vTI
Johann Heinrich
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Sonderheft 357
Special Issue

Newsletter on Enchytraeidae No. 12

Proceedings of the 9th International Symposium on Enchytraeidae, 14-16 July 2010, Braunschweig, Germany

Stefan Schrader and Rüdiger M. Schmelz (Eds.)

Bundesministerium für
Ernährung, Landwirtschaft
und Verbraucherschutz

Bibliographic information published by the German National Library

The German National Library lists this publication in the German National Bibliography; detailed bibliographic data are available in the internet at http://www.d-nb.de/

Bibliografische Information der Deutschen Bibliothek

Die Deutsche Bibliothek verzeichnet diese Publikation in der Deutschen Nationalbibliografie; detaillierte bibliografische Daten sind im Internet über http://www.d-nb.de/ abrufbar.

Johann Heinrich von Thünen-Institut

2012

Landbauforschung
vTI Agriculture and Forestry Research

Johann Heinrich von Thünen-Institut
Federal Research Institute for Rural Areas, Forestry and Fisheries,

Johann Heinrich von Thünen-Institut
Bundesforschungsinstitut für
Ländliche Räume, Wald und Fischerei (vTI)

Bundesallee 50, D-38116 Braunschweig, Germany

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www.vti.bund.de

Preis / Price 8 €

ISSN 0376-0723
ISBN 978-3-86576-083-8

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Stefan Schrader[1] and Rüdiger M. Schmelz[2] (Eds.)

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Editorial

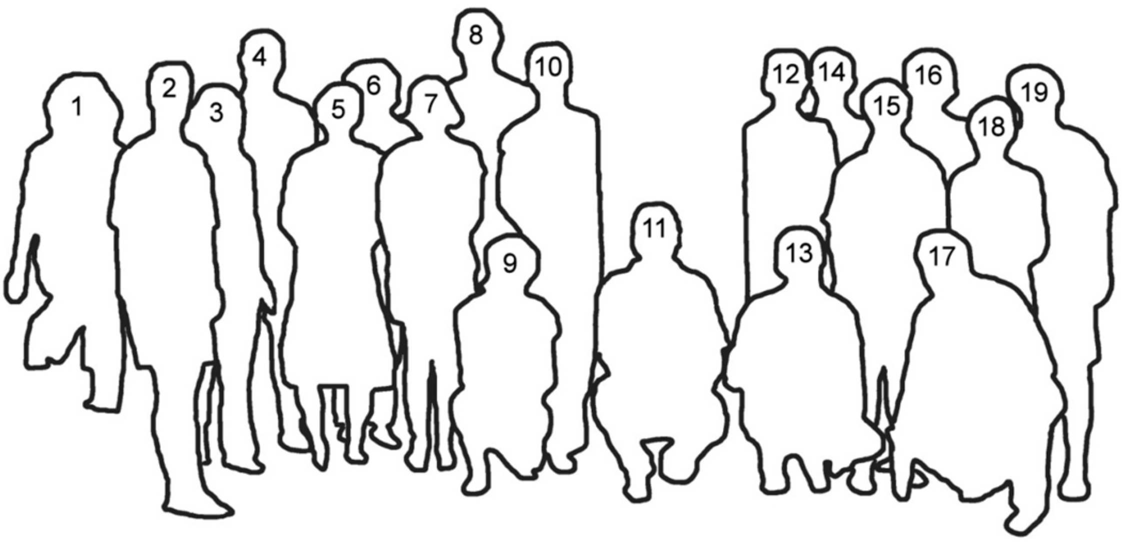
The Newsletter on Enchytraeidae is a series of proceedings, which from issue Nº 4 on present the scientific output of the International Symposia on Enchytraeidae, held every two years from 1994 on. Each issue is traditionally edited by the organizers of the respective conference. The 9th International Symposium on Enchytraeidae was held at the Johann Heinrich von Thünen-Institute (vTI) in Braunschweig, Germany, 14 to 16 July, 2010. In total, twenty participants from eight countries attended the symposium. During the first two days, nine oral and seven poster presentations were given and discussed. Afterwards, a microscopic workshop provided the opportunity for the participants to study interesting or unfamiliar specimens of enchytraeids, using and testing the new identification key on European enchytraeid species, and to discuss open questions in enchytraeid taxonomy. This workshop was supervised by Rüdiger M. Schmelz.

The present Newsletter on Enchytraeidae No. 12 includes most of the scientific contributions that were presented at the 9th International Symposium on Enchytraeidae. These papers cover a wide range of basic and applied research on terrestrial enchytraeids, and they contribute valuable information to the knowledge of soil biodiversity. Additionally, this volume contains a list of currently accepted enchytraeid species, compiled by R.M. Schmelz and R. Collado. All papers included in this volume have been critically evaluated by two reviewers. We acknowledge the effort of all authors and reviewers to make these proceedings possible.

The symposium was organized by Stefan Schrader and kindly hosted by the Institute of Biodiversity at the vTI. Special thanks are due to Sabine El Sayed, who did a lot of work behind the scene to make the symposium a comfortable one. The 10th International Symposium on Enchytraeidae will take place in Coimbra, Portugal, 13-14 August 2012, in association with the XVI International Colloquium of Soil Zoology.

Stefan Schrader
Rüdiger M. Schmelz

9th International Symposium on Enchytraeidae
14-16 July 2010, Braunschweig, Germany



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Relationships between microannelid and earthworm activity

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Abstract

Principal abiotic factors controlling the activity of enchytraeids and earthworms are generally known. Far less information is available on the influence of interactions between both groups. Data from soil monitoring sites in North-Western Germany provide information on earthworm and microannelid activity. Changes in the relation of both groups require interpretation with respect to soil condition changes. The data were analysed with respect to possible patterns in the relationship of earthworm and microannelid activity and influencing factors concerning antagonistic or similar development of both groups. The total abundance of both groups is not significantly correlated for the land-use types forest, grassland and cropland. Stronger correlations are found on the genus or species level. We selected a number of site examples with repeated sampling for opposing trends of both annelid groups as well as similar trends and specify for each case possible determining environmental factors. When abundances of earthworms and microannelids show antagonistic behaviour, this is mostly due to the rise or fall of only one or two species in each group. Probably competition in case of restricted resources plays also a role. Information on land management (fertilization, tillage etc.) and environmental factors (soil properties, climate) are crucial for the interpretation of the results. However, species-specific information on food preferences and behavioural responses on environmental changes are too limited to assess the role of competition between species from the analysed field data.

Keywords: Enchytraeidae; Lumbricidae; abundance; competition; *Dendrobaena attemsi*; land use

1. Introduction

Soil biological investigations at soil monitoring sites aim at detecting long-term changes in the biological soil condition. In three German federal states earthworms as well as microannelids (mainly enchytraeids) are investigated at soil monitoring sites as faunistic indicators of the biological soil condition. This offers the opportunity to assess interrelations between both groups, and thus to add new aspects to the interpretation of activity patterns with respect to soil condition changes. Main soil properties influencing the occurrence of earthworms as well as of enchytraeids are soil moisture, pH and texture (Beylich & Graefe 2009, Krück et al. 2006). In cultivated land also management practices play a decisive role. Some factors affect both groups in a similar way. For example, intensive tillage or drought are known to have negative consequences for the abundance of earthworms and enchytraeids (Langmaack et al. 1996, Plum & Filser 2005), while reduced tillage and organic fertilizer application can have positive effects (Langmaack et al.1996, Pfiffner 1993, Sauerlandt & Marzusch-Trappmann 1959). On the other hand, we find situations where earthworms and microannelids show opposed reactions to environmental changes. Studies on forest soil liming often proved that earthworms profit from increasing pH while enchytraeid abundances decrease (Hartmann et al. 1989, Graefe 1990). In this case, as in most experimental field studies aiming at examining the effect of a certain factor, there is mostly one sampling before the impact and one or more samplings afterwards, documenting the development of the relation between earthworms and enchytraeids as reaction to the environmental changes.

On the other hand, we find field investigations with only one sampling occasion providing us with information on the relationship of both groups as a response to the actual environmental conditions. Also for this latter approach researchers find either antagonistic behaviour (Górny 1984) or both groups showing relatively high abundance, e.g. at some grassland sites (Bauer 2003, Rutgers et al.

2008). The influencing factors are sometimes less obvious in these cases than in experimental setups with defined manipulation of one or a few factors. Thus, data on soil properties and management practices are inevitable for the interpretation of the results.

To assess influencing factors as moisture or liming, but also species composition, laboratory experiments have been conducted. Results are inconsistent. The presence of earthworms can reduce enchytraeid density in mesocosm studies (Räty & Huhta 2003, Räty 2004), but enchytraeids may also increase earthworm mortality in the experiments (Haukka 1987), depending on the experimental setup. The selection of species investigated seems to be essential due to species-specific size differences, habitat requirements and food preferences.

The formulation of reference ranges for the abundance of enchytraeids and earthworms, based on soil monitoring data, showed us general patterns for the relation of both groups, but also deviating behaviour in many cases (Beylich & Graefe 2009). The aim of the current data analysis was to (a) find patterns in the relationship of earthworm and microannelid activity (=abundance) for different land use types, (b) to identify influencing factors concerning antagonistic or similar development of the activity of enchytraeids and earthworms in time series, (c) to detect species-specific effects and (d) to find possible clues for competition for similar resources.

2. Material and methods

The data analysed in this work originate from investigations of 55 soil monitoring sites (Boden-Dauerbeobachtungsflächen, BDF) situated in Germany in the federal states of Schleswig-Holstein, North Rhine–Westphalia and Hamburg. The land-use types were forest (22 sites), grassland (17 sites) and cropland (16 sites). At all monitoring sites earthworms and microannelids were studied. Microannelids in this work comprise mainly enchytraeids, but sometimes also tubificids and polychaetes. For convenience, the terms "enchytraeids" and "microannelids" are used synonymously here. The parameters studied were abundance of earthworms and microannelids, earthworm biomass, vertical distribution of microannelids and species composition of both groups. Ten samples were taken at each monitoring site. Samplings are generally repeated after 5-10 years, so that most sites have undergone two or three samplings so far. Several site-examples with repeated samplings were chosen to demonstrate antagonistic or similar trends in earthworm and microannelid activity.

Earthworm sampling was carried out by a combination of formalin extraction (0.25 m²; ISO 2006), hand-sorting and Kempson extraction. For hand-sorting the samples were taken with a soil corer (250 cm², 20 cm depth cropland, 10 cm depth grassland and forest). The hand-sorted samples were afterwards extracted with a Kempson extractor (heat extraction) to obtain very small specimens that had been overlooked during hand-sorting. Assuming that individuals of similar size might be more likely to compete, the mean individual weight was calculated by dividing mean biomass by mean abundance for some species. For earthworm biomass determination the worms, fixed in formalin, were weighed with a scale-reading precision of 0.01 g (fresh weight with gut contents).

Soil samples for enchytraeids were taken according to ISO (2007) with a split soil corer (diameter 3.8 cm (cropland) or 5 cm (forest, grassland)). Sampling depth was 24 cm at cropland sites and 10 cm at forest and grassland sites. The different sampling depths take into account that the vertical activity reaches farther down in cropland sites due to tillage. Samples were divided vertically into 4 sub-samples of equal height. Soil samples were extracted over 48 h by a wet-funnel technique without heating (following Graefe 1984, as cited in Dunger & Fiedler 1989, p. 301; DIN ISO 2007). The extracted animals were counted and identified alive.

For the interpretation of abundance data, reference ranges according to Beylich & Graefe (2009) were consulted for comparison. For the correlation of the abundances of both annelid groups, of dominant genera or species, significance of correlation was checked with Spearman's rho correlation of SPSS 15.0. In addition to correlation analysis for land-use types, several sites are presented in detail to exemplify certain patterns. The site

Tab. 1. Site characteristics for the forest sites presented in section 3.1. BDF = soil monitoring site; OH = organic horizon. (Data from Haag et al. 2009, Metzger et al. 2005).

Forest site	Soil Type	Humus form	Vegetation	Texture	pH OH ($CaCl_2$)	Remarks
BDF-NW 1.5 Elberndorf	Gleyic Cambisol	Mor	*Picea abies*	silt loam	2.8	-
BDF-NW 1.12 Castrop-Rauxel	Planosol	Mor / moder	deciduous forest, mainly *F. sylvatica*	sandy loam	3.6	clay in subsoil; liming 2001
BDF-NW 1.9 Duisburg Wald	Dystric Cambisol	Mor	*Fagus sylvatica*	sandy loam	4.1	liming 1990; 2001?
BDF-NW 2.1 Mattlerbusch	Gleyic Cambisol	Mor / moder	*Quercus robur*	silt loam, sandy loam	3.6	cropland up to 100 years ago

Tab. 2. Site characteristics for the grassland and cropland sites presented in section 3.2 and 3.3, respectively. BDF = soil monitoring site, GW = groundwater, n.d. = no data (Data from LLUR 2007, Haag et al. 2009, Metzger et al. 2005).

Site	Soil Type	Land use	Texture	pH ($CaCl_2$)	GW influence topsoil	Remarks
BDF-SH 13 Kleihof	Gleysol (marsh)	meadow/pasture	clay loam	5.4	+	
BDF-SH 25 Kudensee	Histosol	meadow/pasture	- (peat)	4.7	+	summer sampling 1995
BDF-NW 3.2 Lütkenberg	Cambisol	meadow	silt loam	5.3		
BDF-HH Amsinckpark	n.d.	park meadow	n.d.	4.3		
BDF-SH 04 Stadum	Gleyic Podzol	maize field	sand	4.7		• dung up to first sampling • no dung 1998-2007, no liming until 2008 • dung again since 2007, liming 2008 • reduced tillage since 2007

Tab. 3. Correlations between the abundance of earthworms and enchytraeids or single taxa of these groups for different land-use types. rs: Spearman's rho rank-correlation coefficient.

Land use	Taxa correlated	significance	r_s
All land uses	earthworms vs. enchytraeids	** p = 0.01	- 0.412
Forest	earthworms vs. enchytraeids	n.s.	- 0.100
	Dendrobaena attemsi vs. enchytraeids	** p = 0.01	- 0.752
	Dendrobaena attemsi vs. Achaeta sp.(affinoides)	n.s.	- 0.528
Wet grassland	earthworms vs. enchytraeids	n.s.	0.169
	epigeic earthworms vs. *Enchytronia* species	n.s.	0.055
	epigeic earthworms vs. *Fridericia* species	n.s.	- 0.130
	Eiseniella tetraedra vs. *Fridericia* species	* p = 0.05	- 0.612
	Aporrectodea caliginosa vs. enchytraeids	n.s.	0.405
	Aporrectodea caliginosa vs. *Fridericia* species	n.s.	0.297
Grassland	earthworms vs. enchytraeids	n.s.	- 0.044
	earthworms vs. *Enchytronia* species	* p = 0.05	- 0.468
	earthworms vs. *Enchytraeus* species	* p = 0.05	0.480
	anecic earthworms vs. *Enchytraeus* species	** p = 0.01	0.651
	earthworms vs. *Fridericia* species	** p = 0.01	0.489
	A. caliginosa vs. *Fridericia, Enchytraeus* and *Enchytronia* species	n.s.	< 0.310
Cropland	earthworms vs. enchytraeids	n.s.	- 0.022
	earthworms vs. *Enchytronia* species	n.s.	- 0.254
	earthworms vs. *Enchytraeus* species	n.s.	0.153
	earthworms vs. *Fridericia* species	** p = 0.01	0.505

characteristics for these examples are given in Tabs. 1 and 2. Soil texture was translated from German into English according to Schrey (2009), soil types were translated according to BGR (2008).

3. Results

The correlation analysis between microannelid abundance and earthworm activity showed a significant trend for enchytraeid abundance to be higher at low earthworm activity (Fig. 1, Tab. 3). However, low to medium microannelid abundance could be found with high as well as with low earthworm activity. To extract possible patterns concerning microannelid and earthworm activity, data were analysed separately for the land-use types forest, grassland and cropland.

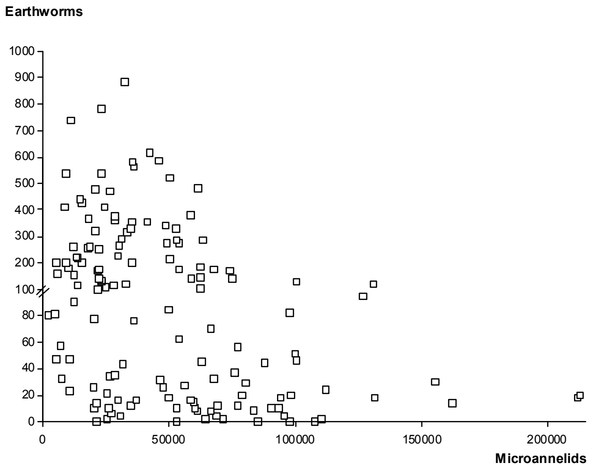

Fig. 1. Correlation of abundances (Ind. m^{-2}) between earthworms and microannelids at 55 German soil monitoring sites (forest, grassland, cropland). N=141 samplings.

3.1. Acid forest soils

All forest soil monitoring sites investigated were characterised by acid soil conditions with pH ($CaCl_2$) < 4.0 in the mineral topsoil (22 sites with 61 samplings). Earthworm abundance ranged from 0 to 520 Ind. m^{-2}, while enchytraeids abundance laid between 21,543 and 212,784 Ind. m^{-2}. There was no significant correlation between both parameters (Tab. 3). In Fig. 2 four examples for acid forest sites are compiled, showing different relations between earthworm and enchytraeid activity. Principal site characteristics are given in Tab. 1. The most acid site (Elberndorf) showed lowest earthworm abundance at all three sampling dates in comparison with the other three examples. In contrast, enchytraeid densities at this site were comparatively high. The sites Castrop-Rauxel and Duisburg Wald had in common (a) abundance of earthworms > 140 Ind. m^{-2}, (b) dominance of the epigeic earthworm species *Dendrobaena attemsi* between 63 and 98%, (c) enchytraeid abundance < 60,000 Ind. m^{-2} and (d) pH ($CaCl_2$) > 3.4. The fourth example, site Mattlerbusch, showed at the first two sampling dates similarities with the most acid site, that is: high enchytraeid abundance, low earthworm abundance and absence or low dominance of *Dendrobaena attemsi*. However, at the third sampling the picture resembled rather that of the other two sites, with a dominance of *D. attemsi* of 85%. The enchytraeid abundance at Mattlerbusch in 2007 amounted to only about one third of the abundance in 2002, which was mainly due to dropping numbers of *Achaeta* sp. (*affinoides*).

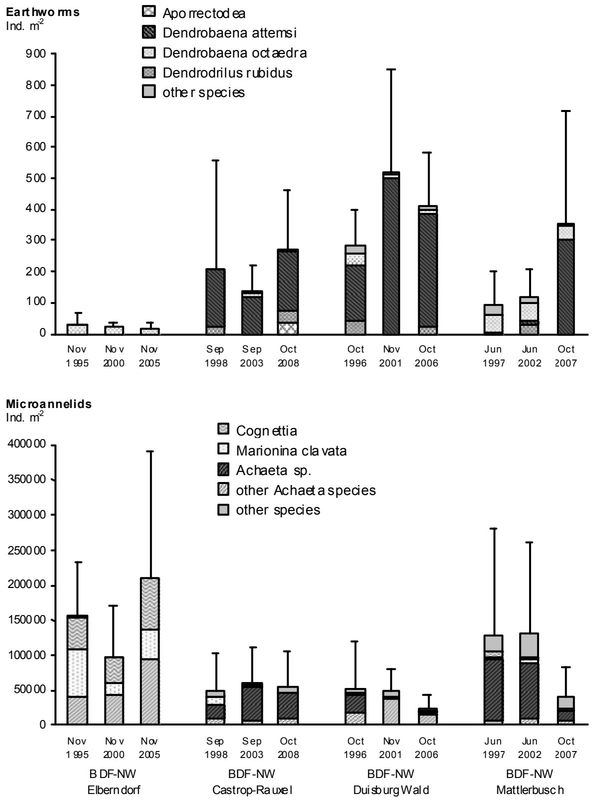

Fig. 2. Abundance of earthworms (above) and microannelids (below) at four acid forest sites. Three sampling dates for each site. Error bars: standard deviation.

The species numbers of Elberndorf were 8-9 for enchytraeids and 1-2 for earthworms respectively. The species numbers for the other three forest sites amounted to 11-20 for enchytraeids and 3-4 for earthworms respectively. If we considered all samplings with *Dendrobaena attemsi* present, we found a significantly negative correlation between the abundance of this earthworm species and the total enchytraeid abundance (Tab. 3). *Dendrobaena attemsi* occurred always in combination with *Achaeta* sp. (*affinoides*). The correlation between both species was also negative but not significant.

3.2. Grassland

Among the grassland sites there were five with groundwater influence in the topsoil at least part of the year. These sites will be termed "wet grassland" in the following, separating them from the rest that will be termed "grassland".

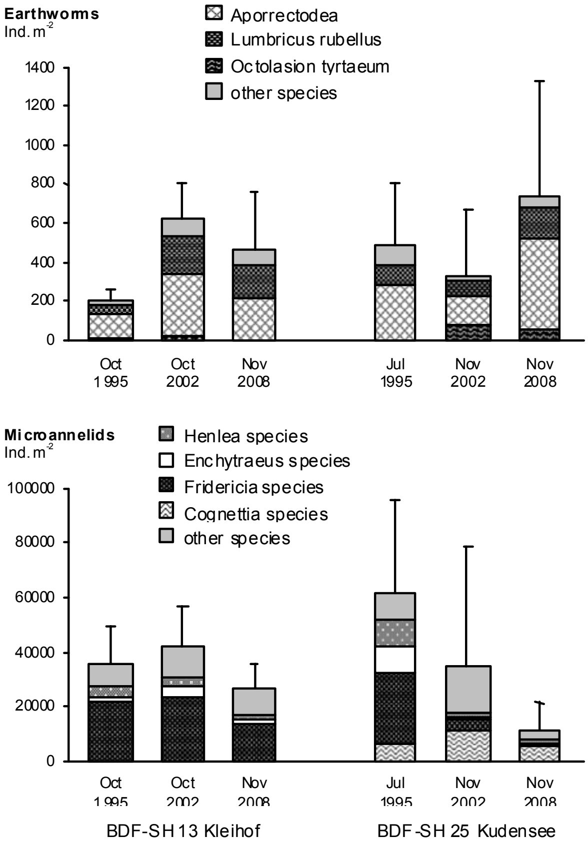

Fig. 3. Abundance of earthworms (above) and microannelids (below) at two wet grassland sites. Three sampling dates for each site. Error bars: standard deviation.

Two examples of wet grassland with different relationships between earthworms and microannelids have been chosen for Fig. 3. Site characteristics are given in Tab. 2. At the site SH 13, abundances of both groups showed parallel fluctuations. A threefold increase of earthworm numbers between 1995 and 2002 did not result in decreased microannelid numbers at the sampling date in 2002. At BDF SH 25, by contrast, the highest earthworm abundance of the three sampling dates coincided with the lowest enchytraeid abundance. Due to high groundwater levels, the microannelids at BDF-SH 13 were concentrating at the topmost 2.5 centimeters at all three sampling dates (Fig. 4). At BDF-SH 25 the vertical distribution of microannelids was much more balanced at the first sampling, which, as an exception, had taken place in summer. As the habitable space reached down to at least 10 cm, total abundance of enchytraeids was higher than at the other sampling dates. The species numbers for earthworms were 4 (BDF-SH 13) and 5-8 (BDF-SH 25) and for microannelids 18-21 (BDF-SH 13) and 18-23 (BDF-SH 25) respectively. Anecic earthworm species were absent.

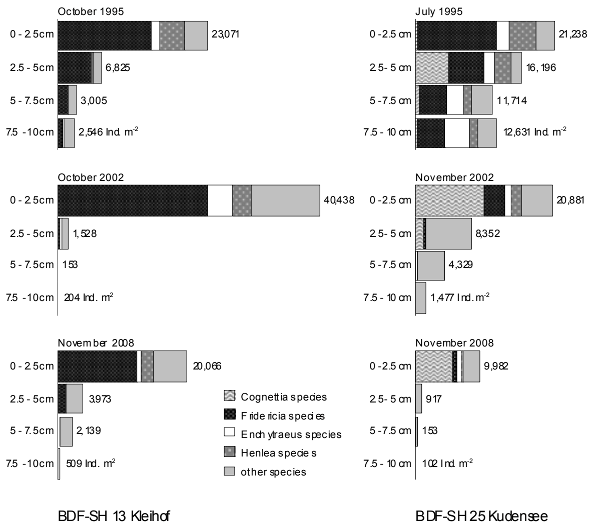

Fig. 4. Vertical distribution of microannelids for two wet grassland sites (same samplings as in Fig. 3).

Earthworm abundance of all five investigated wet grassland sites ranged from 200 to 739 Ind. m^{-2}, while microannelid abundance lay between 8,913 and 63,458 Ind. m^{-2} (14 samplings). Both parameters were not correlated (Tab. 3). Correlations between epigeic earthworms and *Fridericia* or *Enchytronia* species showed no significant relationships, while the abundance of the epigeic species *Eiseniella tetradedra* was significantly negatively correlated with the abundance of the genus *Fridericia*. In contrast, the endogeic species *Aporrectodea caliginosa* showed positive correlation coefficients when correlated with enchytraeids as a whole and with the genus *Fridericia*, but these results were not significant.

While *Eiseniella tetraedra* is a small species (mean individual biomass 0.04 g Ind^{-1}, 4 samplings), *Aporrectodea caliginosa* is of medium size (mean individual biomass 0.17 g Ind^{-1}, 12 samplings). However, individual biomass is naturally variable. At some samplings, high numbers of small (juvenile) individuals of *A. caliginosa* were recorded. This was striking at the third sampling at BDF-SH 25 (Fig. 3), where high numbers of earthworms with individual biomass below average coincided with low numbers of enchytraeids.

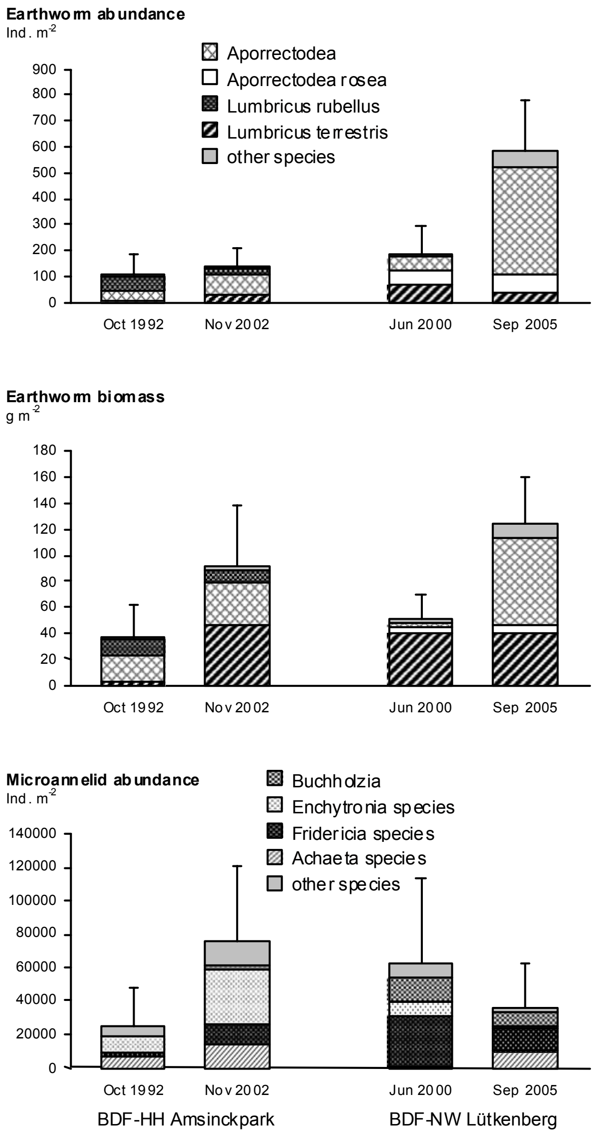

Fig. 5. Abundance of earthworms (above) and microannelids (below) and earthworm biomass (middle) at two grassland sites. Two sampling dates for each site. Error bars: standard deviation.

This situation could also be found on grassland sites not influenced by groundwater (soil characteristics Tab. 2). Fig. 5 shows that an increase of earthworms by a factor of three, mainly caused by *A. caliginosa*, was accompanied by a decrease of microannelids (BDF-NW Lütkenberg). Mean individual biomass of *A. caliginosa* is 0.27 g on grassland sites (27 samplings). At site Lütkenberg mean individual weight of this species was well below average (0.17 g), indicating high numbers of small juveniles. The decline of enchytraeids here was mainly due to falling numbers of the genera *Fridericia* and *Enchytronia*. At the same time, species number of microannelids rose from 20 to 26. However, increased earthworm activity can also coincide with increased microannelid abundance. At an urban park meadow (BDF-HH Amsinckpark), the abundance of earthworms was about 30% higher at the second sampling compared to the first sampling (Fig. 5). This was partly caused by an increase of the big anecic species *Lumbricus terrestris*, so the rise was even more pronounced regarding the earthworm biomass (145 % increase). Microannelid abundance also rose by a factor of three in the same period, *Enchytraeus* species being the most dominant.

Earthworm abundance of all 12 investigated grassland sites ranged from 91 to 886 Ind. m^{-2}, while microannelid abundance lay between 9,218 and 75,274 Ind. m^{-2} (27 samplings). Abundances of microannelids and earthworms were not correlated (Tab. 3). However, at genus level significant correlations were found. The genus *Enchytronia* was negatively correlated with earthworms while the genera *Enchytraeus* and *Fridericia* were positively correlated with earthworm occurrence. The correlation of the genus *Enchytraeus* was even more pronounced with the occurrence of anecic species (*Lumbricus terrestris* and *Aporrectodea longa*). The common species *A. caliginosa* showed no significant correlation with total enchytraeid abundance nor with the abundance of the genera *Enchytraeus*, *Fridericia* and *Enchytronia*.

3.3. Cropland

We chose one example for cropland sites only to show the difficulties in generalizing the relationships between earthworm and microannelid abundance for this land-use type (Fig. 6). At site BDF-SH 04 maize had been grown since the start of our investigations without crop rotation (Tab. 2). Earthworms did not show much variation between the first two samplings, being represented by one single species (*Aporrectodea caliginosa*). At the same time the microannelid community changed completely: while the genus *Enchytraeus* had dominated strongly at the first sampling, it has nearly vanished seven years later. At that time, indicators of moderate acidity belonging to the genus *Achaeta* were dominating the scene. At the third sampling, again the picture has changed completely. In addition to the still dominating *Achaeta* species we found *Enchytraeus* species again, but also representatives of the genera *Fridericia* and *Henlea*. Species number of the enchytraeids rose from five (1996) to twelve (2009). Further, three additional epigeic earthworm species were found in 2009. Total abundance for both annelid groups was highest in 2009.

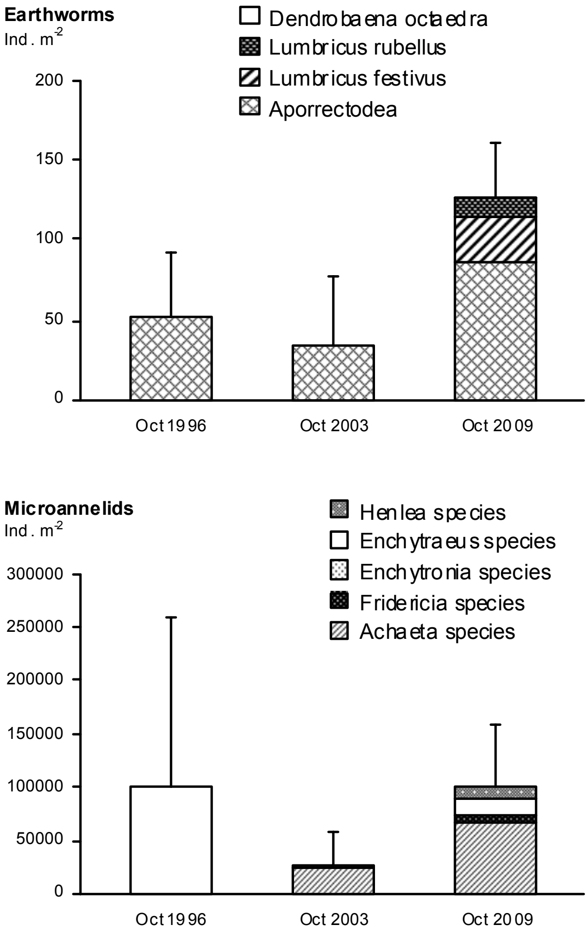

Fig. 6. Abundance of earthworms (above) and microannelids (below) at a maize field in Schleswig-Holstein (BDF-SH Stadum). Three sampling dates. Error bars: standard deviation.

