Available online at www.sciencedirect.com
ScienceDirect
Agriculture, Ecosystems and Environment 124 (2008) 187–192
Agriculture Ecosystems & Environment
www.elsevier.com/locate/agee

Role of tardigrades in the suppressive service of a soil food web

Sara Sánchez-Moreno [a,*], Howard Ferris [a], Noemí Guil [b]

[a] Department of Nematology, University of California Davis, One Shields Avenue, Davis, CA 95616, United States
[b] Department of Organismic and Evolutionary Biology, Harvard University, 16 Divinity Avenue, Cambridge, MA 02138, United States

Received 16 December 2006; received in revised form 19 September 2007; accepted 20 September 2007
Available online 7 November 2007

Abstract

In a previous study, soil suppressiveness to populations of the plant-parasitic nematode *Meloidogyne incognita* was correlated with abundance of omnivore and predatory nematodes. In the current study, performance of the regulatory function switched from nematodes to tardigrades when predatory nematodes disappeared due to predation pressure and/or unfavorable environmental conditions. Two tardigrade species, *Macrobiotus richtersi* and *M. harmsworthi* effectively suppressed nematode populations. While there were significant changes in the soil food web over time, the regulatory function was maintained. Under experimental conditions, *M. richtersi* consumed 61 nematodes per day, indicating that tardigrades may significantly reduce nematode biomass. The impact of tardigrades on soil food web dynamics and their relationships with nematodes are discussed.

Keywords: Trophic interactions; Soil food web; Suppressiveness; Nematodes; Tardigrades

1. Introduction

Nematodes are the most important secondary consumers among the soil microfauna (Mulder et al., 2005) and the most abundant soil metazoans. Some soil organisms, such as arthropods, tardigrades and predatory nematodes can reduce the abundance of nematode populations (Hyvönen and Persson, 1996; Khan and Kim, 2005). Observations of tardigrades attacking nematodes are common, but little is known about the importance of tardigrade regulation of nematode communities. Some positive relationships between tardigrades and nematode abundances have been observed in the field (Hallas and Yeates, 1972) but most of the available information comes from experimental studies (Hohberg and Traunspurger, 2005). Many tardigrade species are predators of other soil animals, but even in extremely short food webs, such as those found in Antarctic soils, relationships between abundances of tardigrades and nematodes are inconclusive (Sohlenius et al., 2004). Tardigrades are reported to feed on a wide range of food sources, including algae, fungi, rotifers, protozoa, bacteria and plant roots (Hallas and Yeates, 1972; Nelson, 2002; Sohlenius et al., 2004).

In this paper, we study the trophic interactions between tardigrades and nematodes and we analyze the correlation between tardigrade abundance and suppression of the pest nematode species *Meloidogyne incognita*. The objectives of this study were to: (a) analyze the relationship between tardigrade abundance and the nematode community trophic composition, and calculate tardigrade predation rate on nematodes under experimental conditions, (b) correlate tardigrade numbers with nematode abundance and soil suppressiveness, (c) determine whether tardigrades exhibit prey preference among nematode taxa, and (d) determine effects of selected agricultural chemicals and physical disturbance on the correlation between tardigrade numbers, suppressiveness, and nematode faunal structure.

2. Material and methods

Soil samples were collected in June, October and November 2005 near Oakville, Napa Valley, California and used to test the effects of chemicals and soil disturbance

* Corresponding author at: Departamento de Protección Vegetal, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, 28040 Madrid, Spain. Tel.: +34 913478710; fax: +34 913471479.
E-mail address: sarasm@inia.es (S. Sánchez-Moreno).

0167-8809/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.agee.2007.09.011

on soil suppressiveness to introduced nematodes. The climate in the study area is Mediterranean, with dry summer and wet winter months. In June, soil suppressiveness was compared in an organic vineyard and in a neighboring undisturbed woodland with oak, laurel, and pine as dominant plant species.

