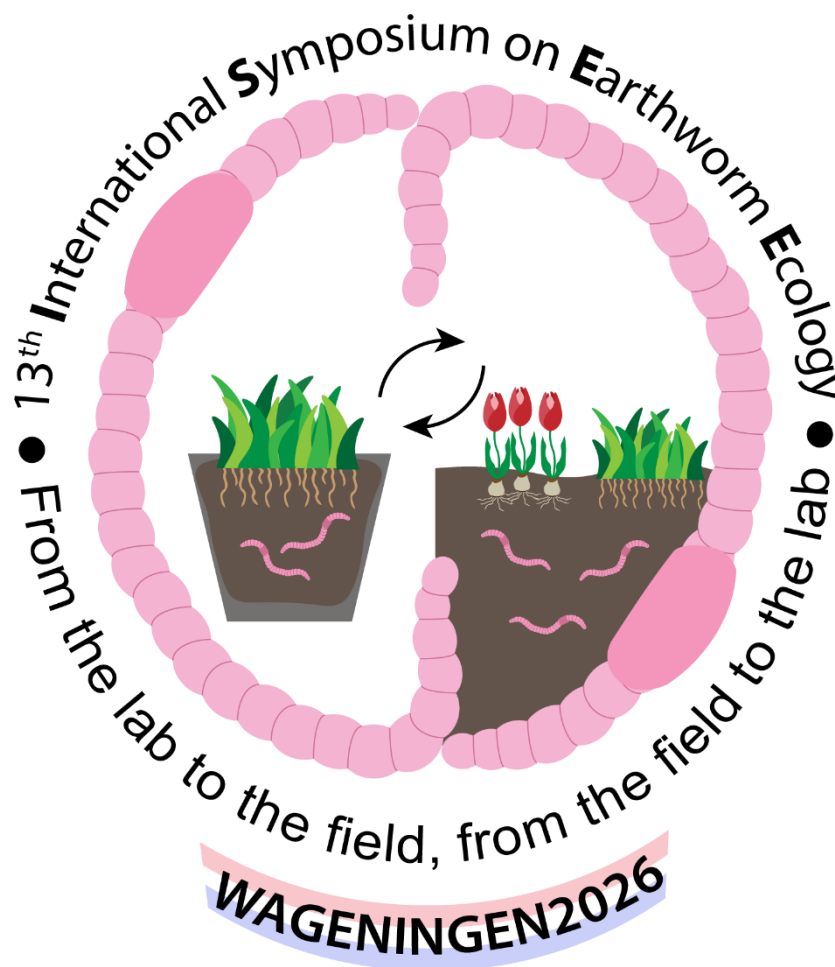




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Book of abstracts

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About this publication

The abstracts in this volume were evaluated by members of the Scientific Committee and the Organizing Committee of ISEE13, resulting in the selection of abstracts and their allocation to oral presentations or poster presentations. This volume of abstracts contains those abstracts that are expected to be actually presented at the conference at the time of compilation. The Organizing Committee takes no responsibility for any error and omission or for the opinions of the authors. This volume contains the selected abstracts as they were received. For the oral presentations, the presenting author is identified in bold.

Scientific committee

For every session of the conference, we have assembled a diverse group of researchers with relevant expertise to serve on the scientific committee. For each session, members of this scientific committee as well as members of the local organizing committee have selected abstracts and allocated them to an oral or poster presentation. The composition of the selection committee is listed below:

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Joann Whalen - McGill University, Canada
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Session 3: Earthworms & Society

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Nicole Schon - Bioeconomy Science Institute, New Zealand

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Session 4: Earthworm Taxonomy

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Esperanza Huerta Lwanga – Soil Physics and Land Management group, Wageningen University, the Netherlands

Matty Berg - Vrije Universiteit Amsterdam, the Netherlands

Session 5: Earthworms Outside the Box & Emerging Methods

External members

Olaf Schmidt - University College Dublin, Ireland

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Emma Belaud - CIRAD Montpellier, France

Markus Maeder - Lucerne University of Applied Sciences and Arts, Switzerland

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Alix Vidal – Soil Biology group, WUR, the Netherlands

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Oral presentations

Opening session

T0.1. Earthworms: ecosystem ambassadors

Ashwood F.

Earthworms spark curiosity; they are among the few soil animals that most people can recognise, having interacted with them as children, or encountered them in gardens. This familiarity makes them powerful ambassadors for the less obvious biodiversity in soil. In this talk, I explore how earthworms can serve as an accessible entry point for engaging broad audiences with the diversity and importance of soil ecosystems. Using examples from public engagement initiatives such as community BioBlitzes, iNaturalist workshops, my own macrophotography and social media outreach, I will explore how focusing on a single, well-known group can open wider conversations about the importance of belowground life. By integrating science communication into all our work, earthworms can be elevated beyond the familiar title of ecosystem engineers, into ecosystem ambassadors, bridging the gap between scientific knowledge and public appreciation of soil biodiversity. As earthworm researchers and enthusiasts, we are ourselves uniquely placed among soil ecologists as ambassadors for soil and its life, and I will explore avenues by which we can all fulfil this great responsibility.

Session 1: Soil Functioning & Biogeography

T1.1. From behaviour to function: how earthworm feeding and burrowing shape soil processes

Hoeffner K., Monard C., Cluzeau D., Pérès G.

