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PROSPECTS IN EARTHWORM ECOLOGY

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Earthworms have been among the first organisms being considered in ecological research. For long much of the research activities have focused on applied aspects, such as employing earthworms for increasing agricultural productivity. Investigations on the role of earthworms in natural ecosystems lacked behind. Also, more principle questions, such as which factors drive earthworm population dynamics in natural ecosystems and the functioning of earthworms in soil food webs remained poorly understood. With the advent of new research tools and an increasing interest in the understanding of belowground systems this situation is changing in recent years. The talk will highlight new approaches for investigating the role of earthworms in terrestrial ecosystems and present recent achievements. Finally, lines of research to achieve a deeper understanding of the functioning of earthworms in belowground systems and implications of earthworm-mediated changes in soil processes for the aboveground system will be presented.

Session 1

Earthworm Biodiversity: Conventional and Molecular Approaches to Systematics

Plenary lecture

EARTHWORM BIODIVERSITY IN LATIN AMERICA: PRESENT STATE OF THE ART

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Around 970 species of earthworms, belonging to 11 families and 125 genera are known from Latin America (LA), although this is less than half the total estimated diversity for the region (>2500 spp.). The most diverse countries are Brazil (306 spp., 260 natives), Ecuador (136 spp., 113 natives), Mexico (133 spp., 83 natives) and Colombia (116 spp., 93 natives). The Caribbean Islands host more than 120 species, of which 95 are native. However, the status of the knowledge on earthworm diversity and ecology in most countries must still be considered poor. For large areas of some countries and several islands in the Caribbean, still nothing is known. Most of the knowledge of the earthworms in LA is due to the work of Gilberto Righi, who described over 220 spp. Other taxonomists (Michaelsen, Cernosvitov, Cognetti, Rosa, Eisen, Beddard, Benham, Cordero, Gates, Sims, Graff, Zicsi, Csuzdi, Fragoso, James, Rodriguez, Borges, Moreno, Jamieson) also contributed greatly to the knowledge of the region's earthworm diversity.

Most of the known (and still unknown) species belong to the Glossoscolecidae family (533 spp.), endemic to LA, and found from Northern Argentina to Central Mexico (native species range). This family is unique in its multiform manners of reproduction, some of which still remain unknown (e.g., *Glossoscolex*). Other diversified families include the Acanthodrilidae (269 spp. in 26 genera) and Ocnerodrilidae (91 spp. in 26 genera), that also include many endemic genera and species. One family is endemic to Colombia (Tumakidae).

Exotic species (total ~70 spp.) are common and widespread throughout the region, although the Lumbricidae are mostly restricted to cooler regions. Acanthodrilids such as some Dichogaster spp. and megascolecids such as Amynthas spp. are found in all tropical regions of LA. Some native species have spread widely throughout LA and must be considered peregrine or exotic invaders outside their native range and place of origin. This is the case for Pontoscolex corethrurus, native to the Guyanan Shield, but invasive throughout the tropics and subtropics. Several ocnerodrilids (especially Eukerria spp.) and acanthodrilids (especially *Microscolex* spp.) also fit in this category. Strategies to curb the spread of exotic species should be studied and adopted to minimize their potential impacts to soils, plants and other organisms. Urgent efforts are also needed to speed descriptions of native earthworms and to promote the study of their biology and ecology, as most studies so far have been performed on exotic or peregrine native earthworm species (very little is known for the vast majority of native species). Given the few trained earthworm taxonomists and ecologists available in LA, the increasing trend in urbanization, intensification of agriculture and pressure on natural resources and native habitats (especially forests and grasslands) throughout LA, this enormous task is daunting!

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A NEW STEP IN USING 16S rDNA AND COI SEQUENCES IN THE MOLECULAR SYSTEMATICS OF EARTHWORMS (ANNELIDA, OLIGOCHAETA)

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An over-view of the positive and negative results of our attempts in using the tools of molecular taxonomy in earthworms is presented. Exemplifications are selected mostly for Lumbricidae, but also on other Crassiclitellata families.

Our preliminary attempts in using 16S rDNA and COI gene sequences (Pop A. A., M. Wink and V.V.Pop 2004, Pop V. V. and A. A. Pop 2004) ended in different ways. For some genera the new molecular data were much encouraging showing plausible species relatedness (i.e. *Octodrilus, Eisenia*), but in others (e.g. *Allolobophora* s.l.) remained hard to understand or even confusing. Nevertheless, the first positive results justified proceeding on the opened ways. In addition to the already published results, new multigene studies were carried out on Lumbricids and other Crassiclitellata families. Partial sequences of ribosomal 16S rDNA and of subunit one of the mitochondrial cytochrome *c* oxidase (COI) were obtained.

Clear and consistent conclusions were formulated regarding the validity of the Lumbricid genera *Octodrilus*, *Octolasion* and *Dendrobaena*, where the patterns of the cladogrames fit the species groupings by means of morphologic and anatomic characters. On the contrary, in the case of the species included in the genera *Allolobophora*, *Aporrectodea*, *Cernosvitovia* and *Proctodrilus*, the scattered aspect of the branches in the cladogrames seemingly does not confirm this genera discrimination on the basis of morphologycal and anatomical characters. According to our 16S rDNA data, the preliminary results in Crassiclitellata families show that Lumbricidae, Eudrilidae, Glossoscolecidae, and Ocnerodrilidae are monophyletic. We have not got similar result for the highly complicated superfamilia Megascolecoidea where only the "Pheretimoid" group formed a well supported clade. The other representatives of this taxon showed a quite variable branching pattern without bootstrap support.

Methodical improvement and new gene approaches are required because there are species (especially ubiquitous or peregrine ones) which can not be properly analyzed by actual techniques. Till now, the 16S sequences gave us the best results, grouping earthworm taxa in agreement with their morphological similarities. COI sometimes fit the 16S data, but frequently does not. We hope that new studies, with more gene frequencies and methodological improvements will allow to recognize a consistent filogenetic pattern of the different earthworm taxa.

ORIGIN AND MEANS OF DISPERSAL OF COSMOPOLITAN PONTODRILUS LITORALIS (GRUBE, 1855) (MEGASCOLECIDAE)

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Origin and mechanisms for global distribution of the genus *Pontodrilus* Perrier, 1895, presently comprising six species, have always been enigmatic. Possible sources were argued for in the region of Australia or New Zealand (Gates, 1972: 47) where congeners occur, or in the Mediterranean (Bouché *et al.*, 2004: 382) whence the genus was first described. The type-species (*P. litoralis*) is circummundane – on shorelines in the tropics and warmer parts of continents and islands in all the world's oceans; one species (*P. primoris* Blakemore, 2000) is littoral in Tasmania; another lacustrine in NZ; others are terrestrial in Sri Lanka and China. Little is known of the life-history nor means of spread of any of the species.

Pontodrilus litoralis is found on beaches, estuaries, and mangroves as part of shore psammon (i.e., organisms growing on, in or moving through sand), or is limnicolous (living in mud), and its being euryhaline (tolerant of saltwater) is unusual for an "earthworm". Sims (1980: 117), remarking on similarlity of distribution of *P. litoralis* and *Pontoscolex corethrurus* (Müller, 1857), proposed primary dispersal by ocean rafting. But Easton's (1984) synonymy of divers forms under *P. litoralis* suggests that either there is frequent global recruitment, or that despite intraspecific variability, it has remarkably conservative morphology, or, possibly, that this synonymy is too broad.

Gates (1972: 48) said "Absence of geographic variation does suggest that transportation may have been recent" although a converse view, especially when *Pontodrilus* is more correctly placed under Megascolecidae rather than Acanthodrilidae where put by some earlier authors, is of a 'primitive' species with precursors close to the original forms of this family, living in a relatively constant littoral habitat. Stephenson (1930: 834) said he "formerly thought that [Sri Lankan] *P. agnesae* might represent the terrestrial ancestor from which the littoral species have descended (Stephenson, '23) but ... the opposite may be the case, and the terrestrial and limnic species of the genus may owe their origin to the survival and modification in a new environment of colonies accidentally transported from the shore". Extension of these ideas is not only of interest for origins of the family Megascolecidae, especially relating to zoogeography and plate tectonics, but also highlights concerns relating to spead of invasive species.

The current review is based on recent ecological survey data and taxonomic studies of *Pontodrilus* specimens. Evidence is presented for dual mechanisms of spread of *P. litoralis*, involving both human transportation and a natural dispersal mode. Probable homeland from options in either Northern or Southern Hemispheres, or in the tropics, will be evaluated on this new information.

INTRODUCTION OF EXOTIC EARTHWORMS IN THE BOREAL FOREST OF WESTERN CANADA

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Recreational and industrial development is expanding rapidly in the boreal forest of western Canada. Concerns exist that such activity is facilitating the introduction and spread of nonnative species such as exotic earthworms. The mechanisms by which earthworms are introduced and spread in forested systems are not well understood. Given the poor innate dispersal abilities of earthworms, we propose four major introduction mechanisms: 1) Direct introduction by humans via abandonment of fishing bait; 2) indirect dispersal by humans via vehicle traffic; 3) transport via waterways; or 4) "natural dispersal" via vertebrate predators. To test the plausibility of these various hypotheses, we sampled earthworms in forest stands near boat launches, linear features (roads & seismic lines), forest interiors, and remote shore lines of lakes in the boreal forest of northern Alberta, Canada. Boat launches and roads had a significantly higher probability of earthworm occurrence compared with the other locations. Species commonly used as fishing bait occurred more often near boat launches than near roads alone. These results suggest that both vehicle transport and bait abandonment are the major mechanisms of earthworm introduction in Alberta's boreal. The consequences of these introductions remain unclear, but suggest that reduced road construction and regulations prohibiting the discarding of bait need to be considered to slow earthworm invasions.

Because the number of individuals introduced and the number of introduction events is emerging as the most consistent predictor of the establishment success of invasive species (Lockwood et al. 2005), we are using genetics to determine whether Dendrobaena octaedra populations are established by single or multiple introduction events. We sequenced the mitochondrial cytochrome oxidase subunit I of Dendrobaena octaedra individuals collected at the locations described above. This information is allowing us to test several predictions about the results expected under different introduction scenarios: 1) if all populations originate from a single source and single invader, one genotype should be detected across the range; 2) if populations originate from a single invader from multiple sources, populations should each contain a unique genotype; and 3) if populations are founded by multiple invaders, either from single or multiple sources, multiple genotypes should be detected in each population (Bohonak et al. 2001). Results indicate that populations may be established by single invaders or multiple invaders. As well, the likelihood of multiple introduction events appears to be greater in areas with greater human activity. If this result is borne out by our continuing research, it would suggest that measures should be taken to reduce human activities associated with greater earthworm introduction rates in Alberta's boreal.

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EFFECTS OF THE INVASIVE EXOTIC EARTHWORM AMYNTHAS AGRESTIS ON NATIVE TAXA: ARE SPECIES INVASIONS A THREAT TO BIODIVERSITY?

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Invasion by exotic earthworm species is a well-established issue in ecology. While the effects of invasions in regions without a native earthworm fauna are well documented, quantitative information about the effects of invasion in regions with a native earthworm fauna is sparse. Since the first reports of these invasions in North America (Eisen 1900, Smith 1928) numerous studies have documented exotic species dominating in locations that were once dominated by native species. No studies have been able to document extirpation of native fauna due to exotic earthworm invasion.

One reason for this may be the paradigm that currently dominates the issue of earthworm invasions in North America. In this theory, which Kalisz and Wood (1995) use to explain exotic and native earthworm distribution patterns, native earthworms are lost due to disturbance before the exotics invade. This disturbance theory predicts that exotics will dominate in disturbed areas and native fauna will dominate in relatively undisturbed areas. This situation does not allow for examination of the effects of invasions on native fauna, because under the current paradigm exotics and natives are not thought to interact.

A recent invasion of *Amynthas agrestis* from a roadside edge of the Great Smoky Mountains National Park (North Carolina and Tennessee, USA) appears to fit into the current paradigm, as the roadside would be certainly be considered disturbed. However, this invasion presents the opportunity to move beyond the paradigm as the invasion proceeds into undisturbed forest. We present preliminary results from a study in which we examine changes in biodiversity of the earthworm community due to *A. agrestis* invasion. We also characterize the movement of the invasion front and use this data to begin to develop a model predicting future spread of this invasive exotic earthworm species.

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Terrestrial earthworm variously reported from China number amount to 306 species with 86 of these, or 28%, non-natives for which 64% are Asiatic Megascolecidae and 21% are Lumbricidae of Holarctic origin. All the 306 species belong to 28 genera in 9 families (1 species of 1 genera in Haplotaxidae, 20 species of 2 genera in Moniligastredae, 245 species of 7 genera in Megascolecidae, 3 species of 3 genera in Ocnerodrilldae, 5 species of 3 genera in Acanthodrilidae, 3 species of 2 genera in Octochaetidae, 2 species of 1 genera in Microchaectidae, 25 species of 8 genera in Lumbricidae and 2 species of 1 genera in Glossoscolecidae). Number of species distributed in Sichuan Province is the highest in China, amounted to 94; Taiwan is the second, amounted to 80; Hongkong, 20; Macao, 3. Reports on earthworm in Shanxi and Ningxia Provinces have not been discovered. Among of them, 59 species are new species or new records after 1992. A defineitive work, however, on the systematics of Chinese earthworm is pending, and the purpose of this paper is only to provide a status quo to track changes from the last revison by Zhong et al. (1992). Subsequently, 240 or so new pheretima names were added by Xu, Qiu and Chang et al. in 1992-2005, most of them are consided valid taxa. While some controversial species had been discussed and new affinities had been given. Amynthas asiaticua (Michaelsen, 1900) was recorded but had no detail description and no specimen, so it was regarded a possible species in China. Chen (1959) named Amynthas carnosus (Goto et al, 1898) as Pheretima pingi for it had 3 or 4 pairs of spermathecae. However, Stephenson (1925) described *Pheretima pingi* as a new species for it had 4 pairs of mature spermathecae. While Goto and Hatai (1899) described Pheretima carnosus for it had 3 pairs of spermathecae. As presented by the description of Goto and Hatai, the spermathecae was vestigial, in additon, the distribution of genital papilla in the two species were completely different. That is, the two species are different species. So we separated Amynthas candidus (Goto et al, 1898) from Pheretima pingi (Stephenson, 1925). Gates named three species, Pheretima tenellula (Gates, 1932), Pheretima glabra (Gates, 1932) and Pheretima vieta (Gates, 1936). Then he combined the three specied into one species in 1972, Pheretima glabra (Gates, 1932), though they were largely different in morphological characters, especially with or without spermathecal pores, the position of papillae in male pores. We examined many specimens from Yunna Province of China by morphological characters, and some of them were wholly identical with Amynthas tenellulus. So we redivided the species into three species, Amynthas tenellulus, Amynthas glabrus and Amynthas vieta. For such uncertainties species, morphological methods and molecular (RNA, DNA) techniques will be combined to determine specific affinities.

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BIODIVERSITY OF EARTHWORMS IN DIFFERENT ECOSYSTEMS OF WESTERN GHATS, TAMIL NADU, INDIA

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Lying between 8° and 21° N latitudes, the Western Ghats, a global 'hottest' biodiversity 'hotspot' are a chain of mountains running along the western edge of the Indian subcontinent, and support one of the major Tropical Evergreen regions in India with exceptional biodiversity and conservation interest. They cover an estimated area of about 160,000 km² and stretch for about 1,600 km from river Tapti south to the southern tip of the peninsula, interrupted only by the 30 kilometers Palghat Gap at around 11° N. The present studies are aimed at assessing earthworm diversity in a region north of Palghat Gap (close to Nilgiri Hills), which is increasingly affected by the human activity. The study sites include a natural forest, an agro-ecosystem and a polluted area in Coimbatore Forest Division, Western Ghats, Tamil Nadu, India

As a result, 40 species of earthworms from different habitats are recorded. Their family wise composition is: 13 species of the Megascolecidae, 10 species of the Moniligastridae, 5 species of the Octochaetidae, 4 species each of the Lumbricidae and Ocnerodrilidae, one species each of the Acanthodrilidae, Almidae, Eudrilidae and Glossoscolecidae. Of these, 22 species are the natives (including *Drawida* sp. nov. and *Megascolex* sp. nov.; to be described somewhere else) of the Indian subcontinent, and the remaining are well known peregrine species of extra Indian origin.

In the natural forest, 18 native and 10 exotic species are found. Native species are represented by *Drawida caenosa*, *D. grandis*, *D. impertusa*, *D. modesta*, *D. pellucida pallida*, *D. sulcata*, *Drawida* sp. nov., *Glyphidrilus annandalei*, *Lampito mauritii*, *Malabaria biprostata*, *Megascolex cochinensis*, *M. filiciseta*, *M. insignis*, *Megascolex* sp. nov., ? *Argilophilus variabilis*, *Perionyx ceylanensis*, *P. excavatus*, and *sansibaricus*, whereas exotics belong to *Amynthas corticis*, *Allolobophora parva*, *Dendrodrilus rubidus*, *Eisenia fetida*, *Dichogaster bolaui*, *D. modiglianii*, *Eukerria kukenthali*, *Gordiodrilus elegans*, *Octolasion tyrtaeum*, *Pontoscolex corethrurus* respectively.

Agro-ecosystems harbour low earthworm diversity in comparison to natural forests but have high proportion of exotic species; 6 native (*Drawida aculeata, D. grandis, D. lennora, D. scandens, Megascolex konkanensis, Lampito mauritii*), and 12 exotic species (*Amynthas corticis, Dichogaster affinis, D. annae, D. bolaui, D. saliens Gordiodrilus elegans, Metaphire houlleti, Polyphretima elongata, P. taprobanae, Pontoscolex corethrurus, Ocnerodrilus occidentalis* and *Eudrilus eugeniae*). In polluted area only one native peregrine species *Lampito mauritii* and 2 exotic species namely *Dichogaster affinis* and *D. bolaui* are found.

Macro and micronutrients of the soil have also been analysed and are discussed in relation to occurrence of earthworms in different habitats. This study indicates that the land use pattern is more severely reflected on biodiversity of earthworms.

BIODIVERSITY OF THE SOUTH AFRICAN MEGADRILE EARTHWORM FAUNA

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Data on present knowledge on biodiversity of megadrile earthworm fauna of South Africa are elaborated. Information on indigenous Microchaetidae and Acanthodlinae, and representatives of Hormogastridae and Ocnerodrilidae recorded in South Africa are given. Species limited ability of dispersal results in a high degree of speciation, and a large number of endemics with restricted distribution ranges is emphasized. Ecological and geographical indigenous species dispersal is highlited. Introduced species as a foregin component in the soils are indicated. Present knowledge on a number of introduced species and their distribution in the country is outlined. Co-existence of indigenous species with exotic element is observed.

The role of protected biotops for preservation of indigenous earthworm fauna is indicated. Conservation of indigenous environment is recommended.

EARTHWORM COMMUNITIES ALONG AN ELEVATIONAL AND CLIMATIC GRADIENT IN NORTHEASTERN PUERTO RICO

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Most of the ecological studies of earthworms in Puerto Rico have been performed in the tabonuco (Dacyodes excelsa) forest; where earthworms are the most abundant group of the soil fauna in terms of biomass and they are important determinants of the decay rates of litter. In this study, we describe 1) earthworm communities along an elevational / climatic gradient of eight forest types in north-eastern Puerto Rico, and 2) relate their abundance, biomass, diversity and developmental stage to climatic, soil physical / chemical properties and /or other biotic characteristics (microbial biomass, root biomass / length). A network of 24 sites was selected for this study (3 sites and 8 forest types) that include: cloud, colorado (Cyrilla racemiflora), palm (Prestoa montana), tabonuco, lowland moist, *Pterocarpus*, and mangrove forests. The gradient compromises 1) a range of 1500 - 5000mm of rainfall and 2) a 5 °C difference in air temperature from the top to the bottom of the Luquillo Mountains. Up until now, 14 worm species belonging to 6 families have been found along the gradient. The density and biomass of worms were higher in the Pterocarpus and cloud forests as compared to the other forest types. In the Pterocarpus forest, only immature individuals of a single species were found. The cloud forest had the highest number of species and presence of native worms. While, the lowland moist forest had the highest presence of exotic worms. The total biomass of worms was significantly and positively correlated to rainfall, soil bulk density and ground litter depth. Meanwhile, the diversity of earthworms was negatively correlated to the air temperature and microbial biomass.

TAXIC DIVERSITY AND DISTRIBUTION OF MICRODRILE OLIGOCHAETES (ENCHYTRAEIDAE, TUBIFICIDAE, NAIDIDAE) IN SECONDARY FORESTS AND PASTURES OF THE MATA ATLÂNTICA (PARANA, BRAZIL)

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Microdrile oligochaetes (Enchytraeidae, Naididae, Tubificidae) were sampled in young, medium and advanced secondary forests and pastures under different soil conditions in the Brazilian Mata Atlântica (coastal plain of the Serra do Mar, "Reserva Natural do Rio Cachoeira"; main soil types: Cambisol, Gleysol; natural vegetation: "Floresta Ombrófila Densa Submontana"). Aim of this pioneering work was to register taxic diversity - so far completey unknown - and to identify factors that explain the distribution and abundance pattern of the species, in order to contribute to the development of a biological soil classification system for the region.

Abundance was low (1000-6000 ind./m²), but diversity was high (ca. 40 taxa altogether). The enchytraeid fauna is dominated by species of the genera *Hemienchytraeus* (ca. 6 species), *Guaranidrilus* (ca. 20 species) and *Achaeta* (ca. 6 species), most of them new to science. Their description requires a large amount of basic taxonomic and revisionary work on the genus and species level. Morphology may prove insufficient to separate and sort all species. Therefore, a pragmatic species concept is favoured here. Rates of endemism cannot be given because the enchytraeid fauna of South America is poorly known. Naididae (genus *Pristina*) and Tubificidae (genus *Bothrioneurum*) are a common component of the soil fauna. These two families can no longer be regarded as exclusively aquatic. Microdrile oligochaete species distribution patterns seem to respond to successional stages. For example, *Fridericia* was found only in pastures, suggesting that in this area the genus is related to land use. We further found soil-type related differences in enchytraeid species number and species composition.

EARTHWORM POPULATIONS IN ATLANTIC FORESTS WITH ARAUCARIA ANGUSTIFOLIA IN THE CAMPOS DO JORDÃO STATE PARK, SÃO PAULO, BRAZIL.

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Araucaria angustifolia, also known as the Paraná Pine is an endangered tree species in Brazil. Forests with predominance of this pine have been reduced to around 3% of their original extent, and are mostly restricted to South and Southeastern Brazil. Little is known of the diversity of soil animals inhabiting these forests and their role in soil properties and processes. As part of a larger project on biodiversity in araucaria forests, the present study was therefore set up to evaluate the density, diversity and biomass of earthworms in natural and reforested A. angustifolia forests, impacted or not by fire and identify the most efficient method to collect earthworms in these sites. The study was conducted in the State Park of Campos de Jordão, in Campos de Jordão, São Paulo, Brazil, at approximately 1560m elevation. The four areas studied included: 1) native forest with araucaria in climax, without little anthropogenic disturbance (NF); 2) A. angustifolia reforestation, planted in 1959 (R); 3) A. angustifolia reforestation planted in 1959, but submitted to an intense accidental fire in July 2001 (RF); and 4) native grass pasture (grazed by animals) with native araucarias and submitted to an intense accidental fire in September 2004 (NPF). In each area a 0,3 ha plot was chosen, in which five samples were taken to evaluate earthworm abundance, biomass and diversity. In August 2005, three sampling methods were tested: a) dillute formol (0,5%), b) monoliths of 25 x 25 cm to a depth of 0-30 cm and c) monoliths of 40 x 40 cm to a depth of 0-30 cm. Six earthworm species were found: the native species Glossoscolex sp., Glossoscolex sp1, Glossoscolex sp2, Glossoscolex bondari and Urobenus brasiliensis and the exotic species Amynthas corticis. The density, diversity and the biomass of earthworms were affected by presence of fire and animals (RF and NPF), and by the sample method used. Formol was more efficient for collecting Amynthas corticis, found in much higher abundance and biomass in NF than the other studied areas (using this method). Larger manually sorted samples were more efficient for capturing Glossoscolex spp., prevalent in RF. Total earthworm abundance ranged from 0 to 16 individuals per m^2 and followed the general decreasing order of importance RF>NF>R>NPF, although the order depended on the collection method employed. For adequate characterization of earthworm abundance and biomass in these forests, both formol and manual sorting should be employed, to guarantee collection of the rapid surface-soil dwelling Amynthas and Urobenus, as well as the endogeic Glossoscolex spp.

COSMOPOLITAN EARTHWORMS– AN ECO-TAXONOMIC GUIDE TO THE PEREGRINE SPECIES OF THE WORLD'' VOL. 2.

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"The importance of taxonomy is clearly recognized by the majority of scientists and without reliable taxonomy, ecological studies are irrelevant." Dominguez, J. et al. (2005). Pedobiologia (Vol. 49: 82).

An updated and upgraded version of "*Cosmopolitan Earthworms*" is newly presented. This Guide to the biology, ecology, and identification of 120+ exotic species found most commonly around the Globe is in CD format. All World families are revised. The original, Blakemore (2002) - first introduced at ISEE7 Cardiff, is now recommended text for species determination by ISO/FDIS 23611 "*Soil quality - Sampling of soil invertebrates*". The format and recommendations of the International Code ICZN (1999) are followed.

Starting with an introduction to the basics of study and sampling methods, taxonomic keys ultimately lead to detailed Family/Genera/Species descriptions, distributions, and illustrations. Included are the commercial vermi-composting species e.g., *Eisenia fetida / andrei, Perionyx excavatus, Eudrilus eugeniae, Dichogaster spp, Dendrobaena / Dendrodrilus*, and various pheretimoids such as *Amynthas* and *Metaphire* spp.

The Guide provides a useful tool for soil ecologists, managers, and students of biodiversity anywhere. Identification is a first step to understanding and, once the exotics are recognized, their global distributions and rates of spread can be plotted, and studies on relationships with native species affecting symbioses and soil processes can progress.

Blakemore, R.J., (2002; 2006). *Cosmopolitan Earthworms – an Eco-Taxonomic Guide to the Peregrine Species of the World*. Pub.: VermEcology, Australia. (Pp. 580 including 120+ figs. IBM CD-Format. Vol. 2).

[ISO 23611-1:2006 Recommended text http://www.iso.org/iso/en/CatalogueDetailPage.CatalogueDetail?CSNUMBER=36914&sco pelist=ALL

See also http://bio-eco.eis.ynu.ac.jp/eng/database/earthworm/].

REVIEW OF FAMILY RELATIONSHIPS OF THE CARIBBEAN GENUS EXXUS GATES, 1959 (OLIGOCHAETA: EXXIDAE)

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The Caribbean earthworm family Exxidae, now containing eight or nine species, was initially proposed to accommodate the "troublesome" *Exxus wyensis* Gates, 1959, a meroic species with an acanthodriline arrangement of male and prostatic pores that complied with the Octochaetidae except for its racemose prostates. Gates (1959: 258) had allowed *Ex. wyensis* to introduce an acanthodriline state into his redefinition of the Megascolecidae, whereas other authors later allowed it to introduce racemose prostates into the Acanthodrilidae. Neither of these schemes was acceptable, and now Exxidae is separated from Octochaetidae *sensu* Blakemore, 2000 primarily by its non-tubular prostates, and secondarily by having two or three oesophageal gizzards. Both Octochaetidae and Exxidae differ from Acanthodrilidae by their non-holoic nephridia. Possibly closely related Neotropical genera are a revived Diplocardi-inae/idae Michaelsen, 1899 (especially those taxa reported with non-tubular prostates) and meroic Benhami-inae/-idea *sensu* Csuzdi, 1996.

A possible junior synonym of *Exxus* is *Neotrigaster* James, 1991 with type *Trigaster rufa* Gates, 1962 that differs mainly in its 3 gizzards [in 5-7 (James, 1991: 348) or 6-8 (Borges & Moreno, 1992)]. Other included genera, in part – not type species, are *Zapatadrilus* James, 1991, *?Trigaster* Benham, 1886, *Cubadrilus* Rodriguez & Fragoso, 2002 (and, doubtfully, Queensland *Torresiella* Dyne, 1997).

Of the names combined in *Neotrigaster* by James (1991), only two belong in Exxidae, the third species, *Trigaster yukiyui* Borges & Moreno, 1991 with tubular prostates, probably requires returning to its original genus in the family Octochaetidae or Benhami-inae/idae. Newly added here are species from the genus *Zapatadrilus* that were recently transferred to *Cubadrilus* as well as other species from this latter genus that have "racemose" prostates (not the type); these appear to merit new combination in *Exxus*. An unauthored taxon, *Trigaster setarmatus* (sic), is tentatively added to Exxidae, and several similar species from Cuba reported by Rodriguez & Fragoso (2002) to be in preparation would possibly also belong.

EARTHWORM PHYLOGENY BASED ON MOLECULAR EVIDENCE

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Traditionally, phylogenetic relationships within Lumbricidae have been investigated mainly using morphological characters. Although the recent use of molecular tools in metazoan phylogeny has rearranged our traditional understanding of animal classification, these techniques have not been yet fully exploited in earthworm evolutionary biology.

Here we aim to use molecular phylogenies to contrast the traditional morphological classification within *Lumbricidae* and to understand the evolution of several morphological characters. We have obtained the DNA sequences of the mitochondrial COI and 16S genes for thirty-three earthworm taxa belonging to the families *Lumbricidae*, *Glossoscolecidae*, *Megascolecidae*, *Acanthodrilidae* and *Eudrilidae*. Preliminary results suggest that the current classification of different earthworm species needs revision. Results will be discussed in relation to several morphological diagnostic characters and the phylogenetic positions of the taxonomic groups evaluated.

DIVERSITY AND DISTRIBUTION OF EXOTIC AND INVASIVE EARTHWORMS IN BRAZIL

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Of the 306 known earthworm species in Brazil, 46 (15%) are exotics, introduced from other countries or continents. They belong mainly to the families Megascolecidae (11 spp.), Acanthodrilidae (12 spp.) and Lumbricidae (13 spp.). The remaining species belong to the Eudrilidae (2 spp.), Almidae (1 spp.) and Ocnerodrilidae (7 spp.) families. Many megascolecids (Amynthas or Metaphire spp.) and some acanthodrilids (espec. Dichogaster spp.) are found throughout the country, while other acanthodrilids (mainly Microscolex spp.) and the lumbricids (except Eisenia fetida & E. andrei that are used in vermiculture) have a more restricted distribution, being found only in the colder part of the country, where the subtropical climate is more conducive to their activities. Amynthas gracilis, the most widely distributed megascolecid in Brazil, was described from specimens of Rio de Janeiro by Kinberg (1867). This species, however, probably arrived much earlier, with the beginning of the exchange of plant and soil material between Asia and the New World. Pontoscolex corethrurus, although native to N Brazil, is the most widespread earthworm species in Brazil, and must be considered an exotic (and often invasive) species throughout most of the country. Most exotic species inhabit mainly disturbed habitats, generally close to human habitations, although they are sometimes found in native forests and grasslands. However, little is known of the effects of their invasion on the soil and its function, and on the populations and activity of soil organisms. In agroecosystems, the effects appear to be mostly positive, but in native ecosystems, the net effect of invasion may be negative. The study of the effects of exotic invasive species on soils, ecosystem function and biodiversity in Brazil are an urgent necessity, considering the extent of invasion, and the increasing human pressure on land use and natural resources.

EARTHWORMS OF AN URBAN CEMETERY

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Cemeteries often present large grass-covered spaces within urban settings. This work set out to obtain data on the earthworms associated with a cemetery in the centre of the city of Preston. Objectives were to unearth which species were present and in what numbers, but more directly to ascertain the status of *Lumbricus terrestris*. This species is of interest as it is reputed to be able to burrow to a depth of 2- 3 metres (Darwin, 1881). Therefore in the given setting, the relationship of such burrows with graves was something to explore, as it has been the subject of folklore for centuries.

General sampling employed a number of methods, such as digging and hand-sorting of soil and mustard vermifuge extraction. Two parts of the cemetery, one older with burials back to Victorian times and the other, brought into use since the 1950s, were both examined. For legal and moral reasons no sampling took place upon or immediately beside any point of human burial. *L. terrestris* burrows were examined following the resin cast method of Shipitalo and Butt (1999) with acrylic paint added for clarity.

Nine relatively common species of earthworms were located. Sampling in September, *L. terrestris* burrows were found to a depth of 0.5 m in the old cemetery in a clay soil, water-logged at depth. These burrows were of the type normally associated with this species, i.e. near vertical and little branched. In the new cemetery *L. terrestris* burrows were very different in form. Greatest burrow depth was to 0.3 m and these were often branched, with a mean of 1.2 divisions below 0.1 m (max. = 8). Nevertheless the mass of the adult animals taken from both sites did not differ significantly.

The branching nature of the burrows was caused by a limited soil layer in the new cemetery. Here sand was extracted historically and the void filled with refuse and rubble before covering with a tough impervious "concrete" layer. This prevented earthworm burrows extending beyond it. The "soil" above was littered with building rubble and made creation of vertical burrow difficult. However *L. terrestris* was able to colonise and survive in this area, demonstrating a remarkable plasticity in burrowing behaviour.

Poster 25 BIOGEOGRAPHY OF THE GENUS DIPLOCARDIA WITH REFERENCE TO THE CALCIFEROUS GLAND

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Although proximate function of the calciferous gland in earthworms is generally well understood, the evolutionary and ecological significance of this gland is still a matter of some debate. The earthworm calciferous gland is manifested in different ways with respect to its complexity and activity, ranging from non-existent to simple evaginations of the esophageal wall to large lamellate vascularized structures.

The North American earthworm genus *Diplocardia* may provide an opportunity to examine biogeographical trends in calciferous gland variation. The *Diplocardia* are especially suitable for this analysis because they have not been reported to be anthropochorous (unlike many European, Asian, and African earthworm taxa), and therefore their present distribution should primarily reflect the influence of evolutionary forces and not anthropic forces.

Using data from original descriptions and other published records, we examine the geographic distributions of all known species within the genus *Diplocardia*, with reference to calciferous gland form and environmental characteristics that might exert selective pressure upon its structure and function. This information is used to develop an ecological framework to explain the occurrence and functional significance of the calciferous gland in this North American genus.

The 8th International Symposium on Earthworm Ecology $4^{th} - 9^{th}$ September Kraków, Poland

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An investigation of the spatial distribution of earthworms across a dune ecotone along the Sefton Coast, Lancashire, NW England, was undertaken to examine species distribution and abundance with respect to coastal accretion, soil physico-chemical conditions and management. Replicated 0.1 m^2 quadrats were taken every 50 m along two transects crossing successional areas from beach strand line to established soils (700 m). In addition, more detailed samples were taken every 10 m where earthworms were first encountered on one transect. A translocation of *Aporrectodea longa* was undertaken in the field to assess the ability of this species to live in soils with a high sand content. In addition a laboratory experiment was set up to test the growth, maturation and survival of this species in soils with a range of sand contents. Treatments used juvenile *A. longa* kept in soils produced by mixing collected sand from the Sefton coast with commercially available loam in combinations of 100, 75, 50, 25 and 0 percent sand.

Within the system examined, a total of 9 earthworm species were encountered. As expected, no earthworms were found within the yellow dunes near the shore, where organic matter (OM) content was negligible (<1%). Earthworms, represented by *Dendrobaena octaedra* and *Lumbricus rubellus*, were first located 300 m from the strand line in grey dunes with an OM content of 3.9 %. Within a dune slack (OM content 11 %) at 350 m, the green morph of *Allolobophora chlorotica* was found as was *Lumbricus castaneus*. Anthropic influences (car park construction) along one transect revealed *Aporrectodea longa*, *A. caliginosa* and *Lumbricus terrestris* at 400 m. Within pine trees planted as a shelter belt (700 m), both *L. castaneus* and *L. rubellus* were present. Neither translocated *A. longa* did extend into the dunes from the parking area half way (25 m) towards the translocation point. Laboratory growth of *A. longa* demonstrated no significant increases in growth (2g to 2.75g) in 0, 25 and 50 % sand, to 16 weeks, but higher sand content produced significantly lower biomasses (p<0.05), less maturation and zero cocoon production.

Earthworm distribution was influenced by dune successional stage. Soil OM content, moisture content and human activity also had a bearing on earthworm number and species present. Laboratory findings of *A. longa* tolerance to sand content of soil were not supported by field observations and experiments.

EARTHWORM DIVERSITY IN THE RUBBER PLANTATION (HEVEA BRASILLIENCIS VAR RRIM 600) OF TRIPURA (INDIA).

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This study presents a survey of earthworm species and their ecological characteristics from the west district of Tripura, a north-eastern state and bio-diversity hot spot of India. Earthworms were sampled by digging and hand sorting method. Temperature, moisture, pH, Oxidizable organic matter and soil texture of 0 – 15cm soil samples were estimated at each sampling site. Following ten species of earthworms belonging to five genera and four families were recorded: Megascolecidae (*Metaphire houlleti*), Moniligastridae (*Drawida nepalensis, Drawida* sp.1, *Drawida* sp.2), Octochaetidae (*Dichogaster bolaui, Dichogaster affinis, Eutyphoeus assamensis, Eutyphoeus gigas, Eutyphoeus comillahnus*) and Glossoscolecidae (*Ponotoscolex corethrurus*) etc. Such a study is necessary with a view to utilizing earthworm resources in vermitechnology.

The 8^{th} International Symposium on Earthworm Ecology $4^{th} - 9^{th}$ September Kraków, Poland

REDESCRIPTION OF ALLOLOBOPHORA DUGESI V. GETICA POP, 1947 AND ITS ALLOCATION TO THE GENUS CERNOSVITOVIA OMODEO, 1956 (OLIGOCHAETA LUMBRICIDAE)

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The earthworm species Allolobophora dugesi var. getica was described by Pop (1947) examining an adult and a subadult specimen from the Tarcu Mountains, Romania. Zicsi (1982) on the basis of literature data raised the varietas to species rank. Allolobophora getica has not been recollected for a long time, until Mršić and Šapkarev (1987) and Mršić (1991) investigated a rich material from Serbia and observed that the specimens they identified as *getica* have the male pore on the clitellar segments. Accordingly, they moved A. getica to the genus Cernosvitovia. They thought that this placement is confirmed by the description of Pop (1947) who was cited as writing: "stated that the male aperture was not visible" (Mršić & Šapkarev 1987: 72.) therefore it might have been on the clitellum as well. Later, Zicsi & V.V. Pop (1991) and Zicsi (1995) considered that Mršić & Šapkarev (1987, 1988) misinterpreted the original text and the following descriptions because Pop (1947: 8) really wrote: "Les pores males sont situes sur les papilles glandulaires" and later (Pop 1948: 60): "Porii masculi incunjurati de arii glandulare" (in Romanian = male pores surrounded by glandular areas). Both sentences mean that the male pores are surrounded by glandular papillae and implicitly visible on segment 15. This led Zicsi (1995) to reset the species to the genus Allolobophora. During a survey of the Pop collection kept in the Zoological Museum of the Babes-Bolyai University, Cluj we discovered the type specimens of Allolobophora getica. A detailed examination of the adult type specimen revealed that the ventral setae of segment 15 are modified to genital setae and those from the setal line b are missing. Only a deep hole, leading to the setal sac could be recognized. This might misled Pop to consider it as a male pore. Dissecting the adult specimen more backwards, it was easy to see that the male duct is running as far as to the clitellum. Consequently, A. getica must be returned to the genus *Cernosvitovia*. Since in the type series there is only one adult specimen, we designate it as lectotype. A new and more complete description is given.

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Poster

EARTHWORMS OF THE CHIHUAHUAN DESERT ECOREGION

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The Chihuahuan Desert stretches from the Rio Grande Valley in southern New Mexico and the San Simon Valley of southeastern Arizona to an area just north of Mexico City. It is approximately 1300 km long and 400 km miles wide. Because of its position in the interior of the continent and its relatively high elevation, ranging from about 600 m to around 1675 m, this desert tends to have hot summers and cool to cold winters. All areas are subject to occasional winter frosts, and northern portions regularly receive hard freezes. The northern portion of the desert in the USA extends into southeastern Arizona, southern New Mexico, and Trans-Pecos Texas and is an area of high elevations with a range between 1000 and 1300 meters. Rainfall varies with elevation, where lower areas receive less than 200 mm per year and higher elevations over 300 mm per year.

The Chihuahuan Desert Ecoregion (CDE) includes not only the desert itself, but also the highland "islands" surrounded by desert and, to a degree, bordering montane areas that are isolated by surrounding, non-forested lowlands. The CDE encompasses some 70 million hectares. The northern region of the CDE (NCDE) supports more than 120 species of mammals, 450 species of birds, 110 species of fish, and more than 170 species of amphibians and reptiles. Among invertebrates, numerous insect and other arthropod species have been described but reports of earthworms from soils associated with the NCDE are rare. Current literature on the biogeography of earthworms commonly dismisses the association of earthworms and desert soils.

In March 2003, the native earthworm species *Bimastos heimburgeri*, *B. longicinctus*, and *Diplocardia minima* were collected by handsorting from the NCDE. *B. longicinctus* occurred in high numerical density and was collected from soil of a north-facing arroyos (shrub-scrub land cover). In 2004 the NCDE in the USA received up to three times the normal annual precipitation that extended beyond the usual July to October wet season. As a result, in March 2005 earthworms were collected by handsorting from a variety of habitats within the NCDE, with several of the soils classified as Aridisols based on characteristic desert vegetation. Range extensions with habitat descriptions are reported for two species of *Bimastos* (Family Lumbricidae) and one species of *Diplocardia* (Family Acanthodrilidae). Several new species of *Diplocardia* are reported for the first time. Among exotic invasive earthworms, range extensions are reported for several Palearctic species (Family Lumbricidae), but none for Oriental or Neotropical species commonly reported in other regions of North America.

EXOTIC EARTHWORMS AS INDICATORS OF DISTURBANCE IN ATLANTIC FORESTS OF SÃO PAULO, BRAZIL

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Exotic earthworm invasion into a native forest ecosystem can cause major changes to the soil, its functioning and local plant, animal and microbial biodiversity. Little is known, however, of the extent of exotic species invasion in Brazilian forest ecosystems, and the potential effects on the soil. In the present study, the effect of anthropogenic disturbance on the presence of surface-active native and exotic earthworms was assessed in two areas of the Atlantic Rain Forest State Park in the State of São Paulo, Brazil. The earthworms were sampled from November 2004 to May 2005, using 30 pit-fall traps placed in forest areas with or without (recent) human disturbance (five traps and six transects per area). Three earthworm species were collected, belonging to two families, Megascolecidae (Amynthas sp., probably A. gracilis) and Glossoscolecidae (Pontoscolex corethrurus and Glossoscolex sp.), totaling 58 individuals overall. In the disturbed area, a significantly ($\chi^2 = 42.6$, p< 0,0001) larger number of earthworms were collected, totaling 53 individuals (91% of total), all of them exotic species (51 Amynthas and two P. corethrurus). In the well-preserved forest area, only five individuals were collected, four Amynthas sp. and one native Glossoscolex sp. The invasion of the exotic species, particularly Amynthas in the disturbed area, is likely due to (among other factors) rapid movements, frequent rising to the soil surface, reproductive strategy (parthenogenesis, small, light-weight cocoons), and ecological plasticity, which permit this species to adapt to and rapidly colonize new areas, even sites relatively unfavorable to native species.

EARTHWORM DISTRIBUTION IN SELECTED ISLANDS OF CENTRAL PHILIPPINE ARCHIPELAGO

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Prior to the works of James (2005, 2004), no systematic collection and taxonomic work has been done on Philippine earthworms.. This work represent the initial findings of the second phase of the project Philippine Terrestrial Annelids Biodiversity Survey that primarily aims to document annelid faunas of the main islands of the Visayas. It includes collection from sixty seven sites involving ten islands representing various biogeographic regions such as Greater Negros-Panay, Greater Mindanao, Siquijor, and Camotes.

Collection sites were chosen based primarily on the Key Conservation Sites identified by Haribon Foundation (Mallari *et al.*, 2001) and the Philippine biodiversity conservation priority areas (Ong *et al.*, 2002).

Earthworms were collected by digging moist blocks of soil and hand sorting them. Photographs of live specimens assumed to be distinct species based on morphological differences were taken. Samples were preserved using phosphate-buffered formalin overnight then drained and replaced with 80% alcohol for storage. Duplicate sets of field-differentiable "species" were preserved in 95% ethanol for DNA extraction. GPS (Magellan) determined latitude, longitude and elevation data were recorded.

Preliminary identification of genera involved determination of the location and number of spermathecae, external markings, and other salient features from the dorsal dissection. Taxonomic keys developed by James (unpublished, 2005, 2004) were used.

Initial results show that the genus *Pheretima* seems to be widely distributed. Diversity of the genus is also high, reaching up to ten possible species in one of the sites in Salvador Benedicto. *Pheretima* is also diverse in other sites such as in Cuernos de Negros, and Madja-as, Antique. *Pleionogaster* seems to be more diverse in Cebu and Bohol though they are as widely distributed as the *Pheretima* group. *Archipheretima* is the least distributed, having been collected only in Samar. *Pithemera*, though present in Samar, Leyte, Cebu and Negros, their diversity and density is comparatively low based on the summary report submitted by James to the DENR in 2004. He suggested the possibility that Luzon is the original home of *Pithemera* owing to the high diversity discovered in the island.

Pheretima, Pleionogaster, Pithemera and *Polypheretima* seem to inhabit a very diverse vegetation type from deep native/primary forest to agro-forests. Data suggest that *Pithemera* and *Polypheretima* could have both exotic and native representative species while *Amynthas* and *Metaphire* could be native species as they were collected from deep native forests.

Vermicomposting is widely practiced in Negros and that *Eudrilus* which is very popular for such purpose has already found its way to the agro-forest strips of the protected areas. *Pontoscolex* proved to be highly invasive as it is present in all islands except Camotes, and its density is extremely high in Hinoba-an, which has already lost more than 95% of its forest cover due to intensive logging.

A total of nine genera were identified, seven belong to family Megascolecidae and two were highly invasive exotics (*Pontoscolex, Eudrilus*). Genus *Pheretima* showed the most diversity and is widely distributed, followed by the *Pleionogaster* group.

ARE NATIVE EARTHWORMS MAINTAINED IN PROTECTED FORESTS OF CENTRAL AND SOUTHERN MEXICO?

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In Mexico there are more than 100 natural protected areas which have been generally selected for the conservation of its biodiversity. It is often assumed that this protection will conserve all kind o animals including invertebrates. However the status of these animals and particularly of earthworms is not often known. In this study it is presented the current status of native earthworms in six natural protected areas (NPA) of Central and Southern Mexico. Earthworms were surveyed in two high altitude NPA of Pine-oak and fir forests (Central Mexico :Sierra Chincua and Rio Frio Mountains; over 2000 m of altitude); in two intermediate altitude NPA of cloud forests (eastern Mexico, El Cielo, 1100-1200 m; Southern Mexico, Montebello, 1480-1550 m); and in two lowland NPA of tropical forests (Southern Mexico, Calakmul and Lacandona reserves; 200-300 m).

Both native and exotic species were found in all these forests, although their domination varied in function of altitude and region. In the three NPA from Southern Mexico natives dominated over exotics, being Montebello cloud forests the only site where no exotics were found inside natural forests. In both tropical forests (Calakmul anad Lacandona) the ratio native spp/exotic spp was the same and relatively high (3.5). In the cloud forests of El Cielo and in the pine-oak forests of Chincua, similar numbers of native and exotic species were found (5 natives and 5-6 exotics). Finally the pine forests of Rio Frio eastern Mexico was the only site where no natives were found (9 exotics).

It is concluded that low and medium altitude NPA are effectively conserving native earthworms; at high altitudes, however, lumbricid exotics invaded the forests- in some situations even completely displacing native earthworms.

EARTHWORM COMMUNITIES FROM LOS TUXTLAS RESERVE: INFLUENCE OF LAND USE SYSTEMS AND LANDSCAPE CHANGES

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The diversity and abundance of earthworms in "Los Tuxtlas" Biosfere Reserve (southern Mexico) were studied. This region of volcanic origin, is now a mosaic of forests and agroecosystems. The study was carried out in three localities that differed by the amount of forested area: 1) López Mateos (LM): 75%, 2) San Fernando (SF): 50% and 3)Venustiano Carranza (VC): 25%. Four different Land Use Systems (LUS) were sampled in each one of the three localities: 1) Tropical rain forests (F), 2) Agroforestry plantations (A), 3) Pastures (P) and 4) Annual crops (C). In each locality, 8 sampling points per LUS were set, making a total of 96 sampling points. In each sampling point earthworms were sampled by three handsorted monoliths.

A total of 13 species of earthworms were found, seven natives and six exotics. The community was dominated by endogeic polyhumics (10 spp); only two epigeics and one endogeic mesohumic were found. The most common species was the exotic *Pontoscolex* .*corethrurus*, (66.6% of frequency). The number of exotic species was higher in VC, the more deforestated window and lower in SF, the intermediate deforestated window. Species accumulation curves indicate that more species should be found, mainly in agroforestry systems, pastures and annual crops.

The main conclusions of the study are: i) local and regional species richness was within expected values, ii) two expected native species were locally extinct, iii) earthworm communities have been modified both by intensive deforestation (domination of exotics in VC) and by local land management (unexpected numbers of native species in of SF pastures).

The 8th International Symposium on Earthworm Ecology $4^{th} - 9^{th}$ September Kraków, Poland

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Amynthas is the major genus in Japanese earthworm fauna. Many species of them show a wide variation in their reproduction organs, which are the key characteristics for taxonomic analysis. Because of their morphological variation, Amynthas worms are divided into many related species (more than 70); two most common worms are Amynthas hilgendorfi (Michaelson, 1892) (approximately 80% in wild population of ~10cm long) and A. corticis (Kinberg, 1867)(approximately 5%). Although A. corticis retains their male pores, more than 90% of A. hilgendorfi lost their male pores and many of them lost spermathecae as well. The incomplete gonad of A. hilgendorfi suggests the dysfunction of usual sexual reproduction in these animals, hermaphroditism. Thus, their taxonimic rationality and their reproductive strategy are still in discussion.

Molecular studies would help rationalize taxonomic analysis and reveal how the major species of wild A. hilgendorfi keep their population. Ribosomal RNA genes were PCRamplified from approximately 120 worms from the the Kanto plate, which belong to at least 7 morphological species of Amynthas worms with several worms from each species, and PCR products were directly sequenced. There was one variation for approximately 500-bps in the 5' region of 18S rRNA gene among worms, suggesting high sequence identity within the genus. The variation was observed only in a minor population lacking male pores. In contrast, mitochondrial 16S rRNA genes have more sequence variations. Molecular phylogenetic analysis was carried out using the NJ and method together with the sequences of Lumbricidae worms including Eisenia fetida and Lumricus rubellus. The analysis suggested that Amynthas worms are divided into two branches. The worms in the first branch constantly retain male pores and some kind of genital markings, but are a minor population in field. The first branch includes A. corticis, A. tamaensis, A. carnosus, and A. robustus. The second brach is a major in the wild population, most worms of them have neither apparent genital marking nor male pore. Th second branch can be divided into two sub-branches, one consists of A. hilgendorfi and A. agrestis and another consists of A. hilgendorfi and A. vittatus. The later sub-branch has a variation in 18S rRNA gene. Taken together, our results suggest that *Amynthas* worms have a wide variation in morphology, are genetically closely related, and can be divided into several groups. Molecular data combined with morphological observation can provide rational classification and further insight how they maintain their population even though the majority of them lost their hermaphroditism. The study was supported (partly) through a grant of LRI by JCIA.

SPATIAL DISTRIBUTION OF EARTHWORMS IN A SPRUCE FOREST IN CENTRAL RUSSIA

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Soil macroinvertebrates were studied in a Norway spruce (*Picea abies*) forest on graybrown podzolic soils near the city of Borovsk, approx. 100 km south of Moscow (Kaluga Region, 55°13' N, 36°22' E). The sampling was carried out in July 2000.

At the site, 144 intact soil cores were taken to a depth of 8-12 cm. Each core had an area of 76 cm². Sample locations formed a grid of 24x6 units in order to determine animal spatial distribution. Samples were placed into separate marked plastic bags and then hand-sorted in the laboratory. The soil pH was acidic (4.19), loss on ignition (LOI) was 6.01% and the water holding capacity 72.0%.

Six earthworm species (*Dendrobaena octaeda, Lumbricus rubellus, L. castaneus, L. terrestris, Allolobophora rosea* and *A. caliginosa*) were noted at the site. The latter genus constituted 73.5% of lumbricid population here. Abundance of earthworms was 187.6 ± 14.8 indiv. m⁻². They constituted about 65.5% of the total macrofaunal abundance. Spatial distribution of eartworms over the studied plot was heterogeneous: areas with 5-6 individuals per sample were neighboring those without animals. By joining adjacent sample units, the size of a sample was artificially increased in order to study soil factors influencing lumbricid distribution in the gray-brown podzolic forest soil. Correlation matrix of sample size vs. environmental variables indicated that sample mass (r=0.21) and pH (r=-0.23) were significantly influencing lumbricid distribution. The raise of sample size led to the increase of correlation value. In the gray-brown podzolic forest soil, the most representative shape of sample for earthworms' collecting is a square one.

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PHYLOGENY OF THE *PHERETIMA*-COMPLEX (OLIGOCHAETA: MEGASCOLECIDAE) USING rDNA ITS

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Various methods of analysis of DNA sequences have been used to elucidate phylogenetic relationships among animal groups. However few earthworm taxa have been studied at the molecular level. The following study attempted to shed a light on phylogeny of *Pheretima*-complex group Megascolecidae using ITS gene in rDNA. This gene is often selected in phylogenetic studies at higher taxonomic categories as well as within genus, species, and population. For 26 species of earthworms from 10 genera and 2 families, a stretch comprising the 3'-end of the 18S rRNA, ITS1, 5.8S rRNA, ITS2, and part of 28S rRNA was PCR-amplified with the primer pair ITS-1 CGC CCG TCG CTA CTA CCG ATT, ITS-2 GTC CCG AAC ACC ACA GTT CCC. Phylograms of 26 species were built with of MP(Maximum Parsimony), NJ(Neighbor Joining), and QP(Quartet Puzzling) on the basis of aligned sequences of the entire ITS.

a. All phylogenetic analyses yielded 5 groups in trees of similar topology. Genus Amynthas was also separated largely into two groups of Korean and Philippine origins. This outcome suggests that comparisons of Philippine and Korean Amynthas species based on morphological characters are likely to be misleading. b. Species grouped into a first clade were Amynthas jirensis, A. agrestis, A. gucheonensis, A. sopaikensis, A. bubonis, A. multimaculatus, A. koreanus, A. dageletensis, A. heteropodus, A. odaesanensis, Pontoscolex sp., Pheretima sp. 1, and Dendropheretima banahawensis. There was no particular disparity in molecular sequences of the species in this group resulting in difficult distance comparison, in spite of the very distant relatedness of Pontoscolex (Glossoscolecidae). c. Amynthas halconensis, A. isarogensis, A. mindoroensis, Pithemera sp. 2, Pithmera sp. 1, and Pleionogaster sp. clustered into one clade forming a second group. Species in this group were all collected in Philippine and not found in the Korean Peninsula. Apart from 3 Korean Amynthas species, 3 of those from Philippine all gathered in this group revealing the influence of differential geographic environment; morphologically heterogeneous but genetically rather homogeneous. Or say: This result indicates the need to re-examine traditional genus definitions, and to obtain more molecular data. d. Polypheretima sp. 1 and Polypheretima sp. 2 stayed closely together representing a separate monophyletic status apart from species in other genera. It appears that most significant cladogram could be obtained from this group by using analysis of ITS gene within the genus.e. Archipheretima sp. falls into the final group. The most distinctive characteristic in the genus is that the clitellum begins at the 13th segment, unlike species in Pheretima-complex where it begins at the 14th. This differential morphological characteristics seemed to lead the Archipheretima sp. branched away from others.

f. *Aporrectodea tuberculata* that has been selected as an outgroup, *Perionyx excavatus* also shows a long branches in the phylogram, which may explain their aparent close relationship.

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Arable land forms approximately a quarter of Estonian territory including pebble rendzinas (9.0% of Estonian arable land), typical brown soils (9.7%) and pseudopodzolic soils (15.1%). Three soil types differ in biological activity. Agricultural management practices affect soil biological and physical properties and habitat of soil organisms altering community composition of soil fauna. Earthworms have influence on decomposition and nutrient cycling in soils, modify soil structure, porosity and aeration, water infiltration and drainage. Their activities have consequences for tillage practices, retention and incorporation of plant detritus, the control of surface runoff and erosion. According to Edwards and Bohlen (1996) there are about 220 species of lumbricids, of which 19 are common in Europe. Thirteen species have been identified in Estonia including species from the genera Lumbricus, Allolobophora, Aporrectodea, Dendrobaena, Dendrodrilus, Eisenia, Octolasion and Eiseniella. Twenty four study areas of three most widespread soil types all over Estonia were selected. In each group of soil type, eight fields were selected for studies of earthworm communities. The mean abundance and fresh biomass of earthworms was the highest in pseudopodzolic soils (107.1±22.4 individuals m⁻², 47.7±14.2 g m⁻²) and lowermost in pebble rendzinas (47.9 \pm 11.3 individuals and 29.0 \pm 12.2 g per m⁻²) and typical brown soils $(73.0\pm15.1 \text{ individuals and } 29.5\pm10.8 \text{ g per m}^{-2})$. In arable soils the moisture 10-20% seems to be most suitable for earthworms, the mean abundance within this range was 68.4 ± 14.6 individuals m⁻². The mean abundance was 19.8 ± 12.8 individuals m⁻² by moisture less than 10%, and 44.0 \pm 16.5 individuals m⁻² by soil moisture more than 20%. It was assumed that pebble rendzinas are suitable habitats for earthworms but periodical drying out decreases the abundance of earthworms. In pseudopodzolic soils the abundance is high if there are optimal moisture conditions, due to increasing abundance of ecologically tolerant endogeic species. In typical brown soils the diversity of earthworms and number of juvenile individuals are high which indicates suitable conditions for habitat in this type of soil. The most tolerant to agricultural practice species Aporrectodea caliginosa was found in all studied fields. Other species were presented as follows: Aporrectodea rosea in 80% of fields, Lumbricus rubellus - 50%, Lumbricus terrestris 44%, Aporrectodea longa – 38%, Allolobophora chlorotica -32%. Three species (Lumbricus species Octolasion cyaneum is not common in Estonia but in some regions the abundance could be quite high, in this case it can be competitor for the most common species Aporrectodea caliginosa because of similar demand for ecological condition. The specific composition of a earthworm community is a good indicator of agricultural practice intensity in the field. The occurrence of species only like Aporrectodea caliginosa, Aporrectodea rosea, Lumbricus rubellus tolerant to disturbance is the result of intensive tillage and agricultural practice or the influence of strong limiting ecological factor (mostly too low moisture). A community including more sensitive species Lumbricus terrestris, Aporrectodea longa and Allolobophora chlorotica indicates more suitable agricultural or ecological factors for habitat.

MOLECULAR AND MORPHOLOGICAL ESTIMATES OF PHYLOGENY IN THE PHERETIMA GROUP OF GENERA (CRASSICLITELLATA: MEGASCOLECIDAE)

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The perichaetine meronephric Megascolecidae consist of the Pheretima group of genera and a few others. The Pheretima group is one of the most speciose among earthworms and is plagued by numerous synonymy problems and by inadequate taxonomic treatment of genera and species groups within genera. To start addressing the latter two difficulties I sequenced the 12s and 16s mitochondrial ribosome genes and the 28s nuclear ribosome gene from many Philippine and some peregrine species, collected morphological data from specimens and literature sources, and found additional relevant sequence data in GenBank. Sequences were aligned with CLUSTALX. Data were analyzed in MRBAYES after selection of sequence evolution models with MRMODELTEST and PAUP*analyses of these data showed several well-supported clades. The 28s nuclear ribosomal gene was too conserved for within-genus phylogenies, but was valuable for deeper branches.

