

Interactions between *Enchytraeus crypticus*, collembolans, gamasid mites and barley plants – a greenhouse experiment

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Abstract

In a greenhouse experiment with soil containing a mixture of Collembola species, the effect of *Enchytraeus crypticus* and *Hypoaspis aculeifer* on growth of barley and nitrogen mineralisation and the interactions between the different mesofauna taxa were investigated over 17 weeks. *E. crypticus* had a negative effect on root biomass and positively influenced shoot/root ratio, both effects being reduced in the presence of gamasids. Enchytraeid abundance increased about eight-fold during the first five weeks and then almost collapsed. Total microarthropods strongly increased for 9 weeks and considerably decreased towards the end whereas the population of *H. aculeifer* grew slowly but continuously. Arthropods tended to react in three different ways to the presence of *E. crypticus*: "pioneers" (*I. anglicana*, *H. assimilis*) were only initially reduced, "mutualists" (*F. fimetaria*, *I. notabilis*, *P. armata*) increased in density, "indirect competitors" (for nutrient resources: *M. macrochaeta*, prostigmatid mites) were considerably reduced during later stages of the experiment. However, except for prostigmatid mites these effects were never significant. *H. aculeifer* reduced most arthropod groups but tended to have a positive impact on prostigmatid mites and sometimes also on certain Collembola. The most probable mechanisms responsible for the observed patterns are resource competition, improvement of food resources due to chemical transformation of the ingested material during gut passage (Collembola and enchytraeids) and polyphagous predation with switching feeding preferences by *H. aculeifer*.

Key words: Preference, competition, predation, feeding ecology, prey switching, ecosystem functioning, plant nutrition, *Hypoaspis aculeifer*.

Introduction

Enchytraeids are important members of the soil decomposer community, increasing in relative abundance and biomass with northern latitude and soil organic matter content. Due to their importance in acidic habitats (Römbke 1991, Laakso & Setälä 1999), they were mostly studied in forest, moor or grassland soils. Sulkava & Huhta (1998) found them particularly abundant in patchy environments with mixed litter. Studies dealing with the effects of enchytraeids on ecosystem functioning (Standen 1978, Williams & Griffiths 1989, Hedlund & Augustsson 1995, Sulkava et al. 1996, Briones et al. 1998, Cole et al. 2000) or their interactions with other soil fauna (Huhta et al. 1998), were restricted to such habitats also.

Enchytraeid abundance and biomass in arable soils is extremely variable, ranging from about 1,000 to 300,000 individuals / m², yet even at moderate densities they may consume a considerable percentage of the annual organic matter input (Kasprzak 1982, Lagerlöf et al. 1989, Friebe 1990, Fründ et al. 1992, Didden 1993, Filser et al. 1999). In patches of organic sludge, Krogh (unpublished data) found extraordinarily high densities, equivalent to 400,000 specimens per m². Most of the aforementioned studies investigated the impact of different management systems on enchytraeids, but - except for effects on soil structure (e.g. Didden 1990, Langmaack et al. 1999) - to our knowledge there are only few experimental studies on their impact on ecosystem functioning in arable soil (Marinissen & Didden 1997). In addition, relatively little is known of the general biology of enchytraeids.

The present study is part of the "COLLEAGES" (Effects of COLLEmbola LEAGUES on ecosystem functioning) experiment which will be described in a forthcoming paper. Here we highlight (i) the effects of *Enchytraeus crypticus* on nitrogen turnover and plant growth in an arable soil dominated by Collembola, (ii) the impact of the predacious mite *Hypoaspis aculeifer* on the investigated parameters, and (iii) population development and interactions of enchytraeids and microarthropods.

Methods

The study was conducted in a greenhouse at 15°C with a 12 h light/dark cycle. Sandy clay soil from an organically farmed field was defaunated by deep-freezing twice and passed through a 7 mm sieve. Plexiglass cylinders (9.4 cm diameter) were filled with 2,500 g (fresh weight) of soil to a height of 24 cm, sown with spring barley (one plant per cylinder) and sampled destructively after 5, 9, 13 and 17 weeks. To the upper 5 cm, 2.4 g (dw) of dried clover were added as a nutrient source. Four replicates each of the following treatments were established:

