Effects of a fungicide on oligochaetes and soil organic matter dynamics under controlled conditions

Pauline Thiel, Céline Pelosi, Sylvain Bart, Joël Amossé

Book of abstracts

13th International Symposium on Enchytraeidae

4-6th of June 2018

INRA Versailles

Foreword

Dear colleagues and friends,

It is a great honor and pleasure to welcome you to the city of the Sun King ‘Louis XIV’ for the 12th International Symposium on *Enchytraeidae*.

ISE is held in every two years since 1994 to bring together people working on enchytraeids all around the world. Just like in previous meetings, we will share and discuss about new research and findings on enchytraeids by addressing different fields of research: ecology, taxonomy, biology, ecotoxicology, agroecology. The interactions between experts, young researchers and students who work or would like to know more about enchytraeids, will be for sure rich and inspiring.

The symposium will include oral presentations, poster sessions, taxonomic workshops, and visits of experimental trials. As usual, a peer-reviewed Proceedings volume, the "Newsletter on *Enchytraeidae*" will be published afterwards.

For its first edition in France, the symposium and all its participants will welcome a guest. Maria J.I. Briones (PhD in Biological Sciences) is currently a Professor of Animal Biology at the University of Vigo (Spain) with special interest on the functional role of soil fauna in terrestrial ecosystems in the context of climate change. Her research has, so far, focused on grasslands, peatlands, forests and agricultural ecosystems in Spain, UK and Sweden, trying to relate enchytraeid activities and the temperature sensitivity of soil organic matter decomposition in response to climate change. She will give a talk to introduce her work and achievements on Monday afternoon.

Once again, thank you for attending this meeting. We hope you will enjoy the scientific and social program!

With kind regards,
Céline Pelosi
On behalf of the Organizing Committee

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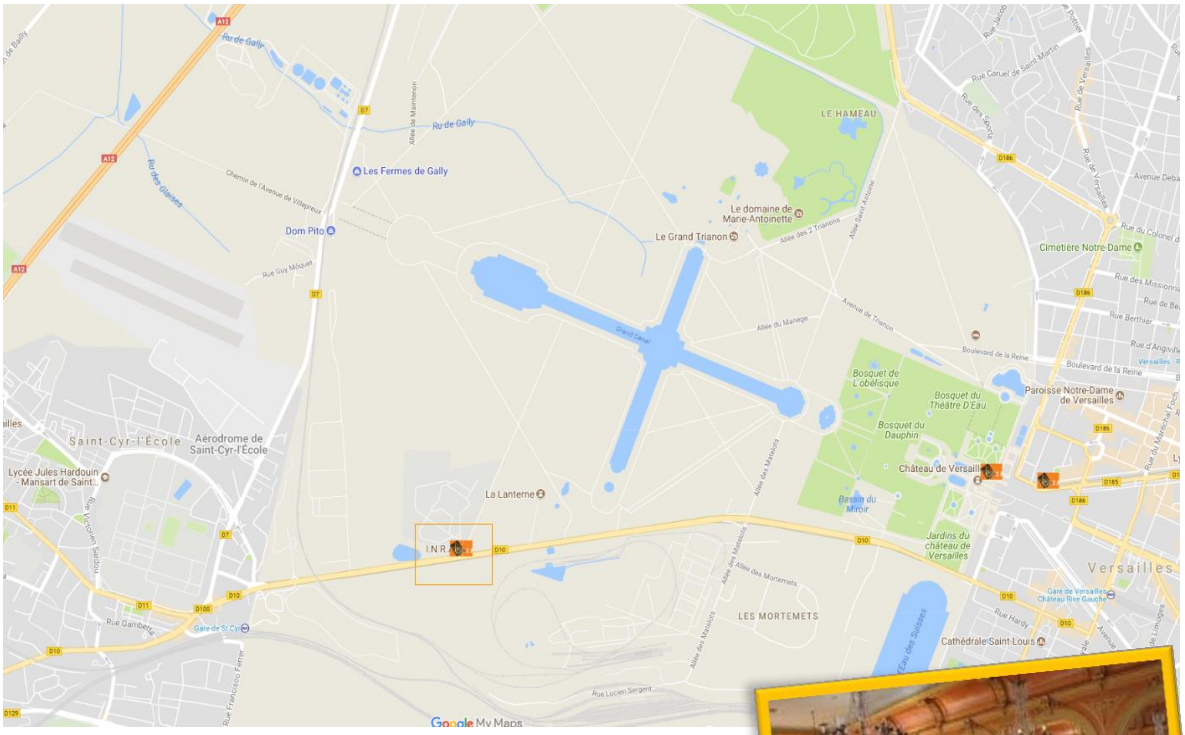

Lunches and Gala Diner

Lunches at the restaurant of INRA of Versailles

- Monday 4th : Cocktail diner at INRA of Versailles
- Tuesday 5th : Gala diner at the Hôtel de France.
  5 Rue colbert - 78000 VERSAILLES - TEL: 00 33 (0)1 30 83 92 23

Meeting Program

Monday 04 June 2018

1:00 pm - 2:00 pm : Registration

2:00 pm - 2:30 pm : Welcome presentation *Isabelle Lamy, head of Ecotoxicology team and Céline Pelosi, on behalf of the organizing committee*

2:30 pm - 3:15 pm : "Enchytraeids, the right honourable members of the mesofauna and not just midget cousins to earthworms"*by Maria Briones Professor of Soil Zoology University of Vigo*

3:15 pm - 3:55 pm : **Session 1 : Ecology , community studies Part 1**

- Tillage effects on enchytraeid diversity: Comparison of field trials on the European scale - *Anneke Beylich*
- The occurrence of Enchytraeidae, Naididae and Tubificidae in four Amazonian forest ecosystems - *Jörg Römbke*

4:00 pm - 4:30 pm : Coffee break

4:30 pm - 5:10 pm : **Session 1 : Ecology , community studies Part 1**

- Enchytraeids in fly ash technosols and sandy mine soils – case study from Poland - *Agnieszka Józefowska*
- Cattle and horse manure increase the abundance and species richness of enchytraeids in pastures - *Gergely BOROS*
-

5:15 pm - 6:30 pm : Visit of la cage experimental trial *By Michel Bertrand*

6:30 pm - 8:00 pm : Cocktail diner

Tuesday 05 June 2018

9:00 am - 9:30 am : Welcome coffee

9:30 am - 10:50 am : **Session 2 : Taxonomy**

- National inventories and species delimitation of Enchytraeidae in Sweden and Norway, using mitochondrial and nuclear genetic markers and morphology - *Christer Erséus*
- Lumbricillus from the Southern Hemisphere - *Mårten Klinth*

- Enchytraeids from Cerrado biome (Brazil): First records - *Rüdiger M. Schmelz*
- Enchytraeids from Thailand: First records - *Rüdiger M. Schmelz*

10:50 am - 1:00 pm : Coffee break - presentation poster. *With vote for the best poster !*

1:00 pm - 2:30 pm : Lunch

2:30 pm - 4:30 pm : Sponsor Presentation and Taxonomy workshop

4:30 pm - 7:30 pm : Visit of gardens of Versailles. *Musical Fountains Show and Visiting by the Little Trains of the Park of Versailles*

7:30 pm - 12:00 am : Gala diner

Wednesday 06 June 2018

9:30 am - 10:00 am : Welcome coffee

10:00 am - 11:00 am : **Session 1 : Ecology, community studies Part 2**

- 25 years of soil monitoring in Germany – are there signs of decline in soil biodiversity? - *Ulfert Graefe*
- Temporal variation in population dynamics and vertical distribution of enchytraeids in peatlands - *Raquel Juan-Ovejero*
- Enchytraeids and other microannelids in spring fens and adjacent grasslands of the Western Carpathians - *Jiří Schlaghamerský, Martina Bílková*

11:00 am - 11:30 am : Coffee break

11:30 am - 12:10 pm : **Session 3 : Ecotoxicology**

- Variability of stress biomarker expression in a model organism for terrestrial ecotoxicology - *Enchytraeus albidus - Andrea Chacon Hurtado*
- A two-year field study to assess the effects of two fungicides and their mixture on oligochaete communities and the soil functioning - *Joël Amossé*

12:10 pm - 1:00 pm : Conference closing and award to the best poster. *By Céline Pelosi and Maria Briones*

1:00 pm - 2:30 pm : Lunch

2:30 pm - 5:00 pm : Visit of the 42 plots assay and Taxonomy workshop

Abstracts of Presentations

Tillage effects on enchytraeid diversity: Comparison of field trials on the European scale

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[(2)] *University of A Coruña, Science Faculty, Evolutionary Biology Research Group (GIBE), A Coruña, Spain*

The project SoilMan funded within the EU-Biodiversa framework aims at uncovering relationships between soil management practices, habitat characteristics and the abundance and performance of functional groups of soil organisms. The understanding of these processes will allow identification of soil management practices that improve soil health and simultaneously sustain soil fertility. As a first step, inventories of major soil fauna groups at field trial sites with different regional and management contexts are currently established.

