Applied Soil Ecology 52 (2012) 1–8

Contents lists available at SciVerse ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

How earthworm activities affect banana plant response to nematodes parasitism

G. Loranger-Merciris[a,*], Y.-M. Cabidoche[b], B. Deloné[b], P. Quénéhervé[c], H. Ozier-Lafontaine[b]

[a] Université des Antilles et de la Guyane/INRA, UR1321, ASTRO Agrosystèmes tropicaux, F-97170 Petit-Bourg (Guadeloupe), France
[b] INRA, UR1321, ASTRO Agrosystèmes tropicaux, F-97170 Petit-Bourg (Guadeloupe), France
[c] IRD, UMR 186 Résistance des plantes aux bioagresseurs, Pôle de Recherche Agroenvironnementale de la Martinique, BP 214, 97232 Le Lamentin Cedex, Martinique, France

ARTICLE INFO

Article history:
Received 19 February 2011
Received in revised form 11 October 2011
Accepted 12 October 2011

Keywords:
Phosphorus uptake
Pontoscolex corethrurus
Radopholus similis
Pratylenchus coffeae
Helicotylenchus multicinctus
Pore size distribution

ABSTRACT

The burrowing nematode *Radopholus similis* and the lesion nematodes *Pratylenchus* spp. are considered as major constraints to banana cultivation worldwide. As an alternative to the use of chemical pesticides in commercial banana plantations, the possible role of the earthworm *Pontoscolex corethrurus* was studied regarding the reducing harmfulness of the main plant-feeding nematodes of banana. The interactions among banana plants, plant-feeding nematodes and earthworms have been tested in a controlled experiment combining treatments including or not earthworms and nematodes, over a complete vegetative banana development cycle. The aim of the work was to evaluate how earthworm activity can affect the pathogenicity of the three main plant-feeding nematodes associated with dessert banana (*Musa* spp. AAA group, cv. Cavendish) in Guadeloupe (French West Indies), *R. similis*, *Pratylenchus coffeae* and *Helicotylenchus multicinctus*.

Results showed that damages caused by plant-feeding nematodes were lowered in the presence of the earthworm *P. corethrurus*; the population of plant-feeding nematodes did not decrease whereas root damage induced by nematodes was significantly reduced (50% reduction of root necrosis in the presence of earthworms). Moreover, in the presence of *P. corethrurus*, we observed a change of soil porosity. On the one hand, the burrowing activity of earthworms destroyed most of the initial structural porosity (mesobiotic pores, 3–300 μm) and on the other hand, they increased the volume of the finest structural pores diameter class (microbiotic pores, 0.3–3 μm). *P. corethrurus* also stimulated phosphorus mineralization.

Soil bioturbation carried out by *P. corethrurus* could have enhanced the efficiency of microbial community that is involved in phosphorus bioavailability. This observation was corroborated by a higher phosphorus availability in earthworm casts ($1386 \pm 38\,\mathrm{mg\,P\,kg^{-1}}$ of dry matter in cast versus $778 \pm 61\,\mathrm{mg\,P\,kg^{-1}}$ of dry matter in the soil compartment not influenced by earthworm activity), and a better plant phosphorus nutrition in the presence of earthworms. Phosphorus uptake resulted in a better plant nutrition and a better tolerance to plant-feeding nematodes. Besides, the reduction of the mesobiotic porosity by bioturbation could also have disturbed the nematodes activities during their soil phase and contributed to the reduction of their damage.

1. Introduction

In most tropical countries, the burrowing nematode *Radopholus similis* (Cobb, 1893) Thorne, 1949 and the lesion nematodes *Pratylenchus* spp. are considered as the major constraints to banana cultivation (Gowen et al., 2005). These plant-feeding nematodes attack primary and lateral roots, causing necroses, affecting plant nutrition, reducing the root anchorage, shortening plantation life and reducing yield (Quénéhervé, 1993). In Guadeloupe, as in the whole Caribbean area, *R. similis*, *Pratylenchus coffeae* Zimmermann, 1898 and *Helicotylenchus multicinctus* (Cobb, 1893) Golden, 1956 are recognized as the major plant-feeding nematodes of bananas, causing extensive damages and yield losses (Quénéhervé, 2009).

From the beginning of the sixties, control strategies against these nematodes in commercial dessert banana plantation have mainly been achieved through the use of nematicides. However, during the last decade, there were many concerns about the side effects of pesticide use on human health and environment, coupled with the fact that these chemicals were generally beyond what resource-poor farmers could afford. This situation has prompted an increasing interest in alternative methods for plant pest control (Akhtar, 1997). Regarding dessert bananas in the Caribbean and in West Africa, these methods are now mainly based on the application of prophylactic practices such as the use of healthy plant materials (tissue cultured plants) on cleaned lands using fallows or rotations (Chabrier and Quénéhervé, 2003; Quénéhervé, 2009).

---

[*] Corresponding author. Tel.: +590 590 25 59 00x5838; fax: +590 590 94 16 63.
E-mail address: gladys.loranger-merciris@antilles.inra.fr (G. Loranger-Merciris).

0929-1393/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.apsoil.2011.10.003

The application of biological control methods such as the use of endophytic bacteria or fungi (Sikora et al., 2003), covering crops with allelopathic properties (McIntyre et al., 2001), or introducing earthworms (Lafont et al., 2007) still remains experimental.

In a recent review, Wurst (2010) analyzed different studies dealing with the interactions between plant-feeding nematodes and earthworms. In this review, two hypotheses explaining possible impact, either direct or indirect, of earthworms on nematodes were put forward. Among the direct effects could be mentioned the ingestion of nematodes by earthworms (trophic interactions). For example, Dash et al. (1980) reported the presence of nematodes in the intestinal content of earthworms. Beside direct ingestion process, indirect effects of earthworms on nematodes are mainly due to physical and chemical changes of soil properties induced by earthworm activities. As a consequence, plant may better tolerate plant-feeding nematodes or better defend themselves against them in the presence of earthworms. The relative importance of these different mechanisms is still unknown and more studies are needed.

In a recent preliminary study, Lafont et al. (2007) reported that the earthworms *Pontoscolex corethrurus* Muller, 1857 were able to reduce the pathogenicity of the burrowing nematode *R. similis*, allowing a greater biomass accumulation of dessert banana plantlets (*Musa* spp. AAA group, cv. Cavendish). However, this experiment had several drawbacks: (i) it only considered the burrowing nematode *R. similis*, whereas in banana plantations, plant-feeding nematodes develop in plurispecific communities, (ii) it was conducted in microcosms (3 L plastic containers filled with 2 kg of soil) and (iii) it was performed for a limited period of time (3 months as the 1/3 of banana crop cycle).

