The 9th International Symposium on Earthworm Ecology

5th – 10th September 2010

Xalapa, Mexico



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Introductory and Magistral Lecture

Earthworms, Gaia's best friends

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It is said that worms went out from the sea 500M years ago and colonised terrestrial environments. Since then, earthworms known for at least 100M years, have developped a large diversity of life forms distributed among several thousands of species able to exploit any ecological niche in the soil and litter environment. They have developped intense interactions with all soil organisms through their remarkable activity as soil ecosystem engineers. They have also become efficient protectors of plants through still little understood mechanisms. We only know so far that very intimate interactions involving plant gene expression are at hand. As a result, earthworms are major providers of soil ecosystem engineers.

Earthworms must have gone through a number of major disturbances and changes. The shift from Gymnosperm to Angiosperms dominant vegetation must have profoundly affected the quality of organic inputs in litter and soils. Closer to us, they lived the great Quaternary glaciations and recolonised the newly formed soils after retreat. The Anthropocene is now at hand. Many earthworm species will likely disappear as a result of conversion or modification of their natural habitat. However, a small number of peregrine species, specially adapted to face great disturbances are already working to limit damages to soils and plant communities, and the remarkable ability of the group to create new species will likely replace the extinct pool of inadapted species. Earthworms will remain Gaias's best friends and help go through one more disturbance.

Session 1

Earthworm Biodiversity and DNA Bar-coding



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Molecular data on the point of revolutionizing earthworm systematics

James Samuel

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From the simple questions of correct application of names to formally described taxa to testing hypotheses of ancestral relationships and origins of faunas, molecular data are bringing clarity and focus to the scientific investigation of earthworm diversity. These data are also upsetting some enduring species concepts, challenging taxonomic opinions regarding synonymy, and may bring precision at the cost of abandoning exclusive reliance on morphology for identification of earthworms. The global earthworm DNA barcoding campaign based at the Canadian Centre for DNA Barcoding has uncovered cryptic diversity within many common earthworm lineages, as have other working groups. These discoveries lead to the need to match lineages to type material, and we can anticipate that some names will be restored from synonymy. This is not always possible and the taxonomic community may need to take action necessary to secure the future of earthworm identification. Bayesian phylogenetic analyses of 18s + 28s sequence data reveal taxon trees that do not correspond to classical earthworm systematics or the implicit homology statements of those systems. These force us to evaluate morphology in different ways and to redefine higher levels of the classification. Earthworms appear to be very old, but we need to do more to calibrate molecular clock(s) in order to estimate dates of divergence and the present and paleo-biogeography of earthworm higher taxa.

Shifting clone diversity and morphometry across northern Europe in the parthenogenetic earthworm *Eiseniella tetraedra* (Sav.) including samples from sites exposed to radioactive pollution

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We have earlier surveyed clone pool structure and variability of morphometrical characters in parthenogenetic earthworms around the Baltic Sea. Here we give results on clone pool diversity and morphometrical variation in the riparian parthenogenetic lumbricid *Eiseniella tetraedra* (Sav.) as indicated by sampled specimens from Norway, Sweden, Finland and the Komi Republic bordering the Ural mountains. A pattern of geographic displacement of clones from west to east was observed and the overall clone diversity decreased towards the east. We also found that fresh weight and posterior body length were lower in adults of the east than in those of the west whereas the number of posterior segments did not differ statistically significantly between the countries. We hypothesize that the decreasing eastward trends may indicate adaptation of populations to the more severe continental climate of the east. There were, however, significant differences between populations within a country perhaps attributable to local factors. As for generative characters no clear-cut geographical west-east trend was observed. In the Vodnyi village (Komi) E. tetraedra populations occupied top soils contaminated by exposure to long-term ionizing radiation resulting from radium extraction from groundwater. The upstream samples of the Ukhta river running close to the contaminated site at Vodnyi (Komi) were made up of several clones, while the samples downstream were almost invariable, including the ones from the sites with the highest levels of background radiation. Further studies are needed to ascertain whether this invariability is related to environmental pollution because clone assemblages in the samples from varying distances of Vodnyi were either variable or monomorphic.

Phylogeographic and adaptive study in the earthworm *Lumbricus rubellus* sampled from locations corresponding to southern European glacial refugia and extending into northern Europe

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Earthworms play key direct and indirect roles in many soil processes, in food web ecology, and ecosystem functioning. For these reasons they are highly significant as bio-monitoring species. Due to their keystone and ecological engineering status, it is essential to achieve a better knowledge and understanding of their population structures, evolutionary histories, and genetic diversity. It is highly likely that during the last glaciation period, the lumbricid fauna of Great Britain was completely eradicated, with the post-glacial fauna having migrated from one or more refugia in mainland Europe extending from Iberia to the Carpathians. A probable consequence of this can be found in the paucity of species in Great Britain, consisting of only ~26 species. In comparison, the number of extant species in southern mainland Europe is ~180 species. Despite the importance of representative lumbricids in ecotoxicology, a large-scale genetic analysis of the patterns of dispersal of certain relatively ubiquitous species from recognized glacial refugia and the subsequent (re)colonization of northern latitudes is conspicuously lacking. The present project ultimately aims to study the phylogeography of the acid-tolerant, cosmopolitan, epigeic species Lumbricus rubellus with regard to the post-glacial colonization of North Europe using mitochondrial DNA markers and next-generation sequencing tools. The project will also investigate the evolutionary processes and metabolic responses of populations that have established sustainable footholds on polluted sites. The Cardiff laboratory has already identified two deeply-differentiated mitochondrial lineages within L. rubellus. The specific purpose of the pan-European collaborative initiative presented here was to explore the mainland European origin(s) of northern including (especially) British L. rubellus populations, and to verify the spatial/temporal clustering of the two presently recognised lineages. We present the preliminary phylogenetic study based on mitochondrial genotype (COI and COII loci) data obtained from specimens from sites in Great Britain, Spain, Portugal, France, Italy, Serbia, Poland, Estonia, Denmark, Austria, Hungary, Sweden, Finland and Norway.

The never ending story of the *Aporrectodea caliginosa* species complex: new insights into its phylogeny

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The taxonomical status of the species included in the *Aporrectodea caliginosa* complex has been a matter of discussion for centuries. Recent phylogenetic analysis added useful molecular information, showing that A. trapezoides is not a subspecies of A. caliginosa as traditionally thought, and that it is genetically more related to A. nocturna and A. longa. In this study, we sequenced more earthworms from the above-mentioned species, and in addition four morphologically very similar species, never considered before to belong to the species complex, were included: A. giardi, Nicodrilus tetramammalis, N. monticola and N. carochensis, the three last ones recently described and considered by taxonomists as synonyms of A. caliginosa. A total of 250 earthworms collected in 47 populations from Spain, France, Portugal, Italy, Greece, Turkey, Egypt and Australia were sequenced. More emphasis was put on the sequencing of A. trapezoides as one of the aims of this study was to reveal the phylogeography of this parthenogenetic species (see Fernández et al., this volume). The second aim was to study in depth the phylogeny of the complex including the abovementioned species. The third one was to check with molecular tools if the three above-mentioned species of Nicodrilus were similar to A. caliginosa. Two mitochondrial (cytochrome oxidase I -456 bp- and II – 564 bp) and two nuclear (28s - 675 bp - and histone 3 -311 bp) genes were chosen as molecular markers. Phylogenetic analyses (Neighbor-joining, Maximum parsimony, Maximum likelihood and Bayesian) revealed two clearly separated and statistically wellsupported lineages in A. trapezoides: lineage A, in the North of Spain and France, and lineage B, in Central and Southern Spain and the rest of the studied countries. Regarding the phylogeny of the whole complex, lineage A of A. trapezoides clustered together with A. nocturna, A. longa and A. giardi, suggesting that from the molecular point of view A. giardi should be included in the complex of species. The lineage B of A. trapezoides clustered together with the newly described species of the genus Nicodrilus. After these results the taxonomical status of A. trapezoides should be reconsidered, as both lineages were genetically more related to different amphimictic species, suggesting a different origin of the two lineages. In addition, there is strong molecular evidence to support that not only the Nicodrilus species cannot be considered as synonymous of A. caliginosa, but also that they should be included in the genus Aporrectodea, at least until a further revision supports the idea of Nicodrilus.

Oral – Session 1

The genome of the earthworm Lumbricus rubellus

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Our understanding of an organism's biology is greatly advanced through the study of its genome. Previously, sequencing a metazoan genome was a major undertaking applied to a handful of model organisms and their close relatives. However, second generation sequencing has made it possible to produce a good-quality genome sequence for any chosen species. There are as yet no genome sequences published for Annelida. Embracing the new sequencing technology, we have generated and assembled a draft genome for the earthworm Lumbricus rubellus. Three wild-caught specimens showing low genomic divergence were chosen for sequencing using Illumina SOLEXA and Roche 454 technologies. From ~40 fold coverage we have assembled an estimate of the 430 megabase genome. We annotated the genome sequence using the extensive expressed sequence tag (EST) resource as well as numerous computational analyses. The genome sequence is likely to be nearcomplete, as we have been able to identify genomic coordinates for 90% of the ESTs. Gene prediction softwares identify about 23,000 protein coding genes. We have used expert manual annotation to explore key metabolic pathways, identify gene families of interest, and to map the age and pattern of expansion of transposable elements. The genome sequence will be a springboard for further analyses, such as genomic biology of metal and other pollutant responses, the dynamics of epigenetic modification of the genome by methylation and definition of novel metabolic pathways.

SeqAWorm: Sequencing a Soil Sentinel

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Next generation sequencing has been harnessed to generate a draft of the 440Mb of the Lumbricus rubellus genome, heralding a new era in our understanding of oligochaete biology. This resource is providing immediate insight into the functional components that drive earthworm biology and ecology. The presence of conserved essential metabolic pathways is contrasted by the identification of homologues to proteins associated with vertebrate hearing and sight which may underlie earthworm sensitivity to vibration and light. Further comparative genetics has identified the gene encoding earthworm chitin which plays an essential role in the structure of chaetae and in providing a non-abrasive surface in the crop and gizzard. We have overlain the genome with a range of cDNA libraries generated from other representative members of the Lumbricidae using classical expressed sequence tag (EST) approaches, subtractive techniques, and next generation technologies. This exercise has yielded novel insights that are significantly enriching the functional aspect of the genome sequence resource, whilst also serving as a framework for intra- and inter- library comparisons and gene-object association. The future assembly of the draft genome into a physical map associated with karyotype, together with the generation of high density SNP maps, will allow the rapid and effective identification of the genetic basis of observed phenotypes. Furthermore, the genome also provides the basis from which to probe the relationship between the epigenome and the environment in organisms with a body plan and mode of existence that contribute to an intimate relationship between the animal and its substrate. However, far from being the end of a process this collaborative earthworm sequencing venture provides the scaffold on which to build a deeper understanding of the molecular mechanisms that drive fundamental aspects of the endocrinology, ecology, evolution and ecotoxicology of a taxon whose ecological engineering and sentinel status is widely acknowledged. Acknowledgement: This initiative was funded by a research grant (NE/F001185/1) awarded by the UK Natural Environment Research Council.

Oral - Session 1

The taxonomic puzzle of Hormogastridae earthworms: molecular insights

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The classical taxonomy of earthworms is somehow limited due to their anatomical simplicity and the variability of their characteristics often used for diagnosing species. In this study we use molecular tools to unravel the phylogeny of Hormogastridae. This family includes four genera and 22 species (and eight subspecies or varieties) of large to middle-sized earthworms distributed almost exclusively in the countries and islands of the western Mediterranean where they play a very important ecological role. The genus Hormogaster includes 18 of the 22 described species and the Iberian Peninsula is host to the highest diversity, with 16 species located in central and eastern Spain. We collected 375 individuals representing the four described genera and 20 species in 46 different locations from the Iberian Peninsula, France, Corsica and Sardinia. Molecular markers included mitochondrial regions of the cytochrome c oxidase subunit I gene (COI), 16S rRNA gene and tRNA Leu, Ala, and Ser, two nuclear ribosomal genes (complete 18S rRNA and a fragment of 28S rRNA) and two nuclear protein-encoding genes (histone H3 and histone H4). Genetic diversity values within the family are very high and similar to those present within lumbricids. The genus Hormogaster is polyphyletic and so is Hormogaster pretiosa, stressing the need of a taxonomic revision. Vignysa could be the ancestral form of the family, although the relations among genera are not clearly defined, as shown by the incongruence of results obtained among the different analyses. Hemigastrodrilus seems to be related to the H. elisae complex, and they are both found in the Atlantic drainage. H. elisae represents one evolutionary lineage with a high genetic diversity and five cryptic species (Novo et al. 2009) that we propose as a new genus within Hormogastridae. Corsica and Sardinia seem to have been under two different genetic influences. The species located in Corsica and Northern Sardinia seem to be more related to Vignysa, whereas H. pretiosa, in the Southern area of Sardinia, where it was originally described and seems to be restricted to, is more closely related to some Hormogaster species from Northeast of Spain, where two different lineages are present. A molecular clock calibration of the tree using the separation of the Sardinian microplate (at 33 MY) indicated that the diversification of Hormogastridae may be very ancient, starting from 97 to 75 MY ago.

Taxonomic chimaera: an overlooked plague exemplified by the common nightcrawler (*Lumbricus terrestris* L.)

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In spite of a critical contribution to global biodiversity, soil organisms have weakly engaged the attention of taxonomists compared with epigeic taxa and, even in groups intensively studied such as lumbricid earthworms, the use of DNA barcodes has recently revealed an unsuspected number of cryptic species often impossible to separate on a morphological basis. In this study, we used morphology and COI sequences to assess phenotypic and genetic variability among: (1) 181 specimens of the common night crawler (Lumbricus terrestris Linnaeus, 1758) freshly collected in European and North American populations; (2) three specimens of the neotype series of L. terrestris collected in Upsala (Sweden) in 1973; (3) one co-type of Lumbricus herculeus (Savigny, 1826) (a species recently synonymized with L. terrestris) collected in Versailles (France) in 1826; (4) specimens of five related Lumbricus species. We obtained full-length barcodes from most of the freshly collected specimens, and a series of smaller fragments (150bp) assembled in a sequence of 480bp for the co-type of L. herculeus. In addition a mini-barcode of 150bp was produced from one of the three specimens of the L. terrestris neotype series. The fresh specimen sequences highlight the existence of two different groups of L. terrestris, which are slightly different by morphology (body size and weight), but have strongly divergent COI sequences (mean distance of 17.6%). A Neighbor-Joining tree of all sequences indicates that L. terrestris s.l. is paraphyletic with L. festivus the sister group to the *L. terrestris* clade containing the *L. herculeus* co-type specimen sequence. The sequence from the specimen of the L. terretris neotype series was in the other L. terrestris clade. These results indicate that L. terrestris as traditionally identified is composed of two cryptic species that have not been discriminated in the literature. One of these species is the now valid L. herculeus. Based on a detailed meta-analysis of the scientific literature dedicated to "L. terrestris", we demonstrate that this taxonomic confusion may dramatically alter the reliability of published data. The attribution to a single scientific name of characteristics measured on two cryptic species may in fact lead to the description of a kind of chimerical organism which finally does not correspond to the true properties of any of the real species.

Genetic diversity and structure in European populations of *Lumbricus terrestris* (L. 1758) (Lumbricidae) as detected by Amplified Fragment Length Polymorphism (AFLP)

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To explore the genetic structure in populations of the anecic earthworm *Lumbricus terrestris* on a European scale we investigated populations at 14 locations in Europe. The study was driven by two main questions: (1) Is genetic diversity of populations decreasing with increasing post glacial migration distance and/or geographic isolations? and (2) Can we identify preferential distribution pathways of L. terrestris by comparing the genetic patterns of different populations. An adapted AFLP method (Amplified Fragment Length Polymorphism) was applied to this species. There was no clear overall pattern of genetic diversity. However, low values in France and Sweden could be explained by specific origins or geographical characteristics of sampling locations. The Monmonier algorithm, the Wombling method and cluster analysis based on different genetic distance measures identified genetic boundaries in Europe, i. e. regions with a sharp change in genetic information. These boundaries indicate several regions in Europe with high genetic similarity, probably due to an origin from the same refuge populations after the Last Glacial Maximum. L. terrestris populations from the Balkans are connected to Northern Europe, i. e. Sweden and Finland, whereas France and possibly Italy are more isolated from the rest of Europe. There is strong evidence that L. terrestris depended on human agricultural activity to spread across Europe and establish new populations. The pattern of spread of agriculture, during the neolithic revolution, approximately 9000 years ago, can help to explain today's patterns found in the genetic structure of L. terrestris populations. Molecular methods, like AFLP, are helpful tools to investigate origin, dispersal, genetic diversity and dynamics of soil organisms which are often difficult to monitor.

DNA bar-coding of New Zealand endemic earthworms

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Endemic earthworms from New Zealand were described by Lee in 1959. Since this date, very few studies have focused on this important group and Lee's taxonomic key remains the only resource available to identify what are thought to be the 170 species endemic to New Zealand. As in most taxonomic keys, it is mainly based on sexual characteristics that are visible only on adult specimens. However, the majority of earthworm individuals collected during ecological studies are juveniles and therefore cannot be identified in this way. This problem is particularly true in communities that contain many species because juveniles from different species can easily be confounded. As part of an opencast coal mine restoration project, an inventory of endemic earthworms communities from the West Coast of New Zealand's South Island was made in various habitats across a 2600 ha area. We used recognizable taxonomic units (RTUs) to separate juveniles collected in the vicinity of a coal mine and we performed DNA analyses to check for RTU reliability and to assess the number of species present. DNA analyses targeting the 16s mitochondrial gene lead to the identification of 17 clades likely to correspond to 17 different species endemic to New Zealand. When adults were collected, both morphological and DNA analyses were conducted, with results indicating that at least eight species could be new to science, because adult morphology did not match that of any of the 170 species described by Lee. The discovery of so many new species in a small area (2600 ha) suggests that the total number of New Zealand endemic earthworm species determined by Lee is likely to have been greatly underestimated. Although Lee's geographical coverage of the country was comprehensive, New Zealand's complex landscape provides many isolated and undisturbed areas where many earthworm species may remain to be collected and identified. This has important implications for earthworm conservation and their role in ecological communities.

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Oral - Session 1

10 years of earthworm molecular phylogenetics: lessons learned and perspectives

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The upcoming 2011 is the 10th year since the publication of the first molecular phylogenetic study of earthworms by Jamieson et al. (2002). After that, in the special issues for the 7th and 8th ISEE, two papers were published by Pop et al. (2003, 2007). With the rapid development on DNA sequencing technology and analytical tools, however, only a few cases have proved this approach successful. In the top journals of molecular phylogeny and population genetics, such as Molecular Phylogenetics and Evolution, Zoologica Scripta and Molecular Ecology, only five full papers focusing on earthworms have been published, a number much lower than both aquatic oligochaetes and leeches, and no genus- or family-level revisions have been achieved using molecular tools. On the other hand, the advances of DNA barcoding seem to be more promising, when used with morphology at the same time, in (dis)proving taxonomic hypotheses. We briefly reviewed published studies, and compared them with some of our unpublished data. All the published sequences of the mitochondrial cytochrome c oxidase subunit 1 (COI) from GenBank were re-examined by reconstructing trees for different families. We found that molecular phylogenetic trees of earthworms are often characterized by short internal branches and long external ones, suggesting rapid diversification at different taxonomic levels, which makes it difficult to achieve a robust tree topology using limited DNA sequence lengths. We further categorized the commonly used genes into four categories based on their evolutionary rates, they are (from fast to slowly evolving genes): 1) COI, cytochrome c oxidase subunit 2 (COII), NADH dehydrogenase subunit 1 (ND1) and internal transcribed spacer (ITS); 2) 12S rRNA and 16S rRNA; 3) 28S rRNA; and 4) 18S rRNA. We then proposed an approach that analyzes a total of >2,000 bp from different genes at the same time for studying earthworm systematics. The genes from groups 1 and 2 are suitable for studying intrageneric phylogeny or related genera, whereas those from groups 2 and 3 are for studying within-family systematics. The 18S rRNA (group 4) is usually used for class- and phylum-level phylogeny and thus is not suitable for studying earthworms. Finally, for DNA sequences in GenBank, some scientific names of the sequences are incorrect, which could be from misidentification, misspelling of names and use of junior synonyms. These data should be carefully used when making taxonomic inferences.

A tribute to the enduring status of Dr Ken Lee's New Zealand earthworm work

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This review is based on the major opus completed over 60 years ago by Ken Lee (1959) as modified by Blakemore in Lee et al., (2000) and in Glasby et al. (2010) based on the information presented at the "Species 2000" meeting held Jan. 2000 at Te Papa Museum in Wellington, New Zealand and finally published in 2010. Lee not only described local species, he also revised all major world families into a system familiar to students of the classical systematics of Michaelsen (1900) and Stephenson (1930). In the current checklist Acanthodrilidae, Octochaetidae and Megascolecidae sensu Blakemore (2000) are all given separate family status, yet much of Dr Lee's taxa are still current and valid. Whereas 193 species were described in Lee (1959), the current list has just over 200 (sub-)spp. The fauna is comparable to adjacent regions, especially Tasmania which at 67,800 Km2 is about one quarter the size of New Zealand (267,000 Km2) yet has 230 known earthworm spp (Blakemore, 2000). However, as much of the north island of NZ is volcanic and the south island is partly alpine less area is available for successful earthworm colonization and perhaps a lesser biodiversity is to be expected. Ken Lee's earthworm work was heavily grounded in applied ecology and soil management. He devised a broad classification of ecological strategies applicable to all major families of earthworms, rather than later systems that were more applicable to just Lumbricidae in Europe. Lee's (1959) prior ecological classification is restated.

An annotated update on the British and Irish earthworm checklist with new records from Botanic Gardens

Blakemore Robert

National Science Museum, Tokyo, Japan.

All known megadrile species reported from the UK and Eire are presented in an annotated checklist including the first record from Ireland of *Amynthas corticis* (Kinberg, 1867) and *Pithemera bicincta* (Perrier, 1875) from the Botanic Gardens, Dublin. That some Lumbricidae (except possibly the North American *Bimastos*) may have originated in the Middle-East, and been introduced along with agriculture since the Neolithic, is supported by several species in common with the 34 Levant species from Pavlicek *et al.*, (2004). Additional reports of megadriles introduced from around the world being recovered from Gardens, such as at Kew and Dublin, attest to one mechanism of accidental introduction (and for certain Lumbricidae, Megascolecidae and Eudrilidae there are deliberate importations for vermi-composting/bait markets). It is unlikely that many of the sub-tropical species from botanic gardens could readily survive temperate winters outside of the glasshouse, unless there is substantial climate change. Records from Sims' & Gerard's (1999) British Isles account were of 48 (sub-species), most of which are commonly found in other countries. The present tally includes recent reports plus some records marked with "?" giving a new total of *ca.* 69 taxa.

Informatics of Asian earthworms based on ICZN types and COI barcodes

Blakemore Robert

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Opportunity to access type material and genetic sequencing has allowed revision and renewal on neglected parts of the Asian fauna. Current species checklists and specimen DNA profiles are uploaded and freely available, e.g. on Annelida Resources (www.annelida.net/earthworm/) and Genbank (www.ncbi.nlm.nih.gov/genbank/) websites. Most recent data will be summarized in this paper, part of a short series of pragmatic works on Japanese earthworm eco-taxonomy and its value to primary production. The first earthworms from the Far East were scientifically described by Kinberg (1867), Horst (1883) and Michaelsen (1891/2) with primary (10?, 100??) types still extant in European institutions. Re-evaluation of these and other Asian species, some uncharacterized by types either by initial oversight or later loss, may require neotypification or topotypic avatars. A combination of specimen availability, online access to primary descriptive literature, standardized genetic barcoding and rapid data distribution via Internet publication will allow the taxonomic service to make rapid, continuously updated progress towards resolving nomenclatural uncertainty. Ancillary considerations are the "Clarity of vision and courage of purpose" (Wheeler 2004: http://www.jstor.org/pss/4142253) needed from individual taxonomists and natural history museums to bring about the revolution of classification into the Bio-Info-Tech Age, especially in 2010 - the UN designated International Year of Biodiversity

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Abundance and Distribution of *Pontodrilus litoralis* (Grube, 1855) on the coasts near Cabo Rojo, P.R.

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The purpose of this investigation was to determine the structure and dynamics of the populations of the oligochaete *Pontodrilus litoralis* on the coasts near the Cabo Rojo Lighthouse in Puerto Rico. During the months of May of 2008 and February 2009, sampling was made at low tide on the sandy littoral shores of two beaches on each side of the lighthouse. Distances from the tide mark and depth stratification in sand were taken to account. Environmental factors such as temperature, pH, soil moisture, organic matter, and surface accumulation of plant debris were registered. Spatial distribution of *Pontodrilus litoralis* was determined to be aggregated in clusters. These clusters were predominant at the high tide mark, where sand was always humid but never completely submerged under seawater. Organic matter and temperature were determined to be the primary factors in the distribution dynamics of this species. Sites with the lowest temperatures seemed to have the greatest number of individuals. Sites with a high accumulation of surface plant debris presented the lowest temperatures as well as the highest organic matter contents. Sites with clusters of individuals recorded the most acidic pH due to heightened cell respiration rates where individuals are numerous. Only a small percentage of the individuals sampled had reached adulthood, indicating a high reproductive output once the worms enter the reproductive stage but a relatively slow growth to reach it. A coastal survey of the island was carried out to examine the presence of *Pontodrilus litoralis* along different coastal habitats. This resulted in a significant increase in Puerto Rican records of Pontodrilus litoralis.

Ken Lee's specimens revisited

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Lee's morphology-based keys, coupled with modern molecular techniques were used to identify earthworm species and estimate species diversity in the vicinity of a coal mine in New Zealand's West Coast. To help species identification and study earthworm phylogeny, we attempted DNA sequencing on 9 museum specimens collected by Lee in the 1950s. This project aims at putting together the taxonomic expertise of Ken Lee and modern molecular techniques for a better understanding of New Zealand earthworm taxonomy and phylogeny. Because museum specimen are more than 60 years old and have not always been stored in ideal conditions for molecular analyses, techniques for analyzing ancient DNA were used an attempt to amplify the DNA. A PCR-based analysis targeting a short internal fragment of the mitochondrial 16s gene was conducted. DNA was successfully amplified and sequenced from some 60 year-old specimens. Results were compared to DNA sequences of recently collected earthworms. This study could be the starting point of systematic DNA barcoding of Lee's museum specimens. Such a project would provide a useful database for the identification of New Zealand endemic earthworms in the future, especially for ecological studies where juveniles can represent 90% of the collected individuals.

Taxonomic characterization of *Lumbricus* species (Oligochaeta: Lumbricidae): comparison of molecular and morphological phylogenies

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Taking into account that earthworms play an important function as key-organisms in terrestrial ecosystems functioning, the present lack of knowledge on phylogenetic relationship between species, weakens study interpretations applied in ecology, biodiversity and evolution. In light of this, the aim of our work is to clarify lumbricid taxonomy: the first objective is to test species distinction based on morphological characters. The second objective is to compare phylogenetic relationships obtained by morphological and molecular approaches. In this work, we focus on the genus *Lumbricus* represented in France by 6 species. First, we use data stored in Lombricien2000 database, representing the earthworm taxa collected and the morphological characteristics described by Bouché (1972) for each species. Species life history traits are included among morphological characteristics (e.g. clitellum and tuberculum position, pores position, Morren's gland, body size). Then, we pursue a molecular approach on individuals sampled in France. Molecular phylogenetic analyses are based on the sequences of nuclear (18S, 28S) and mitochondrial (16S, COI, COII) gene regions and performed with maximum likelihood and bayesian inference. Statistical processings are realized on morphological data to study the contribution of morphological characteristics to separate species (Multiple Correspondence Analysis) and to define interspecific relationships (Hierarchical Ascendant Classification and maximum of parsimony). Further statistical analyses are implemented to compare information from molecular and morphological data to build phylogenetic relationship. Our genetic results corroborate the taxonomy of the different species studied and confirm that morphological characters are pertinent to determinate species in this genus. Our molecular phylogenetic results enable us to propose a hypothesis about the relationship among *Lumbricus* species. Additional work of our study should be developed to test our different hypotheses, notably by applying new molecular markers described in the literature, or going to be developed. A further approach will include measuring morphological characteristics on earthworm individuals sampled and compared to molecular analyses to permit a real comparison between the two types of data.

Spatial Distribution of Primitive Genera in Lumbricidae

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Earthworm paleogeographic studies are limited by a lack of fossil individuals. However, their limited species distributions due to a low dispersion, associated to a low number of families, are relevant to test the geographical history of plates. Indeed, our work is based on the morphological studies initiated by Bouché (1972) on certain genera of Lumbricidae to explain the evolutionary history of the North Mediterranean area. According to their morphology and ecology, species belonging to Prosellodrilus, Zophoscolex and Scherotheca genera are considered as primitive. Several characteristics states present in Lumbricidae firstly appeared in Prosellodrilus genus (e.g. setae distribution, diverse bladder types) suggesting that this genus is the most primitive in Lumbricidae family but the evolutionary meaning remains unclear (Qiu, 1998). Moreover, as Zophoscolex genus, Prosellodrilus genus contains endogeic species. The genus Scherotheca presents adaptations related to the anecic mode of life, such as a strong development of capacities to move in soils (e.g. long body) and to feed on superficial litter (e.g. developed gizzard). Furthermore, this genus seems to diverge from *Prosellodrilus* genus. According to these particular features and their presence in the south of France, the aim of this work is to try to explain the present distribution of these three genera focusing on history of the North Mediterranean area. We use data stored in Lombricien2000 database containing sampling sites (around 2000), the morphological characteristics (around 210) described by Bouché (1972) for each taxon collected and related to each site. We established the cartography of each genus (and subgenus) using ArcGIS software based on sampling sites coordinates in order to have a visual idea of species distribution. Statistical processings are realised to study the contribution of morphological characteristics (e.g. clitellum and tuberculum position, pores position, Morren's gland, typhlosolis, body size) to separate species (Multiple Correspondence Analysis) to interspecific relationship (Hierarchical Ascendant Classification). Results demonstrate a particular distribution of species in south of France, structured and associated to a subgenus classification. The link between species distribution and species phylogeny based on morphological analyses will be presented and discussed in order to develop phylogeographic hypotheses.

Poster - Session 1

Phylogenetic analysis of New Zealand earthworms (Oligochaeta: Megascolecidae) reveals ancient clades and cryptic taxonomic diversity

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We have constructed the first ever phylogeny for the New Zealand earthworm fauna (Megascolecinae and Acanthodrilinae) including representatives from other major continental regions. Bayesian and maximum likelihood phylogenetic trees were constructed from 427 base pairs from the mitochondrial large subunit (16S) rRNA gene and 661 base pairs from the nuclear large subunit (28S) rRNA gene. Within the Acanthodrilinae we were able to identify a number of wellsupported clades that were restricted to continental landmasses. Estimates of nodal support for these major clades were generally high, but relationships among clades were poorly resolved. The phylogenetic analyses revealed several independent lineages in New Zealand, some of which had a comparable phylogenetic depth to monophyletic groups sampled from Madagascar, Africa, North America and Australia. These results are consistent with at least some of these clades having inhabited New Zealand since rifting from Gondwana in the Late Cretaceous. Within the New Zealand Acanthodrilinae, major clades tended to be restricted to specific regions of New Zealand, with the central North Island and Cook Strait representing major biogeographic boundaries. Our field surveys of New Zealand and subsequent identification has also revealed extensive cryptic taxonomic diversity with approximately 48 new species sampled in addition to the 199 species recognized by previous authors. Our results indicate that further survey and taxonomic work is required to establish a foundation for future biogeographic and ecological research on this vitally important component of the New Zealand biota.

Phylogeny of the genus *Balanteodrilus* (Oligochaeta: Acanthodrilidae)

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For more than 70 years the genus Balanteodrilus Pickford 1937 remained monotypic, with Balanteodrilus pearsei as the only described species. B. pearsei is an endogeic polyhumic species that exhibits variation mainly in size and genital markings, and that is widely distributed in southeastern Mexico and Belize. Recently, two species were described from the same genus: B. psamophilus and B. extremus, both of them separated from B. pearsei only by the posterior displacement of prostatic pores (20 and 21 respectively). In one locality B. psamophilus was found coexisting with a small morph of *B. pearsei*, and it was hypothesized that differences in length could be the result of character displacement or the effect of very sandy soils. Recently, however, more populations of the small morph were localized in different localities and in one case it coexisted with large individuals of the same species (*B. pearsei*). Likewise ecological experiments on reproductive isolation suggest that the small morph represents a different biological species. In order to clarify if the three named species (B. pearsei, B. psammophilus and B. extremus) and the small morph of B. *pearsei* are valid phylogenetic species, we will conduct a phylogenetic analysis using sequences of mithocondrial COI (710bp) and 16s (439bp) and nuclear ITS (ca. 600bp) genes of 3 specimens per location of *B. psammophilus*, *B. extremus* and several populations of *B. pearsei* including the small morph. DNA sequences will be analyzed using Maximum Parsimony, Maximum Likelihood, and Bayesian; as outgroups we selected Kaxdrilus silvicola and Lumbricus terrestris. We expect to find two lineages of evolutionary divergence in the genus Balanteodrilus, one related to size and the other one to displacement of prostatic pores.

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Earthworms from Central Mexico: the importance of mountains and undisturbed forests for native species maintenance

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As a part of a general project that intends to characterize earthworms in the plateau and mountains of central Mexico, we sampled earthworms in several, low disturbed sites of the state of Puebla. Sites included pine, oak and fir forests of several important mountains, as well as some lowland pastures and tropical forests. Sampling was mainly qualitative, although in some sites 10 quantitative monoliths were made (50 x 50 cm side and 20 cm depth). Sampling was conducted during the rainy season of years 2007-2009. We found 20 species, 7 native and 13 exotic. All native species belonged to Acanthodrilidae, within subfamilies Acanthodrilinae (five species), Benhamiinae (one) and Octochaetinae (one). Native species were found inhabiting well preserved mountain forests, although they were always less abundant than exotics. From a biogeographic point of view, native earthworms characterized the following regions: Neo-volcanic Axis: *Protozapotecia* sp., *Zapotecia* sp., *Dichogaster eiseni* and *Ramiellona* sp.; Gulf of Mexico: *Balanteodrilus pearsei* and *Diplocardia* sp., and Oriental Sierra Madre: *Protozapotecia aquilonalis*. The native and new species found in this study confirm that Mexican mountains still harbor a rich earthworm fauna, which however is highly threatened by human disturbance and exotic invasions.

Regional earthworm DNA barcode database, BOLD system and their applications: a case study from Taiwán

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The application of DNA barcoding in soil ecology and other disciplines that use earthworms as model organisms requires a DNA barcode database for the regional fauna. However, to the best of our knowledge, no such database has been published for any country. In this study, we present results from Earthworms of Taiwan [EWTW] project, one of the world's earthworm DNA barcoding campaigns registered in the Barcode of Life Data (BOLD) Systems. Our current database comprises 470 sequences of the mitochondrial cytochrome c oxidase subunit 1 (COI) from 82 species, including 30 undescribed species and 52 out of the 75 described species from Taiwan. The results support monophyly of most species. Intraspecific variations were generally high in endemic species but low in cosmopolitan ones. Levels of sequence variation are appropriate for species separation but not suitable for interspecific phylogenetic reconstruction. Furthermore, we demonstrate the application of the Taiwanese earthworm database and the BOLD Systems in three current studies: 1) the parthenogenesis of Amynthas catenus and Amynthas hohuanmontis; 2) the identification of the first domicile earthworm sample from Asia (which is also the first record outside the Europe and the Middle East); and 3) a soil ecotoxicological study using the model animal *Eisenia andrei*. Finally, we show that an integrative taxonomic approach adopting both morphology and DNA barcodes may facilitate the discovery and description of new species.

Phylogenetic relationships of the *Aporrectodea caliginosa* earthworm complex based on DNA sequences

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The Aporrectodea caliginosa species complex includes the most abundant earthworms in grasslands and agricultural ecosystems of the Paleartic region. Historically this complex consisted of the following taxa: A. caliginosa s.s. Savigny, 1826, A. trapezoides Dugés (1828), A. tuberculata (Eisen, 1874), and A. nocturna Evans (1946). These four taxa are morphologically very similar and difficult to differentiate because of their morphological variability. Consequently, their taxonomic status and their phylogenetic relationships have been a matter of discussion for more than a century. To study these questions, we sequenced the COII (686 bp), 12S (362 bp), 16S (1200 bp), ND1 (917 bp), and tRNAsAsn- Asp-Val-Leu-Ala-Ser-Leu (402 bp) mitochondrial and 28S (809 bp) nuclear gene regions for 85 European earthworms from 27 different localities belonging to the A. caliginosa species complex and four outgroup taxa. DNA sequences were analyzed using maximum parsimony, maximum likelihood, and Bayesian approaches of phylogenetic inference. The resulting trees were combined with morphological, ecological, and genomic evidence to test species boundaries (i.e., integrative approach). Our molecular analyses showed that A. caliginosa s.s. and A. tuberculata form a sister clade to A. trapezoides, A. longa, and A. nocturna, which indicates that A. longa is part of the A. caliginosa species complex. Our analyses also showed the presence of highly divergent lineages within A. caliginosa, A. trapezoides, and A. longa, suggesting the existence of cryptic diversity within these taxa.

Molecular discrimination of cytochrome c oxidase in *Eisenia andrei* and *Eisenia fetida*

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Eisenia andrei and *Eisenia fetida* are two closely related epigeic earthworm species. Taxonomic identification of earthworms is often difficult since most of morphological and anatomical characteristics are variable. These two species were first described as a different morphotypes of *E. fetida* according to differences in body pigmentation. Later on they have been considered as subspecies (*E fetida andrei*, *E. fetida fetida*). Therefore we aimed to develop a new approach for objective molecular determination of these species, namely molecular analysis of gene for cytochrome c oxidase was performed. Total RNA was isolated and reverse-transcribed using Superscript II RNase H- Reverse Transcriptase. Resulting cDNA was used for PCR reaction. PCR products were cloned and the complete sequences of cytochrome c oxidase of both species were obtained. Cytochrome c oxidase sequence data sets were aligned. Sequence analyses confirmed their identity as distinct species. Based on these results we have designed sets of specific primers for both species. The simple PCR reaction is then required for reliable, simple and relatively rapid discrimination of *E. andrei* and *E. fetida*.

Genetic variability and population genetics in the parthenogenetic lumbricid *Aporrectodea trapezoides* (Dugès, 1828)

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Parthenogenesis is a common phenomenon among lumbricids. Until the moment, little attention has been paid to shedding light into the genetic diversity of this kind of earthworms. This study examines the genetic variability and population genetics of Aporrectodea trapezoides (Dugès, 1828). All the studied earthworms were morphologically and anatomically identified as A. trapezoides following the taxonomic key in Gates (1972), specially taking into account the position of setal genital tumescence (in segments 9, 10 and 11), the location of tubercula pubertatis (undivided in segments 31 to 33) and male sterility (empty spermathecae and male funnels, both in segments 10 and 11). A previous phylogenetic analysis (Fernández et al., in prep) showed that this species is clearly divided in two main lineages: lineage A in Northern Spain and Europe, and lineage B in the Central and South part of the Iberian Peninsula and other Mediterranean countries. One mitochondrial gene (cytochrome c oxidase subunit I, 456 bp) and one nuclear gene (histone 3, 311 bp) were chosen to analyse in depth the genetic variability and population genetics in both lineages. In total, 189 and 79 individuals (Cox I and H3, respectively) collected in 36 localities from 8 different countries were sequenced. The Cox I data revealed 31 haplotypes, one of them shared by half of the sequenced individuals. The H3 data revealed 8 haplotypes. In both cases, the haplotypes were exclusive to every lineage. Both genes showed higher haplotype and nucleotide diversity than expected in a parthenogenetic species. Genetic divergence between lineage A and B in both genes was very high, suggesting that the two lineages are cryptic species.

Construction of an *Eisenia fetida* Bacterial Artificial Chromosome (BAC) library and the genomic analyses of VCP genes

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Earthworms are established environmental indicators and possess unique biological features; however the detailed knowledgebase of underlying molecular mechanisms remains sparse. Suzuki et al. (Gene 362:11, 2005) previously described the genes which are specifically expressed in the gonads of Eisenia fetida (Savigny, 1826) and isolated two genes, evcp1 and evcp2, with high homology to mammalian valosine-containing protein (VCP) genes. To define the origin of evcp1 and evcp2 and to characterize the genomic structure of *Eisenia fetida*, a bacterial artificial chromosome (BAC) library was constructed containing in excess of a 5 fold genome coverage spread over 50,000 clones. Two clones (20E8 and 91F3) were isolated that contain the entire genomic region of evcp1 and evcp2, respectively. The gene structures including exon-intron junctions were shown to be similar to the mammalian counterparts, suggesting that the genes have evolved from the same origin. Furthermore, the entire genomic sequence for 20E8 (122,785bp) and 91F3 (117,704bp) were determined and the respective GC contents were 41.5% and 41.1%. Genomic informatics analyses revealed that 20E8 contained at least 3 genes, including the malignant T cell amplified sequence 1 (MCTS1), the RIB43A domain with coiled-coils 2 (RIBC2) and evcp1. 91F3 contained at least 2 genes, a gene with cyclin L1 (CCNL1) motif and evcp2. Gene structures analyses confirmed that evcp1 and evcp2 genes occupied approximately 19 kb and 22 kb and consisted of 16 and 17 exons, respectively. Promoter prediction analysis of each gene revealed the presence of common motifs observed in high eukaryotes. Repbase analysis identified 49 and 51 microsatellites in 20E8 and 91F3, respectively. Likewise, many transposons and retroposons were found covering some 6.1% and 6.4% of the sequences. Among them, tRNA derived short interspersed nucleotide elements (SINE) were identified, previously characterized in other animals. To date, comprehensive whole genome analyses have, at large, focused on vertebrates and model organisms. This study provides, for the first time in Annelida, a stretch of continuous genomic sequence in excess of 100 kb, an imperative source for future genome assemblies.

Genomic Sequencing of *Eisenia fetida* and *Lumbricus rubellus* BAC clones containing metallothionein genes

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In order to characterize the structure of the earthworm genome, bacterial artificial chromosome (BAC) libraries were constructed from two Lumbricide earthworms, namely Eisenia fetida and Lumbricus rubellus. Several clones containing the metallothionein (MT) gene were identified from the libraries via a PCR screening approach. The genomic sequences were determined "end to end" by shotgun sequencing for four MT containing BAC clones, 318A10 and 472C1 from E. fetida and 13H12 and 6F14 from L. rubellus. 318A10 was 117kbp in length with a GC content of 40.3%. Blastn confirmed the presence of all 4 exons of the MT gene as previously suggested (Stürzenbaum *et al.*, 2004. Environ. Sci. Technol. 38 (23): 6283 - 6289). In addition, the GeneScan program identified that a gene homologous to SLC25A (solute carrier family 25 member 24) is located on the 5' side of the MT gene. The BAC clone 472C1 consisted of 20kbp of 5' and 75kbp 3' fragment. Restriction/electrophoresis analysis, combined with in silico analysis, indicated that the two fragments were separated by a 3kb un-sequenced gap region located in intron 1 of the MT gene. Unique 500bp repetitive sequences were located in both ends of the gap. Although 472C1 displays a relatively high homology to 318A10 (approx. 70%), it is suggested that 472C1 and 318A10 represent independent loci which may have been generated through an evolutionary recent gene conversion. 13H2 from L. rubellus consisted of 103kbp with a 2 kbp spanning 42 bps repeated sequence. Since the Lumbricus 13H2 and Eisenia 472C1 both contained a similar 500bps inverted repeat regions in intron 1 of the respective MT gene, it is suggested that they are homologous loci. 6F14 from L. rubellus was 123kbp long with 3 short gaps; consisting of a 6kbp TA repeat, a 2kbp TAG repeat and a 500 bp poly G sequence. In 6F14, a gene homologous to ABCA3 (ATP-binding cassette, subfamily A member 3) was located to the 3' side of the MT gene. Since the MT gene in 6F14 was found to be different in structure from those in other BACs, 6F14 was deemed to be a locus distinct from 13H2. Ongoing genomic analyses utilizing the BAC libraries are expected to provide not only the insights into the genomic architecture, but also provide vital information on the evolutionary relationship between earthworm species.

Poster - Session 1

Can mitochondrial genes help identify earthworms?

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Developping a reliable and fast method for species identification is an essential tool for studies dealing with earthworms. However, the lack of morphological characters is the main reason for identification problems as well as for taxonomic inconsistencies. Could molecular methods possibly provide a means to overcome these problems? Here, we compared the four most frequently used mitochondrial gene fragments in earthworm studies - 12S, 16S, COI, and COII - to assess their suitability for species identification. Earthworm specimens of 14 species collected in different countries of the northern hemisphere were included. Sequences were analysed using three phylogenetic methods: (i) Bayesian statistics, (ii) p-distances within species, and (iii) phylogenetic networks. We found high numbers of haplotypes, deep genetic lineages, and high p-distances in all four gene fragments of some species. The network based on the 12S sequences was clearly structured and reflected best the current taxonomic system. Of the four gene fragments analysed, the 12S seemed to be most suitable for molecular identification of earthworm species. However, due to the high number of haplotypes found in several species, more sequencing data of more individuals are needed to detail the picture of the genetic diversity in earthworms. As the results also indicated cryptic diversity, further investigations are required to clarify the taxonomic situation in *Lumbricus* rubellus, Aporrectodea rosea, Octolasion lacteum, and Allolobophora chlorotica.

Earthworm communities on Mount Nimba, a West African mountain

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Mt Nimba is a 1752 mountain m located in Western Africa. Fourty seven earthworm species have been collected on a surface of a few hundred Km² which makes this site the richest ever found until now. A majority of species was found in only one (21) or two (10) of the 87 sites sampled while only two species, Dichogaster leroyi (30 sites) and D. lamottei (40 sites) were the most common species and the Eudrilidae Stuhlmannia zielae the only species with a rather broad regional distribution. Species richness in 50 cm x 50 cm x 30 cm depth samples varied from 1 to 9 and density was up to 1200 ind m² with an average of 250. Correspondance analysis based on species biological traits showed a significant segregation according to altitude (p < 0.05). Eleven species were only found in piedmont areas (< 700m elevation) while 10 were restricted to the grasslands and shrub vegetation of the higher part of the mountain (> 1300m) and 12 were found at all altitudinal stages. No significant effect of vegetation type on communities was observed. Species from the piedmont area were generally large sized, unpigmented with well developed typhlosolis indicating geophagous feeding regimes. On the opposite, species only found at high elevations presented a set of characters (pigmented, relatively small with well developed setae and reduced typhlosolis) that suggest litter feeding habits. Species found at all altitudes presented intermediate characters. This study is a clear example of the high speciation rates that may occur in earthworms when diverse environment conditions are found in a relatively small region.

Cryptic speciation within Hormogaster elisae revealed by molecular data

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Hormogaster elisae (Hormogastridae) is an endogeic earthworm endemic to the central area of the Iberian Peninsula. This species can be very abundant in sandy and poor organic matter soils, with strong erosion and summer aridity. Climate change will presumably favour the extension of this kind of soils, where *Hormogaster* species and specifically *H. elisae* could be the only earthworms capable of thriving. Because of that, their ecological importance could become crucial and hence, it is necessary to look into their genetic variability in order to develop conservation and management programmes. One nuclear (28S rRNA) and two mitochondrial (COI and 16S rRNA) genes were sequenced in individuals from 16 populations of the central Iberian Peninsula where soil properties (texture, carbon, nitrogen and pH) were measured. The morphology of the specimens was carefully studied. The overall genetic diversity was very high and suggested the occurrence of several cryptic species within this region. For instance, the barcoding gene COI showed 80 haplotypes (exclusive to localities) out of 202 individuals and exhibited values of genetic divergence among populations up to 22%. Moreover, the studied genes agreed to cluster the same evolutionary lineages, indicating that different phylogenetic species could be defined. Nevertheless, a morphological constancy was found that led to the conclusion that all studied individuals belonged to the morphospecies H. elisae. These results could indicate discordant patterns of morphological and molecular evolution or a morphological stasis, which seems to be common in non-visual invertebrates and lead to cryptic speciation. The unique morphological distinction among the studied populations was the size of specimens, but its differences were not related to the genetic divergence. C/N exhibited a positive correlation with the weight of the collected specimens. Colonization events seem to have been the main cause of the current scenario as shown by a pattern of isolation by distance. Furthermore, a positive although weak correlation was also found between some soil properties (coarse sand and total loam content) and genetic distances, which indicates that these populations could have been shaped genetically, but not morphologically, by the environment.

Malagasy earthworms

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Madagascar and surrounding islands in the Indian Ocean constitute one of the 25 biodiversity hotspots in the World. These islands are characterized by very important endemisms and many new species are identified every year. However earthworms of Madagascar are poorly studied. The last scientific taxonomic report on a Malagasy earthworm dates from 1931 and was published by Michaelsen. Only 35 species were described at this date. A survey on earthworms and other soil engineers started in 2008. The objectives are to study the taxonomy, ecology and distribution of Malagasy earthworms. For 2 years, earthworms were sampled in different pedoclimatic areas, in different land uses. To date, many species have been sampled and are being identified. They belong to 6 different families: Megascolecidae, Kynotidae, Acanthodrilidae, Eudrilidae, Ocnerodrilidae, Glossoscolecidae. Among the species collected so far 8 are new to science and most of them belong to the endemic family Kynotidae (only 16 species of this family are known). The large size of Madagascar could explain a large number of earthworm species, and especially of endemic species. Other than the Kynotidae family, we found an endemic genus *Howascolex* (Octochaetidae) and three endemic species belonging in the South-African genus *Eodriloides* (Acanthodrilidae). The present work describes the actual knowledge on taxonomy and distribution of Malagasy earthworms.

FISH mapping of rDNA and histone gene clusters in earthworms

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The phylogenetic relationships within earthworms have usually relied on morphological characters which show some limited value due to their enormous variability, even among individuals of the same species, and have led to several taxonomical ambiguities which remain unresolved. Although the recent use of molecular tools in earthworm phylogeny has contributed to clarify some controversial taxonomical aspects, it has also posed new questions. For example, in the case of certain species included in *Dendrobaena, Eisenia* and *Aporrectodea* the resolution provided by the molecular markers is still quite limited. Therefore, in order to provide a better understanding of the evolutionary relationships within Lumbricidae here we provide an analysis of the distribution of the major rRNA, minor rRNA and histone gene families in several species of earthworms. Chromosome preparations were obtained from seminal vesicles after hypotonic treatment and fixation with ethanol/acetic acid. Chromosome regions was analysed and the ribosomal and histone gene families mapped by means of fluorescent in situ hybridization (FISH) using species specific H3, 28S rRNA and 5S rRNA gene probes generated by PCR.
Species richness and density of earthworms in grasslands of western Uttar Pradesh, India

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Earthworm species richness and their density reflects the soil quality of an ecosystem, which was currently unknown in grasslands of Uttar Pradesh. The study was conducted in five regions of western Uttar Pradesh, India, from years 2006 to 2009, with the aim of quantifying species richness and density of earthworms in different seasons. Three samples were taken, from each region in every season, using a metal quadrat of the size 25 x 25 x 20 cm. Worms were identified by observing external and internal morphological body characteristics and their density was quantified in terms of their number/m². Three species of Megascolecidae viz., Metaphire posthuma (Vaillant), Lampito mauritii (Kinberg, 1867) and Perionyx excavatus (Perrier, 1872) and four species of Octochaetidae, Eutyphoeus waltoni (Michaelsen, 1907), E. gigas (Stephenson, 1917), E. orientalis (Beddard, 1883) and E. pharpingianus (Michaelsen, 1907) were identified and recorded. Species richness and density of worms were highest in Moradabad during rainy season (July to October), whereas density was minimal in Saharanpur during the winter months from November to February. Species richness of worms was found to be the lowest in Meerut region during winter and summer months and in Saharanpur only during summers (March to June). M. posthuma was abundantly found in all the regions round the year. In addition, L. mauritii and E. waltoni have been reported from all the regions only during the rainy season. P. excavatus was next in occurrence pattern, as it was recorded from all the regions except from Meerut. Occurrence of E. orientalis, E. gigas and E. pharpingianus was recorded rare. Influence of seasons and climatic conditions in the variation of species richness and density of earthworms are discussed.

Geographical effect on genetic diversity and molecular Evolution of *Eisenia fetida* in Northern China

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Earthworm meta-population is a biogeocenose strongly influenced by fragmentized landscapes. Based on field investigation conducted on summer 2009, in Shandong Province and Liaoning Province, China, this study took DNA sequences of 16S rDNA and COI as molecular markers and analyzed genetic diversity of *Eisenia fetida* sampled from 4 islands, 2 peninsulas and 6 mainland localities. Result shows: i) Different populations of *E. fetida* are divided into 4 clusters, which correspond to populations of Shandong island, Liaoning island, Shandong mainland (including peninsula) and Liaoning and ii) Genetic diversity of island populations is richer than mainland populations. This indicates that seawater, as a kind of geographic barrier, accelerated intraspecific genetic divergence in *E. fetida*.

Diversity and distribution of earthworm communities in easternmost Canary Islands

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Urban and tourist development, still in continual expansion, are probably leading to the disappearance of significant populations of earthworms in the easternmost Canary Islands, up to now little investigated. Along with this, the lack of references in the literature and the evident fragility of the local ecosystems support the urgent need for a study that provides reliable data on the biodiversity and spatial distribution of the lumbricofauna. Up to now this includes 15 taxa belonging to Lumbricidae (60 %), Acanthodrilidae (26.66 %), Megascolecidae (6.66%) and Ocnerodrilidae (6.66 %). Our collection efforts were centered on a minimum of 70 selected sites (with their respective geographical coordinates and elevation data) to include at least the more representative biotopes: sectors of relictual native vegetation, tree plantations, agricultural land, semiaquatic habitats, grassland with dispersed palms, recreational areas (parks, gardens), organic matter dumpsites, watercourse beds and port areas. Earthworms were collected by digging moist blocks of soil, pouring on dilute formalin and hand-sorting; identification of the specimens was made with the aid of a previously compiled taxonomic key. Initially the results reveal that the ubiquitous Pontodrilus litoralis have a non-random spatial distribution and variable abundances; it was found in supralittoral pools and ravine mouths, presently disturbed or in danger of disappearance due to the severe environmental damage. The African Ocnerodrilus occidentalis occurs in semiaquatic habitats of the basal belt facing southeast, while the exotic invasives Dichogaster affinis, Dichogaster bolaui and *Microscolex phosphoreus* have low-density populations spread over anthropic sunny sites, below 100 m a.s.l. Other species are mainly palaearctic peregrines found in diverse microhabitats, from the coast to mid-alttitude areas and even mountainsides; among them Aporrectodea rosea bimastoides and Aporrectodea trapezoides show the highest ecological tolerance, having a wide distribution and average population density. We analyse the invasive front of some species and the different introduction-dispersal pathways, particularly anthropic (human settlements associated with agrocommercial activities), biological (vertebrate transport-vectors and exotic plants) and natural routes (by sea). Our conclusion is that these dispersion patterns have to a large extent determined the present-day composition of the lumbricofauna, and that together they might provide clues for understanding the history of the biota of the Canary Islands. In particular, the case of *P. litoralis* is discussed, considering that its presence should not be related exclusively or necessarily with human interference. Other results point out that: i) an increase of more than 50% in the total species range, ii) Fuerteventura island is more diverse in terms of species richness.

Biodiversity of terrestrial earthworms (Annelida: Oligochaeta) in Shandong peninsula and Liaodong peninsula, China

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A peninsula is a kind of ecotone connecting land and an island with different characteristics, which is suspected are also reflected in earthworm biodiversity. A recent survey of earthworm biodiversity was conducted in China Shandong and Liaodong peninsulas in 2009, which are connected with terrestrial and Korea Peninsula, respectively. In this paper, we report up-to-date checklists of the terrestrial earthworms in Shandong peninsula (SD) and Liaodong peninsula (LD). All materials were collected either by digging and hand-sorting in farmlands, mountains and shoreside of ponds or water reservoirs, or by searching ground litter, mosses and rotting logs. Earthworms were killed in 70% ethanol, and then preserved in 95% ethanol for further research. Meanwhile, a part of them were transferred to 10% formalin for fixation as voucher specimens. Fourteen species are known in SD, mostly of the Megascolecidae (43% of total) and Lumbricidae (36% of total). But seventeen species are recorded in LD, mainly within Lumbricidae (47% of total) and Megascolecidae (35% of total). *Drawida spp.* and *Amynthas spp.* are widespread in Dandong, a locality of LD. *Eisenia fetida* (Savigny, 1826) was intensely harvested for the vermiculture industry. Considering the importance of earthworm activity for soil function, and the existence of two new species to be described from LD, further sampling efforts and taxonomic training of Chinese scientists are urgently needed.

Earthworm biodiversity and paleogeography in Hainan Island, China

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Earthworms can not readily cross seas, rivers, or mountains; therefore, the modes which make their distribution restricted can be very valuable in testifying the geographical history of the world. For example, the paleogeography of Europe can be dovetailed well with the phylogeny of Lumbricidae (Oiu and Bouche, 1998). However, there is no similar research on Megascolecidae while they are also widely distributed in many places, especially in Asia and Australia. Hainan Island is the second largest island of the coast of China. According to the geological history, ingression and regression of the sea occurred several times around this island, followed by a large coastal plain which enabled flora and fauna to migrate back and forth from the mainland to Hainan Island (Su 2004). Finally, Hainan Island was separated from the mainland again in Holocene and became as present (Xing, 1995). In 1938, Chen firstly investigated earthworms in Hainan Island and reported 32 new species (Chen, 1938), followed by Zhong and Quan who reported another 3 new species (Zhong, 1989; Ouan, 1985). However, after that, no research continued in Hainan. In light of these, we supposed that there will be maybe more new species in Hainan Island. Moreover we hypothesized that the phylogeny of Hainan earthworms might support the paleogeographic hypotheses of Hainan Island, even of China and Asia. In order to confirm our hypothesis, the following methods were applied in our study: (1) We investigated earthworm biodiversity in Hainan in 2006 and 2009 separately, and identified them by external and internal morphological characteristics, for example, length, segments, spermatheca, and so on; (2) Molecular methods (COI, AFLP, RAPD as makers) were used to study the phylogeny of earthworms; (3) Correspondence factor analysis, Neighbour-joining and Bayesian inference methods were then applied to analyze the data obtained in step 2. Based on anatomical characteristics, the earthworm biodiversity in Hainan Island was clarified, and ended in 42 earthworm species, including 10 species which we discovered; 38 species belong to Megascolecidae. Results were compared to those from molecular approaches and discussed. Furthermore, those results were used to explain the phylogeny of Megascolecidae in Hainan Island and dovetail the paleogeography of Hainan Island, even with that of Asia.

Session 2

Earthworm Immunology and Physiology



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Earthworm Innate Immunity in the Genomic Era: The Search for the Elusive Recognition Receptors

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The innate immune system is the fundamental immune system of all metazoans and metaphytes. It performs the critical function of pathogen recognition; without it, the vertebrate adaptive response is severely handicapped. The elucidation of mechanisms of immunity in invertebrates has contributed much to our understanding of the innate immune system. Indeed the establishment of the discipline of Immunology was inspired in part by observations of the invertebrate innate immune system. Much of the work in this area has concentrated on the "response" arm of the innate immune system corresponding with identification of humoral factors and phagocytes involved in pathogen clearance. Evidence for the "recognition" function of the innate system and its critical importance has only recently been established in vertebrates yet it has been recognized as important in invertebrates for centuries. The identification and characterization of recognition molecules has, however, lagged behind discovery of the immune response components. Recently, comparative genomics has greatly aided in the unveiling of pattern recognition receptors and their corresponding signaling pathways in several invertebrates. For instance, the immune receptors Toll and Nod and some of their signaling molecules have been detected in representatives of deuterostome invertebrate groups. Innate immune receptors have also been detected in protostomes, however, only two protostome models of invertebrate immunity, namely Drosophila and C.elegans, have been examined, in depth, to reveal both gene identity and receptor function of innate immune system components. Curiously, these studies show that, the major role of Toll, the most widely studied pattern-recognition receptor, may not necessarily involve immune function but instead may play major roles in development and behavior. Recently, Toll has been discovered in the genetic sequences of the annelids Helobdella and Platyneries. The initial analysis of earthworms using anti-Toll antibodies yielded few results; Tolllike receptor 2 and a flagellin binding protein were detected on certain cells in *Eisenia*. In contrast, preliminary data from analysis of newly partially sequenced genome of L. rubellus shows that Toll receptors and other innate immune receptors and their corresponding signaling pathways may be abundant in earthworms affirming that the earthworm immune system is an important model of the innate immune system.

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A model of innate immunity in *Eisenia andrei* earthworms

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Eisenia andrei, like other invertebrates, do not possess anticipatory immune reactions based on the presence of lymphocytes and antibodies but they rely on the innate mechanisms that allow them to survive in often very hostile environments. In the last four decades important cellular and humOral -Session 2 pathways have been described and numerous biologically active compounds have been characterized and often cloned: namely, (i) the pattern recognition molecule CCF that binds to microbe-associated molecular patterns of Gram-negative and Gram-positive bacteria as well as fungi and triggers prophenoloxidase activating cascade, (ii) important antibacterial proteins fetidin and lysenin, and (iii) lysozyme provide a useful tool for description of complex mechanisms. We will present a model of an antimicrobial pathway that begins with recognition of microbe-associated molecular patterns by CCF in the gut, followed by differentiation of coelomocyte precursors in the mesenchymal lining. This results in the release of free effector coelomocytes and expression of immunologically active molecules - fetidin, lysenin, and lysozyme. Simultaneously, CCF triggers prophenoloxidase cascade that leads to the production of melanin with antibiotic properties. Detection of increased microbial pressure by pattern recognition CCF in the gut allows timely mobilization of immune mechanisms to prevent massive uncontrolled overgrowth of microorganisms in the coelomic cavity and maintenance of homeostasis.