General hypotheses are:

- Soil biodiversity at the farm scale depends on local impacts defined by soil management
- Soil biodiversity benefits from reductions in farm-based soil management intensities

In our contribution the hypotheses will be approached by investigations of the enchytraeidae (microannelids) at field trials in Germany (Lower Saxony), Sweden (Uppland), Romania (Transylvania) and Spain (Andalusia) with different tillage treatments. Treatments are 1. conventional (ploughing), 2. minimum (shallow, non-inverting tillage) 3. direct seeding (no tillage).

First data analyses show that the total species numbers vary between locations. Differences between the tillage treatments are not always as distinct as expected. To assess functional aspects, information on strategy types of the species was linked to the data. “Soil dwellers”, i.e. species predominantly occurring in mineral topsoil horizons, strongly dominated at all sites and treatments. However, also “litter dwellers” were detected. Generally, these species rarely occur in agricultural field soils due to the lack of OL- and OF-horizons. In our study, they were detected at the minimum and no tillage treatments, although in low numbers. This indicates that reduced tillage systems can support a higher functional diversity among enchytraeids.

Key words: microannelids, strategy types, vertical distribution, agriculture, direct seeding

For further information on the project SoilMan see: https://www.soilman.eu/

The occurrence of Enchytraeidae, Naididae and Tubificidae in four Amazonian forest ecosystems

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Enchytraeidae are known to be an important group of soil animals in temperate regions of the world but their diversity as well as their contribution to soil functions, especially litter decomposition, in the humid tropics remains largely unexplored (e.g. Schmelz et al. 2013). Therefore, as part of the SHIFT project ENV 52 "Soil Fauna and Litter Decomposition" the species composition, abundance and biomass of enchytraeids were determined in four different forest areas located near Manaus (central Amazonia) between 1997 and 2000. In addition, other microdrilid families like Naididae or Tubificidae were sampled as well, but not in a quantitative way. The aim of the project was to study the regeneration and enhanced use of already degraded forest areas, to diminish the human impact on primary rain forest in Amazonia.

Study sites were two polyculture tree plantations (four different tree species of commercial use in each (POA, POC)) and in two plots of nearby secondary (growing since 1984, SEC) and in undisturbed primary forest (FLO), respectively. Microdrilids (i.e., enchytraeids, naidids, and tubificids) were sampled quarterly for two years by wet-extraction. In the field the samples were divided in 2 depths (litter-layer and mineral soil 0 - 5 cm), meaning that in total 120 samples were evaluated. The enchytraeids were identified in vivo immediately whereas naidids and tubificids were determined after fixation in EtOH. Identification of the microdrilids followed a site-specific key, prepared for the study site based on information from the literature (e.g. Righi, 1978) and own experiences. The ecological results presented here will focus on the genus level. In the case of naidids and tubificids, no distinction beyond the total number could be made. The biomass of larger enchytraeids (mainly from the genus *Guaranidrilus)* was determined via weighing, while the biomass of all other enchytraeid species was estimated by using values previously determined for European species of the same size. In total, 18 enchytraeid species and 14 other microdrilid species have been found at the four study plots (Schmelz & Römbke, 2005; Collado & Schmelz, 2000a,b, 2001, 2002). Most of the enchytraeid species belong to the mainly neotropical genus *Guaranidrilus* (5) and to the cosmopolitan *Hemienchytraeus* (5). Species of genera common in the Northern Hemisphere like *Achaeta* sp. (4) and *Enchytraeus* sp. (2) were also found.

The number of enchytraeids found in the primary forest (1.000 – 10.000 Ind/m2) is comparable to those found at other tropical rain forest sites (Römbke 2007). Numbers in the secondary forests or plantation sites are alse comparable with those obtained in a following study (Römbke et al. 2005, 2007). The number at all four plots is similar whereas the biomass is lower on POA and POC than in FLO and SEC. However, variability between replicates is high. No annual population dynamics were observed, but dry conditions in 1997 had a negative influence on enchytraeids. The four sites are similar concerning species number and

composition, but dominance patterns differ: the dominant genera on FLO is *Hemienchytraeus* but *Guaranidrilus* on all other plots. The litter layer is less inhabited at the plantations than at FLO and SEC. Distinct correlations between climatic parameters (positively: rainfall, humidity; negatively: soil and litter temperature) with enchytraeid biomass were found; some exceptions (esp. on POA) cannot be explained yet. For the first time Naidids were regularly found in terrestrial samples of a tropical rain forest; their species number is high (only about one quarter is known to science). Such microdrilids (esp. naidids in the moist litter of FLO) can reach high numbers under favorable conditions. The same distribution pattern was found when assessing soil samples: naidids are abundant on FLO, rare on SEC and practically not occurring on POA and POC. Thus, the microdrilid community indicates a clear distinction between undisturbed (FL, and partly SEC) and plantations (POA, POC).

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Key words: Microdrilids, Brazil, Diversity, Plantations

Enchytraeids in fly ash technosols and sandy mine soils – case study from Poland

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Technosols are usually characterized by unfavourable conditions for soil biota. Such soils are typically in an early stage of development with an initial organic horizon, lack of nutrients, especially nitrogen, defective water properties, such as water holding capacity, and, very often, extreme acidity or alkalinity. The restoration of biological activity in such technogenic soil is an important indicator of a successful reclamation process. Alder trees (Alnus spp.) are planted on such soils as phytoremediation species to accelerate the soil-forming process and supply nitrogen to the soil. The research questions were: Do enchytraeids occur in technogenic soil-substrates? Is there an effect of alder species (Alnus incana, A. viridis, A. glutinosa) on enchytraeid abundance, species richness and assemblage composition?

Field data were collected in a reclaimed sand pit (Szczakowa), and a fly ash (combustion waste) landfill resulting from lignite combustion in the Bełchatów Power Plant. At the reclaimed sand pit site, samples were collected from plots with A. viridis (A_vir) and A. glutinosa (A_glu) growing on sandy soils (S). On the fly ashes (FA), plots were established on land planted with either A. incana (A_inc), A. viridis (A_vir) or A. glutinosa (A_glu) on two different substrates: pure fly ash (FA) serving as a control and fly ash with lignite culm added in planting holes (FA+L). Samples for enchytraeid and earthworm extraction were collected twice per year (in May and in October 2016). At least six replicate soil cores of 16.6 cm2 sampling area and 10 cm depth were taken. Each core was divided into two 5 cm layers, from which enchytraeids were extracted with O'Connor's wet funnel technique (3 h, with heating) and identified alive to species. Per treatment, three soil blocks, 25×25 cm in area and 15 cm in depth, were collected for earthworm extraction by hand-sorting. Basic soil properties, such as pH, organic carbon (Corg) and total nitrogen (Nt) were measured in the organic layer (Oe) and the 0-5 cm mineral layer. Soil texture was determined in the 0-5 cm layer. Enchytraeid density was also investigated in natural forest with various alder species (A_inc, A_vir or A_glu) as the dominant tree species in the Bieszczady Mts. Here two replicate soil cores (same size as given above) were taken per alder species once in spring.