Earthworm abundance of all 17 investigated cropland sites ranged from 0 to 782.8 Ind. m^{-2}, while enchytraeid abundance lay between 2,037 and 100,607 Ind. m^{-2} (38 samplings). The abundance of earthworms was not correlated with microannelids in general and not with the genera *Enchytreaus* and *Enchytronia*, either (Tab. 3). A significant correlation was found between earthworms and the genus *Fridericia*.

4. Discussion

Earthworms shape the topsoil habitat, e.g. by their burrowing activity, by depositing casts and by influencing microbial activity. These activities can affect enchytraeids, which on the other hand might compete for food resources with earthworms. In addition, both groups are influenced by abiotic and biotic factors independent of their reciprocal interaction. Obviously, we cannot expect a simple relationship between both groups, but rather a network of relations, mostly at species level, influencing the total abundance of both groups in various ways.

4.1. Acid forest soils

The BDF-NW Elberndorf showed a situation as found occasionally in strongly acid forest soils throughout northern and western Europe (Nielsen 1955, Abrahamsen 1972 a, b, Haag et al. 2009). Very few earthworm species are able to exist under these adverse conditions, *Dendrobaena octaedra* often being the only species present in low numbers. Among enchytraeids several species do not only tolerate strongly acid conditions but also reach high population densities. In any case, enchytraeid abundance of > 100,000 Ind. m^{-2} does not seem to be the rule at forest soils with a moder or mor humus layer, and the prerequisites for such very high numbers are not clear. Enchytraeid densities between 50,000 and 100,000 Ind. m^{-2} are most common in combination with earthworm abundance well below 100 Ind. m^{-2} (Fig. 2; Abrahamsen 1972a,b). Whether the low numbers of *D. octaedra* at Elberndorf were mainly due to very low pH or to less successful competition with the numerous enchytraeids for food resources or to other factors, cannot be deduced from the data presented. The pH can have a considerable effect on the competitive performance of species (Hyvönen et al. 1994). Although *Dendrobaena octaedra* is acid tolerant, it reaches higher densities at pH ($CaCl_2$) above 4.0. In laboratory studies Huhta & Viberg (1999) found numbers of *Cognettia sphagnetorum* suppressed in presence of *D. octaedra* at higher pH values (pH 5.5). Liming experiments have shown that the total abundance of autochthonous enchytraeid communities of acid forest soils with mor humus layers, consisting of a few acid tolerant species, is affected negatively by liming while lumbricid activity rises (Bååth et al. 1980, Abrahamsen 1983, Schauermann 1985, Makeschin & Rodenkirchen 1994). This is generally supported by the results of the forest sites NW Castrop-Rauxel and Duisburg Wald, which had been limed in 2001. However, experiments of Pokarzhevskii & Persson (1995) suggest that *C. sphagnetorum*, generally the most numerous enchytraeid species in acid forest soils, is not directly affected by high pH but rather by increased competition of other species. Further, a changed microbial community composition after liming, e.g. concerning the ratio fungi / bacteria, could be an decisive factor.

While the higher pH at the forest sites Castrop-Rauxel, Duisburg Wald and Mattlerbusch could be seen as one principal factor causing low numbers of *Cognettia sphagnetorum* and probably other indicators of extreme acidity among the enchytraeids at these sites, we would expect higher numbers of *Dendrobaena octaedra*. According to Huhta (1979) also *Dendrodrilus rubidus* benefits from pH increase. However, the epigeic earthworm *Dendrobaena attemsi* seems to profit more successful from liming than the other epigeic earthworm species at the sites investigated here. The reason for the increase of *D. attemsi* at site Mattlerbusch is not known. The site was not limed, but had been cropland until roughly 100 years ago. *D. attemsi* here seems to have a competitive advantage compared to the other earthworm species as well as the enchytraeids. Experimental studies to assess interspecific interactions of *D. attemsi* have not been published. In Germany *Dendrobaena attemsi* is not very common and has mainly been found near settlements, often after liming (Beylich 1995, Römbke et al. 2000, Haag et al. 2009). It occupies the humus layer as well as *Dendrobaena octaedra* and the enchytraeid species typical for mor humus layers, as *Cognettia sphagnetorum*. Thus, competition for food resources seems possible.

4.2. Grassland

For the wet grassland sites two examples are presented with similar and antagonistic relationships of microannelids and earthworms respectively. Based on the results of BDF-SH Kleihof we conclude that under generally favourable conditions (pH, food resources) very high numbers of earthworms are compatible with more than average enchytraeid abundance according to the reference range (Beylich & Graefe 2009), even if habitable space is limited to the upper few centimeters due to anoxic conditions in deeper soil layers. In contrast to Kleihof the site Kudensee had peaty soil with lower pH values. Whether the low number of enchytraeids at this site at the third sampling was only caused by severe lack of oxygen or due to further adverse conditions or direct competition with earthworms, is unclear. Adult earthworms, kept in water saturated soil, have been reported to leave their hind end at the soil surface, while the front end remains in deeper layers (feeding?) (Roots 1956). In case also juveniles of *Aporrectodea caliginosa* were capable of this behaviour, this could explain their ability to exist in high numbers in water saturated soil in contrast to microannelids at BDF SH 25. Also our results of other BDF show, that enchytraeids show more often negative short-term reactions to water-logged soil than earthworms. Further, the comparatively low pH might have played a role at the site SH Kudensee. Species number rose from first to third sampling due to an increase of acidity indicators suggesting increasing soil acidity, but not all enchytraeid species occurring at this site, that are tolerating low pH, are simultaneously tolerating wetness. It should be noted that in soils influenced by groundwater the activity may change to a great degree with varying water levels within several months (Beylich & Graefe 2007, Plum & Filser 2005). For the description of the biological soil condition the abundance data are thus of limited informative value and should always be complemented by species composition data.

The fact that single species contribute very differently to changes in total abundance of the whole group explains why we find correlations rather at species or genus level than at family level. A negative correlation between *Eiseniella tetraedra* and *Fridericia* species at wet grasslands suggests direct competition. This small epigeic earthworm species occupies the upper few centimeters, where enchytraeids concentrate as well in times of oxygen deficiency in soil. However, *Eiseniella tetraedra* occurred on two of the wet grassland sites only, so the basis for establishing this relationship is weak.

In contrast to the wet grassland sites, the genus *Enchytraeus* was significantly positively correlated with earthworm abundance at the other grassland sites, which was probably mainly due to a strong relation with the abundance of anecic earthworms, which were not found at the wet grassland sites. Some studies have shown that enchytraeids settle on earthworm casts and consume them, apparently making use of material more easily digestible than the surrounding soil (Dawod & FitzPatrick 1993, Topoliantz et al. 2000, Räty 2004). Also earthworm burrows proved to be more attractive for enchytraeids than the adjacent soil in a woodland study (Dózsa-Farkas 1978). Possibly the opportunistic *Enchytraeus* species profit more from microhabitats created by the deep-burrowing earthworms (casts, burrow coatings), than some other enchytraeid species. Further, both groups prefer slightly acid to neutral soils. Räty (2004) found that earthworms (*Lumbricus terrestris, Aporrectodea caliginosa*) decreased topsoil acidity in a laboratory study. Also the middens of *L. terrestris* showed higher pH than the untreated soil and were preferentially inhabited by an *Enchytraeus* species.

The genus *Enchytronia* showed a quite opposed behavior at the grassland sites, providing an example of antagonistic behavior of earthworms and enchytraeids. This genus was predominantly represented by *Enchytronia parva* at most sites, which is an indicator of moderately acid soils (according to Graefe & Schmelz 1999, Graefe & Beylich 2003), thus indicating conditions not at optimum for anecic and endogeic earthworms. In addition, the study of Dózsa-Farkas (1978) on colonization of earthworm burrows by enchytraeids showed, that the preference of *E. parva* for earthworm burrows is less pronounced than in other enchytraeid species.

The abundance relationship between *Aporrectodea caliginosa* and enchytraeids or enchytraeid genera is difficult to interpret. Sometimes we found examples for antagonistic behavior, especially when small juvenile individuals of *A. caliginosa* occurred in great quantities (BDF-SH Kudensee and BDF-NW Lütkenberg). A meta-analysis conducted by Eisenhauer (2010) on the impact of earthworms on soil microarthropods showed negative effects of endogeic earthworm species on microarthropods, especially at high earthworm densities. The reasons suggested by the author are a) modification of soil structure by burrowing and thereby disturbing microarthropods and their eggs b) higher competitive strength of earthworms concerning competition for food resources. Similar effects on enchytraeids, belonging to the same size range and partly exploiting the same food sources as microarthropods, are imaginable.

4.3. Cropland

In contrast to grassland sites, we found no correlation between the genus *Enchytraeus* and anecic earthworms at cropland sites. One reason might be the fact that anecic species were almost or completely absent at some cropland sites. Especially on sandy cropland sites lumbricid earthworms are often limited to one or few endogeic species (Graefe 1999, Beylich & Graefe 2009). Tillage induced disturbances affect enchytraeids as well as earthworms, reducing the abundance of both groups (Langmaack et al. 1996), but there is also evidence that both groups react differently to tillage measures (Topoliantz et al. 2000). Food supply on cropland sites is variable in amount, frequency and type (organic / inorganic fertilizer), favouring the occurrence of colonizer species. Among earthworms, mainly the epigeic species, normally inhabiting the litter layer, can be considered as colonizers. As epigeic earthworms are mostly missing at cropland sites, earthworms are often not represented with colonizer species here at all. Among enchytraeids colonizers belong mainly to the genus *Enchytraeus*, which is regularly found at cropland sites. Due to their high reproductive ability *Enchytraeus* species can exploit sporadic food supplies more successful than other species. Furthermore, their populations recover easier after management impacts. Consequently, their abundance is not correlated with that of the other genera, which require more time for population stabilization after a collapse (K-strategists). The latter comprise e.g. the genera *Fridericia* and *Henlea* as well as endogeic earthworms, which also share habitat requirements, inhabiting the upper mineral soil, preferring slightly acid to neutral soil. This would explain a positive correlation between endogeic earthworms and *Fridericia* species, at least in case of sufficient food resources.

The fundamental changes in community structure at BDF-SH Stadum illustrate management effects. Dung application in the years before the first sampling brought forward *Enchytraeus* species (first sampling). As liming was neglected, acid tolerant *Achaeta* species dominated at the second sampling. In the years preceding the third sampling the site was limed, dung application was restarted and tillage was reduced. As a consequence, we found colonizers (*Enchytraeus* species), acid tolerant species (*Achaeta* species), but also indicators of neutral to slightly acid conditions (genera *Fridericia* and *Henlea*). In addition, epigeic earthworm species occurred, profiting from crop residues and weeds not being ploughed in. Management measures here improved conditions for many species with some profiting even more (opportunists) than others. Hence, abundance of both annelid groups exceeded the reference range for sandy field soils at the third sampling (Beylich & Graefe 2009).

No site example has been presented for low abundance of both annelid groups. The reference ranges derived from soil-monitoring data (Beylich & Graefe 2009) show that low densities of earthworms and enchytraeids occur more frequently in cropland than in grassland. At cropland sites earthworms and enchytraeids are often exposed to multiple stressors such as tillage, compaction and pesticides (Langmaack et al. 1996). Additional disadvantageous factors, e.g. adverse weather conditions, then might cause populations of both groups to collapse.

4.4. General conclusions

The abundance of earthworms and microannelids in soil is influenced by a complex of abiotic and biotic parameters causing short-term fluctuations and long-term changes. The detection of relationship patterns of the abundance of both animal groups by analysis of the presented field data is difficult due to the heterogeneity of soil properties, land use and management. It was demonstrated that

- correlations between total microannelid and earthworm abundance in general are often missing,
- correlations and especially antagonistic behaviour are species specific,
- small earthworm species or juveniles of medium-sized species (endogeic, epigeic) tend more to show antagonistic relationships with enchytraeids than large, anecic species,
- abundances of species with similar habitat requirements can be positively related if there is no resource limitation,
- conditions favouring both groups can result in increasing activity of both groups (e.g. favourable management measures on cropland).

The field data hardly allow to detect the influence of competition, as abiotic factors are overlaying and influencing possible competition effects. Other biotic factors such as the composition of the microbial community (Huhta 1984) or excretions / secretions (Górny 1984, Haukka 1987) might also play a role. In addition, competition can occur not only between earthworms and microannelids but also between species within these groups (Uvarov 2009) and between microannelids and microarthropods (Huhta et al. 1998). Information at species level on food preferences or strategies to cope with e.g. adverse moisture conditions are missing, but would help to estimate competition effects.

We often find decreasing enchytraeid abundance accompanied by increasing species numbers, e.g. after liming (Graefe 1990) or during acidification of forest or grassland sites respectively. This supports the conclusion that the development of the biological soil condition cannot be interpreted by abundance data alone, as these fluctuate considerably. Interpretations with respect to soil condition evaluation should always refer not only to abundance of both groups but also to species composition, which might show opposed trends.

So far, few publications discussing relationships between earthworm and enchytraeid abundance in the field were primarily designed to investigate interactions. Better understanding of interactions would render insight in changes of soil fauna communities triggered by environmental alterations and their effects on ecosystem services. Soil monitoring data are needed to underpin decision-making and to develop adaption strategies and measures in the fields of e.g. climate and land-use change, invasion/introduction of new species, deliberate release of GMO (genetically modified organisms) or multiple contaminations. However, most soil monitoring sites are not designed for the study of processes and interactions. Thus it would be helpful, to 1) identify information that is needed to interpret monitoring data thoroughly but is missing so far and 2) to target research projects especially to these topics. At present, university research and soil monitoring seem rather detached from each other, although in our opinion both could profit from cooperation.

Acknowledgements. This study was supported by the Landesamt für Landwirtschaft, Umwelt und ländliche Räume Schleswig-Holstein (LLUR), the Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen (LANUV) and the Behörde für Stadtentwicklung und Umwelt, Hamburg.

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Notes on the reproduction, fragmentation and regeneration of *Enchytraeus dudichi* Dózsa-Farkas, 1995 sensu lato (Enchytraeidae, Oligochaeta) found in Paraná State, Brazil

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Abstract

Worldwide, six species of enchytraeids are considered to reproduce by fragmentation (architomy) and two are known from Southern Brazil: *Enchytraeus dudichi* Dózsa-Farkas, 1995 and *Enchytraeus bigeminus* Nielsen & Christensen, 1963. The former species was recently found in Campo Largo City, Paraná State, this being the first record from South America. We found that *E. dudichi* s.l. grows easily in tropical artificial soil, natural soil, and in agar medium. We succeeded in inducing fragmentation in worms subjected to decapitation and subsequent incubation on filter paper, but not on agar medium. Morphologically, the regeneration process is very similar to other fragmenting worms of the same genus. This is the first study on reproductive and regenerative biology of enchytraeids in Brazil, from where little information on this family is available.

Keywords: Enchytraeidae; fragmentation; autotomy; asexual reproduction; Neotropics; Oligochaeta

1. Introduction

Worldwide, six species of enchytraeids are known to reproduce by fragmentation, and most of them are found in European soils: *Buchholzia appendiculata* (Buchholz, 1862), *Cognettia sphagnetorum* (Vejdovský, 1878), *Cognettia glandulosa* (Michaelsen, 1888), *Enchytraeus fragmentosus* Bell, 1959, *Enchytraeus bigeminus* Nielsen & Christensen, 1963, *Enchytraeus japonenis* Nakamura, 1993, and *Enchytraeus dudichi* Dózsa-Farkas, 1995. *E. fragmentosus* has been invalidated (Schmelz et al. 2000) and fragmentation in *E. variatus* as reported in Bouguenec & Giani (1989) has been considered doubtful (Schmelz & Collado 2010). In the Neotropics, knowledge on enchytraeid diversity is still limited. Only recently, the existence of fragmenting species of *Enchytraeus* Henle, 1837 were reported in pasture and forest soils of the Brazilian Mata Atlântica in the State of Paraná (Römbke et al. 2005, 2007) and in rain forest soils near Manaus (Collado et al. 2012), but they were not identified to species level.

The taxonomy of fragmenting *Enchytraeus* species is difficult, and according to Schmelz et al. (2000) and Collado et al. (2012), molecular methods are necessary to recognize species. Collado et al. (2012) suggested combining groups of species that are not distinguishable at the morphological level into species 'sensu lato', and they recognized two such species, *E. bigeminus* s.l. with only two chaetae per bundle, and *E. dudichi* s.l. with three chaetae per bundle. *E. bigeminus* s.l. currently comprises *E. bigeminus* sensu stricto, *E. japonensis*, the invalidated *E. fragmentosus*, and several other unidentified populations (see Collado et al. 2012). The morphological and genetic diversity of *E. dudichi* s.l. has so far not been investigated.

Among the fragmenting enchytraeids in the world, *Cognettia sphagnetorum* (Vejdovský 1878) has been intensively studied in ecological terms because of its relevance in coniferous forests (Lundkvist 1982; Hedlund & Augustsson 1995). On the other hand, the noteworthy capacity to regenerate head and tail in a few days after fragmentation led to the use of *E. bigeminus* and *E. japonensis* as models for the study of reproduction and regeneration. Christensen (1959, 1964, 1973, 1994) studied the regeneration process anatomically and also the biological significance of the asexual reproduction in *E. bigeminus* and other species, while more recently, Myohara et al. (1999) proposed *E. japonensis* as a model for regeneration studies in developmental biology, and launched a molecular investigation to unravel the genes involved in regeneration (Myohara et al. 2006).

It is known that fragmentation (autotomy) can be artificially induced by electric shock (Christensen 1964, Yoshida-Noro et al. 2000), focal compression (Lesiuk & Drewes 1999) and decapitation (Myohara et al. 1999). These techniques are useful for studying the factors that induce or inhibit fragmentation and regeneration. Inomata et al. (2000) showed that fragmentation occurs when signals derived from the brain are interrupted in well-grown *E. japonensis*, while Myohara et al. (2006) identified more than a hundred genes that are differentially expressed in regenerating worms and Takeo et al. (2010) were able to inhibit the expression of the novel gene *grimp* showing its function in cell proliferation in early stages of the regeneration process.

We report here the occurrence of two fragmenting species in the Mata Atlântica biome in Southern Brazil: *E. bigeminus* s.l. and *E. dudichi* s.l. and provide observations on the reproduction, fragmentation induction by decapitation and regeneration of *E. dudichi* s.l.

2. Material and methods

2.1. Sampling and mass rearing

Qualitative samplings were performed at several sites of Parana state, Brazil, in 2008 and 2009. The worms were extracted by the cold method ISO23611-3 (ISO 2007) and reared as described below. Some worms were purchased as fish food from various comercial sources advertised in Brazilian websites. Strains of fragmenting worms from each site (or purchased) were established in agar plates and then identified taxonomically. A culture of each species was started from one individual transferred from natural soil or activated charcoal (in the case of purchased enchytraeids) to agar plates (9 cm diameter) prepared at 1% and raised at 22 ± 1˚C and 90% relative humidity. The worms were fed weekly with autoclaved oatmeal. About once a month, when the agar medium showed a liquefied consistence, smaller batches of worms were transferred to new agar plates. Making a few incisions and providing a thin layer of water on the agar surface helped the worms colonize the new medium. Two or three cultures in agar plates and one in natural soil of each strain were kept for culture maintenance purpose. For the experimental purposes three new agar plates were prepared and new batches of worms reared until desired body size was achieved. Worms of the same plate were preferably used for each experiment described below, otherwise, worms of similar size from each plate were mixed and then used for the experiments.

2.2. Substrates

To compare the reproductive capacity in three different substrates, we used: bacteriological agar 1% (AG), defaunated natural soil from Curitiba City (NS) and tropical artificial soil (TAS). The NS had 22% sand, 62.5% clay, 2.8% organic carbon and pH 5.2. TAS is a substrate used for ecotoxicological tests recommended for the tropics prepared with 70% fine sand, 20% kaolin and 10% powder of coconut fiber and pH 6 (Garcia 2004). NS and TAS were kept at 50% water holding capacity. Five intact individuals of 0.7-1.0 cm length were incubated for up to 40 d at 22 ± 1˚C in 10 g of substrate or the equivalent volume of agar 1%, and 50 mg of oatmeal in Petri dishes (6 cm diameter). The worms were re-fed once at 20 d. Once a week the surface of the agar plates were moistened with a thin layer of water to avoid desiccation and soil substrates weighed to check the humidity and water replenished if necessary. After 20 or 40 d the substrates were submerged in ethanol (96%) and Bengal Rose added to stain the descendents produced, including the intact worms and fragments.

2.3. Amputation and regeneration

The first group of worms was decapitated by laying the worms, one by one, in a Petri dish with a thin layer of deionized water and making an incision in segment VII or VIII - just behind the pharyngeal glands - with a scalpel. Another group had the last 5-7 segments removed, and in a third group the worms were incised in the middle of the body resulting in anterior and posterior halves. The control consisted of intact worms. All worms were previously relaxed in ethanol 0.5% for 5 minutes. The intact and amputated worms were incubated at 22 ± 1°C either on agar plates 1% or wet filter paper and fragmentation was checked after 24 h. Fragments were kept for 7 d on wet filter paper and regeneration was checked and compared with the stages described for *E. japonensis* (Myohara et al. 1999, Myohara 2004). After observations in vivo, the regenerating fragments were fixed in Bouin's fluid, washed in 70% ethanol, rehydrated, mounted in glycerol 80% and photographed. Only intact individuals of 0.7-1.2 cm body length were used for the experiment because this is the range where 100% fragmentation after decapitation had been observed in other species (Inomata et al. 2000).

3. Results

3.1. Species found in Brazil

We found *E. dudichi* s.l. in cultures established on specimens collected from pastures of Itaqui, Campo Largo county in February 2008, and from an *Araucaria angustifolia* plantation in a distrophic latosol at Embrapa Florestas, Colombo county in November 2008. Another species, *E. bigeminus* s. l., was found in vermicompost of the campus of the Federal University of Paraná, in the city of Curitiba in March 2009, and in a paper industry sludge landfill, Piraí do Sul county, in January 2009. All these sites are in the State of Paraná, in subtropical Brazil. Enchytraeid species sold commercially to feed ornamental fish and claimed by the sellers to be *Enchytraeus albidus* Henle, 1837 were actually *E. bigeminus* sensu lato. Species were identified using the number of chaetae per bundle and also the texture of coelomocytes, which differs between *E. bigeminus* and *E. dudichi* (Collado et al. 2012).

3.2. Reproductive performance in different substrates

The reproductive capacity of *E. dudichi* tested with three different substrates showed best performance in TAS and NS after 40 d (Fig. 1). There was no significant difference between these treatments, but the reproductive capacity in agar was 13-14 times smaller than in the other substrates (p< 0,01; Tukey HSD test). This difference was probably due to a high mortality of worms which tried to crawl out of the Petri dish when high densities were reached, since a mass of escaping worms were observed at the Petri dish edge after the 30th day of incubation. In addition, the equivalent number of descendents obtained in TAS and agar in cultures examined after 20 d suggests that population growth was the same independent of the substrate. The period of 40 d was certainly too long to keep a culture in the given conditions. A mixture of fragments in different stages of regeneration and intact worms were found after 20 and 40 d in the substrates showing that asexual reproduction by fragmentation was ongoing. However, in NS and TAS after 40 d, many cocoons with one or two eggs inside were found, suggesting that some worms had mature gonads. Unfortunately, hatching of these eggs was not observed, as they were fixed together with the worms at 40 d. We observed through the transparent cocoon, however, that the fixed eggs were healthy and in very early stages of development.

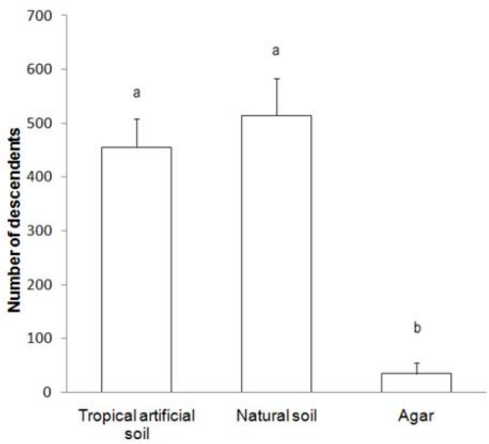

Fig. 1. Number of descendents (fragments, regenerated and intact worms) (±standard deviation) produced by five intact worms of *E. dudichi* s.l. in natural soil, tropical artificial soil and agar after 20 and 40 d at 22 ± 1˚C. Different letters indicate significant differences between substrate types (n = 4-5; Tukey HSD, p ≤ 0.05).

3.3. Induction of fragmentation and regeneration

Decapitated *E. dudichi* s.l. resulted, after 24 h, in a higher number of fragmented worms than tail-amputated, half-worms and intact worms, but only when filter paper was used as substrate (Tab. 1). With agar, not a single worm fragmented after 24 h or even 72 h (Tab. 1). The number of fragments produced per worm varied from 4 to 7.

The fragments developed new heads and tails over the following days (Fig. 2). Externally, on the 5-6th day after decapitation, a new head had already been formed with a more slender

Tab. 1. Percentage of fragmented worms of *E. dudichi* s.l. in different substrates 24 h after amputation. Total number of individuals in each treatment is in parenthesis.

	Agar	Filter paper
Decapitated	0 (36)	70 (23)
Halves	0 (33)	43 (23)
Tail-amputated	0 (30)	0 (13)
Intact	0 (31)	20 (20)

proportion than the older segments and the prostomium, peristomium, and the seven segments of the head with chaetal formation were observed. Internally, the new brain and pharyngeal pad were clearly seen.. The new tail with pygidium and growth zone were observed earlier in fragments on the 3rd-4th day after decapitation and, on the 5th-6th day, new segments had already been added posteriorly. All these fragments regenerated a new head that comprised seven segments and a pygidium with growth zone.

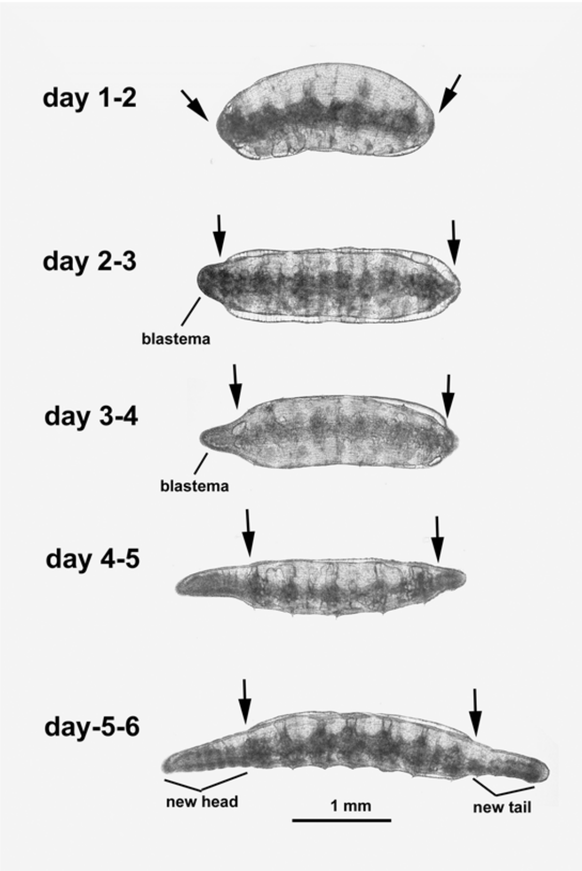

Fig. 2. Fragments of *E. dudichi* s.l. worms from day 1 to day 5 after decapitation showing different stages of regeneration. Anterior end is to the left. Arrows show the approximate amputated site.

The fragments with the old tail regenerated only a new head and the halves with the old head regenerated a new tail. The main events observed during *E. dudichi* epimorphosis were wound healing, blastema formation and elongation, differentiation of organs and segmentation.

4. Discussion

Fragmenting enchytraeids of the genus *Enchytraeus* are relatively easy to find in Brazil, at least in the Southern Mata Atlântica (Paraná State) and the Amazon near Manaus. They had been previously reported by Römbke et al. (2007) in pastures, where the degree of anthropization is higher and cattle dung abundant, conditions that seem to favor rapid population growth. Similarly, the specimens of *E. dudichi* s.l. and *E bigeminus* s.l. reported here were associated with substrates rich in organic matter (vermicompost, landfill). *E. dudichi* was orginally found in garden soil from Iran (Dózsa-Farkas 1995) and later in Hungarian greenhouses (Boros & Dózsa-Farkas 2008). *E. bigeminus* s.l. has several records from Europe and one each from North America (Bell 1959), Iran (Dózsa-Farkas 1995) and Japan (Nakamura 1993) (see Collado et al. 2012). Other species of fragmenting enchytraeids are not yet known from South America.

A number of methods for culturing enchytraeids have been reported using agar as substrate (Gotthold et al. 1967, Dózsa-Farkas 1996, Myohara et al. 1999), moist filter paper (Dougherty & Solberg 1960; Vena et al. 1969) or soil (Christensen 1964). As expected, we easily cultured *E. dudichi* s.l. and *E. bigeminus* s.l. in agar and NS allowing the establishment of long-term cultures of pure strains that can be used for a number of experimental studies in biology. The observed high reproduction in tropical artificial soil (Fig. 1) widens the range of studies also for ecotoxicological purposes. We can infer that asexual cultures can be cultured if lower population density is used (see below). In agar, the crawling of well-grown worms toward the periphery of the culture and Petri dish edges, seems to occur more often in denser populations, eventually causing mass death. This crawling behaviour was also observed by Christensen (1964) and Myohara (personal communication) for worms about to fragment, but the causes are still unknown.

Population density is known to influence reproduction mode in some fragmenting enchytraeids (Christensen 1973, Myohara 1999). Our experiments showed that sexually mature specimens of *E. dudichi* s.l. were present in NS or TAS when population density was high; however, we have not found mature individuals at high density conditions in agar as observed by Dózsa-Farkas (1996). In the mass rearing cultures of *E. dudichi* s.l. and *E. bigeminus* s.l. we also failed to find mature worms in old agar medium with high population density, but the medium might have been renewed for smaller populations before maturation was induced. Low densities, contrastingly, induced maturation for *E. bigeminus* and *E. japonensis* (Christensen 1973, Myohara 1999). Based on the method of Myohara et al. (1999), we have tried to induce maturation of worms starved and then refed plentifully on soil substrates in low densities several times, but have not yet been successful (C.C. Niva, unpublished data).

The nutritional status of the worms might be another important factor stimulating or supressing sexual or asexual reproduction. Dózsa-Farkas (1996) added dried nettle leaves to oatmeal for *E. dudichi* feeding, which may have favored maturation of gonads in the agar medium used. This assumption is supported by results of Myohara et al. (1999) who succeeded in obtaining mature *E. japonensis* by refeeding worms reared in agar on wet leaf mold substrate after starvation or in aged agar. We believe that high density did induce the maturation of some specimens in NS in our experiments, in accordance with Dózsa-Farkas (1996) results. If the same happened in agar, we believe the high mortality of escaping worms after 30 d might have obscured the data, but we cannot discard the possibility of some other factor influencing maturation.

Physical properties may also play a role in reproduction mode, as observed in *E. fragmentosus* which became mature when cultured in wet glass wool (Vena et al. 1969, Hamilton & Hess 1971). Abiotic factors can also influence reproduction mode in other microannelids, such as naidids, where the shift from asexual to sexual reproduction seems to be seasonally controlled (Christensen, 1994; Armendariz 2000) by e.g., photoperiod length (Schierwater & Hauenschild 1990). The factors that induce maturation of fragmenting enchytraeids must still be clarified.

Regarding the mode of gametic reproduction, the spermathecae of *E. dudichi* s.l. specimens examined had sperm inside, as was also reported by Dózsa-Farkas (1995), suggesting that reproduction by cocoons, when it occurs, is not parthenogenetic. The eggs of fragmenting species are not always viable as is the case for *C. sphagnetorum* (Christensen 1959), but the eggs of *E. dudichi* s.l. do hatch (Dózsa-Farkas 1995), and that is probably the case in the present study as well.

We found that the induction of fragmentation after decapitation in *E. dudichi* s.l. was not as efficient as in *E. japonensis*, where 100% of the worms had fragmented in agar after 16 h (Myohara et al. 1999). The difference in fragmentation induction efficiency between *E. japonensis* and *E. dudichi* s.l may be a species-specific trait. However, physiological state could be influencing readiness of a worm to fragment, and yet, the use of diluted ethanol to relax worms before amputation might also have some influence. Anesthesia with menthol showed an inhibitory effect on autotomy after decapitation or electric shock and also caused a higher rate of bipolar head regeneration in *E. japonensis* (Kawamoto et al. 2005). However, we did not find bipolar regeneration in the present study. Fragmentation in *E. japonensis* is believed to be inhibited by brain signals, which travel through the ventral nerve cord posteriorly. When these signals are suppressed, e.g. in the case of decapitation, fragmentation is enhanced (Inomata et al. 2000). A higher rate of fragmentation in posterior halves than in anterior halves observed in *E. japonensis* can also be explained by this suppression of signals (Inomata et al. 2000). Therefore, we could think that some of the intact worms which fragmented on wet filter paper in our experiment, possibly the longest ones, were physiologically more predisposed to fragment naturally, while the fragmented halves may have behaved in response to decapitation.