- C: mixture of six Collembola species (*Folsomia fimetaria*, *Isotoma anglicana*, *Isotoma notabilis*, *Protaphorura armata*, *Mesaphoruramacrochaeta*, *Hypogastrura assimilis*), altogether 108 animals per microcosm (numbers of individual species added are indicated in Figs. 4-9)
- CE: C + 90 *Enchytraeus crypticus*
- CEG: CE + 25 gamasids (*Hypoaspis aculeifer*)

Week		C	CE	CEG	C	CE	CEG
		shoot mass (g dw)			root mass (g dw)		
5	m	0,37	0,43	0,41	0,09	0,13	0,21
5	se	0,06	0,06	0,04	0,02	0,05	0,08
9	m	3,27	2,70	2,83	a 0,78	b 0,52	ab 0,68
9	se	0,25	0,21	0,26	0,04	0,08	0,05
13	m	4,80	4,83	4,54	0,54	0,56	0,61
13	se	0,54	0,64	0,44	0,11	0,09	0,04
17	m	5,47	6,07	5,88	0,53	0,39	0,55
17	se	0,30	0,50	0,69	0,06	0,04	0,06
		grain mass (g dw)			plant / root ratio		
5	m				5,00	5,09	2,79
5	se				0,90	1,72	0,85
9	m				4,21	5,42	4,16
9	se				0,31	0,58	0,29
13	m				10,20	9,39	7,47
13	se				2,44	1,63	0,74
17	m	ab 4,69	a 5,36	b 4,37	a 10,64	b 15,83	a 10,73
17	se	0,37	0,42	0,50	1,19	1,90	0,14

Table 1: Mean values (m) and standard error (se), n=4, of barley dry weight (dw) and shoot / root ratio in microcosms in presence of different mesofauna groups. C = Collembola only, CE = Collembola and *E. crypticus*, CEG = Collembola, *E. crypticus* and *H. aculeifer*. Significant differences (ANOVA followed by LSD test, p<0.05) are printed in bold and indicated by different letters.

At each sampling occasion, plants were harvested and separated into grains (only after 17 weeks), shoot and roots. The latter were thoroughly washed before all plant material was dried at 35°C. The soil of each cylinder was subdivided in three layers (0-5, 5-10, 10-24 cm) and carefully mixed. Plant material was oven-dried and weighed. Soil from each layer was divided into different portions (70 g fresh weight for enchytraeids, 150 g for microarthropods, 40 g for mineral N) for the single extraction procedures. On the basis of these values, numbers (concentrations) for the complete microcosms were calculated. Enchytraeids were extracted by wet funnel extraction (O'Connor 1962), counted and weighed. For microarthropods, a Macfadyen high gradient extraction (collection fluid: benzoic acid) was used, and soil mineral N was measured from a KCl extract (40 g soil / 200 ml 1M KCl) using a QuickChem automatic flow injection analyzer (Lachat, Milwaukee, WI, USA). Statistical calculations were made with SAS. If not stated otherwise, an ANOVA was conducted followed by pairwise comparisons (LSD test).

Results

After 9 weeks, root biomass was lower in CE compared to C (Table 1). When considering all sampling dates, root biomass was positively affected by the presence of gamasids (average dry weight in CE: 0.40, CEG: 0.51 g, $n=16$, $p<0.05$). Conversely, at the end of the experiment, weight and maturity of the barley grains was reduced in the CEG treatment compared to CE. Also in C the grain weight was

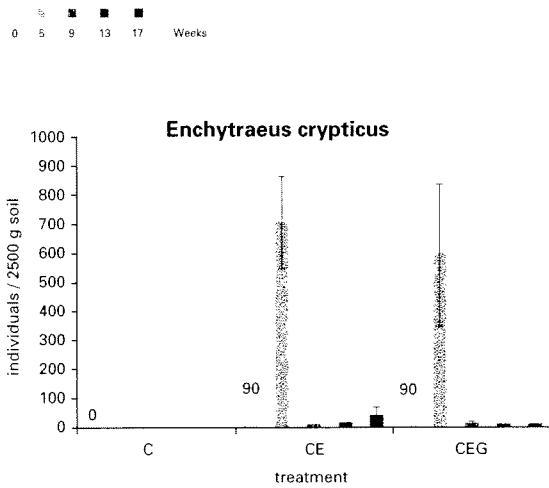


Fig. 1: Development of *E. crypticus* during the course of the experiment (mean values \pm SE, $n=4$). Treatments: C = Collembola only, CE = Collembola and *E. crypticus*, CEG = Collembola, *E. crypticus* and *H. aculeifer*. Initially added individual numbers per microcosm are indicated over the bar at week 0. Mesostigmatid mites were a contamination of incompletely defaunated soil.