Reclaimed sandy soils, both A_vir-S and BA_glu-S, were acid (pHKCl 4.2 and 4.7), Corg content in Oe layers was 340.8 g·kg-1 and 404.5 g·kg-1 in A_vir-S and A_glu-S, respectively. In the 0-5 cm layers Corg content was 2.6 g·kg-1 in A_vir-S and 3.2 g·kg-1 A_glu-S. Nt was

very low – 0.1 g·kg-1 in A_vir-S and 0.2 g·kg-1 A_glu-S, respectively. Combustion waste technosols (A_glu-FA+L, A_glu-FA, A_inc-FA+L, A_inc-FA, A_vir-FA+L, and A_vir-FA) were alkaline (pHKCl 7.3-7.4). Corg content in Oe layer ranged from 265.3 g·kg-1 in A_vir-FA+L to 420.7 g·kg-1 in A_inc-FA+L. In the 0-5cm layers Corg content ranged from 52.1 g·kg-1 to 70.8 g·kg-1 in A_inc-FA and A_glu-FA respectively. The content of Nt was 1.9-2.9 g·kg-1.

No earthworms were found in reclaimed soils. Mean enchytraeid abundance (± SD) varied from 2,827±9,752 ind./m2 in A_glu-S to 9,146±21,626 ind./m2 in A_inc-FA+L. Generally, plots with black alder (A_glu 4,963±8,251 ind./m2) had significantly higher enchytraeid densities than plots with green alder (A_vir 2,653±7,768 ind./m2). The differences in enchytraeid abundance between plots with black and green alder and grey alder (A_inc 5,815±15,728 ind./m2) were not statistically significant. Soil substrate (S, FA or FA+L) was not a differentiating factor. In total, 8 genera and 13 species were recorded. In the natural forests, enchytraeid abundance was 61,747±20,020 ind./m2, 54,518±2,130 ind./m2, 29,518±15,335 ind./m2 in A_glu, A_inc and A_vir respectively.

In combustion waste technosols (FA and FA+L), species tolerating dry conditions and high pH predominated (e.g. Henlea ventriculosa). In sandy mine soils (S), species diversity was very low (Shannon-Wiener index – 0.01), but we recorded Hemifridericia bivesiculata, a species known within Europe only from Hungary.

The study was financed by The National Science Centre, Poland, grant No. 2015/17/B/ST10/02712.

Key words: restoration, sand pit, combustion waste, Alnus, Enchytraeidae, Lumbricidae,

Cattle and horse manure increase the abundance and species richness of enchytraeids in pastures

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Manure as a natural fertilizer impact not only on physical and chemical soil characteristics, but also on soil organisms such as enchytraeids (Annelida: Oligochaeta). Since dung offer food source for saphrophagous worms, we hypothesized an increased abundance under the casts of grazing animal species.

The experiment was carried out in an extensive farm near to Verőce village (Northern Hungary) during 2 months (from May 10 till July 5, 2017). Fresh horse and cattle dung were collected from the barn of the animals, then homogenized to prepare unified samples for each species. 500 g of dung portions were measured and placed randomly in a fenced area in the pasture. Every second week 5-5 soil samples were collected from under the dungs and from control plots. Soil samples were taken with a split soil corer (diameter of 5 cm) to 10 cm depth (volume ca. 200 cm^3) and divided an upper and a lower part to estimate effects of soil depth. Relative humidity, air and soil temperature were measured continuously with MCC USB-502 data loggers during the whole period. Soil moisture was measured weekly using TDR probes (FieldScout TDR 300).

In total, 9 species of 6 genera were found: *Achaeta danica, A. bohemica, Buchholzia appendiculata, Enchytraeus buchholzi* sensu lato, *E. bulbosus, Enchytronia parva, Fridericia bisetosa, F. bulboides* and *Marionina communis.* After 4 weeks the abundance of enchytraeids were slightly higher under the horse manure, but significantly higher under cattle dung compared to the control, however, the increased abundance manifested in mainly juveniles. Between the 4^{th} and the 6^{th} weeks the abundance did not show further rising trend but the species richness under horse manure became higher. In the last two weeks of the experiment, the abundance of worms started to decrease, which might have been caused by the high temperature and drought. The vertical distribution seems to reinforce this ascertainment as it showed that worms migrated to the deeper layers.

Our results revealed that manure is an important factor for enchytraeids in pastures as abundance and species richness are influenced by the quality of dung.

National inventories and species delimitation of Enchytraeidae in Sweden and Norway, using mitochondrial and nuclear genetic markers and morphology

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DNA barcoding has been claimed to be useful for species level taxonomy and identification, but if uncritically used it is a method with pitfalls. For instance, the suggested barcode for animals, Cytochrome oxidase subunit I (COI), shows deep intra-specific divergence in certain groups. Nevertheless, COI is a valuable descriptor for clustering specimens according to their maternal history, which may serve as a first step towards species delimitation. Species boundaries can later be established by integrating also nuclear DNA, morphology, etc. Within the framework of the parallel Taxonomy Initiatives in Sweden and Norway, COI barcoding played a crucial role in nation-wide inventories of the species of potworms, family Enchytraeidae (Annelida: Clitellata), in both countries. These small invertebrates are common in most terrestrial and aquatic habitats, including the sea, and a barcoding approach in such a study is advantageous, as all life stages, and/or even fragments of animals, can be included as records. About 5,500 worms from over 650 sites were barcoded, resulting in a great number of primary species hypotheses (i.e., COI clusters). The number of hypotheses was considerably reduced after comparisons with nuclear (Internal Transcriber Spacer Region [ITS] or Histone 3 [H3]) gene trees, and thus we seem to have recorded about 290 species (i.e., separately evolving metapopulation lineages) of Enchytraeidae in the two countries together. When all of these have been properly identified and described, the known enchytraeid species diversity in Sweden will be 2-3 times higher than that reported before, in Norway 3-4 times higher. This rich fauna includes numerous examples of cryptic taxa, i.e., genetically distinct species that are not easily distinguished by their morphological characters. As many as 100 species are possibly new to science. These surveys have established a baseline library likely to cover a great part of the actual species diversity, and containing detailed information about the known geographical distribution, habitat preferences of all species and their COI barcodes, plus a great deal of other genetic information. The revelation of such a great number of species will add substantially to the resolution of diversity, whenever enchytraeids are part of ecosystem assessment and biomonitoring in Scandinavia.

Lumbricillus from the Southern Hemisphere

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The Enchytraeidae of the Northern Hemisphere have been studied thoroughly since the beginning of the 19th century. Despite this fact, two centuries later there are still major gaps of knowledge regarding species delimitation, phylogeny and ecology of this group. However, these gaps are nothing in comparison to those for the enchytraeids of the Southern Hemisphere, in particular from the Subantarctic, which remains a largely unexplored frontier. Some studies were carried out in the golden days of scientific expeditions such as; Michaelsen (1888) for South Georgia and (1905) for Kerguelen, Ude (1896) for Tierra del Fuego, Benham (1905) for Maquarie Island and Stephenson (1932) for South Georgia. However, these samples were only from a few selected areas and so most of the species diversity from the Subantarctic remains unknown. Furthermore, there is a lack of molecular data from major parts of the Southern Hemisphere and there are few phylogenies comparing the relationship between the species of the South to those of the North, with the exception of a few groups such as *Grania* (Prantoni et al. 2016).

We recently published a couple of papers on the phylogeny and taxonomy of the genus *Lumbricillus* Ørsted, 1844 from the Northern Hemisphere (Klinth et al. 2017a-b). Species of *Lumbricillus* can be found mainly in the intertidal zone of beaches where they live among sand, gravel or under stones and decaying algae. The genus contains some 80 described species and seems to be rich in both species and specimens in the temporal and arctic areas, but not as common in tropical zones. In our estimated molecular phylogeny we found *Lumbricillus* to be closely related to the genus *Grania* Southern, 1913 and, we also found support for four subgroupings within *Lumbricillus* (the *lineatus*, *pagenstecheri*, *buelowi* and *arenarius* groups), where the species within each group shared certain morphological characters.