The objective of the present study was to estimate how the earthworm *P. corethrurus* can affect the pathogenicity of a plant-feeding nematodes community on dessert banana. In the herein study, indirect effects of earthworm activity were hypothesized as the main factors contributing to the decrease of plant-feeding nematodes pathogenicity by reducing root necrosis and increasing nutrient availability. We hypothesized that the indirect effects of *P. corethrurus* on banana root-feeding nematodes may be the result of the combination of two factors: (i) a modification of soil porosity due to the bioturbation activity of earthworms, and (ii) an enhancement of nutrient availability. This hypothesis has been tested in controlled conditions combining treatments including or not both earthworms and plant-feeding nematodes over a complete vegetative banana development cycle.

2. Materials and methods

2.1. Study site and experimental design

The experimentation was carried out in a greenhouse at the INRA research station of Duclos, Petit-Bourg (Guadeloupe, French West Indies). The soil used was an andosol (FAO-UNESCO classification, Driessen et al., 2001). This soil (Total N = 1.2%, Total C = 15.8%, $pHH_2O = 4.78$ and $pH_{KCl} = 4.34$) originated from a tropical humid forest (Manceau, Capesterre-Belle-eau, Guadeloupe). The granulometry exhibited a uniform distribution of loam, silt, fine and coarse sand fractions, with 6% of 0–2 μm “clay” content. It does not reflect abundance of the secondary allophane “clay” mineral, which organization is fractal from “fine sand” micro-aggregate to nanosphere (Chevallier et al., 2008) and remains highly deformable. The soil was collected at 0–25 cm depth and first passed through a 4 mm sieve to eliminate larger gravels. The soil was watered to field capacity and kept at −20 °C for 2 days to eliminate most of the native soil micro- and macrofauna without major alteration of the soil organic matter, which is the main food resource of endogeic earthworms (Gilot-Villenave, 1994). This freezing procedure was repeated twice.

Individuals of *P. corethrurus* (Oligochaeta Glossoscolecidae), widespread in tropical areas (Lavelle, 1983), were hand-collected from the andosol of the tropical humid forest. The earthworms were further reared on the same andosol in plastic containers for 8 weeks. The soil was renewed every 2 weeks so that the earthworms would have fresh organic matter at their disposal. Three nematode species were used in the present study: the burrowing nematode *R. similis*, the spiral nematode *H. multicinctus* and the lesion nematode *P. coffeae*. Six-week-old banana plantlets were inoculated with these nematodes using pieces of infested banana roots collected from a field situation at the CIRAD station (Neufchâteau, Capesterre Belle-eau, Guadeloupe). The banana vitroplants (*Musa* spp. AAA, cv Grande-Naine) free of pests were obtained from the nursery MERISTEM ANTILLES (Petit-Canal, Guadeloupe).

Four treatments were set up: N−E− (absence of introduced fauna); N−E+ (*P. corethrurus* earthworms alone); N+E− (plant-feeding nematodes alone); N+E+ (earthworms plus plant-feeding nematodes). Five replicates were used per treatment. Twenty-five six-week-old banana plantlets were first planted in 3 L plastic containers filled with 2 kg (dry weight) of the de-frozen andosol. Fifteen pots (N+ treatment) were inoculated with plant-feeding nematodes infected banana roots and ten pots were not inoculated. The roots were cut in pieces of 0.5 cm long. Fifty grams of banana root pieces were placed near the trunk of each of the 15 young banana plantlets. Seven weeks later, plant-feeding nematodes were extracted from roots of 5 banana plants previously inoculated in order to determine their mean initial populations. At the same time, the remaining 20 banana plantlets (10 inoculated and 10 not inoculated) were transferred in larger 30 L plastic containers filled with 20 kg (dry weight) of the de-frozen andosol ($T = 0$). Each E+ treatment pot was then inoculated with 60 g of living earthworms *P. corethrurus* (approximately 150 earthworms). This relative high biomass allowed earthworms to mix the entire pot soil during the experiment. The plants were maintained under greenhouse conditions for 337 days after $T = 0$. Soil matrix potential was maintained between −1 and −10 kPa with an automatized irrigation system.

2.2. Measurements at the end of the experiment

Eleven months (337 days) after the experiment set-up, the banana plants were uprooted to remove soil particles from the root system. The dry weight of aerial parts (leaves and pseudo-trunk) was measured on each banana plant, after 72 h drying at 65 °C. The aerial parts of each banana plant were analyzed for their total plant content in C, N, P_2O_5, $N0_3^-$, NH_4^+, exchangeable Ca^{2+}, Mg^{2+}, K^+ and Na^+. For each plant, the severity of root damage induced by plant-feeding nematodes was assessed by indexing the extent of necrosis of the root surface area. Notations were rated with a root necrosis index based on different classes: 0, no lesion; 1, 1–25% of root area is necrotic; 2, 26–50%; 3, 51–75%; and 4, more than 76% (Bridge and Gowen, 1993). Average sums of necrosis notations obtained for each plant were calculated for each treatment. Therefore, plant-feeding nematodes were extracted from an aliquot of the roots (50 g) in a mist chamber (Seinhorst, 1950). The final nematode populations were noted Pf. For each N+ plant, a nematode reproduction rate (Pf/Pi) was calculated. The dry root weight was determined using the remaining collected roots of each plant and extrapolated for the whole root system. Earthworms were hand-sorted from the soil, counted and weighed. Soil samples were analyzed for total soil content in C and N using an auto-analyser. Available P_2O_5 was measured using the Olsen method. Soil mineral N ($N0_3^-$ and NH_4^+) was measured by colorimetry after extraction with a 0.5 M KCl solution. Exchangeable Ca^{2+}, Mg^{2+}, K^+ and Na^+, pH-H_20 and pH-KCl were also measured. Shrinkage curves

Table 1

Number of plant-feeding nematodes per gram of fresh root biomass (density) in the four treatments, at the end of the study (337 days after $T = 0$). N−E−: Absence of introduced fauna; N−E+: *Pontoscolex corethrurus* earthworms alone; N+E−: plant-feeding nematodes alone; N+E+: earthworms plus plant-feeding nematodes. Values are means and confidence intervals are given in parenthesis. The effects of earthworms on nematode final densities were evaluating using mean comparison tests.