Exposure of coelomocytes from *Eisenia hortensis* to oxidizing agents and heavy metals: A study examining effects on phagocytosis, apoptosis and ROS production

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The first part of this investigation analyzed the effect of exogenous hydrogen peroxide (H_2O_2) on phagocytic function and apoptosis in coelomocytes from Eisenia hortensis. Treating coelomocytes with H_2O_2 (0.26 to 8.4 mM) evoked a significant increase in phagocytosis for one or more of the concentrations of H₂O₂ employed in 67% of cases. Using annexin V-FITC we show that H₂O₂ induced apoptosis of coelomocytes in vitro. We found that 100% of viable coelomocyte populations exhibited significant increases in phosphatidylserine translocation for one or more of the concentrations of H_2O_2 tested (8.4 to 67.6 mM). Using a fluorescent inhibitor of caspases (FLICA), we also revealed the presence of activated caspases observing increased caspase activity in 67% of viable coelomocyte populations treated with 33.8mM H₂O₂, and in 100% of cases treated with 67.6 mM H₂O₂. Agarose gel electrophoresis and the TUNEL assay confirmed DNA fragmentation in samples treated with 16.9 and 33.8 mM H_2O_2 . Thus, exogenous H_2O_2 facilitated phagocytosis and promoted oxidative-stress-induced apoptosis, two cellular functions that may play an important function in regulating innate immune responses in E. hortensis. The second part of this study examined the effect of heavy metals on the production of reactive oxygen species (ROS). Oxidation of the fluorogenic substrate dihydrorhodamine 123 was monitored for the detection of ROS in coelomocytes treated in vitro with copper chloride, cadmium chloride or zinc chloride. Our results indicate that copper and cadmium induced a statistically significant, dose-dependent response with ROS production increasing in 40-80% of earthworms treated with copper, and 30-100% of earthworms treated with cadmium at concentrations ranging from 16 - 250 mcM, respectively. Zinc induced an elevated ROS production in only 10% of earthworms treated in this range. This method could be easily adapted for environmental studies investigating contaminated soils using ROS production in earthworms as an indicator of pollutants.

Riboflavin as chemoattractant for earthworm coelomocytes and mammalian immunocompetent cells

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Riboflavin plays an important role in the immunity of plants and animals (as vitamin B2 in the latter) and in quorum sensing of bacteria. We have shown that riboflavin is stored in chloragocytes attached to alimentary tissues and in chloragocyte-derived eleocytes freely floating in the coelomic cavity. It is germane that eleocytes are functionally involved in the formation of multicellular bodies encapsulating soil-derived pathogens. We may assume that riboflavin modulates the balance between pathogens and the earthworm innate immune system. Putatively, upon microbial stimulation active riboflavin metabolites support effectors of immunity, or riboflavin itself is released from chloragocytes/eleocytes intracellular stores as a chemoattractant for immune-competent cells, i.e. amoebocytes and eleocytes with phagocytic and bactericidal properties. The aim of the present investigation was to check if riboflavin acts as a chemoattractant for immune-competent cells from phylogenetically distantly related taxa, namely earthworm coelomocytes, mammalian leukocytes, and mammalian cell lines. Earthworm coleomocytes were retrieved by electric shock from Lumbricus terrestris, Aporrectodea caliginosa and Eisenia andrei. Murine leukocytes were lavaged from the bone marrow of adult Swiss males. Human monocyte U937 cells, murine monocyte/macrophage RAW 264.7 cells, and murine fibroblast L-929 cells were cultured in RPMI medium. Tests were performed in a 48-well chemotaxis chamber. Triplicate wells in the lower part of the chamber were filled with various concentrations (10-8, 10-6, and 10-4 M) of riboflavin as a putative chemoattractant. RPMI medium served as a negative control, while zymosan-activated murine serum (ZAS) or fMLP (chemoattractant of bacterial origin) served as positive controls. Wells of the upper part of the chambers were filled with cell suspensions. Statistical analysis of the numbers of cells migrating through polycarbonate filters was performed by ANOVA with post-hoc Tukey's test. As expected, ZAS and fMLP were efficient chemoattractants for all immune-competent cells and L-929 fibroblasts. Riboflavin acted as an efficient chemoattractant for all cell types involved in immune functions but not for L-929 fibroblast cell line. In conclusion, chemoattractant activity of riboflavin may be one of mechanisms underpinning its immunomodulatory functions. Acknowledgements: Supported by DS/BiNoZ/IZ/773/ZIE and N N303 089834.

A two-dimensional electrophoresis reference map of the earthworm *Eisenia fetida*

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The molecular mechanisms underlying innate immunity in the earthworm *E. fetida* remain unclear. For the recognition of innate immunity in the earthworm *E. fetida*, a detailed knowledge of this proteome is a prerequisite. The absence of a high-resolution *E. fetida* proteome map prompted us to determine *E. fetida* protein spots that can be visualized on 2-D protein gels. Here, we present a preliminary description of the whole earthworm *E. fetida* proteome. A highly detailed 2-DE map of the *E. fetida* proteome was established and approximately 1 500 protein spots were detected from the earthworm sample when applying a 500 µg protein 2-DE in the pH range 3.0-10.0. We present a 2-DE proteome map of *E. fetida*, identifying 76 different proteins by MALDI-TOF/TOF MS analysis. These identified proteins, including Hsp90, fibrinolytic protease 0, gelsolin-like protein, lombricine kinase, coelomic cytolytic factor1 (CCF 1), MnSOD, triosephosphate isomerase, extracellular globin-4, lysenin, and intermediate filament protein, glyceraldehyde-3-phosphate dehydrogenase and et al., are involved in several processes, including transcription, translation, the tricarboxylic acid cycle, the cellular amino acid metabolic process. It will be useful to investigate proteomics in earthworms, which has been rarely reported, despite of their importance in earthworm biology.

Antimicrobial activity of *Lampito mauritii* (kinberg) extract and its probable mode of action

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Studies were made on the antibacterial and antifungal activity of earthworm extract against eight pathogenic bacteria and eight fungi. Earthworm extract exhibited antibacterial activity against four Gram-negative bacteria- *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Vibrio cholerae* and four Gram-positive bacteria- *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Staphylococcus epidermidis* and *Bacillus subtilis*. Earthworm extract also exhibited antifungal activity against four yeast type fungi- *Candida albicans*, *Candida tropicalis*, *Candida krusei* and *Candida parapsilosis*, one dermatophytic fungus- *Tricophyton mentagrophytes* and three mould type fungi- *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus fumigatus*. These results suggest the broad spectrum nature of antimicrobial activity of *L. mauritii* extract. The extract of *L. mauritii*- a compound seems to be composed of low molecular weight proteins (29 kD, 43 kD and 66 kD) amongst the other active principles such as phenolic substances and humic substances found in tissues of the *L. mauritii* and known to be antibacterial and antifungal.

Vermipharmaceuticals isolated from earthworms

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The earthworm is one of the typical saprophagous organisms, which was successfully used to convert animal waste into biomass. Earthworms contribute internationally by vermicomposting. The pharmaceuticals from earthworm, as green biomedicine, have developed fast in recent years. Earthworms have become an international medicine, even though the original utilization of earthworm as traditional medicine has been known for thousands of years. With the development of science, scientists found the medical value of earthworms related to many chemical components. Here we describe vermipharmaceuticals isolated from earthworms, including: fibrinolytic enzymes, lumbrokinase, collagenase, superoxide dismutase, cholinesterase, catalases, glycosidases, metallothionein, calmodulin-binding protein, proteins with proliferation improving activity, lysenin, eiseniapore, antitumor protein, glycoprotein extract, gut mobility regulation peptide, antibacterial peptide, carbamidine, lumbrin, lumbrofobrim, terrestrolumbrolysin, purin, vitamin B, tyrosine, succinic acid, lauric acid, unsaturated fatty acid, and so on. This report mainly summarizes fatty acids, enzyme systems, proteins and peptides.

Mitogenicity of earthworm lectins

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Lectins are non-catalytic proteins or glycoproteins that are capable of recognizing and binding reversibly to specific saccharide moieties of glycoconjugates. Lectins have a variety of biological properties including antifungal, antiproliferative or mitogenic and hemagglutinating activities. The ability of lectins to detect subtle variations in carbohydrate structures on the surface of cells and tissues has made them a paradigm for protein-carbohydrate recognition. Recognition of cell-surface carbohydrates by lectins has broad implications in important biological processes. Earlier lectins were thought to be only of plant origin but later their presence was shown even in animal systems. Animal lectins fulfill a variety of functions. Many could be considered in general terms to be recognition of molecules within the immune system. More specifically, lectins have been implicated in direct first-line defense against pathogens, cell trafficking, immune regulation and prevention of autoimmunity. Invertebrate lectins have highly developed discriminative ability of the clearance mechanisms. Their binding to non-self materials is based on the presence of carbohydrate-specific recognition. Lectins or agglutinins are known to occur in the serum of a variety of invertebrates.. Lectins from Earthworm Eudrilus eugeniae was isolated by a lactose-specific extraction in the absence of detergent and metal ion. EDTA-MEPBS buffer was used for the partial purification of lectin from whole earthworm. After quantifying the protein content in the lectin sample, carbohydrate specificity was determined by DNS method using different sugars. The effect of various saccharides on lectin extraction was investigated and the lectin isolated was found to be galactose binding protein. The mitogenicity of earthworm lectin on vertebrate cells (liver cells) and invertebrate cells (coelomocytes and muscle cells from earthworm) were assessed. On addition of lectin; liver cells and earthworm muscle cells showed increased proliferation and coelomocytes exhibited decrease in cell number. Antimitogenicity of earthworm lectin on cells of the same species was a novel finding, therefore by the same isolation method lectin was isolated from coelomic fluid alone. The lectin showed galactose specificity and showed mitogenicity in coelomocytes. To confirm the results, lectin was isolated from cultured earthworm muscle cells, fluid from outer region of earthworm muscle wall, and fresh vermicompost. Carbohydrate specifity and mitogenicity were found to vary. Lectin from cultured earthworm muscle cells and fluid from outer region of earthworm muscle wall were found to be glucose binding and has no mitogenic effect on muscle cells where as lectin from fresh vermocompost was found to be galactose binding. Since lectin isolated from coelomic fluid and muscular wall cells of earthworm are of different carbohydrate specificity and of different mitogenic effect. The results confirm that the synergistic effect of lectins of different specificity produced by different cells is responsible for the characteristic immune response exhibited by earthworm.

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Oxidative stress caused by UV radiation or heavy metal exposure in earthworms

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It has been demonstrated that either UV radiation or heavy metal can cause severe damage in several earthworm species. UV, especially UV-B which increasingly reaches the earth surface in the past decades due to the breakage of the ozone layer in the atmosphere, can impair the earthworm skin and muscle, or affect the earthworm crawling behavior. Cadmium, one of the major heavy metal pollutants, could be lethal when it is over bio-accumulated into an earthworm. The pathological responses of UV radiation or heavy metal on earthworms have been described and compared to show differences. Generally, the damage effect of UV-B results from oxidative stress which includes reactive oxygen species (ROS) generation. ROS is highly reactive, and can result in significant damage to cell structures or lipid peroxidation due to the presence of unpaired valence shell electrons. Surprisingly, in our lab, it was recently found that the impairments of heavy metal on earthworms also causes oxidative stress. The antioxidant studies have further demonstrated that the damage effects from either UV radiation or heavy metal exposure in earthworms could be significantly reduced when antioxidant such as Vitamin C was present.

Metal mapping and ligand-speciation across the alimentary epithelia of earthworms native to metalliferous field soils: correlative electron microprobe and synchrotron microbeam X-ray analyses on frozen sections

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The capacity of earthworms to bioaccumulate and resist the effects of soil-borne metal contaminants is exceptional, with recorded Pb and Zn dry mass concentrations in whole worms from certain metalliferous soils occasionally exceeding 10,000 μ g/g. Only a small fraction of the equilibrated metal body-burden of earthworm field populations with multi-generation exposure histories can possibly be bioreactive. Thus, defining the compartmentation and ligand-binding speciation of metals in earthworms is as important as it is technically elusive. The main aim of the present study was to use a combination of physic-chemical techniques, electron probe X-ray microanalysis in the transmission and scanning electron microscopes, together with prototype high brightness, synchrotron-based, mini-focus EXAFS on anhydrous cryo-preparations to: (i) map with high spatial resolution the co-distributions of Ca, Cd, Pb, and Zn (as well as P and S) in a linear transect across the absorptive intestinal epithelia and chloragogenous tissue of hyperbarically quench-frozen Dendrodrilus rubidus; (ii) determine the ligand-binding speciation of Zn in situ in cryostat sections of the gut contents and chloragocytes of Lumbricus rubellus quench frozen immediately upon recovery in the field in order to maintain the fidelity of subcellular metal compartments; (iii) to recover the non-destructively analysed micro-focus sections for confirmatory morphological and microprobe assessment in a field-emission scanning electron microscope. We offer three major conclusions of ecotoxicological and ecophysiological consequence. First, Pb and Zn are almost exclusively sequestered by oxygen-donating ligands in earthworm tissues, whilst Cd is sequestered by thiols (i.e S-donating ligands. Second, there is a neglected cell type in the intestinal wall which is distinct from the predominat ciliated absorptive epithelial cells; it possesses the hallmarks of a cell playing a significant role in metal sequestration and possibly excretion. Third, the gut contents of the two epigeic species have different mineral morphologies and compositions. These observations present a challenge in the age of "omics" technologies to determine the transcriptomic profiles of functionally distinct cohorts of cells in complex solid tissues.

Earthworm immunity: a tale of survival by innate but not adaptive immunity

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The immune system of earthworms did not just arise *de novo* nor were the prescient investigations during the early 1960s entirely unique since self-nonself recognition as revealed by transplantation, had been attempted earlier. In Germany about the time of Metchnikoff, there was a prominent school that devoted considerable effort to transplantation in earthworms. Later in the late 1960's simultaneously in France and in my lab, graft rejection was mediated by cells of the immune system. Proliferative responses were detected in free cells during xenograft rejection and stimulation of DNA synthesis was revealed in MLR-like reactions. It is significant that responding and stimulated cells were characterized as lymphocyte-like coelomocytes (8 to 12 μ m). β_2 -microglobulin-like molecules occur on coelomocyte membranes of L. terrestris using polyclonal and monoclonal antihuman β_2 microglobulin antibodies. Only one cell type, which constitutes about 15% was labeled. The Thy-1 gene may be closely related in evolutionary history to the ancestral gene for Ig and MHC. Finding a Thy-1 homolog in invertebrates may establish whether this molecule is more primitive than Ig and MHC. Thy-1 homolog has been found in squid, tunicates, and in earthworms. Immune systems of invertebrates and vertebrates are *innate*, *natural*, *non-specific*, *non-anticipatory*, *non-clonal*, and germ line. Only vertebrates possess adaptive immunity: adaptive, induced, specific, anticipatory, clonal, rearranging genes. Earthworms possess at least two functional leukocytes, small coelomocytes, and large coelomocytes that mediate lytic reactions against tumor cells in vitro. Phagocytosis and natural killer cell responses are effected by distinct coelomocytes. The chlorogogen cell synthesizes and sheds effector lytic molecules. Among them, three have been identified and sequenced (*fetidins*, CCF-1, *lysenin*) and another has been discovered (*eiseniapore*), while three other molecules, H(1) H(2) H(3), share agglutinating and lysing functions. Lumbricin I is the only known earthworm molecule that is antimicrobial but non-lytic. The coelom and its cellular and humoral derivatives as a source of various cell lineages distinguished for their stem cell capability to react with "non-self" is clearly an important component of their capacity to the effect of immune reaction. That earthworms do not develop cancer may be due to their efficient immune capability since there is evidence of a reasonably long life span.

Mucor sp. a Pathogen of Pontoscolex corethrurus and Amynthas corticis earthworms

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Mucor sp. attacks a wide organism spectrum: Beetles (Coleoptera), mammals like *Platypus* (Oberndorf et al., 1993), vegetable species and humans (Pérez et al., 2001). Mucor species have been tested as an efficient biocontrol agent against Nematoda, both by in vivo inoculation and in vitro biotest (Ashoub et al., 2009). However, there are no reports of previous studies of pathogenicity of *Mucor* using annelids. This study aims to evaluate the *Mucor* pathogenicity on two tropical species of earthworms: Pontoscolex corethrurus and Amynthas corticis from the area of Xalapa, Veracruz, Mexico. The fungus was isolated from dead P. corethrurus bodies infected with Mucor sp. and developed one week in PDA medium, at room temperature. For the pathogenicity test, fungus was grown in PDA medium added with cephalosporin antibiotic at 0.5 g/ml in order to guarantee no bacterial contamination. Earthworms were collected in the INIFOR (Instituto de Investigaciones Forestales) area (a patch of pasture land in relict forest mountains), placed in plastic containers and maintained under a greenhouse until the test. The substrate (Ferralitic soil) was collected from the same area and sterilized 1 h. at 120°C. To recover the spores, 10 ml of dH₂O added with 2 drops of Tween 80 were added to Petri dish with Mucor (Barriga et al., 2002). Before the inoculation, earthworms were washed with a solution of Cl at 1 % and then submerged 10 seconds in the spore's solution. The treatments evaluated were: 1) Adult P. corethrurus with fungi. 2) Adult P. corethrurus without fungi. 3) Adult A. corticis with fungi. 4) Adult A. corticis without fungi. Five replicates by treatment were prepared. Dead earthworms were counted at 24, 48 and 72 hours. Preliminary results indicate that the Cl at 1% is lethal, after 72 hours to both earthworm species, reaching values of 95% and 70% of death for P. corethrurus and A. corticis. However, the treatment that involves Cl at 1% and *Mucor* spores inoculation had the highest death range, reaching values of 95% for both species (dead earthworms were infected). Apparently, P. corethrurus resists more the attack of Mucor than A. corticis; that may be due to a difference in the protein cell wall of both earthworm species, since it has been reported that *Mucor* degrades proteins. Further studies are necessary to evaluate if *Mucor* is a factor that mainly attacks earthworms in a healthy state.

In Vitro Assessment of Antioxidant Prospects of Earthworm (Lampito mauritii, Kinberg)

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Antioxidant activity determined by peroxide value, free radical scavenging activity, antioxidant capacity and total phenol content in the coelomic fluid, earthworm paste and earthworm extract in vitro showed that earthworm extract has significantly more antioxidant potency. Results indicate that earthworm extract can donate electrons to reactive free radicals converting them into more stable, non-reactive species and terminating the free radical chain reaction. The prevention of the chain reaction step by scavenging the free radical species seems to be the antioxidant mode of action of the earthworm extract.

Studies on selective therapeutic properties of earthworm *Lampito mauritii* (Kinberg)

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Man's use of medicinals is as old as humans who are constantly concerned about the state of health. This has resulted in many concoctions now known as medicines. The branch of science which deals with medicines obtained from animals and used in the treatment of ailments and diseases is known as zootherapy. In modern civilization, zootherapy constitutes a significant alternative among many other known therapies practiced world-wide. Research attention and actions in the areas of ethnobiology and ethnomedicine have been amplified greatly in the last decade. Since the inception of the disciplines, scientific research in ethnobiology and ethnomedicine has made important contributions to understanding traditional subsistence and medical knowledge and practice. In view of the fact that ancient animals, their parts and their products comprise the inventory of medicinal substances for curing various ailments, cultures continue the diverse tradition even now. In India, since time immemorial, great work exists in Zootheraphy and documented in works like Avurveda, Shidha and Charaka Samhita. All this knowledge has once again come to our attention, as there has been some disenchantment with current allopathic medicine, as it has got its own side effect and in fact has no cure for various diseases. Therefore people are looking for traditional remedies for the treatment of ailments. But in India this traditional knowledge is fast eroding due to modernization. The studies on the therapeutic uses of animals and their body parts have been neglected, when compared to plants. In India, nearly 15-20 percent of the Ayurvedic medicine is based on animal-derived substances. Earthworms are the soil macro invertebrate oligochaete annelid, playing a major role in improving and maintaining soil quality and health for sustainable agricultural activities. We intend to discuss how earthworms have been used in the past by various civilizations and the recent studies in relation to our observations on the many roles claimed to be the cure for variety of diseases by earthworms in different forms- live, dried, powder and extracts. Earthworms apart from being used as food for humans (by Maoris in new Zealand, Japanese, natives in new Guinea, Africa) and animals because of their high protein content, are eaten or applied to humans to cure illnesses or diseases such as piles, fever, small pox, jaundice and removal of bladder-stones. Thus, there is great promise in the use of earthworms as a novel source of the expanding discipline surrounding nutraceuticals.

Life cycle and development of *Pontoscolex corethrurus* (Müller, 1857) in tropical artificial soil

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The earthworm *Pontoscolex corethrurus* is a peregrine species of the Glossoscolecidae family, native to the Neotropics. The aim of this study was to evaluate the life cycle of this species in tropical artificial soil (SAT), the substrate used in ecotoxicological tests, and the influence of food availability and humidity in its growth. The life cycle was evaluated in four treatments: SAT (120 g) without additional food (SAT0) (n = 24), SAT with 5 g of horse manure (SAT5) (n = 24), SAT with 10 g of manure (SAT10) (n = 24) and saturated SAT (with 25% greater soil moisture) with 5 g of manure (SAT5H) (n = 24). Food was provided every 14 days and the containers maintained at room temperature (19.8 \pm 4.1° C). The earthworms used were obtained from 96 cocoons collected near the Embrapa Florestas (Colombo-PR-Brazil), at different stages of development. These were classified into 6 color gradients and compared with the time taken until hatching. On average, white transparent cocoons hatched at 34 days, white opaque cocoons after 33 days, white cocoons with embryo visible to the naked eye at 30 days; white with rosy stripe at 20 days; light pink at 15 days, and dark pink at 6 days. The intensity of the cocoon color indicated the degree of embryo development. The cocoons were placed individually in different treatments and development monitored by registering weekly changes in biometric and morphological characters from hatching to sexual maturation and oviposition. The newly hatched earthworms weighed 0,052 g and measured 1,9 cm in length. By the 13th week all juveniles in SATO had died and reached a maximum of 2,2 cm length and 0,013 g fresh weight. For the other treatments, all earthworms had already played (?) at the 44th week, showing length of 5.4 cm and 0.72 g biomass in SAT10, while in SAT5 had 5.1 cm and 0.61 g in SAT5H, 5,1 cm and 0, 59 g. The growth and weight gain in adults of P. corethrurus responded positively to the offer of food. P. corethrurus was tolerant of excessive moisture in SAT, with eurihigric behavior, surviving both dehydration and flooding, in contrast to other species. The life cycle of *P. corethrurus* was 12 months in SAT. This substrate showed no limitations for the development of the species, provided that the food was offered.

Poster - Session 2

Identification of gene transcripts differentially expressed in the immune competent cells (coelomocytes) of *Eisenia fetida* exposed to metallic pollution

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Anthropogenic activities can lead to the accumulation in topsoils of Metal Trace Elements (MTE) to concentrations often exceeding background levels by orders of magnitude. Excessive MTE exposures can generate degrees of environmental stress that are likely to have deleterious effects (e.g. disturbance of reproductive and immune functions) on resident organisms. Thus, it is imperative that plants and animals living in close contact with metal-contaminated soils are studied in order to better understand their physiological states, the mechanisms of metal sequestration and detoxification, and the metal-specific modes of toxicity. The emergence of molecular genetic "omic" techniques applied to ecotoxicology promises to deliver a more fundamental and holistic understanding of the mechanisms of action of contaminants on receptor organisms. Indeed, gene expression profiles represent the first level of integration between environmental stressors and the genome, responses that subsequently escalate to higher levels of biological organisation. The analysis of changes in gene expression patterns is, therefore, a powerful tool firstly to diagnose the existence of a stress in individual members of a population, and secondly to analyze in exquisite detail the molecular nodes of the stress response. In this context, robust and comprehensive DNA sequence information is a crucial prerequisite. In the present study, Suppression Substractive Hybridization (SSH) was used to construct four cDNA libraries enriched in up- or down-regulated transcripts in the immune circulating cells (coelomocytes) of Eisenia fetida (Annelida; Oligochaeta; Lumbricidae) exposed to metallic pollution. Worms were exposed to artificial soil contaminated by a realistic mixture of MTE (Cd = 40 mg/kg; Pb = 500 mg/kg; Zn = 700 mg/kg), i.e. metal concentrations found in a strongly polluted smelter soil. In total, 20,000 SSH clones (5,000 for each of the 4 libraries) were sequenced, and analysed using the PartiGene analysis suite; they were decorated with functional annotations (GO, EC and KEGG identifiers). Precisely 5897 unique gene objects were obtained and added to the initial database; they are particularly interesting since they have been specifically chosen to be differentially expressed in coelomocytes following metallic stress.

Poster - Session 2

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Canonical Wnt signaling pathway has been proven evolutionarily conserving in several animal phyla. Besides axis establishment during embryogenesis, this pathway also partakes in adult axis homeostasis and re-establishment during regeneration process in both hydra and planarian models. However, little is known about whether there is the same mechanism governing regeneration among annelids that also possess strong regenerative ability. Herein, we introduce a freshwater annelid, Aeolosoma viride, (capable of re-growing its lost tail or head within 3 or 5 days) as a new model animal for analyzing regeneration. To assess the impact of canonical Wnt signaling pathway on A. *viride* regeneration, downstream proteins GSK-3(?) and α/β (?)-catenin were chosen as the target molecules. Alsterpaullone (a novel class of small molecule cyclin-dependent kinase (CDK) inhibitors and LiCl, which can inhibit GSK-3(?) and in turn activate the pathway, thereby significantly prohibiting A. viride head regeneration. On the other hand, Quercetin, an inhibitor of $\alpha/\beta(?)$ -catenin, blocked the pathway but accelerated the speed of A. viride head regeneration. Knocking down of GSK-3(?) and α/β (?)-catenin by their RNAi affected regeneration processes as their inhibitors. Furthmore, after treating A. viride with alsterpaullone and LiCl, cellular α/β (?)-catenin was found to accumulate in the nuclei of A. viride. These results suggest the involvement of Wnt signaling pathway in A. viride head regeneration, and this is the first report concerning Wnt involved in annelid regeneration. Although it is not clear whether Wnt pathway affects A. viride tail regeneration, it is plausible that the pathway governs the anterior-posterior axis in *Aeolosoma* as for other counterparts.

High tolerance to UV-B irradiation in Pontoscolex corethrurus

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Generally, earthworms have been known as nocturnal due to lethal impairment from ultraviolet. Accordingly, UV-B, not UV-A, can induce segmentation, hemorrhage, etc. in some earthworm species such as *Amynthas gracilis*, and *Metaphire posthuma*. Surprisingly, *P. corethrurus* showed less sensitivity to UV-B radiation. The lethal dosage of UV-B to *P. corethrurus* is four times higher than that *A. gracilis*. Because *P. corethrurus* obviously secretes much more mucus on its surface than *A. gracilis* or *M. posthuma*, its mucus was speculated that might serve as UV blocker. In this study, the secreted mucus from *P. corethrurus* can effectively absorb UV-B. Furthermore, UV-B can induce reactive oxygen species (ROS) leading to severe skin damage due to lipid peroxidation. However, the lipid peroxidation induced by UV-B in epidermis and muscles of *P. corethrurus* could be quickly recovered. Therefore, we propose that *P. corethrurus* can serve as a new animal model to study UV-resistant and anti-oxidation.

Coelomocytes: the mediators of cellular immunity in earthworms

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Earthworm cellular immunity is maintained by different coelomocyte subgroups (hyaline amoebocytes, granular amoebocytes and chloragocytes) possessing various immune functions. First, we started to study the expression of conserved molecules (cell surface molecules, enzymes, peptide hormones) in coelomocytes by various immuno-morphological and immunochemical methods using library of mammalian-antigen specific monoclonal and polyclonal antibodies. Among those anti-TNF-alpha and anti-acid phosphatase antibodies proved to characterize coelomocyte subgroups, moreover anti-TSH antibodies showed distinct staining patterns in earthworm neural elements. Second, our aim was to analyze the cytotoxic effects of coelomocytes on mammalian target cells and to provide evidence that the lytic factors originated from coelomocytes. Cell-free coelomic fluid, supernatants of short-term cultured coelomocytes, and lysates from coelomocytes - derived by mechanical and detergent extraction - were used in cytotoxicity assays performed on different standard mammalian tumor cell lines and mouse fibroblasts. Third, because no clusters of differentiation were described previously, we produced and characterized a library of monoclonal antibodies specific in differentiation markers (anti-EFCC clones) raised against coelomocyte (leukocyte) antigens of Eisenia fetida earthworm. Flow cytometry, immunocytochemistry, and immunoprecipitation analyzed and confirmed the specificity of anti-EFCC clones. Anti-EFCC mAbs revealed distinct coelomocyte subpopulations and various staining patterns on tissues. Two functional assays (e.g. phagocytosis and encapsulation) further characterized EFCC clusters revealing a common coelomocyte marker and three subpopulation-specific markers. The comparative studies on different evolutionary stages showed strong earthworm specificity. Moreover, we proved that intracellular calcium plays a major role in the activation of coelomocytes. Immune molecules such as phytohemaglutinin and bacterial formyl peptide induce calcium influx in coelomocytes studied by flow cytometry. Finally, all these facts prove that earthworm coelomocytes are a convenient model to study the evolution of innate immunity in invertebrates.

Poster - Session 2

Aporrectodea trapezoides (Dugès, 1828): life cycle in singletons and twins

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The parthenogenetic earthworm Aporrectodea trapezoides (Dugès, 1828) is widely distributed all over the world due to European agricultural practices. In order to provide baseline life cycle data, cocoons (n = 241) were obtained from field-collected individuals and their features and viability, incubation period, number of hatchlings and mortality rate were recorded. Singleton and twin earthworms were cultured from hatching during a 490-day period under controlled conditions with biomass, survival, reproductive condition and cocoon production being recorded at intervals of 15 days. As a complementary growth measure, instantaneous growth rates (IGRs), assuming that growth proceeds logistically rather than linearly, were calculated for each 15-day growth interval. The growth of A. trapezoides followed a sigmoid curve, with a slow initial phase over 60 days, an exponential phase over 285 days, and a stationary growth phase reached after 345 days. On average, individuals of isolated-reared A. trapezoides reached maturity at day 153 and body weight at maturity was approximately 1 g. Growth rates and fecundity of A. trapezoides were not influenced by the individual status of singletons or twins, although singletons had a mean weight almost twice that of twins. After the first fifteen days, weight was not significantly different between both types of individuals, but mortality rates were higher in twins during this initial period. The addition of twins theoretically would provide an advantageous increase in the population during the colonization of new areas, when the number of individuals would outweigh the advantage of individual size. In A. trapezoides however, things seem to be different: twin/singleton status does not have an influence on individual size, but does influence mortality rate during the first fifteen days of laboratory life. Thus, a trade-off seems to exist between the number of hatchlings per cocoon and mortality rates during the first days.

Poster - Session 2

Parthenogenesis in *Aporrectodea trapezoides* (Dùges, 1828): reproductive traits in field-collected and laboratory-reared earthworms.

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Polymorphic degradation of reproductive structures, as often found in other parthenogens, has not yet been recognized in Aporrectodea trapezoides. Both primary and secondary male sexual characters such as perithecal papillae, tubercula pubertatis, spermathecae, swollen male porophores and seminal vesicles are retained in this species. Two possible hypotheses could be the recent appearance of parthenogenesis or the unusual resistance to mutation in those organs. As reproduction in this species has been poorly studied, neither pseudogamy nor the existence of stimulating requirements to trigger its reproduction have never been proved. Experiments on life cycles have usually been done only with field-collected individuals or with laboratory-reared ones. Hence, some aspects have been poorly tested, such as the existence of putative learning processes in the field or differences between potential and real growth or reproduction. In order to record reproductive traits and differences between field-collected and laboratory-reared individuals of Aporrectodea trapezoides, forty microcosms with an isolated earthworm (20 with field-collected individuals and 20 with laboratoryreared ones) and forty containing groups of three (20 with each type of individual) were maintained during a complete year under controlled conditions. The amount of soil per individual was the same in both types of microcosm. The number of cocoons was recorded every 15 days by replacing the soil and wet-sieving the discarded one. Both all individuals kept in isolation and those cultured in groups produced cocoons, hence completely proving the obligatory parthenogenetic reproduction in this species without copulation or need of any physical-chemical stimulus. In general, isolated earthworms produced a significantly higher number of cocoons than those in groups of three, and the same was recorded for laboratory-reared earthworms when compared with field-collected ones. Not only is A. trapezoides one of the most abundant earthworm species, but it is also very successful under different environmental conditions. Thus, new knowledge about reproduction in a possible key species could be extremely useful in soil management and applied studies.

Detection of pattern recognition receptors (PRRs) and their associated signaling pathways in earthworms

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Pattern recognition receptors (PRR) which play a central role in the recognition response of the innate immune system have been detected in the tissues and immune cells of invertebrates including, C.elegans, Ciona intestinalis, Chlamys farreri, S. purpuratus, Capitella and Helobdella. The most commonly detected invertebrate PRRs can be categorized into three major classes: TLR (Toll-like receptors), NLR (NOD-like receptors) and SRCR (Scavenger receptors). All three receptor classes bind to pathogen associated molecular patters (PAMPs). A soluble PRR, CCF-1 has been isolated from earthworm coelomocytes. In addition, several cell surface PRRs including Toll-like receptor 2 (TLR2) and an uncharacterized flagellin-binding protein have been detected using fluorescent antibody labeling of cell surface components on earthworm coelomocytes. However many antibodies to mammalian TLRs have not detected TLRs on the coelomocyte cell surface. Sequence analysis of the recently elucidated L. rubellus genome show that several homologues of TLRs and SRCRs may exist in L.rubellus. In addition we have been able to detect a variety of intracellular signaling molecules involved in PRR-dependent immune cell activation, including NFkB, IkB and homologues of the entire C.elegans based PKM-1 (map kinase) pathway. Some signaling factors associated with PRRs like MyD88 and others have not been located in the L. rubellus genome. These data suggest that earthworms may possess both an ancient signaling pathway (PMK-1) and mammalian -like signaling pathway as part of the PRR-dependent innate immune response.

Earthworms, Eisenia and 5 more E's: A constructivist approach to teaching invertebrate immunology in an advanced undergraduate curriculum

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The laboratory module describes a flow cytometry exercise appropriate for an advanced microbiology course designed to familiarize students with innate immune mechanisms and acquire quantitative reasoning skills. This activity encourages inquiry-based thinking and incorporates an instructional model based on the 5 E's encompassing engagement, exploration, explanation, elaboration and evaluation. Leukocytes (coelomocytes) isolated from *Eisenia hortensis* (earthworm) are used to demonstrate the ingestion of fluorescent Escherichia coli (E. coli/GFP) prey, and the ease with which this invertebrate model can be adapted for undergraduate teaching of phagocytosis. Students learn how to set up in vitro cellular assays, are introduced to the basic theory of flow cytometry, and employ Cell Quest Pro and WinList software programs for the acquisition and analysis of list mode data files. Single parameter histograms based on fluorescence and dual parameter dot plots based on light scatter and fluorescence are generated for statistical analyses. Gating options permit students to restrict their analyses to particular subsets of coelomocytes in phagocytosis assays. The phagocytosis assay can be modified easily to incorporate hypothesis-driven predictions providing an extension to this module ideally suited for courses interested in promoting an inquiry-based learning approach to teaching at the undergraduate level. Assessment of student learning outcomes is accomplished through a pre-test/post-test evaluation, a student evaluation questionnaire, and a grading rubric used for evaluation of formal laboratory reports. Data is presented that illustrates achievement of specific student learning outcomes prescribed for this activity.

Using dihydrorhodamine 123 to measure reactive oxygen species production in Eisenia hortensis during phagocytosis: A flow cytometric method to study innate immunity in invertebrates

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Phagocytosis is a major mode of immunity for cells (coelomocytes) of the innate immune system of the earthworm *Eisenia hortensis*. Phagocytic cells provide non-specific immunity against environmental hazards and are easily extruded from the dorsal pores for experimental manipulation. Hyaline amoebocytes, one of three different types of coelomocytes, engulf and destroy bacteria via phagocytosis with generation of accompanying reactive oxygen species (ROS). Results of several *in vitro* experiments will be presented investigating the production of ROS in hyaline amoebocytes (also known as large coelomocytes) in response to stimulators such as bacteria including *Bacillus megaterium* and *Pseudomonas stutzeri*, as well as the immune suppressing compound corticosterone (CORT). Large coelomocytes were isolated during analysis based on their size and relative granularity using flow cytometric methods in which the level of ROS production was measured by the fluorescent compound dihydrorhodamine 123 (DHR123). Results indicate that ROS production is best stimulated by introducing bacteria at multiplicities of infection under 100:1 where response rates were as high as 67%. Preliminary finding show that pretreatment with CORT does appear to suppress the production of ROS; however, more experimentation is needed to further investigate this phenomenon.

Earthworm calcium carbonate granule production does not appear to be involved in osmoregulation

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The reason for the secretion of calcium carbonate granules by earthworms is not clear. Previous studies have suggested a variety of functions for the calcite granules including the regulation of excess Ca, the regulation of CO_2 in tissue fluids and the neutralisation of gut pH (e.g. Piearce 1972, J. Animal Ecol 41 167–188). None of these proposed functions have been satisfactorily confirmed. A recent article in Science (Wilson et al. 2009, Science 323 359–362) reported that many marine fish have an osmoregulatory mechanism that culminates in the excretion of small particles of calcium carbonate. Since earthworms are essentially aquatic organisms living in the soil we have carried out experiments to determine whether calcium carbonate granule secretion is part of a similar osmoregulatory mechanism. Two different experiments were conducted in which earthworms were exposed to conditions in which osmotic pressure gradients would have existed between the earthworms and their host soil. Soil from the Hamble soil series (UK) was collected, air dried and sieved to eat between treatments in either the variable soil moisture experiment (1.9 +/- 0.2 mg calcite / earthworm / day, n = 17) or in the variable ionic strength experiment (1.5 +/- 0.3 mg calcite / earthworm / day, n = 18). We conclude that granule production is unlikely to play a significant role in earthworm osmoregulation.

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Response on the levels of proteins, cells and organisms of *Allolobophora chlorotica* exposed dermally to Ni, Cu and Cd ions

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During our previous experiments on earthworms exposed to filter papers soaked with metal chlorides it turned out that the endogeic species, Allolobophora chlorotica, is more susceptible to metal toxicity than the epigeic species, *Eisenia fetida* and *Dendrobaena veneta*. Therefore the aim of the present experiments was to study effects of Ni, Cu, and Cd chlorides on A. chlorotica at the levels of whole organisms, immune-competent cells - coelomocytes, and stress proteins. Adult (clitellate) individuals of A. chlorotica were collected in the garden of the Institute of Zoology in Krakow. Worms were kept in the laboratory in their native metal-free soil samples at 16 °C, 12:12 lightdarkness. For experiments, worms were inserted individually into test tubes lined with filter papers soaked with metal (Ni, Cu, Cd) chlorides, at metal concentrations of 11-176 µg/ml, or with water (controls). Additional control worms were taken directly from the soil. After 3-day exposure, earthworm were inspected for viability and anatomical abnormalities. Survivors were used for coelomocyte retrieval by a mild electric shock (1 min, 4.5V) and/or fixed for morphological studies. Extruded coelomocytes were counted and used for 1) cytospin preparations; 2) flow cytometric analysis of percentages of eleocytes, or for measurement of neutral red uptake during in vitro incubation; 3) preparation of coelomocyte lysates. Immune-blot and immunohistochemistry were applied to estimate expression of heat shock proteins (HSP72) and metallothioneins isoform 2 (MT-2) in cell lysates, on coelomocyte cytospins, and on cryostat cross-sections through posterior parts of worm bodies. The high metal concentrations (especially Ni and Cu) were lethal to worms or induced drastic anatomical changes, including abnormal swelling, lesions, and/or fragmentations of posterior segments. Morphological abnormalities were less drastic at lower metal concentrations. Coelomocytes retrieved from Ni- and Cu-exposed survivors were less numerous and had impaired ability of neutral red uptake. Cd-exposure affected more neutral red uptakes then the cell numbers. Expression of HSP72 and MT-2 was evident not only in coelomocytes of survivors, but also on cross-sections through chloragogen tissue, typhlosole, epithelial cells of intestine, and metanephridia. In conclusion, whole body homeostasis was significantly disrupted in worms exposed to toxic chemicals. Acknowledgements: Supported by PB 3502/PO1/2007/32.

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We have shown previously that riboflavin (vitamin B2) accumulates in chloragosomes of chloragocyte-derived eleocytes freely floating in coelomic cavity of several - but not all - earthworm species. Riboflavin plays an important role in immunity, thus its storage in immune-competent cells may have an adaptive value for earthworms vulnerable to soil-derived pathogen invasion. Thus, it is important to determine whether riboflavin stores are also present in earthworm species with low eleocyte counts, especially in chloragocytes. Experiments were performed on adult (clitellate) earthworms from several species, some of them with an abundance of eleocytes (Eisenia andrei, Dendrobaena veneta, Allolobophora chlorotica) and others with few eleocytes (Lumbricus terrestris, L. rubellus, and Apprectodea caliginosa). Freely floating coelomocytes (consisting of amoebocytes plus species-specific numbers of eleocytes) were extruded by mild electric shock (4.5V, 1 min). The worms were then anaesthetized by cooling and the chloragocytes were mechanically detached into buffer. Both cell suspensions were analyzed by flow cytometry (for cell size, complexity, and autofluorescence) and spectrofluorometry (for riboflavin content). Transverse cryostat sections posterior to the clitellum of some worms were prepared for fluorescence microscopy, scanning confocal microscopy, or transmission electron microscopy. Ultrathin sections revealed that chloragogen tissue of L. terrestris is more compact than that of E. andrei. Fluorescence microscopic observations of sections and analyses of cell suspensions revealed distinct riboflavin-derived autofluorescence in free eleocytes but not in chloragogen tissue of E. andrei. Riboflavin signatures were also detected in detached chloragocytes, but not in the coelomocytes (i.e. almost exclusively amoebocytes) expelled from coelomic cavities of L. terrestris, L. rubellus, and A. caliginosa. Riboflavin was also a prominent constituent of free coelomocytes (i.e. amoebocytes plus eleocytes) and chloragocytes of D. veneta and A. chlorotica. In conclusion, all investigated earthworm species have the ability to store riboflavin in chloragocytes and/or chloragocyte-derived eleocytes. Acknowledgements: This work was supported by a grant (N N303 089834) from the Ministry of Science and Education (Poland) (for BP and AIM) and by the Jagiellonian University (K/ZDS/000784).

RSC96 Schwann cell proliferation and survival induced by earthworm extract through PI3K/Akt signaling mediated by IGF-I.

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Schwann cell proliferation is critical for the regeneration of injured nerves. Earthworms are widely used in Chinese herbal medicine to remove stasis and stimulate wound-healing functions. Exactly how this Chinese herbal medicine promotes tissue survival remains unclear. The aim of the present study was to investigate the molecular mechanisms by which earthworm extract promotes neuron regeneration. Our results show that treatment with earthworm extract induces the phosphorylation of the insulin-like growth factor-I (IGF-I)-mediated phosphatidylinositol 3-kinase/ serine-threonine kinase (PI3K/Akt) pathway, and activates protein expression of cell nuclear antigen (PCNA) in a time dependent manner. Cell cycle analysis showed that G1 transits into the S phase in 12 to 16 h, and S transits into the G2 phase 20 h after exposure to earthworm extract. Strong expression of cyclin D1, cyclin E, and cyclin A occurs in a time-dependent manner. Small interfering RNA (siRNA)– mediated knockdown of PI3K significantly reduced P13K protein expression levels, resulting in Bcl2 survival factor reduction and a marked blockage of G1 to S transition in proliferating cells. These results demonstrate that earthworm extract promotes the proliferation and survival of RSC96 cells via IGF-I signaling. The mechanism may be primarily dependent on the PI3K protein.

Cadmium Affects PKC Activity by a Zinc- or Calcium-Independent Mechanism in Earthworms

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Earthworms have been recognized as an important model animal on soil toxicology. Earlier researches revealed that earthworms have many behavioral and physiological changes in response of heavy metal such as cadmium. Expression of metallothionein (MT), a small cystein rich divalent metal ions-binding protein, is among one of those responses induced by cadmium. In vertebrates, induction of metallothionein caused by protein kinase C (PKC) activator has been demonstrated, but inductive effects of PKC inhibitor have also been reported. PKC is a very important relay protein of the signaling pathways which mediate responses such as cell proliferation, apoptosis and smooth muscle contraction. These responses can be affected by cadmium exposure. However, few experiments have been done to elucidate how the activity of PKC is affected by cadmium. In this study, earthworm *Eisenia andrei* treated with 75ppm cadmium (w/w) for 48 hours significantly reduced its PKC alpha activity compared to control group. This result was confirmed in vitro by using 0.04 mM cadmium to treat earthworm tissue crude extract. Furthermore, both calcium and zinc ions are crucial for PKC's activity, and both similar as cadmium ion are divalent cations. However, PKC alpha activity reduced by cadmium could not be rescued by calcium or zinc ion. This result suggests that the PKC alpha activity reduction is not caused by competition of cadmium to calciumor zinc-binding sites in PKC.

Schwann cell migration induced by earthworm extract via activation of PAs and MMP2/9 mediated through ERK1/2 and p38.

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The earthworm, which has stasis removal and wound healing functions, is a widely used Chinese herbal medicine in China. Schwann cell migration is critical for the regeneration of injured nerves. Schwann cells provide an essentially supportive activity for neuron regeneration. However, the molecular migration mechanisms induced by earthworms in Schwann cells remain unclear. Here, we investigate the roles of MAPK (ERK1/2, JNK and p38) pathways for earthworm-induced matrix-degrading proteolytic enzyme (PAs and MMP2/9) production in Schwann cells. Moreover, earthworm induced phosphorylation of ERK1/2 and p38, but not JNK, activate the downstream signaling expression of PAs and MMPs in a time-dependent manner. Earthworm stimulated ERK1/2 and p38 phosphorylation was attenuated by pretreatment with U0126 and SB203580, resulting in migration and uPA related signal pathway inhibition. The results were confirmed using small interfering ERK1/2 and p38 RNA. These results demonstrated that earthworms can stimulate Schwann cell migration and upregulate PAs and MMP2/9 expression mediated through the MAPK pathways, ERK1/2 and p38. Taken together, our data suggests the MAPKs (ERK1/2, p38) -PAs (uPA, tPA)- MMP (MMP2, MMP9) signaling pathway of Schwann cells regulated by earthworms might play a major role in Schwann cell migration and nerve regeneration.

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The tale of a lazy worm: Reproductive behaviour of *Hormogaster elisae* revealed by microsatellite markers

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Endogeic earthworms inhabit the sub-surface layers of the soil, rarely reaching the surface, which makes their observation and consequently their behavioural study extremely difficult. That is the reason why in sexual selection studies epigeic and anecic earthworms have been chosen as model organisms. Nowadays, the development of genetic tools such as microsatellite markers gives the opportunity to investigate the mating behaviour and sexual selection of these earthworms without the need for observation. In this study, we address some questions on precopulatory and postcopulatory sexual selection of Hormogaster elisae, an endogeic earthworm, such as what is the average number of partners, whether worms choose partners taking into account their size, whether mating success depends on their size or their genetic variability or whether they use the different spermathecae to store sperm from different partners. By sampling different points within a population we also tried to detect the genetic structure of *H. elisae* at a microgeographical scale. Six to ten mature individuals were collected from nine different 1m² plots, each one separated by 8 m, within a square of 16 m². The 75 individuals collected were dissected, and five different samples were taken from each specimen: a portion of their tegument and their four spermathecae, resulting in a total of 375 samples. Four microsatellite markers (Novo et al. 2008) were used to analyze all the samples separately. The results showed that overall genetic diversity values were high, and departure from Hardy-Weinberg Equilibrium was detected. H. elisae individuals have multiple mates, normally maintaining the sperm of two individuals mixed in their four spermathecae, with no signs of differential storage of sperm from different partners. A negative correlation was found between weight differences and mating success, which seems to indicate that *H. elisae* individuals select partners with a similar weight. Their mating success was not dependent on their weight or degree of heterozygosty. The significant result of a Mantel test indicated isolation by distance, which could mean that H. elisae does not need long-distance dispersion to seek a partner, and moderate inbreeding was detected although there was not genetic structuring within the studied area.

Effects of soil quality on coelomocytes from eco-physiologically contrasting lumbricid species

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We have shown previously that coelomocytes non-invasively retrieved from earthworm coelomic cavity may serve as bioindicators of soil pollution: eleocytic riboflavin content in Dendrodrilus rubidus was high in worms from unpolluted reference soil but low in worms living in, or experimentally transferred to, either Zn/Pb-contaminated or Ni-contaminatedfield soils. The aim of the present study was to determine whether coelomocytes in eco-physiologically contrasting lumbricid species (Eisenia andrei, Dendrobaena veneta, Allolobophora chlorotica) are also affected by exposure to various metalliferous mine-associated field soils from the UK and Poland, or to metalfree soils of different physicochemical character. At the end of 3 to 8 week exposures, coleomocytes were counted and analysed by flow cytometry (for cell size/complexity/autofluorescence), and by spectrofluorometry (for riboflavin content). Investigated metals accumulated avidly in whole-worm tissues in species-related manners. Despite metal bioaccumulation, the spectrum of cytotoxic effects caused by elevated soil metal content on the immuno-competent cells were consistently less severe than those observed previously in D. rubidus. In particular, the coelomocytes of E. andrei were slightly affected after 8-week maintenance on Zn/Pb-polluted mine soil from the UK; A. chlorotica cells were slightly affected after 3-week exposure to the same Zn/Pb-rich soil, but died after short exposure to aerially contaminated Ni-smelter soil. Ni-smelter soil was not conspicuously harmful to E. andrei or D. veneta during 3-week exposure. Most revealing were 4-week exposures of the three species to either Zn/Pb/Cd-contaminated soils from sites in Poland, or to an unpolluted commercial soil, or to unpolluted field soils of differing geochemistry. D. veneta coelomocytes were unaffected by maintenance in a variety of soils; in contrast, the riboflavin content was increased (E. andrei) or decreased (A. chlorotica) in coelomocytes retrieved from worms kept either in metalliferous Polish (Bukowno) soils, or in unpolluted sandy-clay and loamy-sand soils. In conclusion: (a) effects of soil quality on earthworm coelomocytes are species-specific; (b) riboflavin content is the most sensitive of the investigated coelomocyte parameters; (c) riboflavin content is affected not only by soil metal (Cd, Ni, Pb, Zn) pollution, but putatively also by other "natural" edaphic variables. Acknowledgements: Supported by PB3502/PO1/32.

Species-specific sensitivity of earthworm coelomocytes to heavy metal pollution

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Earthworm immune-competent cells (coelomocytes) are affected by soil quality, including metal contamination. Thus it is important to study the effects of individual components of the pollutant cocktails that are often found in metalliferous field soils. Aporrectodea caliginosa versus Dendroabena veneta and Allolobophora chlorotica are representatives of two contrasting groups of earthworms with respect to coelomocyte traits. In A. caliginosa they contain almost exclusively amoebocytes; both amoebocytes and chloragocyte-derived, riboflavin-storing, eleocytes are well represented in A. chlorotica and D. veneta. Adult (clitellate) worms of the three investigated species were exposed for 2 days to filter paper soaked with chlorides of Ni, Cu, Zn, Cd, or Pb at the same molar concentration (1 mM) (equivalent to 58.7, 63.5, 65.4, 112.4, and 207.2 g/ml, respectively). Coelomocytes were expelled, counted and analysed ex vivo after electro-stimulation. In A. caliginosa the total number of amoebocytes was increased in Pb-treated worms; they exhibited enhanced autofluorescence when retrieved from both Cu- and Pb-treated worms; in vitro neutral red uptake revealed that pinocytosis was more prevalent (peaking after 15 minutes incubation) in metal-exposed A. caliginosa than in the controls. In D. veneta, amoebocytes were unaffected by metal exposure; Cuexposure caused a minor reduction of eleocyte numbers and of their riboflavin content. In A. chlorotica, the number of coelomocytes (especially eleocytes) and the riboflavin content of coelomocyte lysates were significantly lowered in Cu-exposed worms, moderately reduced in response to Ni, Zn, or Cd exposures, and unaffected by Pb. In conclusion, the amoebocytes of the three species were relatively resistant to acute dermal exposure to divalent metals. However, eleocyte numbers and their riboflavin content in the endogeic species A. chlorotica appear to hold some promise as sensitive biomarkers of metal exposure under laboratory conditions. Acknowledgements: This work was supported by a grant (No PB3502/PO1/32) from the Ministry of Science and Education (Poland).

Isotopic analysis (C, N, S) of setae and soft tissues of *Lumbricus terrestris* reared on isotopically distinct diets

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This project had two objectives: 1) to establish if lumbricid setae are permanent or semi-permanent tissues that could act as biochemical archives of life histories and 2) to measure the isotopic fractionation (tissue-diet spacing) for C, N and S in earthworms raised on controlled diets. Newly hatched Lumbricus terrestris were raised individually for 26 weeks on one of two cattle dung diets that had distinct C, N and S compositions. Two treatment groups (4-5 individuals each) were fed exclusively on one of the two diets, and three other treatment groups were initially fed with one diet and switched to the second diet at 2 g body mass, at maturity or one month after maturity. Setae from anterior and posterior body segments were collected by digesting body segments (without gut content) in 10% KOH for 4 h, rinsed repeatedly, transferred into a watch glass with some distilled water and picked out manually under a light microscope. Soft bulk tissue from segments 1-5 were also collected and freeze-dried. Diets, setae and soft worm tissues were analysed by EA-IRMS (Elemental Analysis - Isotope Ratio Mass Spectrometry). C and N isotope results of setae from worms that had been switched between diets showed that setae are not permanent but turned over at similar rates to soft tissues. This turnover or replacement could be due to wear and tear, breakage or growth. This non-permanency implies that lumbricid setae cannot be used as an archival tissue for biochemical analysis, for example to age-date individuals or detect ontogenetic diet changes. Measurement of the isotopic composition of soft tissues of the group on the constant control (natural abundance) diet made it possible, for the first time, to determine isotopic fractionation in earthworms. The tissue-diet spacing for C, N and S was 2.47, 2.83 and -1.03 per mil, respectively, for the control diet. These figures will be useful for the interpretation of future studies on the feeding ecology in earthworms based on natural abundance stable isotope measurements.

Reproductive seasonality of the Taiwanese montane earthworm *Amynthas hohuanmontis* Tsai et al., 2002 (Oligochaeta, Megascolecidae)

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Reproductive seasonality of a field population of *Amynthas hohuanmontis* Tsai et al., 2002, a Taiwanese montane earthworm belonging to the Pheretima complex of the family Megascolecidae, was investigated. Its reproductive organs show various stages of degeneration in size, number and structure. The spermathecae vary from absence (athecal) to three pairs in VI-VIII (sexthecal), and the prostate glands from large, asymmetrical, to totally absent. For the specimens examined so far, there were 56.7% athecal, 23.3% sexthecal and 20% between. The number of mature ova of athecal individuals peaks in summer, whereas that of sexthecal individuals is highest in winter. Spermatogenesis occurred throughout the year, with highest activity in summer and autumn for athecal and sexthecal individuals, respectively. Sperms were present in testes/seminal vesicles but no sperm was found in the histological sections of the spermathecae for all specimens examined, which is an evidence of parthenogenetic reproduction of this species.

Different expression of fetidin and lysenin in *Eisenia andrei* and *Eisenia* fetida earthworms

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The earthworms are regarded as a model organism of comparative immunology since 1960s when transplantation experiments were performed and initiated extensive studies of earthworm immunity. Moreover, official protocols involving screening of earthworm immunological parameters as markers/indicators of impaired environmental conditions were accepted. The effect of microbial environment on innate immunity of model earthworms is thus of particular interest. Eisenia andrei and *Eisenia fetida* are two closely related epigeic earthworms. Until recently they have been considered as subspecies (E. fetida andrei, E. fetida fetida). However, molecular analyses of cytochrome c oxidase have confirmed their identity as distinct species. As these earthworms live in completely different environment - E. andrei lives in compost and manure, while E. fetida is found in litter layer in forests with much lower quantity of microorganisms as well as their heterogeneity - our work was focused on the differences in their antimicrobial immunity. The coelomic fluid of Eisenia exhibits numerous biological activities including bactericidal and/or bacteriostatic activities against soil bacteria. Formerly, two major antimicrobial proteins - fetidin and lysenin - were described in E. andrei. Both proteins are closely related and share biochemical analogies. Here we show that E. *fetida* express neither fetidin nor lysenin, although sequences of both molecules were found in their genomic DNA. Moreover, the expression is not detectable upon the stimulation by microorganisms isolated from E. andrei worms or the substrate where they live in. The unique occurrence of fetidin and lysenin expression in E. andrei could reflect their different living conditions. They could have developed distinct defense mechanisms to better tolerate a wide range of environmental conditions and their fluctuations.

Characterisations of an antibacterial peptide isolated from skin secretions of the earthworm (*Eisenia fetida*)

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Earthworm Slime Peptide (ESP) was isolated and purified from the skin secretions of earthworm (*Eisenia fetida*) by gel filtrations using Sephadex G-75, and G-50 followed by G-25. The Earthworm Slime Peptide-1 (ESP-1) was derived from ESP, using C-18 reversed-phase high-performance liquid chromatography (HPLC). It was found that the ESP was composed of 50 residues of sixteen different amino acids, among which ten residues were serine. Molecular weight of ESP -1 was 5814 D. The minimum inhibitory concentrations (MIC) of ESP to *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* was found to be 6.25mg/ml, 6.25mg/ml, and 12.5mg/ml respectively, whereas the MIC of ESP-1 to the same bacteria was 6.25mg/ml, 12.5mg/ml and 50mg/ml respectively. Therefore, the antibiotic function of ESP was better than that of ESP-1. It follows that from the skin secretions of earthworm (*Eisenia fetida*) antibacterial peptide can be isolated and purified; the antibiotic function of earthworm's antibiotic peptide was a coordinated function from different components.

Function of mucilaginous secretions in the antibacterial immunity system of *Eisenia fetida*

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The function of skin secretions in the antibacterial immunity system of the worm *Eisenia fetida* was confirmed for the first time in this study. These earthworms were infected in a bacterial bath with *Escherichia coli* to induce antibacterial skin secretions of *E. fetida*. These antibacterial skin secretions were found to be selective. After induction with *E. coli*, the bacteriostasis of worm skin secretions on exposure to *E. coli* and *Staphylococcus aureus* increased significantly P secretions. Our results suggest that mucilaginous secretions are an important defense in the antibacterial immunity system of *E. fetida*.

Detection and preliminary characterization of Toll-like Receptors (TLRs) in *Lumbricus rubellus*

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Toll-like receptors (TLRs) are an ancient family of pattern recognition receptors, which show homology with the Drosophila TOLL protein. There is mounting evidence that TLRs are essential for the development of innate immune reactions to pathogenic microbes in mammals. As with other immune responses, genome-wide analyses strongly support the view that TLRs or related genes are ubiquitously conserved in the genomes of non-mammalian organisms. TLRs have been reported from a variety of aquatic invertebrates, but very little information on TLR distribution in terrestrial invertebrates is known. Previously, we identified TLR homologues from the leech Helobdella and polychaete Capitella (Davidson et al. 2008). To identify potential TLR homologues in earthworms, we searched for homologues of human TLR-2 (AAC34133) in the genome of Lumbricus rubellus (sequencing progress) BLAST server LumbriBASE in using the at (http://xyala.cap.ed.ac.uk/Lumbribase/index.shtml). We found 20 contigs (a set of overlapping DNA segments derived from a single genetic source) with significant similarity to the human TLR-2 (E)[?].

Cadmium induced abnormal muscle contraction in Eisenia andrei

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Cadmium (Cd), a common heavy metal existing in our environmental soil, is a useful material and has been widely used in our life such as rechargeable nickel-cadmium battery, oil paints, plastics, etc. However, bio-accumulation of Cd can cause severe toxicity in many organisms, especially terrestrial animals. Earthworms have been accepted as a bio-indicator for evaluating soil pollution including heavy metal contamination. In this study, Eisenia andrei was used to analyze Cd-induced acute toxic response. After exposure to 200ppm Cd, around 60% of E. andrei began to show abnormal morphology, including skin wrinkle, partial hyperemia, or abnormal muscle contraction of body wall in 12 hrs. And most of the Cd-treated earthworms died in 24 hrs. In some serious cases, the earthworm body wall which might be caused by muscle cell lysis resembling a chain that links the exposed intestine. The posterior part of the chain-like body could be shed later. Based on the data from flame atomic absorption spectrometry, the chain-like part of the *E. andrei* obviously accumulated higher concentrations of Cd than the normal part of the same earthworm. It is not clear why the absorbed Cd was accumulated in the posterior part of the body to cause the shedding behavior in E. andrei. On the other hand, because the symptom of the abnormal muscle lysis in E. andrei caused by Cd shows some similarity as rhabdomyolysis in human, it will be interesting to study whether the Cd-induced abnormality in E. andrei could serve as an animal mode to study rhabdomyolysis, the breakdown of fibers and consequent release of its contents into the bloodstream, which if untreated can result in kidney damage.