Soil functioning emerges from the activity of organisms, yet linking individual behavior to soil-scale processes remains a major challenge in soil ecology. Earthworms provide a striking example of this gap: they are unambiguous ecosystem engineers that profoundly modify the physical, chemical, and biological soil properties, and yet the behavioral mechanisms underlying these effects are still only partially resolved. This keynote approaches earthworms through their feeding and burrowing behavior, considered as a central link between species autoecology and soil functioning. By focusing on how earthworms interact with organic matter and how they create burrows, the talk will explore the consequences for organic matter dynamics, soil porosity, and water movement, as well as for the structure and activity of microbial communities. Particular attention will be paid to earthworm burrows as microscale environments where soil porosity, resource availability, and microbial processes are tightly coupled, offering a window into how individual behavior translates into functional outcomes. As knowledge progresses, ecological categories once considered functionally homogeneous are increasingly shown to include interspecific variability, although they may remain relevant for specific processes. This process-dependent variability challenges classical ecological groupings and highlights the limits of static functional categories, calling instead for a more mechanistic and behavior-based view of earthworm functioning. Finally, from a forward-looking perspective, the talk will reflect on major knowledge gaps that still constrain prediction of earthworm-mediated processes in real soils, such as the limited consideration of species interactions, strong context dependency across soil types, and the gap between controlled experiments and field complexity.

T1.2. Global patterns of earthworm diversity

Phillips, H.R.

Despite ongoing research, our understanding of the global distribution of species remains limited for all but a small number of charismatic groups, and is particularly pronounced for belowground organisms. As many soil organisms provide vital ecosystem functions and services, understanding where they are distributed, why those patterns exist, and where we should be prioritising conservation actions is important.

In this talk, I will focus on work that my collaborators and I have been doing to understand the global distributions of earthworm diversity. By using two different global datasets, we illuminate patterns across two different spatial scales. The first dataset synthesised over 10,000 sites across the world, where earthworms had been sampled using a consistent methodology. The second dataset compiles species lists of earthworms 195 countries and territories, that have been reviewed by in-country experts.

I will discuss some of the contrasting patterns that were found when analysing earthworm diversity patterns at two different scales, and what that means for our understanding of earthworm diversity. Given the gaps in our data and our knowledge, I will also present the different approaches we are taking in order to fill those gaps. As climate and human activities are consistently being shown as the main drivers of the current patterns, drivers which are predicted to continue changing in the future, such global mapping is especially important. It can identify areas that may have high diversity and therefore should be prioritised for future research, as well as areas with high conservation value.

T1.3. Earthworm contribution to the soil carbon pump

Whalen, J.K.

The soil carbon pump describes the processes whereby carbon moves from the atmosphere into the deep subsoil, analogous to the marine carbon pump. In soil, this process begins when CO₂ is captured by plants and is transferred from plant tissues (leaves, roots, root exudates) into physically occluded organic matter or microbial necromass, which gradually descends through the soil layers or is transported by burrowing animals. Burial of carbon in the deep subsoil may effectively sequester carbon from the atmosphere for centuries or millennia, thus acting as a vital climate regulator. Earthworms as key actors in plant decomposition and burrow formation, are expected to accelerate the soil carbon pump. The objective was to estimate the earthworm contribution to the soil carbon pump in a corn agroecosystem based on an ecological energetics framework. The framework considered the physiological, population and community processes affecting the physical transfer of plant carbon to deep subsoil (e.g., via litter comminution and dissolved organic carbon movement through earthworm burrows). Direct carbon storage in earthworm casts, as organic carbon from partially decomposed plant materials plus inorganic carbon in excreted calcite granules, is also included. Furthermore, there is consideration of earthworm-microbial interactions that affect the soil microbial carbon pump's conversion of plant carbon from labile to persistent anabolic forms. Due to their multiple roles in carbon cycling, earthworms could be considered climate heroes in the soil carbon pump and for soil carbon sequestration.

T1.4. Earthworm gut and cast microbiota as drivers of plant growth promotion

Álvarez-Jiménez M., Reverchon F., de los Santos Villalobos S., Blouin M., Guevara-Avendaño E., Casas-Godoy L., Cortazar Murillo E. M., Zamora-Briseño J. A., **Barois I.**

Although the effects of earthworms on soil physical and chemical properties are well documented, their impacts on biochemical and microbial properties are less studied. In this work, we focused on bacteria known for producing signal molecules that strongly influence plants, namely Plant Growth-Promoting Bacteria (PGPB). Specifically, we evaluated the effect of the PGPB obtained from the gut and casts of the earthworm *Pontoscolex corethrurus*. Bacterial strains were selected from the foregut, hindgut, casts, and surrounding soil, and then screened for key PGP traits, including indole production, phosphate solubilization, siderophore production, and nitrogen fixation. Multivariate clustering allowed the selection of the most promising strains, which were subsequently evaluated in vitro using *Arabidopsis thaliana* seedlings. Co-cultivation assays revealed that bacterial strains isolated from the hindgut and casts induced the strongest plant responses, significantly increasing lateral root formation, primary root elongation, and both root and shoot biomass compared to controls. In contrast, strains from the soil and foregut showed weak or inconsistent effects. Multivariate analyses confirmed a positive association between hindgut- and cast-derived bacteria and overall plant performance. These results demonstrate that *P. corethrurus*, particularly through its hindgut and casts, harbors bacterial communities with high PGP potential, highlighting earthworm-associated microbiota as a valuable and underexplored resource for sustainable agriculture.

T1.5. Niche differentiation and interaction between earthworm functional groups shape distinct soil carbon fate

Ma K., Huang C., Huang J., Wei Y., Du Z., Cao J., Zhang W., Capowiez Y., Yuhui Qiao Y.