Fortunately, we have since been able to sequence DNA from a number of specimens collected in the Southern Hemisphere and the Subantarcic region, mainly from South Georgia, Marion Island, South Africa and the Antarctic Peninsula. Although the sample size remains very small we still were hoping that this could give insight into how the species of the Southern Hemisphere are related to those in the North. A total of seven genetic markers, three mitochondrial, three nuclear ribosomal and one from the histone complex, were used to estimate a species tree for the new species together with those used in our previous phylogeny (Klinth et al. 2017a). Specimens of the Southern species were also stained and mounted for morphological studies.

Preliminary results do not find support for a separate clade containing all species from the Southern Hemisphere in the species tree. Instead, one group of two species with black pigmentation is supported as sister to the *arenarius* group, and these together make up the sister

group of the remaining *Lumbricillus*. Another group of Southern species was found within the *lineatus* group (containing the type species *L. lineatus* (Müller, 1774)) where the Southern species make up a sister clade to the species from the Northern Hemisphere. These species match the morphology of the *lineatus* group well, having the lobed testis sacs that characterize most species of the genus. The two species with black pigmentation share some morphological traits with the *arenarius* group such as lacking the lobed testis sacs of the aforementioned *lineatus* group. However, the pigmented species are still clearly separated by having unusually high numbers of chaetae per bundle (>10), two prostate glands associated with the penial bulbs and by their pigmentation, and are suggested to be categorized into a new morpho-group containing other darkly pigmented *Lumbricillus* species from the Southern Hemisphere.

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Lumbricillus, Southern Hemisphere, Subantarctic, Phylogeny

Enchytraeids from Cerrado biome (Brazil): First records

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Enchytraeids from Cerrado biome (Brazil): First records

The Cerrado is a large tropical savanna biome in the center of Brazil, covering 21% of the land's area. It is the second largest biome of the country, after the Amazonian rainforest, and one of the richest tropical savanna regions in the world, with high levels of endemisms. In the last century, most of its area has been converted to fields for intensive agriculture. Knowledge of the Cerrado's soil fauna is still poor but may be useful for sustainable agriculture. Records of enchytraeids have so far been only sporadic.

Here we report on results of sampling campaigns carried out with the objective to register the diversity and density of Enchytraeidae fauna from different habitats of the Cerrado biome adjacent to the Embrapa Cerrado Research Station in Brasilia and in Goias State. We sampled in gallery forests, fields, and open savanna in protected and unprotected areas. Worms were extracted from soil with ISO standard methods and identified to genus level *in vivo*. Selected specimens were fixed in ethanol and re-investigated as whole mounts.

We distinguished morpho-species of the genera *Guaranidrilus* (8 taxa), *Xetadrilus* (3 taxa), *Achaeta* (3 taxa), *Marionina* (1 taxon), *Fridericia* (1 taxon), and an as yet unspecified number of *Hemienchytraeus* taxa. Most taxa cannot be assigned a name; they are probably new to science. Noteworthy are records of *Xetadrilus pitucus* (Righi, 1974) and *Marionina schreiberi* Righi, both originally described form the Cerrado region and recorded here for the first time after. These putatively typical and common Cerrado species are redescribed here in detail.

Key words : Clitellata, Oligochaeta, Enchytraeidae, Neotropical Region, Biodiversity, Taxonomy

Enchytraeids from Thailand: First records

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The diversity of tropical enchytraeids is poorly known; research up to now has focused on colder regions, where enchytraeids are supposedly more diverse and more abundant. As regards South-East Asia, records of enchytraeids are only sporadic; in Thailand, for example, not a single enchytraeid species has been recorded.

The species diversity of enchytraeids in Thailand is currently being explored covering different habitat types, among them rice fields and adjacent soils, hardwood forests, riverbanks, and urban parkland. Since many new species are to be expected, first diagnoses focus on the recognition of genera and the distinction of morpho-species, to be characterized and described as nominal species in a second step, using morphological and molecular data.

Species of five genera have been distinguished so far: *Achaeta* (3 species, one of them probably *A. piti*), *Fridericia* (4 species, one of them probably *F. peregrinabunda*), *Hemienchytraeus* (number unkown), *Enchytraeus* and *Marionina*. Fragmenting *Enchytraeus* spp. identifiable as *E. bigeminus* Nielsen & Christensen, 1963 and *E. dudichi* Dózsa-Farkas, 1995 were commonly found, together with *Enchytraeus* species of the *buchholzi* group but with nephridia beginning in more anterior segments (4/5 instead of 6/7), as in *Enchytraeus indicus* Stepenson, 1912, a species with only one (doubtful) record after the original description. Two new species of *Marionina* share the chaetal pattern of *Marionina seminuda* Xie & Rota, 2001 from Southeastern China (lateral chaetae absent, 2 chaetae in ventral bundles, absent in II), and suggest a regional, possibly monophyletic, species group. Some species cannot be assigned known genera – for example a form with only one pair of pharyngeal glands.

Abundance was low in general, except for organically enriched sites (e.g., patches of cowdung manure). In the dry season sexually adult specimens were rare, suggesting a seasonal life cycle. Some sites were without enchytraeids even though earthworms were present, e.g. some ricefields and urban "lawns". *Enchytraeus* and *Fridericia* spp. were common even at supposedly natural sites. Apart from the description of species, the investigation aims at distinguishing native and exotic species.

Key words : Clitellata, Oligochaeta, Enchytraeidae, Tropics, Biodiversity, Taxonomy

25 years of soil monitoring in Germany – are there signs of decline in soil biodiversity?

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In Germany a net of about 800 Permanent Soil Monitoring Sites (BDF = Boden-Dauerbeobachtungsflächen) is established to observe creeping changes in soil quality that cannot be detected at short time scales. The biological part of the programme has a focus on soil biodiversity using predominantly earthworms and microannelids as indicator groups (Barth et al. 2000). Having started in the early nineties, we can now present time series of 25 or more years, which embrace several phases of environmental stress and varying modes of agricultural practices. The observed changes in soil biodiversity will be considered separately here for each land-use type.

In forests the condition of soils slowly improved since the 1990ies through the reduction of atmospheric deposition particularly of sulfur (S) and lead (Pb), resulting amongst others in an increase in pH values primarily of the upper soil layers (Wellbrock et al. 2016). Simultaneously, a trend towards higher diversity of the annelid community could be observed at many sites. Especially limed forest soils show a considerable increase of annelid diversity. However, the effect of the reduction of soil acidification and pollutant input is also evident at unlimed places.

At ploughed agricultural sites the annelid community resembles the community occurring in the A-horizon of forest mull, but generally exhibits lower species diversity due to the lack of litter dwellers. The increasing practice of reduced tillage during the observation period at first stimulated soil biodiversity, but then was more and more counteracted by the intensified application of herbicides.

The collected data may be used as benchmarks for the threat ‘decline in soil biodiversity’.

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Key words: soil monitoring, long-term observation, soil biodiversity, earthworms, microannelids, forest soils, agricultural soils

Temporal variation in population dynamics and vertical distribution of enchytraeids in peatlands

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Peatland formation requires certain climatic conditions (i.e. cold temperatures and high precipitation distributed throughout the year) so that organic matter accumulation exceeds decomposition (White et al. 2008; Erwin 2009). As a result, peatlands are very sensitive to climate change. Although they cover a very small percentage of the Earth's land, they store 15-30% of the global soil carbon (Limpens et al. 2008). They also contain a highly diverse soil fauna communities and specifically, enchytraeids contribute up to 70% of the total animal biomass in these areas and are a keystone invertebrate group in these ecosystems (Briones et al. 2007). This is because these organisms are very responsive to changes in abiotic factors and for example, they migrate down to deeper peat layers when soil moisture contents decrease at the surface (Briones et al. 1997; Holmstrup et al. 2015). Different vegetation types can modulate soil climatic conditions and also respond to climate changes (Kardol et al. 2010; Reinsch et al. 2017). Therefore, it is crucial to understand how plant-soil interactions can influence the abundances and vertical distribution of enchytraeids to make better predictions on the functioning of these vulnerable ecosystems. We hypothesised that enchytraeid abundance and vertical distribution will vary differently among seasons and across peatland habitats.