The Pheretima complex is monophyletic in trees rooted with Dichogaster sp as the outgroup, and the sister group to the Pheretima complex was composed of Pontodrilus litoralis plus the Australian taxa Spenceriella and Heteroporodrilus. Within the Pheretima complex, Pleionogaster, Archipheretima and Dendropheretima are monophyletic. All other genera of the in-group appear to be paraphyletic or polyphyletic. The Australo-Papuan Begemius though morphologically close to Dendropheretima is not closely related, instead showing affinities to Pithemera. Pithemera and Polypheretima, are intermingled, suggesting that the acquisition or loss of intestinal caeca has happened more than once and therefore the presence of caeca cannot be assumed homologous across the perichaetine meronephric Megascolecidae. The species group within Pheretima s.s. defined by spermathecal pores in 7/8 only are probably monophyletic. The large octothecal darnleiensis group is clearly para- or polyphyletic, but is also diverse in species, in contrast to published statements placing all known included species in the synonymy of Ph. darnleiensis. There is good support for some geographical units within Pheretima and Pleionogaster. In other words, within a related set of taxa, those found in the same region of the Philippines are generally more closely related than to clade members from more distant locations. This suggests a strong role for geographical isolation in earthworm speciation.

THE USE OF 16S RDNA AND COI SEQUENCES FOR THE IDENTIFICATION AND PHYLOGENETIC ANALYSES OF *DIPLOCARDIA* (GARMAN, 1888) AND OTHER SOUTHEASTERN USA EARTHWORMS

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DNA sequence data for the genus Diplocardia (family Acanthodrilidae) has several potential applications. Unlike many other genera of earthworms where external characteristics are sufficient to identify species, a thorough dissection of specimens in the genus Diplocardia is required for species verification. Many of the Diplocardia species are less than 2 mm in diameter and necessitate tedious dissections under a microscope. Also, two or more species of Diplocardia can be identical in overall size and external features and nearly identical in their internal anatomy, making the challenge of species identification all the more difficult. For earthworm population studies, verification of species for sub-adult specimens is not possible since several couplets in *Diplocardia* keys are based on genitalia. There has been much recent interest from megadrile systematists to utilize DNA data to help parse earthworm phylogeny. The Nearctic earthworm genus Diplocardia has over 40 described species, yet DNA sequencing has not been thoroughly compared to classical systematics hypothesized for the genus. Since systematic questions still remain in higher-level earthworm taxonomy, such as families, it is not surprising that there are even more unresolved phylogenies for many earthworm genera.

In this research at least 15 earthworm species from the southeastern USA, most belonging to the genus Diplocardia, were collected and identified by traditional morphological methods. 16S rDNA and COI sequences were determined for each and 34 sequences were submitted to the NCBI database. According to both 16S rDNA analysis and morphological determinations specimens identified belonging to the genus Diplocardia formed a distinct group from specimens identified belonging to the family Megascolecidae (genus Amynthas and an unknown parthenogenic pheretimoid) and the family Lumbricidae (genera Lumbricus, Octolasion and Eisenia). A constructed phylogenetic tree based on these data indicates the genus Diplocardia has distinct genetic differences from other earthworm genera and identifies 16S rDNA species signatures for numerous specimens of Diplocardia collected from a variety of soils across the Gulf Costal Plain (Florida to Texas) in the SE USA. Among specimens collected from multiple states there is sometimes a dramatic size difference between adults of the same species. Through 16S rDNA analysis, microscopic species determinations for D. caroliniana (LA, MS, and TX) and D. pettiboneae (LA and AL) were confirmed for adult specimens that varied considerably in size. Gates (1977) separated all species within the genus Diplocardia into three groups. DNA sequencing corroborates the sexthecal group delineated by Gates, whereas the other two groups based on the presence or absence of calciferous lamellae are not supported by gene sequencing analysis.

EARTHWORM DIVERSITY FROM RIO GRANDE DO SUL, BRAZIL, WITH A NEW NATIVE CRIODRILIDI GENUS AND SPECIES (OLIGOCHAETA: CRIODRILIDAE)

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Earthworm taxonomy research has been practically absent in Rio Grande do Sul state of Brazil since the end of 70-ties of last century. Earthworm diversity literature for this state is also incomplete, scattered and outdated. This paper has the objective to present the state of knowledge of the earthworm diversity in Rio Grande do Sul state, south Brazil, and a description of a new native criodrilid genus and species. It includes data of a recent survey from 21 rice fields and 8 adjacent areas in Camaquã region. Samples from rice fields were taken from April to July 2004 after harvesting and samples from adjacent areas were taken in July 2004 and June 2005. Protocols of the Tropical Soil Biology and Fertility (TSBF) programme were applied, involving hand-sorting of 30 x 30 x 30 cm square soil monoliths. The sampled worms were fixed and killed directly in a formalin solution (5%). The list of earthworm diversity from Rio Grande do Sul was completed by reviewing all available literature regarding this state. Current earthworm diversity in this state shows a total of 37 species and 21 genera, belonging to 7 families. Glossoscolecidae is the most diverse (10 species) and represent 27% of the total fauna. Approximately 33% of the genera and 40.5% of the species are natives, while 67% of the genera and 59.5% of the species are exotics. Native species are dominated by Glossoscolecidae whereas exotic species are Lumbricidae and Megascolecidae. In this study, 9 species are new for the Camaquã region, 2 species are reported for the first time for Rio Grande do Sul state and a new native genus and species of criodrilid is described for science. The potential biodiversity of the state of Rio Grande do Sul is highlighted.

EFFECTS OF CROPPING AND TREE DENSITY ON EARTHWORM SPECIES DIVERSITY IN SOUTHERN CAMEROON

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<u>Abstract</u>:

Earthworms can have positive effects upon crop growth in the tropics. So if these soils are to be managed so that their biological capacity for nutrient cycling and maintenance of soil structure is retained then more attention should be paid to the effect of cultivation and cropping practices upon earthworms. When trees are removed from land-use systems, such as when land is cleared, slashed, burned and tilled in slash and burn agriculture, earthworm density, diversity and activity are reduced. Conversely retaining trees in systems may maintain earthworm populations during the cropping phase.

Here we assessed the impact on earthworm species diversity and densities of crop cultivation in the understorey of timber plantations thinned to two tree densities and compared this with uncropped, undisturbed timber plantation controls. The plots were reassessed after the end of the cropping phase to assess whether populations had recovered. The experiment was in southern Cameroon. Sampling was conducted, using the formol explusion method in a circular frame of 50cm diameter. The experiment was a randomised complete block design in four replicates.

Twenty-three earthworm species were recorded from Eudrilidae sub families Eudrilinae and Parendrilinae, Ocnerodrilidae and Octochaetidae, most of which were endemics. This included two new species from two new genera from the sub family Parendrilinae, one new species from one new genus of Ocnerodrilidae, two new species of Dichogaster and one new species of Legonodrilus. Twelve species were endogeic, ten epigeic and one anecic.

We found no significant differences in earthworm diversity and densities between the different tree density treatments. Gernerally earthworm densities were decreased by cropping compared to the undisturbed plantation control. The most abundant species was a *Legonodrilus* sp novum with average densities in the undisturbed control plantation of 68 individuals m-2, across sampling periods. Densities in cropped plots were reduced to 15% of the control densities, however, populations later recovered as densities during the fallow phase were not significantly different from those in the undisturbed plantation. The densities of the epigeic Octochaetidae were significantly reduced from 42 m⁻² to 12 m⁻² to 20% of the densities of the undisturbed plots and did not recover during the fallow phase. Two Dichogaster species, *Dichogaster kunguluensis* and *Dichogaster* sp. novum were eliminated by cropping and populations did not recover during the fallow phase. The effects of cropping were species-specific and more work is required to identify which of these endemics are the ecosystem engineers in the system.

EARTHWORM BIODIVERSITY IN VARIOUS LAND USE SYSTEMS IN THE REGION OF JAGUAPITÃ, PARANÁ STATE, BRAZIL.

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Little information is avaliable on earthworm populations and biodiversity in the State of Paraná, southern Brazil, where most of the native vegetation (predominantly Atlantic Forest) was transformed into agroecosystems over the last 50 years. In the present study, earthworm biodiversity was evaluated in various land use systems near Jaguapitã, Paraná, where pastures predominated. Samples were taken during the rainy and dry seasons of 2004 and 2005. Seven sites were sampled: old pastures (n = 2), newly established soybean (n = 2)2) and sugarcane (n = 2) fields and a native forest (secondary) fragment (n = 1). Twentyfive samples (25×25 cm soil blocks) were taken at each site using a grid pattern; 20 samples to a depth of 10 cm, and five up to 30 cm depth, located on the diagonal of the grid. Earthworms were manually sorted from the soil and preserved in formaldehyde (4%). Fourteen earthworm species were found: the exotics Pontoscolex corethrurus (Glossoscolecidae) (the dominant species), Dichogaster affinis, D. bolaui and D. saliens (Acanthodrilidae), E. saltensis and Ocnerodrilus occidentalis (Ocnerodrilidae), and Amynthas sp. (Megascolecidae) (aclitellate sub-adult); the native species Glossoscolex n. sp. and Fimoscolex n. sp. (Glossoscolecidae), Eukerria eiseniana, Belladrilus n. sp.1 and an unidentifiable Ocnerodrilidade n. sp.1 (probably a Belladrilus sp.), as well as two unidentified Eukerria spp. (of unknown origin) (all of the Ocnerodrilidae family). The study revealed that some native earthworm species can be maintained, even in degraded pastures, but that exotics tend to predominate. This is particularly the case in cultivated agroecosystems and degraded forests, where both earthworm abundance and species diversity tend to be lower than in pastures.

NEW DATA AND AN UPDATED VIEWPOINT ON THE EARTHWORM COMMUNITIES WITH THE *DENDROBAENA ALPINA* SPECIES GROUP (OLIGOCHAETA, LUMBRICIDAE) FROM THE SOUTH-EASTERN CARPATHIANS.

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Dendrobaena alpina (Rosa, 1884) has been considered to be the most characteristic lumbricid species of the coniferous belts from the South-Eastern Carpathians (V. V. Pop, 1987, 1992, 1997). New morphologic and molecular taxonomy researches (V. V. Pop, Csuzdi, Antonia Pop, Zicsi, 2005) proved that the Carpathian earthworm populations formerly attributed to D. alpina should be splited in four closely related, but different species, namely: Dendrobaena alpina alpina (Rosa, 1884), D. a. alteclitellata (Pop, 1938), D. attemsi (Michaelsen, 1902), and a newly described *Dendrobaena sp.* These species are characteristic for the so called "earthworm community with Dendrobaena alpina" found in rocky soils from the subalpine belt of Mugo pine and Juniperus nana, Vaccininium or Rhododendron scrubs and in the montane belt of fir tree or of mixed beech-fir forests. With less density and biomass, they also occur in the "community with Octodrilus compromissus - D. byblica" from acid brown soil under beech forests, and in the "community with O. friwaldszkyi" in rendzinas under beech forests. As a consequence of splitting the old Dendrobaena alpina in the above taxa, the structural patterns of the Carpathians earthworm communities should to be reconsidered and redefined accordingly. The paper presents the species structure and synthetic quantitative data of the newly defined earthworm communities.

DIVERSITY AND DISTRIBUTION OF EARTHWORMS ON THE KII PENINSULA, WEST JAPAN

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The ecosystem function of earthworms is prominent and gives strong influence to the soil ecosystem. But information of specific diversity is lacking in Japan. Family Megascolecidae is a major group in Asia including Japan and Oceania (Stephenson, 1930). The Japanese Islands spread lengthwise southwest from northeast and provide various climate zone such as sub-tropical, temperate and sub-arctic zone. A quite different earthworm fauna in each zone is to be expected. However past reports on earthworm are only from Central and Northeast Japan. Little is known about diversity and distribution of the fauna in West Japan. Kii Hanto, the largest peninsula in Japan, in the southwestern part of Honshu (main island of Japan) is one of the areas that can provide new discoveries. The purpose of this study is to elucidate the diversity and distribution pattern of earthworms on Kii Hanto.

In 2004-2005, our surveys and 263 collecting sites on Kii Peninsula, included various environments such as city parklands, meadows, artificial plantations and natural forests. Site elevations ranged from sea level to 1660m.

This report deals with the distribution of the following species that show high frequency on the Kii Peninsula; *Amynthas micronarius* (Goto & Hatai, 1898), *Metaphire acincta* (Goto & Hatai, 1899), *Metaphire agrestis* (Goto & Hatai, 1899), *Metaphire hilgendorfi* (Michaelsen, 1892), *Metaphire megascolidioides* (Goto & Hatai, 1899), *Metaphire sieboldi* (Horst, 1883). Distribution pattern of these 6 species were analyzed with environmental data (e.g. evolution, artificial disturbance degree, vegetation, geology, etc.). Two distinct patterns emerged among the three most widespread species, *M. hilgendorfi*, *M. acincta* and *M. sieboldi*.

M. hilgendorfi, is mostly restricted to the urban environment, in contrast to *M. acincta* and *M. sieboldi* which occur mainly in natural forest. Three other species also showed characteristics of distribution. These different distribution patterns suggest that Japanese earthworms are good indicators of various environmental conditions.

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Earthworm invasion in North America was facilitated by large scale land use change, primarily forest clearing. The Smithsonian Environmental Research Center in Maryland, USA, is a mosaic of young and mature secondary upland forests, grasslands and wetlands. Since European settlement the area has experienced multiple shifts of agricultural practices from tobacco to corn and wheat, and, before abandonment, dairy operations. Today the forests are of different maturity reflecting different time of abandonment. To assess whether earthworm composition and abundance of earthworm fauna changes during succession we sampled young (40 yr), mature (80 yr) and uncut stands. The earthworms were extracted in spring, summer and fall using a variety of techniques. Soil characteristics were also determined.

The total of ten species consists of a mixture of native (3) and introduced (7) earthworms. The latter were all common European introductions with the exception of *Lumbricus friendi*, the only anecic species in the assemblage. Interestingly, *L. terrestris* was not present. Native species were mostly found in riparian areas, except *Eisenoides lonnbergi* which occurs in the mature forest, as well. Earthworms were most abundant in the young forest stands regardless of the season. The highest density and biomass values were obtained in April 1999: 433 ± 70 ind m⁻² and 155 ± 20 g m⁻², respectively. The two undisturbed sites did not have earthworms at all. This is likely due to different local conditions such as presence of well drained soil and physical isolation of the sites. These sites also had higher soil organic matter content (9 % and 14 %) than the disturbed sites (>7%). Leaf litter disappears faster in the young forests than in the mature ones indicating high soil invertebrate activity. If earthworms were abundant after abandonment, their densities declined during later stages of secondary succession. This might be related to changes in resource quality i.e. dominance of less palatable leaf litter.

Poster EARTHWORMS EVERGREEN LAUREL FOREST OF LA PALMA AND HIERRO (CANARY ISLANDS)

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The objective of this paper is to present original faunistic, ecological and biogeographic data on earthworms of the evergreen laurel forests of the two western most Canary Islands. For this we studied a total of 712 specimens collected in the most representative biotopes of this vegetation formation, a relict of the Tertiary era, nowadays restricted to the Macaronesian Region. Significant differences have already been found, since in the most shaded virgin microhabitats, the specimen density is usually high in contrast to their scarcity in the sunnier more deforested spots. A total of 18 species and 1 subspecies are identified, 7 for the first time in La Palma: Allolobophora moebii, Aporrectodea rosea bimastoides, Dendrobaena cognettii,, Lumbricus rubellus, Microscolex dubius, Microscolex phosphoreus and Ocnerodrilus occidentalis. Another 3 are new records for Hierro, while Aporrectodea trapezoides is a novel find in autochthonous evergreen laurel forest; in addition Amynthas corticis are scarce and recent fortuitous introductions.

The fauna of these forests is composed mainly of native species of the family Lumbricidae, with different ranges of distribution, and by peregrine species with wide ecological valency, such as A. corticis, A. trapezoides, M. dubius, M. phosphoreus and O. occidentalis.. These last ones were introduced by man and once on each island have been able to occupy niches less attractive to native species, owing to their arid and anthropochoric conditions. The most evident faunistic affinities are with the islands of Tenerife, Gomera, Madeira and Azores, together with European and African Mediterranean zones and the northwestern Maghreb; especially those areas occupied by lush forests before the glacial period of the Quaternary, which probably had similar climatic conditions and floristic composition.

REGIONAL VARIATION OF ARABLE SOIL EARTHWORM COMMUNITIES IN FINLAND – PRELIMINARY RESULTS

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From the viewpoint of field crop cultivation, earthworms are a worthy biological resource which contributes in a number of ways to the ecosystem services provided by the soil. Earthworm assemblages in arable soils are determined by a combination of regional and local processes. In Finland regional earthworm communities of cultivated soils are poorly known. Also, no information is available on the influence of inherent soil variability to ranges of species. However, this knowledge is needed for the prediction of earthworms' responses to field management changes in different parts of the country as well as for assessing earthworms' usefulness as soil quality indicators.

To close the knowledge gaps, a countrywide survey of arable soil earthworms is carried out in 2003-2007. The sampling campaign was executed during the autumns of 2004 and 2005 to obtain a geographically extensive study material. In the sampling we utilized a previously set up network of arable soil monitoring sites at experimental stations situated in different parts of the country. The comprehensive soil and cultivation information from these sites will be related to the presence of the species and species assemblages. Altogether 53 fields, situated at 11 separate localities (typically 4-6 fields per locality) were sampled. The fields were cultivated either for cereals or silage or used as a pasture. At each sampling node three combined hand-sorting – formalin extraction samples were taken. For comparison a set of three samples were taken from each field margin, close to the cultivated site. Here we present preliminary results from the first leg of the survey (Autumn 2004; 5 localities, 29 fields).

Earthworms were present in all the places except for one inside-field sampling node. The nine species found and their corresponding proportions given in brackets are as follows: endogeics, Allolobophora chlorotica (1%), Aporrectodea caliginosa (45%), A. rosea (9%), and Octolasion tyrtaeum (2%), epigeics, Dendrobaena octaedra (5%), Dendrodrilus rubidus (1%), Lumbricus. castaneus (1%) and L. rubellus (4%) and anecic L. terrestris (32%). The number of species per field site ranged from 2 to 8. The mean number of species was estimated to be 2 species more in the field margin than inside the field. Under cereals or silage, the earthworm community inside the field was strongly dominated by endogeics compared with the field margin community (92% vs. 65% of individuals, correspondingly). In pastures, the dominance of endogeics was less clear and inside the field and in the field margin the dominances were almost identical (73% vs. 70%, correspondingly). The overall mean density of earthworms was 245 ind. m⁻² and biomass 160 g m⁻², the figures in the field margin were higher than inside the field (e.g. for density $304 \text{ vs. } 192 \text{ ind. } \text{m}^{-2}$). In pastures the total earthworm biomass was about two times higher than in the other type of sites but the earthworm biomasses in field margins and inside the pastures did not differ. The earthworm densities in the field margin and inside the field correlated positively which indicates that site specific factors are affecting the numbers.

Poster

A CHECKLIST OF OLIGOCHAETES FROM TAIWAN AND ITS ADJACENT ISLANDS

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The oligochaete study in Taiwan has progressed rapidly since 1990. Discoveries of new species and new records in recent years have made great contributions to our understanding of the earthworm biodiversity of this island and its adjacent islands. This checklist consists of 71 nominal species, including 7 aquatic species and 64 terrestrial species and subspecies. For the former all are peregrine. For the latter 35 species and subspecies (54.7%) are endemic and 29 species (45.3%) are peregrine. Several species with taxonomic problems were re-evaluated.

Session 2

Earthworm Ecology and Global Change

Plenary lecture

INTER- AND INTRASPECIES RELATIONSHIPS IN EARTHWORMS

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The role of earthworms as keystone animal group regulating activities of soil microorganisms, plant growth and interactions within soil communities has been extensively investigated. Less is known about the interrelationships among earthworms themselves. Earthworm associations are ecologically diverse and comprise the major part of invertebrate biomass in soil; hence, interactions within the associations must have a profound significance for functioning of soil systems. Summarising a very restricted number of studies all belonging to the latest decade (by Butt, Lowe, Capowiez, Baker, Uvarov, Scheu and some others), several conclusions can be made on variation of interactions between earthworms: (a) both intra- and interspecies relationships were documented; (b) both types of relationships were shown for representatives of any ecological group (epigeic, anecique, endogeic); (c) interrelationships between the species belonging to different ecological groups were documented; (d) various types of interactions are common, i.e. positive, negative, visibly neutral, one-sided or mutual; within species populations, (e) density-dependent effects on ecophysiological processes and activities of earthworms (respiration, reproduction and growth, feeding and burrowing activity, mortality) were found; (f) interactions between adult and juvenile earthworms were revealed.

However, little is known about mechanisms regulating the interrelationships in earthworms. E.g., negative relationships were often explained in terms of competition for space and/or resources (or even by cocoon predation) though it was rarely shown directly, and the data are contradictory. Lowe & Butt (2002) related the intensity of interactions to the degree of species niche overlap but also stated that the outcome of interactions could not be predetermined simply by reference to ecological groups of the earthworms. In general, interspecies interactions often seem to be situation-specific. In some cases, their character is affected by the environmental factors, such as soil compaction, temperature regime or winter conditions. Within species populations, at higher densities reproduction and growth are generally reduced and mortality increased.

As an example, complex relationships between two epigeic species regularly occurring together in two- or multispecies earthworm natural associations (litter-dwelling *Dendrobaena octaedra* [Savigny] and litter/topsoil dweller *Lumbricus rubellus* Hoffmeister), are analysed based on the authors' data. Under long-term joint maintenance *L.r.* tended to outcompete *D.o.*: offspring production, density and biomass in *D.o.* populations were significantly decreased. In contrast, *L.r.* rather gained in the presence of *D.o.*; however, no predation of *L.r.* on cocoons of *D.o.* was documented. In summer reproduction rates of *D.o.* were higher at constant than diurnally fluctuating temperature regime, at lower population density and higher litter supply. In winter populations of *L.r.* deadly suffered from occasional frosts tolerated by *D.o.*, which also had higher recovery potential due to parthenogenesis. These characteristics contribute to the undestanding of mechanisms regulating coexistence in these earthworm species.

The study was funded by the Deutsche Forschungsgemeinschaft and the Russian Foundation for Basic Research.

INVASION OF EUROPEAN EARTHWORMS INTO A NORTH AMERICAN ASPEN FOREST: EFFECTS ON SOIL CHEMISTRY, MICROFLORA, FAUNA, AND VEGETATION

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After the last glaciation earthworms, which had taken refuge in the southern parts of the continent, did not recolonize North America. They have invaded the region which today is Canada since 1500 AD when European settlers colonized the continent and were distributed by inadvertent or intentional human activities.

Earthworms are known to alter soil structure, nutrient cycling and the complex interactions between soil fauna and microflora and therefore likely affect aboveground plant communities. However, the impact of these ecosystem engineers on soil microbial and soil mesofauna communities has only been studied recently.

In this study the effect of invasion of earthworm species (*Dendrobaena octaedra*, *Lumbricus terrestris* and *Octolasion tyrtaeum*) into a western Canadian aspen forest on soil fauna, microbial and plant community and soil chemistry were investigated.

Along three transects (200 m) starting at the edge of the forest, densities of earthworm species were determined. Plants were harvested, and separated into three plant functional groups (herbs, grasses, legumes) and the dry weight was measured. In addition, soil cores were taken to investigate the effects of burrows of *L. terrestris* on soil fauna, microflora and soil chemistry.

The invasion of *L. terrestris* und *O. tyrtaeum* in the investigated aspen forest is still progressing. *O. tyrtaeum* was very abundant in the middle of the forest. As shown previously, invading species initially may reach high abundances due to high resource availability. In contrast to *D. octaedra, L. terrestris* and *O. tyrtaeum* did not invade from a forest road close to the aspen forest. Probably, they invaded laterally from a little creek nearby the aspen site.

Chemical and microbial parameters were altered only in the litter layer in *L. terrestris* burrows. *L. terrestris* incorporated litter into the soil and *O. tyrtaeum* mixed it with the mineral layers. Consequently, the organic layers disappeared where both species occurred together. Microbial biomass was reduced where *L. terrestris* and *O. tyrtaeum* were present. This was probably due to resource competition and mechanical disturbances. Structural and chemical properties of the soil and the plant community were altered when *L. terrestris* and *O. tyrtaeum* were present. Some plant species benefited from earthworm presence, others did not. The presence of *O. tyrtaeum* reduced microarthropod densities (-75 %) and diversity (-29 %) through mechanical disturbances, increasing compactness of the soil and resource competition.

The invasion in the investigated aspen forest by mineral soil dwelling earthworm species presumably is slow due to the climatic restrictions on growth, reproduction and activity of these species. However, this study indicates that earthworm invasions can have dramatic impacts on vegetation, soil microarthropods, microorganisms and soil chemistry.

IMPACT OF EUROPEAN LANDSCAPE PATTERN ON EARTHWORM COMMUNITIES

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Earthworms are ecosystem engineers that influence directly or indirectly resource availability by modification of physical environment. The negative relationship between land use intensity and biodiversity impacts on earthworm communities, leading to a commensurate reduction in the ecosystem services they provide. However, at landscape scale, the nature of this change remains poorly documented, and with it the understanding of which landscape and habitat features are responsible for the shift in community composition. Aims of the paper are to analyse the response pattern of earthworm community to the composition and structure of landscapes as part of the 5th PCRD-EU BIOASSESS project. In Europe, landscapes range from semi natural forests to highly intensive cropped land with a number of intermediates that associate natural or planted forests with a mixture of pastures and crops managed at different levels of intensity. In order to understand the influence of landscape on earthworm communities, we defined 6 levels of landscape intensification and replicated them in 8 different European countries representing a climatic gradient. Our basic sampling unit was a square 1km² land use mosaic in which 16 points regularly distributed 200m apart were sampled for earthworms. Each km² was the basic sampling unit for which we assessed earthworm species richness and abundance in 2001 and 2002. We tested three hypotheses. H1. The landscape composition (proportion and diversity of different land use type in the landscape) has an effect on earthworm community structure. We found that earthworm have different communities in crop landscapes, grassland landscapes, forests landscapes and heterogeneous landscapes. H2. The landscape structure (size, shape and dispersion of fragments etc.) has an effect on earthworm distribution. We found that earthworm communities are different in connected and forested landscapes, fragmented landscapes, grassland contiguous landscapes and crop contiguous landscapes. H3. Land use managment determines the diversity and abundance of earthworm communities. We found significantly different earthworm communities in landscapes with mowing, organic, permanent grassland, landscapes with changing land uses, fertilised crops and grazing pastures, and managed forest landscapes. The variance partitioning between the three sets of environmental variables showed that management factors are the most influencing, followed by landscape composition and landscape structure.

EARTHWORM COENOSES OF CALCAREOUS GRASSLAND SITES IN SOUTH-WESTERN GERMANY - INFLUENCE OF MANAGEMENT PRACTICES AND SUCCESSION

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For more than twenty years earthworm coenoses were monitored on five experimental grassland sites with different management practices on calcareous sites in South-western Germany. The earthworm coenoses of the plots "Natural succession" (without any management) were investigated at five sites, the plots "Mulching twice a year" (mowing in June and August without removal of the phytomass) at four sites, the plots "Sheep grazing" at three sites and the plots "Prescribed Burning" at two sites. Vegetation dynamics and soil properties e.g. pH, potassium, phosphorus and soil organic matter were monitored.

In the beginning of the monitoring period earthworms were collected by formalin extraction. later on oktett method combined with hand sorting was used. Hence, only species composition and its changes over 20 years are presented. More detailed analyses of the earthworm coenoses are done in the last years, comparing the coenoses of different managed sites. During the first years, earthworm coenoses at all plots included anecic species, epigeic species and endogeic species. At the mulched, grazed and burned plots, the species composition did not change during 20 years. At the succession plots, the occurrence of species changed in dependence on plant succession. At the succession plots without woody plants, anecic earthworms disappeared. At one of these succession plots, the stenoecious epigeic earthworm Dendrodrilus rubidus immigrated. On one succession plot an ash stand became established. At this plot, anecic earthworms are abundant, while epigeic species disappeared. After 20 years, the abundances and the biomasses of earthworms at the managed plots are higher than at the succession plots. The managed plots and the succession plot covered by an ash stand tend to mull conditions with a high decomposition rate, while the other succession plots tend to moder conditions with inhibited litter decomposition. The Influence of changing soil and vegetation conditions on earthworm coenoses are discussed.

VARIATION IN MEGASCOLECIDAE EARTHWORM CASTING UNDER DIFFERENT NATIVE VEGETATION COMMUNITIES IN SOUTH-EAST AUSTRALIA

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Megascolecidae earthworm casts (size up to 125 cm^3) form a conspicuous layer in undisturbed basalt soils in South-East Australia. These fertile, well structured clay soils form a couplet with native mesic vegetation communities within sheltered sites in the humid zone. Casting is of potential importance to nutrient cycling at these sites since phosphorus fixation is a well established constraint in basalt soils. To explore this, we have initiated the present study, which seeks to determine spatial variability of earthworm activity (as proxied by casting) within and between adjacent native vegetation communities, and the influence of soil moisture and surface litter on casting.

This study, conducted during August 2005, is based on 84 permanent 0.25 m^2 sample plots, constructed within four well defined vegetation communities in the Blue Mountains of South-East Australia (dry sclerophyll, wet sclerophyll, rainforest and an ecotone between the last two which is distinctive at this site). Each vegetation community contains 16 randomly distributed sample plots. To assess the transition between these communities, four sub-zones were created, with 5 random sample plots between each community.

The total amount of surface casts increased as the vegetation community became more mesic (dry sclerophyll – rainforest). This trend coincided with an increase in soil moisture of the cast layer and a decrease in the surface litter. The areas dominated by sclerophyll forest produced approximately 3200 g/m² of surface cast material, contrasting to the ecotone and rainforest communities which produced 5139 g/m² and 6888 g/m² respectively. This represents a two fold increase between sclerophyll and rainforest communities. Future work will address the rates of casting to explore seasonal effects.

THE EFFECT OF BIODIVERSITY ON ECOSYSTEM FUNCTION: A MICROCOSM EXPERIMENT USING EARTHWORMS

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The effect of biodiversity on ecosystem function is a subject open to debate. The increasing impact of man on biodiversity makes this an important and topical question. However, there is much difficulty involved in studying this due to flawed experimental designs. Many traditional experimental approaches that attempt to quantify community interactions introduce a size bias. This paper presents work which uses the Simplex design and thus manages to avoid the problems associated with many experiments examining the relationships between ecosystem function and community structure.

A six-month experiment was carried out using earthworms as model organisms. Sixty-eight microcosms were inoculated with mixtures of earthworms, Anecic earthworms and the Endogeic species Aporrectodea caliginosa and Allobophora chlorotica, in various combinations. These were incubated under two temperature regimes, stable and variable. Food was added every week in the form of dry cattle manure labelled with stable isotopes: 15N, 13C and 34S. The microcosms were watered every week and leachate was sampled every second week. After six months the microcosms were destructively harvested and earthworm biomass assessed. Due to the importance of earthworms in agro-ecosystems and their effect on nutrient cycling, the ammonium and nitrate content of leachate and soil were determined as was pH. This has shown that there is a significant effect of temperature regime on nitrate dynamics, with significant species interactions occurring only at the stable 12°C temperature. At this temperature there was no functional redundancy. Significant interactions were detected between the two Endogeic species (p<0.0028), which lead to a reduction in the amount of nitrate leached while the presence of the Anecic species increased the amount of nitrate leached (p<0.0097).

DENITRIFICATION COUPLED TO THE HETEROTROPHIC ACTIVITY OF EARTHWORMS: A GUT-ASSOCIATED PROCESS WITH CONSEQUENCES AT THE ECOSYSTEM LEVEL.

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Earthworms are known to accelerate the decomposition of organic substrates, thus affecting nitrogen mineralization, nitrification and denitrification. Despite several scientific advances in the effects of earthworms in long term studies, especially in agroecosystems, little is known about the processes related to the earthworm digestion, the black box, which are the origin of most of their ultimate effects.

In a microcosm experiment, we studied how mineral N availability and the litter-feeding earthworm density (Eisenia andrei) affected N₂O emissions during organic matter decomposition. Fluxes of CO₂, N₂O and ancillary soil properties were analysed during 21 day incubation. Our objectives were (i) to determine how nitrate availability interact with the earthworm density in N₂O emission, and the density-dependency of these effects; (ii) to identify if the N₂O flux come from denitrification activity; (iii) to identify whether the earthworm's gut with his associated microflora is the hotspot of denitrification. The CO2 and N₂O fluxes were highest in the first 4 days and decreased through time. The N₂O flux was as much as 1.5 times greater in microcosms with earthworms than those without earthworms, especially when the mineral N concentration in the substrate was high. Denitrification potential was greatest in microcosms with the highest number of earthworms and the greatest mineral N concentration. Our results indicate that earthworm should be considered by itself for potential emission of this greenhouse gas. Earthworms were identified as key regulators of denitrification in the organic substrate, and the gut of epigeic earthworms was revealed as the functionally active microsite for denitrifying bacteria. Denitrification by the earthworm gut microbiota is a Gut-Associated Process (GAP) with proxy effects at the earthworm scale, but may have important consequences at the ecosystem-level that remain to be quantified.

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BIOLOGY AND ECOPHYSIOLOGY OF THE ENDEMIC EARTHWORM DENDROBAENA MRAZEKI, AND ITS EFFECTS ON SOIL AND SOIL ORGANISMS IN XEROPHILOUS OAK FORESTS

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Dendrobaena mrazeki is an endemic earthworm distributed across a small part of Central Europe. It shows preference for dry habitats hostile for other earthworm species, where produces large amount of characteristic casts composed of well recognisable pellets. To improve our knowledge about the physiology, ecology and the effects of *D. mrazeki* on soil and other soil organisms, the research was carried out in thermophilous oak forests located in the Pálava Biosphere Reserve, Czech Republic, in 2003-2005. In addition to field research, sets of laboratory experiments were performed.

The results showed that

- life-span of *D. mrazeki* seemed to be 2 yrs or longer. Cocoon production was low (0.8 per wk) in laboratory conditions, as well as the mean number of hatchlings produced per cocoon (1.2). Both juvenile and adult worms became quiescent during drought;
- metabolic rate of *D. mrazeki* was low (10 70 μ l O₂.g⁻¹.h⁻¹) within the whole temperature range measured (from 5 °C to upper lethal limit of 35 °C), significantly lower and less temperature sensitive than that of other epigeic and endogeic earthworms studied;
- composition of digestive enzyme complex of *D. mrazeki* did not differ from that of other earthworms. Amylolytic (1950 mg.g⁻¹.h⁻¹), laminarinolytic (200) and celulolytic (20) activities were the highest;
- population of *D. mrazeki* produced monthly up to 883 g of surface casts per square meter. The number of vertically oriented burrows reached a maximum of 29 m⁻². There was however significant seasonality in both casting and burrowing activity with peaks in May and/or June;
- concentrations of C_{ox}, K, Na, Ca and available and water-soluble P were higher in casts of *D. mrazeki* than in uningested soil, while pH and total P did not differ significantly;
- species number of soil fungi was higher in casts of *D. mrazeki* (92) than in surrounding soil (60) and litter (47). Their CFU numbers in soil were much lower than those in casts and litter, however, length of fungal mycelium did not differ between substrates. Community structure of soil fungi in fresh casts was similar to that in soil, however, it changed markedly during cast ageing;
- community of oribatid mites was significantly richer in litter (55 species; average density 12188 ind.m⁻²) than in drilosphere (25; 7638) and casts (24; 4656) of *D. mrazeki*. Microphytophagous *Tectocepheus velatus* predominated in both litter and casts, while small euedaphic *Microppia minus* and *Ceratozetes minutissimus* belonged to most common species in drilosphere. *Zygoribatula exilis, Eniochthonius minutissimus, Parachipteria punctata* and *Quadroppia quadricarinata* were exclusively recorded from earthworm casts.

The study suggests that *Dendrobaena mrazeki* could not be easily classified into any of the major ecological groups of earthworms and has a potential to affect significantly both soil parameters and the spatial distribution of soil microbial and faunal communities in thermophilous oak forests.

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COLONIZATION AND ASSIMILATION OF ISOTOPICALLY DISTINCT CATTLE DUNG BY TEN SYMPATRIC EARTHWORM SPECIES

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The objective of this research was to investigate the colonization of cattle dung pads by a species-rich earthworm community and to quantify carbon, nitrogen and sulphur assimilation from this dung by individual species using a natural abundance stable isotope approach. A field experiment was conducted during the active season in spring 2005 in a set-aside, unfertilized grassland known to have >10 lumbricid species. Twenty cages each enclosing a 50 by 50 cm area were constructed with wire and timber frames, nylon mesh 'wind barrier' sides and bird netting on top. The cages were installed in the field, at least 5 m apart, in a randomized block design. Urine-free bovine dung was collected from two beef cattle fed an experimental 'isotopic' diet containing maize, urea and seaweed ingredients, which introduced natural, distinct sources of ¹³C, ¹⁵N and ³⁴S, respectively. The bulk isotopic spacing of this dung compared to local pasture-derived dung was about 10% for δ^{13} C, 8‰ for δ^{15} N and 7‰ for δ^{34} S. In late March, one artificial dung pad was placed into each cage by applying 900 g reconstituted dung onto an area of 30 by 30 cm cleared of vegetation. Earthworms were sampled from the dung pad and the core 25 by 25 cm area beneath it (25 cm deep) by hand sorting after 2, 4, 6 and 8 weeks (5 pads per date) and also at the start of the experiment from unaffected areas. Stable isotope ratios in gut content free, whole-body worms were measured by EA-CF-IRMS. Sulphur results are not yet available. Earthworms colonized dung pads extremely rapidly, with abundances (expressed as mean individuals per 25 by 25 cm pad area ±SD, n=5) increasing from initial levels of 31±16 to 169±27 after 2 weeks, peaking at 230±19 after 4 weeks and declining again to 55±23 after 8 weeks. Colonizing species with the largest increase in numbers were the epigeic Satchellius mammalis and Lumbricus castaneus, juvenile Lumbricus spp. of all size classes, but also the endogeic Allolobophora chlorotica. These taxa assimilated ¹³C and ¹⁵N fastest from the dung, as indicated by rapid shifts in their isotopic composition. For example, in large juvenile *Lumbricus* spp., whole-body δ^{13} C and δ^{15} N shifted by 4‰ and 2‰, respectively, in the first two weeks. By contrast, strictly endogeic species (Aporrectodea rosea and Murchieona minuscula) occurring beneath dung pads did not exploit them nutritionally at all over the 8 week experimental period. A complementary lab experiment was conducted in which L. castaneus and S. mammalis were fed the same isotopic dung under controlled conditions for up to 4 weeks. Using resultant timeenrichment relationships, isotopic enrichments measured on individuals of these fast colonizing species from the field experiment were used to estimate their exact arrival date in dung pads.

In conclusion, this research gives new, species specific insights into the colonization and nutritional exploitation of dung pads by undisturbed earthworm populations.

Oral

DARWIN'S EARTHWORMS REVISITED

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Charles Darwin made Down House in Kent his home from 1842 until his death in 1882 and this is where in 1881, he wrote "The Formation of Vegetable Mould through the Action of Worms". The work described here was based upon passages drawn directly from this book and also grew from descriptions and observations recorded in his papers and letters, that made reference to earthworms at Down House (Darwin correspondence). In addition, as part of a bid to make the area around Down House a World Heritage Site, further sampling was undertaken at sites identified in Darwin's papers which made reference to earthworms and their activities.

At Down House, general observations were made in addition to systematic sampling in areas of interest. Seleccted areas were either determined from signs of earthworm activity, habitat type or in direct relation to Darwin's documented work. Sampling for earthworms employed digging and hand sorting of soil, electrical stimulation, use of a mustard vermifuge and observations under objects located on the soil surface. Fifteen earthworm species were found representing all three ecological groupings. Greatest species richness was found in pasture below an ash (*Fraxinus*) tree (n = 9 species). Greatest earthworm density was present in Darwin's Kitchen Garden (715 m⁻²) with the largest associated biomass (261g m⁻²).

The two most abundant earthworm species recorded were *Aporrectodea caliginosa* and *Aporrectodea longa*. This was determined from direct sampling and, in the case of the latter, from observations of surface casting across most grassy areas. *Lumbricus terrestris*, described by Darwin in terms of its behaviour, but not directly named, was located in the grounds of Down House in relatively low numbers. Nevertheless, its diagnostic middens and associated burrows were easily located. At areas surrounding Down House, other investigations confirmed the findings of Darwin over a century before.

GROWTH OF *PONTOSCOLEX CORETHRURUS* IN AN OXISOL MIXED OR NOT WITH DIFFERENT AMOUNTS OF SAND AND/OR ORGANIC MATTER

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Pontoscolex corethrurus is a pantropical geophagous endogeic earthworm species native to the Guyana Shield area in Northern Brazil. It is found throughout the country, mainly in disturbed ecosystems, although it also may inhabit natural ecosystems far from its original habitat, where it must be considered an exotic invasive species. Its distribution has been associated with a wide-ranging tolerance to different soil types and environments (Lavelle et al., 1987). However, the species is rare in agricultural fields of Northern Paraná State, Brazil. Therefore, the present study was undertaken to evaluate possible soil limitations for the colonization of this species at the Embrapa Soybean experiment station (Londrina, Brazil). P. corethrurus were incubated at 27°C in the laboratory, using the Rhodic Hapludox from the station and different amounts of added organic matter (OM; composted city organic wastes) and sand. The following treatments were used: 100% soil; soil+2% (weight) OM; soil+4% OM; soil+10% OM; soil+10% sand; soil+30% sand; soil+60% sand. A potting mixture used in the greenhouse (70% soil, 20% sand, 10% OM) was also tested. Mean C content of the mixtures ranged from 1.2 to 4.3% and sand contents from 8 to 76%. Each container (10 cm square plastic box) received about 140g air-dry substrate, and water at 70-80% field capacity. Each box had 1 specimen and each treatment was replicated 14 times. The worms were cultivated for 395 d, and the substrates changed approximately every 20 d. After 1 yr, only 5 worms were alive in the soil+60% sand treatment and>9 individuals in the remaining treatments. A negative relationship was observed between sand content and earthworm biomass ($R^2 = 0,60$). Worm biomass and soil C content showed a polynomial relationship (R2 = 0.89), with maximum values at 2.8% C. Positive linear correlations ($R^2 = 0.5$) with soil P and Mg were also observed. Worms grew best in soil+2% OM, followed by soil+10% sand, soil+4% OM, soil 100%, and soil+30% sand. These results indicate that the soil from Embrapa Soybean is adequate for the development of *P. corethrurus*, and that some other factor must be limiting colonization by this species. Furthermore, a small increase in soil OM (from 2.3-2,6% C) could greatly benefit the populations of this species.

A FIELD TECHNIQUE FOR TAGGING EARTHWORMS

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Laboratory experiments undertaken over a 12 month period demonstrated that it is possible to apply Visible Implant Elastomer (VIE) tags to earthworms. These tags have previously been used with fish, amphibians and crustaceans (Brennan et al, 2005) but appear to be equally effective for tagging earthworms. The tags are made from a medical grade material which is injected into the animal as a liquid but within 24 hours cures into a soft, biologically-compatible polymer. Different colours, including fluorescent yellow, are available and if injected into muscle are visible to the naked eye, or further defined using a LED blue torch, filtered through amber glasses. Subsequent field work was undertaken at Papadil on the Isle of Rum, a National Nature Reserve in Scotland. Papadil was chosen as it is a remote location which suffers minimal human disturbance and also because it has an isolated *Lumbricus terrestris* population (Butt and Lowe, 2004).

L. terrestris middens were located within defined areas of woodland and animals were removed from associated burrows using a mustard vermifuge. Washed earthworms were then weighed and VIE tagged in the field, before some animals were returned to their burrows, after flushing the burrow with fresh water. Others were translocated to an area of grassland nearby where *L. terrestris* was not present. Areas where the tagged animals were released were fenced with plastic sheeting 0.2 m above and 0.1 m below the soil surface to prevent major over-surface movements. Objectives of this work were to ascertain the viability of VIE tagging earthworms in the field, along with *L. terrestris* burrow fidelity. Results suggest that VIE tagging is a valuable addition to the tools available to earthworm researchers and may prove useful in mark-release-recapture experiments concerned with ecological and ecotoxicological investigations of earthworm populations.

MIDDEN USE BY LUMBRICUS TERRESTRIS L. AND OTHER EARTHWORMS

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Lumbricus terrestris burrows are often easy to locate in the field as each has an associated surface midden. There is speculation as to the function(s) that the midden may perform, but collection of organic matter, inorganic objects and careful maintenance through cast addition/incorporation suggests that it is essential for successful existence of the resident earthworm. Previous researchers have looked at the *L. terrestris* midden as a hot spot for microbial activity and as a residence for soil mesofauna (e.g. Hamilton and Sillman, 1989), but this initial study examines use of *L. terrestris* middens by earthworms, other than the large resident individual.

Over the course of a year, samples were collected from two wooded sites, one at Kinloch, on the Isle of Rum, Scotland and one at Preston, England, with sites examined every 4 and 2 months respectively. Sampling protocol involved location of *L. terrestris* middens; removal of the intact midden (sample A), removal of a 7.5 cm diameter core, to a depth of 4 cm taken immediately below the midden (sample B). A control core of the same size (sample C) was also collected within a distance of 15 cm from sample B, but avoiding any obvious middens. An attempt was then made to remove and examine the resident L. terrestris from the burrow below sample B using a mustard powder vermifuge (5g in 1 litre water). All soil samples were taken to the laboratory and hand-sorted for the presence of earthworms. Any mature individuals located were preserved in formaldehyde and identified. Any cut heads were also identified if possible, but tails were discarded. Immature earthworms were recorded as such, but then kept in sample groups in 600 ml pots. Under optimal culture conditions (Lowe and Butt, 2005) they were allowed to grow to maturity for identification purposes. Each pot was examined bimonthly and animals preserved and identified as they matured. In this way a more complete picture of which species were utilising L. terrestris middens and surrounding soils was established.

Ten earthworm species were found associated within and directly beneath middens. Epigeic species, typified by *L. rubellus* dominated within the midden itself whereas endogeic species, such as *Aporrectodea caliginosa*, were often located in the soil below the midden. Total earthworm number associated with the midden (A+B) was significantly greater than control (C). Juvenile *L. terrestris* were found in middens during spring sampling. Time of year had an effect on numbers of earthworms, species composition and location across the *L. terrestris* midden burrow interface.

LUMBRICUS TERRESTRIS: A MODEL FOR EARTHWORM BEHAVIOURAL AND POPULATION ECOLOGY

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The lob worm, dew worm, nightcrawler or *Lumbricus terrestris* L. (1758) has been the subject of countless scientific investigations since its inclusion in Linné's taxonomic system 250 years ago. The species is known universally as it appears in numerous biology textbooks, as a typical example of the annelid group and is even referred to by many as "the common earthworm". Since Linné's days *L. terrestris* has also widely expanded its global distribution from Eurasian origins.

Much early research on the species centred upon morphology and physiology but the ecological importance of L. terrestris in soil systems was also noticed and today many soilrelated activities of this ecosystem-engineer are well documented. Although the earliest L. terrestris research had a strong behavioural leaning thanks to Charles Darwin's wide interests, earthworm ethology has not been a particularly strong field. However, during the last ten years L. terrestris research has been increasingly seeded with concepts and approaches adopted from evolutionarily-oriented behavioural ecology. Due to this development L. terrestris is, for instance, emerging as a model organism in the study of mate choice and sexual conflict in hermaphrodites (Butt & Nuutinen 2005). Those studies, together with field and laboratory studies on individual distribution and dispersal patterns are creating an image of spatially organised populations where intricate and fascinating intraspecific interactions are governing the fate of individuals. We feel that this is only a starting point for a new type of study of *L. terrestris* with significant potential for growth. Undoubtedly many elements of sexual conflict are still to be fully explored and in the area of population biology many fundamental questions remain unanswered. To list a few: What endogenous population processes are central to the creation of individual distributions at different spatial scales? What is the importance of ecological inheritance through burrow recycling for the fitness of L. terrestris individuals? And how important is the burrowmidden complex as a potential nursery for juveniles prior to dispersal? Could there indeed be parental care in L. terrestris? Research questions of this type would obviously benefit from the application of marking techniques and molecular ecological tools which so far have not been widely adopted in earthworm ecology. Further, observations of behaviour have revealed that individuals possess delicate, possibly chemosensory means for navigating on the soil surface and use them for tracking their own trails (during homing) and conspecifics (in mate search). Studies on L. terrestris sensory ecology would seem most worthwhile for revealing the adaptations that are in play when the species is searching for resources in its heterogenous environment.

Our poster is an invitation for all interested conference delegates to meet and consider *Lumbricus terrestris* as a focus for future research efforts and collaborations.

EARTHWORM COMMUNITIES IN RELATION TO TREE DIVERSITY IN THE HAINICH NATIONAL PARK (THURINGIA, GERMANY)

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The understanding of belowground biodiversity is still rather incomplete and interactions between above- and belowground systems have rarely been looked at when explaining belowground biodiversity patterns. The present study investigates the influence of tree diversity on the earthworm community in a central European mixed forest that characterises large areas of the Hainich National Park. Three replicate sites were chosen within three levels of tree diversity ranging from one-species (beech) to three- (beech, lime, ash) to fivespecies stands (beech, lime, ash, hornbeam, maple). Soil samples of 20cm ID were taken at three sampling dates from six replicates of each site and soil fauna was extracted using a high gradient canister extractor. In addition, four samples per site were sorted by hand and anecic earthworms were extracted using a mustard solution. Generally, earthworm densities were positively correlated with tree diversity. This correlation was most pronounced in spring when mean densities were <100 ind./m² in one-species stands and >500 ind./m² in five-species stands. In summer mean densities of earthworms were low in all site (100 ind./ m^2) while in fall densities in five-species stands were highest again (170 ind./ m^2). Changes of species composition between sampling dates and tree diversity as well as environmental parameters influencing earthworm communities will be discussed.

A REPORT ON HATCHING OF A 'THREE-TAILED' EARTHWORM (POLYPHERETIMA ELONGATA) FROM INDIA

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The anomalies of structure and malformation in Oligochaeta with reference to bifurcation and twining, segmentation, the translation or absence of external sexual characters and of internal organs are not uncommon (Stephenson,1930).During our laboratory observation on cocoon hatching one juvenile with three tails of *Polypheretima elongata* (Perrier). Probably this is the first report on the occurrence of extra tails in *P.elongata*. The cocoon that hatched had no abnormality externally compared to other cocoons except that its development time was 17 days more than the average development time (49 days) of *Polypheretima* cocoon ... The abnormal juvenile, 26 mm in length, had 3 slenden cylindrical body parts(called tails) radiating from a slightly swollen portion of the worm- body so that the worm looked like 'Y'.This monstrous juvenile lived for 21 days only and was a case of compound bifurcation due to congenital abnormality as found in other Oligochaetes

NITROGENOUS WASTE EXCRETION BY EARTHWORMS UNDER THE MIMIC SITUATION OF DROUGHT AND FOLLOWING A HEAVY RAIN

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In order to realize the excretion of nitrogenous wastes by earthworms under drought condition, earthworms, *Amynthas robustus* and *Metaphire posthuma*, were treated under a mimic drought condition and following water submergence to mimic a heavy rain. Under the mimic drought condition, earthworms gradually lost their body moisture resulting in weight lost. For lack of sufficient water to excrete nitrogenous waste including ammonia and urea, the drought-suffered earthworms were forced to accumulate either ammonia or urea in their blood. When the drought-suffered earthworms submerged into sufficient water, they quickly recovered their body weight by water absorption and excreted accumulated nitrogenous wastes in one hour.

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EFFICIENCY AND SIDE EFFECTS OF FIVE SAMPLING METHODS FOR SOIL EARTHWORMS

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In this study, we investigated five commonly used methods for sampling earthworms from soil - handsorting, Kempson extraction, electrical octet method, and application of formalin and allyl isothiocyanate (AITC). The study was conducted in May 2005 in an experimental area in Styria, Austria. Additionally to assessing the efficacy of the methods, we determined the short- (one week after sampling) and long term (five months after sampling) side effects on microarthropod abundance, soil respiration, microbial fatty acids (PLFA) and vegetation. The chemical expellents formalin and AITC were tested in five dosages each (formalin: 0.1%, 0.2%, 0.4%, 0.8% and 1.6%; AITC: 0.25, 0.5, 1.0, 2.0, 4.0 mmol). The Kempson extraction yielded the greatest number of individuals, followed by the octet method and handsorting. Formalin and AITC showed lower efficacy, but AITC 1.0 mmol and formalin 0.2 and 0.4% expelled almost the highest numbers of adult earthworms. The octet method predominantly produced small juvenile earthworms, but performed poorly with respect to collecting adults. The abundances of the investigated microarthropod groups were decreased one week after application of the chemical expellents. Collembola numbers were affected by both substances, regardless of dosage. Gamasid mites were decreased only by the highest dosages. Oribatid mites were generally affected by AITC, but only by the highest formalin dosage. Soil respiration and the PLFA patterns were unchanged by the chemical expellents. The octet method did not have any detectable short term effects on the parameters measured. Five months after the sampling, there was no remaining effect of formalin and AITC on collembolan numbers, and the abundances of Gamasida and Oribatida were depressed only in the highest AITC dosage. Soil respiration did not differ between the treatments and the control. The PLFA patterns were severely affected by the formalin applications, especially by the high dosages, but not by AITC. The highest AITC dosage and the higher formalin dosages extensively erased the aboveground vegetation. This was reflected in a significant reduction of shoot dry mass in these treatments, whereas root dry mass did not differ between control and treatments. Again, the octet method did not have any measurable side effects. In conclusion, the electrical octet method seems to be the method of choice for non-destructive sampling of earthworms from soil. During the whole study period, no side effects of this method were observed. It yielded the highest total numbers of worms, but underestimated the abundance of adults. Thus, for biomass estimation and species inventories, the use of AITC is more appropriate. It was as efficient as formalin in this study, and had only minor side effects. If formalin is to be employed, a dosage of as low as 0.2% should be applied, since it was as efficient as the double dosage, but did not have severe and long lasting side effects.

INFLUENCE OF EARTHWORM ACTIVITY ON SPATIAL DISTRIBUTION OF SURFACE CROP RESIDUES

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Lumbricus terrestris (Linnaeus 1758) or the nightcrawler has an important role in the decomposition of surface crop residues especially for minimum tillage management systems in south central Canada particularly for regions of the St. Lawrence Lowland with fine textured soils. A characteristic behaviour of these earthworms is to come out of their burrows at night to forage for food sources. The result is that leaves, small stems and even pebbles are pulled into the opening of the burrows creating rounded piles of debris mixed with soil material; that is, middens. The middens are very noticeable on the soil surface and because of this can be used as a measure or indicator of the extent of activity for *L. terrestris* for accessing crop residue materials.

This behaviour was taken advantage of in a comparison study that examined the influence of agroforestry buffers (i.e. native vs planted hedgerows) on the adjacent cropland. The hypothesis was that the presence of the treed hedgerows affected earthworm populations by supplying additional food resources from leaf litter and shading temperatures and as a consequence there would be increased activity whereby crop residues would be noticeably removed. The second hypothesis was that the extent of this influence into the adjacent crop would decrease with distance from the hedgerows.

A 100 m grid with 10 m intervals was set up at 2 field sites with four transects at the 0, 30, 60 and 90 meter points along the hedgerows. On each of these transects, earthworm activity was assessed by counting of middens at following distances into the adjacent field: close to treed buffer, at 30, 60 and 90 meters. At each assessment point, a 60 x 60 cm wooden frame was placed on the soil surface at positions, north, east, south and west. The number of middens and a visual estimation of the percentage area still covered by residues was recorded. The mean number of middens and mean per cent of residues remaining on the surface was plotted for the sampling area with contour positions determined by kriging option using Surfer^{7®} software.

An inverse relationship was observed between areas with increasing midden numbers and per cent residues remaining on the surface. The implications of the spatial distribution of middens and residues will be discussed.

EFFECT OF EARTHWORM INTERACTION OVER SELF DEMOGRAPHIC PARAMETERS, MICROBIAL FUNCTIONAL GROUPS, NUTRIENT STATUS AND BIOTURBATION

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In order to evaluate the effect of earthworm interaction on demographic parameters, microbial functional groups, nutrient status and bioturbation, the endogeic Pontoscolex corethrurus and the epigeic Amynthas corticis, were kept in laboratory microcosms in single and interaction treatments. Artificial two layer soil profiles were set up, bottom layer consisted of mineral subsoil, whereas top layer was an organic substrate (mixture of soil, sawdust and legume Mucuna pruriens). Growth rates and fecundity of earthworms, microbial counts (cellullolitical and proteolitical fungi and bacterial), nutrient content and mineralization in cast and control soil, and burial of organic matter into the subsoil were quantified in four treatments over 24, 48, 72 and 96 days. Growth rates of P. corethrurus were not affected by the presence of A. corticis, whereas an increase in growth rates of A. corticis was observed in presence of P. corethrurus. Similarly, fecundity of P. corethrurus was not affected significantly, but a great variability in cocoon production was observed in the interaction treatment. A. corticis had a higher cocoon production in presence of P. corethrurus than in its absence. It is concluded from both, growth rates and fecundity, that intraspecific competition is more intense for A. corticis than interespecific competition. P. corethrurus stimulated higher cellulolitical bacterial and fungi populations than A. corticis, whereas the last species stimulated higher proteolitical fungi and bacterial populations than P. corethrurus. It was interpreted as an adaptation of P. corethrurus to compensate a deficiency in their enzyme system, whereas A. corticis, as a consequence of r-selection. Contrary to other studies, nutrient contents (N, P, C and K) were lower in cast than in control soil, whereas Ca was higher in cast than in control soil. More attention to the effect of earthworms to nutrients, different to C, N or P, is necessary in order to explain these contradictory results. Mineralization was more rapid in interaction treatment and slower in control soil; therefore, more frequently addition of organic matter is recommended in presence of earthworms to support continual productivity. Burial of organic matter was higher for P. corethrurus than A. corticis, whereas burial in the interaction treatment was similar to that for *P. corethrurus*. It was showed the ability of this species for forming mull profiles.

Poster

A GEOSTATISTIC STUDY OF THE SPATIAL DISTRIBUTION OF EARTHWORMS IN EL MOLAR, MADRID

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In the study of the spatial distribution of soil organisms, geostatistic methods are more efficient and more robust than other available alternatives (such as dispersion indices) – the latter are limited to the analysis of distribution within the samples taken (Rossi *et al.*, 1995). The present study made use of data collected in a sampling campaign undertaken in El Molar during the spring of 2001 (partially examined in an earlier publication Hernández *et al.*, 2003), plus information collected in the spring and autumn of 2002. Geostatistic tools were used to determine the spatial distribution of a number of soil variables and of the three dominant earthworm species. Canonical correlation analysis was also performed to detect relationships between both groups of variables.

The earthworm community of the experimental plot was composed of six species, of which *Hormogaster elisae*, *Allolobophora caliginosa* and *Allolobophora rosea* were the most abundant. The aggregation indices used showed an aggregate type distribution for these species. The semivariograms obtained for their abundance basically fitted a spherical model. The dominant species, *H. elisae*, appeared aggregated in patches with a mean diameter of 45.55 m in spring and 100 m in autumn, largely in the top right hand area of the experimental plot. *Allolobophora rosea* was distributed in patches with a mean diameter of 22.3 m in the upper central and left areas of the plot. The patches containing *A. caliginosa* had a diameter of 38.3 m, all located by the side of a stream that crossed the plot.

A spatial distribution was seen for all the soil variables studied except porosity (uniform throughout the plot) and the soil moisture content of 2001. A strong nugget effect was seen for the latter, perhaps due to the distance between the sample sites (14 m) being too great to detect any spatial distribution.

Canonical correlation analysis showed a linear correlation (r = 0.759; p < 0.01) between the group of dependent variables (abundance of species) and the group of independent variables (soil factors) that explained 45.61% of the variance of the former. A positive correlation was seen between the abundance of *H. elisae* and the percentages of total sand and coarse sand; a negative relationship was seen with respect to clay content, nitrogen content, carbon content and the amount of coarse silt. Exactly the opposite relationships were seen for *A. rosea*. Given the low variance of the group of dependent variables explained by the group of soil factors (26.28%), other biotic and/or abiotic factors must also influence the abundance of earthworm species in EL Molar.