Soil organic carbon (SOC) dynamics are strongly shaped by biological regulation, with earthworms acting as key ecosystem engineers. Yet how different ecological groups jointly mediate SOC redistribution and stabilization remains unclear. Here, a full-factorial macrocosm experiment combined with ^{13}C tracing was used to evaluate the effects of epigeic (*Eisenia fetida*), anecic (*Amyntas carnosus*), and mixed assemblages under straw input. Earthworms did not simply increase or reduce SOC but redistributed carbon from active pools (DOC, MBC) to more stable pools (POC, MAOC). Mixed earthworms achieved the highest SOC accumulation (4.33 g kg^{-1}), 18 - 27% above single-species treatments, with ~23% derived from straw. SOC showed a decrease-then-increase pattern, indicating initial priming followed by stabilization. Epigeic species accelerated surface decomposition, whereas anecic species enhanced deep-soil stabilization through aggregation and organo - mineral associations. Their coexistence generated complementary effects coupling rapid turnover with retention. Earthworm casts contained up to 5.53 g kg^{-1} SOC-20 - 35% higher than bulk soil-and shifted from DOC/MBC toward POC/MAOC, indicating their central role in stabilizing labile organic matter. Overall, this study provides quantitative evidence that earthworm functional diversity and cast-specific processes co-regulate SOC redistribution and persistence, highlighting the need to integrate such biogenic mechanisms into SOC models.

T1.6. How does the interaction between a mole and earthworms affect soil surface changes and water infiltration patterns? Insights from a mesocosm experiment

Loreggian M., Baartman J., Larsen A., Commelin M., Bravo-Peña S., van Schaik L., Wallinga J., Ritsema C.

Animal bioturbation shapes terrestrial landscapes by altering water infiltration, storage, and soil surface dynamics. While moles (*Talpa europaea*) construct surface mounds and extensive tunnels, their primary prey - earthworm (i.e., *Lumbricus terrestris*) - simultaneously engineer deep burrow networks. Despite their interaction, the combined impact of these organisms as soil hydro-physical engineers remains poorly understood.

To address this, we conducted a densely-monitored mesocosm experiment (single box, $W=0.5$ m, $L=2$ m, $H=1$ m), sequentially increasing bioturbation complexity across three treatments: bare soil (B), earthworms only (EW), and earthworms plus mole (EW+M). Ten rainfall events were applied at a constant intensity of 0.8 mm min^{-1} . Photogrammetry was performed before and after each event to quantify surface roughness and soil redistribution. Spatiotemporal soil moisture was monitored through 24 sensors installed at 5, 15, 30, and 60 cm depths.

Preliminary results indicate that: (i) EW activity promoted fast infiltration to deeper soil layers and shorter response time compared to B and EW+M; (ii) mole increased the spatial heterogeneity of soil moisture dynamics and surface microtopography through contrasting burrowing strategies; and (iii) mole's formation exhibited limited erosion but underwent progressive subsidence, resulting in a longer wetting period of the upper layers. Additionally, high earthworm escape rates following mole introduction likely altered hydrological processes.

These findings demonstrate that increasing bioturbation complexity enhances heterogeneity in soil water dynamics. This provides insights into both soil hydro-physics and soil fauna behavioural interactions, highlighting the need for further study to better understand their implications for landscape evolution.

T1.7. Consequences of colonization of the arctic tundra by burrowing earthworms for soil organic carbon stability

D'Hervilly C., Krab E., Jonsson H., Garamszegi P., Monteux S., Klaminder J., Bellot A., Blume-Werry G.

Arctic ecosystems store a huge amount of undecomposed soil organic carbon. Recent colonization by burrowing earthworms raise concerns regarding its stability in a context of climate change. In a mesocosm experiment, we investigated the effect of four years of burrowing earthworm addition on carbon stability and microbial and mesofauna community in the arctic tundra. Carbon stability was approached through two methods: the amount of carbon associated to different soil organic matter fractions and soil aggregates sizes, and the effect of an increase of the temperature by 10°C on CO₂ emissions during a four-months incubation (Q₁₀ ratio). Our results show that patterns differ according to the type of tundra ecosystem. In a meadow-type tundra, earthworm addition decreased both carbon stocks and the proportion of readily available carbon in the organic layer. This was associated to an increase in the proportion of large macroaggregates in the mineral layer, suggesting a protection of the carbon from further decomposition. Surprisingly, this carbon was however less resistant to mineralization following an increase in temperature. In a heath-type tundra, earthworm presence increased the proportion of readily available carbon in the mineral layer, with a simultaneous decrease in the proportion of large macroaggregates. The bacterial community, evaluated through DNA analyses, was not affected by the presence of earthworms in any soil horizon and any type of tundra, while the mesofauna community was positively affected by the presence of earthworms in the mineral layer of a heath-type tundra.

T1.8. Soils worm-worked, arthropod-altered, and mammal-modified: faunal contributions to pedogenesis at continental scale

Looker N., Wackett A., Mathieu J., Brown G.G., Willenbring J.