The absence of fragmentation following decapitation in *E. dudichi* s.l. worms incubated on agar is likely due to the better physical support of filter paper for autotomization and separation of the fragments. Christensen (1964) and Inomata et al. (2000) proposed that physical support is needed for fragmentation to occur. Nevertheless, *E. dudichi* s.l. did fragment spontaneously stock cultures on agar, but due to the deficient physical support, autotomy might take longer to happen in agar after induction stimuli.

Spontaneous fragmentation in oligochaetes occurs by a transverse fissure caused by strong contractions of the muscles of the body wall, which ruptures the segment approximately in the middle and consequently separates the anterior and posterior portions (Christensen 1964, 1994, Lesiuk & Drewes 1999). Autotomy is reported to occur at a fixed position just anterior to the second narrow band of the circumferential neuromuscular junction between the first and second of the six annuli of each segment (Yoshida-Noro et al. 2000, Kawamoto et al. 2005). The nerves of each segment are connected to the ventral nerve cord and, consequently, to the brain. Drewes & Fourtner (1991) and Lesiuk & Drewes (1999) demonstrated that electrophysiological signals were different in pre-fission and non pre-fission aquatic fragmenting oligochaetes and that cholinergic synaptic signals at neuromuscular junctions could be controlling autotomization. In addition, immunoreactivity of antibodies of the neuropeptides FMRFamide, substance P and GLWamide have been shown in brain, ventral nerve cord and nerve fibers in the body wall (Yoshida-Noro et al. 2000, Kawamoto et al. 2005). However, the nature of signals regulating fragmenting/not fragmenting states in *E. dudichi* s.l. and other species needs more investigations.

Epimorphic regeneration of *E. dudichi* s.l. was similar to that described for *E. bigeminus* (Christensen 1964) and *E. japonensis* (Myohara et al. 1999, Myohara et al. 2004), showing the same sequence of events, at least when observed in live specimens or whole-mounts (Fig. 2). Differences in regeneration speed are known from the literature (Christensen 1964, Dózsa-Farkas 1996, Myohara et al. 1999), but may be a result of the different temperatures used. More detailed studies using immunohistological techniques and/or molecular markers are needed to better compare regeneration in fragmenting species.

Although significant advances have been made in the study of regeneration mechanisms of *E. japonensis* (e.g., Myohara et al. 2006, Takeo et al. 2010), the fragmentation and regeneration process in enchytraeids and oligochaetes in general continues to be little studied, and the ecological and phylogenetic importance remains unknown. The findings presented here are a first step in the development of future studies on the biolgy and ecology of fragmenting enchytraeids in Brazil.

5. Conclusions

E. dudichi s. l. and *E. bigeminus* s. l. are found in Brazil. Decapitation can induce fragmentation in *E. dudichi* s.l. when incubated on filter paper. Epimorphic regeneration of head and tail fragments of *E. dudichi* s.l. is very similar to other fragmenting species of the same genus. *E. dudichi* s. l. can be reared in agar for asexual cultures but population density should be kept low.

Acknowledgements. We dedicate this paper to Dr. Maroko Myohara with esteem, gratitude and admiration in honor of her retirement. She idealized a fragmenting enchytraeid as a model organism for regeneration studies in developmental biology dedicating 17 years for these studies. First author is thankful to her for the guidance into a new research field. The authors acknowledge financial support of Programa Nacional de Pós-doutorado (PNPD), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Financiadora de Estudos e Projetos (Finep) for C.C. Niva and G. Brown and support of the Empresa Brasileira de Pesquisa Agropecuária – Centro Nacional de Pesquisa de Florestas (Embrapa – CNPF).

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Isotopic labelling of enchytraeids under FACE conditions: A possible way to analyse the residue-enchytraeid-soil system considering elevated atmospheric CO_2 concentrations

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Abstract

A soil microcosm experiment was conducted in the laboratory with enchytraeids to assess the carbon (C) and nitrogen (N) turnover when feeding on barley straw. The straw originated from a field experiment where the crop was cultivated under elevated atmospheric CO_2 conditions (FACE = Free Air Carbondioxide Enrichment). The CO_2 concentration amounted to 550 ppm compared to ambient air with 380 ppm. CO_2 applied to the crop in the enrichment plots was depleted in ^{13}C; its $\delta^{13}C$ value was -21‰. Additionally, one subplot of plants was labelled with ^{15}N in both ambient air and elevated CO_2 treatments. The aim of our study was to evaluate if straw from plants grown under elevated CO_2 conditions and treated with labelled fertilizer can be used to trace carbon and nitrogen from the plants to the enchytraeids. Microcosms (n = 5) were filled with a previously defaunated silt loam soil, topped with ground barley straw and inoculated with a mixture of the two enchytraeid species *Enchytraeus crypticus* and *E. buchholzi* s.l.. One treatment without enchytraeids served as control. After 50 days of incubation at constant 20°C in darkness, the remaining straw was collected; the enchytraeids were extracted and counted. Samples of soil, straw and enchytraeids were analysed for C and N contents as well as ^{13}C and ^{15}N signatures. While the C/N ratio in the remaining straw material was significantly reduced, no change was observed in the enchytraeids. Under ambient air conditions, $\delta^{15}N$ values of the enchytraeids were 27.6‰ in animals from plots with non-N-labelled straw, while those from labelled-N-treatments showed 52.2‰. In the FACE treatments $\delta^{15}N$-values of 15.7‰, and 29.3‰ were measured for animals from unlabelled and labelled treatments, respectively. The $\delta^{13}C$-values of the enchytraeids were as well significantly different reflecting isotope signatures of the consumed straw.

Keywords: soil mesofauna; ^{13}C; ^{15}N; microcosm experiment; carbon turnover; nitrogen turnover

1. Introduction

The analysis of the stable isotopes ^{13}C and ^{15}N provides a promising technique to trace consumed organic matter in food webs and to get insights into the structure of consumer communities (DeNiro & Epstein 1978, Robinson 2001). With respect to belowground communities, this technique has been successfully applied to various taxa of the makro- and mesofauna in forest soils (Scheu & Falca 2000) and arable soils (Briones et al. 2001, Schmidt et al. 2004). More recently, the abundances of ^{13}C and ^{15}N of soil fauna and their food sources in special environments like vermicomposts were analysed (Sampedro & Dominguez 2008).

In the present study, ^{13}C and ^{15}N isotope analysis was used to trace the carbon (C) and nitrogen (N) translocation from crop residues to enchytraeids. Barley straw derived from a field experiment on effects of elevated CO_2 concentrations on the plant-soil-system (Free Air Carbondioxide Enrichment = FACE) was used. Increasing atmospheric CO_2 concentrations are known to affect vegetation through enhanced photosynthetic rates and biomass production above- and below-ground, increase of plant water use efficiency (Ainsworth & Long 2005), change in C/N ratios (e.g., Ehleringer et al. 2002), and modified rhizodeposition (Phillips et al. 2006). Direct influences of elevated atmospheric CO_2 concentrations on soil fauna can be excluded because of its adaptation to higher CO_2 concentrations in soil (Whalen & Sampedro 2010). However the soil food web may be indirectly affected by elevated atmospheric CO_2 concentrations through changes in litter quantity and quality like reduction of N concentrations (Cotrufo et al. 1998), as well as shifts in root turnover rates and nutrient exudation into the rhizosphere (Coûteaux & Bolger 2000). Previous studies showed changes in the stable ^{13}C-signatures for collembolans (Sticht et al. 2008) and nematodes (Sticht et al. 2009), which were living in the rhizosphere and feeding on straw originating from crops cultivated under elevated CO_2 conditions in the same FACE treatment mentioned before. Stable isotope analysis of ^{13}C and ^{15}N offers the opportunity to test to which extent the isotopic signatures in soil fauna resemble those of their food (Schmidt et al. 2004, Sampedro & Dominguez 2008). The aim of our study was to analyse whether straw, which was produced under CO_2 enrichment conditions with a ^{13}C label and which was additionally labelled with ^{15}N, can be used for tracing both carbon and nitrogen from food to enchytraeids. In case of FACE straw as food, C/N relationships in food webs under elevated CO_2 conditions can be analysed, additionally. Our hypotheses were (1) Changes in C/N ratio of the food affect the C/N ratio of enchytraeids; (2) The ^{13}C and ^{15}N label of the straw affects the isotopic signature of enchytraeids.

2. Material and methods

2.1. Soil, litter and enchytraeids

Topsoil was sampled from an agricultural field located at the Johann Heinrich von Thünen-Institute (vTI) in Braunschweig, Lower Saxony, Germany (10°26' E 52°18' N, 79 m a.s.l.). It was a Luvisol derived from loess with a pH value of 7.3 and a mean organic matter content of 2.1%. The soil texture is characterised by 12% clay, 85% silt and 3% sand resulting in a silt loam. The soil was defaunated by freezing at –20°C for 24 h followed by thawing at room temperature for 24 h. This freezing-thawing cycle was repeated three times. This procedure is known to significantly reduce the number of soil microarthropods and annelids (Wright et al. 1989). The soil was macroscopically cleared of organic plant residues like straw or roots and sieved (mesh size 2 mm). At the beginning of the experiment the soil moisture was 12%.

Straw from winter barley (*Hordeum vulgare* cv. Theresa) was obtained from the same field as the soil, where a FACE (Free Air Carbondioxide Enrichment) experiment had been running for four years. The FACE equipment was constructed according to an arrangement developed by the Brookhaven National Laboratory in New York, USA: In circular plots, the standing crop was supplied with atmospheric air enriched in CO_2 up to 550 ppm (FACE treatment). Control plots under ambient air conditions revealed atmospheric CO_2 concentration of 375 ppm (ambient air treatment) (for details see Hendrey 1992, Weigel et al. 2006). The CO_2 used for the enrichment was depleted in ^{13}C resulting in a $\delta^{13}C$ of atmospheric CO_2 in the in the FACE treatment of -21.0‰ compared to a $\delta^{13}C$ of -9.8‰ in the ambient air treatment (for details see Sticht et al. 2008). Furthermore, in either treatment subplots were fertilised with ^{15}N labelled ammoniumsulfate, ^{13}C depletion and ^{15}N labelling were used to trace C and N from litter in different compartments of the soil system. Straw from FACE and ambient air plots at natural ^{15}N abundance as well as straw labelled with ^{15}N from both treatments was collected, air-dried and ground. The initial C/N ratios of barley straw from FACE and ambient air plots were 69.8 and 58.0, respectively.

Two different enchytraeid species (*Enchytraeus crypticus* and *E. buchholzi* s.l.) were obtained from our own laboratory cultures. The enchytraeids were bred in petri dishes on solid agar at 20°C in darkness. Chopped oatmeal was offered for feeding. *E. crypticus* is common in fields and a standard test species; *E. buchholzi* s.l. is a widespread species complex (Schmelz & Collado 2010).

2.2. Soil, litter and enchytraeids

Perspex cylinders (6 cm in height and 4 cm in diameter) were used as microcosms, which were filled with moist soil up to a height of about 4 cm with a bulk density of 1.2 g cm^{-3}. The soil of each microcosm was covered with 250 mg ground barley straw and inoculated with both enchytraeid species in a mixed population (30 individuals). A total of 50 microcosms (25 with enchytraeids; 25 without enchytraeids as a control) were set up with 5 replicates of the following treatments: (1) FACE straw ^{15}N labelled; (2) FACE straw non-labelled; (3) Ambient air straw ^{15}N labelled; (4) Ambient air straw non-labelled; (5) No straw as control. All microcosms were covered with Parafilm and closed with nylon-gauze (20 µm mesh size) at the bottom. The microcosms were randomly placed on moist sand-baths to maintain soil moisture and kept in a climate chamber at 20°C in darkness.

After 50 days soil and remaining straw were sampled and dried. The enchytraeids were extracted according to Graefe (1984), collected in petri dishes with water for gut clearance, counted and stored in ethanol (96%). It was not distinguished between the two enchytraeid species initially inoculated. C and N contents of soil, straw and enchytraeids were measured by combustion in a TruSpec CN-Analyser (LECO). Furthermore, $\delta^{13}C$ and $\delta^{15}N$ signatures of soil, straw and enchytraeids were measured with a mass spectrometer „Deltaplus“ (Finnigan MAT GmbH) coupled with an elemental analyzer FlashEA 1112 (ThermoQuest) via a „Continuous Flow Interface“ (ConFlo III, Thermo Finnigan MAT GmbH). The initial data for $\delta^{13}C$ and $\delta^{15}N$-values of soil, straw and enchytraeids in FACE and ambient air treatments are given in Tab. 1.

Tab. 1. Mean initial $\delta^{13}C$ and $\delta^{15}N$-values of enchytraeids, straw and soil in FACE (elevated CO_2 concentration) and ambient air (control) treatments.

Treatment	Parameter	Enchytraeids	Straw	Soil
Ambient air	$\delta^{13}C$ [‰]	-23.38	-29.91	-27.09
FACE			-40.49	
Ambient air	$\delta^{15}N$ [‰]; non-labelled	20.69	4.46	6.90
FACE			5.29	
Ambient air	$\delta^{15}N$ [‰]; ^{15}N-labelled		70.99	
FACE			31.54	

2.3. Statistical analysis

All data were tested with the Kolmogoroff Smirnov-test for normal distribution. In case the data were not normally distributed the data were log-transformed to get an approximation to normal distribution. Normally distributed data were tested for significance with a Student-t test. Statistical analysis was done with the program SPSS for Windows.

3. Results

3.1. Enchytraeid abundance

At the end of the experiment, fewer individuals were extracted than inoculated before. Most enchytraeids per microcosm were found in the ambient air treatment (17.9 ± 28.1 individuals) followed by the control without straw (15.8 ± 4.0 individuals). In microcosms, where FACE straw was offered, 10.5 ± 13.0 individuals were found which significantly ($P < 0.01$) was less than found in the control without straw.

3.2. Carbon and nitrogen

The C/N ratio in remaining straw samples from the FACE treatments (with enchytraeids and control without enchytraeids) was significantly ($P < 0.001$) lower than in those of the ambient air treatments (Tabs. 2 and 3). In both, enchytraeids as well as the corresponding soil samples, differences were not significant. Furthermore, the C and N contents of enchytraeids, remaining straw and soil both in the enchytraeid and the non-enchytraeid treatment did not differ significantly between FACE and ambient air treatment samples (Tabs. 2 and 3). In enchytraeids, C contents increased with increasing N contents in the FACE ($y = 1.26x + 29.77$; $R^2 = 0.84$**) as well as in the ambient air ($y = 1.17x + 31.58$; $R^2 = 0.84$**) treatment.

Tab. 2. Means (±SD) of C and N contents of enchytraeids, remaining straw and soil samples as well as C/N-ratios in FACE (elevated CO_2 concentration) and ambient air (control) treatments with enchytraeids after 50 days experimental time.

Treatment	Parameter	Enchytraeids	Straw	Soil
Ambient air	C [mg g^{-1}]	527.77 ± 65.23	145.28 ± 53.42	11.04 ± 0.31
FACE		550.08 ± 82.12	103.65 ± 63.24	10.95 ± 0.28
Ambient air	N [mg g^{-1}]	181.14 ± 42.73	4.85 ± 1.81	1.17 ± 0.05
FACE		183.07 ± 34.47	4.59 ± 1.80	1.16 ± 0.03
Ambient air	C/N ratio	2.95 ± 0.45	31.84 ± 5.16	9.44 ± 0.31
FACE		2.89 ± 0.19	19.81 ± 4.37	9.45 ± 0.21

Tab. 3. Means (±SD) of C and N contents of remaining straw and soil samples as well as C/N-ratios in FACE (elevated CO_2 concentration) and ambient air (control) treatments without enchytraeids after 50 days experimental time.

Treatment	Parameter	Straw	Soil
Ambient air	C [mg g^{-1}]	118.64 ± 36.76	11.23 ± 0.46
FACE		96.10 ± 61.54	10.87 ± 0.37
Ambient air	N [mg g^{-1}]	3.26 ± 0.54	1.18 ± 0.02
FACE		3.93 ± 2.21	1.16 ± 0.02
Ambient air	C/N ratio	36.32 ± 9.07	9.55 ± 0.31
FACE		21.98 ± 7.49	9.35 ± 0.15

3.3. δ^{13}C and δ^{15}N signatures

Mean δ^{13}C and δ^{15}N signatures of enchytraeids, remaining straw and soil collected at the end of the experiment are presented in Fig. 1. Samples from the FACE straw treatment were significantly depleted in δ^{13}C values ($P < 0.05$) compared to those from ambient air straw treatment. Furthermore, enchytraeids and soil from both straw treatments showed δ^{13}C values significantly ($P < 0.05$) more negative compared to the control without straw. Samples of remaining straw and soil harvested from microcosms inoculated with enchytraeids did not differ in δ^{13}C values compared to those from non-enchytraeid control microcosms.

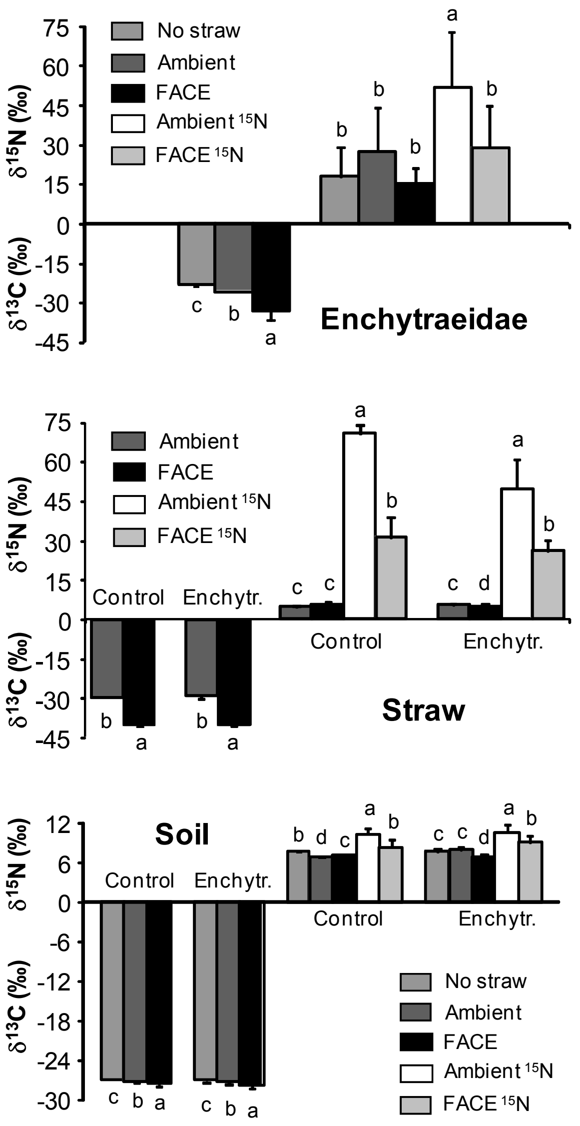

Fig. 1. Mean δ-values (± SD) of remaining barley straw, enchytraeids and soil samples from treatments with enchytraeids (Enchytr.) and the non-enchytraeid control after 50 days of incubation. δ^{13}C and δ^{15}N values are presented for treatments applied with straw grown under ambient air and FACE conditions, additionally labelled with ^{15}N and a non-straw control treatment. Different letters within the same group of columns indicate significant differences ($p < 0.05$).

After 50 days of experimental time, ^{15}N label was present in all compartments, which were in contact with ^{15}N (Fig. 1). Hence, the δ^{15}N values here were significantly higher ($P < 0.05$) compared to unlabelled treatments. The highest δ^{15}N values were found for enchytraeids, remaining straw and soil in the ambient air treatment where ^{15}N labelled straw had been applied (Fig. 1). The δ^{15}N-value of enchytreids amounted to 52.2‰ for labelled and 27.6‰ for non-labelled straw from ambient air treatment. δ^{15}N-values of enchytraeids in the FACE treatments were less enriched, they read 29.3‰ for labelled and 15.7‰ for non-labelled straw.

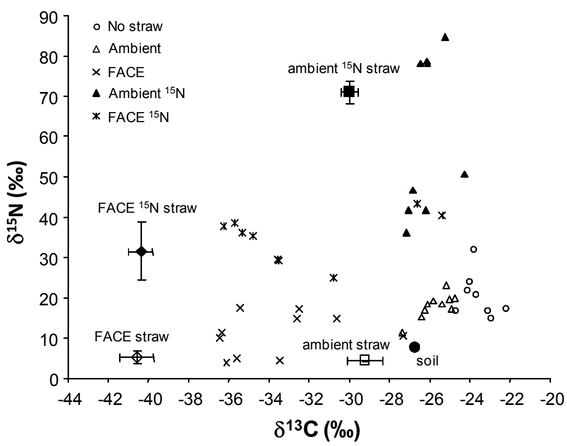

Fig. 2. Dual plot of δ^{13}C and δ^{15}N patterns of enchytraeids depending on their food source (barley straw) after 50 days of incubation. Additionally, mean (±SD) δ^{13}C and δ^{15}N signatures of soil as well as non-labelled and ^{15}N-labelled barley straw produced under ambient air and FACE conditions are given.

The C and N isotopic composition of the enchytraeids from all treatments gave insight into the translocation of C and N from straw, which had been initially offered for feeding. Enchytraeids, which were kept in soil without straw, showed δ^{15}N and δ^{13}C values slightly enriched copmpared to soil (Fig.2). Those animals, which fed on straw produced under ambient air conditions, gave slightly more negative δ^{13}C values than before, indicating the uptake of C from the straw. The δ^{15}N values remained more or less the same. The C isotopic composition of straw cultivated under FACE conditions significantly differed from straw under ambient air conditions. So did the enchytraeids: their δ^{13}C values significantly shifted towards the isotopic composition of their food source to more negative values (Fig. 2). $\delta^{15}N$ again remained in the range of all other individuals so far. With respect to the ^{15}N labelled straw, a clear shift in $\delta^{15}N$ was measured in enchytraeids - again towards the label of their food source. Since straw produced with ^{15}N label under ambient air condition contained much more ^{15}N than did straw produced under FACE conditions, the N isotopic signature in enchytraeids grown on ^{15}N straw from ambient air showed the most profound change in $\delta^{15}N$. The ^{13}C values, however, were always in the range of the respective straw. These results indicate enchytraeids as consumers of the straw as food source within the experimental time of 50 days.

4. Discussion

The initial individual density of enchytraeids was reduced in all treatments at the end of the experiment. Although refaunation of defaunated soils can be restricted in terms of recolonization (Wright et al. 1989), the laboratory conditions of the present study were chosen according to recommendations of Römbke et al. (2005) for the use of enchytraeids in standardized tests and hence should have been appropriate. It might be possible that a lack of adaptation to soil conditions after breeding the enchytraeids in solid agar led to a general decline of individual numbers. Nevertheless, a treatment effect was found with lowest enchytraeid numbers in microcosms containing FACE straw. Recently, Maraldo & Holmstrup (2010) summarized results on indirect effects of elevated CO_2 concentrations on enchytraeids. According to this review, increased CO_2 might affect the reproduction of enchytraeids. Furthermore, it is discussed that the composition of their food source (e.g. the balance between fungi and bacteria) might alter (Maraldo & Holmstrup 2010).

Concerning the N content of remaining straw, Cotrufo et al. (1998) reported a mean N reduction of 11% in leaf litter of C3 plants grown under elevated atmospheric CO_2 concentrations. In our study, we also observed a less profound reduction in N content of 5.4% (enchytraeid treatments). The C/N ratio in the straw material originating from cultivation under FACE conditions was also altered. Although a reduction of C/N was visible in the straw, the C/N ration in the enchytraeids was not affected. Thus, our first hypothesis, that changes in C/N ratio of the food affect the C/N ratio of enchytraeids, cannot be confirmed.

According to Didden (1993) enchytraeids mainly feed on microorganisms which colonize dead organic material und utilize simple organic compounds. This indicates an indirect role of enchytraeids in decomposition processes. According to an analysis of the trophic structure of soil fauna communities in beech forests enchytraeids are classified as secondary decomposers (Scheu & Falca 2000). Our result presented in this study, however, revealed that enchytraeids incorporated part of the labelled organic material. The results of our ^{13}C and ^{15}N isotopic analyses confirm our second hypothesis, that ^{13}C and ^{15}N label of the straw affects the isotopic signature of enchytraeids. This indicates crop residues (besides soil microorganisms) being an important food source for enchytraeids. Hence, enchytraeids are directly involved in decomposition processes feeding on fragmented litter. However, so far this conclusion is restricted to the two species *E. buchholzi* s.l. and *E. crypticus*, which were used in the present study. Further studies on food selection and feeding behaviour of enchytraeids on the species level is needed.

Acknowledgements. We wish to thank Sabine El Sayed, Martina Heuer, Detlef Koch and the BayCEER lab-team for their excellent assistance in the laboratory.

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Ecotoxicological evaluation of selected forest plots in Baden-Württemberg (Germany): Influence of emissions of potential toxic substances from a highway on oligochaetes

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Abstract

The influence of anthropogenic stress on soil ecosystems is difficult to assess because their "normal" status is often unknown. Therefore, based on literature data from undisturbed sites the soil biological classification concept (BBSK) was developed, using Enchytraeidae and Lumbricidae as an example. This BBSK approach was applied at a site (Bruchsal) with four sampling plots, which differ in vegetation (two deciduous, two coniferous) and contamination, i.e. in distance to a highway. Based on five site properties (e.g. pH) the expected oligochaete species composition was predicted. Afterwards, the actual species composition was investigated. Two questions were studied: 1. How did anthropogenic stress such as highway emissions influence structure and function of soil organisms? 2. Is it possible to assess the quality of this site as a habitat for soil organisms by using the BBSK approach? The plots near the highway differed from the plots in the inner forest in enchytraeid species composition but not regarding earthworms and organic matter breakdown. Therefore, the soil of all Bruchsal plots is assessed as being conspicuous in its ability to act as a habitat for soil organisms. It is recommended to further develop the methods used here, preferably as part of the TRIAD concept.

Keywords: Soil quality; Lumbricidae; Enchytraeidae; PAHs; decomposition

1. Introduction

In an industrialised region like Baden-Württemberg soils can be highly disturbed. At the same time the natural community of soil organisms is poorly known. Based on a literature study the "normal" abundance range and species composition for a representative number of more or less undisturbed forest sites was determined in an earlier project for the same spnosor, the Landesanstalt for Umwelt (LfU) Baden-Württemberg (Karlsruhe) (Römbke et al. 1997). Using this information as a reference, it should be possible to assess the influence of anthropogenic impacts on soil organism communities. The central idea of this approach is to determine (mainly qualitatively) the expected soil community living at a specific site, using the relationship between soil and site properties and the occurrence of individual species. By comparing this expected community with the one really living at that site it is possible to evaluate the biological quality of this specific site. This approach became known as BBSK (soil biological site classification concept; Ruf et al. 2003, Breure et al. 2005).

The BBSK approach was applied at a site (Bruchsal) with four sampling plots, which differ in vegetation (two deciduous, two coniferous) and contamination, i.e. in distance to a highway (Beck et al. 2001). Based on five site properties (= ecological determinants), i.e. land use, pH, organic matter content, texture and moisture, the species composition of enchytraeids and lumbricids was predicted. Afterwards, the actual species composition of these organisms as well as a functional parameter (litter decomposition) was investigated for a period of two years. In particular, two questions were studied:

1. How did anthropogenic factors such as emissions of potentially toxic substances from a highway influence structure and function of the soil organism community?
2. Is it possible to assess the soil quality of this site as a habitat for soil organisms by using the soil biological classification concept (abbreviated as BBSK)?

Referring to these questions it should be noted that the work described here follows two designs:

Firstly, and very simple, it is asked whether the structure and function of the soil organism community is influenced by emissions from the highway. For this purpose, study plots differing in their distance to the highway were set up and their biological characteristics were compared (oligochaete communities and litter decomposition). Secondly, the data gained in the comparison just described were used to apply the BBSK approach. This second part can be seen as part of a TRIAD approach, i.e. a combination of chemical residue analysis, ecotoxicological testing and monitoring in the field, and it is considered to be the future of site-specific risk assessment (Jensen & Mesman 2006). In order to make things even more complicated it has to be pointed out that determination of functional parameters in this kind of studies is an accepted part of the first design (i.e. comparison between control and treatment plots) but not of the second one: functional parameters are not (yet) part of the BBSK approach. In any case, the results of such functional tests are important for an overall assessment of anthropogenic impacts on soil organism communities as exemplified in the recent discussion on the ecosystem services provided by soil organism communities (e.g. Elmquist et al. 2009, Turbé et al. 2010).

2. Material and methods

2.1. Literature review

The Soil Biological Site Classification Concept, abbreviated as BBSK (in German: Bodenbiologische Standortklassifikation), was developed as a literature review sponsored by the State of Baden-Württemberg (Römbke et al. 1997). In searching and evaluating the literature, several proposals using individual organism groups for classification purposes were compiled like the ideas of Volz (1962) or Graefe (1993). In particular, already Stork and Eggleton (1992) discussed all the theoretical questions (e.g. the relationship between structure and function of the community, the need of integration of zoological and microbiological parameters) and practical problems (e.g. the most suitable parameter, the advantage of keystone species, the need for interactive taxonomy programs), which are still valid today. During the review it was realized that very similar ideas have been developed in limnology and in vegetation science (for a compilation of these ideas see Breure et al. 2005). Combining existing ideas, the two basic characteristics of the BBSK concept are as follows:

1. Classification: There is a limited number of reference sites (= site types or ecotypes; in German: “Standorttypen”) (Breure et al. 2005) in a certain region, each with a characteristic soil organism community. The site types can be defined by a few parameters, mainly soil properties and land use, but also climatic or geographical factors.
2. Assessment: The evaluation of a soil, in particular regarding potential anthropogenic stress, is possible by comparing the observed (i.e. sampled) community with the community expected for this site with its specific properties. The expected community has to be defined using the occurrence of species at “undisturbed” reference sites.

The most important precondition of this approach is that the occurrence of soil organisms (i.e. the structure of the community and therefore, indirectly, their functions (= processes)) is determined by soil and site parameters. Each site, including the community occurring there, is characterized by a combination of biologically important parameters. The problem is to select those parameter combinations (out of a very large number) which are relevant for certain regions. These relevant combinations can be called reference sites (= site types or ecotypes; in German: “Standorttypen”) (Breure et al. 2005). Due to practical reasons their number should be neither too high nor too low. With a low number of reference sites, the whole system does not work due to its low ability to detect any deviations between observed and expected community. With a high number of reference sites, the approach is simply not manageable.

In the latest version of the BBSK concept, reference sites (= ecotypes) are classified in five steps (Römbke et al. 2002, Breure et al. 2005). In the first step, land use is the most important criterion. Since this outline is aiming to cover large regions, a differentiation according to climatic zones (e.g. the “ecozones” of the European Union) has to follow (step 2). In steps numbered three and four soil properties are the main focus, followed by a plausibility check. A fifth step, site management (including measures) is not included here since this is done mainly based on socio-economic criteria. In addition, four further very important pre-conditions have to be fulfilled in order to get reliable results in an efficient way when using this concept:

1. Sampling methods for soil organisms must be standardized (e.g. Römbke et al. 2006b).
2. The species identification of soil organisms must be relatively easy (e.g. good keys should be available for the important groups).
3. The “normal” occurrence of soil organisms must be known, preferably by sampling many “undisturbed” reference sites in one large monitoring program as it has already been done for fresh water sites (Wright 2000). For soils, large scale field studies sponsored by the German Environmental Agency (UBA) as well as literature compilations were performed (Römbke et al. 2002).
4. Finally, an agreement is necessary how to evaluate (e.g. statistically) and to assess (i.e. classifying the habitat function as “good” or “disturbed”) the results obtained when comparing the observed and expected community at a given site.

When using the BBSK approach for a specific site to be assessed, the same four main steps as described above for the definition of reference sites have to be taken into consideration. Concerning the measurement parameters to be used, qualitative parameters are better than quantitative ones since the latter are usually much more variable in time and space, mainly due to climatic events (Fründ 1995). Therefore, biodiversity remains the main assessment parameter (Fig. 1). In theory, also functional parameters could be included into this concept, but details how to do it have still to be clarified.

