positive bacteria related to Arthrobacter appeared to be favoured by grazing. In a study in sediments, De Mesel et al. [4] also found that the composition and the relative importance of different members of the pool of bacteria was severely modified by the grazing activities of the bacterivorous nematodes, even at relatively low nematode densities. Moreover they suggested that the effect of nematode on the bacterial community composition was dependent of nematode feeding behaviour. Modifications of the microbial community obtained in treatment inoculated with Monhysterids were explained by species-specific food preferences whereas other mechanisms must explain effects of *Panagrolaimus paetzolti* as it is supposed to feed unselectively on the bacterial community [4].

The two types of bacterial-feeding nematodes inoculated in the soil experiment are particularly important in soil ecosystems. Indeed, enrichment opportunists (Ba1 according to Bongers's classification [2]) of these main families: Rhabditidae, Panagrolaimidae, Diplogasteridae can increase drastically in density further to an enrichment of the environment [7,29]. General opportunists and particularly Cephalobidae (Ba2) are very common and generally the most abundant bacterial-feeding nematode of the soil [30]. While under enriched conditions such as fertiliser or manure input in soil or breeding on nutrient agar, Rhabditids multiply more than Cephalobidae [1,7,29]. However on the long term and in limited resources conditions as in natural soil, Cephalobidae may present higher populations than enrichment opportunists [28,32]. In our experiment when Cephalobidae and Rhabditidae were inoculated simultaneously the final density of Rhabditidea was significantly lower than when it was inoculated alone. Moreover, *Cephalobus* sp. tended to lead to more intense modifications of the structure of the microbial community than *P. oxycerca* with our experimental conditions. Furthermore, when the two nematodes were inoculated simultaneously, the effect of *Cephalobus* sp. dominated. Direct observations of Rhabditidae and Panagrolaimidae indicate that these nematodes may consume food particles non-selectively at high rates as they show a constant pumping activity of the oesophageous. Within the general opportunists as Cephalobidae, bacterial selection could occur at the level of food selection.

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