A SIMULATION MODEL OF EARTHWORM INVASIONS: THE IMPACTS ON SOIL NUTRIENT DYNAMICS

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Recently, "species invasion" has become an important issue, because invasive species could completely change ecosystem structures and functions. Peregrine earthworm species have been introduced mostly by human activities (e.g., horticultural plant imports, habitat disturbance, and land-use changes) and distributions of these exotic earthworms now appear to overlap the range of native earthworms in many regions. Previous references indicated that native earthworm species have already disappeared in several areas that are now colonized by exotic species. Hence, the invasion of exotic earthworms could alter native earthworm communities and have an impact on nutrient cycling.

A simulation model has been developed to evaluate the potential impacts of earthworm invasions on nutrient cycles by considering three combinations of earthworm community structure: native earthworms only; exotic earthworms only; and native and exotic earthworms coexisting. This model also takes account of feeding and casting activities of the earthworms. Responses of several variables, including litter/soil organic matter, microbial biomass and earthworm population, have been evaluated. Preliminary results show that exotic earthworms induce slightly higher microbial biomass carbon and activities after one-year simulation time. However, this effect on microbes is suppressed when exotic and native earthworms coexist. For wider application in terrestrial ecosystems, more experimental data will be applied to calibrate and validate the model.
Poster

LIFE FORM PATTERNS OF JAPANESE MEGASCOLECIDAE; MORPHOLOGICAL AND FUNCTIONAL VIEW

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Ecological studies on native earthworms in Japan are still rather limited. The dominant families for natives in Japan are Megascolecidae, Moniligastridae, and Lumbricidae. So far around 70 Megascolecidae species are named, all pheretimoids, for which Ishizuka (1999, 2001) found patterns in the morphology of their intestinal caeca. These patterns seemed to correspond to the life form of the species classed as: litter-feeding epigeics, topsoil anecics, and geophagous subsoil endogeics. We analysed body stable isotope C and N ratios of native Megascolecidae worms and, from estimations of their food sources, have largely confirmed the patterns proposed by Ishizuka (see Uchida *et al.* 2004).

Megascolecidae epigeics are mostly annual species that overwinter as eggs in resistant cocoons, whereas endogics are perennial surviving at depth. Growth rates in epigeics are much higher than endogeics and these ecological characteristics should be closely linked to their food resource use.

In this presentation we will compare body nitrogen contents, nitrogen use efficiency and growth rates for further characterization of the life forms of Japanese Megascolecidae earthworms. The various morphologies of the intestinal caeca in relation to their function will be briefly discussed.

EARTHWORM POPULATIONS IN THREE ATLANTIC RAINFOREST FRAGMENTS WITH DIFFERENT DISTURBANCE LEVELS NEAR LONDRINA, PARANÁ, BRAZIL

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Earthworms are known to be good indicators of soil conditions, due to their susceptibility to management practices, vegetation types, disturbance, and other environmental properties. Little is known of the earthworm communities of Paraná State, Brazil, and therefore the present study was undertaken to evaluate the earthworm communities in Atlantic Rainforest fragments in the vicinity of Londrina, Paraná State, Brazil, and the effect of forest disturbance on their populations. Earthworms were sampled from three forest fragments at 1) the Godoy Forest State Park (GF); 2) the Arthur Thomas Municipal Park (AT); 3) the State University of Londrina (UEL) campus. GF is a moderately well preserved primary forest, AT is a secondary forest, at advanced level of succession, while UEL is a secondary forest at the incipient level of succession. Disturbance levels could thus be classified as UEL>AT>GF. Samples were taken in the wet and dry seasons (March and July) of 2005, and consisted 25 square soil blocks of 25 x 25 cm (1/16 m²), taken in a grid format, with 10 m distance between samples. Twenty samples were taken to 10 cm depth and five to 30 cm depth (diagonally in the grid). In the dry season, highest earthworm density was observed at AT (475 ind.m⁻²), followed by UEL (326 ind.m⁻²) and GF (24 ind.m⁻²). Earthworm biomass followed the order UEL (15.8 g.m⁻²) > AT (6 g.m⁻²) > GF (0.8 g.m⁻²). In the rainy season, highest populations were again observed at AT (1069 ind.m⁻²), followed by UEL (149 ind.m⁻²) and GF (139 ind.m⁻²). Earthworm biomass again followed the order UEL (23.1 g.m⁻²) > AT (18 g.m⁻²) > GF (3 g.m⁻²). Therefore, abundance and biomass were generally higher in the rainy season. Cocoon abundance and biomass was also greatest at all sites in the rainy season. In the dry season, more worms were found in the 20-30 cm layer compared to the top 10 cm, with the exception of the UEL forest. In the wet season, most earthworms inhabited the top 10 cm layer. Earthworm abundance was greater in the disturbed (UEL and AT) sites than the primary forest (GF). Species composition in the forests was also different. GF had only native earthworms (Urobenus brasiliensis, Urobenus sp. and Glossoscolex sp.), while both UEL and AT had both native (Urobenus brasiliensis, Fimoscolex sp., Glossoscolex sp., Belladrilus sp.) and exotic (Amynthas gracilis, Dichogaster spp. and Pontoscolex corethrurus) species, although exotics predominated in terms of abundance and biomass.

EFFECTS OF ENDOGEIC EARTHWORMS (*OCTOLASION TYRTAEUM*, LUMBRICIDAE) ON DECOMPOSITION OF PLANT RESIDUES DIFFERING IN LITTER QUALITY IN AN ARABLE AND FOREST SOIL

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decomposition and nitrogen mobilisation.

Litter decomposition is influenced by internal factors, such as nitrogen and lignin content, and external factors including soil type and soil biota. We studied the effects of the endogeic earthworm species Octolasion tyrtaeum on the decomposition of litter materials of different quality, i.e. forming a gradient from high to low C-to-N ratio, in an arable and forest soil. Fragmented rye, maize and rape litter were separately mixed into arable soil, and maple, beech and woodgarlic litter into forest soil. Soil with and without addition of litter was incubated in microcosms at 20°C for 282 days. Rates of carbon mineralization of high quality litter (low C-to-N ratio) were high in the first months but decreased rapidly to low levels later in the experiment. Decomposition of low quality litter started slower but decomposition rates remained high throughout the experiment. Carbon mineralization was increased by earthworms in the first half but decreased in the second half of the experiment. Stabilisation of soil organic matter by O. tyrtaeum later in the experiment was more pronounced in the forest soil than in the arable soil, possibly due to higher content of silt and clay. Leaching of mineral nitrogen was reduced by low quality litter. Earthworms counteracted the immobilisation of N by low quality litter, possibly by interrupting hyphal connections between litter and soil or by competing with microorganisms for easily available carbon resources. Earthworms lost body mass (treatments with litter) or died (treatments without litter) in the arable soil whereas they did not grow (treatments without litter) or increased in biomass (treatments with litter) in the forest soil. Microbial biomass was increased by the addition of litter, in particular by high quality litter. Earthworms in general slightly reduced microbial biomass. Microbial biomass nitrogen and ergosterol content was differentially affected by the type of litter and the presence of earthworms. The results suggest that soil organic matter may be stabilised in casts of endogeic earthworms in the long term but the stabilisation depends on the soil material in which the litter is incorporated. Early during litter decomposition earthworms uniformly increase litter decomposition irrespective of its quality. Litter quality strongly affects the growth of

endogeic earthworms and this likely feeds back to earthworm-mediated changes in litter

EFFICIENCY OF ELECTROSHOCKING AND FORMOL EXTRACTION OF EARTHWORMS COMPARED TO HANDSORTING IN AGRICULTURAL AND NATURAL ECOSYSTEMS NEAR LONDRINA, BRAZIL

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Sampling of earthworms is generally a labor intensive and time-consuming activity and means of reducing the time and effort necessary to sample earthworm populations are always welcome. Therefore, in the present study, we compared the efficiency of two "quicker" and "easier" alternatives (electro-shocking and dilute-formalin expulsion) in relation to the traditional hand-sorting method. Sampling was performed in four ecosystems: 1) Native forest (secondary vegetation); 2) edge of a cattail marsh; 3) sorghum cropping (after harvest); and 4) a >10 yr old Cynodon spp. pasture grazed by horses. Electro-shocking (Thielemann octet method) was performed over an area of approximately 0.4 m^2 for 20 min and immediately thereafter, earthworms were handsorted from 0.16 m² soil monoliths, to a depth of 30 cm, directly underneath the electro-shocked area. Formalin (0.5%) was applied to a 1 m² area, 5-m away, and the surfacing earthworms collected. Eight samples were taken in the forest and the marsh and 5 samples were taken in the agroecosystems. Earthworms were killed in alcohol (30%) and then fixed in formalin. All individuals collected with each method were counted (separately into age classes), weighed and identified to species level (when possible). Earthworm diversity was always higher with the handsorting method (forest = 8 spp., crop system = 6 spp., swamp = 3 spp., pasture = 2 spp.). Formol and electro-shocking yielded at most 3 species only, generally the fastermoving epigeics and poly-humic endogeics. Amynthas gracilis and Urobenus brasiliensis predominated in the forest, Pontoscolex corethrurus dominated in the marsh and various Dichogaster spp. dominated in the agroecosystems. Earthworm abundance was highest in the cropping system, followed by the forest, swamp and pasture. Efficiency of the sample methods depended on the ecosystem, the species and the measurement in question. At all sites, electro-shocking yielded the fewest earthworms, and the extraction efficiency, was generally low for the total earthworm population: from 0% in the pasture, where the soil was compact and drier, to around 20-30% in the remaining ecosystems. Formalin expulsion was also less efficient than handsorting (10-25%), except in the forest, where it yielded 5 times as many earthworms, primarily due to its efficient sampling of Amynthas and Urobenus spp. Electro-shocking and formalin appear to be interesting methods to sample mostly large, surface-active earthworms, whereas handsorting appears to be better suited for smaller, mostly endogeic earthworm species. Ideally, a combination of both methods should be attempted, to adequately sample both large and faster moving earthworms often missed by handsorting, and smaller, endogeic earthworms, not adequately sampled with formol or electricity.

SEASONAL CHANGES IN EARTHWORM DENSITIES AND BIOMASS ALONG ALTITUDINAL GRADIENT OF THE SILESIAN BESKID MOUNTAINS.

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The Silesian Beskid range is the westernmost part of the Carpathians. The highest peak reaches 1257 m a. s. l. The climate is characterized by high precipitation (1200 mm per year) and long lasting snow cover. The mountains are densely forested with a clear altitudinal vegetation zones, namely: foothills (250 - 670 m a. s. l.), lower forest zone (beech-spruce, 670 - 980 m a. s. l.) and higher forest zone (spruce, above 980 m a. s. l.). The study on earthworms was carried out in three vegetation zones – both in the meadows and in the forested sites (beech, spruce, oak-hornbeam, alder-ash carr). In the spring, summer and autumn of 2004 and 2005 earthworm communities were checked on 11 study plots. On every sampling occasion and on every study plot 5 samples (20x20x20 cm) were dug out. Earthworms were separated from soil by hand sorting.

Eight earthworm species were found: Aporrectodea caliginosa, Ap. rosea, Dendrobaena octaedra, Dendrodrilus rubidus, Eisenia lucens, Lumbricus castaneus, L. rubellus and Octolasion lacteum. The changes in density and biomass were observed according to season and study year.

Poster HOW TO SAMPLE EARTHWORM POPULATIONS? A COMPARATIVE STUDY WITH TWO CHEMICALS AND TWO SAMPLE SIZES

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Earthworms conduct important agro-ecologic functions since they participate to the evolution of organic matter and soil structure; they are also good soil quality biological indicators. The most efficient method to sample earthworm populations combines an extraction by spraying the soil with a chemical and hand-sorting. Formaldehyde is the most widespread chemical used but it is toxic for the earthworms and their environment, as well as for the manipulator because it is cancer-producing (Gunn, 1992). Hot mustard is alternately used as an expellant (Lawrence and Bowers, 2002) but this method is difficult to standardize because the component concentrations are not known. The aim of this study is to test the efficiency of two repulsive chemicals (diluted hot mustard and formalin) on earthworm extraction.

Six plots of a long term field trial were sampled in autumn 2005 in Northern France. Eight samples (four with mustard and four with formalin) on each plot were drenched with the repellents deposited within a 40x40 cm² frame. Earthworms emerging from the soil were collected within a twenty minutes timeframe. The soil was then excavated to 30 cm and the earthworms were hand-sorted. The sample size was studied by excavating a 25x25 cm² and 15 cm deep central block inside the highest.

The results showed that formalin is more effective in extracting anecic and endogeic earthworms from the soil than do hot mustard. In the ploughed system, the density (number of individuals per unit area) reaches 62.5 m^{-2} with formalin and only 51.3 m^{-2} with mustard. Concerning the weight per unit area, it is 12.6 g.m⁻² with formalin and 10.3 g.m⁻² with mustard. Excluding hand-sorted earthworms, mustard and formalin density ranges were 4.7 and 16.2 m⁻² respectively and biomass per unit area were 1.0 and 6.8 g.m⁻² respectively. In the unploughed system, we measured the same trends and we counted 2.5 times more worms with formalin, when we excluded hand-sorted. Concerning the sample size, the most the sample surface is, the most the density and the biomass per unit area increase.

Chan and Munro (2001) found that mustard extract produced a 67% higher abundance than the formalin but we showed in this study that formalin is the most optimal chemical to extract earthworms. The diluted hot mustard method should be interesting if the composition of the commercial product was more stable. The Allyl Isothiocyanate (AITC) is a molecule contained in the mustard; its efficiency as a repellent for earthworms is proved in the literature (Zaborski, 2002) and it appears to be a good compromise between a "noxious" chemical and a method difficult to reproduce.

Poster

EARTHWORM RESPONSES TO LIMING OF ACIDIC WOODLAND SOIL

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Liming of acidic woodland soils has been shown to increase earthworm numbers, biomass and species richness, apparently raising the availability of nutrients to earthworms. Transit of acidic soil through the gut neutralises acidity so that earthworm casts may represent localised "hotspots" of elevated pH and nutrient availability. In this study responsiveness of the acid-tolerant lumbricids *Dendrobaena octaedra*, *Lumbricus rubellus* and *L. terrestris* to addition of calcium carbonate to acidic woodland soil (pH 4.3) was examined by assessment of burrowing rates, distribution in soil choice chambers, avoidance of soil extracts and soil palatability tests. All species responded positively to CaCO₃ addition, interspecific differences in responsiveness corresponding to the field distribution of these species in relation to soil pH. The earthworms produced near-neutral casts in acidic soil. The *Lumbricus* species consumed moderate amounts of 14d and 35d old casts, showing a preference for the latter, but ingested acidic soil more readily. Cast respiration rate was substantially greater than that of parent soil and declined over time.

DIVERSITY AND ECOLOGY OF SOIL ANNELIDS IN URBAN GREENS OF A MEDIUM-SIZED CITY - PROJECT OUTLINE AND PRELIMINARY RESULTS

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The soil annelid community (mainly Lumbricidae and Enchytraeidae) is being studied in urban greens of Brno - a medium-sized city located in the south-east of the Czech Republic since spring 2006. Three pairs of isolated parks of known history, situated in the central part of the city and belonging to three size-classes (large, medium and small), were chosen for the study. The main project objectives are to investigate potential links between (1) the community structure and the plot size, (2) earthworm and enchytraeid populations, (3) soil characteristics and annelid communities, (4) contamination of urban soils (mainly due to air-born pollutants emitted by motor vehicles and due to the management of greens) and soil annelids, (5) soil compaction by pedestrian activities and soil annelids, and (6) exotic trees and shrubs and soil annelid communities. The sampling scheme has been designed to provide basic data on the species composition, dominance, density and biomass of soil annelids and on the corresponding urban soils. Heavy metal and polycyclic aromatic hydrocarbon content shall be measured both in the tissue of the major annelid species and in the upper soil of the study plots. The impact of soil contamination, soil compaction and of exotic plants shall be investigated by sampling along gradients (transects from the outer edges to the inner area of the parks; various distances from individual exotic trees or shrubs) or in pairs of affected and unaffected sampling points (foot path versus adjacent lawn). Detailed laboratory experiments will be carried out to verify results of field measurements and observations. In spring 2006, a set of unofficial pedestrian paths was selected in the individual parks. To assess the effects of pedestrian activity on both qualitative and quantitative parameters of soil annelid communities, sampling of both animals and soil was done simultaneously on these paths and in their vicinity (0.5 m distance). Earthworms were extracted by the electrical octet method, using the Worm-Ex III device with a working area of 1250 cm^2 (= sampling point). To assess the community of enchytraeids, soil cores were taken down to ca. 12 cm using a soil corer of 17 cm² working area. The soil cores were sub-divided into four layers and enchytraeids were collected by wet funnel extraction. To measure soil parameters, three soil cores (depth 0-15 cm) were taken in each sampling point using Kopecky soil sample rings. The cores were used for the measurement of soil physical parameters (moisture, bulk density and conductivity). Then, the cores were divided into three sub-samples (depth 0-5, 5-10 and 10-15 cm) and used for measurement of basic chemical characteristics. Worm density and biomass on and outside of pedestrian paths were compared using ANOVA and the Wilcoxon nonparametric test. The effects of changes of individual environmental parameters (soil compaction, moisture, organic matter content, etc.) on soil annelids were evaluated using multidimensional statistics (CANOCO programme). Preliminary results are presented and discussed in this contribution.

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THE LIFE CYCLE OF THE EPIGEIC EARTHWORM, *EISENIA FETIDA* AS INFLUENCED BY SEASONAL ENVIRONMENTAL FACTORS

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Seasonal abiotic factors play very important role on different aspects of various soil organisms. We have conducted an experiment to know the influence of seasonal environmental factors such as room temperature (RT) and percent relative humidity (%RH) etc on the life cycle of the epigeic earthworm, Eisenia fetida in semiarid region of peninsular India. One week old hatchlings of Eisenia fetida were cultured (in triplicate) on cattle manure at room environmental conditions with sufficient moisture (70-75%) and food with the onset of summer (February-May), monsoon (June-September) and winter (October- January) seasons prevailing in this region. Observation were made with respect to all life activities such as growth, attainment of sexual maturity, commencement of cocoon production, cocoon weight, incubation period, percent hatching and number of hatchling per cocoon to find out the influence of prevailing seasonal environmental factors on the life activities of this worm. Results revealed that overall life activities of the epigeic earthworm, Eisenia fetida were significantly more during winter followed by monsoon season. Least activities of this worm was observed during summer season. Vermiculture and vermifarming with Eisenia fetida could be undertaken effectively during winter and monsoon seasons in semiarid region of peninsular India.

COMPETITION BETWEEN *H. ELISAE* ÁLVAREZ, 1977 AND OTHER EARTHWORMS IN EL MOLAR, MADRID (SPAIN)

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An unusual distribution of earthworm species was observed in an experimental plot in El Molar (Madrid). The three most abundant species, *Hormogaster elisae*, *Allolobophora caliginosa* and *Allolobophora rosea*, appeared

in three, relatively isolated areas. A possible explanation for this might be competition. Competition between species often centres on food; the capacity to obtain food, the efficiency of its use, and resistance to a lack of food etc., all play their part. However, the observed distribution might also be explained by the occupation of space or other factors (Margalef, 1974). The aim of the present work was to detect the possible intra- and interspecific relationships between theses three species in the laboratory.

Three types of microcosms were prepared: <u>Monospecific</u> (controls). These contained either *H. elisae*, *A. caliginosa* or *A. rosea*, but always alone. Two types of control were set up - single (Control A) and double (Control B). The double controls contained twice the initial biomass of the single controls, thus allowing intraspecific competition to be studied.

Polyspecific. These cultures contained either H. elisae plus A. caliginosa, H. elisae plus A. rosea or A. caliginosa plus A. rosea. Soil from each of the three areas of the experimental plot (i.e., the H. elisae, A. caliginosa and A. rosea areas) was used as the culture medium, rendering a total of nine treatments (6 replicates per treatment) for each soil type. The soil was renewed every 14 days. The experiment lasted four months. At each soil change the earthworms were weighed and their state of maturity recorded; the numbers of dead and quiescent individuals were also registered. Cocoons were isolated by passing the soil through a sieve column. Mortality for the three species in each treatment was practically zero, with no significant differences between treatments. The results show that, in the microcosms containing soil from the plot area where H. elisae was the sole species (more sandy and poorer in organic material), intraspecific competition occurs in H. elisae and A. rosea as well as interspecific competition between these two species. This is possibly due to the lack of food resources owing to the scanty organic material available. Allolobophora caliginosa, however, appeared to benefit from the presence of the other species and also grew more quickly in the double controls, perhaps because earthworm activity (production of mucus and casts etc.) improves the poor soil. In microcosms containing soil from the A. rosea area, intraspecific competition was seen in H. elisae and in A. rosea. Interspecific competition between these two species was also seen. In contrast, the growth of A. *caliginosa* was not affected by the presence of the other species, although the presence of A. caliginosa did affect the growth of A. rosea. In the soil from the A. caliginosa area, A. caliginosa itself showed intraspecific competition and interspecific competition with H. elisae. No differences in growth were seen between H. elisae and A. rosea in the monospecific and polyspecific treatments. Together, these results show interspecific competition exists between H. elisae and A. rosea in soils where these species are dominant, but in the A. caliginosa area all three species could coexist.

The results for *A. caliginosa* varied depending on the soil type. In poor soils the species benefited from the presence of the other species, but in the *A. caliginosa* area soil the presence of *H. elisae* negatively affected its growth.

SAMPLING OF EARTHWORMS WITH NON-STANDARD METHODS: SURPRISING RESULTS FROM GERMAN FOREST SITES

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In 1990 the provincial government of Hesse, Germany in cooperation with the Senckenberg Institute started a longterm survey of the succession in forest reserves. 31 formerly managed areas covering all different forest types in Hesse, are allowed to mature without direct human influence. A broad faunistic inventory is conducted step by step in all of these reserves using a wide range of traps and hand sampling over a period of two years. The first three sites, which have been studied are covered by different types of beech (Fagus sylvatica) wood. 13, 21 and 22 sites (depending on the richness of different structures in the reserve) were studied with a triplett of pitfall traps; 4 living and 4 standing dead beech-tree trunks were studied with trunk eclectors in 1.80 m height.

Although no special research program was run for the earthworm fauna, pitfall traps and eclectors at tree trunks provided an overview of the qualitative spectrum of the eathworm biocoenoses. Most worms were found in the pitfall-traps, but an astonishing high number did enter eclectors - not only on dead wood but also on living trees. The number of lumbricid species varied between 9 and 13 at the three sites while the composition was comparable. However, when comparing pitfall-traps and eclectors, both showed completely different sets of lumbricids. In the pitfall-traps species from all three ecological groups were found (mainly Lumbricus rubellus but also Aporrectodea caliginosa and Lumbricus terrestris). In fact, more or less all species either expected from these sites or already found in monitoring studies were identified in the pitfall-traps. In the tree eclectors the number of species was lower (4-5) and the most dominant species was by far Lumbricus eiseni (recently moved to the new genus Allolobophoridella). This worm classified as an epigeic species and often considered as rare in Germany, seems to be a true cortical species which was simply overlooked in most studies focused on the soil inhabitants. It seems that L. eiseni is a common worm of forest sites (in particular with moderately acid soils and beech wood) in Central Europe which can provide an important part of the total invertebrate biomass due to its high numbers at these sites. In lesser extent also Dendrodrilus rubidus and Dendrobaena octaedra were present in the eclectors, which documents that an even larger part of our Central European lumbricids fauna is climbing up tree trunks. Unpublished results from collectings in the crown region of Bavarian forests show, that even in this stratum lumbricids are present Thus, specific sampling programmes focusing on these species (and also enchytraeids) should be initiated in order to clarify the ecological role of these oligochaetes outside their "normal" habitat.

FROM MOWING TO GRAZING: DOES THE CHANGE IN GRASSLAND MANAGEMENT AFFECT SOIL ANNELID ASSEMBLAGES?

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Changes in agricultural policy in the last decades have led to alterations in the management of Central European grasslands. In managing grasslands, extensive pasturage represents today a cheap alternative to mowing. It has been implemented even in traditionally mowed meadows of high conservation value, disregarding its possible negative effects on these grassland ecosystems.

Soil annelid assemblages were investigated within a comprehensive research project to assess the impact of cattle and sheep grazing on formerly mowed meadows. The study was carried out in 2004-2005 in the White Carpathians Biosphere Reserve, Czech Republic. Three pairs of grassland plots representing plant communities characteristic of the area (*Polygalo-Cynosurenion* and *Arrhenatherion*) were chosen for the study. In each pair of plots grazing was taken up in one plot while mowing continued on the other. After this management had been established, earthworms, enchytraeids and other small annelids were studied based on two years of sampling in spring and autumn. Annelids were extracted from soil cores (heat and wet funnel extraction, respectively) and identified to species.

Earthworm density was significantly higher in the *Arrhenatherion* plot (120.8 ind.m⁻²) than in the *Polygalo-Cynosurenion* plots (67.2 and 19.2 ind.m⁻²). The shift from mowing to grazing had a negative impact on the assemblages of earthworms, significantly reducing their density at all sites under study (densities in mowed vs. grazed plots were 137.6 vs. 104.0; 92.8 vs. 41.6 and 35.2 vs. 3.2 ind.m⁻², respectively). The number of species per plot was 7-8, with an only weak effect of grazing observed at two sites. At the third site (*Polygalo-Cynosurenion*), however, grazing led to a reduction of species number from 7 to 2. There were significant effects of grazing on the structure of earthworm assemblages at all sites, populations of the epigeic *Dendrobaena octaedra* and the anecic *Fitzingeria platyura* being most affected.

Enchytraeid species numbers ranged from 12 to 23 per plot; the species-richest plot was the only one harbouring also non-enchytraeid annelids of small body-size, i.e. *Rhyacodrilus falciformis* (Tubificidae) and *Aeolosoma hemprichi* (Aeolosomatidae). In two pairs of plots species numbers were higher in the meadows, in one case the pasture had the species-richer assemblage. Enchytraeid densities per plot and sampling date ranged between ca. 3 500 and 27 000 ind.m⁻². There was no consistent trend indicating an effect of grazing on enchytraeid densities. Regarding vertical distribution enchytraeids seemed to be more accumulated in the uppermost soil layer in pastures when compared to the mowed meadow plots, but this trend was again not very consistent.

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TOPOLOGICAL VARIATIONS IN THE SEASONAL DYNAMIC OF A. caliginosa SUBPOPULATIONS UNDER DIFFERENT FOREST STANDS

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Assessments of effects of the recent climatic trend on soil biotic communities requires precise investigations of the population dynamic of keystone pedobiont groups and their spatial-temporal variations. This task is of actual interest for polytopic species of earthworms with wide geographic ranges.

Comparative studies of the seasonal dynamic of 2 subpopulations of *Aporrectodea caliginosa* Savigny were carried out in 2 forest types (*Piceetum oxalidosum* and *Tilietum caricosum*) belonging to one forest massif (Moscow region). These forest types are characteristic of the coniferous-broad-leaved forests of Russian Plain. A comparatively short distance between these forest plots allowed exchanges by soil animals. Local microclimatic differences in soil (loamy luvisols) in experimental forest stands were determined by litter content and decomposition dynamic. Earthworms were numerous in both forest plots (170-220 Ind./m²), *A. caliginosa* predominating among them (25 % - in *Piceetum* and 80 % – in *Tilietum*).

The seasonal dynamic of the abundance, production and population structure of *A.caliginosa* were studied during 2 subsequent years using regular quantitative samplings (8 times from April to October) of standard soil cores ($25 \times 25 \times 20 \text{ cm}$, 10 parallels). Growth rates of different weight groups were studied in field and laboratory experiments. Results obtained were used to determine the age structure of *A.caliginosa* local subpopulations. The growth rates of *A.caliginosa* from forest habitats were a little lower than those in experiments of Nowak (1975).

In the spruce stand the abundance curve of *A. caliginosa* subpopulation showed two maxima - in April-June (46-42 Ind/m²) and in late October (27,2 Ind./m²) with the minimum (17,6 Ind./m²) in August. Mature earthworms of 2-4 years old (500-900 mg) predominated in April-June. At that time an active growth of separate age groups was recorded. The maximum emergence of juveniles from cocoons was observed in midsummer-autumn, and the population structure became bimodal with the predomination of two cohorts of juveniles - hatched in the late autumn (160-240 mg) and in summer (40-120 mg). Relative significance of these groups in the population averaged 30 and 28% in August and 20 and 55 % - in October. Old individuals in August-October were practically absent in samples. After aestivation they transited to hibernation in inactive condition.

In the lime forest a high population numbers of *A. caliginosa* were supported during the spring-summer (145-133 Ind./m²) with the autumn decrease to 110 Ind./m². The population structure preserved the bimodal form during the whole period. Young specimens of 1-4 months old were presented in samples from April to late October, consisting 22-33 % of the total numbers. The other predominated group was represented by mature individuals of 2-4 years old (350-700 mg). The cocoon production and hatching of juveniles were observed in the lime plot practically during the whole warm season.

Results of field investigations showed that local microclimatic conditions determine cardinal differences in seasonal activity and reproduction rhythms and population structure of earthworms. This ecological resilience of can be considered as the adaptive population strategy of *A. caliginosa*, determined the polyzonal distribution of this species.

VERTICAL SEED TRANSPORT BY EARTHWORMS: IMPLICATIONS FOR THE DIVERSITY OF PLANT COMMUNITIES

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Earthworms are supposed to play an important role in altering the dynamics of the soil seed bank and to have a close association to the distribution of certain plant species. To evaluate the impact of the earthworm species Lumbricus terrestris on the vertical transport of seeds in the soil, we conducted a full-factorial greenhouse experiment where we placed a defined number of seeds in different depths of a soil column (diameter 15 cm, length 40 cm; n = 3) and added four adult individuals of L. terrestris. The seeds distributed in the soil column were of a grass species (Dactylis glomerata), two non-leguminous grassland herbs (Rumex obtusifolius, Taraxacum officinale) and a leguminous herb species (Trifolium repens). Preceding palatability tests showed that seeds of these species were eaten by earthworms. Results demonstrated a significant bi-directional and species-specific translocation of the seeds by earthworms after a period of three months. During the course of the experiment up to 2150 seeds m^{-2} of a single species (*T. officinale*) were translocated by earthworms. Interestingly, seeds of different species were translocated in different directions and over different distances. In order to link the findings from the greenhouse experiment to the field situation, we also monitored seeds present and germinating on earthworm surface casts collected in a perennial grassland over five weeks. During the sampling period we recorded around 500 seedlings m⁻² of 32 plant species in surface casts. These results showed that earthworm activity has a substantial impact on the vertical distribution of seeds in the soil and hence possibly also on the plant recruitment in this species-rich ecosystem.

NIRS SPECTRAL SIGNATURES OF CASTS OF APORRECTODEA CALIGINOSA FED DIFFERENT ORGANIC SUBSTRATES

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Immature individuals of *A. caliginosa* were kept for 16 weeks in laboratory cultures and fed soil added with respectively 0, 10 or 50% of a natural *Populus nigra* leaf compost. Growth was maximum in the 10% organic matter treatment and cocoon production occurred. Soil ingestion rates decreased from 1.4 to 2.2 g.g⁻¹ fresh weight of worm.day⁻¹ in control soil to respectively 0.78 -1.28, and 0.24-0.46g respectively in treatments with 10 and 50% natural compost. Surface casts never comprised more than 10% of total cast production. Near Infra Red Spectrometry signatures of digested and non-ingested soil significantly differed and showed a rather constant effect of digestion, independent from the organic matter content (p<0.01). These results confirm the value of NIRS spectral signatures as indicators of the origin of soil aggregates.

Session 3

Immunology and Physiology

COELOMIC FLUID PROTEINS AS BASIC COMPONENTS OF INNATE IMMUNITY IN EARTHWORMS

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The coelomic fluid (CF) of earthworms contains cells and many molecular components involved in innate immunity of these species. Among those are glycoproteins of lectin character which contribute to the recognition of foreign material in the coelomic cavity by binding to carbohydrates. Moreover they cause its immobilisation by agglutination and destruction by membrane lysis. Further humoral components phenoloxidase, proteases and other enzymes are mainly involved in the elimination of foreign material.

Based on the analysis of several earthworm species (Allolobophora chlorotica, Apporectodea caliginosa, Dendrodrillus rubidus, Eisenia fetida, Lumbricus rubellus, L. terrestris) we showed that wounding as well as the injection of foreign material into the coelomic cavity increases coelomocyte numbers and the activity of some easily measurable humoral immune factors like proteases, agglutinins and lysins. Moreover newly synthesised proteins were identified within the protein pattern of CF after challenging, suggesting their involvement in immune reactions. Differences in protease activity become obvious especially by comparing enzyme patterns of untreated and stimulated CF samples separated by electrophoresis. Earthworm CF proteases are relatively heat stable, posses an activity optimum at neutral to basic pH and are classified mainly as serine proteases, presumably involved in melanization and phenoloxidase activation. The naturally occurring titre of the hemagglutinating activity varies among the earthworm species investigated but was generally increased after stimulation. An enhanced lytic activity was evident for some species after stimulating the immune response. Taking all these facts it can be summarised that disturbance of the homeostasis in earthworms results in an increased immune response. Some of these immune proteins might be considered as suitable bio-markers in monitoring environmental conditions. Protease pattern and protease activity may assumed to be promising candidates in this regard and are easy to analyse.

NEW INSIGHTS INTO THE STRUCTURE OF THE EARTHWORMS' CALCIFEROUS GLANDS

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The oesophageal glands of earthworms are commonly referred as calciferous glands or glands of Morren. They are present in all species of the family Lumbricidae and, despite the number of biological roles attributed to these glands (egg formation, pH buffering of the blood and the ingested food, excretion and respiration) their true function is still unsolved. In the past several authors have tried to describe the structure and activity of these organs with special emphasis on certain species (e.g. *Lumbricus terrestris*), however many structural aspects are still unknown and the significance of their secretions remains uncertain.

In this study we provide new insights into the morphology and histology of several earthworm species belonging to six genera (*Allolobophora, Aporrectodea, Lumbricus, Dendrobaena, Eisenia, Octolasion*) collected from different locations in Europe. Earthworms were hand-sorted in the field and taken back to the laboratory for removal of the glands by dissection. For the histological and histochemical study several staining techniques were used: (i) some thin sections of the glands were stained with the standard hematoxylin-eosin methods, (ii) other sections were treated with either periodic acid-Schiff reagent (PAS), alcian blue (AB) at pH 2.5 or PAS-AB to detect the presence of mucosubstances, and (iii) the presence of calcium in the gland was determined by the von Kossa technique.

The morphological study of the different genera showed that the anatomy of the gland falls into three types: (i) pouches and oesophageal enlargements present (*Lumbricus, Octolasion*), pouches present but no enlargements (*Allolobophora, Aporrectodea*), pouches absent but enlargements present (*Dendrobaena, Eisenia*). Calcium secretion usually appears initially to be a "milky fluid" and in those species with pouches the calcium carbonate crystals aggregate resulting in bigger concretions of different sizes. Pouches open to the oesophageal lumen allowing the release of these big granules to the gut. Calcium secretion into the lumen is also likely to occur through the gland's inner epithelium as the milky fluid is often seen wrapping the ingested material. The greatest gland activity was observed in the genus *Lumbricus* which showed the largest concretions inside the pouches as well as a great number of crystalline structures between the lamellae. These structures, mostly consisting of neutral mucosubstances, were also observed to a lesser extent in *Allolobophora, Aporrectodea* and *Octolasion* species.

These results highlight the differences in the morphology and activity of these structures among different species.

IDENTIFICATION OF VALOSINE-CONTAINING PROTEINS (VCP) AS A NOVEL SPERMATOGENESIS RELATED FACTOR IN *EISENIA FETIDA* (SAVIGNY, 1826)

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It is of particular interst to investigate the mechanism of reproductive organ differentiation and gametogenesis in annelida. In an attempt to isolate gonad relatedgenes from the common Lumbricidae earthworm Eisenia fetida (Savigny, 1826), we have isolated two members of AAA family (ATPase-Associated with diverse cellular Activity) with sequence similarities to mammalian valosine-containing proteins (VCP). The primary sequences, referred to as eVCP-1 and eVCP-2, display a similarity of 74%. The predicted amino acid sequence reveals that eVCP-1 contains two AAA domains, one amino-and one carboxylproximal. eVCP-1 also contains a VCP/P97/Cdc48 consensus carboxyl-terminal motif including a stretch of acidic residues and a carboxyl-terminal tyrosine (DDDLY). Likewise, eVCP-2 also contains two AAA domains, but does not have the carboxylterminal DDDLY motif. The deduced amino acid sequence of eVCP-1 is 58.9% identical to eVCP-2. The intron/exon organization of evcp-1 and evcp-2 was deduced from fragments amplified by genomic PCR. Both contain 14 introns and 15 exons with 10 conserved intronic splice sites. All introns, apart from intron 9 in evcp-1, comply with the GT-AG rule, an observation that is analogous to human vcp where intron 14 fails to obey the rule. At least eight introns are conserved in earthworm and mammalian VCPs, strongly suggesting that the VCPs originate from the same ancestral gene. However, based on phylogenetic analyses, there is evidence that eVCP-2 may be distinct, and possibly an invertebrate specific member in the VCP/P97/Cdc48p family. An investigation by reverse transcription-polymerase chain reaction (RT-PCR) revealed that, whilst evcp-1 was ubiquitously expressed during all developmental stages, evcp-2 was specifically expressed in the anterior segments of mature (ciliated) earthworms. In situ hybridization clearly demonstrated that evcp-2 is expressed in the seminal vesicles, the location of spermatogenesis, and more precisely within the cytophores surrounded by secondary spermatocytes or spermatides. Taken together, this evidence leads to the notion that eVCP-2 is a likely component involved in the final modulation of spermatogenesis.

EARTHWORM *EISENIA FOETIDA* AS A SOURCE OF MACROMOLECULES WITH DIFFERENT BIOLOGICAL ACTIVITIES

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The glycolipoprotein extract (G-90) obtained from tissue homogenate of eartworm Eisenia foetida has many biological characteristics, which are usfull in different biological processes. This extract showed interaction with biological materials from evolutionary more developed species, such as serums, erythrocytes, normal and transformed cells in culture of birds and mammals. The extract has also lectine activity. Depending on the concentration, it acts as agglutinin, hemagglutinin, mitogen, anticoagulant, fibrinolytic and intermediate agent in cell impulse transmission. Part of the structures responsible for described activites are isolated and characterized (chemically and biologically). These structures are growth factors belonging to insulin superfamilly, adhesins - integrins from immunoglobulin superfamilly and serine proteases with tripsin code with fibrinolytic and anticoagulant activities. Based on the our research work and those from literature data about regenerative ability of earthworm, we found that G-90 contains the structure and qualities required for "in vivo" tissue regeneration process. The macromolecular mixture G-90 in nanograms and micrograms concentration stimulates proliferation of the cells, depending on the type. In terapeutic concentrations G-90 is not toxic, allergenic, mutagen nor carcinogen. It has strong antibacterial activity "in vivo" and "in vitro". G-90 protected cells in culture from oxidative damage, caused by hydrogen peroxide. G-90 stimulates cellular immune system. As fibrinolytic and anticoagulant it can participate in an active maintenance of homeostasis. G-90 has an ability to stimulate the synthesis of growth factors and helps in tissue regeneration. It stimulates proliferation of fibroblasts and epithelial cells, as well as their differentiation.

EFFECT OF EARTHWORM PASTE (*LAMPITO MAURITII*, KINBERG) ON THE ANTI-INFLAMMATORY, ANTI-OXIDATIVE, HAEMATOLOGICAL AND SERUM BIOCHEMICAL INDICES OF RAT (*RATTUS NORVEGICUS*)

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From time immemorial earthworms have been used as a therapeutic agent. Recently earthworm protein and its coelomic fluid were reported to have cytolytic, agglutinating, proteolytic, haemolytic, mitogenic, anti-pyritic, tumorstatic and antibacterial activities. Vohora and Khan found earthworms to have healing effect on wounds, chronic folds, piles and sore throat. Earthworm's anti-pyretic properties were reportedly tried in China and Japan in reducing fever. Hori et al have reported significant anti-pyretic activity of earthworms Lumbricus spp and Perichaeta spp. Bhatnagar and Palta have reported that earthworms when ingested in to our body system, increase body heat and are of value in neural disorders, bronchitis and tuberculosis and in curing rheumatism. Mihara et al have reported the earthworm, Lumbricus rubellus to be potentially very useful in treating thrombosis and infact, orally administrated earthworm powder was capable of digesting intra vascular fibrin clots. The anti-inflammatory activity of earthworm paste (EP) and its extracts in different solvents were studied in carageenin induced oedema and cotton pellet granuloma in rats. It was found that the anti-inflammatory activity of earthworms was similar to that of aspirin on carrageenan induced oedema. Experiments were conducted to understand the therapeutic properties such as anti-inflammatory, anti-oxidative, haemataological and serum biochemical markers of earthworm paste (EP) from an indigenous species Lampito mauritii (Kinberg), in comparison with the standard antiinflammatory drug- aspirin, on Wistar albino rat (Rattus norvegicus). Our results showed that the administration of aspirin (75 mg/kg) or different doses of EP (20, 40, 80, 160 and 320 mg/kg), reduced inflammation and restored to a normal condition, both in acute as well as in chronic phase. We found that the effects were more significant when 80 mg/kg EP was administered compared to aspirin or other doses of EP. Under this condition, in the liver of acute phase inflamed rats, the levels of reduced glutathione, glutathione peroxidase, superoxide dismutase, catalase and thiobarbituric acid reactive substances were found to be normal. Also in the liver and muscle of chronic phase inflamed rats, levels of reduced glutathione and glutathione peroxidase were normal. Furthermore, in chronic phase inflamed rats, the following parameters were normal: haematological parameters e.g., red erythrocyte corpuscle, white leukocyte corpuscle, differential levels of neutrophils, lymphocytes, eosinophils, haemoglobin and serum biochemical contents e.g., protein, albumin, glucose, cholesterol, acid and alkaline phosphatase, electrolytes e.g., sodium, potassium and chloride. These results reflect the presence of anti-inflammatory effects of EP, which may scavenge the free radicals /and stimulates the activities of anti-oxidative enzymes, and normalizes the haematological and serum biochemical characteristics. Cooper EL. Bioprospecting: a CAM Frontier. Evid Based Complement Altern Med 2005; 2:

1-3.

Oral A NEW ANTIBACTERIAL VERMIPEPTIDES FAMILY FROM EARTHWORM ESENIA FETIDA

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By using a simple and effective method of earthworm peptide inducement, separation and purification we established in our laboratory and achieved the patent in 2004, five antibacterial peptides (EP1 to EP5) were isolated and purified from earthworm tissue homogenate liquid and coelomic fluid in this research, which contained 5-7 amino acids with the same or similar amino acids sequence Ala-Met-Val-Ser-Gly, was named the antibacterial vermipeptides family (AVPF) according with the structure characteristic of these antibacterial peptides. The resistance of EP2 and EP3 to MGC803 cell was found in this research. The AVPF exist wide antibacterial speciality, for example of EP3, the results of anti-tumor activity of EP3 were that: EP3 could cause apoptosis of HeLa cell. The apoptotic activity was dose dependent. And the threshold value of EP3 to cause apoptotic on HeLa cell was 0.75mg/mL, with an apoptotic rate of 48.12%. The mechanism of antiviral activity of EP3 was inhibiting the duplicate of virus DNA.

AVPF concentrations and activities varied with the relation between the variation of survival environmental and the activity change of antibacterial peptides. In this research, we used environmental factors induced antibacterial peptides, compared with methods both damage inducement (shearing-off) and no damage stimulates (the treatment of shake and heavy metal). Results showed the biology and electricity induced method was the effective induction to the production of earthworm antibacterial peptides. The ecology mechanism principle of induction expression to the earthworm antibacterial peptide named trigger theory was proposed firstly in the research. The results from this paper and our former researches provided the foundation of earthworm's ecology adaptation to the stressing environment and proved the theoretical hypothesis that peptides may be the antibacterial component in the antibacterial system of earthworm coelomic fluid.

IDENTIFICATION OF EARTHWORM COELOMOCYTE SUBGROUPS WITH IMMUNOLOGICAL AND MOLECULAR BIOLOGICAL APPROACHES

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Coelomocytes are immunologically active cells playing a major role in cellular immune functions of earthworms. Functional investigations and microscopy reveal various subgroups of coelomocytes, which have different morphological and functional properties. Chloragocytes are important in nutrition, heavy metal sequestretation, production of antimicrobial (cytotoxic) molecules- and respiratory pigments. Hyaline coelomocytes, granular coelomocytes are essential in cellular effector functions such as phagocytosis and encapsulation. Our aims include the characterization of earthworm coelomocyte populations

using modern immunological methods and identification of specific molecular markers characteristic of coelomocyte subgroups. Using flow cytometry, coelomocytes show three groups according to physical parameters. A set of earthworm coelomocyte specific monoclonal antibodies (anti-EFCC mAbs) was produced against *Eisenia fetida* coelomocytes. Anti-EFCC1 (3/C2) clone labels the whole coelomocyte pool. Anti-EFCC2 (1/B1) marks specific intracellular structures in the chloragocyte population. Intracellular staining was observed in the hyaline amoebocyte group using anti-EFCC3 (2/B1) mAb. A third cell population was labeled with anti-EFCC4 (3/C1). A few EFCC4 positive cells have also been identified in the coelomic cavity. Several antibody-producing clones with a similar reaction

pattern were raised within the EFCC1 category. To identify the target sequences of the anti-EFCC mAbs we used a random peptide library, expressed on the VIII. coat-protein of filamentous M13 bacteriophage. After repeated enrichment with anti-EFCC1 mAb, several phages were analyzed with indirect ELISA. According to the ELISA results high positive phage clones were sequenced providing a 6 AA long (SLSDSC) target sequence of anti-EFCC1 mAb. Phage inhibition ELISA was used to verify the epitope of a-EFCC1 mAB. Interestingly, this anti- EFCC mAb, but no other, shows reactions with cells of other earthworm species, and by comparison tissues of the freshwater snail (*Planorbarius corneus*).

In summary we developed immunological tools for specific identification of morphologically and functionally distinct coelomocyte clusters. Using a powerful molecular biological

methods we identified an epitope of our a-EFCC1 mAb, however the epitope mapping of the other anti-EFCC mAbs are still in progress.

THE SEARCH FOR INNATE IMMUNE RECEPTORS ON EARTHWORM COELOMOCYTES

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Pattern recognition receptors are known to play major roles in modulating both innate and adaptive immune responses. Much attention has focused on a family of receptors called the Toll-like receptors (TLR). TLR expression has been detected in a variety of different vertebrate and invertebrate species as well as in plants. In some invertebrate species, TLR gene expression has been detected but their cell surface expression on immune cells has not been noted. TLRs as well as several other innate immune receptors have not yet been detected on earthworm coelomocytes.¹ We have employed a group of commercially available polyclonal and monoclonal antibodies (mAb) directed against mammalian TLRs It is expected that many mAb directed against mammalian TLRs may not interact with invertebrate TLRs since mAbs typically identify only a single epitope on a given antigen and it is very possible that TLRs from different species only share a small subset of epitopes. Using fluorescence microscopy, we recently obtained some preliminary data that suggests that a mAb directed against TLR2 binds to roughly 20% of the coelomocytes of Eisenia foetida. Meanwhile, we did not detect expression of TLR4, 4, or 9. We are in the process of confirming the expression of TLR2 using flow cytometry. In positive control experiments, CD 90 and a series of recently developed mAb (EFCC) directed against coelomocyte surface markers were detected on the surface of coelomocytes from both earthworm species.² (CD-90 has been shown to be a surface marker present on Eisenia coelomocytes but to our knowledge it has not been shown to be a surface marker on Lumbricus terrestris coelomocytes). To further demonstrate that earthworm coelomocytes possess unique TLRs (largely undetectable with currently available mAB) or receptors which bind to TLR ligands we have constructed a fluorescent flagellin probe (TLR 5 ligand) and a fluorescent peptidoglycan probe (TLR 2 ligand). Our preliminary results show that a subset (10-20%) of both Lumbricus and Eisenia coelomocytes bind the flagellin probe. Taken together our results suggest that invertebrate coelomocytes may express pattern recognition receptors capable of detecting pathogens and providing a mechanism of host defense.

THE EARTHWORM EXPRESSED SEQUENCE TAG (EST) PROJECT

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Expressed Sequence Tags (ESTs) are short (300-700 bp) sequences derived from cDNA libraries. They are a powerful resource to acquire large scale information on gene content and expression levels. The earthworm (*Lumbricus rubellus*) EST project was initiated in the year 2000 and now contains over 17000 sequences from libraries synthesised from dissected late cocoon embryos, juveniles, adults, anterior sections, and adult earthworms exposed to atrazine, cadmium, copper, fluoranthene or lead. Following the masking of poly A tails and the removal of vector sequences, high quality sequences were submitted to the EMBL/GenBank databases. All sequences were clustered, annotated and integrated into LumbriBASE (see http://www.earthworms.org), a relational database designed to facilitate searches by key words and/or sequence information within the entire dataset or limited to specific libraries. In addition, predicted protein translations with domain and signal peptide identification, pI, molecular weight and putative cellular locations were included with a dedicated Peptide Mass Fingerprint (PMF) search function. In total, over 7500 unique gene objects were identified and have allowed the creation of earthworm micro-arrays, a resource that has recently been exploited in numerous gene expression experiments.

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In recent years, antimicrobial peptides have become recognized as important contributors to nonspecific host defense for both vertebrates and invertebrates. The value of antimicrobial peptides lies in their ease and speed of synthesis, their broad specificity against prokaryotic cells and their general lack of toxicity for eukaryotic hosts. Thus, they serve as a first-line defense against microbial invasion, supplementing the humoral and cellular immune system. Many different kinds of antimicrobial peptides, which possess antimicrobial activity against bacteria, fungi and enveloped viruses with little or no cytolytic activity, have been isolated from diverse sources [1,2]. In the 700 million years of existence, earthworms have evolved in the environment replete with microorganisms, some of which threatening their existence. To survive in such an environment, they have developed efficient defense mechanisms against invading microorganisms. Such defenses are present in the coelomic fluid of earthworms. The antimicrobial activity in the coelomic fluid is partly attributed to some proteins, such as lysozyme and fetidins. Antibacterial peptides must provide function in earthworm innate immunity, which has been reported [8,9,10]. Those research focus on the identification of antibacterial peptides, mechanism of expression and the molecular character and activity. Though progresses have got in the studying of antibacterial peptides from earthworm, more detail biological role in vivo, the structure-function relationship, extention, activity and toxicity to human or other mammals are unknown. More research needs attention. Firstly, it is essential to obtain enough sample peptides for detailed analysis. Because of the difficulties to purify natural peptides from earthworm directly, for example, the total amount of purified Lumbricus I recovered was 0.1?g per g earthworm, expression of the antibacterial peptides in E. coli or yeast by efficient methods. Secondly, the detail biological role, also the toxicity should be considered, for example, the antitumour activity has been found in several antibacterial peptides. On earthworm, the antitumour activity of F-1 and F-2 were probed with MTT assay and by scanning electron microscopy. F-1 and F-2 played antitumour activity to MGC803 cells (p< 0.05) and morphological changes are observed. Analysis of toxicity is necessary before molecular and clinical investigations. Finally, the molecular design and protein engineering will be the important research interests after analysis of structurefunction relationships. Earthworm, as a natural resource, is easy to obtain and a convenient animal model. It will provide clinical therapy and a source of antibacterial peptides in the future and a theoretical contribution to understanding innate immunity.

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Multicellular organisms including invertebrates and vertebrates live in various habitats that may be aquatic or terrestrial where they are constantly exposed to deleterious pathogens. These include viruses, bacteria, fungi, and parasites. They have evolved various immunodefense mechanisms that may protect them from infection by these microorganisms. These include cellular and humoral responses and the level of differentiation of the response parallels the evolutionary development of the species. The first line of innate immunity in earthworms is the body wall that prevents the entrance of microbes into the coelomic cavity that contains fluid in which there are numerous leukocyte effectors of immune responses. When this first barrier is broken, a series of host responses is set into motion activating the leukocytes and the coelomic fluid. The responses are classified as innate, natural, non-specific, non-anticipatory, non-clonal (germ line) in contrast to the vertebrate capacity that is considered adaptive, induced, specific, anticipatory and clonal (somatic). Specific memory is associated with the vertebrate response and there is information that the innate response of invertebrates may under certain conditions possess specific memory. The invertebrate system when challenged affects phagocytosis, encapsulation, agglutination, opsonization, clotting and lysis. At least two major leukocytes, small and large mediate lytic reactions against several tumor cell targets. Destruction of tumor cells in vitro shows that phagocytosis and natural killer cell responses are distinct properties of these leukocytes. This has prompted newer searches for immune function and regulation in other systems. The innate immune system of the earthworm has been analyzed for more than 40 years with every aspect examined. However, there are no known entire sequences of the earthworm as exists in these other invertebrates. Because the earthworm lives in soil and has been utilized as a successful monitor for pollution, there are studies that reveal up and down regulation of responses in the immune system after exposure to a variety of environmental pollutants. Moreover, there are partial sequences that appear in earthworms after exposure to environmental pollutants such as cadmium and copper. There are now attempts to define the AHR receptor crucial for intracellular signaling after exposure to pollutants, but without linking the signals to changes in the immune system. There are several pathways for signal transduction, including JAK/STAT, TOLL, TRAF PIP3, known in invertebrates and vertebrates. For resistance to pathogens, conserved signal transduction components are required and these include a Toll/IL-1 receptor domain adaptor protein that functions upstream of a conserved p38 MAP kinase pathway. This pathway may be an ancestral innate immune signaling pathway found in a putative common ancestor of nematodes, arthropods and even vertebrates. It could also help us to link pollution, innate immunity and transduction in earthworms.

LYSOZYME-TYPE ACTIVITY IN COELOMIC FLUID AND IN EGGS OF

DENDROBAENA VENETA

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A natural immunity of annelids is formed by anatomical and chemical protective barriers preventing damage of underlying tissues, body fluid losses and microbial infections of the body cavity. The internal defence mechanisms of the invertebrates involve phagocytosis, nodulation, encapsulation, blood coagulation, wound repair and antibacterial immune proteins activation. Antibacterial and antifungal activity of annelids is mainly associated with the action of lysozyme-like substances, inducible humoral molecules and cytolityc proteins. Lysozyme activity is directed against Gram-positive bacteria and against fungi. In this paper lysozyme-like activity in coelomic fluid and in the extract of Dendrobaena veneta eggs was analysed. Activity against indicator bacteria Micrococcus luteus was higher in coelomic fluid than in the extract from eggs of D. veneta. After immunobloting using antibody against egg white lysozyme as a standard, single protein with molecular mass about 15 kDa was recognized in coelomic fluid and in the extract of eggs. After analysis by electrophoresis at pH 4.3 and subsequent bioautography, two lytic bands were observed in coelomic fluid and three - in the extract of eggs. This results can indicate the presence of several forms of lysozyme in D. veneta. It was observed that, lysozyme activity has increased 5-6 times after infection with E. coli.

EFFECTS OF HEAVY METALS ON COELOMOCYTES OF ALLOLOBOPHORA CHLOROTICA

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We have previously shown drastic effects of soil pollution on general condition and impairment of coelomocyte counts and activity in adult individuals of Allolobophora chlorotica (Homa et al. 2003) and Dendrobaena veneta (Wieczorek-Olchawa et al. 2003). In particular, adult animals transferred from the heavy-metal free soil to heavily polluted soil samples from the industrial area (rich in zink, cadmium and lead) exhibited inhibition of reproduction and high mortality, connected with massive bacterial invasion corresponding with impairment of immune functions. In order to examine the effects of the known concentration of particular metals on functioning of the immune system, we used methods recommended by OECD (1984), i.e. dermal exposure to metal chloride-soaked filter papers. Experiments on Eisenia fetida (Homa et al. 2005) and D. veneta (Olchawa et al. 2006) revealed that metals accumulate in animal tissues and affect coelomocyte functioning, leading to the disrupted balance between immunocompetence and bacteria. Coelomocytes extruded from metal exposed animals were less numerous and showed upregulated expression of stress proteins. The aim of investigations presented here was to perform the same sort of experiments on A. chlorotica, which differs from E. fetida and D. veneta in the preferred habitat. Adult individuals of Allolobophora chlorotica were collected in the experimental garden of the Institute of Zoology (Krakow) and kept in the laboratory in the commercial metal-free soil. The animals were exposed for 1-3 days to metal ions (Zn, Cu, Pb, Cd chlorides, 44 mg/L) or to distilled water (controls) via filter papers (heavy metals exposure concentrations up to $1.32 \,\mu g/cm^2$). Heavy metal accumulation in the animal body was measured as previously (Homa et al. 2003, 2005), coelomocytes were expelled by the electric shock (1 min, 4.5 V), counted, and used for cytospin preparations subjected to immunoperoxidase staining with monoclonal antibodies against human heath shock proteins (HSP70 or HSP72), caspase-3, or rabbit polyclonal antibodies raised against metallothionein 2 (w-MT2) of Lumbricus rubellus (Morgan et al. 2004), as described previously (Homa et al. 2005).

During 3-day dermal exposure only Cu was lethal at 44 mg/L, thus lower doses of this metal were used. Cu and Cd significantly accumulated in the animal body, while Zn and Pb were efficiently eliminated. The number of coelomocytes was significantly decreased after Cu, Pb, or Cd treatment. Expression of HSP70, HSP72 and w-MT2 proteins was either induced or significantly enhanced in coelomocytes from metal-exposed animals. Metal exposure up-regulated expression of metallothioneins in coelomocytes, mainly amoebocytes, in the order: Cd>>Zn>Pb>Cu. Heat shock proteins HSP70 and HSP72 were induced mostly in the sequence Cd>Pb>Zn>Cu; HSP70 was also detectable in the control cells. The results show that earthworms *A. chlorotica* are more sensitive then *E. fetida* and *D. veneta* on contamination with Cu ions; *A. chlorotica* coelomocytes are sensitive biomarkers of Cu, Cd, and Pb pollution.

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PHARMACOLOGY OF THE EARTHWORM CROP-GIZZARD

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The digestive tract of the earthworm is innervated by a myenteric plexus which controls smooth muscle contractility. Various studies have shown that neurotransmitters including, acetylcholine, biogenic amines, and FMRFamide-related peptides are present in the nerves that innervate the annelid digestive tract. To determine whether these neurotransmitters play a role in controlling smooth muscle movement we have examined the effects of acetylcholine, serotonin, dopamine, octopamine, epinephrine, norepinephrine, FMRFamide and several of their agonists on the isolated earthworm crop-gizzard. The crop-gizzard was removed from, specimens of *Lumbricus terrestris*, attached to a smooth muscle transducer, and challenged with increasing concentrations of neuropeptide. A log-concentration curve was used to determine the neurotransmitter threshold concentration for changes in rate and amplitude of beat. Acetylcholine increased the contraction rate with a threshold between 10^{-8} and 10^{-7} M and caused a biphasic change in contraction amplitude. Amplitude rose with a threshold between 10^{-9} and 10^{-8} M and decreased with a threshold between 10^{-6} and 10^{-5} M. Serotonin decreased the contraction rate with a threshold between 10^{-8} and 10^{-7} M and the amplitude with a threshold between 10^{-8} and 10^{-7} M. Dopamine caused a biphasic response in both the contraction rate and amplitude. There was a decrease in both contraction rate and amplitude with a threshold of 10^{-6} M and an increase in both contraction rate and amplitude with a threshold of 10^{-5} M. Octopamine initiated a biphasic response in rate, characterized by an increase with a threshold of 10^{-10} M and a decrease with a threshold of 10^{-7} M, and a decrease in overall amplitude (threshold at 10^{-8} M). Both epinephrine and norepinephrine initiated increases in amplitude with a threshold of 10^{-8} M and a slight increase in contractile rate throughout the concentration range. FMRFamide caused a decrease in contraction amplitude with a threshold between 10^{-9} and 10^{-8} M and a biphasic change in contraction rate. The contraction rate increased with a threshold between 10^{-8} and 10^{-7} M and decreased with a threshold between 10^{-6} and 10^{-5} M. To determine whether the earthworm receptors for serotonin, epinephrine, norepinephrine and FMRFamide were similar to any other known receptors crop-gizzards were challenged with a series of agonists. The effects of the serotonin agonists suggested that the earthworm serotonin receptor is pharmacologically related to the mammalian serotonin type 2 receptor, whereas the actions of the adrenergic agonists showed that the earthworm adrenergic receptor only slightly resembled the mammalian alpha adrenergic receptor. The earthworm FMRFamide receptor was unlike any FMRFamide receptor characterized in molluscs or arthropods. Thus it appears that acetylcholine, serotonin, dopamine, octopamine, epinephrine, norepinephrine, and FMRFamide may all play a role in regulating the movement of the digestive tract.