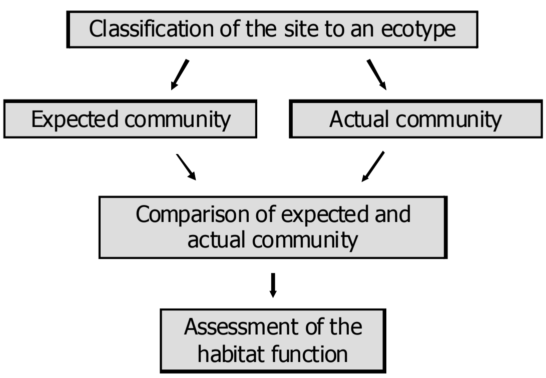

Fig. 1. Schematic overview of the BBSK-concept (Soil Biological Site Classification Concept; in German: Bodenbiologische Standortklassifikation).

2.2. Field study

2.2.1. Study site

For a practical investigation of this concept a forest site near the town of Bruchsal in Northern Baden-Württemberg was selected at which the influence of contamination along a “mini-transect” starting at a highly frequented highway (A5) was studied. In detail, four plots with a size of 10 * 10 m were fenced in, and they were characterized pedologically and chemically (Tab. 1). Two sites were situated in a deciduous forest (BRA and BRL) and two in a coniferous forest (BRB and BRK). In each case, one plot served as treatment (distance to the A5: 13 m) and one as a control (distance to the A5: 140 m).

Tab. 1. Site and soil characterization of the four study plots at Bruchsal. The four study plots are abbreviated as follows: BRA = Treatment plot in the deciduous forest; BRL = Control plot in the deciduous forest; BRB = Treatment plot in the coniferous forest; BRK = Control plot in the coniferous forest.

Parameter	BRA	BRL	BRB	BRK
Distance to highway	13 m	140 m	13 m	140 m
Vegetation	Beech, oak, hornbeam		Pine, beech	
Ø Temperature	10.2 °C			
Ø Precipitation	640 mm			
Texture class	Loamy sand (Sl)			
pH value (soil)	4.0	3.4	3.4	3.2
Organic matter	13.1%	14.1%	12.8%	19.3%
Humus form	F-Mull	Moder	Moder	Raw
pH value (litter)	4.3			

2.2.2. Residue analysis

Soil and litter samples were taken at all four plots in order to verify the assumption that treatment and control plots differ in terms of soil contamination. Concentrations of selected heavy metals and organics as mean values of three samples per plot are presented in Tab. 2. With the exception of Cadmium, concentrations are higher on the treatment compared to control plots – and they are always higher in the litter layer than in the mineral soil. This is not surprising since these chemicals entered the plots via the air. Copper (BRA, BRB), Zinc (BRA, BRB) and lead (all 4 plots) concentrations are higher than trigger values indicating that there is concern about negative impacts of these metals on soil organisms (Römbke et al. 2006a). These trigger values have been defined in the German Soil Protection Law (1998) as “values which, if exceeded, shall mean that investigation with respect to the individual case in question is required, taking the relevant soil use into account, to determine whether a harmful soil change or site contamination exists". In addition, it has to be mentioned that all four plots, especially the litter layer, were mechanically disturbed, mainly by the digging and feeding activities of wild pigs.

Tab. 2. Concentrations of 4 heavy metals and 3 organics in litter and soil samples of the 4 study plots at Bruchsal. Concentrations in bold are higher than the trigger values for the pathway soil; soil organisms as proposed by Römbke et al. (2006a). For study plot abbreviations see Tab. 1.

Chemical	BRA		BRL		BRB		BRK	
[mg/kg soil dw]	Litter	Soil	Litter	Soil	Litter	Soil	Litter	Soil
Lead	**258**	119	**186**	56	**667**	79	**217**	25
Cadmium	1.7	0.6	4.1	0.6	5.3	0.5	4.6	<0.3
Copper	**109**	16	43	6.0	**145**	6.6	49	4.5
Zinc	**395**	71	**163**	27	**413**	23	132	25
Mineral Oil	133	77	117	55	212	58	123	<50
PAH (total)	1.3	4.5	1.4	1.3	1.5	2.0	1.1	0.8
Benz[a]pyren	0.1	0.4	0.1	0.1	0.1	0.1	0.1	0.1

2.3. Sampling of soil organisms

All four plots were sampled four times: October 1997, May/October 1998, and May 1999. Lumbricids were sampled using the standard ISO method, i.e. a combination of hand-sorting and formalin extraction (ISO 2006). Each of the four sampling spots was randomly distributed on the respective plot. They had a size of 50 * 50 cm and a depth of ca. 25 cm. Worms were determined to the species level (Sims & Gerard 1999), fixed in 70% alcohol, followed by a short storage in 4 % formalin before they were finally stored again in 70% alcohol. Four enchytraeid samples per plot and date were randomly taken using a soil corer (diameter: 5.3 cm), which is divided by in two layers (litter and 0 – 5 cm mineral soil) (ISO 2007). Worms were driven out from the soil by wet extraction and finally were determined to the species level alive Nielsen and Christensen (1959). For both organism groups, abundance and species composition were used as parameters.

2.4. Measurement of litter decomposition

Litter decomposition was determined using the litter-bag method (Dunger & Fiedler 1997), meaning that 1120 bags made of inert material were filled with litter (mainly beech leaves for BRA and BRL or pine needles for BRB and BRK). Bags had three different mesh sizes: 20 µm (exclusion of fauna), 250 µm (exclusion of macrofauna) and 10mm (no exclusion of invertebrates). Bags were laid out in October 1997 and were brought in every three months until autumn 2000. Per sampling date, plot and mesh size, ten litter-bags were taken. Main measurement parameter was the ash-free dry weight of litter. Decomposition rates are given as DT50-values (= degradation time of 50% of the original amount of organic matter).

2.5. Statistical evaluation

The results of the litter-bag test were statistically evaluated in detail. Ash-free dry weight was used as measurement parameter. Ash free dry mass values from the different retrievals were submitted to regression using the exponential decay model (SigmaPlot 8.2., Mt=Mo e-k; Olson 1963). Statistical analyses (ANOVAs) were made with Statistica 8.0 (StatSoft, Inc. (2007)).

3. Results

3.1. Lumbricidae (earthworms)

Abundance, species number and species composition of earthworms at the four plots at Bruchsal (mean of four sampling dates) are given in Tab. 3. Only three species were found and they occurred always in very low numbers.

Tab. 3. Number [Ind m^{-2}], species number and species composition of earthworms (mean of 4 sampling dates) found at the 4 plots at Bruchsal (* = only juveniles). For study plot abbreviations see Tab. 1.

Parameter	BRA	BRL	BRB	BRK
Ind. m^{-2}	1.0	8.5	0.5	0.5
Spec. No.	2	2	1	1
Species	*L. rubellus* *O. tyrtaeum*	*L. rubellus* *D. rubidus*	*Lumbricus* sp.*	*Lumbricus* sp.*

Referring to the BBSK concept, at three of the four plots (BRA, BRB, BRK) three species are expected to occur: *Lumbricus rubellus, Dendrodrilus rubidus, Dendrobaena octaedra*. At the fourth plot, the deciduous control plot (BRL), these species plus another three ones should occur: *Dendrobaena attemsi, Allolobophoridella eiseni, Lumbricus castaneus.* However, in reality in both cases the species number is lower than expected. In addition, the species composition is not in line with the one expected according to the BBSK approach. The species *Octolasion tyrtaeum* is very uncommon in such acid forest soils. Probably, it has been introduced anthropogenically via road-building material.

3.2. Enchytraeidae (potworms)

Abundance [Ind m^{-2}], species number and species composition of potworms at the four plots at Bruchsal (mean of four sampling dates) are given in Tabs. 4 and 5 and Fig. 2. There is no significant difference in abundance at the four plots.

Tab. 4. Number [Ind. m^{-2}] of potworms (individual dates plus mean of the 4 sampling dates) found at the 4 plots at Bruchsal. For study plot abbreviations see Tab. 1.

Date	BRA	BRL	BRB	BRK
Oct. 1997	11,100	23,200	8,100	7,800
April 1998	14,300	16,600	17,900	11,300
Oct. 1998	22,300	42,100	16,000	25,800
April 1999	30,900	30,500	22,900	19,600
Mean	19,700	28,100	16,200	16,100

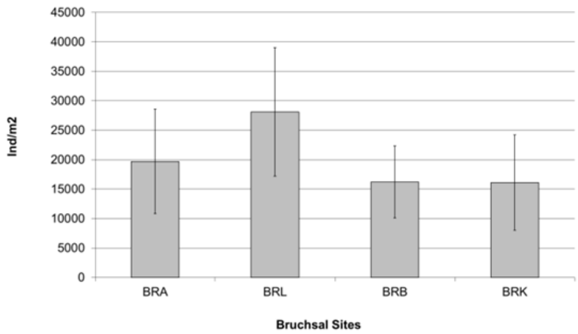

Fig. 2. Mean number and standard deviation of potworms [Ind. m^{-2}] on the 4 plots at Bruchsal. For study plot abbreviations see Tab. 1.

Tab. 5. Species list and dominance [%] of potworms given as mean of the 4 sampling dates found at the 4 plots at Bruchsal (number missing to 100% = non-determinable rests). For study plot abbreviations see Tab. 1.

Species	BRA	BRL	BRB	BRK
Achaeta sp.	12.9	9.6	17.3	11.4
A. affinis sensu lato	12.3	5.0	17.6	15.0
A. bohemica	1.8	0.0	0.5	0.2
A. camerani	1.7	0.4	1.3	2.4
A. eiseni	0.9	0.3	7.2	4.6
A. urbana	0.0	0.0	3.6	0.9
B. appendiculata	3.6	0.0	1.0	0.1
C. sphagnetorum	28.8	44.3	26.6	32.8
Enchytraeus sp.	2.4	0.0	0.3	0.1
E. buchholzi sensu lato	0.5	0.7	0.0	0.0
E. norvegicus	1.4	1.1	0.0	0.9
E. parva	1.0	0.4	0.1	0.0
Fridericia sp.	1.2	2.6	1.3	1.0
F. cf. caprensis	0.0	0.0	0.3	0.0
F. galba	0.0	0.4	0.0	0.0
F. striata	0.4	0.4	0.1	0.0
M. clavata	5.7	5.6	2.8	2.8
M. glandulosus	0.2	0.7	0.0	0.0
O. cambrensis	11.2	19.2	7.6	11.5
S. niveus	0.5	0.0	0.0	0.0

Also the number of species occurring at the four plots did not differ: in the deciduous forest 14 (BRA) and 13 (BRL) and in the coniferous forest 12 (BRB) and 10 (BRK) species were found. However, the number was higher at both treatment plots compared to the controls as well as at both deciduous forest plots compared to the coniferous forest plots. Please note that in some cases species names were changed compared to the original report (Beck et al. 2001) in order to be in line with Schmelz & Collado (2010).

The parameter dominance spectrum was evaluated on the genus level in order to counteract high variability on the species level (Tab. 6). Out of ten genera found at Bruchsal, two (*Mesenchytraeus, Stercutus*) were only found in the deciduous forest, but with less than 1% of the total number. In tendency, the percentage of *Achaeta* species increased at the treatment plots while the dominance of *Cognettia* species decreased there compared to the control plots. No difference of *Marionina* was found between treatment and control in both forests.

Tab. 6. Dominance spectrum (genus level) of potworms (in % as mean of the 4 sampling dates) found at the 4 plots at Bruchsal. For study plot abbreviations see Tab. 1.

Genus	BRA	BRL	BRB	BRK
Achaeta	29.6	15.3	47.5	34.5
Buchholzia	3.6	0.0	1.0	0.1
Cognettia	28.8	44.3	29.6	32.8
Enchytraeus	4.3	1.8	0.3	1.0
Enchytronia	1.0	0.4	0.1	0.0
Fridericia	1.6	3.4	1.7	1.0
Marionina	5.7	5.6	2.8	2.8
Mesenchytraeus	0.2	0.7	0.0	0.0
Oconnoriella	11.2	19.2	7.6	11.5
Stercutus	0.5	0.0	0.0	0.0

In addition, the species level was addressed by looking at selected indicator species. In Tab. 7, those species are shown which occur with different percentages in both forest types. The respective five species might be classified as "typical" deciduous or coniferous species. Three species prefer the former and two the latter forest type.

Tab. 7. Dominance spectrum of selected indicator species of potworms (in % as mean of the 4 sampling dates) found in the deciduous and coniferous plots at Bruchsal, respectively. For study plot abbreviations see Tab. 1.

Species [%]	BRA	BRL	BRB	BRK
„Deciduous" species				
F. striata	0.4	0.4	0.1	0.0
M. glandulosus	0.2	0.7	0.0	0.0
M. clavata	5.6	5.7	2.8	2.8
„Coniferous" species				
A. urbana	0.0	0.0	3.6	0.9
A. eiseni	0.9	0.3	7.2	4.6

Again referring to the species level, it is asked whether there are differences between the occurrences of selected stress indicators, i.e. those species which occur with higher or lower percentages at the treatment plots (Tab. 8). According to these numbers, *Buchholzia appendiculata*, *Enchytraeus buchholzi* sensu lato and *Achaeta affinis* sensu lato (in the original report (Beck et al. 2001) named *A. cf. affinoides*) occur more frequently at the treatment plots. On the other hand, *Cognettia sphagnetorum* and *Oconnorella cambrensis* are less dominant at the treatment plots. It should be mentioned that these differences are partly influenced by the forest type; i.e. *Achaeta affinis* and *Cognettia sphagnetorum* seem to react stronger in the deciduous forest than in the coniferous forest. In addition, *Fridericia* sp. (at least two species of the genus) could be classified as a stress indicator in the deciduous forest and as a sensitive species in the coniferous forest.

Tab. 8. Dominance spectrum of selected indicator species of potworms (in % as mean of the 4 sampling dates) found with different percentages at the treatment and control plots at Bruchsal, respectively. * In the original report named *A. cf. affinoides*. For study plot abbreviations see Tab. 1.

Species [%]	BRA	BRL	BRB	BRK
„Stress indicators“				
B. appendiculata	3.6	0.0	1.0	0.1
E. buchholzi s.l.	2.9	0.7	0.3	0.1
A. affinis s.l.*	12.3	5.0	17.6	15.0
„Sensitive“ species				
C. sphagnetorum	28.8	44.3	29.6	32.8
O. cambrensis	11.2	19.2	7.6	11.5

Referring to the BBSK concept, at three of the four plots (BRA, BRB, BRK) six species are expected to occur: *Achaeta affinis, Cognettia sphagnetorum, E. norvegicus, M. clavata,* and *O. cambrensis,*. At the control plot (BRL), all of these species plus *Mesenchytraeus glandulosus* and *Stercutus niveus* should occur. In reality, more species were found than expected (in particular *Buchholzia appendiculata* and *Fridericia* sp.). Therefore, expected and found occurrence does not fit together.

3.3. Organic matter decomposition

The ash-free mass loss over time (i.e. about four years) in the deciduous forest is shown in Fig. 3. In all cases a continuous decomposition with neither an obvious lag-phase nor an accelerated mass loss at the beginning of the process is visible. According to the statistical evaluation,

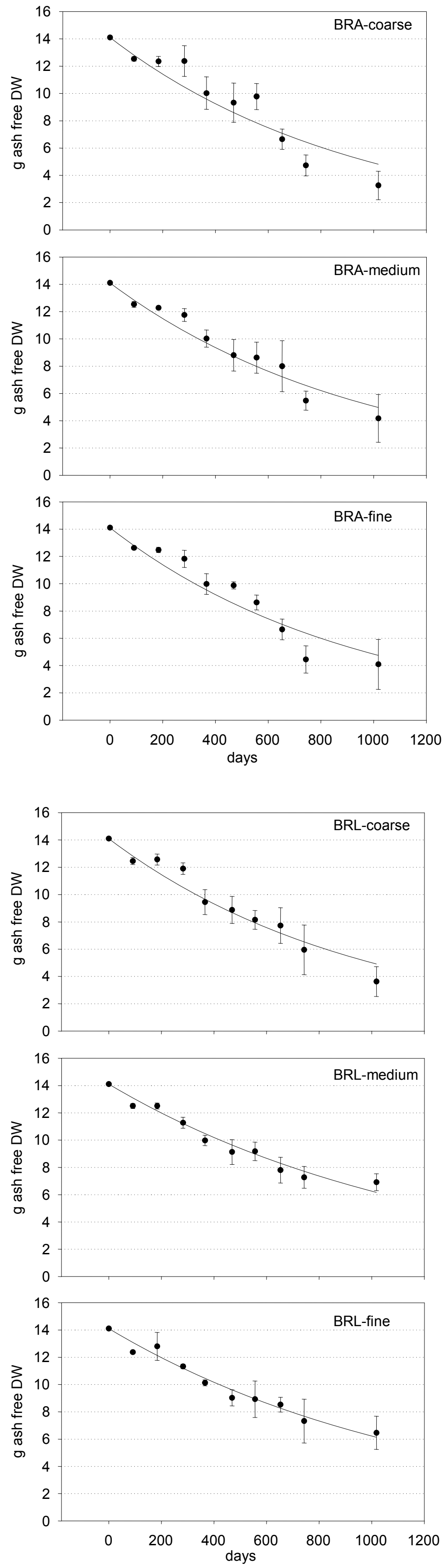

Fig. 3. Ash-free mass loss (plus standard deviation) over time (i.e. about 3 years) in the deciduous forest, separately for the 3 mesh sizes of the litterbags. For study plot abbreviations see Tab. 1.

there was no significant difference between the three mesh sizes but decomposition was significantly slower in the control compared to the treatment plot (Fig. 4).

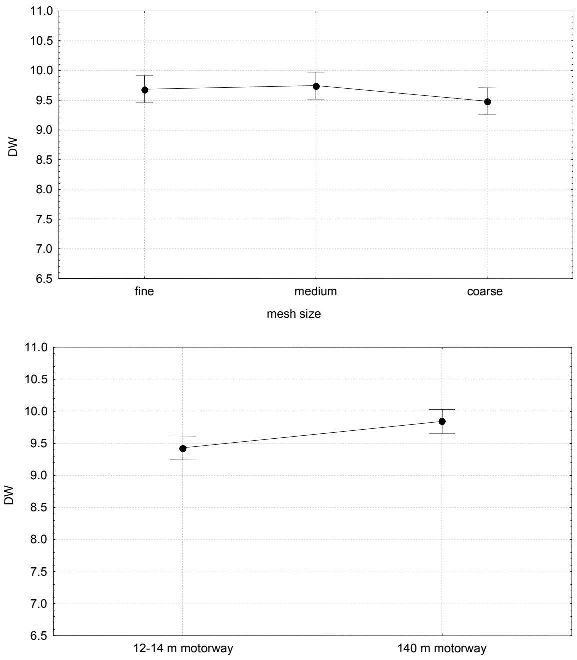

Fig. 4. Statistical comparison of the effect of mesh size (above) and distance to the motorway (below) in the deciduous forest plots at Bruchsal.

The ash-free mass loss over time (i.e. about three years) in the coniferous forest is shown in Fig. 5. Again, in all cases a continuous decomposition with neither an obvious lag-phase nor an accelerated mass loss at the beginning of the process is visible. According to the statistical evaluation, there was neither a significant difference between the three mesh sizes nor between the control and the treatment plot when looking at mass loss of organic matter in the litterbags (Fig. 6). However, there is a slight tendency that decomposition is quicker in bags with coarse mesh size at both coniferous plots.

The mass loss of organic matter at the control and treatment plots as well as between the two forest types can also be expressed as DT50 values (= time in weeks when 50% of the initial amount are decomposed), which gives even more detailed results (Tab. 9). However, the main outcome is confirmed: in the deciduous forest litter decomposition is slower at the control plot than close to the highway, mainly caused by a delayed mass loss in the litterbags with fine and medium mesh size. No such difference is visible in the coniferous forest. However, there is a tendency that decomposition is quicker in

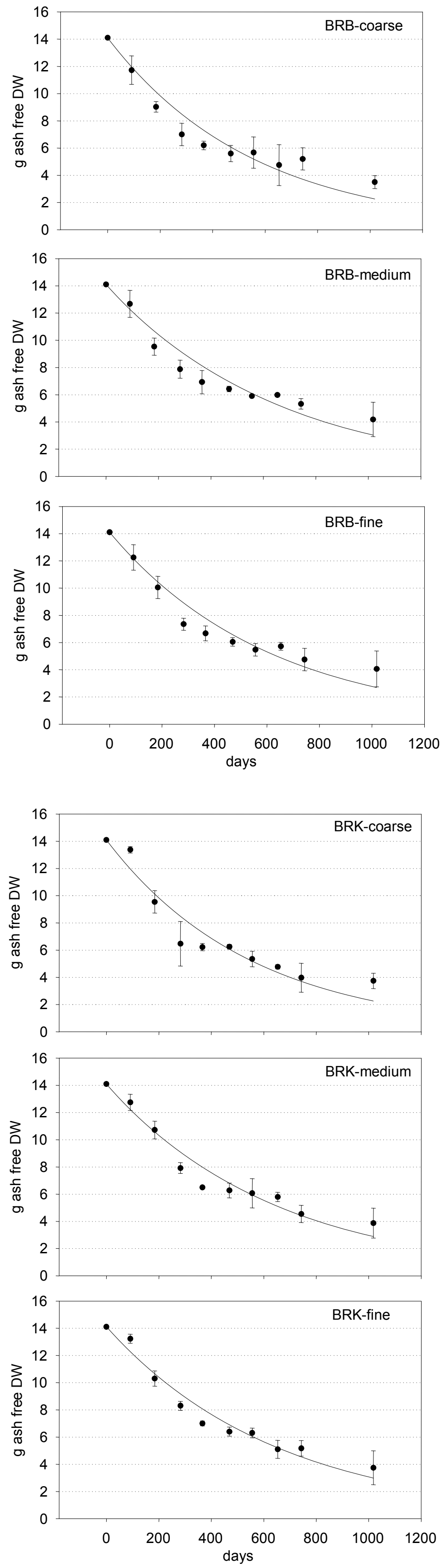

Fig. 5. Ash-free mass loss (plus standard deviation) over time (i.e. about 3 years) in the coniferous forest, separately for the three mesh sizes of the litterbags. For study plot abbreviations see Tab. 1.

coarse litterbags than in the other ones at three plots (BRA is the exception). While not being a major aim of this study data show that litter mass loss is clearly higher in the coniferous compared to the deciduous forest.

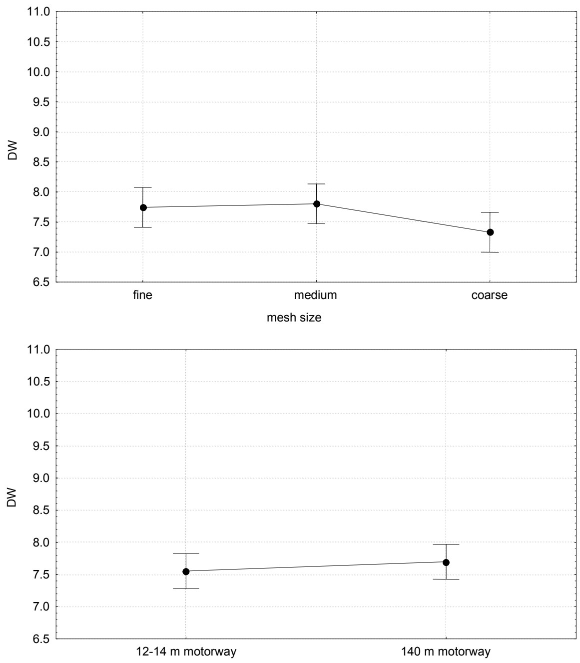

Fig. 6. Statistical comparison of the effect of mesh size (above) and distance to the motorway (below) in the coniferous forest plots at Bruchsal.

Tab. 9. DT50 values in weeks (= time in weeks when 50% of the initial amount were decomposed) at the 4 plots at Bruchsal. For study plot abbreviations see Tab. 1.

Mesh size	BRA	BRL	BRB	BRK
Coarse	99	99	55	55
Medium	99	124	66	62
Fine	90	124	62	66

4. Discussion

4.1. Lumbricidae (earthworms)

The concentrations of lead and (partly) copper and zinc in the litter layer at the Bruchsal site are higher than those concentrations assumed to harm the soil organism community in general (Römbke et al. 2006a). However, when comparing these concentrations with those causing a decrease in earthworm reproduction in the laboratory, it seems that only at BRA and BRB negative impacts on earthworms can be expected (Tab. 10) (Bengtsson et al. 1986, Spurgeon et al. 2000). More detailed comparisons between metal concentrations occurring in the laboratory tests and those determined in the field do not make sense since the former were performed in a soil-sand mixture or in artificial soil with an organic matter content of 4.5 – 10% and a pH of about 6.0 and the latter in the almost purely organic litter layer with a pH of 4.3.

Based on the metal concentrations in the litter, it was expected that earthworm numbers and diversity are lower close to the highway than in the inner forest. However, no consistent difference between BRA, BRB on one side and BRL, BRK on the other side was found. This result is partly caused by the fact that on all four plots abundance and diversity were very low; i.e. there were not enough earthworms in order to allow a differentiation between plots. In addition, it was expected that reference numbers (BBSK approach) can be used for the evaluation of the biological soil quality of the four plots at this site. Based on this approach the earthworm community was so poor in species number that all four plots have to be classified as disturbed. Since independently from the distance to the highway the litter layer of the whole site was strongly contaminated by lead (and partly zinc), it seems that metal contamination determined abundance and species number of earthworms. If this is true, plots located even further away from the highway but still belonging to the same site and thus (probably – no measurements are available) having lower metal concentrations should host a richer earthworm fauna. Actually, at a deciduous forest plot about 300 m away from BRL with the exception of *A. eiseni* all expected earthworm species were found with a mean abundance of about 100 Ind m^{-2} in 1994/1995 (Ruf & Römbke 1996), which seems to support the assumption that the two metals are causing the poor status of the earthworm community at the Bruchsal site.

4.2. Enchytraeidae (potworms)

Despite that fact that metal concentrations in the litter layer are likely to cause harm for soil organisms (Römbke et al. 2006a), only lead and zinc concentrations of the two plots close to the highway are higher than EC50 values determined in laboratory tests with enchytraeids (Tab. 10). These tests were performed using OECD artificial soil and the species *Enchytraeus albidus*, which is not among those species found in the field (Lock & Janssen 2002). In general,

Tab. 10. Comparison between the results of earthworm reproduction tests in the laboratory, given as EC50 values [mg kg^{-1} soil] and the heavy metal concentrations in the litter layer [mg kg^{-1} litter]. Concern: plots at which the EC50 was lower than the actual concentration. For study plot abbreviations see Tab. 1.

Metal	EC50	Species	Reference	Litter				Concern
				BRA	BRL	BRB	BRK	
Copper	100	*D. rubidus*	Bengtsson et al. 1986	109	43	145	49	BRA, BRB
Lead	500	*D. rubidus*	Bengtsson et al. 1986	258	186	667	217	BRB
Zinc	599	*L. rubellus*	Spurgeon et al. 2000	395	163	413	132	-

Tab. 11. Comparison between the results of earthworm reproduction tests in the laboratory, given as EC50 values [mg kg^{-1} soil] and the heavy metal concentrations in the litter layer [mg kg^{-1} litter]. Concern: plots at which the EC50 was lower than the actual concentration. For study plot abbreviations see Tab. 1.

Metal	EC50	Species	Reference	Litter				Concern
				BRA	BRL	BRB	BRK	
Copper	305	*E. albidus*	Lock & Janssen 2002	109	43	145	49	-
Lead	320	*E. albidus*	Lock & Janssen 2002	258	186	667	217	BRB
Zinc	345	*E. albidus*	Lock & Janssen 2002	395	163	413	132	BRA, BRB

the sensitivity of earthworms and enchytraeids did not differ strongly (cf. Tabs. 10 and 11). According to the heavy metal concentrations in the litter layer a negative effect on enchytraeids is expectable at the BRB and, less strong, at the BRA plot. In reality, no difference was found when comparing enchytraeid abundance or species numbers at the four plots. On the genus level, the percentage of *Achaeta* species, mainly living in the mineral soil, increased at the treatment plots while the dominance of *Cognettia* species, typical inhabitants of the litter layer, decreased there compared to the control plots. However, most genera did not show any preference between the different plots.

However, on the species level, clear indications of different conditions were found: Species known as “stress indicators” such as *B. appendiculata* or *E. buchholzi*s.l. did occur more frequently at BRA and BRB (e.g. Pizl et al. 2009), while species such as *C. sphagnetorum* or *O. cambrensis* showed the opposite behavior. Unfortunately, the effects of heavy metals or PAHs on enchytraeids in the vicinity of highways or in urban areas are difficult to compare at different sites since the number of confounding factors can be very high (e.g. Bengtsson & Rundgren 1982, Kapusta et al. 2003). In summary, it seems that on the species level an impact of the highway is occurring, but at the same time – and on higher taxonomic levels - all four plots seem to be affected, as indicated by the overall low and similar numbers (see below).

The enchytraeid community differed in the two forest types: few species were only found in one of the two types; e.g. *M. glandulosus* in the deciduous or *A. urbana* in the coniferous forest. More often, the relative frequencies of species changed. In general, the two communities were still quite similar, which is probably caused by the fact that important soil properties did not differ. Enchytraeid abundance is almost similar at all plots, ranging between 10,000 and 30,000 Ind m^{-2}. These numbers are clearly lower as those found in comparable Central European forests: on average, 50,000 – 60,000 Ind m^{-2} are found in forests with pH values between 3.2 and 4.8 (Römbke et al. 1997). This impression is confirmed by the results of a study performed in a deciduous forest plot at Bruchsal but further away (about 300 m behind BRL) from the highway: at this plot enchytraeid abundance varied within one year between 70,000 and 170,000 Ind m^{-2} (Beck et al. 1999).

Based on the BBSK approach all four plots have to be considered as conspicuous since the number of species was higher than expected. A comparable result was found at another plot already mentioned (Römbke et al. 1997). On that plot a mixture of typical acidophil species such as *M. clavata, C. sphagnetorum* and various *Achaeta*-species on one side and typical basophil species, mainly belonging to the genus *Fridericia,* was found. This result was in line with the humus form determined at that plot: a mull moder.

4.3. Organic matter decomposition

In general, there was no indication of an impact of the location (including contamination) of the plots on litter decomposition. In fact, in the deciduous forest mass loss was quicker at the treatment plot BRA, mainly in those bags in which only mesofauna and microbes (medium mesh size) or only the latter (fine size) were active. Maybe the microbial community (and indirectly via feeding the mesofauna) was favored by the conditions of the forest edge (higher temperatures?). Strangely, decomposition was also slow in bags with medium mesh size in the BRL plot despite the fact that the number of enchytraeids was highest at this plot. In the coniferous plots, the difference (not statistically significant) in mass loss between bags with different mesh size is astonishing; especially because it is not known which organisms are responsible for this fact. Earthworms surely can be excluded due to their low number and feeding preferences; so, arthropod macrofauna such as diplopods could be responsible. In general it seems to be difficult to relate the results of the litterbag test with the occurrence of oligochaetes. Interestingly, litter decomposition as a quite integrating functional parameter is sometimes not strongly impacted even in polluted soils; for example, no correlation between diversity of soil microbes and litter mass loss in a copper-contaminated soil was found (Griffiths et al. 2000).

Litter decomposition at Bruchsal is difficult to evaluate. In comparison to another deciduous forest plot at the same site but even further away from the highway, decomposition was too slow at the BRA and BRL plots except in the medium and fine bags at the BRA plot (Paulus et al. 1999). Slightly further away at a site on the foothills of the Black Forest (i.e. at higher altitude and lower mean temperature), similar DT50 values but significant differences between mesh sizes were found (Beck et al. 1988). In both cases, referring to low numbers of lumbricids but high numbers of enchytraeids, the latter were considered to be responsible for mass loss. In the two coniferous plots, decomposition of pine needles was in the same range as for example described from a litterbag study performed in a coniferous forest in Eastern France (Berg et al. 1993). The quick decomposition of pine needles might be explained by the more constant moisture conditions in the litterbags compared to the “normal” situation on the forest floor where such needles can stay for up to ten years (Kurz-Besson et al. 2005).

Summarising the results of the functional part of the Bruchsal study there was no obvious impact of metal emissions indicated by differences in mass loss between plots close to the highway and those in the inner forest. However, in the two deciduous plots there is a delay in litter decomposition in comparison to unpolluted sites in the same region, indicating that other factors not yet identified may play a role here.

5. Outlook

In this study data from three different areas have been compiled: chemical analysis (i.e. determination of the concentrations of known or suspected contaminants, here heavy metals and some organics), ecotoxicological laboratory tests (e.g. reproduction tests with selected earthworm and enchytraeid species) and field monitoring (i.e. identification of the species composition of an important part of the soil organism community followed by an assessment of the data using the BBSK-approach). Despite being not optimally designed the additional value of such a broad approach is clearly visible: while heavy metal concentrations indicated concern for all plots, only the monitoring data confirmed that they had a biological effect, In fact, if the laboratory tests had been performed with soil sampled from the four plots, the whole study would be in line with newest recommendations for using the TRIAD approach when assessing potentially contaminated sites (ISO 2010). However, the problem of a proper control in the monitoring part of the TRIAD is not solved. As exemplified in this study it is recommended to use at least two approaches: (hopefully) non-contaminated plots at the same sites but also reference values determined for the whole region (BBSK).