Session 3

Earthworms as Indicators of Change on a Local, Regional and Global Scale



The 9th International Symposium on Earthworm Ecology 5th to 10th September 2010, Xalapa, Mexico

Are earthworms good bioindicators of human activities?

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Vincenzo Tanara in 1644 and Charles Darwin 1881 were among the first modern supporters of the earthworm activity, function and indication in times in which such invertebrates were not much popular in crop-fields and appreciated only as baites for line fishing and or for chickens. Today earthworms have increased their rank among most people perception as beneficial for soil, garden and as fertility promoters. Luckily they have been chosen among 54 potential bioindicators as one of the key parameters to assess organic versus conventional farming in one European founded project Bio- Bio. However their snakelike shape and their place of thriving- in the soil make them "suspicious" and "dirty" creatures. For these reasons most earthworm popularization is made with head and legs making the worms imagine more like vertebrates or humanized pets. These perceptions are important when considering or promoting earthworms as bioindicators among farming community or citizens-consumers. The current knowledge of these creatures being very limited among the large majority of people no matter about the different taxa and or different behaviours. I will discuss some points of earthworms bioindication in rural and seminatural landscapes. Different ecological roles and earthworms importance as quality indicators in different landscapes. Organic Farming activity and earthworms. Earthworms local knowledge and their use as human food (the cases of New Zealand and The Alto Orinoco). Bioindication limits will be also discussed for earthworms. The cases in which some anecic species are referred as potential invaders or pests or are targeted as potential new colonizers to b introduced for tropical countries "in which apparently" no anecic native species are active.

The use of earthworms as tool for soil monitoring, characterization and risk assessment. Example of a Bioindicator Programme developed at National scale (France)

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Soil can be considered as a non-renewable resource that must be protected because it is essential to our livelihood (it provides food, drinking water, biomass and raw materials) but its degradation is still accelerating (e.g., erosion, contamination, compaction). Thus, protecting and managing soils require a set of indicators able to judge the degradation and/or remediation of its properties and functions. Considering the lack of knowledge on the best bioindicators to describe soil quality, a national research programme has been set up to develop such indicators (2006-20012). This "Bioindicator Programme" is the first in Europe. The main objectives are to provide new tools for soil monitoring, characterization and risk assessment, based on soil biological properties. In the first step of this programme (2006-2009), research teams developed and tested their indicators (n=80) in a few given situations. After selection, the most relevant indicators (n=22), including earthworms, are presently being tested and compared in a large number of common contexts involving: 13 sites all over France (forest, agricultural and contaminated sites) and 51 contexts differing in terms of land use, contamination origin and pollution level. Earthworms are being studied at community level (abundance, biomass, species and functional structures) as well as organism level (applying metallothionein coding gene expression in earthworms). Moreover, the relationships between earthworms, assessed at functional level (epigeic, endogeic, anecic), and other soil fauna (collembola, mites, nematodes) are being analysed using multi-parameter analysis. The results presented in this communication are those obtained during the spring of 2009. They clearly show the value of the earthworm community as indicator of land use, especially of the impact of different organic matter inputs, as well as tillage vs. no tillage systems. Moreover, earthworm communities indicate strongly the level of different sources of pollution (metal or HAP). Furthermore, the first results obtained on *L.terrestris* are encouraging as they show that the expression of metallothionein increases in contaminated soil. The multi-parameter analysis (HAC) revealed 4 groups which defined specific indicators (indicator of sites, land uses, pollution). These first results are very interesting because they reinforce the interest of using earthworms in soil monitoring, characterization and risk assessment.

A conceptual model to understand the role of earthworms as bioindicators in the Argentine Pampa steppe

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We present a conceptual model for the response of different earthworm species to soil conditions. The model considers two main axes: the first represents the soil organic matter content (OM), associated to the fertility and agricultural aptitude of the soil; the second axis represents the intensity of disturbance (DI) due to tillage and other agricultural practices. We used information obtained over the last 25 years from the Pampa region of Argentina under different land uses, from agriculture to forestry. In this region there are both indigenous and introduced earthworm species. The chosen axes allow us to define four soil groups (one per quadrant). 1) Lightly disturbed and fertile, located in the fourth quadrant (high OM - low DI); these are soils under grasslands used for cattle production or forested soils. 2) Fertile agricultural soils, located in the first quadrant (high OM, high DI). 3) Explored and poor soils in the second quadrant (low OM, high DI). 4) Poor and undisturbed soils in the third quadrant (low OM, low DI). Starting with this framework and knowing the ecophysiological responses of the more frequent earthworm genera, we defined the following characteristic groups: genera Amynthas and Microscolex for type [1] soils; genus Aporrectodea for type [2] soils; genus Eukerria for type [3] soils, and genera Octolasion and Bimastos for type [4] soils. Soils having low OM values contained mainly endogeic and oligohumic species; soils with high OM values showed poly- or meso-humic species. On the other hand, soils with high DI had earthworms with small body size and low variance; soils with low DI had large earthworms with high variability in body size. This conceptual model is a first approximation in an effort to use earthworms as bio-indicators of the soil history and ecological status. Moreover, the model helps explain field results. This kind of framework can be represented by a soil mandala using the similar concept of Margalef to explain the presence of algae groups in marine and freshwater ecosystems.

Quality in semi-deciduous forest areas (Centre-West, Ivory Coast)

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Although soil quality has been used as an indicator of sustainable land management for several decades, there is no common agreement about its evaluation and monitoring. We used here a multifunctional indicator of soil quality that combined a set of 4 sub indicators associated with different soil ecosystem services to assess the variation of soil health along a gradient of forest disturbance in Centre-West Ivory Coast. The sampling method consisted in digging out 3 monoliths (50 x 50 x 20 cm) at 5 m intervals, collecting soil samples and measuring chemical parameters, bulk density and resistance to penetration at each sampling point along a gradient of forest disturbance. The land-use types comprised semi-deciduous forests (as a baseline), tree plantations, fallow, perennial and annual crops. The calculation procedure was based on a sequence of Principal Component Analysis (PCA) by which physical, chemical, soil organic matter content and earthworm diversity sub indicators were combined into a single general indicator of soil quality (GISQ). In parallel, earthworms were used to test the concept of bio-indicators of soil quality and we sought to test the theory of species indicators as well. Results showed a significant variation of GISQ along the gradient of forest disturbance, with the highest values $(0.78\pm0.05-0.93\pm0.03)$ obtained in natural forests, the lowest (0.24±0.05) in recurrent fallows and mixed-crop fields, while tree-based systems showed intermediary values $(0.40\pm0.03-0.58\pm0.06)$. There were significant relationships between earthworm density (R2=0.27, F=17.2, P=0.0005), biomass (R2=0.35, F=20.2, P=0.0000) and the GISO, showing that earthworm communities can be used as indicators of soil quality. Moreover, we found 6 indicator species among which 4 (Dichogaster terrae-nigrae, Chuniodrilus zielae, Dichogaster baeri and Millsonia lamtoiana) were associated with tree-based systems and 2 (Millsonia omodeoi and Hyperiodrilus africanus) with recurrent fallows and mixed food crops. The present study thus revealed that a GISQ can be used to assess forest disturbance and confirmed that earthworm communities can be used as indicators of soil quality.

Earthworm communities in deciduous forest and productive systems in Yucatan, Mexico. Changes, disturbance responses and indicator species

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In Yucatan, Mexico, we studied earthworm communities (biomass, abundance, species composition and dominance) in deciduous tropical forest and four livestock production systems established through slash and burn practices, with increasing management disturbance: silvopastoral systems -SP-, 2 year-old and 12 year-old Taiwan grasslands -TP2 and TP12-, and Star-grass pastures -SG12. The earthworms were collected in 10 monolith-sampling points per system, in order to detect sensitive species with potential as indicators of karstic soil health. We also performed discriminant analysis to assess the biological validation of management practices according to earthworm communities, and provide a disturbance response index for both native and exotic species. We found seven endogeic species: Balanteodrilus pearsei, Dichogaster affinis, Dichogaster bolaui, Diplotrema Dichogaster (Acanthodrilidae), murchiei. *Pontoscolex* corethrurus SD. (Glossoscolecidae) and a newly recorded Ocnerodrilidae species. The last four species are new records for the state of Yucatan. B. pearsei, D. murchiei and the new Ocnerodrilidae species were native; the remaining species were exotic. B. pearsei was the only species found in every production system and was the most abundant, dominant species, accounting for 87.5% of the total earthworm biomass. TP2 had the highest biomass of Oligochaeta, and disturbance response indexes confirmed that the earthworm community responded positively to intermediate levels of management disturbance (TP2). A numerical classification of the production systems showed that 62% of the earthworm species recorded were correctly assigned to production systems and explained 42.2% of total variance in the classification (Wilk's lambda = 0.117, p<0.05).

Change trajectories of earthworm communities after conversion of mixed semi-natural broadleaved forest to spruce monoculture along a soil trophic gradient

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The effect of tree species choice and tree species conversion on soil acidity, humus type and earthworm communities through the effect of their decomposing litter has been thoroughly studied. However, the size of such change trajectory as a function of initial soil trophicity has not been looked at in a systematic way, nor is it well understood whether earthworms play an active role in this environmental change. The objective of this study was to better understand the change trajectories of topsoil, humus type and earthworm communities by multiple pair comparisons along a soil trophic gradient. In a context of acid deposition sensitivity mapping it is common understanding that poorly buffered soils are more sensitive to acidification, so we hypothesized longer trajectories in poorer soils. Along a trophic gradient in a large (about 20,000 ha) temperate semi-natural forest in Southern Belgium we selected 60 plots, half of which in patches of 1-5 ha of Picea abies (Norway spruce) of approximately 50 year of age, and 50 plots at short distance from each conifer plot in the broadleaved matrix (main species Carpinus betulus, Ouercus robur, Fagus sylvatica, Ouercus petraea and Fraxinus excelsior). In all plots topsoil and substrate chemistry were described, humus form determined and earthworm communities characterized. Causal relations between factors were tested with path analysis using structural equation modeling. In general, spruce monocultures acidified soils, increased ectorganic horizons, decreased earthworm biomass and eliminated endogeic earthworm species. Interestingly, the change trajectories were larger in richer soils with higher buffer capacity. This can be interpreted as a regime shift between soil buffer ranges, which is supported by path analysis suggesting a forward-feeding mechanism of increasing litter accumulation through the production of organic acids toxic for burrowing earthworm species (endogeics and anecics).

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Earthworm and other soil invertebrate communities along an altitudinal gradient in the Silesian Beskid mountains (Southern Poland)

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The Silesian Beskid range is the western-most part of the Carpathian Mountains. The highest peak in the range reaches 1,257 m a.s.l. The climate is characterized by high precipitation (1,200 mm per year) and long-lasting snow cover. The Carpathians are densely forested with a clear altitudinal zonation of vegetation including: uplands (200-550 m a.s.l.), lower forest zone (dominating beechspruce, 550-1,100 m a.s.l.) and higher forest zone (dominating spruce, 1100-1,350 m a.s.l.). The study on earthworms was carried out along an altitudinal gradient (500-1000 m a. s.l) in three vegetation zones – both in the meadows and in the forested sites (beech, spruce, oak-hornbeam, alder-ash). In spring, summer and autumn of 2004, 2005 and 2006 soil animals were studied in 12 plots. On every sampling occasion and in every plot 5 samples $(20 \times 20 \times 20 \text{ cm})$ were taken. Earthworms and other soil invertebrates were separated from soil by hand sorting and preserved. In the laboratory, earthworms were identified to species level and the other invertebrates to higher taxonomic levels. The biomass of earthworms and other invertebrates was estimated. Eight earthworm species were found: Aporrectodea caliginosa, A. rosea, Dendrobaena octaedra, Dendrodrilus rubidus, Eisenia lucens, Lumbricus castaneus, L. rubellus and Octolasion lacteum. The highest earthworm density, diversity (H') and biomass was in foothill meadows. The earthworm and other invertebrate community structure was compared between sites and sampling seasons. The impact of site, season and altitudinal gradient on soil animals communities was analyzed.

Using earthworm behaviour (avoidance, cast production and burrowing activities) to assess the effects of liming on the functioning of anthropogenically acidified forest soils

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Liming of acidified ecosystems can represent a valuable alternative to promote the recovery of biodiversity impoverished by anthropogenic acidification. For the first time in France, two forested catchments (2x120 ha) located in the Vosges Mountains (NE France) were limed (2.5 t ha-1) by helicopter in the autumn of 2003. A preliminary study demonstrated higher earthworm abundance in the limed soils than in the surrounding acid soils, five years after the liming operation. In order to determine the detailed effects of liming, we carried out three laboratory tests to follow the possible modifications in the behaviour of three ecological groups of earthworms. Three modalities were tested: (i) acid soil (pH = 3.9), (ii) in situ limed soils (pH = 4.2) (both of them sampled in the catchments) and (iii) in vitro limed soils composed by acid soil mixed with liming concentrations (1, 2.5, 10 and 20 t ha-1). First, avoidance tests were performed using *Eisenia fetida* and *Lumbricus* castaneus sampled on the in situ limed site. Ten earthworms were introduced for 24h in plastic boxes, half composed of acid soil and half by each limed soil. Secondly, L. terrestris were introduced in Petri dishes to compare the earthworm body mass change and cast production after an exposure for 7 days in these three soils. Finally, Aporrectodea caliginosa, A. giardi or L. castaneus were incubated for 9 weeks in soil cores filled with acid, in situ or in vitro limed soils and X-ray tomography was used to assess their burrowing activities. A significant repellence was observed for the acid soil while the in vitro and in situ limed soils were attractive: $94 \pm 5\%$ of E. fetida and $48 \pm 27\%$ of L. castaneus were found in the in situ limed soil. Similarly, 100% of E. fetida and $80 \pm 10\%$ of L. castaneus were found in the invitro limed soil at 2.5 t ha-1 (pH = 6.2). Cast production of L. terrestris significantly increased with soil pH when a rational concentration of lime (2.5 t ha-1) was mixed with acid soil (pH 5.5). Finally, earthworms significantly increased their burrowing activities in the surface layers of soil cores in the in vitro soil at 2.5 t ha-1 (pH = 5.4). Thus, these studies demonstrated that earthworm behaviour is a relevant indicator of liming restoration. A study under field conditions with a local liming operation on an acid catchment is in process to validate these laboratory results.

Resilience to inundation in earthworm communities in river floodplain grassland: trait attributes and diversity

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Alterra, Wageningen UR

The relationship between biological diversity and ecosystem functioning can be studied in terms of ecological traits in species. We studied ecosystem functioning in terms of resilience to stressors, using as an example an earthworm community in a frequently flooded floodplain in the Netherlands. Our general hypothesis was that ecological resilience is determined by the distribution of traits between species within the community: a greater diversity of traits to overcome the adverse effects of flooding will allow the earthworm community to better respond to inundation and therefore be more resilient. Whilst earthworms can occur in high densities in floodplains, periodic occasions of flooding restrict population growth and species diversity. The different mechanisms employed by earthworm species for coping with this stressor can be described by a combination of ecological traits. For 10 earthworm species encountered along an inundation gradient in the Duursche Waarden floodplain area flanking the river IJssel we compiled a dataset of 11 relevant ecological traits. Trait frequency analysis confirmed the presence of flood tolerance traits at more frequently inundated plots, such as active dispersal, hydrophily, and parthenogenetic reproduction. The greater diversity of flood tolerance trait attributes in low-elevation plots suggests that earthworm communities at these sites are more resilient to flooding. Ordination analysis using trait composition instead of species composition somewhat improved the proportion of variance explained, suggesting that trait composition yields more information on community resilience than species composition. The combination of the two approaches improved our understanding of community resilience.

Earthworm biological traits, soil and landscape characteristics

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This study analyzes the links between the occurrence and frequency of biological traits in earthworm communities and soil and landscape characteristics in Amazonia. Biological traits are a powerful attribute to consider in order to understand the links between biodiversity and ecosystem processes. The present consensus is that diversity expressed in the nature and range of traits, rather than species numbers, determines ecosystem functioning. Biological traits are also important characters that determine communities in a given environment. Few studies have ever considered earthworm biological traits and their ecological significance. Therefore, we sampled earthworms at 135 points distributed among 3 localities, 9 sub localities and 27 farms in the South of Pará state (Eastern Brazilian Amazonia). At each point, 3 samples 25 x 25 cm in size were hand-sorted for earthworms (TSBF method). Traits (10) of 1256 earthworms in total were measured. They were individual (length, post and pre-clitellar diameter, length and type of caudal setae, pigmentation) or species specific (gizzards, thickness of septa, type of typhlosole, ecological category), multiqualitative, semi quantitative or fully quantitative. All the earthworms were determined at the morpho-species level. The landscape in a 100 m radius centered on each point was described with a set of metrics that basically described the composition, diversity and fragmentation pattern of the landscape on a matrix of 20 x 20m pixels. At each point 41 chemical and physical soil parameters were measured. Each data set (trait frequency matrix, landscape metrics and soil data) was analyzed using PCA and coinertia analyses were performed to search for co variations. Significant co-inertias were measured between the biological traits matrix and landscape structure (p=0.047, RV=0.05), landscape composition (P=0.015, RV=0.12) and soil characteristics (p=0.01, RV=0.26), respectively. These results show a strong link of earthworm traits with soil characteristics and landscape structure and composition.

An integrated assessment of metal adaptation in the sentinel earthworm Lumbricus rubellus

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A central premise of the use of sentinel organisms such as earthworms in toxicity testing is that their presence or abundance reflects the level of contaminant to which they are sensitive and predicts that individuals of a given sentinel species are less likely to survive at a contaminated site due to the physiological consequences of contamination. However, it is well known from pest management and evolutionary ecology research that organisms are capable of rapid adaptation to toxins via a variety of mechanisms, enabling them to survive and reproduce in environments where levels of toxicity would eradicate native populations. Such adaptation has been shown to be a mix of phenotypic plasticity and adaptive evolution through molecular processes such as gene amplification. If adaptive evolution is a common feature of sentinel species, it potentially confounds their use in toxicity testing and makes generalizations among sites and populations problematic. We examined this question in the earthworm Lumbricus rubellus, using a population adjacent to a long-established Nickel smelter in Clydach, Wales and two control populations as a model system. We tested the alternative hypotheses that populations closest to the Ni source were maintained by immigration from less contaminated sites versus the hypothesis that this population had become adapted to Ni contamination by rapid evolution. To examine this question we measured gene-flow, genetic diversity, soil chemistry and metabolite differences between populations of differing distance to the source and compared these results with 'natural' variation due to soil chemistry and agriculture at two uncontaminated sites. I will synthesize the results of this study examining the correlation between patterns of diversity and differentiation using DNA-based molecular markers, the chemical environment and the metabolome revealed through multivariate NMR spectrum analysis. Preliminary results indicate that soil chemistry has a strong effect on metabolome profiles but that the classical patterns of evolutionary adaptation (genetic erosion and limited gene-flow between populations close to the Ni source) are not evident and that populations in contaminated sites are more likely to be sustained by immigration. Further analyses are ongoing and will be presented.

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Understanding the ecology of soil is crucial for its sustainable management, however, given its complexity, little is known about the dynamics of the variables under different land uses (Anderson 1975). Agriculture is undoubtedly one of the activities that require attention in the field of species conservation, since large land areas have been transformed to become growing areas. The international project "Conservation and Sustainable Management of Below Ground Biodiversity" propose solutions to conserve species of organisms which live below ground and maintain the ecological functions of soil. This study, located within the former context, aimed to assess the population density of Oligochaeta in an experimental plot located in the biosphere reserve "Los Tuxtlas" in the state of Veracruz, Mexico, under different systems of maize production. In order to evaluate the relative abundance of earthworms, different treatments and control were used as follow: Maize-Mucuna (Mucuna pruriens) -Fertilizer (MMF); Maize-Mucuna (GM); Maize-Fertilizer (MF); Maize (M); Control (C) and uncultivated land (S). The experiment was conducted during a production cycle of maize in the summer of 2008. A higher density of earthworms was observed in the uncultivated land, far exceeding all other treatments, reaching a value of 10.34% in relative abundance of earthworms with respect to the other macrofauna accounted; the treatment that followed, the Maize without fertilizer, had a value of 3.0%; and finally, Maize-Mucuna with 1.33%. Results suggest that treatments without management seem to be preferred by the earthworms, showing a tendency to decrease their populations in the presence of agrochemicals.

Earthworm density and biomass in various land use systems in Northern Paraná State, Brazil**

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Soil alterations, such as oscillations in temperature, moisture, and organic matter contents, mainly resulting from agricultural activities, can impact earthworm abundance. The objective of this paper was to evaluate earthworm density and biomass in various land use systems in Northern Paraná State, Brazil. The study was conducted in Rolândia and Arapongas municipalities, in five areas: 1) no-till (36 yr) - NT; 2) subsoiled no-till - SNT (every 3 yr); 3) pasture - P (> 30 yr); 4) coffee plantation -C (> 30 yr); and 5) native forest – NF. Soils of the area were Oxisols: Rhodic Hapludox in NT, SNT, C and NF and a transition between Rhodic Hapludox and Rhodic Kandiudox in P. Nine samples were taken in each area over a one-year period (march 2008 to march 2009) every three months, using the TSBF (Tropical Soil Biology and Fertility) methodology (hand sorting of 25×25 cm monoliths from the 0-20 cm layer of soil). The earthworms were removed from the soil and preserved in 4% formaldehyde, and then counted (ind. m⁻²) and weighed (g m⁻²). The mean density in the year was higher in P (105 ind m⁻²), followed by NF, NT and SNT with 15, 13 and 12 ind. m⁻², respectively. Lowest mean density (4 ind. m⁻²) was found in C. Mean biomass followed the same order: P>NF>NT>SNT>C, with 7.15, 1.07, 0.21, 0.09 and 0.03 g m⁻² in each area, respectively. In the five samples dates (mar/08, jun/08, set/08, dec/08 and mar/09) highest densities and biomasses were observed in sep/08, in the end of the dry season, in P, C, SNT and NF. In NT, highest values for density and biomass were observed in mar/08, and then decreasing over the sampling period. All areas showed a large decrease in density and biomass in dec/08 and mar/09 which was attributed to climatic conditions during the study period which affected both, the water balance and soil moisture, determining factors for the survival and reproduction of earthworms. **Financed by Capes (scholarship for the first author) and Fundação Agrisus (divulgation of the results)

Earthworm species in various land use systems in Northern Paraná State, Brazil

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Earthworms are present in almost all terrestrial ecosystems, and are one of the most important components of the soil invertebrate macrofauna. Species identification is essential in biological, ecological, and behavioural studies, as well as to characterize the benefits of these organisms in various land use systems (LUS). This study aimed to identify earthworm species in four LUS in Northern Paraná State, Brazil. The study was conducted in the counties of Rolândia and Arapongas, in five areas: 1) no-till (36 yr) – NT; 2) subsoiled no-till – SNT (every 3 yr); 3) pasture – P (> 30 yr); 4) coffee plantation -C (> 30 yr); and 5) native forest -NF. Soils of the area were Oxisols: Rhodic Hapludox in NT, SNT, C and NF and a transition between Rhodic Hapludox and Rhodic Kandiudox in P. Nine samples were taken in each area over a one-year period (march 2008 to march 2009) every three months, using the TSBF (Tropical Soil Biology and Fertility) methodology (handsorting of 25 \times 25 cm monoliths from the 0-20 cm layer of soil). The earthworms were preserved in 4% formaldehyde, and then they were separated in juveniles and adults and were identified at the genus or species level when possible. In the five samples dates (mar/08, jun/08, sep/08, dec/08 and mar/09) highest diversity was founded in P (7 species): Fimoscolex sp. and Glossocolex sp. (native and new species), Pontoscolex corethrurus, Dichogaster gracilis, D. affinis, D. saliens (exotics) and NIsp.1 (not identified species - native?). In NT, 6 species were founded: P. corethrurus, D. affinis, D. saliens (exotics), Fimoscolex sp. and Belladrilus (Santomesia) emilianii and Belladrilus sp.1 (natives). In NF, 5 species were found: P. corethrurus, D. affinis (exotics), Urobenus sp., Belladrilus sp1 and sp2 (natives). Lowest diversity was found in SNT (3 species): D. saliens (exotic), B. (S.) emilianii and Belladrilus sp1 (native), and in C (2 species): D. saliens (exotic) and B. (S.) emilianii (native). Therefore, several native species were able to survive in the agro ecosystems, though disturbance was high, although these tended to be of small size and higher diversity was associated with lower disturbance, allowing comparable diversity with the native forest. **Financed by Capes (scholarship for the first author) and Fundação Agrisus (divulgation of the results)

Earthworm abundance and diversity in no-till systems in SW Paraná State, Brazil*

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No-tillage is a widely practiced conservation agriculture system in Brazil, with over 22 million ha currently in no-till. This management system, which includes permanent soil cover (straw), minimum soil physical disturbance and crop rotation with green manures, linked with integrated pest management, is known to promote, among other factors, the role of soil organisms in soil fertility. Therefore, the present study was undertaken to assess earthworm populations in 6 watersheds (Mineira, Ajuricaba, Facão Torto, Buriti, Pacurí and Toledo), in SW Paraná State, Brazil, aiming towards a ranking of earthworm density and diversity in no-till systems. Soils in the region are mainly Red clayey Latosols (Oxisols), derived from cretaceous era basalt flows. Earthworms were collected in 37 farms with no-tillage aging 3 - 27 yr of age, in February 2010, using an adaptation of the TSBF method (hand sorting of five 20×20 cm holes to 20 cm depth). In each watershed, earthworms were also sampled in one native forest fragment, used as a control. In the 37 no-till systems, earthworm abundance ranged from 0 - 24.2 individuals/hole (0 - 605 indiv./m²) and in the forests, from 0.2 - 11.4 indiv./hole (5 - 285 indiv./m²). Most of earthworms encountered were exotic species of the genus Dichogaster (D. saliens, D. gracilis, D. bolaui and D. affinis, Acanthodrilidae family) and native Ocnerodrilidae (mainly Belladrilus sp.), all of small individual size. In a few sites, individuals of the native Glossoscolecidae (P. corethrurus, Glossoscolex sp., Fimoscolex sp.) and exotic Megascolecidae families (Amynthas gracilis) were also encountered, in low densities. Urobenus brasiliensis (glossoscolecid) was found only in the forest fragments. Diversity per site ranged from 1 to 6 sp. and the totals per watershed were: Mineira, 4 sp.; Ajuricaba, 8 sp.; Facão Torto, 4 sp.; Buriti, 7 sp.; Pacurí, 6 sp.; and Toledo, 8 sp. In the forests, diversity was, 1, 4, 4, 2, 2, and 6 sp. per watershed, respectively. Based on this study and results from previous studies by the authors, a ranking of earthworm abundance and diversity in no-till systems is here proposed for the red Latosol, warmer climate regions (Cfa, Koeppen) of Paraná State: poor = 2 indiv./hole and 1 sp.; moderate = > 2 to 6 indiv./hole and 2 sp.; good = > 6 to 10 indiv./hole and 3 sp.; excellent = > 10indiv./hole and > 4 sp.*Financed by Itaipu-Binacional

Effect of afforestation and landscape position on the stable isotopic signatures of soils, vegetation, and earthworms on the Isle of Rum, Scotland, UK

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The Isle of Rum, in the Inner Hebrides off the northwest coast of Scotland, is a 10,000 ha nature preserve administered by the Scottish Natural Heritage (SNH). Historical land-use for subsistence farming and sheep grazing, and more recent private management of the site as a hunting preserve for red deer, have each contributed to the near total loss of native forest cover on the Island. However, after the SNH assumed management of the site, one goal has been to restore forest vegetation to large areas of the island. Afforestation efforts on Rum to date have consisted of experimental plantings of different tree species in fenced areas at various landscape positions across the island. Previous sampling for earthworms in these plots revealed that earthworms were found in much greater abundances under tree cover than in open moorland. In an effort to understand how increasing cover of forest vegetation and associated changes in soil faunal communities might be expected to influence nitrogen dynamics, we sampled soil, vegetation and earthworms for stable isotope analysis from beneath different tree species in several of the experimental forest stands as well as from older, well established woodlands surrounding the village of Kinloch (the only continuously inhabited human settlement on the island). Several patterns emerged in delta-¹⁵N signatures of soil, vegetation, and earthworms, with clear differences between tree species and elevation. Samples collected at higher elevations were less enriched with ¹⁵N than those at lower landscape positions, and soil and vegetation collected under pines (Pinus sylvestris) was less enriched than that collected under oaks (*Quercus robur*). We also observed inter-specific differences in delta-¹⁵N signatures for earthworms at a given site, as well as intra-specific differences in delta-¹⁵N in earthworms collected at different landscape positions, and in earthworms collected under different tree species. ¹⁵N signatures were always lowest in vegetation, and bulk soil usually 1.5-2.5% enriched relative to vegetation. When Aporrectodea caliginosa were collected they were always more enriched than bulk soil, and earthworms in the genus Lumbricus were always depleted in ¹⁵N relative to bulk soil. Dendrobaena spp., Dendrodrilus rubidus, and Octolasion cyaneum had variable delta-¹⁵N tissue signatures relative to bulk soil. These results will be interpreted in terms of earthworm feeding behavior, and nitrogen biogeochemistry.

Invasion of Asian *Amynthas* species and increase of earthworm abundance in Mid-Atlantic deciduous forests of the USA during 1999-2010

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Over 90% of the forests in the Mid-Atlantic USA are secondary forests at different successional stages. Soil macrofauna in these forests is often dominated by cosmopolitan earthworms, while native species are still present. In 1999 we surveyed the earthworm fauna at the Smithsonian Environmental Research Center (SERC), Maryland. We recorded eight species in the forests, including Allolobophora chlorotica (Savigny, 1826), Aporrectodea caliginosa (Savigny, 1826), Aporrectodea rosea (Savigny, 1826), Eisenoides loennbergi (Michaelsen, 1894), Lumbricus friendi Cognetti, 1904, Lumbricus rubellus Hoffmeister, 1843, Octolasion cyaneum (Savigny, 1826) and Octolasion lacteum (Orley, 1881). Four additional species, Bimastos palustris Moore, 1895, Dendrobaena octaedra (Savigny, 1926), Dendrodrilus rubidus rubidus (Savigny, 1926) and Diplocardia caroliniana Eisen, 1899, were found only in non-forest habitats. Earthworm abundance was significantly higher in young (70 yr) than in old (150 yr) forests. Species composition was also different. In addition, smaller, uncut forest stands had no earthworms. The objective of this study was to assess if this pattern holds ten years later. Between 2006 and 2010 we re-sampled the original plots and added new ones. Earthworms were sampled using formalin extraction from three randomly selected 50 cm X 50 cm quadrates at each plot. In addition to the eight species recorded in 1999 two cosmopolitan species, Lumbricus terrestris Linnaeus, 1758 and Amynthas hilgendorfi (Michaelsen, 1892) were found in the forests. Another exotic earthworm, Amynthas hupeiensis (Michaelsen, 1895) was newly recorded in the grassy areas. Species compositions in old forests differ from that in young ones by the presence of the native E. loennbergi and the absence of Ap. rosea, Al. chlorotica, and O. lacteum. Earthworm densities were significantly lower in old forests than in young ones in 2006, but subsequent sampling revealed an increase in overall abundance. The difference between young and old forests is diminishing. The uncut sites are still earthworm-free. Densities were lowest in the summer with the lowest ratio of mature (clitellate) to total individuals and the possible absence of Ap. caliginosa. We conclude that the earthworm fauna in the SERC forests is stable in terms of community composition but dynamic in terms of abundance. The presence and possible establishment of the three newly recorded species needs to be monitored.

Integration of soil biodiversity in soil quality monitoring: earthworm results of RMQS BioDiv program

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A French program "RMQS BioDiv" was developed in Brittany (Western France) in order to classify at a regional scale soil biodiversity in relation to land use and pedoclimatic parameters. The sampling area covered 27 000 km² and 109 sites were studied, in the Spring of 2006 and 2007, using a systematic sampling (regular grid 16 km x 16 km). The strength of this program was to propose a large panel of geological and pedological contexts combined to land uses (pastures, cultures, rotations). A large national research network (12 research teams) supported this program that undertook the study of several biological parameters such as macrofauna (earthworms, total macroinvertebrates), mesofauna (acarina and collembola), microfauna (nematodes) and other microorganisms (microbial biomass, community structure and functional genes). This program was connected to another soil monitoring network "RMQS" which covered the whole French territory and monitored physicochemical soil parameters and land-use management. The link between these two programs, working on the same sites, permitted the study of biological parameters in the light of soil and agricultural characteristics, and thus the definition of biological descriptors of soil quality. In this work we will especially focus on earthworm results of the RMQS BioDiv program. First, we will present abundance (global and by ecological categories), biomass, richness and diversity index results. These parameters were placed into a large dataset and have the advantage of geographical illustration at regional scale. The species assemblages will be also described thanks to the 23 earthworm species sampled in the area. Then, the relationship between earthworm parameters and explanatory variables such as land use and agricultural practices will be put in evidence. All combinations will be summarized in a table, in which we can distinguish, for example, that tillage practices greatly influence anecic abundance regardless of the pedological context. Moreover, spatial analyses conducted on earthworm parameters and species distribution will be shown. Actually, few species (e.g. Lumbricus terrestris, Aporrectodea nocturna) displayed spatial patterns at a regional scale. Finally, links existing between earthworm and other biological groups sampled in the RMQS BioDiv program will be shown, especially the significant co-structure that exists between nematodes and earthworm communities.

The need for meta-databases to store and manage soil biological data

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One of the eight major threats on soil identified in the EU's Thematic Strategy for Soil Protection (COM 2002, 2006) is the decline in soil biodiversity. Moreover, the pertinence of some biological groups as indicators is now largely admitted, and the "top 3 indicators" have been defined during the ENVASSO Program as the minimum set of indicators for assessment of biodiversity decline: 1) Earthworm diversity and biomass; 2) Collembola diversity; and 3) Microbial respiration. In order to study soil biodiversity (species and function), numerous long term monitoring programs are being carried out at different scales (local, regional, national, European and International scale) and on various agricultural systems by the EcoBio team of the University of Rennes (France). Research mainly addresses earthworm communities in regard to soil parameters and agricultural practices, and their relationship to the other biological groups, from microbes to macrofauna. Since vast data sets are generated by these different programs, and in order to facilitate data analysis, it became evident that an efficient tool of storage and management was required. Thus, since 2007, a data base model has been developed. Originally dedicated to a regional program "RMQS BioDiv" which produced more than 80 000 data points, it has been progressively adapted to be compatible to store data from all soil biodiversity monitoring studies and resulted in the creation of a relational meta-database. It consists of standardized modules, each module storing data from a particular program. Moreover, the meta-database was designed to be interoperable with other databases for instance with the national DONESOL database (Infosol INRA, Orléans), thus permiting analysis of earthworm data in relation to soil physicochemical data. Furthermore, it is interoperable with the Lombricien2000 database of the CNUSC (INRA, Montpellier) which contains all the data generated by the studies of Marcel Bouché since the 1960s, including anatomo-morphologic descriptions of earthworm species as well as data from pan-European inventories. A user-friendly interface has been designed, and will be soon accessible over the web, stimulating information sharing and the building of a real biodiversity observation system. The aim of such a poster is to present the structure of this meta-database, its components and functions, and examples of possible applications.

Use of earthworm traits to understand links between species assemblages and their environment

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Species' functional characteristics strongly influence ecosystem properties. Functional characteristics operate in a variety of contexts, including effects of dominant species, keystone species, ecological engineers and interactions among species. Most studies in community ecology are based on species diversity and relative abundance but these parameters alone are not always a good predictor of species importance at the ecosystem-level. Recently, numerous studies are attempting to use functional traits to link global change drivers, functional diversity and ecosystem processes, in order to develop indicators or generalize ecological patterns. An understanding of how changes in species richness and composition, and biodiversity in general, influence ecosystem properties requires knowledge of the functional traits of the species involved. By definition, functional traits are those that influence ecosystem properties or species' responses to environmental conditions. Many studies have addressed the link between plant species traits and their environment, but few studies have involved soil fauna. In this work we focus on temperate earthworm species, because as soil ecosystem engineers, they play an important role in, and interaction with their environment. In this context, our aim was to determine which environmental variables control community assemblages using earthworm traits. To reach this aim, we used data stored in the Lombricien2000 database. These data include earthworm taxa and environmental variables collected in France by Marcel Bouché (1972). Environmental variables included C, N, pH, Ca, altitude, soil texture and land use from about 1000 sites. It included artificial surfaces, agricultural areas, forest and semi natural areas. Species life history traits were selected among anatomical and morphological characteristics (e.g. calciferous glands, bladder shape, typhlosole, body size, body shape, pigmentation, resistance form). We used a three-table ordination method (RLQ analysis) to identify the relationship between environmental variables and species traits. Results demonstrated a gradient within the environmental data (C and N opposed to pH and Ca). The relevance of anatomical and morphological traits used to link community structure and environmental variables will be presented and discussed.

Earthworm community response to a disturbance gradient

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The aim of this study was to determine the earthworm community response to a soil deterioration gradient. The gradient was determined according to different management practices as follows: 1) a system with 40 years of uninterrupted agriculture, under no tillage practice during the last 16 years, 2) a 16 year old grazing field under feedlot management since two years ago, and 3) an abandoned pasture that has not been disturbed during the last 50 years. The samples were taken every three months between August 2008 and August 2009. For each system, five samples 25 x 25 x 25 cm were taken. Numbers of individuals and species composition were measured. Physical, chemical, and microbiological variables were measured at the same sampling points. The relation between these variables and earthworm presence was analyzed. Usage history showed differences between variables, through discriminant analysis. These differences were mirrored by earthworm species composition and abundance. A Mann-Whitney analysis (p< 0.05) showed that earthworms preferred environments with P levels higher than 20 ppm, Ca levels higher than 11 meg/100g, K below 1.4 meq/100g, total N content above 0.25%, soil humidity above 15%, low compaction values (<1,2 g/cm^3) and low mechanical resistance. Species distribution was, in turn, associated to some of the variables mentioned. In this way, the abandoned pasture had soils with neutral pH, the lowest levels of K, was slightly compacted and had high activity of N2-fixing bacteria. In these soils the native Microscolex dubius was dominant, with the presence of Aporrectodea and Octolasion sp. The longterm agricultural system had slightly acidic soils, high Ca and Mg, intermediate K level, low total N and low activity of N2-fixing bacteria. In these soils, the exotic Aporrectodea was the dominant genus, followed by Octolasion and Microsocolex. The old grazing field was different from the agricultural system by having lower P, Ca, and Mg, and high levels of K and total N. However, earthworm presence in this environment was associated to high soil humidity, with the native Eukerria stagnalis present, indicating flooding problems not detected by the chemical and physical analyses. Even though the analyzed variables showed statistically significant differences, from an agricultural point of view (INTA agronomic indicators) the systems did not differ from each other nor showed signs of deterioration. But earthworm species composition and abundance indicated present environmental conditions and the risk of resource deterioration.

Diversity and abundance of earthworms in land use systems in Central-Western Colombia

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The Neotropical landscape of the Colombian Andes is characterized by a mosaic of agroecosystems with diverse land cover in which the adaptation ability of soil fauna is still unknown. To analyze the diversity and abundance of earthworm species in relation to changes in selected physical and chemical parameters (bulk density, C & N stocks, %C, %N, 13 C, 15 N, C:N ratio) the systems were classified in five categories according to land-use intensity: non-intensive (NI), low intensity (LI), medium intensity (MI), high intensity (HI) and highest intensity (IN). There were significant differences (p<0,05) in C and N contents of IN systems (pastures), compared to the other categories. In total, 26 earthworm species were recorded of which 16 showed a high adaptation capacity to natural (NI) and to low and medium disturbance (LI, MI) systems. Depending on the use intensity, there were also significant differences (p<0.05) in the origin (native vs. exotic) and ecological category of earthworms, mainly in the variables native species diversity, endogeic species diversity, native species abundance, native species biomass, and exotic species abundance. The results allow deduction of which mosaic systems are more favourable for agrobiodiversity conservation and for which subsystems special management is required to resolve problems of habitat degradation.

Impact of floodplain restoration on earthworm communities

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Floodplains have been intensively embanked, dammed or otherwise engineered in industrialized countries, but are now increasingly being restored, often at high cost. Increasing biodiversity is a major goal of these projects. There is however much confusion over the impact of such transformation on below-ground communities. Even a functionally essential group such as earthworms remains understudied in this context. Using a functional process zones (FPZ) approach, we aimed at assessing the differences along a perturbation gradient (fluvial dynamic) of a recently restored floodplain in terms of earthworm diversity and community structure, defining the relations among earthworms and ecosystem functions and among earthworms and other soil fauna communities. The study took place along the Thur River in western Switzerland. The site was divided into six FPZ showing different fluvial dynamics. Six replicates were used in the most dynamic ones (from gravels to willow bushes) and four in the most stable ones (forest). A non restored section of the river (pasture) was sampled as a pre-restoration control. Site (coordinates, elevation, vegetation structure and soil morphology) and functional (litter input and decomposition rate, C and N cycling, enzymatic activity, microbial biomass and soil respiration) variables were measured. Earthworms were sampled in early October 2008 by mustard powder extraction. Their functional role was assessed by calculating the fraction of variance explained by each explanatory variable. The relations with other communities (vegetation, spiders, testate amoebae and bacteria) were investigated using multiple factor analysis. Earthworm diversity and species richness increased along the gradient. The highest values were obtained far from the river in the most stable FPZ. The composition of earthworm communities differed between the dynamic and stable FPZ and between the restored and control areas. This pattern was best correlated to litter input, lateral distance to the river and plant community composition. Restoration changed earthworm communities that are now structured into FPZ. Litter input is of primary importance for this pattern. As a result, changing plant communities may change the quality and quantity of litter inputs as well as the related earthworm communities which may in turn influence organic matter cycling. This highlights the importance of below-ground communities for the success of restoration projects.

Passive invasion of earthworms in rehabilitated open mined soils: the effect of time, topography and vegetation types

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The extraction of primary materials in open cement mines eliminates the entire soil biotic community. To recover ecosystem function and biodiversity, rehabilitation and restoration practices include soil inoculation from neighbouring or foreign sites, the introduction of different plant types and other ecological strategies. In this study we evaluated the passive invasion (no inoculation implemented) of earthworms in open cement mined soils in central Mexico, rehabilitated 17 years ago with four main strategies/treatments: slope with trees (ST), slope with grasses (SG), flat clay banks with trees (FT) and flat clay banks with grasses (FG). Earthworms were sampled in 1995 (2 years after rehabilitation), 1998 (5 years after) and in 2009 (16 years after). Control sampling of earthworms was made in a nearby tropical undisturbed forest. Our results indicate that species richness, abundance and vertical distribution increased with time, and that some natives were capable of re-colonizing rehabilitated soils. In some treatments, species richness and abundance were even higher than in tropical forests; vertical distribution in all treatments, however, was always more superficial than in forests.

Earthworm abundance and biomass in native Brazilian Atlantic forest and Araucaria angustifolia and Pinus elliotti plantations collected using two sampling methods

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Earthworms are important indicators of environmental quality and management, such as the conversion impacts of native to planted forests. Nevertheless, little research has been conducted in this field in Latin America. Therefore, the present work was undertaken to determine the effect of native and exotic tree plantations on earthworm populations in the Atlantic Forest region of Southern Brazil. Earthworms were sampled in sixteen areas at the Embrapa Forestry Research Station in Colombo, Paraná State, Brazil, including five areas with Ombrophyllous mixed forests (Atlantic forest with native Araucaria angustifolia trees), five A. angustifolia plantations and six Pinus elliotti plantations (PP). All the Araucaria and Pine plantations were established between 1979-1987. Two sampling methods were used: a) excavation of 40×40 cm monoliths and manual sorting of the soil at the depths of 0-10 and 10-20 cm, and b) application of 20-L of diluted formaldehyde (0,5%) on an area of 1m2. Earthworm species density and biomass obtained with each sampling method were assessed and compared across the areas. The most abundant species were *Pontoscolex corethrurus*, a peregrine species and Amynthas gracilis, an exotic. A few individuals of Amynthas corticis and Metaphire schmardae (exotics) and of the native species Urobenus brasiliensis and Andiorrhinus sp., were also found. The highest abundance of earthworms was encountered using hand-sorting in Pine plantations (116 ind/m2), followed by the Araucaria plantations (64 ind/m2) and the lowest abundance was found in the native forests (38 ind/m2). On the other hand, biomass was similar in the Araucaria and Pine plantations (around 25-26 g/m2), and the lowest biomass was found in the native forests (12 g/m2). With the formaldehyde sampling, earthworm abundance was much lower, ranging from 11 (Araucaria plantations) to 20 ind/m2 (native forests). Biomass ranged from 6.6 (Araucaria plantations) to around 9 g/m2 (native forests and Pine plantations). Therefore, although all species were collected using the formaldehyde method, hand-sorting for earthworms was much more efficient for extracting earthworms from the studied forest ecosystems.
Diversity of earthworm communities as indicators of flood regime on coastline

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The salinity of sea water, periodical flooding and dry-off of habitat as well as unfavourable soil texture has strong negative impacts on earthworm communities. In spite of this, most Estonian earthworm species are present on the coast of the Baltic Sea and the community composition can be used as indicator of flood regime. The grasslands of the Western Estonian coast are on saline littoral soils. Distribution and abundance of earthworms are influenced by flooding duration and character. The salinity in the Baltic Sea water is low (6-8‰), the salinity of Matsalu and Liivi Bays being lower (5-6‰) or nearly fresh at the river mouths. The brackish conditions of the Baltic Sea create special habitats for earthworms. We studied earthworm communities on the coast of Matsalu Bay and Liivi Bay, in different zones divided on the basis of degree of influence of the sea and characterized by plant species that tolerate various levels of salinity and humidity. In Estonia 13 species of earthworms have been founded so far, all peregrine cosmopolitans. Earthworm abundance on the coastal areas ranged from 0 to 84 individuals m-2. Epigeic species are more tolerant to limiting factors and they recolonize coastal areas very quickly after unsuitable conditions. Endogeic species are more sensitive to submersion and unfavourable soil texture. Only the semiaquatic earthworm Octolasion lacteum was able to inhabit coastline areas mostly flooded by seawater. Coastal areas are impossible habitats for anecic species because of high groundwater table. The most tolerant species to extreme conditions was Lumbricus rubellus being dominant in the subsaline zone of the coast. This is generally an area underwater that dries up when water level decreases. Here O. lacteum was also abundant. Epigeic earthworms, Dendrobaena octaedra, Eiseniella tetraedra, Dendrodrilus rubidus and Lumbricus castaneus were present in both subsaline and saline zones of the coast. In the suprasaline zone a small number of Aporrectodea caliginosa and A. rosea were present on areas where the soil layer was developed. Five peregrine species (endogeic and anecic) were not present in the zones under influence of sea water. In conclusion, we found five epigeic and three endogeic species living in habitats with periodic flooding by brackish sea water. These peregrine species were able to recolonize the habitats on the coast after flooding and can be used as indicators of changes in salinity and flood character of coastal areas.

Poster - Session 3

Impact of flood deposits on earthworm communities, humus forms and soil parameters in alder forests from a subalpine floodplain (Kandersteg, Switzerland)

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Floodplains are areas of land adjacent to rivers and streams that are subject to recurring inundations. As a result of this dynamic, vegetation changes along a gradient of flooding frequency and duration, and usually consists of many small patches of vegetation with different species composition and succession of stages often related to fluvial landforms. Regarding soils, they are highly variable and also strongly related to fluvial dynamics; the coarsest sediments being deposited on the natural levee, immediately adjacent to the stream channel, while finer soil particles are progressively deposited with increasing distance from the stream. In this context, we studied a floodplain at the subalpine level (1200 m) that presents visually a flow-rate gradient over approximately 3 km. We hypothesized that biological and physicochemical soil parameters varied along this river gradient. In order to eliminate the variation of vegetation, and because forests of alder shrubs and trees were recovered from the highest flow regime to stagnant waters, we described humus forms and soil types in seven alder phytocenoses. Soil samples were removed at two depths when possible (0-10 and > 10 cm) and extraction of earthworms was performed at each of the seven stations. Soil analyses included pH (water and KCl), cation exchange capacity (CEC), amount of organic matter (loss on ignition, carbon and nitrogen), amount of CaCO3 (total and active) and soil texture. Fungal mycelia was also qualitatively observed. Earthworms were weighed and identified to species level. First results demonstrated a high variation of soil organic matter and active CaCO3 in the first 10 cm, as well as a great difference in soil texture. Thus, at the soil surface, coarse sands were positively correlated with high flow regimes, while the inverse tendency was observed for fine loam and clays. No strong variation was observed for pH, CEC and total CaCO3. Studies on the earthworm communities are still in progress, as well as humus forms and total C and N.

Invasion of *Pontoscolex corethrurus* (Glossoscolecidae, Oligochaeta) in Amazonian landscapes

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Pontoscolex corethrurus (Glossoscolecidae, Oligochaeta) is an invasive endogeic earthworm that has colonized most land transformed by human activities in the humid tropics. When installed, populations can change soil physical properties, biogeochemical processes and microbial communities. The aim of this study was to determine whether P. corethrurus establishment is a result of: 1) a competitive exclusion of native species, or 2) the exploitation of a new niche created by disturbance that native species cannot use. We tested these hypotheses by performing a survey of earthworm communities in 270 sample points that covered the diversity of land use systems encountered in two contrasted regions of the Amazonian arc of deforestation, in Brazil and Colombia, respectively. Independence between the processes that determine each group density, indicating an absence of competitive exclusion, was verified by their respective locations on orthogonal (Colombian points) or quasi-orthogonal (Brazilian points) axes of a Principal Component Analysis of their abundance across sampling points and land uses. Pontoscolex corethrurus densities co-varied with soil N content and pH (all points). Our results thus suggest the latter hypothesis, i.e., that P. corethrurus, unlike native species, is able to feed and develop in environments where litter resources are severely decreased and soils have been enriched in C and nutrients by deforestation and burning.

Dew worms in the white nights

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Lumbricus terrestris L. (the dew worm) forages, mates and migrates on the soil surface during the night. Its distribution covers a broad latitudinal gradient and variation in day length conditions. Since soil-surface activity is crucial for the survival and reproduction of dew worms, it is conceivable that northern populations that experience light nights in the summer have adapted to remain surface active in twilight and show less sensitivity to light compared with more southerly populations. We evaluated this hypothesis experimentally. Adult dew worms were collected from a wide latitudinal gradient in May 2009, from field sites in Jokioinen (Finland; 60°48'N, 23°28'E), Preston (UK, 53°46'N, 2°42'W) and Coshocton (USA, 40°16'N, 81°50'E). In Jokioinen there is a 3-week period without a dark-night interval (twilight prevails throughout the night) in the middle of summer, while in Preston even the shortest midsummer night has 5 hours of darkness and, respectively, 8 hours in Coshocton. Using field-collected animals, an experiment was conducted in Jokioinen from June 6 to June 21 - midsummer. Groups of three individuals from a particular geographic origin were housed in soil-filled PVC-tubes. Before the experiment they were kept in darkness and then under an artificial light-cycle to equally distort any inherent daily activity pattern. Three replicate tubes were exposed to either ambient natural light or artificially induced darkness during the night. Earthworm surface activities were recorded from above using low-light video cameras from 22:30 to 04:30 hrs, which covered the period between sunset and sunrise. The main response variable was surface activity (activity vs. no activity in 0.5 hr increments). Preliminary analysis of the data has shown that under artificial darkness, surface activity was high throughout the night for all three dew worm origins. Under ambient light, surface activity increased gradually after sunset, peaked after midnight and then declined towards sunrise. Giving tentative support for the initial hypothesis, under natural light Finnish earthworms began their surface activity earlier than the others and remained active for the longest period. In the statistical analyses currently underway, we are using air temperature and moisture together with an index of cloud cover as covariates. Our presentation will report the full results and discuss their importance and implications.

Tillage practices and organic fertilization influence endogeic earthworms

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In agricultural soil, it is widely recognized that reducing soil tillage and organic fertilization can, independently, improve earthworm communities. On one hand, conservation tillage management promotes anecic species thus increasing total earthworm biomass. On the other, organic fertilization improves the whole earthworm community. However, the response of earthworm communities to a combination of reduced tillage and organic fertilization is less well documented, despite the potentially favourable aspects of this agricultural management system. Our study was based on an experimental site in Britain (France) that combined three tillage managements: conventional tillage with plough (CT); superficial tillage without plough (ST) and No tillage (NT), with mineral fertilization (M), poultry manure (PM) and Pig slurry (PS). After eight years, earthworm communities were sampled by formaldehyde extraction. Complementarily, earthworms were sampled at different depths (0-5 cm; 5-10 cm and 10-20 cm). In mineral fertilization, ST (102 ind.m-2) and NT treatments (196 ind.m-2) were more favourable to earthworm abundance (and biomass) compared to CT treatment (62 ind.m-2). With organic fertilization, this trend was also verified in term of biomass, but not in term of abundance. Indeed, anecic earthworms were supported by the reduction of tillage (increasing total earthworm biomass) whereas endogeic earthworms were supported by organic fertilization (increasing total abundance). Therefore, endogeic abundance reacts differently to soil tillage reduction according to organic fertilization. Although no significant difference was observed, superficial tillage (ST) affected more endogeic species (91 ind.m-2) compared to CT (121 ind.m-2) with pig slurry; abundance being equivalent in CT (165 ind.m-2) and ST (152 ind.m-2) with poultry manure. In conventional tillage (CT), endogeics were regularly distributed on the whole ploughed layer (0-20 cm) whereas they were concentrated in the first 5 centimetres in ST and NT. This study indicates that the superficial tillage can be as harmful as traditional ploughing because endogeic species live near the soil surface and are more susceptible to superficial soil disturbance. Just like reduced soil tillage, organic fertilization appears to be a good practice for soil restoration, but acts differentially on the functional component of the earthworm community.

The effects of production systems on earthworm assemblages in vineyards and apple orchards

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Three different production systems in vineyards and apple orchards, i.e. biological (BP), integrated (IP) and conventional (CP), were compared for their effects on earthworm communities. Commercial vineyards and apple orchards were studied in three wine and three fruit production areas in the Czech Republic. In each area, plots under BP, IP and CP, and a nearby natural site (control), were chosen for the study. In spring and autumn 2009, soil samples were taken in two vine rows or tree lines and adjacent alleys of each plot and in transects across natural sites. Earthworms were extracted by heat using a modified Kempson apparatus. In total, 10 earthworm species were found, 6 in vineyards and 7 in orchards. In nearby natural sites 8 species were recorded and 3 of them were exclusives. The average species number per-site was higher in BP and IP than CP vineyards and in BP than in IP and CP orchards. The community structure in IP vineyards and in IP and CP orchards was similar in all production areas, while those in BP vineyards and orchards differed strongly between production areas. Earthworm density and biomass were higher in IP than in CP vineyards, whereas both parameters in BP did not differ significantly from those in IP and CP vineyards. In apple orchards, the earthworm density was higher under CP and IP than under BP. However, there was no difference in earthworm biomass among differently managed orchards. In vineyards, earthworm density and biomass were significantly lower in the vine rows than between them. In contrast, both parameters were higher in the tree lines than in the alleys of orchards. The results are discussed with reference to management practices (soil tillage, mulching and pesticide applications) used in plots under different production systems.

Poster - Session 3

Edaphobase – an online soil organism data warehouse for taxonomy, literature and ecological data management: The example of earthworms

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Edaphobase is a database project aiming to bring together information concerning taxonomy, literature references and ecological requirements of important soil taxa in a combined database system. At present, Collembola, Enchytraeidae, Gamasina, Lumbricidae, Myriapoda, Nematoda and Oribatida will be included. Data are to be extracted from literature, collection data and unpublished material from research institutions with a focus on Germany and its neighbouring countries. Linking such data on a wide range of soil-organism groups from several trophic levels with climatic, habitat and geographical data will allow detailed subsequent analyses on geographical distribution as well as ecological requirements of single species such as, e.g., preferences for temperature, pH, moisture or nitrogen content or effects of anthropogenic perturbation. In addition, prognostic tools will be provided to discern changes within soil biocenotic assemblages as a reaction to, e.g., modifications in land-use techniques or effects of climatic changes. The final data set will be available for public access via a web-based application, including investigations tools for construction of fine-scaled distribution maps, analysis results regarding parameter preferences or estimations of threats to soil-organism distribution. First experiences with earthworms are presented and discussed.

Ecological stoichiometry in earthworms (Oligochaeta, Lumbricidae) belonging to different ecological groups

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The growing interest in applying stoichiometry approaches to community ecology has resulted in numerous studies concerning aquatic systems, while terrestrial (soil) biota are still poorly known in this respect. Usually, species closely related taxonomically and belonging to the same functional group may be expected to have similar stoichiometric body composition. On the other hand, from ecological niche theory, we can expect that closely related species of soil fauna coexisting in relatively homogenous environments should realize different niches that may be reflected in different stoichiometry. Conversely, species characterized by similar stoichiometry should be competitively excluded. The aims of our study were to check: (i) if earthworms belonging to various ecological groups differ in body composition and stoichiometry, (ii) if earthworms belonging to the same ecological groups are homogenous with respect to body composition and stoichiometry. The study was conducted on earthworms belonging to three different ecological groups: anecic (Lumbricus terrestris), endogeic (Aporrectodea rosea) and epigeic (Dendrobaena octaedra, Dendrodrilus rubidus). Contents of macroelements (C, N, S, P) and microelements (Mg, K, Ca, Fe, Cu, Mn, Zn) were analyzed in earthworms bodies, using the following methods: C, N and S with CHNS analyzer, P colorimetrically using molibdenian blue method (FIA), and Mg, K, Ca, Fe, Cu, Mn, Zn by AAS using standard procedures. We found that in earthworms the mean concentration of macroelements was: C 45.9%, N 10.2% and P 1.1%. The ratio C/N was 4.5, C/P 40.3 and N/P 9.0. The average contents of microelements in earthworms were: Ca 9664.4 mg/kg, Zn 284.7 mg/kg, Mn 107.8 mg/kg, Mg 695.4 mg/kg, Fe 564.9 mg/kg, Cu 22.9 mg/kg, and K 9111.1 mg/kg. We found significant interspecies differences in body composition. D. rubidus differed from D. octaedra in the N content, and D. rubidus differed from D. octaedra and L. terrestris in C content. Interspecies differences were also been found in the content of Ca, Zn and Mn, while the concentration of P was similar in all species. The obtained results are discussed.

Soil-dwelling annelids during meadow restoration on arable soil using different restoration schemes

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Earthworms, enchytraeids and other annelid taxa present (Rhyacodrilinae; Aeolosomatidae) were studied during a field experiment comparing several approaches to meadow restoration on arable soil. The study site was situated in the White Carpathians (Czech Republic), where meadows, rich in herb species and of high conservation value, have been one of the traditional land-uses, providing protection to the local erosion-prone soils on flysch. The experiment was conducted on a former meadow that had been used as an arable field from 1970 to 1998. The soil was luvisol (clay-loam; pH 6.0-7.0; highly variable soil moisture). In spring 1999 the site was subjected to different treatments in a randomized block design. Our study focused on three treatments: (1) sowing with regional seed mixture, (2) sowing with commercial grass seed mixture, and (3) natural regeneration (old field succession directed by annual mowing). All experimental plots were mown each year in summer. Samples were also taken in an adjacent arable field, representing the pre-restoration situation, and in a well-developed herb-rich meadow, representing the approximate restoration target. Soil-dwelling annelids were studied from 2000, a full sampling programme with three samplings per year covering all replicates of the above treatments was conducted in 2002-2004, another sampling followed in Nov. 2009. In total, 4 earthworm species were founded in the field, 7 in the restoration plots, and 8 in the well-established meadow. Octolasion lacteum, Aporrectodea trapezoides and A. caliginosa dominated in the initial phase of meadow restoration. A. trapezoides had almost disappeared by 2004, A. rosea, one of two dominant species in the meadow, became dominant in all restoration plots. Annual densities were below 7 individuals/m2 in the field, about 15 ind./m2 in the sown plots, over 40 ind./m2 in the natural regeneration plots, and 50-60 ind./m2 in the meadow. In total, 30 enchytraeid species and 3 other small annelids were identified. Their assemblage in the arable field was impoverished, that of the meadow the richest. All assemblages were dominated by Fridericia spp. and Enchytraeus spp. Annual densities ranged from 700 to almost 19 000 ind./m² in the restoration plots. Neither earthworm nor enchytraeid densities showed a trend in the course of succession, possibly because of a drought in 2003. Enchytraeids showed a shift in vertical distribution towards the upper 3 cm of soil during the experiment.