COMPARATIVE STUDIES ON MORPHOLOGY, FLOW CYTOMETRIC CHARACTERISTICS, AND ACTIVITY OF COELOMOCYTES EXTRUDED FROM ADULT INDIVIDUALS OF FOUR EARTHWORM SPECIES: ALLOLOBOPHORA CHLOROTICA, DENDROBAENA VENETA, EISENIA FETIDA, AND LUMBRICUS TERRESTRIS

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Heterogeneous earthworm immunocompetent cells, coelomocytes, were for the first time classified by Cooper and Stein (1981) in *Lumbricus terrestris* cells. Morphology of coelomocytes of *Eisenia fetida* were described by Hamed et al. (2002), and those in *Dendrobaena veneta* by Adamowicz (2005). In general, the earthworm coelomocytes consist of three apparent cell populations: eleocytes, granular amebocytes, and hyaline amoebocytes. We have previously shown that the number and composition of coelomocytes are different and undergo distinct seasonal changes in the four earthworm species: *Allolobophora chlorotica, Dendrobaena veneta, Eisenia fetida*, and *Lumbricus terrestris* (Kurek and Plytycz 2003). The objective of our present studies was to extend the interspecies comparisons using wider spectrum of techniques.

Coelomocytes were isolated from four earthworm species: *E. fetida, D. veneta* (from the commercial supplier) and *A. chlorotica* and *L. terrestris* (collected in the field in vicinity of Institute of Zoology, Jagiellonian University, Krakow). Coelomocytes were extruded by the dorsal pores of animals subjected to the electric shock (1 min., 4.5V). Extruded coelomocytes were counted and used for: 1) observations in fluorescent microscope; 2) *in vitro* incubation and assessement of plastic adherence or neutral red pinocytosis (Kurek and Plytycz 2003); 3) MGG stained cytospin preparations; 4) glutaraldehyde-fixed for electron microscopy: SEM or TEM; 5) freshly prepared or formalin-fixed for flow cytometry (Beckton-Dickinson).

Present results fully confirmed previous observation (Kurek and Plytycz 2003), that all investigated species possess the rich population of coelomocytes, consisting amoebocytes (granular or hyaline) and eleocytes, but they differ considerably in the number of cells per body mass, and proportion of eleocytes (both being the lowest in *L. terrestris*). Moreover, flow cytometry and fluorescence microscopy revealed the high proportion of autofluorescent cells (eleocytes) in *A. chlorotica, D. veneta*, and *E. fetida*, while autofluorescence of some (but not all) earthworm species collected in Cardiff (Wales) (Cholewa et al. 2005). In all species a large proportion of cells was strongly plastic-adherent and avidly pinocytosed neutral red; in all species TEM revealed 3 types of coelomocytes: agranular (hyaline) and granular amoebocytes and eleocytes filled with electron-luced granules (chloragosomes). Eleocytes and amoebocytes were also easy to distinguish by SEM.

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RESTORATION OF COELOMOCYTES IN *DENDROBAENA VENETA* AFTER EXPERIMENTAL EXTRUSION

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Coelomocytes (amoebocytes and eleocytes), the earthworm immunocompetent cells, circulate in fluid-suspension in the earthworm coelomic cavity. The earthworms irritated mechanically or chemically are able to extrude coelomocytes through the dorsal pores of the body wall but the animals depleted of coelomocytes are fully vital and able to rebuild to total pool of these cells. The aim of present studies was to investigate the rebuilding process of the total pool of coelomocytes of Dendrobaena veneta following experimental depletion. Experiments were performed on adult (clitellate) individuals of D. veneta obtained from the commercial supplier (Ekargo, Slupsk) and maintained in the Institute of Zoology at high $(22^{\circ}C)$ or low $(10^{\circ}C)$ temperature or shifted from the cold to the room temperature. At the required time points coelomocytes were extruded from the animals subjected to the electric shock (1 min., 4.5 V), counted, and analysed by immunohistochemistry or flow cytometry (Beckton-Dickinson) without or with staining with propidium iodide. Some animals were injected behind clitellum with 30 1 of BrdU $(50 \square M)$ and sacrificed 24 hours later for *in situ* detection of proliferating cells on paraffinembedded cross sections stained with H&E or used for immunohistochemical localisation of proliferating cells with monoclonal mouse anti-human Ki-67 antibodies (DAKO, CA, USA) or monoclonal mouse anty-BrdU (ICN Biomedical, USA) followed by peroxidaseconjugated secondary swine anti-mouse antibodies (Labelled Streptavidin Biotin method, DAKO, CA, USA), diaminobenzidine (DAB, DAKO) 'staining' and hematoxylin counterstaining. No-primary antibody and no-secondary antibody controls were performed. Electric shock induded the expulsion of more then 95% of coelomocytes suspended in the coelomic fluid. Almost half of the initial cell number (mainly amoebocytes) was restored within few hours perhaps from the existing spare pool while the total pool of coelomocytes was restored only within 4-6 weeks at the room temperature; the number of coelomocytes was still low in the cold-maintained individuals even 7 weeks after expulssion. Flow cytometric analysis indicated that proliferating cells were seldom among free-floating coelomocytes in the intact control animals while they were common in those experimentally depleted coelomocytes and/or shifted from the low to higher temperature. Immunohistochemistry of the cross-sections through the animal body confirmed that proliferating cells were apparently absent in the control animals while they were detected both by anti-BrdU and anti-Ki-67 antibodies in animals with experimentally forced coelomocyte proliferation, mainly in typhlosole and metanephridial region of the posterior part of the body. Co-localisation of the cells immunostained by anti-BrdU antibodies and those stained by antibodies raised against proliferation-related human Ki-67 antigens strongly supports an assumption that the latter anti-human antibodies bind the same or very similar molecules in the nuclei of proliferating earthworm cells. It indicates an evolutionary stability of the Ki-67 molecules and their usefulness for investigations of proliferating cells in evolutionary distant species. In conclusion, earthworms are able to restore coelomocytes extruded from the coelomic cavity both from the spare pool of coelomocytes and by the cell proliferation, the latter in a temperature-dependent rate.

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RIBOFLAVIN-INDUCED AUTOFLUORESCENCE OF ELEOCYTES FROM SEVERAL EARTHWORM SPECIES

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Two main lineages of coelomocytes, namely amoebocytes and eleocytes, are present in various proportions in different earthworm species (Kurek and Plytycz 2003). Analysis by phase contrast/fluorescence microscopy and flow cytometry has demonstrated a high percentage (>10%) of autofluorescent eleocytes in 6 out of 12 investigated earthworm species, namely in Dendrobaena veneta (Olchawa et al. 2006), Allolobophora chlorotica, Dendrodrilus rubidus, Eisenia fetida, Octolasion spp. (O. cyaneum, O. tyrtaeum tyrtaeum and O. tyrtaeum lacteum). In contrast, less then 1% of autofluorescent coelomocytes was recorded in representatives of Aporrectodea spp. (A. caliginosa and A. longa) and Lumbricus spp. (L. castaneus, L. festivus, L. rubellus, L. terrestris) (Cholewa et al. 2006). Then we have shown by spectrofluorometry (excitation and emission spectra, and fluorescence lifetime) and HPLC analysis that riboflavin is the main fluorophore responsible for fluorescence in Eisenia fetida eleocytes (Koziol et al. 2006). Recently we quantified riboflavin content in autofluorescent coelomocytes from several earthworm species combining the results of flow cytometry and spectrofluorometry. Spectrofluorometry of coelomocyte lysates showed that riboflavin was apparently absent in coelomocytes of Aporrectodea spp. and Lumbricus spp., while was distinct in the species with autofluorescent eleocytes. In the latter case, riboflavin content (in pg) calculated per one autofluorescent eleocyte was highest in E. fetida, then in Octolasion spp., low in A. chlorotica, and lowest in D. rubidus. These results fully correspond with autofluorescence intensity measured by flow cytometry and that visible in fluorescent microscope. This is a significant observation in the context of cellular immunity in earthworms (Dhainaut and Scaps, 2001), because riboflavin (vitamin B2) plays a potentiating role in immune responses. For example, riboflavin increases neutrophil migration (Verdrengh and Tarkowski, 2005), count, and activation (Osame et al., 1995) in mice and cattle, and displays a potent ability to incapacitate the human malaria parasite Plasmodium falciparum (Akompong et al., 2000).

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Retention of supravital dye, neutral red, in lysosomal vesicles of healthy cells, is impaired in cells retrieved from animals exposed to environmental stressors, due to increased membrane permeability. Svendsen et al. (2004) reviewed and critically examined the neutral red retention ecotoxicity assay applied for earthworm coelomocytes. The aim of the present work was to adapt an automated version of the method with a flow cytometer.

Coelomocytes expelled by electric shock (1 min, 4.5 V) from adult individuals of *Dendrobaena veneta, Eisenia fetida, Allolobophora chlorotica, Lumbricus castaneus, Lumbricus rubellus,* and *Lumbricus terrestris* were incubated *in vitro* in HBSS (controls) or HBSS with copper chloride (experiments) at the required concentrations (up to 44 \square g/ml) for the required time points (up to 90 min) either without or with post-incubation (minimum 5 min) with neutral red (NR) solution (final concentration 40 \square g/ml) (Maboeta et al., 2003). Samples (10000 thresholded events per sample) were collected with a FACScalibur flow cytometer (BD Biosciences), with side scatter (for cell complexity/granularity) and forward scatter (for FL-2H and FL-1H fluorescence) and analysed using WinMDI 2.8 software. Samples were also examined in transmission bright field and by fluorescence microscopy and photographed.

Both amoebocytes and eleocytes of all investigated species accumulated neutral red in lysosomal vesicles, as evidenced by the bright field, fluorescence microscopy, and flow cytometry, in the latter case by the induction (in Lumbricus spp.) or enhancement (in *D. veneta, E. fetida,* and *A. chlorotica*) of FL-2 fluorescence. Differences between control and copper-treated coelomocytes were detectable by flow cytometry in the samples before NR addition, but they were much more profound after NR uptake. The resulted profiles were relatively simple and easy for interpretation in a case of coelomocytes from Lumbricus spp. A complexity of flow cytometric profiles of *E. fetida, D.veneta, and A. chlorotica* coelomocytes was related to the presence of abundant population of riboflavin-derived autofluorescence of eleocytes of these species (Cholewa et al. 2006, Koziol et al. 2006, Plytycz et al. 2006). Effects of copper were species-specific, while within the species they were dependent on a time and a dose of copper exposure. The results strongly indicate that a flow cytometry may serve as a suitable tool for a precise quantitative assessment of lysosomal membrane stability in the neutral red retention ecotoxicity assay.

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EARTHWORM POLYSACCHARIDES AND ITS ANTIBACTERIAL FUNCTION IN VITRO

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The experiment was conducted to study earthworm (Eisenia fetida) polysaccharides and its antibacterial function to plant diseases in vitro. Results showed that: (1) Polysaccharides isolated from common earthworms and induced earthworms had broad-spectrum antibacterial activities to plant diseases in vitro. (2) Antibacterial activities of earthworm polysaccharides were assayed by Disk method. The diameter of bacteriostasis circle to Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Pseudomonas solanacearum Smith, Sclerotinia sclerotiorum, Collectotrichum lagenarium, Fusarum oxysporum, Phytophthora boehmeriae Sawada, Rhizoctonia solani Kühn and Botrytis *cinerea* was 2.10±0.08, 2.33±0.05, 3.30±0.08, 4.28±0.10, 4.15±0.06, 1.53±0.05, 2.15±0.06, 4.58 ± 0.10 , 2.40 ± 0.08 , and 2.48 ± 0.10 centimeter respectively. The antibacterial function of earthworm polysaccharides isolated from induced earthworms increased significantly for all the testing bacteria (P<0.01, Phytophthora boehmeriae Sawada: P<0.05) except Escherichia coli. (3) Antibacterial function of mucopolysaccharide for Pseudomonas aeruginosa and Staphylococcus aureus increased significantly (P<0.05) after earthworm was induced by Escherichia coli, while minimum inhibitory concentrations (MIC) did not change for Escherichia coli. (4) The antibacterial component of polysaccharides isolated from both earthworms and induced earthworms were composed by five molecules which were determined by the HPLC-MS. The composition at retention time 5.3 min was Glu-Xyl-Glu-Xyl, which molecular weight was 533 D. That of 6.0 min was 452 D, which was composed by Glu-Rha-Rha or Rha-Glu-Rha. And molecular weight of the 6.7 min composition was 678 D, which was composed by Glu-Glu-Glu-Xyl-Xyl. Other two compositions were not analyzed in the research.

AN ANTIMICROBIAL PEPTIDE OF THE EARTHWORM PHERETIMA TSCHILIENSIS: CDNA CLONING, EXPRESSION AND IMMUNOLOCALIZATION

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A cDNA encoding a putative antimicrobial peptide (named PP-1) was obtained using a rapid amplification of cDNA ends from the Asian earthworm, Pheretima tschiliensis. PP-1 showed 77.6% homology with the antimicrobial peptide lumbricin I isolated from the earthworm Lumbricus rubellus. PP-1 lacked an obvious signal peptide sequence. RT-PCR analysis demonstrated that this gene was expressedmainly in the body wall. PP-1 was expressed in Escherichia coli as a fusion protein with a maltoze-binding protein. A polyclonal antiserum was raised in mice using this recombinant fusion protein as antigen. Immunohistochemical studies showed that PP-1 was only in the mucus of the epidermis. Large numbers of antimicrobial peptides, which are key components of the innate immune system of most multicellular organisms, have been isolated from various sources (Boman 1995). Most of these peptides differ considerably in basic features, such as their sequences, structuralmotifs and the presence of disulfide bonds despite their generally cationic character (Boman 1995). These peptides can be divided into five chemically distinct groups: (1) linear peptides lacking cysteine, often forming amphipathic helices; (2) linear peptides with a high proportion of certain amino acid residues such as proline; (3) loopforming peptides with one disulfide bond; (4) peptides with two or more disulfide bonds and normally forming β -sheet structures; (5) peptides derived from larger molecules with other known functions (Boman 1995). Earthworms possess two kinds of immunodefense systems, cellular and humoral. Many proteins are involved in immunodefense of earthworms, such as lysozyme, lysenin, fetidins, CCF-1 (Cooper et al.2002). However, it is not known whether antimicrobial peptides contribute to the innate defense of earthworms. To date only one antimicrobial peptide, lumbricin I, has been isolated from the earthworm Lumbricus rubellus (Cho et al. 1998). Lumbricin I, which belongs to proline-rich antimicrobial peptides group, kills both bacteria and fungi without hemolytic activity in vitro (Cho et al. 1998). Its physiological function and cellular localization are unknown. Cooper et al. (2002), after analyzing bacteriostatic activity of earthworm coelomic fluid and choloragogen cells secretion, hypothesized the existence of families of antimicrobial peptides within the oligochaete annelids. In this study, we have isolated and characterized a cDNA encoding a novel proline-rich antimicrobial peptide (named PP-1) from the Asian earthworm, Pheretima tschiliensis. The distribution of Pp-1 mRNA in earthworms tissues was examined by RTPCR analysis. The histological location of PP-1 was detected by immunohistochemical studies.

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Session 4

Interactions With Other Organisms

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WHY DO EARTHWORMS INCREASE PLANT GROWTH?

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The positive effect of earthworms on plant growth has been clearly demonstrated in two recent papers reviewing more than 300 experiments. Several hypotheses have been proposed to explain this positive effect, but not properly tested. Earthworms could increase plant growth because they modify the soil (i) chemically, by mineralizing the soil organic matter, (ii) physically, by modifying soil structure (porosity, aggregation, infiltration, runoff...) and (iii) biologically, by activating, dispersing or controlling some plant parasites or symbionts. In three laboratory experiments, we studied the impact of the earthworm Millsonia anomala, originating from the Lamto wet savannah (Côte d'Ivoire), on the growth of rice (Oryza sativa L., cv. Moroberekan). The most common hypotheses addressed in the literature to explain the positive effect of earthworms were refuted in these experiments. The increase in primary production in the presence of *M. anomala* is not due to the increase in the mineralization of nitrogen, since M. anomala produces an equivalent positive effect in an environment limited or saturated with nitrogen. This worm neither increases primary production by improving the availability of water; indeed, stomatal closure, typical of a water deficit, appears earlier in the presence of this compacting earthworm, because of a reduction of the readily available water in the soil. In addition, the worm made rice tolerant to a severe attack of plant parasitic nematodes, without reducing their population. The last observation also led us to reject the hypothesis of a control of plant parasites by M. anomala to explain its positive effect on plant. A modification of rice physiology in the presence of earthworms, revealed by the study of photosynthesis and stress-responsive gene expression, suggests that the impact of this worm on rice could be due to the last mechanism proposed in literature, namely earthworm activation of symbiotic bacteria. Just like Plant Growth Promoting Rhizobacteria known to be activated by root exudates (PGPR), soil bacteria stimulated by earthworm mucus could act on plant physiology by emitting phytohormones in the soil, which induces modifications of root system morphology and/or systemic resistance to parasites.

INFLUENCE OF EARTHWORMS ON THE GROWTH OF COTTON AND WHEAT PLANTS IN CONTRASTING SOIL TYPES

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Cotton (*Gossypium hirsutum*) production in Australia was, until recently, heavily dependent upon the use of non-selective insecticides (e.g. endosulfan) to control key pests. This practice also reduced the abundance of several non-target invertebrate species. However, with the advent of transgenic (Bt) cotton (which targets lepidopteran pests), pesticide use has been drastically reduced. This has provided a greater opportunity for biodiversity to flourish, including populations of soil animals such as earthworms. But, whilst the influence of earthworms on the growth of various crop and pasture plants (e.g. wheat, clover, ryegrass) is well documented and known to vary between plant species, the response of cotton to earthworms has attracted little study. This paper explores the impact of two earthworms, *Aporrectodea caliginosa* and *A. longa* (Lumbricidae), with contrasting burrowing and feeding behaviours, on the growth of cotton in glasshouse experiments, using two soils (a grey clay and a red-brown earth), commonly used for cotton production in Australia, and also a commercial sandy loam.

The earthworms survived in the "cotton" soils, but their growth was generally less than in the commercial sandy loam. In the absence of fertiliser, the earthworms increased the nitrogen content of leaves and the biomass of cotton bolls on plants grown in the two cotton soils, but the plants generally grew poorly and the earthworm effects that were recorded were confounded with mortalities of earthworms that would have contributed some additional nutrients to the soils, compared with the control treatments. A further experiment, however, suggested the influence of the dead earthworms as a source of fertiliser was probably unimportant. The first experiment failed to show an earthworm effect on the general vegetative growth of the cotton plants, but another experiment, in larger pots and using only the grey clay soil, did suggest a significant effect. In an additional experiment (with relatively cool conditions), earthworms did not influence early cotton growth using the grey clay soil (with fertiliser) and the commercial sandy loam (with and without fertiliser). At the same time, the earthworms had a strong, positive influence on the early growth of wheat plants in the commercial sandy loam and a similar (but not significant) effect on wheat growth in the grey clay soil. Overall, the results suggest that earthworms can influence cotton growth, but that they do not enhance early cotton growth to the same extent as that of wheat, and their influences on plant growth can vary with soil type.

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The decomposition process of organic matter in terrestrial ecosystems involves a diverse assemblage of soil organisms that interact in a complex way. Among these organisms, earthworms play a key role in determining decomposition rates because of their ability to influence distribution and activity of primary decomposers (bacteria and fungi). Earthworms also affect soil fauna, so that they shape the entire community and change its structure and function. In order to examine the effect of earthworms on the decomposer community we conducted three separate and complementary experiments using the vermicomposting process as an accelerated model of the decomposition process that occurs in soil systems. We tested whether a) the changes in the decomposer food web structure along an age gradient simulating a soil profile depend on the presence of earthworms and on the availability of resources; b) the indirect effects of earthworms modify the composition of the microfaunal community because of changes in biotic and abiotic characteristics of the substrate; c) the transit of the organic substrate through the gut of the earthworms modifies the presence of the different groups of decomposer microfauna. We performed all the experiments in laboratory conditions using pig slurry as substrate. In the first experiment, we compared the presence of functional groups of microorganisms, protozoa, nematodes, enchytraeids and microarthropods in continuous feeding vermireactors with two different doses of slurry, with and without presence of the earthworm *Eisenia fetida*. In the second experiment, instead of using earthworms, only different amounts of vermicompost were inoculated in plastic pots containing fresh pig slurry, and changes in the presence of different groups of protozoa and nematodes were recorded. In the third experiment, we analysed the content of the posterior part of the gut of different earthworm species fed with pig slurry. The results of the first experiment showed that the presence of earthworms modified significantly the structure and function of the decomposer community, increasing diversity of functional groups of micro- and mesofauna and increasing nutrient flux through the fungal energy channel of the decomposer food web. Earthworms also increased microbial biomass and activity, but not faunal biomass. Similar changes in the presence of microfauna were obtained in the inoculation experiment, showing that indirect effects of earthworms play an important role in determining the composition of the faunal community. Despite this, the absence of nematodes in the substrate after transit through the gut of the earthworms revealed the existence of strong direct effects of earthworms on the abundance of some specific groups.

ENDOGEIC EARTHWORMS REDUCE THE FUNGAL STABILIZATION OF LITTER DERIVED CARBON AT THE SOIL LITTER INTERFACE

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The soil litter interface is a hot-spot of microbial activity and regarded as one important linkage of carbon flow between above- and belowground. Presumably, fungi play the most decisive role in these processes by transporting and stabilizing litter derived carbon in their networks in the upper mineral soil. Although this layer, the upper few centimetres of the mineral soil, is the main habitat of endogeic earthworms, the knowledge about their influence on these processes is scanty. Therefore the objective of the experiment was to investigate the influence of endogeic earthworms on fungal biomass and fungal carbon stabilization in the upper layer of the mineral soil.

In a full factorial design 174 g fresh weight arable soil (equivalent to 150 g dry weight), 0.3 g dry weight plant material, (¹³C and ¹⁵N labelled rye leaves, dried and chopped by hand to < 20 mm) and one individual of the endogeic earthworm species *Octolasion tyrtaeum* were added to sixty microcosms (height 135 mm, diameter 60 mm). Labelled rye leaves were placed on plastic rings covered with gaze (64 μ m), which enabled fungi to grow from litter into the soil and vice versa, but avoided direct incorporation of rye leaves into the mineral soil by *O. tyrtaeum*. Half of the rings were placed on the soil surface, the other half were adjusted 3 cm above to distinguish between active translocation of carbon and nitrogen by fungi and passive translocation by leaching. During the experiment CO₂ and ¹³C signature of CO₂ were determined. At the end of the experiment the incorporation of ¹³C and ¹⁵N in the soil and earthworms were analysed. Microbial parameters were determined by measurement of ergosterol concentration and analysis of fatty acid composition in soil and litter.

At the end of the experiment the earthworms were significantly enriched in ¹³C and ¹⁵N and responded to the position of litter with higher incorporation in treatments with soil litter contact. Additional, the position of the litter significantly affected the ¹³C concentration in soil with an average ¹³C enrichment of 2.5% and 1.4% in treatments with and without soil litter contact, respectively. Independent from the position of litter *O. tyrtaeum* reduced the ¹³C enrichment in the soil. In treatments with soil litter contact *O. tyrtaeum* significantly increased mineralization of ¹³C. Overall, fungal and bacterial biomass in the soil negatively responded to the presence of *O. tyrtaeum*.

We conclude that endogeic earthworms reduce the fungal stabilization of litter derived carbon by feeding and disrupting fungal networks at the soil litter interface and thereby degrade an important mechanism of carbon stabilization in the upper soil layer.

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EARTHWORM CASTS AS HOTSPOTS OF SOIL NUTRIENT DYNAMICS IN A PASTURE ECOSYSTEM

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We studied the effect of digestion and cast deposition of the earthworm *Aporrectodea caliginosa* on the microbial community of a pasture ecosystem as well as the changes on microbial activity and, consequently, on nutrient cycling.

In a pasture in NW Spain, one hundred casts newly deposited by *A. caliginosa* were selected and flagged randomly in autumn of 2005 when the first rainfalls started after a severe summer drought. Casts were monitored daily to check their growth and after one month sampled destructively by taking randomly ten paired samples of casts (C) and soil below (SB) to asses the existence of nutrient transference between them. Samples of soil surrounding the casts (SS) at a distance of 20 cm were also taken as controls.

One month later, other ten casts were sampled as well as SB and SS to evaluate the effect of ageing in natural conditions. Fresh samples of casts and soil were analyzed for their organic matter and moisture content, pH and electrical conductivity, different C and N forms, microbial activity (hydrolysis of fluorescein diacetate, β -glucosidase, cellulase, protease and phosphatase activities). Microbial communities were characterized and quantified on lyophilized samples by analysis of the phospholipid fatty acids (PLFAs).

A. caliginosa significantly modified the microbial community structure by digestion of the soil as showed by the PLFAs analysis. In addition, casts had also higher microbial activity and consequently nutrient cycling and mineralization was enhanced as compared to SB and SA as showed by the higher levels of mineral nitrogen and dissolved organic carbon. Cast deposition increased the microbial activity of the soil below but no other differences were found between SB and SS. Nutrient content (N-NH₄⁺, DON, DOC) increased further in two-month old casts as well as the β -glucosidase and protease activities suggesting the existence of newly deposited soil. However microbial activity was reduced with time. Again, we found little differences between SB and SS, which would mean that in spite of the high density of earthworms in the pasture, the effect they exert on soil properties was restricted to the dynamics of ingested-egested soil.

Oral

THE SENSITIVENESS OF SOIL BACTERIA AT THE DIGESTIVE FLUID IN THE GUT OF THE EARTHWORM *APORRECTODEA CALIGINOSA*

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Earthworms can directly regulate microbial populations by consuming large amount of soil. This leads to elimination of some microbes and creation favorable conditions for others to successfully grow in a digestive tract and in excrement. In earthworms, the gut transit time of soil material is very high, 2-3 hours. It is believed, therefore, that worms should kill and digest the consumed soil micro-organisms quickly. Earlier we showed that digestion of microorganisms in intestines of millipedes and wood lice began with fast death of cells under action of specific non-protein killing agents (Byzov et al., 1998). To date no data exist on earthworm digestive activity. The aim of this study was to test whether the digestive fluid of the earthworm, *Aporrrectodea caliginosa*, may act as a selective killing agent towards soil bacteria.

The earthworms were collected from soddy-podzolic soil. They were kept on sterile sand for several days to clean up the digestive tracts. Then the worms were desiccated on a friezing stage (- 16°C). The digestive tracts were divided into midguts and hindguts. Then the gut material was centrifuged at 12000 rpm for 15 min for separation of gut fluid and gut tissue, blood cells and the majority of microbial cells. One portion of the fluid was heat treated (98°C,10 min) to inactivate proteins and then it was centrifuged. Bacteria were isolated from soddy-podzolic soil. The bacterial clones were identified by sequencing PCRproducts of 16S rDNA gene. Among them there were found Alphaproteobacteria (6 strains), Betaproteobacteria (3 strains), Gammaproteobacteria (12 strains), Actinobacteria (12 strains) and Bacilli (9 strains). Bacterial suspensions were mixed with the gut fluids (1:2 v/v) and volumes of 3 μ l of the mixture were placed on a thin nutrient-agar film. The CFU numbers were estimated by counting micro colonies under microscope.

For the first time it has been shown that midgut extracts inhibit bacterial growth. Seven bacterial strains belonging to Beta- (*Alcaligenes faecalis*) and Gammaproteobacteria (*Kluyvera ascorbata, Pseudomonas reactans*), Actinobacteria (*Microbacterium sp., Arthrobacter sp.*) and Bacilli (*Bacillus megaterium, 2 strains*) have been found to be the most sensitive to the digestive fluid. Their CFU numbers decreased by two orders of magnitude upon 20-30 sec. of exposition with the digestive fluid. The supernatant of the pre-heated fluid exhibited the killing activity, too. Thirty-five strains, belonging to all the groups tested, have been found to be not sensitive to the digestive fluid. No bactericidal activity has been found in the hindgut fluid. The killing effects do not correspond to taxonomic position of the bacteria. Possible mechanism of the killing effect is a destruction of cell membranes as it was demonstrated earlier (Byzov et al., 1998).

The digestive fluid of earthworms could be significant factor for selective modification of microbial communities in soil.

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EARTHWORM ACTIVITY MODIFIES SOIL NEMATOFAUNA AND BIOFUNCTIONING ACCORDING TO QUALITY AND LOCALIZATION OF CROP RESIDUES (MADAGASCAR)

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Soil provides a wide range of ecosystem services that are essential for human life and well being, e.g., food and fibre production, nutrient cycling, carbon sequestration, erosion control, water quality, detoxification, and regulation of climate. Soils are also probably one of the most species-rich habitats of terrestrial ecosystems. Decades of studies have demonstrated the importance of soil fauna and microorganisms in the functioning of soils. This role is now well recognized, so that soil biota is seen as an essential resource in sustainable systems of agricultural production. Nevertheless the role of soil biological activity and diversity in regulating ecosystem services and the way it can be valued, maintained and managed in agricultural systems is poorly understood. In the tropics, direct seeding mulch-based cropping (DMC) systems are extending, especially in South America. These systems characterized by no-tillage and permanent soil cover reduce soil disturbance and often result in a significant accumulation of soil carbon, a decrease in erosion, and an increase in soil biological activity and biodiversity. Studies in Brazil, Benin and Madagascar showed a strong increase in the density and biomass of soil macrofauna, especially earthworms in DMC systems. The interactions between agricultural practices, soil macrofauna and other soil organisms (mesofauna and microorganisms) are known as the main determinants of soil biofunctioning at a local scale, although not well understood. Among mesofauna, nematodes are of main importance as the relative distribution of their trophic groups is a recognized indicator of soil functioning.

Our experimental study aims at analysing the effect of endogeic earthworms on nematode diversity in tropical DMC systems (Madagascar) according to the quality and localization of crop residues. Different (10) experimental treatments were studied in mesocosms, crossing: presence or absence of earthworms (*Pontoscolex corethrurus*), presence or absence of residues (soya or rice), localization of crop residues (incorporated into the soil or left at the soil surface). Nematode community was studied after 5 months at a genus level.

Nematode density was more important in presence of earthworms than in their absence irrespective of litter quality and localization. Nematodes were also more abundant in presence of soya residues than in presence of rice residues and were more abundant when residues were incorporated in the soil. ANOVA showed a significant effect of earthworms and of residue localization on nematode density. Earthworms especially enhanced the bacterial-feeders Cephalobus sp. in all treatments and enhanced the fungus-feeders Aphelenchoididae in presence of soya residues. Plant-feeders nematodes were not modified by the presence of earthworms.

Considering modifications in the nematode community structure, it appears that earthworms modify soil functioning especially through an increase in bacteria and subsequently in bacterial-feeders nematodes.

Oral EARTHWORMS AS PREY OF CARABID BEETLES: THE USE OF MOLECULAR MARKERS TO ANALYSE PREDATION IN THE FIELD

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Carabid beetles, such as *Pterostichus melanarius*, are major predators of crop pests such as slugs and aphids¹. Earthworms, however, are a major alternative prey, helping to sustain these predators within arable crops. Quantification of predation on earthworm can be achieved using molecular markers to analyse the gut contents of carabids trapped in fields. This was first achieved using general earthworm-specific monoclonal antibodies which could detect earthworm proteins in the guts of predators for up to 64 h after they were consumed². Although unable to distinguish between earthworm species, monoclonal antibodies revealed that 36 % of *P. melanarius* collected from a field of wheat contained earthworm proteins². These data strongly suggested that earthworms were a major prey item.

Subsequently work has concentrated upon the development of PCR-based techniques to analyse predators for the presence of earthworm DNA in their guts. Our first approach was to develop general earthworm-specific PCR primers to the mitochondrial 12S ribosomal gene, which proved successful. Earthworm could be detected in the guts of the carabids for an extended period, with 50 % still testing positive 88 h after feeding. Analysis of *P. melanarius* trapped in a crop of field beans, using multiplex PCR, showed that 49 % contained earthworm DNA and that earthworms were consumed more frequently than any other invertebrates^{3 and in prep}. However, TGGE (temperature gradient gel electrophoresis) and multiplex PCR analysis of earthworm DNA failed to separate all earthworm species, mainly because divergent lineages resulted in as much variation within, as between, species^{3,4}.

We have now developed species-specific, and in some cases lineage-specific, PCR primers for seven of the earthworm species at our field site. Extreme genetic divergence amongst two species (see Abstract by King *et al.*), *Allolobophora chlorotica* and *Apporrectodea rosea*, necessitated the development of different primers for each lineage. The primers for all species and lineages were combined within two multiplex PCRs to simultaneously detect all species of earthworm in the guts of each predator. The application of this approach to the analysis of spatial and temporal interactions between carabids and multiple earthworm species will be presented.

EARTHWORMS AND MICROORGANISMS: RELATIONSHIPS AND CONSEQUENCES ON DECOMPOSITION OF ORGANIC MATTER

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Microorganisms are largely responsible of organic matter decomposition, but earthworms may also affect to rates of decomposition directly by feeding on and digesting organic matter, or indirectly affect them through their interactions with the microflora, basically involving stimulation or depression of the microflora biomass and activity. Here we present the results of three experiments which tried to separate between the direct and indirect effects of earthworm on decomposition of organic matter. We used the vernicomposting process as a model to study the relationships of earthworms and microorganisms. The first experiment studied the whole process of vermicomposting, using *Eisenia fetida* and pig manure in continuous feeding vermireactors; these vermireactors were made of independent layers which allowed us to date them. Earthworms promoted significant increases in both microbial biomass and activity when they were present in the layers, and after that, in aged layers where earthworm were absent, microbial stabilization (i.e. low microbial biomass and activity) occurred. Further, community physiological profiles (Biolog Ecoplate) revealed that earthworms promoted significant alterations in the physiological capabilities of microorganisms. As consequence, carbon mineralization was significant enhanced. In a second experiment, we inoculated pig manure with two doses of vermicomposts (2.5 and 10%) obtained from laboratory cultures of E. fetida, E. andrei and Eudrilus eugeniae. By doing this, we were able to test the indirect effect of earthworms, that is, whether an earthworm processed substrate can modify organic matter decomposition or whether it is necessary the earthworm presence to achieve the same results of the first experiment. We found that inoculation of vermicompost produced the same increases in microbial biomass and activity, and although these increments were not as higher as in presence of earthworms, we can state that indirect effects of earthworm are strong enough to alter the dynamics of organic matter decomposition. In the third experiment, we analyzed the gut content of four earthworm species (E. fetida, E. andrei, E. eugeniae and Octodrilus complanatus) which were fed with pig slurry. We determined the number of bacterial cells by fluorescence microscopy to understand the direct effect of earthworms on microorganisms. We found that only O. complanatus produced a significant decrease in the number of bacterial cells, whereas there were no differences between pig manure and the gut content of the other earthworm species.

INTERACTIONS BETWEEN EARTHWORMS AND MICROORGANISMS INCREASE PLANT PRODUCTIVITY THROUGH PRODUCTION OF PLANT GROWTH REGULATORS

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It has been well-documented in recent years, that soil microorganisms produce significant amounts of hormones and plant growth regulators which can influence plant growth. Interactions between earthworms and microorganisms, in the degradation and stabilization of organic wastes to produce vermicomposts, can increase the potential production of plant growth regulators, since this process increases microbial diversity, populations and activity, by several orders of magnitude. Fully-processed vermicomposts contain large amounts of humic substances, which themselves can induce plant growth responses at low concentrations and act as plant growth regulators. We have shown that solid humic acids, extracted from pig manure and food waste vermicomposts, applied to a soil-less plant growth medium (MetroMix360) at rates of 50-500 mg kg⁻¹ humic acids increased growth rates of tomato, pepper, cucumber, marigold and strawberry plants significantly, but there were decreases in growth, at concentrations above 500-1000 mg kg⁻¹, even when all nutrients needed for growth were supplied. Aqueous extracts, of plant growth hormones such as auxins (IAA) from vermicomposts, extracted and applied daily to marigold, pepper and strawberry seedlings, at rates of 10% and 20% by volume, increased plant growth significantly above that of controls when all needed nutrients were available. In other experiments, commercial humic acids, or those extracted from vermicomposts, were applied at a rate of 500 mg kg⁻¹ dry wt of Metro-Mix 360, either singly or in combination with IAA, to pepper seedlings. The commercial humic acids and IAA mixtures increased plant growth more than the materials did individually but not as much as the solid extracts from vernicomposts containing both humic acids and IAA. We hypothesize that auxins, which are very transient, soluble and readily broken down in UV light, becomes adsorbed onto humates, persist longer in soils and are released slowly to plants. In our experiments, the amounts of N in plant tissues did not differ significantly between treatments showing clearly that any increases in plant growth were additional to the effects of plant nutrients.

DIFFERENCES OF THE INTESTINAL MUCUS AND SOIL MICRO-ORGANISMS IN THE INTESTINE AMONG EXOTIC AND NATIVE EARTHWORMS FED ON DIFFERENT SOIL QUALITIES

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The mutualistic hypothesis between earthworms and micro-organisms to digest soil organic matter had been studied. The earthworms produce a good intestinal quality mucus to stimulate the soil micro-organisms' activity which can decompose the soil organic matter and make it digestible for the earthworms. This mechanism needs to be more detailed with new experimental data, particularly to answer the questions: Do the earthworms produce the same mucus quantity and quality when fed with a different soil organic matter quantity? Do exotic earthworms have a more intense mutualistic interaction with micro-organisms than native earthworms? Are the soil micro-organisms the same as the ones that inhabit the earthworms' intestine?

Thus we have studied three tropical geophagous earthworm species, two exotic *Pontoscolex corethrurus* and *Polypheretima elongata* and the native *Balanteodrilus pearsei* from southeast Mexico. These 3 species are grown in their original soil where they were collected and in two other treatments where the respective soils were amended with rich organic matter (grinded green leaves of *Mucuna pruriens*, 1% and 6 % respectively). In 5 samples: the non-ingested soil, the anterior, mid and hind intestine and the 24-hour-old casts; the following parameters are going to be measured: 1) hydro-soluble material or mucus production, 2) the C and N content in the mucus and 3) the microbial DNA and the most probable number MPN).

Up to now the measurements are in process, and we expect less mucus content in the intestine when the earthworms are fed with more organic matter and with a higher C/N ratio; and that there is a quantitative and qualitative selection of the soil micro-organisms during the intestinal course.

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EFFECT OF EARTHWORM INTERACTION (*AMYNTHAS CORTICIS* AND *PONTOSCOLEX CORETHRURUS*) ON THE DIVERSITY FUNGAL ALONG TIME

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In order to evaluate the relationships between earthworms interaction and the structure of the microfungal community and the diversity, the endogeic *P. corethrurus* and the epigeic A. corticis, were kept in laboratory microcosms in single and in interaction treatments. The fungal communities and the diversity were evaluated in cast and in Control Soil over 24, 48, 72 and 96 days using the washing and plating methods. Richness of the fungal community was greater in worm cast that in control soil in both species. The greatest richness was in interaction treatment, following in order P. corethrurus, A. corticis and Control Soil. Differences in the dominance structure were appreciated between treatments, but mean frequencies were not significantly different in most of the dominant species. C. merdarium, H. grisea and Trichosporiella were dominant species during all time in most of treatments. Dominance structure of the fungal community was more similar between control soil and A. corticis being the fungal community in interaction treatment the most differentiated in most of dates. Differences between endogeic and epigeic species were subtle and were clearer on first 48 days. P. corethrurus consumed differentially soil fungi as H. grisea, Paecilomyces carneus, Pestalotiopsis maculans whereas A. corticis preferred litter fungi as Trichosporiella sp, F. oxysporum, and C. cladosporoides. Interaction treatment was the most differentiated treatment and P. glabrum and A. niveus were discriminative species. On day 72 control soil and singles treatments were very similars and F. solani, F. trichoteciodes and F. moniliforme became dominant fungi; to difference, P. glabrum, P. citrinum and P. simplicissimum were preferentially in interaction treatment. No differences between treatments were found on day 96 but they were a mixture of fungi considered as the latest decomposition stage and early stages. Growth rates of earthworms were better on first 48 days in association with the greatest fungal richness and fungal species as C. merdarium, H. grisea, H. fuscoatra and Trichosporiella which were dominant. Diminished performance of earthworm was in association with the greatest richness and dominance of Fusarium spp and toxic Penicillium spp.

THE ROLE OF ANIMAL DECOMPOSER DIVERSITY IN DEBRIS DECOMPOSITION

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Compared with microorganism, little is known about the role of soil fauna diversity in decomposition processes. Ample evidence suggests that effects of soil animals on soil processes are nearly related to their body size. The relationship indicates that at least among keystone soil-animal species which modify the structure and function of the microbial community, diversity has an important impact on decomposition and nutrient cycling. For example, evidence indicates that the different ecological groups of earthworms differentially affect the activity of soil microorganisms and decomposition processes. Experimental manipulations suggest that the loss of both functional groups and species diversity within functional groups of earthworms alters the ability of soil microorganisms to use organic substrates. Whereas, the relationship between animal decomposer species diversity and soil processes remains controversial. Some evidence shows a weak relationship between soil-animal diversity and ecosystem processes. These observations indicate high redundancy among soil animals. In line with these findings, the trophic structure of the decomposer community examined with stable isotopes also indicates a high redundancy in soil-animal communities. Still other evidence supports the functional significance of animal diversity for soil processes. However, these studies are still too few and are limited to narrow diversity gradients and low species numbers. Therefore, a microcosm experiment explain macrofauna biodiversity effects on leaf litter-mass loss and soil respiration by functional dissimilarity rather than the number of species. The presence of certain keystone species and the number of functional groups of soil animals may be more significant for decomposition processes than is simply species diversity.

DO ENGINEERING EARTHWORMS (APORRECTODEA CALIGINOSA) AND PLANTS DIVERSITY AFFECT ORIBATID MITES?

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The aim was to study how geophagous earthworms and grass species diversity affect the abundance and species composition of soil oribatid mites.

A field experiment was started in May 2004. Plastic mesocosms were filled with sieved and mixed arable loamy soil and inserted into the soil. Mesocosms had a surface of 450 cm^2 and depth of 20 cm. A half of mesocosms were sown with one grass species – red fescue (*Festuca rubra*), the other with a mixture of 8 grass species. In the next year, when the grass sward was established 6 individuals of geophagous *Aporrectodea caliginosa* were introduced to the half of mesocosms in each plant variant – monoculture and mixture. The second half of mesocosms without earthworms was used as a control. In that way four variants were established – monoculture with and without *A. caliginosa* and mixture with and without earthworms. In each variant 150 mesocosms were prepared. The sample were taken 60 days after the introduction of the earthworms.

A total of 10 species of oribatid mites was found. As so far oribatid mites community is dominated (app. 95%) by one species: *Microppia minus*, because of it there are slight differences between terms and variants. But it is noticeable that higher grass production causes higher oribatid mites abundance but lower diversity. After the earthworm introduction substantial differences in abundance of oribatid occurred: the abundance is higher in mixture with and without earthworms as well, than in monoculture in which this effect is not observed. Experiment is not finished yet, and analyses are in process.

THE INFLUENCE OF *HORMOGASTER ELISAE* ON THE COLONIZATION CAPACITY OF SOIL MICROARTHROPODS IN EL MOLAR (MADRID)

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The effect of earthworms on microarthropod abundance and distribution has been studied by several authors (Marinissen and Bok, 1988; Brown, 1995; Loranger *et al.*, 1998; Salmon and Ponge 1999, 2001; Maraun *et al.*, 1999; Migge, 2001), but the results obtained have been variable. In laboratory work involving the use of soil microcosms (Gutiérrez et al., 2003), we showed that the presence of *Hormogaster elisae* from a plot in El Molar (Madrid, Spain) reduces in the size of mite and collembolid populations. The present work attempted to confirm this effect and to determine the capacity of soil microarthropods to colonise new areas in the presence and absence of earthworms.

Microcosms composed of plastic boxes with a central 2 mm mesh jail were prepared. Earthworms were then placed inside this jail. In one experiment, the jail contained defaunated natural soil from El Molar, while the soil outside was non-defaunated. In a second experiment, the soil outside the jail (where there were no earthworms) was defaunated, while that inside the jail was non-defaunated. Defaunation was performed using a method based on those of Huhta *et al.* (1989) and Wright *et al.* (1989). This consisted of freezing the soil at -32° C for 24 h and then heating it to 60°C for a further 24 h. Control microcosms were set up without earthworms. All microcosms were kept at 13°C and 20% humidity for 21 days before extracting the microarthropods from the soils. These were identified and enumerated by standard methods. The data obtained were analysed by ANOVA.

In the first experiment, the majority of microarthropod groups were incapable of colonising the defaunated soil containing earthworms, but were able to colonise it when the earthworms were absent. In the second experiment, nearly all the microarthropod groups left the inside of the jails containing earthworms, but in control experiments without earthworms the majority stayed inside the jail. The results appear to confirm that the presence of *H. elisae* has a negative effect on microarthropod numbers and on their ability to colonise new zones.

128EFFECT OF EARTHWORMS ON SOIL MICROARTHROPODS DISTRIBUTION IN A PLOT IN EL MOLAR (MADRID)

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Several authors have investigated the effect of earthworms on microarthropod abundance and distribution in field and laboratory (Marinisen and Bok, 1988; Hamilton and Sillman, 1989; Loranger et al., 1998; Maraun et al., 1999; Shrader and Seibel, 2001; Tiunov, 2003). The results obtained, however, have been variable. The present work simultaneously examined the horizontal distribution of different earthworm species and several groups of soil microarthropods in a plot in El Molar (Madrid). The aim was to determine the relationships between the distributions of these organisms and to explain their causes.

In April 2002, the earthworms and microarthropods at 42 points uniformly distributed around the experimental plot were sampled. Earthworms were extracted from $0.5 \times 1 \text{ m}$ quadrats using formol; microarthropods were extracted from 400 g soil samples using the Berlese-Tullgren method.

Distribution maps were made according to the earthworm and microarthropod abundance data obtained. The mean abundance of microarthropods at points with and without earthworms was compared using the Kruskal-Wallis and Mann-Whitney U tests. Principal components analysis was also performed to determine the distribution trends of both types of organism. Cluster analysis was performed to determine the different types of sampling point in the plot in terms of microarthropod abundance; their relationship with earthworm presence was then determined.

The greatest abundance of many types of microarthropod was recorded in areas where earthworms were absent. Principal components analysis showed opposing trends with respect to microarthropod and earthworm distribution. Cluster analysis distinguished two types of sampling point: one characterized by large numbers of microarthropods but low numbers of earthworms, the other with low microarthropod numbers and larger numbers of earthworms. This inverse relationship between microarthropod and earthworm abundance suggests there may be a negative relationship between their distributions in natural conditions. However, the influence of other factors cannot be ruled out.

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Poster

EARTHWORMS MODIFY THE SOIL FAUNAL COMMUNITY STRUCTURE

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Decomposer communities were analysed at nine deciduous forest sites in central Finland: three birch stands planted on spruce forest (mor) soil, three birch stands on arable soil, and three naturally developeded deciduous stands (mainly silver birch). All major faunal groups were identified to species, and their biomasses were estimated (Huhta and Räty, 2005; Huhta et al., 2005). Populations of Lumbricus terrestris and Aporrectodea caliginosa were present in all arable soils, two natural forests and one birch stand after spruce. L. terrestris was also found at another "birch after spruce" site, while Dendrobaena octaedra alone or no earthworms at all were recorded in one natural and one planted forest (Räty and Huhta, 2004). Numbers of nematodes, enchytraeids and microarthropods were lowest at the sites with the strongest populations of L. terrestris and A. caliginosa. Biomasses of earthworms and those of all other fauna together were inversely correlated. Laboratory experiments revealed that the presence of burrowing earthworms will reduce the populations of nematodes and enchytraeids (Räty and Huhta, 2003), and field observations indicate that the same is true for microarthropods (Maraun and Scheu, 2000). It was concluded that the presence or absence of burrowing earthworm species plays a decisive role in determining the structure of the whole soil faunal community.

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DOES EARTHWORM ACTIVITY ALTER PLANT DIVERSITY EFFECT ON SOIL MICROFAUNA?

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The effects of two factors: plant species richness and presence of the earthworm species, *Aporrectodea caliginosa* on the nematode abundance and community structure was studied in a field experiment. The aim of the study was to find out if the activity of *A. caliginosa* can modify the plant diversity effect on soil nematodes.

The experiment was established in spring of 2004. Six hundred plastic isolators (mesocosms) were dug into the ground and filled with previously sieved and mixed arable soil. The mesocosms (450 cm² in surface area and 20 cm deep) were sowed with grasses, one half – with one species (*Festuca rubra*), and the second half – with a mixture of 8 grass species. One year later (after one vegetation season) 6 individuals of *A. caliginosa* were introduced into the half of mesocosms in each treatment – monoculture and mixture. Some preliminary results, concerning nematode abundance, generic diversity and nematode functional structure, sixty days after the introduction of *A. caliginosa* were presented.

The total number of nematodes in the monoculture treatment was significantly lower than in the mixture treatment. The presence of earthworms did not influence the nematode density in monoculture, while in the mixture treatment the total number of nematodes significantly increased due to the activity of earthworms.

The generic diversity of nematodes was lower in the mesocosms with one grass species, than in mesocosms with the diverse grass species composition. A total of 18 genera were found in monoculture treatments and 25 in mixture mesocosms. The presence of the earthworms did not have significant effect on the total number of genera.

Two trophic groups dominated nematode communities in all treatments: bacterial and plant feeders, but their proportions differed between the treatments. While the proportion of bacterial-feeding nematodes (mainly genus *Acrobeloides*) was higher in monoculture, that of plant feeders (mainly genus *Paratylenchus*) was higher in the mixture treatments. The presence of earthworms did not change the proportion of that two groups.

COMBINED EFFECTS OF EARTHWORMS AND ARBUSCULAR MYCORRHIZAL FUNGI (AMF) ON SOIL STRUCTURE PROCESSES: A MICROCOSM STUDY

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Biotic influence, mainly earthworms and arbuscular mycorrhizal fungi (AMF), plays a major role on soil structure. Soil structure is very important in agricultural managment and has been widely studied since the 1900s. Soil aggregation and soil organic matter (SOM) are major components of soil fertility. According to the conceptual model of aggregate hierarchy, microaggregates ($<250\mu$ m) and organic residues are bound into macroaggregates. Aggregates protect SOM physically by making it not accessible for mineralisation. Factors influencing the formation, stabilization and degradation of soil aggregates, involve physical, chemical and biological processes. Despite the existence of a lot of studies highlighting the importance of earthworms or AMF on aggregation, their combined effects have not been studied yet, except on plant growth parameters. Thus, this study will contribute to better understand the role of earthworm and AMF on soil structure.

The general aim of this project (part of "NCCR-Plant Survival"), is an integrative study aiming at better understanding plant-AMF-soil interactions. Here, in particular, we study the consequences of biological activity of AMFs and earthworms on soil structuring processes and their complementary or antagonistic effects on soil structure dynamic. Thus, we focus on their role on aggregation and incorporation of SOM at different scales (clayhumus complex, micro- and macro-aggregate) in order to distinguish and compare their single or combined effects. A plant species (*Petunia hybrida*) has been used for mycorrhization and different amount of phosphorus (P) supply (high P concentration induces low mycorrhization) has been used in a second phase in order to compare aggregation dynamics.

In order to control a maximum of parameters (i.e. temperature, humidity, light/dark cycle) microcosms in climatic chamber have been used. Microcosms consist of PVC tube (30cm high and 15cm diameter) separated in two egal parts with a nylon membrane (mesh of 25μ m) that allows extraradical mycelium (ERM) to pass through but stop roots. The soil used has been first sterilised by gamma-irradiation and re-inoculated with a known amount of fresh filtered soil suspension in order to standardise microorganisms content in soil. This experimental device permits to obtain eight treatments depending on the presence or absence of plant, earthworms (*Aporrectodea caliginosa*, an endogeic species) and AMF (*Glomus intraradices*). Four repetitions were made for each treatment.

Different physicochemical analyses have been performed on mycorhizosphere, drilosphere (casts and faecal pellets) and distant soil. Organic matter has been examined (fractionation, degree of humification, biochemical content), as well as organo-mineral aggregation and stability. Thin sections have also been used.

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In 2004-2005 a lysimetric experiment was designed to determine whether and how plant diversity and/or engineering earthworms could influence on diversity and densities of collembolan communities. We compared two grass communities: a single-species community, represented by a monoculture of Festuca rubra L. and a polyculture made up of the mixture of grasses (8 species) commonly used in agriculture. In addition to two plant communities of different diversity we used in our experiments an engineering species of earthworm - Aporrectodea caliginosa (Sav.). The experiment was initiated in May 2004 when 300 lysimeters were sown with Festuca rubra and 300 lisimeters by mixture of grasses. Next year, in May 2005, we added 6 individuals of earthworm - Aporrectodea caliginosa (Sav.) to half of the lisimeters sown with Festuca rubra and mixture of grasses. In experiments we used plastic lysimeters, 450 cm^2 in area and 20 cm deep, filled with alluvial coarse sandy soil. Soil samples were taken in 2005 at depths of 0-5cm, 5-10cm, 10-15cm and 15-20cm., four times per season: in April (before introduction of earthworms) and in July, September and November (after introduction of earthworms). On each occasion samples were taken from six lysimeters of each variant of experiment. Soil samples were taken from central parts of lysimeter to avoid the edge effect. For statistical analysis multivariate analysis of variance MANOVA/ANOVA was used. Species diversity of Collembola communities was calculated according to Shannon-Wiener equation using logarithms at a base of 2. Hutcheson's test was used to determine the statistical significance of differences between the H indices. Three groups were distinguished in Collembola communities basing on their preference to soil layer.

We found in our experiments that soil layer had an important effect on densities of collembolan communities independently of plant diversity and presence of earthworms (F=7,675; p=0,0000). But this phenomenon was changing during experimental season. After introduction of earthworms we noticed a positive effect of earthworms on densities of Collembola (F=4,824; p=0,03), and this effect was more intensive in polyculture of mixed grass (F=3,924; p=0,01). However, after 4 months, we found no effects of both plant diversity and presence of earthworms on Collembola. We found 19 species of springtails and number of species decreased with soil layer. The dominants were pioneer species Cryptopygus bipunctatus (Axels.) and Isotomodes productus (Axels.).

Our suggestion is that engineer earthworms probably have an effect on diversity and densities of collembolan communities but it is probably connected with plant diversity and time in season.

Poster 133 THE INFLUENCE OF EARTHWORM EXCRETES ON MICROBIAL ACTIVITY OF SOIL

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A number of studies have shown that earthworms affect biological activity of soils. In drilosphere, a two-fold increase in the abundance of bacteria was observed. The length of fungal mycelium decreased by one third. The quantity of the total nitrogen, carbon and humidity noticeably increased in comparison with native soil. These features give the basis to believe that earthworms actively participate in turnover of biogenic elements and regulate microbial communities of soil (Tiunov, Scheu, 1999). The aim of this study was to test the hypothesis whether excretes (mucosa and excrement) of earthworms may act as regulator of microbiological activity of soils.

Excretes were obtained by placing 50 worms in 0.5 kg of sterilized sand for 2 days. Then the excretes were extracted from sand with distilled water and added to soddy-podzolic soil. Dynamics of soil respiration and mineral nitrogen were measured. Influence of excretes in quantity equivalent to volume, that comes from one worm in 10 grams of soil (8 μ g C/g, 2.5 μ g organic N/g and 0,17 μ g NH₄⁺/g), led to increased respiration of microorganisms in 2 hours after treating the soil. Emission of CO₂ was found to be 2-3 times higher when soil was treated with excretes. Addition of glucose in quantity equivalent to carbon that comes with earthworm excretes did not increase respiration. Considering the small amount of the carbon from the excretes and the fast reaction of the microbial community, it is possible to assume that excretes stimulate respiration of microorganisms on the stressful mechanism. The reaction proceeds and disappears in a day after treating excretes. Dilution removes effect. This means that there is some threshold concentration of substances which cause stressful influence on microbial respiration.

Results of plating have shown that quantitative changes in bacteria and fungi have not occurred, therefore we concluded that worm excretes do not change the structure of microbial community.

We have found for the first time that earthworm excretes stimulated mineralization of compounds of nitrogen in soil. For two days after treating the concentration of $\rm NH_4^+$ increased by 19 % and that of $\rm NO_3^-$ by 2 times. It corresponds to 70 times and 20000 times more ammonium and nitrate ions, respectively, than has come with excretes. The concentration of nitrate ion remained higher even on day 30 of the addition of excretes. Dilution removes the effect. Therefore, there is some threshold concentration of substances, which cause stressful influence. Ammonium chloride added at a concentration equivalent to that of the excretes stimulated nitrification, too. This led us to assume, that earthworm excretes could act as promoter (priming effect) of mineralisation of organic nitrogen and nitrification in soil. Ammonium as the major component of earthworm excretes could stimulate nitrification in soil, causing long-term cumulative effects disproportional to the direct action. Possibly, complex organic substances are utilized through so-called cometabolism.

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Poster

TROPHIC TRANSFER OF FATTY ACIDS FROM THE GUT MICROBIOTA TO THE EARTHWORM *LUMBRICUS TERRESTRIS* L.

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The diet of earthworms includes soil organic matter, soil microbes and other microfauna, but the relative contribution of these dietary components to earthworm nutrition is not well known. Analysis of fatty acid (FA) profiles can reveal trophic relationships in soil food webs, leading to a better understanding of the energy and nutrient flows from microbiota to earthworms. The objective of this study was to determine the origin of FAs assimilated by the earthworm Lumbricus terrestris L. We analysed the pattern of FAs in (i) the bulk soil, (ii) soil in the earthworm gut, (iii) the absorptive tissue of the earthworm gut wall, and (iv) the muscular layers of the earthworm body wall. Multivariate analyses performed on the FA profiles suggest that the microbial community in the earthworm gut differs from that in bulk soil. Diverse bacterial and fungal derived FAs, which earthworms cannot synthesize, were found in the earthworm gut wall and body wall, and in the neutral lipids (storage lipids) of the gut wall. The major compounds isolated were 20:4ω6, 20:5ω3 and 18:2ω6, followed by the monoenoic 18:1007 and 18:109c, and the saturated 18:0. The microbial FA assemblage in the gut wall resembled the gut soil more than the bulk soil, and the body wall of L. terrestris showed the same microbial derived FA pattern as the gut wall, although at reduced concentrations. We propose the existence of a specific microbial community in the earthworm gut that provides FAs to the earthworm. It appears that L. terrestris may derive more of its energy and nutrients from gut specific microbiota than from microbiota already present in the ingested soil, based on the trophic relationships revealed through FA analysis.

Poster 135 CHARACTERIZATION OF POSSIBLE ENDOSYMBIONTS IN ONYCHOCHAETA BORINCANA

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The presence of endosymbionts in the gut of *Onychochaeta borincana* had been suggested but never characterized. The intestinal tissue of this species was examined using electron microscopy and microbiological and molecularly using genetic engineering techniques, in order to identify the possible endosymbiont microorganisms. Four gram-positive, endospore-forming and β -hemolytic strains remained adhered to the intestinal wall even after intense cleansing. Biochemical and physiological characterization proved their similarity with *Bacillus cereus*, which is typically found in soils and has been reported as a possible intestinal symbiont in arthropods. These results were corroborated by SEM that showed bacillary structures adhered to the intestinal wall. However, amplification of bacterial 16S rRNA encoding gene from the total genomic DNA extracted from the gut was not possible and FISH trials were negative maybe due to the reduced number of these proposed gut-associated-microbes that remains after the intensive cleansing procedure.

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Session 5

Behavioural and Evolutionary Biology

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EXTREME GENETIC DIVERGENCE AND MULTIPLE CO-OCCURRING LINEAGES WITHIN THE EARTHWORM ALLOLOBOPHORA CHLOROTICA

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Colour dimorphism occurs in the earthworm *Allolobophora chlorotica*, with green and pink morphs. The two morphs appear to have different ecological preferences (the green morph preferring moister condition), although mixed populations are common. Previous work, including hybridisation experiments, suggested that these morphs may be genetically isolated and possibly represent separate species (Sims & Gerrard 1985). Preliminary work by Harper *et al.* (in press), using TGGE analysis of the mitochondrial 12S gene, also showed that divergent haplotypes may be present within the green morph collected from a single population.