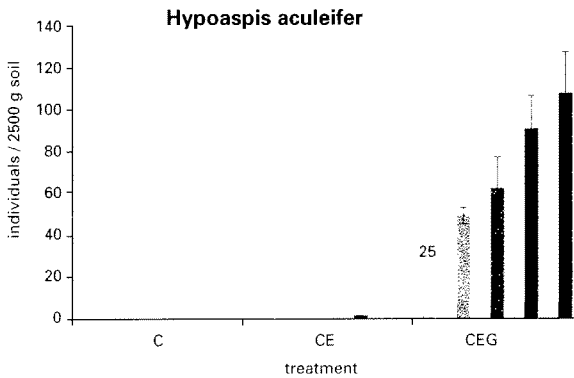


Fig. 2: Development of *H. aculeifer* during the course of the experiment. Further explanations see Fig. 1.

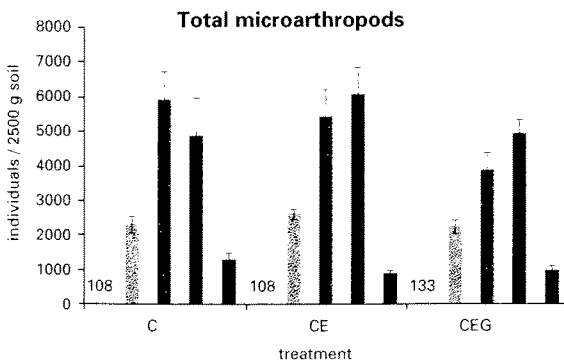


Fig. 3: Development of total microarthropods during the course of the experiment. Further explanations see Fig. 1.

0 5 9 13 17 Weeks

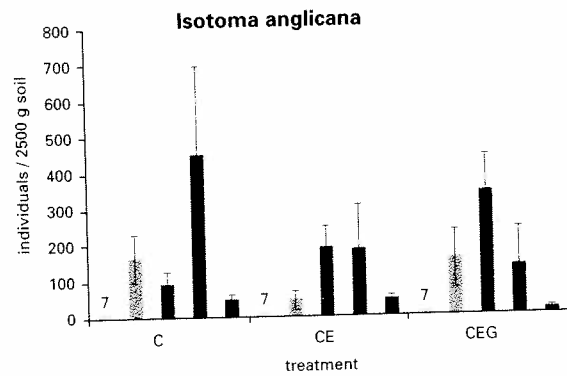


Fig. 4: Development of *I. anglicana* during the course of the experiment. Further explanations see Fig. 1.

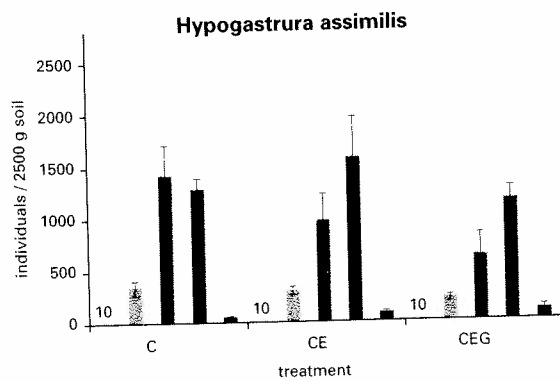


Fig. 5: Development of *H. assimilis* during the course of the experiment. Further explanations see Fig. 1.

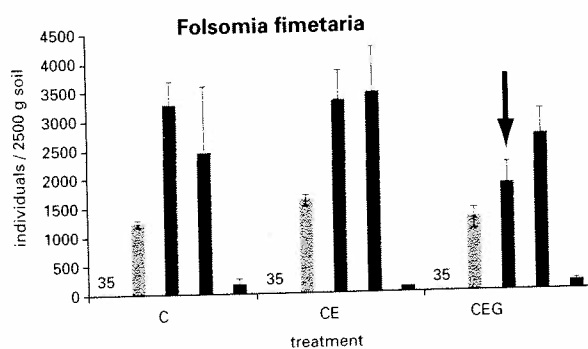


Fig. 6: Development of *F. fimetaria* during the course of the experiment. Further explanations see Fig. 1. The arrow indicates a significant effect of *H. aculeifer* (ANOVA, LSD-test, $n=4$, $p<0.05$).

lower than in CE but this difference was not significant. At that sampling date, also the plant/root ratio was by far highest in CE. There were no differences between the treatments in shoot biomass or soil mineral N (data not shown).