Earthworm population dynamics under the impact of agricultural practices and meteorological factors in the most common soil types in Latvia

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The data of the State Agricultural Land Monitoring program were used in the present analysis. These data included long term observations of agricultural soil properties and analysis of earthworm population dynamics under the impact of intensive agricultural practices and fluctuations in meteorological factors at four monitoring sites, representing the most common soil types in Latvia: sod podzolic sand and loamy sand soils, sod podzolic glevic loam soils, sod calcareous loam soils and fen peat soils. Sample plots included arable lands used for growing different crops over long time periods. Earthworms were sampled from 1992 to 1998 every year in autumn. In total six earthworm species were found in the study sites: Aporrectodea caliginosa, A .rosea, Allolobophora chlorotica, Lumbricus terrestris, L. rubellus and Dendrobaena octaedra. Only two species were present in all sampling plots: A. caliginosa and L. rubellus. In permanent pastures there was a tendency for 2-3 earthworm species associations. In fields with intensive use there was a distinct dominance of A. caliginosa; other species were found in low numbers or not at all. The most favourable soils for earthworms were the sod podzolic loam and gleyic loam. In general, the greater abundance of earthworms corresponded to the more beneficial weather conditions characterized by warm and moderate wet season. After dry and hot periods in summer, a substantial reduction in number of worms occurred. In pastures with low intensity of soil disturbance, the impact of meteorological factors had less effect on the year-to-year variability of the earthworm communities. Perennial vegetation reduced the influence of unfavourable climatic factors and enabled a more even distribution of worms in the soil. The density and composition of earthworm communities were good bio-indicators for assessment of the agricultural soil quality. Mechanical soil disturbance like intensive tillage significantly affected abundance and diversity of earthworms and resulted in reduction in earthworm numbers and species diversity. In general earthworm communities including less than 3-5 earthworm species are indicators of possible damage of agricultural soils. A considerable factor determining earthworm populations was also relief. On the top of the hills earthworms were usually less abundant than at the base because of more intense soil erosion. In the depressions the ground-water level often was closer to the soil surface. During dry summer months, this last factor is of particular importance for earthworms.

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Relationship between Oligochaeta and plant communities: a case study from North-central Uruguay

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Community composition of terrestrial Oligochaeta depends on climatic, edaphic, topographic and vegetations conditions. The aim of this study was to describe the relationship between plant communities of the Basaltic region of Uruguay and earthworm communities. In the spring 2007, plant communities present in the study area were surveyed: Meso-xerophytic grasslands (PMX, 11 sites); Lithophytic steppes (EL, 3 sites) and Meso-hydrophytic grasslands (PMH, 8 sites). We used the standard Tropical Soil Biology and Fertility (TSBF) method that consists of hand-sorting earthworms from soil monoliths 25 x 25 cm and 30 cm depth. In each site eight samples were taken every 5 meters on two parallel transects of four points separated by 20 m. Oligochaeta from Ocnerodrilidae, Acanthodrilidae and Glossoscolecidae families were collected. PMH had the highest density of individuals (181 ind/m²) and Ocnerodrilidae was the dominant family (94%). PMX had a density of 20 ind/m² with Ocnerodrilidae and Glossoccolecidae as the most abundant families. These represented 49 and 42% of the total individuals, respectively. EL had a density of 50 ind/m2 with Ocnerodrilidae (83%) being the most abundant family. In this plant community, Acanthodrilidae (15%) was the second most important family. Results suggest a relationship between the earthworm communities and vegetation, allowing the determination of biological assemblages.

Session 4

Earthworms and Ecosystem Services



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Plenary Lecture - Session 4

Earthworm activities, soil functioning and ecosystem services

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Earthworms have been described as ecosystem engineers that act as powerful regulators of soil functioning at different scales of space and time. At a local scale, they produce large amounts of organo-mineral structures (burrows, chambers and casts) that have specific impacts on soil physical, chemical and biological processes. At higher scales, their aggregated effects may significantly impact ecosystem functioning and the providing of important environmental services for human societies. Here I present a state of the art review of what is currently known about the impacts of these organisms on soils and how these impacts may affect services delivering by terrestrial ecosystems. The approach considers several observation levels, allowing the scaling of processes from the species functional domain to their impacts at the ecosystem scale.

Anecic earthworms in New Zealand sheep- and dairy-grazed pastures

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Anecic earthworms play an important role both the development and maintenance of soil structure and in the incorporation and initial mixing of plant litter. A previous study, has found the distribution of anecic earthworms patchy in New Zealand pastoral - Session 4 land, limiting their potential contribute to ecosystem services. In this study earthworm populations were estimated as part a larger project examining soil invertebrates in New Zealand pastures. Nine different management regimes in sheep-grazed pastures and 17 in dairy-grazed pastures across ten locations in the North and South Islands were investigated. Other factors which were assessed included stocking rates, fertiliser use, soil fertility, and soil structure. Peregrine anecic earthworms (Lumbricus terrestris, Aporectodea longa) were present in most flat and rolling, dairy-grazed pastures, with a biomass of up to 400 g wet weight/m2. The response of anecic earthworms to increasing live-weight loading (i.e. dairy cow stocking rates) and nutrient inputs was observed in a well-structured Allophanic soil. Anecic earthworms tended to increase as pasture production and other inputs increased (14 to 155 g wet weight/m2). This probably reflects both increased food resource input into the soil environment and the lower susceptibility of anecic earthworms to stock treading pressure; with their ability to burrow deeper to avoid physical damage from livestock hooves gives them a competitive advantage over epigeic earthworms. At sites with poorly structured soils such a response was absent, indicating that the physical environment was a limiting factor. The ability of anecic species to feed on surface litter yet to escape to depth to avoid treading pressure suggests that they may offer an option for maintaining the service of litter incorporation in the soils where stock treading limits epigeic earthworm populations. This study confirmed, what the early study showed, that anecic earthworms have a patchy distribution in New Zealand pastures. The absence of peregrine anecic species in sheep-grazed hill-country pastures was notable. Some indigenous anecic species (especially Octochaetus multiporus) were present in low fertility hill-country, but appear to play little role in the provision of soil services once soil fertility and stocking rate are increased.

Manipulated earthworm populations decrease runoff and erosion rates in arable land with distinct soil tillage treatments

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Earthworms as physical ecosystem engineers may affect key erosion-controlling soil (surface) characteristics (e.g. soil cover and roughness, surface depression storage capacity, water infiltration capacity, porosity) through the formation and maintenance of biogenic structures such as casts, middens, and burrows. Although many studies observed decreased erosion rates under conservation tillage systems as compared to conventional ploughing, and numerous others showed increased earthworm abundance and related macroporosity under conservation tillage, the net effects of earthworm bioturbation on soil erosion in arable land have not yet been systematically studied, at least not at an appropriate scale. The objectives of this study were to gain insights in the relationships between (1) soil tillage system, (2) earthworm biomass and species composition, and (3) sediment loss through water erosion, thereby disentangling the much acclaimed net positive effects of earthworms on soil erosion control in arable land. A large field experiment on moderately sloping sandy loam soil in central Belgium, representative for much of the intensive crop culture in the Western European erosion-prone loam belt, was carried out during 2005-2008. In each of three different soil tillage treatments - shallow reduced (non-inversion) tillage (RTs), deep reduced (noninversion) tillage (RTd), and conventional (inversion) tillage (CT) – earthworm populations were either reduced by capturing using electro-shocking, increased by introducing commercially obtained Lumbricus terrestris L., or remained unmanipulated as controls in 8×8 m² plots. Earthworm fluctuations were monitored biannually. Erosion and runoff rates were measured at two scales through rainfall simulations and experimental runoff discharges in spring 2007 and 2008, together with relevant physical soil (surface) variables. Anecic earthworm biomass was identified as an important independent variable in runoff and erosion regression models, next to plot slope, soil moisture content and rainfall intensity. Erosion rates exponentially decreased as a function of anecic earthworm biomass. Path analysis using structural equation modelling revealed that soil tillage had a profound effect both on soil erosion and earthworm abundance, but also that anecic earthworm biomass in itself contributed to soil erosion reduction. This study underlines the importance of earthworms as conservators of the valuable soil resource against degradation by erosion in the studied agro-ecosystem, and the need for promotion of appropriate soil ecosystem management by the farmer supportive of populations of anecic earthworm species (non-inversion tillage, direct drilling).

Nitrous oxide emission as a function of earthworm feeding strategy

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Recent studies suggest that earthworm activity leads to increased emissions of the potent greenhouse gas nitrous oxide (N_2O) . This is especially salient as, in terms of global warming potential, it may negate the supposed capacity for earthworms to increase carbon stabilization in soil organic matter. However, the exact pathway behind this effect is not clear. In a series of three experiments, we studied how N₂O emissions differ between earthworm species with different feeding strategies. In a first experiment, dried grass residue (Lolium perenne) was applied on top of a loamy soil or mixed through the soil, and N₂O-emission was measured for three months. Treatments included presence of the epigeic earthworm Lumbricus rubellus or the anecic earthworm Aporrectodea longa. Cumulative N₂O-emissions increased significantly for both species. The strongest effect was measured for L. rubellus, where N₂O-emissions significantly increased from 56 to 789 μ g N₂O-N kg-1 soil, but only when residue was applied on top of the soil. In a second experiment we determined the effect of epigeic (L. rubellus) and/or endogeic (Aporrectodea caliginosa) earthworms on N_2O -emissions in the presence of ¹⁵N-labeled radish residue (Raphanus sativus subsp. oleiferus). Both species showed significant increases in N2O-emissions, which differed with residue application method. The strongest effect was measured for A. caliginosa when residue was mixed into the soil, increasing emissions from 1350 to 2223 µg N₂O-N kg-1 soil. L. rubellus only resulted in elevated N₂Oemissions when residue was applied on top. In a third experiment, we determined whether the earthworm-induced N_2O effect can be explained by the depth they can mix in the residue from the top. In a mesocosm (50 cm depth, sandy soil) study we confined individuals of L. rubellus to the top 10 cm depth with a nylon mesh, or individuals of the anecic L. terrestris (L.) to 10, 30 or 50 cm depth. Cumulative N₂O emissions from maize residue were highest from the L. rubellus treatment at 10 cm confinement depth, but did not differ significantly with L. terrestris at 10 cm confinement depth. Lowest emissions were found for L. terrestris confined to 30 cm. We conclude that earthworm-induced N₂O emissions differ considerably between earthworm species, which is related to feeding strategy. Differences between epigeic and anecic earthworms can at least partly be explained by the fact that the latter mix residue deeper into the soil.

Effect of earthworms on priming effect intensity and involved microbial communities

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Priming effect is defined as a stimulation of the mineralization of soil organic matter due to fresh organic matter supply. This process is likely based on a competition between microorganisms for the access to the "fresh" carbon and is depending on the availability of soil nutrients. This poorly studied process can have important consequences on the fate of soil organic matter and on the management of residues or amendments in agricultural soils. The understanding of this process is thus of particular significance in southern countries where soil fertility is essentially based on the management of organic matter. Earthworms are ecosystem engineers known to affect the dynamics of soil organic matter. Geophagous endogeic earthworms ingest large amounts of soil and assimilate a part of organic matter it contains. During gut transit, microorganisms are transported to new substrates and their activity is stimulated by (i) the production of limited amounts of readily-assimilable organic matter (earthworm mucus), and (ii) the possible presence of fresh organic residues in ingested soil. The objective of our study is to see (i) whether earthworms impact on the priming effect intensity when a fresh residue is added to the soil, and (ii) whether this impact is linked to a stimulation/inhibition of bacterial taxa. To achieve this goal, a tropical soil from Madagascar was incubated in the laboratory, amended with a ¹³C highly enriched, finely ground wheat residue, in the presence or absence of a peregrine endogeic tropical earthworm, Pontoscolex corethrurus. Emissions of ${}^{12}\text{CO}_2$ (respiration from the soil) and ${}^{13}\text{CO}_2$ (respiration from the residues) were followed during 24 days. The coupling between DNA-SIP (Stable Isotope Probing – separation between ${}^{12}C$ and ${}^{13}C$ labelled bacteria) and pyrosequencing (both on ¹²C and ¹³C labelled bacteria) on samples with or without earthworms was realized. Results showed that the activity of earthworms led to a stimulation of the mineralization of wheat residues, increased priming effect intensity and modified its dynamics. Several bacterial phylogenetic groups stimulated by earthworms were identified, some of taxa feeding on fresh residues and others on soil organic carbon.

Anecic earthworm removal in the field: Putting keystone species to the test using stable isotopes

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One of the greatest challenges of soil biodiversity-functions research is to conduct experiments under realistic field conditions. Previous field studies have extracted the entire earthworm community first and then reintroduced ambient or elevated communities. The main disadvantages of this approach are that the survival of reintroduced earthworms is unknown and that the functions cannot be ascribed to particular species or ecological groups. The objective of this research was to test experimentally the status of anecic earthworms as keystone species, by conducting a field experiment in which anecic species were removed selectively, with minimum disturbance to the soil-plant system and remaining earthworm community. Anecic earthworms at the study site (mainly Lumbricus terrestris) were expelled from removal treatment plots (by injecting dilute mustard oil solutions into burrows) at the start of the experiment and repeatedly thereafter for 2 years. Stable isotope tracers were then used to quantify the effect of removal on the incorporation and decomposition of green cover crop residues into plant functional groups and co-occurring earthworm species. Specifically, anecic functions were assessed in terms of the fate of ¹³C and ¹⁵N from surface-applied, dual-labelled plant residue, measured 39 days after residue application. Addition of mustard residues strongly stimulated aboveground plant biomass production and residue-derived ¹⁵N was taken up by grasses, herbs and, to a lesser extent, legumes. However, removal of anecic earthworms did not affect plant biomass or N uptake, neither did it have systematic effects on the assimilation of residue N and C by other earthworm species. Some ecologically similar species responded differently, for instance, L. rubellus was much more enriched in removal plots, but L. festivus was less enriched. This could reflect reduced food competition from L. terrestris, but it could also reflect differences in migration behaviour. N and C isotopic compositions of the 8 earthworm species studied here clearly reflected typical endogeic (soil feeding) and anecic/epigeic (litter feeding) feeding behaviours. While selective removal of anecic earthworms was successful, mustard residues acted as strong attractants to earthworms in this unfertilised grassland system and removal plots were quickly recolonized by anecic conspecifics as indicated by lower isotopic enrichment levels in removal plots.

Can earthworms help us sequester C? Production and dissolution rates of earthworm secreted calcium carbonate

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Earthworms secrete granules of calcium carbonate. These are potentially important in the terrestrial carbon cycle and are routinely recorded in archaeological studies of Quarternary soils.

Production rates of calcium carbonate granules by the earthworm Lumbricus terrestris L. were determined over 27 days in a range of soils with differing chemical properties. Production rate varied between soils, lay in the range 0 - 4.3 mg_{CaCO3} ·earthworm⁻¹ ·day⁻¹ with an average rate of 0.8 mg_{CaCO3} ·earthworm⁻¹ ·day⁻¹ and was significantly correlated (r = 0.83, p < 0.01) with soil pH. In a longer term experiment earthworms repeatedly produced comparable masses of granules though over time the earthworm granule production rates into masses of C precipitated per hectare of land per year depends heavily on estimates of earthworm numbers. Using conservative values suggests a rate of 18 mol_{CaCO3} ·ha⁻¹·yr⁻¹. Upper limit estimates are in the order of 42 000 mol_{CaCO3} ha⁻¹ yr⁻¹.

Data obtained from flow-through dissolution experiments conducted in NaHCO₃ solutions to buffer the effects of atmospheric CO₂ were fitted to the empirical calcite dissolution relationship $r = k(1-SI)^n$ where r = dissolution rate, k = a rate constant, SI = saturation index and n = the reaction order. For granules we obtained values of $k = 1.72 \times 10^{-10}$ and 3.51×10^{-13} mol· cm⁻²·s⁻¹ for rates normalised to BET and geometric surface areas respectively and n = 1.8. Geometric surface area normalised dissolution rates were similar to those reported elsewhere for both inorganic and biogenic calcite indicating that, for calcite at least, geometric surface area is a good proxy for reactive surface area. Leachate chemistry from leaching column experiments using soil containing 1% by mass granules was unaffected by granule dissolution though, as assessed by changes of granule mass, dissolution did occur. Trends in dissolution rate referenced to soil chemistry corresponded to predictions made by the SLIM model for dissolution of limestone in soil and indicate that if Ca concentration builds up to critical levels in soils, granule dissolution will slow or even stop and granules be preserved indefinitely.

The standing mass of granules in soil will be a function of both production and dissolution rates and the above data need to be combined to allow an assessment of the importance of granules in the soil C cycle to be made.

Earthworm ecosystem services in organic and biofuel agriculture

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Although Charles Darwin's last book emphasised the importance of earthworms in soil systems, the importance of these soil animals has been known for hundreds of years. Organic agriculture usually delivers a much higher level of ecosystem services than conventional farming but the economic role of earthworms in organic systems has rarely been quantified. This presentation will include a combination of field experimentation, value-transfer and other resource economics techniques to explain and quantify the high value of earthworms in organic agriculture and its potential for conventional systems. Although biofuel (ethanol) and biodiesel (crops) are increasing in area rapidly worldwide, there is considerable concern about the effects these crops may have on the environment, including ecosystem services such as biological control, pollination and soil processes. This work has evaluated a range of potential biodiesel crops for yield and economic benefit. The latter includes a resource economics approach which evaluates the external costs of biodiesel crops on soil ecosystem services.

Are earthworms good or bad for the soil greenhouse gas balance? A review

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The soils of the world are an important source of all three major greenhouse gases (GHG's). Terrestrial ecosystems have contributed as much as half of the anthropogenic carbon dioxide (CO_2) emission over the past two centuries; soils are by far the dominant source of nitrous oxide (N_2O) emissions; and wetlands and landfills dominate global methane (CH₄) fluxes. Yet, soils can also be a sink for these three GHG's through carbon sequestration, biological reduction of N₂O, and increased methanotrophy. The GHG balance of the soil is therefore dependant on soil management. This raises the question to what degree soil ecosystem engineers such as earthworms can affect the soil GHG balance. It has been suggested that earthworms can stabilize fresh organic C in soil organic matter. Furthermore, several studies have reported effects of earthworms on emissions of N₂O or CH₄. Here we review the available literature covering the effects of earthworm activity on the soil GHG balance, and identify knowledge gaps that need to be addressed. With regard to C, many studies suggest earthworm-induced C stabilization in soil organic matter. However, measurements were almost exclusively indirect (measuring new C in supposedly more stable soil fractions), had a very short duration (less than 4 months), or earthworm effects were confounded by other effects (e.g. tillage). For N_2O , a growing body of literature indicates that earthworm activity can significantly increase N₂O emissions. This was mostly measured under controlled conditions and only when a food source was added to the system. Few studies discussed the earthworm effect on CH₄ emission. Both positive and negative effects have been reported, however, CH_4 emissions appeared to be mainly measured in landfills and contaminated soils. We conclude that available data on the effect of earthworms on the GHG balance of the soil are fragmentary and it has certainly not been conclusively proven that earthworms increase soil organic matter stocks. The most important knowledge gaps appear to be (i) direct measurement of C stabilization over longer time periods; (ii) field-based studies on N₂O effects; and (iii) integral studies measuring all three effects simultaneously. Options to fill these knowledge gaps will be discussed.

The ecology of earthworm impact on ecosystem services

Blouin Manuel¹, Cluzeau Daniel², Aranda Delgado Eduardo³, Baker Geoff⁴, Brussaard Lijbert⁵, Butt Kevin R.⁶, Dai Jun⁷, Dendooven Luc⁸, Hodson Mark E.⁹, Lavelle Patrick¹⁰

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Effect of *Pontoscolex corethrurus* on aggregate formation, water infiltration and phosphatase activities over a maize-mucuna crop

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The aim of this study was to evaluate the effects of the earthworm *Pontoscolex corethrurus* in the: i) enzymatic activity of acid and basic phosphatases from the leachate; ii) leachate pH; iii) water infiltration; iv) leachate volume and v) soil macroaggregates, classified into two categories: 1) biogenic A (3 cm < x), B (1 cm < x < 3 cm), C (0.5 cm < x < 1 cm), and 2) physicogenic D (3 cm < x), E (1 cm < x < 3 cm), F (0.5 cm < x < 1 cm). Sources of variation were: *Pontoscolex corethrurus* (P), Mucuna pruriens (M) and Zea mays (Z) with their different combinations, making a total of eight treatments: PZM, ZM, PM, M, PZ, P, Z and C (control). Data were analyzed using STATISTICA 8.0, using nonparametric analysis (Kruskal-Wallis). Results indicated significant differences (p <0.05) in infiltration between P (0.03 cm³s⁻¹) and Z (0.002 cm³s⁻¹) treatments at 30 days, where P was the highest. At 60 days, differences were observed between treatment C (0.053 cm^3s^{-1}) and PZ $(0.0011 \text{ cm}^3 \text{s}^{-1})$. The volume of leachate was higher in M (2.30 L) treatment. In regards to leachate pH, there was significant difference (p <0.05) at 60 days between M (pH 7.74) and PZM (pH 6.69) treatments. On the other hand, at 120 days, basic phosphatase activity was higher in the P (0.682 nkat/mL) treatment and acid phosphatase activity was higher in the PZ (6.79 nkat/mL) treatment. Finally, macroaggregates type C (0.5 cm < x < 1 cm) showed significant difference (p <0.05) between PZM (108.61 cm²) and M (0.0 cm²) treatments; this possibly due to the formation of aggregates in fine roots (PZM), and the lack of earthworms (C). The earthworm P. corethrurus promotes infiltration by a short period of time and may promote enzyme activity in the leachate and the formation of aggregates associated with fine roots.

The role of New Zealand endemic earthworms in mine restoration Darwin's dream comes true: NIRS allows quantifying the impact of earthworms on soil and Carbon dynamic

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Among the soil-dwelling species that are likely to be important early in mine restoration, earthworms are particularly good candidates. They provide ecosystem services that are likely to facilitate and accelerate the restoration of a functional ecosystem. These services include increasing topsoil fertility, providing food resources for a wide range of predators and the recycling of waste organic materials. Despite their ecological importance, earthworms have been poorly considered in mine restoration. Most restoration plans focus only on vegetation and above-ground macro-fauna, while the aim should be to restore functioning ecosystems above and below ground. With the aim of developing recommendations for better management of earthworm communities after opencast mining, we evaluated the response of an endemic earthworm community to mining activities and rehabilitation treatments currently used in an opencast coal mine in New Zealand. The main treatments were vegetation direct transfer (VDT) and vegetation replanting in soil that has previously been stockpiled. VDT consists of removing pieces of land, including the vegetation and the topsoil and transferring them to another area where the land 'jigsaw' is reconstituted. The present work showed that this method was efficient in conserving earthworm communities, both in terms of biomass and species diversity. Soil that has been stored in high stockpiles, on the other hand, was mostly anaerobic and contained very few earthworms. When re-spread and replanted, such soil contained a depauperate earthworm community and required enhancement of its fauna to restore its ecosystem functions. Stockpiling, re-spreading and replanting is a common practice in opencast mine rehabilitation. However, based on our study it does not provide a satisfactory outcome for earthworm communities and is likely to be detrimental for most soil invertebrates as well as the ecological communities that depend on them.

Can reduced tillage and field margins improve soil water dynamics through stimulation of earthworms?

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Concern is growing among Dutch farmers about water logging and soil compaction given that climate change scenarios for The Netherlands predict exacerbated extreme rainfall events and drought periods (MNP, 2005; KNMI, 2006). Conservation tillage practices, such as non-inversion tillage, are being implemented to reduce compaction, improve the infiltration and water storage capacity of soils, and stimulate biological activity. The ability of the soil to absorb and retain water is considered an important ecosystem function. After initiating reduced tillage practises there may be an increase in earthworm populations and diversity due to reduced disturbance, but this process will depend on their presence in the arable field and near-by landscape elements, and on their dispersal rates (Hoogerkaamp et al., 1983; Mather and Christensen, 1988). These non-productive landscape elements (e.g. field margins) are managed to promote above-ground farmland biodiversity but can also have an impact on soil macrofauna (Smith et al., 2008). This project is investigating the complementarity of soil and landscape management strategies for the enhancement of ecosystem services. A replicated field experiment was initiated in 2009 to investigate the relationships of noninversion tillage and earthworm populations and diversity with water infiltration and retention under conventional and organic farming practices. A second trial investigates earthworm populations in field margins and distributions in adjacent arable land. Earthworm species diversity and soil physical property data will be presented from two field seasons. References: -Hoogerkamp, M., Rogaar, H., Eijsackers, H.J.P., 1983. Effect of earthworms on grassland on recently reclaimed polder soils in the Netherlands. In: Satchell, J.E. (Ed.), Earthworm Ecology. Chapman and Hall, London, pp. 85-105. - KNMI, 2006. Klimaat in de 21e eeuw, vier scenario's voor Nederland. In: De Bilt. -Mather, J., Christensen, O., 1988. Surface movements of earthworms in agricultural land. Pedobiologia 32, 399-405. - MNP, 2005. Effecten van klimaatverandering in Nederland. In: Milieu- en Natuurplanbureau. -Bilthoven Smith, J., Potts, S.G. Woodcock, B.A., Eggleton, P., 2008. Can arable field margins be managed to enhance their biodiversity, conservation and functional value for soil macrofauna? Journal of Applied Ecology 45, 269-278.

Biological Alternatives to the use of pesticides in banana plantations of Colombia

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Productivity and sustainability in Banana plantations is currently threatened by a large number of pests and diseases. The continuous use of nematicides and other pesticides is only solving the problem in short term and creates serious problems of human and ecosystem health. Our project evaluates the manipulation of different biological components as alternatives to the use of pesticides. We experimented the effect on phytoparasitic nematodes of 4 different treatments: 1. application of lixiviates from banana trunks; 2. application of lombricompost; 3. Inoculation of mycorhizae in roots and 4. application of the FBO (Fertilization Bio Organique) patented technique that creates islands of high fertility with application of low (Gliricidia branches) and high quality (lombricompost) organic materials in trenches inoculated with local endogeic earthworms. Before the experiment, we assessed soil aggregation (Velasquez et al., 2007 method) and population of mycorhizae, saprophytic and plant parasitic nematodes and we found a significant relationship among i) aggregates of biological origin (earthworm casts) and mycorhizal spores; ii) Endogeic earthworms and non aggregated soil; iii) polyhumic earthworms and organic matter content and iv) nematodes with root and physical aggregates. Treatments were applied to 93 plants. Six months after, we measured mycorhizal colonization frequency, the occurrence of arbuscules in the whole root system, the number of mycorhizal spores in 100g of soil and densities of phytoparasitic and saprophytic nematodes in roots and soil. PCA analysis on soil communities of the 31 plants that survived an unexpected drought showed 1) very low numbers of all nematode groups and mycorhizae in the control treatment as opposed to our biological treatments, with FBO having the highest numbers of organisms and the lombricompost and mycorhizae treatments with intermediate values (axis 1 of the PCA analysis); 2) relatively larger numbers of mycorhizae and saprophytic nematodes in the FBO treatment as opposed to other biological treatments.

Earthworms in the Quesungual agroforestry system and their role in aggregate-associated soil organic matter and nutrient dynamics.

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The Quesungual agroforestry system from the mountains of western Honduras has been promoted as a viable alternative to traditional slash-and-burn agriculture in tropical dry forest regions of the Americas. Although mulch application and the lack of burning in this system can have notable impacts on soil macrofauna, the influence of Quesungual management on earthworm populations has not been fully quantified. Even less is understood about the role earthworms in regulating soil organic matter (SOM) and nutrient dynamics in the Quesungual system. We present a summary of 3 field studies conducted in Honduras aimed at 1) quantifying the influence of the Quesungual management on earthworm abundance and diversity and 2) understanding how earthworms in this system interact with agroecosystem management to influence soil aggregation, SOM and nutrient cycling. The first study examined earthworm populations and soil properties within a replicated field trial comparing the Quesungual system with traditional slash-and-burn agriculture and undisturbed secondary forest. Quesungual management significantly increased earthworm populations relative to slash-and-burn agriculture (with intermediate values for secondary forest). A second experiment manipulated earthworms (Pontoscolex corethrurus) using microcosms within the replicated field trial mentioned above. Earthworms were found to reduce total soil C in the surface soil across all management systems and to facilitate the loss of fertilizer N within both the Quesungual and slashand-burn plots. A third study employed field mesocosms (each containing a growing maize plant) to manipulate earthworms (*P. corethrurus*) and residue additions at a nearby study site. In the presence of active plant roots, earthworms improved aggregation and C storage within stable aggregate fractions, but only when residues were added to the soil surface. Earthworms also increased the uptake of fertilizer N by maize plants and reduced total and available P across all residue treatments. Considered together these studies suggest that earthworms are promoted by Quesungual management, but that their beneficial impacts on SOM and nutrient cycling depend on the proper management of plants and the addition of sufficient residue inputs.

Earthworm-induced N₂O emissions in a sandy soil with surface residue application

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Earlier research with endogeic and epigeic earthworm species in loamy soils has shown that both earthworm groups can increase N₂O emissions when crop residues are incorporated into the soil by epigeic earthworms. This effect was related to changes in soil organic matter (SOM) distribution in a loamy soil, characterized by a well-aggregated stable soil structure. The current study focused on sandy soil which typically contains less SOM and stable soil aggregates. We aimed to i) quantify N₂O emissions in sandy soil as affected by endogeic and/or epigeic earthworm species and ii) relate changes in N₂O emissions to earthworm-induced changes in OM distribution and physical soil fractions. A 90 day ¹⁵N-tracer mesocosm study was conducted using sandy soil, with the endogeic earthworm species Aporrectodea caliginosa (Savigny), the epigeic species Lumbricus rubellus (Hoffmeister) and both species combined. ¹⁵N-labeled radish (Raphanus sativus cv. Adagio L.) residue was placed on top the soil. An additional treatment was included with residues manually incorporated into the soil and no earthworms. L. rubellus significantly increased N₂O emissions from 228 to 859 µg N₂O-N kg⁻¹. A. Caliginosa did not significantly affect N₂O emissions and no significant interactions between earthworms species were observed. When residues were manually incorporated, N₂O emissions were significantly (p<0.008) lower (509 μ g N₂O-N kg⁻¹) compared to incorporation by L. rubellus. The amount of C and N in the size fraction >250 µm was significantly increased by the presence of L. rubellus and/or A. Caliginosa, but the ¹⁵N enrichment of that same fraction was only increased in the presence of L. rubellus. We conclude that earthworm-induced N₂O emissions in a sandy soil with surface residue application are affected by L. rubellus, but not by A. *Caliginosa*, reflecting the feeding strategies of the different ecological groups. In contrast to earlier studies in loamy soil, no interactive effects of the two species on N_2O emissions were found. The higher N₂O emissions with L. rubellus compared to manual mixing, could be due to earthworminduced changes in OM distribution or microbial processes in the soil.

Impact of the earthworm *Pontoscolex corethrurus* on the porosity of the soil, observations in a pot experiment by CT scanning and standard methods

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Earthworms as ecosystem engineers have an important impact on the soil porosity and the water flux; although standard methods of soil porosity do not allow to observe clearly this impact. In order to study in a detailed form this effect a pot experiment was set. Treatments included: 1) P. corethrurus with maize and Mucuna (PZM), 2) P. corethrurus with Mucna (PM) 3), P. corethrurus with maize (PZ) and 4) the control which is the soil alone (T). The Mucuna was put as crushed litter on the surface at the beginning of the experiment. The soil is repacked disturbed luvisol from 0-10cm already ground to 5 mm and dumped into 25 cm lower diameter, 35 cm higher diameter and 40 cm height truncated cone shaped plastic pots. In treatments PZM, PM and PZ and 10 adult earthworms were introduced in the pot at the beginning of the experiment. The experiment was conducted for a whole growing season for maize. At the end of it, CT Scan images were acquired with a 64 multislice helicoidal CT Scanner (GE. BCT light speed). Macroporosity 3D reconstruction and analysis was conducted using a digital crop monolith of 15.0x15.0x10.0 cm from each treatment to avoid unnatural preferential burrow paths at the soil-pot contact. Image analysis was done by means of AMIRA 4.x software (Visage Imaging). After scanning, soil cores were extracted with a cylinder (5.0 cm diameter x 5 cm height) to calculate Bulk Density at two depths (0-10 cm and 20-30 cm) in each pot and from it obtain total porosity. Results indicate that 3D images of treatments with earthworms (PZM, PM, PZ) present a complex and large macro porosity network. Treatments with earthworms developed 6,162.85 to 7,468.43 mm³ of macropore space $(2.36 \times 10^{-1} \text{ to } 3.32 \times 10^{-1} \text{ \%})$ while treatments without earthworms developed only 7.5-9.9 mm³ of macropore space $(3.33 \times 10^{-4} \text{ to } 3.98 \times 10^{-4} \text{ \%})$. The difference in soil macropore volume between treatments with earthworm and those without earthworms is over 700 times The core technique did not evidence any differences in total porosity among the treatments. The porosity range was between 48 and 49 %. These results suggest that the core method may have few sensitivity to significant changes in macropore space. As mentioned earlier in literature, our analysis allowed us to corroborate that earthworms had a dramatic impact (two orders of magnitude) in soil macropore space and structure. CT Scan image processing and 3D image reconstruction of macropore soil space is a powerful tool in the field of earthworm ecology and soil bioengineering. This work was partly supported by the project *Conservation and Sustainable* Management of Below Ground Biodiversity financed by GEF/UNEP and executed by CIAT/TSBF.

Field assessment of burrow reuse by Lumbricus terrestris L.

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A short term field experiment was set out to investigate evidence of burrow inheritance or re-use by Lumbricus terrestris. This was established in a temperate broadleaved woodland and involved removal of L. terrestris from a number of 1 m^2 plots with subsequent monitoring of colonisation in these areas. Manipulation of L. terrestris number in plots resulted in 4 treatments. 1. Removal of L. *terrestris* while preserving their burrows (Rem) from (a) 1 m² open plots (RemO) to examine re-use and inheritance of vacated burrows by other conspecifics and offspring (b) 1 m2 enclosed plots (RemE) to examine inheritance of vacated burrows by offspring. 2. Removal of L. terrestris and cocoons through soil sterilisation and the destruction of burrow systems (Ster) from (a) 1 m² sterilised open plots (SterO) to examine colonisation of empty space (b) 1 m^2 sterilised enclosed plots (SterE) used as a control for the sterilisation method. Each of the treatments was replicated 3 times resulting in a total of 12 plots. Enclosure was achieved by construction of a plastic fence which extended 0.3 m below and 0.3 m above the soil surface. The cumulative mean percentage of vacated burrows being re-used by conspecifics was compared in plots of all treatments on a monthly basis over a period of 10 months. A significantly (p<0.01) greater proportion of vacated burrows were re-used in RemO plots (mean±S.E. 50.27%±3.57%) compared with RemE plots (38.48%±3.49% m⁻²). A similar number of L. terrestris middens was observed in plots of SterE treatment (mean±S.E. 29.33±2.33) and SterO treatments $(23.57\pm1.28 \text{ m}^{-2})$, over the entire duration of the experiment. The spatial distribution of L. terrestris middens was examined using Ripley's K function and midden permanence using the bivariate Ripley's K function. Point patterns were found to be regularly distributed at mean(±S.E.) distances ranging from 0.084±0.029 m to 0.130±0.063 m in 43.27% (n=45) of instances. Patterns of vacated and reused burrows in Rem treatment were found to be aggregated in all six plots at distances ranging from 0.022±0.015 m to 0.132±0.043 m. These observations suggest that L. terrestris burrows could be recycled or inherited. Such behaviour could be a means of minimising energy expenditure for burrow construction. Results from this experiment are significant as they provide evidence of burrow reuse by conspecifics and further support the views of other researchers, on possible niche inheritance in L. terrestris.

Poster - Session 4

Earthworms and soil ecosystem services at homegardens of Tabasco Mexico

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Home gardens have been defined as agroforestry land-use systems with multipurpose trees and shrubs, under the management of family labour (Fernándes and Nair 1986). The main questions addressed in this study were: plant diversity or vegetation structure in home gardens may enhance soil earthworms diversity and abundance? Earthworms diversity and abundance may enhance soil ecosystem services? At Tabasco, southeast Mexico we measured the diversity and abundance of earthworms at 50 homegardens of the 5 physiographic regions of the State. 5 monoliths of 25x25x30cm in a random designed per homegarden were made, where we measured earthworms abundance, biomass, diversity and soil physicochemical properties. We found significant correlations (Pearsons and Spearman rho) between earthworms and infiltration (R: 0.3 p<0.05), earthworms and soil organic matter (r2: 0.6 p<0.05), and earthworms and total nitrogen (r2:0.7 p<0.05). We observed a significant effect of precipitations and clay content on the presence of earthworms. The Mountain region was the region with the highest abundance of endogeics (118.4 ind.m⁻²), represented mainly by the Megascolecidae individuals (66.9 ind.m⁻²). We observed that total basal area of plants is the main characteristic that influences earthworms abundance.

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Effect of *Pontoscolex corethrurus*, *Zea mays* and *Mucuna pruriens* on the respiration rate of soil

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Soils are involved in the provision of many important environmental services to maintain the ecosystems and human societies (Velasquez, 2004). Changes in land use are known as contributors to increase atmospheric CO₂ levels (Wisniewski et al., 1993), and may also have an influence on earthworms, which play an important role in regulating processes to be held on the ground (Liu and Zou. 2002), they are also part of the functional groups of the soil biota that contribute to the regulation of vital processes to the functioning of ecosystems. Soil respiration is one of the most common method to infer the level of biological activity including soil microorganisms, roots and soil fauna. The presence of exotic earthworms significantly accelerates the decomposition of organic matter and soil respiration, which increase the release of CO_2 from soils to the atmosphere (Liu and Zou 2002) and the incorporation of organic matter increases the respiration rate. In this study, the aim was to evaluate the effect of *Pontoscolex corethrurus*, *Mucuna pruriens* var. *utilis* and *Zea mays* on the respiration rate of a ferric Luvisol soil type, cultivated with maize under green house conditions, using a multifactorial design at two levels (presence-absence), with eight treatments and three replications each: T (Control), PZM (P. corethrurus + Z. mays + M. pruriens); ZM (Z. mays + M. pruriens); PM (P. corethrurus + M. pruriens); M (M. pruriens); PZ (P. corethrurus + Z. mays); Z (Z. mays) and P (P. corethrurus). Respiration rate was monthly monitored using an environmental gas monitor (PP Systems). Results indicate that the respiration rate of treatments Z, T and P had average values of 0.66, 0.41 and 0.3 g CO₂ \cdot h⁻¹ m⁻², respectively, corresponding to those treatments with a relatively low respiration rate. In contrast, average values of treatments PZM, ZM, PZ, PM and M were: 1.5, 1.1, 1.0, 0.76 and 0. g CO₂ \cdot h⁻¹·m⁻², respectively, corresponding to those which had an increased in respiration rate during the experiment. Combined treatments PZM, ZM and PZ positively affect respiration rate over time compared with control; there was a combined effect between organic matter, earthworms and plant on the modification of the respiration rates.

Control of manure odorous compounds by adding *Lactobacillus* and earthworm meal into piglet diet

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Many effective microorganisms lived in earthworm gut have strong interaction for functioning digestion and other physiological activities of earthworm. If some microorganisms including lactobacillus spp. in vermicompost can be used to other animal waste management? It is little reported. In this experiment, we added lactobacillus and earthworm meal into piglets' diet to investigate their effect of on the pig performance and odorous compounds in feces. 144 piglets (Landrace X Duroc) pigs were randomly divided into four groups, and fed with Corn Soybean Meal diet (CS), CS+2×10⁹cfu, CS+0.45% and CS+2×10⁹cfu+0.45% earthworm respectively; Piglet's performance, feces pH, the counts of E. coli, Lactobacillus, Clostridium, Eubacteria, ammonia emission, ammonium, total nitrogen and main odorous compounds in the feces were determined. It was turned out that when the diets was supplemented with lactobacillus, feces pH, E.coli counts, the concentration of valeric acid, p-cresol in the feces were reduced greatly (P<0.05), while the concentration of acetic acid, propionic acid in feces increased significantly (P<0.05). Dry material of feces, feces pH and the concentration of indole were decreased greatly (P<0.05), whereas the concentration of 3-methylindole in the feces was increased (P<0.05), when the piglets fed with diets containing earthworm; the concentration of p-cresol, indole in the feces also were decreased (P<0.05), increase of ammonia emission from slurry and no significant effect on feces pH, volatile fatty acids and 3-methylindole in the feces (P>0.05) were observed when piglets fed with diets containing with both Lactobacillus and earthworm.

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Spatial earthworm casting activity in connection with the structure of the vegetal cover in a tropical rainforest of the Guyana Shield

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In a primary rainforest of the Guyana Shield (French Guiana), the earthworm surface casting activity was quantified and mapped in connection with the distribution and size of a range of vegetal cover types. A 50 plots regular quadrat was settled within a one hectare sloping parcel (BRIDGE project) at the Paracou research site. Each plot corresponded to a 4m diameter circle in which number, volume and position (according to various structure of vegetal cover) were measured. Results showed that, at the scale of a one hectare gentle slope, surface earthworm casts were not homogeneously distributed but significantly concentrated in middle slope, thus reaching 2.55 casts.m-2 and 2.7 dm-3. In addition, the density of trees (of all diameter classes) and seedlings (dicotyledons), palms (monocotyledons), dead trunks or branches, lianas on soil, emerging roots or herbaceous did not influence the density of earthworm surface casts. However, within areas of high density of casts, these latest were significantly more localized at the foot or directly under hard vegetal structures than within the litter.

Settlement and spread of an introduced earthworm (*Lumbricus terrestris* L.) population in relation to field management in boreal clay soil

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To study the feasibility of earthworm introduction in increasing the macroporosity and permeability of arable clay, the deep burrowing earthworm Lumbricus terrestris L. was inoculated into an experimental leaching field in Jokioinen, S-W Finland in autumn 1996. The species was initially absent from the field and its immediate surroundings. The soil of the sloping (2%), sub-drained field is Vertic Cambisol with clay content ranging from 61% in topsoil to 90% in subsoil. The field consists of four adjacent plots (33 m x 140 m) surrounded and separated by permanent grass strips. Inoculation took place inside the plots and in the grass strips, close to the upslope end of the field using Earthworm Inoculation Unit technique. Mustard extraction of L. terrestris was used to follow population trends. The first follow-up (1998) showed that the population had persisted in low numbers, but only in the grass strips outside of the cultivated area. At second monitoring (2003), median density was 9 ind. m⁻² in the grass strips where there was evidence for dispersal to 8 m from the inoculation points. This time a few L. terrestris were also found within the inoculation area in the field but the median density was only 1 ind. m⁻². The third monitoring was in autumn 2009 until which two of the plots had remained unploughed (from 2003: either grass-covered or direct drilled). The other two plots had been ploughed the previous autumn but otherwise had similar management. In the grass strips, population density had increased and the mean density peaked at 36 ind. m^{-2} close to the points of inoculation, gradually declining down-slope with mean density of 6 ind m⁻² at 55 m distance from inoculation. Inside direct drilled plots, L. terrestris were abundantly found, with a similar trend, from 21 ind. m⁻² close to the inoculation to 2 ind. m⁻² at 50 m distance. In ploughed plots the numbers were much lower, with maximum density of 3 ind. m⁻² upslope and no individuals found beyond 34 m from inoculation. Inside the field the densities were consistently higher above the sub-drains than between them (overall mean 6 vs. 2 ind. m^{-2} , respectively). It seems that the eventual establishment of a population inside the field depended on the preceding population growth at the plot margins which acted as sources of dispersers into the field. Low physical disturbance together with sub-drainage were factors favouring settlement and spread of the introduced population.

Poster - Session 4

Population densities for the growth and cocoon production of the earthworm *Pontoscolex corethrurus* (Annelida: Oligochaeta, Glossoscolecidae)

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In spite of the potential of the earthworms to conserve the fertility of the soils and to increase the agricultural and forest production, biotechnologies for the massive production, of earthworms do not exist. In the present study was evaluated the effect of the population density of the exotic earthworm *Pontoscolex corethrurus* in the cocoon production. In conditions of greenhouse, during a period of 135 days, the earthworm culture under three population densities, high, medium and low (340, 227 and 114 worm m-2, respectively) each with three replicates. Every 15 and 30 days was registered the number and biomass of the produced cocoon and the growth of progenitor earthworms, respectively. The results this study suggested that until the 90 days of established the experiment of the three densities of *P. corethrurus* maintained a constant density and increased significantly their biomass; nevertheless, at the end of the study (135 days) the densities were similar to the initial population being 58.73%. The total cocoon production (71.8) was not significant between the three densities and the major cocoon production happened to the 60 days. The preliminary conclusions suggest that the cocoon production is determined by the environmental conditions and the greater number of cocoons for each earthworm occurs in a low population density.
Modelling earthworm populations and traits of earthworm activities under contrasted soil tillage management

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Tillage practices greatly affect soil physical characteristics and substitute natural soil aggregation (pedoclimatic and biological) by soil fragmentation. In other hand, conservation tillage is favourable to soil biological activities and notably earthworm populations. An organic management system tends to increase the density of earthworm community due to organic fertilization (as trophic resources). If reduce tillage can be practiced in organic farming management, we can considered that this type of management is favourable to biological activities notably to earthworm biological activities (and thus earthworm activities (macroporosity and aggregation)), but a few work confirms this hypothesis. In this work, two tillage managements are compared using a completely randomised block design with three replicates (6 experimental plots): (1) traditional mouldboard ploughing (tilling to a depth of 30 cm), (2) reduced tillage with tined tools (15 cm depth) We studied the structure of earthworm functional groups (according to ecological groups), and quantified biological soil pattern (generated by earthworms). So we developed a model to predict earthworm population by superficies of soil structure patterns according to Partial Least Squares regression.

Poster - Session 4

Soil water movement is affected by two anecic species (*Lumbricus terrestris* and *Aporrectodea giardi*)

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Earthworms modify soil physical properties and thus affect soil hydraulic conductivity. Anecic group describes large earthworm species that built some semi-vertical burrows. So, anecic burrows are widely recognized to facilitate soil infiltration. However, Jégou et al. (2001) have shown some pronounced differences of burrow systems (length and connection) for the two species Lumbricus terrestris L. and Aporrectodea giardi. The aim of this study was to assess the water movement according to these two anecic species. So, artificially packed soil columns were inoculated with two individuals of Lumbricus terrestris (LT) or Aporrectodea giardi (AG). During 12weeks, we have weekly measured the percolation and more, we have study the kinetic of percolation after a rainfall simulation. Compared to soil columns that were no inoculated, our results indicate that earthworm species have improved the water movement in soil columns. Similar percolated volumes were measured with the two earthworm species, these volumes being twice these measured under control columns. After a rainfall simulation, the percolation begins more late in the columns of control, only 1,5 % of water have percolated during the first hour. Respectively with LT and NG, 61,2 % and 57,8 % of water have percolated during the same time. So, the kinetics of percolation differs slightly between the both earthworm treatments during the first hour. In LT, we observed a phase of very fast percolation (< 5min) and then residual flows. In AG, two phases of percolation were observed: very fast (< 5min) and then a steady percolation during 55min. In conclusion, the contrasted burrow systems constructed by anecic does not affect significantly the percolation ratio but the rapidity of the water movement in soil column.

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Alluvial ecosystems are good models to understand the establishment of earthworm populations. These ecosystems exhibit a mosaic of habitats containing strong different pedologic characteristics as soil texture, profile depth, carbonate and organic matter contents, humus forms and vegetation cover. Furthermore, the age of soils is very young in such fluvial systems (from a couple of years to around a hundred years); hence, soil structure is not widespread developed and pedogenetic processes are continuously in progress. So, the various roles of earthworms as ecosystem engineers in these ecosystems are very interesting to investigate because of the pioneer nature of soils. In this context, we proposed to study earthworm populations and their roles in the first steps of soil structure formation. We hypothesise that it may vary according to both altitude and elevation from the river bed. Thus, our three main study sites were located in Switzerland at three altitudinal levels (subalpine, mountain and hill level). Perpendicular to this altitudinal gradient, a vegetation gradient was also chosen reflecting the duration of soil stabilisation. Sampling was performed in carbonaterich alluvial soils. A total of 45 soil profiles were studied for their structure (porosity, aggregate stability and mean weight diameter of aggregates) and their physico-chemical characteristics in the 30 first centimetres. For each soil profile, earthworms were collected twice a year; they were counted, weighted and identified to species level. First results revealed a biomass ranging from 1g m-2 to 160g m-2, with strong differences between and within altitudinal levels. A total of 27 species and sub-species were recorded along the two gradients. In addition, strong differences were found comparing ecological categories: for example, no anecic species were found at the subalpine level. Statistical treatments of the data are still on-going to i) characterize the specific habitats of earthworm species performing multivariate analyses (clustering and redundancy analysis) that compile soil characteristics, vegetation units and altitudinal levels, and ii) to point out the role of earthworms as ecosystem engineers. For this latter part, our results reveal that anecic and endogeic species in less extend are determinant in the development of soil structure through their burrowing and casting activities and their biomass is highly correlated with the thickness of the structured soil layers.

Biomass energy crop impacts on earthworms and associated cast microbial activity

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Biomass energy crops have the potential to contribute to sustainable energy targets. In the UK, consideration is being given to their widespread planting. However, some of the crops are exotic so it is important to consider potential ecological impacts. Litter from established field trials growing various *Salix* and *Miscanthus* hybrids was collected and fed to *Lumbricus terrestris* in mesocosm trials with and without a pre-decomposition phase. Earthworms were monitored for health and growth, litter consumption rates and surface casting. Casts were further analysed for C, respiration and microbial biomass. Decomposition and litter type (including different *Miscanthus* hybrids) affected earthworms differently. There were also clear differences in cast microbial indices which were not explained solely on the basis of C contents. Results suggest that at least some of the favoured biomass energy crops may significantly alter below ground ecology and C cycling.

Feeding ecology of Lumbricidae: the other species

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Most of our knowledge on food selection and consumption by earthworms is based upon studies carried out with a small number earthworm species. These species are usually synanthropic, cosmopolitan species. Moreover, soil food webs are often considered to be highly redundant, when one species in the same functional group can replace another without altering ecological functions. Here we compare the feeding ecology of a total of 31 lumbricid species. Most of the lesser known species are endemic in Central Europe where many of them dominate temperate deciduous forests. Five large anecic species [Lumbricus polyphemus (Fitzinger, 1833), L. terrestris L. 1758, Fitzingeria platyura montana (Cernosvitov, 1932), F. platyura depressa (Rosa, 1893), F. platyura platyura (Fitzinger, 1833) and *Dendrobaena clujensis* Pop, 1938] were kept in 150 cm tall laboratory monoliths and fed hornbeam (Carpinus betulus) leaf litter. Leaf consumption rates and cast production varied between 16 and 37 mgDM gFM-1 day-1 and 314 and 30 mgDM gFM-1 day-1, respectively. In most cases at least 75% of the cast was deposited in the soil, but the distribution of casts with depth varied with species. Smaller litter feeding species were kept in 50 cm tall mesocosms and included the peregrine Lumbricus. rubellus Hoffmeister, 1843, L. castaneus (Savigny, 1826), Dendrodrilus rubidus (Savigny, 1826), Dendrobaena octaedra (Savigy, 1826), and the Eastern-Alpine D. auriculata (Rosa, 1897). Here both consumption and cast production rates were smaller: 17-24 mgDM gFM⁻¹ day-1and 2-8 mgDM gFM⁻¹ day⁻¹, respectively. Endogeic species [Allolobophora chlorotica (Savigny, 1826), A. leoni Michaelsen, 1891, A. dacica (Pop, 1938), A. mehadiensis Rosa, 1895, A. hrabei (Cernosvitov, 1935), Aporrectodea georgii (Michaelsen, 1890), Ap. jassyensis (Michaelsen, 1891), Ap. handlirschi (Rosa, 1897), Ap. rosea (Savigny, 1826), Ap. caliginosa (Savigny, 1826), Ap. dubiosa (Örley, 1881), Proctodrilus antipai (Michaelsen, 1891), P. tuberculatus (ernosvitov, 1935), Octodrilus transpadanus (Rosa, 1884), Oc. gradinescui (Pop, 1938), Octolasion lacteum (Örley, 1881), Oc. montanum Wessely, 1905)] were fed cattle dung. Cast production values in this experiment varied by an order of magnitude. Again, species differed on the proportion of cast deposited on soil surface vs. deeper layers. The experiment highlights similarities and differences in feeding and casting behaviour at the species level, and thus their contribution to decomposition and nutrient cycling. This information needs to be taken into account when evaluating changes in soil community composition and food web structure.

Effects of different types of litter on earthworms belonging to three ecological categories

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The invasion of species could be a cause of deep changes in structure and function of native soils. The composition of European forests is changing from monospecific formations to mixed forests including exotic species. This fact has an influence on the physico-chemical characteristics of the soil and on its fauna and decomposing microorganisms, by changing the nutrients cycle and the decomposition of litter (Yelenik et al. 2007). Litter is considered as the most important source of allelochemicals (Reigosa et al. 1999); several properties of the litter, such as a high proportion C:N, lignin, phenols and other secondary components, are associated to a slow decomposition rate. Given the fact that vegetal and edaphic invertebrate diversity are related to each other, the vegetal invasions could cause important changes in the structure of the ecosystem, having an influence on the communities and behaviour of detritivores (Mayer et al. 2005). It is known that the quality of the litter has an influence on trophic selection, reproduction and biomass of the earthworms (Cesarz et al. 2007). At the same time, earthworms have an influence on the decomposition of the organic matter, by improving the availability of resources for the microflora and the transfer of organic particles to the deep layers of the soil. Any effect on the earthworms activity will indirectly influence the soil properties. The objective of this work is to assess the effects of the litter from Acacia nigra, exotic species in Spain, and the native species *Quercus pyrenaica* on the growth and reproduction of three earthworm species belonging to the three known ecological categories Eisenia fetida, Aporrectodea trapezoides and Hormogaster elisae. Three types of microcosms were set up (controls without litter, treatments with acacia's litter and problems with oak's litter) where earthworms with a similar initial weight were introduced. The microcosms were checked weekly. The earthworms and number of coccons were counted and weighted. Furthermore several abiotic factors were analyzed in every microcosm at the beginning and in the end of the experiment. Starting from the ninth week, E. fetida exhibited a more pronounced growth when cultured in litter from oak and in the controls than when cultured in litter from acacia. A. trapezoides growed slightly more when cultured with the two types of litter than in the controls. No significant differences were detected in the growth of *H. elisae*.

Ecosystem services of earthworms in agro-ecosystems

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It is now well admitted that by their engineering activities, earthworms are able to alter key soil properties responsible for improving soil quality and sustainability in low-input agricultural systems. However, very few studies have investigated their impact on ecosystems services such as food production, water infiltration capacity, soil structure regulation and carbon sequestration in agroecosystems. Short-term demonstration and experimental studies based on earthworm inoculations was undertaken in Centre-West Côte d'Ivoire to: (i) improve maize production in farmer fields, (ii) validate the concept of integrated soil fertility management (ISFM) using earthworms as biological component in field mesocosms and, (ii) assess the impact of these organisms on water infiltration capacity, soil structure regulation and carbon sequestration in field mesocosms. Results showed that in farmer fields, earthworms are able to increase maize grain yield, cobs and biomass production respectively at 21%, 26.9, and 27.6% relative to control and fertilizer treatments. In field mesocosms, by significantly increasing maize phosphorus and water use efficiency 50 days after planting, earthworms improved maize growth in treatments characterized by coffee wastes and fertilizer applications. Maize grain rates of increase ranged from 96.3 to 266.8% in earthworm-based treatments and from 58.8 to 111.04% in fertilizer-based treatments showing the positive impact of earthworms in the ISFM trials. Moreover, the significant improvement of water infiltration capacity and soil structure regulation is due to the presence of earthworms with contrasting functional attributes in the agro-ecosystems, even if there was no significant impact of earthworms on carbon sequestration. More field and laboratory researches are needed to confirm these preliminary observations and promote the positive impact of earthworm in agro-ecosystems.

Contrasting effects of soil fauna on the nitrogen dynamics of tillage and no-tillage soils

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Managing the soil fertility of an agricultural ecosystem for sustainable production can be problematic. Although soil biodiversity is presumed to maintain soil fertility, the exact mechanisms of this control have not been elucidated. Certain agriculture practices, such as no-tillage (NT) or leguminous plant production, increase soil fauna diversity and may also improve soil nutrient retention and plant production. NT cropping systems usually increase soil aggregation and organic matter content relative to conventional tillage (CT). However, little is known about the separate effects of tillage or soil biodiversity on plant production, as tillage affects both plant production and soil biota. Hence, determining if the effect of a practice on plant production is direct or mediated by soil biota change is difficult. In this study, we investigated the contribution of soil fauna and agricultural practices to nitrogen dynamics, using manipulation experiments. To distinguish the effects of NT, legume planting, and soil fauna presence, we conducted a laboratory full factorial experiment combining CT, NT, legume (Lotus corniculatus) presence and absence, and soil fauna presence and absence. We measured the nitrogen (N) concentration in water leaching from soil to determine the N loss of agricultural soil systems. After 90 days, the experiment was terminated, and the dry weight and N content of aboveground plant biomass were determined. General linear model (GLM) analysis was used to compare the effects of soil practices, soil animal manipulations, and their interactions on N concentration in leached water and plant biomass. Tillage significantly increased leached nitrate and total N leached into water. While the presence or absence of fauna alone showed no significant effect, the interaction between tillage and fauna had a significant effect on N leaching. Faunal presence increased total N leaching in CT soil, but decreased it in NT soil. In the faunal presence, NT soil could conserve more N than did tilled soil. The aboveground plant N biomass of NT soil was significantly higher than that of tilled soil. Plant N was increased by fauna in CT soil but not in NT soil. These results suggest that soil fauna in tilled soil may possibly accelerate N cycling between the soil and plants, whereas the opposite is true of NT soil.

Key messages for earthworm management to control soil erosion in arable land

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Currently, soil erosion by water is one of the major threats on agricultural land throughout the world, and both its local (e.g. decreased crop yield) and downstream consequences (e.g. river eutrophication and sedimentation, increased flooding risk) may intensify due to climate change. A large set of technical (e.g. non-inversion tillage); vegetational (e.g. grass buffers), structural (e.g. sedimentation pond) or land use related (e.g. set aside land) erosion control measures have been proposed, most of them expensive end-ofpipe interventions. Up to now the potential of within-field ecological erosion control, i.e. through the action of ecosystem engineers such as earthworms, has been overlooked. The ECOWORM project (2005-2009) (Erosion control in arable land through the management of earthworm communities) tried to gain insights in the relationships between (1) arable field management (soil cultivation, green manure), (2) earthworm biomass and species composition, and (3) sediment loss through water erosion through diverse experiments and observations in the field and in the lab for West-European conditions. Based on these results, a toolkit was developed to inform policy makers, extension workers, and farmers about the significant role earthworms play in sustainable field management. The toolkit provides information about the different ecological groups of earthworms and their respective functions in the soil, and gives practical advice concerning earthworm sampling, identification and interpretation of results. Also the importance of earthworms in ecological erosion control is demonstrated. Finally, the toolkit gives recommendations to arrive at sustainable and well-considered field management, one of the key elements to successfully control erosion at the source. Seven key messages form the backbone of the toolkit: Key message 1 Within the boundary conditions set by climate and soil type, farmers' management is the key factor controlling earthworm abundance and species composition in arable land. Key message 2 Suitable habitat conditions (minimal soil disturbance, food of adequate quantity and quality) are the key determinant for the restoration and maintenance of diverse earthworm communities and abundant earthworm populations. Key message 3 Reduced soil disturbance – through non-inversion tillage in particular - promotes the abundance of deep-burrowing earthworm species, of high importance for erosion control. Key message 4 Earthworm populations need one to five years to recover after years of intensive soil management, depending on initial conditions. Key message 5 Use of the widely grown cover crop Yellow mustard (Sinapis alba L.) does not harm earthworm populations. Key message 6 Earthworms - in particular deepburrowing species such as the night crawler (Lumbricus terrestris L.) - significantly reduce runoff and soil loss in arable land. Key message 7 Weather conditions and soil status cause large variability in the erosion-controlling effects of reduced tillage and deep-burrowing earthworm species. These key messages underline the importance of good soil ecosystem management by the farmer and the need for the promotion of agricultural practices supporting populations of deep-burrowing earthworm species (noninversion tillage, direct drilling). Farmers who stimulate the occurrence of deep-burrowing earthworm species also significantly reduce the erosion risk.

Do earthworms stimulate nitrous oxide emissions at the ecosystem level?

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Earthworms are well known for their contribution to nitrogen mineralization, which is beneficial for soil fertility and crop production. However, earthworms may also stimulate gaseous nitrogen losses in terrestrial ecosystems. The earthworm gut is an anaerobic fermentation system that favours facultative anaerobic microorganisms such as denitrifiers that produce nitrous oxide, a potent greenhouse gas. Earthworm structures such as surface and subsurface casts (fecal pellets), middens (soil-litter mixture at the soil surface) and burrows are also a favourable habitat for microbial nitrifiers and denitrifiers that produce nitrous oxide. The objective of this presentation is to summarize what we know about earthworm-microbial interactions that lead to nitrous oxide production, to identify ecosystems that may be "hot spots" for such interactions, and to determine when are the "hot periods" of nitrous oxide emission in these ecosystems. Laboratory studies demonstrate that earthworms stimulate nitrous oxide production in plant-free soil, leading us to hypothesize that earthworm-microbial interactions lead to nitrous oxide production at small scales (microcosms, small field plots) and may contribute to nitrous oxide emissions at the ecosystem level. Agroecosystems with perennial vegetation, such as grass- and legume-based hayfields, support large earthworm populations because soils are undisturbed by cultivation and senescing plant residues are a food source for earthworms. Moist forests, riparian buffers and floodplains are also important because many earthworm species have adaptations that permit them to thrive in wet soils and tolerate flooding. These ecosystems constitute "hot spots" for nitrous oxide emissions attributable to earthworm activities. The "hot period" for emissions in these ecosystems may correspond to times of maximal earthworm activity (e.g., spring and fall in temperate climates), although earthworm structures may be a persistent source of nitrous oxide during the growing season, when climatic conditions are favourable for microbial nitrifiers and denitrifiers. We conclude by proposing a modelling framework to interpret small-scale biotic interactions and extrapolate them to the ecosystem level.