In October 2005, soil was sampled only in the woodland and used to test the effects of nitrogen fertilizers (ammonium nitrate (NH_4NO_3) and calcium nitrate $Ca(NO_3)2{\cdot}4H_2O$)), herbicides (diuron (®) and simazine (®)) and simulated tillage. Nematodes were extracted and identified before the application of the treatments (T0), and 10, 20 and 30 days (T1–T3) after they were applied to 96 microcosms (6 treatments × 4 replicates × 4 sampling dates) of the woodland soil. Twenty-four samples were collected from the field, and each sample was carefully homogenized and divided into 500 g subsamples that were used to fill four clay pots, the replicates for each takedown period of each of the six treatments. To simulate tillage, soil was mixed in a cement mixer for 20 min before the establishment of the microcosms. Chemical treatments were applied by adding 50 ml of a solution of fertilizer or herbicide in distilled water. Herbicides were applied at 2.68 kg a.i./ha of simazine and 1.79 kg a.i./ha of diuron (as recommended by the University of California Integrated Pest Management Program—http://www.ipm.ucdavis.edu/PGM/r302700211.html). The nitrogen fertilizer treatments were applied at 11.2 kg/ha. The sixth treatment was a control treated with distilled water (see details in Sánchez-Moreno and Ferris, 2007). The control and time zero (T0) microcosms received 50 ml of distilled water. Microcosms were kept in a growth chamber (12 h light, 12 h dark at 20 °C). Soil moisture was maintained at 10% adding water daily.

At each sampling time, microcosms were destructively sampled for assessment of soil properties and soil suppressiveness tests. For analysis of soil properties, 8 g of each microcosm was mixed with 40 ml of 2 M KCl and shaken for 1 h. Each sample was centrifuged at 5 × *g* for 5 min at room temperature. The supernatant was filtered and submitted to the ANR analytical laboratory for NO_3^-–N and NH_4^+–N determination by an automated flow injection analyzer method (Hofer, 2003; Knepel, 2003). Twenty grams of soil was used to measure soil pH by the saturated paste method (Hesse, 1971).

Each pot (of the 96 microcosms) was subsampled in two portions of 100 g soil to test soil suppressiveness. One portion was used as a control to determine that the target nematode for the suppressiveness study, *M. incognita*, was not present in the soil. The second portion was used to study the influence of the soil food web on the target nematode survival. This replicate received 500 *M. incognita* juveniles and was incubated for 5 days at room temperature in the dark. Then the whole 100 g sample was used for nematode extraction. Nematodes were counted and identified. Soil suppressiveness against *M. incognita* was tested in all treatment and time combinations (for details, see Sánchez-Moreno and Ferris, 2007). After determining that *M. incognita* juveniles were not naturally present in the soil, soil suppressiveness was calculated as the percentage of inoculated nematodes that did not survive after 5 days of incubation in the sample.

In November, another set of samples was collected from the woodland to test tardigrade predation on nematodes and to study soil food web characteristics through soil food web indices (Enrichment, Structure, Channel and Basal Index, Ferris et al., 2001, see Section 2.2). Simulated agricultural treatments and suppressiveness tests were not applied to these samples. Analyses of soil properties were not performed for November samples.

From both experimental (microcosms, soil collected in October) and field (November) samples, tardigrades and nematodes were extracted using a combination of sieving and Baermann funnels (Barker, 1985). Nematodes and tardigrades were stored in a refrigerator in water at 4 °C for 24 h in glass vials. Total numbers of tardigrades and nematodes were counted at low magnification (×50). Nematode suspensions were centrifuged, each concentrate spread on a slide, and at least 200 living nematodes from each sample were identified to genus or family level. Nematodes were categorized as dead or alive on the basis of movement and internal structural integrity. When possible, dead nematodes also were identified. As many tardigrades as possible were hand-removed with a Pasteur pipette and fixed in Carnoy's solution (3 parts ethanol 96%:1 part acetic acid).

Tardigrades were mounted on microscope slides in Hoyer's medium (Ramazzotti and Maucci, 1983) and identified to species level based on morphological characteristics using the most recent classification (Guidetti and Bertolani, 2005).

2.1. Sand experiments

To extend the study of the influence of tardigrades on the nematode community, 12 samples were collected in November at the same location as the October samples. At each sampling point, 1 kg soil sample was collected between 0–20 cm depths. Soil was stored at 4 °C and nematodes and tardigrades were extracted by Baermann funnels. Nematodes and tardigrades were identified and soil food web indices were calculated.

Nematodes extracted from November samples were used to test tardigrade predation. Between 67 and 263 nematodes were used in each of six tests. Sterile sand (0.4 g) was placed in small glass vessels (13 mm diameter and 4 cm high). Nematodes were extracted from 200 g of woodland fresh soil and identified under a dissecting microscope. Then the nematode suspension was divided in two aliquots and each 0.2 ml aliquot was added to one vial with sand. One single adult tardigrade was added to one of the two vials and the other, without a tardigrade, was used as a control. Each pair of vials comprised one experiment. Vials were kept in the dark at room temperature and after 24 h of incubation the

sand from both vials was washed to recover the nematodes and the tardigrade. Nematodes were again identified under a compound microscope at the end of the experiment. Tardigrades were fixed in Carnoy's solution for further identification.