This investigation was carried out at the Serra do Xistral (Lugo, NW Spain) where we selected four different peatland habitats based on the dominance of different plant communities: an active blanket bog dominated by sedges (*Eriophorum angustifolium* and *Carex durieui*), a blanket bog covered by the purple-moor grass (*Molinia caerulea*) and two valleyside bogs, one dominated by heather (*Calluna vulgaris* and *Erica mackaiana*) and another one by a moss carpet of *Sphagnum* spp. The area is characterised by having an oceanic climate, with a mean annual temperature of 10.6 ºC (from 6.0ºC in February to 16.0 ºC in August) and 1533 mm of rainfall. Sampling at each habitat was performed every two months from January 2016 to November 2017. On each sampling occasion, ten intact soil cores (10 cm diameter x 14 cm depth) were collected and horizontally sliced in 2 cm layers down to a depth of 10 cm in the field (4 habitats x 10 replicates x 5 soil layers x 12 sampling periods). Two subsamples of 20 cm^2 were obtained from each layer and one used for the extraction of enchytraeids (wet extraction method) and the other for soil moisture determinations (oven-drying the samples at 105 ºC for 48 hours or until constant weight and re-weighing). Once extracted, the animals were preserved in 70% ethanol and then counted.

Our results showed that enchytraeid abundance was significantly different between habitats, soil layers and sampling periods. On average, the graminoid habitat had the highest enchytraeid abundance during the investigated period when compared with the other three habitats, and with the habitat dominated by sedges showing the lowest values. This could be related to soil

moisture conditions at these sites, with the peat under *Sphagnum* moss and sedges showing the highest water contents, and those under graminoids and heather being the driest ones.

Soil moisture also showed important temporal variations during the two investigated years, but in this case it was not possible to find a direct relationship between soil water content and changes in enchytraeid populations. Generally, the abundances were greater in 2017, a warmer and drier year, than in 2016 and peaked in the late summer of 2017 in those habitats dominated by vascular plants. In contrast, in the habitat dominated by mosses, population increases were associated with drier conditions in 2016, whereas the hot and dry summer of 2017 resulted in severe reductions of enchytraeid numbers.

At the four peatland habitats, the majority of the individuals were concentrated in the top layer (0-2 cm), although they also colonised deeper layers in response to changes in soil moisture. The most pronounced migrations along the peat profile occurred in summer. For example, at the sedge habitat, they migrated to the 2-4 cm layer in July 2016 when the moisture content was 72% at the top layer. In the habitats dominated by graminoids and heather, higher mortality rates were recorded at the uppermost layer in spring and summer when soil moisture reached values of 70% and 54%, respectively, and resulted in the surviving individuals colonising all four deeper layers. Under *Sphagnum*, enchytraeid abundance was significantly higher in the 2-4 cm layer in the summer of both years, when soil moisture decreased to 86% in the 0-2 cm layer.

These findings indicate that enchytraeid responses to changes in abiotic conditions greatly differ between peatland habitats. However, soil moisture played an important role in controlling their vertical distribution at the four sites. Enchytraeid survival and activities are highly dependent on water availability and consequently, adverse conditions can result in mortality and migration to deeper layers when the peat became drier (moisture contents below 60%). Previous studies have already shown that these vertical migrations could lead to significant C loses and a greater turnover of older organic matter (Briones et al. 2010; Carrera et al. 2011) so plant-soil-enchytraeid interactions are determinant in predicting changes in C storage in peatlands under future climate change scenarios.

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Keywords: soil moisture, vertical migration, peatland habitats, population abundance

Enchytraeids and other microannelids in spring fens and adjacent grasslands of the Western Carpathians

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Although wetlands have received substantial attention by researchers, knowledge on the invertebrates of the transition zone between aquatic sediments and terrestrial soils remains poor. The present study was conducted to bridge this gap as far as annelids in micro-wetlands and adjacent grasslands are concerned. A typical but rather threatened type of micro-wetlands in the flysch zone of the Western Carpathian mountain range of Central Europe was studied, i.e. spring fens ranging from calcareous mineral-rich ones with tufa formation to somewhat acidic mineral-poor Sphagnum fens. Thus comparisons of annelid assemblages included the comparison of proximate habitats highly different in moisture (and organic matter) content as well as sites (both grassland and fen habitats) differing in mineral richness and soil pH.

In 2015-2017, 27 pairs of treeless spring fens and grasslands in close vicinity were sampled in the hills, mountains and valleys (420-820 m a.s.l.) of the Western Carpathians in eastern Moravia (Czechia) and western Slovakia. All four types of spring fen, which can be distinguished along the mineral richness gradient, were covered. The object of interest were assemblages of annelids, represented by several families and of ecological importance in both types of habitat. The present contribution reports on microannelids ("microdriles" and "polychaetes") only, focusing on the dominant group in terrestrial soils – potworms (Annelida: Clitellata: Enchytraeidae). Microannelids were sampled with a cylindrical corer of 17 cm^2 working area to 10-15 cm depth. Per site and sampling date, five cores were taken in the fen and five in the grassland. Most sites were sampled twice, in spring and autumn of a single year (either 2015 or 2016). At some sites (where low numbers of microannelids had been obtained), additional samples were taken in autumn 2017. The soil or sediment cores (including live vegetation such as moss) were subdivided into 3-cm layers, stored in the cold immediately after sampling and subjected to wet funnel extraction (for 48 hours, no heating, cooling water bath, water exchange and first annelid retrieval after 24 hours of extraction). As microannelid densities in the spring fens showed to be substantially lower than in the grasslands, all fen samples were analysed (yielding 17 to 6186 specimens per fen site; almost 97% of the latter number were *Aeolosoma hemprichi* specimens), whereas a minimum of five grassland soil cores, representing both the spring and autumn season, were deemed sufficient when enough microannelids were obtained (in general, soil core extraction continued until at least 150 annelids were obtained or until all 10 soil cores were subjected to enchytraeid extraction). The microannelids were identified alive under a light microscope (a high power compound microscope with Nomarski contrast was used when required for reliable enchytraeid identification).

In total, ca 20 000 microannelids were processed, of those ca 9000 individuals from grassland samples and ca 12000 from fen samples. However, the latter included ca 8000 representatives of Aeolosomatidae ("Polychaeta": Aphanoneura) with a very uneven distribution among sites and sampling dates. The grassland soils were dominated by enchytraeids, whereas the microannelid assemblages in the fens were a mix of representatives of several families: the clitellate Enchytraeidae, Naididae (sensu Erseus et al., 2008), Haplotaxidae, Lumbriculidae and the non-clitellate ("polychaete") Aeolosomatidae. Two further "polychaetes", *Parergodrilus heideri* (Parergodrilidae) and *Hrabeiella periglandulata* (Hrabeillidae), were present at some sites, the former in both habitats, the latter in terrestrial soils only (Schlaghamerský & Bílková, 2017). All studied fens together yielded ca 55 microannelid species (of those ca 33 enchytraeid species), all grasslands together ca 70 species, of which ca 65 species belonged to Enchytraeidae. Species identity could not be resolved with absolute certainty in the case of some species complexes and a few specimens (often juvenile or damaged ones). However, morphological investigation combined with molecular barcoding confirmed, for instance, several species of the *Cognettia/Chamaedrilus sphagnetorum-glandulosa* species complex. Many enchytraeid species were recorded for the first time in Slovakia and/or Czechia. At the time of abstract submittance, ecological data analyses is still underway.