Adsorption and degradation of vermicompost on five phenolic acids in watermelon soils: experiment in laboratory

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Vermicompost can decompose some poisonous compounds, but the detailed mechanism of that on phenolic acids is not clearly understood. Under this object, the effect of vermicompost on phenolic acids in continuous cropping soil was detected in laboratory. Contents of five phenolic acids, 4hydrobenzoic acid, vanllic acid, syringic acid, ?-coumaric acid, frulic acid, extracted by water and methanol were determined in continuous cropping soil with and without watermelon seedlings. The proportion of sterilized and fresh vermicompost were 0% (control), 10%, 25%, 50% (volume ratio) in mixture of continuous cropping watermelon soil and vermicompost with and without watermelon seedlings, respectively. The data showed the amount and the concentration of phenolic acids in water extracts were less than that in methanol extracts. So the organic extracted solution was better choice than water for phenolic acids in soil. The methanol extracted 1-3 phenolic acids in soil while only one by water. Sterilization of vermicompost and plant also affected phenolic acids amounts and concentration in soil. Amount and total concentration containing sterilized vermicompost without plant were more than those of containing fresh vermicompost, which indicated the adsorption of vermicompost was the main mechanism in decomposition in sterilized condition. And microbe degradation of fresh vermicompost stimulated phenolic acids decomposition, so the adsorption and degradation was the main mechanism of decomposition with fresh vermicompost. The plant root exudation had important effect on decomposition. The amount of phenolic acids in soil with sterilized vermicompost with plant was less than that of without. Total concentration of phenolic acids in soil with sterilized vermicompost with plant was more than that of without. So plant root exudates maybe the reason that increased amounts of phenolic acids in sterilized condition. While the concentration containing fresh vermicompost with plant was decreased. And the more decomposition rate the more addition of fresh vermicompost. Decomposition test of pure phenolic acids in vermicompost demonstrated the interaction of adsorption and degradation in fresh vermicompost. It was concluded fresh vermicompost decomposed the allelopathic matters such as phenolic acids in continuous cropping soil by adsorption of particle and degradation of microbe. The decomposition rate was influenced by addition content of vermicompost into continuous cropping soil.

Influence of vermicompost on seedling growth and microbial community in watermelon monocropping soil: experiment in greenhouse

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Continuous cropping system led to plant yield decrease, and pathogen increase in soil. In order to find a biological solution to deal with continuous cropping problems, cow manure vermicompost was chosen in this experiment for its nutrients and resistance to microbe. The tested soil was 3-year continuous cropping watermelons. Four treatments: 0(control), 10%, 25%, 50% (volume rate), were set. Three watermelon seedlings were left after seeds germination in each pot with 4kg of substrate mixture in a greenhouse condition. After 40 days, plant height, stem width, leaf area and amount, root length, biomass of shoot and root were measured. Populations of bacteria, fungi, actinomycetes, *Fusarium oxysporum* were detected by plate culture. The data showed that vermicompost had more stimulation on plant growth. For example, the plant height, stem width, leaf area, root length of seedlings grown in mixture with vermicompost were 129.5%-147.9%, 37.0%-44.4%, 59.1%-83.9%, 61.9%-114.8% respectively higher than that of seedlings grown without vermicompost. Differences of each factors between vermicompost treatments and control were significant Besides, reasonable addition of vermicompost, such as 25%, was benefit for microbial community stabilization in rhizosphere and decreased the incidence of watermelon wilt.

Session 5

Earthworms interaction



The 9th International Symposium on Earthworm Ecology 5th to 10th September 2010, Xalapa, Mexico

Diversity of bacterial symbionts in earthworm nephridia

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Nearly all multicellular organisms form partnerships with bacteria that confer adaptive advantages in many different forms and environments. Earthworms are no exception and many harbour specific bacteria within their excretory nephridia. Using a combination of microscopy and 16S rRNA gene sequence analysis, we identified species harbouring nephridial bacteria and what types. Out of the 15 earthworm families we have screened, seven harbour nephridial symbionts. Members of the Lumbricidae harbour Verminephrobacter spp, related to the genus Acidovorax. Although nearly all Lumbricidae members (19 genera screened) contained nephridial Verminephrobacter, this symbiont was not detected among the other families. However, 19 species (representing 6 families) were positive for other types of nephridial bacteria. In addition to the Verminephrobacter spp., two or more other bacterial types that were consistently found included a Flexibacter-related bacteria (60 % of the Lumbricidae and in five genera outside of Lumbricidae) and Alphaproteobacteria from Rhodospirillales (Azospirillum) and Rhizobiales. Colonization patterns suggested that both specific symbioses and transient colonizations of certain types of bacteria occur in earthworm nephridia. The function of these symbionts is unknown. In the lab, Eisenia fetida serves as a symbiosis model for these widespread earthworm-bacteria associations. The entire lifecycle is easily maintained and the Verminephrobacter is cultivable outside of the host. E. fetida transfers the nephridial bacteria, V. eiseniae, Flexibacter and an Actinobacteria, into the egg capsules where they are recruited into the developing embryo. Theorized functions of these symbionts include nitrogen conservation, ammonia detoxification and chemical protection of the egg capsules. Antibiotic treatments to the eliminate egg capsule bacteria led to fungal overgrowth suggesting that the egg capsule bacterial community may provide protection for the capsule while it incubates in the soil. These nephridia-associated bacteria likely influence the success of earthworms within the complex soil system.

Determinants of gut wall bacterial communities in earthworms

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We investigated interactions between lumbricid earthworms and their intestinal microorganisms. First, we tested the effects of DNA extraction and field-based earthworm preservation methods on the quality of DNA obtained from earthworm gut contents and gut walls. We assessed several DNA extraction methods that are known to yield high quality DNA from soil samples. Field-based preservation methods assessed were absolute ethanol, chloroform and liquid N, with fresh earthworms as a control. Bacterial communities were detected using automated ribosomal intergenic spacer analysis (ARISA) of 16S and 23S genes, and ribotype data was used to assess diversity and community composition. Second, in a series of studies, we tested the hypothesis that differences in burrowing and feeding habits of anecic and endogeic earthworms are reflected by the existence of specific bacterial communities attached to the gut wall. Using soil and earthworm samples collected from adjacent wheat-barley and grass-clover fields, we found that the anecic Lumbricus terrestris and L. friendi, the endogeic Aporrectodea caliginosa and A. longa (traditionally defined as anecic, but shown here to possess endogeic characteristics) contain ecological group-specific gut wallassociated bacterial communities. The abundance of specific gut wall-associated bacteria (identified by sequence analysis of ribotype bands), including Proteobacteria, Firmicutes and an Actinobacterium, was ecological group dependent. A microcosm experiment, conducted using A. caliginosa and L. terrestris and five different feeding regimes, indicated that food resource can cause shifts in gut wall-associated bacterial community, but the magnitude of these shifts did not obscure the delineation between ecological group specificity. In the final study that used A. caliginosa and A. *longa* samples collected from six different arable fields, we deduced that, within an ecological group, habitat was a more important determinant of gut wall-associated bacterial community composition than was host species. We conclude that the determinants of the selection of bacteria associated with the gut wall of earthworms are in the order ecological group > habitat > species. Thakuria D. *et al.*, 2009. Field preservation and DNA extraction methods for intestinal microbial diversity analysis in earthworms. J. Microbiol. Methods 76(3), 226-233. Thakuria D. et al., 2010. Gut wall bacteria of earthworms: a natural selection process. ISME J. 4(3), 357-366.

Lactobacillus spp. maybe helps earthworms against dangerous pathogens in the environment?

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When some dangerous pathogens, like E. coli O157:H7, Salmonella typhimurium and Staphylococcus aureus were added in the living environment, six strains of Lactobacillus isolated from the earthworm casts, which were marked as WP, HF1, HF2, 5HF, MHF, and RP respectively and proved that these six bacteria were Gram-positive and Contact -negative and preliminarily determined these were *Lactobacillus*. They were identified by 16S rDNA sequence analysis: WP as Weissella; HF1, HF2 as Lactobacillus brevis; 5HF, MHF as Lactococus garvieae; RP as Pedicoccus acidilacticii. These strains of Lactobacillus have broader spectrum, they show significant antimicrobial effect on Escherichia coli, Staphylococcus aureus and Salmonella typhimurium under certain conditions. Not only playing a role against Gram-positive bacteria, but also for Gramnegative bacteria, so it can be considered as broad-spectrum antibacterial substance. Changes in environmental conditions affect the antibacterial activities of the supernatants of *Lactobacillus* spp., present diversified effects to different pathogens. Changes in environmental conditions not only affect the production of Lactobacillus and bacteriocin production, it also affects bacteriocin activities and the adhesion to bacteria, thereby affecting the concentration and the activities of bacteriocins in the supernatant. In stress conditions due to some dangerous pathogens, such as E.coli O157:H7, Salmonella typhimurium and Staphylococcus aureus, Lactobacillus spp living in earthworm alimentary canal may strengthen its antibacterial functions to help its host against those dangerous pathogens. Much further work is needed to be done to confirm this interaction.

Biotic interaction between earthworms and pesticide degrading bacteria -Impact on the detoxification service of soil

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Earthworms are efficient soil engineers that change the physical and chemical properties of soil and act in turn on soil microbial communities. Earthworm bioturbation will thus impact microbial processes in soil and also the generated ecosystemic services such as detoxification function. By using RNA based Stable Isotope Probing we demonstrated that the bacterial communities responsive to ¹³C enrichments in earthworm casts were different from those in bulk soil resulting in different degradation kinetics of ¹³C labelled substrates. From these results we focused on the impact of earthworm soil bioturbation on pesticide degradation by using atrazine as a model. This pesticide has been widely used leading to soil bacteria adaptation to its degradation and the degradative pathway involved has been elucidated (atzA, B, C, D, E and F genes). We hypothesized that earthworms, by generated spatial heterogeneity, modify the abundance and activity of atrazine-degraders in the soil microsites they engineer and act on the atrazine depollution service sustained by the adapted soil bacteria. Atrazine degradation genes as well as their expression were quantified by qPCR during in situ degradation of atrazine in the soil drilosphere (burrow-linings and casts) and bulked samples from two soils with different abilities to mineralize atrazine. The kinetic of ¹⁴C-atrazine mineralization was measured to link the atrazine-degraders abundance and activity changes to the degradation function. We observed different results depending on the initial soil but the atrazine mineralisation was always related to the atrazine degradation gene amount and expression. In the soil that slowly mineralised atrazine, we observed a positive "burrow-lining" effect on atrazine degrader abundance and activity compared to bulk soil whereas a negative impact was detected in earthworm casts. In the soil that quickly mineralised atrazine, opposite results were obtained. It seemed that depending on the soil physico-chemical properties and the inhabiting bacterial communities the impact of earthworm soil bioturbation on atrazine mineralization was different. However, in both soils atrazine mineralization was increased in certain microsite generated by earthworm bioturbation indicating their special feature regarding microbial activity and thus pollutant degradation.

Interaction between earthworms and soil fungi: volatiles attraction

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Earthworms and microorganisms are two major representatives in soil. Several interactions are known between these both actors of soil. But principally, microorganisms are an unavoidable constituent of earthworms' diet, in particular soil fungi are assumed to be a major food source for earthworms. Some fungi are presents in guts and casts of earthworms whereas some others are completely digested. For example, Eisenia fetida totally digests the fungi Geotrichum candidum and not Aspergillus fumigates. Geotrichum candidum is found in various habitats such as the soil and is a saprophyte in gut of humans and other animals as earthworms. Eisenia fetida is an important ecological earthworm species that is commonly used in industrial vermiculture and vermicomposting and is the model species for all scientist ecotoxicological researches. Chemical ecology and close association with soil fungi of this earthworm are poorly study. Therefore this information is essential to understand E. fetida life and to enhance our biological knowledge. As a first step to achieve such understanding, we focus our study on the behaviour of E. fetida in presence of filtrate of G. candidum culture in 4-arm olfactometer. We show for the first time, that E. fetida is attracted by filtrate of G. candidum culture. Volatile molecules emitted by this filtrate are analysed and identified by SPME-GCMS. Each identified molecule is tested in 4-arm olfactometer in order to find which are responsible for *E. fetida* attraction. We find that this attraction is due to ethyl pentanoate and ethyl hexanoate.

Earthworm - denitrifier interactions play a role in increased nitrous oxide emissions from soil mesocosms amended with crop residue

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Recent studies suggest that earthworm activity increases emissions of nitrous oxide (N_2O), a potent greenhouse gas, from arable soils. Understanding this mechanism is of great importance to sustainable agricultural management. Earthworm feeding strategy has been suggested as a driving factor, as it determines their access to fresh organic carbon for denitrification. Here, we determined whether interactions between earthworm feeding strategy and the soil denitrifier community can predict N₂O emissions from the soil. We set up a 90-day mesocosm experiment in which ¹⁵N labeled maize (Zea mays L.) residue was applied either on top or manually incorporated into the soil; additional treatments included presence or absence of the epigeic earthworm species Lumbricus rubelllus and/or the endogeic earthworm Aporrectodea caliginosa. On days 6, 21, and 90 we tested the bulk soil for denitrification enzyme activity as well as the abundance of total bacteria (16S rRNA) and denitrifier genes *nirS* and *nosZ* through real-time qPCR. Cumulative N₂O emissions after 90 days were significantly enhanced by the presence of L. rubellus (76%; p = 0.014) in the top-applied treatment. There was no significant effect of A. caliginosa, nor a species interaction on cumulative N_2O after 90 days. However total emissions in the treatment with both species had comparable emissions to mesocosms with manually incorporated residue (159.9 vs 156.3 mg N₂O-N kg⁻¹ soil, respectively). On day 21 A caliginosa presence significantly increased N₂O emissions (117%; p =0.005) and decreased microbial biomass (-28%; p = 0.004). The presence of L. rubellus caused a significant increase in denitrification enzyme activity in top-applied residue treatments on days 21 and 90 (p = 0.034 and 0.001, respectively). No changes in 16S rRNA or *nirS* abundance were observed. However, total *nosZ* abundance was significantly increased (100%; p < 0.05) on day 90 in the top-applied residue treatment containing only L. rubellus. On day 21 the presence of L. rubellus significantly decreased the proportion of soil bacteria containing the *nirS* gene (p = 0.010), indicating a possible shift in the denitrifier community.

We conclude that the effect of *L. rubellus* on increased N_2O emissions is related to its ability to enhance denitrifier community activity and affect composition through incorporation of fresh organic matter into the soil. The *A. caliginosa* effect appeared to be linked to an increase in general microbial turnover.

Oral – Session 5

Using molecular diet analysis to evaluate the role of earthworms in food webs

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Ecosystem services provided by earthworms include increasing soil fertility and recycling organic material. As well as their key role in improving soil structure and chemistry, and their overall positive influence on plant communities, earthworms often represent a substantial biomass, which supports large communities of vertebrate and invertebrate predators. Estimating the role of earthworms in food webs is essential for a thorough measurement of the ecosystem services they provide. This requires identifying all predators feeding on earthworms. Because their soft body is rapidly digested, the assessment of earthworms in predators' diet often relies on the detection of chaetae in the faeces of the predators. But earthworm chaetae are not species-diagnostic and which species is predated remains largely unknown. The development of molecular techniques targeting prey DNA remaining in predators' guts or faeces is a promising tool for identifying earthworm species. The techniques used here are usually based on the polymerase chain reaction (PCR). This study proposes a method for species identification of predated earthworms using predator faeces. We conducted molecular analyses on the faeces of a carnivorous landsnail endemic to New Zealand (Powelliphanta augusta). Earthworm group-specific primers and next-generation sequencing (454pyrosequencing) were used to extract and sequence earthworm DNA. Although earthworm tissue was not detectable in snail faeces, earthworm DNA was still present in sufficient quantity for molecular tools to detect and analyse it. Results showed that the studied landsnails feed on at least six species of New Zealand endemic earthworms. The method developed here is particularly appropriate for investigating the diet of potential predators for which feeding behaviour is difficult to observe. Such studies are required to draw a complete picture of the ecosystem services provided by earthworm communities. In this case it also helps conservation of rare and endangered predator populations.

Importance of earthworm – seed interactions for the composition of plant communities: a review

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Soil seed banks are crucial elements for the understanding of plant population and community ecology. Among animal species, earthworms are increasingly recognised as important dispersers and predators of seeds. Through direct and indirect effects, they might affect either positively or negatively the distribution, survival and establishment of seeds in plant communities. Thereby, earthworms may affect seedling establishment by a variety of mechanisms such as selective ingestion and digestion of seeds, acceleration or delaying of seed germination, and downward or upward seed transport. Seed displacements might for instance reduce exposure to harsh environmental constrains and to aboveground seed predators. Surface earthworm casts and middens often contain many viable seeds and might be important regeneration niches for plant seedlings and therefore likely favour specific plant strategies. Nevertheless, the role of earthworm as seed dispersers and mediators of seed bank dynamics has seldom been considered in past studies, especially in case of natural plant communities. Many aspects of the processes induced by earthworm activity remain obscure. Thus, we propose in this review to discuss the potential consequences of earthworm-mediated impacts on soil seed banks, plant community dynamics and potential outlooks in plant evolution, restoration, and conservation ecology.

Secondary seed dispersal of *Ambrosia trifida* by *Lumbricus terrestris*: Implications for Earthworm – Weed Interactions

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Lumbricus terrestris L. is a naturalized earthworm in North America that actively collects and caches seeds of Ambrosia trifida in its burrow. As a native annual plant of North America, A. trifida has become an important agricultural and allergenic weed of the U.S. within the past 30 years. Burial of A. trifida seeds in L. terrestris burrows reduces seed exposure to predators and influences depthdependent seed germination and seedling recruitment. We conducted field experiments to determine the interaction of L. terrestris and vertebrate seed predators on A. trifida seed fate and seedling recruitment in different habitats. A Bayesian model was fit to the data to assess the effects of L. terrestris on A. trifida in the presence and absence of seed predators. Burial of A. trifida seeds by L. terrestris increased the probability of seedling recruitment in areas where seed predation intensity was high, but decreased the probability of recruitment when predators were excluded or when predation intensity was low. In areas with high vegetative cover, seed predators consumed more seeds than were cached by L. terrestris. Conversely, L. terrestris cached more seeds than were consumed by seed predators in areas with low vegetative cover. Results from field studies provide evidence that the relationship between L. terrestris and A. trifida is a conditional mutualism wherein L. terrestris benefits A. trifida survival by increasing seedling recruitment in habitats where seed predation intensity is high. Furthermore, seed caching by L. terrestris may also increase A. trifida survivorship by burying some seeds below the germination depth limit, thereby maintaining seed dormancy and increasing seed bank longevity. Laboratory experiments designed to investigate L. terrestris nocturnal foraging behavior revealed that the earthworms preferentially collected real seeds over plaster-cast seed duplicates and debris, and that they were able to detect and forage the seeds from below as well as above the soil surface. This feeding behavior suggests that the earthworm utilizes olfactory sensing to forage seeds surreptitiously, thus limiting its exposure to predators while promoting the mutualism with A. trifida.

Do non-native earthworms cause invasional meltdowns?

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Invasional meltdowns, in which facilitation between species causes an accelerating increase in the number of introduced species or their impacts, can result in larger changes to invaded systems than otherwise expected. Earthworm invasions of northern forests have been suggested as an example of an invasional meltdown, with epigeic and epi-endogeic species altering soil structure and facilitating the establishment of endogeic or anecic earthworms that may be less capable of invading intact forest floors. We used intact boreal forest soil cores to examine facilitation between an epigeic species (Dendrobaena octaedra) and an anecic species (Lumbricus terrestris). Cores were inoculated with either L. terrestris alone or both L. terrestris and D. octaedra and maintained in a greenhouse. After 3.5 months, we determined earthworm abundance, biomass, cocoon production, and organic horizon depths in the different treatments. There were no significant differences in L. terrestris survival, biomass, or average cocoon production between treatments. As well, the two species appeared to have an additive rather than a synergistic effect on organic horizon depths. These results suggest that D. octaedra does not facilitate reproduction or survival of L. terrestris and that the two species do not cause a synergistic effect on leaf litter as would be expected in an invasional meltdown. Since D. octaedra does not appear to greatly increase the success of L. terrestris, management in boreal forest ecosystems may be most effective if focused on L. terrestris, which has larger impacts on its own than D. octaedra.

Spatial distribution of earthworm species assemblages in pasture of northwestern France

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Earthworms represent one of the more important groups of soil fauna in many terrestrial ecosystems. They are involved in the regulation of critical soil processes and in the providing of key ecosystem services. Understanding the spatial patterns of species assemblages and their impact on soil functioning at different scales has become an important goal in soil ecology. Earthworm populations often exhibit conspicuous patterns, specimens being aggregated in patches which relative arrangement responds to both habitat constraints and species interactions. In temperate regions, these patterns have been the subject of a relatively low number of studies, particularly in context of agricultural pastures. In this study, we investigated the spatial distribution of lumbricid earthworm assemblages at the scale of pastures plots of different ages (one permanent pasture and two temporary pastures with different agricultural management). Study site was located in Upper-Normandy in France. Earthworms were collected each 10m on a regular grid and identified at species level. Data were analysed with multivariate and geostatistical tools in order to assess if earthworm communities will present some typical patches distribution. At this moment data are still under analysis. Our main hypothesis is that species assembly rules during pasture progressively drives assemblages towards a dynamic-equilibrium stage in which competitive interactions are reduced by niche complementary and spatial segregation.

Seed consumption and burial by the earthworm *Lumbricus terrestris* favours the establishment of *Lolium perenne* over *Agrostis capillaris*

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We studied the effects of the anecic earthworm *Lumbricus terrestris* L. on seed germination of two plant species to understand the mechanisms underlying earthworm-seed interactions. We set up microcosms with seeds of *Lolium perenne* L. (large seeds) and *Agrostis capillaris* L. (small seeds), in a loam based compost, with and without earthworms. We buried seeds at three depths and recorded their germination, we also examined to what depth earthworms buried seeds, and we studied seed viability after transit through the gut of earthworms. Earthworms dramatically reduced the numbers of *A. capillaris* seeds germinating, but did not modify the germination of *L. perenne* seeds. Earthworms, however, increased the biomass of *L. perenne* seedlings. When we buried the seeds, those from *A. capillaris* rarely germinated when they were placed below 7 cm, unlike *L. perenne* seeds. Earthworms buried the seeds of *L. perenne* to a depth of 15 cm. Passage of seeds through the gut of earthworm shad a detrimental effect on *A. capillaris* seed viability, while *L. perenne* seeds remained unaffected. While our results show that earthworm activity can strongly influence grass seed germination and plant establishment in microcosm experiments, further work is needed to assess the implications of earthworm-seed interactions for vegetation dynamics in the field.

Pre-copulatory and male reproductive allocation in relation to inbreeding, outbreeding and non size-assortative mating in the earthworm *Eisenia andrei* (Oligochaeta: Lumbricidae)

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In simultaneous hermaphroditic animals like earthworms, the division of reproductive resources into male and female function is an important strategy for optimizing fitness. Thus, the earthworm Eisenia andrei (Oligochaeta: Lumbricidae) reduces cocoon production under inbreeding, outbreeding and non size-assortative mating. However, we do not know whether this reproductive adjustment (post-copula) comes from a female effort decision or from a decrease in the sperm received (i.e. male effort decision). In order to clarify this question we tested whether the genetic similarity (inbreeding and outbreeding) and the relative size of partners influence the volume of sperm donated. We also explored the possibility of pre-copula adjustment via mate choice decision. Mate choice decision was evaluated setting up mating trios in which a focal individual can choose between other two earthworms: one sibling, and one unrelated (inbreeding); one from the same population, and one from a different population (outbreeding); and one of the same size, and one of a different size (sizeassortative). Male effort decision, measured as the volume of sperm donated, was studied setting up the following mating pairs: inbreeding pairs, mating two fullsiblings and two unrelated earthworm from the same population; outbreeding pairs, mating an earthworm with another earthworm from a distant or the same population; and size-assortative pairs, mating an earthworm with another of different or similar weight. Our results revealed that: (1) inbreeding but not outbreeding affects to the mate choice decision reducing the probability of copula, (2) neither inbreeding nor outbreeding affects to the volume of sperm donated during mating, and (3) earthworms have a trend to a non sizeassortative mating. Thus, small earthworms prefer to mate with large partners whereas large earthworms are less choosy. Further, small earthworms donate more sperm to large than to small partners, however large earthworms do not significantly change the volume of sperm donated.

How to reduce the number of *Meloidogyne paranaensis* galls in tomato using earthworms?

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The objective of the study was to evaluate the incidence of *Meloidogyne paranaensis* galls in Solanum lycopersicon roots after inoculating Amynthas sp. and Pontoscolex corethrurus. The experiment was conducted in the greenhouse of the Nematology Laboratory of "Marcos Enrietti" Diagnosis Center of the Department of Agriculture and Supply of the state of Paraná (SEAB), using randomized blocks, with eight treatments and four replicates: T1- standard; T2-Meloidogyne paranaensis; T3-Amyntas sp.; T4-Pontoscolex corethrurus; T5- Amynthas sp. + M. paranaensis; T6-P. corethrurus + M. paranaensis; T7- Amynthas sp. + P. corethrurus; T8- Amynthas sp. + P. corethrurus + M. paranaensis. Initially, six adult of Amynthas sp. or P. corethrurus earthworms were inoculated, individually or in the same proportion (3:3) with fresh weight previously determined, in a 4L polyethylene pot containing three litres of sterilized soil. After one week, tomato seedlings (cultivar 'Rutgers') were transplanted into the pots and 5 mL of egg suspension of M.paranaensis containing 5,000 eggs and/or juveniles per pot were inoculated. At 65 days after inoculation, they were assessed the number of remaining earthworms by hand-sorting, the fresh weight by direct weighing, and the number of galls on tomato roots by direct counting using a stereoscopic microscope. The results showed a decrease in the number of galls/plant in those treatments where worms were inoculated, ranging from 44.8% to 60.8% for Amynthas sp and P. corethrurus, respectively. Yet, the incidence of galls decreased 54% with the combination of earthworms. The remnant rate of worms ranged from 16.7% to 62.5%, with higher values for the genus Amynthas. However, understanding the interaction "nematodes x earthworms" need to be studied further.

Genetic identification of bacteria isolated from the digestive tract of the earthworm Pontoscolex corethrurus

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Invertebrates and soil microbes establish interactions or relations through which they make use of minerals and organic resources. There is little knowledge of the interaction which takes place between earthworms and soil bacteria. The bacteria are transported to the inside of the digestive tract when these invertebrates ingest soil. It is known that within the intestines, bacteria degrade complex organic compounds such as cellulose, lignin, tannins and humic complexes, by means of enzymes which they secrete. The objective of this study was to genetically characterize the populations of bacteria which inhabit the digestive tract of the earthworm Pontoscolex corethrurus. The sampling of the species P. corethrurus was carried out in two localities within the state of Veracruz, Mexico: "Plan de las Hayas", which bears cattle livestock pastures and "La Mancha", a conservation zone with a section assigned to banana production. The earthworms were superficially sterilized, and under aseptic conditions, the intestinal content was placed in a Brain Heart Infusion (BHI) liquid medium. The resulting suspension was inoculated in Petri dishes containing nutrient agar, Tripticasein soy agar, King medium, Pseudomonas and BHI agar. DNA was extracted from the purified bacterial isolations for PCR amplification of the region corresponding to the 16S rDNA gene. The amplified region was partially sequenced with the aim of identifying bacterial isolates. A total of 8 bacterial genera were identified within the digestive tracts of P. corethrurus isolated from both sampling zones: Bacillus, Bacterium, Terribacillus, Pseudomonas, Acinetobacter, Massilia, Aeromonas and Citrococcus. The sample taken from "Plan de las Hayas" presented 11 bacteria species: Pseudomonas aeruginosa, Massilia timonae, Bacterium sp, Acinetobacter sp., Aeromonas sp., Citrococcus sp., Bacillus sp., Bacillus subtilis subsp. subtilis, Bacillus mycoides, Pseudomonas sp., and Bacillus cereus. In contrast, 6 bacteria species were identified from "La Mancha": Bacillus sp., Bacillus megaterium, Bacillus horikoshii, Aeromonas punctata, Bacterium, and Terribacillus sp. The most predominant genus was Bacillus with 5 species in both sampling sites from the digestive tract of P. corethrurus: Bacillus megaterium, B. horikoshii, B. mycoides, B. subtilis subsp. subtilis and B. cereus. The present research demonstrates the existence of the genetic diversity of bacteria which inhabit the digestive tract of the earthworm *P. corethrurus*. It is possible that this bacterial diversity is due to the soil type and management. The "La Mancha" site presents sandy soils with banana cultivation, meanwhile "Plan de las Hayas" has clayed soils which supports pastures. Future research should focus in knowing the transfer time and permanency of the bacterial species in the different parts of the digestive tract of the earthworms, likewise determine the role of bacteria in their passage through the intestines of these invertebrates.

Poster - Session 5

Influence of body size and geographic distance in reproductive isolation of the genus *Balanteodrilus* (Acanthodrilidae, Oligochaeta)

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Balanteodrilus pearsei is a native species widely distributed along southeast Mexico. In contrast to the majority of Mexican native species, its distribution has not been reduced by human activities, probably due to its high adaptative plasticity and wide morphological variability. This variability is partially expressed by different patterns of genital markings present in several populations and, more conspicuously, in the length of body size. Two morphs have been recognized that strongly differ in the last characteristic: a small, narrow distributed morph mainly restricted to sandy soils, and a larger one, widely distribute and with no clear soil texture restrictions. The aim of this study was to evaluate reproduction between two populations of each one of the two morphs, located at different geographical distances. Laboratory cultures of intrapopulation and interpopulation treatments were carried out with virgin individuals from each population and morph. Reproduction was evaluated by the number and viability of produced cocoons. Preliminary results reveal that the small morph is reproductive isolated from the larger one, and also, that reproductive success within the same morph is inversely related to geographic distance. As far as reproductive success is concern, this study brings out ecological evidence that suggests that the small morph is reproductive isolated from the larger one, and thus that it represents a valid (new) biological species. Further anatomical and molecular studies should support this founding. Finally it is discussed the importance of size and distance in the separation of congeneric earthworm species.

Importance of direct earthworm-seed interactions along a succession gradient in chalk grasslands

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Many researches showed the beneficial role of earthworms in plant productivity through their positive impact on soil physical, chemical and biological properties. Nevertheless, the role of earthworms on the composition and the dynamic of plant communities had seldom been considered in past studies. Many aspects of the processes induced by earthworm activity on natural community remain obscure. This study focuses on the interactions between earthworms and seeds. The leading question is: do earthworms affect the composition and the dynamic of chalk grasslands via their direct effects on the seed bank? Three hypotheses structured the study: (i) Earthworms (EW) modify the repartition of seeds by deeply burying seeds and by taking buried seeds out to the soil surface, (ii) the ingestion of seeds is "seed-EW" dependent and related to species traits. This "seed-EW" specificity favours and/or inhibits the germination of some species, (iii) the extent of these EW-seeds interactions varies along the secondary vegetation succession. The study site was located on chalk grasslands of Upper-Normandy (North-West of France) on a diversity-rich protected site. This fragmented habitat is partly managed by grazing and that is part of the regional patrimony. Soil and earthworm casts --that contain seeds- were sampled in four different stages of the secondary succession from open grasslands to bushes in order to create a gradient. Each soil sample was divided into three depths (0-2 cm; 2-5 cm; 5-10cm) to evaluate each seed composition and to compare it with cast seed composition. Species were identified at the seedling stage. Naturally expressed vegetation was later identified in situ. Results were analysed using multivariate techniques (Co-inertia Analysis) to detect the effect of EW on seed banks composition and dynamic. Additionally, seed selectivity by earthworms and effects on germination success were tested in microcosms. Fifteen plant species identified on-site and/or in EW casts and two EW species commonly found in the site were selected: Allobophora chlorotica and Aporrectodea caleginosa. Preliminary results of this study suggest that the similarity between soil seed banks and casts changes along the successional gradient. We will discuss the potential consequences of EW-seeds interactions on soil seed banks, plant community dynamics and potential outlooks in conservation ecology.

Earthworm communities along an altitudinal gradient in Central Mexico highest mountain: Influence of local soil type, regional weather and human disturbance.

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High mountains are important as endemic hotspots and as a refugee for native species. Central Mexico is characterized by a high altitude plateau (2000 m over sea level), with several isolated peaks over 3000 m of altitude. The "Pico de Orizaba" region (PO), currently the highest peak in the country (5675 m), belongs to this group of mountains within the Mexican Transvolcanic Belt. Hitherto, Mexican earthworms have been well studied in lowlands of the southern region, but there are few records in the central mountains, which are expected to harbour a rich still unknown diversity. In this study we characterized earthworm communities (species richness, abundance, biomass, ecological categories, origin and diversity) along four altitudinal gradients in PO (2000-3500 m), two located in the east-humid slope and two in the west-more dry slope. In each transect four sites were sampled at varying altitudes. Vegetation types included pine, oak and fir forests, high altitude grasslands and cultivated fields. In each site qualitative (five monoliths of 0.25 m2 surface and 20 cm depth) and qualitative methods were used. Results indicate that exotic earthworms were mainly found in altitudes below 3000 m, in oak forests, cultivated areas and pine tree plantations, whereas natives were limited to undisturbed forests and grasslands at or over 3000 m of altitude. Abundance and diversity were higher in the east-humid mountainside. We discuss if the narrow altitudinal gradient were natives were found, is a natural pattern or a response to local human disturbance, exotic invasions or global climate change.

Predominant Microbial Populations Associated with Earthworms Living close to Extreme Environments of Volcanic Origin: A Comparison Between Azorean and Mexican Populations of *Pontoscolex corethrurus*

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The extant volcanic activity in certain terrestrial locations in São Miguel, one of the nine islands comprising the Azores archipelago, provide good "field-laboratories" to study life in extreme environments of volcanic origin. Microorganisms are believed to play an essential role in earthworm's adaptation process, especially the ones passing through the guts and thereby facilitating digestion among other features. In this study we compared by using a genomic approach the dominant bacterial populations present in two distinct populations of P. corethrurus, one living inside an hydrothermal field in Azores and the other, a population resident in pastures in Veracruz, Mexico. For each studied population a genomic library was created and clones were successfully sequenced and analysed. There were few numerically dominant species as well of intermediated abundance but a large number of rare species. In both genomic libraries the microbial relative abundance was dominated by the subclass of the class Proteobacteria. Azorean population was found to have higher species richness and diversity than those from Mexico. The most dominant genera in Mexican samples were found to be Bradyrhizobium, Methylobacterium, Nitrobacter, Paracoccus, Pectobacterium, Rhizobium, Rhodopseudomonas, Burkholderia, Pseudomonas, Stenotrophomonas, Blattabacterium, Dickeya, Edwardsiella, Escherichia and Serratia. In Azorean populations dominant microbial groups belonged to genera such as Agrobacterium, Azorhizobium, Bradyrhizobium, Caulobacter, Mesorhizobium, Methylobacterium, Methylocella, Nitrobacter, Rhizobium, Rhodobacter, Rhodopseudomonas, Acidovorax, Bordetella, Burkholderia, Verminephrobacter, Anaeromyxobacter, Desulfovibrio, Haliangium, Acinetobacter, Pseudomonas, Xanthomonas, Arthrobacter, Clavibacter, Conexibacter, Corynebacterium, Eggerthella, Serratia, Cyanothece and Gloeobacter. The implications of possible mutualistic relationships and the different metabolic groups are further discussed.

The role of bacterial swimming and twitching motility during recruitment of earthworm symbionts

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Earthworms harbor bacterial symbionts in their excretory nephridia. Most members of the Lumbricidae contain a Verminephrobacter species in addition to one to three additional bacterial types. Isolates of Verminephrobacter eiseniae from Eisenia fetida have been brought into cultivation and a full genome sequenced and annotated. Targeted genetic transformation of the V. eisenia enable studies of genes, mechanisms and functions involved in this host-symbiont system. The nephridial symbionts are deposited into the egg capsule albumin by the parent, and bacteria colonize the embryos beginning mid development. Recruitment of the bacteria to the specific tissue sites is a multi-step process beginning with aggregation at a pore, and migration of bacterial cells into a canal leading to the nascent nephridia. We hypothesized that motility was required for early recruitment of the symbionts. To begin to elucidate bacterial recruitment mechanisms, we targeted flagellar motility and twitching motility for study. Twitching motility uses pili, anchored in the cell membrane, that extend from the cell to bind a target then retract to pull the cell forward. The pilus tip often possesses adhesins for specific binding to targets. Using the suicide vector pLVCD for directed mutagenesis, we generated flagellar (flgK-) and type IV pilus (pilBC-) mutants of V. eiseniae. Disruption of V. eisenia pilus genes eliminated synthesis of type IV pili, twitching motility and aggregation of cells in liquid medium. Mutation of the flgK gene eliminated flagellar synthesis, resulting in loss of swimming motility. We tested these mutants for their ability to colonize E. fetida embryos by injecting bacterial cells into egg capsules lacking their V. eiseniae symbionts, and waiting for embryos to develop. The flgK- mutants were able to migrate partially, but not completely, into the nephridia. The pilBC- mutants did not appear in any portion of the hatchling nephridia. These findings suggest that surface adhesion is critical for initiation of this association, and cell surface recognition may play an important role for selection of the proper bacteria. The ability of nonswimming V. eisenia to migrate partially, but not completely, indicates swimming motility is not necessary for the initial entrance, but is needed to complete the journey. Future studies will investigate the targets of the adhesins also found in the V. eiseniae genome that likely direct symbionts to their specific colonization sites.

Life cycle and reproductive biology of the earthworm Allolobophoridella eiseni (Oligochaeta, Lumbricidae)

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The earthworm *Allolobophoridella eiseni* (Oligochaeta: Lumbricidae) is an epigeic species often found underneath leaves or rotting trunks and is widespread in Western Europe. In order to determine the characteristics of its life cycle, earthworms of this species belonging to three different populations were cultured from hatchling until maturity in isolation. Development rates, cocoon production, and incubation period were recorded under controlled conditions. On average, individuals of *A. eiseni* reached maturity after day 43, with a weight ranging between 175 and 184 mg. Virgin earthworms started to lay cocoons after reach maturity indicating uniparental reproduction. Cocoon production was 7 cocoons per month, the incubation period was 24 days, hatching 3 individual per cocoon, with a mean hatchability of 80%. Copulation occurs in the field; we found several individuals (35% of captured earthworms) carrying spermatophores with spermatozoa. Our results showed that the characteristics of the life cycle of *A. eiseni* are typical of an epigeic earthworm species; it means high growth rates and high fecundity. More studies are necessary to identify what kind of uniparental reproduction takes place.

Understanding the fate and linkage of N and S in earthworm engineered peat soil using NanoSIMS

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Earthworms, by changing soil physical properties, are efficient engineers that play a key role on the soil nutrient dynamics but their biological processes at the micrometric scale are still misunderstood. The nano-scale secondary ion mass spectrometry (NanoSIMS) is a new tool in the study of biophysical interfaces in soils with its ability to operate at high mass resolution, while maintaining both excellent signal transmission and high spatial resolution (down 50 nm). Based on this new technology, the aim of this study was to visualize at the micrometric scale the burrow-linings of Lumbricus rubellus L. and to determine the fate and linkage of nitrogen (N) and sulphur (S) deriving from this soil engineer. Earthworms were first triple labelled with isotopic tracers (^{15}N and ^{34}S) prior to introduction into unlabelled peat mesocosms for 24 days. Then, the mesocosms were embedded in a polyester resin to carry out the NanoSIMS analysis (NanoSIMS 50[™]; Cameca, Gennevilliers, France). Burrow-linings were analysed with a transect of images increasingly far of the biophysical interface (from poral space to inner of the burrow-lining) and we assessed the linkage of ¹⁵N and ³⁴S with the soil micro-architecture by using the linescan technique. Isotopic compositions were determined by using image processing with the ImageJ software (W.S. Rasband, US National Institutes of Health) and thus nutrients transfers from labelled earthworms to soil burrow-linings were detected. We had a visible gradient with a decrease of percentage of 15 N and 34 S (2.00 to 0.38 % and 10.0 to 4.4 % respectively) and no transfer was detected beyond 1mm, which defined clearly the area of earthworm's influence (i.e. the drilosphere). Interestingly, the occurrence of spatially anticorrelated hotspots of ¹⁵N and ³⁴S suggested either i) two kinds of earthworm's excretions (an excretion rich in N and poor in S, and an excretion poor in N and rich in S) or ii) two kinds of microbial activity linked with N or S cycle (¹⁵N or ³⁴S microbial uptake). The speciation of these enriched hotspots are currently under analyzes. By using the NanoSIMS tool coupled with isotopic tracers, we bring out new understandings of the impact of earthworms on the nutrients and thus their ability in sustaining hot spots of microbial activity in peat.

Role of interacting soil engineers in the transfers of C-N-S in a regenerating cutover peatland

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Soil organisms, because they change soil physical and chemical properties as well as microbial communities, are efficient engineers that play a key role on the soil nutrient dynamics. Although the coupling of carbon (C) and nitrogen (N) in soil have been well studied, such studies in peat soils are scarce and even unavailable for sulphur (S). The aim of our study was to assess the impact of peat bioturbation by an epi-anecic earthworm (Lumbricus rubellus L.) and/or a plant (Eriophorum angustifolium L.) in the coupling of C-N-S in a regenerating cutover peatland, by using simultaneously three isotopic tracers (¹³C-¹⁵N-³⁴S). We thus focused on C-N-S transfers in the drilosphere and rhizosphere that are representative of the earthworm and/or plant activities. Labelled organisms (three earthworms or two plants) were introduced in unlabelled peat mesocosm (2 litres). Earthworm tissues, plants, representative peat samples of the drilosphere and the rhizosphere and bulk peat were analyzed for their $(\%)^{13}$ C, 15 N and 34 S by using an elemental analyser-isotope ratio mass spectrometry (20-20 IRMS, Europa Scientific) after 3 and 18 days of incubation in a climate chamber. We demonstrated rapid C, N and S enrichment of casts (x 1.3, x 70 and x 1.6 respectively) and burrows (x 1.2, x 30 and x 1.2) derived from labelled earthworms and of rhizosphere (x 1.1, x 10 and x 1.1) derived from labelled plants compared to the bulk soil. With regard to Nitrogen, a significant N transfer from labelled earthworms to unlabelled plants was shown, which reached up 4 % of 15 N (40 µg) within 18 days. Our study highlighted the key role of soil engineers in the cycling of C-N-S in regenerating cutover peatlands by enhancing C-N-S transfers and providing nutrient enriched peat hot spots that are expected to sustain higher microbial activities. Further studies will therefore focus on microbial communities and C-N-S microbial uptake in the rhizosphere and the drilosphere.
Short-term effects of epigeic earthworms on the structure and activity of microbial decomposer communities

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Epigeic earthworms live in organic horizons, in or near the surface litter, where they interact closely with other biological components of the soil system, and in turn can affect the structure and function of microfauna and microflora. In the present study we investigated the short-term effects of three epigeic earthworm species (Eisenia andrei, Eisenia fetida and Perionyx excavatus) on the microbial community structure and activity of three animal manures (cow, horse and rabbit), in comparison with those in a control treatment. We evaluated the impact of these earthworm species on the abundance of bacteria and fungi and their corresponding growth rates and on total microbial activity (basal respiration) after one month. We performed a two-factor experiment with each combination of manure and earthworm species. For this, plastic containers (2 L) were filled to three quarters of their capacity with moistened and sieved vermiculite and inoculated with ten mature earthworms. Two hundred grams (fresh weight) of manure was placed on a mesh (5 mm pore size) on the surface of vermiculite. The containers were covered with perforate lids and stored at random in an incubation chamber at 20 °C and 90% relative humidity. We also included a control treatment that consisted of 100 g of manure incubated without earthworms. Each treatment was replicated three times. The relatively rapid gut transit time of epigeic earthworm species resulted in the residues being completely processed by the earthworms in one month. The discriminant analysis of the PLFA data clearly distinguished between the animal manures after being processed by the three different epigeic earthworm species, irrespective of what manure type was used. The separation between the earthworm-processed manures and controls was also very clear. The earthworm species E. andrei reduced in a large way the abundance of bacterial and fungal PLFAs relative to the control, although this effect varied in function of the type of manure. The same pattern was observed with respect to the bacterial growth rate and total microbial activity; however, no such changes were found in the fungal growth rate as a result of earthworm activity. These findings provide a new insight into how detritivorous earthworms affect the microbial ecology of the substrates that they inhabit and highlight the potential of *E. andrei* in the biodegradation of organic residues.

Effects of the transit through the gut of epigeic earthworms on the structure and activity of microbial decomposer communities

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Earthworms can interact with the soil microbial community either directly or indirectly through their feeding, burrowing and casting activities. Digestion of the ingested material, which occurs in a time scale of hours, is the first step in this interaction process. Most of the current knowledge regarding interactions between earthworms and microorganisms relates to soil-dwelling endogeic and anecic species; as such, in the present study we evaluated the changes in the microbial community structure (PLFA profiles) and activity (fluorescein acetate hydrolysis) of three animal manures (cow, horse and pig) after passing through the gut of the epigeic earthworm species *Eisenia andrei*. Plastic containers (250 mL) were filled to three quarters of their capacity with moistened and sieved vermiculite and inoculated with twenty-five mature earthworms. One hundred grams (fresh weight) of manure was placed on a mesh (5 mm pore size) on the surface of vermiculite. The containers were covered with perforate lids and stored at random in an incubation chamber at 20 °C and 90% relative humidity. We also included a control treatment that consisted of 100 g each manure incubated without earthworms. Each treatment was replicated five times. After three days, the earthworms were removed from the containers, washed with sterile distilled water, and placed into a Petri dish to allow them to shed their gut contents on moistened tissue paper for 24 h. After this time the gut contents from earthworms from the same containers were pooled for analysis; the same amount from the control containers was also collected. The passage of the three animal manures through the gut of the earthworm species E. andrei led to a reduction in the viable microbial biomass measured as total PLFAs relative to the control, irrespective of what manure type was used. A decrease in the abundance of bacterial and fungal PLFA biomarkers was also observed as a result of gut transit, although this effect was more pronounced in horse manure with respect to fungal populations. Moreover, we detected a reduction in microbial activity relative to the control when *E. andrei* fed on cow and horse manure rather than pig slurry. These findings reveal that epigeic earthworms play a key role in the decomposition of organic matter via their intense interactions with microbial communities through the gut associated processes.

Do earthworms prey on nematodes? Laboratory food-preference experiment

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Soil nematodes and earthworms perform important functions in the soil system, but the relations between these organisms are still unequivocal. In the presence of earthworms, nematode abundance usually decreases. Common conclusion is that nematodes are digested by earthworms, but whether it is accidental or selective preying is still ambiguous. Do earthworms choose nematodes selectively and more often than the other available food types? The food preference of different species of earthworms to different food types were studied in a laboratory experiment. The tested species of earthworms were: Lumbricus terrestris, Lumbricus rubellus and Aporectodea caliginosa. As a food source were chosen: bacteria cultured on agar, fungi cultured on agar, agar with a known number of nematodes, agar with sterile oak-birch litter and as a control agar with fine-grained sand. One specimen of earthworm was introduced to a centre of Petri dish (diameter 21 cm) with prepared food sources. Cultures on agar were arranged near the circumference of a Petri dish, in equal distance from the centre of the dish and other food types. Experiment was conducted for 72 hours. Presence of animals on food during experiment was observed. The number of nematodes was evaluated after termination of experiment. A methodology of this experiment, some difficulties connected with the experimental design and observation procedure are discussed and preliminary results are presented. The study was supported by the grant of Polish Ministry of Science and Higher Education (N N304 402038).

Response of nematodes to earthworm abundance in the litter

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Nematodes are important component of soil food web. Presence of earthworms or changes in the earthworm abundance affects abundance of nematodes. In a mesocosm experiment, the response of nematodes in the gradient of earthworm population density in litter was investigated. Five-month experiment was conducted in beech-oak forest. Each mesocosm was filled with 10L of soil and 100 g of litter. Tested earthworms' species represented three ecological groups - epigeic (Dendrobaena octadera, Lumbricus rubellus), endogeic (Allolobophora chlorotica, Aporrectodea caliginosa) and anecic (Lumbricus terrestris). Each earthworm species was introduced to mesocosms in three different densities - minimal, medium and maximal. Number of earthworms in each density level varied between earthworms' species due to differences in the biomass. Results for litter may be summarized as follow: 1) All investigated earthworm species reduced significantly the number of nematodes, except D. octaedra. The most distinct effects were observed with maximal earthworms' density, which is related to increase of earthworm biomass in those treatments. The most severe reduction in nematode abundance was observed in mesocosms with L. terrestris. 2) Considering the ecological groups, epigeic and endogeic earthworms reduced nematodes number at similar level (on the border of the significance). Impact of the anecic L. terrestris on nematode density was far stronger in comparison to the earthworms' species from the other ecological groups. 3) In mesocosms with high earthworms' biomass decrease in amount of litter was noted. In treatments with low quantity of litter lower abundance of nematode was found. 4) At maximal density L. *rubbelus*, an epigeic earthworm, reduced number of nematodes in litter only slightly stronger than A. caliginosa, endogeic, soil dwelling earthworm. Impact of endogeic earthworms on nematodes inhabiting litter should receive more attention. The study was supported by the grant of Polish Ministry of Science and Higher Education (2P04F03030).

Importance of ammonium content in the attraction or repulsion of Collembola to the excreta of earthworms

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Previous studies have shown different reactions of several collembolan species to the excreta of earthworms belonging to different families and ecological categories (Salmon 2001, 2004; Salmon and Ponge, 2001; Salmon et al., 2005; Gutiérrez et al., in preparation). Trophic causes related with the ammonium concentration were proposed. The objective of this study was to measure the ammonium concentration in two types of excreta (casts and mucus-urine) of two earthworm species in order to explain the contrasted response of collembolan species. The earthworm species used in the experiments were the anecic Aporrectodea giardi (Ribaucourt, 1900) (Lumbricidae) and the endogeic Hormogaster elisae Álvarez 1977 (Hormogastridae). The $NH4^+$ concentration was determined in fresh casts and in the mix of epidermial mucus and urine, following the Kjeldahl method (IPLA, 1984). To determine NH_4^+ concentration in mucus-urine, half-discs of filter paper impregnated with the excreta of each earthworm species were analyzed. Earthworms had previously voided their digestive tube by keeping them for 3 days in Petri dishes with moistened filter paper. They were then placed in Petri dishes with six half disks of filter paper to saturate them with mucus-urine during 4 hours. NH4⁺ concentration was referred to fresh weight of mucus urine. The casts produced by earthworms during these 3 days were collected daily and maintained at -20° C until analysis. NH₄+ concentration in casts was referred to dry weight of casts. Means of NH4₄⁺ concentration in casts and mucus-urine of both earthworm species were compared by a t-test for independent samples. NH_4^+ content in casts was significantly ten times higher in A. giardi than in H. elisae. The rate of cast production was 0.0755 g fresh body $g^{-1}d^{-1}$ for *H. elisae* and 0.0425 g fresh body $g^{-1}d^{-1}$ for *A. giardi*. Conversely, the NH_4^+ content in mucus-urine was significantly ten times lower in A. giardi than in H. *elisae*. The rate of mucus-urine production was 0.0007 g fresh body $g^{-1}h^{-1}$ for *H. elisae* and 0.0015 g fresh body $g^{-1}h^{-1}$ for A. giardi. These results indicate differences in the nitrogen excretion way between these anecic and endogeic earthworm species. Contrasted responses of collembolan species to the excreta of earthworms (Gutiérrez et al., in preparation) may thus be attributed to different nitrogen excretion way between earthworm species, and to variable sensitivity among collembolan species to levels of ammonium concentration.

Earthworm mating success: dependence on environmental conditions and individual properties

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Reproductive success is an important element of individual fitness and mating frequency is one of its key components. In earthworms the factors affecting mating success of individuals have remained largely unexplored. The aim of this study was to find out how likelihood of mating in two earthworm species with different ecology is related to i) environmental conditions, ii) population density, and iii) the size of an individual. We utilized preserved earthworm samples that had been collected during autumns of 2004 and 2005 from arable fields in 11 localities of Finland. The samples had been first preserved in 1:1 mixture of formalin and ethanol and subsequently stored in 85% ethanol. The presence of spermatophores on individuals was recorded and used as an indicator of recent mating preceding the sampling. Spermatophores are formed on the body surface of the individuals during the mating but in soil dwelling worms they detach in a few days. The study species were endogeic Aporrectodea caliginosa Sav. and anecic Lumbricus terrestris L.. We used logistic regression to model the probability of the occurrence of spermatophores. As explanatory variables we used i) environmental conditions (rainfall, relative humidity and air temperature in different time periods preceding the sampling and soil moisture during sampling), ii) number of adult individuals in the sample and iii) weight of preserved individuals. Preliminary results show that the mating probability of L. terrestris is positively related to the size of the individual and the cumulative rainfall during the week before the sampling. We found no evidence for any relation between L. terrestris mating success and population density, which ranged from 1 to 16 adults in the 0.25 m² samples. High likelihood of mating in large individuals may be due to their higher efficiency in mate searching and/or assortative mating. The response to rainfall is likely to be explained by the mating behaviour of the species: L. terrestris copulates on the soil surface and moist weather conditions provide favourable conditions for surface activity. In A. caliginosa the statistical modelling is still in progress, the likely presence of two subspecies in the material making the modelling more challenging than in L. terrestris. We conclude that the present approach offers a useful tool for the study of reproductive ecology of earthworms.

Earthworm-mediated carbon and nitrogen losses via leaching and gaseous emissions in riparian buffers

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Riparian zones occur at the transition between terrestrial and aquatic ecosystems, thereby regulating nutrient flow across ecosystem boundaries. The hydrologic regime, vegetation and soil conditions in riparian buffers may favour soil foodweb organisms like earthworms. It is well documented that earthworms' feeding and burrowing activities stimulate carbon (C) and nitrogen (N) transformations, leading to nutrient leaching and production of greenhouse gases (GHG) such as CO₂ and N₂O. The objective of our research was to identify how earthworms affect these processes in riparian buffers. A literature review revealed that in agroecosystems, the aforementioned earthworm-mediated processes have variable effects on nutrient leaching and GHG emissions depending on land-use practices. There is limited information about how these processes are affected by the presence of earthworms in riparian buffers. This led to a microcosm experiment on the effects of earthworms and riparian plant litters on soil C and N cycling. Earthworms, soil and plant litter came from riparian buffers along the Riviere-aux-Brochets, Quebec. The microcosms were made of PVC pipe (10cm dia. x 35cm tall), hand packed with soil to a bulk density of 1.0 g cm-3 and wetted to 20% soil moisture content. The experiment was a replicated (n=4) complete factorial design with twelve treatments comprising three earthworm treatments (Lumbricus terrestris, Aporrectodea turgida, no earthworm) and four plant litter treatments (soybean, switchgrass, deciduous tree, no litter). The 48 microcosms were kept in a controlled temperature chamber at 12°C. At days 1, 3, 5, 7, 10, 14 and then every two weeks during the 20 wk experiment, soil in each microcosm was moistened with 150 mL water, cores were covered with a vented lid equipped with a septa and incubated 24 h at 20°C. Headspace air in each core was analyzed for CO₂and N₂O, and soil leachate was analyzed for DOC and NO₃⁻. A 10 g soil sample was removed from each core and analyzed for NH₄⁺, NO₃ and microbial N. At week 20, earthworms were removed and soil was mixed with 1.6 g cm⁻³ Na-polytungstate to isolate the light fraction of organic matter and assess the effect of treatments on C humification. Preliminary data indicate higher NO3leaching and CO₂ production in microcosms with L. terrestris, compared to microcosms without earthworms. Greater CO₂ production was observed in microcosms with soybean litter, compared to switchgrass or deciduous tree litters. These results highlight the important contributions of earthworms and plant litter quality to soil C and N transformations in periodically flooded soils. Results from this study will be linked with in-field measurements of earthworm demographics, soil properties, and gas flux measurements to develop a landscape-level model of earthworm-mediated nutrient cycling in riparian buffers.

Microbial communities associated with three populations of *Eisenia* earthworms - polyphasic approach

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The study was focused on the complex microbial changes during the passage of food material through the gut of two earthworms, Eisenia fetida collected from forest soil and two populations of Eisenia andrei originated in garden compost and large scale vermiculture plant, respectively. Microbial community composition and biomass was determined in the gut and faeces of earthworms as well as in feeding substrates. Phospholipid fatty acid analysis (PLFA) was used to assess total microbial biomass, aerobes/anaerobes density and main community taxa composition. Bacterial and Archaeal diversity was evaluated using polymerase chain reaction - denaturing gradient gel electrophoresis (PCR-DGGE) and plate cultivation of bacteria completed with species screening of dominant isolates (MIS Sherlock System). The passage of raw material (soil or organic compost) through the intestine of all three earthworms under study resulted in a significant increase of total microbial biomass and in enhanced density of anaerobes. Selective effect of the gut transit was demonstrated by the increase of Gammaproteobacteria and microeukaryotes and the decrease of Actinobacteria and Firmicutes in worm guts and faeces as compared with undigested food. The diversity of cultivable bacteria decreased during the gut passage in all earthworms whereas the bacterial counts were significantly (3 orders) higher in the gut and faeces of E. andrei than in those of E. fetida. Shifts in the bacterial and Archaeal community composition were more apparent during the passage of compost or vermicompost through the gut of E. andrei than during the passage of forest soil through E. fetida. The polyphasic approach based on different views into the complex microbial community added to the knowledge of the structural and diversity changes of the microflora during the passage through the intestine of *Eisenia* earthworms.

Earthworm-mycorrhiza interaction on growth of the Camedor palms

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Management of the earthworms, arbuscular mycorrhiza fungus AMF) and plant roots are key components of the below-ground agroecosystems, but their interacting effects on plant growth are still poorly understood. An experiment was conducted in greenhouse to evaluate the influence of presence or absence of native tropical earthworm *Balanteodrilus pearsei* on the development of AMF on camedor palms (*Chamaedora elegans*) roots and the contribution of the symbiosis to palm growth. When plants had the first leaf pair they were inoculated with AMF (Micofer-UV consortium). The plants were harvested after 327 days (140 days after worms added) of growth. The formation of external mycelium, vesicles and colonization were measured. The results suggested that the earthworms were added, the formation of vesicles and hyphae of AMF significantly increased. However, the presence of earthworm had no effect on camedor palms root and shoot biomass. This finding suggested that the earthworm activity affects AM root colonization by consuming soil, building tunnels or feeding on extraradical mycelium.

Interaction between earthworms (*Eisenia fetida*? and mycorrhiza (*Glomus intraradices*) take positive effects on maize growth

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Arbuscular mycorrhiza fungi and earthworms are important components of the belowground part of agricultural ecosystem. However, their interacting effects on crops growth are poorly understood. A pot experiment was conducted to investigate the influence of epigeic earthworm Eisenia foetida, Abuscular mycorrhizal fungi Glomous intraradices and their interactions on the growth and nutrition uptake of maize. Arbuscular mycorrhizal fungi and earthworms significantly affected the performance of maize, and these effects were not independent of each other. With the mycorrhiza or earthworms lonely, the shoots biomass was significant higher compared with the control treatment. Moreover, shoots biomass was higher when both AM and earthworms were present in the microcosm. In the absence of earthworms the mycorrhizal association reduced the amount of N in leaves, but when earthworms were present, this effect disappeared. It is possible that mobilized soil N by earthworms reduced G. intraradices competed with the roots for available N. Available phosphorus and ammonium-N in the soil were significantly lower when AM and earthworm were added respectively. AM and earthworm had synergistic effect in the promoting soil organic carbon. The formation of mycorrhizal structures in roots and hyphal length density in soil was measured at harvest too. Earthworms caused an increasing in hyphe frequency and hyphal length density of AM which might acquire more nutrition for mycorrhiza. Management of mycorrhiza-earthworm interactions may be of value, particularly in low-input and organic agricultural systems, and deserves further investigation.

Interactions between invasive earthworms, arbuscular mycorrhizal fungi and diazotrophic bacteria on native araucaria pine seedling performance

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The Araucaria angustifolia is a tree of high lumber and resin values, a source of food, and the most important tree in the Mixed Subtropical Ombrophilous forest in Brazil, a rich ecosystem with many endemic species. However, due to intense exploration for decades, the Araucaria is presently considered a critically endangered species, and the understanding of this ecosystem is fundamental for conservation and regeneration purposes. Soil organisms such as earthworms, arbuscular mycorrhizal fungi (AMF) and diazotrophic bacteria play an essential role in nutrient cycling and can increase plant performance. Many studies have evaluated the impact of each of these organisms independently, but few have studied their interactions. Therefore, the aim of this study was to evaluate the inoculation effects of invasive earthworms (Amynthas corticis), AMF (Gigaspora rosea) and an isolated of diazotrophic bacteria, and the interactions between each of these, on the performance of A. angustifolia seedlings. The plants were grown in greenhouse conditions in a blocktype randomized design with a factorial layout of 2x2x2, being: presence and absence of earthworms (A. corticis), presence and absence of AMF (G. rosea) and presence and absence of diazotrophic bacteria (isolated \$32274), in eight replicates, evaluated at 150 and 250 d after transplanting. The following parameters were evaluated: plant height and diameter, shoot and root biomass, shoot nutrient content (P, N), number of AMF spores, root mycorrhizal colonization rate and earthworm survival, reproduction and fresh biomass. Plant biomass and shoot nutrient content were affected by the three-way interaction of earthworms, AMF and bacteria. AMF inoculation enhanced A. angustifolia performance, while bacterial inoculation alone had no significant effect on plants, and earthworms had low survival rates and tended to reduce plant biomass. Microbial-faunal interactions can lead to important changes in plant growth and should be considered in future research and development of sustainable Araucaria forest management practices.