To investigate levels and patterns of genetic diversity, and possible speciation within *A. chlorotica*, we screened samples from 14 British populations for sequence variation at a 585 bp segment of the mitochondrial cytochrome oxidase I (COI) gene. The samples included populations monomorphic for colour morph and also those possessing both morphs.

A total of 23 haplotypes were found, belonging to a total of eight distinct haplogroups or lineages. There were four pink and four green haplogroups. Within haplogroups, divergence ranged from 0% - 1.4%, while between haplogroup divergence ranged from 4.5% - 16.9%. Within single populations, haplotypes from several divergent lineages were found with as many as five haplogroups co-occurring at single field sites

Preliminary analyses, using scanning electron microscopy, showed no morphological differences between samples belonging to divergent lineages. There were no clear cases of one colour morph possessing the mtDNA of the other colour morph, suggesting that some at least of these lineages are genetically distinct and are not hybridising with each other, even where they co-occurr. Theories that could account for both the extreme divergence and mixing of different lineages within populations will be presented, along with evidence that similar genetic structuring may be found within populations of other common earthworm species.

Oral

VARIABILITY AND DIFFERENCES IN EUROPEAN POPULATIONS OF LUMBRICUS TERRSTRIS AS DETECTED BY MOLECULAR-GENETIC FINGERPRINTING USING THE AFLP-TECHNIQUE

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The earthworms of the Lumbricidae family are widely distributed across Europe following the extending vegetation in south-north direction after the last ice ages. In Europe (and today also in other temperate areas) they successfully occupied a high number of different habitats.

Taking *Lumbricus terrestris* as one of the most common earthworm species we asked for its genetical variability within and between populations from different geographical regions. Due to the support and collaboration of a high number of European earthworm scientists we were able to cover an area from the Alps in the south to Scandinavia in the northeast and Scotland in the northwest. We applied the AFLP (Amplified Fragment Length Polymorphism, Vos et al. 1995) technique to quantify the genetical indices of the populations.

This talk will cover a short but detailed description of the method, its application on earthworms and the presentation of results representing the current status and progress of our project.

The populations genotyped until now exhibit a comparatively high genetic diversity but only a weak differentiation among the populations. Thus, there is no clear association between geographic distribution and genetic differentiation of *L. terrestris* populations. Also a loss of genetic diversity in northern populations due to a founder effect during migration after the last glaciations was not detectable. Taking into account that *L. terrestris* is a widely spread organism with limited individual mobility our results do not match the expectations of a restricted gene flow between distance-isolated populations. On the other hand the high genetic diversity of the populations is maybe a key to understand the strength of *L. terrestris* in occupying new environments

Until the Krakow-meeting we will increase the number of individuals and populations in the study and we will verify our results from the anonymous AFLP markers by analysing mt-DNA of *L. terrestris*.

Oral 141 FOOD REQUIREMENTS AND PREFERENCES OF ENDOGEIC EARTHWORMS

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Endogeic earthworms ingest large amounts of organic matter enclosed in mineral soil and are often the most abundant ecological group of Lumbricidae in cultivated soils. During the gut passage, soil organic matter (SOM) is mobilised from which only a small part is assimilated by the earthworm itself. Only little is known about the origin and age of the organic matter pool which is mobilised and used by the earthworms to build up biomass. The presented studies were conducted to gain insight on the food requirements and preferences of two common endogeic earthworm species – *Octolasion tyrtaeum* Savigny and *Aporrectodea caliginosa* Savigny.

Six experiments were performed in microcosms and planar vessels, filled with arable soils with different contents of SOM, and with and without the availability of different litter types. Controlled environmental conditions in microcosms enabled us to investigate burrow constructions and changes in live biomass of single specimens during the incubations.

The ability of *O. tyrtaeum* to mobilise old soil organic carbon pools was shown by the ¹³C signatures of CO_2 evolved from soil as an indicator of mobilized SOM. Soil derived from a maize field, where cultivation had changed from wheat (C_3 plant) to maize (C_4 plant) 23 years ago. Microbial biomass carbon in earthworm worked soil derived from younger carbon sources than in soils without earthworms. Soils from a long-term fertilization experiment were used to study the effects of SOM contents on earthworm performance in the same type of soil, consisting of the same soil texture and pH (Marhan and Scheu 2005). The results document that mostly the labile organic matter pool of SOM in arable soil is essential for soil microorganisms as well as for endogeic earthworms. *O. tyrtaeum* decreased the microbial biomass, which suggests that earthworms and soil microorganisms compete for food resources derived from labile organic matter pools. In a food choice experiment with the same soils, *A. caliginosa* constructed significantly more burrows in the soil with higher SOM content. These findings question the use of different soils in the substrate avoidance response test which is commonly used in ecotoxicological tests.

In another 9 month lasting experiment *O. tyrtaeum* benefited from the addition of litter to microcosms consisting of arable soil. At the end of the incubation the animals lost less weight in treatments with low quality litter (rye straw). This indicates that in the long term, low quality litter as rye straw may better serve the need for food resources of endogeic earthworms than high quality litter which is decomposed rapidly by microorganisms. In contrast to the positive effects of plant residues on endogeic earthworm performance, no positive effects of living annual plants could be detected in three experiments which we performed.

In conclusion, the presented studies demonstrate that the origin a minimum amount of SOM is crucial for the establishment of endogeic earthworm populations in arable soils.

DEMONSTRATION OF THE ROLE OF EARTHWORMS ON THE REMOVAL OF HIGHLY COMPACTED ZONES IN FIELD CONDITIONS

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By their excavation activities, earthworms are thought to contribute to the removal of highly compacted zones ("delta" zones) but the quantitative measurements of their impact is lacking. A first experiment was carried out in semi-field conditions (caging experiment). Adult earthworm from the most common species (Lumbricus terrestris, Aporrectodea giardi, A. caliginosa and A. rosea) were in presence of different compacted zones: (i) horizontal, (ii) vertical layer and (iii) isolated zone representing different natural situations (under wheel or timmage compaction). Depending on the species (and their behaviour) we observed different impacts on compacted zones (L. terrestris is more prone to cross horizontal layer whereas A. caliginosa is more prone to colonize vertical ones). A second experiment was carried out in field conditions to characterise the dynamics of the recolonisation by earthworms after a compaction (through a 8t vehicle). Under and between wheels zones were followed during 2 years. We studied earthworm bulk density, earthworm community, 2D vertical macroporosity and water infiltration measurements were done. We observed that earthworm recolonisation is a rapid process (less than 6 months to recover similar abundances) whereas macroporosity and water infiltration are much slower processes. This illustrates the difficulty of using earthworm abundance as way to estimate the physical consequences of earthworm activity. Moreover, 3D soil cores were collected at different dates under and between wheels and analysed through X-ray tomography. This enables us to visualize the (slow) recolonisation of the soil with earthworm macropores and to discuss the effect of the different species present in the field.

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Oral

SEED FORAGING AND CACHING BY LUMBRICUS TERRESTRIS

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We recently discovered that the European earthworm species, Lumbricus terrestris, which takes and stores plant litter as a food source in its permanent burrows, interacts with seeds of weedy species in its introduced range in the Midwestern and other parts of the United Lumbricus terrestris caches the seeds of the North American annual weed, States. Ambrosia trifida (giant ragweed), while foraging at the soil surface by gripping the large seeds with its mouthparts and pulling them inside its burrow. The function of caching seeds remains unknown but the outer covering of A. trifida seeds decays rapidly and may provide nourishment to the earthworms. Field and laboratory experiments were conducted to determine seed foraging preferences of L. terrestris, namely if the earthworm forages in a generalist fashion for large seeds and if it forages for seeds when other plant residue is available. Lumbricus terrestris collected seeds of six large seed species tested, but cached higher percentages (77% to 86%) of A. trifida, Helianthus annuus (common sunflower), and Sicyos angulatus (bur cucumber) compared to Xanthium strumarium (common cocklebur), Zea mays, and Glycine max (23% to 59%). These data confirm that the earthworm forages as a selective generalist for seeds and fruits within the size range of 7-22 mm. The basis for seed selectivity by L. terrestris is unknown, but may involve physical aspects such as seed size, shape, and texture that determine ease of handling along with chemical cues such as nitrogen and phenol content that may indicate the suitability of the material for decomposition. Field experiments were conducted to determine if seed foraging activity by L. terrestris was affected by the presence of other plant litter. Ambrosia trifida seeds were dispersed to the soil surface in plots with and without G. max leaf litter. There was no significant effect of leaf litter on seed collection by *L. terrestris*; 84% of A. trifida seeds were collected when plant residue was present and 94% when residue was absent. The data from these experiments support the hypothesis that large weed seed species constitute a regular component of materials foraged by L. terrestris and are attractive to the earthworms even when other plant litter is available. Seed foraging and caching by Lumbricus terrestris may be an important biotic seed dispersal activity with the potential to influence seed survival and seedling establishment by large weed seed species.

Poster THREE-D SIMULATIONS OF BURROW SYSTEMS MADE BY EARTHWORM COMMUNITIES AND STUDY OF SOME PHYSICAL CONSEQUENCES

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In the past decade, X-ray tomography was successfully adopted as a mean to study earthworm burrow systems under natural or artificial conditions. However this method has some limitations such the size of the soil core, the existence of a border effect, and cost. Recently a new apparatus, called the "Colonne Gamma", was developed that enabled to study the trajectory of radio-labelled earthworm belonging to different species in soil cores. The accurate knowledge of (i) the burrowing behaviour of earthworm and (ii) the 3D characteristics of the burrow systems gave the necessary information to develop a simulator. The input parameters are: (i) the earthworm community (and principally the ratio anecic/endogeic), (ii) the interaction between earthworms (avoidance or not) and (iii) the rate of burrow refilling. Hundred simulations were made for each parameter set and several output parameters were computed such as (i) volume and area of burrows connected to the surface and (ii) the verticality of the burrow systems. The first set of parameters was used in another model to simulate water flow through the burrow systems. Interestingly, we observed that increasing interactions between earthworms increased the verticality of the burrow systems. Regarding water flow, we observed that the key parameters were unsurprisingly the proportion of anecic earthworm and the intensity of the burrow refilling. These examples illustrate the potential use of our simulator to study the effect of behavioural parameters on the resulting burrow systems.
EARTHWORMS TAILOR THEIR EJACULATES IN RESPONSE TO PARTNER MATING STATUS

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In many animals, individuals may mate repeatedly within their reproductive cycle, producing competition among the ejaculates to fertilize available eggs. Although most studies have been restricted to organisms with separate sexes, sperm competition is likely common in hermaphrodites and an important selective agent shaping life-history traits. Simultaneous hermaphrodites have both functional female and male reproductive organs, and it is assumed that they have a limited amount of reproductive resources to both sexual functions and they are likely selected to adjust the resource allocation in an opportunistic way. When multiple matings are common, animals must invest more in sperm to outnumber that of their rivals and as a consequence influences sex allocation. Despite of a strong theoretical basis, empirical studies of sperm competition in simultaneous hermaphodites are rare. In this study we manipulated earthworm mating history performing a double-mating experiment. Spermathecae were dissected using a stereomicroscope and the total sperm volume received in the matings was estimated by the sum of the volume of the four spermathecae. We found that donated sperm volume to virgin partners was not affected by the number of previous copulations and, in comparison, earthworms triplicate the donated sperm when paired with a previously mated partner. Moreover, after two copulations, the total sperm volume in the spermatecae correlated with the receiver body mass, but not after a single copulation, suggesting that the amount of sperm transferred by the second donor was related to receiver body mass. Overall, these results strongly suggest that earthworms can evaluate their partner and adjust the ejaculate accordingly.

INBREEDING AND OUTBREEDING REDUCES COCOON PRODUCTION IN THE EARTHWORM EISENIA ANDREI

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Earthworms are animals with reciprocal insemination. *Eisenia andrei* Bouché, 1972 is a simultaneous hermaphroditic earthworm that lives in manure heaps at high densities, with low opportunities of dispersal, thus very close inbreeding is expected. As the negative effects of inbreeding and outbreeding may be severe, we studied whether *E. andrei* adjusts its breeding effort according to the degree of mate relatedness. To test this, we performed laboratory experiments in which earthworms were mated with their sibs and with non-sibs from the same population and no-sibs from a geographically isolated population. Inbreeding and outbreeding matings caused a strong reduction of cocoon production, especially in genetic lines with high reproductive rates. As far as we know, this is the first study that indicates reproductive adjustment in earthworms according to the genetic divergence of their partners. Optimal outbreeding should be considered a crucial point in the management of breeding populations for applied purposes.

LUMBRICUS TERRESTRIS: SETTLEMENT AND DISPERSAL OF ADULTS

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The aim of this study was to investigate the influence of earthworm density and resources availability on the settlement and distribution of adult Lumbricus terrestris. A field experiment, set up in a mixed broadleaved woodland, involved manipulation of L. terrestris number and leaf litter amount in 1 m² enclosures, formed from plastic sheeting extending 20 cm above and 15 cm below the soil surface. The treatments included 3 earthworm densities (ambient, $+30 \text{ worms/m}^2$, $+60 \text{ worms/m}^2$) and 2 leaf litter quantities (300 g dm/m^2 , $100g \text{ dm/m}^2$). These were replicated 5 times and were randomly allocated to the enclosures. Midden number was used to estimate the number of settled L. terrestris. Destructive sampling of 1 replicate per treatment allowed confirmation of equivalence of one earthworm per midden. Individuals trying to disperse from the enclosures were captured using tunnel traps, located on the soil surface at the enclosure sides. Initial results of the ongoing experiment suggest that midden formation increased after 1 month in plots where earthworms were added and this increase was greater in plots with the higher leaf litter supply. Increase in midden formation, and therefore in the number of L. terrestris settled in the enclosures, was significantly greater in the plots with high food availability (p<0.01). Finally, the number of dispersing earthworms was significantly greater in the treatments of earthworm addition for both food availabilities (p < 0.05). Greater number of L. terrestris dispersed from plots with low leaf litter quantity but the difference was not marked.

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EVIDENCE FOR THE ADOPTION OF ALLOLOBOPHORA VIRESCENS (SAVIGNY, 1826)

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The temperate earthworm species *Allolobophora chlorotica* (Savigny, 1826) exists as two colour morphs, green (g) and pink (p). It is widely accepted that the two morphs have different ecological preferences, linked to soil water potential. Field observations have suggested that the green morph dominates in wet soils and the pink morph in dry soils. Breeding experiments (Kalmus et al., 1955; Satchell, 1967) have questioned the status of these two morphs. If they become regarded as separate species then the name *Allolobophora virescens* (Savigny, 1826) is available for the pink morph (Sims and Gerard, 1999).

Laboratory-reared stocks of both morphs (collected from observed single morph field populations) were established to further investigate the status of this species. Life cycle traits (growth rate, fecundity and cocoon viability) were assessed, firstly in standard culture conditions, and then in wet and dry soil moisture regimes (29 and 21 % soil moisture, respectively). A breeding experiment was established to determine cocoon production and viability from p x g, g x g, and p x p pairings. Offspring from these pairings were cultured to maturity, status determined and progeny of p x g pairings backcrossed with those of p x p and g x g.

Under standard culture conditions the green morph exhibited significantly higher (P<0.05) growth rates, fecundity (3.17 and 1.5 coc worm⁻¹ month⁻¹ for g and p respectively) and cocoon viability (87 and 58 % for g and p respectively). Growth rates of the pink morph were not significantly influenced by soil moisture levels. In contrast, growth and maturation rates of the green morph were significantly (P<0.05) reduced in dry soil. All p x p and g x g pairings bred true. Cocoon production was low in p x g crosses, where both pink and green individuals produced cocoons but only those from the pink morph were viable.

It is proposed that in wet soil conditions (≥ 25 %), higher growth rates, fecundity and cocoon viability provide the green morph with a competitive advantage resulting in the pink morph occupying, and having adapted to, drier soils conditions. It is suggested that soil moisture is acting to isolate these 2 morphs, providing in extremes, a barrier to intermorphic mating. Further evidence for this isolation can be drawn from the low fecundity and cocoon viability of p x g pairings.

DO EARTHWORMS TRADE SPERM?

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Earthworms are simultaneous hermaphrodite animals and consequently the allocation decisions of the reproductive resources on the male and female functions influence their fitness. It has been proposed that the sexual conflict between the two genders in simultaneous hermaphrodites could be resolved by gamete trading. Theory predicts that sperm trading should occur in hermaphrodites in which the female role controls fertilization, for example in earthworms with sperm storage in spermathecae such as *Eisenia andrei*. To see whether sperm trading occurs, we examined whether individuals of *E. andrei* adjust the volume of sperm they release to the volume they receive from their mating partner. One hundred hatchlings of *E. andrei* were individually bred in plastic Petri dishes until sexual maturity. Once sexually mature, randomly established couples of earthworms (n=50) were housed in plastic Petri dishes and revised daily until the apparition of spermatophores, indicatives of copula. Then earthworms were fixed and conserved with formaldehyde 10% in plastic tubes until their later dissection. Spermathecae and seminal vesicles were dissected using a stereomicroscope and total volume of sperm measured to check whether sperm trading occurs.

PARENTAL CARE OF ENDOGEIC EARTHWORM COCOONS: IS CLEANING, CONSTRUCTION, AND CAST SURROUNDING OF CHAMBERS RELATED TO HATCHING AND SURVIVAL OF JUVENILE WORMS?

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Earthworms inhabiting soil must face an environment full of bacterial and fungus activity. Antibiotic and antimicotic strategies have been developed by different soil organisms, including the formation of specific glands to counteract the attack of these microorganisms. As far as endogeic earthworms lay their cocoons underground, it is expected to found some kind of chemical and physical protection of them. Some of the last included the construction of chambers and, in some worms, deposition of cocoons within burrows of other worms.

In this study we compared the cocoon care strategies of two endogeic tropical earthworms: the exotic *Pontoscolex corethrurus* and the Mexican native *Balanteodrilus pearsei*. *P. corethrurus* is a worldwide common parthenogenetic worm currently found in disturbed and natural tropical ecosystems, widely tolerant towards edaphic and climatic changes. *B. pearsei* is a native species widely distributed in southern Mexico and with wide tolerances towards edaphic changes.

Observations were carried out in a glass chamber 10 cm wide and 35 cm heigth. Worms (sexual mature) were feed with soil and the legume *Mucuna pruriens*, in a dark room at 26° C. Activity was recorded each hour, during 30 days. We found that both species build up a chamber where a single cocoon is deposited; in the two species the beginning of reproduction is negatively related to surface cast production. Deposition of cocoon within the chamber differs between both species: *P.corethrurus* lays the cocoon in the air fixed by several mucus filaments, whereas *B. pearsei* fixed the cocoon with only two vertical filaments. Both *P.corethrurus* and *B. pearsei* surround the chamber with fresh casts and periodically clean inside the chamber. In another experiment currently on the way, we are testing the effect of this "parental care" behaviour over hatching and survival of juvenile earthworms.

COMPETITION BETWEEN H. ELISAE ÁLVAREZ, 1977 AND OTHER EARTHWORMS IN EL MOLAR, MADRID (SPAIN)

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An unusual distribution of earthworm species was observed in an experimental plot in El Molar (Madrid). The three most abundant species, *Hormogaster elisae*, *Allolobophora caliginosa* and *Allolobophora rosea*, appeared

in three, relatively isolated areas. A possible explanation for this might be competition. Competition between species often centres on food; the capacity to obtain food, the efficiency of its use, and resistance to a lack of food etc., all play their part. However, the observed distribution might also be explained by the occupation of space or other factors (Margalef, 1974). The aim of the present work was to detect the possible intra- and interspecific relationships between theses three species in the laboratory.

Three types of microcosms were prepared: <u>Monospecific</u> (controls). These contained either *H. elisae*, *A. caliginosa* or *A. rosea*, but always alone. Two types of control were set up - single (Control A) and double (Control B). The double controls contained twice the initial biomass of the single controls, thus allowing intraspecific competition to be studied.

Polyspecific. These cultures contained either H. elisae plus A. caliginosa, H. elisae plus A. rosea or A. caliginosa plus A. rosea. Soil from each of the three areas of the experimental plot (i.e., the H. elisae, A. caliginosa and A. rosea areas) was used as the culture medium, rendering a total of nine treatments (6 replicates per treatment) for each soil type. The soil was renewed every 14 days. The experiment lasted four months. At each soil change the earthworms were weighed and their state of maturity recorded; the numbers of dead and quiescent individuals were also registered. Cocoons were isolated by passing the soil through a sieve column. Mortality for the three species in each treatment was practically zero, with no significant differences between treatments. The results show that, in the microcosms containing soil from the plot area where *H. elisae* was the sole species (more sandy and poorer in organic material), intraspecific competition occurs in H. elisae and A. rosea as well as interspecific competition between these two species. This is possibly due to the lack of food resources owing to the scanty organic material available. Allolobophora caliginosa, however, appeared to benefit from the presence of the other species and also grew more quickly in the double controls, perhaps because earthworm activity (production of mucus and casts etc.) improves the poor soil. In microcosms containing soil from the A. rosea area, intraspecific competition was seen in H. elisae and in A. rosea. Interspecific competition between these two species was also seen. In contrast, the growth of A. caliginosa was not affected by the presence of the other species, although the presence of A. caliginosa did affect the growth of A. rosea. In the soil from the A. caliginosa area, A. caliginosa itself showed intraspecific competition and interspecific competition with H. elisae. No differences in growth were seen between H. elisae and A. rosea in the monospecific and polyspecific treatments. Together, these results show interspecific competition exists between H. elisae and A. rosea in soils where these species are dominant, but in the A. caliginosa area all three species could coexist.

The results for *A. caliginosa* varied depending on the soil type. In poor soils the species benefited from the presence of the other species, but in the *A. caliginosa* area soil the presence of *H. elisae* negatively affected its growth.

THE STUDY OF ABNORMAL MOVEMENT BEHAVIOR IN EARTHWORM AMYNTHAS GRACILIS CAUSED BY UV-B RADIATION

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Earthworms, Amynthas gracilis, are often observed lying on the soil surface or in a puddle after a heavy rain in Taiwan. In this study, ultraviolet-B (UV-B) exposure was found to be correlated to the dying on earthworms. Under the UV-B exposure, the earthworms showed the abnormal behaviors, the S-shape movement or the jumping behavior. Furthermore, their crawling was retarded after UV-B exposure. In this study, we found that the oxygen consumption of the UV-B treated A. gracilis significantly decreased and the metabolic production of anaerobic respiration, lactate, in those earthworm muscles increased after UV-B exposure. It inferred that the UV-induced crawling retardation was caused by muscle lactate accumulatation in the earthworms. On the other hand, it is well known that muscle contraction is controlled by nerve coordination. Some cells in earthworm ventral nerve cords were shown Fos-labelled after the UV-B radiation, which is a molecular marker of neuron activation. The UV-B radiated nerves in earthworms were found to have higher protein kinase Ca (PKCa) activity. The PKC might regulate the opening of the calcium channel(s) in presynaptic cells to increase the intracellular calcium concentration, which promotes acetylcholine (ACh) releasing. Therefore, the treatment of PKC activators, either PMA or calcium ionophore, A23187, enhanced the two abnormal behaviors caused by UV-B radiation. Contrarily, calcium channels blocker, verapamil, blocked the jumping behavior caused by UV-B radiation. Besides, the UV-B radiation also decreased the activity of acetylcholinesterase (AChE), a key enzyme in the synaptic cleft for ACh hydrolysis. AChE inhibitor, chlorpyrifos, prolonged Ach activity could enhance the earthworm jumping behavior. Together, all of those results support that PKC α and AChE are involved in the regulation of earthworm movement caused by UV-B radiation.

EFFECT OF EXPOSURE OF *EISENIA FETIDA* TO CERTAIN COLORED LIGHTS ON ITS BODY GROWTH AND COCOON PRODUCTION

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Effect of some visual range colored lights on the body growth of *Eisenia fetida* and its cocoon production was assessed. The goal of the present study was to investigate the influence of particular light radiation on the body morphology of the earthworms in terms of their length and weight and the rate of reproduction.

Six sets of buckets, each of 20 litre capacity, in replicate of five, were taken. Each bucket was filled with 2.5 cm base layer of broken pieces of bricks followed by a layer of the similar thickness of coarse sand and a third layer of loamy soil of 3 cm. The top layer of 20 cm thickness was of air dried, sieved cattle dung. The moisture level of the medium was maintained by sprinkling 250 ml of tap water everyday in each bucket. A compact fluorescent lamp (220V, 50Hz, 18W) was fitted hanging from 15 cm height of the central top of each bucket. CFLs of the1st set were singly wrapped by red cellophane paper; while of 2^{nd} , 3^{rd} , 4^{th} and 5^{th} sets by blue, yellow, green and plain transparent (without color) cellophane papers, respectively. The last set of buckets was used as control (without light system). Ten pre-weighed mature worms, *Eisenia fetida* were introduced in each bucket and light was given 12 hours a day for two months. Body length and weight of the worms were taken and number of cocoons counted after experiment.

Body growth of the worms was recorded the maximum in the buckets provided with red light and the minimum in the buckets with no light system. The maximum body length, body weight and number of cocoons were 11.2 cm, 1.81 g and 80, respectively in red light system; while these values were the minimum i.e. 5.2 cm, 0.57 g and 29, respectively, in no light system. The number of cocoons were 2.76 times more in red colored light system with respect to the buckets devoid of light. Worm's body length and weight were found positively correlated with respect to their rate of reproduction (r = +0.993, r = +0.978, respectively). Difference in the body length and weight of the worms was highly significant (F- value = 25233, CD =0.003; F- value =3442.86, CD =0.011, respectively). The progressive order of increase in the body growth of earthworms and the number of cocoons produced in different color radiations was as: no light < transparent < blue < green < yellow < red

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Lumbricus terrestris is an obligate outcrossing hermaphrodite and the largest earthworm in northern Europe. Infection of *L.terrestris* by *Monocystis sp.* is a well studied host-parasite system. Molecular markers are essential to understand the evolutionary dynamics of this relationship and to assess the importance of other processes such as mate choice and habitat fragmentation, historical processes (e.g. bottlenecks, range expansions), and direct and indirect selective forces that shape genetic variation in natural populations. Conducting such studies requires availability of highly variable molecular markers. Therefore we successfully isolated several polymorphic microsatellites from this earthworm species. Our primary use of these microsatellites will be to study population structure and fertilization in a mate preference context in *L.terrestris*.

Session 6

Earthworms and Soil Properties

The 8^{th} International Symposium on Earthworm Ecology $4^{th} - 9^{th}$ September Kraków, Poland

CHANGES IN PLANT COMMUNITIES INFLUENCE SOIL MACROAGGREGATION THROUGH EARTHWORM ACTIVITIES IN BRASILIAN AMAZONIA

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Soil macro-aggregation was assessed in the 16 different treatments of an experiment where all possible combinations of 4 plant species had been implemented in three replicated blocks. Communities comprised a grass (*Arachis pintoi*) and shrub (*Leucaena leucocephala*) legume and non legume African grass (*Brachiaria bryzantha*) and a local shrub (*Solanum nigrum*). A visual method derived from Topoliantz and Ponge (2000) was used and 15 different classes of soil macroaggregates (biogenic or physical of different sizes) and other soil features were quantified. Significant differences were observed and attributed to changes occurred in soil macro-invertebrate communities, with special influence of earthworms. Biogenic aggregates associated to populations of one small sized endogeic species were dominant in sites covered with the herbaceous legume *Arachis pintoi*, alone or in association. The African grass *Brachiaria bryzantha* favoured macro-invertebrate communities dominated by termites and macro-aggregates were mainly made by roots. Finally, legume and non-legume shrubs had relatively limited communities of earthworms and their macro-aggregate fractions mostly comprised elements of physical origine.

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EARTHWORM INDUCED MINERAL WEATHERING – PRELIMINARY RESULTS

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The role of organisms in mineral weathering has been investigated by several authors. Studies have shown that bacteria (Bennett et al. 2001), fungi (Burford et al. 2003), lichen (Banfield et al. 1999) and plants (Augusto et al. 2000) all have a role in mineral weathering. The role of annelid worms in mineral weathering has been little studied. Mineral weathering has been demonstrated in marine annelids (McIlroy et al. 2003) and there is some indication that earthworms may also play a role in mineral weathering (Suzuki et al. 2003). Earthworms interact intimately with the mineral soil and have a major influence on the physical structure and chemistry of soils. They increase the porosity and the water holding capacity of soils (Shipalto & Le Bayon 2004). Their casts have more available plant nutrients than the bulk soil (Edwards & Bohlen 1996). They transport material from the lower horizons to the soil surface and they are responsible for the incorporation of organic matter into the lower horizons (McInerney & Bolger 2000). A series of experiments were run to investigate the effect of an epigeic earthworm (Dendrobaena The minerals anorthite, biotite, olivine, attemsi) on soil minerals weathering. montmorillonite and kaolinite were mixed with a sterilized manure substrate. Four treatments were used to investigate the effect of the earthworms on the minerals; earthworms and minerals, earthworms and no minerals, no earthworms and minerals, and no earthworms and no minerals. The earthworms were left to process the substrates for 1, 2, 4 and 6 months. Four sacrificial replicates were used. Changes in mineralogy were analysed using x-ray diffractometry, scanning electron microscopy and chemical methods. Results for these experiments will be presented. Weathering of olivine (formation of Fe oxyhydroxides) and biotite (release of K) appears to be accelerated by earthworm activity, but more resistant minerals such as hornblende and anorthite show no significant alteration over the shorter timescales of these experiments.

MODELLING THE CONTRIBUTION OF EARTHWORMS TO SOIL N POOLS AND PLANT N UPTAKE IN QUEBEC AGROECOSYSTEMS

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Earthworm activity increases the rate of nitrogen cycling in soils and is generally associated with an improvement in plant nutrient uptake and primary production. The contribution of earthworms to soil N pools ranges from negligible to 363 kg N ha⁻¹, for a range of agroecosystems and climatic zones. Many of these estimates are based on laboratory experiments under optimal conditions for earthworm activities. There is a need to further refine these estimates using field data.

The scientific literature documenting earthworm contributions to soil N pools uses one of two approaches. The first determines the quantity of N that is released from earthworms as the total N consumed by earthworms minus the N that is assimilated into body tissues. The second determines the quantity of N that is released in urine, mucus and casts, and from dead earthworm biomass. However, the proportion of N released from earthworms and transferred to plants is poorly understood. Earthworm activity may cause some losses of N by increased leaching, immobilisation in the microbial biomass and denitrification losses, thereby decreasing the amount of soil N available for plants.

We have developed a model to refine current estimates of earthworm contributions to soil N by calculating a nitrogen mass balance around a soil system. The model determines that the total input of N to soil should equal all outputs of N (plant N uptake, leaching and denitrification losses) plus any change in soil N pools (inorganic N, microbial biomass-N, and other organic N fractions). The model provides improved estimates of earthworm contributions to soil N pools and what proportion of that N may be taken up by plants. The key parameters, equations and the development of the model will be discussed. Furthermore, model simulations will be presented and compared to other estimates from the scientific literature.

THE EFFECTS OF AN ANECIC AND AN EPIGEIC EARTHWORM SPECIES ON SOIL AMINO ACIDS; A SIGNIFICANT SOURCE OF ORGANIC NITROGEN

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The availability of nitrogen (N) in soluble forms that can be taken up by plants limits primary production in most terrestrial ecosystems. However, the majority of N in soils is organically combined, and up to half of this is in the form of amino acids (AA). The free AA (FAA) are increasingly being recognised as an important part of hte soil N pool, for both plants and the microbial population. In some systems, the FAA make up a significant part of the plant's N budget. The efficiency of the decomposers is the principal regulator of the rate of return of nutrients from litter to soil, from where they can be taken up again by plants. Earthworms are keystone species because of the important role they play in many soil processes, particularly in decomposition and nutrient cycling. This is in no small part due to their high abundance and their widespread distribution. There is a large temporal and spatial variation in availability of AA in soils, with a high turnover for individual FAA. Systematic differences in FAA composition of soils are due to ongoing soil processes and earthworms can be a significant regulatory factor (Striganova *et al.*, 1989). Because of their 'ecosystemivorous' nature (Pokarzhevskii *et al.*, 1997) their AA profile could also reflect the soil habitat in which they live.

The specific objectives of this work were:

(1) To see how earthworms from two different feeding groups – the epigeic *Lumbricus rubellus* and the anecic *Lumbricus terrestris* – affect soil FAA profiles;

 $\left(2\right)$ To compare the relative effect of different food types on earthworm and soil FAA composition, and

(3) To compare the FAA profile of the earthworms against the FAA profile of the soils.

We cultured *L. terrestris* and *L. rubellus* in individual mesocosms filled with a standard soil (Kettering loam), with either beech litter or horse manure as a food source; a control group had no food. After six weeks the mesocosms were dismantled. We used High Performance Liquid Chromatography to obtain FAA spectra from the earthworms, soil and cast material. Principal components analysis of these data showed that the FAA profiles differed significantly. We discuss the effect of feeding group and food type on FAA composition, and the implications of this on the cycling of organic N in soil systems.

EARTHWORM SPECIES SPECIFIC AND ORGANIC MATTER EFFECTS ON BURROW PROPERTIES AND SOIL WATER MOVEMENT

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Earthworms modify soil chemical and physical properties by their burrowing activity and by incorporating organic matter (OM) in the soil profile. Their burrows have a major impact on water infiltration by creating preferential pathways for water transport. Previous studies on the subject showed that properties of burrow networks that influence water flow are related to (i) the morphology and geometry of burrow systems, (ii) the earthworm ecological groups (endogeic, anecic, epianecic) which create different burrow networks, and (iii) the trophic richness of the site which influence the burrowing activity. Because of their location between the macropore itself and the soil matrix, burrow walls may play an important role in water exchanges in the soil. We hypothesized that the chemical and physical characteristics of the burrow walls may also influence the water transport into the soil matrix. The aim of this study was to quantify the effects of three earthworm species (L. terrestris - epianecic; N. giardi - anecic; A. caliginosa - endogeic) in relation to two organic fertilization treatments (cattle manure and pig slurry) on the functional role of the drilosphere by investigating (i) physical (distribution of pores) and chemical (carbon and nitrogen) characteristics of the burrows and (ii) water movement (infiltration through the burrow and diffusion through the burrow wall into the soil matrix).

The study was carried out in microcosms (soil columns 25 cm high with a diameter of 12 cm in Plexiglas tubes) under controlled laboratory conditions (10°C). The different organic fertilizers were applied to the soil surface and compared to a non-fertilized control. The earthworm species were introduced into the columns: one species per treatment. In order to characterize burrow properties at small scale, water infiltration was assessed by using a mini-infiltrometer equipment: considering different water tension potentials (0.05; 0.2; and 0.6 kPa), this original equipment measures the hydraulic conductivity of soil and allows to characterize different pore size classes of the burrows. In each column, we distinguished an "infiltrated zone" and a "non-infiltrated zone". Chemical characteristics of burrows, i.e. C and N contents, were measured (i) in two subzones: inner layer (from the lumen of the burrow up to 3 mm) and the outer layer (from 3-10 mm) (ii) at three depths of the soil columns: 0-4 cm, 4-8 cm, 12-16 cm. Chemical measurements were also done with soil samples obtained under the infiltrated zone and the non-infiltrated zone in order to assess the influence of the chemical characteristics on water movement. For all fertilizer treatments, the introduction of L. terrestris or N. giardi increased the soil water infiltration (0.05 kPa), whereas in the case of A. caliginosa (endogeic) this increase was only observed under cattle manure. The pore size distribution of both large species differed: L. terrestris burrows are characterized by large pores (0.05 pKa), whereas large and medium pores (0.05 and 0.2 pKa) characterized the N. giardi burrows. The vertical distribution (depth) and horizontal distribution (from burrow lumen into soil matrix) of C and N were strongly related to the earthworm species and to the fertilizer type.

Oral

LINKING EARTHWORM POPULATIONS TO SOIL PROPERTIES IN LANDSCAPES: POTENTIAL OF THE STATE-SPACE APPROACH

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Knowledge about the relationships between earthworm populations and soil properties is important because of several reasons, such as for the estimating faunal abundances from surrogate variables (soil properties), for estimating functional consequences of faunal activities in the field, or for identifying effects of management. Yet, information about quantitative relationships is not readily available, partly due to a lack of adequate methodologies. Relations based on randomly fluctuating values of faunal abundances or soil properties, detected by classical statistics, are often not satisfying (Nielsen & Wendroth 2003). Therefore, new approaches are necessary for detecting solid relationships between soil fauna and soil properties.

In this context, spatial studies were carried out on agricultural sandy soils in north-east Germany, in order to determine relationships between earthworm and enchytraeid populations and soil properties at different scales. The data were analysed with state-space analysis. The results indicated close relationships between earthworm populations and soil properties However, relationships differed between sites, seasons and scale studied. From the results we conclude that no universal relationship exist between earthworm or enchytraeid populations and soil properties. The results further indicate, that state-space analysis is a promising tool for elucidating soil fauna-soil relationships at different scales and landscapes.

EFFECTS OF WINTER COVER CROPS, REDUCED TILLAGE AND WEED MANAGEMENT ON EARTHWORM POPULATIONS

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Winter cover crops are being investigated as a strategy to reduce nitrate leaching in spring barley cropping systems in Ireland. The objective of this study was to assess the effects of a mustard winter cover crop and different soil tillage and weed management options on earthworm abundance, biomass and species diversity.

A randomised-block field experiment was set up on a sandy loamy soil at the Oak Park Crops Research Centre, Carlow, Ireland, in 2003. There were six treatment combinations in a spring barley main crop system, comparing a mustard cover crop with no cover crop, reduced tillage with conventional spring ploughing, and natural regeneration with autumn herbicide application. Each of the six treatment combinations was replicated four times, with individual plots measuring 12 m by 30 m. Earthworm populations were assessed by hand sorting of soil blocks (two per plot) at three selected stages of the second/third barley cropping cycle, namely after sowing of the main crop (May 2005), after cover crop establishment (October 2005) and before spring ploughing for the next main crop (March 2006).

Data for May and October 2005 suggest that mustard cover crop treatments, with or without ploughing, supported smaller earthworm populations than treatments in which natural vegetation regeneration was allowed to occur. Use of an autumn herbicide to maintain bare soil over the winter for experimental purposes was associated with the lowest earthworm population levels. Ploughing caused a reduction in earthworm population sizes compared to reduced cultivation treatments.

It is concluded that mustard as a winter cover crop, even though it provides organic matter inputs and affords soil protection between main crops, does not *per se* result in larger earthworm populations. This may be because it reduced water availability on this lighttextured soil or because mustard is not a palatable food source for earthworms, possibly related to its glucosinolate content, which has been shown to have suppressive effects on root-feeding nematodes.

EARTHWORM CASTING AND BURROWING IN CONVENTIONAL AND ORGANIC FARM SYSTEMS

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Earthworm casting and burrowing activities affect the distribution of nutrients/organic matter within the soil profile influencing the location of root development and microbial activity. Water infiltration and drainage are also greatly influenced by burrowing patterns. There is evidence from previous studies that land management practices associated with conventional and organic farming affect the size and composition of earthworm populations. Less information is available on the consequences of these differences for casting and burrowing patterns and on whether such differences can be attributed solely to population changes.

Three pairs of adjacent organic and conventional farms practicing similar grass-arable rotations on similar soil types were compared; several rotation stages were investigated within each farm. Population and activity data were recorded on three occasions over two years. Abundance and weight of surface casts, surface burrow openings and burrows at 30 cm depth were recorded on each occasion. Relationships between population and activity data were investigated and these relationships compared for different rotational stages and farm systems. Various soil and agronomic factors were also recorded.

On two of the three farm pairs, earthworm populations were generally higher on the organic farm though not at all stages of rotations. Surface casting was low on all arable compared to grassland phases, regardless of farm system; high densities of casting were observed within tilled layers during arable cropping. Organic sites had markedly higher densities of surface burrows at most stages of rotations compared and these densities were at least an order of magnitude higher than for casting on all but longer-term grassland. Burrow densities at 30 cm depth varied less between rotational phases but tended to be higher on conventional farm systems.

Casting indices and burrow densities at depth generally correlated poorly with population data. Surface burrows in contrast showed close relationships with most population classes. There was a general tendency for levels of surface burrowing to be higher in organic systems for a given population density, particularly during arable rotation phases. Burrowing at depth showed the opposite tendency.

Findings suggest some modification to earthworm behaviour in response to management practices, in addition to population changes. Implications for soil fertility and function will be considered.

FUNCTIONAL RELATIONSHIP BETWEEN EARTHWORM ACTIVITY AND SELECTED SOIL PROPERTIES CONSIDERING DIFFERENT SOIL TILLAGE SYSTEMS

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It is well documented that reduced tillage systems compared to ploughing cause positive effects on abundance and biomass of earthworms. However, there is still a lack in knowledge concerning the direct relation between earthworms, functional soil properties and applied tillage in arable soils. For this reason, we measured functional abiotic parameters related to earthworm activity to go deeper into the role of earthworms as important providers of ecosystem services.

Within the experimental field site of the Federal Agricultural Research Centre different tillage systems are applied for long terms since more than 10 years: conventional tillage with ploughing, reduced tillage without ploughing like conservation tillage with harrowing and mulching and direct drill as a no-till treatment. All fields sampled were cropped with maize in 2004. Sampling for earthworms and soil moisture occurred at three dates some weeks before and after harvest and finally after soil tillage. In case of soil functional properties like air permeability, water tension, bulk density, pore volume, pore size distribution, percentage of water stable aggregates and organic carbon samples were taken only once between harvest and tillage.

Earthworm abundance and biomass were highest in the direct drill treatment followed by the conservation tillage treatment. Two species were found in all treatments: *Aporrectodea caliginosa* and *Lumbricus terrestris*. Additionally, *A. rosea* was determined under direct drill. *L. terrestris* dominated under reduced and no tillage. For most soil properties, conservation tillage showed the most beneficial results. Here the proportions of water stable aggregates and coarse pores, organic carbon content and air permeability were highest, bulk density was lowest. The results indicated conservation tillage to be the best way to promote earthworms to gain ecological benefits from their activity. The results will be discussed in the light of measures for soil protection in agricultural management.

THE LOCATION OF ORGANIC MATTER IN SOIL MATRIX AFFECTS GROWTH RATES OF APORRECTODEA ROSEA

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Earthworms are widely used in soil reclamation programs in order to increase the incorporation of organic matter, and stimulate biological activity in restored soils. Surface-feeding species translocate litter into deep horizons, whereas endogeic earthworms play a key role in carbon stabilization, formation of stable aggregates, and development of soil profile. The availability of food for earthworms of different ecological groups depends of its location in the soil matrix.

We investigated the effect of plant litter location on the growth of juvenile *A. rosea* and *L. rubellus* in a microcosm experiment. Poplar litter cut to 1×2 cm pieces and mineral urban soil were used. Five treatments with six replicates were set up: (1) mineral soil and litter thoroughly mixed; (2) litter placed on the soil surface; (3) litter covered by mineral soil; and (4, 5) litter only and mineral soil only, respectively. Three specimens of *A. rosea* (mean fresh wt 60 mg) or two specimens of *L. rubellus* (mean wt 110 mg) were placed in each microcosm. The experiments lasted six weeks.

The location of litter did not affect the weight increase of *L. rubellus*. The growth of *A. rosea* was at a maximum $(13\pm1 \text{ mg week}^{-1})$ in treatments with mixed litter and mineral soil (1), and with buried litter ($19\pm2 \text{ mg week}^{-1}$). The lowest growth ($5\pm1 \text{ mg week}^{-1}$) was recorded in soil without litter and in litter without soil (4, 5). In the treatment with litter atop of mineral soil (2) the growth was $9\pm1 \text{ mg week}^{-1}$.

In order to estimate the importance of the litter location for earthworms of different size, we compared the correlation between relative growth rate (% of initial wt) and initial weight of animals in each treatment. The results suggest that smaller specimens of *A. rosea* had a relative advantage in the use of food sources in the treatment with litter on the surface (2), whereas in the treatment with buried litter (3) larger worms grew faster than small ones. Larger specimens of *L. rubellus* were seemingly more successful than smaller specimens in all combinations of soil and litter.

Thus, the distribution of organic residues in restored soils can affect dynamics and eventually the overall success of inoculated earthworm populations. Endogeic earthworms utilize litter buried compactly or dispersed through soil matrix more effectively than litter concentrated on the soil surface. This suggests that preliminary soil preparation (e.g. turning under) is necessary to ensure a successful use of endogeic earthworms in soil restoration programs.

THREE-D SIMULATIONS OF BURROW SYSTEMS MADE BY EARTHWORM COMMUNITIES AND STUDY OF SOME PHYSICAL CONSEQUENCES

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In the past decade, X-ray tomography was successfully adopted as a mean to study earthworm burrow systems under natural or artificial conditions. However this method has some limitations such the size of the soil core, the existence of a border effect, and cost. Recently a new apparatus, called the "Colonne Gamma", was developed that enabled to study the trajectory of radio-labelled earthworm belonging to different species in soil cores. The accurate knowledge of (i) the burrowing behaviour of earthworm and (ii) the 3D characteristics of the burrow systems gave the necessary information to develop a simulator. The input parameters are: (i) the earthworm community (and principally the ratio anecic/endogeic), (ii) the interaction between earthworms (avoidance or not) and (iii) the rate of burrow refilling. Hundred simulations were made for each parameter set and several output parameters were computed such as (i) volume and area of burrows connected to the surface and (ii) the verticality of the burrow systems. The first set of parameters was used in another model to simulate water flow through the burrow systems. Interestingly, we observed that increasing interactions between earthworms increased the verticality of the burrow systems. Regarding water flow, we observed that the key parameters were unsurprisingly the proportion of anecic earthworm and the intensity of the burrow refilling. These examples illustrate the potential use of our simulator to study the effect of behavioural parameters on the resulting burrow systems.

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EARTHWORM RESPONSES TO LONG TERM AGRICULTURAL MANAGEMENT PRACTICES.

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Crop production with different tillage practices and subsequent crop residue management are important factors for sustainable agriculture world-wide. Soil structure, degradation, soil erosion, organic matter loses and changed in sensitive biological properties are major issues that have brought about renewed interest in tillage and residue management practices for crop production. Tillage and other soil management practices affect earthworm populations by affecting the food supply, mulch production and chemical environment. Earthworm populations are almost always higher under no-till than under conventional practices (Parmelee & Crossley 1988, Aslam *et al.* 1997). Numerous examples from research plots and producers' fields support the conclusion that reduction of tillage intensity encourages earthworm populations. Moldboard ploughing and no-till represent two extremes of tillage systems, and systems with intermediate levels of soil disturbance and surface residue usually have populations intermediate between the two extremes.

The specific objectives of this project were to examine the effects of long term agroecosystem management practices on: (1) earthworm abundance and diversity and

(2) soil properties.

We sampled plots that had been under the same cultivation for 23 years. All were annually sown with winter wheat, and there were two treatments: soil disturbance (ploughed, tined and direct drilled) and crop residue (straw residues left *in situ* or removed). The replicated plots were sampled for earthworms (hand sorting of soil monoliths, 25 cm x 25 cm x 10 cm deep), and the soil from these pits were analysed for C and N. Other soil properties, including stable isotope ratios, soil enzyme activity, microbial biomass and bulk density were also recorded but will be reported in a companion paper.

The degree of disturbance had a significant effect on earthworms, with both a higher abundance and biomass in the direct drilled plots. The addition of straw also increased the number of earthworms. The degree of disturbance and addition of straw increased the %C in the 0-10 cm depth of soil, but had no significant effect on the %N. The earthworm species composition also varied according to the two treatments. We will discuss these findings with reference to sustainable agroecosystems.

EFFECTS OF DIFFERENT TILLAGE PRACTICES ON SOIL PROPERTIES AND EARTHWORM POPULATIONS

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Intensive agricultural practices (ploughing, fertilisation, burning of the residues...) strongly affects the biodiversity and fertility of soils. The introduction of conservative agricultural techniques, including reduced tillage systems (e.g. no tillage and conservation tillage), provide favourable conditions for the soil populations due to an increase in the amount of organic residues as available food and the reduction of the mechanical alteration of the surface horizons.

The aim of the study presented here was to compare the effects of different tillage systems on soil properties (N, K, P, SOM, electrical conductivity and soil pH) and earthworm's diversity and abundance. The site selected was a barley monoculture located at a semi-arid area in Olite (Navarra, Spain). In 1994 a complete random block design of sixteen experimental plots including three tillage systems and one control system was established with four replicates per treatment: (i) conventional tillage with a mouldboard plough (MT), (ii) reduced tillage (RT), (iii) no tillage with stubble burning (NTSB) and no tillage (NT). Fertilisers and pesticides are commonly applied to all plots. Two soil samples (20 x 20 cm) per treatment were taken to a depth of 20 cm for earthworms in May and November 2004. Additional soil samples from three different depths (0-5, 5-15 and 15-30 cm) were taken for chemical analyses in November 2004. Earthworms were hand-sorted in the field, fixed in a 10% formalin solution and identified in the lab to species level.

The earthworm community consisted of three species: Allolobophora rosea, Scheroteca gigas and Prosellodrilus praticola. Significantly more worms were collected in autumn, three months after the cultivation preparations, although *A. rosea* was only recorded in May. Conventional tillage had a detrimental effect on *A. rosea* and *S. gigas* populations and no single specimen of these two species was found at both sampling dates. *P. praticola* was the dominant species in all treatments and the only species present at the MT treatment, although its mean density was severely reduced (p < 0.05). Treatment effects were only significant in November when the two no tillage treatments (NT and NTSB) supported a higher earthworm population than the RT and MT treatments (p < 0.05). No significant differences between treatments were detected for any of the soil characteristics measured, with the exception of nitrogen which was significantly lower in the MT treatment. Furthermore, significantly higher N, K and SOM contents were present in the upper soil of all treatments when compared with the deeper layers.

We conclude that conventional tillage has a negative effect on earthworm communities and that different agricultural practices have a different effect on different species. Climatic conditions may also play an important role on earthworm activities especially in semi-arid areas and, consequently, harvesting the crop and removing the agricultural residues from the top soil could result in sudden exposure of soil populations to extreme temperatures, especially in the summer months.

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DEMONSTRATION OF THE ROLE OF EARTHWORMS ON THE REMOVAL OF HIGHLY COMPACTED ZONES IN FIELD CONDITIONS

Poster

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By their excavation activities, earthworms are thought to contribute to the removal of highly compacted zones ("delta" zones) but the quantitative measurements of their impact is lacking. A first experiment was carried out in semi-field conditions (caging experiment). Adult earthworm from the most common species (Lumbricus terrestris, Aporrectodea giardi, A. caliginosa and A. rosea) were in presence of different compacted zones: (i) horizontal, (ii) vertical layer and (iii) isolated zone representing different natural situations (under wheel or timmage compaction). Depending on the species (and their behaviour) we observed different impacts on compacted zones (L. terrestris is more prone to cross horizontal layer whereas A. caliginosa is more prone to colonize vertical ones). A second experiment was carried out in field conditions to characterise the dynamics of the recolonisation by earthworms after a compaction (through a 8t vehicle). Under and between wheels zones were followed during 2 years. We studied earthworm bulk density, earthworm community, 2D vertical macroporosity and water infiltration measurements were done. We observed that earthworm recolonisation is a rapid process (less than 6 months to recover similar abundances) whereas macroporosity and water infiltration are much slower processes. This illustrates the difficulty of using earthworm abundance as way to estimate the physical consequences of earthworm activity. Moreover, 3D soil cores were collected at different dates under and between wheels and analysed through X-ray tomography. This enables us to visualize the (slow) recolonisation of the soil with earthworm macropores and to discuss the effect of the different species present in the field.

Poster

OLIGOHUMIC ENDOGEIC EARTHWORM INDUCED MINERAL WEATHERING – A FIELD STUDY

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It is hypothesised that the different ecological types of earthworms will have different effects on the weathering of soil minerals, due to the proportion of the mineral soil ingested by these different types. Endogeic earthworms are found deeper in the soil horizon and feed on the mineral soil (Edwards and Bohlen, 1996). In Africa, the endogeic earthworms are further separated according to the amount of organic matter in the soil. Oligohumic endogeic earthworms feed on mineral soils which have very little organic matter in them. Field work was carried out in the Republic of Congo to assess the influence of oligohumic endogeic earthworms on soil mineral weathering. Mesocosms where employed to assess the impact of earthworms on the weathering of a mineral spike to the native soil. Four treatments were used: spiked soil with earthworms; spiked soil without earthworms; native soil with earthworms. The experiment was run for 5 weeks. Changes in soil mineralogy were analysed using x-ray diffractometry and chemical analyses. Results from these field experiments will be presented.

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Poster

EARTHWORM ACTIVITY AFFECTS SOIL AGGREGATION AND SOIL ORGANIC MATTER DYNAMICS IN DIRECT MULCH-BASED CROPPING - AN EXPERIMENTAL STUDY

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Soil provides a wide range of ecosystem services that are essential for human life and well being, e.g., food and fibre production, carbon sequestration, erosion control, water quality, detoxification, and regulation of climate, These services are based on ecosystem functions such as organic matter dynamics, nutrient cycling, etc. Decades of studies have demonstrated the importance of soil fauna and microorganisms in the functioning of soils, and particularly in organic matter dynamics. Nevertheless the role of soil biological activity and diversity in regulating ecosystem functions such as organic matter dynamics, and the way it can be valued, maintained and managed in agricultural systems is poorly understood. In the tropics, direct seeding mulch-based cropping (DMC) systems are extending, especially in South America. These systems characterized by no-tillage and permanent soil cover reduce soil disturbance and often result in a significant accumulation of soil carbon, a decrease in erosion, and an increase in soil biological activity and biodiversity. Studies in Brazil, Benin and Madagascar showed a strong increase in the density and biomass of soil macrofauna, especially earthworms, in DMC systems. Earthworms are known to modify soil structure through soil ingestion and cast production, and to modify the living community of the drilosphere. These changes lead to modifications in soil organic matter dynamics, a key factor in soil functioning and in the durability of agricultural practices. However, the links between earthworms' density increase and changes in organic matter dynamics in such systems has not been clearly elucidated.

Our experimental study aimed at analyzing the effect of endogeic earthworms on soil aggregation, carbon localization and protection against mineralization according to the quality and localization of crop residues, in a clayey Inceptisol in the highlands of Madagascar. Ten experimental treatments were studied in mesocosms, crossing: presence or absence of earthworms (Pontoscolex corethrurus), presence or absence of residues (soybean or rice), localization of crop residues (incorporated into the soil or left at the soil surface). The experimental setting was maintained during five months. Then soil samples were wet-sieved, and earthworm casts were manually separated from non ingested soil. Total soil and fractions were analyzed for carbon concentration, and carbon mineralization was measured during a 28 day incubation at 28°C.Earthworm addition had significant effect on soil aggregation, particularly increasing the proportion of > 2 mm water-stable aggregates. Litter addition, quality and localization had no effect on aggregation. Total soil carbon was not significantly affected by earthworm addition, but it was slightly higher when litter was added, especially when it was incorporated. Carbon concentration was higher in casts than in not ingested soil. Moreover, carbon located within the casts was more protected against mineralization compared to carbon in not ingested soil.

These results show that in the studied system, earthworms play a role in the location of carbon and in its protection. However, relative differences between treatments may have been partly hidden by the naturally high carbon concentration of the studied soils.

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INTERACTIONS BETWEEN MORPHOLOGICAL CHARACTERISTICS OF EARTHWORM BURROWS NETWORK AND WATER DIFFUSION IN SOIL : SIMULATION & MODELISATION

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Currently, the development of new methods of soil management aims to limit the physical disturbances of the arable layer, and has as a consequence and interest a notable increase in the functional diversity of soil fauna. In particular, this new agriculture involves an increase of earthworm populations, major responsible of the biological porosities of soil (tubular and packing voids). The long term objective is thus to quantify the influence of this porosity increases on the processes of water circulating in soils (initiation, diffusion, macropore and matrix flow).

The aim of the study was to create a simulator of earthworm burrows network, thanks to knowledge on the earthworms burrowing behaviours so as to, as well as possible, adjust the simulation of anecics earthworms to biological reality. This simulation allowed (i) to obtain geometrical data on the burrows and (ii) to simulate a great number of networks in order to carry out a typology of these networks according to species. Then, a second stage was to put in parallel the simulation of the networks and a model of water infiltration in order to identify the more important morphological parameters of the earthworm networks according to the hydrodynamic properties. In conclusion, this study should permit to quantify the influence of the earthworms bioporosity on soil water diffusion.

SOIL FRACTAL DIMENSION, PERTURBATION AND EARTHWORMS COMMUNITIES

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During the last 20 years the surface of annual culture in the pampean region of Argentina $(34^{\circ} \text{ S and } 59^{\circ} \text{ O})$ has increased, especially with transgenic soybean (*Glicine max*).

This process has produced a reduction in earthworm populations, changes in their community structure, and modifications in soil structure. Rotation with pastures allow for soil recovering and fertility maintenance. Soil structure, in particular soil porosity, may be characterized by measuring their fractal dimension (an indicator of pore system complexity).

The aim of this work was to analyze the relationships between earthworm community structure and soil fractal dimension in relation to the recovery process that occurs during the pasture cycle.

In the experimental dairy farm of National University of Luján (northeast of Buenos Aires province, Argentina) three fields were selected by pasture age and use history. The first, sowed in 1997 (P97), the second one in 1998 (P98) and the third one in 1999 (P99). In a neighbouring city a reference field that presents 30 years without cultivation (SN) was studied.

Samples were taken in autumn 2000. Soil and earthworm samples were randomly taken between intersection points of a 1 ha grid, equally spaced at 25 m. Fifteen earthworm samples were hand-sorted from a 25 x 25 x 20 cm soil portion. We registered total biomass, number of individuals and species composition. Maximum species richness (Smax) was estimated by means of the Chao index. The Shannon index of diversity (H) was calculated, and the confidence interval estimated by jacknifing method. Relative abundance of species were plotted against rank, and fitting to geometric series (Motomura) and "broken stick" (Mc Arthur) models were evaluated.

Five undisturbed samples were taken for fractal analysis; impregnated with a mix of a polyester resin and a fluorescent dye. Images of polished blocks were taken with a digital camera and converted to binary. Soil mass fractal dimension (Dms), pore mass fractal dimension (Dmp) and soil surface dimension (Ds) were calculated by box-counting method.

Results for biomass (g/m^{-2}) and number of individuals (ind/m^{-2}) are greater in the three-year pasture (P97: 72,1; 702,9) followed by SN. The smaller value was found in P99 (17,8; 426,7). Species richness and H were greater in SN: 9 species and 2,48; followed by P97 and P98. Species' distribution functions show better fitting to Motomura model indicating an intermediate level of perturbation with some hierarchy in resources' competition. Dms was smaller in SN and P97 (1,92), Dmp and Ds were greater in the SN site (1,80). We found that earthworm populations do recover in soils with pastures, and at the same time an improvement of soil structure measured by means of fractal dimensions is observed; these effects were observed after 1 year of pasture implantation.

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ECOLOGICAL GROUPS OF EARTHWORMS – THEIR BIOHYDROLOGICAL IMPLICATIONS AND SENSITIVITY TO SOIL PROPERTIES AND MANAGEMENT

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Earthworms are functionally classified into epigeic, endogeic, and anecic ecological group. These ecological groups of earthworms (epigeic, endogeic, anecic) are different in their feeding behavior, in their burrowing behavior, and in their distribution in the soil profile. From this it can be inferred that they are influencing the hydrological soil properties in different ways. This is discussed for the three ecological groups with respect to water infiltration, preferential flow and soil hydrophobicity.

The influence of soil properties and management on the distribution of functional groups of earthworms is elaborated from case studies with recultivated soils, soils with toxic horizons and soils varying in their tillage regime systems. Additionally we present results from mesocosm studies.

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THE EFFECT OF EARTHWORMS ON ACCUMULATION OF SOIL ORGANIC MATTER DURING POST MINING SOIL DEVELOPMENT

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The effect of macrofauna on soil organic matter accumulation (carbon content in soil), microbial respiration, and microbial biomass in post mining soil was studied in field and laboratory microcosm experiments. Field experiment was carried out at colliery heaps located in the Sokolov brown-coal mining area (Czech Republic). Two sites were chosen, the first reclaimed by planting alder (*Alnus glutinosa*) and the second un-reclaimed, naturally overgrown by shrubs among which *Salix caprea, Betula pendula* and *Populus tremula* dominated.