Acknowledgements. We thank the LfU Baden-Württemberg (Karlsruhe) for the analytical determination of heavy metals and organics in litter and soil samples collected at the study site in Bruchsal. In addition, special thanks for helping both in the field and in the laboratory go to Astrid Neßler, Franziska Meyer, Wolfgang Hohner and Stephan Regner. Finally, we thank Roland Paulus and Jörg Spelda for statistical advice.

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Terrestrial assemblages of small annelids (Clitellata: Enchytraeidae, Naididae) in beech-fir old growths of the Beskids Protected Landscape Area (Czechia) – results of a rapid assessment

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Abstract

In 2008, enchytraeids and other annelids of small body size were investigated through limited sampling of soil in four West Carpathian montane beech-fir old growth reserves in the Beskids Protected Landscape Area (NE Czechia): Smrk, Mionší, Razula, and Salajka. In the past decades all four sites have been subjected to soil acidification, albeit to different degrees; soil pH (H_2O) values in upper soil ranged between 3.5 and 4.8 at the four sampling sites. In total, 19 enchytraeid species were found. At sites of higher soil pH and with partially waterlogged conditions *Rhyacodrilus falciformis* (Naididae: Rhyacodrilinae) was also part of the assemblages. Only two species were found at the most acidic site (Smrk), *Cognettia sphagnetorum* comprising over 90 percent of individuals. At the other sites, 10–11 species were found, with *Achaeta danica* and the genus *Fridericia* reaching higher proportions at the least acidic ones (Salajka, Razula). Mean enchytraeid densities ranged between 2,000 and 13,000 ind. m^{-2}, increasing with decreasing soil pH. These results are compared with the few published data on enchytraeids in the Beskids, in particular from a quantitative study in spruce monocultures.

Keywords: Enchytraeids; Beskydy; Western Carpathians; montane forest

1. Introduction

In the Beskids Protected Landscape Area (Chráněná krajinná oblast Beskydy; BPLA), situated in north-eastern Moravia (Czech Republic) on the border to Slovakia (Fig. 1), several remnants of the original montane forests dominated by beech and fir have been preserved in reserves. These are usually referred to as virgin forest or old growth reserves, although there has been some human impact (logging, pasturage) in practically all of these stands (Vrška 1998, Vrška et al. 2000, 2001). Still they surely represent habitats closest to the natural state of most of this montane region, which is part of the western Carpathians (Šamonil & Vrška 2007). Elsewhere, the original vegetation cover has been either replaced by monocultures of spruce (mostly), beech or pine, or the forest was cleared to produce pasture and arable land. Before the present study, the only information published on enchytraeids from the BPLA was a single record of *Mesenchytraeus ogloblini* Černosvitov, 1928 from the range's highest mountain, Lysá hora, reported as *M. moravicus* n. sp. (without further description except nephridial anatomy) by Vejdovský (1905), see Chalupský (1988), and a quantitative study from two spruce forests affected by acid rain on the Kněhyně Mountain conducted in 1988–1991 (Chalupský 1992, 1995). In 2008, I used the opportunity of repeatedly visiting four old growth reserves in the BPLA while working on a research project on saproxylic beetles, to conduct limited soil sampling for a rapid assessment of the terrestrial assemblages of enchytraeids and other annelids of small body size at these sites. Access to these sites required a special permit that was granted for the above-mentioned project. The objectives of the present study were to enhance our poor faunistic knowledge, to provide information on the species richness and structure of the assemblages, and to compare the site-specific assemblages in light of available data on the site conditions and the ecological preferences of the species found. Three of the reserves had been the subject of very detailed research of forest dynamics, including pedological investigations, which provided relevant background data (see Material and Methods).

2. Material and methods

The mean long-term annual air temperature and mean annual precipitation of the sites range between 5–6 °C and 1050–1370 mm (Šamonil & Vrška 2007), respectively. The area's bedrock is West-Carpathian flysch, comprised of Cretacious sandstones accompanied by claystones. Skeletic and haplic cambisols are the predominant soil types (Šamonil & Vrška 2007). Due to the proximity of the Silesian black coal mining and associated heavy industry, such as iron smelters, to the north-west of the mountain ranges, the area has a long-term history of exposure to air pollution. Acid deposition was most severe in the 1970s and 1980s; since then deposition loads have decreased substantially, whereas effects of past soil acidification persist (Háněl 1996; Novotný et al. 2008).

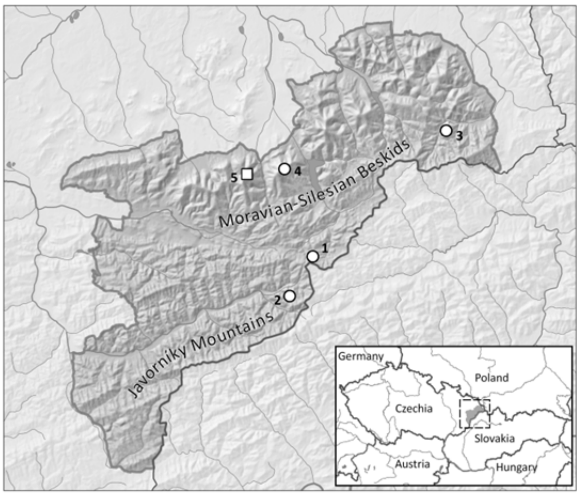

Fig. 1. Situation of the present study sites (1 – Salajka National Nature Reserve, 2 – Razula National Nature Reserve, 3 – Mionší National Nature Reserve, 4 – Smrk Nature Reserve) and the compared sites with formerly published data on enchytraeids (5 – Kněhyně Mountain).

Soil samples were taken in the National Nature Reserves Mionší (171 ha; 620–950 m a.s.l.), Salajka (22 ha; 715–815 m a.s.l.) and Razula (23 ha; 660–812 m a.s.l.) and in the Smrk Nature Reserve (161 ha; 620–1276 m a.s.l.). All reserves were situated in the Moravian-Silesian Beskids (Beskydy) mountain range, except Razula, which lies farthest to the south (but only 5 km from Salajka National Nature Reserve) in the Javorníky Mountains (Fig. 1). The largest reserves, i.e. Mionší and Smrk, include also other habitats, such as abandoned pastures and spruce dominated forests at the highest elevations. As the sampling was to remain of low intensity and comparable data were to be collected from all localities, soil samples were from a single habitat most typical of the mountain ranges' old growths, which are stands dominated by beech with a substantial presence of fir (the representation of fir has substantially decreased over the last decades). Detailed data on soil, stand composition and site history are available for the first three sites, which are all well-known "virgin forest reserves" (Vrška 1998, Vrška et al. 2000, 2001). Such data are lacking for the Smrk Nature Reserve. The top of the Smrk mountain (1276 m a.s.l.), now covered by spruce forests, had been severely affected by acid deposition in the 1970s–1980s, but our sampling site at the southern slope at ca. 1000 m a.s.l. was one of the best places of fir regeneration within the entire Protected Landscape Area (T. Myslikovjan, administration of the BPLA, pers. comm.). The composition of its tree layer was similar to that studied in the other reserves, however, with higher representation of spruce. Only soil pH and organic carbon content in the upper 10 cm were measured here (three soil samples taken in the area of enchytraeid sampling; mean values are presented). An overview of relevant data on the sampling sites is given in Tab. 1.

Six to twelve soil cores (each of 17 cm^2 surface area, down to ca. 15 cm including organic layer) were taken per site (see Tab. 2) at elevations of 700–800 a.s.l. or, in case of the Smrk Nature Reserve, 1000 m a.s.l. The highest number of cores was taken at Mionší, the largest reserve, covered to a large extent by the habitat of interest (beech-fir stands). The lowest number was taken at Smrk, where beech–fir stands covered only a small part of the reserve and a poorer enchytraeid assemblage was to be expected due to more acidic conditions. The soil cores were subdivided according to soil horizons, and annelids were extracted by wet funnel extraction without heating for 48 hours. Soil samples prior to extraction and extracted annelids in water-filled Petri dishes were stored at 8 °C. Enchytraeids and other annelids of small body size (i.e. earthworms excluded) were sorted out under a dissecting microscope and identified alive under a high power transmission microscope with Differential Interference Contrast (Nomarski). Additional to standard literature and individual species descriptions, species identification was based on a draft version of Schmelz and Collado (2010).

Tab. 1. Soil characteristics of the studied reserves (beech-fir stands) and the commercial spruce stands (more or less affected by acid deposition) studied by Chalupský (1992, 1995). Based on published data (Salajka, Razula, Mionší – Vrška 1998, Vrška et al. 2000, 2001; Kněhyně – Háněl 1996) and own measurements (Smrk; from upper 10 cm of mineral soil).

Site	pH (H_2O)	pH (KCl)	C_{org} [%]	C:N	Humus Form	Texture	Soil Type
Salajka	4.8	3.9	13.2	57.4	mull moder	clayey loam or sand	cambisols (pellic, pseudogleyic)
Razula	4.7	3.7	3.6	11.1	mull to mull moder	sandy loam	mesotrophic cambisol
Mionší	4.3	3.6	14.5	27.4	mull moder	clayey sand or sandy loam	mesotrophic cambisol
Smrk	3.5	2.8	76.7	?	moder to mor	?	?
Kněhyně - less	4.18	?	10.0	?	?	?	dystric cambisol to leptic podzol
Kněhyně - more	3.98	?	15.0	?	?	?	dystric cambisol to leptic podzol

Tab. 2. Mean enchytraeid densities (± standard error of the mean) per sampling date at the four study sites; data on two spruce stands at Kněhyně mountain, affected to a different degree by acid deposition, based on a study conducted in 1988–1991 (Chalupský 1995), are given for comparison (densities as minimum and maximum values per sampling date)

Study Site	Sampling Date	Number of Soil Cores	Density ± SE (ind. m^{-2})
Salajka	May 7, 2008	3	1,667 ± 1,135
	Sept. 25, 2008	6	3,922 ± 1,026
Razula	May 7, 2008	3	1,862 ± 2,429
	Sept. 25, 2008	6	1,961 ± 690
Mionší	May 7, 2008	6	7,843 ± 656
	Sept. 25, 2008	6	10,981 ± 4,826
Smrk	Sept. 26, 2008	6	13,039 ± 3,514
Kněhyně – less acidic	June 1988 – Nov. 1991	5 x 10	10,900 – 68,900
– more acidic	June 1988 – Nov. 1991	5 x 10	20,200 – 69,600

3. Results

Enchytraeid densities ranged from ca. 2,000 ind. m^{-2} at Razula to ca. 13,000 ind. m^{-2} at Smrk; with ca. 8,000 to 10,000 ind. m^{-2} (depending on the sampling date) Mionší had an intermediary position also in respect to enchytraeid density (Tab. 2). In total, 19 enchytraeid species and *Rhyacodrilus falciformis* (Naididae: Rhyacodrilinae) were identified (Tab. 3). Enchytraeid species numbers per site ranged from 2 (Smrk) to 11 (Razula). The assemblage at Smrk, the site poorest in species, was dominated by *Cognettia sphagnetorum*, *Achaeta affinis* being the second species found here. The enchytraeid assemblages at the other sites included a broad range of species from various genera: *Achaeta*, *Buchholzia*, *Cernosvitoviella*, *Cognettia*, *Enchytraeus*, *Fridericia*, *Henlea*, *Mesenchytraeus*, and *Stercutus*. The sites Salajka and Razula hosted also *R. falciformis.*

4. Discussion

In line with the limited scope of the study, and the incomplete environmental data on some of the compared sites, the ecological interpretation of the results was also limited. Abiotic data show that of the four present study sites Smrk was by far the most acidic and had the thickest soil organic layer (however, the extremely high organic C content – see Tab. 1 – might be misleading, being the result of measuring the upper 10 cm of mineral soil only, whereas

Tab. 3. Inventory and percentage representation (dominance) of enchytraeid and rhyacodriline species at the four study sites (beech-fir old growths within the given reserves); presence/absence data from two spruce stands at Kněhyně Mountain, affected to a different degree by acid deposition, based on a study conducted in 1988–1991 (Chalupský 1995) are given for comparison, species not found in the present study put in [ ].

Site Species	Salajka	Razula	Mionší	Smrk	Kněhyně, less acidic	Kněhyně, more acidic
Achaeta affinis Nielsen & Christensen, 1959	-	-	-	5.3	+	+
[*Achaeta brevivasa* Graefe, 1989]	-	-	-	-	+	+
[*Achaeta eiseni* Vejdovský, 1878]	-	-	-	-	+	+
Achaeta danica s.l. Nielsen & Christensen, 1959	16.7	30.8	-	-	-	-
Achaeta unibulba Graefe, Christensen & Dózsa-Farkas 2005	1.9	2.6	-	-	-	-
Achaeta spp.	1.9	10.3	3.3	0.8	-	-
Buchholzia appendiculata (Buchholz, 1862)	-	-	2.0	-	-	-
Buchholzia sp.	-	2.6	0.7	-	-	-
Cernosvitoviella minor Dózsa-Farkas, 1990	-	2.6	-	-	-	-
Cognettia cognetii (Issel, 1905)	5.6	-	-	-	+	+
Cognettia glandulosa (Michaelsen, 1888)	1.9	-	0.7	-	-	+
Cognettia sphagnetorum (Vejdovský, 1878)	16.7	2.6	57.5	77.4	+	+
Cognettia spp.	-	10.3	17.0	16.5	-	-
Enchytraeus buchholzi s.l. Vejdovský, 1879	1.9	2.6	4.6	-	+	+
[*Enchytraeus norvegicus* Abrahamsen, 1969]	-	-	-	-	+	+
Enchytraeus spp.	-	2.6	1.3	-	-	-
[*Enchytronia parva* Nielsen & Christensen, 1959]	-	-	-	-	+	-
Fridericia benti Schmelz, 2002	-	2.6	-	-	-	-
Fridericia isseli Rota, 1994	-	-	0.7	-	-	-
Fridericia maculata Issel, 1905	1.9	-	0.7	-	-	-
Fridericia perrieri (Vejdovský, 1878)	-	7.7	-	-	-	-
Fridericia sylvatica Healy, 1979	7.4	-	0.7	-	-	-
Fridericia waldenstroemi Rota & Healy, 1999	-	2.6	-	-	-	-
Fridericia spp.	22.2	5.1	3.9	-	-	-
Henlea perpusilla	3.7	-	-	-	-	+
[*Marionina* sp.]	-	-	-	-	-	+
Mesenchytraeus armatus (Levinsen, 1884)	-	2.6	-	-	-	+
[*Mesenchytraeus flavus* (Levinsen, 1884)]	-	-	-	-	+	+
[*Mesenchytraeus gaudens* Cognetti, 1983]	-	-	-	-	+	+
Mesenchytraeus glandulosus (Levinsen, 1884)	3.7	-	2.6	-	+	+
Mesenchytraeus spp.	3.7	2.6	-	-	-	-
[*Oconnorella cambrensis* (O'Connor, 1963)]	-	-	-	-	+	-
Stercutus niveus Michaelsen, 1888	-	5.1	4.6	-	-	-
Rhyacodrilus falciformis Bretscher, 1901	1.9	5.1	-	-	-	-
Total Numbers (enchytraeids + *R. falciformis*)	53 + 1	37 + 2	153 + 0	133 + 0		
Species Number (enchytraeids + *R. falciformis*)	10 + 1	11 + 1	10 + 0	2 + 0	12 + 0	14 + 0

corresponding literature data for the other sites were based on soil taken down to greater depth). This certainly resulted from the most severe exposure to acid deposition as well as of the higher representation of conifers, in particular spruce, at the Smrk sampling site, compared to the other sites studied. Mionší was in an intermediate position, whereas soil acidity, humus form and thus also organic carbon content indicated the least acidic conditions at Salajka and Razula. However, even at these sites, soil pH values were rather low as a result of preceding acidification (Šamonil & Vrška 2007). The observed trend, i.e. total densities decreasing and species numbers increasing with increasing soil pH, is in general agreement with established knowledge on enchytraeid ecology (Didden 1993). The semiaquatic *Rhyacodrilus* *falciformis* was collected at those sites (Salajka, Razula), where wet soil in terrain depressions and along brooks was also covered by the sampling. This was the case in those reserves in which brooks contributed substantially to the character of the site. However, the other study sites also included some small watercourses and this species could potentially be present in their vicinity as well. At Smrk this is improbable, as *R. falciformis* prefers sites with higher soil pH (Graefe & Schmelz 1999). The presence of *Cernosvitoviella minor*, *Mesenchytraeus armatus* and *Fridericia perrieri* at Razula and *Cognettia glandulosa* at Salajka also indicate the effect of wet conditions prevailing in a part of the sampled area (Graefe & Schmelz 1999).

In the light of the soil pH values and the overall favourable moisture conditions the enchytraeid densities found at all sites seem very low. The low values of the September sampling date might have been the consequence of a reduction of enchytraeid populations during the drier summer. The species compositions at Razula, Salajka and Mionší corresponded to soils with medium pH values and as the actual pH values found were all rather low, one might speculate that the present assemblages reflected a situation with higher pH in the past and that at present many species were surviving under suboptimal conditions. However, one might wonder why present species with higher tolerance of or even preference for lower pH, such as *C. sphagnetorum*, do not immediately exploit the situation. In fact, at Mionší *C. sphagnetorum* comprised almost 80% of all individuals (including *Cognettia* specimens not identified to species). The extremely low number of just two enchytraeid species found at the Smrk site was very probably a consequence of small sample size. However, there is no doubt that these species were indeed the dominant ones and that the assemblage was much poorer in species than assemblages at the other sites. The sampling site on the Smrk mountain (Fig. 1) was situated in ca. 5 km distance from Kněhyně Mountain, where Chalupský (1995) had found 16 enchytraeid species (17 when his two “forms” of *C. sphagnetorum* are counted separately) in two commercial spruce stands (Tab. 3). Lysá hora, from where *Mesenchytraeus moravicus / ogloblini* had been reported (Vejdovský 1905, Chalupský 1988) was situated in only ca. 7 km distance. Only *Mesenchytraeus armatus* and *M. glandulosus* were found in the present study, and none of these at Smrk. Chalupský (1995) reported *M. flavus* and *M. gaudens* from the Kněhyně sites, species that were not identified in the present study. Considering the low soil pH at Chalupský’s sites, the species numbers reported by him (12 and 14 per site) are remarkably high. However, he also reported that *C. sphagnetorum* made up for ca. 80 percent of individuals, and densities ranged between ca. 11,000 and 70,000 ind. m^{-2}. He found no *Fridericia* species at his sites, which corresponds with their preference for less acidic soils. Chalupský’s data are thus in no contradiction with the results of the present study. On the one hand, they show that even enchytraeid assemblages that seem to be composed of very few species are in fact often richer. To prove this, however, high numbers of specimens have to be examined to find the scattered representatives of scarce species (see also Schlaghamerský 2002). In terms of functional importance, such species can probably be neglected. On the other hand, one might ask if the assemblages found at Kněhyně mountain by Chalupský have been preserved to the present day or if the acidic soil conditions persisting over another 15–20 years, with probably ongoing acidification, have not by now impoverished them further. The species-rich state found by him, with few dominant species and many others represented by very small numbers, might well have been a transitional one. Nevertheless, the species list for the sites investigated in the present study cannot be considered complete, because the sampling effort was low (additional species might also be present in habitat types other than beech-fir stands as far as such are present in the reserves) and as Chalupský’s data indicate higher species richness under the local conditions, even in spruce monocultures.

Acknowledgements. The research was conducted under the Masaryk University’s Research Plan MSM 0021622416 funded by the Czech Ministry of Education, Youth and Sports. The Administration of the Beskids Protected Landscape Area and the administrations of the corresponding forest districts of the Forests of the Czech Republic, state-owned company (Lesy České republiky, s. p.), provided logistic support. Rüdiger Schmelz and Rut Collado provided a draft version of their enchytraeid key (then under preparation) which greatly facilitated state-of-the-art species identification. Maxwell Barclay (The Natural History Museum, London) kindly corrected the English.

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The impact of conventional and reduced tillage on the Enchytraeidae population in sandy soil and their correlation with plant residue and earthworms

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Abstract

Enchytraeids are a significant part of soil biota especially in arable land; it is therefore important to know their sensitivity to management. Reduced tillage (RT) is a cultivation method that benefits many groups of soil fauna, but the effects RT has on enchytraeids remains unclear. The aim of this study was to ascertain how the enchytraeid abundance at a research site on sandy soil in Northeast Brandenburg was affected by RT and conventional tillage (CT) during a ten year observation period and what reasons there may be for any observed effects of cultivation methods on enchytraeids. Works of several authors led to our hypothesis that a change in food availability and possible antagonistic relations to earthworms are likely to be the reasons for a negative effect of RT on enchytraeids abundance. Therefore, we examined the correlation between enchytraeids and plant residue as well as earthworms using Spearman's rank correlation coefficient. Our study showed that RT had a distinctly negative effect on the abundance of enchytraeids at our research site. The results of the study also showed that their abundance correlated significantly positive with plant residue under CT while under RT no correlation was ascertained. These findings support the thesis of Hendrix et al. (1986), which states that the bacteria-based food webs developing under CT favor organisms with high metabolic activity like enchytraeids, whilst the fungi-based food webs that occur under RT favor other soil animals such as earthworms. While earthworms were indeed favoured by reduced tillage at the experimental site, no clear relationship, neither positive nor negative, was found with respect to enchytraeid vs. earthworm abundances.

Keywords: Enchytraeidae; earthworms; conventional tillage; reduced tillage; plant residue

1. Introduction

Soil organisms are of great importance for agriculture. They affect soil structure and the breakdown of plant residue and therefore have a considerable impact on soil fertility (Whalley et al. 1995, Heisler 1998). In arable land soil organisms are more influenced by human activity than in any other ecosystem (Heisler 1998). For these reasons one aim of the German Federal Soil Protection Act is to improve the biological activity of arable soil by using site-specific management methods (BBodSchG 1998).

Reduced tillage (RT) is a management method that has positive effects on many groups of soil organisms (Didden et al. 1994, Kladivko 2001, Holland 2004, Joschko et al., 2009), in particular earthworms, which belong to the most important biota in agricultural soils (Lee 1985). This study investigates the effects that conventional tillage (CT) and RT have on enchytraeids.

Enchytraeids are good indicators of biological activity in soil (Jänsch et al. 2005, Graefe & Beylich 2005) and have been the subject of many studies concerning arable land. They are also part of many processes which occur in arable soil and thus have an impact on the decomposition of plant residue (Golebiowska & Ryszkowski 1978, Hendrix et al. 1986) and on the soil structure (Didden 1990, Van Vliet et al 1995, Graefe & Beylich 2005). Hendrix et al. (1986), Golebiowska & Ryszkowski (1978) and Novak (2004) suggest that enchytraeids are even of greater importance than earthworms where conventionally cultivated arable land is concerned because they are less sensitive to ploughing and more active metabolically.

The effect RT has on enchytraeids is not entirely clear. Some studies suggest that enchytraeids react negatively to the reduction of tillage (House & Parmelee 1985, Didden et al. 1994, Zwart et al. 1994), others suggest a positive reaction (Parmelee et al. 1990, Röhrig et al. 1998). One study found that the abundance of enchytraeids was higher under CT or under RT depending on the season of the year (van Vliet et al. 1995).

In this study the impact of CT and RT on the enchytraeid population of a research site on sandy soil in Northeast Brandenburg was investigated over a time span of ten years. Previous studies at this research site led to the hypothesis that enchytraeid abundance reacts negatively to reduced tillage. To find the reasons for the reaction of enchytraeids to RT we first examined the correlation between enchytraeids and plant residue. Plant residue and microorganisms decomposing plant residue are the main food source of enchytraeids (Whitfield 1977, Didden et al. 1994, Van Vliet et al. 1995). This led to the hypothesis that a change in food webs occurring under CT and RT would be visible in the relationship between enchytraeids and plant residue. Additionally we tested the hypothesis that enchytraeids and earthworms have an antagonistic relationship.

2. Material and methods

2.1. Study site experimental design

The study was carried out on a 74 ha heterogenous field belonging to the Komturei Lietzen in the federal state of Brandenburg, Germany (Joschko et al. 2009). The dominating soil type is Luvisol (FAO/ISRIC/ISSS 1998; Seyfarth et al. 1999). The site is characterised by a 9.6 °C mean annual temperature and 472 mm of mean annual precipitation (1992–2004). The field was under conventional tillage until 1996. Following the harvest in September 1996, non-inverting, ploughless tillage was established in one half of the field, whilst the other half continued to be tilled conventionally. Residue cover at the time of sowing was <15% for both systems. The ploughless system is referred to as ‘‘reduced tillage’’ throughout this article because the energy input and depth of the soil disturbance is reduced (Cannell 1985). The amount of fertiliser and pesticides used was the same in both tillage systems except in 1997 and 1998, when additional herbicides were applied to the reduced tillage system. Further details are found in Joschko et al. (2009).

42 monitoring plots (2m x 15m) on four transects were permanently installed in the field. The transects follow the main slope and tillage direction, with 21 plots for each tillage system. (Fig. 1). The distances between the 42 plots were irregular, with a mean distance of 70 m (Joschko et al., 2009).

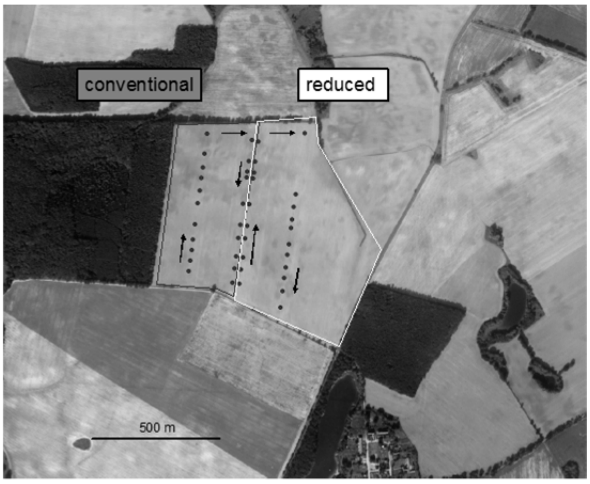


Fig. 1. Experimental design Lietzen: a 74 ha field under conventional and partly under reduced tillage with 21 plots each.

The crop rotation was as follows: winter wheat (1996), winter rye (1997, 1998), oilseed rape (1999), winter rye (2000, 2001, 2002), oilseed rape (2003), winter wheat (2004), grain maize (2005) and peas (2006).

2.2. Enchytraeid sampling and species identification

In September 1996 enchytraeids were sampled at each of the 42 plots prior to the installation of the two tillage variants and subsequently in spring (April, May) or autumn (August, September, October, November) of the following years up to 2006. In 1998, 2000, 2001 and 2006 sampling was carried out both in spring and autumn. Two soil cores with a 4.1 cm diameter were taken up to a depth of 20 cm from each plot. The soil cores were divided into four equal sections (0-5 cm, 5-10 cm, 10-15 cm, 15-20 cm).

In 2003 and 2004 only the top 15 cm of the soil were sampled.

Enchytraeids were extracted from soil using a modification of the wet-funnel method without heating (following Graefe 1984, as cited in Dunger & Fiedler 1997, p. 420). The soil sample was immersed in water by placing it into a sieve (mesh size 1.5 mm) in a water-filled bowl. After 2-3 days of extraction the sieve with the remaining soil was removed and most of the water in the bowl was emptied. The remaining water containing the enchytraeids was filtered through a fine sieve (mesh size 20 µm), and washed into a petri dish. Then the enchytraeids were counted using a binocular microscope. The total number of soil cores analysed for enchytraeid abundances was 1260 with a total of 4872 single samples.

Enchytraeid species identification was carried out at one soil core each (0-20 cm) from 5 plots sampled in November 2005 after maize (one under conventional tillage, 4 under reduced tillage) and at one soil core each (0-20 cm) from 8 plots sampled in September 2008 (four of each tillage variant). Species identification was based upon Nielsen & Christensen (1959), Schmelz (2003) and Schmelz & Collado (2010).

2.3. Earthworm sampling

Earthworms were also assessed at each of the 42 plots in September 1996 prior to the installation of the two tillage variants and subsequently in spring (April/May) of the following years up to 2006 (see Joschko et al. 2009). In 2000, 2003, 2005 and 2006 additional sampling was carried out in autumn (September, October, and November). Earthworms were collected at each of the 42 plots and were hand sorted from one 50 cm x 50 cm x 20 cm (w, l, depth) soil block immediately after sampling. Hand sorting has proved to be the optimal sampling method for the dry soils sampled at this location.

The earthworms were counted and identified to species level according to Sims & Gerard (1985) and Graff (1953).

2.4. Plant residue sampling

After extracting the enchytraeids the amount of coarse plant residue in the soil sample was ascertained using a modified Fenwick-can (Dunger & Fiedler 1997). The air-dried soil was placed on the upper sieve (5 mm mesh size) of the Fenwick-can. With the water flow coming from below and above, the coarse organic material floated and was caught in a finely woven sieve (ca. 200 µm). Then the organic material was dried for two days at 60°C, sieved again (1 mm mesh size) and weighted to scientific precision.

Plant residues were collected from 0-20 cm; under conventional tillage plant residues were distributed to a depth of 25 cm however; this bias explains slightly lower values for plant residue amounts in 0-20 cm soil depth.

2.5. Statistical analysis

For data analysis we used the software StatSoft STATISTICA 7.1. With STATISTICA we tested for normal distribution using q-q plots and carried out correlation analysis. Because the data were not normally distributed we used Spearman's rank correlation coefficient. This correlation coefficient requires only ordinal data and can also be used for metric data which are not normally distributed (Rudolf & Kuhlisch 2008). The software automatically tested for significance ($p < 0.05$). When small amounts of data were concerned no automatic test for significance was conducted. We also used STATISTICA for the creation of graphics and Sigma Plot.

2. Results

3.1. Effects of tillage on enchytraeid abundance and species composition

The average abundance of enchytraeids collected at all 21 CT plots and 21 RT plots during the 10 year observation period indicate a strong positive effect of tillage on the enchytraeid population. The average enchytraeid abundance under CT was considerably higher with 22567 ind. m^{-2}. Under RT the enchytraeid abundance was lower with 12318 ind. m^{-2}.

The changes over the course of time in the average enchytraeid abundance under CT and RT (Fig. 2) indicate that the increase and decline of the enchytraeid abundance under both cultivation systems occurred mainly during the same years. It also shows that the enchytraeid abundance was considerably higher under CT at most sampling dates. Enchytraeid abundances at single plots were not spatially autocorrelated (data not shown). While under conventional tillage enchytraeids were distributed evenly over the sampled soil depth of 0-20 cm, they concentrated in the upper soil (0-10 cm) in plots under reduced tillage. The enchytraeid species determination from soil samples in 2005 and 2008 yielded 15 species in 2005 (Tab. 1) and 9 species in 2008 (Tab. 2).

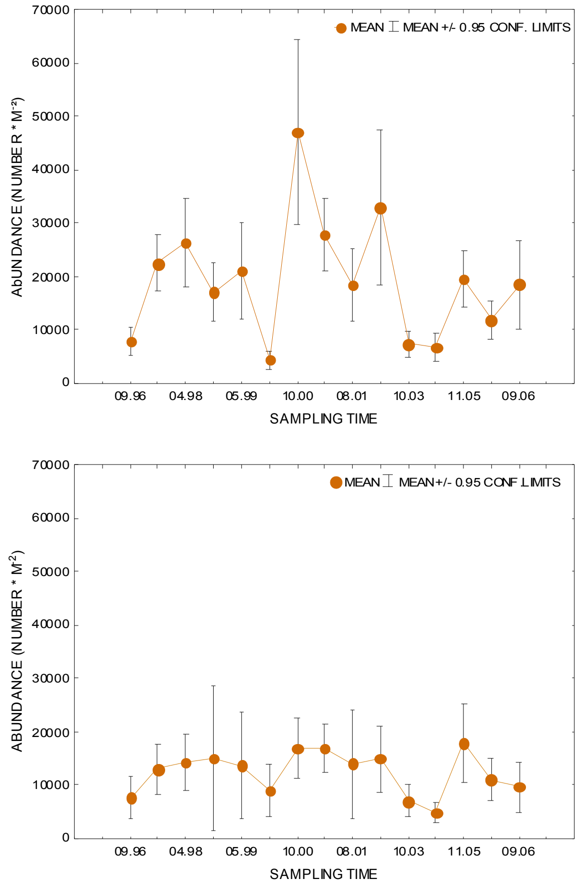


Fig. 2. Enchytraeidae abundance under conventional (above) and reduced (below) tillage over a ten year period.

Tab. 1. Enchytraeid species composition and abundance (ind. m^{-2}) found in five plots under conventional (No. 10) and reduced (Nos. 29, 30, 31, 42) tillage in Lietzen in 2005.