	N−E−	N−E+	N+E−	N+E+	F	P
Radopholus similis	0.00	0.00	31.5 (52.8)	29.3 (46.1)	$F_{1,8} = 1.34$	0.34
Pratylenchus coffeae.	0.00	0.00	0.10 (0.50)	0.00 (0.00)	n.c.	n.c.
Helicotylenchus multicinctus	0.00	0.00	124 (75.1)	81.1 (18.4)	$F_{1,8} = 16.9$	0.009
Nematodes (total)	0.00	0.00	156 (56.2)	118 (51.1)	$F_{1,8} = 1.18$	0.44

n.c., non calculated due to missing data

of centimetric aggregates (earthworm free soil aggregates and aggregates originated from soil with earthworms) and casts were established after 1-week succion equilibrium of −1, −10, −100 kPa using the ultrafiltration apparatus of Tessier and Berrier (1979), and 3-weeks at −1000 kPa using a pressure plate. The bulk specific volumes were determined by dipping the aggregates in petroleum by the technique of Monnier et al. (1973). Specific volume of solids was measured by water pycnometry. Specific volumes of water were measured after desiccation at 105 °C in an oven. According to Laplace's law, the maximum diameter of pores necks filled with water is 300, 30, 3 and 0.3 μm at −1, −10, −100 and −1000 kPa succion respectively. The difference of specific water volumes between two succion values is equal to the specific volume of pores that necks range between the corresponding diameter values. Because andosol's allophane exhibits a fractal organization of its glomerules (Woignier and Pochet, 2006), we assumed that the pore neck diameters were not different from the diameters of chambers between necks. According to Van Weerdt (1958), *R. similis* varies in size from 12 μm in width for the smallest juveniles to 27 μm in width for the largest females while the other considered species, *H. multicinctus* and *P. coffeae* exhibit body widths ranging from 18 to 35 μm (Fortuner, 1991; Loof, 1991). These nematodes thus need capillaries with a diameter ranging from 30 to 300 μm to be able to enter and to move actively through soil (Wallace, 1958, 1960; Otobe et al., 2004). We therefore distinguished two pore diameter classes: (i) 3–300 μm pores diameter which contains the pore diameter range which can host nematodes and were called mesobiotic pores, and (ii) 0.3–3 μm diameter pores which can host only bacteria, and were called microbiotic pores. All pores whose diameters were larger than 0.3 μm have been considered as structural pores. Any pore smaller than 0.3 μm in diameter has been considered as an abiotic matric pore.

2.3. Data analyses

Dry shoot and root biomass data were subjected to two-way factorial (earthworms and nematodes) analyses of variance. Mean values were separated at $P < 0.05$ with a Tukey test for post hoc procedure. Earthworm biomass was subjected to a one-way analysis of variance (comparing N+E+ and N−E+ treatments). The effects of earthworms on nematode final abundances, densities, reproduction rates (Pf/Pi) and on the severity of root damage were evaluated using one-way analyses of variance or mean comparison tests (comparing N+E+ and N+E− treatments). Nematode data were $\log(x+1)$ transformed to normalize variance prior to analysis. The effects of earthworms on soil and shoot chemical contents and on pore specific volume were evaluated using one-way analyses of variance (comparing E+ and E− treatments). Linear regressions were performed in order to test (i) the correlation between pore specific volume, (ii) the correlation between plant mineral content and pore volume ratio, and (iii) the correlation between nematode populations and pore specific volume. All statistical analyses were performed using STATBOX statistical software.

3. Results

3.1. Earthworms and nematode densities

At the end of the experiment, an average of 61 ± 7 earthworms were collected in N−E+ treatments with a corresponding biomass of 13.6 ± 1.79 g. In N+E+ treatments, 72 ± 5 earthworms were collected with a biomass of 20.8 ± 3.66 g. There was no significant difference in earthworm number (one-way ANOVA, $F_{1,8} = 1.71$, $P = 0.24$) and biomass (one-way ANOVA, $F_{1,8} = 3.11$, $P = 0.12$) between the two treatments.

There was no significant decrease in final abundances of plant-feeding nematodes (in whole fresh root system) in treatments with earthworms (mean comparison test, $P > 0.05$). The reproduction rates varied from 3 for *P. coffeae* nematode populations, 7 for *H. multicinctus* and 8 for *R. similis*. There was no significant decrease in reproduction rates in treatments with earthworms (mean comparison test, $P > 0.05$). Table 1 showed the number of nematodes per gram of fresh roots 337 days after planting. The whole plant-feeding nematodes density slightly decreased (Table 1). There was only a significant decrease in *H. multicinctus* population in treatments with earthworms (mean comparison test, $P = 0.009$, Table 1).

3.2. Root necrosis at the end of the experiment

Root necrosis did not occur in N− treatments. Earthworms significantly decreased root necrosis in N+E+ treatment compared to N+E− treatment (Table 2, one-way ANOVA, $F_{1,8} = 25.6$, $P = 0.001$).

3.3. Growth patterns of banana plants

At the end of the experiment, the dry biomass of aerial parts was significantly enhanced in the presence of earthworms (two-way ANOVA, $F_{1,16} = 17.8$, $P = 0.001$, Table 2). There was no significant

Table 2

Severity of root damage induced by plant-feeding nematodes, banana dry shoot biomass per plant and dry root biomass per plant in the four treatments, at the end of the study (337 days after $T = 0$). N−E−, Absence of introduced fauna; N−E+, *Pontoscolex corethrurus* earthworms alone; N+E−, plant-feeding nematodes alone; N+E+, earthworms plus plant-feeding nematodes. Values are means and standard errors are given in parenthesis. *Root necrosis index*: 0, no lesion; 1, 1–25% of root area is necrotic; 2, 26–50%; 3, 51–75%; and 4, more than 76%.

	N−E−	N−E+	N+E−	N+E+
Root necrosis	0.00 (0.00)	0.00 (0.00)	3.20 (0.21)	1.60 (0.21)
Dry shoot biomass	90.5 (11.9)	198 (25.8)	133 (11.9)	211 (31.3)
Dry root biomass	20.7 (3.37)	34.6 (5.75)	14.1 (2.45)	19.8 (3.65)

Table 3
Soil analyses in the four treatments, at the end of the study (337 days after $T = 0$). N−E−, Absence of introduced fauna; N−E+, *Pontoscolex corethrurus* earthworms alone; N+E−, plant-feeding nematodes alone; N+E+, earthworms plus plant-feeding nematodes. Values are means and standard errors are given in parenthesis.