Species segregation and distance-related decline in the similarity of earthworm communities

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Earthworms have a major impact on soil structure and nutrient dynamics. The type and intensity of changes caused by these soil invertebrates depend on their ecological category —epigeic, endogeic, anecic- and therefore on their community composition. Although earthworm communities differ between sites, little is known about the factors controlling the spatial distribution of earthworms and the structure of their communities. We used data from 107 locations in northwestern Spain to investigate the degree of similarity of earthworm communities over distances ranging between 10 and 200 km. The locations were exhaustively sampled using the formalin - hand sorting method and the collected earthworms were identified at the species level. Community similarity indexes were calculated using pair-wise comparisons between locations. The correlation between these indexes and geographic distance was calculated using a Mantel test. We found a significant decline in community similarity with distance. This decline was not associated with distance-related gradients in soil pH and organic carbon, suggesting that factors other than soil chemistry, e.g. dispersal limitations or competitive interactions, may be involved in the decline of community similarity. We examined the co-occurrence patterns of earthworm species in our sampling region and the results showed that some species were spatially segregated. These results support the idea that species interaction processes were also shaping earthworm communities. Overall, we found that geographic distance is a significant factor accounting for differences in earthworm community composition, and that the observed decline in similarity with distance may be related to differences in dispersal abilities and to competitive interactions between earthworm species.

The midden sites of *Lumbricus terrestris* L. as a source of greenhouse gas emissions in arable no-till soil

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Due to enhanced carbon sequestration, no-till is regarded as a useful method for reducing carbon dioxide (CO_2) emissions from arable lands. However, no-till has also been shown to elevate nitrous oxide (N_2O) emissions in some soils, which could offset the atmospheric benefits of no-till. The role of earthworms in gas emissions from arable soils has recently received interest and earthworm activity has been shown to contribute for instance to N_2O emissions. No-till often leads to increased population densities of the large, deep burrowing earthworm Lumbricus terrestris L. We therefore established a field and a laboratory experiment to find out if the presence of L. terrestris can significantly affect the emissions of the greenhouse gases N₂O, CO₂ and CH₄ from arable no-till soil. In the long-term no-till field in S-W Finland, gas emissions were measured five times in two weeks on L. terrestris midden and adjacent non-midden sites (n=10) in October 2008 using plastic chambers (diameter=15 cm). The laboratory experiment (duration 15 weeks) was established in November 2008, with the soil, L. terrestris individuals and straw collected from the same no-till field. The soil was sieved and packed to PVC-tubes (diameter 15 cm, height 45 cm), and one large L. terrestris individual was placed to half of the tubes, the tubes without a worm serving as controls (n=15). Gas emissions were measured 12 times in approximately one week intervals. Although statistical analyses are still underway, preliminary results from the field experiment suggest that N_2O and CO_2 emissions were significantly higher on L. terrestris midden than on adjacent non-midden sites. Methane (CH_4) emissions differed only at one measurement, and then the non-midden sites emitted more CH4 than the midden sites. At the last measurement, when the gas emissions were measured with and without middens or residues in the sampling area, L. terrestris sites were found to emit more N_2O , CO_2 and CH₄ than the non-midden sites both before and after the removal of middens / residues. There was even an indication of N_2O emissions at *L. terrestris* sites being higher after than before the removal of middens. In the poster, we will present a comprehensive summary of the results of the field and the laboratory experiment. We will also try to draw the first conclusions of the net impact of L. terrestris activity on greenhouse gas emissions from no-till soils.

Effect of the moisture of soil in position of cocoons of earthworms *Pontoscolex corethrurus* (Annelida; Oligochaeta, Glossoscolecidae)

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The ability of *Pontoscolex corethrurus* to colonize an ample variety of agroecosystems is wellknown and to understand the factors that take part are of great relevance to design new practices that include their handling, to improve soil fertility. The present study had as objective to investigate if the earthworm *P. corethrurus* has a preference for certain soil moisture to deposit its cocoons and their influence in the incubation period. The study was divided in two stages. First, in terrariums modified (0.35 x 0.45 x 0.005m) with an adult earthworm (clitellum), manipulating (50 days) in a combined form, the moisture content (25, 33 and 42%; with 5 replicates each) in three depths of ground. Second, in order to eliminate the possible effects of the interaction of three moist within the terrariums, manipulating (60 days) in separated from the soil moisture content (25, 33 and 42%, with 5 replicates each) registering the number, position and the incubation duration of cocoons deposited by *P. corethrurus*. The preliminary results indicate that the humidity of soil, independent of the depth, had a significant effect in the cocoon position. *P. corethrurus* deposited cocoons in the three moist of the soil; nevertheless, the major and minor numbers of cocoons were deposited with a humidity of 33 and 42%, respectively. In addition, the youthful earthworms emerged in minor and major time with an intermediate and high moist, respectively. Poster - Session 5

Two parasitic mites in the cocoons of the pantropical earthworm *Pontoscolex corethrurus* (Annelida: Oligochaeta, Glossoscolecidae)

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It is well documented that some mites parasitize cocoons of the European lumbricid species. In a previous study, damage was observed in developing cocoons of the tropical earthworm *Pontoscolex corethrurus* associated with the presence of mites. In this study we assessed whether the parasitic mites the cocoons of the *P. corethrurus*. In the garden of INBIOTECA for a period of 70 days (from September to December 2008) at a distance of 100 m, each week (10 times) 20 cocoons were collected randomly by manually (depth of 0-20 cm). In the laboratory, the cocoons were individually placed in Petri dishes with filter paper and deionized water under 23 ± 5 °C. During the incubation was recorded the number of earthworm hatched, number of cocoons parasitized and taxonomic identification of species mites that feed on the cocoons incubated. The results showed that 12% of cocoons were parasitized by mites. Two species of mites were identified belonging to the families Anoetidae (genus *Histiostoma*) and Glycyphagidae. It is concluded that mites are natural enemies to parasitic cocoons of *P. corethrurus*. Future studies should determine whether the distribution of *P. corethrurus* is limited by the geographical distribution of mites and if they also parasitized cocoons of the other tropical species (exotic and native).

Poster - Session 5

Impact of anthropic pressure gradient on earthworms in saline soil in China forest. Consequences on ecosystems services as soil structuration and hydraulic conductivity

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Since two years ago, China has noted the increase of environmental problems as the same time as an increase of economic and demographic growth. Chinese government decided to take some measures to resolve environmental problems. Thus, it's now a challenge to fight against environmental problems as decline in biodiversity, soil pollution or soil erosion, and thus many local programs as those proposed in Shanghai was developed in order to find solutions. This study is part of a Frenchchinese cooperation, the "ARCUS" Project, which is an ambitious program from Shanghai municipality for Chongming Island. The objective is to create the first eco-island with sustainable development in terms of eco-agriculture, water management, waste recycling, renewable energy and eco-tourism. This island should be used as a model for Chinese town management in the future. The first goal of this study is to assess the impact on earthworm communities of an anthropic pressure gradient under forest and cultivated fields in a saline soil; earthworms are studied through their bioindicators state. The second goal is to assess the consequence on ecosystem services as soil structuration (bioturbations) and soil hydraulic conductivity (Kh). The originality of this study was to study both biological and physical at the same time and the same place. Four treatments were compared (forests of Metasequoia, 8 years old, 15 years old, 30 years old and a wheat field, 400 m² each). Earthworms were sampled using formaldehyde extraction combined to hand sorting. Soil structuration was mapped and quantified applying a new field methodology based on the description of bioturbations created by earthworms and vegetation. Conductivity was measured using infiltrometers at 3 different water potentials. Results showed low earthworm species richness in all treatments, explained by salinization combined to anthropic actions, especially under wheat treatment leaded in the erosion of earthworm density. The quantity and quality of bioturbations observed at the 40th first centimeters of soil appeared strongly influenced by anthropic gradients. Soil water conductivity (Kh) in wheat was very low (impermeable soil) while it was strongly higher under forest due to important macroporosity; biological activity (macrofauna and roots) was probably the main factor that explained this structuration of soil forest. All these investigations established an interesting perspective of tool transfer towards stakeholders.

The effects of earthworm addition on nitrous oxide and carbon dioxide fluxes in clover and grass enclosures

Prieto Roberto, Whalen Joann

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Earthworms are the dominant macrofauna in perennial forage systems. They profoundly impact soil in situ conditions and consequently microbially-mediated processes. The earthworm gut and structures (casts, burrows and middens) are proposed as hotspots of carbon dioxide (CO_2) and nitrous oxide (N_2O) production, mostly based on laboratory studies in the absence of plants. A field study was conducted to determine how increasing the population of two earthworm species would affect the CO₂ and N₂O flux from red clover (*Trifolium pratense*) and grass (*Elytrigia repens*) forage systems. The experimental site was in Ste. Anne de Bellevue, Quebec, Canada where adjacent red clover and grass systems were found on a Chateauguay clay loam (pH = 6.5, 78.12 g organic C kg⁻¹). Enclosures were 1 m^2 soil blocks surrounded by polyethylene plastic to a depth of 30 cm. Earthworm species added were the anecic Lumbricus terrestris L. or the endogeic Aporrectodea turgida (Eisen). The experimental design was a factorial arrangement of earthworm species (anecic alone, endogeic alone, anecic plus endogeic) and population levels (250, 500 and 1000 earthworms m⁻²) plus a control with no earthworm addition. These 10 treatments were randomly assigned and replicated in 4 blocks in each forage system, for a total of 80 experimental enclosures. Gas samples were collected in the fall of 2009 using a vented closed chamber system, the CO₂ and N₂O concentrations were determined with gas chromatography and gas fluxes (mg m⁻² h^{-1}) were calculated. Grass enclosures with added L. terrestris had significantly (P<0.05) greater CO₂ fluxes than with A. turgida or mixed species. Earthworm species did not affect the CO_2 flux in the clover system, nor did the population level. The CO₂ flux was up to 2.5 times greater from grass than clover enclosures, which we attributed to the denser and more extensive rhizosphere under grass that promoted soil respiration. The N₂O flux in the clover enclosures increased linearly ($R^2 = 0.993$) as the number of added earthworms increased (0, 250, 500 and 1000 m⁻²); thus, earthworms stimulated N₂O emission in the clover system. No effect of earthworms was observed in the grass enclosures. Moreover, N₂O flux was up to 11 times greater in clover than in grass, likely due to greater mineral nitrogen availability from decomposing clover residues. In conclusion, increasing earthworm populations had little effect on CO₂ flux but stimulated N₂O flux from a perennial clover system.

Direct and indirect effects of deer on the native earthworm, *Eisenoides* carolinensis

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Invasive earthworms are known to benefit from the high quality organic matter provided by deer fecal pellets, but little is known about the effects of deer on native earthworms in the Southeastern United States. We proposed that native earthworms would also benefit from increased organic matter provided by deer fecal material. We examined potential interactions between a common aboveground herbivore, the white-tailed deer (Odocoileus virginianus), and earthworms using a laboratory experiment and field exclosures. For the laboratory experiment, our hypothesis was that a native earthworm, *Eisenoides carolinensis*, would consume deer fecal pellets at a similar rate to an invasive earthworm, Lumbricus terrestris. Both species fared better in treatments with deer pellets alone and with deer pellets plus leaf litter in relation to the treatment with leaf litter alone. For the field component of our study, we set up 18 field plots: exclosures, controls, and high deer fecal density plots; deer exclosures were constructed in 2006. Octet electroshocking was used to evaluate the densities and total mass of earthworm populations relative to deer fecal pellet densities. Deer were found to negatively influence large juvenile E. carolinensis in mass and number (no invasive earthworms were found on the field plots). The field and laboratory portions of our study appeared to give contradictory results: in the field, earthworm densities were negatively related to deer fecal pellet counts, but native earthworms gained more mass in the presence of fecal pellets (compared with leaf litter) in the laboratory. However, we also measured soil and vegetation characteristics on all field plots and used path analyses to explore how deer might negatively influence native earthworms. The results of our path analyses and model selection support the hypothesis that deer negatively influence E. carolinensis via indirect effects on understory plants. This study highlights the importance of above- and below-ground interactions in forest ecosystems.

Poster - Session 5

Effect of the intestinal transit and mucus of the endogeic earthworm *Pontoscolex corethrurus* on bacterial communities from two tropical soils

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Endogeic earthworms are the most active ecosystem engineers in most tropical soils. Their energy and nutrient needs are thought to derive from a mutualistic relationship with ingested soil microorganisms. This assumption is supported by the observation of significant changes in microbial activity during gut transit and in casts of different ages. For the first time, changes in bacterial communities during transit through the gut of a tropical endogeic earthworm, *Pontoscolex corethrurus*, were analyzed by PCR-DGGE fingerprinting. The bacterial community in the gut content was different from that in the soil, demonstrating a selection effect. The native soil of the earthworm was shown to have a significant effect on the structure of the ingested bacterial population and no gut-specific taxa were observed. In vitro incubations of soil with added extracted intestinal mucus were compared with incubations with added water or glucose instead of mucus. The mucus had a specific effect on the composition of the bacterial communities.

Earthworms Ecology in the Soil of Estação Experimental Cascata, Embrapa Clima Temperado, Pelotas, RS, Brazil

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In the world, earthworm diversity is estimated to approximately 5900 species. However, little is known about the diversity of earthworms in Brazil. The present study had the objective to estimate the population density and diversity of earthworms in the Estação Experimental Cascata (EEC), Pelotas, RS, Brazil. The study was realized in the EEC, the site has an area of 150 ha, with the definition of seventeen sampling sites, from an area image with their geographical coordinates. After the characterization of the site, the points were grouped in ecosystems: plantation area, secondary forest, riparian forest, plantation area of exotic trees and area of initial successional stages. The samples were realized in the spring of 2008 and in the summer of 2009, with two complementary methods of collection; each sites had a collect area of $0,4375 \text{ m}^2$. The soils were taken to the laboratory, in which the soil was made manual sorting. Then, the earthworm specimens were preserved, counted and identified. Each treatment had four replicates, with the exception of AE and SI. For data analysis, principal component analysis (PCA) was used. Three earthworm families were found: Glossoscolecidae, Megascolecidae and Acanthodrilidae. In the spring collection five earthworm species were identified: Glossoscolex sp.1, Glossoscolex sp.2, Urobenus brasiliensis, Amynthas gracilis, Microscolex dubius and juvenile unidentified earthworms. In the sample of summer four earthworm species were found: Glossoscolex sp.1, Glossoscolex sp.3, Urobenus brasiliensis, Amynthas gracilis, one unidentified species and juvenile earthworms. The main correlation was between the plantation areas and the specie Amynthas gracilis. This demonstrates that the inadequate management in agroecosystems can increase the population density of exotic earthworm species.

Ecology of Soil Macrofauna of Estação Experimental Cascata, Embrapa Clima Temperado, Pelotas, RS, Brazil

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The soil is an habitat for a wide variety of organisms, mainly, invertebrates that act in different ways in this environment. The soil invertebrates are classified as: microfauna, mesofauna and macrofauna. This study had the objective to evaluate the density, biomass and diversity of groups from the soil macrofauna, in the area of Estação Experimental Cascata (EEC), Embrapa Clima Temperado, Pelotas-RS. In order to understand the ecological processes in natural, anthropogenic ecosystems and in the agroecosystems. The study area has about 150 ha. The sampling points were defined without choosing specific areas, using an overlapping grid of points, 300 m distant from each other, in the air image of the EEC. After the points were located in the field, from the pre-defined geographic coordinates, using GPS and grouped by ecosystems. The ecosystems found were planting area, secondary forest, riparian forest, plantation area of exotic trees and area in the initial stage of succession. Samples were collected in the spring 2008. For the survey of macrofauna, two methods of collecting were used: the method TSBF (Tropical Soil, Biology and Fertility) and extracting solution of formaldehyde to 0.5%. The planting areas and the areas in initial successional stage comprised 13 taxonomic groups. However, secondary forest, the riparian area and the planting of exotic trees had 15 taxonomic groups. In plantation areas, the most representative taxonomic groups were Hymenoptera, Isopoda and Isoptera. In the remaining forest and the area in initial stage successional, the groups with higher density were Hymenoptera, Isopoda and Isoptera. In the area of riparian forest, the most representative groups were Isoptera, Hymenoptera, Chilopoda and Oligochaeta. The plantation area of exotic trees was represented by groups Hymenoptera, Amphypoda, Isopoda and Isoptera. Therefore, this work emphasize the importance of the orders Hymenoptera, Isopoda and Isoptera.

Poster - Session 5

Comparative ecology of earthworm population between natural and manmade conditions

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In the natural ecosystem, the species composition, abundance and biomass of earthworm population, apparently were influenced by vegetation and soil types, and varied essentially in relation to soil organic matter content and anthropocentric actions, and soil temperature, moisture and pH value. Some optimum ecological factors for worm growth are not best for worm reproduction, for instance, temperature 25oC, moisture 70% and pH 6 are most suitable for E. fetida to cocoon production, while temperature 18oC, substrate moisture 65% and pH 8-9 are most suitable to increase body weight. In cultivated fields, most earthworms live within a 20 cm depth tillage layer due to the presence of rich nutritional elements and favourable pH value; whereas in artificial substrate E. fetida can descend to the 70 cm depth, or even deeper, owing to even-distributed nutrients and uniform feed texture. But 99.2% of earthworms dwelled within the first 50 cm depth of substrate. It was suggested that the substrate layer could be extended to a 50 cm depth. Vertical distribution of earthworms was related to population density. In fact, earthworms mainly concentrated on the upper soil layer, whereas in substrate, when the spatial competition between worm individuals intensifies with population density increase, worms tend to go down to lower substrate layers. There may exist a lower threshold density of ca. $60,000 \pm 10,000$ ind.m², over which earthworm migration would occur. Whether in natural soils or in substrate, most earthworm cocoons were deposited in the upper 15 cm layer; this characteristic possibly evolutes over a long period of time. Worms, however, dwelled in deeper layer in most time, which may be termed "non-uniformity of egg-laying and living in vertical space". A depth of 15-20 cm may be sufficient for breeding worms. Under those experimental conditions the clitella of E. fetida appeared 60-70 days after cocoon appearance. The maximum individual weight was reached between 90-100 days. The worm density was not relative to the time in which earthworms start to breed or individuals were harvested. E. fetida is a species of higher reproduction rate and fast-growth. It may be more favourable to rear cocoon-laying and bodyfattening worms in different worm beds on the basis of the characteristics of E. fetida mentioned above. On the base of substrate preference and vermiculture needs, the earthworms can be grouped in compost-preferring species and soil-preferring species.

Session 6

Earthworms and Soil Fertility



The 9th International Symposium on Earthworm Ecology 5th to 10th September 2010, Xalapa, Mexico

Soil improvement and earthworms: Production and behavioural results since ISEE4

Butt Kevin R.

University of Central Lancashire

Over the past twenty years a host of experiments have been conducted in these research areas. A personal view is presented to demonstrate developments which may assist promotion of soil fertility in given soils.

Earthworms as new pests of irrigated rice in Brazil

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Earthworms have been reported as pests of rice in various countries, mainly in SE Asia and Australia, but only recently have they been accused of damage to rice cultivation in Brazil. Nevertheless, few studies have been performed on earthworm communities in rice fields in Brazil and little is known of the ecology of the species encountered thus far. Therefore, the present study was undertaken to: a) evaluate the species associated with rice damage in three sites, and b) assess the relationship between earthworm populations and rice yields at two sites. Earthworms were collected in Miranda and Rio Brilhante, Mato Grosso do Sul and in Santa Maria, Rio Grande do Sul. At the two former sites, rice production was also assessed in a $1m^2$ area, and in its center, earthworm abundance assessed using the TSBF method (25 x 25 cm monoliths). The relationship between rice yields and earthworm abundance was assessed and correlation coefficients tested using Student's T-test. In Santa Maria, the species associated with yield losses was a small, thin Ocnerodrilid, Eukerria eiseniana. In Miranda, a new species of earthworm in the Criodrilidae family was found, and the relationships between abundance of this species and rice grain yields (r = 0.35; P=0.13) or number of stalks (r = -0.08; P=0,71) were not significant. In Rio Brilhante, two species predominated, a Eukerria sp., and a moderate-sized Glossoscolecid (Anteoides sp.). The relationship between earthworm abundance and rice grain yields or number of stalks was also non-significant. Therefore, earthworm abundance does not appear to be directly negatively related to rice production. Nevertheless, yield losses may result from high infestation due to plant lodging, which impedes adequate machine-harvesting of the rice grains. Further research is needed to evaluate the extent of the problem in rice fields throughout Brazil, and find environmentally-friendly means of reducing potential damage.*presenting author

Relationship analysis between earthworm population and soil fertility in different land-use types

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Soil biological fertility was influenced earthworms and land use. A field survey was carried out in Quzhou County, Hebei Province of China, for studying relationship between earthworm population and soil fertility in different land-use types. In the survey we investigated earthworm population characteristics, including species abundance, population density, biomass, age-structure and H'index, which can indicate the biodiversity pattern of earthworm--were employed to investigate the effects of different Land--use types on earthworm biodiversity pattern. The relationship between Land--use types and soil character was discussed after measuring soil character under ways of soil using. The results indicated that: The number of earthworm species, its population density, biomass, and age structure and diversity index were obviously related to most indices of soil chemistry and biological characteristics as the evaluation index, the result corresponded with that of index evaluation using soil biological characteristics and soil chemistry. Therefore, it is practical to synthetically appraise soil biological fertility using earthworm population characteristics.

Effects of mouldboard ploughing on changes with time in earthworm abundance, in an organic farming system in Northern France

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In cultivated fields, earthworms are soil fertility major actors. Adopting respectful agricultural practices is necessary to protect, promote and optimize their activities. Mouldboard ploughing is often recognised to have a greatest effect on earthworm abundance and activity. However, this real impact is not well known and seems to be very variable as a function of the earthworm ecological type. Moreover, the mechanisms are still debated: direct mortality, food resource decrease, reproduction capacity reduction or habitat destruction? Literature gives contradictory answers to those questions. To contribute to this issue, we set up a field trial in which changes in earthworm abundance and community composition were monitored during two month after ploughing (November to December 2009). The experiment was located in Northern France (49°7'N, 1°42'E) on an organic field converted since 2001. After a chisel plough operation over the whole surface, ploughing was performed on one part of the field (ploughed Pl) the other remaining unploughed (N-Pl). Barley was sown the same day (12th of November). Initial population was counted the day before ploughing, and then evaluated 1, 14, 35 and 55 days after ploughing. Ten samples were taken in the Pl zone and ten in the part left N-Pl. The community structure exhibited a strong dominancy of the endogeics (anecics <10%). In the N-Pl area, earthworm abundance followed a positive trend, showing a 20% increase after two months of monitoring. At the opposite, in the Pl area, the initial density was, after 55 days, nearly divided by two. During the first two weeks, no difference appeared between the Pl and N-Pl areas. Ploughing response was delayed. Taking the age classes, ploughing was detrimental either for the juveniles and the adults. Concerning the species, results show surprisingly a difference between the two most abundant endogeic species: Apporectodea caliginosa were dramatically reduced while Allolobophora rosea exhibited the same evolution in the Pl and N-Pl areas. We hypothesized that: 1) ploughing direct mortality was, in this experiment, negligible. However, no birds (e.g. common gulls) followed the tractor during the operation and 2) ploughing had a detrimental effect on survival or reproductive capacity, for example by decreasing the viable cocoon stock, exposing them to desiccation and predation. Taking the species results, further investigations will be needed to understand the different responses of the endogeics.

Effect of management practices and earthworm biostructures on soil hydraulic conductivity

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This study aimed to evaluate the effect of management practices on soil hydraulic conductivity (Kh) by studying earthworm biostructures. Two types of tillage were compared (moldboard plowing: MP and no-tillage: NT) and two types of fertilization (mineral and poultry manure), seven years after their establishments. Measurements were carried out under wheat crop during four sampling periods at 2- to 7-cm depth. We measured on soil thin sections (i) three indicators of earthworm activity: the area of individual casts, coalescent casts and burrows and (ii) soil macroporosity. The Kh (at -0.05 kPa) was measured at the same times and in the same locations than soil macroporosity. At all four sampling dates, the soil macroporosity and Kh were the lowest under NT while earthworm activity was the highest. Poultry manure, which was applied at agronomic rates, affected neither the macroporosity nor Kh nor the amount of earthworm casts but increased slightly burrows abundance. Regardless of the tillage treatments, Kh increased in spring and decreased in summer. Part of this variation was attributed to rainfall events and earthworm activity which acted on the macroporosity (shape and size) under MP and on the pore connectivity under NT. This study highlighted: (i) that earthworm activity is not capable to replace the effects of the mouldboard ploughing even after seven years of no-tillage under poultry manure and (ii) the type of tillage determines how the earthworm activity promotes Kh.

Oral - Session 6

Evidence of the involvement of the auxin pathway in the impact of earthworm on plants

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Earthworms are susceptible to affect plant growth through a variety of mechanisms such as trophic relationships, physical engineering, modifications of microbial community or the emission of signal molecules. The relative importance of these different mechanisms has not yet been elucidated. Extracts from earthworm's casts have been shown to produces auxin-like effect on plants. Here, we investigated the hypothesis that earthworm effect on plant growth was due to these auxin-like molecules. In a laboratory experiment in microcosms, we compare the effect of earthworm presence either on a wild plant genotype or on a mutant plant genotype with a deficient gene implicated in the auxin pathway. We choose the Arabidospis thaliana double mutant aux1-7;axr4-2, which has a strongly altered auxin transport. This results in a reduced sensitivity to auxin which in turn induce a dwarf phenotype. As earthworms are susceptible to produce auxin-like compounds, they should by this way compensate the deficient auxin transport towards the roots and revert the mutant phenotype to the wild one. Earthworm presence induced a negative effect on Arabidopsis thaliana wild type (shoot biomass decreased by 39%), this negative effect on the wild type was probably due to an inhibitory effect of high auxin concentration but a huge positive effect on the aux1-7;axr4.2 Arabidopsis thaliana double mutant with an impaired auxin transport (shoot biomass increased by 780%). The positive effect on the mutant was likely due to the compensatory effect of earthworms on auxin concentration in roots, as stated in the hypothesis. Root length and root surface area of the wild type decreased by 55% and 51% respectively in the presence of earthworm. For the mutant, root length and root surface area of increased by 474% and 550% respectively in their presence, for a final surface uptake equivalent to that of the wild type in earthworm presence. This absence of difference between root system of the mutant and wild type suggested that root development, which is under hormonal control, was at the origin of the phenotype reversion in the presence of earthworms. Our results validated the idea that signal molecules emitted from earthworm casts can affect plant growth. This stresses on the importance of signals as an important but neglected mechanism as compared with trophic relationships and physical engineering.

Darwin's dream comes true: NIRS allows quantifying the impact of earthworms on soil and Carbon dynamic

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Far less famous than his work on Natural Selection, Darwin was also a great soil ecologist who stressed the importance of earthworms in soil and landscape evolution. To date, however, we are still unable to precisely quantify the impact of earthworms on soil processes. In this communication, we review several studies that aimed to determine the specific signature of cast aggregates and to quantify the importance earthworms play on soil erosion. Soil erosion was studied in different sloping lands in Vietnam and Near Infrared Reflectance Spectroscopy (NIRS) was used to generate signatures corresponding to different soil surface aggregates (aboveground soil casts produced by earthworms vs. surrounding topsoil). The NIRS-signature of eroded soil sediments was compared to those of casts and of the surrounding soils. Our results confirm that NIRS spectra might be used as "fingerprints" to identify the origin of soil aggregates. Although earthworms are assumed to play a favourable role in promoting soil fertility and ecosystem services, our results showed that sediments might be almost entirely constituted of cast aggregates. Two hundred years after Darwin's birth, we now have of a relevant method to quantify the true impact of earthworms on landscape evolution.

Fungal Diversity in the Burrow Wall Soil of Two Earthworms Species

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Earthworm burrow wall creates a favourable microhabitat for the soil microflora with generally enhanced microbial activity in the burrow wall soil as compared to bulk soil. Nitrogenous wastes excreted through the body surface of earthworms accumulate in the burrow wall and affect the soil microbial community in those areas. Much of the work regarding earthworm effects on microorganisms has focused on the functional significance of microbial-earthworm interactions. Virtually nothing is known about the possible effect of burrow wall on the taxonomic composition of communities of soil microorganism's particularly filamentous fungi. The present study focuses on the comparison of the taxonomic structure of the fungal community and fungal diversity in the burrow wall soil of two species of earthworms, viz., Lampito mauritii and Pontoscolex corethrurus, both of which are abundant in most areas in and around Bangalore, India. The soil sample was generated in the laboratory by maintaining earthworms in chambers with a dimension of 30 cm height, 40 cm length and 10 cm width. Soil sample were collected by the destructive method at an interval of 30 and 45 days. The frequency of occurrence of fungal taxa as percentages was calculated and the community of filamentous microscopic fungi was studied. DMRT showed a significant increase in the total fungal count in the burrow wall soil P. corethrurus (both 30 and 45 days) compared to control, where as the reverse was true with L. mauritii. In both the species of earthworms the 45 day burrow wall soil showed higher total fungal count compared to 30 day soil sample. A total of 55 fungal species which included 29 genera were isolated during this study. Six and seven fungal species were isolated only from the burrow wall soil of *P. corethrurus* and L.mauritii respectively. Higher species diversity and species evenness was observed in the burrow wall soil of L. mauritii compared to P. corethrurus.

Vermicompost as biocontrol agent in suppression of three soil-borne plant pathogens at field level

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Three serious soil-borne plant pathogens vis-à-vis Fusarium oxysporum, Xanthomonas campestris and Ralstonia solanacearum are causing considerable damage to vegetable crops. These pathogens are difficult to manage by chemical methods. The current interest is to promote their biological management. The present study was carried out to evaluate the effect of seed treatment with 10% aqueous extracts of vermicompost prepared from different substrates [agricultural waste, leaves of Azadiractina indica (neem) Parthenium hysterophorous (Parthenium) and Lantana camara (Lantana)] and application of vermicomposted neem to soil. The test crops were tomato (Lycopersicon esculentum) and egg plant (Solanum melongena). Vermicompost was prepared by mixing the respective substrates with cow dung slurry (9:1 ratio w/w) separately. The seeds of tomato and egg plant were soaked for one hour in different aqueous extracts of vermicomposts before sowing. The seedlings were raised in separate nursery beds of size 1m X 1m to which vermicompost was applied at the rate of 5 tonnes/ha. From the nursery beds seedlings were transplanted to plots and each plant received 250 g of vermicompost (spot application). The number of plants was 50 per plot. Seed treated with water and Farm Yard Manure served as control. Plots were chosen where the incidence of pathogens was high (R.solanacearum - 22 X 10³ CFU/g; F. oxysporum - 19 X 10³; X. campestris - 34 X 10³). The microplots in triplicate for each of the treatments were monitored for both survival of transplanted seedlings and yield. The results revealed that the best treatment for suppression of bacterial and fungal wilt diseases in tomato and egg plant is seed treatment (1 hour) with 10% aqueous extract of vermicomposted neem coupled with application of vermicompost to the soil both during sowing as well as on transplantation. This treatment reduced the incidence of both fusarial and bacterial wilt by 100%. Better biomass and yield were observed on application of vermicomposted neem. In tomato crop, the survival was 100% when seeds were soaked in aqueous extract of vermicomposted neem and in the field receiving vermicomposted neem. The least survival was found in controls where the seeds were soaked in water before transplanting them in to fields receiving either vermicompost (70 %) or Farm Yard Manure (58%) respectively. The total yield was recorded for three harvests at time duration of fifteen days. Among different treatments, the seed treatment with vermicompost extract followed by application of vermicompost to field resulted in maximum yield (385kg/plot) and least in plot supplied with Farm Yard Manure (87.46 kg/plot). It was found that the application of vermicompost alone is not enough to protect the plants against the disease but coupling it with seed treatment is important to achieve complete disease suppression and to get better yield. Similar treatments were given to egg plant seeds before transplanting the seedlings to respective plots. The survival was 98% in treatment where seeds were soaked in aqueous extract of neem based vermicompost that received neem based vermicompost after transplantation. The least survival of 22 % was encountered in the control treatment with seeds soaked in water and transplanted to field receiving Farm Yard Manure. The total yield was recorded for two harvests at time duration of 20 days. The lowest yield was in plot supplied with Farm Yard Manure.

Growth and reproduction in *Pontoscolex corethrurus* (Muller) – a dominant species of rubber plantation

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Pontoscolex corethrurus, an exotic geophagous earthworm with wide range of tolerance to environmental factors, is the most dominant earthworm species in the soils of rubber plantations in Tripura (India). Under laboratory condition, it showed highest growth and reproduction rates compared to native geophagous species of earthworms when subjected to same diet (unpublished data). So laboratory experiments were set up in order to evaluate the effects of different food substrates (collected in and around the rubber plantation sites) on the growth and reproduction of this tropical earthworm. Decomposed five different experimental diets used were cowdung, rubber leaf litter (Hevea brasiliensis), rubber saw dusts, bamboo leaf litter and mixture of these plant residues. All the experimental diets included mineral soil as the main substrate. The chosen materials were mixed with soil in different proportions to provide five treatments (dry weight proportions in brackets): 1) Soil-cowdung (30:2); 2) Soilcowdung-rubber leaf litter (30:1:1); 3) Soil-cowdung-rubber saw dusts (30:1:1); 4) Soil-cowdung-bamboo leaf litter (30:1:1) and 5) Soil-cowdung-mixed diet of rubber leaf litter, rubber saw dusts and bamboo leaf litter (30:1:1). Soilcowdung mixture was considered as control and the rest as experimental diets. Cowdung was introduced in all the treatments to increase the suitability of the culture media, as suggested by Lowe and Butt (2005). In each treatment (three replicates) one pair of young individuals (P. corethrurus) of similar weight and age group was inoculated randomly per plastic pot $(15 \times 15 \times 8 \text{ cm})$ containing 1600 g substrate. The culture pots were kept in BOD at a temperature of 27°C and 25% moisture of the substrate was maintained. The experiment continued for 105 days and the culture media kept unchanged till the end of the experiment. Biomass (fresh weight) and juvenile number were recorded every 15 days. In the first fortnight, biomass value increased to more than double with the appearance of clitellum in all the experimental diets. Maximum weight for the endogeic P. corethrurus was observed in both soilcowdung-bamboo leaf litter and the diet mixture having rubber leaf litter, rubber saw dusts and bamboo leaf litter, whereas minimum weight was obtained in the soil-cowdung treatment (control). Highest growth rate was achieved in the mixture with rubber saw dust (21.14 mg worm⁻¹ day⁻¹) which however was not significantly different (p>0.05) from that in the mixture having rubber leaf litter, rubber saw dusts and bamboo leaf litter (20.92 mg worm⁻¹ day⁻¹), bamboo leaf litter (20.27 mg worm⁻¹ day⁻¹) and rubber leaf litter (18.81 mg worm⁻¹ day⁻¹). Lowest growth rate was noticed in the soil-cowdung mixture (13.36 mg worm⁻¹ day⁻¹) which was significantly lower (p<0.05) than that in all other treatments. Lowest and highest fecundity rates were obtained in the soil-cowdung (0.35 juveniles adult⁻¹ week ¹) and in the mixture with rubber saw dust (1.21 juveniles adult⁻¹ week⁻¹) respectively. Fecundity rate in the latter was however not significantly different (p>0.05) from that in the mixture with bamboo leaf litter (0.94 juveniles adult⁻¹ week⁻¹) and diet mixture of rubber leaf litter, rubber saw dust and bamboo leaf litter (0.89 juveniles adult⁻¹ week⁻¹). Fall of biomass of *P. corethrurus* in most of the diets after 90 days was due to reproductive costs as reflected by dramatic increase in juvenile production in all the diets after 105 days. In fine, P. corethrurus showed higher rate of growth and reproduction in the treatments with saw dust or bamboo leaf litter or in a combination of both. Because of least growth and reproduction rate in cowdung, it is not considered to be a food of choice for its culture. Thus our results indicate that for vermiculture of P. corethrurus to be used as a source of protein or for its introduction and systematic use in low-input tropical agriculture (as suggested by Lavelle et al.1987), it is necessary to add suitable bulking agents like saw dusts, bamboo leaf litter etc. in the soil in order to maintain its successful field population.

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Soil enzymatic activity on presence of *Pontoscolex corethrurus* (Oligochaeta: Glossoscolecidae), *Cyclocephala barrerai* and *Phyllophaga setifera* (Coleoptera: Melolonthidae)

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Soil enzyme activity in the presence of adult earthworms of *Pontoscolex corethrurus* Muller and late instars of Cyclocephala barrerai Martínez and Phyllophaga setifera Burmeister ("white grubs") was evaluated. The substrate was an agricultural soil mixed with 1.5% of dried mucuna (Mucuna pruriensis var. utilis, Fabaceae) as an organic matter. The experimental test was completely randomized, with four treatments and six replications of each: treatment I, control, without individuals; treatment II, with three individuals of C. barrerai; treatment III, with three individuals of Ph. setifera and treatment IV, with three adult earthworms of P. corethrurus. All the containers stayed to at $24 \pm 2^{\circ}$ C, constant illumination (25 watts) and relative humidity of 65 ±5%. Enzymatic activity was evaluated from each treatment, at initial (t_0) and final $(day 30^{th}; t_{30})$ time. Soil samples (50 g) were taken from 10 cm depth of each container, placed under plastic bags and stored at 4°C until analysis. The activity of fluorescein diacetate hiydrolase (FDA, total esterase test) was determined according to Alarcón-Gutiérrez et al. (2008). No significant difference (Mann-Whitney test, p > 0.05) was observed between treatments when different times were evaluated; although there was a tendency to major activity in treatment II (0.0041nkat/g DM) with respect to the other treatments. Nevertheless, when sampling time was compared (t_0 versus t_{30}), in the same treatment, significant difference (Mann-Whitney test, P<0.05) was detected. Results indicate that soil enzymatic activity in the presence of *Pontoscolex corethrurus* ($0,0006 \pm 9.3 \times 10^{-5}$ nkat/g DM) is similar to that observed in the soil of white grub species: Cyclocephala barrerai (0,00049 \pm 1.5 x 10⁻⁴ nkat/g DM) and *Phyllophaga setifera* $(0,00059 \pm 1.8 \times 10^{-4} \text{ nkat/g DM})$ after 30 days of experiment.

Evaluation of the effect of fodder trees and earthworms on the growth of sorghum

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In Yucatan, Mexico, the land that initially had tropical deciduous forest is now grassland and now about 1.5 million hectares are disturbed. The extensive livestock farming by slash and burn are of low productivity due to shallow soils and climate of humid tropics and high degree of disruption of ecosystems. Sylvopastoral systems (SSP) are an agroforestry management option for livestock purposes its settlement is affordable and promotes conservation and several environmental services which can coexist with traditional livestock, although to promote greater technical knowledge is required. There are about 211 forage species used by the Mayans, which can be used for the design of SSP prior recognition. In addition, are well known the benefits of earthworms in soil fertility modifying many soil properties, which affect plant growth and therefore its production. This work was done in search of design options for agroforestry systems where forage species benefit to plants and the macroinvertebrate community for evaluating the effect of the quality of litter species of fodder trees combination with the earthworm on growth of sorghum (Sorghum vulgare) as an indicator of soil fertility. Thus, under conditions of greenhouse, it was analyzed the effect of the quality of the foliage of six species of trees, G. floribundum (G), B. simaruba (B), P. piscipula (P), D. cuneata (D), C. gaumeri (C) and R. longiloba (R) and the earthworm Dichogaster bolaui in the growth of the sorghum and the earthworms populations. Plastic pots were used containing 2 kg of soil, mixed with 100 g of dry foliage of each used tree and 1.2 g of earthworms biomass was applied to the pot. The design was completely randomized and the information was analyzed by analysis of variance and principal components analysis to know the relation among the quality of the foliage and the growth of the sorghum and the biomass of earthworms. The foliage of B and G that contain the highest amounts of phosphorus allowed a better production of dry matter of the sorghum, though the better height of this plant was correlated with the highest amounts of neutral detergent fiber. The foliages of B and G with or without earthworms benefit the growth of the sorghum. The application of earthworms produced an increase of the total biomass and the height plant of sorghum. The foliages of R and C favour the populations of D. bolaui but not the growth of the sorghum. In the group of treatments with earthworms, the foliage of P. piscipula and D. cuneata benefited earthworms populations, but they did not benefit the growth of the sorghum. This study also found that there are tree species such as R and C that can benefit the soil fauna, as the worms, immediately by releasing nutrients more easily helping to initiate the cycle of organic matter. Thus, when designing agroforestry systems is the combination of tree species to encourage diversity in the quality of the substrates for systems to be environmentally stable.

Estimating automatically the abundance of belowground earthworm casts: dream or reality?

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Belowground earthworm casts have a profound influence on soil processes. To predict the effects of earthworms on soil quality, ecologists need adequate methodologies to assess earthworm casts. We attempted to estimate belowground earthworm casts by image analysis. First, we compiled a learning set of three types of pores organized between: (i) individual casts (PIC), (ii) coalescent casts (PCC) and (iii) unknown aggregates (control pores). Then, each type of pore was classified according to two shape indexes. Second, we applied this model on soil blocks sampled two weeks after sowing maize under moldboard plowing (MP) and no-tillage (NT), at 2- and 12-cm depths. Third, we compared results from our new model with results obtained by point counting on individual casts. According to the model, The PCC were well discriminated from the other pore types, while the PIC were confounded with the control pores. Hence, our model was thereafter only used to assess the PCC abundance under the two tillage treatments. Results indicated that just after tillage almost all PCC were destroyed under MP; as consequences the PCC abundance was much higher under NT compared to MP for the both depths. The PCC abundance was positively correlated with the abundance of individual casts (r= 0.68, p<0.01) demonstrating that our model is relevant to automatically estimate the abundance of belowground earthworm casts. This automatic method may allow for more effective evaluation of earthworms influence on soil quality.
Life history studies of Lumbricus friendi (Cognetti, 1904)

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The objective of this work was to obtain baseline data on the life cycle of the anecic earthworm Lumbricus friendi, which although known to science for over a century is little studied. Fieldcollected specimens from NW Spain (where this species is widespread) were grown to maturity and maintained under controlled laboratory conditions. Thereafter experiments were undertaken to determine its mode of reproduction, reproductive output, cocoon development and juvenile growth rate. Effects of density and temperature on earthworm growth were also examined. Plastic culture vessels containing a substrate of Kettering loam were incubated in temperature-controlled chambers, with dried horse manure as food. Paired, mature animals were sampled monthly over a period of 9 months for cocoon production. Cocoons were incubated on moist filter papers in Petri dishes and hatchability and hatchling growth rates were recorded. Results showed that at 15 °C individuals matured at a live mass of 3.5 g. Zero cocoons were produced by virgin animals maintained in isolation but recently-matured, paired L. friendi produced 2.16 cocoons worm⁻¹ wk⁻¹. Cocoons were lemon-shaped with a smooth pale yellow-coloured outer surface and a mass ranging between 17 and 37 mg. At 15 °C, cocoons required around 3 months to hatch and hatchability from the first five months of cocoon production was 85 % successful. The majority of cocoons produced a single hatchling although twins were also occasionally recorded. Growth to maturity of these hatchlings was achieved within a period of 5 months, with tubercular pubertatis recorded after 3 months. Increased temperatures in the range of 5 - 15 °C led to significantly increased growth rates, whereas increased earthworm density led to a significant decrease in rate of growth. These findings permit comparisons with closely related, but more closely studied species such as Lumbricus terrestris.

Earthworms from an alkali industry waste site in North West England

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Soils at this post-industrial site have developed over the past century from waste alkaline materials (pH 12) dumped to a depth of some 10 m. These soils, now suitable for plant growth, were further contaminated historically by acidic boiler waste in some areas and retain a pH of 4. The site, near Bolton, is designated a Site of Special Scientific Interest (SSSI) for the well documented flora, dominated in spring by orchids. However, practically nothing is recorded of the site's soil fauna. The objectives of this research were to determine which earthworm species were present, to measure densities and biomasses and how the community composition varies horizontally along the pH gradient. Finally we aimed to determine potential earthworm origin of the colonising animals. The shape of the 9 ha site, surrounded on 3 sides by the confluence of 2 rivers and a disused canal, meant that this could be assessed by sampling in linked, adjacent habitats of woodland, grassland and further ex-industrial areas. Preliminary investigations were undertaken in autumn 2008 to assess earthworm community composition by sampling (hand-sorting of soil plus mustard vermifuge application) at areas differing in soil pH and site management: (i) acidic, (ii) alkaline unmanaged and (iii) alkaline managed by scrub-cutting to retain the desired flora of the SSSI. The following autumn, a 110 m transect was sampled between extremes of soil pH. Initial results revealed a total of 7 earthworm species including the two anecics, Aporrectodea longa and Lumbricus terrestris, of interest as the depth of habitable soil, above the weathered alkaline material is less than 20 cm. Scrub management had a positive effect on earthworm density (204 m⁻²) compared with unmanaged areas (134 m⁻²) in the alkaline soils. Acidic soils (pH 4.7) supported a lower diversity of earthworms, consisting mainly of L. rubellus and L. castaneus - average density 64 m⁻². Four further earthworm species were revealed in subsequent work, including Dendrodrilus rubidus in the acidic areas. A. caliginosa accounted for more than half of the earthworms located across the site and was found in all but the most acidic soils. Sampling in soils surrounding the site showed all but two of the (least common) species found on site, thereby acting as a potential source. Results show that earthworms are able to colonize and survive in the shallow soils above the inert material of what was once an uninhabitable environment.

Reproductive Strategies of Tropical Earthworms from Tripura (India)

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Some data on the reproductive biology of thirteen tropical earthworm species under fluctuating laboratory conditions are presented. The culture pots (4.5 L) received one pair of clitellate earthworm species and varying amounts of air-dried ground and sieved (0.05 mm mesh) field soil mixed with 15-day old cow dung dusts (particle size < 2mm) as food substrate except for *Perionyx excavatus*, where only air dried cow dung dusts were used. The moisture level of the substrate was maintained around 70% and 40% for *P. excavatus* and other species respectively by periodical spraying of water. Cocoons were collected by gentle wet sieving through 0.05 mesh sieve. The number of cocoons produced was calculated on a per individual basis. After isolation, cocoons were kept on moist filter paper spread over water soaked cotton (85% moisture) inside a petri-dish (one cocoon per dish) to study the incubation period and hatching pattern of juvenile worms. The newly hatched juveniles (F_1) of the studied earthworm species were kept individually in fresh culture and reared until they became clitellate and started to produce cocoons. Copulation is not prerequisite for production of viable cocoons in Pontoscolex corethrurus, Dichogaster affinis, Metaphire houlleti, Octochaetona beatrix and Polypheretima elongata, indicating that these worms may be parthenogenetic. Continuous breeding with high fecundity (cocoon production worm⁻¹ year⁻¹) was recorded in peregrine earthworms viz. P. excavatus (156 cocoons worm⁻¹ year⁻¹), P. corethrurus (118), Dichogaster modiglianii (68), D. affinis (46), Lampito mauritii (43) and O. beatrix (40). Native earthworms are either semi-continuous breeder with moderate fecundity (Drawida nepalensis 29, Drawida assamensis 11, Drawida papillifer papillifer 11) or discrete breeder with least fecundity (Eutyphoeus gammiei 1, Eutyphoeus comillahnus 2). Incubation period (days) was low in P. excavatus (13), D. modiglianii (14), L. mauritii (15), D. affinis (21), D. papillifer papillifer (21), D. nepalensis (24), D. assamensis (29), P. corethrurus (29); moderate in P. elongata (49), O. beatrix (49) and high in E. comillahnus (51), M. houlleti (64) and E. gammiei (110). Hatching success (%) was high in P. corethrurus (91), M. houlleti (91), D. affinis (81), O. beatrix (81), D. modiglianii (63), L. mauritii (60), P. excavatus (52) and moderate to low in P. elongata (40), D. nepalensis (37), D. assamensis (33), E. gammiei (20) and D. papillifer papillifer (18). More than one hatchling per cocoon was recorded in L. mauritii, D. nepalensis, P. elongata, D. assamensis and M. houlleti. There was a dramatic increase in cocoon production by most earthworm species during summer and monsoon in contrast to the winter. With rise in temperature, incubation period increased in *P.corethrurus*, *P.* elongata, D. nepalensis, E. comillahnus and O. beatrix and decreased in P. excavatus, M. houlleti, D. affinis, D. modiglianii and D. assamensis. High rate of cocoon production, short incubation period with higher hatching rate as well as continuous breeding strategies in P. excavatus, P. corethrurus, L. mauritii, D. affinis, D. modiglianii, O. beatrix indicate their possible usefulness in vermiculture either for waste degradation or for soil reclamation depending upon the species.

Conventional tillage and sampling date effects on earthworm population in a maize monoculture in Central Mexico

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An earthworm population of Aporrectodea caliginosa was analysed on three sampling dates (maize emergence in spring, crop harvest in autumn and post-harvest in winter) under four tillage systems: 1) shallow tillage; 2) normal ploughing; 3) ripper decompaction (one pass); and 4) ripper decompaction (two passes). We also considered two soil depths (0-15, 16-30 cm). The soil was a clay loam in under irrigation in Central Mexico. The tillage systems were applied during four consecutive vears on experimental plots (400 m^2 area) cultivated with maize. The tillage treatments did not provoke differences in juvenile, mature and total earthworm individuals. In contrast, the sampling date had a significant effect (P<0.05) on the earthworm population: individuals were < 10 m⁻² at maize emergence (in April); 135 m⁻² at harvest (in November); and 45 m⁻² at post-harvest (in January). Sampling date also affected the earthworm biomass. Earthworm abundance showed differences caused by soil depth, but only on the post-harvest date, when individuals apparently migrate to the deep layer; individual populations were higher in the 16-30 cm soil layer than in the 0-15 cm soil. Burrowing activity was not affected by tillage treatments or by the sampling date. Burrows were constant in three sampling dates, suggesting that the bio-structures created by earthworms resist the erosive impacts of irrigation, rainfall, and tillage implements. Soil moisture and soil resistance changed in the three sampling dates, while the soil organic matter fractions remained constant. Juvenile and adult individuals on the maize emergence date were significantly associated (P<0.05) with heavy macro-organic matter. The lack of negative effects of intense tillage sequences on the earthworm population suggests that other factors acting together (2-5 ton ha-1 year-1 of maize stover restitution, clayey texture, high soil water content due to irrigation) determine the dynamics of earthworm population.

Are vermicompost and the stimulation of endogeic earthworm activities relevant alternatives to chemical fertilizers?

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Soil degradation and water pollution are widespread land degradation problems in Vietnam. There is therefore an urgent need for sustainable agricultural practices. Presently, farmers and researchers are investigating different strategies to maintain soil fertility and avoid off-site effects. Within this context, direct (i.e., the drilosphere) and indirect (vermicompost) earthworm activities could be considered as interesting ecosystem services suppliers. The aim of the present study was to examine the interactions between an endogeic earthworm species, Metaphire khami, compost, and vermicompost produced by *Eisenia andrei*, an epigeic earthworm species, in a degraded tropical soil. A year-long experiment was carried out in greenhouse with a corn-tomato-corn cycle. Following organic fertilizer inputs earthworms survived and soil properties were improved; the soil's carbon and nutrient contents were higher. For the first corn crop, the same yields were obtained with vermicompost inputs as with chemical fertilizer. However, this positive effect did not continue with time and no difference was observed between vermicompost and compost for the second corn crop, one year later. The effect of *M. khami* on corn growth was unexpected. While we observed a positive interaction between M. khami and vermicompost, the earthworms had a negative impact on plant growth in combination with compost. In conclusion, this experiment demonstrated that organic matter amendments do show interesting potential for the rehabilitation of tropical soils. However, negative interactions can occur between local endogeic earthworms and compost.

Changes in soil quality after passage through the gut of *Pontoscolex* corethrurus (Oligochaeta: Annelida)

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Earthworms and a few other groups of large invertebrates have the ability to ingest soil altering its physical and chemical characteristics. To evaluate the effect of earthworms on soil properties in a regenerating mine-site, three soil types (Neossolo Litólico - RL, Cambissolo Háplico - CX and Neossolo Quartzarênico - RQ), differing in clay and organic matter contents were collected from a lead-mining area in Adrianópolis-PR, Brazil, at two depths: 0-10 and 20-40 cm. Earthworms (Pontoscolex corethrurus) were collected from the Federal University of Paraná Farm and five adults were incubated per container (n=3 replicates) in the three soils (300 cm³), maintained at 70% field capacity and at room temperature. Castings and control soil were collected every two days using tweezers and the analyses were performed on uningested soil (control) and the casts. Parameters analyzed were: pH-H₂O, exch. acidity, CEC, exch. Ca, Mg and Al, available K and P, total organic carbon (TOC) and nitrogen (TN), soil texture and water dispersible clay. The results were submitted to ANOVA using SAS and the means were compared by Tukey test (p < 0.05). The worms selected soil fractions to ingest, increasing fine sand and silt, reducing total sand contents. They also altered soil fertility, increasing levels of available P and K, CEC and Ca+Mg. Few effects on TN were observed, TOC increased only in the castings from the poorer soil (selection of C-rich particles) and pH was generally higher in the casts. The only side effect observed due to gut passage, was the increase of clay dispersed in water, which can increase soil erodibility.

Laboratory and field studies on the lifecycle of *Dendrobaena alpina* (Rosa, 1884)

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Distribution of Dendrobaena alpina covers the mountainous region of central Europe. It is considered an epigeic or intermediate earthworm with a high dominance and frequency index. In the field it is easily recognized among other species because of its violent escape response. This work describes data from field studies conducted over an annual cycle in the Bieszczady National Park in different types of beech (Fagus) forest. Additionally we set out to obtain baseline data on the life history of D. alpina under controlled environmental conditions. Animals were collected from forest soils near Lutowiska in the Carpathian Mountains, SW of Poland in June 2008 and taken to the laboratory for investigation. All were initially housed individually in native soil within plastic containers, but over time soil was replaced with a standard Kettering loam. Food was supplied in the form of dried horse manure. Mature individuals were kept in isolation and cocoon production monitored on a 2 monthly basis. Immature individuals were kept until they reached maturity, at which point they were put into pairs and again combined output of cocoons regularly monitored. Cocoons themselves were collected, had masses determined and were incubated on moistened filter papers in Petri dishes. Hatchability was recorded, as was estimated duration of incubation and growth to maturity. Field-collected adults had a mean mass of 1.40 g. Results over 21 months at 15 °C, revealed that these individuals (n=6) produced an average of 1.83 cocoons worm-1 month-1. Zero cocoons were produced by further animals, collected as immatures and grown to maturity and maintained in isolation. However, recently-matured (mean mass 1.32 g), paired D. alpina produced an average of 1.65 cocoons worm⁻¹ week⁻¹ (9 pairs over 15 months). All individuals and pairs were kept in 0.4 litre pots. Cocoons had a mean mass of 19 mg (range 14 - 26 mg). At 15 °C, cocoons produced by recently matured adults during the first 2 months, hatched after approximately 80 days (hatchability of 82 %). Thereafter the majority of cocoons, although showing visible development of a viable earthworm, failed to hatch which may be an anthropogenic function. Hatchling growth from an average of 18 mg to maturity (at 1.2 g) required 7-11 months in the given substrate with Tubercula pubertatis seen after 5 months. This work presents both field and laboratory-based data on this particular species.

Earthworm abundances under different agricultural managements in a kiwi fruit plantation

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Sustainable agriculture improves soil fertility and health. Consequently, it is becoming a feasible alternative to current intensive cropping practices. However, in northern Spain kiwi fruit (Actinidia *deliciosa*) is still mostly produced in intensively managed plantations. Earthworms play an important role in soil fertility; however, agricultural practices have detrimental effects on their populations and higher mortality rates have been often recorded. In this study, we investigated the effects of four different management practices, typically used for producing kiwi fruits in Galicia (namely conventional (CT), ecological (ECO) and two integrated systems (INT- and INT+)), on earthworm's diversity and abundance and soil properties (soil pH, organic matter and soil moisture contents). Sampling was performed at three different growth stages of the kiwi plant: (i) resting period, (ii) sprouting and (iii) fruit growth. On each sampling occasion, 15 intact soil cores (11 cm diam.x16 cm deep) were taken from each of these management treatments. The cores were sliced horizontally into 2 cm layers down to a total depth of 10 cm; sub-samples from each layer were used for chemical analyses (%C and %N) and soil moisture determinations. In addition, three soil samples (50x50x10cm) were also randomly taken for earthworm and pH determinations. In total, eight earthworm species were identified: Lumbricus friendi (average density of 63m⁻²), Octolasion lacteum (25m⁻²), Aporrectodea caliginosa (19m⁻²), Dendrobaena octaedra (6m⁻²), D. madeirensis (4m⁻²), Amynthas sp. (4m⁻²), L. rubellus (3m⁻²) and Eisenia fetida (1m⁻²). Results also showed that INT- and ECO treatments supported the highest species number and abundances. In addition, abundances of L. friendi and O. lacteum showed a positive relationship with soil pH (R=0.55 and 0.48, respectively), whereas D. octaedra preferred lower values (R=0.39). Furthermore, L. rubellus populations were negatively affected by nitrogen content. Finally, soil moisture did not play a significant role possibly be due to the fact of all treatments having automatic irrigation systems. In conclusion, our results confirm that agricultural managements have important impacts on earthworm populations. In particular, at the sprouting period when the plots (excepting the ecological) received herbicides and consequently, organic matter dynamics and soil pH were altered, earthworm's abundances and diversity were mostly affected.

Production of tomato saladette in greenhouse: a combined effect of earthworms and green manure

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It has been documented the activity of earthworms and green manure promoted the growth and increased the annual and perennial cultures production. Nevertheless, the knowledge in protected culture is limited. In these intensive production systems pesticides are applied in the ground and on the plants. However that can be toxic for the survival and development of earthworms. Also, it is known that the nitrogen fertilizers can be replaced by green manure to incorporate to the soil organic matter rich in nitrogen, and to reduce the production costs. Because of its ample distribution and tolerance to disturbed atmospheres, the earthworm *Pontoscolex corethrurus* has been the tropical species more studied and handled to foment the growth and agricultural production. The present study had as objective to study the effect of the presence/absence of the exotic earthworm P. corethrurus and the leguminous named picapica (Mucuna pruriens var. utilis) in production of tomato saladette. In greenhouse, during a period of 230 days, 80 plants of tomato, 20 plants were cultivated by treatment. The evaluated treatments were: a) conventional handling without earthworms and mucuna (-E-M); with earthworms and without mucuna (+E-M); without earthworms and with mucuna (-E+M) and with earthworms and mucuna (+E+M). At the end of the experiment the earthworm and its interaction with the green manure (mucuna) affected the number significantly (F = 18.49, P < 0.0001; F = 4.16, P <0.04) and biomass (F = 27.86, P < 0.0001; F = 11.67, P < 0.001) of the fruits. The greater production of tomato (number and biomass) was registered in the treatments with absence of earthworms (-E-M) and (-E+M). Nevertheless, when they incorporate together (+E+M) to the soil an intermediate production was obtained. We conclude that the addition of green manure and earthworms can be an alternative for an organic and low costs production..

Impact of management of the production systems of maize in the populations

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The key factors for the earthworms indirect management is the continual supply of organic matter, reduced tillage, and avoiding the use of pesticides and fertilizers are toxic to earthworms. This study was performed in the southern of Mexico in the summer cycle and aimed to assess the impact of the management of four maize production systems (Fallow, Mechanical tillage, Transition and Organic) in populations of earthworms. It registered the diversity, abundance and biomass of earthworms in each production system of maize. Each system had three replicates based on a randomized design. The results showed that the earthworm populations consisted of two native species (*Larsonidrilus orbiculatus* and *Lavellodrilus sp.*) and an exotic (*Pontoscolex sp.*). In all system, the dominant species were natives. The total biomass was significantly higher in Transition Systems and Organic maize than Fallow and Mechanical tillage. We conclude that mechanical tillage had a negative influence in the populations of earthworms.

Influence of the earthworm *Pontosocolex corethrurus* in the survival and growth of *Abies religiosa* in the nursery stage

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In the forest nursery of Mexico the plastic bags are still widely used for the massive production of seedlings. It is suggested that the constant irrigation alters the soil conditions (for example, the compaction) that affect the growth and development of the roots. It is known that the earthworms can improve the quality of the soil (physical, chemical and biological) and are of easy management. Nevertheless, there are no studies on the effect of earthworms on the survival and growth of seedlings *Abies religiosa*. In the present research the effect of earthworms *Pontoscolex corethrurus* was evaluated in the survival and growth of seedlings *Abies religiosa*. In greenhouse conditions, during a period of 365 days, seedlings were cultivated in plastic cubs under three treatments (0, 1 and 2 juveniles earthworms), with 25 replicates by treatment. Every 15 days registered the height, diameter of the stem, maximum and minimum cover and number of branches. In addition, when finishing the experiment it was determined the total dry matter production and by component of plants (root, stem, branches and leaves). The results showed that the presence of *P. corethrurus* had a significant effect when increasing height of the plant and dry matter production (total, root and stem). It is concluded that the presence of earthworms promotes the growth of seedlings.