Species \ Plot	10	29	30	31	42
Achaeta pannonica	1136	12498	31812	379	-
Buchholzia appendiculata	379	-	11361	-	-
Enchytraeus buchholzi agg.	-	379	-	-	-
Enchytraeus christenseni agg.	6059	757	3408	-	-
Fridericia bulboides	-	-	379	-	-
Fridericia christeri	1136	-	-	757	-
Fridericia galba	-	1136	-	-	-
Fridericia maculatiformis	-	2272	3408	3030	-
Fridericia sp. (trid)	-	757	-	-	-
Fridericia sp. juv.	1136	3030	2651	3030	-
Henlea jutlandica	1515	757	-	-	-
Henlea perpusilla	-	-	-	-	379
Henlea ventriculosa	379	379	1136	-	-
Marionina communis	-	-	4166	379	-
Marionina minutissima	-	-	-	-	379
Oconnorella tubifera	-	-	5681	-	-
Total	11740	21965	64003	7574	757

Tab. 2. Enchytraeid species composition and abundance (ind. m^{-2}) found in eight plots under conventional (Nos. 10, 19, 20, 21) and reduced (Nos. 29, 30, 31, 42) tillage in 2008.

Species \ Plot	10	19	20	21	29	30	31	42
Achaeta pannonica	757	1515	3787	757	2272	11361	-	-
Buchholzia appendiculata	-	-	-	-	-	1515	-	-
Enchytraeus buchholzi agg.	-	-	757	757	1515	5302	1515	-
Enchytraeus christenseni agg.	-	-	5302	1515	3030	15906	6059	12119
Fridericia bulboides	-	-	-	-	757	-	-	-
Fridericia christeri	-	757	-	-	-	-	-	-
Fridericia maculatiformis	-	-	757	-	1515	2272	-	-
Fridericia singula	-	757	-	-	-	-	-	-
Fridericia sp. juv.	757	1515	2272	-	2272	1515	2272	-
Henlea ventriculosa	-	-	-	-	1515	-	757	-
Total	1515	4545	12876	3030	12876	37871	10604	12119

3.2. Plant residue and its correlation with enchytraeid abundance

The average amount of plant residue in the upper 20 cm of soil was considerably higher under RT and a distinct accumulation in the upper 5 cm could be seen. Under CT the plant residue was evenly distributed over the sampled depth.

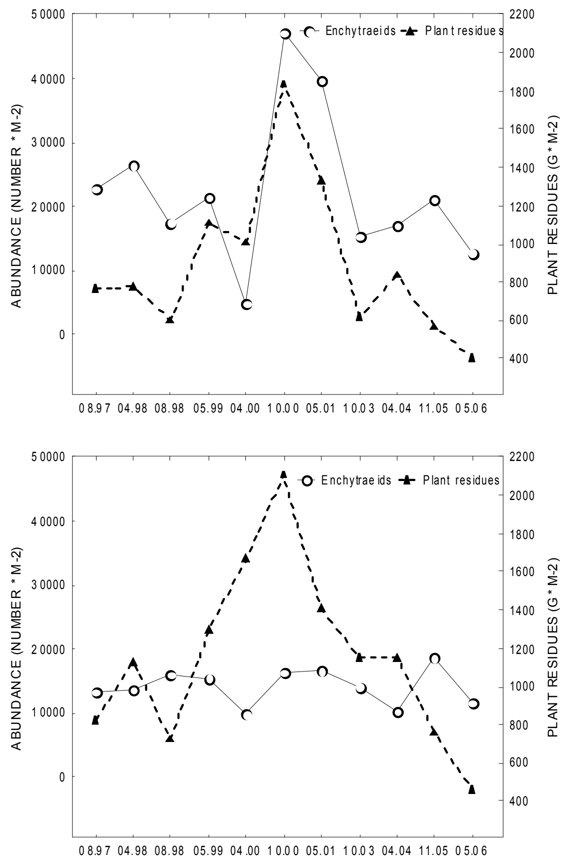


Fig. 3. Relationship between Enchytraeidae and plant residue during the observation period. Above: conventional tillage; below: reduced tillage.

The relationship between the total amount of enchytraeids and plant residue sampled each year, showed considerable differences between CT and RT on the 21 plots per cultivation system (Fig. 3). The relationship was markedly closer under conventional tillage. Under CT, the correlation was positive at 10 out of 11 sampling campaigns; four of these correlations were statistically significant (Tab. 3). Under reduced tillage, in contrast, the correlation coefficient did not indicate any positive or negative trend and was never significant.

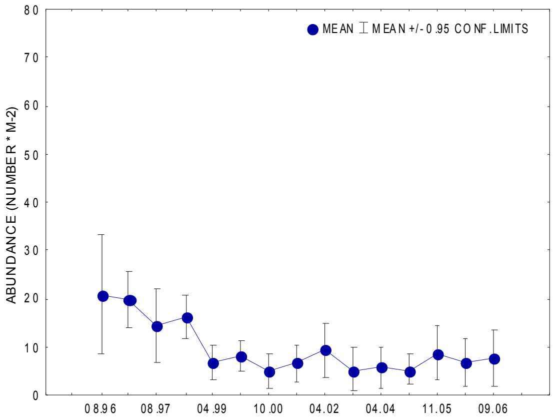
Tab. 3. Rank correlation coefficient (Spearman) between enchytraeids and plant residue for the 42 sample plots under conventional (CT) and reduced (RT) tillage (bold: significant).

Sampling date	CT	RT
Aug. 97	-0.099	0.158
Apr. 98	**0.546**	0.112
Aug. 98	**0.453**	-0.399
May 99	**0.625**	0.071
Apr. 00	0.199	-0.107
Oct. 00	**0.578**	-0.001
May 01	0.092	0.283
Oct. 03	0.104	-0.164
Apr. 04	0.007	0.077
Nov. 05	0.326	0.208
May 06	0.055	-0.073

3.3. Earthworm species composition and abundance and its correlation with enchytraeid abundance

Main earthworm species at the Lietzen site was the shallow-working *Aporrectodea caliginosa* (Savigny), especially *A.*c. forma *trapezoides* (Severon et al. 2007, Joschko et al. 2009). Besides this dominating species, *A. rosea* (Savigny) and the deep-burrowing *Lumbricus terrestris* L. was found. The proportion of *L. terrestris* was higher under reduced tillage compared to conventional tillage. The average earthworm abundance, assessed between 1997 and 2006 at 21 plots under both CT and RT was low, characterized by a mean abundance of 12 ind. m^{-2}. The average abundance of earthworms, shown in Fig. 4, indicates a strong negative effect of tillage on earthworm abundance. Under RT the average earthworm abundance was considerably higher than under CT. The data showed a remarkable spatial variability of abundances which were related to soil properties (Joschko et al. 2009).

As stated above, the two cultivation systems had the opposite effect on enchytraeids, with increased enchytraeid abundances under conventional tillage, suggesting an antagonistic relationship between the two families. However, no significant negative correlation between average enchytraeid and earthworm abundance could be established when the abundance data of both groups from 42 plots at 12 sampling dates between 1997 and 2006 were compared. The only significant correlation was positive and was found under reduced tillage (Tab. 4).



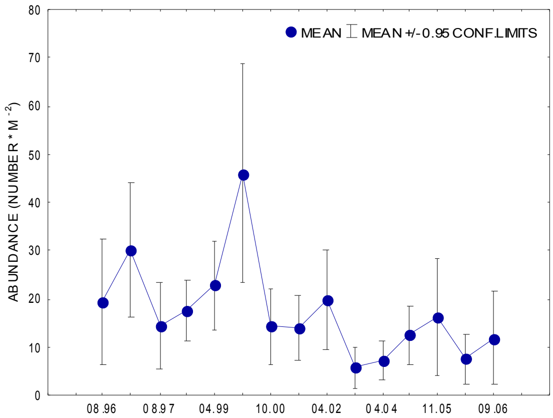


Fig. 4. Earthworm abundances under conventional (above) and reduced (below) tillage over a ten year period.

Tab. 4. Rank correlation coefficient (Spearman) between enchytraeids and earthworms at 42 plots and 12 dates under conventional (CT) and reduced (RT) tillage (bold: significant).

Sampling date	CT	RT
Aug. 97	0.43	-0.27
Apr. 98	0.10	-0.08
Aug. 98	-0.12	0.24
May 99	0.09	0.43
Apr. 00	0.11	**0.55**
Oct. 00	-0.27	-0.18
May 01	-0.21	0.02
Aug. 01	-0.02	0.00
May 02	-0.24	0.01
Nov. 05	-0.35	-0.04
May 06	0.11	-0.38
Sep. 06	0.08	-0.09

4. Discussion

The long-term field experiment Lietzen, installed in 1996 on a 74 ha field, enabled to monitor enchytraeid abundances on tilled soils as influenced by conventional and reduced tillage. The research site is characterized by sandy soils typical of the dry Northeast of Brandenburg; corresponding to the suboptimal conditions, faunal activity such as earthworm activity, is usually low and spatially highly variable (Joschko et al. 2009).

Average enchytraeid abundance in Lietzen was 22567 ind. m^{-2} under CT and 12318 ind. m^{-2} under RT. These values are high but not unusually high compared to other studies carried out on arable sites; the majority of the enchytraeid species found here are typical of arable land (Didden et al. 1997).

Our main research question related to the effect of reduced, i.e. non-inverting tillage on the enchytraeid population compared to conventional tillage. Our data clearly showed that enchytraeid abundance was higher under conventional tillage. While under reduced tillage the "original" enchytraeid abundances found in autumn 1996 stayed more or less at the same level, enchytraeid numbers apparently increased under conventional tillage with, however, considerable fluctuations (Fig. 4).

The changes in cultivation management from CT to RT in Lietzen thus had a distinctly negative effect on enchytraeid abundance. This supports the findings of House and Parmelee (1985), Didden et al. (1994), and Zwart et al. (1994), who also noted that enchytraeids were negatively affected by RT.

Next, we found that under conventional tillage there was a close relationship between enchytraeid abundances and plant residues, with increasing enchytraeid numbers with increasing food supply. Plant residue and the microorganisms, which primarily decompose the plant residue, are the main food source for enchytraeids (Whitfield 1977, Diddern et al. 1994, Van Vliet et al. 1995).

Interestingly, the amount of plant residues in the soil was slightly higher in plots under reduced tillage compared to conventional tillage (Severon 2008). Since annual yields were more or less the same under both tillage systems (Barkusky et al. 2007), there must be other reasons for this result. First, the amount of plant residues under conventional tillage may be slightly underestimated due to a sampling depth of 0-20 cm only, while plowing depth was 20-25 cm. Second, higher amounts of plant residues could be due to reduced decomposition processes under reduced tillage, including sparser grazing by enchytraeids due to reduced abundances. This latter explanation refers mainly to the data from spring sampling, since harvest and incorporation of plant residues happened in autumn.

Obviously, under conventional tillage with high numbers of enchytraeids, the food resource was limiting for enchytraeids in the studied soil, reflected in the close relationship between plant residues and enchytraeid numbers. Under RT no correlation was detected.

But what could be the reason for enhanced enchytraeid numbers under conventional tillage? Next we addressed potential antagonistic relations between enchytraeids and earthworms, considering the striking contrast between our enchytraeid results and the earthworm abundances which were increased under reduced tillage between 1996 and 2006 (Fig. 4., Joschko et al. 2009). A possible reason for the negative affect that RT had on enchytraeid abundance could be the higher abundance of earthworms under RT. Some studies suggest an antagonistic relation between the two families (Schaefer & Schauermann 1990, Zwart et al. 1994) whilst others state that this antagonistic relationship occurs only at the species level (Haimi & Boucelham 1991, Didden 1993, Hyvönen et al. 1994, Huhta & Viberg 1999, Yli-Olli & Huhta 1999). The results of this study indicate however, that the negative effect of RT on the enchytraeid abundance is not due to an antagonistic relationship between enchytraeids and earthworms. No negative correlation between earthworms and enchytraeids were found (Tab. 4). Also, earthworm abundances were generally low at the site, with only some plots with earthworm abundances above 150 ind. m^{-2}. Therefore, antagonistic effects of earthworms can be excluded as a possible cause for our findings. At sites with larger earthworm populations, however, they may well have a stronger effect on the enchytraeid population.

Our study does not allow to identify a clear cause, nevertheless speculations are possible. The conventional tillage may itself be beneficial to enchytraeids. Our results are compatible with the theory that under CT there exists a bacteria based food web as suggested by Golebiowska & Ryszkowski (1977), House & Parmelee (1985) and Hendrix et al. (1986). It is possible that higher microbial activity under CT benefits the enchytraeids, because rapid decomposition and mineralization of the plant residue leads to a faster availability of nutrients for the enchytraeids. Thus a change in the amount of plant residue affects the availability of food resources for the enchytraeids via a bacteria-based food web. Under RT a fungi-based food web, as suggested by Hendrix et al. (1986), causes a much slower decomposition and mineralization of the plant residue. The nutrients are stored in the residue for a longer amount of time and so a change in the amount of plant residue does not directly affect the availability of food resources for the enchytraeids. This would explain why no positive correlation was found between enchytraeids and plant residue under RT. Also, differences in soil moisture between RT and CT may be important. The mixing in of plant residues under CT might prevent the deeper soil layers from drying out, and thus enhances microbial activity and food availability and fosters the survival of drought sensitive enchytraeids. Unfortunately, no data are available for soil moisture under reduced compared to conventional tillage at the studied site. The relationship between soil water and enchytraeid abundance in differently tilled sandy soils should definitely be studied in more detail.

As stated in the introduction, the aim of the German Federal Soil Protection Act is to improve the biological activity in arable soil by using site-specific management methods (BBodSchG 1998). This study suggests a higher level of enchytraeid activity and indicates higher microbial activity under CT at the sandy soil studied. Further research is necessary to substantiate our findings in other soils and climates. The development of fungi-based food webs under RT, when substantiated for other soils and climates, can however have many advantages, such as a slower and steadier release of nutrients as well as the promotion of earthworms. Thus the effect of cultivation management on the biological activity of soil has to be viewed differently, depending on the groups of soil organisms favored by the cultivation system and the impact they have on processes in soil.

Acknowledgements. We thank Gebhard Graf Hardenberg, Lietzen, for the possibility to carry out this field experiment. The technical assistance of Doris Beutler and Ursula Fuhr, ZALF Müncheberg, the Forschungsstation Landwirtschaft, Standort Müncheberg, especially Brigitte Krüger, Matthias Lemme and Sven–Äge Schnabel, is gratefully acknowledged. We also thank the organizers of the 9th International Symposium on Enchytraeidae at the Johann Heinrich von Thünen-Institute (vTI) in Braunschweig (Germany) from 14 to 16 July 2010 where we could present some of our results. We expecially thank Jiři Schlaghamerský for valuable comments during the Discussion in that Symposium. The study was financially supported by the BMELV and MELF Brandenburg, and the Landwirtschaftliche Rentenbank, Frankfurt/Main.

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Guide to European Terrestrial and Freshwater Species of Enchytraeidae (Oligochaeta): First supplement

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Abstract

Supplementary notes on the "Guide to European Terrestrial and Freshwater Species of Enchytraeidae (Oligochaeta)" (Schmelz & Collado 2010) are presented. The meaning of the informal categories of "species groups", "species sensu lato" and "species sensu stricto", used in the Guide, is explained. New species to be included are dealt with, notably *Enchylea heteroducta* Nielsen & Christensen, 1963, *Cernosvitoviella longiducta* Dumnicka, 2010, *Cognettia valeriae* Dumnicka, 2010, and *Enchytraeus dudichi* Dózsa-Farkas, 1995. Several changes in the keys and the species diagnoses are proposed, to improve operability and to include new evidence. Regarding the problem of *Enchytraeus crypticus* and *E. variatus*, we propose a classification that differs from the one given in Schmelz & Collado (2010). Instead of synonymizing both species under the name of *E. crypticus*, we propose to maintain *E. variatus* at the morphological level. This species sensu lato comprises *E. variatus* sensu stricto and *E. crypticus* at the molecular level. Finally we give a page index to species in Schmelz & Collado (2010).

Keywords: Clitellata, taxonomy; species sensu lato; species sensu stricto; species groups

1. Introduction

The present paper contains supplementary notes on the recently published "Guide to European Terrestrial and Freshwater Species of Enchytraeidae (Oligochaeta)" (Schmelz & Collado 2010), referred to in the following, for convenience, and without author and year, as "Guide". The supplementary notes are partly results of our ongoing research with enchytraeids, but also results of first uses of the Guide by students and researchers, who drew our attention to some points that need improvement or correction. In a first chapter we specify the meaning of the informal categories of "species groups", "species sensu lato" and "species sensu stricto", used in the Guide. Secondly we deal with new species to be included. The following chapter deals with changes in the diagnoses of some species, and makes proposals how to change the text accordingly. After a series of further corrections we give a species index to the Guide.

2. Informal categories: species groups and species sensu lato / sensu stricto

Apart from genus and species, the Guide uses several informal categories, in order to deal with the taxonomic uncertainty in many groups. The informal categories may be briefly explained here.

A **species group**, e.g. "*Enchytraeus buchholzi* -group" unites several named species of the same genus that are distinctly more similar to each other than to the remaining species in the genus, thereby assuming a common evolutionary history separate from the rest of the species. It is roughly equivalent to the subgeneric level, but it avoids the obligation to assign a subgenus to all other species of the genus, which may be heterogeneous or without a patterns of similarity that allow meaningful subgeneric divisions. 'Species group' may be replaced by '**species complex**', when the distinction among these species is difficult or questionable, or when intermediate forms occur.

The use of '**species sensu lato**' and '**species sensu stricto**' means that two taxonomic concepts are applied to one and the same species name: A species name can denote a heterogeneous group of forms ('species sensu lato') and at the same time a well-circumscribed species with a narrow morphological diagnosis ('species sensu stricto'). The sensu lato / sensu stricto distinction is a flexible tool and especially helpful in groups with unresolved taxonomy or phylogeny. It allows to identify at the same time specimens that agree closely with the original description and those that differ from the original description to a degree that makes it difficult to decide whether it belongs to the same species or to a different one - without throwing both into the same basket.

There are two alternatives to this procedure, both unsatisfactory: (1) Enlargement ("emendation") of the species diagnosis in order to include the deviant forms. This has been common practice in many enchytraeid species (see for example the history of *Hemienchytraeus stephensoni* in Schmelz & Collado 2007), with the result that almost anything fitted into it. (2) Exclusion of the deviant forms, with the result that they remain unidentified. This has been practised several times in Schmelz (2003, see e.g. *Fridericia singula*, p. 320 ff.).

The sensu lato / sensu stricto distinction is also useful in the case of cryptic or molecular species, see below our example of *Enchytraeus crypticus* and *Enchytraeus variatus*.

Generally speaking, informal categories are useful to describe patterns of diversity that are incompletely known or difficult to interpret, and they have the advantage of not being restricted by nomenclatural regulations. With the accumulation of further evidence, they may later be integrated into the traditional system of categories.

Comparing the concepts dealt with above, 'species group' is more inclusive than 'species sensu lato / sensu stricto'. For example, the *Enchytraeus buchholzi*–group includes many species of *Enchytraeus*, all characterized by meandering oesophageal appendages, compact male glandular bulb and not more than 3 chaetae per bundle, for example *E. bulbosus*, *E. luxuriosus*, *E. coronatus*, and others. *Enchytraeus buchholzi* sensu lato has a much narrower morphological circumscription, comprising several named species and also forms that in later studies may turn out as good species. *Enchytraeus buchholzi* sensu stricto has not yet been defined.

3. Species new to Europe

3.1. *Enchylea heteroducta* Nielsen & Christensen, 1963

Species known only from laboratory cultures and of unknown geographical origin were not included in the Guide. One of these species was *Enchylea heteroducta* Nielsen & Christensen, 1963, described from a laboratory culture of unknown origin. Recently we found *Enchylea heteroducta* in soil samples from an experimental field of the University of Coimbra, Portugal (Schmelz, unpublished), this being the first record after the original description and the first record from a habitat outside the laboratory. The three specimens that we found, one juvenile, one subadult, one adult, fit the original description almost perfectly. In the following brief revised description, traits not originally mentioned but present in our material are marked with "(N)".

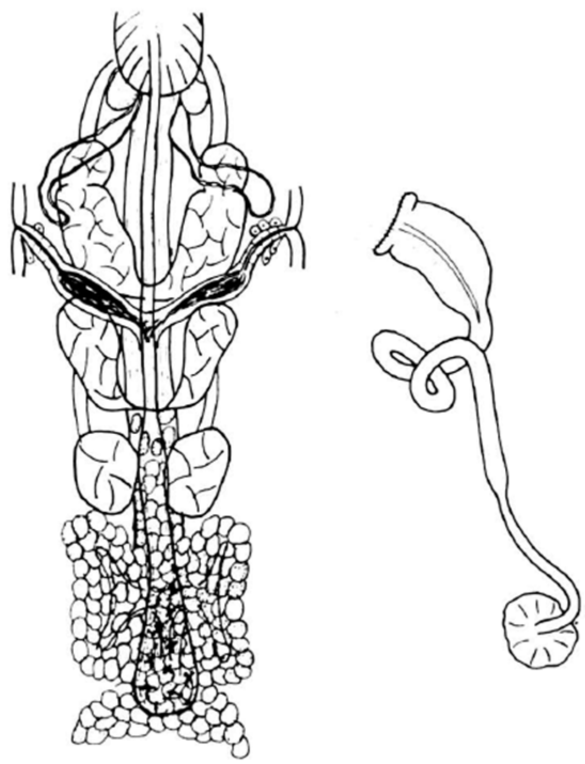

Fig. 1. *Enchylea heteroducta*. Left: segments III-VII, dorsal view. Right: Male efferent apparatus. From Nielsen & Christensen (1963, Figs 14, 17).

Length c. 5 mm. Segments 25-35. Chaetae straight distally, 2 and 3 per bundle, ventrally mostly 3. Head pore at 0/I. Brain rounded posteriorly. Pharyngeal glands in IV-VI, decreasing in size from IV to VI, separate or with narrow dorsal connection, no secondary glands. Oesophageal appendages in III-IV, a pair of short, sac-like tubes attached to oesophagus dorsally behind pharyngeal pad. Intestinal diverticula in VII, a pair of dorsolateral pouches connected with intestine by a short, common, dorsal duct. Intestine widening abruptly at VII/VIII. Chloragocytes with dense layer from VI, covering also intestinal diverticula. Dorsal blood vessel from VII, blood colourless. Nephridia with anteseptale that consists only of the funnel, efferent duct with terminal vesicle. Preclitellar nephridia 6 pairs (N), from 4/5 to 9/10. Coelomocytes one type, only mucocytes, c. 20 µm long, filled with small, slightly refractile vesicles. Clitellum girdle-shaped, cells in dense rows, hyalocytes present also mid-ventrally (N). Testes and developing sperm enclosed by a common membrane, i.e. sperm developing in testis sacs and not freely in the coelom (N). Sperm funnel small, c. 1.5x as long as wide: Vas deferens short, proximal part wider than distal part. Male copulatory organ with rounded, compact gland. Subneural glands absent. Spermathecae simple, without diverticula, united mid-dorsally and attached to oesophagus, ampullae drop-shaped, longer than ectal duct, ectal glands inconspicuous or absent.

The species combines a unique set of characters especially as regards chaetae, nephridia, oesophageal appendages and intestinal diverticula. Therefore a new genus had been erected for it. Nielsen & Christensen (1963) suggested an affinity of *Enchylea* to *Enchytraeus* because of the shape and position of the oesophageal appendages. The presence of testis sacs, one of the traits not mentioned originally but present in our material, supports this hypothesis.

Some slight differences in our material to the original description concern body length, segment number, chaetal pattern, and other traits; they will be dealt with in a redesription, to be published elsewhere.

In the genus key of the Guide, *Enchylea* keys out at couplet 25 (p. 45), which should accordingly be replaced by the following two couplets:

25 Oesophageal appendages present in III-IV, between pharyngeal pad and first pair of pharyngeal glands, unbranched tubes or sacs **25A**
25* Oesophageal appendages absent **26**

25A Gut widening gradually in VII-X; no intestinal diverticula; origin of dorsal blood vessel in or near clitellar region; preclitellar nephridia from 6/7 (rarely from 5/6 or 7/8) .... *Enchytraeus*
25A* Gut widening abruptly at 7/8; intestinal diverticula dorso-laterally in VII, origin of dorsal blood vessel in VII; preclitellar nephridia from 4/5 .... *Enchylea*

3.2. *Cernosvitoviella longiducta* Dumnicka, 2010

This new species, described from high mountain streams in the Italian Alps, keys out between couplets 11 and 13 (Guide p. 61), provided that the coelomocytes of this species do not have strongly refractile granules (comp. Guide p. 59, couplet 7). As the species was described on ethanol-preserved material, it is unknown whether refractile granules are present or absent. However, absence may be inferred from the shape of the cells, described as oval or spherical. Strongly refractile coelomocytes are usually spindle-shaped in *Cernosvitoviella*. We suggest the following amendments in the key to *Cernosvitoviella* species (Guide p. 61):

11 Vas deferens widened in distal half (if enlarged in proximal half, see 4 or 7*) .. **12**
11* Vas deferens without widenings or widened olny close to male pore **12A**

12A Spermathecae usually extending beyond V **13**
12A* Spermathecae confined to V .... *Cernosvitoviella longiducta* Dumnicka, 2010

L 2.5-3.5 mm (fixed), S 24-29. Up to 7 chaetae per bundle, dorso-laterally 4-6. Length of chaetae 26-43 um, largest in posterior segments. Pharyngeal glands with 2+2 pattern. Dorsal blood vessel origin unknown. Coelomocytes oval or spherical. Seminal vesicle small. Sperm funnel cylindrical, 2x as long as wide, collar as wide as funnel body. Vas deferens 12-15x as long as sperm funnel, slightly widened proximally near sperm funnel, and distally near male pore; here an atrium-like expansion, surrounded by glands. Spermathecae confined to V, ampulla elongately oval, ectal duct shorter than ampulla, of same diameter throughout.

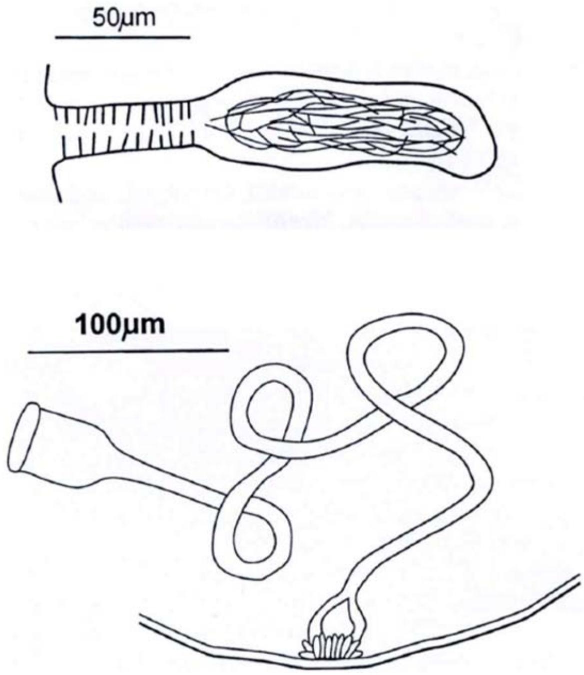

Fig. 2. *Cernosvitoviella longiducta*. Top: Spermatheca. Bottom: Male efferent apparatus. From Dumnicka (2010, Figs. 5, 4).

In case that our inference concerning the coelomocyte texture is incorrect, the species keys out together with *C. aggtelekiensis* (Guide p. 61, couplet 10), a species with strongly refractile granules in the coelomocytes. This species differs in several traits from *C. longiducta*: some coelomocytes spindle-shaped, sperm funnel 3x as long as wide, vas deferens swollen distally, spermathecal ectal duct canal widened distally (comp. Guide p. 58, Fig. 21E).

3.3. ***Cognettia valeriae*** Dumnicka, 2010

This new species, described together with the foregoing one from high mountain streams in the Italian Alps, is within the range of variability of "*Cognettia sphagnetorum* sensu lato" as conceived in the Guide, although reproduction by fragmentation is not mentioned in the original description of *C. valeriae*. It is similar especially to *C. paxi* and *C. anomala*, species synonymized with *C. sphagnetorum* in the Guide, but accepted by Dumnicka. Additional notes on *C. anomala* are given in Dumnicka (1976). A critical appraisal of the synonymy is pending, and the taxonomic status of *C. valeriae* can only be dealt with in a revision of *C. sphagnetorum*. The following brief description of *C. valeriae* is a summary of the original account.

L 12-15 mm, S 49-52. Chaetae 3 per bundle, laterally 2 or 3 in anterior and posterior bundles. Pharyngeal glands in 5 segments, from IV to VIII; first two pairs dorsally united; secondary glands present in VII and VIII. No nephridia in segments anterior to gonadal segments. Clitellum not developed, seminal vesicle and sperm funnel in IX, male pores in X. Sperm funnels 3x as long as wide, shorter than body diameter. Spermatheca in V or V-VI, with small ectal gland, ampulla with ectal dilatation and large spherical ental reservoir, not connected with oesophagus.

The pattern of pharyngeal glands seems identical with the one illustrated in Fig. 30C (Guide, p. 78). It is also the same as illustrated for *C. anomala* in Dumnicka (1976: Fig. 2). The 'secondary pharyngeal glands' mentioned in the original description are ventral lobes of the primary glands (comp. Guide, p. 20).

3.4. ***Enchytraeus dudichi*** Dózsa-Farkas, 1995

A fragmenting population of *Enchytraeus* sp. with 3 chaetae in many bundles was found by Annemariet Vanderhout in a compost heap in the Netherlands, and cultures are currently maintained in the labs of the finder and the authors, respectively. Sexual specimens have so far not been obtained. The coelomocyte texture agrees with the one described for *Enchytraeus dudichi*, cultures of which are unfortunately no longer available. *E. dudichi* has also been found in South America, see Niva et al. (2012). So far *E. dudichi* is the only fragmenting *Enchytraeus* species with 2 and 3 chaetae per bundle, all others have only 2. Collado et al. (2012) suggest naming all specimens of fragmenting *Enchytraeus* with 3 chaetae per bundle "*Enchytraeus dudichi* sensu lato". In a preliminary molecular study they showed that *E. bigeminus* sensu lato (i.e. fragmenting *Enchytraeus* with only 2 chaetae per bundle) comprises several cryptic lineages diverse enough to be recognized as species, and the same may apply to *E. dudichi* sensu lato. Fragmenting *Enchytraeus* spp. appear to be fairly common in soils at lower latitudes, and finds in temperate or more northern climates should be recorded carefully.

E. dudichi keys out at 2* (Guide p. 84); the section should be changed as follows:

2* Three chaetae in several, many or all ventral bundles **4A**

4A Never more than 3 pairs of pharyngeal glands, in IV-VI, sexual specimens with egg and clitellum common **5**

4A* Often four pairs of pharyngeal glands, in IV-VII; reproduction mainly by fragmentation (i.e. sexual specimens usually absent) .... *Enchytraeus dudichi* Dózsa-Farkas, 1995 sensu lato

L 2-22 mm, S up to 100 and more, fragments 10 segments or less. Specimens often with incomplete anterior or posterior end. 2 and 3 chaetae in lateral bundles, 3 ventrally (occasionally 4 in single bundles). Coelomocytes with few refractile granules embedded in a conspicuously vesicular matrix; aggregations grey. Sexual specimens rare, not yet recorded from Europe. Gonadal segments shifted anteriad, male pores in VIII or IX. Large testis sacs and sperm funnels. Spermatheca similar to *E. christenseni* but larger, ampulla as large as pharyngeal gland lobes. – The Netherlands, a compost heap.

4. Revised diagnoses and further changes

4.1. *Enchytraeus crypticus* Westheide & Graefe, 1992 and *E. variatus* Bouguenec & Giani, 1987

In the Guide, we have synonymized *E. crypticus* and *E. variatus*, and we maintained the name of the junior synonym *E. crypticus*, arguing that the species is established under this name as standard species in ecotoxicological tests under the name of *E. crypticus*. Here we present a different solution, using the sensu lato / sensu stricto concept of species, shown in Figure 3. We use two levels, a morphological (light-microscopical) level and a molecular level. At the morphological level, *E. crypticus* and *E. variatus* are united as *Enchytraeus variatus* sensu lato. *E. variatus* s.l. is further distinguished at the molecular level into *E. crypticus* and *E. variatus* sensu stricto. One of the reasons for this classificatory change is taxonomic uncertainty: We do not know whether *E. crypticus* and *E. variatus* are different species. Further reasons are listed below. Before, we review the taxonomic history of *Enchytraeus crypticus* and *E. variatus*, in order to explain the taxonomic uncertainty.