	N−E−	N−E+	N+E−	N+E+
C%	14.7 (0.16)	14.9 (0.26)	14.7 (0.16)	14.0 (0.88)
N%	1.00 (0.01)	0.99 (0.01)	0.94 (0.04)	0.94 (0.04)
P (mg kg^{-1} MS)	1187 (16.7)	1194 (14.9)	1190 (36.8)	1333 (32.1)
N-NH$_4$%	0.01 (0.001)	0.004 (0.001)	0.004 (0.001)	0.01 (0.004)
N-NO$_3$%	0.21 (0.01)	0.12 (0.03)	0.10 (0.01)	0.15 (0.01)
Ca%	1.03 (0.06)	1.15 (0.07)	1.16 (0.1)	1.42 (0.06)
Mg%	0.24 (0.02)	0.23 (0.01)	0.33 (0.02)	0.26 (0.01)
K%	0.20 (0.04)	0.08 (0.01)	0.17 (0.02)	0.13 (0.02)
Na%	0.08 (0.004)	0.08 (0.004)	0.08 (0.01)	0.08 (0.004)
pH-H$_2$O	4.41 (0.03)	4.70 (0.05)	4.89 (0.08)	4.75 (0.06)
pH-KCl	4.19 (0.01)	4.17 (0.01)	4.25 (0.01)	4.22 (0.002)

effect of nematodes on dry biomass of aerial parts 337 days after planting (two-way ANOVA, $F_{1,16} = 1.61$, $P = 0.23$, Table 2).

Dry biomass of roots measured at 337 days after $T = 0$ (Table 2) was significantly lower in treatments with nematodes (two-way ANOVA, $F_{1,16} = 7.12$, $P = 0.02$) but was enhanced in the presence of earthworms (two-way ANOVA, $F_{1,16} = 5.93$, $P = 0.03$).

3.4. *Soil and plant nutrient balance*

337 days after $T = 0$, soil available P content significantly increased in the presence of earthworms (one-way ANOVA, $F_{1,18} = 4.92$, $P = 0.04$) while there was a decrease of soil exchangeable K content (one-way ANOVA, $F_{1,18} = 9.71$, $P = 0.01$), Table 3. Analysis of casts showed that available P was almost twice more important in fresh casts (1386 ± 38 mg P kg^{-1} dry matter) than in the soil compartment which was not influenced by earthworm activity (778 ± 61 mg P kg^{-1} of dry matter).

Plant analysis revealed high and not biomass-related K content values (2.77–7.04% of K in banana plant at the end of the experiment). Thus, K was not a limiting factor for plants. Nitrogen dilution curves indicated a slight N deficiency in the treatment with nematodes alone. On the contrary, P uptake was the main limiting factor of biomass yield (0.01–0.09% of P in banana plant at the end of the experiment). Total P of banana plants significantly increased in the presence of earthworms, 337 days after $T = 0$ (one-way ANOVA, $F_{1,18} = 10.3$, $P < 0.001$), Table 4.

3.5. *Soil physical features*

The shrinkage curves of aggregates and casts are shown in Fig. 1. A low decrease of bulk specific volume, as water content decreases, indicates a rigid behavior of soil material. Conversely, a path line parallel to the saturation line indicates a perfectly deformable material, into which each water loss induces an internal re-organization resulting in equal contraction of bulk specific volume. The shrinkage curve of earthworm free soil aggregates showed a low deformability to all water contents. The decrease of the bulk specific volumes, as water left the pores, was mainly compensated by air entry into these pores. Despite the dispersion of values, the specific volumes of these earthworm free aggregates are significantly higher than the other aggregate and cast ones, for matric potential lower than −1 kPa. Contrary to aggregates of soil without earthworms, earthworm casts appeared highly deformable, as for the highest water content each water loss resulted in a contraction, without additional air entry. The deformability of casts was associated with an increasing range of water content between −1 and −10^3 kPa (−1.2 g g^{-1} against −1.0 g g^{-1} for aggregates). The volume of intra-aggregate matric pores remained steady; it was not affected by earthworm activity. The aggregates of soil with earthworms exhibited an intermediate behavior, but were not different from casts even in their highest water content range.

The porosity system of earthworm free soil showed a uniform volume distribution of pore diameter ranges (Table 5). The aggregates of soil with earthworms showed an increase of the finest pore class volume and a decrease of the > 3 μm structural pore volume (Table 5, Fig. 2). The poral class which was the most affected by reduction was between 30 and 300 μm diameter (Table 5).

Table 4
Plant nutrient analyses in the four treatments, at the end of the study (337 days after $T = 0$). N−E−, Absence of introduced fauna; N−E+, *Pontoscolex corethrurus* earthworms alone; N+E−, plant-feeding nematodes alone; N+E+, earthworms plus plant-feeding nematodes. Values are means and standard errors are given in parenthesis.

	N−E−	N+E−	N−E+	N+E+
C%	38.6 (0.52)	40.0 (0.40)	40.5 (0.74)	40.6 (0.55)
N%	3.63 (0.11)	1.87 (0.09)	2.43 (0.30)	2.58 (0.21)
P%	0.06 (0.01)	0.03 (0.01)	0.07 (0.01)	0.07 (0.01)
N–NH$_4$%	0.01 (0.001)	0.004 (0.001)	0.005 (0.001)	0.01 (0.004)
N-NO$_3$%	0.21 (0.01)	0.12 (0.01)	0.10 (0.03)	0.15 (0.01)
Ca%	0.88 (0.06)	0.80 (0.02)	0.94 (0.06)	0.88 (0.04)
Mg%	0.47 (0.03)	0.37 (0.01)	0.38 (0.03)	0.40 (0.02)
K%	5.86 (0.37)	5.35 (0.31)	4.47 (0.59)	5.50 (0.31)
Na%	0.07 (0.01)	0.08 (0.01)	0.06 (0.004)	0.07 (0.01)