Enchytraeid population size increased almost eight-fold within the first five weeks and then rapidly decreased (Fig. 1). After that, the population almost collapsed but slowly increased towards the end of the experiment in the treatment without

gamasids. *H. aculeifer* increased linearly in abundance throughout the experiment (Fig. 2). In their presence, the population of *E. crypticus* seemed to be about 25 % lower on average (Fig. 1), however, due to the high variation in enchytraeid numbers this difference was never significant. The pattern of development of total microarthropods was again different (Fig. 3): population size strongly increased until week 9 (except for CEG). Between week 9 and 13, microarthropod population growth was low, followed by a strong decrease until week 17 in all treatments.

Populations of single microarthropod taxa differed in their reactions to the presence of *E. crypticus*. Two species tended to be initially reduced in CE compared to C, namely *I. anglicana* after 5 and 13 weeks and *H. assimilis* after 9 weeks (Figs. 4 and 5); later on slightly positive effects occurred. *E. crypticus* seemed to exert a positive influence on the population size of *F. fimetaria*, *I. notabilis* and *P. armata* (Figures 6-8). However, non of the effects on Collembola was significant. Only

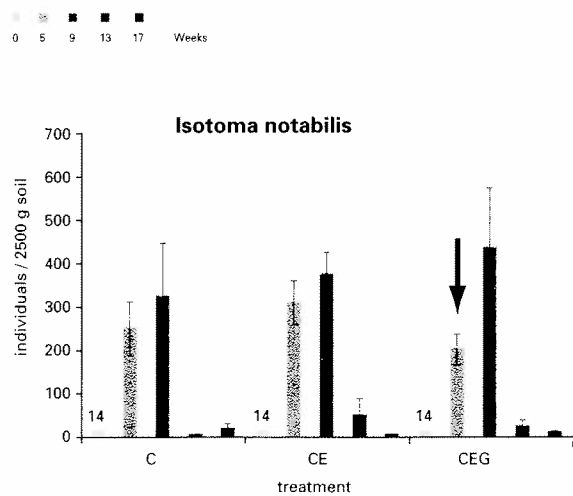


Fig. 7: Development of *I. notabilis* during the course of the experiment. Further explanations see Fig. 1. The arrow indicates a significant effect of *H. aculeifer* (ANOVA, LSD-test, $n=4$, $p<0.05$).

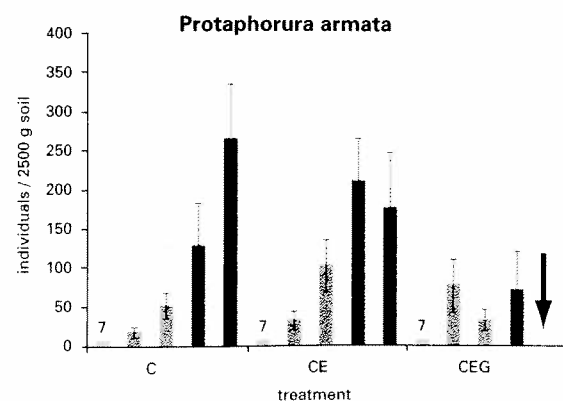


Fig. 8: Development of *P. armata* during the course of the experiment. Further explanations see Fig. 1. The arrow indicates a significant effect of *H. aculeifer* (ANOVA, LSD-test, $n=4$, $p<0.05$).

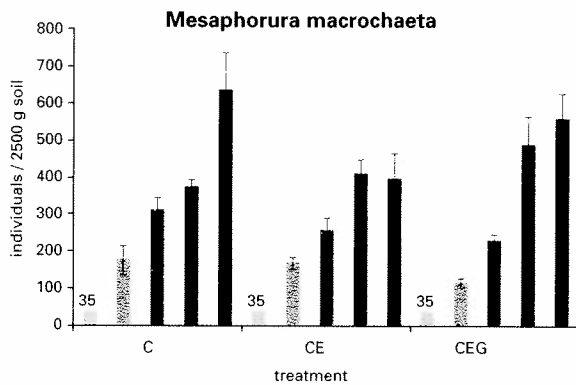


Fig. 9: Development of *M. macrochaeta* during the course of the experiment. Further explanations see Fig. 1.