Temporary effect of burning on earthworm communities and physicochemical properties of soil in areas of *Eucalyptus grandis*

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The town of Huimanguillo, Tabasco, counts on an extensive zone of eucalyptus (Eucalyptus grandis) plantations, because it generates significant revenue sources for the small producer, to lease their land to transnational corporations in the area of wood and cellulose. However, several studies conducted in other tropical areas suggest that by introducing this crop acidify this soil, compete with native flora, produce allelopathic effects, absorbs large quantities of water to the soil, etc. This study aims to evaluate the temporal effect of burning in management of Eucalyptus grandis sites on the communities of earthworms and soil properties, because the presence of these worms indicates the fertility of soils. The collection of earthworms was carried out at the end of the rainy season in the month August 2007. Sites were selected to evaluate four treatments: T1 = E. grandis with five years of age (Euc), T2 and T3 = sites burned 15 to 60 days post-harvest (Q15 and Q60 respectively) and T4 = acahual with more 15 years of abandonment (Acah). The sites had an area of 1-2 ha, in each one made three quadrants of 50 x 20 m^2 , and each quadrants six monoliths of 25x25x30cm (TSBF method). The soils of the area are Acrisols, showed a pH of 4.5-6.0 in the first 30 cm deep. The nutrients in the soil decreased in the recently burned sites (organic matter, 6-8%, N, 0.19-0.22%) but once you set the vegetation tends to increase their levels (MO, 9-11%; the N, 0.27-0.33%). Only the specie found *Pontoscolex corethrurus* throughout the area, their populations were found in juvenile stage with highest densities and biomass in the culture of Euc (164.4 ind.m⁻² and 36.8 g.m⁻² respectively), followed by Acah (138.7 ind.m⁻² and 19.1 g.m⁻² respectively), while the Q15 and Q60 sites had small populations (80% and 77% with respect to Euc and Acah). In the post-harvest, the use of burning is part of the techniques used to eliminate the vegetal remainders more easily, however, its use has negative effects when increasing the risk of losing the "pool" of soil nutrients for the following cultivation cycle, to compensate this decrement must apply external inputs (chemical fertilizers) periodically to maintain the crop production, in addition, the management from the ecologically significant impact on the oligoquetofauna native for the loss of species diversity, soil fertility decreases and moves and controls the native flora. The state forest industry should considered to maintain prolonged periods of rest in these areas, which would retain nutrients in the soil that would result in greater profitability in the medium and long term.

Food and habitat preference of *Lumbricus terrestris* L. for cover crops indicate short-term soil biofumigation effects of the cruciferous Yellow mustard (*Sinapis alba* L.)

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Worldwide, cover crops are used because of their multiple roles in soil erosion control, nitrogen loss reduction, weed suppression, and soil fertility in agro-ecosystems. Recently, (cover crop) species of the Brassicaceae family have been reported to act as biofumigants against a wide variety of soilborne pests (e.g. fungi, nematodes, and slugs) through the release of isothiocyanates (ITC's) from glucosinolate precursors upon cell tissue disruption (e.g. after soil cultivation, freezing). Although these allelochemicals have the potential to reduce the need for application of synthetic pesticides, their effects on beneficial soil ecosystem engineers such as earthworms (Lumbricidae) have not been assessed yet. The common use of mustard powder (variably composed of grinded seeds of Brassica juncea, Brassica nigra, Sinapis alba) in earthworm sampling may suggest adverse effects on earthworms. Food preference for five commonly used cover crops (ryegrass (Lolium multiflorum LAM.), phacelia (Phacelia tanacetifolia BENTH.), oats (Avena sativa L.), and the cruciferous Yellow mustard (Sinapis alba L.) and rapeseed (Brassica napus L.)) were studied both in food choice chambers in the lab for 10 days and by litter bags (mesh sizes 0.5 and 4 mm) in a no-till field during 12 months. The effects of growing plants and freshly incorporated residues of three-months-old Yellow mustard and oats on the habitat preference of adult L. terrestris were studied in simple habitat preference units consisting of flowering pots connected through a central corridor area in which earthworms were introduced. Preferences were analyzed by compositional analysis. In general, ryegrass residues were a preferred food resource over mustard, phacelia or rapeseed residues, and these were in turn more fed on than oats. Litter disappearance from the 4 mm bags indicated the same trends for the first two weeks, but then food preference in the field became variable. Between 24 and 48h after incorporation of mustard residues, release of isothiocyanates affected habitat selection by L. terrestris, but 72h after incorporation these negative effects were neutralized again by ITC breakdown and volatilization. Habitat selection was not affected by growing mustard plants during the 12-day study period. The experimental results indicate that *Brassicaceae* residues are used as a food resource by earthworms in amounts comparable to other cover crops. Further, neither surface applied residues nor the living root system of the commonly used cover crop Yellow mustard have lasting negative impacts on earthworm habitat selection. However, it is strongly advised that mustard residues are only superficially worked and not ploughed into the soil to stimulate volatilization of detrimental isothiocyanates.

Improvement of vermicompost on main nutrients and microbial population in tomato soil under continuous cropping system: experiment in laboratory

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The continuous cropping system always led to nutrients disequilibrium in soil and inhibited plant growth. In order to find a biological solution to deal with continuous cropping problems cow manure vermicompost (CMV) was used into continuous cropping (over 10 years) tomato soil in laboratory with consideration of ecology effect. The rate of CMV into soil were 0% (control, CK), 10%, 20%, 30% (in volume). The data showed that CMV accelerated plant growth rate. The dry weight of shoot and root grown with CMV (10%, 20%, 30%) were more 31.6%, 15.3%, and 92.4% and 60.3%, 63.8%, and 112.1% than those of CK, respectively. The total N content in soil contained 20% CMV after seedlings harvested decreased 1.9% than before planting. The total P content after planting in soil with CMV (10%, 20%, 30%) reduced 93.2%, 49.2%, 45.8% than that of before planting, respectively. On the country, the total P content in the CK after planting increased 14.5% than that of before planting. It was to say that CMV stimulated plant absorption on P. Plant utilization for K in three vermicompost levels increased, too. The content of total K in 10%, 20%, and 30% vermicompost after planting reduced 45.4%, 40.9%, and 40.0% than that of before planting, respectively. The difference of factors above were significant (P < 0.05). The microbial populations in soil with CMV were more than that of in CK. The fungi population in soil with vermicompost of 20% and 30% after planting was little or no change than that of before planting. It was indicated that reasonable CMV application can control fungi population in continuous cropping soil. It was concluded that CMV improved nutrients supply and maintained the microbial community stable in continuous cropping tomato soil. It can be a modifying agent in continuous cropping soil.

Session 7

Earthworms, Soil Contamination and Bioremediation



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Experiences with the earthworm avoidance test as a new tool for the environmental risk assessment of individual chemicals and contaminated soils

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The earthworm avoidance test using (mainly) the species *Eisenia fetida* or *Eisenia andrei* is a relatively new ecotoxicological laboratory test which is characterized by its short duration and high practicability (i.e. simple performance and low need of resources). Despite the fact that it is not required in any regulatory context so far, it is becoming more and more popular as a screening tool in lower tiers of site specific risk assessment schemes as well as for the evaluation of the effects of chemicals, in particular pesticides. This popularity is partly caused by its simplicity, partly because it has already been standardized by ISO (Guideline No. 17512-2, 2008). In this contribution, a brief overview on the experiences made so far with this test is given. In particular, two issues will be tackled: Firstly, when avoidance tests are used with site contaminated soils, the avoidance behaviour of the test organisms can be influenced by soil properties which can act as confounding factors creating highly biased results and misinterpretations. This can be problematic particularly when using a reference soil (either an artificial or site soil) with different properties of the test soils. Using data of a case study from Brazil, we will present the development and application of a GLM model to correct for the influence of these confounding factors, thus decreasing the uncertainties associated to this type of ecotoxicological data. Secondly, the toxicity of the worms observed in avoidance tests will be compared with results gained in reproduction tests with the same soils. Here, we will use experiences made in a case study from Germany in order to discuss the pro's and con's of the earthworm avoidance test in order to evaluate the relevance of such test results for the environmental risk assessment of contaminated soils.

Interaction of lead with earthworm secreted calcium carbonate granules

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The influence of soil organisms on metal mobility and bioavailability is not currently fully understood. We conducted experiments to determine whether the calcium carbonate granules excreted by the earthworm *Lumbricus terrestris* L. could incorporate and immobilize lead in an artificial soil amended with lead and calcium. Individual *Lumbricus terrestris* were each exposed to 300 g air dried OECD-style artificial soil (comprising 10 wt % peat, 20 wt % kaolinite and 70 % quartz sand, all sieved to Average granule production rates of 0.39 +/- 0.04 mg calcite / earthworm / day did not vary with soil lead concentration. The lead: calcium ratio in granules increased significantly with that of the soil ($r^2 = 0.81$, p = 0.015) with lead concentrations in granules reaching 1577 mg / kg. X-ray diffraction (XRD) and X-ray absorption spectroscopy (XAS) detected calcite and aragonite in the granules. X-ray absorption spectroscopy indicated that some lead was present in the granules as Pb₃O₄, possibly as a result of beam damage, cerrusite (PbCO₃), hydrocerrusite (2PbCO₃.Pb(OH)₂) and adsorbed to calcite and aragonite,. Despite their high lead concentrations the likely mass of granules in soil of 3 to 30 g / m³ means that they are unlikely to account for significant masses of lead in lead contaminated soil.

The effects of earthworm activities (*Aporrectodea giardi*) on the distribution of geogenic arsenic and soil nutrients in the Jura Mountains (Switzerland)

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Environmental pollution due to arsenic (As) has been well studied and most investigations focused on inorganic As coming from industrial areas. However, the natural rock-borne contamination of this metalloid was underestimated, as well as the impact of arsenic on soil biota. Focusing on annelids, only one study addressed the question of geogenic arsenic distribution in earthworm casts and arsenic speciation in tissues. Moreover, to our knowledge, no research was conducted on the impact of earthworms on soil arsenic behaviour. As a consequence, this study aimed to look at the influence of earthworms on the mobilization (availability, transfer) of arsenic coming from a geogenic source. In this context, we assumed that: 1- geogenic As should differ in biogenic structures (faeces, burrows) compared to non-ingested soil, 2- the distribution and the bioavailability of geogenic As should be modified due to earthworm bioturbation activities and gut processes, thus influencing the potential As mobilization, 3- earthworms should favour the transfer of As in the soil profile through their vertical movement, 4- earthworms may accumulate geogenic As in their own tissues, leading to a modification of their biological traits. To test our hypotheses, microcosms in controlled conditions were filled with soil containing 157 mg As kg-1 from a geogenic origin. The soil was collected from a meadow situated at an altitude of 1330m in the Jura Mountains. As a result, our first assumption was not verified: while earthworm surface-casts were enriched in carbon, nitrogen and available phosphorus compared to non-ingested soil, no enrichment was found for arsenic. The second hypothesis about the As-distribution showed no significant difference comparing soil texture in surface-casts and non ingested soil. Arsenic predominated in the coarse sand fraction. Focusing on arsenic potential mobilization, less As was released from earthworm surface-casts suggesting that the humification of organic matter in the digestive tract of earthworms may help the formation of Ashumic/fulvic complexes. The third assumption supposed that earthworms should favour the transfer of as in the soil profile. In our experiment using artificially enriched soil, almost 50 % of worms died and some of the survivors entered into diapauses. As a consequence, the expected mixing between the As-enriched soil section and the upper part of the column did not occur. Finally, we did not demonstrate any geogenic arsenic effect on earthworm survival and growth. As a general conclusion, it appeared that geogenic as slightly affects biological components and environmental aspects. These findings are particularly positive taking into account the fact that most of the environments at an altitude of 1330 m in the Jura Mountains present rocks coming through the soil, especially in meadows that are regularly under the influence of the cattle.

Impact of earthworms on the remediation efficiency of contaminated soil

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Pollutant pathways have been identified at the Parys Mountain mining site, Anglesey, UK. Ochre ponds at the site are un-vegetated, with conditions inhibiting natural revegetation. The aim of this study is to test the impact of compost and biochar, with or without earthworms, on the mobility of metals and revegetation of the contaminated soil and see if the addition of earthworms compromises remediation efforts. Contaminated ochre (1343 mg/kg Cu and 262 mg/kg Zn) was remediated with compost and/or biochar in mesocosms in order to create a substrate suitable for plant growth. Lumbricus terrestris (L.) were added to a treatment that was remediated with both biochar and compost in order to determine the impact that they had on the mobility and availability of the metals in the remediated soil. Mobility was determined by measuring the water soluble metals and availability by measuring uptake into Common Bent (Agrostis capillaries). The combination of biochar and compost decreased the mobility of Cu and Zn in the contaminated soil and increased the pH from pH 2.7 to pH 6.7 with L. terrestris and 6.6 without. In the soil amended with both biochar and compost the water soluble Cu was significantly decreased from 5.6 mg/kg to 0.2 mg/kg, a factor of 248 and Zn was significantly decreased from 3.3 mg/kg to 0.05 mg/kg, a factor of 65. The addition of L. terrestris however had no significant effect on the mobility of the metals in the remediated soil. Common Bent did not germinate in the unamended control soil but both compost and biochar increased the germination with the compost treatment producing the greatest growth (8 times greater than the next highest treatment). The combination of compost, biochar and L. terrestris produced the lowest concentrations of Cu (8.1 mg/kg) and Zn (35.7 mg/kg) in the aboveground biomass of the grass; significantly lower than the compost treatment (15 mg/kg Cu and 126 mg/kg Zn) by a factor of 1.9 and 3.5 respectively. The addition of both biochar and compost, individually and together, was effective at reducing the mobility and availability of metals and created a medium that is suitable for revegetation and earthworm survival. The addition of L. terrestris did not re-mobilize previously sequestered metals.

Suitability of *Dendrodrilus rubidus* and *Lumbricus rubellus* coelomocytes for monitoring soil pollution: mini-review

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Abandoned Zn/Pb mine-sites and aerially-contaminated Ni-smelter sites in the UK are inhabited by limited number of earthworm species, including the epigeics Dendrodrilus rubidus and Lumbricus rubellus. Phylogenetic trees based on the COII mitochondrial gene of both species sampled from two unpolluted reference sites and from four Pb/Zn-polluted sites in Wales revealed that although L. rubellus comprises two distinct genetic lineages these are not differentially restricted to polluted or unpolluted soils; our limited D. rubidus samples were genetically clustered into two separate clades corresponding with polluted and reference sites, respectively. D. rubidus accumulated metallic soil contaminants, especially Zn and Pb, much more efficiently than L. rubellus, and was found by ex vivo counting after non-invasive extrusion from the coelomic cavity to possess a relatively higher number of immune-competent cells (coelomocytes). These two lumbricid species belong to groups of worms with contrasting coelomocyte communities and biochemistry: in L. rubellus coelomocytes are almost exclusively amoebocytes; D. rubidus contain both the small non-granular amoebocytes and large, granular, autofluorescent chloragocytes/eleocytes. Eleocytes contain fluorescence emitting riboflavin stored in cytoplasmic vesicles. In L. rubellus, interpopulation differences in numbers of amoebocytes and their pinocytic activity (established by flow cytometric analyses of neutral red uptake) did not correlate with soil pollution intensity. The coelomocytes of D. rubidus from unpolluted and metal-polluted soil samples were subjected to flow cytometry for determining the percentage autofluorescent eleocytes, and to spectrofluorometry for riboflavin assays in coelomocyte lysates. In D. rubidus, riboflavin content was a sensitive molecular biomarker of soil pollution: riboflavin-derived autofluorescence was high only in the cells of worms inhabiting unpolluted soil but was virtually extinguished in their counterparts inhabiting or transferred to field soils contaminated with Zn/Pb or Ni/Cu. In conclusion, coelomocyte-connected parameters investigated so far in L. rubellus can be modulated by soil quality perturbations, but they are not such sensitive and robust biomarkers of soil metal pollution as is the easily measured riboflavin content of D. rubidus coelomocytes. Acknowledgements: Supported by a grant (No PB3502/PO1/32) from the Ministry of Science and Education (Poland).

The worm that turned? Inferring possible genetic adaptation of *Lumbricus rubellus* (Hoffmeister) populations along a Ni-pollution gradient, and in adjacent non-polluted geochemically contrasting soils with microsatellite markers

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Earthworms are particularly suitable for monitoring contaminated land because of their ecophysiological characteristics, their role in modifying the physic-chemical properties of soils, and their capacity to bio-accumulate contaminants, occasionally to concentrations exceeding those in the native polluted soil. For these reasons, a range of biomarkers of sub-lethal stress have been developed in certain species. Whilst these cover the spectrum of biological levels of organization, the advent of contemporary "omics" technologies have facilitated an intense interest in field-based environmental diagnostics using earthworms. These studies are, however, potentially confounded by site-specific micro-evolutionary events whereby resistant genotypes may not conform to the doseresponse predictions of laboratory toxicity tests. Moreover, the ecotoxicological implications of the fact that several lumbricid species are differentiated into distinct genetic lineages have not yet been fully assimilated. This study had two major aims: (i) to determine whether populations of the cosmopolitan epigeic species, Lumbricus rubellus, are genetically differentiated along a gradient of nickel contamination in the vicinity of the largest Ni-smelter in western Europe; and, (ii) whether the genetic structure of L. rubellus populations native to adjacent calcareous and acidic non-polluted soils are distinct. Gene flow was examined using microsatellite markers, the majority of which were developed from expressed sequence tag sequences of L. rubellus, whilst two were amplified using primers developed for a congeneric species. Preliminary results indicate a degree of genetic heterogeneity to exist between populations of L. rubellus at some of the most heavily contaminated sites along the Ni gradient. This could be indicative of adapted populations at these sites. Contrastingly, little evidence of genetic heterogeneity was found between populations from unpolluted soils of different pH, indicating that this edaphic parameter has little impact upon gene flow. In conclusion, the presence of intraspecific genetic heterogeneity at contaminated sites could present a problem for ecotoxicological studies because such studies implicitly assume L. rubellus to be genetically homogenous, and thus display variations in genetically-determined traits reflecting site-specific pollution intensities. Acknowledgement: We are grateful to the Levehulme Trust for funding this project (Ref. F/00407/AI).

Earthworms, are a means to accelerate removal of hydrocarbons from soil? A review

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Contamination of soil with hydrocarbons occurs frequently in Mexico, one of the most important petroleum producers in the world. Restoring contaminated soils is costly and time consuming. Earthworms might help to accelerate the removal of hydrocarbons as they burrow through soil, feeding on the organic matter, improving soil structure and aeration. Polycyclic Aromatic Hydrocarbons (PAHs) are of the most recalcitrant hydrocarbons that can contaminate the environment and can thus serve as models to study the factors that control the removal of petroleum from soil. We report on the effect of earthworms on the removal of phenanthrene, anthracene and benzo(a)pyrene from soil and dynamics of C and N. It was found that earthworms have the capacity to accelerate the removal of PAHs from soil and increase the mineral N content. However, their use in a contaminated field will require a high density of the earthworms, which might be expensive and the provision of sufficient organic material as feed while a high soil water content must be maintained to stimulate burrowing.

Genetic diversity of Lumbricus rubellus in unpolluted and polluted habitats

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Earthworms have been extensively used in terrestrial ecotoxicology due to their well-known ecological engineering functions and ecophysiological characteristics. Micro-evolutionary events, such as local population adaptation to elevated concentrations of soil contaminants may, however, confound the deployment of biomarkers in field-based ecotoxicological assessments. In order to assess the utility of biomarker data we evaluated and compared genomic variation and dispersal in populations of the sentinel earthworm Lumbricus rubellus, in geochemically diverse unpolluted and polluted habitats. Specimens of L. rubellus were collected from sites along a Nickel (Ni) gradient downwind and upwind from a working Ni smelter at Clydach (Wales, UK), and also along transects centred on two unpolluted reference sites with contrasting soil types (Dinas Powys and Rudry Common). The chemical composition of soil samples from each site was analyzed comprehensively for 23 elements by ICP-OS, and all earthworms collected (30 individuals from c. 10 stations per site) were genotyped using mitochondrial cytochrome oxidase II (COII) sequences. 210 specimens from twelve selected sites were further screened at the whole genome level using Amplified Fragment Length Polymorphism (AFLP). These analyses resolved L. rubellus into at least two mitochondrial lineages ('A' and 'B') with an average p distance of 11% between them. A differential distribution of lineages along the Ni-pollution gradient suggests that each lineage may express different levels of Ni tolerance. Ni contaminated sites show the same level of genetic diversity as non-polluted sites, suggesting that the populations closer to the contamination source may represent "demographic sinks", sustained by the recruitment of non-adapted individuals from adjacent less contaminated land, which may possibly adapt to their new relatively stressful surroundings through phenotypic plasticity. Our observations did not support the notion of genetic erosion in the vicinity of a pollution point source. The role of this soil-dwelling species as a sentinel organism in laboratory- and (especially) field-based ecotoxicological studies should assimilate the knowledge that it is differentiated into at least two deeply distinct genetic lineages, each lineage possibly comprising ecotypes of significantly different metal sensitivity. Acknowlegement: This study was supported by a research grant (F/00407/AI) awarded by The Leverhulme Trust.

Molecular toxicology of cadmium on earthworms in low polluted soil

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An experiment was carried out in order to accurately reflect the actual exposure or the potential biological toxicity of heavy metals to earthworm in the polluted soil under low-dose cadmium concentrations. In this study, changes of physiological related genes expression of earthworms were detected using real time PCR under long-time of 70 days' pollution. The physiological genes selected were MTs, Annetocin, Calreticulin and Anti-microbial peptide and two types of soil; natural soil with Cd content of 0 and 1.46 mg/kg (pollution-free soil and polluted soil) and artificial soil with Cd content: 0, 1, 5, 10, 25, 50 mg/kg, were studied in the experiment. The results showed that the expression of metallothionein gene (MTs) increased significantly with the increase of soil cadmium concentration. For artificial soil treated earthworms, when the cadmium concentrations come up to 50 mg/kg, the MTs expression was 12.68 times of that of the CK. It showed a significant positive correlation of dose-response relationship. For natural soil treated ones, MTs expression of "polluted soil" groups was 2.4 times of that of the "pollution-free soil". The expression of earthworms' production hormone gene (Annetocin) was gradually reduced with the increase of the soil cadmium concentration. For artificial soil treated earthworms, the Annetocin gene expression of "50mg/kg" groups was 0.243 time of the CK groups. It manifested a significant negative correlation of doseresponse relationship. In the meantime; expression of "Polluted soil" treated earthworms was 0.214 time of "pollution-free soil" treated ones. There was no significant correlation between the soil cadmium concentration and the Calreticulin expression of the treated earthworms. For artificial soil treated earthworms, the Calreticulin expression level of "1 mg/kg" groups was the highest. For natural soil treated ones, Calreticulin expression of "polluted soil" groups was also higher than that of the "pollution-free soil". Gene expression of Anti-microbial peptide was not changed obviously for artificial soil treated earthworms under low-dose cadmium. The existence of a higher dose of cadmium (10 mg/kg) may increase the expression level. However, if the dose was too high, the existence of cadmium may also have inhibiting effect on the genes expression of Anti-microbial peptide. For natural soil treated ones, there was no significant difference between the "polluted soil" groups and the "pollution-free soil" groups.

Metabolomics in earthworm biology

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Metabolomics — the untargeted profiling of small-molecule metabolites — is a powerful approach to measuring subtle phenotypic differences. We have been applying metabolomics to a number of different problems in earthworm biology, including understanding responses to toxic chemicals and other environmental stresses. In addition, it can be used to study genotype/phenotype differences, and how these interact with environmental factors. Here we will present data from a number of studies using nuclear magnetic resonance (NMR) spectroscopy as an analytical tool, including controlled lab-based exposures to single toxic chemicals, field studies at contaminated sites, and assessing functional differences between populations. The results demonstrate some of the potential advantages of the NMR metabolomic approach: for instance, chemicals with different toxic modes of action lead to differing metabolite responses, implying that potentially soil contaminant effects could be identified by 'fingerprinting'. In addition, an example is given where the observed metabolite responses lead to clear and directly testable biochemical hypotheses. Extending this to the field level, we could identify a toxic metal (zinc) as the most probable cause of actual functional effects, even across sites with very different underlying soil type, vegetation, etc. Finally, we show how metabolomic phenotyping can be used to help the interpretation of genotype information in natural populations.

Mixture interactions and mechanisms of joint effects of stressors in earthworms exposed to stressor combinations

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In soil ecosystems, earthworms are exposed to combinations of stressor including toxic chemicals, oxygen depletion, climate extremes and pathogens. Describing and understanding the nature of these combined effects requires the integration of statistical modelling approaches with methods that elucidate the mechanisms responsible for joint effects. Established concepts used in mixtures pharmacology (notably the concentration addition and independent action models) can be used to predict the effects of both mixtures of chemicals and combinations of chemicals with other stress factors. In many cases, such as mixtures of neonicotinamide pesticides, metals and PAHs when exposed in increasingly dry soils, these standard models have been found to effectively describe joint effect. In other cases, however, the standard models fail to account for actual effects; responses being either synergistic or antagonistic. In cases where the available models fail, the assumption must be that interactions between the chemicals and/or other stressor mean that the conceptual basis (that of strict and independent additivity) of the models are not met. Where such interactions occur, approaches to address the temporal nature of exposure (e.g. toxicokinetics) and/or mode of action can be used to investigate the basis of the interactions in earthworms. Temporal approaches to address exposure include toxicokinetic models based on simple and more complex compartment models that can be utilized in earthworms. For mode of action assessment, the emergence of functional genetic tools has allowed a deeper investigation of the common and unique transcriptional responses that delineate the common response and distinct mechanistic effects of different chemicals in a mixture.

Bioaccumulation of cypermethrin in earthworms *Eisenia andrei* from different soils

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The pyrethroid insecticide cypermethrin has been widely used in agriculture and public health campaigns due to it is efficiency in controlling insects, short persistence in the environment and low toxicity to birds and mammals. However, a large portion of the applications of cypermethrin may leave toxic residue in the soil and, organisms such as earthworms, may be exposed to these contaminants in soil, which are absorbed by earthworms or bioaccumulated in their tissues. As earthworms are at the base of the food chain serving as food for other organisms, studies on bioaccumulation of pesticides in these organisms may indicate the potential for contamination along the food chains. This study determined the bioaccumulation factors -FBA ¹⁴C-cypermethrin on earthworm Eisenia andrei from three soil types: Typic Hapludox (LV), Mollic Hapludalf (PV) and Typic Humaquept (GM). Four replicates of 100 g of each soil type were treated with 0.05 mg and 0.056 kBq of ¹⁴C-cypermethrin g⁻¹ soil. The controls received 400 mL of acetone. After solvent evaporation, five earthworm specimens with clitellum and more than 300 mg each were placed in each sample. The study was conducted in a temperature-controlled chamber at 22 °C \pm 2 °C and continuous light for 14 days. At the end of 14 days, 10 g of each type of soil were extracted with methanol by mechanical agitation. The earthworms in each chamber were sacrificed by freezing at -18 °C for 20 minutes and ¹⁴C-cypermethrin was extracted from their tissues with methanol for 30 cycles of 15 seconds and 160 W of microwave energy. The bioaccumulation factors (FBA) was calculated by the following formula: Ctm = FBA / Cs, where Ctm: concentration of $[^{14}C]$ in the tissues of earthworms (kBq g^{-1}) and Cs: Concentration of $[^{14}C]$ in soil (kBq g^{-1}). The values of FBA showed that cypermethrin was bioaccumulated by earthworms, regardless of soil type; therefore the FBA was 3.73 ± 0.37 in LV, 3.52 ± 1.09 PV and 2.11 ± 0.73 in GM. It was verified less FBA in soil with high organic content (GM), which is the factor that usually determines the pesticide residuesbounds formation, and therefore less available to organisms. However, it was found that cypermethrin is bioaccumulated and this contamination can occur throughout the food chain from earthworms.

Reducing the toxicity of bone meal to earthworms so that it can be used to remediate metal-contaminated soil

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Bone meal is an organically derived source of phosphorus (P) that could potentially be used as a P amendment to remediate metal-contaminated soil. Despite successful laboratory trials using a variety of sources of P to immobilize metals, there are relatively few studies that investigate impacts of P amendments on earthworms or other soil biota. Toxicity tests indicate that bone meal could have a significant impact on earthworm mortality. It is hypothesized that the organic component of bone meal is the source of toxicity. The aim of this study was to establish whether bone meal toxicity could be reduced by simple, cost-effective treatments. Standard OECD methodology was adapted for all toxicity assessments. Individual Eisenia fetida were exposed to 50 g of meal, no treatments showed 100% mortality. Experiment 2: The time between bone meal amendment and earthworm introduction was varied for soils amended with bone meal at a concentration of 3500 mg/P kg, i.e. approximately the LC_{50} for untreated bone meal. Earthworms were introduced immediately after amendment and then 7, 14, 21, 28 and 35 days after amendment. Delaying the introduction of earthworms into bone meal amended soil reduced mortality rates. Experiment 3: Soils were amended with both green waste compost (GWC) at rates of 5 to 25 % by weight and bone meal at 3500 mg P / kg. Addition of GWC reduced earthworm mortality though there was no increase in this reduction with increasing amounts of GWC. Our results are consistent with bone meal toxicity being associated with release of organic components to the soil from the bone meal. The toxicity is transient and can be reduced by simple treatments.

Bioavailability and Ecotoxicity of altered TiO₂ nanocomposite on the earthworm, *Eisenia fetida*

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The quantity of nanomaterials produced annually is in constant increase. This trend may induce an increase of the release of nano by-products in air, water, soils, and therefore in contact with organisms. Up today, ecotoxicological risks associated with nanoresidues from alteration and aging of nanomaterials are poorly documented. The aims of the present study were firstly to identify and characterize altered TiO₂ nanocomposite suspensions derived from particles usually included in sunscreen cream, and secondly to evaluate their potential toxicity on the earthworm, Eisenia fetida. In order to simulate the alteration of the nanocomposite, an accelerated aging process was performed by mixing TiO₂ nanocomposite with water during 48h. Then, a stable solution of TiO₂ rich nanoresidues was obtained, and chemically and physically characterized. Earthworms were exposed to altered nanocomposite solutions at 4 concentrations $(0, 1, 5 \text{ and } 25 \text{ mg.L}^{-1})$ during 24h in the dark. The different biological parameters included survival, bioavailability, immunotoxicity and detoxication system for which metallothionein, superoxide dismutase and pi-class Glutathione-Stranferase mRNA expressions on earthworm's gut were measured by real-time PCR and cocoon production. Results showed that the altered TiO₂ nanocomposite had no effect on survival rate and did not modify gene expression in the earthworm's gut. At contrast, cocoon production seems to be reduced at all conditions. Observations of titanium absorption with X-ray fluorescence microscopy show titanium embedded with residual soil ingested by earthworm's gut but not in the tissue. Results on total metal concentrations (Al/Ti) in tissues show a significant increase of titanium in earthworm's tissue. immunotoxicity (phagocytosis and cellular viability).

Pontoscolex corethrurus (Müller, 1857) and Eisenia andrei, Bouché, 1972 as

Cristhy Buch Andressa¹, Brown George²

bioindicators of soil contaminated by pesticides

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The indiscriminate and excessive use of pesticides has been constantly increasing due to the demand for greater agricultural production. This intense use of soil impacts the environment and soil biota. To indicate the extent to which these chemicals are harmful and how and where their effects occur, you can make use of ecotoxicological tests with earthworms. These animals are essential for the maintenance of chemical and biological processes in the soil and are useful to evaluate impacts of pesticides through behavioural, acute and chronic tests. Some of these tests were standardized by ISO (International standardization organization) using the earthworms Eisenia fetida and Eisenia andrei, both native to temperate climate. However, these species may be of little relevance for ecotoxicological seraphic studies, because they live in the soil litter (epigamic), and consume mainly fresh organic matter. The species *Pontoscolex corethrurus*, native to tropical regions, can be an alternative to an ecotoxicological most ecologically important species, because it is a true soil living organism and consume soil organic matter (endogeic). Little is known about their sensitivity to pesticides though. Therefore, in the present study, the sensitivity of E. andrei and P. corethrurus when exposed to three pesticides often used in fruit and grain in Brazil - carbendazim, carbofuran and glyphosate – were tested in a behavioural test ISO 17512-1, 2007 and an acute mortality test, ISO 11268-1, 1993. The results showed that carbendazim and carbofuran were toxic to both species. For carbendazin, *E. andrei* showed EC_{50} of 76.12 mg i.a. kg⁻¹ and LC_{50} of 19.74 mg i.a. kg⁻¹, while for P. corethrurus, EC₅₀ of 65.81 and LC₅₀ of 15.32 mg i.a. kg⁻¹. For carbofuran, E. andrei showed EC_{50} of 9.74 and LC_{50} of 13.50 mg i.a. kg⁻¹ while for *P. corethrurus* EC_{50} of 7.26 and LC_{50} of 9.28 mg i.a. kg⁻¹. Glyphosate, on the other hand, showed no toxic effects at the tested concentrations for both species. The EC₅₀ obtained for both species were very similar, 44.52 mg i.a. kg⁻¹ for P. corethrurus, and 45.52 mg i.a. kg⁻¹ for *E. andrei*. The sensitivity of *P. corethrurus* seems to be statistically similar when compared with the standard species E. andrei for the pesticides evaluated.

Poster - Session 7

To reveal the relationship of earthworm and UVC: from a platform for assessment of dynamic DNA damage/repair capacity in fresh-prepared earthworm cells

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DNA is a stable molecule which is highly sensitive to all kinds of DNA damage and even a subtle alteration can be detected via specific assays. Therefore, the assessment of DNA damage is considered important in toxicity testing. However, few studies on the assessment of genotoxic risks of DNA damage agent for earthworms due to the difficulties in quantifying exposure levels, bioavailability, and effects. The specific aim of this study is to determine dynamic DNA damage and repair process in earthworms exposure to different DNA damage agent (including: UVC, 4NQO, BPDE, cisplatin, MMC, MMS, H_2O_2 , SNP, or SIN⁻¹), and to reveal the relationship between earthworm and UVC.

Molecular mechanism associated with heavy metal tolerance in three epigeic earthworms

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Tolerance to heavy metals is the salient feature exhibited by earthworms. Earthworms have the mechanism for accumulating heavy metals in the chloragogenous cells that prevent them to creep into blood stream with the help of metal binding protein namely metallothionein. Metallothionein is secreted in the earthworm body in response to heavy metal exposure as a detoxification mechanism. The present study is aimed to find out the limit of tolerance to heavy metals and associated molecular mechanism exhibited by three species of epigeic earthworms, namely, Eudrilus eugeniae, Perionyx excavatus and Eisenia fetida. These earthworms were exposed to Lead (Pb) and Cadmium (Cd) at the concentration range of 1mg to 10 mg/kg of partially decomposed feed. After 30 days of composting period, the heavy metal levels in the earthworm body were analyzed using atomic absorption spectrophotometer. The results revealed that the tolerance to lead among the species was 2 mg/kg for Eudrilus eugeniae, 3mg/kg for Perionyx excavatus and 6mg/kg of feed for Eisenia fetida. The accumulation levels in their bodies were found to be 112, 225 and 350 ppm respectively. The tolerance levels for Cadmium in the same order of species were 4 mg/kg, 6 mg/kg and 8 mg/kg of feed with accumulation levels in the body ranges from 160, 252 and 390 ppm. The amount of metallothionein recorded on exposure of these earthworms was in the order 40, 60 and 120 µg/g of body tissue for both Lead and Cadmium for Eudrilus eugeniae, Perionyx excavatus and Eisenia fetida. The metallothionein was purified using affinity column chromatography with sephadex G200. Purified protein is subjected to N & C terminal sequencing in a Shimadzu sequencer by automated Edman degradation method. A sequence of 9 amino acids namely Ala-Gly-Ser-Tyr-Thr-Pro-PheAla-Val-Leu-Isoleu was found to be responsible for metal binding property of metallothionein that is stored in the chloragogenous tissue. The present study revealed that Eisenia fetida exhibited maximum tolerance to heavy metals followed by *Perionyx excavatus* and *Eudrilus eugeniae*. All the three species together can be used for bioremediation of Pb and Cd contaminated soil since they have tolerance.

Changes in forms of Lead and Manganese with passage through the intestinal tract of *Pontoscolex corethrurus* (Oligochaeta: Annelida)

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Mining and industrialization are major sources of environmental pollution and soil contamination, thus affecting soil food webs. Earthworms are sensitive to soil contaminants and can be used as bioindicators of soil pollution with heavy metals. On the other hand, their bioturbating activities in soils can alter soil chemistry and the availability of various elements. However, little is known regarding the capacity of tropical earthworms to alter the forms of heavy metals in soils. Therefore, the present study was undertaken to evaluate the effect of the passage of soil containing different amounts of heavy metals through the intestinal tract of *Pontoscolex corethrurus* on the forms of lead and manganese. The transformation of labile forms to more stable forms was assessed using the sequential extraction of Pb and Mn on five soils from a lead mining site in Adrianópolis country, Parana State, Brazil. Five adult worms were incubated in each plastic container with 300 cm³ of each soil type (n=3 replicates), maintained at 70% field capacity and room temperature until enough castings were produced for the analyses. Castings and control soil were collected every two days using tweezers and the analyses were performed on uningested soil (control) and casts. Statistical differences between uningested soil and castings were tested using ANOVA and Tukey-tests.

Synergic Effect of Heavy Metals in Automobile Waste on the Earthworm Eudrilus eugeniae

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In most of the developing countries including India, soil pollution due to industrial activities and urbanization is of great concern, affecting adversely soil organisms especially earthworms. The epigeic earthworm *Eudrilus eugeniae* was exposed to 100%, 75%, 50%, 30%, 25%, 15% and 5% of automobile service station waste mud. Each concentration level was tested with three replicates using 10 animals. In flow-through experiments life history parameters such as biomass, clitellum development, and cocoon production were measured. After exposing the earthworm to the service station waste mud, mortality was observed in the 100%, 75%, 50% and 35% concentrations. So, further experiments were conducted within 25%, 15% and 5% levels. At the end of the experiment, the control group had a mean biomass of 2.814 ± 0.235 mg and, in the 25%, 15% and 5% exposed group, the mean biomass 2.531 ± 0.094 mg, 2.712 ± 0.123 mg and 2.926 ± 0.276 mg, respectively.

Apoptotic activity as a toxicity response in the earthworm *Eisenia fetida* (Oligochaeta) after exposure to TiO₂ nanoparticles

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In parallel to a dramatic increasing production and use of engineered nanoparticles (ENPs) in nanotechnology applications, investigations on potential physiological and ecotoxicological effects are currently emerging. However, still few it is known about potential impacts of nanoparticle releases on soil macrofauna, and in particular on earthworms, a key component in soil structuration and decomposition processes. Beyond the complex interactions between ENPs and the soil matrix, this is partly due to the lack of suitable toxicological endpoints in organisms exposed to ENPs. In this work, we exposed the earthworm *Eisenia fetida* to various concentrations (1-25 mg 1^{-1}) TiO₂ nanoparticles in water solution under three different pH values (pH 5.5 to pH 6.5). After exposure, we measured the programmed cell death activity in various tissues using two staining techniques to determine apoptosis: TUNEL and Apostain. A dose-dependent response was detected with respect to apoptotic activity in a range of tissues, mainly cuticule, intestinal epithelium and chloragogenous matrix, at the most acute concentration. Apoptosis thus seems to be a sensitive endpoint for detection of adverse effects of TiO₂ nanoparticles, and potentially a powerful tool for quantifying environmental hazards posed by ENPs.

Mode of exposure and nanoparticle size determine toxicity of silver nanoparticles measured as apoptosis in the earthworm *Lumbricus terrestris*

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In terrestrial ecotoxicology there is a serious lack of data for potential hazards posed by engineered nanoparticles (ENPs). This is partly due to complex interactions between ENPs and the soil matrix, but also to the lack of suitable toxicological endpoints in organisms that are exposed to ENPs in a relevant manner. Earthworms are recognized as key organisms in terrestrial ecosystems, but so far only physiological endpoints of low sensitivity have been used in ecotoxicity studies with ENPs. We exposed the earthworm *Lumbricus terrestris* to different concentrations (1-100 mg 1^{-1}) silver nanoparticles of two contrasting sizes under three different conditions: in water, in food and in soil. After exposure, we measured the impact on apoptosis in different tissues using two staining techniques: TUNEL and Apostain. A dose-dependent response was detected with respect to apoptotic activity in a range of tissues both at acute and sub-lethal concentrations (down to 4 mg kg soil⁻¹). Comparing exposure in water and soil showed that reduced bioavailability in soil is reflected in the apoptotic response. Apoptosis thus seems to be a sensitive endpoint for detection of adverse effects of silver nanoparticles, and potentially a powerful tool for quantifying environmental hazards posed by ENPs.
Remediation of Cadmium Contaminated Soil and its affective factors by earthworm casts

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With the development of industry and agriculture, the contamination of soils and crops by heavy metals has became more and more serious, especially for Cadmium deposited in soils, which is very harmful to plants and human beings. In this paper, soil culture experiment was carried out to study the remediation of cadmium contaminated soil by earthworm casts. In this experiment, different proportions (0%, 10%, 25%, 40%, 50%) of earthworm casts were put into those soils which were polluted by different concentrations of Cd (1.0, 5.0 and 20mg/kg). Different fractions of Cd in soils were determined by BCR (Community Bureau of Reference) method in order to verify the inactive function of earthworm casts and possible affective factors like pH, Cation exchange capacity and Humus acid in those treated soils were determined by traditional methods in order to find out the crucial factors for remediation function of earthworm casts. And then, pot experiment and analysis of crucial factors were carried out to validate the inactive effect of earthworm casts and do some elementary studies on the mechanism of amendment by earthworm casts. The mainly results from this paper showed as follows: (1) The addition of earthworm casts could significantly (p<0.05) reduced the Ex-Cd and Oxides-Cd contents extracted by HAc and NH₂OH·HCl in soil polluted by low content of Cd (1.0 mg/kg), and the more earthworm casts added the lower bioavailability of Cadmium was. Soils contaminated by 5.0, 20mg/kg Cd had the same situation as the soil polluted by 1.0 mg/kg Cd, but the interaction is not more significant than that in lower polluted soils. (2) pH value in soils polluted by Cd (1.0, 5.0, 20mg/kg) located between 7.210 and 7.473 after the addition of earthworm casts. Compared with the control, CEC and humus acid had significantly (p<0.05) increased by about 30% and 200% respectively by adding 10% earthworm casts. In addition, CEC and humus acid had significant (p<0.05) and negative correlations with the content of Ex-Cd in soils. Therefore, CEC and humus acid were two crucial affective factors influencing the immobilization of Cd by earthworm casts. (3) The addition of earthworm casts in 5.0 and 20mg/kg Cd polluted soils could promote the growth of Chinese cabbage significantly (p<0.05). The biomass of Chinese cabbage treated by earthworm casts was $2\sim3$ times than that in control. In addition, no matter which kind of Chinese cabbage, the content of total Cd absorbed by plant tissues decreased by about 50%. Cd concentration decrease from 9.386mg/kg to 4.506mg/kg in high accumulated Chinese cabbage and from 3.356mg/kg to 1.583mg/kg in low accumulated Chinese cabbage in 5.0 mg/kg Cd polluted soils after adding earthworm cast. Therefore, Cadmium in soils were immobilized significantly (p<0.05) by earthworm casts.

Biochemical responses of the earthworm *Eisenia andrei* exposed to contaminated natural soil, effects of glyphosate

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We investigated the effects of a commercial formulation of glyphosate (N-(phosphonomethyl)glycine) herbicide, at different exposure times on biochemical responses of *Eisenia andrei* to (1) elucidate the mechanisms of action of glyphosate and (2) investigate the potential for using these responses as biomarkers to monitoring pesticide contaminated soils. Thus, earthworms were exposed a glyphosate (100 g/kg natural soil) for 7 and 21 d using the standard soil test method. After exposure period, a group of earthworms were depurated for 30 days. Reduced glutathione (GSH) concentrations and activities of glutathione peroxidase (GPx) and glutathione-Stransferase (GST) were measured in muscle tissue as antioxidant defences. Malondialdehyde (MDA) tissue levels were determined as biomarker of lipoperoxidation in cellular membranes. Earthworms exposed to glyphosate showed increased MDA production in muscle tissue at both exposure times. Similarly, GPx and GST activities increased during of exposure to herbicide. GPx and MDA were reduced in earthworms depurated. These results showed that glyphosate and its other chemical compounds altered cellular antioxidant defences and induced oxidative stress in E. andrei. Oxidative damage was reverted in depuration phase indicating that the effects observed in exposure period were due to experimental treatment. The biochemical parameters measured can be used as herbicide toxicity indicators considering environmentally relevant concentration.

Mapping the distribution of Ni and Cu in surface soils and biota (lumbricid earthworms and a grass) in the vicinity of a working smelter

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Ni is an essential trace element whose bioavailability, and consequent toxicity toward terrestrial plants and animals, is both dependent on edaphic factors (notably pH) and on the ecophysiology of receptor organisms. Ni is a recognized genotoxic metal. Some plants are efficient Ni accumulators, but there is no evidence that any soil-dwelling invertebrate has a similar trait. Earthworms are acknowledged macro-accumulators of several different essential and non-essential metals, and yet regression analysis of the rather meagre body of literature data indicates that earthworm [Ni]: soil [Ni] relationship has a modest negative slope. Thus, there is considerable uncertainty about Ni bioaccumulation by an invertebrate taxon whose ecological engineering functions has conferred upon it a prominent status in terrestrial ecotoxicology. The uncertainties concerning the Ni relationships of earthworms arise mainly from the limited number of field studies that have been undertaken and the limited range of soil Ni concentrations that have been examined. The soils around the longestablished (1902) and still working INCO Ni smelter in the Swansea Valley (UK), the largest in Western Europe, therefore presents an excellent biomonitoring opportunity. We have analyzed and mapped (with 'Surfer' software) the spatial distribution of Ni in soil (conc. HNO₃ digests and or 1M ammonium acetate extractions), lumbricid earthworms, and the grass Agrostis capillaris across 36 locations up- and down-wind of the point source. Soils adjacent (~100 m) to the smelter stack were found to contain c.3000 µg Ni/g dry mass (compared with Soil Guideline Value for residential soils of 75 μ g Ni/g). The slope of the downwind Ni gradient was steep and the edaphic heterogeneity (e.g. soil pH ranging from 3.91 to 7.69) was noteworthy. Mapping revealed an unsuspected Ni-pollution 'hotspot' coinciding with an historical loading area which bears no current physical remnants. The Ni biconcentration factor (earthworm [Ni : soil 'total' [Ni]) was found to be exceptionally low, with this transition metal behaving akin to another, Cu. Acknowledgement: This study was completed during the tenure of a research grant (F0 0407/AI) awarded by The Leverhulme Trust to AJM.

Micro-focus X-Ray Absorption Spectroscopy of Pb and Zn in different cell types in two earthworm species (Dendrodrilus rubidus and Lumbricus rubellus)

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Station I18 at the Diamond Light Source Ltd (UK) has established an X-Ray Absorption Spectroscopy facility capable of performing metal speciation by the micro-XAFS (Extended X-ray Absorption Fine Structure) mode in heterogeneous structures at the ?m-level of spatial resolution. This capability has been exploited in the present study to characterize the atomic environments surrounding sequestered Pb and Zn centres, respectively, in the chloragocytes, intestinal epithelia, and nephridial cells of two epigeic earthworms inhabiting a heavily contaminated derelict Pb/Zn mine, where the local soil concentrations (Pb = 16300 mg/g d.w., Zn = 48800 mg/g d.w.) comply with the Kelly Indices Guidelines definition of "unusually heavy contamination" and exceed the UK's Soil Guideline Values for domestic soils by orders of magnitude. We and others have previously observed that chloragocytes not only have a phenomenal capacity to sequester and partition metals into specific organelles, but must transport the metals to the organelles with impressive efficiency whilst retaining sufficient functionality in, for example, the Pb-susceptible haem biosynthesis pathway. Understanding how earthworms tolerate metals, and to better interpret global transcriptome responses to metal exposures in terms of modes of action, would be promoted by determining metal speciation in individual target cell types. But this goal presents a dilemma: how to retain both the structural and compositional fidelities of the cells in close approximation to their in vivo states? Cryo-preparative procedures offer analytical advantages but are less useful for critical morphological examination of relatively large, complex, systems. We have exploited a promising compromise technique involving gentle fixation of whole-earthworm pieces in 70% alcohol, followed by methacrylate embedding and transverse sectioning in the range of 2 to 5 m? Sections were mounted on films suspended across holes in aluminum planchettes, and imaged without staining prior to metal mapping and micro-EXAFS. These high-resolution studies under a synchrotron source of high coherence and brightness confirmed that chloragocytes (within the typhlosole and those facing the coelomic cavity) are the main foci of Pb and Zn sequestration in earthworms. The metallospeciation findings were verified where possible with analyses of unfixed cryostat sections. The methacrylate protocol produces 'permanent preparations' offering the possibility of correlative studies. Whilst synchrotron-based techniques will never achieve the status of being truly routine, micro-focus techniques yield spatially resolved assessments of metal partitioning that compliment the recent drive to fractionate cellular metal content in homogenised tissues. Acknowledgement: This study was supported by a beamtime award (Expt. No. 4693) from Diamond Light Source Ltd.

Effects of earthworm *Dendrobaena veneta* and low metals concentrations on soil bacterial activity and functional diversity

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It is well known that soil microbes and earthworms are main drivers of soil processes, but the interaction between these two groups is still poorly understood. We have shown that Dendrobaena veneta exposure to natural soil polluted with heavy metals led to disrupted balance between bacteria inhabiting the earthworm coelomic cavity and the earthworm immune system based on coelomocytes (amoebocytes and riboflavin-storing eleocytes), and that metal contamination affects also soil bacterial flora. Therefore, it seems reasonable to continue studies on mutual interactions between soil-inhabiting microbes and earthworms, which may be additionally affected by metal pollutions. We also aimed to evaluate the influence of *D. veneta* in unpolluted or slightly metal polluted organic soil on soil bacterial activity and the functional diversity. Soil samples from the commercial supplier were soaked either with deionized water or with low concentrations of Ni, Zn or Pb ions (metal concentrations of 2, 2 or 5 mg/kg, respectively). Adult D. veneta were introduced to half samples of each metal treatment and kept unfed for 4 weeks. At the termination of experiments: 1) earthworm coelomocytes and riboflavin content were analyzed by flow cytometry and spectrofluorometry; 2) soil bacterial activity (average color development-AWCD), richness (R) and the functional diversity (Shannon's index- H') were estimated using BIOLOG® Ecoplates; 3) metal concentrations were measured in soils and in earthworm bodies. The results showed that Ni and Pb accumulated significantly in worm bodies. Earthworm eleocytes and riboflavin content were affected by Ni and Pb treatments. The bacterial activity (AWCD) was generally higher in soil temporarily inhabited with worms than in soils without them. Presence of worms changed significantly microbial richness. The functional diversity H' increased in soils with worms but the values did not differ significantly in comparison with H' index for soils without worms. Metals have engendered different changes in the bacterial parameters. The highest toxic effect was observed for lead treatment which significantly reduced activity, richness and bacterial functional diversity. Different effects of worms and metals on activity, richness and soil bacterial functional diversity need more comprehensive study of soil macro-and microfauna interaction. Acknowledgements: Supported by No PB3502/PO1/32 and DS 759/INoS/2009.

Bioreclamation of acidgoldmine tailings using Pontoscolex corethrurus

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Earthworms are considered to be bio-engineers. They are implied in pollution control, land reclamation, bio-remediation, and as biomarkers of eco-toxicity. The studies have primarily concentrated on the metal uptake and accumulation by Lumbricus, Eisenia and Pheretima species. *Pontoscolex corethrurus* needs to be explored for restructuring the soil. This earthworm is a voracious geophagous feeder and the physico-chemical properties of the soil ingested is transformed considerably during its passage through gut. P.corethrurus also processes three pairs of calcium glands which release or resorbed ions to/from the environment and change the pH of the medium and make itself better adapted to the hostile soils. The present study enumerates the role of *P*.corethrurus in bioremediation of the acidic gold mine tailings. Kolar gold mines were one of the three major gold mining areas of India and gradually closed operations from 2001. The abandoned mine site now has about 32 million tonnes of tailings dumps lying in the midst of township and occupying 10% of the land area. The dust and leachate from dumps resulted in eco-toxicity and respiratory problems to the ex-miners living in the vicinity. Hence, the present study was undertaken for restructuring of the dump waste. The success of earthworm survival in the highly acidic tailings will enable restructuring and converting the 'spoil to soil' and support revegetation of the area. With this, the toxicity of the area can be reduced and surface erosion and contamination of adjacent cultivation area prevented. Mine tailings are highly acidic (pH 3.5-5.5) with zero organic matter. Pot culture with sandwich layering of mine tailings with red soil and farmyard manure supported the survival of *P.corethrurus* for the study period of 16 weeks. The earthworms ingested the minetailings as they burrowed through changed from 5.5 to 7.5 and %OM from 0.031 to 1.86. Water holding capacity, available phosphate and potassium increased and the bulk density decreased following P. corethrurus activity in the mine soil. Thus, there was not only the mixing of layers through burrowing but also the restructuring while passing through the earthworm gut. Thus, the physico-chemical characters of the toxic tailings were brought closer to support plant viability. Increase in organic matter and water holding capacity is a measure of improved soil fertility tailings but not lead. The metal concentration in the mine tailings recorded a reduced concentration following earthworm activity. It can now be inferred that the locally inhabiting P. corethrurus can effectively accumulate the metals and restructure the gold mine tailings for reclamation and revegetation and thus alleviate ecotoxicity and health hazards.

Heavy metal (Cd, Cu, Pb, Zn and Mn) bioaccumulation by the earthworms (*Eudrilus eugeniae*, *Eisenia fetida* and *Perionyx excavatus*) during vermicomposting of urban waste

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The present study revealed bioaccumulation of Cd, Pb, Zn, Cu and Mn by the mature (clitellate) specimens of three earthworm species - *Eudrilus eugeniae, Eisenia fetida* and *Perionyx excavatus* during the vermicomposting processes of three different urban wastes – Municipal Solid Waste (MSW), Vegetable Market Waste(VMW) and Flower Waste (FW), carried out for sixty days. The metals of earthworm tissue and the substrates were analyzed at every 15 days interval. The metal concentrations in earthworm tissue and its bioaccumulation factor (BAF) were significantly higher in *E. eugeniae* than that in *E. fetida* and *P. excavatus* cultured in the three substrates seperately (P<0.05); and were low at the beginning i.e., at 15 days of the vermicomposting process and increased gradually towards the end of the procees in the ranking order of 15 days<30 days<45 days<60 days. The concentritions of Cd and Cu were higher in the earthworms cultured in MSW and that of Pb, Zn and Mn were higher in those cultured in VMW. The BAF of the metals followed the ranking order of Cd>Zn>Pb>Cu>Mn in the earthworm species, across the substrates and the time intervals; while, it was more than one in case of Cd.

Metals in earthworm casts are more mobile but earthworm mucus reduces mobility

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Human activities have resulted in an increase in the concentrations of metals in both urban and rural soils which can lead to toxic effects on flora and fauna. Generally earthworms appear to increase the mobility and availability of metals but the mechanisms for this are unclear. Earthworms burrow in the soil and create casts that are chemically, biologically and physically different to the surrounding soil. In addition, they also release mucus into the soil solution which has been shown to increase the uptake of metals into plants when added to hydroponic solutions (Zhang et al. Env. Poll. 157, 2009). In order to explain the mechanisms behind the impacts of earthworms on metal mobility in soils it is necessary to separate the effects that are due to passage through the gut from the effects due to the excretion of mucus. We used Lumbricus terrestris (L.) and soil that was amended with Cu (120 mg/kg), Pb (988 mg/kg) and Zn (241 mg/kg) fifteen years ago. One experiment aimed at determining the effect of earthworm mucus on metal mobility compared metal extractability from soil using a dilute solution of earthworm mucus and a control solution of deionised water. A second experiment compared water soluble metals in earthworm casts with bulk earthworm inhabited soil and earthworm-free control soil. Passage of soil through the earthworm gut significantly increased the concentrations of water soluble Cu (from 0.8 to 2.3 mg/kg), Pb (from 1.7 to 3.7 mg/kg) and Zn (from 1.3 to 2.9) by a factor of 2.9, 2.2 and 2.1 respectively. However, significantly less Cu (from 0.26 to 0.25 mg/kg) and Pb (from 0.51 to 0.34 mg/kg) were extractable with the mucus solution than with deionised water, although this effect is small compared to that of the gut. The greater mobility of metals in the casts is probably due to the release of organically bound metals during organic matter degradation in the gut. It is not clear what process is responsible for the reduced extractability of metals by the mucus solution. Before one can comment on the environmental significance of these findings, more information is required concerning the longevity of each of these effects and how diluted they become in the soil environment.

Earthworm survival in oil contaminated soil

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In the southwest of Mexico the contamination of soil by hydrocarbons due to the extraction conduction and transport of crude petroleum is frequent. In diverse studies on environmental risks, the earthworms have been used like toxicity indicators. The earthworms are associated with the health of soil and its absence can be an indicator of degradation of the soils. The knowledge about the sensitivity of earthworms to the contamination of soil by crude petroleum and its ability to survive in bioremediated soil is limited. The work objective was to evaluate the populations of earthworms in a contaminated soil (Arenosols) with crude petroleum. In the low basin of the Tonala-Tabasco River a contaminated arenosols soil was selected; it was because of a crude petroleum spill. In a contamination gradient, from the spill, 15 monoliths were realized in the three transects (50 m of length and 5 m of separation). In each, 5 monoliths became (25x25x40 cm). The monoliths separated in four layers (10 cm) and in each the earthworms were collected manually (diversity, distribution, abundance and biomass) and samples were taken from soil to determine the physical and chemical properties and the total hydrocarbon content. The Pontoscolex corethrurus earthworm was the dominant species. The distribution of the earthworms to the contamination gradient was similar with an abundance and biomass average of 296.5 worms/m² and 55.9 g/m², respectively. The vertical distribution (profile) varied with the contamination gradient. The preliminary conclusion suggests a tolerance of the earthworms to inhabit contaminated soil with intemperate crude petroleum exceeding the official norm.

Increasing temperature enhances Cd toxicity and accumulation in *E. Andrei*

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To determine whether the toxicity of cadmium to earthworms is affected by temperature, adult specimens of Eisenia andrei were exposed in OECD soil to a series of Cd concentrations (0, 250, 500, 750, 1000 g/kg) and incubated for 28 days in three different temperatures (15, 20 and 25 °C). During the exposure period, earthworms' biomass, reproduction and survival were assessed weekly. Results indicated that, at each temperature, the biomass decreased as metal concentration increased. However, higher temperature led to higher biomass loss. The highest biomass loss was observed at 1000 mg/kg Cd for all temperatures. These losses were 42.12% at 15 °C; 50.98% at 20 °C and 87.72% at 25 °C (means for all replicates). Survival data also indicated that temperature enhanced cadmium toxicity. At 15°C, mortality (3.3%) only occurred at 1000 mg/kg Cd. At 20°C mortality was recorded at 1000 (3.3%) and 750 mg/kg Cd (6.6%). At 25°C, mortality occurred at 1000 (66.6%), 750 (56.6%) and 500 mg/kg Cd (13.3%). An LC₅₀ of 725.46 mg/kg, for *E. andrei* exposed to Cd could only be determined at 25 °C because the mortality rates in the other temperatures were too low. Reproduction was completely inhibited in all Cd exposures. The incubation period of cocoons (that were only recorded in the control treatments) decreased as temperature increased (84 days at 15 °C, 21 days at 20°C, and 13 days at 25 °C). Inversely, the mean number of hatchlings per cocoon increased as temperature increased (0.5 at 15 °C, 1.56 at 20 °C, and 1.79 at 25 °C). The earthworms were analyzed to determine their Cd body contents at the end of the exposure period. These results revealed a dose response accumulation pattern and also an increase of Cd body burden in earthworms as temperature increased from 15 °C to 20 °C and to 25 °C. It is concluded that increasing temperature enhances Cd toxicity and accumulation in E. andrei.

Impacts of earthworms (*Eisenia fetida* and *Amynthas morrisi*) and organic matter on the availabilities of Zn, Cu, Cd and Pb in contaminated soils by heavy metals in Southern China

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Laboratory cultures experiments were conducted to evaluate the impacts of earthworm and organic matter on the availabilities of Zn, Cu, Cd and Pb with multiple heavy metals contaminated soils in Southern China for 60 days. Two species of earthworms, *Eisenia fetida*, and a native earthworm, Amynthas morrisi were introduced into the contaminated soil, which were taken from Dabaoshan mine in Southern China. And the total metals contents of the soil are $394 \text{ mg} \cdot \text{kg}^{-1}$, $405 \text{ mg} \cdot \text{kg}^{-1}$, 439mg·kg⁻¹, and 0.64 mg·kg⁻¹ for Cu, Zn, Pb, and Cd respectively. All the following treatments combinations were implemented in six treatments: (1) 100% soil, (2) 100% soil + Eisenia fetida, (3) 100% soil+ Amynthas morrisi, (4)10% organic matter (mixture of coco bran and cattle dung) + 90% soil, (5) 10% organic matter +90% soil+ Eisenia fetida, (6) 10% organic matter + 90% soil + Amynthas morrisi. Both of surface and internal casts of two species earthworm were separated from soil and collected respectively. pH, total contents of Zn, Cu, Cd, Pb and their DTPA-extractable fractions were measured respectively. Results showed that soil pH ranged from 4.71 to 6.50 and they increased significantly in 100% soil treatment with earthworm at the end of the culture (p<0.05), however, no variation has been observed in 10% OM treatments. For total heavy metal, cast of E. fetida have higher Cu and Pb compared to A. morrisi, while lower Zn and Cd. For DTPA-extractable heavy metal, results showed DTPA-Cd in cast of A. morrisi >E. fetida > control soil (p<0.05), while DTPA-Zn, Cu, Pb in cast of control > or $\approx E$. fetida > or $\approx A$. morrisi. As conclusion, our study confirmed different effects of two epigeic earthworm species on total heavy metal contents and their availabilities in contaminated soil with/without organic matter and proved A. morrisi could be a more superior to be used in the bio-remediation of heavy metal contaminated soil.