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Key words : wetlands, Czechia, Czech Republic, Slovakia, Annelida, Clitellata, Enchytraeidae, Oligochaeta,Polychaeta, soil fauna, aquatic oligochaetes

Variability of stress biomarker expression in a model organism for terrestrial ecotoxicology-*Enchytraeus albidus*

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Enchytraeid (Annelida, Oligochaeta) are ecologically relevant terrestrial organisms involved in the litter decomposition, bioturbation, nutrient cycling (Zhang et al, 2017), and the porosity and soil structure, etc. (Gomes et al, 2012). The enchytraeid are present in a wide range of ecosystems (Didden et al 2001) and come into contact with the different soil constitutants: the soil solution, the solid phase, and the gaseous phase (Didden et al, 2001), hence the importance to take them into account in the soil quality assessment. Moreover, anthropogenic pressures may alter the organism's health and thus, affect the ecological functions assuring by enchytraeids. That is why the development of tools for soil quality assessment requires to understand the enchytraeid sensitivity to environmental factor variations.

Many studies have shown the sensitivity of these organisms to different chemical contaminants in soils. Nevertheless, in most studies the soil used was a standard laboratory soil (Kuperman et al, 2006). The present study aims to analyze the natural variability of stress biomarker expression in an enchytraeid species exposed to 20 natural soils collected in different ecosystems: urban, forest, and agricultural. This makes it possible 1) to characterize the variability of biomarker expression, 2) to identify the soil edaphic parameters influencing the biomarker expression, and thus 3) to define the sensitivity of these tools for the soil chemical stress diagnosis.

The species used for this study is *Enchytraeus albidus*, a model species for terrestrial ecotoxicity tests, with a short life cycle (42-days for the ecotoxicity test) (ISO 2004) and present in various ecosystems (Kasprzak, 1982, Rombke et al, 2002). They are easy to use for assessing toxic and chemical stress (Didden et al, 2001), reproduce rapidly, and can be maintained in a variety of substrates (Rombke et al, 2002). The experimental device is designed according to ISO 16387 norm (2014). The biomarkers measured during this study are life history traits (survival and reproduction) and energy reserves, the latter of which provides more detailed information on the organism's health. In parallel, the pedo-edaphic parameters, enzymatic activities and metal contamination levels of the 20 soils are characterized.

Finally, multifactorial statistical analysis of the data allow us to distinguish the predominant environmental parameters involved in the variation of stress biomarkers studied in *E. albidus*, and to establish the relevance of these biomarkers as tools for the soil quality assessment.

Keywords: Enchytraeus albidus, soil quality assessment tools, life history traits, energy reserves, soil edaphic parameters.

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A two-year field study to assess the effects of two fungicides and their mixture on oligochaete communities and the soil functioning

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Agriculture intensification and in particular pesticide use can affect soil fauna and its affiliated ecological functions (e.g. organic matter decomposition). Adapted from the ISO standard method for earthworm field studies (ISO 11268-3, 2014), we tested the effects of two fungicides, i.e., Cuprafor Micro® and Swing Gold® - used respectively in organic and conventional agriculture - on oligochaetes (Lumbricidae and Enchytraeidae) and the soil functioning (i.e., feeding activity and organic matter decomposition with the bait lamina and tea bag methods, respectively). The trial consisted on four replicates of six experimental treatments (control, Cuprafor Micro® and Swing Gold® at x 1 and x 10 the recommended dose, and a mixture of both pesticides at the recommended dose, 1,5 $l.ha^{-1}$ of Swing Gold® and 4 $kg.ha^{-1}$ of copper) in a meadow located near the Palace of Versailles (France). After 1, 6, 12, and 18 months of experiment (i.e., t1, t6, t12, and t18), we compared the species composition of annelids and functional endpoints between treated and non-treated plots. A lower Shannon diversity index was found for earthworms in the treatment with the mixture of both pesticides at t1 and t6. However, no effect on earthworm abundance was observed in the mixture treatment mainly explained by the compensation of earthworm species. We also found a lethal effect of Swing Gold® on anecic earthworms at t1, while an effect of copper on annelids at ten times the recommended dose (40 $kg.ha^{-1}$ of copper) was observed only after 12 months. These results could be explained by the various pesticide mechanisms on life history traits (i.e., growth, reproduction). However, we showed no overall significant difference in total feeding activity, enchytraeid density and diversity between treatments with or without pesticide at t1, t6, t12 and t18. In the Swing Gold® treatment, earthworm community did not fully recovered 18 months after pesticide application. This study underlines the need to study pesticide effects on non-target organisms as well as functional endpoints for more than one year after pesticide application. Another sampling campaign will be performed in spring 2018.

Key words : enchytraeids, earthworms, Cuprafor Micro®, Swing Gold®, agroecosystems, feeding activity

Abstracts of Posters

A new limnic *Chamaedrilus* species from Scandinavia

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Data on a new species of *Chamaedrilus*, *Cognettia* sensu Nielsen and Christensen (1959), are presented. The species is so far only found in three Scandinavian streams, two in Norway and one in Sweden.

The species is morphologically closest to *Chamaedrilus chalupskyi*, and has the same chaetal arrangement, pharyngeal gland pattern and similar posterior origin of the dorsal blood vessel. However, genetically the new species is well separated from *C. chalupskyi*, and the two species are not even found as sister-species in any of the four genetic markers (16S, COI, ITS2 and H3) analyzed, instead the new species seems to be more closely related to *C. glandulosus* and *C. varisetosus*.

Unfortunately no sexually mature specimens of the new species have been found, but specimens regenerating part of their body have been found. It is likely that the species like some other species in the genus mainly reproduces by fragmentation, and that sexually mature specimens are rare. If no differences in the spermatheca or male genitalia exists between the new species and *C. chalupskyi*, they must be considered a cryptic species pair, and in any case, as sexually mature individuals of *C. chalupskyi* are rare, they will in most cases still be impossible to separate using morphological characters.

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Key words : Chamaedrilus, Cognettia, DNA-barcoding, cryptic species

Cryptic species from the genus *Bryodrilus*: morphologically similar *B. ehlersi* specimens from Korea and Europe

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There are such evolutionary lineages in Enchytraeidae which differ genetically but their morphology is very similar, so they are classified as the same species (Martinsson et al. 2014, Martinsson & Erséus, 2017). These species are the cryptic species, which cannot or can only be hardly differentiated with the traditional morphological methods (Schmelz et al. 2017). In Enchytraeidae, cryptic species were found in more genus already, for instance the *Enchytraeus crypticus* Westheide & Graefe, 1992 and the *Chamaedrilus pseudosphagnetorum* Martinsson et al. 2014.

In one of our previous research projects, the enchytraeid fauna of South Korea was investigated, and an enchytraeid species was found which was identified as *Bryodrilus ehlersi* based on its morphological characters. *Bryodrilus ehlersi* has not been found on the Far East yet. Molecular analysis using mitochondrial cytochrome c oxidase subunit I gene, the nuclear histone H3 gene and the nuclear ribosomal ITS region showed unambiguously that the specimens collected in South Korea were different from *Bryodrilus ehlersi* common in Northern and Central Europe, so they can be considered as a new species.

In our poster we give the description of this new species based on morphological and molecular analysis.

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Key words: cryptic species, Bryodrilus, South Korea, cytochrome-c subunit I gene

Toxicity assessment of pesticides of natural origin on soil non-target organisms

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The development of new pesticides of natural origin is the main objective of several European and National research projects. Extracts or isolated compounds derived by plants, seaweeds or yeasts exhibit promising pesticide activities and they can be used as alternative pesticides in a more natural products-based pest management system. These compounds are intended to be less- toxic since they are of natural origin and thus potentially have minor adverse environmental impacts. However, this needs to be proven by ecotoxicological studies on non-target organisms. In the frames of the present study, the toxicity of certain alternative compounds on non-target soil organisms have been investigated. Furthermore, a comparative toxicity assessment on different non-target soil organisms was carried out.