2.2. Data analyses

Once identified, nematode taxa were assigned to trophic groups (Yeates et al., 1993), colonizer-persister groups (Bongers, 1990) and functional guilds (Bongers and Bongers, 1998). Absolute abundance (number of nematodes in taxon i in 100 g of fresh soil) and relative abundance (number of nematodes in taxon i/total number of nematodes in 100 g of fresh soil) of each nematode taxon and trophic group were calculated. Soil food web indices (Ferris et al., 2001) were used to infer soil food web characteristics. High Enrichment Index values indicate organically enriched soils in which opportunistic bacterial-feeding nematode species predominate. The Basal Index is an indicator of communities composed mainly by tolerant taxa that became dominant under stressed soil conditions. The Channel Index is an indicator of the relative flow of organic matter through fungal- and bacterial-mediated decomposition pathways. Finally, the Structure Index is an indicator of soil food web length and connectance (Ferris et al., 2001).

Spearman rank order correlation coefficients were calculated to infer relationships between continuous variables. Kruskal–Wallis ANOVA was used to determine the effect of treatments on nematode community descriptors, soil properties and tardigrade abundance. Mann–Whitney test was used posthoc. All statistical analyses were performed with the STATISTICA software package (StatSoft, 1996).

3. Results

Two species of the tardigrade genus *Macrobiotus* were found in the woodland soil. *M. richtersi* and *M. harmsworthi* were found in the samples collected in October, while only *M. richtersi* was found in November and was used in the predation experiment. Details on the characteristics of the soil food web and nematode community in the samples collected in October have been previously published (Sánchez-Moreno and Ferris, 2007).

At T0, before applying any treatment, there was a negative relationship between the number of tardigrades per sample and the number of live nematodes, and between tardigrade abundance and absolute abundance of bacterial-feeding nematodes (Table 1). Number of tardigrades was positively correlated with the number and proportion of dead nematodes (Fig. 1), relative abundance of fungal-feeders, and with the Channel Index. Tardigrade abundance was positively correlated with pH. There was no relationship between soil mineral nitrogen and tardigrade abundance.

Table 1
Spearman rank order correlation coefficients between tardigrade abundance and soil properties, nematode community descriptors, and soil food web indices[a]

	T0	T1	T2	T3	All
Live nem.	**−0.50**	−0.40	−0.25	−0.27	**−0.38**
Dead nem.	**0.48**	**0.82**	**0.62**	0.15	**0.54**
Dead/total	**0.73**	**0.74**	**0.71**	0.12	**0.58**
NH_4^+–N	−0.06	−0.38	−0.08	−0.05	−0.17
NO_3^+–N	0.22	0.07	−0.36	−0.03	−0.09
pH	**0.46**	**0.55**	0.16	0.38	**0.44**
Supp.	**0.59**	−0.02	0.40	0.12	**0.29**
Ba	−0.32	**−0.43**	−0.19	**−0.42**	**−0.24**
Fu	**0.51**	0.40	0.21	0.31	**0.23**
Pp	0.31	**0.61**	0.19	0.22	**0.34**
O	−0.15	**−0.59**	−0.39	−0.21	**−0.25**
P	−0.18	−0.32	−0.30	−0.39	**−0.30**
Ba Abs.	**−0.50**	−0.27	−0.17	−0.34	**−0.25**
Fu Abs.	−0.20	0.19	0.20	0.25	0.04
Pp Abs.	−0.31	0.18	0.09	0.09	0.05
O Abs.	−0.17	**−0.57**	−0.31	−0.21	**−0.24**
P Abs.	−0.17	−0.39	−0.39	−0.39	**−0.34**
EI	−0.16	−0.24	0.06	0.24	0.03
SI	−0.03	−0.34	−0.34	0.05	−0.16
CI	**0.46**	**0.50**	0.36	0.09	**0.24**
BI	0.20	**0.42**	0.31	−0.16	0.10
N	**−0.42**	0.01	−0.11	−0.06	−0.11
S	−0.18	−0.27	−0.06	−0.10	**−0.22**

Pooled data from the six treatments were used. Bold numbers indicate significant correlation coefficients ($P < 0.05$).