4.1.1. Taxonomic history of *E. crypticus* and *E. variatus*

Enchytraeus crypticus was detected in the framework of the joint project "Solving zoosystematic problems with biochemical and morphological methods" (Westheide & Schminke 1991). Different groups of organisms, among them culturable strains of *Enchytraeus* spp., were investigated and compared with several methods that addressed the sub-morphological level, namely: spermatozoal ultrastructure (Westheide et al. 1991), isoenzyme and total protein patterns (Brockmeyer 1991a), lipids (Jacob et al. 1991), DNA restriction fragment patterns (Schlegel et al. 1991), and immunulogical distances (Gabrich et al. 1991). Background for choosing *Enchytraeus* spp. as one of the model groups - the other group were copepods of the genus *Tisbe* - was the notoriously difficult taxonomic situation in enchytraeids and especially in *Enchytraeus*, where morphology often does not provide sufficient evidence for clearcut species separations (Westheide & Schmelz 1997). All in all, twenty-one cultures of *Enchytraeus* spp. raised on agar plates in the laboratory, were compared. The geographical origin of the starter specimens was known in most of the cultures. More than half of the cultures were not identified to species level.

One result of the concerted comparisons was the separation of a group of seven unidentified cultures from the rest. These seven, in turn, could be separated into two subgroups by minute but constant differences in the protein patterns (Brockmeyer 1991a), the spermatozoal ultrastructure (Westheide et al. 1991), and DNA restriction fragment length polymorphisms (Schlegel et al. 1991). Within each subgroup, no differences among specimens could be detected. The two subgroups were indistinguishable from one another at the morphological level, i.e. using light-microscopy.

In order to assess the taxonomic status of these two subgroups, cross-breeding tests were carried out (Brockmeyer 1991b). Because many *Enchytraeus* species are able to reproduce by self-fertilization, offspring alone did not provide evidence for interbreeding. Instead, the offspring was analyzed for evidence of heterozygoty, using isozyme markers that differed among the two subgroups (all cultures were homozygous with respect to all isozyme systems tested). Heterozygote specimens would prove interbreeding and the existence of only one species, their absence would indicate reproductive isolation of the subgroups and establish their status as two separate species. Two juveniles, one of each subgroup, were placed on an agar plate, and the first offspring generation (F1) was screened for possible heterozygotes, choosing an appropriate enzyme. The test was repeated at least 10 times (the exact number is unknown), and 4-5 descendants per replicate were analyzed electrophoretically. Heterozygotes were absent in the first generation, and this was taken as evidence that the two strains do not interbreed (Brockmeyer 1991b). Evidently, these reproduction tests were based on the assumption that the test specimens would prefer interbreeding over self-fertilization when given the choice.

Light-microscopical investigation and comparison of specimens of both subgroups revealed congruence with the description of *Enchytraeus variatus* Bouguenec & Giani, 1987, a species found in garden mould in southern France. Since all strains of one of the subgroups came from the type locality of *E. variatus*, these were identified as *E. variatus*, and the other strains were recognized and described as representing a new species, *E. crypticus* (Westheide & Graefe 1992). The species name refers to its being morphologically indistinguishable from *E. variatus*.

As a further result of these investigations, another group of unnamed cultures of investigated *Enchytraeus* sp. was described as representing a new species, *E. doerjesi* Westheide & Graefe, 1992. *E. doerjesi* differed at all levels, including the light-microscopical one. Other cultures remained undescribed. A following study using RAPDs (Schirmacher et al. 1998) confirmed the similarity of *E. crypticus* and *E. variatus*. The calculated genetic distance of both species (0.17) was in the range usually considered as intra-specific, while distances to *E. doerjesi* were larger (0.83 and 0.84, respectively).

4.1.2. Critical assessment of the reproduction tests

As is evident from the foregoing, the species-status of *E. crypticus* is based on the cross-breeding tests and not on the molecular differences. The cross-breeding tests, however, published in the doctoral thesis of Brockmeyer (1991b), rely implicitly on two assumptions: (1) Interbreeding among conspecific specimens is possible. (2) Specimens raised in the laboratory will prefer interbreeding over self-fertilization when given the choice. However, these two assumptions may be incorrect.

(1) Up to now, the oligochaete-typical cross-wise mode of copulation (documented cinematographically in Westheide & Müller 1995, using *E. albidus*) has not been observed in *E. crypticus* and *E. variatus*, whereas self-fertilization has been demonstrated by the reproduction tests themselves. This means that interbreeding may, for some unknown reason, be impossible at all and self-fertilization may be the only mode of reproduction. Under this scenario, the species-distinguishing criterium of reproductive isolation would be obsolete, because the so-called biological species concept applies only to bisexually reproducing populations. Using alternative species concepts, however, such as those that use genetic distances as yardstick, the two species would rather be considered as two lineages or strains of the same species, considering their high similarity. Evidence in favour or against the hyothesis of 'obligate automixis' in *E. crypticus* and *E. variatus* is pending - a video-based observation program to observe the reproductive behaviour of specimens would be helpful.

(2) Nevertheless, the first assumption underlying the reproduction tests may be correct, and interbreeding may indeed occur within a given population of *E. crypticus* or *E. variatus*. However, even then self-fertilization and not interbreeding may be the preferred mode of reproduction in these species, especially in new environments, such as freshly cast agar plates not yet modified by the worms' activities – the type of substrate used in the reproduction tests. As a consequence, heterozygotes may not appear in the F1 generation but several generations later. Considering this, we carried out a long-term reproduction test with *E. variatus*

and *E. crypticus* (Collado & Schmelz, unpublished): We reared both cultures together in one soil-filled dish over a longer period of time, and after 6 months and 2 years, respectively, we searched for heterozygotes using an appropriate enzyme system, here PGI, phospho-glucoisomerase. Interestingly, we did find heterozygotes, albeit at a low percentage (c. 5%). Percentage was about the same after 6 months and 2 years. This would mean that both species do occasionally interbreed, but that some reproductive barrier would still exist that prevents panmixy. However, the original cultures were not analyzed in detail, and there was some indication that the heterozygous genotype was already present in the original culture of *E. variatus*.

As a result, the status of *E. crypticus* and *E. variatus* as two separate species is questionable, but evidence to unite them into one species is insufficient as well.

4.1.3. Advantages of the new classificatory proposal

As already mentioned above, the new proposal makes use of the sensu lato / sensu stricto concept of species, and it considers two classificatory levels, a morphological (light-microscopical) level and a molecular level (Fig. 3). At the morphological level, *E. crypticus* and *E. variatus* are united as *Enchytraeus variatus* sensu lato. *E. variatus* s.l. is further distinguished at the molecular level into *E. crypticus* and *E. variatus* sensu stricto. The advantages are as follows:

(1) In view of the taxonomic uncertainty explained above, it is a stable solution, irrespective of results of future investigations into the reproductive relationships of *E. crypticus* and *E. variatus*. If species status of both species in question is confirmed, *E. variatus* sensu lato may be maintained as a phylogenetically meaningful entity: both species, though closely related, certainly form a monophyletic group. If on the other hand partial interbreeding or obligatory automixis is confirmed, both species may be considered as subspecies, and the classificatory architecture will remain the same.

(2) It further considers practical needs: Both levels, the morphological and the molecular level, should be covered by names. The morphological level is necessary for the identification of specimens found in the field. The molecular level is necessary for the laboratories worldwide that maintain cultures of a well-identified species (*E. crypticus*), all derived from the same strain as far as we know, and identifiable by several molecular methods, including DNA sequences (see Erséus et al. 2010). Reservation of the name *E. crypticus* to this strain will facilitate inter-comparability of results and meet standardization requirements of test procedures especially in the field of exotoxicology.

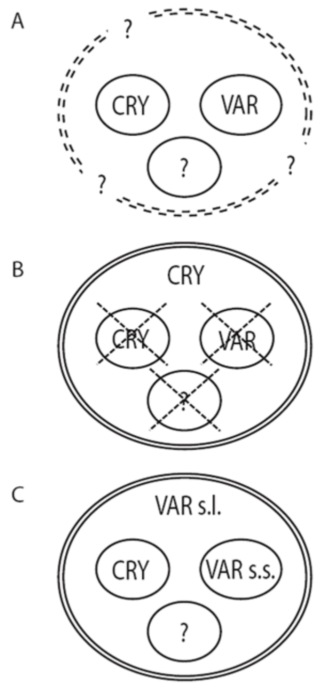

Fig. 3. Taxonomic and nomenclatural change in *Enchytraeus crypticus* and *E. variatus*. Double line: morphological level, equivalent to "species sensu lato". Single line: molecular level, equivalent to "species sensu stricto". cry = *crypticus*, var = *variatus*. A. Situation as established in Westheide & Graefe 1992: Two cryptic species, leaving the morphological level vacant. B. Situation as established in the Guide: Synonymy of *E. crypticus* and *E. variatus*, leaving the molecular level vacant; *E. crypticus* maintained as valid name against nomenclatural regulations. C: Solution as proposed here: *Enchytraeus variatus* sensu lato, defined at the morphological level, includes both *E. crypticus*, *E. variatus*, defined at the molecular level, and further cryptic species to be discovered (question mark). For further details, see text.

(3) Our new proposal is the best way to cover the pattern of diversity as currently known. In contrast, the scenario of the Guide (one name for both species) leaves the molecular level vacant, whereas the scenario previous to the Guide (maintenance of two separate species) leaves

the morphological level vacant. The latter may surprise, but if two species can be separated only with molecular methods, *both* of them – and not only the new species – are 'molecular' or cryptic species. The old one (in this case *E. variatus*) undergoes a change in definition or circumscription, because morphology no longer suffices to identify the species: it becomes a molecular species as well. As a consequence, specimens that fit the original description of *E. variatus* can no longer be identified, and this not only because they may belong to *E. crypticus* or to *E. variatus*, but also because they may belong to one of several other still unknown molecular species (see Fig. 3, question marks).

(4) Finally, a proposal towards the Commission on Zoological Nomenclature for conservation of the junior synonym *crypticus* as valid name, as suggested in the Guide, is no longer necessary.

4.2. *Fridericia perrieri* (Vejdovský, 1878) **and *F. dozsae*** Schmelz, 2003

Fridericia perrieri is a taxonomically difficult species. At first sight, identification is easy and straightforward because of the proximal spiral loop of the spermathecal ectal duct canal; this trait is unique in enchytraeids. Unfortunately, this trait is not present in all specimens. Furthermore, a few morphological variants seem to close the 'morphological gap' that separates *F. perrieri* from other species. For example, in Schmelz (2003, p. 376f.), a form is described with traits that mediate between *F. perrieri* and *F. ulrikae*. Recent finds of *F. dozsae* and *F. perrieri* from the Northwest of the Iberian peninsula (Schmelz, unpublished) go in a similar direction: Maximum number of chaetae is 6-8 in *F. perrieri* and 4-6 in *F. dozsae*, according to the Guide, following Schmelz (2003). However, some Iberian specimens of *F. perrieri* have not more than 5 chaetae per bundle and some specimens of *F. dozsae* have up to 7 chaetae per bundle. As distinguishing traits remain spermathecae and male glandular bulb. In *F. dozsae*, spermathecal ampulla and diverticula have a rugose, granular texture, and the male glandular bulb is about twice as long as wide. In *F. perrieri*, spermathecal ampulla and diverticula are smooth and pale, and the male glandular bulb is almost as wide as long. It needs to be shown whether these traits hold for all specimens. To adjust the recent finds to the keys in the Guide, we suggest the following changes:

P. 143 couplet 16*: description of *F. dozsae*: “A maximum of 4, 5, or 6 chaetae per bundle **(rarely 7)** ... Seminal vesicle **up to** 2 segments.”

P. 150 couplet 5: description of *F. perrieri*: Add behind the chaetal formula: "Occasionally not more than 5 chaetae per bundle."

P. 150 couplet 6*: “Up to 6 chaetae per bundle**, rarely 7** ...”

4.3. *Fridericia gamotheca* Issel, 1905

Maximum number of chaetae in *F. gamotheca* varies from 4 to 6, but this species has erroneously been omitted in group F, the group of *Fridericia* species with two spermathecal diverticula and a maximum number of 5 or more chaetae per bundle. We suggest the following changes in the key of the Guide, group F, p. 148:

1 Spermathecal ectal glands absent **1A**
1* Spermathecal ectal glands present **7**

1A Spermathecae separate, not fused **2**
1A* Spermathecal ampullae completely fused; see group E, couplet 13, p. 142 .... *Fridericia gamotheca* Issel, 1905c

4.4. *Marionina hoffbaueri* Möller, 1971

In the Guide key to species of *Marionina*, the absence of chaetae at some positions is one of the principal distinguishing traits. *M. hoffbaueri* is originally described with chaetae absent laterally in VIII-XII, but the trait seems to be variable. We found, in samples taken near Frankfurt, specimens that fit the diagnosis of *M. hoffbaueri* but with chaetae in all positions (except I and XII, of course), those laterally from VIII-XI included. The same holds for the single specimen of "*Marionina* sp. 1" in Schlaghamerský & Pizl (2009), collected in urban parkland of Brno, Czech Republic. Finally, Xie & Rota (1999), re-investigating the type series of *M. hoffbaueri*, found a continuous row of chaetae from II to XI on one side in one of the type specimens, so this variation is also present in the type series. In the key to species of *Marionina*, *M. hoffbaueri* with lateral chaetae in all positions keys out between couplets 14 and 15 of the Guide (p. 104), and we suggest the following adjustments:

14* Pharyngeal glands 3 pairs, dorsally connected in IV and V, separate in VI **14A**

14A Oesophageal appendages present ventro-laterally in IV, paired, hollow, with wide lumen; see 12 .... *Marionina hoffbaueri* Möller, 1971

14A* Oesophageal appendages absent **15**

The two additional finds of the species now circumscribe a Central European area of distribution.

4.5. *Achaeta hallensis* Möller, 1976

According to Schlaghamerský (2010: 160), the pattern of epidermal gland cells in *Achaeta hallensis* is more variable than indicated (Guide p. 66 couplet 6; p. 67 Fig. 26C). There may be an additional pair of epidermal gland cells, either dorsally or ventrally.

4.6. *Achaeta bifollicula* Chalupský, 1992 **and *A. urbana*** Heck &Römbke, 1991

Both species are very similar. *A. bifollicula* is distingiushed by two dorsal pyriform glands per segment (four in *A. urbana*, two dorsal and two ventral) and by peculiar knob-like bodies in front of and behind the spermathecal ectal pore (not described in *A. urbana*). We reinvestigated the type series of *A. urbana* (deposited at the Zoological Museum Hamburg, Cat. Ns. ZIM OL 13526, 13527) and found that these peculiar bodies are also present in the type series of *A. urbana*, which leaves the ventral row of pyriform glands as the only distinguishing trait. The types of *A. urbana* are ill-preserved, and ventral pyriform glands were difficult to distinguish and not seen in all specimens. We therefore recommend to check this trait carefully before assigning *Achaeta* specimens to either *bifollicula* or *urbana*, and to preserve reference specimens. It seems possible that both species are synonymous, in which case the name *urbana* would have priority.

5. Further amendments, corrections, or comments

P. 21, Fig. 8, and p. 23, Fig. 9: Figures 8 and 9 give schematic representations of the taxon-specific distribution and shape of oesophageal appendages and intestinal diverticula. Pharyngeal glands are shown as well but here only the most common constellation is represented, i.e. some species may differ from the scheme, especially regarding the dorsal connection of the dorsal lobes. Besides, some errors need rectification:

Fig 8C *Fridericia*: Dorsal lobes are shown separate in IV and V and united in VI. This constellation does not occur. Dorsal lobes are either (1) all separate dorsally (e.g. *F. striata*), (2) all united dorsally (e.g. *F. maculata*) or (3) lobes of IV and V are united while those of VI are separate (many species).

Fig. 8D bottom, *Achaeta*: The dorsal pharyngeal gland lobe in V is missing.

Fig. 9G top, *Hemienchytraeus*: the dorsal pharyngeal gland lobes should be united in IV and V, and not separate.

P. 35: *Achaeta parva* Nielsen & Christensen, 1959 has not been placed in alphabetical order.

P. 37 and P. 88: Order of synonymy should be reversed, with *Enchytraeus crypticus* Westheide & Graefe, 1992 as junior synonym and *Enchytraeus variatus* Bouguenec & Giani, 1987 sensu lato (!) as senior synonym.

P. 40: "*Henlea perpusilla*" is listed twice; the entry after *Henlea nasuta* should be eliminated.

P. 43, couplet 16: In the key to genera, the only way to get to *Fridericia* is by recognizing the presence of two types of coelomocytes, mucocytes and lenticytes. However, in some species mucocytes may be almost absent (*F. profundicola*) or difficult to see (*F. benti*, *F. paroniana*), or lenticytes may be almost absent (*F. minor*, *F. nix*) (see Schmelz 2003: 40). In order to avoid larger changes, we suggest that the reader who arrives at 16* first studies number 17 before continuing with number 18.

P. 46, Tab. 1: Oesophageal appendages in *Guaranidrilus* are not situated in VII but in VI, as shown in Figs. 9H, 29B.

P. 57, couplet 4*: Not "Distal half of mid-part of vas deferens inflated ..." but "Distal half or mid-part of vas deferens ..."

P. 66, couplets 2, 3 and 3*: Add at the end: "or spermathecae absent"

P. 74, The two forms of *A. danica* dealt with in the last paragraph are distinguished by other characters than the ones mentioned (U. Graefe, pers. com.).

P. 88, couplet 15, 15*: Add to 15, as first distinguishing character: "Mostly 2 chaetae in ventral preclitellar bundles". Add to 15*, as first distinguishing character: "Three chaetae in ventral preclitellar bundles"

P. 96, couplet 6*: Insert "35B bottom" after "Fig. 36G". The nephridium shown in Fig. 35B is that of *Lumbricillus arenarius*, quite different from the the upper one, which is typical of the rest of the *Lumbricillus* species dealt with here. Erséus et al (2010), in a molecular phylogeny of Enchytraeidae, found *L. arenarius* placed in a clade different from the other *Lumbricillus* species; they suggest that it should be removed from the genus.

P. 106, paragraph on *Marionina riparia*: Not Timm (2005) but Timm (2007).

P. 116, remarks on *Henlea puteana*: Friend's record of this doubtful species with two pairs of spermathecae is not completely without morphological information, as stated in the Guide, but the details are insufficient and partly incongruent with the original description. - Apart from *Henlea udei*, there is one more species in *Henlea* with a dorsal blood vessel origin in X (and not in VIII or IX as in all the other species). Piper et al. (1982) and Christensen & Dózsa-Farkas (1999) found *Henlea diverticulata* Cejka, 1912 close to the original locality in North-Eastern Siberia, and they observed a dorsal blood vessel origin in X in their specimens. However, according to the original description (Cejka 1912), the origin is in VIII. Piper et al (1982) suggest erroneous observation in the original description, but the subsequent records may also refer to a new species.

P. 118, chapter heading of *Buchholzia*: Year of publication of the generic name is 1886, not 1887.

P. 119, couplet 1: For unequivocal identification of all specimens of asexually reproducing *Buchholzia appendiculata*, the couplet should run as follows:

1 Intestinal diverticula present (Fig. 48E, F); in adults more than 4 chaetae in several ventral bundles (juveniles may have not more than 3 or 4) **2**

1* Intestinal diverticula absent (Fig. 48L); not more than 4 chaetae per bundle (check ventral bundles) **3**

P. 128, couplet 8: Not B4 but B5.

P. 131, couplet 14*: "... coelomo-mucocytes with pale or refractile vesicles at cell periphery ..." Comment: Mucocyte vesicles in *F. striata* are occasionally pale and not refractile, see Guide p. 129, Fig. 54E.

P. 136, bottom, description of *F. maculata*: Three different chaetal patterns are listed. The first one should be changed from "4 chaetae throughout" into "2-4 chaetae per bundle".

P. 156, couplet 5: There is a new record of *Fridericia digitata* Cognetti, 1901 in Erséus et al. (2010) from Sardinia, possibly close to the original locality. Actually, this is the first record after the original description. The species was put in synonymy with *F. galba* in Schmelz (2003), and we await a detailed redescription in order to include the species in the Guide.

P. 161, line 7: Delete "B *F. unisetosa*". This species is known only from China, not included in the key.

P. 170, References: Friend 1913b ("Some Jersey oligochaets") should be eliminated. Citations in the text of "Friend, 1913a" or "Friend, 1913b" should be reduced to "Friend, 1913" (pp. 82, 130, 154, 158, 169).

6. Page index to species in Schmelz & Collado (2010)

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An updated checklist of currently accepted species of Enchytraeidae (Oligochaeta, Annelida)

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Abstract

An updated checklist of all currently accepted species of Enchytraeidae (Oligochaeta, Clitellata, Annelida) is presented. The list comprises 676 species, which add up to 715 species-group taxa when subspecies and controversial species are included. Species-richest genera are *Fridericia* (99 species) and *Marionina* (96 species), followed by *Lumbricillus* (80 species), *Mesenchytraeus* (76 species), and *Grania* (71 species). Six genera are monospecific. Given the generally poor knowledge of Enchytraeidae in most regions on earth, these numbers most probably represent only a minute fraction of the actual diversity, but reasonable estimates as to the actual number of enchytraeid species are not possible at present. For reasons of homonymy, *Henlea dicksoni* (Nurminen, 1980) and *Henlea irkutensis* (Nurminen, 1980) are renamed into *Henlea dicksoniana* and *Henlea irkutiana*, respectively.

Keywords: Clitellata; taxonomy; species list

1. Introduction

Checklists of species are important for any kind of question or investigation related to biodiversity. With this paper we present a checklist of currently accepted species of Enchytraeidae at a global scale. Lists of new taxa of enchytraeids have been an integral part of the 'Newsletters on Enchytraeidae' from the very beginning. Actually the series of Newsletters started (Graefe & Römbke 1985) with a compilation of names and references of enchytraeid taxa described after Nielsen and Christensen's seminal monography on enchytraeids (Nielsen & Christensen 1959, 1961, 1963). Ulfert Graefe, Jörg Römbke, and Klára Dózsa-Farkas were the contributors that kept the lists up-to-date in subsequent issues of the Newsletter; later on we continued the traditon. In the near future, we will shift to a web-based presentation of lists and commented bibliographies, covering all available species-group names of enchytraeids (http://www.enchytraeids.org).

Apart from the Newsletter issues, a global checklist of enchytraeid species has been compiled by Nakamura (2000, updated in Nozaki & Nakamura 2005). The contribution has a more detailed bibliography than the one presented here, with page and figure numbers specified to the original descriptions, but several taxon names are misspelled and the revisionary literature is represented incompletely. However, we recommend considering this source as well. Other updated lists of enchytraeid species are regional (Schmelz & Collado 2010, see also http://www.faunaeur.org) or focus on names rather than species (ION, index of organism names: http://www.organismnames.com).

In this paper, 'accepted' means that the species has not been questioned, rejected, or put in junior synonymy. In the best of all cases, it has been confirmed in several revisionary studies carried out by different authors. However, very often 'accepted' means simply that the species has never been revised at all, due to the general scarcity of revisionary studies in enchytraeids, or because the description is of recent origin. 'Accepted' means roughly the same as 'valid', but we prefer to use 'accepted' to avoid confusion with valid/invalid names in nomenclatural terminology.

Species considered as doubtful by any author are omitted, unless stated otherwise, or unless revision or redescription has led to re-acceptance. If nothing but the generic affiliation is considered doubtful ("*species incertae sedis*"), these species are included as well, together with a comment. Recent taxonomic opinions are included in the list, because they may not be shared by all researchers; diverging taxonomic opinions are presented as well. Opinions in Schmelz (2003) are listed only, when they concern frequently cited names, or when they have been questioned subsequently. Species names preceded by an asterisk are rejected here, but they may be controversial, i.e. accepted by other authors. Species names in square brackets should not be counted. Species names without asterisk or square brackets are those accepted by us. Synonyms and other taxonomic details such as type species and type specimens are omitted. A complete taxonomic and nomenclatural account of all enchytraeid species is in preparation (http://www.enchytraeids.org, forthcoming.)

In the case of subspecies (e.g., *Mesenchytraeus solifugus rainierensis*) we also list the nomino-typical subspecies (e.g., *Mesenchytraeus solifugus solifugus*), although they were rarely made explicit in the literature.

Regarding names, combinations, and signatures (i.e. author plus year of publication), we made the following changes:

1) We accept *Marionina litterata* (Hesse, 1893), a doubtful species according to Nielsen & Christensen (1959). A type-based redescription is in preparation.
2) *Enchytraeus christofferseni* is placed in *Fridericia*, as a result of the synonymy of *Timmodrilus* with *Fridericia* in Schmelz et al. (2005).
3) We agree with Tynen & Coates (1991) that *Punahenlea* Nurminen, 1980 should not be maintained as a genus separate from *Henlea* Michaelsen, 1889. The synonymy of both genera, however, creates two pairs of homonyms: (1) *Henlea irkutensis* Burov, 1929 and *Henlea irkutensis* (Nurminen, 1980) (originally *Punahenlea*), (2) *Henlea dicksoni* (Eisen, 1878) (originally "*dicksonii*" but now to be spelled "*dicksoni*" following prevailing usage, see ICZN 1999, Art. 33.3.1.; 58.14) and *Henlea dicksoni* (Nurminen, 1980) (originally *Punahenlea*). Here we give new names to Nurminen's species: *Henlea irkutiana* is the replacement name for *Henlea irkutensis* (Nurminen, 1980), and *Henlea dicksoniana* is the replacement name for *Henlea dicksoni* (Nurminen, 1980). *Henlea dicksoniana* and *Henlea dicksoni* (Eisen, 1878) are excluded from our checklist, because *Henlea dicksoniana* (Nurminen's *Punahenlea dicksoni*) has been synonymized with *H. tolli* Michaelsen by Christensen & Dózsa-Farkas (1999), and *Henlea dicksoni* (Eisen) has been questioned ("species dubia") by Nielsen & Christensen (1959).
4) We believe that the synonymies of *Henlea ochracea* (Eisen, 1878) in Nurminen (1973b) need reappraisal, therefore the junior synonyms are listed completely.
5) The ending in the species name of *Bryodrilus arctica* has been accomodated to *Bryodrilus arcticus*.
6) In "*Buchholzia* Michaelsen, 1887" the year is erroneous. The genus was erected one year earlier (Michaelsen 1886a: "Über Chylusgefäβsysteme bei Enchytraeiden"). The error occurs in Nielsen & Christensen (1959) and has been repeated since then down to Schmelz & Collado (2010). Michaelsen 1887 ("Enchytraeiden-Studien") is a different publication (see references).

Furthermore, there is some uncertainty regarding the year of publication of some species names, probably because papers were actually ("effectively") published the year after the official date of publication. According to the ICZN, the effective year of publication is to be cited with a species name. However, when the effective year is unknown, the official year is to be cited (ICZN 1999, Art. 21.2). Since in most cases we do not know the effective year of publication, we adhere to the official year, unless stated otherwise. A known and documented difference between effective and official year or publication is indicated here as, for example: Vejdovský 1878 ("1877").

Finally, we do not include *Enchytraeus tylidis* Barnard, 1932, a species name that has passed unnoticed until most recently, when data of the Zoological Records were made available online (see http://www.organismnames.com). The species is described in a monograph on South African crustaceans. Worms were found commensalic on pleopods of the beach sand isopod *Tylos granulatus* near Cape Town, South Africa (Barnard 1932). The known details are insufficient at the species and genus level, but they suggest that the worms are enchytraeids:

body colour whitish, body length 2.5-3 mm, three simple chaetae in four bundles per segment. These details are repeated in Kensley (1974), who cites the original description in Barnard (1932) and gives data on host-specifity and number of worms per host. Kensley's misspelling of the species name as *tylidus* has continued in the Zoological Records. More information on morphology and life-style of these oligochaetes would certainly be desirable.

The following new taxa or taxon names, published in 2010 and 2011 after the most recent list of new enchytraeid taxa (Schmelz & Collado 2009) are included in the list:

Genus level:
Xetadrilus Schmelz, Collado & Römbke, 2011

Species level:
Cernosvitoviella longiducta Dumnicka, 2010
Cognettia valeriae Dumnicka, 2010
Enchytraeus dichaetus Schmelz & Collado, 2010
Guaranidrilus andreolii Schmelz, Collado & Römbke, 2011
Guaranidrilus cingulatus Schmelz, Collado & Römbke, 2011
Guaranidrilus hoeferi Schmelz, Collado & Römbke, 2011
Hemienchytraeus jeonjuensis Dózsa-Farkas & Hong, 2010
Hemienchytraeus koreanus Dózsa-Farkas & Hong, 2010
Hemienchytraeus quadratus Dózsa-Farkas & Hong, 2010
Marionina biwaensis Torii, 2012
Mesenchytraeus megachaetus Shen, Chen & Xie, 2011
Mesenchytraeus anisodiverticulus Shen, Chen & Xie, 2012
Xetadrilus aphanus Schmelz, Collado & Römbke, 2011
Xetadrilus fabryi Schmelz, Collado & Römbke, 2011
Xetadrilus maacki Schmelz, Collado & Römbke, 2011

2. Checklist of enchytraeid species

2.1. *Achaeta* Vejdovský, 1878 ("1877")

aberrans Nielsen & Christensen, 1961
abulba Graefe, 1989
affinis Nielsen & Christensen, 1959
afolliculata Sesma & Dózsa-Farkas, 1993
antefolliculata Dózsa-Farkas & Boros, 2005
**becki* Schmelz & Collado, 2005a (Schmelz et al. 2008: jun. syn. of *A. neotropica* ?)
bibulba Graefe, 1989
bifollicula Chalpuský, 1992
bohemica (Vejdovský, 1879b)
brevivasa Graefe, 1980
bulbosa Nielsen & Christensen, 1961
camerani (Cognetti, 1899)
christenseni Prabhoo, 1966
danica Nielsen & Christensen, 1959
diddeni Graefe, 2007
eiseni Vejdovský, 1878 ("1877")
**etrusca* Rota, 1995 (Schmelz & Collado 2010: jun. syn. of *A. iberica*)
gigantea Dózsa-Farkas, 2000
hallensis Möller, 1976
hanagarthi Schmelz, 2008
iberica Graefe, 1989
indica Prabhoo, 1960
iridescens Christoffersen, 1979
littoralis Lasserre, 1968
maorica Benham, 1903
macrocyta Christensen & Dózsa-Farkas, 1999
matritensis Sesma & Dózsa-Farkas, 1993
**microcosmi* Heck & Römbke, 1991 (Schmelz & Collado 2010: jun. syn. of *A. bohemica*)
minima Southern, 1907
neotropica Černosvitov, 1937a
nielseni Prabhoo, 1960
nurmineni Dash & Thambi, 1978
pannonica Graefe, 1989
paranensis Schmelz, 2008
parva Nielsen & Christensen, 1961
**petseri* Dózsa-Farkas, 1998 (Schmelz et al. 2005: jun. syn. of *A. pannonica*)
pigmentosa Christensen & Dózsa-Farkas, 2007
piti Bittencourt, 1974
segmentata Prabhoo, 1966
seminalis Kasprzak, 1972a
**silvatica* Nurminen, 1973a (Schmelz et al 2008: species inquirenda)
singularis Schmelz, 2008
unibulba Graefe, Christensen & Dózsa-Farkas, 2005
urbana Heck & Römbke, 1991
vesiculata Nielsen & Christensen, 1959

2.2. *Aspidodrilus* Baylis, 1914

kelsalli Baylis, 1914

2.3. *Barbidrilus* Loden & Locy, 1980

paucisetus Loden & Locy, 1980

2.4. *Bryodrilus* Ude, 1892

archipelagicus Christensen & Dózsa-Farkas, 2006
arcticus (Bell, 1962)
borealis Cejka, 1912
cejkai Nurminen, 1980
chernovi Nurminen, 1980
cockerelli (Bell, 1947)
diverticulatus Černosvitov, 1929
ehlersi Ude, 1892
[*ehlersi glandulosus* Dózsa-Farkas, 1990 (Schmelz & Collado 2010: elevated to species rank)]
fuscistriatus Chen & Xie, 2006
glandulosus Dózsa-Farkas, 1990
librus (Nielsen & Christensen, 1959) (Schmelz & Collado 2010: ex *Marionina*)
longifistulatus Xie, Liang & Wang, 2000d
macrotheca Xie, Liang & Wang, 2000d
novaescotiae Bell, 1962
parvus Nurminen, 1970a
tunicatus Dózsa-Farkas & Christensen, 2002

2.5. Buchholzia Michaelsen, 1886a

africana Černosvitov, 1933
appendiculata (Buchholz, 1862)
fallax Michaelsen, 1887
simplex Nielsen & Christensen, 1963
subterranea (Černosvitov, 1937b) (Nielsen & Christensen 1959: doubtful species. Schmelz & Collado 2010: accepted)