More specifically, the toxicity of compounds of natural origin has been assessed on *Enchytraeus albidus* and on earthworm *Eisenia fetida.* The aim of the study was to determine the adverse effects of test compounds on the survival and reproduction of *Enchytraeus albidus*. In parallel, the possible (sub-) lethal effects such as mortality, growth and reproduction output of the test compounds on earthworm *Eisenia fetida* has been investigated as well. The tests were performed according to the recommendations of the OECD Guidelines 222 (2004) and 220 (2004) for *E. fetida* and *E.albidus* respectively. Since no information on the toxicity of the test compounds were available, range –finding tests were conducted prior to the definitive tests. The results of these tests revealed the low toxicity to soil organisms; therefore, the definitive tests were carried out as limit tests at 1000 mg test item/kg dry soil.

The results indicated that no significant mortality occurred (EC_{50} > 1000 mg/Kg dry soil) and no adverse effects on growth and on reproduction were observed for most of the tested compounds on *Eisenia fetida* (NOEC values ≥ 1000 mg/Kg dry soil). In addition, no significant mortality occurred for all tested compounds on *Enchytraeus albidus* (EC_{50} > 1000 mg/Kg dry soil). Furthermore, no significant adverse effects on reproduction of *Enchytraeus albidus* were recorded for the tested compounds at the highest tested concentration of 1000 mg/Kg dry soil (NOEC values ≥ 1000 mg/Kg dry soil). Only one compound showed a slight reduction in the number of juveniles of *Eisenia fetida.* Therefore, the results of this study confirmed the low toxicity of the pesticides of natural origin on soil organisms and indicated that under these specific experimental conditions, *E. fetida* was slightly more sensitive than *E. albidus.*

Part of this research work was conducted in the frame of the FP7 Project CO-FREE 'Innovative strategies for copper-free low input and organic farming systems', which is funded by the E.U.

Key words: soil organisms, pesticides, Eisenia fetida , Enchytraeus albidus, toxicity

The role of varied organic additives and earthworms on structure formation in soils differing in texture - preliminary results of the experiment

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Soil structure is a key factor in soil functioning that affects the soil quality (Bronick and Lal, 2005). Stability of aggregates is an indicator of soil structure (Six et al., 2000). The factors, that create a well developed and stable structure are well recognized. The content of organic matter and the soil texture, in addition to biological factors, are the most important elements affecting the stability, size and shape of soil aggregates. Despite the fact that it is known what factors created soil structure, it is still an open question what is the role of varied organic additives on structure formation in soils, and how mentioned organic additives works in soil differing in texture. Unquestionable is that soil organisms, e.g. earthworms, are creating soil structure. However, the question how the type of organic additives and varied soil texture is related to the soil engineers – earthworms is still open.

Eighty-four microcosms were prepared in plastic boxes (80x110x160mm, 1200cm3). They were filled with 400g of mineral soil with sandy (S), silty (Si), silty loam (L), and clayed (C) texture. The content of sand fraction was 99%, 31%, 14% and 4% and clay fraction 1%, 21%, 15% and 67% in S, Si, L and C, respectively. Into each box one variant of additives: 0- control without additives, S- straw; P- peat (garden soil); C- compost; S+A- straw and humic acid; S+A+Bio+Em- straw, humic acid and microbial components; C+Em- compost and microbial components was added. The amount of organic components corresponded to the soil with the content of organic matter equal 2% in each box (without control). Into each box six earthworms were introduced, two of each : Dendrodrilus rubidus, Aporrectodea caliginosa, Aporrectodea rosea. After first and second week of experiment dead earthworms were replaced with living one. Microcosms were kept in darkness at 10°C, for six months (from October 2017 until April 2018). The soil moisture was monitored monthly, and was kept in 50±5% of water hold capascity for each soil type.

After the experiment the following soil properties will be mesured: the stability of agregates using lasser difraction method (Bieganowski et al. in press) and wet sieving method (Kemper and Rosenau 1986, Saygin et al. 2017); organic carbon content (LECO® CNS 2000); pH in H2O and 1 M KCl; content of microbial biomass carbon (fumigation-extraction method, PN-ISO 14240-2, 2001); content of dissolved organic carbon (Zsolany 2003); and quantitative characteristics of soil organic matter by the method recommended by International Humic Substances Society (Swift 1996).

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Key words: microcosm, aggregate stability, straw, humid acid, compost, peat,

The influence of soil moisture content on feeding activity of earthworms and enchytraeids using the bait lamina method

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Feeding activity of soil invertebrates decreases due to human disturbance (e.g. contamination, compaction), but it is also influenced by abiotic parameters such as soil moisture. The bait-lamina test is a simple and functional method, which allows measuring the feeding activity of soil invertebrates in the laboratory as well as in the field. It can be used to assess the effect of contaminants or to monitor the long term biological quality of soil, but the influence of soil moisture content on the outcome of the test is still poorly described.

This study aimed at characterizing the relationship between soil moisture content and feeding activity of earthworms (*Eisenia andrei*) and enchytraeids (*Enchytraeus albidus*). Both species were exposed to a range of different moisture contents using natural LUFA 2.2 soil as substrate. Feeding activity was assessed using the bait-lamina method, under laboratory conditions.

Bait consumption increased with increasing soil moisture until reaching a peak, which was around 60% of the maximal water holding capacity (WHC) for earthworms and 60-70% WHC for enchytraeids. Also, earthworms consumed bait faster than enchytraeids. The increase in feeding rate with soil moisture up to the optimum was well described by a linear regression, having similar slopes for both organisms. The moisture contents at which the feeding activity was optimal were in the range of field capacity for the same soil texture class as the LUFA 2.2. At moisture contents higher than the optimum, feeding activity was reduced and seemed to be less dependent on soil moisture.

Our results show that the bait lamina laboratory test is an easy and efficient method to test the feeding activity of some soil organisms and has allowed the establishment of a first model describing the influence of soil moisture on faunal activity. Using such a model could improve the interpretation of the impact of soil contaminants on feeding activity of earthworms and enchytraeids under field conditions.

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Key words: Bait-lamina, feeding activity, soil moisture, earthworms, enchytraeids

Forestry treatment effects on enchytraeid worms (Annelida, Oligochaeta) in a temperate zone sessile oak-hornbeam forest

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Forest microclimate and soil conditions are essential regarding soil invertebrate communities. Enchytraeid worms (Annelida: Oligochaeta) are important decomposer organisms in forested landscapes, but very little is known about the effects of forest site conditions on their assemblages, especially in the temperate zone. It is hypothesized that applying a less intensive forest harvesting method, lower changes will be observed in this belowground decomposer community.

This experiment investigates the effect of different forest management practices through forest site conditions on the abundance and diversity of enchytraeids. The following treatments were carried out in a mature temperate sessile oak-hornbeam forest using six replicates in a randomized complete block design: preparation cutting, gap-cutting, clear-cutting, retention tree group within the clear-cut area and control. Microclimate and soil conditions were measured in the experimental plots Enchytraeid worms were monitored two times per year in the plots collecting soil samples divided to three vertical layers (0–4 cm, 4–8 cm, 8–12 cm). Each mature enchytraeid individuals were identified on species level. The field survey was carried out before (in 2014) and after the forestry treatments in 2015 and 2016.

Our results revealed that enchytraeids were quite sensitive for a part of treatments. All measured variables (abundance, species richness and assemblage composition) of this group have changed considerably in clear-cutting and retention tree group and differed significantly from other two methods (preparation-cutting, gap-cutting) and control. The study of vertical distribution showed that worms did not migrate downwards in soil as there was no ascending abundance or species number from upper layers to lower ones. This phenomenon can be explained by the low dispersion abilities of enchytraeids, which may presume that this invertebrate group especially sensitive for any habitat alternation and they response immediately to any changes. Consequently, they can be candidate for early-warning signallers in Central European forests.

The study was supported by the OTKA 111887 project and the Pilisi Parkerdő Ltd.