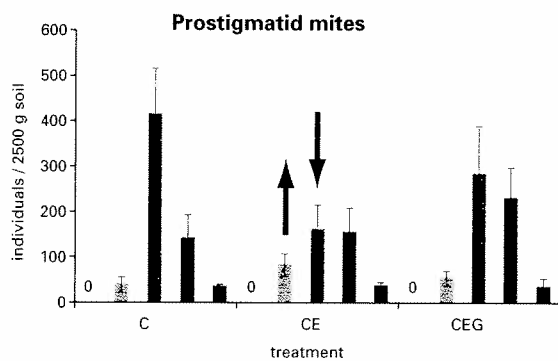


Fig. 10: Development of mesostigmatid mites during the course of the experiment. Further explanations see Fig. 1. The arrows indicate significant positive (bold) or negative effects of *E. crypticus* (ANOVA, LSD-test, $n=4$, $p<0.05$).

prostigmatid mites were clearly affected by enchytraeids, namely slightly positive after 5 weeks and strongly negative after 9 weeks, as the enchytraeids population had collapsed (Fig. 10).

The predatory mite *H. aculeifer* mostly negatively affected microarthropods (Figs. 4-10), in particular *I. notabilis* at week 5, *F. fimetaria* at week 9 and *P. armata* which was completely extinct in the end of the experiment. However, positive effects also were found, namely for Sminthuridae at week 5 ($n=4$, $p<0.05$, data not shown), for *I. anglicana* at week 5 and 9 (Fig. 4), for *M. macrochaeta* at week 13 and 17 (Fig. 9), for prostigmatid mites at week 9 and 13 (Fig. 10), and even for *P. armata* at the first sampling date (Fig. 8). Enchytraeids were not affected, although the difference between CE and CEG at week 17 (Fig. 1) hinted at predation by *H. aculeifer* which could not be confirmed statistically due to high variation and overall low enchytraeid numbers at that time.

Discussion

Effects on plants

E. crypticus exerted no significant effect on barley shoots, yet had a negative effect on root biomass, which together revealed a strong positive effect on shoot/root ratio. Although they are mainly saprophagous/microbivorous (Didden 1993), the enchytraeids may have fed on living roots. Kurir (1964) reviewed a number of studies in which enchytraeids had detrimental effects on a variety of plants. In our case, shoot biomass was not affected, and we also hypothesise that the observed effects were indirect. First, *E. crypticus* may have increased microbial activity and nutrient mineralisation (Williams & Griffiths 1989, Didden 1993, Hedlund & Augustsson 1995, Sulkava et al. 1996, Marinissen & Didden 1997). At high nutrient availability, plants produce less roots, the shoot/root ratio generally decreases. This explanation would be supported by the high grain weight in the enchytraeid compared to both other treatments. Yet more complex mechanisms could have occurred. For example, resource competition may have forced Collembola to feed on living roots in the enchytraeid treatments, this effect being reduced by *H. aculeifer* preying mainly on Collembola (Filser & Krogh, unpublished).

Interactions between mesofauna taxa

To our knowledge, predation or egg predation by enchytraeids on arthropods has never been reported. Therefore, any negative effects of *E. crypticus* upon Collembola are unlikely, except indirectly through resource competition. The two species that tended to be negatively affected in the beginning of the experiment when enchytraeids were abundant, are known to feed on fresh litter (*I. anglicana*; Filser 1999) or be abundant in decaying organic matter (*H. assimilis*; Gisin 1960). Thus, they may have competed with the enchytraeids for the clover material.

There are several possible explanations for positive effects of *E. crypticus* on microarthropods. Enchytraeid faeces are enriched in soluble carbohydrates, proteins, enzymes and nitrogen compared to the ingested soil or litter material (Kasprzak 1982), thus providing a high-quality nutrient resource for Collembola and mites (of which many species are known to feed on faeces of other soil animals). Most *E. crypticus* died between week 5 and 9, probably because of exploitation of the clover litter. The carcasses of the enchytraeids are a protein-rich food resource, either for microarthropods directly (at least *I. anglicana* and *P. armata* are known to be omnivorous, feeding also on other living or dead soil animals; Mebes & Filser 1998, Filser 1999, and references therein), or indirectly, for the bacteria and fungi growing on them. Collembola may also have inhibited enchytraeid population growth through predation on eggs or - at least the large *I. anglicana* - on juveniles, but our experimental design was not appropriate to test this hypothesis.