Alteration of soil structure and biogeochemistry by earthworms and other soil fauna can lead to major changes in the flow of carbon, nutrients, and water through soils, as evidenced by lab and field experimentation. Natural and inadvertent experiments (e.g., pedologic gradients, species introductions) have provided insights into the cumulative effects of fauna on soil functioning over longer time scales. However, this work has generally been restricted geographically and/or taxonomically, and the extent and magnitude of faunal effects on soil development and functioning remain unclear. To constrain the influence of fauna on key soil properties at the continental scale, we analyzed soil morphological descriptions and associated laboratory data from soil surveys conducted across the U.S. over the past century. We leveraged surveyors' observations of zoogenic features to infer environmental controls on faunal occupancy across sampling scales (e.g., horizon, pedon, county levels). Attribution of soil morphological features to fauna varied systematically across survey areas (e.g., infrequent mention of fauna in Southeast, despite high levels of faunal endemism). Earthworms were the most frequently mentioned driver of zoogenic features, but were recorded in fewer than 5% of profiles. Maximum depths of zoogenic features increased with distance from the southern limit of the Laurentide icesheet and were generally greater for crustaceans and mammals than for earthworms. Cumulative effects of bioturbation were evident in deeper penetration of organic carbon in fauna-modified pedons. These results highlight that improving models of soil formation and digital soil mapping efforts requires explicit consideration of fauna as agents of soil development.

T1.9. Earthworm ecology and biodiversity in Brazil: recent advances and future challenges

Brown G.G., Demetrio W., Ferreira T., Dudas R., Bartz M., Rousseau G., Hernández L., Sousa S., Marichal R., James S., Nadolny H., Oliveira V., Baretta D., Zeiss R., Eisenhauer N., Guerra C., Peres C., Cunha L., Feijoo A., Pereira J., Tapia-Coral S., Niva C., Decaëns T., Cooper M., Mathieu J.

Over the last 10+ years, a major dataset on earthworm communities, sampled mostly using standardized hand sorting has been constructed with the contribution of many (>130) colleagues. Qualitative samples including soil, litter, epiphytes, logs, streambanks and other niche sampling were also included. The file has ca. 13 thousand lines, combining >100 individual datasets from >2,000 sampling events, representing >11,500 monoliths (quantitative samples) and ~1,900 qualitative samples. All sites (and many individual monoliths) are geolocated, and extensive primary environmental and soil metadata are available for many sites, allowing for ample data exploration. Preliminary assessments showed significantly higher densities in agroforestry systems, pastures and annual crops without tillage (all ~140 ind.m⁻²) than in tilled crops (70 ind.m⁻²), and native vegetation (90 ind.m⁻²). However, overall species richness was higher in native vegetation (~900 sites, ~250 spp.), than pastures (~200 sites, ~90 spp.) and annual crops (~400 sites, ~100 spp.), but site-level richness was higher in disturbed than native vegetation due to peregrine species invasion. More than 300 species were found, including many still undescribed, though the most frequently encountered were the cosmopolitan *Pontoscolex corethrurus*, small African *Dichogaster* spp., and jumping Asian worms (mainly *Amyntas* spp.). However, there are still many neglected areas, biomes and land uses, and further attention to soil parameters are needed in order to better assess the true potential of earthworms as soil health bioindicators in Brazil. Interactions between species and with the environment will be explored and some examples shown in the presentation.

T1.10. Intraspecific variation in earthworm functional traits across sites and land uses in Southern Vietnam

Lam D.H., Decaëns T., Mathieu J., Nguyen A.D., Bottinelli N.

Trait-based approaches in earthworm ecology are still constrained by the limited quantification of intraspecific variability and its spatial structuring, which hampers identifying the most variable traits and the environmental contexts to which they respond. To address this gap, we measured morphological and anatomical traits in 454 individuals (8 species) sampled in Southern Vietnam across five land-use types. Measurements spanning the whole organism were summarized into four trait sets: (i) body size, (ii) digestive, (iii) body wall/musculature, and (iv) microstructures. We used hierarchical models to partition trait variation into fine-scale (within-site) heterogeneity, among-site differences, and land-use effects. Across trait sets, variance was largely explained by fine-scale heterogeneity and among-site differences, with only a small contribution of land use. Fine-scale heterogeneity accounted for 76.0% (body size), 67.9% (digestive), 77.0% (body wall/musculature), and 89.7% (microstructures), compared with 9.9–30.7% for among-site differences. Land use explained 0.7% (body size), 1.4% (digestive), 4.8% (body wall/musculature) and 0.4% (microstructures), indicating weak land-use effects mainly expressed in body wall/musculature traits. Integrating site-level soil properties (e.g., texture, moisture, organic matter, pH) will help identify the edaphic drivers associated with the strong fine-scale heterogeneity and among-site differences observed here.

T1.11. Changes in earthworm populations in grazed hill country pastures with contrasting phosphorus fertiliser histories since 1975

Schon, N.L., Mackay, A.D.

Sheep-grazed hill-country farmlets were established in 1975 in the Hawke's Bay region, New Zealand to explore the effect of phosphorus (P) fertiliser on pasture and livestock production. Superphosphate was applied, with one farmlet receiving 11 kg P/ha/yr from 1975-1979 and none since (NF); a low fertility farmlet receiving 11 kg P/ha/yr since 1975 (LF); and a high fertility farmlet receiving 56 kg P/ha/yr from 1975-1979 and 34 kg P/ha/yr since (HF). Earthworm populations were monitored on medium slopes (15-25°) from 1979.

Four years after the farmlets were established, earthworm abundances were correlated to pasture production, being lower in the NF and LF farmlets (510 ± 74 and 550 ± 100 m⁻²) than the HF farmlet (640 ± 75 m⁻²). In 2024, differences in soil fertility were more pronounced, with Olsen P levels ranging from 8 µg/ml at NF, 13 µg/ml at LF, and increasing to 38 µg/ml at HF. Sampling the same locations, earthworm abundance increased from 230 ± 103 , 315 ± 76 to 430 ± 112 m⁻² in the NF, LF and HF farmlets, respectively. On medium slopes earthworm abundances were more reflective of pasture production than Olsen P levels.