Field microcosms consisting of autochthonous litter and mineral (tertiary clay from a pioneer site) layers were exposed at the sites for one and three years. The microcosms were either accessible or non-accessible to soil macrofauna. The access of soil macrofauna did not increase significantly carbon mineralization (the loss of organic matter from the whole microcosms) but increased the translocation of organic matter into the mineral layer. This effect seemed to be more pronounced at the reclaimed site, apparently due to higher density of earthworms. Accumulation of organic matter in the mineral layer resulted in higher microbial respiration and biomass.

Laboratory microcosms were constructed consisting of mineral soil (alkaline clay spoil substrate) and two types of litter: taken from an un-reclaimed site and site reclaimed by planting alder. Both types of microcosms were inoculated with five different types of decomposer community, originating from the same field site as the litter: (1) microflora only, (2) microflora + mesofauna, (3) microflora + mesofauna + litter feeding macro-arthropods, (4) microflora + mesofauna + litter dwelling earthworms and (5) microflora + mesofauna + *Lumbricus rubellus*. Four microcosms of each type were incubated in the dark at 15° C for 90 days. The presence of macrofauna resulted in more intensive litter removal from the litter layer. Litter removal correlated with the accumulation of C in the mineral layer of the spontaneous sites, while it correlated with carbon loss from the system at the reclaimed sites. Considering both litter types together it was evident that litter fragmenting macro-arthropods were responsible for carbon loss and higher actual microbial respiration, while the presence of soil mixing *L. rubellus* resulted in higher potential respiration and carbon accumulation in the mineral layer.

Comparison of both field and laboratory microcosms indicated that the presence of litter mixing earthworms may be more important for soil organic matter accumulation than the type of litter.

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EFFECTS OF NATIVE AND EXOTIC EARTHWORMS ON SOIL PROCESSES IN THE NORTH AMERICAN PACIFIC NORTHWEST

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As many as 100 species of Nearctic earthworms may occur in wildland ecosystems in the Pacific Northwest of North America, but little is known about their influence on soil processes compared to the exotic earthworms that are invading forests and grasslands in the region. We studied native and exotic earthworm populations in and around old-growth Douglas fir forests in central Oregon and oak-grassland savannas in northern California to assess their relative abundance and effects on soil organic matter dynamics. Field surveys showed that native earthworms were primarily endogeic, occurred mostly in undisturbed habitats, and were highly clumped in their distributions; densities ranged from 0 to 32 (mean=5) m- $^{-2}$ in forests and from 0 to 96 (mean=60) m- $^{-2}$ in grasslands. Exotic earthworms occurred mostly in disturbed areas (e.g., pastures, golf courses) and along stream banks in forested areas, reaching highest densities in fertilized pastures (over 500 per m⁻²). Field experiments showed significantly greater effects of exotic than native species on cast production, surface litter incorporation and microbial mineralization, even at equal population densities. Because native earthworms often occur at lower densities than exotic species, exotics should exert greater effects than natives do on soil processes; where they occur together, competitive interactions between native and exotic species may alter these patterns.

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EARTHWORMS UTILIZATION AND THEIR POTENTIAL USE IN TROPICS, FOR INCREASING SOIL FERTILITY

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At the southeast of Mexico, Tabasco we are searching the potential use of earthworms in order to increase soil fertility. We studied 24 sites (7 natural and 7 managed sites) where we applied TSBF method (25x25x30 cm monoliths) in order to identify earthworm species and their relationship with soil properties, in natural and managed sites (some of them also in spatial way). 10 or 50 monoliths were made by site. We found the highest diversity of earthworms (2.58 Shannon index) in natural sites (Tropical rain forests) where organic matter content (3-15%), P available (13-24 mg.kg⁻¹) and total nitrogen (0.14-0.43%) were also high. We contributed with 3 new registers for the state. We found Lavellodrilus bonampakensis and Balanteodrilus pearsei, as native species important for being used in agro ecosystems, because of their tolerance to inhabit in managed sites (sugar cane culture and crops). We have cultured tropical epigeic earthworms (Dichogaster saliens) with manure, cacao wastes and Mucuna pruriens var. utilis leaves in order to find their potential use for vermicomposting, after 90 days of the experiment: the highest individual fresh weight of 360 ± 127 mg, with the highest growth rate of 3.54 ± 5.03 mg day⁻¹, cocoons with most biomasses (8.88 mg), and casts with the highest content of organic matter (4.81%), N (0.24%), available P (29.3 mg kg⁻¹) and k (2 cmol kg⁻¹) were found in substrates with 1.5 % Mucuna pruriens var. utilis. Also we have cultured in spite of their ecological features, endogeic earthworms as Lavellodrilus bonampakensis, Pontoscolex corethrurus, and Balanteodrilus pearsei in substrates rich in fresh organic matter (1.5% Mucuna pruriens *var. utilis*) where they have increased their biomass twice in one month, so as they can be reintroduced in agro ecosystems with leguminous in order to promote soil fertility. We have reintroduced earthworms (Glossoscolecidae) to a traditional backyard culture, we observed the highest organic matter content (5.45%) at plots with earthworms and leguminous, 1.2% higher than control plots (plots without earthworms and leguminous).

EARTHWORM ECOLOGY AND INFLUENCE ON SOIL PROPERTIES IN BAHIAGRASS/PEANUT/COTTON CROPPING SYSTEMS

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Growers are encouraged to adopt diverse cropping systems. Diverse cropping systems generally increase yield and promote greater and more diverse soil fauna and flora. Numerous and diverse soil fauna including microbial species, beneficial arthropods and earthworms are essential for the productivity of agroecosystems. Studies were conducted in Headland, AL and Quincy FL, in 2005 and 2006 to evaluate earthworm species and their influence on soil properties including water infiltration in bahiagrass based peanut/cotton cropping system compared to the traditional peanut/cotton cropping system. We also monitored the growth and development of earthworms through the season. The sod rotation had greater earthworm densities than the conventional systems at both sites. So far, identification of the Microscolex phosphoreus (Acanthodrilidae), Amynthas loveridgei (Megascolecidae), Amynthas cortices and Diplocardia eiseni has been made at the Florida site and Aporrectodea trapezoides (Lumbricidae) and Ocnerodrilus occidentalis (Ocnerodrilidae) species were found at the Alabama site. Most of the earthworm species identified are exotic to the Southeast United States. The higher earthworm population densities in the sod rotation increased water infiltration at both sites. Soils in the coastal region of the United States have a natural compaction layer between the 15-30 cm soil depths. This layer restricts root growth. Earthworm channels were visible even in the compaction layer. The roots of the subsequent cotton crops were able to grow through the channels to reach deeper soil depths. The rotations with higher earthworm densities also had the greater cotton root development in terms of root biomass and root crown diameter. Earthworm juveniles were mostly found in winter and more adults observed in summer. Earthworms are important to the cropping systems because they increase aggregate stability, water holding, and aeration and reduce surface crusting.

EFFECT OF EXOGENOUS ORGANIC MATTER ON EARTHWORM POPULATIONS IN SANDY AGRICULTURAL SOIL: A SPATIAL APPROACH

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It is known that biodiversity in sandy agricultural soils is strongly related to soil organic matter content, especially in soils under dry climatic conditions. Positive effects of organic matter additions on different soil organisms have been frequently documented. However, most of these results are from small field trials and may not be applicable to large, spatially variable fields. Therefore, an experiment was carried out at a 74 ha heterogeneous field site in north-eastern Brandenburg (Lietzen), where exogenous organic matter originating from limnic sediments was applied in November 2005. Sampling of earthworms was carried out on 42 permanent plots which were characterized by different soil organismic community to organic matter additions would be found, but spatially different reactions of organisms at the plots. We expected that earthworms reacted positively especially at those sites which already supported a thriving earthworm population during recent years. The results of the first sampling period after the manuring in spring 2006 are reported and discussed.
A CLASSIFICATION SCHEME FOR EARTHWORM POPULATIONS (LUMBRICIDAE) IN CULTIVATED AGRICULTURAL SOILS IN BRANDENBURG, GERMANY

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Earthworm activity is observed at long-term monitoring sites as an indicator of soil function to assess changes resulting from environmental and management conditions. In order to assess changes, characteristic values of earthworm populations under different site conditions have to be known. Therefore, a classification scheme for site-specific earthworm populations was developed for soil in agricultural use, taking interactions between earthworm populations and soil properties into account. Characteristics of sites grouped by means of a cluster analysis after principal component analysis served as a basis for the derivation of the classification scheme. Soil variables found to characterise site differences with respect to earthworm populations were the texture of the topsoil, the texture of the subsoil, and the soil organic matter content. The textural classes of the topsoil were divided into five groups comprising sandy soils (Ss), silty sand soils (Su), slightly loamy sand soils (Sl2), medium to strongly loamy sand soils (Sl3/Sl4), and loam and clay soils. Soil organic matter was divided into grades of equal size in a range from < 1, 1 - 2 up to > 6 %. The variables earthworm abundance and earthworm species were selected to represent earthworm populations and were divided into six groups ranging from very low to extremely high. Defined groups of earthworm populations showed a clear structure in relation to soil texture groups and the content of soil organic matter. From this distribution a classification scheme was derived as basis for prognostic values of site specific earthworm populations, thus enabling the interpretation of changes over time.

Soil properties as well as management were accountable for variation in earthworm populations on the arable sites in Brandenburg. Though management affects the soil organic matter content, this aspect can have a greater influence on earthworms than on the soil carbon content, which leads to deviations in the classification scheme. The same effect can be assumed for sites with conservation tillage. To cover further sites and land use systems with the classification scheme it may become necessary to add further explanation variables to the scheme. However, the presented classification scheme was suitable for the prediction and interpretation of earthworm population structure and size for cultivated agricultural soils in Brandenburg in a first evaluation carried out for sites reassessed after 10 years as well as for sites which were not included in the original database.

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INFLUENCE OF SOIL TYPE AND CROP ON EARTHWORMS AND GROUND BEETLES IN AGRICULTURAL LANDSCAPES

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Out of all dry land ecosystems, the biological diversity is the biggest in soil. Soil biota is of fundamental importance in the cycling of nutrients, decomposition processes, movement of matter and energy. Soil biota plays central role in the development of biological diversity of agricultural landscape; several soil biota groups represent approved indicators of land use and agricultural activities. Earthworms are the most important decomposers of organic matter in the soil, being at the same time connected to the processes taking place in the ecosystem. The main carnivorous insects living on the soil are ground beetles. Among the more important genera from agricultural perspective are *Carabus, Pterostichus, Harpalus* and *Amara*.

Agricultural activities change the diversity of the ecosystem directly, influencing the survival of individuals; or indirectly, changing the level of resources. Agricultural activities such as soil tillage, turning the soil while plowing, fertilization, irrigation, using pesticides, planting hedges, tree lines or small forest etc influence very strongly the soil biota, affecting the organisms living on the surface of the soil as well as underneath it. Mechanical cultivating of the soil with different agricultural machines influences especially strongly all the invertebrates of the soil. Earthworms are influenced by soil cultivation, the way of crop husbandry, fertilization, plant protection, in dry areas also irrigation. Soil cultivation influences the abundance of earthworms directly (through mechanical disturbance) and indirectly (decreasing the availability of nutrients, destroying the habitat, taking plant litter fall into deeper layers, rearranging the reserves or organic matter between soil layers, opening the population of earthworms to birds). The ground beetles are very sensitive to plant protection products, especially pesticides, through direct contact as well as through eating poisoned prey.

The aim of the research is to evaluate the influence of soil types and crops on earthworms and ground beetles in the soil of different agricultural landscapes in Estonia. Twenty four study areas of three most widespread soil types (pebble rendzinas, typical brown soils and pseudopodzolic soils) all over Estonia were selected. In each group of soil type, eight fields with different management practices were selected for studies on 2003-2004. Earthworms were collected during autumn period when the earthworms are most active and when they can be found in the upper soil layers, by hand sorting method. Collecting the samples of ground beetles on the soil the pitfall-traps were used.

The mean abundance of earthworm communities was the highest in pseudopodzolic soils and in 2003 the hay fields turned into the most suitable habitat for the earthworms, in 2004 they preferred the abandoned fields. Ground beetles did not show significant prefers for soil types and crops, probably abundance of ground beetles in agricultural landscapes depend on different factors.

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SOIL FOOD WEB, C AND N TRANSFORMATIONS AND SOIL STRUCTURE: INTERACTIONS AND FEEDBACK MECHANISMS AS A FUNCTION OF THE QUALITY OF EXOGENOUS ORGANIC MATTER

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Applying organic matter (OM) to the soil is crucial for (i) maintaining the organic carbon content and (ii) soil structure, (iii) the biodiversity, (iv) functioning of the soil food web and (v) protection against erosion. It is widely known that the quality, i.e. the composition, of the applied OM has a significant influence on the different functions of the soil (e.g. soil physical properties, disease suppression, nutrient availability); however, not all kinds of organic amendments are of equal value.

The aim of our research is to determine how changes in soil physical properties and soil food web structure induced by the different qualities of OM interact with each other and how they influence the C and N mineralization in soil.

Therefore we designed a field experiment in which several organic amendments are applied, starting in spring 2005. The field trial is a randomised complete block design with 4 replicates and compares 8 treatments: farmyard manure, cattle slurry, vegetable, fruit and garden waste (VFG) compost, two types of farm compost, mineral fertilizer, and two treatments without fertilization (one with and one without a crop). All plots (8 x 6 m²) receive the same amount of organic C (4000 kg C/ha) once or twice a year. Due to a different nutrient content, applying the same amount of organic C induces the application of unequal amounts of plant available N. Consequently extra mineral N is applied on organically amended plots. The experiment has the following crop rotation: fodder beet, winter wheat, a catch crop, potatoes and ryegrass.

Soil physical properties, C and N mineralization, distribution of C and N over different soil fractions (soil physical fractionation), and changes in composition of the soil fauna and flora are monitored over time. Three major components of the soil food web are investigated: the microbial population and the population of nematodes and earthworms.

Earthworms are sampled with mustard powder and subsequent handsorting. The day before the sampling, 6g of mustard powder is mixed with 15ml of water to produce a paste. Immediately prior to sampling, this mustard paste is mixed with 0.8l of water. A metal frame of 20 x 20cm² is placed on the soil and all crop litter within the frame is cleared. The mustard solution is then poured evenly across the frame. After 10 minutes, while collecting the emerging earthworms, this treatment is repeated. After another 10 minutes, the soil in the same quadrant is excavated using a higher metal frame to a depth of 20cm, spread on a white plastic and handsorted to detect remaining earthworms. Per plot two samples are investigated.

At the start of the experiment, samples were taken to identify the initial earthworm population. Species present were *Lumbricus terrestris* and *Lumbricus rubellus* (anecic) and *Allolobophora rosea*, *Allolobophora chlorotica* and *Aporrectodeae caliginosa* (endogeic). We will monitor the influence of the different treatments on the earthworm population.

EARTHWORMS AND NITROGEN COMPOUNDS: SADIE ANALYSIS OF DISTRIBUTION IN A "STUDY POINT"

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Spatial distribution of earthworms and activity of water-soluble NH_4 and NO_3 and protein content (Bradford assay) in soil were studied in July 2000 in a grid (6 x 24) of adjacent samples in a spruce forest near Borovsk (Kaluga region, 55°13' N, 36°22' E, Russian Federation). A sample size was 76 cm² area and 15 cm depth. Six species of earthworm (Dendrobaena octaedra, Lumbricus castaneus, L.rubellus, L.terrestris, Aporrectodea rosea, A.caliginosa) were extracted from soil by hand sorting. Mean density of earthworms was 188 per m² with dominating of A.caliginosa (72 per m²). Activity of NH₄ and NO₃ and protein content was measured in a 5 g-probe from every sample after 7day-conditioning (each probe was moistening by 2.5 ml of distilled water and kept during conditioning at 22°C). Mean activity of NH₄ was 0.53 mg/g soil, NO₃ – 0.45 mg/g and protein content – 5.1 mg/g. Pearson correlation r=.17 was significant p=004 (Dutilleul (1993) modified) between protein content and total number of earthworms but was insignificant for any separate species number and any nitrogen compound. SADIE analysis (Perry & Dixon (2002) was used to determine spatial association (or dissociation) of earthworms and different nitrogen compounds. Positive SADIE X index points out an association between parameters under study, and negative one indicates dissociation between them. Strong and significant (p<0.025) association was shown for NH₄ activity and: total number of earthworms (X= 0.425, p<0.0001), A.caliginosa number (X= 0.26, p=0.013), juvenile Apprectodea number (X= 0.222, p=0.004), D.octaedra number (X= 0.373, p<0.0001). Strong assotiation was also observed for protein content and: total number of earthworms (X = 0.382, p < 0.0001), A.caliginosa number (X = 0.248, p = 0.002), juvenile Aporrectodea number (X= 0.23, p= 0.007), D.octaedra number (X= 0.293, p= 0.0003). Strong and significant (p>0.975) dissociation was revealed only for NH₄ activity and Lumbricus species number (X= -0.17, p= 0,98). There was no association (or dissociation) between NO₃ activity and earthworm number (total or separate species). Pearson correlation (r= 0.18) between NH₄ activity and protein content was significant p = 0.04 (Dutilleul (1993) modified) while SADIE association was more strong (X = 0.291, p = 0.0004). It is obvious that relations between both the compounds are strong because NH₄ activity is a marker of microbial processes and protein content is a marker of microbial biomass. Both the compounds exhibits very strong dissociation with pH value: NH_4 activity (X= -0.555, p >0,999) and protein content (X= -0.246, p=0.997). This study confirms hypothesis that earthworm distribution and activity is connected strongly with microbial activity in soil.

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EARTHWORM COMMUNITIES IN SYSTEMS OF REDUCED TILLAGE IN AN ORGANIC ROTATION

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Tillage reductions in organic farming are not as easy to work out as for conventional farming since weed control is very limited except for mechanical inputs. Chisel plough or no-till practises are clearly attributed to sustainable farming for the support of soil organisms and soil biological functioning. For good reasons, namely the exclusion of agrochemicals and mineral fertilizers, also the organic growers raise a claim on sustainable land use. In organic farming it is important to develop and establish gentile soil tillage practises to benefit from the support of soil organisms, especially from ecosystem engineers like earthworms.

A project to test systems of reduced tillage in organic farming was established at the Domain Frankenhausen (research farm of the Univerity of Kassel) in Germany in fall 2002. Three tillage systems were compared on a silty loess derived soil in a 6 crop rotation with 4 replicates in a randomized design. The tillage systems established were (1) ECOMAT,

a shallow (6 - 10 cm) inversive ploughing; (2) a ridge culture system and (3) conventional tillage down to 30 cm as a NIL control. In fall 2005 three years after establishment of the field plots earthworm communities were investigated using formalin extraction.

In total 6 species were detected covering the endogeic and anecic ecological groups. In general, the earthworm abundance was low covering a range from zero to 84 individuals m-2. It turned out that shallow ploughing using the ECOMAT technique didn't make a difference to conventional ploughing in earthworm populations while the ridge culture system was significantly reduced in earthworm numbers and biomass compared to conventional ploughing. This was true for both endogeic and anecic species. The highest abundances of anecic earthworms (dominated by L. terrestris) were recorded for the ECOMAT-treatment. Detailed results for abundance and biomass will be presented on the level of ecological groups.

We conclude that tillage reductions are not effective for the support of earthworm communities as long as ploughing is just reduced in depth and disturbance stays on as a permanent characteristic of the agro-ecosystem. Ridge culture systems, however, detract earthworms even more than conventional tillage. This is maybe due to an increased number of measures (eg. weed control) and/or due to less favourable habitat conditions in the ridges (microclimate like moisture and temperature amplitudes). More detailed investigations on the habitat quality of the ridge culture systems for key groups in soil fauna are needed to put value on these systems as favourable tillage in organic rotations.

THE IMPACT OF CULTIVATION TECHNIQUES ON EARTHWORM POPULATIONS IN NORTHERN EUROPE

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The economics of cereal production following the review of the Common Agricultural Policy has made farmers evaluate the methods used to establish a crop. Concerns about soil erosion, water-runoff and reduced biodiversity in farm systems have prompted research into the benefits of conservation tillage. Earthworm populations are regarded as important indicators of soil health and increase gradually under conservation tillage. They play a pivotal role in maintaining soil structure through the incorporation of soil organic matter, redistribution of soil nutrients and aiding water infiltration (Tebrügge and Düring, 1999).

Conservation tillage refers to non-inversion tillage which leaves 30% or more crop residues on the soil surface after planting (Köller, 2002). The number of operations is decreased or a tillage implement that requires less energy per unit area is used to replace an implement typically used in a conventional tillage system. Conventional tillage denotes mouldboard ploughing typically to a depth of 25 cm followed by secondary operations. The intensive manipulation and destruction of the soil structure coupled with a reduction of valuable food resources reduces earthworm numbers and biomass when compared to conservation tillage (Gerard and Hay, 1979).

Limited work has been carried out across northern Europe with regard to the impact of conservation tillage on earthworm populations. The results presented in this paper form part of a research project looking at the effect of cultivation techniques on earthworm populations. This research is part of a 3 year EU-Life demonstration project (2003-2006) looking at improved ways of managing the land, utilising conservation tillage techniques in order to minimise soil and water erosion. Experimental sites have been established in temperate northern European countries in the United Kingdom, Belgium and Hungary. The primary hypothesis was that conservation tillage, when compared to conventional tillage was beneficial to earthworm numbers and biomass. Earthworm populations were sampled using both mustard extraction and soil coring techniques in the autumn and spring. The trends from the demonstration sites show that despite temporal fluctuation in data the conservation tillage techniques were more beneficial to populations than mouldboard ploughing. The dominant species in soil subject to conventional tillage techniques were more abundant.

EARTHWORM AND ORGANIC INPUT MANAGEMENT IMPROVE THE QUALITY OF SOILS AND TEA PRODUCTION IN SOUTHERN CHINA

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In disturbed ecosystems, soil fertility decreases and increased chemical fertilizer inputs do not result in a proportional increase in crop yield. More nutrients are lost and soil degradation is accelerated.

Four treatments were tested in order to improve the quality of soils and tea produced in the area of Yingde in the Guangdong Province (China) using the Organic Biofertilisation (FBO®) technique (Senapati et al., 1999). This method is based on the combined effects of the introduction of earthworms and a specifically designed application of organic inputs that result in improved nutrient inputs and specific growth stimulation processes. These effects also have significant effects on soil quality, particularly its physical structure and the many properties associated to it.

We installed the systems in trenches 45x15x30cm, using inorganic (NPK) and organic inputs and several local earthworm species among which *Amynthas corticis* (Kinberg, 1867)was dominant. Soil and tea quality were evaluated before the onset of the experiment and respectively 6 and 12 months after. Increases in tea quality and organic matter content and physico-chemical properties of soil were observed.

The abundance and diversity of soil macro-invertebrate communities significantly increased. Their activity, measured by a visual evaluation of soil morphology done with a modified version of Topoliantz and Ponge (2000) technique, was also improved. This technique isolates and quantifies aggregates according to their origin and other soil components; a significant co-variation between soil aggregation patterns and soil macro-fauna communities was observed.

Mutivariate analyses (PCA and Co-Inercia) among macrofauna, soil morphology, organic matter, physical and chemical data have allowed testing the positive effect of the FBO technology on tea quality and soil properties whereas chemical fertilizer inputs were decreased or suppressed allowing a soft transition towards organic production systems.

MINERALOGICAL CHANGES IN THREE COMMON SOILS BY TWO ENDOGEIC SPECIES OF EARTHWORMS AND POSSIBLE SOURCE OF 'Fe' IN THEIR BLOOD

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Mineralogical studies were carried out for three most common soils of the region before and after passing through the gut of two species of earthworms, Metaphire posthuma and Eutyphoeus waltoni. The goal was to investigate the changes in the soil mineralogical spectrum due to earthworms. Samples were exposed to X-ray diffractometer (PW-1730) fitted with vertical goniometer and the diffraction peaks were recorded and interpreted. The study revealed that the Illite mineral, present in all three soils, was reduced in amount as a consequence of its passage through the gut of both the worms. Feldspar was also reduced when the soils having pH 5.9 and 8.4 was ingested by both the worms. However, its amount was increased when soil having pH 7.8 was takn up by both the species of worms. Muscovite mineral was decreased when the soil of pH 5.9 traversed through both the worms and also when other two sils passed through the gut of E.waltoni but its content was unchanged when the soil of pH 7.8 passed through M. posthuma but increased when the soil having pH 8.4 was ingested.

Mineral Quartz was passed as such without any change in its amount in the sils by both worms; while Hematite- an oxide of iron, whenever present in the parent soil, was found missing in the castings of both the worms. The results of the study indicate earthworms significant role in altering and translocating the mineralogical spectrum of the soils.

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Earthworms modify the soil environment by mixing organic residues into soil and create aggregates and macropores through their casting and burrowing activities. Generally, this stimulates microbial growth and thus increases decomposition rates and soil respiration. Some of the structures created by earthworms (e.g., casts, middens and burrows) may possess anaerobic microsites that favour the growth of denitrifying bacterial populations in the short term. Yet, it is not known whether the increase in aerobic and anaerobic microbial populations due to earthworm activities would induce CO_2 and N_2O losses from soil. The objectives of this study were to determine 1) whether earthworm-microbial interactions increase CO_2 and N_2O emissions from an agricultural soil and 2) whether CO_2 and N_2O emissions were influenced by the number of earthworms and the earthworm species present. The field site was located on the Macdonald Campus Research Farm, Ste. Annede-Bellevue, Quebec, Canada. Steel enclosures $(3m^2)$ were permanently installed to a depth of 40 cm in the soil and corn was planted in each to simulate a corn agroecosystem. Natural earthworm populations were reduced by repeated applications of a carbaryl insecticide before earthworm treatments were added. The seven treatments included: no earthworms (control), Lumbricus terrestris L. at two population levels (1x and 2x the ambient population level at the site), Aporrectodea caliginosa (Savigny) at the two population levels, and a mixed *L. terrestris-A. caliginosa* treatment at the two population levels. Each treatment was replicated 4 times for a total of 28 enclosures. Once a week, for 14 weeks between June and September 2005, gas samples were collected from closed chambers (polyvinyl chloride cylinder, 20 cm internal diameter by 13 cm height) during a 2-h period. Soil temperature, water-filled pore space, extractable and microbial biomass carbon and nitrogen pools were also measured on each sampling date. Seasonal variation in CO₂ and N₂O fluxes related to rainfall events and temperature fluctuations. Soil water-filled pore space, microbial biomass N, dissolved organic C and NO₃-N were positively correlated with the CO_2 flux from enclosures during the season, but only microbial biomass N was positively correlated with the N₂O flux. The cumulative CO_2 flux was greater (P<0.05, Tukey-Kramer test) from the L. terrestris 1x treatment and the A. caliginosa 2x treatment than the control, but earthworm treatments did not affect the cumulative N_2O flux. We found that A. caliginosa populations in enclosures at the end of the season were positively correlated with the cumulative CO_2 flux (r=0.366, Spearman coefficient) but not with the cumulative N₂O flux. The L. terrestris populations were not correlated with CO₂ or N₂O fluxes, possibly because many died during the summer, which was uncharacteristically hot (many days with >30°C temperatures). These results suggest that earthworms, especially endogeic species, stimulated CO₂ emissions from our experimental corn agroecosystem. Earthworms did not influence N2O emissions at the temporal and spatial scales sampled in this study.

VARIABILITY OF EARTHWORM COMMUNITIES IN RELATION WITH SOIL BULK ELECTRICAL CONDUCTIVITY IN A LOAMY SOIL UNDER DISTINCT CROPLAND MANAGEMENT

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The spatial variability of the earthworm communities in a conventionally plowed and a non-inversion tilled arable field located in the loam belt in central Belgium was assessed and compared with soil bulk electrical conductivity. In autumn 2003 earthworms were sampled in 100 locations within a sampling area of 105×75 m² in each field using a combined sampling technique (mustard extraction followed by hand sorting). 48 samples were taken on a regular grid (15×15 m) while the remaining 52 sample locations were randomly chosen in the sampling area. Soil bulk electrical conductivity (EC) was assessed in the same sampling area through magnetic induction measured by an EM38DD device (Geonics Ltd., Ontario) connected with a GPS with a density of 720 measures per hectare. In each field soil samples were taken (0-30, 31-60 and 61-90 cm) in five locations spanning the range of interpolated EC values. Texture, pH, moisture content and organic carbon were determined for each depth fraction.

Geostatistical analysis was used to detect spatial structures in the soil bulk EC and earthworm data (numbers, biomass). Maps of the spatial distributions of soil properties and earthworm populations were computed with the point kriging interpolation method.

In the plowed parcel 7 earthworm species were observed (Allolobophora chlorotica, Aporrectodea caliginosa, Ap. longa, Ap. rosea, Dendrodrilus rubidus, Lumbricus terrestris and Octolasion cyaneum). Densities reached 448 earthworms per m² and biomass was 93 g m⁻². Only 5 species were observed in the non-inversion tilled parcel: Ap. caliginosa, Ap. limicola, Ap. longa, Ap. rosea and L. terrestris and abundance was also lower than in the plowed parcel: 201 m⁻² and 70 g m⁻². Soil bulk EC was higher and more variable in the plowed parcel. The plowed parcel also had slightly higher pH values and contained more moisture at a depth of 60-90 cm but had a lower clay content than the non-inversion tilled parcel.Earthworm populations occurred in distinct species clusters between 20 and 40 m in diameter. Similarities between earthworm populations and soil EC were high in the plowed parcel but less pronounced in the non-inversion tilled parcel. In both parcels however, anecic species (Ap. longa and L. terrestris) occurred in zones with low clay content at a depth of 30-90 cm while the reverse was true for the endogeic species (Al. chlorotica, Ap. caliginosa, Ap. rosea, and Octolasion cyaneum). Despite the preference of both anecic species for low clay content at greater soil depth, they lived in separate clusters. Locations with high L. terrestris biomass and low numbers appeared next to clusters with low biomass and high numbers suggesting the occurrence of juvenile and adult L. terrestris in spatially separated patches. Ap. caliginosa and Ap. rosea showed similar spatial patterns but Ap. caliginosa clearly was more abundant. D. rubidus, an epigeic species, was present in a zone neighboring a pasture from where it probably migrated into the cropland.

This study demonstrated that earthworm populations are spatially structured in patches with varying diameters (20-40 m). These spatial patterns seem to be driven both by species interactions as well as soil conditions.

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EFFECTS OF ORGANIC MATTER CONTENT ON EARTHWORMS AND NITROGEN MINERALIZATION IN GRASSLAND SOILS

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Earthworms play an important role in the nitrogen cycle in the soil. Through their activities they affect the mineralization of organic matter directly and indirectly. In the Noordelijke Friese Wouden (NFW) in the Netherlands, due to decades of continuous differences in land use and management, soil phenoforms differing in quantity and quality of soil organic matter are present.

In a field study we wanted to determine consequences of different organic matter contents for earthworm population dynamics and nitrogen cycling. We expected a higher mineralisation rate and a larger number of earthworms at grasslands with a higher organic matter content.

To test this hypothesis we selected nine dairy farms located on sandy soils in the northern part of the Netherlands. At each farm two grasslands, which differed in organic matter content, were selected. At 4 farms the slurry manure, used to fertilized the grasslands, was enriched by the additive Euromestmix[®] (Mx).

Earthworm and soil samples were taken in April, June and September 2004. Triplicate pits of 20x20x20 cm (WxLxH) were sampled for earthworms in each grassland. Soil from each pit was collected on a plastic bag and examined for earthworms, and 500 mL of 0.2 % formaldehyde was poured into each pit to recover earthworms occurring below 20 cm. All earthworms found, were taken back to the laboratory, weighed (fresh weight) and identified to species (adults and juveniles). Numbers were expressed on a m² basis. The species were allocated into the categories anecic, epigeic or endogeic. Cocoons were washed from separate soil samples taken to a depth of 15 cm. Plant yield and root biomass were also determined. Soil samples for the determination of mineral nitrogen, potential mineralization and actual nitrogen mineralization were taken to a depth of 10 cm.

Results were analyzed within each farm using paired t-tests (high organic matter content versus low organic matter). For an analysis for all data combined we used an ANOVA with manure quality (Mx and controle) as a categorical variable and organic matter content as a continous variable.

At each farm the grassland with the highest organic matter content had the highest root biomass. These grasslands also contained the largest abundances of earthworms and had the highest numbers of cocoons. Due to differences in soil moisture during the season, abundances of earthworms and fractions of epigeic, endogeic and anecic earthworms changed during the growing season. With an increase in organic matter in the soil, the potential nitrogen mineralization increased.

In this presentation the organic matter content of the soil, manure quality, abundances of earthworms, the species distribution of earthworms, and the fractions of epigeic, endogeic and anecic earthworms will be related to the potential and actual mineralization in the different grasslands.

Session 7

Earthworm Ecotoxicology and Risk Assessment

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IN SITU COMPOSITIONAL 'FINGERPRINTS' OF EARTHWORM CHLORAGOCYTES: INTER-POPULATION DIFFERENCES IN FIELD WORMS WITH MULTI-GENERATIONAL METAL EXPOSURE HISTORIES.

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Determining the distribution, speciation, and cellular effects of metals on earthworms in the field is essential for gaining insights into the bio-reactive fraction of accumulated body burdens and toxicosis. Recently, X-ray absorption spectroscopy (XAS) [1], and subcellular fractionation [2] were used to determine the coordination chemistries and partitioning, respectively, of metals in whole-earthworm preparations. Moreover, an NMR-platform assay has been used to evaluate metabolite profiles in indigenous and microcosm-restrained introduced earthworms under potentially stressful field conditions [3]. Assessing in situ metal speciation and multivariate biochemical effects at spatial resolutions approximating the dimensions of single cells represent formidable technical challenges. This paper describes the novel applications of two high-energy, synchrotron-based, physical techniques (micro-EXAFS [4] and Fourier Transform Infrared Microspectroscopy, FTIR [5]) to detect, map, and characterise the metal environment and global biochemical consequences of metal exposures in the chloragogenous tissues of earthworms (Lumbricus rubellus) native to one uncontaminated reference site and to three sites whose mineassociated soils are contaminated to different degrees with Pb and Zn. Both microfocus techniques were applied to thick (nominally 50 µm), unfixed and unstained, cryostat sections of earthworms that had been quench frozen in the field to minimize the effects of non-environmental stressors. We conclude that: (i) the speciation of Pb and Zn in gut contents and in chloragocytes are different; for example, Pb is probably surrounded by a shell of S scatterers in the gut lumen but is surrounded by shells of O and S in the cell; (ii) the FTIR spectra displayed qualitative tissue-specific characterisitics; (iii) FTIR revealed site-specific, inter-population, phenotypic differences (particularly in the wavenumber range 1800-800 cm⁻¹) in the biochemical profile of chloragocytes. This is the first time to our knowledge that earthworms have been subjected to these forms of analyses, where the compositional and structural fidelities of an efficient metal-sequestering tissue have been preserved in approximately in vivo states. The implications in terms of metal toxicoavailability and cyto-toxicity will be discussed.

Oral CLONING AND REAL-TIME PCR TESTING OF 14 POTENTIAL BIOMARKERS IN EISENIA FETIDA FOLLOWING CADMIUM EXPOSURE

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Metal pollution causes disturbances at various levels of biological organization in most species. Important physiological functions could be affected in the exposed individuals and among the main physiological functions, immunity may provide one (or more) effector(s) whose expression can be directly affected by a metal exposure in various macroinvertebrates. As many proteinic effectors showed a high degree of homology between species, determination of primers allowing the cloning by RT-PCR of selected effectors in the laboratory model, *Eisenia fetida* was done. After cloning, the level of expression of each gene was analysed following exposures (80 and 800 mg.Kg⁻¹) to cadmium using real time PCR. The implemented approach made it possible to test quickly potential biomarkers in *Eisenia fetida*. Selected effectors were calmodulin, heat shock proteins, superoxide dismutase, catalase, metallothionein, β -adrenergic receptor kinase, pyruvate carboxylase, trancriptionally controlled tumor protein, protein kinase C and ubiquitin.

HAS METAL CONTAMINATION AFFECTED CLONAL DIVERSITY OF THE EARTHWORM DENDROBAENA OCTAEDRA?

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Metal concentrations and genetic (i.e. clonal) diversity of the parthenogenetic epigeic earthworm species *Dendrobaena octaedra* were studied in three metal contaminated areas in Finland. Main harmful contaminants were Cu and Zn although several other metals were also increased close to the contamination sources which were the Cu-Ni smelter (Harjavalta), the steel mill (Imatra) and the Zn production plant (Kokkola). In Harjavalta, two contaminated sites (1 and 2 km from the smelter) and an uncontaminated site (8 km) were sampled. In Imatra and Kokkola, one contaminated (1 and 2 km, respectively) and one uncontaminated site (4 km in both areas) were sampled. More than 40 specimens of *D. octaedra* were sampled from each site. Total (rigorous ultrasound-assisted aqua regia extraction) and estimated bioavailable (acetic acid extraction) soil metal concentrations were analytically determined for the soil sampled from each site. Metal concentrations of each earthworm specimen were analysed (aqua regia extraction). All metal measurements were performed with ICP-OES. Genetic variation in *D. octaedra* populations was evaluated by analysing variation in allozymes using cellulose acetate electrophoresis. Basing on preliminary surveys, eleven different enzymes were assayed in the study.

Metal concentrations (both the total and estimated bioavailable values) were elevated in the soils in the vicinity of the contamination sources. Further, concentrations of Zn and Cu in *D. octaedra* were significantly higher at the sites close to the contamination sources compared to the uncontaminated sites indicating clearly increased exposure of earthworms to metals. Zn became accumulated in the earthworms more than Cu.

In Harjavalta, clonal diversity was lowest at the 2 km site, and was similar at the 1 and 8 km sites. The proportion of unique genotypes was lowest at the 2 km site, and unexpectedly highest at the 1 km site. Concomitantly clonal dominance was highest at the 2 km site. In Imatra, genetic variation was similar at the sample sites and no clear reduction of clonal diversity was observed at the 1 km sample site compared to the 4 km site. In Kokkola, clonal diversity value and proportion of unique genotypes were higher at the 4 km site although the difference to the 2 km site was small.

Our results showed that high metal exposure levels are needed to significantly affect genetic (clonal) diversity and variation in parthenogenetic *D. octaedra*. It was shown in a separate experiment that *D. octaedra* avoid Cu and Zn contaminated soil more sensitively compared to few other earthworm (both epigeic and endogeic) species (Lukkari and Haimi, 2005). As the contamination is very heterogeneous in the field we suggest that *D. octaedra* can reduce its exposure by avoiding the most contaminated soil patches and food resources. However, other factors seem also to counteract harmful effects of metals because body burdens of the earthworms living close to the contamination sources were clearly elevated.

Oral 198 ECOTOXICOLOGICAL RESPONSES OF EARTHWORMS TO ULTRAMAFIC SOILS

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Besides a number of other unfavourable living conditions such as drought and poor organic content, ultramafic soils are characterised by extremely high concentrations of a range of heavy metals of natural origin known to be toxic to organisms living in and on these soils. The aims of the study were to investigate the availability and effects of these heavy metals on earthworms and the ability of earthworms to survive under these conditions. Soils from the Barberton area, Mpumalanga, South Africa were collected and different fractions representing different levels of (bio-)availability were analyzed for arsenic, chromium, cobalt, copper, manganese and nickel. Two ecophysiologically different species, Eisenia fetida and Aporrectodea caliginosa were long term exposed to these ultramafic soils and to a pristine control soil and responses to stress were measured on different levels of earthworm organization. The endpoints on the subcellular level were DNA integrity as measured by the Comet Assay, cell membrane integrity as measured by the neutral red retention assay, and cellular proliferation as measured by the MTT colouring assay. As endpoints of sublethal effects, cocoon production, fecundity and viability, affecting population development and growth were investigated. On the individual level, heavy metal body burden and tissue distribution were evaluated. In relation to non ultramafic or anthropogenically contaminated soils, the proportion of heavy metals in the DTPA and CaCl₂-extractable soil fraction, representing mobile and mobilisable amounts of heavy metals was far lower. Significant responses were found in all earthworm bioassays, but the sensitivity of the different bioassays was not predictable by the level of organization. No resistance of earthworms towards the metals was observed, but both earthworm species showed different strategies for metal sequestration. The toxicity of ultramafic soils is not necessarily related to the total or bioavailable amounts of the selected heavy metals. It can be speculated that ultramafic soils either contain unidentified toxicants or that interactions between toxicants play a role or that earthworms are able to remobilize a certain amount of heavy metals. It was shown that earthworms are useful tools for the assessment of the toxic stresses emerging from these ultramafic soils, but that the application of a single endpoint does not give reliable results when studying effects of soils with such complex composition.

EFFECT OF TIME AND MODE OF DEPURATION ON TISSUE COPPER CONCENTRATIONS OF THE EARTHWORMS EISENIA ANDREI, LUMBRICUS RUBELLUS AND LUMBRICUS TERRESTRIS

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Eisenia andrei, Lumbricus rubellus and *Lumbricus terrestris* were exposed to 250, 250 and 350 mg kg⁻¹ Cu in Cu(NO₃)_{2(aq)} amended soil for 28 days. Earthworms were then depurated for 24 to 72 hours, digested and analysed for Cu and Ti, or, subsequent to depuration were dissected to remove any remaining soil particles from the alimentary canal then digested and analysed. This latter treatment proved impossible for *E. andrei* due to its small size. Regardless of depuration time, soil particles were retained in the alimentary canal of *L. rubellus* and *L. terrestris*. Tissue concentration determinations indicate that *E. andrei* should be depurated for 24 h, *L. rubellus* for 48 h and *L. terrestris* should be dissected. Ti was bioaccumulated and therefore could not be used as an inert tracer to determine mass of retained soil. Calculations indicate that after 28 days earthworms were still absorbing Cu from soil.

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EFFECTS OF CHROMIUM (III) AND CHROMIUM (VI) ON THE GROWTH AND REPRODUCTION OF THE EARTHWORM *EISENIA FETIDA*.

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The effects of Cr(III) and Cr(VI) on the growth, maturation, number of cocoons produced, hatching success of the cocoons and number of hatchlings produced per worm in 10 different soils and organic substrate (cow dung) have been assessed and the EC₅₀ and NOEC values were determined in soils. The statistical analysis using two way ANOVA showed that there were significant differences in the growth and maturation rate of worms. The results of factorial design experiment showed that not only in a soil but also among soils there were significant differences in the growth rate but not in the maturation rate of worms. The difference between EC50 and NOEC values of Cr(III) and Cr(VI) on the growth of the earthworm was more than five times. The NOEC values for the effect of both species of Cr in soils on reproduction were with exemptions half or less than that recorded on growth. The NOEC values for the effect of Cr in soils on the number of hatchlings produced were equal or less than those values recorded for cocoon production. The NOEC values of Cr(III) and Cr(VI) on growth and reproduction show that growth is less sensitive than reproduction. The body concentration of Cr in worms generally showed an increasing trend. Among soils the body concentration of Cr varied. Though the difference between the concentration ranges of Cr(III) and Cr(VI) to which the earthworm exposed was five times, when the mean body concentration of earthworm in a soil was compared, the difference was around 1 mg/kg only. The stepwise multiple regression analysis predicted strong dependence of bioavailable concentration of Cr on Cr(III) and Cr(VI) added and is only slightly modulated by the soil characteristics such as pH, EC, CEC, OC and clay.

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Oral

TOXICITY INFLUENCE OF OLAQUINDOX ON EARTHWORM

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Recently, veterinary medicine and bioactive metabolites are released continually to soil environment by excretion and promote adverse effects on non-target organisms utilizing the excrement. Olaquindox is the growth promoter and antibacterium medicine of animal and utilize widely in livestock production. Olaquindox is assimilated rapidly and excreted after administration, about 85% of olaquindox with urine and 15% of parent medicine with feces are directly or indirectly discharged in the soil in most cases, raising the concern over possible adverse effects on soil dwelling organisms. In the present study, standard earthworm acute toxicity concluding filter paper test and solution test were used to assess the toxicity of olaquindox, and earthworm growth rate was tested weekly with sublethal exposure, catalase (CAT) activity of whole earthworm and its various segments were measured to determine further the toxicity of olaquindox. In the growth rate and CAT activity measure test, the olaquindox concentrations in substrate were control (0 mg. kg^{-1} dry weight) and four exposures (500, 1000, 3000 and 5000 mg. kg⁻¹ dry weight). The results showed: the 48-h median lethal concentration (LC50) was 1.02 mg.cm⁻² on filter paper and above 4000 mg.L⁻¹ in solution, and morphological changes of earthworm, especially the anterior segment, showed a progression of damage with increasing olaquindox concentrations, which was conformed with the result in solution test. In addition, earthworms growth rate revealed no significant effect (p>0.05) with 7d or 14d expose of lower concentrations (500 mg.kg⁻¹, 1000 mg. kg⁻¹), while reduced significantly (p<0.01) with 7d or 14d exposure to higher concentrations 3000 mg. kg⁻¹ and 5000 mg. kg⁻¹ compared to the control. CAT activity in whole earthworm and the anterior and midpart was inhibited significantly at two higher concentrations after 14d exposure. However, only the anterior exhibited the significant (p<0.05) reduce of CAT activity at two lower concentrations compared to the control, which suggested CAT activity of the anterior was more sensitive to Olaquindox than other segments.

VALIDATION OF CHOLINESTERASE INHIBITION AS A BIOMARKER OF EXPOSURE FOR EARTHWORMS IN APPLE ORCHARDS

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Earthworm communities were estimated using mustard in 17 apple orchards at the regional scale in Provence: 5 orchards were in conventional farming, 5 in IPM (Integrated Pest Management), 5 in organic farming and 3 abandoned (since at least 7 years) orchards as control. Conventional and IPM orchards use many organophosphate and carbamate pesticides. Allolobophora chlorotica and Lumbricus terrestris were very common in these orchards and were chosen for cholinesterase (ChE) characterisation. L. terrestris exhibits a two to three-fold higher specific ChE activity than A. chlorotica. Based on substrate and inhibitors specificities it is likely that in L. terrestris, ChE activity is a true acetylcholinesterase (AchE). Characterization of cholinesterase from A. chlorotica is uncertain and cannot be classified as true AchE as an important part of the cholinesterase activity seems to be related to butyrylcholinesterase. To follow ChE activity in natural conditions, ten earthworms of each species were collected in each earthworm in April for L. terrestris and in April, May, August and November for A. chlorotica. ChE inhibition was observed in A. chlorotica at each date in most of the orchards under IPM or conventional protection strategy and never in organic or abandoned orchards. Surprisingly, inhibition was still observed in November, i.e. 2 months after the last pesticide application. For L. terrestris, ChE inhibition was only observed in 2 orchards under conventional protection strategy. The difference between the two earthworm species could be due to (i) their different forms of ChE or (ii) to difference in exposure related to difference of behaviour. To gain insight into the duration of ChE inhibition, a laboratory experiment was set up using parathion as a model organophosphate pesticide. We observed that ChE inhibition could last more than two months under laboratory conditions for A. chlorotica. This is in agreement with our field observations.

EFFECTS OF DIFFERENT FUNGICIDE APPLICATION RATES OVER FOUR YEARS UPON EARTHWORM CAST PRODUCTION IN CACAO SYSTEMS IN SOUTHERN CAMEROON

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Copper-oxide based fungicides to control cacao blackpod disease are the main external input used in cacao systems in Cameroon and elsewhere. The cacao tree trunks, pods and leaves are sprayed manually during the rainy seasons using a knapsack sprayer. However, losses of fungicide to the soil surface are inevitable, from dripping during the spraying, and during subsequent rainfall. Earthworms are known to be sensitive to copper. For example, studies in vineyards in Europe have shown that long-term use of copper fungicides has increased soil copper concentrations and where concentrations exceed 80 mg kg⁻¹ earthworms are eradicated. However, there are very few in-situ studies on toxicology from the tropics.

An experiment was established in smallholder farmers' 35 y old cacao farms in southern Cameroon to assess the effects of different fungicide application rates (none, reduced, and manufacturers recommended rate) upon earthworm cast production over four years and this was also compared with cast production in a forest fallow of the same age. The experimental design was a replicated randomised complete block design with imposed spray treatments and three replications.

While initially there were no significant differences in cast production between cacao spray treatments, by the third year, there were significant differences in cast production between treatments with highest production in the zero spray treatment (21.1 Mg ha⁻¹) compared with 14.4 Mg ha⁻¹ in the reduced spray treatment and 10.2 Mg ha⁻¹ in the recommended rate. This trend continued in the fourth year with 19.2 in the zero spray, 12.1 in the reduced and 4.5 Mg Mg ha⁻¹ in the recommended spray regime.

Casts and soil were analysed for texture and chemical parameters. Casts derived from the high fungicide application plots had a higher percentage clay than those derived from the low fungicide application and zero-spray treatments. This suggests that the earthworms may be feeding more in the deeper soil layers where the clay content was higher to avoid the soil with higher Cu concentrations near the surface. However that low term spraying at high application rates does, in the long run have a negative impact upon earthworm activity.

THE USE OF EARTHWORMS AS A TEST ORGANISM IN ECOTOXICOLOGY: A CRITICAL REVIEW

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Earthworms have played a major role in acute (lethal) toxicity testing for over 20 years. Recently there has been a shift from lethal to non-lethal testing, with more sensitive and ecologically relevant tests designed to monitor life cycle traits (e.g. growth rate and fecundity). Due mainly to its short life cycle and ease of culture, *Eisenia fetida* is the most widely used test species, but use of this epigeic earthworm, especially in chronic tests, has been widely questioned. *E. fetida* is more tolerant than most earthworms to soil contaminants, does not inhabit mineral soil and is uncommon in most natural environments. Continued reliance on this species is often attributed to time constraints and perceived difficulties in culture and maintenance of more appropriate soil dwelling earthworms.

A critical review of earthworm use in non-lethal ecotoxicological studies is presented. In laboratory studies, species selection has often been based on commercial availability and where non-commercial species were used there was a heavy reliance on field-collected earthworms. The validity of results employing earthworms of unknown origin or previous exposures must be questioned. Many non-lethal studies have relied heavily on methodologies adopted from acute testing where earthworm maintenance was not a priority. In reporting experimental design, abiotic and biotic conditions were largely overlooked. A number of studies have been published without reference to critical factors such as earthworm density, feed, temperature or soil moisture levels. Earthworm senescence has been identified as a source of error especially in reproduction tests where adult number is directly related to cocoon production. This has been partially addressed by culturing individuals separately (for growth studies) or in pairs for reproduction studies. It is recommended that a further improvement could be made by the use of parthenogentic species (e.g. Octolasion cyaneum) allowing reproductive output to be assessed on an individual basis. The use of such species would also permit tests to be undertaken on genetically identical cohorts

It is widely recognised that there are problems with extrapolation from laboratory to field scale. However, field tests remain uncommon due to difficulties of experimental design and interpretation of results. Using laboratory-reared earthworm cohorts and new mark-release-recapture techniques may allow for development of a standard comparative procedure that can be employed across different sites. It is suggested that the wider use of established earthworm culture techniques allied with reference to ecological requirements and behaviour of test species will increase the consistency and validity of sub-lethal toxicity tests.

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RECOVERY OF CHOLINESTERASE ACTIVITY IN THE EARTHWORM EISENIA FETIDA SAVIGNY FOLLOWING A SHORT-TIME EXPOSURE TO CHLORPYRIFOS

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Organophosphorus (OP) insecticides cause irreversible inhibition of cholinesterase (ChE) activity. ChE are essential enzymes in the nervous system of almost all animals. Reestablishment of active enzymes is slow and depends on elimination of the insecticide from the body, followed by two lengthy processes: reactivation and/or biosynthesis of new enzyme molecules. The results presented here demonstrate that recovery of the activity of one of the cholinesterases (E2) in the earthworm *Eisenia fetida* (Savigny) is virtually absent after acute exposure to the OP insecticide chlorpyriphos, while another cholinesterase (E1) slowly re-establishes its activity. Chemical analysis showed that the elimination of chlorpyriphos-oxon) was not detected in any of the samples.

The activity of the two cholinesterases was discriminated by selective inhibition with carbaryl (5 μ M in one minute), which inhibits E1 completely, without influencing the activity of E2. Earthworms were exposed to chlorpyrifos (240 mg kg⁻¹) in soil for 48 hours and were subsequently transferred to clean soil in three independent replicates. Controls were kept under similar conditions, and were not subject to chlorpyrifos treatment. After 7-14 days of recovery, the appearance and behavior of the exposed worms were indistinguishable from that of the control worms. The chlorpyrifos content of the worms was determined by gas-liquid chromatography. It disappeared almost completely in a firstorder elimination rate during seven days with a half-life of approximately 24 hours. The activities of E1 and E2 were measured spectrophotometrically in homogenates from whole specimens at regular intervals for 12 weeks by using acetylthiocholine as substrate. Immediately after exposure, the E1 and E2 activity were 0.479 ± 0.074 nkat/mg protein (mean \pm SEM, n=3) and 0.45 \pm 0.06 nkat/mg respectively, compared to control values of 1.57 ± 0.178 nkat/mg and 0.951 ± 0.07 nkat/mg, respectively. After three weeks E1 had regained activity comparable with the activity in controls, whereas E2 stayed low throughout the 12 week monitoring period. It was therefore concluded that there is no reactivation or de novo biosynthesis of E2 whereas E1 is slowly reactivated. The nonrecovery of E2 suggests that historically exposed earthworms have low levels of E2 even when OP insecticides can no longer be traced in their body. Provided a reproducible baseline for E2 control activity in is established, the E2 level could be considered as a tool to detect previous episodes of exposure to OP pesticides or warfare agents in earthworms.

INTEGRATING SYNCHROTRON INFRARED MICROSCOPY (SIM) METABOLOMICS WITH METAL SPECIATION IN EARTHWORMS

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Metal bioavailability has traditionally been estimated indirectly through chemical extractions of soil. However, a true assessment of risk requires an accurate measure of organism exposure and an alternative means of expressing toxicity is provided through organism-based estimates of metal absorption and intracellular accumulation. Tissue fractionation methods can be used to demonstrate the subcellular partitioning of metals into operationally defined active and detoxified fractions and provide information regarding metal bioavailability and toxicity (Vijver et al, 2004). The use of organisms in risk assessment bioassays does however raise the question of whether they can evolve genetically based resistance through processes of natural selection as this would lead to confounding estimations of the contaminant in question and render the species unsuitable for monitoring. In this study the subcellular partitioning of Pb, Zn and P was investigated in Lumbricus rubellus (Annelida: Oligochaeta) resident in metalliferous soils associated with two disused Pb and Zn mine-sites. The experiment design involved: (a) maintaining mine-site earthworms (fully clitellate adults) on their native soils and, (b) maintaining earthworms collected from an uncontaminated reference site on both metalliferous soils in the laboratory for two weeks. Earthworms from both of the 'treatment groups' were sampled at 0, 1, 3, 14, 28 and 70 day intervals and fractionated into a C (cytosol), D (metalrich granules) and E (tissue fragments) fraction (Wallace et al, 1998). The morphological distinctions of the sub-cellular fractions were confirmed by transmission electron microscopy (TEM). Furthermore, the effects of sequestered metals on the biochemical 'fingerprint' of the major site of metal accumulation, the chloragocytes, was assessed by synchrotron-based Fourier Transform Infrared microspectroscopy (FTIR).

THE EFFECT OF EDTA AND THE DPASV LABILE COPPER CONCENTRATION ON THE TOXICITY OF COPPER TO *EISENIA ANDREI* BOUCHÉ

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The most significant route of uptake of metals by earthworms is that of passive adsorption of the metal dissolved in the soil interstitial water through the dermal membrane, or via an uptake route that is related to the soil pore water metal concentration. The concentration of the free cupric ions (the most available form of copper) and the easily dissociable and exchangeable inorganic complexes of copper present in the soil solution, are considered to govern uptake. However, causal relationships between metal speciation and terrestrial biological toxicity / bioaccumulation are limited due to inherent difficulties in measuring chemical speciation in the aqueous and solid phases of soils.

This series of experiments aimed to determine the relationship between the speciation of copper in solution and the mortality and tissue copper concentrations of *E. andrei*. A novel technique was used which exposed the earthworms to reconstituted ground water solutions treated with both copper nitrate and the chelating agent ethylenediaminetetraacetic acid (EDTA). Differential Pulse Anodic Stripping Voltammetry (DPASV) was used to determine the labile copper concentration. In the first experiment mature E. andrei were exposed for 14 d to reconstituted ground water treated with concentrations of Cu(NO₃)₂ such that the total dissolved copper concentrations were 0.006 - 0.125 mg Cu l⁻¹. The 14 d LC50 was 0.046 mg Cu l^{-1} (95 % CI were 0.032 – 0.066 mg Cu l^{-1}). There was a significant positive correlation between earthworm mortality and the bulk copper concentration in solution (Pearson Product Moment Correlation Coefficient (PPMC), R = 0.95, P < 0.001), and a significant positive correlation between earthworm tissue copper concentrations and the copper concentration in solution (PPMC, R = 0.98, P < 0.001). In a second experiment three concentrations of EDTA (0.15 mg l^{-1} , 0.35 mg l^{-1} , 50 mg l^{-1}) were added to solutions of reconstituted ground water containing 0.099 mg Cu 1⁻¹, 0.049 mg Cu 1⁻¹ and 0.125 mg Cu 1^{-1} , and *E. andrei* were exposed to the solutions for 14 d. The most marked difference in both the mortality and tissue copper concentration occurring as a result of the addition of EDTA solution was evident in solutions containing 0.125 mg Cu l⁻¹. There was 100 % mortality of *E. andrei* exposed to solutions treated with 0.125 mg Cu 1^{-1} and 0 mg EDTA 1^{-1} , a figure which was reduced to 60 %, 50 % and 25 % in solutions containing 0.15, 0.35 and 50 mg EDTA 1^{-1} respectively. There was a corresponding decrease in the DPASV labile copper concentration from 1.06 – 0.001 μ g l⁻¹ following the addition of 0.15 mg EDTA l⁻¹ and 50 mg EDTA l⁻¹ respectively. The decrease in mortality and earthworm tissue copper concentrations following the addition of the EDTA is therefore attributed to the complexation of the free copper ions with EDTA molecules, and the non bioavailable nature of the resultant Cu-EDTA complex.

It is anticipated that quantifying the effect of copper speciation in solution would greatly aid the understanding of the importance of chemical composition and speciation of metals, the interpretation of comparable experiments using soils, and the development of a biotic ligand model predicting the toxicity of metals to earthworms.

IMPACT OF EARTHWORM DENSITY ON METAL MOBILITY AND AVAILABILITY

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It has been suggested that earthworms may be of use in enhancing phytoremediation or alternatively that earthworms may enhance metal mobility at contaminated sites thereby increasing risk of exposure for receptors. In this study earthworms were cultivated for 28 days in two different metal contaminated soils at differing earthworm number to soil mass ratios. Swaledale soil contained 16 ppm Cd, 28 ppm Cu, 5164 ppm Pb, 3433 ppm Zn, 6 % organic matter, had a pH of 6 and a sandy texture. Wisley soil contained 5 ppm Cd, 110 ppm Cu, 910 ppm Pb, 260 ppm Zn,10 % organic matter, had a pH of 5.4 and was a loamy sand. Earthworm number to soil mass ratios ranged from 1 :200 to 1 :5. After 28 days earthworms were depurated on filter paper and metal body burden was determined. Soil solution was extracted from soils using rhizon soil solution samplers and analysed for metals and dissolved organic carbon. Cold and hot water extractable carbon were measured on the soils. Soils were extracted with K_2SO_4 to estimate microbial numbers. The European 3-stage sequential extraction was applied to the soils.

Earthworm metal body burden and BCR Step 1 extractable metal decreased with increasing earthworm density. Metal and organic carbon soil solution concentrations and cold and hot water extractable soil C increased with increasing earthworm density. There were no significant changes in microbial activity with earthworm density.

The data are consistent with earthworm-derived organic compounds complexing metals and bringing them into solution off soil particle sorption sites. Thus metal concentration in soil solution and therefore metal mobility increases with earthworm density. However, the metals present as organo-metal complexes are not available for uptake by the earthworms so that metal body burden is unaffected or decreases, i.e. metal bioavailability decreases.