2.6. *Cernosvitoviella* Nielsen & Christensen, 1959

aggtelekiensis Dózsa-Farkas, 1970
ampullax Klungland & Abrahamsen, 1981
atrata (Bretscher, 1903)
briganta Springett, 1969
bulboducta Martínez-Ansemil & Collado, 1996
carpatica Nielsen & Christensen, 1959
celere Nurminen, 1973a
christenseni Dash, 1970
crassoductus Dózsa-Farkas, 1990
[*estaragniensis* Giani, 1979 (Healy 1980, Schmelz & Collado 2010: jun. syn. of *H. palustris*)]
**goodhui* Healy, 1975 (Schmelz & Collado 2010: jun. syn. of *C. aggtelekiensis*)
immota (Knöllner, 1935b)
longiducta Dumnicka, 2010
**microtheca* Rota & Healy, 1999 (Schmelz & Collado 2010: jun. syn. of *C. atrata*)
minor Dózsa-Farkas, 1990
omodeoi Rota, 1995
palustris Healy, 1979b
parviseta Gadzinska, 1974
pensau Timm, 1994
pusilla Nurminen, 1973b
sphaerotheca Healy, 1975
tatrensis (Kowalewski, 1916)
tridentina Dumnicka, 2004

2.7. *Christensenidrilus* Dózsa-Farkas & Convey, 1998

blocki Dózsa-Farkas & Convey, 1997
[*georgiana* (Michaelsen, 1888b) (Rota et al. 2008, Schmelz & Collado 2008: *Marionina*)]

2.8. *Cognettia* Nielsen & Christensen, 1959

**anomala* (Černosvitov, 1928b) (Schmelz & Collado 2010: jun. syn. of *C. sphagnetorum*. Dumnicka 2010: accepted species)
bisetosa Christensen & Dózsa-Farkas, 1999
clarae Bauer, 1993
cognettii (Issel, 1905c)
floridae Healy, 1996b
glandulosa (Michaelsen, 1888a)
hayachinensis Nakamura, 2001
hibernica Healy, 1975
lapponica Nurminen, 1965b
**paxi* (Moszyński, 1938) (Schmelz & Collado 2010: jun. syn. of *Cog. sphagnetorum*. Dumnicka 2010: accepted species)
piperi Christensen & Dózsa-Farkas, 1999
quadrosetosa Christensen & Dózsa-Farkas, 1999
sphagnetorum (Vejdovský, 1878 ("1877")
valeriae Dumnicka, 2010
zicsii Dózsa-Farkas, 1989

2.9. *Enchylea* Nielsen & Christensen, 1963

heteroducta Nielsen & Christensen, 1963

2.10. *Enchytraeina* von Bülow, 1957 (Kasprzak 1984: doubtful genus)

lutheri von Bülow, 1957

2.11. *Enchytraeus* Henle, 1837

albidus Henle, 1837
athecatus Wang, Xie & Liang, 1999
australis Stephenson, 1932c
berhampurosus Dash & Thambi, 1978
bigeminus Nielsen & Christensen, 1963

bohemicus Dumnicka, 1996
bonus Shurova, 1978
buchholzi Vejdovský, 1879a
bulbosus Nielsen & Christensen, 1963
capitatus von Bülow, 1957
carcinophilus Baylis, 1915a
chaoyangensis Xie, Liang & Wang, 2000a
christenseni Dózsa-Farkas, 1992
citrinus Eisen, 1904 (Nielsen & Christensen 1959: *Marionina*. Coates & Ellis 1981: generically indeterminate)
coronatus Nielsen & Christensen, 1959
crypticus Westheide & Graefe, 1992
dichaetus Schmelz & Collado, 2010 (new rank and new name for *E. christenseni* ssp. *bisetosus* Rota & Healy, 1994)
doerjesi Westheide & Graefe, 1992
dominicae Dumnicka, 1976
dudichi Dózsa-Farkas, 1995
**florentinus* Bell, 1947 (Schmelz & Collado 2010: jun. syn. of *E. buchholzi* s.l.)
fonteinensis Michaelsen, 1933
**fragmentosus* Bell, 1959 (Schmelz et al. 2000: species inquirenda)
gillettensis Welch, 1914
globuliferus Nielsen & Christensen, 1963
gordioides Černosvitov, 1942 ("1941")
harurami Stephenson, 1914
indicus Stephenson, 1912
irregularis Nielsen & Christensen, 1961
issykkulensis Hrabě, 1935
japonensis Nakamura, 1993
kincaidi Eisen, 1904
lacteus Nielsen & Christensen, 1961
liefdeensis Stephenson, 1924
luxuriosus Schmelz & Collado, 1999
mariae Kasprzak, 1973
mediterraneus Michaelsen, 1926
metlakatlensis Eisen, 1904 (Nielsen & Christensen 1959: *Marionina*. Coates & Ellis 1981: generically indeterminate)
multiannulatus Altman, 1936
multiannulatoides Altman, 1936
norvegicus Abrahamsen, 1969
parasiticus Baylis, 1915b
platys Semernoy, 1972
**polonicus* Dumnicka, 1977 (Schmelz & Collado 2010: jun. syn. of *E. buchholzi* s.l.)
przewalskii Hrabě, 1935
rupus Coates, 1980a
simulans Benham, 1903
subitus Nurminen, 1970a
syracussus (Dash & Mitchell, 1981)
thomasi Rodriguez & Giani, 1986
variatus Bougenec & Giani, 1987
varithecatus Bougenec & Giani, 1987

2.12. ***Enchytronia*** Nielsen & Christensen, 1959

annulata Nielsen & Christensen, 1959
baloghi Dózsa-Farkas, 1988b
christenseni Dózsa-Farkas, 1970
hellenica Dumnicka, 1979
longispermatheca Chalupský, 1991
**minor* Möller, 1971 (Coates 1989, Schmelz & Collado 2010: jun. syn. of *E. parva*)
oligosetosa Sesma & Dózsa-Farkas, 1993
parva Nielsen & Christensen, 1959
pratensis Chalpuský, 1994

2.13. ***Fridericia*** Michaelsen, 1889

agilis Smith, 1895
agricola Moore, 1895
alata Nielsen & Christensen, 1959
anomala Košel, 1975
**antensteineri* Bauer, 1996 (Schmelz 2003: doubtful. Bauer 2004: accepted)
argillae Schmelz, 2003
armenica Schmelz, 2003
asymmetricoides Kasprzak, 1972b
aurita Issel, 1905c
auritoides Schmelz, 2003
baskini Černosvitov, 1937c
benti Schmelz, 2002
berkeleyensis Bell, 1936
berninii Dózsa-Farkas, 1988a
bisetosa (Levinsen, 1884)
brachiata Rota, 1994
bretscheri Southern, 1907
brunensis Schlaghamerský, 2007
bubalus Sesma & Dózsa-Farkas, 1993
[*bulbosa* (Rosa, 1887). Schmelz 2003: nomen dubium]
bulboides Nielsen & Christensen, 1959
callosa (Eisen, 1878)
[*caprensis* Bell, 1947. Schmelz 2003: jun. syn. of *F. pretoriana*]
chongqingensis Xie, Liang & Wang, 1999
christeri Rota & Healy, 1999
**christiani* Bauer, 1998 (Schmelz 2003: doubtful. Bauer 2004: accepted)
christofferseni (Righi, 1975)
composti Schmelz, 2003
conculcata Dózsa-Farkas, 1986
connata Bretscher, 1902
crassiductata Dózsa-Farkas & Cech, 2006
cusanica Schmelz, 2003
cylindrica Springett, 1971

deformis Möller, 1971
dianchiensis Chen & Xie, 2008
**digitata* Cognetti, 1901 (Schmelz 2003: jun. syn. of *F.galba*. Erséus et al. 2010: accepted species)
discifera Healy, 1975
douglasensis Welch, 1914b
dozsae Schmelz, 2003
dura (Eisen, 1879)
[*eiseni* Dózsa-Farkas, 2005 (Schmelz & Collado 2010: jun. syn. of *F. ratzeli*)]
firma Smith & Welch, 1913
florentina Dequal, 1914
galba (Hoffmeister, 1843)
gamotheca Issel, 1905a
gigantea Dequal, 1912
glandifera Friend, 1913
**glandulosa* Southern, 1907 (Schmelz 2003: jun. syn. of *F.galba*. Rota, Zalesskaya et al. 2003: accepted species)
globuligera Rota, 1995
[*gracilis* von Bülow, 1957. Schmelz 2003: jun. syn. of *F.minor* Friend, 1913]
granosa Schmelz, 2003
healyae Schmelz, 2003
hegemon (Vejdovský, 1878 ("1877"))
heliota Zalesskaya, Petushkov & Rodionova, 1990
humicola Bretscher, 1900
ilvana Issel, 1905b
isseli Rota, 1994
lacii Dózsa-Farkas, 2009
larix Schmelz & Collado, 2005b
[*leydigi* (Vejdovský, 1878). Schmelz 2003: nomen dubium]
lenta Schmelz, 2003
liangi Chen & Xie, 2009
loretensis Schmelz, 2003
losangelensis Bell, 1936
maculata Issel, 1905c
maculatiformis Dózsa-Farkas, 1972
magna Friend, 1899
minor Friend, 1913
monochaeta Rota, 1995
monopera Cognetti, 1903b
montafonensis Schmelz, 1998
multisegmentata Wang, Xie & Liang, 1999
nanningensis Xie, Liang & Wang, 2001
nemoralis Nurminen, 1970b
nielseni Möller, 1971
nix Rota, 1995
oconeensis Welch, 1914b
omeri Stephenson, 1932a
parasitica Černosvitov, 1928a
parathalassia Schmelz, 2002
paraunisetosa Xie, Liang & Wang, 2000c
paroniana Issel, 1904
peregrinabunda Michaelsen, 1913a
perrieri (Vejdovský, 1878)
pretoriana Stephenson, 1930
profundicola Dózsa-Farkas, 1991b
pyrenaica Giani, 1979
ratzeli (Eisen, 1872)
reducata Dózsa-Farkas, 1974
regularis Nielsen & Christensen, 1959
**renatae* Möller, 1971 (Schmelz 2003: jun. syn. of *F. maculata*. Erséus et al. 2010: accepted species)
rendsinata Dózsa-Farkas, 1972
sardorum Cognetti, 1901
schmelzi Cech & Dózsa-Farkas, 2005
semisetosa Dózsa-Farkas, 1970
sima Welch, 1914b
singula Nielsen & Christensen, 1961
**sohlenii* Rota, Healy & Erséus, 1998 (Schmelz 2003, Schmelz & Collado 2010: jun. syn. of *F. cylindrica*. Erséus et al. 2005, 2010: synonymy not accepted)
stephensoni Moszyński, 1933
strenua Rota, 1995
striata (Levinsen, 1884)
sylvatica Healy, 1979b
terrarossae Sesma & Dózsa-Farkas, 1993
tuberosa Rota, 1995
tubulosa Dózsa-Farkas, 1972
ulrikae Rota & Healy, 1999
unisetosa Xie, Liang & Wang, 2000c
viridula Issel, 1904
vixdiverticulata Sesma & Dózsa-Farkas, 1993
waldenstroemi Rota & Healy, 1999

2.14. ***Grania*** Southern, 1913

acanthochaeta Rota & Erséus, 1996
algida Rota & Erséus, 1996
alliata Coates & Stacey, 1993
americana Kennedy, 1966
angustinasus Rota & Erséus, 1996
antarctica Rota & Erséus, 1996
aquitana Rota & Erséus, 2003
ascophora Coates, 1990
atlantica Coates & Erséus, 1985
bermudensis Erséus & Lasserre, 1976
breviductus De Wit, Rota & Erséus, 2009
bykane Coates, 1990
canaria Rota & Erséus, 2003
carchinii Rota & Erséus, 1996
cinctura De Wit & Erséus, 2007

colorata De Wit, Rota & Erséus, 2009
conjuncta Coates & Stacey, 1993
crassiducta Coates 1990
curta De Wit & Erséus, 2007
darwinensis (Coates & Stacey, 1997)
dolichura Rota & Erséus, 2000
ersei Coates, 1990
eurystila Coates & Stacey, 1997
fiscellata De Wit & Erséus, 2007
fortunata Rota & Erséus, 2003
fustata De Wit & Erséus, 2007
galbina De Wit & Erséus, 2007
hastula Coates, 1990
hirsuticauda Rota & Erséus, 1996
homochaeta De Wit, Rota & Erséus, 2009
hongkongensis Erséus, 1990
hylae Locke & Coates, 1999
hyperoadenia Coates, 1990
incerta Coates & Erséus, 1980
inermis Erséus, 1990
integra Coates & Stacey, 1997
lasserrei Rota & Erséus, 1997
laxarta Locke & Coates, 1999
levis Coates & Erséus, 1985
longiducta Erséus & Lasserre, 1976
longistyla Coates & Stacey, 1993
macrochaeta (Pierantoni, 1901)
maricola Southern, 1913
mauretanica Rota & Erséus, 2003
mira Locke & Coates, 1998
monochaeta (Michaelsen, 1888b)
monospermatheca Erséus & Lasserre, 1976
novacaledonia De Wit & Erséus, 2007
ocarina Rota, Erséus & Wang, 2003
ovitheca Erséus, 1977
pacifica Shurova, 1979
papillata De Wit & Erséus, 2007
papillinasus Rota & Erséus, 2003
parvitheca Erséus, 1980
paucispina (Eisen, 1904)
postclitellochaeta (Knöllner, 1935a)
principissae (Michaelsen, 1907)
pusilla Erséus, 1974
quaerens Rota, Wang & Erséus, 2007
reducta Coates & Erséus, 1985
regina De Wit, Rota & Erséus, 2009
roscoffensis Lasserre, 1967
sperantia Rota, Wang & Erséus, 2007
stephensoniana Rota & Erséus, 1997
stilifera Erséus, 1990
tasmaniae Rota & Erséus, 2000
torosa Rota & Erséus, 2003
trichaeta Jamieson, 1977
vacivasa Coates & Stacey, 1993
variochaeta Erséus & Lasserre, 1976
vikinga Rota & Erséus, 2003

2.15. *Guaranidrilus* Černosvitov, 1937a

andreolii Schmelz, Collado & Römbke, 2011
athecatus Christoffersen, 1977
atlanticus Christoffersen, 1977
cernosvitovi Healy, 1979a
cingulatus Schmelz, Collado & Römbke, 2011
columbianus Michaelsen, 1913b
europeus Healy, 1979a
finni Christoffersen, 1977
glandulosus Černosvitov, 1937
hoeferi Schmelz, Collado & Römbke, 2011
joanae Christoffersen, 1977
lamottei Omodeo, 1958
marquesi Schmelz, Collado & Römbke, 2011
mboi Righi, 1975
oiepe Righi, 1974b
oregonensis Coates & Diaz, 1988
rarus Černosvitov, 1937
sawayai Righi, 1973 (Coates & Diaz 1988: jun. syn. of *G. rarus*?)

2.16. *Hemienchytraeus* Černosvitov, 1934b

africanus Černosvitov, 1935
bifurcatus Nielsen & Christensen, 1959
brachythecus Xie, Wang & Liang, 1999
cipoensis Righi, 1973
csuzdii Dózsa-Farkas, 1989
guineanus Omodeo, 1958
inversus Omodeo, 1958
jeonjuensis Dózsa-Farkas & Hong, 2010
khallikotosus Dash & Thambi, 1978
koreanus Dózsa-Farkas & Hong, 2010
loksai Dózsa-Farkas, 1989
makusi Righi, 1988
mauriliae Righi, 1981a
patricii Schmelz & Römbke, 2005
planisetosus Xie, Wang & Liang, 1999
quadratus Dózsa-Farkas & Hong, 2010
rixae Righi, 1974b
shirensis Bell, 1954
siljae Schmelz & Römbke, 2005
solimoensis Righi, 1978
stephensoni (Cognetti, 1927)
tanjae Schmelz & Römbke, 2005
theae Prabhoo, 1960

2.17. *Hemifridericia* Nielsen & Christensen, 1959

bivesiculata Christensen & Dózsa-Farkas, 2006

parva Nielsen & Christensen, 1959
varanensis Lal, Singh & Prasad, 1981

2.18. *Henlea* Michaelsen, 1889

adiverticulata Christensen & Dózsa-Farkas, 1999
africana Bell, 1954
andreae Rodriguez & Giani, 1986
**arctica* Welch, 1919b (Nurminen 1973b: jun. syn. of *H. ochracea*)
**birulae* (Cejka, 1910) (Nurminen 1973b: jun. syn. of *H. ochracea*)
californica Eisen, 1904
californica californica Eisen, 1904
californica monticola Eisen, 1904
conchifera Christensen & Dózsa-Farkas, 1999
diverticulata Cejka, 1912
ehrhorni Eisen, 1904
eiseni Bell, 1942
ghilarovi Nurminen, 1980
glabra Altman, 1936
glandulifera Nurminen, 1970a
guatemalae Eisen, 1904
helenae Eisen, 1904
heleotropha Stephenson, 1922
irkutensis Burov, 1929
irkutiana replacement name for *Henlea irkutensis* (Nurminen, 1980) non Burov, 1929
jutlandica Nielsen & Christensen, 1959
**moderata* Welch, 1914b (Nurminen 1973b: jun. syn. of *H. ochracea*)
moderatoidea Altman, 1936
montana Rota, 1994
nasuta (Eisen, 1878)
nivea Černosvitov, 1929
ochracea (Eisen, 1878)
palmeni (Nurminen, 1980)
perpusilla Friend, 1911 augm. Černosvitov 1937c
**pertoserica* Popchenko, 1988 (Schmelz & Collado 2010: doubtful species)
**puteana* (Vejdovský, 1878) (Schmelz & Collado 2010: doubtful species)
scharffi Southern, 1910
**sibirica* (Cejka, 1910) (Nurminen 1973b: jun. syn. of *H. ochracea*)
similis Nielsen & Christensen, 1959
taimyrensis (Nurminen, 1980)
**tenella* (Eisen, 1878) (Nurminen 1973b: jun. syn. of *H. ochracea*)
tolli Michaelsen, 1901
**tubulifera* Welch, 1914a (Nurminen 1973b: jun. syn. of *H. ochracea*)
udei (Eisen, 1904)
urbanensis Welch, 1914b
ventriculosa (d'Udekem, 1854)
welchi Bell, 1942
yukonensis Tynen & Coates, 1991

2.19. *Isosetosa* Zhi-Fang, De-Ning & Jian-Ming, 1989 (Schmelz 2003: genus doubtful)

**minxianensis* Zhi-Fang, De-Ning & Jian-Ming, 1989 (Schmelz 2003: species doubtful)

2.20. *Lumbricillus* Ørsted, 1844

aegialites Stephenson, 1922
aestuum (Stephenson, 1932c)
alaricus Shurova, 1974
algensis Erséus, 1977
americanus (Ude, 1896)
annulatus Eisen, 1904
antarcticus Stephenson, 1932c
arenarius (Michaelsen, 1889)
balticus von Bülow, 1957
belli Tynen, 1969
benhami Stephenson, 1932c
brunoi Martínez-Ansemil, 1982
buelowi Nielsen & Christensen, 1959
charae (Tynen, 1970)
christenseni Tynen, 1966
colpites (Stephenson, 1932c)
corallinae Shurova, 1977
crymodes (Stephenson, 1922)
curtus Coates, 1981
dubius (Stephenson, 1911)
eltoni (Stephenson, 1924)
enteromorphae (von Bülow, 1957)
eudioptus (von Bülow, 1955)
fennicus Nurminen, 1964
franciscanus Eisen, 1904
georgiensis Tynen, 1969
griseus (Stephenson, 1932c)
healyae Rodriguez & Rico, 2008
helgolandicus (Michaelsen, 1934)
horridus Finogenova, 1988
ignotus Shurova, 1977
imakus Nurminen, 1970a
immoderatus Finogenova, 1988
incisus Wang & Liang, 1997
insularis (Ude, 1896)
intricatus Finogenova, 1977
**kalatdlitus* Nurminen, 1970a (Coates & Ellis 1981: jun. syn. of *L. pagenstecheri*?)
kaloensis Nielsen & Christensen, 1959
kamtschatkanus (Michaelsen, 1929)
knoellneri Nielsen & Christensen, 1959
kurilensis Shurova, 1974
lentus Shurova, 1978

lineatus (Müller, 1774)
macquariensis Benham, 1905
macrothecatus Erséus, 1976a
magdalenae Nurminen, 1965a
mangeri (Michaelsen, 1914)
maritimus (Ude, 1896)
maximus (Michaelsen, 1888b)
  maximus maximus (Michaelsen, 1888b)
  maximus robinsoni Michaelsen, 1905a
minimus (Černosvitov, 1929)
minutus (Müller, 1776) (Nielsen & Christensen 1959: sp. dub.; Nurminen 1973c: accepted sensu Michaelsen 1911)
mirabilis Tynen, 1969
murmanicus Finogenova & Streltsov, 1978
muscicolus (Stephenson, 1924)
nielseni Nurminen, 1965a
niger Southern, 1909
nipponicus (Yamaguchi, 1937)
ochotensis Shurova, 1979
orientalis Shurova, 1974
pagenstecheri (Ratzel, 1868)
parabolus Shurova, 1978
parvus (Ude, 1896)
pinquis Shurova, 1977
pumilio Stephenson, 1932a
pygmaeus (Michaelsen, 1935)
pseudominutus Timm, 1988
qualicumensis Tynen, 1969
reynoldsoni Backlund, 1948
rivalis Levinsen, 1884
**rubidus* Finogenova & Streltsov, 1978 (Dózsa-Farkas 1992: jun. syn. of *L.. enteromorphae*)
rufulus Shurova, 1974
rupertensis Coates, 1981
rutilus Welch, 1914b
sadovskyi Marcus, 1965
santaeclarae Eisen, 1904
sapitus Shurova, 1979
scoticus Elmhirst & Stephenson, 1926
semifuscus (Claparède, 1861)
similis Shurova, 1977
taisiae Shurova, 1978
tenuis (Ude, 1896)
tsimpseanis Coates, 1981
tuba Stephenson, 1911
viridis Stephenson, 1911
werthi (Michaelsen, 1905a)

2.21. *Marionina* Michaelsen, 1890 (in Pfeffer 1890)

aberrans Finogenova, 1973
achaeta Hagen, 1954
aliger (Michaelsen, 1930)
antipodum (Benham, 1905)
appendiculata Nielsen & Christensen, 1959
arenaria Healy, 1979b
argentea (Michaelsen, 1889)
biwaensis Torii, 2012
brendae Rota, 1995
brevis Finogenova, 1972a
bulbosa Finogenova, 1994
cana Marcus, 1965
canadensis Dash, 1970
changbaishanensis Xie, Liang & Wang, 2000b
charlottensis Coates, 1980
clavata Nielsen & Christensen, 1961
coatesae Erséus, 1990
communis Nielsen & Christensen, 1959
diazi Coates & Erséus, 1985
dirksi Bell, 1942
ecuadoriensis Righi, 1981a
eleonorae Rota, 1995
elgonensis (Černosvitov, 1938)
elongata Lasserre, 1964
exigua Ude, 1896
falclandica Michaelsen, 1905b
filiformis Nielsen & Christensen, 1959
forbesae Smith & Welch, 1913
gabiae Healy & Coates, 1997
georgiana (Michaelsen, 1888b)
glandulifera Jansson, 1960
graefei Koβmagk-Stephan, 1983
hoffbaueri Möller, 1971
indica Dash & Thambi, 1978
insignis Ude, 1896
istriae Giere, 1974
kinangopensis (Černosvitov, 1938)
klaskisharum Coates, 1983
levitheca Erséus, 1990
[*libra* Nielsen & Christensen, 1959 (Schmelz & Collado 2010: transferred to *Bryodrilus*)]
limpida Shurova, 1979
litterata (Hesse, 1893)
macfadyeni Dash & Thambi, 1978
macgrathi Healy, 1996a
macrobulbi Christensen & Dózsa-Farkas, 1999
magnaglandulosa Nurminen, 1970b
magnifica Shurova, 1978
mandorae Healy & Coates, 1997
mangle Healy & Coates, 1997
mesopsamma Lasserre, 1964
mica Finogenova, 1972a
micula Finogenova, 1972b
miniampullacea Shurova, 1978
minutissima Healy, 1975

nea Marcus, 1965
neroutsensis Coates, 1980
nevisensis Righi & Kanner, 1979
nordica Christensen & Dózsa-Farkas, 1999
normani (Michaelsen, 1907)
oligosetosa Koβmagk-Stephan, 1983
paludis Healy, 1994
patua Righi, Ayres & Bittencourt, 1978
paucispina (Eisen, 1904)
pituca Righi, 1974a
preclitellochaeta Nielsen & Christensen, 1963
[*righiana* Xie & Rota, 2001 (Schmelz et al. 2011: transferred to *Xetadrilus*)]
riparia Bretscher, 1899
rubens Rota, 1995
sacculata Xie & Rota, 2001
schreiberi Righi, 1975
schrijversi Healy, 1997
scintillans Boros & Dózsa-Farkas, 2008
seminuda Xie & Rota, 2001
**serbui* Botea, 1984 (Schmelz & Collado 2010: jun. syn. of *Buchholzia simplex*)
sexdiverticulata Dózsa-Farkas, 2002
simillima Nielsen & Christensen, 1959
singula Ude, 1896
sinica Xie & Rota, 2001
sjaelandica Nielsen & Christensen, 1961
southerni Černosvitov, 1937d
spartinae Healy, 1994
spicula (Leuckart, 1847)
spongicola Rota & Manconi, 2004
subachaeta Shurova, 1979
sublitoralis Erséus, 1976b
subterranea (Knöllner, 1935b)
subtilis (Ude, 1896)
swedmarki Lasserre & Erséus, 1976
tica Righi, 1981b
transunita Coates, 1990
triplex Matamoros, Yildiz & Erséus, 2007
tumulicola Healy & Coates, 1997
ulstrupae Healy, 1996a
vancouverensis Coates, 1980
vesiculata Nielsen & Christensen, 1959
waltersi Healy, 1994
weilli Lasserre, 1964
welchi Lasserre, 1971

2.22. *Mesenchytraeus* Eisen, 1878

affinis Michaelsen, 1901
altus Welch, 1917
americanus Bell, 1942
anisodiverticulus Shen, Chen & Xie, 2012
antaeus Rota & Brinkhurst, 2000
arcticus Bell, 1962
argentatus Nurminen, 1973b
armatus (Levinsen, 1884)
  armatus armatus (Levinsen, 1884)
  armatus kananaskis Dash, 1970
asiaticus Eisen, 1904
atriaphorus Altman, 1936
beringensis Eisen, 1904
beumeri (Michaelsen, 1886b)
bungei Michaelsen, 1901
cejkai Cernosvitov, 1937d
celticus Southern, 1909
chaunus Piper, MacLean & Christensen, 1982
chromophorus Altman, 1936
crenobius Timm, 1994
diplobulbosus Bell, 1949
diverticulatus Piper, MacLean & Christensen, 1982
eastwoodi Eisen, 1904
eltoni Stephenson, 1925
falciformis Eisen, 1878
flavidus Michaelsen, 1887
flavus (Levinsen, 1884)
fontinalis Eisen, 1904
  fontinalis fontinalis Eisen, 1904
  fontinalis gracilis Eisen, 1904
franciscanus Eisen, 1904
[*franzi* Nurminen, 1977. Schmelz & Collado 2010: syn. of *Cognettia clarae*]
fuscus Eisen, 1904
  fuscus fuscus Eisen, 1904
  fuscus inermis Eisen, 1904
gaudens Cognetti, 1903a
gelidus Welch, 1916
glandulosus (Levinsen, 1884)
grandis Eisen, 1904
grebnitzkyi Michaelsen, 1901
groenlandicus Nielsen & Christensen, 1959
hamiltoni Healy, 1996b
harrimani Eisen, 1904
hydrius Welch, 1919a
johanseni Welch, 1919b
kincaidi Eisen, 1904
kontrimavichusi Piper, MacLean & Christensen, 1982
konyamensis Michaelsen, 1916
kuehnelti Dózsa-Farkas, 1991a
kuril Healy & Timm, 2000
lusitanicus Collado, Martínez-Ansemil & Giani, 1993
macnabi Bell, 1942
maculatus Eisen, 1904
magnus Altman, 1936
megachaetus Shen, Chen & Xie, 2011

melanocephalus Christensen & Dózsa-Farkas, 1999
minimus Altman, 1936
mirabilis Eisen, 1878
monochaetus Bretscher, 1900
monothecatus Bell, 1945
multispinus (Grube, 1851)
nanus Eisen, 1904
obscurus Eisen, 1904
ogloblini Černosvitov, 1928b
orcae Eisen, 1904
pedatus Eisen, 1904
pelicensis Issel, 1905c
penicillus Eisen, 1904
**primaevus* Eisen, 1878 (Nielsen & Christensen 1959: doubtful species; Piper et al. 1982: type species of genus)
rhithralis Healy & Fend, 2002
sanguineus Nielsen & Christensen, 1959
setchelli Eisen, 1904
solifugus (Emery, 1898)
solifugus solifugus (Emery, 1898)
solifugus rainierensis Welch, 1916
straminicolus Rota, 1995
sveni Christensen & Dózsa-Farkas, 1999
svetae Piper, MacLean & Christensen, 1982
tetrapodus Timm, 1978
torbeni Christensen & Dózsa-Farkas, 1999
tundrus Piper, MacLean & Christensen, 1982
unalaskae Eisen, 1904
variabilis Cejka, 1914
vegae Eisen, 1904
viivi Timm, 1978
vshivkovae Timm, 1994

2.23. *Oconnorella* Rota, 1995

cambrensis (O'Connor, 1963)
cheni Chen, Xie & He, 2006
globula Chen, Xie & He, 2006
tubifera (Nielsen & Christensen, 1959)

2.24. *Pelmatodrilus* Moore, 1943

planariformis Moore, 1943

2.25. *Randridrilus* Coates & Erséus, 1985

codensis (Lasserre, 1971)
quadrithecatus Coates & Erséus, 1985
westheidei (Koβmagk-Stephan, 1983)

2.26. *Sinenchytraeus* Liang & Hsü, 1979

glacialis Liang & Hsü, 1979

2.27. *Stephensoniella* Černosvitov, 1934a

marina (Moore, 1902)
sterreri (Lasserre & Erséus, 1976)
trevori (Coates, 1980)

2.28. *Stercutus* Michaelsen, 1888a

niveus Michaelsen, 1888a
[*ugandensis* Bell, 1954 (Schmelz et al. 2011: transferred to *Xetadrilus*)]

2.29. *Timmodrilus* Dózsa-Farkas, 1997 (Schmelz et al. 2005: jun. syn. of *Fridericia*; Dózsa-Farkas 2010: accepted genus)

[*oligoseta* Dózsa-Farkas, 1997 (Schmelz et al.: 2005: jun. syn. of *F. maculata*)]
[*christofferseni* (Righi, 1975), originally *Enchytraeus*, here transferred to *Fridericia*]

2.30. *Tupidrilus* Righi, 1974b

gei Righi, 1974b
lacteus Righi, 1974b
marcusae Righi, 1974b
wilsoni (Righi, 1973)

2.31. *Xetadrilus* Schmelz, Collado & Römbke, 2011

aphanus Schmelz, Collado & Römbke, 2011
fabryi Schmelz, Collado & Römbke, 2011
maacki Schmelz, Collado & Römbke, 2011
righianus (Xie & Rota, 2001) (Schmelz et al. 2011: ex *Marionina*)
ugandensis (Bell, 1954) (Schmelz et al. 2011: ex *Stercutus*)

3. Discussion

According to this list, there are currently 676 accepted species of Enchytraeidae. If we add to these the subspecies and controversial species, the number of species-group taxa adds up to 715 (nomino-typical subspecies excluced). The number in Jänsch et al. (2005) of ca. 900 enchytraeid species described worldwide seems, therefore, too high, but the authors may have included the doubtful species. The number of ca. 700 in Erséus et al. (2010) is in better agreement with our list. The species are placed in 31 genera, 3 of them of uncertain status. Species-richest genera are *Fridericia* (99 species) and *Marionina* (96 species). *Marionina* is polyphyletic and likely to be split in the near future

(Matamoros 2011). Other species-rich genera are *Lumbricillus* (85 species), *Mesenchytraeus* (76 species), *Grania* (71 species), *Enchytraeus* (48 species), *Achaeta* (40 species), and *Henlea* (33 species). Six genera are monospecific.

Given the generally poor knowledge of Enchytraeidae in most regions of the earth, these numbers most probably represent only a minute fraction of the actual diversity, but reasonable estimates as to the actual number of enchytraeid species are in our opinion not possible at present.

Acknowledgements. Special thanks are due to Tarmo Timm (Rannu, Estonia), Klára Dózsa-Farkas (Budapest, Hungary) and Joel Hallan (Austin, Texas), for scrutinizing the lists and eliminating errors. Any remaining errors are our own.

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Sonderheft 357
Special Issue

Preis / Price 8 €

ISBN 978-3-86576-083-8