[a] Number of live (Live nem.) and dead (Dead nem.) nematodes; number of dead nematodes as a proportion of total nematodes (Dead/total); ammonium (NH_4^+–N); nitrate (NO_3^+–N); suppressiveness to target nematode, *Meloidogyne incognita* (Supp.); trophic groups (relative and absolute (Abs) abundances: Ba, bacterial-feeders; Fu, fungal-feeders; Pp, plant-feeders; O, omnivores; P, predators), soil food web indices (EI, Enrichment Index; SI, Structure Index; CI, Channel Index; BI, Basal Index), total number of nematodes (excluding the target nematode) (N) and taxa richness (S) after 0 (T0), 10 (T1), 20 (T2), and 30 (T3) days of incubation after treatment application; over all sampling times (All).

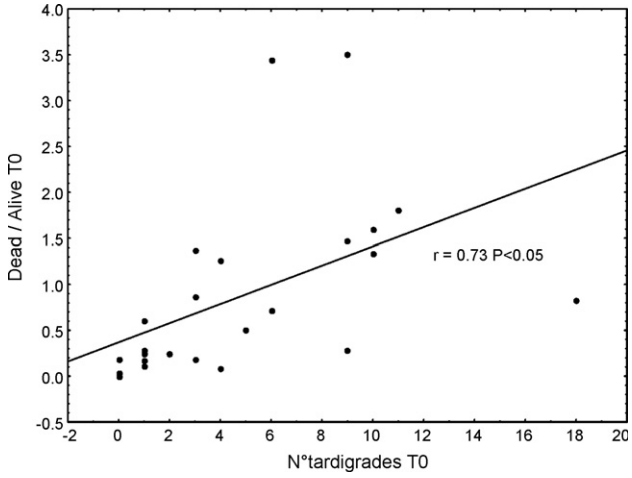

Fig. 1. Scatter plot of number of tardigrades and the ratio of number of dead nematodes to total nematodes at T0 (before the application of any treatment).

Some of the significant relationships detected at T0 disappeared at subsequent time periods after the treatments were applied. At T1, 10 days after the start of the experiment, number and proportion of dead nematodes averaged across all treatments was still positively correlated with number of tardigrades, but number of live nematodes was no longer related to tardigrade abundance. Soil suppressiveness at T1 was not related to tardigrades either. However, relative abundance of bacterial-feeding and omnivore nematodes was negatively correlated with tardigrade abundance (Table 1).

At T2, most of the significant relationships had disappeared, and tardigrades appeared correlated only with abundance and proportion of dead nematodes. Thirty days after the application of the treatments (T3) the only significant correlation coefficient was found between number of tardigrades and bacterial-feeder abundances.

The number of significant correlations between tardigrade abundance and the nematode assemblage increased when all four sampling times were included in one analysis. Overall nematode abundance and taxa richness decreased in the presence of tardigrades. Abundances of bacterial-feeders, predators and omnivores were negatively correlated with tardigrade abundance, while there was a positive correlation between tardigrades and relative abundances of plant- and fungal-feeding nematodes. The strongest correlations were found between tardigrade number and number of dead and live nematodes (Table 1).

Disruptive treatments did not strongly affect tardigrade abundance (data not shown). Only ammonium nitrate fertilizer seemed to decrease tardigrade abundance in T1, but it recovered in T2. In the control treatment, number of live nematodes per sample was greater at T1–T3 than at T0. In the samples treated with ammonium nitrate, number of live nematodes was greatest at T3. In the tillage treatment, number of live nematodes was highest at T3, when number of dead nematodes was least. Averaged among all sampling dates and treatments, correlation coefficients between number of tardigrades and number of dead, live, and dead/total number of nematodes remained significant (Fig. 2). Comparisons among treatments showed that tardigrade abundance at T0 was greater in the ammonium nitrate treatment and lowest after tillage, while at T1 numbers of tardigrades and dead nematodes were greater in ammonium nitrate and lower in the control and calcium nitrate treatments, respectively (data not shown).