EFFECTS OF THE ALTHEMINTIC ABAMECTIN ON THE THREE SOIL INVERTEBRATE SPECIES

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Abamectin, an antiparasitic agent with high efficiency to prevent parasites, is widely used in livestock production. It can be excreted in the faeces of treated animal and enter the environment by amending the soils with manure. Possible sub-lethal effects of the drug on soil fauna are very important to environmental risk assessment of veterinary medicines. Very little information on effects of Abamectin on soil fauna was known. The effects of Abamectin on the survival, growth and reproduction of three species soil invertebrate F.femintaris, Enchythraeis and earthworm, have been studied in the laboratory to evaluate the sublethal toxicity of Abamectin on different soil invertebrate animals and predict the possible environmental risk of veterinary medicines. The results showed that Abamectin was toxic to adult survival of springtails at the concentration above 0.5 mg/kg. Estimated EC₁₀ and EC₅₀ values was found in the range of 0.48 mg/kg and 0.81mg/kg respectively. Reproduction was a more sensitive parameter than survival. The number of juveniles was reduced significantly at the soil concentration above 0.25 mg/kg. They showed a good correlation between survival and reproduction of springtail. The adult survival of Enchytraeids was a less sensitive endpoint to Abamectin. There was no toxicity to survival of Enchytreides , whereas the number of juveniles was reduced at the concentration above 25 mg/kg. Abamectin showed significant toxicity on the growth of earthworm at concentrations above 2.5 mg/kg. The mean biomass in the group exposed to above 2.5 mg/kg Abamectin was reduced. The mean number of cocoons produced at the concentration 1 mg/kg was reduced by 48 % and there was no cocoons produced at the highest concentration of 5 mg/kg. There was significant difference of the number of hatchling between the control (19.5±5.2) and the exposure at the concentration over 1 mg/kg (5.75±3.5). Among the three test animals, springtail was the most sensitive species to the exposure of Abamectin. Reproduction was the most sensitive endpoint to all tested animals following the exposure of Abamectin. It is concluded that there was direct toxic effects of Abamectin to non-target soil invertebrate such as springtail, enchytraeids and earthworms. Further field study on sublethal toxicity of Abmectin should be conducted.

EARTHWORM EXPOSURE TO THE FUNGICIDE CARBENDAZIM -INVESTIGATING THE EFFECT OF SOIL PROPERTIES ON CARBENDAZIM BIOAVAILABILITY AND TOXICITY TO *EISENIA FETIDA*

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Current ecotoxicological guidelines for the determination of the effects of chemicals on earthworms under field conditions recommend the use of a reference substance in order to validate that the field study is capable of detecting an effect on earthworm populations. The reference substance must cause a >40% and <80% reduction in earthworm populations in order for the study to meet the validity criteria. Carbendazim is currently recommended as a reference substance following the withdrawal of benomyl. However, current studies with carbendazim have shown a high variability in response (10-80% population reduction) which often invalidates studies. Additionally the level of variability in response observed amongst studies using carbendazim is greater than that observed amongst previous trials using benomyl.

The wide variation in earthworm response to carbendazim may result from a range of different factors such as earthworm behaviour, population structure and soil chemical and physical properties. In order to gain a better understanding of the reasons for the varied response of earthworms to carbendazim, these different factors are being investigated as part of a larger study.

Organic matter, pH and clay content are key components of the soil which influence fate and transport of chemicals. Therefore this poster focuses on how these soil properties influence carbendazim bioavailability and its subsequent toxic effects on earthworm populations.

The preliminary experiments reported here used the earthworm *E. fetida* because of the large number of existing toxicity studies carried out on this species. Initially an OECD acute toxicity test was performed using a soil similar to the standard OECD soil (with pH = 6.6 organic matter content = 7% and a clay content = 12%) in order to determine an LC50 concentration for carbendazim. Subsequent experiments used this concentration of carbendazim in the same soil but with soil pH, organic matter content and clay content systematically varied.

Soil pH was varied between 5 and 8 using calcium carbonate (to increase pH) and HNO_3 (to decrease pH). Organic matter content was varied between 1 and 10 % through the addition of sphagnum moss peat. Clay content was varied between 10 and 30% by addition of kaolinite clay. Toxicity of carbendazim was assessed after 7 and 14 days. Bioavailability of the carbendazim was assessed by analysis of the body tissues of the surviving earthworms. Statistical analysis was carried out to determine the key soil parameters controlling toxicity and bioavailability of the carbendazim.

The 8^{th} International Symposium on Earthworm Ecology $4^{th} - 9^{th}$ September Kraków, Poland

THE COMET ASSAY AS A BIOMARKER TO DETERMINE EARTHWORM SPECIES SENSITIVITY DIFFERENCES TO CADMIUM GENOTOXICITY

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The concept of species sensitivity differences is becoming important in ecotoxicology, but testing usually focuses on lethality of toxicants. The effects on the suborganismal level are mostly ignored; therefore the present study compared species sensitivities using a biomarker of genotoxicity. Five earthworm species (*Amynthas diffringens, Aporrectodea caliginosa, Dendrodrilus rubidus, Eisenia fetida* and *Microchaetus benhami*) were exposed for 48h to cadmium sulphate in artificial soil water, and DNA integrity was assessed with the comet assay. The comet assay proved to be useful and cadmium induced significant amounts of DNA damage in three (*A. caliginosa, D. rubidus* and *E. fetida*) of the five species, with *E. fetida* the most sensitive and therefore an appropriate representative earthworm species. All exposed earthworms accumulated Cd, but these body loads did not correspond to the amount of DNA damage; most of the Cd was probably sequestrated and did not cause DNA damage.

TOXICITY OF ALBENDAZOLE ON ADENOSINE TRIPHOSPHATASE AND ACID PHOSPHATASE ACTIVITIES OF EARTHWORM

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In recent decades, because of incorrect or excessive utilization in domestic animals, a substantial quantity of veterinary medicine and its bioactive metabolites have been released continually in the soil environment by excretion, and promoted adverse effects on soil ecosystem. Albendazole (ABZ) exhibits broad spectra of gastrointestinal anthelminthic activity and have been used widely in livestock. Several studies suggested that carcinogenic potential and embryotoxicity in vertebrate were induced during long-term use of ABZ. There is, however, no clarity of the effects that the use of ABZ might have on soil organisms. The aim of the present study was to determine the toxicity of ABZ on whole body and different segments (the anterior, the mid-part and posterior) of earthworm, Eisenia foetida, during 2, 7 and 14 days of expose to ABZ by measuring adenosine triphosphatase (Na⁺, K⁺-ATPase and Mg²⁺ -ATPase) activities and acid phosphatase (AP) activities. The ABZ concentrations in substrate were control (0 mg. kg⁻¹ dry weight) and four exposures (100, 200, 400 and 600 mg. kg⁻¹ dry weight). The results indicated that both ATPase and AP activities varied in relation to the exposure time and ABZ level as well as the segments of earthworm. ABZ inhibited significantly ATPase activities in the mid-part at all exposure duration, especially after 7 days and 14 days of exposure. Even with the lowest concentration (100 mg.kg⁻¹), both ATPase activities were inhibited significantly at 14 days exposure compared to control. The ATPase activities, however, enhanced significantly in the anterior with highest concentration at 14 days, which might be caused by the special organs in the anterior (calciferous glands, gizzard, esophagus, stomach, or reproductive apparatus). In posterior segment, only after 14 days, Na⁺-, K⁺- and Mg²⁺-ATPase activities were reduced significantly as ABZ concentration increased. On the other hand, significant decrease of AP activity was expressed in the anterior and mid-part of earthworm with exposure to 14 days compared to whole body and the posterior, even on the lowest concentration AP activity was significant decreased. Our results suggested that obvious response of ABZ on the AP activity was mainly caused in the anterior and mid-part of earthworms after exposure to 14 days, while ATPase activity varied significantly in the mid-part and posterior of earthworms with 14 days exposure. Being sensitive to low concentrations, these enzymatic activities could serve as early warning signals of soil contamination.

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EFFECT OF FLUIDIZED BED COMBUSTION ASHES USED IN METAL POLLUTED SOIL REMEDIATION ON LIFE HISTORY TRAITS OF THE OLIGOCHAETA <u>EISENIA ANDREI</u>

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The use of Fluidized Bed Combustion (FBC) ashes as immobilising agents for metals in contaminated soils is a recent and promising approach. To date, only few studies focused on the possible impact of such treatment on soil fauna. The aim of the present work was to investigate the effects of silico-alumineous and sulfo-calcical ashes on the earthworm *Eisenia andrei* life history traits in OECD artificial soil.

An important mortality of either juveniles or mature worms was observed following silicoalumineous (>60% mortality) or sulfo-calcical (100% mortality) ashes adjunctions. This effect was due to a transient increase of soil pH. When worms were exposed to supplemented soils several weeks after ashes adjunctions, allowing pH to decrease, no mortality occurred. However, adverse effets affecting life history traits were observed after pH stabilization. Indeed, growth rate was significantly reduced for both treatments.

-Silico-alumineous ashes decreased significantly cocoon production, hatching success and increased the incubation period.

-Sulfo-calcical ashes decreased drastically cocoon production and abolished hatching success.

Consequently, both ashes exhibited sub-lethal effects likely to affect population dynamics. Most of the harmful effects observed on growth and reproduction of worms could be attributed to an increase of pH and/or an osmotic disturbance caused by FBC ashes , especially sulfo-calcical ones.

To conclude, a compromise must be found between positive and negative effects of a remediation process. It is essential to evaluate its impact on biota. Complementary studies may provide information regarding remediation processes. In case of field application, the disposal strategy must consider the adverse effects on fauna.

THE EFFECTS OF MERCURIC CHLORIDE ON SURVIVAL, GROWTH, REPRODUCTION, BURROWING SPEED AND GLUTATHIONE CONCENTRATIONS IN THE EARTHWORM SPECIES *EISENIA FETIDA* SAVIGNY

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The earthworm *Eisenia fetida* was exposed to various concentrations of mercury in a wellcharacterized agricultural soil obtained from a local field (Ås, Norway), and the effects on survival, reproduction and glutathione levels were recorded. Internal concentrations of mercury were also measured. For each treatment, four replicates containing groups of five or ten clitellate earthworms were used. Mercury was added to soil in the form of mercuric chloride (HgCl₂).

Reproduction (cocoon production) was arrested in intermediate and high concentrations after four weeks (EC₅₀ for reproduction was approximately 7.4 mg Hg/kg soil). Glutathione (a non-protein thiol) was analyzed by standard spectrophotometric methods. Earthworms exposed to mercury (22 to 740 mg Hg/kg soil for four weeks) experienced a threefold increase in glutathione levels from background levels of approximately 0.62 μ mol/g earthworm wet weight.

Pre-exposure to a non-toxic mercury concentration (22 mg/kg soil for one week) strongly increased survival in a subsequent standard test carried out one week after the termination of pre-exposure. Calculated LC_{50} -values for pre-exposed and non pre-exposed worms were 545 and 170 mg Hg/kg soil, respectively.

The difference in cocoon production between pre-exposed and non pre-exposed worms was small, but significant (t-test, p<0.05). Mercury exposure increased glutathione levels in a concentration-related manner, but at a similar rate in pre-exposed and non pre-exposed earthworms, suggesting that the increased tolerance was not caused by the GSH increase.

Internal concentrations of mercury followed a one-site binding hyperbola reaching a maximum at 139 ± 9 and $165 \pm 16 \ \mu g \ Hg/g$ earthworm wet weight in two independent experiments. The shape of the accumulation curves suggested that glutathione levels in earthworms were closely related to internal mercury concentrations.

HEAVY METAL CONCENTRATIONS IN SOIL AND EARTHWORMS COLLECTED FROM AGRICULTURAL AND INDUSTRIAL HABITATS IN EGYPT

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The important role of earthworms in the biomagnification of heavy metals in terrestrial ecosystems and its role in the transfer of heavy metals toward higher trophic levels are widely recognized. In the present study the bioconcentration levels of essential (Cu, Fe, Mg, Mn, and Zn) and non-essential (As, Cd, Hg, Pb, and Sn) heavy metals were investigated in soil and earthworm samples to evaluate soil contamination in different habitats and the validity of earthworm as a bioindicator of soil contamination and the selected ecosystems health status. Both soil and earthworms were sampled from three sites: Fyoum (about 100 km south of Cairo) and Abu Rawash (about 8 km to the North of Giza Governorate) representing areas polluted by agricultural activities; Mostorod (about 10 Km to the North of Cairo) representing area moderately polluted by industry to examine whether earthworms populations originating from differently polluted terrestrial habitats differ in their tendency to accumulate heavy metals or not. For each of the metals, evidence suggests that bioconcentration depends on the metal concentrations in the soil; bioconcentration is greater at lower soil concentrations. Eearthworms collected from industrial area exhibited different storage capacity and different concentration factors (concentration in animal/concentration in soil), effective bioaccumulation of some heavy metals by earthworm, revealed a strong influence from industrial pollution on the biotic community. This study for the first time presents data on the impact of heavy metal pollution on various earthworms in Egypt. The positive correlation between some heavy metals concentration in the soil and in earthworms is noteworthy. In general the variation in heavy metals concentration in soil and earthworms in different sites may be significant depending on soil properties and pollution status. It will also be of interest for the future to analyze certain effects of metal concentration in soil on innate immune response of resident earthworms and to compare those with results from other geographical populations.

DISRUPTED HOMEOSTASIS IN COELOMOCYTES OF EISENIA FETIDA EXPOSED DERMALLY TO HEAVY METALS

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We have previously shown that *Eisenia fetida* dermally exposed to heavy metals accumulates some of them avidly in the body, what is accompanied by upregulation of stress proteins in coelomocytes (Homa et al. 2005). As heavy metals accumulate also in immunocompetent cells and affect their elemental composition (Morgan and Morgan 1998; Fischer and Molnar 1992), the aim of present studies was to follow day-by-day the metal content, elemental composition, and stress protein expression in coelomocytes extruded from animals exposed to heavy metals via filter papers.

Mature earthworms *E. fetida* were exposed via filter paper for 1, 2 and 3 days to water (controls) or zinc, cooper, lead and cadmium chlorides (44 mg/L) at concentrations 1.32 μ g/cm². Coelomocytes were extruded from animals subjected to the electric shock (1 min., 4.5 V) and used for analysis of expression of heat shock proteins (HSP70, HSP72), caspase-3, and metalotionein MT-2 by immunocytochemistry, western blotting and dot blotting (Homa et al. 2005). The contents of heavy metals and elemental composition (Ca/P and S/P ratios) in coelomocytes were measured by X-ray microanalysis combined with a scanning electron microscopy (SEM) (Banach et al. 2004) in the Department of Cytology and Histology, Institute of Zoology, Jagiellonian University.

During 3-day dermal exposure, copper, lead and cadmium accumulated in the earthworm body while excess of Zn was efficiently eliminated. Nevertheless, coelomocytes retrieved from coelomic cavity were affected by each of them. Copper accumulated gradually mainly in amoebocytes, while lead in both amoebocytes and eleocytes. Zink appeared in coelomocytes transitionally on day 1, while cadmium on day 2. All heavy metals affected in various ways the Ca/P and S/P balance in coelomocytes. Apoptotic pathway was activated by Cd, Pb, and mainly Cu, but not by Zn or water exposure. HSP-72 and MT-2 were upregulated by metal exposure, HSP72 being more sensitive to lead contamination. Constitutive expression of HSP70 make it less attractive as biomarker of metal pollution. In conclusion, coelomocytes homeostatis in *E. fetida* is significantly disrupted, at least transitionally, in animals exposed to environmental pollution, thus these cells may serve as sensitive biomarkers of soil quality.
THE EFFECT OF CHELATOR (EDTA) AMENDMENT ON THE ACCUMULATION OF METALS (PB, ZN, MN, NI) BY EARTHWORMS (*EISENIA FETIDA*) MAINTAINED IN THE LABORATORY ON DIVERSE METALLIFEROUS FIELD SOILS

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Abstract: Metal contaminated terrestrial sites represent major environmental problems in many industrialised countries. A number of in situ and ex situ clean-up protocols are available, but there is currently an increased interest in phytoremediation [1]. In many cases, and especially where Pb is a major contaminant, amending soils with a chelator increases the availability and uptake of metals by plants. For example, the commonest chelator for this purpose, EDTA, has been shown to enhance the phytoextraction of Cd, Mn, Ni, Pb, and Zn [2, 3], but with some risk of metal leaching. To our knowledge, very little work has been published on the effects of chelator-mediated increases in metal availability on the survival, reproduction, and metal accumulation (and, by implication, potential trophic transference) of ecologically important, 'non-target', soil organisms, such as earthworms. Therefore, the primary aim of the present study was to assess, under laboratory conditions, the effect of a moderate EDTA application on the performance of Eisenia fetida exposed for 4 weeks to four different metal-contaminated soils: (i) a circumneutral, heavily Pb+Zn-contaminated mine soil ('Draethen'); (ii) an acidic, moderately Pb+Zn-contaminated mine soil ('Stream'); (iii) a heavily Mn-contaminated, pyrolusite-rich, mine soil ('Barmouth'); (iii) a soil near a working Ni smelter and contaminated by atmospheric deposition ('Clydach'). Earthworms were maintained in pairs of five ('Draethen') or eight ('Sream', 'Barmouth', 'Clydach') replicates per treatment.

The main findings were:

1. EDTA amendment did not significantly reduce earthworm survival rates in Draethen, Barmouth, and Clydach soils below the 100% survival all non-EDTA-amended contaminated soils. Survival in EDTA-amended 'Stream' soil was significantly reduced to approximately 55%, but without depressing pH in this already acidic soil.

2. Reproduction rates (cocoon counts) were very low in both Pb+Zn-contaminated soils ('Draethen' and 'Stream'), whether amended or not. EDTA-amendment did not affect cocoon counts in the Ni-contaminated 'Clydach' soil, but did significantly reduce reproductive output in the Mn-contaminated 'Barmouth' soil (where, in the absence of EDTA, reproduction was particularly high).

3. EDTA did not alter the CaCl₂-extractable metal fraction(s) of 'Barmouth' (Mn), 'Clydach' (Ni), 'Draethen' (Zn), and 'Stream' (Zn); it did increase the Pb fraction in both 'Draethen' and 'Stream' soils.

4. EDTA treatment of field-derived soils significantly increased the earthworm concentrations of Ni ('Clydach'), and Pb ('Stream' but not 'Draethen'). Mn ('Barmouth') accumulation showed an upward, albeit non-significant, trend; Zn was unaffected in both 'Stream' and 'Draethen' soils.

Poster 218 UPTAKE OF MERCURY BY THE EARTHWORM LUMBRICUS TERRESTRIS IN A UK CREMATORIUM

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440,000 people are cremated in the UK every year with an estimated discharge of 1300 kg of mercury (3g Hg per cremation) arising mainly from the thermal decomposition of dental amalgam It has been predicted that by the year 2020 crematoria will account for between 11 and 31 % of mercury emissions to air in the UK. New statutory guidance published by the UK Government in 2005 has recommended that Crematoria should install equipment to cut mercury emissions by 50 % by 2012. Only a few studies (e.g. Nieschmidt and Kim, 1997) have been conducted on the local environmental impact of crematoria emissions and have concentrated on mercury and lead concentrations in soils in the vicinity (crematoria grounds) of the crematorium stack. Earthworms are known to accumulate metals in their tissues and are also used as bio-indicators of soil health.

This preliminary study was conducted at Preston Crematorium in north-west England. A transect was established in the direction of the prevailing wind from the stack, and sampling points marked at distances of 5, 10, 15, 30, 60, 90 and 200 m from the stack. At each distance soil cores (n = 3) were taken and adult (clitellate) *L. terrestris* were collected (using mustard extraction) for lead and mercury analysis on an ICP-MS. Earthworm populations at each distance were assessed by collecting (using mustard solution vermifuge) earthworms from 3 x 0.25 m² squares. These were preserved (in 0.37 % formalin solution) and identified in the laboratory.Earthworm populations did not differ significantly in their abundance or species ratios. Initial results of lead and mercury uptake into soil and earthworm tissues indicate that distance from the stack is primarily important when determining potential negative environmental impacts of crematoria emissions. Results suggest that earthworms provide a good indicator of the bioavailability and possible bioaccumulation in the food chain of mercury (in soils) emitted by the cremation process. Further investigations are recommended.

THE IMPACT OF CADMIUM AND MERCURY CONTAMINATION ON REPRODUCTION AND BODY MASS OF THE EARTHWORMS *EISENIA FETIDA* (SAV.)

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The accumulation of heavy metals in the tissues of earthworms is a helpful indicator of environmental contamination. The degree of substrate contamination can be additionally evaluated on the basis of survivability, reproduction and body mass of earthworms. In this study, in laboratory conditions, *Eisenia fetida* (Sav.) earthworms were exposed to a series of increasing concentrations of cadmium and mercury (2, 4, 6, 8, $10\mu g/g$ dry mass of substrate). The numbers of animals and their body mass were checked after 4 and 8 months. The strongest impact of substrate contamination was exerted upon the number of young individuals and cocoons. The cadmium contamination did not affect adversely the mass of earthworms, whereas in the mercury-contaminated group the decline in body mass was evident. After 8 months of experiments, the heavy metal content in the bodies of earthworms was determined using atomic absorption spectrometry. An evident relationship between the cadmium and mercury contents in the substrate and their accumulation in earthworms' tissues was found. The concentration in the bodies exceeded the level in the substrate.

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EARTHWORM (EISENIA FETIDA) BIOASSAY TO ASSESS THE POSSIBLE EFFECTS OF PLATINUM TAILINGS DISPOSAL FACILITIES ON THE ENVIRONMENT ALONG A GRADIENT

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Platinum mines produce large amounts of inorganic tailings containing high levels of metals which are disposed of on tailings disposal facilities (TDFs) and there is no information available on their possible effects on the surrounding terrestrial environment. The aim of this study was to do an earthworm bioassay of soils along a gradient from a TDF over a period of 28 days in terms of growth, reproduction and metal accumulation. After 28 days the earthworms in the soil collected up to 1 km away from the TDF showed a significant (P < 0.05) decrease in bodyweight and in the soil 2-5 km away showed no effect. The earthworms in the soil collected 15 km away from the TDF showed a significant (P < 0.05) increase in bodyweight. The mean hatching success of cocoons was significantly (P < 0.05) higher in the soils further away from the TDF viz. 15 km > 3-5 km > 2-0 km. Cr, Cu, Ni and Zn concentrations in the soils collected in the soils on the platinum TDF (TDF - 15 km in the case of Zn) were higher, while Cd, Co and Pb were lower when compared to screening benchmarks proposed by the U.S. Dept. of Energy. The presence of these metals in a mixture, however, makes it extremely difficult to assess their effects. All of the metals had low bioconcentration factors (BCFs) viz. < 0.01 (CR, Ni and Pb), 0.01 (Co), 0.33-0.5 (Cd), 0.01-0.08 (Cu) and 0.18-0.29 (Zn). It can be concluded that platinum mining, with TDFs as source of contamination, has negative effects on the environment but further studies are needed to assess the exact extent of these effects.

Poster

EFFECTS OF METALS IN GROWTH, REPRODUCTION AND GAMETOGENESIS IN *EISENIA ANDREI* INHABITING IN ARTIFICIAL CONTAMINATED SOILS. FIRST RESULTS

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The aim of this study was to check the effect of six metals (Cd, Cu, Ni, Pb, Se and Zn) on growth, reproduction and gametogenesis in *Eisenia andrei* inhabiting in artificial contaminated soils. Of the six metals chosen in this study, four of them, Cd, Cu, Pb and Zn have been tested in earthworm frequently for different authors but about effects of Ni and Se there are little information for terrestrial invertebrates.

For the study about the effect of metals on survival, growth and reproduction preclitellated earthworms (*Eisenia andrei*) were cultivated in artificial soils containing different concentrations of six heavy metals (Cd, Cu, Ni, Pb, Se and Zn) and one control soil during 195 days.

All cultures were supplied weekly with horse manure and were checked each 15 days, and cocoons were separated, weighted and incubated individually and examined twice weekly.

For gametogenesis couples of preclitellated earthworms (*Eisenia andrei*) were cultivated in similar artificial soils. Earthworms were weekly revised until the first copula and in this moment worms were fixed until their later dissection for estimating the effect of metals on total volume of sperm in seminal receptacles, the remaining sperm in seminal vesicles and the mean number of mature ova.

The first results indicated significant differences in the effect of the different heavy metals in almost all the studied parameters.

Poster

EFFECTS OF PARATHION AND IMIDACLOPRID ON THE CROP/GIZZARD COMPLEX OF APORRECTODEA CALIGINOSA

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Earthworm is a relevant indicator of environmental changes and is currently used as a biomarker for assessing chemical pollution. Organophosphorus agents and pesticides are know to induce in some cases, a weight loss and an alteration of the burrowing behaviour. Earthworm gut represents one of the two contamination pathways with the skin.

In this study, we focused our attention on the crop/gizzard complex, a part of the earthworm gut equivalent to the stomach of vertebrates and we investigated the effects of pollutants on its biochemical (cholinesterases (ChE), glutathion-S-transferase (GST)) and physiological (motility) parameters. The crop/gizzard preparation is dissected away from the animal and immersed in physiological solutions containing different concentrations (10^{-3} , 10^{-6} and 10^{-9} M) of parathion (Oleo-Bladan, Bayer) and imidacloprid (Confidor, Bayer).

The biochemical results show a 70% decrease of the ChE (cholinesterase) specific activity for parathion at 10⁻³M concentration. We observed no effects of parathion on the GST activity and no effect of imidacloprid on the ChE and GST activities. Considering these results, we tested the contractile responses of preparations pre-treated by immersion in parathion 10⁻³M. We observed a loss of the spontaneous contractions and a diminution of the area under the curve (AUC) after application of KCl rich solution. Applications of acetylcholine induced also a diminution of AUC. These physiological results seem to indicate a dysfunction of the contractile machinery.

Parathion at high concentration affected biochemical and physiological parameters of the crop/gizzard complex. These additional data could explain the physiological and behavioural alterations previously observed with this organophosphorus pesticides. However, further investigations are needed to compare these biomarkers in other conditions; for example crop/gizzard complex of earthworms collected in mesocosms or polluted fields.

BIOTIC CONTROL OF ATRAZINE DEGRADATION IN SOILS: IMPACTS OF EARTHWORMS ON ATRAZINE DEGRADING BACTERIAL COMMUNITIES

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Enhanced atrazine degradation is observed in cultivated soils frequently treated with this herbicide. Different soil bacteria such as *Pseudomonas* sp. strain ADP and *Chelatobacter heintzi*. have acquired the capacity to metabolise this herbicide. The genes involved in Atrazine catabolism are carried onto a plasmid and are *atzA*, *B*, *C*, *D*, *E* and *F* in *Pseudomonas* sp. ADP, and *atzA*, *B* et *C* in *C*. *heintzii*.

As efficient soil engineer, earthworms modify soil physical and chemical properties, along with microbial communities and activities. They may change the distribution and degradation of organic compounds such as pesticides.

We have analysed interactions between earthworms (*Lumbricus terrestris*) with indigenous and atrazine degrading bacterial communities. Representative soil biostructures of earthworm activity *i.e.* ingested soil, excreted soil, bioturbated soil and uningested soil in presence of earthworms were sampled. Soil microcosms were treated with Atrazine and inoculated or not either with *P*. sp. ADP or *C. heintzii*. In all soil microsites we have estimated the atrazine degrading activity of bacterial community (CO₂) along with 16S and *atzA* genes quantities by real time PCR.

We demonstrate i) a significant decline in the number of *C. heintzii* thirteen days following inoculation but not for *P.* sp. ADP, ii) an enhanced atrazine mineralization potentiality in all microsites except in excreted soil and iii) a negative impact of earthworms on indigenous bacteria but a stimulation of atrazine degrading strains in burrow-linings formed by *L. terrestris*.

Other objectives are to assess the microbial fraction able to degrade Atrazine and to analyse the earthworms impact on this degrading communities. Another molecular strategy based on SIP RNA will be performed using ¹³C labelled Atrazine and preliminary assays are currently running with ¹³C-Glucose.

Poster 224 INTERPOLATED NICKEL MAPS FOR EARTHWORMS AND SOILS AROUND A NICKEL-SMELTING POINT SOURCE

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<u>Abstract</u>: Ni is an essential trace element whose bioavailability, and consequent toxicity toward terrestrial plants and animals, is both dependent on intrinsic edaphic factors (notably pH) and on the ecophysiology of receptor organisms. Ni is listed on the European Commission List II (Dangerous Substances Directive), and Ni compounds are classified by the World Health Organisation in Group 1 (human carcinogens) [1]. Some plants are efficient Ni accumulators (e.g. *Vaccinium myrtillus*), but there is no evidence that any soil-dwelling animal species have similar properties. Earthworms are acknowledged macro-accumulators of several different essential and non-essential metals; and yet, there is considerable uncertainty about the Ni relationships of earthworms [2, 3] due to a number of factors, including a very limited number of field studies on populations living in heavily contaminated, geochemically diverse, soils.

Ni production at the INCO Ltd. Ni smelter in the Swansea Valley (UK), the largest working Ni smelter in western Europe, started in 1902 and continues to the present, processing feed material imported from Sudbury, Canada. Soils (0-10 cm depth), plants (the grass, *Agrostis capillaris*) and earthworms (*Lumbricus rubellus*) were hand-sampled at 36 stations within an area of about 2 x 3.5 km around the smelter, and analysed for Ni concentrations (conc. HNO₃ digests). The resolution of sampling was highest in the immediate vicinity of the source. Sampling-station positions were recorded with a GPS navigator (Jarmin ETREX). Interpolated metal-distribution maps for abiotic and biotic samples were plotted with Surfer Software.

The main findings were:

- Soils near the point-source contain c.3000 μ gNi/g dry mass, compared with the UK Soil Guideline Value (SGV) for 'residential soils without plant uptake' of 75 μ gNi/g) [4]. The Ni concentration in a high proportion of the soils exceeded the coccon production EC50 value (85 mgNi/kg) recorded for *Eisenia veneta* exposed for 4 weeks to spiked soil in the laboratory [4].

- The slope of the downwind Ni gradient in soil and earthworms is steep.

- The Ni bio-concentration factor (BCF = worm_[Ni] \div soil_[Ni]) in earthworms was very low across the entire sampling area, indicating efficient regulation.

- Soil pH varied considerably across the sampling area (ranging from 3.91 to 7.69); soil pH did not exert a strong influence on Ni accumulation by earthworms.

- Ni was not partitioned to any particular tissue fraction of the earthworm.

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THE MANGANESE RELATIONSHIPS OF ECOPHYSIOLOGIALLY CONTRASTING EARTHWORM SPECIES (*LUMBRICUS RUBELLUS* AND *APORRECTODEA CALIGINOSA*) INHABITING MANGANESE-MINE SOILS

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The transition metal Mn is biologically essential. It has multiple redox states. Mn phytoavailability is highest in the reduced state; hence, Mn mobility is relatively low at circumneutral and alkaline pHs, but relatively high in acidic and anoxic soils. Unlike other essential transition metals, Mn does not have a dominant storage protein in animal tissues. Observations indicate that biogenic manganese oxide nanoparticles play important nutritient-regulatory roles in soils, mainly by surface-coating and masking minerals [1]. Similarly, manganese oxides are ecotoxicologically important because they not only scavenge and reduce the bioavailabilities of certain essential metals (Cu, Fe), but they also suppress the accumulation of non-essential metals (e.g. Pb, Cd) by receptor organisms [2]. Despite the geochemical, eco-physiological, and biochemical prominence of Mn and its minerals, surprisingly little is known about the uptake and fate of the transition metal in field populations of (even) earthworms, whose metal-relationships are generally well-studied.

The Harlech Dome area of N.W. Wales is renowned for Mn mineralization which was mined over much of the locality up to about a century ago [3. In this study, two earthworm species with differing Ca physiologies (the epigeic *Lumbricus* rubellus with 'active' mineralizing calciferous glands and the endogeic *Aporrectodea caliginosa* with 'inactive' glands) were collected and analysed at 10 sites across the Dome. In addition, a number of invertebrate taxa (slugs, snails, earthworms, isopods, millipedes, and centipedes) were analysed at one site, 'Cell Fechan' O.S. Map Ref. = SH614166, to gain insight into the distribution of Mn at different trophic levels.

The main findings were: (i) soil Mn ranged from 9,000 μ g/g to 45,000 μ g/g dry weight (conc. HNO₃-digests) across the study sites, values exceeding the 'natural' or 'background' global range of <1 to 4000 μ g/g (mean around 300-600 μ g/g) [4]; (ii) gastropod molluscs accumulated Mn in their soft tissues much more efficiently than all other macro-invertebrate species; (iii) consistently low Mn bio-concentration factors (BCF) in earthworms (e.g. BCF_{Mn} for *L. rubellus* at 'Cell Fechan' = 0.04) indicate tight homeostasis; (iv) shallow earthworm-to-soil('total') Mn regression slopes confirm the ability regulate Mn; (v) no significant differences were found in the Mn burdens of the two earthworm species. Finally, electron spin resonance spectroscopy of frozen-hydrated, powdered, whole-worms showed that Mn (IV) is the predominant species.

EFFECTS OF IVERMECTIN CONTAINED-CATTLE DUNG ON THE EARTHWORM, EISENIA FETIDA

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The effects of faecally excreted ivermectin on survival and growth of the earthworm, *Eisenia fetida* (Savigny), have been studied in the laboratory. Dung was collected 1, 2, 3 and 7 days after dosing ivermectin. The mortality of worms provided with untreated dung was 0% whereas mortality in ivermectin within a week was high as 73.7%, 98.3% in 1 and 2 days dung, respectively. The growth rate of the worms fed dung from 1 day excreted dung from cattle given ivermectin was 75% lower than that recorded for the control. In total number of hatchling earthworm in each treatment, 1 day after treatment produced the lowest compare with untreated control. However, dung from cattle treated with ivermectin did not prevented the rate of hatchling from each cocoon of earthworm. The results show that ivermectin is less toxic to 2nd generation of earthworm.

We concluded that ivermectin have adverse effects on the survival and growth of *E. fetida* when exposed through dung under laboratory conditions. This results will be discussed in relation to the effects of anthelmintics on non-target organism in our ecosystem.

UPTAKE AND EXCRETION OF METALS BY *EISENIA FETIDA*, IN NATURAL POLLUTED SOILS

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Numerous studies have evaluated the accumulation of metals by Eisenia fetida, but most of them where performed on artificial soils, or on soils artificially amended with metals. The aim of this study is (1) to investigate soil metal bioavailability and toxicity to the OECD standard earthworm *Eisenia fetida* on the basis of soil physical and chemical properties and (2) to test existing, and produce new kinetic models for metal accumulation and excretion in earthworms. Ten sites (2 unpolluted soils, 7 mines, and 1 industrial site) were selected and soils were sampled (0-10 cm depth). For each soil, pH, CEC, organic matter, texture, and metals were analysed. Then, 500g of each soil were put into plastic boxes and 12 adult earthworms were added. To evaluate the rate of metal uptake, earthworms were sampled from each soil (6 earthworms per boxes, 5 boxes) after 3, 7, 14, 21, 28, and 42 days. After the earthworms had been in the metal contaminated soil for 42 days they were transferred to uncontaminated soil in order to evaluate the rate of excretion of metals; earthworms were sampled (6 earthworms per boxes, 5 boxes) 7, 14, 21, 28, and 42 days after being transferred to the uncontaminated soil. Earthworms from the microcosms were analysed for metals burden; additionally, metal concentrations in the soil solution were analysed. There were significant differences in rates of metal uptake and excretion between different metals and between different soils. Texture, pH and total metal content were key parameters accounting for variability of uptake and excretion between soils. These parameters also had a significant effect on earthworm parameters such as weight, cocoon production and lethal effects. In this presentation results will be used to test existing models on earthworm metal uptake rates and new kinetic models for metal accumulation and excretion will be presented.

Poster BIOCHEMICAL AND BEHAVIOURAL EFFECTS OF PARATHION ON APORRECTODEA CALIGINOSA UNDER LABORATORY CONDITIONS

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Ethyl parathion is a widely used organophosphate pesticide in Mexico. The sublethal effects of this pesticide were studied using A. caliginosa as a model earthworm since it is very common in agricultural soils in France but also in Mexico. In a first experiment, classical endpoints (i.e. mortality, weight loss and cholinesterase activity) were measured in earthworms exposed to parathion sprayed on four different soils, three Mexican soils (two vertisols and one andosol differing in their organic matter content) and one French soil (eutric cambisol). All earthworms were collected in France in an abandoned orchard close to Avignon. The Predictive Environmental Concentration (PEC) for parathion is approximately 0.625 mg/kg of dry soil (or ppm). Earthworm mortality was between 80 and 100% for concentrations in parathion of 100 ppm for the three Mexican soils but was only 50% in the French soil. Weight loss was significant different from the control for concentrations in parathion of 10 ppm and no difference was observed between the four soils. Significant cholinesterase inhibitions were observed in the four soils for concentration in parathion of 1 ppm. No important differences in effects were observed between soils suggesting that exposition was not directly related to sorption coefficients of the active ingredient but to available concentrations after soil ingestion. Another experiments were carried out with the French soil to evaluate behavioural effect (avoidance and burrowing capacity) of parathion. A. caliginosa did not avoid any of the tested concentrations (10,1 and 0.1 ppm) of parathion. The burrowing behaviour of A. caliginosa was significantly affected in 2D terrarium for concentrations as low as 1 ppm showing that behaviour is a sensitive biomarker.

CORRELATING EFFECTS OF LONG-TERM HEAVY METALS EXPOSURE ON LIFE-CYCLE PARAMETERS AND NEUTRAL RED BIOMARKER RESPONSES IN *EISENIA FETIDA*.

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Earthworms were exposed in a long-term experiment to heavy metals in our laboraatory. They have been exposed to different heavy metals over a period of 10+ years at sublethal concentrations of the respective metals in an organic substrate. A number of studies have been conducted using specimens from these cultures, e.g. looking at the possible development of resistance by methods such as allozymes, metallothioneins, the MTT assay, the comet assay and DNA polymorphism. Since it has already been established that good correlations exist between the Neutral Red biomarker responses and life-cycle parameters in earthworms, it was the aim of the present study to determine whether this could be shown for worms from these long-term cultures and to compare these results with exposures of previously unexposed earthworms. Specimens of earthworms (Eisenia fetida) obtained from control (non polluted) and long-term heavy metal (Cd, Zn, Cr, Cu, Pb) polluted substrates kept in the laboratory, were exposed to the respective metals in OECD soil. Five exposures were done for each group - Worms from control cultures were exposed to a high and a low concentration of the metal and worms from the pre-exposed cultures also to the same high and low concentrations as well as to a clean substrate. Control worms were also exposed to a clean substrate as experimental control.Growth was monitored weekly over eight weeks and cocoons collected. Cocoons were allowed to hatch in distilled water and hatching was monitored. After eight weeks of exposure, the neutral red retention times were determined and worms and substrates were analysed for metals by AA spectrometry after acid digestion. The results were very varied with clear correlations for some but not for other metals between biomarker responses and traits. So far it seems that different heavy metals exert different influences on these parameters.

CHANGES IN THE OVARY CELLS STRUCTURE IN THE EARTHWORM DENDROBAENA VENETA (ROSA) IN NEUROSECRETION DEFICIENCY AND AFTER EXPOSITION TO CADMIUM

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Earthworms living in soil polluted with cadmium accumulate its ions in some tissues of their bodies. Cadmium accumulated in the ganglia of nerve system inhibited the synthesis of neurosecretion and its release from the cells of supra- and subpharyngeal ganglia (Siekierska, 2003). Cadmium ions also caused distinct degenerative changes in the earthworm ovary structure and affected oogenesis (Siekierska, Urbańska-Jasik, 2002). But it is not known whether those changes result from the direct cadmium effects on the ovary or they are the manifestations of disturbances in neurosecretion synthesis and release. In the absence or shortage of the released neurosecretion, the processes, which are regulated by it may get distorted. The aim of the study was to determine whether deficiency in neurosecretion may induce changes in the ovary and oocytes structure in the earthworm species *Dendrobaena veneta* and define possible correlations between those changes and the ones induced in the ovarian structure by cadmium ions present in soil.

The experiment was conducted on decerebrated sexually matured earthorms *D. veneta* bred in uncontaminated soil. Their head parts were cut off at segment 6, to deprive them of the nerve cephalic ganglia. The ovaries of experimental and intact specimens were isolated after 20 days of decerebration, fixed and processed for electron microscopy.

The experiment indicated that decerebration deprived the earthworm of the substances produced in the supra- and subpharyngeal ganglia and disturbed oogenesis considerably. In these cases also the structure of the ovary in the earthworm *D. veneta* was affected. Within the ovarian stroma irregularly shaped, elongated and broadened spaces were visible. Ooplasm of large oocytes was distinctly shrunk and their surface undulating. In oocytes the number of well-developed Golgi dictyosomes increased and endoplasmic reticulum formed circular patterns. In trophocytes, Golgi complexes and endoplasmic reticulum cisternae were strongly vacuolised and were similar to those that were manifested in ovarian cells in case of cadmium occurrence. Cadmium disturbed oogenesis and induced degenerative changes in the ovaries in somatic cells as well as in oocytes and trophocytes (Siekierska, Urbańska-Jasik, 2002; Siekierska, 2003). After the analysis of the obtained results it was possible to conclude that cadmium effects on the ovary of earthworm *D. veneta* indirectly by suppressing neurosecretion synthesis. Such conclusion may be confirmed by the fact that the structure of oogonia did not seem affected neither in neurosecretion deficiency nor after exposition to cadmium.

CADMIUM EFFECT ON THE SEMINAL VESICLES STRUCTURE AND SPERMATOGENESIS IN THE EARTHWORM *DENDROBAENA VENETA* (ROSA).

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Cadmium ions inhibit cocoons production and decrease fertility in earthworms living in cadmium contaminated soils. However, it is not known whether reproductive disturbances are caused only by cadmium induced degenerative changes in the earthworm ovary and which affect oogenesis (Siekierska, Urbańska-Jasik, 2002), or they are manifestation of disturbances in spermatogenesis too. The aim of this study was to determine what changes in the testes, seminal vesicles structure and spermatogenesis occurred after Cd exposure and whether those changes were dose dependent. The seminal vesicles structure and spermatogenesis were tested in *Dendrobaena veneta* earthworms exposed to cadmium ions at concentrations: 10 and 50 mg Cd kg⁻¹ of soil for 20 days and in the controls. In this species spermatogenesis took place in testes and seminal vesicles. D. veneta had 2 pairs of small testes lying in segments 10 and 11 and 3 pairs of seminal vesicles in segments 9, 11 and 12 of the body. The testes contained spermatogonia which divided mitotically. Divisions were synchronous and resulted in the development of groups of cells interconnected by cytoplasmic bridges forming clusters (morula). The last mitotic division of spermatogonia gave primary spermatocytes which were released from testes to seminal vesicles. Spermatogenesis continued within the seminal vesicles and the subsequent spermatogenic stages were observed - clusters of primary and secondary spermatocytes, spermatids and spermatozoa. Cytoplasmic bridges connected germ cells in a cluster to an acellular central mass - cytophore. During spermatogenesis cytophores enlarged and in spermatid morulae reached the maximal size. The cytoplasmic mass of cytophores included mitochondria, lysosomal bodies, ribosomes and endoplasmic reticulum. D. veneta had 3 pairs of seminal vesicles. Among the germ cells clusters the somatic tissue was distributed, which filled the inside of seminal vesicles.

Light and electron microscopy revealed that cadmium ion in both concentrations caused distinct degenerative changes in the seminal vesicle structure and affected spermatogenesis. Young stages of spermatogenesis were most susceptible to cadmium. Differently shaped membranous structures were located within nuclei of some primary spermatocytes and the cytoplasm of those cells was strongly vacuolized. The number of clusters within the seminal vesicles decreased and the volume of somatic tissue among them increased. Degenerative changes occurred in cytophores. They became smaller in size and irregular in shape and their cytoplasm was vacuolized. Cadmium inhibited spermatogenesis.

Poster 232 EFFECT OF CADMIUM AND MERCURY ON THE SURVIVAL, MORPHOLOGY AND BURROWING BEHAVIOUR OF THE EARTHWORM *LAMBITO MAURITII* (KINBERG)

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The earthworm, *Lambito Mauritii* (Kinberg) were exposed to various concentrations of cadmium and mercury in soil and water medium. The 24hrs LC ₅₀ value of cadmium and mercury in water environment was 1.1 mg/L and 0.09 mg/L respectively and in soil medium it was found to be 800.7 mg/kg and 336.6 mg/Kg. The various behavioral changes observed were burrowing, fast movement, lifting of the body, curling and coiling. The morphological changes were rupture of the cuticle, blood accumulation, constriction, bulging, oozing out of blood and oozing out of coelomic fluid. In all the concentrations tested the time taken for the initiation of morphological changes decreased.

ACUTE AND CHRONIC ECOTOXICITY AND BIOAVAILABILITY OF PHENANTHRENE IN CONTAMINATED SOILS BY *EISENIA ANDREI* BOUCHÉ AND *EISENIA FETIDA* SAVIGNY

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Several protocols, such as OPTTS and OECD, assess the acute toxicity of pollutants indifferently on *Eisenia fetida fetida* or Eisenia *fetida andrei*, considering a similar physiology of the two subspecies. On the contrary, very recent biomolecular and spectrophotometric investigations have demonstrated that they are two well defined species (Albani *et al.*, 2003). Therefore, they might show specific tolerance and sensitivity to the same xenobiotics.

The aim of the present contribution is to compare physiological responses of *E. fetida* and *E. andrei* in the presence of a specific pollutant in order to evaluate the validity of the use of the two species indifferently in ecotoxicological studies.

The toxicity of the Polycyclic Aromatic Hydrocarbons (PAHs) is usually associated to the chronic risks that their exposure involves. Relevant is the correlation of these studies with the acute toxicity and with the pollutant fraction actually accumulated in the body of a test organism (actual bioavailability).

The acute toxicity test has been carried out on an artificial soil with increasing concentrations of phenanthrene as representative of PAHs. After 20 days of aging, *E. fetida* and *E. andrei* have been added to the soil and incubated under controlled environmental conditions for up to 28 days. LC_{50} were assessed for both species. Moreover, the bioavailable fraction of phenanthrene was evaluated by means of its accumulation in earthworm body at the end of the incubation period (actual bioavailability).

The response of the immunological system of *E. fetida* and *E. andrei* in the presence of the pollutant has been studied by means of the lysosomal membrane stability of the coelomocytes every 7 days within 28 days from the beginning of the experiment, applying the Neutral Red Retention Time (NRRT).

POSSIBLE RESISTANCE TO CADMIUM IN THE EARTHWORM *EISENIA FETIDA*? USING A BIOMARKER TO DETERMINE LONG-TERM EFFECTS

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The MTT Assay was used to assess the effect of cadmium (Cd) on the activity of the mitochondria of Eisenia fetida. Adult earthworms from a long-term cadmium exposed and a control culture were exposed to a range of Cd concentrations (0, 2.5, 5, 10, and 20 mg/l) in artificial soil water for two days. Coelomic cells were thereafter extracted and transferred to microtiter plates and the tetrazolium salt (MTT) was added to the medium. The reduction of the MTT into a blue formazan product, measurable by a multiwell scanning spectrophotometer (at 570 nm) reflected the mitochondrial activity of the cells. Analysis of variance across treatments and cultures revealed significant differences between the different groups. Post-hoc analysis showed significant differences amongst rather than within the cultures. When absorbance values for all treatments within each culture were added together a very highly difference between the two cultures was found. A third population (an outdoor population) of E. fetida was added to the study and the MTT assay was similarly carried out as for the long-term laboratory cultures. It was found that all three populations differed significantly from one another in terms of their absorbance values. The laboratory control culture and the outdoor population however did not show any differences at the untreated exposure samples.. Differences in absorbance appeared when these groups were exposed to cadmium. Both the outdoor and the laboratory control culture however, were significantly different from the long-term cadmium exposed culture across all exposure samples. Differences in absorbance at the treated exposure samples, between the outdoor population and the long-term laboratory control culture could have been caused by differences in feeding regimes and other ecological factors. Whereas differences between the long-term Cd-exposed and the long-term control cultures were directly related

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MECHANISMS BY WHICH EARTHWORMS INTERACT WITH PLANT PATHOGENS, PLANT PARASITIC NEMATODES AND INVERTEBRATE PEST ARTHROPODS

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It has been demonstrated that earthworms can influence plant diseases, by spreading infective spores into plant rhizospheres, changing their viability; and affecting the susceptibility of crop plants to plant pathogens. Earthworms interact strongly with nematodes in soils, by changing the viability of nematode cysts that they ingest; influencing the relative balance of trophic levels of: plant parasitic, bacteriovorous, fungivorous, and omnivorous nematodes; and by moving nematodes into the rhizospheres of plants. Examples of these interactions and their importance will be discussed.

Recent research in our laboratory has demonstrated clearly, that most organic wastes can be processed rapidly into humic materials (vermicomposts) by interactions between earthworms and microorganisms; these materials are rich in microbial activity and diversity. Our research has shown that vermicomposts, produced commercially, from food waste, paper waste, or cattle manure, can suppress populations and damage by a range of species of plant parasitic nematodes including: Meloidogyne hapla, and Heterodera glycines, on a range of ornamental, vegetable, and fruit crops, in both the greenhouse and field. Possible mechanisms of nematode suppressions are: (i) competition between the main trophic groups of nematodes, plant parasites, fungivores, bacteriovores, predators and omnivores, (ii) increased populations of fungal parasites of cyst nematodes, (iii) increased populations of nematode-trapping fungi, (iv) build up of bacterial parasites of plant parasitic nematodes. Any of these mechanisms are feasible because, passage of organic wastes through the earthworm gut promotes increased microbial activity, particularly fungal activity, by several orders of magnitude. We have demonstrated that small amounts of vermicomposts can suppress attacks by plant pathogens, such as Pythium, Rhizoctonia, Plectosporium, Phytophthora and Fusarium in the greenhouse, and Verticillium wilt, Phomopsis, Uncinula necator and bacterial rots in the field. Possible mechanisms for pathogen suppression include: (i) a general pathogen suppression by competition with other microorganisms for energy and nutrients (ii) specific suppression due to multiplication of particular microbial antagonists (iii) production of fungistatic substances by microorganisms. We have also demonstrated clearly that vermicomposts can suppress populations and damage by tomato hornworm (Manduca quinquemaculata), cucumber beetles (*Diabiotica undecimpunctata*), cabbage white butterflies (*Pieris brassicae*), mealy bugs (Pseudococcus spp.), spider mites (Tetranychus urticae), and aphids (Myzus persicae) significantly. Possible mechanisms of pest invertebrate suppression include: (i) changes in plants' nutritional status, (ii) altered rates of nutrient release, (iii) uptake of phenolics into plants.

Oral

RELATIONSHIPS BETWEEN EARTHWORM POPULATIONS AND MANAGEMENT INTENSITY IN CATTLE-GRAZED PASTURES IN IRELAND

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While an earlier survey of earthworm populations in sixty-eight pasture fields in Ireland had revealed a significant, positive relationship between management intensity and earthworm biomass (Muldowney et al. 2003), the data exhibited a great deal of inter- site variability, probably reflecting local management conditions. This paper reports new results from randomised, large field plot experiments conducted on three grassland research farms located on medium-textured mineral soils at Solohead Co. Tipperary, Grange Co. Meath and Johnstown Castle, Co. Wexford. The experiment at Solohead involved four levels of fertilizer application (80, 175, 225 and 350 kg N ha-1), and the plots were rotationally grazed for appropriate intervals by dairy cows. Three levels of fertilizer were compared at Johnstown Castle (0, 225 and 390 kg ha-1) and two at Grange (100 and 225 kg N ha-1), and the plots at these sites were rotationally grazed by suckler cows and followers or dry cattle.

Between 10 and 15 species were recorded per site, with Allolobophora chlorotica and Aporrectodea caliginosa being dominant. Other abundant species included Aporrectodea rosea, A. longa, Lumbricus terrestris, L. friendi, L. festivus, L. rubellus and Satchellius mammalis. Mean earthworm densities per treatment ranged from 198 to 324 ind. m-2 and biomass from 60 to 176 g m-2. ANCOVA revealed a significant overall relationship between management intensity as indicated by N application rate and earthworm density (F = 18.24, P = 0.024) and biomass (F = 26.14, P = 0.015). There was considerable variation between the three sites, but in no case was earthworm populations adversely affected by intensive management.

It is likely that any adverse effects of trampling on earthworm populations were compensated for by increased dung and plant litter inputs. The presence of high earthworm populations is potentially an important factor in countertacting adverse effects on pasture of soil compaction at high stocking levels, especially on heavy soils liable to poaching.

MODELLING AT REGIONAL SCALES THE POTENTIAL AVAILABLE RESOURCES FOR SUSTAINING EARTHWORM POPULATIONS

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The extent of population abundance of different earthworm species across agricultural landscapes at regional scales is in direct response to variations in environmental properties and influences from cropping and tillage management. Environmental attributes such as soil, climate, and topography define the set of natural available resources or inherent conditions that exist across the landscape. For the different earthworm species one can from field observations and literature sources determine and identify the ecological preferences to various soil types, temperature and moisture ranges and influences from changing topographic position in the landscape. From this knowledge of the environmental resources required, one can in general identify landscape areas at regional scales that would be conducive to either supporting or limiting earthworm population growth; that is, the inherent natural potential. In addition environmental attributes, cropping patterns and tillage management will affect the extent or amplitude to which the existing inherent natural potential can sustain earthworm population abundances. Depending on crop type and tillage system, there will be differences in the amount of crop residues and the extent of disturbance from tillage operations that will affect both availability of food resources and soil habitat. By recognizing these interacting influences from both environmental properties and agricultural activities provides a means by which to identify across regional scales the set of resources that are potentially available for sustaining earthworm populations. A modelling approach based on Geographic Information Systems (GIS) methodologies is being proposed for identifying at regional scales the potential extent of available resources.

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A lot of articles deal with the effect of soil macrofauna (termites and earthworms) on soil properties and ecosystems functioning. These soil animals are usually considered to positively affect soil organic matter decomposition and nutrient cycling, then increasing the concentration of nutrients in their biogenic structures (casts, sheetings, nests, galleries...) and promoting the growth and diversity of plants. They are also considered as good indicators of soil quality in term of soil structural stability. However, there is a paucity of data concerning their impacts on tropical ecosystems with steep slopes.

This study is part of the MSEC project (Management of Soil Erosion Consortium), which examines the effects of land use changes on soil erosion on a regional scale in southern Asia. The aim of this study is to analyse the effects of land use change on (1) earthworms, in term of diversity and abundance, and (2) dynamic of soil aggregates and nutrients, in an experimental watershed in Vietnam (Dong Cao Village, Hoa Binh Province).

Land use management had a major effect on the size, composition and diversity of earthworms, probably due to changes in the quantity and quality of available litter. Annual crop plantations (cassava, Bracharia and fallow) were characterized by endogeic species (mainly *Pheretima californica*) that has little effects on soil surface. Conversely, the forest and eucalyptus plantations were characterized by epigeic species (mainly *Pheretima leucocirca*) that produced large, globular and very water stable casts on the soil surface. The activity of *Ph. leucocirca* led to a typical granular soil structure that increased surface roughness, and infiltration, as compared to the annual plantations. As a consequence, earthworm activity led to an increase of water infiltration, a decrease of water runoff and soil erosion. Although soil cover plays an undisputed role on soil conservation, our data clearly show that other biological parameters, and especially earthworm populations, must not been neglected in studies of the determinants of soil erosion.

EFFECTS OF AGROCHEMICALS (FERTILIZERS AND INSECTICIDES) ON JUVENILE EARTHWORM POPULATION DENSITIES IN SEMI-ARID TROPICAL RICE (ORYZA SATIVA L.) PADDIES (INDIA

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Earthworms are most important beneficial soil animals ameliorating the physico-chemical and useful microbial characteristics of the soil. Various contemporary agricultural practices such as application of different agrochemicals affect the earthworm populations and their activity either adversely or favorably. These agrochemicals are used indiscriminately in agriculture in the tropics. Therefore, the effects of farmer's doses of different chemical [Urea and Diammonium phosphate (DAP)] and organic [Farm Yard Manure (FYM)] fertilizers and organochlorine [Antio, Dimecron ans Ekalux] and organophosphate [Methyl Parathene, Monocrotophos and Dimethiote] insecticides on densities of three different agegroups such as juveniles, pre-adults and adult of earthworm populations were monitored and assessed during three crop seasons in the semi-arid tropical wetland rice agriculture fields at Gadwal in Mababoonagar district (15 55' N and 17 20'E) of Andhra Pradesh in India, which showed that the fertilizers, both chemical and organic did not show any significant effect on the population densities of Octochaetona phillotti (Michaelsen) after 30 days of broadcasting in the rice nursery systems. However, its juvenile population reduced (38 times) significantly in densities in the nurseries treated with Urea and DAP and increased in the FYM treated nursery systems after 60 days of broadcasting compared to the control ones [ANOVA: P<0.01]. Further, it was found that the response of the juvenile population densities of Lampito mauritti Kingberg to different doses of Urea in rice fields (after transplantation of rice plants from the nurseries) showed two to six fold increase in the population densities in treated paddies compared to the control ones (ANOVA: P<0.05]. The response of population densities of different age-groups of O. phillotti after 30 days of broadcasting of FYM in the fields depicted a two fold increase of juvenile populations in the paddies compared to that of the control ones [ANOVA: P<0.05].

The response of population densities of the three age groups of *L. mauritti* after 30 days of treatment of different organochlorine insecticides showed that the densities of its juveniles decreased about two folds under Ekalux and three folds under both Antio and Dimecron treated paddies leading to half to two fold decrease of the total population of the earthworm compared to that of Control ones [ANOVA: P<0.01]. However, the population densities of juveniles, pre-adults and adults of both *O. Phillotti* and *L. mauritii* though decreased in response to the organophosphate insecticide application after seven and 30 days, but such decreases were not statistically significant. Therefore it is concluded that these contemporary agricultural amendments showed significant effects on the juvenile earthworm populations in the rice agro-ecosystems; the application of nitrogenous and organic fertilizers significantly increased the juvenile population densities of O. phillitti and L. mauritii, whereas the persistent organochlorine insecticides significant effect on their abundance while organophosphate insecticides did not show any significant effect on their densities.

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ACIDIFICATION AND SOIL REHABILITATION OF ATLANTIC BROADLEAVED FORESTS ON LOAMY SOILS USING EARTHWORM REINTRODUCTION: AN EXAMPLE OF ALTERNATIVE STABLE STATES?

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This paper hypothesizes that Atlantic broadleaved forests on loamy soils as they occur in Central Belgium and elsewhere are examples of alternative stable states (sensu Scheffer et al., 2001; Scheffer and Carpenter, 2003). According to this theory many aquatic and terrestrial ecosystems show multiple equilibria, each characterized by a specific ecosystem state in terms of biomass, productivity, species diversity, or other ecosystem performances. The ecosystem may move from one equilibrium to another as a consequence of degradation or rehabilitation. The lower ecosystem state being the stronger attractor, shifting from a higher to a lower stable state as a consequence of a degradation process is more likely than the opposite through a restoration process. Using data from literature and own observations we recognize this pattern in Atlantic broadleaved forests on eolic loess, where the original highest stable state, being a plant species-rich forest on a carbonate-buffered soil with vermimull humus has disappeared since long when the upper layers ran out of carbonate. Depending on the degradation history (level of biomass extraction, dominant tree species, level of acid precipitation) most of these forests reside in the mesotrophic stable state, being a rather plant species-rich forest on a base cation-buffered soil with an acid mull humus, or in the oligotrophic stable state, being a plant species-poor forest on an aluminium-buffered soil with a moder humus. There are different reasons why the oligotrophic state is a stronger attractor and thus a more stable state than the mesotrophic one, among which the fact that acid cations are more strongly bound on the exchangeable places than base cations, and the fact that the disappearance of endogeic and especially anecic earthworms in the pH_{H2O} range 4-4.5 is a semi-irreversible process due to the slow migration rates in this spatially continuous hostile environment. It is also hypothesized that shifting back from the oligotrophic to the mesotrophic state is only possible through an active restoration process, in which several driving forces are modified simultaneously. This is illustrated by comparing results of soil rehabilitation efforts from literature, where only one factor was corrected (mostly by liming) with results from our own integrated rehabilitation experiments, where tree species change, fertilization and earthworm reintroduction were combined.