However, earthworm abundances in 2024 were lower than in 1979 across all three farmlets. This reflects declines in pasture production seen during this period, despite increases in soil P fertility. This is contrary to what is expected under a climate of increasing atmospheric carbon dioxide and associated warming. We explore the implications of a changing climate on earthworm communities in grazed pastures.

T1.12. Assessing earthworm diversity and abundance in UK agricultural soils using molecular approaches

Zhao Y., Daniell T., Watt P.

Earthworms as key ecosystem engineers, play an essential role in nutrient cycling, soil structure maintenance, and organic matter turnover. Their high sensitivity to changes in environmental conditions makes them well suited as biological indicators of soil health. Despite their ecological importance, studies of earthworm community responses to different agricultural regimes remain limited.

This study investigated how different agricultural management regimes – conventional, regenerative, transitional systems changing from conventional to regenerative practices – affect earthworm abundance and diversity across 25 farm fields in two different clusters from south of the UK. Earthworm communities were assessed by integrating DNA barcoding of individual specimens with soil environmental DNA (eDNA) metabarcoding based on field surveys.

Our study revealed clear differences in earthworm abundance and diversity among management regimes. From 2022 to 2025, regenerative fields show significant increases in earthworm abundance while transitional systems show mixed responses and conventional systems show weaker changes. In addition, regenerative fields support more diverse and more evenly structured earthworm community. This study establishes a scalable framework for monitoring earthworm biodiversity during different agricultural management regimes and provides evidence-based ecological insights for agricultural transition and long-term soil health assessment.

Session 2: Ecotoxicology & Stress Ecology

T2.1. Earthworms in terrestrial ecotoxicology, from standardised tests to integrative approaches

Pelosi C.

For several decades, numerous chemical contaminants have been routinely used in industry and agriculture. These substances, including pesticides, PFAS, microplastics, and heavy metals, are now widely disseminated in the environment, leading to persistent contamination of waters, soils, and living organisms. It is well established that such contaminants can have adverse effects on biodiversity, particularly on soil organisms such as earthworms, which play a central role in terrestrial ecosystem functioning and ecosystem service provision. However, it is neither feasible nor realistic to test all existing chemicals and their degradation products on all living organisms. This challenge is further exacerbated by differences between commercial formulations and pure substances, by cocktail effects, and by interactions with other environmental stressors such as climate parameters. Current regulatory risk assessments rely on standardized tests conducted on a limited number of model species. Growing evidence demonstrates the limited ecological relevance of many of these approaches, due to unrealistic exposure conditions, low species sensitivity, restricted consideration of life stages, and poorly informative endpoints. Despite these limitations, the need for robust and predictive risk assessment remains critical. I will illustrate the effects of selected contaminants on earthworms. I will also review the limitations of traditional terrestrial ecotoxicology and stress ecology, with a focus on earthworms, and highlight emerging approaches such as theoretical and modelling frameworks based on a mechanistic and hierarchical representation of biological responses, adaptations of regulatory testing schemes, and integrative approaches of exposure and risks for a better assessment of current risks for earthworms in natural ecosystems.

T2.2. Visualization and quantification of earthworm three dimensional burrowing behavior for ecotoxicological study

Li Y., Xu Y.

Earthworms are pivotal bioindicators of soil health, with their behavioral biomarkers providing sensitive and comprehensive early-warning signals for environmental contaminants, complementing deficiencies in existing biomarkers. However, soil opacity has limited understanding of continuous three-dimensional (3D) earthworm burrowing. In this study, we developed an integrative method combining a transparent medium with a deep learning-based trajectory algorithm to quantitatively investigate species-specific burrowing strategies. Earthworms thrived in the transparent medium, shown by biomass increase (0.62 g to 0.72 g) and > 80% survival over the 28 days, while algorithm stability (83.65% coordinate extraction rate) allowed for continuous tracking of the earthworm spatial coordinates in 3D locations. Comparative analysis across five earthworm species reveals species-specific patterns: *E. fetida*, *A. pecteniferus* and *E. eugeniae* prefer surface layers and move laterally, whereas *P. excavates* and *M. guillelmi* create vertical burrows. These findings are valuable for quantifying soil health assessments using different earthworm species. Moreover, this method captured dose-dependent avoidance behavior in arsenate-contaminated soils (0, 25, 50, 100, 200 As mg/kg). With increasing arsenic concentrations after 28-day exposure, earthworm trajectory points shifted from high-density clustering toward a dispersed spatial distribution, and cumulative burrowing length decreased by 5.60% - 51.66%. These results validated the efficacy of this integrated approach for pollutants toxicity assessment in ecotoxicological research. This study establishes a critical tool for advancing ecological risk assessment and management of soil pollutants by offering visual and quantitative insights into 3D earthworm burrowing dynamics, and provides potential applications in ecology, environmental pollution monitoring and remediation, and sustainable agroecosystem contexts.

T2.3. Mechanistic underpinning of earthworm sensitivities to metals

Spurgeon D., Djdhorajiwalar R., Balan S., Eagles E., Short S., Robinson A., Kille P.

Trace metals are some of the highest-risk environmental pollutants, because of their widespread occurrence, potential to transfer in food webs and inherent toxicity. Earthworms are notably vulnerable to the impacts of metal contamination. Many anthropogenic activities release metals to soils, where they have long residence times leading to sustained exposures for below-ground species. The construction of species sensitivity distributions for earthworms for different metals (e.g. Cu, Cd, Pb, Zn, Ag, Ni, Cr) has highlighted the extent to which different species vary in metal sensitivity. Currently, however, the phylogenetic distribution and mechanistic causes of these differences are not well understood.