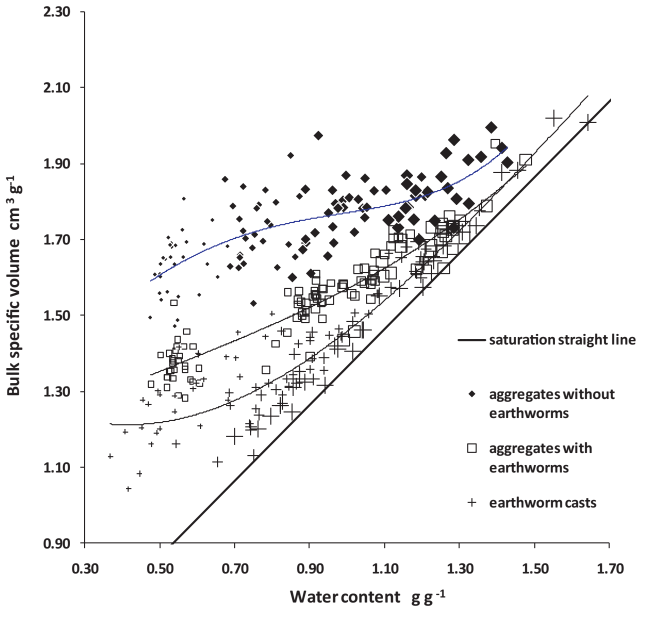

Fig. 1. Shrinkage curves obtained on 0.8–5 cm^3 aggregates and casts at four matric potential values: −1, −10, −100, −1000 kPa, following decreasing size of points. Each fine line represents a third degree polynomial regressor which underlines the shrinkage behavior of each kind of aggregate.

Table 5

Pore specific volumes ($cm^3\ g^{-1}$ soil) at the end of the study (337 days after $T = 0$). E−, treatments without earthworms; E+, treatments with *Pontoscolex corethrurus* earthworms. Values are means and standard errors are given in parenthesis.

	E−	E+	$F_{1,18}$	P
0.3–3 μm diameter pores ($cm^3\ g^{-1}$ soil)	0.21 (0.01)	0.35 (0.01)	78.5	<0.001
3–30 μm diameter pores ($cm^3\ g^{-1}$ soil)	0.22 (0.02)	0.17 (0.03)	1.71	0.22
30–300 μm diameter pores ($cm^3\ g^{-1}$ soil)	0.26 (0.02)	0.15 (0.02)	14.5	<0.001
>300 μm diameter pores ($cm^3\ g^{-1}$ soil)	0.21 (0.02)	0.09 (0.01)	28.7	<0.001

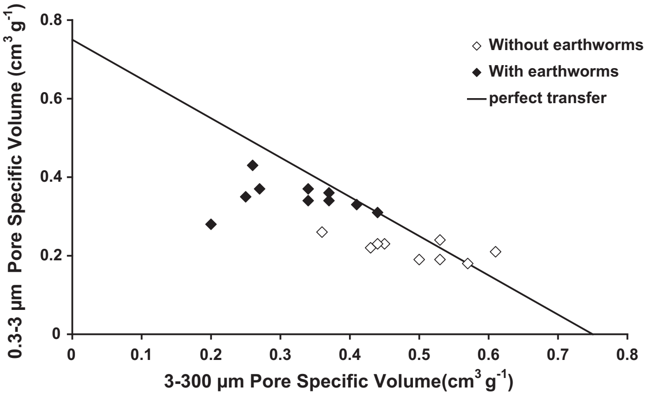

Fig. 2. Specific volume of mesobiotic (3–300 μm) and microbiotic (0.3–3 μm) pores in the presence or absence of the earthworm *Pontoscolex corethrurus* at the end of the experiment (337 days after $T = 0$). The dotted line indicates exact volume transfer from one class to the other.

Total P uptake by plants was positively correlated to the ratio between microbiotic and mesobiotic pore volumes (Fig. 3, ANOVA, $F_{1,18} = 29.6$, $P < 0.001$).

H. multicinctus density (number of nematodes per gram of fresh root biomass) was positively correlated with the mesobiotic pore specific volume (Fig. 4, ANOVA, $F_{1,18} = 5.31$, $P = 0.05$).

4. Discussion

4.1. Effects of earthworms on banana growth

As Lafont et al. (2007), we found that overall growth of banana plants was significantly increased in the presence of earthworms *P. corethrurus*. This observation is probably due to the burrowing and feeding activities of these earthworms which had two main consequences.

The first consequence is a change in soil porosity. It is well known that endogeic earthworms play a crucial role in influencing soil structure and related soil physical properties. Through their burrowing and feeding activities, they maintain specific patterns in the amount, connectivity and size distribution of pores in the soil (Blanchart et al., 1997, 2004; Hallaire et al., 2000). In our study, the effect of *P. corethrurus* activity on aggregates pore size distribution was dramatic. Their burial activity resulted in a mixing of soil material under high water potential conditions. On the one hand, earthworms destroyed most of the initial structural porosity and on the other hand they increased the volume of the finest structural pore diameter class. Barois et al. (1993) also showed that soil initial structure was totally destroyed in *P. corethrurus* gut and then reconstructed in the casts. Besides, our results corroborate the fact that *P. corethrurus* is a compacting species as showed by several studies (e.g., Barros et al., 2001). Our results are also consistent with those of Görres et al. (2001), which showed a similar effect of *Lumbricus terrestris* Linnaeus, 1758 on soil porosity using mercury intrusion porosimetry on casts and burrows obtained in a silt loam. This increase of the finest structural pore volume was not due to a general intensification of the matrix porosity, because the water contents under a matrix potential of -10^4 kPa were the same for both aggregates and casts. As a consequence, the only difference between water contents at the same water potential was observed at -10^2 kPa, where casts were wetter. We noted a slight rise of the swelling capacity of the matrix, as both water content and specific volume were greater for casts than for initial aggregates at −1 kPa, and their specific volume was lower at -10^3 kPa. The functioning of aggregates of soil with earthworms and casts were identical and differed significantly from the earthworm-free aggregates one.