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APPLICATIONS OF VERMICOMPOST AND VERMICOMPOST TEA IN THE BIOREMEDIATION OF PAH CONTAMINATED SOIL

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The bioremediation of soil contaminated with polycyclic aromatic hydrocarbons (PAHs) often involves the amendment of the soil with a source of organic matter, with the aim of enhancing the existing soil microbial community and increasing the rates of hydrocarbon degradation. Vermicomposts and their extracts ('teas') are a rich source of a wide range of microorganisms, which seem to provide a suitable soil amendment for enhancing rates of polycyclic aromatic hydrocarbon (PAH) biodegradation, as was demonstrated by a recent field study in Illinois. Our bench-top experiments examine the effectiveness of a cattle manure vermicompost, and liquid extracts of vermicompost (teas) amendments in enhancing the rates of degradation of pyrene (PYR) and phenanthrene (PHE) in a natural soil.

The experimental design comprised four soil treatments, with each treatment amended with a mixture of PYR (250 mg/kg) and PHE (500 mg/kg). Three control treatments (Webster clay loam (WCL) soil (pH - 5.5, organic matter - 2.4 g C/kg, cation exchange capacity -21 cmol./kg, clay - 36%), vermicompost, and WCL amended with 240 ml of 50% (v/v) vermicompost tea) were also included. The treatments treated with PAHs included: WCL + vermicompost (5% w/w), WCL + vermicompost (50%, w/w), WCL + vermicompost tea (240 ml, 5% solution (v/v), added to soil field capacity), and WCL + vermicompost tea (240 ml, 50% solution (v/v), added to soil field capacity). Three replicates of each soil treatment were incubated at 20°C and sampled for chemical and microbial analysis 0, 4, 8, 16, 32, and 64 days after treatment. The dissipation of PYR and PHE was assessed by measuring total PAH levels using a hexane extraction of soils, and the potentially bioavailable fraction of PAHs using solid-phase microextraction fibres (SPMEs) (30 um C18 coating) and gas chromatography. The microbial community structure and activity was monitored by measuring substrate-induced respiration (SIR), total microbial biomass, and phospholipid fatty acid (PLFA) composition. Dissipation curves for the PAHs and associated microbial parameters will be presented and discussed. These provide evidence of the potential of vermicomposts and vermicompost teas in the bioremediation of polluted soils.

MICROBIAL COMMUNITY CHANGES DURING THE VERMICOMPOSTING OF SPENT GRAPE MARC

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During the vermicomposting process, earthworms can modify the diversity and abundance of the microbial communities directly, by selective feeding or by stimulation of particular taxa of microorganisms, and indirectly, fragmenting and conditioning the substrate, increasing surface area for microbial activity, and altering its biological activity, and also by microbial dispersion and release of additional food resources in their casts. In our study, specific phospholipid fatty acids (PLFAs) were used to characterize and quantify the microbial communities during the vermicomposting process of spent grape marc.

Spent grape marc was turned and watered until the earthworms were capable of eating it. After this, 1 kg of spent grape marc (65% moisture, 50% C, 3.5% N) was introduced into each of ten plastic containers (24 cm diameter and 12 cm height). Juvenile and adult specimens of *Eisenia andrei* (250 g per container), were added into five of the containers, while the other five remained without earthworms and served as controls. All the containers were randomly introduced into a culture chamber at $25\pm2^{\circ}$ C. After 15 days, the control and spent grape marc (SGM) vermicompost samples were collected and sieved (<5 mm) in order to separate the stalks and seeds. Five replicates of the initial spent grape marc were also analysed for comparison. All the samples were lyophilized before being subjected to the PLFA analysis.

The canonical discriminant analysis of the thirty-nine identified PLFAs was sensitive enough to discriminate between the treatments with and without earthworms, and those from the initial spent grape marc. Using PLFAs as biomarkers, we found that Grampositive bacteria, identified by terminally-branched PLFAs (i14:0, a15:0 and a17:0), and Gram-negative bacteria, by monounsaturated PLFAs (16:1 ω 5, 16:1 ω 7 and 18:1 ω 9c) decreased after the vermicomposting process. Fungal biomass, estimated from the concentration of the biomarker 18:2 ω 6c, was also lower in the SGM vermicompost, whilst the protozoa, estimated by PLFAs 20:2c, 20:3 ω 6c and 20:4 ω 6c, were not present in any sample. Thus, the activity of earthworms had a negative effect on the abundance of Grampositive and Gram-negative bacteria, and also on fungi.

In summary, our results provide a new insight into how earthworms modify the microbial communities using PLFAs profiles and could also help in the difficult task of understand the interactions between earthworms and microorganisms.

ANTIMICROBIAL POTENCY OF *EUDRILUS EUGENIAE* EXTRACTS ON CERTAIN PLANT PATHOGENS

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Use of vermicompost as an organic amendment to agricultural lands is practiced by many Indian farmers and they opine that the application of vermicompost brings down the incidence of many soil borne diseases in crops. To investigate the facts about this view the laboratory research was carried out to study the effect of different preparations from the body of *Eudrilus eugeniae* earthworms on selected bacterial and fungal pathogens.

Since earthworms are soil residents and they play an active role in improving soil quality, it was hypothesized that worm extracts may have inhibitory/suppressive effect on soil-borne pathogens. Hence 3 bacterial and 5 fungal soil-borne plant pathogens were selected for the study. The result showed that the body wall and gut extract had both antibacterial and antifungal activities while coelomic fluid was found to have only antibacterial activity.

Body wall and gut extracts were found to have inhibitory effect on Xanthomonas campestris, Ralstonia solanacearum and Fusarium oxysporum. Erwinia carotovora was inhibited by gut extract and coelomic fluid. Botryodiplodia theobromae, soil-borne fungal plant pathogen, was suppressed by body wall extract, but at slightly higher concentrations. There was no inhibitory effect recorded by any of the worm extracts at the used concentrations during this study on *Rhizoctonia solani*, Alternaria solani, and Sclrotium rolfsii. Only delayed sporulation was observed in all the cases, but this was only a visual observation. There was no further work like hyphal length measurements or germination study conducted for these fungal pathogens due to various limitations.

Further studies are in progress to assess the concentration effect of extracts on response of organisms to the earthworm extracts.

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Preparations made of material of animal origin (placenta, snake and bees poison, dags, sea and river hydrocoles) were used extensively in folk and modern medicine for treating many human diseases. These preparations are also very important nowadays, though some of them are not used in modern medicine because of resource deterioration. Renewable natural medical material of animal origin and pharmaceutical preparations on their basis as well as biologically active food supplements are the future. This area of human activity and economics must be developed and improved in every possible way.

Earthworms (Oligochaeta) are the most ancient invertebrate animals on Earth. Over 3100 Oligochaeta species are known, but at present only 12-15 species are used as vermiculture in different countries of the world. The vermiculture is particularly good for bioconversion of organic wastes into organic fertilizer (vermicompost) and earthworm biomass as the source of proteins, peptides, enzymes and physically active substances production.

In many Southeast Asia countries earthworms have already been used for 2300 years to cure different human diseases. In Vietnam a dry earthworm powder produced according to different techniques that guarantee its purity is the compulsory ingredient of pharmaceuticals or "magic medicines that save life within 60 minutes". These pharmaceuticals are often used to treat various organ dysfunctions – the result of bacterial and viral infections.

Already in 1883 Charles Darwin observed that tissue fluid can dissolve fibrin. In 1986 Japanese researchers extracted a fibrin dissolving enzyme from *Lumbricus rubellus*. It consisted at least of six enzymes, collectively named lumbrokinase. Commercial fibrinolytic preparations are produced in China and Canada accordingly named Plasmin and Bolouake.

The vermicultivation technology is practically wasteless. Potential and perspectives of this state-of-art biotechnology may play am important role in three vital spheres of human activity on the Earth, i.e. environment protection, agriculture, health care.

Thus, vermicultivation is a modern biotechnology able to turn plant organic wastes into valid animal proteins effectively. Moreover, the earthworm biomass is a unique and renewable natural source to produce different preparations of biologically active substances.

Poster

EARTHWORMS AS ECOENGINEERS IN THE RESTORATION OF OIL AND BRINE-IMPACTED SOILS FOLLOWING REMEDIATION

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The restoration of soil ecosystems following remediation of oil and brine spills can be a l engthy process. As eco-based standards are implemented, the domestic oil and gas industry (and especially the small independent oil and gas producers) will need low-cost methods of accelerating the restoration of these sites. The key to this restoration will be the rebuilding of soil structure, which in turn stimulates soil microbial communities and results in revegetation to pre-spill levels of biomass and species diversity. We propose that re-introduction of earthworms to remediated sites can be a cost-effective technology to rebuild soil structure and restore disturbed soil ecosystems.

In a new project initiated in 2005 we seek to determine the efficacy of the re-introduction of earthworms to sites which have undergone remediation for crude oil or brine spills in order to accelerate the restoration of these sites in terms of soil quality and plant biomass and species diversity. Specifically we are examining the effects of three treatment variables: earthworms, organic matter, and fertilizer, on the restoration of the two previously remediated sites, a brine-impacted site and a crude oil-impacted site. Microbial community structure and diversity in terms of phospholipid fatty acid analysis, soil nutrient concentrations, numbers of nitrogen cycling bacteria, nematode numbers and trophic diversity, and earthworm numbers and reproductive status have been monitored.

This paper will describe the experimental protocol and discuss preliminary results. The most interesting result thus far has been the observation that these damaged areas do not have to be inoculated with earthworms. When hay cover and sufficient moisture were provided we observed that indigenous earthworms quickly invaded the site and that their impact on bioavailability of nutrients was evident in just 30-60 days.

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EVALUATING THE GROWTH CHARACTERISTICS OF LETTUCE IN VERMICOMPOST AND GREEN WASTE COMPOST

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A vermicompost trial was carried-out using commercially produced green waste-derived compost as the feed material. Replicated growth trials were undertaken with lettuce using pure worm casts, green waste compost and mixture of the two to determine the extent of growth enhancement gained through the vermicomposting process.

The mixture comprised of 50/50 (v/v) and 20/80 (v/v) of worm casts and green waste feedstock. Fresh and dry weights of the plants at the end of growth period are reported and it was observed that the plant growth was retarded whilst using pure casts, whereas, 20/80 (v/v) showed better results when compared with other treatments.

Leaf chlorophyll content is a key indicator of plant health and also helps in identifying the amount of nitrogen uptake by the plant. Using pure vermicompost for the plant growth yielded the lowest chlorophyll content, whereas, the others contained similar amounts of chlorophyll. The concentration of bio-available nutrients and heavy metals for each treatment, before and after the plant growth are also reported.

The root zone for each plant has been examined to determine the presence of suitable microbial symbionts (e.g. mycorrhizas) and other key microbial communities in various growth media.

Poster 249 VERMICOMPOSTING-ENHANCING THE QUALITY OF COMPOST DERIVED FROM GREEN WASTE?

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A vermicomposting trial was conducted for 18 weeks using commercially produced green waste-derived compost as the feed material. The aim of the study was to investigate the extent to which a combination of vermicomposting and the traditional windrow composting of green waste may be performed, in order to enhance the value of the final product.

Two identical reactor beds (A and B) were constructed and fed with mature screened compost (<10 mm) that was re-hydrated to a moisture content of 60 ± 2 % before vermicomposting using *Denbrobeana veneta*. The objective was to enhance the product quality at maximum throughput and waste processing capacity, therefore, a high stock density 7 kg/m² of earthworms was used. The reduction in earthworm biomass obtained is reported at various stages during the trial.

An average mass throughput of 32.6 kg/m²/week was achieved and overall mass reductions of 24 % and 20 % were observed for reactor A and B respectively at the end of trial. The rate of organic matter decomposition was also determined by measuring volatile organic matter of feedstock and casts material. The greatest reduction (35%, m/m) was observed during the initial stages of trial. However, no significant reduction was noted towards the end of vermicomposting process, when worm mass had reduced to approximately 1.2 kg/m².

Minor changes were observed for pH, electrical conductivity and redox potential. The temperature within the reactor bed remained within optimum range i.e. 15 to 23 °C. Chemical analysis of the total and bio-available nutrients and heavy metals concentrations of the substrate and casts materials before and after the treatment are also reported.

CARBON DIOXIDE DYNAMIC DURING THE PROCESS OF COFFEE PULP VERMICOMPOSTING TO MONITOR THE MATURATION OF THE VERMICOMPOST

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The carbon dioxide emission (CO_2 production) will be measured during the whole process (4 months) of coffee pulp vermicomposting in order to 1) Determine the contribution of earthworms (*Eisenia andrei*) to reach the stabilization of coffee pulp vermicompost, during and after the worm transit. 2) Use this parameter, CO_2 production, to calibrate the maturity level of vermicompost. The aforementioned is to have a quality control of the final product, which will show that the vermicompost is stabilized.

In Laboratory conditions (constant water content and temperature 25° C) hermetic boxes with 1 kg of fresh coffee pulp without and with 50 *Eisenia andrei* are incubated. The CO₂ production in the boxes will be measured by the classic method: the CO₂ is captured with NaOH and titulate with HCl. The CO₂ measurements will be performed 15 times during the days 2, 5, 8,11, 14, 19, 24, 29, 36, 43, 50, 57, 64, 77 and 90.

Simultaneously temperature, pH and C/N ratio of coffee pulp will be determined to correlate them with the CO_2 dynamic organic matter. Also the earthworm population (biomass, cocoon production) will be monitored. Therefore 84 experimental boxes will be settled and there will be for each measurement 4 replicates.

It is expected that the CO_2 dynamic will be more important during the first month of incubation and then the CO_2 production will decrease until it will become low and constant. When this phase is attained the pH will be neutral and the C/N ratio near to 10. The vermicompost is estimated to reach the stabilization phase before than compost.

EFFECTS OF SLUDGE AND MUNICIPAL SOLID WASTE COMPOSTS DISPOSAL ON EARTHWORM COMMUNITIES UNDER CROP FIELD CONDITIONS

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Before using different solid waste composts at large scale in agricultural fields, it is necessary to prove that these composts do not have harmful effects on soil fauna, especially on earthworms that play important roles in the soil ecosystem. A study was carried out the effect of two particular composts (a municipal solid waste compost (OMG) and a sludge compost (DVB)) on earthworm communities under field conditions. This study was conducted on the "QualiAgro" site (near Paris, France), an agricultural field where these composts are disposed every two years since 6 years and compared to controls (with (T+N) or without (TSA) N fertilisation). Earthworm communities were sampled in each treatment at different dates (a month before and then 2, 7, 9 and 13 months after) using mustard extraction followed by a manual sorting. Avoidance tests were carried out under laboratory conditions to determine whether earthworm surface migrations between treatments occurred. OMG had significant and lasting effects increasing earthworm abundances and biomass whereas DVB had limited and transitory positive effects (Figure 1). No effect was observed on species distribution except for TSA where less L. terrestris were sampled (Figure 2). The positive effects observed on abundance were not due to either (i) difference in the number of immature earthworms or to avoidance/attraction of soils with composts (Table 3). Moreover we found that in OMG (and DVB for one of the date), adults of A. caliginosa species had higher weights. Globally only positive effect on earthworm communities after disposal of these composts were observed. However only OMG seems to have effects that last between two successive disposals.

PERFORMANCE AND ENVIRONMENTAL IMPACT OF A COMBINED IN-VESSEL COMPOSTING AND VERMICOMPOSTING SYSTEM

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There is a growing interest in the use of combined composting systems designed to comply with legislation and/or to achieve specific technical objectives, such as producing high specification composts. However, there is little published data on the effectiveness of combined systems, especially relating to vermicomposting systems. In England, the use of commercial-scale vermicomposting to process and add-value to kitchen wastes is prohibited without first subjecting the waste to thermophilic composting, under enclosed conditions. Hence, a combined system is required if vermicomposting is to be utilised. The aim of this study was to investigate the performance and environmental impact of a combined system of composting comprising in-vessel thermophilic composting and vermicomposting, compared with a system comprising in-vessel thermophilic composting and windrow composting. Source segregated household waste was first subjected to invessel composting for 14 days to comply with the Animal By-Products Regulations (2003). After this, the partially stabilised waste was matured for 92 days using either a large-scale vermicomposting system or a windrow composting system. The maturation phase was replicated five times. The mature windrow compost and vermicompost were then subjected to bioassay evaluation using the PAS100 (2005) method. During all stages of processing, physico-chemical characteristics of the waste were recorded, stabilisation rates were measured using respirometry and greenhouse gas emissions were monitored.

During the initial stage of in-vessel composting, the waste lost 12% of its carbon content and 8% of its nitrogen content. The in-vessel composting system generated CH_4 and N_2O . During the 92 day maturation process, respirometric analysis of the windrow compost and vermicompost was undertaken. This showed the two processes to be equally effective at maturing the waste. In terms of greenhouse gas emissions, the vermicomposting process emitted N_2O mainly while the windrow composting process emitted CH_4 . At the end of maturation, the physico-chemical characteristics of each compost were compared. The nitrate (NO₃) content for the vermicompost was 43% greater than for the windrow compost while the mass of material in the smallest particle size fraction (<10 mm) was significantly higher for the vermicompost. Also, the electrical conductivity (EC) of the vermicompost was approximately 50% less than the windrow compost. When subjected to the PAS100 bioassay test, the vermicompost, compared with the windrow compost, produced significantly increased yields for one out of the three plant types tested.

It may be concluded that the combination of thermophilic in-vessel composting and vermicomposting produced a horticultural grade compost with enhanced physico-chemical qualities compared with the combined windrow system. However, there was no evidence to suggest that the vermicomposting system increased compost maturation time compared with the windrow system. Equally, the enhanced characteristics found for the vermicompost only resulted in increased yield for one plant type out of three tested. The study demonstrated that all composting and vermicomposting systems investigated had the potential to generate greenhouse gases (CH₄ or N_2O) and the implications arising from this are discussed in the paper.

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COMBINING CO-COMPOSTING AND VERMICOMPOSTING FOR THE EFFECTIVE TREATMENT OF PINEAPPLE AND SHEEP RESIDUES.

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Green waste derived from pineapple (Ananas sp.) processing has been used as forage for cattle by farmers in the Papaloapan area of Mexico (Aw2 climate). The work described here explores an alternative use of this pineapple-derived green waste as a potential soil ameliorant to enhance soil quality. Traditionally, farmers in the study area have used sheepderived manure as a fertilizer to enhance pasture quality. However, due to the environmental and sanitary consequences associated with manure accumulation in sheep housing (e.g. risk of zoonotic disease) this is not the best option. The main aim of this work is to combine the precomposting of pineapple and sheep residues with subsequent vermicomposting to obtain a stable organic fertilizer. Precomposting of sheep manure and pineapple green waste was done in three ratios (1:1; 2:1; and 3:1), over a period of 12 weeks followed by vermicomposting for a further 12 weeks. Time trials were employed to determine the growth and reproduction of Eisenia foetida in material precomposted for various periods of time. Also, earthworm preference trials were undertaken in triplicate. Our results showed that E. foetida was capable of achieving good rates of growth and reproduction in the co-composted pineapple green waste and sheep manure substrates. Combining traditional composting practices with vermicomposting may provide an option to produce an organic fertilizer of higher quality (e.g. reduced pathogen load and better nutrient content and balance). Further work is therefore necessary to test the organic fertilizers in vegetable production.

CHANGES IN THE MICROBIAL COMMUNITY STRUCTURE AND ACTIVITY DURING VERMICOMPOSTING OF PIG MANURE

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We studied how the epigeic earthworm *Eisenia fetida* modifies the microbial community during the vermicomposting process of pig slurry. We analyzed the microbial biomass and activity (microbial biomass carbon and basal respiration, respectively), substrate utilization patterns (Biolog Ecoplate) and fungal biomass (comparison between ergosterol content and PLFA 18:2w6c); moreover, specific phospholipid fatty acids (PLFAs) were used to characterize and quantify the microbial communities. We set up a batch of six vermireactors, three without earthworms (control) and three containing 500 mature specimens of Eisenia fetida each. Vermireactors were initially composed of one module containing vermicompost, in which earthworms were placed, and another module containing a layer of fresh pig slurry (1.5 kg fresh weight). New modules containing the same amount of fresh pig slurry were added when required; this procedure allowed us to date the addition of each module within vermireactors. At the end of the experiment, the vermireactors comprised 12 modules with an increasing gradient of age, resembling a soil profile, from upper to lower layers as follows: 2, 4, 7, 8, 11, 18, 21, 25, 27, 29, 33 and 36 weeks. Microbial biomass and activity were clearly enhanced in vermireactors with earthworms, and in particular in the upper layers, which contain the highest density of earthworms. Fungal growth, estimated by the ergosterol content, was also stimulated in vermireactors with earthworms and the highest fungal growth was observed in the upper layers. The community level physiological profiles obtained from Biolog Ecoplate data revealed that the presence of earthworms and the age of layers modified the microbial community structure of pig slurry. Our results provide a better knowledge of the modifications in the microbial community structure in a vermicomposting system, as well as permitted us to determine the ability of the different approaches to describe these changes.

ROLE OF EARTHWORMS AND NITROGEN ADDITIONS ON THE SOIL FERTILITY OF AN INTENSIVE CULTIVATION SYSTEM

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Earthworms have a positive effect on soil fertility as a result of their effects on both soil physical properties and nutrient turnover. Intensive agricultural practices have a dramatic impact on the soil system by reducing soil biodiversity, water infiltration and the amount of organic matter susceptible to be decomposed and incorporated into the soil.

Here we investigated the effect of the earthworm Lumbricus friendi in combination with nitrogen additions on the chemical properties of a soil obtained from a Kiwi fruit (Actinidia deliciosa (A. Chev.)) intensive plantation. 60 intact soil cores were obtained in December 2005 from one of the plots using PVC cylinders (11 cm diameter x 16 cm deep) and then brought to the laboratory where the surface vegetation was removed and the soil was watered to field capacity. Individuals of L. friendi were obtained from a grassland soil by hand-sorting and once in the lab transferred to the agricultural soil for acclimation prior their use in the experiment. Nine earthworms (average weight = 8.5 g) were inoculated in 45 replicates and designated as +Earthworm treatments (+E) with the remaining 15 being referred as control treatments (C). For the nitrogen additions two sources were considered: (i) white clover (*Trifolium repens* L.), a N_2 fixing plant, was planted in 15 cores containing earthworms and labelled as +Earthworm and Clover treatments (+EC), (ii) cow manure (7.5 g dwt) was added to another 15 of these worm replicates and used as +Earthworms and Manure treatments (+EM). The cores were incubated in a climate controlled chamber at 18 °C and 70% RH and with a 16/8 h day/night cycle for 15 weeks. Three random replicate cores from each of the four treatments were destructively sampled every three weeks. On each sampling occasion plant and earthworm biomass, CO₂ fluxes and dissolved organic carbon (DOC) and pH of the leacheates were measured.

Results showed that manure addition had a positive effect on earthworm biomass during the course of the experiment whereas no significant differences were detected between the +EC and +E treatments. This positive effect of the addition of organic fertiliser was also reflected in the C release from these cores (both CO_2 and DOC) when compared with the rest of the treatments. In addition, the acidity of the leachates was also significantly reduced by adding cow manure to the soil. Although the presence of earthworms in the +E and +EC treatments certainly improved the physical properties of the soil by soil porosity through their burrowing activities, their lower biomass in these treatments did not significantly affect any of the chemical parameters measured.

From the above, it can be concluded that in the short-term organic fertilisers provide a suitable food source ready to be used by soil populations (both earthworms and microorganisms), whereas that nitrogen sources from symbiotic fixing plants could possibly have an effect in the long term. The implications of this work have relevance to the potential role of soil fauna and N fertilisers in a more sustainable use of the soil.

THE EFFECT OF FARMYARD MANURE APPLICATION ON EARTHWORM BIOMASS ON LOWLAND WET GRASSLAND AND THE IMPLICATIONS FOR WADERS

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Surveys suggest a marked decline in the populations of many breeding waders in Britain, with formerly widespread species becoming increasingly concentrated, particularly in areas with directed management. Reduced breeding success is believed to be the underlying cause in some species. Low chick survival rates have been observed in several studies for which one hypothesis is a reduction in invertebrate prey availability. This research is part of an ongoing three-year project to assess the potential of low-level farmyard manure (FYM) application to increase the availability of the invertebrate biomass to foraging wader adults and chicks on lowland wet grassland. Earthworms are a particularly important food source for wader adults and older chicks. In this study, the specific response of earthworms (Lumbricidae) to FYM applied at rates of 5 tonnes ha⁻¹,10 tonnes ha⁻¹ and 15 tonnes ha⁻¹ is considered. Results to date show an application rate of 15 tonnes ha⁻¹ produced a significant increase in earthworm biomass availability, as measured by soil cores in the spring following an autumn FYM application, suggesting that this practice may have a role in habitat management directed towards improving foraging conditions for breeding waders.

THE EFFECT OF WILLOWS (SALIX VIMINALIS) AND EARTHWORMS (EISENIA FETIDA) ON SEWAGE SLUDGE TREATMENT.

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The production of municipal wastes is an increasing problem. In wintertime the sewage sludge cannot be used directly for fertilization and until spring it accumulates to high amounts. The aim of the study was check the suitability of willows (*Salix viminalis*) and earthworms (*Eisenia fetida*) for sewage sludge management. The study was conducted in Tarnów city sewage plant. In April 2004 81 pots were filled with concentrated sludge, 54 of which were planted with willows. In May, mature earthworms (20 individuals per pot) were introduced into 27 pots with willows. The remaining 27 pots with sludge only were controls. Every month the growth of willows was measured . In November 2004 the density of earthworms, the size of willows, the moisture and structure of sewage sludge and their chemical properties were estimated. The following observations have been made:

- the moisture content of the sludge was lower in the pots with willows (49%) and with willows + earthworms (37%) than in controls (67%);

- the mass increase of willow shots and roots was higher in pots with earthworms by 38% and 41%, respectively;

- the introduction of willows and earthworms changed the structure of sludge.

No effect of willows and earthworms on pH, nutrient, heavy metals and nitrogen forms concentration was observed.

VERMICOMPOSTING OF ORGANIC PART OF WASTES AT THE PLACE OF WASTE GENERATION: IN AN OFFICE ROOM AND A STUDENT HOSTEL

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The objective of this study was to analyze the possibilities for converting the organic wastes thrown into refuse baskets in office and student hostel areas, into a nutrient-rich vermicompost, at the place of residue generation.

Earthworm ecology boxes in the office room and student hostel corridor were functioning over a considerable period. *E. fetida* was fed on organic waste produced in that and adjacent rooms for ten months. During vermicomposting, *E. fetida* population grew by about 120-180% (50-70% growth of biomass).

E. fetida produced vermicompost which was rich in nutrients vital for flowerpot plants (pH 6.1-7.5; N-NO₃ 680- 1950; P 284- 370; K 2200- 4200; Ca 2600-3000; Mg 314- 550 mg \cdot dm⁻³ of the fresh mass). However it had a drawback of high salination (6.2-12.0 g NaCl \cdot dm⁻³).

The highest growth in earthworm number was noticed in the office's earthworm ecological box, what could result from the fact that large quantities of paper were also vermicomposted with the other organic rests, there. When waste thrown into waste backets was analysed in randomly selected office rooms in a yearly scale, it was found that paper waste had a dominating share (60%) in it. The share of compostable organic waste was 20%.

Between vermicomposted wastes in the office room wide diversify was observed (citrus fruit skin, skin of banana, cucumber, tomato, apple, bakery products, spent tea bags, dry leaves and small branches, nut shells, plum stones, used paper sheets, paper bags, sandwich wrapping paper, coffee filters, tissue paper, tissue handkerchiefs, envelopes, paper trays, etc.).

Students vermicomposted more food wastes.

VERMICOMPOSTING FOR SUSTAINABLE WASTE MANAGEMENT AND EDUCATION

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2005-2014 is the period of the Decade for Education for Sustainable Development announced by UNESCO. Sustainable development has three pillars: economic, social and ecological. Large-scale presentation of the new ideas, especially within the third pillar, is immensely important. To be a responsible citizen we should read a lot, get acquainted with new possibilities of actions and discuss them with our families and friends.

Using an earthworm ecological box will help to develop and practice many skills needed in every-day sustainable activities. When using earthworms for ecological activities in favor of sustainable development we can easily find that being responsible can be enjoyable for teachers and pupils or for children and their parents! By introducing vermicomposting to kindergarten, schools, universities, offices and enterprises it is hoped that a great environmental awareness will develop. Education of the children is the most important. It has to be stressed that well educated children will take care of education of their adult relatives.

In that paper major benefits of introducing earthworm ecological boxes for the youngest are presented. Working with worms in a "kindergarten /class /home vermi-recycling system" we can introduce a lot of various activities to the pupils. In order to increase their motivation we can grant them skills after a well performed task. The most obvious would be the skill of a worm breeder but apart from that we can introduce an activity that involves utilizing the humus produced by earthworms in order to acquire skill of a gardener. Another activity, this time on examining the earthworms, could give the pupils a skill of a scientist.

This system of skill reward can be made even much more complex and is limited only by the creativity of the teacher. There is no reason why pupils should not be able to obtain such skills as a skill of a journalist who reports on the value of vermicomposting to other classes and schools and other skills that at first might not seem relevant to the issue. Moreover, they should be informed that even a journalist should live up to the expectations of a sustainable society.

What is more, it is possible to earn money using the earthworm ecological box and its products. The class that breeds the earthworms may earn money by selling flower seedlings potted into vermicompost medium, breeding and selling aquarium fish fed with earthworm biomass (that are usually beautiful specimens, valuable to aquarium fish breeders), or selling earthworm population to an ecological box in a neighbouring class or schools as well as fishermen to use the earthworms as bait.

The whole experiment can be incorporated with many courses, namely, on mathematics pupils can be taught how to calculate the vermicomposting rate, the biomass growth rate etc; elements of economy may be taught by preparing a business plan of the entire enterprise, financial social and environmental benefits should be pointed out and made clear to the children.

Again it is only the creativity of the teachers that limits the enterprise's scale. Pupils may learn how to make vermicomposting boxes of various types during a manual training course, which is extremely beneficial as they may need to know how to make them in case they manage to encourage their parents to perform vermicomposting at home or at work.

VERMICOMPOSTING OF DUCKWEED (LEMNA MINOR) BIOMASS

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Since composting of the duckweed biomass is progressing slowly, an attempt was undertaken to vermicompost it with the help of *Eisenia fetida* Sav. earthworms.

The experiment presents the state of *E. fetida* earthworm population during vermicomposting of biomass of duckweed *Lemna minor*, in laboratory conditions (in an average temperature 25 ± 5^{0} C).

Ten-liter test pots (in 3 replications for each combination) were filled with one liter of garden soil. Than 100 individuals of *E. fetida* of known age structure and biomass were introduced into each. The duckweed biomass was given as a feed to the earthworms during vegetation period in two combinations:

1/ fresh duckweed + cattle manure (1 : 1),

2/ fresh duckweed.

The number and biomass dynamic of tested populations after 4 months (from June to October) were determined and it was found that *E. fetida* in containers with duckweed and manure, had better average parameters [the average numbers -121 ± 5 ind./container on duckweed and manure, 57 ± 6 ind./container on duckweed alone; total biomass -25.8 ± 1.12 g and 9.8 ± 1 g respectively].

Numbers of cocoons produced, were also higher on the same conditions. Weaker characteristics were observed in populations fed on duckweed without mixing with cattle manure (55 ± 13 and 231 ± 37 respectively).

The features of duckweed vermicompost were also found:

vermicompost 1 (produced from fresh duckweed + cattle manure): dry matter 84.1- 85.0%; organic substance 62-63.5%; ash 37.2-39.0%; pH 5.5-6.0; N- 25-28; P 5.4-6.0; K 12.0-17.5; Ca 20-25; Mg 4.1-5.3 g \cdot kg⁻¹ of dry mass, Na 600-1111; Fe 2000-3000; Mn 600-680; Zn 90-120; Cu 40-60; Ni 6.0-7.2; Co 0.7-1.05; Pb 7.8-10.0; Cd 0.25-0.90; Cr 4.0-7.0 mg \cdot kg⁻¹ of dry matter *vermicompost 2* (produced from fresh duckweed): dry matter 86.8-87.9%; organic substance 64-66%; ash 35-37%; pH 4.3-4.6.0; N- 23-26; P 4.0-4.8; K 3.5-4.7; Ca 2.6-3.1; Mg 2.4-3.0 g \cdot kg⁻¹ of dry mass, Na 540-1055; Fe 2600-3200; Mn 635-726; Zn 70-92; Cu 22.5-65; Ni 5.0-6.8; Co 1.20-2.34; Pb 7.02-9.27; Cd 0.20-0.45; Cr 2.55-5.02 mg \cdot kg⁻¹ of dry matter

EFFECTS OF VERMICOMPOST ON THE GROWTH OF TWO ORNAMENTAL PLANTS UNDER OPTIMAL GREENHOUSE CONDITIONS

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We studied the performance of two different vermicomposts as amendments into the potting media of two ornamental plants: *Viola wittrockiana* Delta (pansy) and *Primula acaulis* Oriental (primula), grown under optimal inorganic fertilization and chemical fumigation for pathogen control conditions in a commercial greenhouse. We hypothesized that vermicompost may show a beneficial effect beyond nutrient-mediated or pathogen control processes.

Pansies and Primulas were grown in 500 ml pots containing conventional greenhouse medium substituted with 5%, 15%, 25% and 50% (v/v) commercial and self-produced vermicompost. There were 5 replicates for each treatment and controls were made with no vermicompost addition. A continuous pesticide treatment was applied in the greenhouse during the experiment using different pesticides in order to avoid the appearance of resistant strains and a inorganic fertilizer was supplied to the plants by fertirrigation to avoid nutrient limitations. After 56 days for pansies and 117 days for primulas, different growth parameters were measured on the plants (root biomass, aerial biomass, number of leaves, leave biomass, number of flowers, flower biomass) as well as the photosynthetic efficiency (PEA). We determined also the pH and electrical conductivity of the substrates.

Both types of vermicompost had similar effects on plant growth. We observed a general reduction of growth on both species with increasing concentrations of vermicompost. Highest percentages of vermicompost showed between 20-60% of plant mortality. However, no effect or little reduction was appreciable in 5% vermicompost. Plants growing in 25% and 50% vermicompost showed high levels of stress and damage on the photosynthetic apparatus as showed by the PEA analysis, as well as a significant reduction on number and biomass of leaves. Reproduction of both plants was also affected with a decrease in the number and biomass of flowers.

An excessive nutrient input into the pots might have resulted into salt accumulation and physiological drought in the treatments with higher percentages of vermicompost. Consequently, the addition of vermicompost would allow the reduction on the amount of fertilizers normally used for ornamental cultivation.

We suggest that further investigation is necessary to determine whether vermicompost's effects are merely physical and nutrient mediated or, on the contrary, biological-mediated effects.

DEVELOPMENT OF INOCULATED AND NATURALLY COLONISED EARTHWORM POPULATIONS ON LANDFILL CAP AMELIORATED WITH COMPOSTED GREEN WASTE

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Large-scale green waste processing facilities are now common-place on landfill sites throughout the UK. The product, composted green waste (CGW), has commercial value as a soil conditioner, but surplus, lower quality material may also be used on site as additional daily cover. A more environmentally sensitive and cost effective use for the latter is incorporation into landfill cap to improve soil quality. A proven method of incorporating organic matter into soils is through the action of earthworms. The role of earthworms in the process of soil formation and maintenance of soil fertility is widely recognised and has led to their inoculation into degraded land to enhance soil restoration. However, development of sustainable populations is often limited by a deficit of organic matter. Therefore earthworm inoculation allied to CGW application may be considered as an effective route towards final restoration of a site.

This study was conducted at two landfill sites in the UK (Calvert and Clifton Marsh). At both sites, waste filled cells had been capped with (1-2 m) compacted clay (engineering cap). At Calvert this engineering cap was covered with a layer of less compacted clay but at Clifton Marsh a layer of soil / subsoil mixed with CGW was applied. At Calvert (1999), a range of surface application and mechanical incorporation treatments of CGW were established in 50 m^2 plots where earthworms had been introduced 7 years earlier. Monitoring showed that surface application of CGW promoted earthworm numbers and biomass. In 2003, 3 species of earthworms (Apporectodea caliginosa, Apporectodea longa and Octolasion cyaneum) were inoculated into 400 m² plots with a treatment of surface applied CGW versus control. Results indicated that addition of CGW increased earthworm survival and enhanced cap integrity. At Clifton Marsh (2005), earthworm populations were monitored from sites representing a chronosequence (1.5, 3.5 and 5.5 years after capping) and in undisturbed pasture (control) adjacent to the Landfill site. Natural colonisation was rapid with earthworm number and biomass in the 5.5 yr cap comparable with the control. Species number (4-6) was consistent across the 4 sites but community composition varied with location and was attributed to specific ecological requirements. Percentage of mature worms was inversely related to age of cap with 85, 52 and 23 % mature in the 1.5, 3.5 and 5.5 yr old sites respectively compared with 24 % in the control.

Results indicate that at sites lacking topsoil, surface application of CGW coupled with earthworm inoculation is an alternative and sustainable method of land restoration. In addition, incorporation of CGW into landfill capping material can promote the development of earthworm populations and increase site amelioration.

EARTHWORM *EISENIA FETIDA* (SAV.) BIOMASS AS AN ALTERNATIVE FOOD FOR BREEDING AQUARIUM FROG *HYMENOCHIRUS CURTIPES* (SOKOL, 1959)

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The aim of this research was to find the possibility for a cheaper way for feeding aquarium frog *Hymenochirus curtipes* (Sokol, 1959). The alternative usage of *Eisenia fetida* and *Enchytraeus albidus* biomass was chosen. Earthworm' and enchytraeid' biomass have been taken from the earthworm ecological box, which was also maintained.

Hymenochirus curtipes is a small amphibian (3–4 cm), with a long lifespan (up to 10 years), but relatively difficult to breed. It results from the fact that, as the literature points out, it has specific feeding requirements. It prefers living animal food that moves.

The frogs breeding was carried out in laboratory conditions, with 12 hours lighting cycle and constant temperature of 22° C (water pH = 8). In the experiment, very young specimens, from one litter were used (average weight: 1.052 ± 0.141 g).

Those amphibians were bred in three groups, with 5 specimens each. The controlled group was made up by frogs fed on standard food (living gnat larvae). The second group of frogs was fed on frozen earthworm biomass, and the third one was fed on living enchytraeids. Frogs were weighed every 7 days. The presented experiment lasted for 4 months.

As an effect of the study, it was noticed that the biomass of the earthworms and enchytraeids produced in earthworm ecological boxes, can be used for feeding this species of aquarium frog.

It is an interesting achievement that, after suitable preparations, dead food of frozen earthworm biomass was accepted.

Mean increase biomass per 7 days was the biggest in frogs fed on enchytraeids (0,0417 g), lower in frogs fed on living gnat larvae (0.0227 g), and the lowest in frogs fed on earthworms (0.0074 g) (no significant difference), but frogs fed on earthworms were more active.

EARTHWORM POPULATIONS IN PERMANENT MARGINS AND CREATED GRASS MARGIN STRIPS OF ARABLE FIELDS

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Field margins are recognized as important habitats for a wide range of animals in agricultural landscapes. However, little is known about their importance as a refuge for earthworms in intensively cultivated fields and as a potential source for recolonisation by earthworms of such fields following conversion to conservation management. The objective of this study was to assess earthworm populations in permanent field margins, newly created, uncultivated grass margin strips and in-field in the same arable fields.

In autumn 2004, five arable fields on different farms in south-eastern Ireland were selected and prepared for winter cropping, involving conventional ploughing in three fields and minimum tillage in the two other fields. In each field, one crop edge was selected for the creation of a new grass strip adjacent to the permanent field margin. These strips were 100 m long and between 2.5 and 4.0 m wide, depending on the drilling machine employed on each farm. The newly created strips were split into four sub-strips (25 m in length); two sub-strips were allocated at random to be either sown with a grass/wildflower mixture or left unsown to regenerate naturally. In each field, earthworm biomass, abundance and species richness were estimated in the permanent margin (hedge-base, fence-base or grass margin), the newly created margin strips and in-field (1, 3, 10 and 50 m along a transect perpendicular to the new margin strips). Starting in autumn 2004, earthworms were sampled in the autumn and spring of each year using a hand sorting method.

Earthworm populations were considerably (2-5 times) larger in the permanent margins than in the cropped in-field areas in all five fields studied on all sampling dates. Population sizes in the newly created, uncultivated margin strips increased rapidly, doubling within 6 months and approaching levels in the permanent margins 12 months after the creation of the strips, regardless of whether they had been sown or not. The transect in-field data suggest that earthworm population levels were not related to proximity to the newly created margin strips, not even in the two fields under minimum tillage.

In conclusion, while earthworm populations were larger in permanent margins and 12 months old grass margin strips than in the cropped area of five arable fields, there was no evidence that these refuges are a source for earthworm recolonisation of fields.

BIOTRANSFORMATION OF WINERY WASTES BY *EISENIA ANDREI* IN SOIL ORGANIC AMENDMENTS: A SEMIPILOT-SCALE STUDY

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Wine production is a major food industry in the world, especially in countries with a Mediterranean climate. Viticuture and the winery industry generate huge amounts of wastes and by-products and their disposal is regarded as a serious environmental issue in the main grape-growing regions in the word. The main wastes from the winery industries are vine shoots (VS), spent grape marc (SG), lees cake (L), vinasse (V), and after depuration of vinasse, biosolids vinasse (BV). In general, these wastes have a recalcitrant lignocellulosic nature and high content of toxic compounds, reason why its direct application to soils would have to be prohibited.

In this study we present the results of vermicomposting with *Eisenia andrei* of these winery wastes (SG, SG+L, BV+SG) in order to obtain suitable organic amendments to soils. The effectiveness of these substrates for vermicomposting was assessed by monitoring, monthly, earthworms growth and reproduction, as well as four enzyme activities (dehydrogenase, β -glucosidase, phosphatase and urease) over the course of a six-month semipilot-scale experiment. The chemical composition of initial substrates and obtained vermicomposts were also determined.

No mortality of earthworms was observed in any substrate. Total earthworm biomass increased in all substrates, reaching the maximum weight in BV+SG (68 g kg⁻¹ in the third month), SG + L (50 g kg⁻¹ in the second month) and SG (13 g kg⁻¹ in the second month). Maximal number of clitellated earthworms was reached between the second and third month in all substrates. After six months of vermicomposting, a low biomass was recorded in all winery substrates assayed. Changes in dehydrogenase activity and hydrolytic enzymes during the vermicomposting process led the biodegradation of the winery substrates and resulted in the disappearance of the initial toxicity of them. Vermicomposting caused a decrease in total organic carbon, available organic carbon, salinity and K₂O. In contrast, increases of nitrogen, P₂O₅, humic substances and micronutrients were observed at the end of the vermicomposting process. As consequence of these changes, the vermicomposts had lower C/N ratio and high humification rate, which would imply a higher degree of stability, humification, and polimerization of the organic matter contained in these final products. These characteristics, together with the appreciable amounts of plant nutrients, make these vermicomposts as soil organic amendments for being used in conventional, integrated and biological agriculture.

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STABLE ISOTOPE NATURAL ABUNDANCES (Δ¹³C AND Δ¹⁵N) OF THE EARTHWORM *EISENIA FETIDA* AND OTHER SOIL FAUNA LIVING IN TWO DIFFERENT VERMICOMPOSTING ENVIRONMENTS

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In nature, epigeic earthworms live in fresh organic matter such as litter mounds, manure heaps and herbivore dung. These environments are hotspots of heterotrophic activity where earthworms intensively interact with other soil fauna, bacteria and fungi deeply affecting decomposition rates. Strong biotic interactions and the rapid changes both in substrate quality and functional diversity are main properties of these systems. Vermicomposting systems, as a particular case of the manure heaps commonly found in agroecosystems, support a high detritivore biomass conformed by protozoa, rotifera, mites, nematodes, collembolans, enchytraeids and earthworms. It is known that epigeic earthworms accelerate decomposition processes, but where they obtain their energy inputs (i.e. decaying organic matter, microorganisms, microfauna or a combination of them) remains unknown; they may utilize from non-selective substrate feeding to grazers strategies, and have the ability to shift between living and non-living carbon sources. In this study we performed a first approach to the vermicomposting food web using stable isotope ratios in order to evaluate the suitability of this tool for studying this heterotrophic system. We studied the natural abundance of C an N stable isotopes in 66 samples of soil fauna and the substrates where the animals live in of two contrasting typical vermicomposting systems: (i) high feedingrate vermireactor, feed with pig slurry, and (ii) a cattle farm manure vermicomposting heap. Our aim was specifically (i) to test at what extent isotopic signal in the earthworms resemble those of the substrates were they live in, (ii) to advance in the knowledge of the ontogenic changes in resource utilization of the earthworm *Eisenia fetida*, and (iii) to get insight about the relative trophic position of the food web evolving in the vermicomposting systems. Tissues of earthworms were significantly enriched in ¹⁵N regarding to fresh and mature manures in both vermicomposting systems by 4 to 8‰. The δ^{13} C values of adult earthworms were not different from those of the fresh animal wastes in both vermicomposting systems, supposedly tracing the origin of their carbon source. The little but significant enrichment in ¹⁵N observed in hatchlings living in the pig slurry vermicomposting bins relative to adult tissues likely reflect different feeding strategies, not observed in the cattle manure heap. Hatchlings in the cattle manure heap appeared markedly depleted in 13 C relative to the adult earthworms by ~5‰, evidencing the use of a different source of carbon in the early stage. Based on the shifts in δ^{15} N, the taxa in the pig slurry vermicomposting bins could be assigned at least to three relative trophic positions separated by a Δ^{15} N shift of 2‰, with Enchytraeida clearly in the lower position, adults and hatchlings of *E. fetida* and nematodes in an intermediate level, and Colembolla at the higher position showing an enrichment of 9‰ relative to the substrate. In the cattle manure heap three trophic levels could be identified as well, with larvae of Diptera and Coleoptera as the less ¹⁵N enriched level, a general detritivore group in intermediate position, and finally a predatory taxa with a +9‰ shift comprised by Staphylinidae.

A STUDY ON THE EFFECTS OF COMPOSTING OF PARTHENIUM PLANT AND NEEM LEAVES IN THE PRESENCE AND ABSENCE OF AN OLIGOCHAETE, *EISENIA FETIDA*

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Parthenium plants and neem leaves were composted using the epigeic earthworm, Eisenia fetida and to study the growth and reproductive indices of earthworm involved in the process of composting. Efficacy of the resulting compost in supporting the growth of plant has also been tested with Vigna radiata Co3. There is no significant difference of growth and reproduction rate in all the treatments of neem amendments compared with control. Parthenium has significantly reduced the growth and reproductive efficiency of *E. fetida* in the amendment concentration of 100 gms. Increase in the quantity of parthenium caused not only significant reduction in growth rate but also mortality of earthworm (<10%). The statistical analysis employing two way ANOVA showed a significant difference in the growth rate of worms between parthenium plant and neem leaf treatments. Significant increase was recorded in the EC, K, organic carbon and C/N ratio and significant reduction was recorded in the P, Cu and Zn contents of parthnium plant vermicompost. Significant increase was recorded in the EC, K and Fe and significant reduction was recorded in the pH, N, Cu, Zn and organic contents of neem leaves vermicompost. The C/N ratio of the parthenium plant and neem leaves vermicompost remained almost the same as that of the control (cow dung) vermicompost except highest amendment concentration of 100 gms of parthenium plant. Between parthenium plant composts and neem leaves composts significant differences were not observed in any of the plant biometric parameters.

THE TEN-YEAR RESULTS OF TREATMENT WITH THE EXTRACT OF EARTHWORM TISSUES (PREPARATION «VERMIVIT»)

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Preparations made of material of animal origin (placenta, snake and bees poison, dags, sea and river hydrocole) were used extensively in folk and modern medicine for treating many human diseases. These preparations are also very important nowadays, though some of them are not used in modern medicine because of resource deterioration. Renewable natural medical material of animal origin and pharmaceutical preparations on their basis as well as biologically active food supplements are the future. This sector of human activity and economics must be developed and improved in every possible way.

Earthworms are ancient invertebrate animal on Earth. They play an important role not only in the food chain of ecosystem, but they also influence physical chemical properties of soil composition and enforce microbiological processes in soil itself. About 4000 kinds of earthworms are known to humanity, but nowadays only 10-15 are used as vermicultures in different countries. Manure (compost) worm *Eisenia fetida* (Savingy, 1826) is particularly good for "multi goal" usage. Earthworms are grown for two main reasons – to turn organic wastes into organic fertilizers (vermicompost/biohumus) with maximum effect and to increase the biomass of earthworms as the source of proteins, peptides, enzymes and physically active substances. Thus vermiculture is a modern biotechnology that helps turn organic wastes of plant origin into animal proteins of full value effectively. Moreover, the biomass of earthworms is a unique and renewable natural material for producing different preparations of biologically active substances.

The medical value of earthworms has been known for a long time as is evident from the history of the ancient medicine of South-Eastern Asia (Japan, China and Vietnam). The ointments and extracts prepared from earthworm tissues have been used for the treatment of numerous diseases.

The preparation "VERMIVIT" is an alcoholic extract from the earthworm *Eisenia fetida* tissues after a special treatment: density 0.939, protein concentration 8 g/l, amino acids concentration 1.7 g/l, 17 microelements, vitamins, essential amino acids. The course of treatment was 1 teaspoonful of the preparation "VERMIVIT" dissolved in 50 ml of boiled water 3 times a day before meals during 3 weeks. After a short break for laboratory tests the course of treatment was repeated.

The comparative examination has been performed in two groups of patients: one of which consisted of 31 and another of 25 persons (a control group), including 11/7 men and 20/18 women at the age from 22 to 72 years old. Affections: shotty breast – 11/10, malignant swellings of the 4th clinical group – 5/5, adiposity – 3/5, osteochondrosis – 9/3, psora – 2/2, other diseases – 1/0. The treatment by the agent lasted from 1 to 3 months for 15 patients, up to 1 year for 14 ones and up to 3 years for 2 patients. The results of a 4-year follow-up observation revealed the recovery of 9/0, improvement in 17/7, without effect – 2/15, death of 3/3 of progressive tumoral process. No pathologic changes have been revealed in patients taken the extract from the earthworm over a long period of time. There is no medicine to cure shotty breast, it is treated now only surgically. Further researches are necessary to extract a specific substance from earthworm tissue to make a medical preparation against this disease.

COMPARATIVE STUDY OF EARTHWORMS IN COCOA SYSTEMS OF THE CHONTALPA, TABASCO, MÉXICO

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In the state of Tabasco the economic activity is dominated by the agricultural products, being the plantations of cocoa (*Theobroma cocoa* L.) the ones that provide important foreign currencies in the national and international market. Mexico in 2003 produced 1.9% of international cocoa production, being in the 11th place as an international producer. In Mexico, Tabasco is the first producer of cocoa. The cocoa production of Tabasco is settled down in the Chontalpa region where three types of handling are applied: traditional, intensive and organic management. This agroforestry system has ecological importance because of its vertical structure and due to the varieties of plants that are being used for the establishment of this culture, it serves as refuge for a great diversity of species either to the conservation of the soil. That makes favorable the development of the soils organisms as earthworms (oligoqueta). Their functions according to its ecological classification are: to transport, to mix and to bury the vegetable residuals from the surface to the interior of the soil, with their galleries promote the aeration and infiltration of the soil, they produce effects in soil structure when they are producing small or big casts depending on the species.

The aim of this study is to know if the handling of the cocoa systems can or can not modify the structure and composition of earthworms species, for what this study helps to settle down which are the conditions of fertility in the studied soils. On the other hand, they have been carried out few studies directed to manipulate endemic species that demonstrate their application to transform organic wastes, and to use them as vermicomposters in order to increase soil fertility with their vermicompost. That's why the earthworm selection of the cocoa system can help cocoa systems producers.

VERMICOMPOST IN SEEDLING POTTING MEDIA CAN AFFECT GERMINATION, BIOMASS ALLOCATION, YIELDS AND FRUIT QUALITY OF THREE TOMATO VARIETIES

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Commercial potting media often contain substantial amounts of peat that is harvested from endangered bog and fen ecosystems. The main aim of this study was to assess whether vermicompost produced from food and cotton waste by Eisenia fetida Sav. can be used as a peat substitute. The specific objectives were to assess (1) whether the amendment of 0, 20, 40, 60, 80 and 100% (vol/vol) of vermicompost to a fertilized commercial peat potting substrate has effects on the emergence, growth and biomass allocation of three tomato varieties (Lycopersicon esculentum Mill.) under greenhouse conditions, and (2) whether possible impacts on seedlings are manifested in altered tomato yields and fruit quality when transplanted into equally fertilized field soil. Results showed that vermicompost amendments affected seedling emergence, growth and shoot and root production specifically for each variety. Marketable yield and total yield of field grown tomatoes was not affected by vermicompost amendments of seedlings. However, morphological (circumference, dry matter content, peel firmness) and chemical parameters (contents of C, N, P, K, Ca, Mg, L-ascorbic acid, glucose, fructose) of fruit quality were significantly affected by the amount of vermicompost amended to the substrate used to raise seedlings. These effects were different between varieties and there was across varieties no correlation between the proportion of vermicompost amended and fruit quality. Overall, results showed that vermicompost could be an environmentally and economically sound substitute for peat in potting media with favourable effects on tomato fruit quality. However, variety-specific responses should be considered when giving recommendations on the optimum proportion of vermicompost amendment to horticultural potting substrate.

SESSION 1 - EARTHWORM BIODIVERSITY: CONVENTIONAL AND MOLECULAR APPROACHES TO SYSTEMATICS

NEW PATTHERN OF DISTRIBUTION OF EARTHWORM (ANNELIDA, OLIGOCHAETA) IN COLOMBIA

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An effort was made to construct the patterns of distribution of earthworms in Colombia taking into account the information from collections made from 1872 to 2006. The information includes fragmented isolated data, sporadic registers with only taxonomic information of the species (Michaelsen, 1900; 1913; 1918) and systematic studies conducted in some hydrographic basins and savanna regions (Feijoo, 1993; 2001; Jiménez 1999), highlighting the high diversity in mountainous areas of the Andes. Most (93.2%) collections were carried out in the Andean region. The Colombian Oligochaeta fauna comprises 125 species placed in nine families, 30 genera and six subgenera, of which 99 are native and 26 exotic. Most of the species are endemic and belong to the Glossoscolecidae family (16 genera). *Andiodrilus* is represented by the greatest number of species (25), while *Martiodrilus* with 21 species, and shows the highest habitat diversity, from forests to pastures and even the Páramo. Two species in the family are cosmopolitan (*P. corethrurus* and *O. elegans*) currently inhabiting anthropic environments. Excepting *Tumak hamenni* and *Dichogaster medellina*, the remaining species of the other families are eurytopic,.

SESSION 1 - EARTHWORM BIODIVERSITY: CONVENTIONAL AND MOLECULAR APPROACHES TO SYSTEMATICS

RELATIONSHIPS BETWEEN LAND USE AND EARTHWORM COMMUNITIES IN THE LA VIEJA WATERSHED, COLOMBIA

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In La Vieja river basin (Colombia) samples of earthworm communities were taken from the plot to the landscape levels, six windows of 1 km2 with 16 sampling sites and 96 monoliths located 200 m apart from each other were were used to predict and to describe the conditions of the different agroecosystems. The sampling method used was the Tropical Soil Biology and Fertility Program (TSBF) in 51 farms and 22 different land uses.

To analyze the influence of human activities, the differences in abundance, biomass, number of species and some physical and chemical parameters of the soil were evaluated among the different landuses of the landscape, this was done using principal component analysis with the interaction of variables and the determination of the main correlations and the IndVal (Dufrêne, M. and P. Legendre, 1997) analysis were used to find indicator species and groups of species that characterized the sampling.

Thirty two earthworm species were found, these were located in four families and 13 genera, of these, thirteen (13) were native, seven (7) exotic and eleven (11) unknown. The species belonged to the following ecological categories: epigeas (22) and engoeas (9) and the mayority (14) were found in the 0-20 cm earthlayer with an average vertical distribution at 7.5 cm of depth.

The abundance values within the earthworms permitted the separation of landuses into conserved and degraded with earthworm species indicators of change identified with de IndVal. There were statistically significant (p < 0.05) differences in the abundance and number of earthworm species between land use. It is concluded that soil bioindicators are suitable for monitoring human pressure as well as the effects of introduction some land use may increase the abundance and diversity of earthworms.

THE DEVELOPMENT OF EARTHWORM POPULATIONS ON SELECTED FLOODPLAIN SITES OF RIVER ELBE AND RIVER MULDE AFTER GREAT (CENTURY) FLOODING IN 2002

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The great flooding on the river Elbe and some of its tributaries in August 2002 was an incident of extraordinary violence and extreme devastation which has not been observed for more than hundred years ("century flooding") in Central Europe. On selected floodplain sites, different in sedimentation regime (soil texture), flooding frequency and vegetation (grassland and forest) the development of earthworm populations (species composition, abundance, biomass, dominance structure and population structure) has been studied from autumn 2002 until spring 2004.

The flooding led to a patchwork of different sedimentation and the same heterogeneity could be observed with earthworm populations. Some communities were extinguished totally, some reduced to single specimen, but there were also some populations so well adapted to flooding conditions that they survived rather well and increased in autumn 2002 and spring 2003. The summer 2003 was characterized by extraordinary drought which prevented an optimal development of the earthworm communities and reduced the potential of recolonization. The regeneration of the earthworm populations is discussed under the aspects of soil conditions, flooding frequency of the sites and its vegetation.

Poster

SESSION 3 - EARTHWORM IMMUNOLOGY AND PHYSIOLOGY

DEVELOPMENT OF A FLOW CYTOMETRIC, NON-RADIOACTIVE CYTOTOXICITY ASSAY IN *EISENIA FETIDA*: AN *IN VITRO* SYSTEM DESIGNED TO ANALYZE IMMUNOSUPPRESSION OF NATURAL KILLER-LIKE COELOMOCYTES IN RESPONSE TO 7,12 DIMETHYLBENZANTHRACENE (DMBA)

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Traditional methodology for measuring cytotoxicity involves the use of radioactive chromium $({}^{51}Cr)$ in a ${}^{51}Cr$ -release assay, which is costly and imposes safety considerations. We have developed a non-radioactive cytotoxicity assay to measure cytotoxicity mediated by natural killer (NK)-like effectors in the earthworm Eisenia fetida using a two-color fluorescence assay. This assay has been reported to correlate well with results obtained using conventional ⁵¹Cr-release assays (Kroesen et al., J. Immunol. Methods 156, 47-54, 1992). A human tumor cell line, K562, was used as the target cell population and NK-like coelomocytes isolated from the coelomic cavity of E. fetida were used as the effector cell population. In order to differentiate targets from effectors, the targets were labeled with 3,3'-dioctadecyloxacarbocyanine (DiOC₁₈(3) or DiO) which is a green fluorescent dye that stains the plasma membrane. The targets were then incubated with effector cells together with the membrane impermeable red dye propidium iodide (PI) which labels the DNA of only those cells with a damaged plasma membrane. Targets killed by NK-like effector cells were double-labeled exhibiting coincident green membranes (DiO) and red-nuclear (PI) staining. Intact targets and dead effectors were single-labeled, exhibiting only green or red fluorescence, respectively. Fluorescence was detected and measured using flow cytometry. Specific cytotoxicity was determined by gating on the DiO-positive population (targets), and determining the percentage of green target cells exhibiting both DiO and PI fluorescence in the gated population. This methodology was employed to analyze the immunosuppressive effects of the pollutant 7,12 dimethylbenzanthracene (DMBA) on earthworm coelomocytes with NK-like activity. Coelomocytes were incubated in vitro with DMBA for different periods of time (0.5-16 hours), different temperatures (9-28°C), and at a range of DMBA concentrations (0-100µM), before adding DiO-labeled targets and PI. Flow cytometry was carried out at the end of the assay, and data was analyzed using WinList5.0 software and WinList's Interative N-Color Compensation System. Between 25-60% of earthworms used demonstrated a significant decrease in the ability of their coelomocytes to kill targets following DMBA exposure in all assays performed compared to control. These experiments demonstrate how flow cytometry can be used to measure cytotoxicity by earthworm coelomocytes cultured and exposed to DMBA in vitro to study the immunosuppressive effects of environmental pollutants such as the polycyclic aromatic hydrocarbon DMBA on NK-like effector cells of E. fetida.

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