In this presentation, we will show that earthworm sensitivity to metal exposure is not randomly distributed among different species, but instead has a clear phylogenetic signature. This means that some species are more vulnerable to the effects of some metals than they are to others. We show how these differences in sensitivity can be linked to key metal-handling traits, including the toxicokinetics of how different species take up different metals; and how these traits influence tissues, internal distribution and sequestration states. Focussing on a species-specific case study of differential sensitivity to a range of metals in earthworms compared to other invertebrate species species, we show how traits that are linked to the presence and extent of uptake and the physiological capacity of earthworm to tolerate external exposure act as key driver of difference in sensitivity to different metals.

T2.4. Characterizing climate vulnerability and stress tolerance in the riparian earthworm *Allolobophora molleri*: an integrative physiological and transcriptomic approach

Martínez Navarro A., Frutos I., Tilikj N., Trigo D., Marchán D.F., Novo M.

Earthworm physiological performance is tightly bound to soil hydro-thermal regimes, making them highly sensitive to environmental fluctuations. This dependence is particularly critical for riparian species, with narrow hydrological niches. In the Mediterranean region, these specialists face an existential threat as climate change drives increasingly frequent droughts and heatwaves. To evaluate the resilience of such vulnerable taxa, this study dissects the stress responses of the hygrophilous *Allolobophora molleri* using a three-tiered integrative approach. First, physiological boundaries were explored in laboratory microcosms under combinatorial regimes of temperature (16°C and 21°C) and soil moisture (5–30%). Second, to decipher aestivation mechanisms, transcriptomic profiles and oxidative stress (MDA) were analysed under specific hydric constraints (10% vs 30%). Third, thermal resilience was assessed across a latitudinal gradient by comparing transcriptomic responses to acute heat (26°C) versus controls (16°C) in Northern, Central, and Southern populations. Results identified soil moisture as the primary driver of survival, with mortality being higher at extreme dryness and saturation. Aestivation was restricted to $\leq 10\%$ moisture at 16°C; however, 21°C triggered dormancy even at intermediate soil moisture levels. Physiologically, aestivators experienced biomass loss but retained higher tissue water content compared to active individuals, a retention capacity enhanced by higher temperatures. Arousal tests confirmed a facultative diapause. Lipid peroxidation levels remained stable during aestivation, indicating efficient oxidative regulation. Ongoing transcriptomic analyses are characterizing the gene regulatory networks underlying these responses. Altogether, these findings offer crucial insights into local adaptation and the future persistence of this soil engineer in a changing climate.

T2.5. Effect of higher groundwater levels on earthworms in agricultural peat meadows

van Eekeren N., van der Laan A., Bestman M., van Dijk J.

In the Netherlands, agricultural drainage of peatlands causes major problems, including CO₂ emissions, soil subsidence, and biodiversity loss. Raising groundwater levels using active water infiltration systems (AWIS) is a promising mitigation strategy, but its effects on soil biota remain poorly understood. We therefore conducted a controlled mesocosm experiment and a field study to assess the effects of higher groundwater levels on earthworms in agricultural peat soils.

In the mesocosm experiment, 40 intact fen meadow peat cores were exposed to four groundwater levels (0, 20, 40, and 60 cm below the peat surface) and two nutrient application levels. After 15 months, earthworm abundance and biomass were highest at intermediate water levels (~40 cm). Nutrient addition had no effect under low water levels but increasingly reduced earthworm abundance and biomass at higher water levels

In the field, groundwater level effects were less pronounced. Earthworms were sampled in 2022 and 2024 in fields with AWIS (~20 cm below peat surface) and reference fields, half of which were grazed and half mown. In 2022, earthworm abundance was lower under AWIS, mainly due to fewer juveniles, while biomass was unaffected. Grazing showed a much clearer effect with a higher earthworm abundance and biomass under grazed conditions. In 2024, no treatment effects were detected, likely due to uniformly wet conditions across all fields during that year.

Our results suggest that earthworm numbers may be affected when raising groundwater tables, which can also have consequences for other aspects such as soil structure, water infiltration and food for meadow birds.

Session 3: Earthworms & Society

T3.1. Challenges in integrating earthworms into large-scale survey assessments and conservation policies

Briones M.J.I.

Despite the increasing efforts in taxonomy, monitoring, data compilation and recent policy instruments for ensuring soil health, ecosystem services delivered by earthworms are mostly unknown to the general public, stakeholders and policy makers. This together with substantial geographical data gaps, the time-consuming population estimation methods, and the narrow focus of current legislations hampers their full acknowledgement as reliable biological indicators. Here, I will describe these challenges and possible ways of overcoming such impediments through (i) establishing meaningful assessment frameworks (science), (ii) incorporation of earthworm awareness and welfare into people's daily lives (society). (ii) integration of abundance, biomass, and functional diversity metrics into binding legal instruments (policy). Essential research "dos" are to disentangle their "direct" from their "indirect" effects on soil processes together with a more complete understanding of their functional dissimilarity and the modulating effects of the soil abiotic conditions. Advanced technologies in species identification (molecular, AI) could empower taxonomists and public audiences but should be limited until we resolve the methodological flaws and build databases of verified specimens. The current spatial and temporal sampling bias of species occurrence records, and their high sensitivity to environmental variables, also makes results difficult to replicate and integrate in large scale surveys. This could be alleviated by contributory science platforms that generate massive quantities of valuable records for research but needs strategic planning. Finally, integrating these key soil organisms into national and international regulations requires that earthworm receives legally binding protection, enabling early detection of potential declines and trigger restoration obligations.