The second consequence of earthworm bioturbation activity was the enhancement of P availability. Earthworm free aggregates, where 30–300 μm in diameter pore volume were abundant, appeared favoring nematodes activity. Conversely, cast deriving aggregates were less favorable to the microfauna after structural

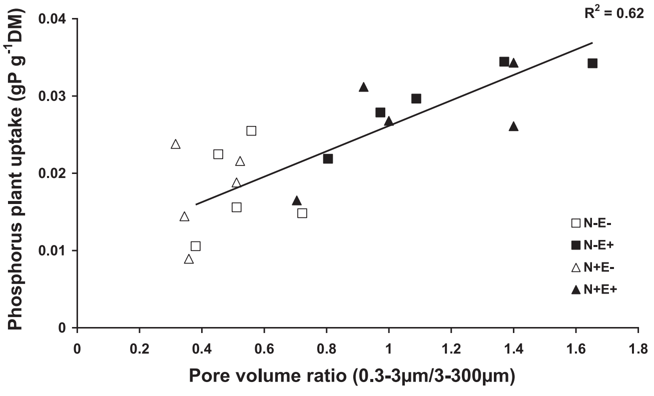

Fig. 3. Relationship between P uptake and pore volume ratio (microbiotic pores/mesobiotic pores) at the end of the experiment (337 days after $T = 0$). N−E−: Absence of introduced fauna; N−E+: *Pontoscolex corethrurus* earthworms alone; N+E−: plant-feeding nematodes alone; N+E+: earthworms plus plant-feeding nematodes. A linear regression was performed to test the correlation between total P uptake and the pore volume ratio.

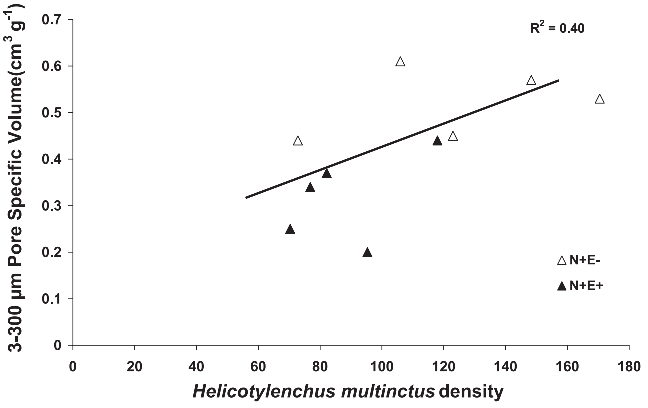

Fig. 4. Relationship between specific volume of mesobiotic pores (3–300 μm) and the density of *Helicotylenchus multicinctus* (number of nematodes per g of fresh root) at the end of the experiment (337 days after $T = 0$). A linear regression was performed to test the correlation between specific volume of mesobiotic pores and *H. multicinctus* density.

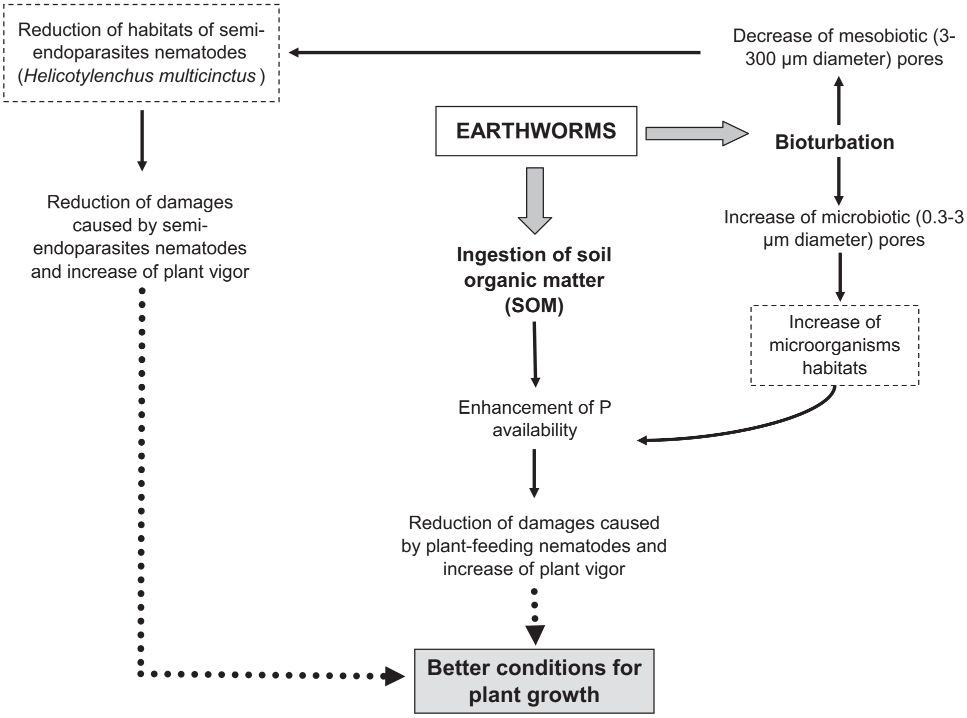

Fig. 5. Systemic action of *Pontoscolex corethrurus* on banana and phytoparasitic nematode regulation. Hypotheses that must be verified by further studies are mentioned in dashed frame (⬚).

pores had been rebuilt, but they could shelter high microbial activity in the increased 0.3–3 µm pore class. Barois et al. (1993) showed that ingested soil was completely dispersed during transit in *P. corethrurus* gut. Soil particles were thus completely reorganized, resulting in formation of new aggregates like microbial colonies surrounded by polysaccharides and clays. We hypothesized that this destructuration and restructuration of the soil have also activated some dormant microorganisms, stimulating mineralization of organic elements that were protected. In particular, in our study, *P. corethrurus* activity, by increasing the microbiotic pores, may have contributed to the increase of a microbial community which stimulated P mineralization, as suggested by the greater P availability in earthworm casts. This hypothesis is also consistent with the increasing carbon mineralization measured by Görres et al. (2001) in *L. terrestris* casts and burrows compared to bulk soil. Kuczak et al. (2006) also showed that earthworm casts of the large earthworms (Family Glossoscolecidae) contained more labile P pools than soils and suggested that earthworm casts are a significant source of P for plant growth.

The greater availability of P in the presence of earthworms resulted in a greater P content in banana plants, and probably in the significant vigor strengthening of the plants. In our case, plant-feeding nematodes reduced the biomass and the strength of banana roots. In the presence of earthworms, this negative impact was mitigated. One of the best defenses against root diseases is a vigorous and well-developed plant root system. It is well known that P plays an important role in promoting rapid root development in young plants (Grant et al., 2001). Thus, the negative impact of plant-feeding nematodes on root development may be reduced by the input of P induced by earthworms.