Session 8

Vermicomposting and Waste Management



The 9th International Symposium on Earthworm Ecology 5th to 10th September 2010, Xalapa, Mexico

Effects of Vermicomposts and Aqueous Extracts from Vermicomposts ('Teas') on Plant Diseases and Pests

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Vermicomposts are produced by interactions between earthworms and microorganisms, in breaking down food and animal organic wastes, to produce finely-divided, fully-stabilized soil amendments or plant growth media, containing available plant nutrients and plant growth regulators. They have dramatic effects on crop germination, growth, flowering and fruiting. Vermicompost aqueous extracts ('teas') were produced in commercial brewing equipment, from 20 parts vermicompost to 80 parts water (v.v.), to produce a 20% extract, that was diluted to 10% and 5% and compared with a water control. Substituting vermicomposts into equal amounts of a plant growth medium (MetroMix 360) decreased attacks by the root pathogens Pythium ultimum, Rhizoctonia solani, Verticillium sp., and powdery mildew almost completely, in laboratory and field experiments. Applying soil drenches of vernicompost aqueous extracts('teas') to plants, suppressed attacks by the root pathogens, Fusarium oxysporum, Phytophthora capsici, Rhizoctonia solani and Pythium ultimum, on tomatoes and cucumbers very dramatically. Leaf sprays with 'teas' decreased attacks by foliar pathogens, such as Plectosporium tabacinum, Botrytis cinerarea, Sclerotinia rolfsii and Verticillium sp., on tomatoes and cucumbers almost completely, in greenhouse experiments. The pathogen suppression must have been microbially-mediated, because sterilization removed the suppressive activity. The suppression mechanism could have been due to: microbial competition, antagonism, anti-fungal activity or antibiotics produced by bacteria. Substitution of 10%, 20% or 40% vernicomposts into the plant growth media (MetroMix 360), suppressed attacks by arthropod pests such as aphids, two-spotted spider mites, mealy bugs and cabbage white caterpillars, caged over cucumbers and tomatoes, almost completely. Vermicompost aqueous extracts ('teas') applied as to the plants as soil drenches suppressed numbers of aphids, mealy bugs, two-spotted spider mites, cabbage white caterpillars, tobacco hornworms and cucumber beetles, caged over the plants, almost completely in greenhouse tests. The degree of suppression was correlated with the 'tea' concentration. The most likely mechanism for the arthropod suppression appeared to be the uptake of water-soluble phenols from vermicomposts and 'teas' into the plant tissues, making them very unattractive to the pests, decreasing pest reproduction and killing pests.

Comparison of co-composting with and without *Eisenia fetida* for bioremediation of distillery sludge

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The aim of the present study was bioremediation of toxic distillery sludge into a soil enriching material. The sludge produced from the spent wash of distilleries has a high BOD, COD and nutrient imbalance and depresses soil flora and fauna of the disposal sites and pollutes environment for a long duration. This sludge was mixed with a complementary waste the cattle dung and subjected to cocomposting with (V) and without (T) Eisenia fetida. The sludge was mixed with cattle dung in the ratio of 0:100 (V1, T1), 12.5: 87.5 (V2, T2), 25: 75 (V3, T3), 50:50 (V4, T4), 75:25 (V5, T5) and 100:0 (V6, T6) w/w on dry weight basis and efficiency of vermicomposting (with E. fetida) and aerobic composting (without *E. fetida*) was adjudged with respect to time taken for biodegradation and quality of the end products from the two technologies. The distillery sludge was observed to be very toxic to earthworms even after precomposting of three weeks but the proportion of cattle dung in the mixture enhanced its acceptability for *E. fetida*. Survival rate, growth rate, onset of maturity, cocoon production and population buildup decreased with decreasing concentration of cattle dung in the mixture. Minimum mortality and maximum population buildup were observed in the 12.5: 87.5 mixture of the sludge and cattle dung. On the basis of response surface design the concentration giving highest number of worms, cocoons and hatchlings came out to be 21.11%, 24.51% and 17.19% respectively. The weight of cocoons as well as hatchlings also showed a negative correlation (r = -0.78) with the amount of distillery sludge in the mixture. Organic carbon declined significantly (p<0.05) in the vermicompost as compared to a small decline in the products of traditional aerobic composting. Nitrogen, phosphorous, sodium and pH increased in the products of vernicomposting but decreased in the products of traditional aerobic composting. However, electrical conductivity and potassium showed opposite trends in the products of these two techniques. An increase was observed in the contents of transition metals in the products of both the techniques but these were with in the international limits of heavy metals in the manures. The comparison clearly indicated that vermicomposting was a better technology over traditional aerobic composting for bioremediation of toxic distillery sludge as it gave a better product in five months time.

Extraction condition influenced vermicompost tea function

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In recent years, "vermicompost tea" was proved to have the function to accelerate crop nutrient absorption, decompose harmful toxins, and restrain soil-borne pathogen microbes. What components in vermicompost tea to bring these functions? Microorganisms, special nutrients or comprehensive substances? We try to give answer in this paper. The results showed vermicompost tea made by raw material from cow manure contained higher necessary nutrients and trace element and easily absorbed by plant, for example, soluble P, NO₃ - N and exchangeable Mg, K, Ca,, etc. In addition, soluble salt content, cationic exchange properties and humus acid content were significantly increased in vermicompost tea. By the joint action of microbes, organic matter converted into the stable humus complex. Some soluble humus acid can not only affect plant nutrition absorption, and can affect protein synthesis. In the microflora vermicompost was rich in bacteria, fungi and actinomycetes. These microbes plays a very important role, not only make the complex material mineralized into effective nutrients easily absorbed by plants, but also some produced bioactive substances, such as sugar, amino acid, vitamin etc in it. Microbial flora composition and content of nutrient levels have significant influence with different extraction temperature and extraction time from fresh vermicompost. On the conditions of 25 °C extraction temperature and 36h extraction time, the solution has the highest content of microorganisms and then the microbe quantity fell down rapidly. While the higher extraction temperature, bacteria and fungi vermicompost tea had significant adverse effects.

Oral - Session 8

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Polyacrylamide (PAM) is a general designation of homopolymer and multipolymer of Acrylamide (AM). Owing to own characteristic of molecular constitution, PAM is apt to produce modifier, through the charge neutralization and inter-particle bridging action, So It has been extensively used to in every field and was extolled as multipurpose product. Because of good flocculation capability of PAM, it is still the water treatment agent which was used much in water treatment filed at home and abroad, especially for wastewater treatment. The total usage of PAM was 65×104 t all over the world in 2005, and 40 percent of it has been used in water treatment field. Research indicates that the concentration of PAM in sewage sludge can reach 1000mg/kg (dry weight). In the past the PAM was considered as stable, safe and nontoxic substance. In fact, the PAM will produce various oligomer and monomer, under influence of physical, chemical and biologic factor in nature environment, and AM monomers have been shown clinically to act as animal carcinogens and neurotoxins. Therefore this study addressed the possible role of PAM to earthworm during vermicomposting through acute and subacute toxicity testing. The result shows the PAM and AM have effect on not only the living and growth but also reproduction of the earthworm when they reached a certain concentration.

Vermitechnology for fast conversion of toxic wastes of a soft drink industry into quality manure

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Large quantities of highly toxic spent carbon and chemical sludge are generated during the production of soft drinks. The industries generally dump them in or out of the premises in low lying areas. An attempt has been made to convert these wastes into nutrient rich manures through vermicomposting with *Eisenia fetida*. Both spent carbon and chemical sludge were very toxic to earthworms so these were mixed with cattle dung in various proportions. But 100% mortality of worms was observed even in 20:80 % mixture of spent carbon with cattle dung and 50:50 % mixture of chemical sludge with cattle dung. Therefore mixtures with higher percentage of each waste were discarded. Two mixtures per 2 Kg of each waste i.e 5% and 10% for spent carbon and 20% and 40% for chemical sludge were selected and inoculated with 20 worms. These mixtures were degraded after a long time of 6 months so another experiment was run with a variation in weight of earthworms (g) per Kg waste for reducing the time of recycling the wastes. The worms were inoculated at 7.5, 12.5 and 25 g/ Kg waste. It was noticed that recycling time and decline in organic carbon showed a positive correlation (r = 0.98) with weight of worms/ Kg in both the wastes. Mixture with 5% spent carbon and 20% chemical sludge were degraded respectively after 70 and 95 days with 25 g worms/ Kg mixture. However, chemical analysis showed that in final products nitrogen, phosphorous and sodium content was maximum in 12.5 g worms/ Kg feed mixture. Maximum increase in electrical conductivity was observed in 12.5 g worms/ Kg spent carbon mixture but in chemical sludge maximum increase was in 7.5 g worms / Kg mixture. pH declined significantly from the initial feed mixture (p < 0.05) and maximum decrease was in 25 g worms/ Kg mixtures. Although potassium and transition metals showed a decline over initial in all the products but the decline showed a positive correlation with the weight of worms in the mixtures.

Mechanisms of reduction of total coliform numbers during vermicomposting

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During vermicomposting of organic waste, the interactions between epigeic earthworms and the detrital microbial community lead to decreases in the abundance of some potentially pathogenic microorganisms. Despite its importance, little is known about the mechanisms involved and the factors that affect the intensity of this effect. We carried out three experiments to test the effect of the earthworm *Eisenia fetida* on total coliform numbers in pig slurry. We firstly applied low and high doses of pig slurry to small scale vermireactors with and without earthworms. We found that E. fetida significantly reduced total coliform numbers after two weeks, but only in the low dose vermireactors. In a subsequent feeding experiment in mesocosms, we observed that the coliform population was reduced by 98% after passage through the earthworms' guts, which suggests that digestive processes in the gut of E. fetida are the main factors involved in the decrease in total coliforms observed in the low dose vermireactors. Decreases in total coliform numbers were not related to decreases in bacterial biomass, which indicates a specific negative effect of earthworms on the coliforms. In the third experiment, we tested the indirect effect of earthworms on total coliforms by inoculating pig slurry with either 2 or 10% vermicompost. The addition of vermicompost did not affect the number of coliforms either after 15, 30 or 60 days, which supports the idea that this bacterial group is more affected by the passage through the gut of *E. fetida* than by interactions with the earthworm-shaped microbial community.

Efficacy of vermiwash-smeared mulberry leaves on cocoon characters of multivoltine hybrid mulberry silkworm *Bombyx mori* L.: Kolar Gold (K.G.) Race

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The effect of vermiwash and vermicompost in augmenting the growth process in a variety of plants is amply documented. Biological role of feed supplements like semi-synthetic diets and mulberry grown through vermicompost administration on the growth and cocoon characters of silkworms is also equally well known. In the present investigation, the effect of fresh and two week old vermiwash of two epigeic earthworms Eudrilus eugeniae and Perionyx sp. on the cocoon characters of the mulberry silkworm, multivoltine hybrid Kolar Gold race was studied. Mulberry leaves were collected from a field where different treatments were given to the existing three year old mulberry crop. The treatments to mulberry plants included spot application of 1 and 2kg, vermicompost, 30g NPK (300N:120P:120K) and Farm yard Manure (1kg) respectively. One set of the plants were sprayed with vermicompost extract (1kg. fresh vermicompost suspended in ten litres of fresh water and left for 72hr. before decanting to use as spray), one more set of plants received vermicompost brew (1kg. Vermicompost was treated with water at 1000C and slowly filtered through a gauge filter to get the brew to use as spray to plants) and finally spraying of water to third set served as control. The leaves collected from these treatments were used as feed to different batches of silkworms of fourth instar immediately after the third molt. The silkworms were divided into two groups and one was fed with the leaves collected from the plants exposed to different treatments smeared with vermiwash [1kg. earthworms were immersed in one litre warm water for 30 sec. in a tray and later transferred to another tray containing water at room temperature (26 to 280C) before releasing them back into bins. The fluid in both the trays were mixed and this served as vermiwash] and the other group was fed on the leaves without smearing with vermiwash (untreated leaves). The parameters recorded included weights of larvae, silkglands, cocoons, pupae, shell and floss. Vermiwash- smeared mulberry leaves grown with vermicompost and sprayed with brew showed significant influence on the cocoon characters when compared to other treatments. The data is analysed using SAS program to show the levels of significance.

Earthworm effect on gaseous emissions during vermifiltration of animal wastewater

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Vermifiltration is used to treat wastewater. As wastewater of animal farms is rich in available nitrogen and carbon, treatment of wastewater can be a source of ammonia (NH_3) , nitrous oxide (N_2O) and methane (CH_4). Earthworms have the advantage of mixing and transforming nitrogen and carbon without consuming additional energy. However this technology should not increase gaseous emissions. The objective of this paper is to analyze the specific role of earthworms on emissions of NH₃, N₂O and CO₂ during vermifiltration of animal wastewater. The experiment used fresh slurry of swine wastewater and the material of a vermifilter running with woodchips. Three levels of slurry input were added on eighteen mesocosms, with or without earthworms, during one month. The earthworm abundance was measured three times, and the gas emissions five times, with the closed chamber method. The results showed that earthworms decrease the NH₃ and N₂O emission when slurry levels are normal or high. A small increase of N₂O emission was observed with the earthworms associated to the lowest input of slurry. These effects are attributed to the role of earthworms in mixing the fresh organic matter after input and maintaining a connected free air space inside the porous media. We suggest that earthworms abundance could be used as a bioindicator of low energy and low green house gas emissions when slurry systems are converted to fresh slurry with water recycling.

Vermicomposting in Businesses and Institutions in N. America

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Vermicomposting converts organic waste materials, otherwise destined for landfills or incineration, into a valuable soil amendment called vermicompost that promotes plant growth and prevents plant diseases and pests. Thus, vermicomposting has environmental and economic potential. The primary organic components of waste streams for businesses and institutions are food residuals, paper, and yard debris. Some agencies already have their paper and yard waste collected for recycling (by their municipality or local businesses) and they want to focus on food waste recycling. Others desire to recycle all three streams on site. Businesses and institutions are becoming environmentally-conscious and looking for ways to cut costs. Vermicomposting is a viable method of diverting the organic portion of their waste streams away from disposal and instead converting it to a value-added product. More and more businesses and institutions are vermicomposting to manage their organic waste. Vermicomposting helps them save solid waste collection and disposal fees, and wastewater processing costs. Organic materials are vermicomposted on-site or transported to a centralized facility. Medium-scale vermicomposting takes place at businesses and institutions, such as prisons, universities, hospitals, office buildings, schools, and military bases. Vermicomposting provides a cost-effective and environmentally beneficial solution for managing food waste, paper products, and yard debris on-site. Vermicomposting helps businesses and institutions save solid waste collection and disposal fees, water usage costs, and wastewater disposal expenses. Brief case studies of a variety of businesses and industries that are vermicomposting food and paper waste will be presented.

Vermicomposting of biosolids: earthworm population growth and agronomic characteristics

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The biosolids that came from the wastewater treatment are a severe source of environmental contamination due the lack of an adequate disposal management. The vermicomposting can be used to enrich and mineralize biosolids in order to amend agricultural soils. The aim of this study was to estimate the adaptation of the earthworm *Eisenia foetida* to different mixtures of biosolids with cow manure and evaluate the quality of the vermicompost as fertilizer. Five treatments with three replicates were prepared in plastic containers (3 L) perforated in the base. The treatments contained biosolids and cow manure in a proportion of 70:30 (A), 80:20 (B), 90:10 (C), 95:5 (D), and 100:0 (E), with a moisture content of 60-70%, and were incubated at an average temperature of 15 ° C. The dried mass was 0.8 kg per container, with 50 mature earthworms each. Following 50 days of composting, cocoons, juveniles and mature earthworms were counted. Vermicompost was sampled and pH, bulk density, organic matter, organic C, total N, inorganic N and available P and C/N were determined. E. foetida could not tolerate the treatments D and E, likely due to ammonification processes and lack of oxygen of the biosolids. The treatment B had the higher survival (100%) and reproduction (77 mature, 18 juveniles and 150 cocoons). In the treatment C were detected the highest amounts of N and P available (1950.89 and 2203.96 mg kg-1, respectively) and a C/N mineralized product of 12.46, making it the better product as fertilizer; the values of bulk density (0.64 g cm-3), organic matter (54.83%), organic C (31.80%) and total N (2.55%) were within the ranges established for a vermicompost. With the exception of P (1882.15, 1929.18 y 2310.63 mg kg-1 in A, B and C, respectively), the differences between the physical and chemical parameters of the treatments were statistically significant (p < 0.05), which indicated that different proportions of biosolids and cow manure had different vermicompost characteristics. Therefore, the vermicomposting of biosolids and cow manure with E. foetida can produce amendments to improve soil fertility and nutrient availability in agricultural soils.

Suppressive compost: Vermicompost enriched with biocontrol agents

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Soil-borne diseases result from a reduction of biodiversity of soil organisms. Restoring beneficial organisms that attack, repel or otherwise antagonize disease causing pathogen will render soil disease suppression. Plants growing in disease- suppressive soil resist diseases much better than in soils low in biological diversity. The introduction of a food base with the biocontrol agent that supports its activity without stimulating the activity of pathogens can be a recent approach to govern rhizosphere competency. The application of organic wastes directly to soil leads to many problems such as immobilization of nutrients, phytotoxicity etc. Compost quality determines its effectiveness at suppressing soil-borne plant diseases. Vernicomposting is the use of earthworms for composting of organic residues. The information on vermicompost enriched with biocontrol microorganisms (suppressive vernicompost) is meager. The analysis of how microbial consortia in complex or compost amended substrates interact with one another, the pathogen, or the host to facilitate disease suppression has always been a challenge. In the present study following three objectives was investigated: a) the shelf life of biocontrol agents in vermicompost maintained at three different moisture levels. b) Influence of gauge size of polybags on the colony forming units of bio- inoculants in vermicompost and c) to compare sterilized vs. unsterilized vermicompost as the carrier for biocontrol agents. The biocontrol agents used were Pseudomonas fluorescens, Streptomyces sp. and Trichoderma viride; they were multiplied on King's B, Ken Knight's and Trichoderma specific medium respectively. The vermicompost was taken in 100g lots in polythene covers, sealed and thoroughly mixed . Three to four pin holes were made with sterile needle and incubated at room temperature. Initial population and the population at fortnightly intervals up to fifteen months were determined by serial dilution pour plate method. To determine the favourable moisture level for suppressive compost Phosphate buffer was added to obtain three different moisture levels viz. 15, 20, and 25 %. The results showed that the biocontrol agents at 20 and 15% moisture levels increased up to 30 days of incubation, thereafter it declined. The cfu/g of the all the biocontrol agents was significantly higher at 25% moisture level at all intervals of sampling. Thus it was concluded that 25% moisture level is more suited for longer shelf life of all the three biocontrol agents under study. To study the influence of gauge size of polybags on the vermicompost enriched with biocontrol agents the moisture content of vermicompost adjusted to 25% was taken in 100g lots in polythene covers of three different gauge size viz., 39, 47 and 50. The result obtained showed that the biocontrol agents had a longer shelf life with higher cfu/g in the thickest polybags i.e., 39 gauge. Thus it was concluded that 39 gauge polybag is better suited for longer shelf life of the suppressive vermicompost. Finally sterilized vs. unsterilized vermicompost were compared for the ability to harbor biocontrol agents using 39 gauge polybags and vermicompost at 25 % moisture level. The initial population of Pseudomonas fluorescens, Streptomyces sp. and Trichoderma viride were 1.42 x 10⁸, 6.00 x 10⁶, and 16.75 x 10^5 cfu/g respectively. According to the results obtained using sterilized and unsterilized vermicompost, the colony forming units of biocontrol agents were significantly higher in case of sterilized vermicompost as compared to unsterilized vermicompost with respect to all the three biocontrol agents. It can be concluded that 25% moisture level, sterilized vernicompost packed in 39 gauge polybags is best suited for the longer shelf life of suppressive vermicompost.

Opening the black box of vermicomposting: relationships of earthworms and microorganisms

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Microorganisms are the main decomposers, but earthworms also affect decomposition directly by feeding on and digesting organic matter, or indirectly through their interactions with the microflora, by 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 vermicomposting 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 reduced bacterial counts, whereas there were no differences between pig manure and the gut content of the other earthworm species.

Enzymatic activity decreases associated with the fall of microbial biomass during aging of vermicomposting

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Vermicomposting is the biooxidation and stabilization of organic matter involving the joint action of earthworms and microorganisms, thereby turning wastes into a valuable soil amendment called vermicompost. Studies have focused on the changes in the type of substrates available before and after vermicomposting, but little is known on how these changes take place, especially those changes related with maturation of vermicompost. In the present study, the effects of vermicompost aging especially patterns of changes in microbial biomass and enzyme activities were investigated because these parameters can control the quality of the resulting vermicompost. To do this we analyzed the pig slurry once earthworms have initially processed it (fresh vermicompost). We incubated 16 weekold vermicompost (fresh vermicompost) and sampled it after 15, 30, 45 and 60 days analyzing microbial biomass and activity (assessed as microbial biomass-N and basal respiration respectively), and four enzymatic activities (β -glucosidase, cellulase, protease, alkaline phosphatase). Aging of vermicompost resulted in decreases of microbial biomass and activity. Three of the four enzymes analyzed also showed decrease. An initial increase followed by a rapid decrease in alkaline phosphatase was also recorded. High and significant correlations between microbial biomass and β glucosidase (r = 0.62, P<0.001), cellulase (r = 0.56, P<0.01) and protease (r = 0.82, P<0.001) were found. Results suggest that there may be two steps involved in the aging dynamics of vermicompost with regards to extracellular enzyme activity; the first step was characterized by a decrease in microbial populations, which resulted in a reduction in the synthesis of new enzymes. The second step was the degradation of the pool of remaining enzymes. This dynamic does not seem to be affected by earthworms because similar decaying patterns of microbial biomass and activity were found in substrate where earthworms were present.

Earthworms enhanced cellulose decomposition during vermicompost by promoting of fungal biomass

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Cellulose is the most abundant polymer in nature and constitutes a large pool of carbon for microorganisms, the main agents responsible for organic matter decomposition. Cellulolysis occurs as the result of the combined action of fungi and bacteria with different requirements. Earthworms influence decomposition indirectly by affecting microbial population structure and dynamics, and also directly because the guts of some species possess cellulolytic activity. Here we assess whether the earthworm Eisenia fetida (Savigny 1826) digests cellulose directly (i.e. with its associated gut microflora), and also whether the effects of E. fetida on microbial biomass and activity lead to a change in the equilibrium between fungi and bacteria. By enhancing fungal communities E. fetida would presumably trigger more efficient cellulose decomposition. To evaluate the role of E. fetida in cellulose decomposition we carried out an experiment in which pig slurry, a microbial-rich substrate, was treated in small-scale vermireactors with and without earthworms. Five samples of substrate per module were taken at random and gently mixed for biochemical analyses, i.e. i.e. cellulose and hemicellulose content, microbial biomass C (Cmic), ergosterol content, and β -glucosidase and cellulose activities. The presence of earthworms in vermireactors significantly increased the rate of cellulose decomposition (0.43% and 0.26% cellulose loss day-1, with and without earthworms respectively). However the direct contribution of E. fetida to degradation of cellulose was not significant, although its presence increased microbial biomass (Cmic) and enzyme activity (cellulase and β -glucosidase). Surprisingly, as fungi may be part of the diet of earthworms, the activity of E. fetida triggered fungal growth during vermicomposting. We suggest that this activation is a key step leading to more intense and efficient cellulolysis during vermicomposting of organic wastes.

Carbon loss and microbial stabilization of substrates during vermicomposting depended on rate of manure application

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The decomposition systems depend on inputs of resources, that is, the components of the system do not have any control on their availability (regeneration). Vermicomposting is the process whereby organic residues are broken down by earthworms and microorganisms. Addition of manure has been shown to be of critical importance and determines most of the changes that take place during vermicomposting. Here we study how the rate of manure applied affects microbial biomass and activity and carbon losses. Five samples of substrate per module were taken at random and gently mixed for biochemical analyses, i.e. organic matter content, total C, microbial biomass C and basal respiration. We found that earthworms increased microbial biomass and were more active in reactors fed with 3 kg of slurry. However the differential rates of respiration were not reflected in C losses. The results thus showed that loss of C was not affected by the rate of pig slurry applied. We conclude that despite the strong effect that the rate of manure has on microbe-earthworm relationships, it did not affect carbon losses. We therefore recommend the use of low application rates of manure when the objective is the microbial stabilization of the residue.

Earthworms enhanced microbial populations triggering enzymatic activities during vermicomposting of pig slurry

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We assessed whether there is a relationship between earthworm activity, microbial biomass and the activation and dynamics of several enzyme activities during vermicomposting. Since decomposition systems are donor controlled we also study the effect of different rates of application of manure. For this, we carried out an experiment in which low and high rates (1.5 and 3 kg respectively) of pig slurry were applied to small scale reactors with and without earthworms. Five samples of substrate per module were taken at random and gently mixed for biochemical analyses, i.e. microbial biomass C (Cmic), microbial activity (basal respiration), β-glucosidase, cellulose, alkaline phosphatase and protease activities. In both rates of pig slurry applied, earthworms stimulated microbial growth which decreased once earthworms left the slurry. This increase was related to the initial activation of the microbial enzymes studied: β -glucosidase, cellulase, alkaline phosphatase and protease as correlations between microbial biomass and activity and enzymes showed. These strong relationships indicated an increase of intracellular enzyme activity. In the aged slurry, the pattern of activity of the four enzymes assayed depended on the rate of pig slurry applied. Thus, in low rate reactors, enzymatic activity through layers appeared to be related to microbial biomass, but in high rate reactors the activity of enzymes was more or less continuous. Further, these differences in overall enzyme activity agree with the variation found in extracellular enzyme activity suggesting certain dependence on substrate availability. We found that extracellular enzyme activity increased with rate of pig slurry, indicating a baseline of enzyme activity through decomposition. Thus, rate of pig slurry should be chosen carefully if vermicompost is going to be used as organic amendment, since earthworm activity did not decreased it.

Changes in structure and function of microbial communities improve decomposition during vermicomposting of pig manure

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Although microorganisms are largely responsible of organic matter decomposition during vermicomposting, earthworms may also affect to rates of decomposition directly by feeding on and digesting organic matter and microorganisms, or indirectly affect them through their interactions with the microorganisms, basically involving stimulation or depression of the microbial populations. We tested the general hypothesis that microbial populations, and especially fungi, are enhanced by earthworm activity, and also whether earthworms are able to modify the biodiversity of microbial populations, and its relation with the function of the system. In addition we examined the metabolic quotient and the effect of labile organic C to assess the relationships between earthworm and microbes. Five samples of substrate per module were taken at random and gently mixed for biochemical analyses, i.e. microbial biomass-C (Cmic), ergosterol content, basal and substrate induced respiration and Biolog® Ecoplate analysis. We found that decomposition of pig manure has two stages characterized by the presence or absence of earthworms. Thus, the presence of earthworms was related with increases in overall microbial biomass and activity which decreased when earthworms left the substrate; the same pattern was observed for fungi. Furthermore, earthworms modified the physiological profiles of microbial communities of pig manure, increasing the diversity of substrates utilized. In addition, earthworms promoted a more efficient use of energy of microbial communities as the metabolic quotient showed. The rate of carbon loss was almost twice in the presence of earthworms, revealing faster decomposition. Our data match with the recent findings that for maintaining essential processes the functional properties of present species are at least as important as the number of species per se. This is in accordance with the "insurance hypothesis" which states that a large number of species is probably essential for maintaining stable processes in changing environments, as presence of earthworms would have promoted in pig manure.

The Mexican Standard of vermicompost; scope and limitations

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Since July 2005, the "Humus Working Group" was formed, to reach the formal document of the Mexican Standard NMX-FF-109-SCFI-2008, published on June 10th, 2008. Regular meetings were conducted to establish the quality specifications to be met by vermicompost (solid state only) that is produced or marketed in Mexican territory. These meetings were attended by earthworm cast producers, researchers, associations and government institutions dedicated to the standardization and certification, all coordinated by the National Standardization Technical Committee of Agricultural Products, Livestock and Forestry, belonging to the SAGARPA (Ministry of Agriculture, Livestock, Rural Development, Fisheries and Food). The group established the initial references, the objectives and specific areas of application, definitions, descriptions and classifications of product quality. This was defined by sensorial (smell and color) and physicochemical specifications, including the presence of impurities, not digested and foreign materials, seeds and live earthworms. The parameters selected to verify their quality were: pH, electrical conductivity, water content, ashes, organic matter content, organic carbon, total nitrogen, C/N ratio, cation exchange capacity and bulk density. Not even with the existence of all these test and specifications, it cannot be assured, -without the possibility of error-, the certainty that some material is actually vermicompost; that means, without no doubt, the material processed by earthworms. Trying to assure this indisputable certainty, some representative samples of compost and vermicompost from different organic sources were requested to test them with the Near Infrared Reflectance Spectroscopy methodology, NIRS. This work showed the great potential of using this methodology, because apparently, it was not only able to recognized the difference between compost and vermicompost, but it was also able to discern the origin of organic matter used to feed the worms, even in mixtures. However, it remains to do more comprehensive and complete studies, using more samples from different organic materials, paired on both processing techniques, -with or without earthworms-, to discriminate those NIRS wavelength values (nm) characteristic of the earthworm cast (earthworm signature) and perhaps, some other useful quality or quantity organic matter parameters.

Consolidation of the Vermicomposting Technique at the Organic Solid Waste Program in the Municipality of Teocelo

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Teocelo municipality is located 15 miles from Xalapa, the state capital of Veracruz, in the coffee region of the state's central highlands. Teocelo has a population of 14,900 inhabitants, 9,062 of which live in the municipal center and the rest in 20 rural towns scattered in a vicinity of 21 square miles. From 2001 to 2003 and from December 2008 till now, the municipality took the alternative to operate a vermicomposting module to transform organic wastes into fertilizer. Daily, approximately 7 tons of agricultural, agro-industrial, forest and domestic organic waste are collected, transformed into vermicompost and distributed among local producers through agricultural promoting programs. The challenge of this program is not only the organic conversion by the worms, but it also consist to know how to combine low cost environmental technology, citizenship participation and economic development promotion. Up to now these are poorly articulated in local government policies in Mexico. Although there are very little implementation of equipment, techniques and methodologies which are rudimentary and little systematized, besides the difficulty to modify behaviour of the public's patterns for solid waste separation, the result of the vermicomposting treatment are comparatively improved and process costs are lowered to the half. At this level of municipal implementation, a multidisciplinary group has been formed, in order to technify the vermicomposting treatment, to rationalize and improve the general operational methodology, implementing quality control standards and build a procedure's manual for a wider application, in other municipal solidwaste processing facilities. Beyond the consolidation of the vermicomposting technique and the regional impact of the program, the scope of the multidisciplinary group, is to replicate and escalate many more processing facilities, to many other rural, small size municipalities in Mexico, which have equivalent economic constraints; thus discouraging the expansion of sanitary landfills, toward a better, cost-effective and environmentally-beneficial solution. This project has the financial support of the National and State Council of Science and Technology, (CONACYT and COVECYT) with the Fordecyt fund.

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Physicochemical characterization of vermicompost from rumen content and hen manure

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Handle and final disposition from solid residuals generated by municipal slaughterhouses (rumen content) and hen manure generally do not receive a suitable treatment; therefore causes a very serious contamination problem in the environment and life quality of human being. The aim of this study was to produce vermicompost using *Eisenia foetida* for recycling rumen content and hen manure, as well as to characterize physicochemically both vermicomposted. In order to do recycling process; eight beds settled down, four were for recycling rumen content and the rest for hen manure, the beds had the following measures: three meters in length by one meter in width. The physicochemical characterization of the vermicompost was made according to the methodologies recommended by the NMX-FF-109-SCFI-2008 (Mexican Standard). Both substrates were stabilized in a lapse of 45 days. The vermicomposting process is a good alternative for handle and disposition of slaughterhouses residuals and hen manure from farms as long as it is fulfilled four fundamental requirements: a) Superficial and underground waters are protected of contamination, b) reduce the risk of propagation of animal diseases, c) avoid de prescence of flies, larvae, noxious fauna, etc. and c) keep the air without of bad odors. Vermicomposting is a simple, economic and very effective procedure for recycling organic residuals. We obtained a mature and stable vermicompost, we can conclude that vermicompost from rumen content and hen manure can be used as biofertilizer or to enhance degraded soils.

Ecofriendly Treatment of Biomedical waste Using Epigeic earthworms

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Hospital wastes are hazardous and need to be disinfected before disposal. Biomedical waste (BMW) contains spilled liquid orals, antibiotics in wound dressing and tissues resulted during surgery constitute 30% of the urban waste (Walsh et al 2000). Land filling and incineration are the present methods. Former method leading to soil pollution and the latter is an expensive and energy consuming process. The present study was conceived using earthworms Eisenia fetida, Eudrilus eugeniae, and Perionyx excavatus to compost the bio-degradable part of infected BMW. The Bio Medical Waste was primarily treated with 5% NaCl for chemical sterilization process and the same was later subjected for detection of pathogens by pour plate method with specific media. The pathogens analysed in the BMW were Escherichia Coli, Staphylococcus aureus, Salmonella typhii, Pseudomonas auriginosa, Bacillus cereus, Clostridium sp., and Klebsiella. They were in the order of $2x10^6$ cfu/g using bio-rad microbial colony counter. Initial processing of the waste was done by adding cow dung slurry in 1:1 ratio. A biomass of 200g of each species of earthworms was released into the containers filled with 5kg of waste and a control was maintained without earthworm. After 30 days of composting period, increase in earthworm biomass, compost yield and pathogen load in the compost were analysed. The study revealed that *Eisenia fetida* exhibited better performance in conversion of BMW with 40% increase in biomass with 60% compost yield. The biomass increase in Perionyx excavatus was 25% with 45% compost yield and in Eudrilus eugeniae, it was 15% increase in biomass with 40% compost yield. It is clear from the results that *Eisenia fetida* is more suitable species for composting of BMW than Perionyx excavatus and Eudrilus eugeniae. There was decrease in pathogen level when compared to control during composting compare to control from $2x10^6$ cfu/g to 1×10^2 cfu/g. The composted material is subjected storage in polythene bags. There is no further increase in pathogens level inferring the ability of Vermicompost in suppressing the growth of pathogens of BMW.

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Vermicomposting of sugar factory pressmud using two different species of earthworm via., *Eudrilus eugeniae* and *Lampito mauritii* was prepared. Plant nutrient contents of these vermicompost and their effects on black gram (*Vigno mungo*) growth in relation to the effects of sole soil were investigated. Vermicompost produced by the two species of earthworms differed in their nutrient concentrations. But possessed higher concentration of total N and Ca than that of the control. The results showed that effects of vermicompost are more efficient for the vigorous production of black gram. Vermicompost had significant positive effects on flower number, leaf growth, shoot length, root length, number of leaves, leaf area index, wet weights and dry weights compared to control. It is also suggested that pressmud compost is more favourable for vigorous production of black gram and maintenance of soil environment but vermicompost can be economically and environmentally suitable.

Earthworms modify microbial community structure and activity and accelerate decomposition during vermicomposting

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The vermicomposting process includes two different phases regarding the activity of earthworms, (i) an active phase during which earthworms process the organic waste, thereby modifying its physical state and microbial composition, and (ii) a maturation-like phase marked by the displacement of the earthworms towards fresher layers of undigested waste, during which the microbes take over the decomposition of the earthworm's processed waste. In the present study we investigated the direct and indirect effects of the epigeic earthworm species Eisenia fetida on the microbial community structure (bacterial and fungal PLFA biomarkers) and activity (basal respiration), and on organic matter decomposition (total and dissolved organic carbon content) during the vermicomposting process of pig slurry. We set up a batch of twelve reactors, six with a low dose of pig slurry and the other six with a high dose. For each dose, three reactors were inoculated with 500 mature specimens of E. fetida and three remained without earthworms (control). Each reactor was initially composed of one module containing vermicompost from pig slurry, in which earthworms were placed, and another module containing a layer of fresh pig slurry (1.5 or 3 kg fresh weight, low and high rate, respectively). New modules with the same amount of fresh pig slurry were added sequentially following the feeding activity of the earthworm population. At the end of the experiment, the reactors 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. The principal component analysis of the 25 identified PLFAs clearly distinguished between samples in function of earthworm presence and age of layers in both high and low rate reactors. The activity of epigeic earthworms reduced the abundance of bacterial and fungal PLFA biomarkers relative to the control during vermicomposting; although their impact on bacterial populations was more pronounced in low than in high rate reactors, probably due the fact that the greatest losses of carbon as a result of earthworm activity occurred in the reactors fed with 1.5 kg of pig slurry. These findings indicate that detritivorous earthworms play a key role in the decomposition of organic matter during vermicomposting via their intense interactions with decomposer communities, which may have important implications for the stabilization of the organic residue.

Bulb formation in *Lilium sp* from hypogeous bulblets grown in different proportions of vermicompost

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There are a technological dependence of Mexico in the ornamental horticulture, especially in the acquisition of vegetative material; thus is needed research of appropriate, sustainable technologies that allow to obtain good quality bulbs, adapted for satisfactory crops, specially for producers with low input resources. In the majority of the cases these producers do not have access to imported materials, high cost technology, surely resulting in good quality vegetative materials. The formation of bulbs of *Lilium sp.* with a technology adapted to zones and low input resources, does not imply the violation of rights of possessing one, since this technology is realized by themselves, cultivating free-access varietes, not protected by the rights of possessing. In this context, the aim of the present work was the production of bulbs of *Lilium sp.* from hypogeal bulblets grown in different proportions of vermicompost. The work was realized in the Faculty of Agricultural Sciences of the UAEM, in greenhouse conditions; we can conclude that the best depth of sowing the hypogeal bulblets is around 15 cm., the best time of refrigeration to induce vernalitation in the hypogeal bulblets is around 90 days at 2°C. The mixture formed by 60 % of vermicompost, 10 % litter soil and 30 % perlite was the best option in terms of leaf area , fresh weight and length for the initial bulb.
Effects of biological conversion on winery waste using Eisenia foetida

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Our observations and literature search have shown that vermicomposting is a very suitable method for utilization of waste from wine production. This organic mater is usually used as an agricultural fertilizer, however, overproduction of grape pomace from wine production in recent years has led to its direct use in rural areas and due to the release of tannins and phenols, this trend has resulted negative effects on growth of plant roots. Not only this negative effect of tannins, but also some of adverse qualities of matter could be reduced by the treatment of wine waste using biological convertion of matter (vermicomposting) as an alternative to their disposal or use for direct application. The paper summarizes results of experiments aimed at utilization of wine waste using Eisenia foetida in laboratory and outdoor conditions. There are described also technological aspects of the process of biological conversion and main significant changes in product quality, especially increase of the agronomic value, reducing of the C:N ratio, increase of pH, content of nutrients and higher microbiological activity.

Effect of Aloe vera composted residues on reproduction of Eisenia foetida

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Aloe vera is a succulent plant introduced to America and widely cultivated for medicinal uses. Aloe industry that processes the leaves and remove the gel, which made cosmetics and therapeutic drinks, discard the mesophyll (M) enveloping the hydroparenchyma, representing almost 30% of wasted tissue. Moreover, on large plantations with low expansion, separation of suckers (S) from mother plants, is also a vegetal biomass that is lost if they are not planted in new areas. The aim of this work was to evaluate the reproductive performance of the Californian red worm (*Eisenia foetida*) experimentally raised in different composts made from a mixture of horse manure (HM), dry chaff (DC) of *Phragmites australis* and aloe fresh residues (M and S). Preliminary composting lasted 90 days, using heaping with roll over method. Multiplication rate (MR) of earthworms (EW) was evaluated, set initially at a density of 15 EW/L in plastic crates of 40 L. Treatments were established: T1) composting of the mixture 90% HM + 10% DC; T2) 60% HM + 30% M + 10% DC; T3) 30% HM + 60% M + 10% DC; T4) 60 % HM + 30% S + 10% DC; T5) 30% HM + 60% S + 10% DC, with five repetitions, humidity 65-80% and temperature 22-26 °C. The experiment ended after 120 days, and significant differences between treatments (ANOVA).

Growth and reproduction of the earthworm, *Perionyx ceylanensis* Mich. during vermicomposting of different substrates

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It is absolutely essential to study the growth and development of earthworms in order to promote vermiculture based biotechnology. This may help in understanding the organism, selecting prolific breeders, predicting the population characteristics. *Perionyx ceylanensis* Mich. is a purple-red coloured epigeic earthworm, mainly found in biogas slurry, dung pats, composting heaps and decomposing leaf litter heaps and has been recently reported for its efficiency in vermicomposting. But its growth and reproduction during vermiconversion of organic materials is not studied in detail. Hence the present study has been carried out to assess the growth and reproduction of P. ceylanensis during vermicomposting of pressmud, pressmud+leaf litter, cowdung and cowdung+leaf litter. trials in $45 \times 35 \times 15$ cm plastic troughs containing 2 kg of substrates. The vermibeds were maintained at $27\pm1^{\circ}$ C with a moisture content of $70\pm5\%$. Sixty clitellate *P. ceylanensis*, were placed in each trough covered with wire mesh. The cocoons were collected from the vermibeds after 10th of inoculation up to 60 days and the worms were weighed at the termination of study on 60th day. The highest individual biomass of 780 mg/worm was recorded in pressmud followed by cowdung and cowdung+leaf litter (1:1). The cocoon production rate of 0.95, 0.98, 0.89 and 0.92 cocoon/worm/day was observed in pressmud, cowdung, pressmud+leaf litter and cowdung+leaf litter. The present study shows that the earthworm species, P. ceylanensis is a prolific breeder comparable to that of P. excavatus.

Eradicating flies (Diptera) in earthworm ecological boxes

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A consumerist lifestyle generates great amount of waste, including organic waste. One of the ways to manage it on-site - in a place it is produced, is "earthworm ecological box". Vermicomposting in those small containers can be problematic as relatively small earthworm population loses its dynamics of growth easier in ecological boxes, it can also become dominated by competing Enchytraeidae and, what is more uncomfortable to operate, flies Sciaridae can proliferate in ecological boxes. The aim of the experiment, which concerned bio-preparation "Owinema" and a xenobiotic DAR 2.5 GR (active substance: chlorfenvinphos), was to observe the impact of the products eradicating flies larvae on the state of earthworm Eisenia fetida (Sav.) which vermicomposted (the same amount and quality) of kitchen waste. The doses of the preparations used were suggested by the producer. Before experiment the number of flies had been increased (by multiplying it for 4 weeks on mushroom mycelium infected with larvae of Sciaridae). In each of 20 containers (capacity of 2 dcm³) 50 earthworms were bred for 5 months. The dynamics of the population properties was being checked on regular basis by hand sorting. The rate of vermicomposting and the quality of the fertilizers produced were also determined. It was observed that the bio-preparation "Owinema" had a positive impact on the average number and biomass of earthworms when compared to the control.

Short term effects of vermicompost on soil microbial and biochemical properties in a sweet corn (*Zea mays*) crop

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Organic fertilizers have shown to improve significantly the soil status through the amelioration of its physical and biological properties. Therefore they allow an increase in soil fertility which could result in better crop growth or quality. However these changes are normally produced in the long term therefore questioning the profitability of this type of amendments in the short term as compared to synthetic mineral fertilizers. The short-term effects of the partial substitution of mineral fertilizers by vermicompost on the soil biological properties of a sweet corn crop were evaluated in a field experiment. The effects of the equivalent non-processed organic matter (manure) were also evaluated in order to determine if earthworm processing produced any biological gain in the fertilizing capacity of this material. Full inorganic fertilization was used for comparison and two doses (normal: 80/24/20 and high: 120/36/30) of the three fertilizing treatments were added. Experimental units consisted of 10m2 plots each with one combination of fertilizer and dose. Plots were overplanted and thinned to obtain a final density of approximately 60000 plants ha-1, and arranged in the field following a randomized block design. At harvest, three months after sowing, the concentration of available plant macronutrients (N-NH₄, N-NO₃, K₂O, PO₄), microbial community structure (PLFAs), and activity (protease, phosphatase and glucosidase activity, basal respiration), were evaluated in soil samples collected from the plots. Macronutrient content was similar among the different fertilizing treatments. Significant differences were only observed in the case of PO₄ which was significantly higher in vermicompost plots. Partial substitution of mineral fertilizers by vermicompost produced also significant changes in the structure of the microbial community as evidenced by the discriminant analysis of the phospholipid fatty acid (PLFA) profiles of the soil samples. Changes were due to an increase in gram negative bacteria with vermicompost and manure as compared to full mineral fertilization, especially in the case of manure. These changes were accompanied by an increase in soil metabolic activity with significant increases in phosphatase and glucosidase activities with vermicompost and protease activity with both organic fertilizers. The integrated use of organic and inorganic fertilizers significantly improved the soil biological properties as compared with full inorganic fertilization while maintaining the concentration of available plant macronutrients. Effects were observed in a three month growing period showing that these types of practices do not risk crop productivity and allow a short-term amelioration of soil properties. Vermicompost enhanced the release of PO_4 although no other beneficial effects were observed as compared to manure.

Assessment of fertilizer power of vermicompost obtained by utilization of municipal slaughterhouse wastes

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The common problem in municipal slaughterhouses in Mexico is the absence of processes for management and final disposal of solids and liquid wastes. Approximately 20% of the total animal weigh slaughtered are solid wastes, such as rumen, manure and liming. Today, these are not used for food consumption and have no commercial value. The aim of this research was to assess the fertilizer power of a vermicompost and leachate obtained from slaughterhouse waste. The rumen and manure of slaughterhouse were mixed with vegetable wastes and pre-composted for eight days. The vermicomposting process was performed in a laboratory with 8 kg of total wastes and 0.5 kg of Eisenia fetida earthworms (200 individual m-2 density) with four replicates during two months. Vermicompost and leachates from the process were recovered and characterized by physicochemical parameters, microbiological and phytotoxicity tests. An experimental design (32) in triplicate was used to investigate the vermicompost and leachate fertilizer power with a tomato crop in a greenhouse during three months. These were compared with a commercial fertilizer. Three dose levels of fertilization were used: high (300 kg N·ha⁻¹), medium (200 kg N ·ha⁻¹) and low (100 kg N ·ha⁻¹). Height, number of leaves, chlorophyll, photosynthesis, total leaf area and total weight of fruit were evaluated. The yield of transformation of waste by vermicomposting process was from 55% to 65% and 1.9 L of leachate was recovered. The N/C, P/C K/C ratios of waste have a direct relationship with fertilizer power from the vermicompost and leachate. Those have a significant effect on plant growth and yield from a tomato crop. The vermicomposting process was successful in converting the municipal slaughterhouse waste. The vermicompost and leachate obtained had potential characteristics for use as organic fertilizer.

Characterization of Organic Alternative Substrates for the Production Of Potato Tuber- Seed

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Mixtures of organic and inorganic substrates for food production in greenhouses need to be evaluated physically, chemically and agriculturally in order to know their properties and thereby adjust the ferti-irrigation system. Thus to be used in intensive production systems as the seed potato production in greenhouses. We evaluated six mixtures of substrates by replacing only the peat component of the mixture used traditionally by producers, which consists of 40% coir, 40% peat and 20% perlite, all in a relationship v/v, materials. The substrates to test were peat (control), pine sawdust, rice hulls, compost from agave tequila, vermicompost from coffee pulp and sugar cane bagasse. The potato varieties used were Alpha and Atlantic. The substrate mixtures were placed in beds divided into squares 0.5 m x 0.5 m, with a height of 15 cm and a density 69 plants m^{-2} . Each treatment had four seed tubers, with 12 repetitions, having a total of 144 experimental units l, 288 tubers of each variety wasused. The results indicate that the largest number of mini-tubers per plant in the Alpha range were obtained in the screening substrate mixture of coffee pulp (9.5) was statistically peat treatment (control) 5.75 and for the variety Atlantic sieving treatments coffee pulp (5.9), compost of agave tequila (4.7), peat (4.4) and rice husk (4.4). The aeration capacity (AC) was higher in treatments with 15.9% sawdust, coffee pulp screening (15.8%) and rice bran with 12.8%. In the total pore space (which is the sum capacity of aeration and moisture holding capacity) processing peat (76.0%) and bagasse from sugar cane (75.3%), where the latter is not allowed in irrigation water beginning of the experiment (hydrophobicity), and this present compaction. On both Alpha and Atlantic range submitted diseased tubers with common scab (Streptomyces scabies), although with a low incidence per plant.

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Quality of humus produced by *Eisenia andrei* from three organic substrates. Utility in agriculture and ecotoxicology

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This study examined physical chemical characteristics of humus produced by earthworm *Eisenia* andrei from three organic substrates prepared with a mixture of horse manure (HM), pruning wastes (PW) and kitchen wastes (KW) in the following treatments: T1 (60% HM + 40% KW), T2 (60% HM + 20% KW + 20% PW) and T3 (60% HM + 40% PW). *E. andrei* was used due to their biological characteristics conducive to biotransformation of organic wastes into fertilizer. Humus samples were sieved (that humus produced from these wastes have high levels of macro and micronutrients and heavy metals concentrations below the limits set by environmental legislation, making it an optimal choice for fertilizing crops.

Bio-remedial measures of a monocot weed, Saccharum munja using Eisenia fetida and impact of vermicompost on the shoot length of Vigna radiata

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Saccharum munja (munj) – a cosmopolitan weed, is known to develop barren conditions in the soil, has been taken for its bio remedial measures by inoculating epigeic worm, *Eisenia fetida*. Weed was collected from nearby University area, chopped into small pieces of 2 cm size. The cut pieces were mixed with cattle dung in 1:3, 1:1 and 3:1 ratio separately. The experimental sets of each ratio were prepared in triplicate in rectangular plastic tubs, each having weight of 4 kg. The control sets, devoid of earthworms, were also put aside with respective experimental sets. Ten g matured E. fetida were introduced in each of the experimental sets after 15 days of pre-decomposition period. Physicochemical parameters of weed: dung media and vermicompost were analysed by using standard techniques. The experiment was conducted for successive two years. It was noticed that 1:3 weed: dung medium transformed earlier into vermicompost than that of other two media (1:1 and 3:1). Rate of reproduction of worms was recorded 2.47 times faster and their total biomass was 2.15 times more in 1:3 medium than that of 3:1. Vermicompost transformed from 1:3 weed: dung medium when used with soil in the ratio of 1:1 showed excellent results in the shoot length (26.0 cm) of pulse plants, Vigna radiata, although increase in shoot length (15.5 cm) was also noticed when vermicompost transformed from 3:1 weed: dung medium was used with the similar ratio of soil. It may be concluded that by using epigeic earthworms, the weed may easily be transformed into biomanure that could be used in improving soil health for the better crop yield.

Effect of the type and proportion of physical conditioners on the biology of earthworms and chemical properties of the humus

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The use of crop residues with high C/N ratio mixed with the food offered to the earthworms can be useful to improve environment drainage and aeration, promoting the earthworm development and reproduction of the. The objective of this work was to evaluate the effect of two materials for physical conditioners of the food on biological aspects of earthworms and their impact on the chemical properties of the produced humus. The study was conducted at the "Estação Experimental Cascata", "Embrapa Clima Temperado", in Pelotas, RS, Brazil, using cattle manure as the main food and oat straw chopped and crushed peanut hulls as physical conditioners. Plastics pots of 100 mm diameter were filled with cattle manure (CM) and mixtures of CM with chopped oat straw (OS) or crushed peanut hulls (PH), at 2:1 (OS33 and PH33) and 1:1 (OS50 and PH50) in volume/volume ratios. In each pot were placed 10 adults of Eisenia andrei and weekly evaluated their biomass progress, cocoon production and food volume evolution. After 68 days the chemical characteristics of humus were analyzed. A completely randomized design, with five treatments and three replicates was used. The means were compared with the Scott-Knott test at the probability level 5%. Physical conditioners did not affect the biomass of earthworms. However, there was a tendency of the earthworms in the OS50 treatment to produce larger biomass, and the PH50 treatment to produce smaller biomass. The OS50 and CM treatments did not differ amongst themselves in relation to the total average cocoon production, ranging between 300.52 and 285.26 cocoons, but they were statistically higher than other treatments. The lowest value occurred in the PH50 treatment, with only 256.02 cocoons. The final volume of produced humus in the OS50 and OS33 treatments was 47.9% and 52.4% of the original volume, respectively. PH33, PH50 and CM treatments did not differ amongst themselves in relation to the final volume, ranging between 65.6% and 77% of the original volume. PH33 and PH50 treatments showed smaller levels of N, P, K, Ca, and Mg, while OS33, OS50, and CM had similar values amongst themselves. The results indicate that the use of chopped oat straw in a 1:1 ratio by volume with cattle manure favours the reproduction of the earthworms without affecting their growth and the chemical properties of humus.

Production of biofertilizer (*Rhizobium*) enriched vermicompost from pressmud using *Eudrilus eugeniae* and *Perionyx ceylanensis*

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In the present study, a trial has been made to prepare Rhizobium enriched vermicompost from a sugar-industry by-product, pressmud so as to get dual benefit from a single product. Vermibeds were prepared in plastic trays of $45 \times 30 \times 15$ cm size with 3 kg of 20 days pre-decomposed pressmud in each tray with a moisture content of 65-75%. After 24 hrs stabilization clitellate adult worms (30 numbers of Eudrilus eugeniae Kinb./trough and 60 numbers of Perionyx ceylanensis Mich./trough) were introduced. Rhizobium leguminosarum (MTCC 99) procured from Microbial Type Culture Collection (MTCC) Chandigarh was mass multiplied in Yeast Extract Mannitol Salt Agar (YEMA) media and used for the study. R. leguminosarum at the rate of 30, 35 and 40 ml/100 g of substrate was inoculated on 0, 10, 20 and 30 days of vermicomposting. Each experimental set-up was maintained in triplicate. After 40 days of vermicomposting, the vermicompost was harvested and analysed for total NPK, organic carbon, C/N ratio and total viable population of R. leguminosarum. The viability count of *R. leguminosarum* in vermicompost was carried out by spread plate method using YEMA medium. Once in 15 days, the vermicompost was analysed for the total viable count of R. leguminosarum up to 75 days to assess the survivability upon storage. The C/N ratio of pressmud vermicompost produced using E. eugeniae and P. ceylanensis was 13.4 and 14.2 respectively. The total NPK contents of the vermicomposts produced by both the worms showed increase due to mineralization. On 75th day of storage, the pressmud vermicompost of E. eugeniae showed 4, 7 and 6 CFU x 10^7 viable cells of *R. leguminosarum* respectively in 30, 35 and 40 ml/100 g inoculated on Oth day of vermicomposting. The inoculation of R. leguminosarum at the rate of 35 ml/100 g substrate on 30th day of vermicomposting showed higher viable counts on 75th day of vermicompost storage. The same trend has been observed in the pressmud vermicompost of P. ceylanensis. It is concluded that the amount and time of inoculation of R. leguminosarum in vermicomposting systems are important for the long term survival of the biofertilizer organisms to get multiple benefits when the vermicompost is applied to soil.

Preference of Eisenia foetida for vegetable waste of kitchen

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The use of earthworms is an alternative to organic waste management, especially in urban areas, in addition to adding vermicompost to the soil they will not only improve in nutrient content but also in structure. The objectives of this study were to quantify the acceptance of the California red earthworm (*Eisenia foetida*) to bovine manure and organic waste from kitchen, both composted. The bovine manure came from the livestock facility and the waste plant material from the dining room both from Instituto Tecnologico de Roque. The treatments were: (I) composted manure EC, (II) organic waste DO, (III) combination of EC + DO 1:1 proportion (IV) combination EC + DO 2:1 proportion and (V) combination EC + DO 3:1 proportion. Each treatment had four replicates, were placed in plastic containers of 20 litres. The moisture content was 80%, the experimental units were placed in a greenhouse and inoculated with the earthworm, 50 per experimental unit. The substrate temperature was measured every day and checked that the humidity in the substrate was 80%. A sample of substrate by repeated to quantify the density of earthworms, total biomass and was determined the pH of substrate. The study was done with a design in complete blocks at random. The population counts of young and adult earthworms were performed at 30, 60 and 90 days. According to statistical analysis, highly significant differences exist for treatment. The Tukey test shows that the treatment (II) DO, than the other treatments since it significantly increased the population of earthworms. It was also noted that the number of cocoons exits differences in treatment, increasing these one in the treatment II. Differences in pH and electric conductivity were detected in the substrates, for treatment II the values of these variables were the lowest.

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An eco-engineering of integration of crop-vermiculture to treat organic waste in situ

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We designed an intercropping system with vermiculture in the field to process organic waste in situ. the study was done using winter-wheat, summer-maize rotation cropping system, with rows of vermicomposting beds in between to transform organic wastes such as cow manure, municipal sludge and mushroom residuals, comparing with conventional agriculture. We evaluate the effectiveness of the model considering different organic wastes convertion, the crop production, the environmental effects and the economic benefits. The results showed that the social and economic benefits of the cropping-livestock system of corn/wheat- earthworm were higher than that of the traditional cropping pattern of corn wheat: (1)The system is an effectively waste processing method; the waste reduction and the nutrient content obtained was different because of different types of organic waste used. Manure treatment not only get a 70% reduction but also get a more high-quality organic fertilizer, which total nutrient reached 4.3%, in contrast to this values, mushroom waste treatment only obtain a 55% reduction and 2.8% total nutrient content. (2) In this system, through the rational close planting, we can get a higher corn yield than traditional, but a half when grow wheat because of the decreased planting density. But the overall seed weight cropped, size of the grain, nitrogen, phosphorus, potassium, protein content, is better than of the traditional. (3)In the harvest season, we not only get corn or wheat, but also earthworm and organic fertilizer, with no chemical fertilizers input and mechanical cultivation reduction, we get an increased revenue form crop-vermiculture system than traditional agriculture. (4)The earthworm activity certainly improved the soil physical structure, maintained and increased the soil nutrients, playing a fertililizer role. (5) We found that the treatment with sludge -with available heavy metals, results in a slight enrichment of Zn content in soil; In the harvested grain was also higher, but no more than the National Security Standard. In a word, the organic waste produced on the farm was transformed by earthworms and at the same time, kept a good soil quality for sustainable organic farming system. The cropping-livestock system could not only improve the soil fertility and structure, but also controled the weeds in winter. Therefore, there was a big decrease in amount of herbicides and pesticides, and the field ecology and environment could be protected well.

Interaction of vermicompost and peanut in iron mine tailing ecological restoration: laboratory experiment

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In order to improve poor fertility and microbial environment of iron mine tailing in its ecological restoration, cow manure vermicompost was applicated into iron mine tailing, and peanut (Arachis hypogaea) as restoration species was sown in mixture of iron mine tailing and vermicompost in laboratory condition. Four application rates of cow manure vermicompost (in volume), 10% V(1:9), 20%V(2:8), 30%V(3:7), were designed in this experiment, and 0%(0:10) was control, CK. Three peanut seeds were sown in each pot contained 1kg iron mine tailing or mixture of iron mine tailing and cow manure vermicompost. Three replicates were tested for each treatment. Each pot was watered with 150mL tap water every day. The germination, plant height, biomass of plant were recorded during experiment. 35-day after germination the seedlings were harvested and three nutrients elements, such as N, P, and K and microbial population, bacteria, fungi, actinomycetes, in iron mine tailing were determined. The data showed that cow manure vermicompost stimulated peanut seedlings height, root length, and dry weight of shoot. For example, plant heights grown in pot with 10%, 20%, and 30% vermicompost were higher 83.3%, 67.6%, 63.5% than that of CK, respectively. And seedlings root length and dry weight of shoot grown in pot with vermicompost(10%, 20%, 30%) were longer 11.0%, 3.23%, and 2.91% and more 13.7%, 5.8%, -6.8%, than those of CK, respectively. Peanut seedlings root exudate was beneficial for microbial thrive. The population of bacteria in rhizosphere of 35-day seedlings was more than that of CK. The sequence of bacteria population or fungi population in rhizosphere of four treatments was 10%V > 20% V > 30% V > CK. While the actinomycetes population sequence was 10% V > 30% V > 20% V > CK. After peanut seedlings were harvested the nutrient contents, such as N, P, and K, increased than before peanut planting. The increment percentages of N and P elements were 10%V(243.8% and 204.5%) > CK (100% and 172.2%) > 20%V(52.3% and 72.0%) > 30%V(-26.5% and 39.1%) as comparing the value of mixture after seedlings harvested to that before planting, respectively. While the K element increment percentage was CK(187.7%) > 10%V (106.2%) > 20%V(45.2%) >30%V(23.4%). It was concluded that cow manure vermicompost stimulated peanut seedlings growth as it was used into iron mine tailing during plant restoration. The 10% vermicompost in mixture was an appropriate application for this experiment. It was indicated that vermicompost supplied nutrients for peanut seedlings and plant root exudates stimulated microbial thrive, and microbial activities stimulated nutrients release from iron mine tailing and vermicompost.

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