T3.2. How big dataset can support policy makers: example of French dataset for earthworm criteria provisioning

Pérès G., Hoeffner K., Briones M.J.I., Butt K.R., Decaëns T., Gérard S., Guillocheau S., Hedde M., Le Bayon R.C., Muys B., Phillips H.R.P., Pelosi C., Cluzeau D.

In the context of the application of the Soil Monitoring Law and Resilience, European Member States will have to analyse and survey soil health in order to fully restore soil health across Europe by 2050. For the first time, biological indicators will be part of the set of parameters of soil health evaluation. In order to assess soil biodiversity erosion, criteria values (such as target or threshold values) are expected. However, these criteria need to be based on a huge biological dataset, as they can strongly vary depending on agro-pedo-climatic contexts (Riggi et al., 2025). Taking advantage from a huge earthworm dataset (>2300 sites) collected at national scale in France, the research program Landworm was able to provide these criteria for several earthworm descriptors (abundance, biomass, ecological groups i.e. anecic, endogeic, epigieic, and species richness). By crossing several land uses (e.g. grassland, annual crop, perennial crop such as vineyard, forest), soil properties (e.g. texture) and climate-geology-geomorphology which define "eco-region", it was possible to provide criteria for all these earthworm descriptors at a very fine scale. In addition to presenting these criteria at a very fine scale, we assess and highlight the consequence of changing scale, from the national scale to the scales of soil units and soil district, two scales identified for the application of the Soil Monitoring Law and Resilience, and provide some guidance addressed to European policymakers on the application of these expected earthworm criteria.

T3.3. Earthworms as engagement facilitators for discussions on soil health

Pommeresche R., Rittl T.F.

Earthworms provide a practical and intuitive tool for engaging farmers in discussion about soil health. By observing earthworms and traces from their activity directly in the field, farmers can explore key soil health aspects in practice. Soil structure becomes visible through the presence of earthworm channels and casts as aggregates. Decomposition, biological activity and nutrient recycling can be illustrated by examining earthworms, and the location and color of casts.

Across several projects, an on-farm visual soil assessment method Soil Certificate (Jordlappen) was applied to evaluate soil health. Among the ten assessed indicators, earthworms (abundance and diversity) as well as visible earthworm traces (e.g. tunnels and casts), emerged as particularly effective indicators for engaging farmers in discussions on soil properties, tillage practices and management.

Counting earthworms within a soil cube proved to be a practical and tactile entry point for evaluating soil structure and biological activity. This hands-on approach facilitated dialogue around soil compaction, aggregation, and biological functioning. Furthermore, earthworm diversity could be assessed without detailed species identification by categorizing individuals according to skin pigmentation and behaviour. Linking to their respective ecological groups and functions in the soil.

Within on-farm events that brought together farmers, advisers, and researchers to jointly dig in the soil, earthworms - assessed through simple measures of abundance, diversity, and visible activity -functioned as effective and engaging facilitators for discussions on soil structure, biological functioning, soil health and management practices. Making soil health concepts more accessible and relevant for on-farm learning.

T3.4. SoilRise - monitoring of earthworm communities by tutor-based citizen science

Euteneuer, P., Potthoff, M., Józefowska, A., Hervé, M., Nicolai, A., Schmidt, O., Fertil, A., Hoeffner, K., Gailing, O., Sturm, L., Müller, M., Randecker, L., Greifenstein, N., Amirhosseini, K.

SoilRise is a project to study the biodiversity of earthworms and contributes to the ongoing efforts to enlarge and optimise biodiversity monitoring in Europe. Earthworms play a crucial role in soil functioning and are considered important bioindicators. Our focus is on their distributions and diversity in agricultural production, (small) gardens and urban areas. With the help of a specific tutor-based concept of citizen science (CS), the SoilRise project (2024-2027) has collected data in France, Ireland, Poland, Austria and Germany on earthworm abundance, community composition through traditional identification methods and DNA barcoding. Our research aims are to: i) build an interactive network between scientists, the public and stakeholders to raise awareness of the importance of soil biodiversity, ii) evaluate our tutor-based concept, iii) fill barcoding gaps in DNA databases, iv) evaluate potential differences of earthworm species in DNA between project-based countries and v) harmonise earthworm sampling and identification methods. We will present our concept of tutor-based CS, selected results such as new earthworm species to some of the countries, stakeholder and CS engagement and lessons learned from the first campaigns in 2024 and 2025 for agricultural habitats and gardens.

SoilRise (03LW0510) was funded through the 2022-2023 Biodiversa+ COFUND call, under the European Biodiversity Partnership programme, and with the funding organisations: National Science Centre 2023/05/Y/ST10/00098

T3.5. Earthworm diversity in urban parks: a transnational citizen science study on anthropogenic soils

Józefowska A., Pacanowski P., Potthoff M., Greifenstein N., Randecker, L., Euteneuer P., Sturm L., Stuhler, J., Schmidt O., Clune S., Fertil A., Le Callonec L.