4.2. *Effects of plant-feeding nematodes on banana growth*

Contrary to what might be expected, the presence of plant-feeding nematodes was not always associated with crop damage. In fact, in our study, the N−E− treatment showed performances similar to the N+E− for shoot development. These results support the hypothesis that nematode damages may be related to the balance between species within plant-feeding nematodes community (Villenave and Cadet, 1998; Cadet et al., 2002). These authors showed that the ectoparasitic species such as *Helicotylenchus dihystera* (Cobb, 1893) Sher, 1961 can mitigate the pathogenicity of other potentially more harmful nematodes species in millet and sugarcane.

4.3. *Effects of earthworms on banana plant-feeding nematodes*

Plant-feeding nematode abundances were not modified in the presence of earthworms, but their negative impact on bananas was significantly reduced. These results may be compared to those obtained by Boyer et al. (1998) on maize, Blouin et al. (2005) on rice and Lafont et al. (2007) on banana. These authors also observed a positive effect of earthworm activity on plant growth, without a diminution of total plant-feeding nematodes. Changes in biomass and quality of roots initiated by earthworms may induce the plant to compensate the damage caused by nematodes. In our case, as the root biomass increased and the number of nematodes remained the same, this can be a simple dilution effect as suggested by Wurst (2010). However, in our case, the reduction of nematode devastation on banana root systems in the presence of earthworms was probably due to the greater availability of P resulting in the significant strength of bananas. Thus, these plants can better tolerate nematode attacks. This mechanism was probably the main factor involved in the regulation of the damaging effect of strict migratory endoparasites (*R. similis* and *P. coffeae*), the life cycles and activities of which occur almost exclusively within root tissues. Besides, we identified a second mechanism which could take place in regulation of the damaging effect of *H. multicinctus*. Our results showed that the burrowing activity of *P. corethrurus* reduced the number of mesobiotic pores. In the same way, the soil density of *H. multicinctus* is correlated with the number of mesobiotic pores. By destroying microhabitat of this semi-endoparasite, the earthworms may have reduced the negative impact of these nematodes on bananas.

The effect of earthworms on banana may also be of different nature, not explored in our study. In a recent publication, Jana et al. (2010) showed that phytohormone-like compounds are stimulated by earthworms. Organic matter mineralization and release of phytohormone-like compounds by earthworms may induce a systemic action of earthworm on plant growth driven by a modification of the expression of genes that code for tissue reparation (Blouin et al., 2005).

In our study, the other original effect of earthworm inoculation was the reduction of root necrosis, which was also observed by Lafont et al. (2007). These necroses are mainly caused by the introduced plant-feeding nematodes but also by opportunistic associated fungi and bacteria. The reduction of root necrosis was a consequence of the increase of banana growth and was also due to the slight reduction of nematode density (e.g., *H. multicinctus*).

4.4. Conclusion

We can conclude that the soil bioturbation by the earthworm *P. corethrurus* can stimulate phosphorus mineralization. The bioturbation that was attested by modifying soil pore size distribution (transformation of mesobiotic pores to microbiotic ones) might have enhanced the efficiency of microbial community which is involved in phosphorus bioavailability. At least, the increase of microbiotic pore volume indicated that the physical habitat of microorganisms was enhanced and the bioturbation did not provoke severe compaction. Phosphorus uptake resulted in a better plant nutrition and lighter damages from plant-feeding nematodes. Besides, the reduction of the mesobiotic porosity by bioturbation could also disturb the nematodes activities during their soil phase and contribute to the reduction of their damage (Fig. 5). Compared to previous studies, this work opens new ways to the understanding of the complex interactions occurring in the soil biota – soil – plant continuum. The measurement of microbial activity to support the hypothesis stipulating that bioturbation creates better conditions for microorganisms activity (Fig. 5) will be one of the main challenges of future researches.

Acknowledgements

This work has been supported by INRA. Authors wish to thank J. André, T. Bajazet, F. Burner, L. Desfontaines, C. Flèreau, A. Mulciba, C. Palmier and F. Razan for their technical help during field and laboratory experiments.

References

Akhtar, M., 1997. Current options in integrated management of plant-parasitic nematodes. Integr. Pest Manage. Rev. 2, 187–197.

Barois, I., Villemin, G., Lavelle, P., Toutain, F., 1993. Transformation of the soil structure through *Pontoscolex corethrurus* (Oligochaeta) intestinal tract. Geoderma 56, 57–66.

Barros, E., Curmi, P., Hallaire, V., Chauvel, A., Lavelle, P., 2001. The role of macrofauna in the transformation and reversibility of soil structure of an oxisol in the process of forest to pasture conversion. Geoderma 100, 193–213.

Blanchart, E., Lavelle, P., Braudeau, E., Le Bissonnais, Y., Valentin, C., 1997. Regulation of soil structure by geophagous earthworm activities in humid savannas of Côte d'Ivoire. Soil Biol. Biochem. 29, 431–439.

Blanchart, E., Albrecht, E., Brown, G., Decaens, T., Duboisset, A., Lavelle, P., Mariani, L., Roose, E., 2004. Effects of tropical endogeic earthworms on soil erosion. Agric. Ecosyst. Environ. 104, 303–315.

Blouin, M., Zuily-Fodil, Y., Pham-Thi, A.-T., Laffray, D., Reversat, G., Pando, A., Tondoh, J., Lavelle, P., 2005. Belowground activities affect plant aboveground phenotype, inducing plant tolerance to parasites. Ecol. Lett. 8, 202–208.

Boyer, J., Michellon, J., Reversat, G., 1998. Interactions between earthworms and plant parasitic nematodes in various crops on Reunion island. In: Agrobiological Management of Soils and Cropping Systems, Paris, France. Cirad colloques, pp. 323–333.

Bridge, J., Gowen, R., 1993. Visual assessment of plant nematode and weevil damage on bananas and plantain. In: Gold, C.S., Gemmill, B. (Eds.), Biological and Integrated Control of Highland Banana and Plantain Pest and Diseases. Cotonou, Benin, Africa, pp. 147–154.

Cadet, P., Spaull, V.W., McArthur, D.G., 2002. Role of plant parasitic nematodes and abiotic soil factors in growth heterogeneity of sugarcane on a sandy soil in South Africa. Plant Soil 246, 259–271.

Chabrier, C., Quénéhervé, P., 2003. Control of the burrowing nematode *Radopholus similis* (Cobb) on banana: impact of the banana field destruction method on the efficiency of the following fallow. Crop Prot. 22, 121–127.