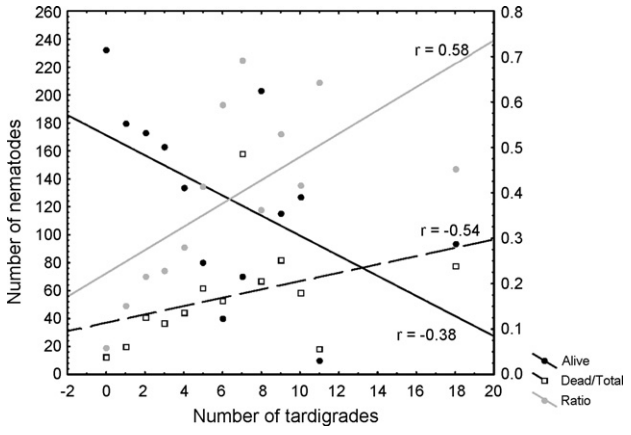

Fig. 2. Scatter plot of number of tardigrades and number of dead, live and the ratio of dead to total nematodes. Significant correlation coefficients are indicated ($P < 0.05$).

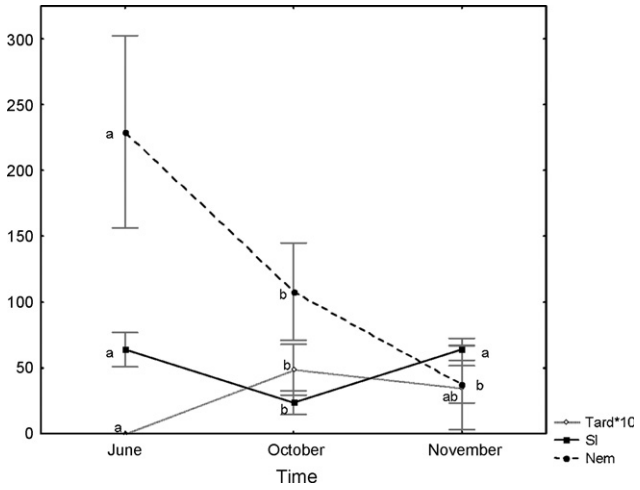

Fig. 3. Number of nematodes (Nem), tardigrades (Tard*10) and Structure Index (SI) values for June (Sánchez-Moreno and Ferris, 2007), October and November. Different letters mean significant differences at $P < 0.05$. Number of tardigrades multiplied by 10 to facilitate visualization.

Tardigrade abundance, nematode abundance and food web structure changed with time (Fig. 3). Tardigrade abundance was highest in October, when the Structure Index values were lowest. Nematode abundance decreased from June to November.

In glass vials, nematode abundance was reduced substantially in 24 h by tardigrade predation (Table 2). To avoid

Table 2
Percentage of nematodes of each nematode taxon recovered after 24 h in the presence (T) or absence (NT) of a tardigrade (Δ = difference in percentage of nematodes recovered with and without tardigrade, NT-T)

Taxa	T	NT	Δ
Mesorhabditis	40.0%	62.1%	22.1%
Panagrolaimus	43.4%	52.8%	9.3%
Acrobeloides	36.56%	54.2%	17.7%
Plectus	63.3%	66.1%	2.8%
Aphelenchoides	18.5%	40.0%	21.56%
Tylenchidae	39.4%	56.7%	17.3%
Tylencholaimus	33.0%	80.0%	47.0%
Qudsianematidae	38.67%	86.1%	47.4%
Tripyla	0.0%	83.3%	83.3%
Prionchulus	65.23%	68.0%	2.7%
Average %	37.8%	64.9%	27.1%
Average	101.2	39.8	61.4

In the last rows, average percentage (Average %) and average number (Average) of nematodes recovered in each treatment and difference between treatments are indicated.

false interpretations, only the most abundant nematode taxa are included in the table. On average, 27% (61.4 individuals) fewer nematodes were recovered in the samples after 24 h of incubation with a tardigrade. Nematode recovery in vials with a tardigrade was significantly lower than in vials without a tardigrade ($U = 11.5$, $P = 0.002$).