Keywords: Clitellata, clear-cutting, retention tree group, decomposers

Terrestrial Oligochaeta communities in a world's unique long-term bare fallow experiment

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The use of fertilizers can impact physical and chemical soil characteristics, but also soil organisms such as Oligochaeta (Clitellata, Annelida) and their activity. We here assessed the impacts of 89 years of different fertilization practices (N, P, K fertilizers, basic and organic amendments, or no input) on the species composition and abundance of earthworm and enchytraeid communities at INRA's 42-plot design in Versailles (France). This long-term bare-fallow experiment on silty Luvisols, developed from aeolian loess, includes 16 duplicated treatments that received continuous annual applications of 16 different amendments, leading to highly diverging physical and chemical properties in the soil's surface layer. Enchytraeids (with soil cores) and earthworms (with octet method) were sampled in spring 2017 in these 32 plots and in 5 reference plots that had received no amendments. We also used bait lamina sticks to assess the feeding activity in this trial.

Only 14 earthworm individuals were sampled from the 37 plots, belonging to three different species i.e., *Aporrectodea giardi, Allolobophora chlorotica,* and *Allolobophora icterica*. Eight individuals of *A. chlorotica* were found in soils with a pH ranging from 6.5 to 9. The highest number of earthworms (4 individuals of *A. chlorotica*) was sampled from the plot receiving lime. The other two species were found in plots with a pH between 4.5 and 7.5.

For enchytraeids, 256 individuals were found, belonging to 13 species of 6 genera. The most abundant genus was *Enchytraeus* (95 specimens), then *Achaeta* (72), *Buchholzia* (65), *Fridericia* (23), and lastly *Enchytronia* (2) and *Marionina* (2). The most abundant species was *Buchholzia appendiculata* (Buchholz, 1862) which is common and widespread in neutral to acidic soils (Schmelz and Collado 2010). This species was found in most of the treatments (calcium carbonate, potassium chloride, natural phosphate, lime, ammonium sulfate, calcium nitrate, sodium nitrate, fumier, diammonium phosphate) and reached the highest density (4 840 individuals m^{-2} in calcium carbonate plots). *Enchytraeus bulbosus* (Nielsen and Christensen, 1963) was also a widespread species that represented about 7% of the relative abundance. The individuals of this species were mainly present in horse manure treatments, but also in lime, natural phosphate, superphosphate and control treatments. The highest abundance for this species was 1 020 individuals m^{-2} in horse manure fertilized plots. *Achaeta affinis* (Nielsen and Christensen, 1959) was also found (6% of the relative abundance) but only in control and horse manure treatments, where the highest density was 3 060 individuals m^{-2}. In natural habitats this species is often found in moderately acidic soils (Schmelz and Collado, 2010).

The other species, representing less than 5 % of the total abundance, were: *Achaeta bohemica* (Vejdovský, 1879); *Achaeta eiseni* (Vejdovský, 1878); *Achaeta pannonica* (Graefe, 1989); *Buchholzia fallax* (Michaelsen, 1887); *Enchytraeus buchholzi* s.l. (Vejdovský, 1879); *Enchytronia parva* (Nielsen and Christensen, 1959); *Fridericia bulboides* (Nielsen and Christensen, 1959); *F. isseli* (Rota, 1994); *F. perrieri* (Vejdovský, 1878) and *Marionina communis* (Nielsen and Christensen, 1959).

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Key words: Long-term experiment, fertilization, earthworms, enchytraeids

Enchytraeids as ecotoxicological models – overview of available tools – towards a systems toxicology approach

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Enchytraeids are important soil inhabitants and have been used as a model for ecotoxicology for many years (ISO and OECD guidelines). During the last decades there has been considerable development of additional tools to assess the mechanisms underlying biological responses (e.g. omics) within the organism, the effects on the organism during the full life cycle and how organisms interact with other species. Here we give an update and overview of the tools developed, showing the potential usage of this organism group for a vast range of ecological assessments.

The developed tools include various omics i.e. (1) a full transcriptome implemented onto a microarray high-throughput tool (4x44000 genes, Agilent) used to assess differential gene expression (Gomes et al., 2017, 2018), (2) identification methodologies for the metabolome, and (3) identification methodologies for the proteome (Maria et al., 2018a,b). A range of oxidative stress biomarkers identification techniques have also been optimized and implemented (Howcroft et al., 2009), including catalase, superoxide dismutase, acetylcholinesterase, glutathione, lipid peroxidation, metalothionein, comet assay, etc. and the energy budget measurements with lipids, proteins and carbohydrates. Finally, histopathological analysis and embryotoxicity test methods have been developed (Gonçalves et al., 2015).

At the life cycle, population and community level we have developed a full life cycle (Bicho et al. 2015) and multigenerational test (Bicho et al., 2017), which complement the standard ecotoxicity test where one-time-point effects on survival and reproduction are measured. Enchytraeids are also part of multi-species, e.g. the soil multispecies test system (SMS) where species/community interactions can be assessed under stress impact (Mendes et al. 2018).

The overarching goal is to better understand the adverse outcome pathways in the full life cycle of an organism, aiming to also improve the risk assessment for the terrestrial environment.

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Key words : Omics, Population, Full life cycle, Multigenerational, Multispecies

Effects of a fungicide on oligochaetes and soil organic matter dynamics under controlled conditions

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Pesticides are used in agriculture to protect crops and ensure satisfying yields. However, they can affect non-target organisms and the related ecological functions (e.g. organic matter decomposition, soil structure). Earthworms (macrofauna > 2 mm in diameter) and enchytraeids (mesofauna < 2 mm and > 0.2 mm in diameter) are Annelida Oligochaeta involved in organic matter transformation. These organisms are considered as ecosystem engineers because they modify environmental conditions for other organisms through their bioturbation activity. For example, Huang and Xia (2018) had shown that earthworm mucus promoted microbial activity that increased organic matter mineralization by specific bacteria. If this process has been quite well studied for earthworms, much less is known about the effects of enchytraeids on soil organic matter dynamics, and even less after pesticide applications.

We conducted a laboratory experiment with a natural soil to test the assumption that the application of a commercial formulation of fungicide (Swing® Gold, composed of 50 g.l-1 epoxiconazole and 133 g.l-1 dimoxystrobin) would lead to direct effects on terrestrial Oligochaeta (mortality, weight gain) and indirect effects on organic matter mineralization (CO_2 release). We also tested whether these effects were in the same magnitude for earthworms and enchytraeids. For that, we used different concentrations of fungicide (no fungicide, one, and three times the recommended dose RD) and four conditions related to soil fauna (no fauna, earthworms *Aporrectodea caliginosa* alone, enchytraeids *Enchytraeus buchholzi* alone, a mix of earthworms and enchytraeids) during 26 days. To estimate the mineralization of organic matter, CO_2 release was measured at days 1, 5, 8, 12, 15, 19, 22 and 26. The total mineralization was calculated on the entire experiment duration. For trapping CO_2, NaOH was placed in a dish and inserted in each microcosm for 24 hours, then titrated with HCl to determine the CO_2 content.

No mortality was recorded at the end of the experiment, whatever the treatment. We found no significant effect of the pesticide on animal weight gain. Organic matter mineralization decreased with increasing concentration of pesticide until day 19. After this period, the contrary occurred since the release of CO_2 was higher in the treatments with the fungicide at 3 times the RD than in treatments at the RD and without fungicide. This can be explained by some bacteria species which can use pesticides as energy sources, thus enhancing their activities, such as mineralization. Fauna assemblage had no effect on mineralization until day 22. Then, CO_2 release was higher when earthworms or enchytraeids were present (no difference between these treatments) than in the treatment without animals. The treatment involving earthworms and enchytraeids had a middle level of mineralization. This could be explained by a competition between earthworms and enchytraeids for food resources.

At the end of the experiment, the highest mineralization rate was recorded in the treatment with earthworms and without pesticide. This result confirms the positive role of earthworms in soil organic matter mineralization. We also found an effect of the interaction between the pesticide and fauna treatments on mineralization at days 19 and 22. With no pesticide, enchytraeids were less efficient than earthworms but, probably withstanding higher pesticide contamination, they displayed a higher rate of mineralization than earthworms in conditions with pesticide at 1 and 3 times the RD.

Key words: pesticides, enchytraeids, earthworms, organic matter turnover

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