Chevallier, T., Woignier, T., Toucet, J., Blanchart, E., Dieudonné, P., 2008. Fractal structure in natural gels: effect on carbon sequestration in volcanic soils. J. Sol–Gel Sci. Technol. 48, 231–238.

Dash, M.C., Senapati, B.K., Mishra, C.C., 1980. Nematode feeding by tropical earthworms. Oïkos 34, 322–325.

Driessen, P., Deckers, J., Spaargaren, O., Nachtergaele, F., 2001. Lecture Notes on the Major Soils of the World. FAO, Rome.

Fortuner, R., 1991. The Hoplolaiminae. In: Nickle, W.R. (Ed.), Manual of Agricultural Nematology. Marcel Dekker, Inc., New York, pp. 669–719.

Gilot-Villenave, C., 1994. Determination of the origin of the different growing abilities of two populations of *Millsonia anomala* (Omodeo and Vaillaud), a tropical geophageous earthworm. Eur. J. Soil Biol. 30, 125–131.

Görres, J.H., Savin, M.C., Amador, J.A., 2001. Soil micropore structure and carbon mineralization in burrows and casts of an anecic earthworm (*Lumbricus terrestris*). Soil Biol. Biochem. 33, 1881–1887.

Gowen, S.R., Quénéhervé, P., Fogain, R., 2005. Nematodes parasites of bananas and plantains. In: Luc, M., Sikora, R.A., Bridge, J. (Eds.), Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. , 2nd edition. CABI Publishing, UK, pp. 611–644.

Grant, C.N., Flaten, D.N., Tomasiewicz, D.J., Sheppard, S.C., 2001. Importance of early season phosphorus nutrition. Better Crops 85, 18–23.

Hallaire, V., Curmi, P., Duboisset, A., Lavelle, P., Pashanasi, B., 2000. Soil structure changes induced by the tropical earthworm *Pontoscolex corethrurus* and organic inputs in a Peruvian ultisol. Eur. J. Soil Biol. 36, 35–44.

Jana, U., Barot, S., Blouin, M., Lavelle, P., Laffray, D., Repellin, A., 2010. Earthworms influence the production of above- and belowground biomass and the expression of genes involved in cell proliferation and stress responses in *Arabidopsis thaliana*. Soil Biol. Biochem. 42, 244–252.

Kuczak, C.N., Fernandes, E.C.M., Lehmann, J., Rondon, M.A., Luizão, F.J., 2006. Inorganic and organic phosphorus pools in earthworm casts (Glossoscolecidae) and a Brazilian rainforest Oxisol. Soil Biol. Biochem. 38, 553–560.

Lafont, A., Risède, J.M., Loranger-Merciris, G., Clermont-Dauphin, C., Dorel, M., Rhino, B., Lavelle, P., 2007. Effects of the earthworm *Pontoscolex corethrurus* on banana plants infected with the plant-parasitic nematode *Radopholus similis*. Pedobiologia 51, 311–318.

Lavelle, P., 1983. The structure of earthworm communities. In: Satchell, J.E. (Ed.), Earthworm Ecology, from Darwin to Vermiculture. Chapman and Hall, London, pp. 449–465.

Loof, P.A.A., 1991. The family Pratylenchidae Thorne, 1949. In: Nickle, W.R. (Ed.), Manual of Agricultural Nematology. Marcel Dekker, Inc., New York, pp. 363–421.

McIntyre, B.D., Gold, C.S., Kashaija, I.N., Ssali, H., Night, G., Bwamiki, D.P., 2001. Effects of legume intercrops on soil-borne pests, biomass, nutrients and soil water in banana. Biol. Fertil. Soils 34, 342–348.

Monnier, G., Stengel, P., Fies, J.C., 1973. Une méthode de mesure de la densité apparente de petits agglomérats terreux, application à l'analyse des systèmes de porosité du sol. Ann. Agron. 24, 533–545.

Otobe, K., Itou, K., Mizukubo, T., 2004. Micro-moulded substrates for the analysis of structure-dependent behaviour of nematodes. Nematology 6, 73–77.

Quénéhervé, P., 1993. Nematode management in intensive banana agrosystems: comments and outlook from the Côte d'Ivoire experience. Crop Prot. 12, 164–172.

Quénéhervé, P., 2009. Integrated management of banana nematodes. In: Ciancio, A., Mukerji, K.G. (Eds.), Integrated Management of Fruit Crops and Forest Nematodes. Springer Serie: Integrated Management of Plant Pests and Diseases, vol. 4, pp. 3–61.

Seinhorst, J.W., 1950. De betekenis van de toestand van de grond voor het optreden van aantasting door het stengelaaltje (*Ditylenchus dipsaci* (Kühn) Filipjev). Tijdschr. PlZiekt 56, 289–348.

Sikora, R.A., Niere, B., Kimenju, J., 2003. Endophytic microbial biodiversity and plant nematode management in African agriculture. In: Neuenschwander, P., Borgemeister, C., Langewald, J. (Eds.), Biological Control in IPM Systems in Africa. CAB International, Wallingford, UK, pp. 179–192.

Tessier, D., Berrier, J., 1979. Utilisation de la microscopie électronique à balayage dans l'étude des sols. Observations des sols humides soumis à différents pF. Bull. l'A. F.E.S. 1, 67–82.

Van Weerdt, L.G., 1958. Studies on the biology of *Radopholus similis* (Cobb, 1893) Thorne, 1949, part II, morphological variation within and between progenies of single females. Nematologica 3, 184–196.

Villenave, C., Cadet, P., 1998. Interaction of *Helicotylenchus dihystera*, *Pratylenchus pseudopratensis* and *Tylenchorhynchus gladiotus* on two plants from the soudano-sahelian zone of West Africa. Nematropica 28, 31–39.

Wallace, H.R., 1958. The movement of eelworms. I. Influence of pore size and moisture content of the soil on the migration of larvae of the beet eelworm, *Heterodera schachtii* Schmidt. Ann. Appl. Biol. 46, 75–85.

Wallace, H.R., 1960. The movement of eelworms. IV. The influence of soil type, moisture gradient and host plant roots on the migration of the potato-root eelworm *Heterodera rostochiensis* Wollenweber. Ann. Appl. Biol. 48, 107–120.

Woignier, T., Pochet, G., 2006. Use of the supercritical drying to preserve the andosols porous features. Clay Sci. 12, 255–260.

Wurst, S., 2010. Effects of earthworms on above- and belowground herbivores. Appl. Soil Ecol. 45, 123–130.