4. Discussion

Although not detected in high abundances in a sampling at the same site in June 2005, tardigrade densities in the soil were high in October 2005 (Sánchez-Moreno and Ferris, 2007). The two tardigrade species found in the samples, *M. richtersi* and *M. harmsworthi* are broadly distributed throughout the world (Kaczmarek et al., 2005; Tumanov, 2005; Hohberg, 2006), exhibit carnivorous trophic habits and can be cultured on bacterial-feeding nematodes as prey (Li and Wang, 2005; Hohberg, 2006). Hohberg (2006) suggests that high abundances of *M. richtersi* may appear in soils depauperate in predators and competitors. The absence of tardigrades four months before the October sampling time, together with the disappearance of the significant relationship between tardigrade abundance and suppressiveness in the microcosm experiments detected at T0 at subsequent sampling dates, suggest that tardigrades may behave as r-strategists and respond rapidly to favorable environmental conditions. Correlation between number of nematodes and number of tardigrades disappeared when the manipulative treatments were applied. In this study, *M. richtersi* populations may have responded to a temporal niche provided by environmental conditions unfavorable to other predators, as found previously in leaf litter (Guidetti, 1999). Also, in agreement with previous studies (Meyer, 2006), the distribution of tardigrades in the soil was patchy (average S.D. 98%).

Soil suppressiveness to plant-feeding nematodes was significantly correlated with predator and omnivore nematode abundances in June 2005 (Sánchez-Moreno and Ferris, 2007). This relationship was not observed in October, but the (limited) suppressive service of the soil food web was preserved through the high abundances of tardigrades, exemplifying temporal functional redundancy in the system. Significant negative correlation between *M. incognita* suppression and total nematode abundance (Sánchez-Moreno and Ferris, 2007) suggests that predators consumed more *M. incognita* juveniles when other nematode prey were at low densities and *M. incognita* represented the most abundant prey. Indeed abundant tardigrades not only killed more nematodes, but also killed a larger proportion of the available prey. Tardigrade predation rates depend on prey density (Hohberg and Traunspurger, 2006), and may be facilitated by aggregation mechanisms; when nematodes (Bilgrami and Jairapuri, 1988) and mites (Bilgrami, 1994) feed on plant-parasitic nematodes, the presence of injured prey strongly attracts more predators.

Hohberg and Traunspurger (2005) conducted the first study to quantify tardigrade predation on nematodes. They found that, per individual, tardigrades ate from 38.2 nematodes per day on agar plates to 56.8 nematodes per day in sand. Predation rates in sand in our study were in a similar range (61.4 nematodes per day per tardigrade). Not every prey individual is completely consumed by tardigrades; some injured nematodes escape from tardigrade attacks but die later as a result of damage to the cuticle (Hohberg and Traunspurger, 2005; Doncaster and Hooper, 1961). When many prey are available or food quality is high, tardigrades attack nematodes but do not consume them completely, a foraging behavior described by Hohberg and Traunspurger (2006). Injuries to the cuticle may also provide infection ports for fungi (personal observation).

In October, the Structure Index (based on the abundance of predatory and omnivore nematodes) in the woodland soil was very low, but it recovered in November (data not shown), reaching the higher values measured in June. This increase may have been due to predator and omnivore nematode migration to the surface from deeper soil layers when soil temperatures decreased, or tardigrades may have preyed primarily on predator and omnivore nematodes

Acknowledgements

This work was supported by the National Science Foundation Division of Environmental Biology (grant no. DEB–0120169). N. Guil was supported by a Fulbright postdoctoral fellowship financed by the Ministry of Education and Science of the Spanish Government (BOE/21/05/2005) at Harvard University. The authors thank two anonymous reviewers for helpful comments that greatly improved this manuscript.

References

Barker, K.R., 1985. Nematode extraction and bioassays. In: Barker, K.R., Carter, C.C., Sasser, J.N. (Eds.), An Advanced Treatise on Meloidogyne, Vol. 2 Methodology, North Carolina State University Graphics, Raleigh, N.C., pp. 19–35.

Bilgrami, A.L., 1994. Predatory behavior of a nematode feeding mite *Tyrophagus putrescentiae* (Sarcoptiformes: Acaridae). Fund. Appl. Nematol. 17, 293–297.

Bilgrami, A.L., Jairapuri, M.S., 1988. Attraction of *Mononchoides longicaudatus* and *M. fortidens* (Nematoda: Diplogasterida) towards prey and factors influencing attraction. Rev. Nématol. 11, 195–202.

Bongers, T., 1990. The maturity index: an ecological measure of environmental disturbance based on nematode species composition. Oecologia 83, 14–19.

Bongers, T., Bongers, M., 1998. Functional diversity of nematodes. Appl. Soil Ecol. 10, 239–251.

Doncaster, C.C., Hooper, D.J., 1961. Nematodes attacked by protozoa and tardigrades. Nematologica 6, 333–335.