The predator under study, *H. aculeifer*, is highly omnivorous, feeding on both enchytraeids and Collembola (Chi 1981, Schlosser & Riepert 1992). Therefore, it probably preyed also on *E. crypticus* (although the differences were not significant), particularly at the end of the experiment, when Collembola densities were comparatively low. The positive effect of *H. aculeifer* on some taxa is surprising only at first sight. *I. anglicana* is very large compared to the small gamasid, and, being able to escape quickly, is thus less attractive than smaller, blind, slow-moving prey such as the clearly preferred *P. armata*. *M. macrochaeta*, on the other hand, is so small that it is probably a less attractive prey than any of the other microarthropod taxa present. Towards the end of the experiment, when resource availability in the microcosms was generally low, *M. macrochaeta* must have profited from reduced resource competition by other Collembola which were all less abundant at that stage in the presence of *H. aculeifer*. Also *I. anglicana* may have profited from the gamasids preying upon resource competitors in the beginning of the study. However, towards the end, at low arthropod density, it became a prey as well. Interestingly, the only taxon never negatively affected by the gamasid was the one most closely related to it, namely the prostigmatids (which were a contamination of the not completely defaunated soil). At approximate taxonomic level we refrain from discussing this commensalistic effect.

Our experiment indicated a positive effect of *E. crypticus* on plant / root ratio of barley under experimental conditions. Whether this holds true under field conditions remains to be tested. The interactions between enchytraeids and microarthropods were relatively weak, probably because a large worm population was only found in the first weeks of the experiment. Enchytraeids exerted both positive and negative effects on the numbers of mesostigmatid mites. Despite its very low population size (3-30 % of all other arthropod species), *H. aculeifer* had a strong negative impact on most Collembola, in particular on *P. armata* which was completely extinct in its presence. Other taxa appeared to be not or even positively affected, probably through indirect effects such as reduced competition. The COLLEAGUES experiment had been mainly designed for studying different Collembola taxa, which is why our discussion on enchytraeids had to remain speculative in many aspects. Despite this, we hope to have contributed to knowledge of the ecology of *E. crypticus*, Collembola and *H. aculeifer*, and we would be happy if some of our data stimulated colleagues to conduct more detailed experiments on these interactions.