Ferris, H., Bongers, T., de Goede, R.G.M., 2001. A framework for soil food web diagnostics: extension of the nematode faunal analysis concept. Appl. Soil Ecol. 18, 13–29.

Guidetti, R., 1999. Nuovi approcci all'ecologia e alla sistematica dei tardigradi. PhD Thesis. Department of Animal Biology, Università di Modena e Reggio Emilia, Modena.

Guidetti, R., Bertolani, R., 2005. Tardigrade taxonomy, an updated check list of the taxa and a list of characters for their identification. Zootaxa 845, 1–46.

Hallas, T.E., Yeates, G.W., 1972. Tardigrada of the soil and litter of a Danish beech forest. Pedobiology 12, 287–304.

Hesse, P.R., 1971. A Textbook of Soil Chemical Analysis. J. Murray, London.

Hofer, S., 2003. Determination of Ammonia (Salicylate) in 2 M KCl Soil Extracts by Flow Injection Analysis. QuikChem Method 12-107-06-2-A. Lachat Instruments, Loveland, CO.

Hohberg, K., 2006. Tardigrade species composition in young soils and some aspects on life history of *Macrobiotus richtersi* J. Murray, 1911. Pedobiology 50, 267–274.

Hohberg, K., Traunspurger, W., 2005. Predator–prey interaction in soil food web: functional response, size-dependent foraging efficiency, and the influence of soil texture. Biol. Fertil. Soils 41, 419–427.

Hohberg, K., Traunspurger, W., 2006.In: Abstracts of the 10th International Symposium on Tardigrada, Catania, Italy, June 18–23, p. 20.

Hyvönen, R., Persson, T., 1996. Effects of fungivore and predatory arthropods on nematodes and tardigrades in microcosms with coniferous forest soil. Biol. Fertil. Soils 21, 121–127.

Kaczmarek, L., Michalczyk, L., Diduszko, D., 2005. Some tardigrades from Siberia (Russia, Baikal region) with a description of *Macrobiotus garynahi* sp nov (Eutardigrada: Macrobiotidae: richtersi group). Zootaxa 1053, 35–45.

Khan, Z., Kim, Y.H., 2005. The predatory nematode, *Mononchoides fortidens* (Nematoda, Diplogasterida), suppresses the root-knot nematode, *Meloidogyne arenaria*, in potted field soil. Biol. Control 35, 78–82.

Knepel, K., 2003. Determination of Nitrate in 2 M KCl Soil Extracts by Flow Injection Analysis. QuikChem Method 12-107-04-1-B. Lachat Instruments, Loveland, CO.

Li, X.C., Wang, L.Z., 2005. Effect of temperature and thermal acclimation on locomotor performance of *Macrobiotus harmsworthi* Murray (Tardigrada, Macrobiotidae). J. Thermal Biol. 30, 588–594.

Meyer, H.A., 2006. Small-scale spatial distribution variability in terrestrial tardigrade populations. Hydrobiology 558, 133–139.

Mulder, C., Schouten, A.J., Hund-Rinke, K., Breure, A.M., 2005. The use of nematodes in ecological soil classification and assessment concepts. Ecotoxicol. Environ. Safety 62, 278–289.

Nelson, D.R., 2002. Current status of the tardigrada: evolution and ecology. Integr. Comp. Biol. 42, 652–659.

Ramazzotti, G., Maucci, W., 1983. Il phylum Tardigrada. III edizione riveduta e aggiornata. Memorie dell'Istituto Italiano di Idrobiologia Dott. Marco de Marchi 41, 1–1012.

Sánchez-Moreno, S., Ferris, H., 2007. Suppressive service of the soil food web: effects of environmental management. Agric. Ecosyst. Environ. 8, 75–87.

Sohlenius, B., Bostrom, S., Jonsson, K.I., 2004. Occurrence of nematodes, tardigrades and rotifers on ice-free areas in East Antarctica. Pedobiology 48, 395–408.

StatSoft, I., 1996. STATISTICA for Windows (Computer Program Manual). Tulsa, OK, 74104, USA.

Tumanov, D.V., 2005. Notes on the Tardigrada of Thailand, with a description of *Macrobiotus alekseevi* sp nov (Eutardigrada, Macrobiotidae). Zootaxa 999, 1–16.

Yeates, G.W., Bongers, T., de Goede, R.G.M., Freckman, D.W., Georgieva, S.S., 1993. Feeding habits in soil nematode families and genera—an outline for soil ecologists. J. Nematol. 25, 315–331.