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References

- Brones, M. J. I., J. Carreira & P. Ineson, 1998: *Cognettia sphagnetorum* (Enchytraeidae) and nutrient cycling in organic soils: a microcosm experiment. - *Appl. Soil Ecol.* 9: 289-294.
- Chi, H., 1981: Die Vermehrungsrate von *Hypoaspis aculeifer* Canestrini (Acarina, Laelapidae) bei Ernährung mit *Onychiurus fimatus* Gisin (Collembola, Onychiuridae) unter verschiedenen Temperaturen. - *Mitt. dtsh. Ges. allg. angew. Ent.* 3: 122-125.
- Cole, L., R. D. Bardgett & P. Ineson, 2000: Enchytraeid worms (Oligochaeta) enhance mineralization of carbon in organic upland soils. - *Eur. J. Soil Science* 51: 185-192.
- Didden, W. A. M., 1990: Involvement of Enchytraeidae (Oligochaeta) in soil structure evolution in agricultural fields. - *Biol Fertil Soils* 9: 152-158.
- Didden, W. A. M., 1993: Ecology of terrestrial Enchytraeidae. - *Pedobiologia* 37: 2-29.
- Filser, J., 1999: Habitat requirements and ecology of *Isotoma viridis* Bourlet, 1839 and *Isotoma anglicana* Lubbock, 1862 (Insecta, Collembola). - *Braunschweiger Naturkundliche Schriften* 5/4: 905-911
- Filser, J., A. Dette, H. Fromm, A. Lang, K. H. Mebes, J. C. Munch, R. Nagel, K. Winter & F. Beese, 1999: Reactions of soil organisms to site-specific management: the first long-term study at the landscape scale. - In: Windhorst, W., Enckell, P.H. (eds.): *Proceedings of the conference "Sustainable Landuse Management - The Challenge of Ecosystem Protection"*, 28.9.-1.10.99, Salza Federal Cultural Center, Organizers: University of Kiel/European Ecological Federation. *Ecosys Suppl.* Bd. 28: 139-147.
- Friebe, B., 1990: Die Besiedlung von Ackerböden durch die Meso- und Makrofauna in Abhängigkeit von der Bodenbearbeitung. - *Verh. d. Ges. f. Ökologie* XIX/II: 246-252.
- Fründ, H.-C., U. Necker & T. Kamann, 1992: Bodenbiologische Untersuchungen auf konventionell und organisch-biologisch bewirtschafteten Ackerflächen. - II. Erhebungen zur Bodenfauna. *Schriftenreihe VDLUFA* 35: 567-570.
- Gisin, H., 1960: Collembolenfauna Europas. - *Museum d'histoire naturelle*, Genf.
- Hedlund, K., A. Augustsson, 1995: Effects of enchytraeid grazing on fungal growth and respiration. - *Soil Biol. Biochem.* 27: 905-909.
- Huhta, V., P. Sulkava, K. Viberg, 1998: Interactions between enchytraeid (*Cognettia sphagnetorum*), microarthropod and nematode populations in forest soil at different moistures. - *Applied Soil Ecology* 9: 53-58
- Kasprzak, K., 1982: Review of enchytraeid (Oligochaeta, Enchytraeidae) community structure and function in agricultural ecosystems. - *Pedobiologia* 23: 217-232.
- Kurir, A., 1964: *Fridericia galba* (Enchytraeidae) als Fichtenschädling in einem Forstgarten. - *Pedobiologia* 4: 269-280.
- Laakso, J., & Setälä, Reindeer, 1999: Sensitivity of primary production to changes in the architecture of belowground food webs. - *Oikos* 87: 57-64.
- Lagerlöf, J., O. Andrén, K. Paustian, K., 1989: Dynamics and contribution to carbon flows of Enchytraeidae (Oligochaeta) under four cropping systems. - *Journal of Applied Ecology* 26: 183-199.
- Langmaack, M., C. Wiermann, S. Schrader, 1999: Interrelation between soil physical properties and Enchytraeidae abundances following a single soil compaction in arable land. - *J. Plant Nutr. Soil Sci.* 162: 517-525.

- Marinissen, J. C. Y., W. A. M. Didden, 1997: Influence of the enchytraeid worm *Buchholzia appendiculata* on aggregate formation and organic matter decomposition. - *Soil Biol. Biochem.*, Vol. 29, No.3/4: 387-390.
- Mebes, K.-H., J. Filser, 1998: Does the species composition of Collembola affect nitrogen turnover? - *Applied Soil Ecology* 9: 241-247.
- O'Connor, F. B., 1962: The extraction of Enchytraeidae from soil. - In: Murphy, P.W. (Ed.): *Progress in soil zoology*, Butterworth, London, p. 279-285
- Römbke, J., 1991: Estimates of the Enchytraeidae (Oligochaeta, Annelida) contribution to energy flow in the soil system of an acid beech wood forest. - *Biol. Fertil. Soils* 11: 255-260.
- Schlosser, H. J., F. Riepert, 1992: Entwicklung eines Prüfverfahrens für Chemikalien an Bodenraubmilben (Gamasina) Teil 1: Biologie der Bodenraubmilbe *Hypoaspis aculeifer Canestrini*, 1883 (Gamasina) unter Laborbedingungen. - *Zool. Beit. N.F.*, 34: 395-412.
- Standen, V., 1978: The influence of soil fauna on decomposition by microorganisms in blanket bog litter. - *Journal of Animal Ecology* 47: 25-38.
- Sulkava, P., V. Huhta, 1998: Habitat patchiness affects decomposition and faunal diversity: a microcosm experiment on forest floor. - *Oecologia* 116: 390-396.
- Sulkava, P., V. Huhta, J. Laakso, 1996: Impact of soil faunal structure on decomposition and N-mineralisation in relation to temperature and moisture in forest soil. - *Pedobiologia* 40: 505-513.
- Williams, B. L., B. S. Griffiths, 1989: Enhanced nutrient mineralization and leaching from decomposing Sitka spruce litter by enchytraeid worms. - *Soil Biol. Biochem.* 21: 183-188.