Urbanization fundamentally transforms soil ecosystems, yet below-ground biodiversity in cities remains poorly understood. Earthworms are key soil engineers that improve structure, nutrient cycling and ecosystem services essential for urban environments. This study examines earthworm diversity and density in urban parks across five European countries, focusing on anthropogenic soils typical of urban green spaces. Within the SoilRise framework we applied a mentor-based citizen science protocol to standardise earthworm sampling in parks of Poland (Kraków), Austria (Vienna), Germany (Göttingen and Osnabrück), France (Rennes) and Ireland (Waterford, Galway, Wexford, Dublin, Meath, and Kilkenny). At each site we took five soil blocks (20 × 20 × 25 cm), extracted earthworms, identified them to species and assigned them to ecological groups (epigeic, endogeic, anecic). The same blocks yielded five subsamples that were pooled for analysis of pH, organic matter content, texture and other properties. Data include earthworm density, biomass, species richness and diversity indices (Shannon, Simpson). Preliminary results show distinct community patterns linked to land management regimes, soil conditions and climatic gradients. Comparative analysis relates earthworm assemblages to site-specific characteristics and measured soil parameters. Despite intensive management, urban parks host functionally diverse earthworm communities that can sustain critical ecosystem services. This trans-national assessment clarifies the role of below-ground biodiversity in urban soil quality and supports evidence-based strategies to enhance ecosystem resilience across European cities. The citizen science approach also illustrates how community involvement can advance large scale biodiversity monitoring.

The research is funded by the Biodiversa+ COFUND programme (2022 2023 call) within the European Biodiversity Partnership and by the National Science Centre (grant 2023/05/Y/ST10/00098).

T3.6. Drivers of earthworm community patterns in urban private gardens

Feng C., Lubbers I.M., Vasenev S., Vink T., Stoorvogel J.

Earthworms are well-recognized soil ecosystem engineers that contribute substantially to soil ecosystem services. However, in urban environments, particularly in private gardens, the drivers of earthworm community patterns remain poorly understood.

This study examines how garden and soil characteristics and management practices influence earthworm communities in urban private gardens.

We conducted the Wageningen Green Space Survey to collect information on garden characteristics, soil conditions, and management practices. A spatially representative subset of gardens was selected for soil and earthworm sampling. Earthworm abundance and functional composition were quantified and used as biological indicators, alongside laboratory-measured physical and chemical indicators, to evaluate soil health. A second questionnaire explored gardening motivations to classify garden owner types, analyze their relationships with soil conditions and earthworm community structure, and provide soil health feedback to participants.

We collected 94 survey responses and 37 gardens were sampled between October 13th and November 14th, 2025. Our findings indicate that garden management intensity increases with the age of garden owners until around 60 years and that management intensity is higher in larger gardens. Endogeic earthworms dominate the sampled communities (63.7%), followed by anecic (24.3%) and epigeic species (7.3%); juveniles account for 57.2% of all sampled individuals. Higher mean earthworm abundances were observed in the largest gardens. However, we have not found evidence of parent material being driving factors for earthworm community structure.

These analyses will reveal whether, in urban private gardens, management-related factors outweigh soil characteristics in driving earthworm community patterns.

T3.7. Integrating earthworm ecology into public understanding: challenges and insights from translating research into public community education in Hong Kong

Law M.A.M.

A comprehensive earthworm community survey was conducted across diverse land uses and land covers in Hong Kong in 2019, generating the most extensive local baseline information on species diversity, distribution patterns, and ecosystem functions. Building on this scientific foundation, the resulting knowledge has been actively transformed into large scale public education efforts in recent years. These initiatives include a city-wide community outreach project, public and media engagements, and a series of school talks designed to introduce students to earthworm ecology and the importance of soil biodiversity. Such efforts aim to bridge the persistent gap in soil ecology coverage within Hong Kong's formal education system. Despite the significant role of soil biodiversity in ecosystem functioning, topics related to soil ecology remain limited or absent in both youth curricula and tertiary environmental studies.

In this talk, I will explore this educational gap and discuss how findings from the scientific research were translated into accessible and engaging community outreach materials. I will also reflect on the challenges encountered—from limited public awareness, insufficient funding supports, and curriculum constraints to logistical and cultural barriers unique to Hong Kong's dense, urban, and culturally complex setting. By sharing these experiences, the presentation highlights opportunities for integrating soil biodiversity into mainstream environmental education and fostering broader public appreciation of earthworm ecology.

Session 4: Earthworm Taxonomy

T4.1. Go big or go home: evolutionary drivers and molecular mechanisms behind European giant earthworms

Leal-Cardín M., Piris A., Hackenberger D., Szederjesi T., Gérard, S., **Marchán D.F.**

An evolutionary trend towards gigantism has been observed within several tropical earthworm families, but also within the European Lumbricidae and Hormogastridae. The latter are an ideal opportunity to understand the evolutionary mechanisms behind the origin of large body size. This complex trait could have evolved convergently as a response to similar or different environmental pressures, and at different moments of geological history or synchronically. To answer these questions, we followed a dual approach combining phylogenomics and transcriptomics. The first objective was to establish a robust phylogenetic background based on Anchored Hybrid Enrichment as a base for an explicit spatio-temporal frame. The second objective was to characterize the genes involved in the evolution of giant body size by identifying selection signatures and differences in gene expression (using transcriptomes). Seventy-two species (with emphasis on *Scherotheca*, *Octodrilus* and Hormogastridae) were sequenced and incorporated into Marchán *et al.* 2022 AHE dataset, resulting in a robust time-calibrated tree which suggested the role of past global climate changes on giant earthworm evolution. More than 600 loci were tested for selection signatures exclusively present in giant species. Differential gene expression was tested between two species pairs (small and giant *Scherotheca* and *Boucheona*), accounting for age (juvenile vs adult) and tissue (digestive, circulatory and body wall) to identify biologically relevant genes for giant earthworms. All these findings provide an integrated perspective on when, why and how these impressive animals evolved, and serve as a base to understand their ecological relevance in the soil.

T4.2. Mating of the giant earthworm *Lumbricus badensis*: an enigma?

Butt K.R., Lang F., Ehrmann O., Healey R., Nuutinen V.

The giant earthworm *Lumbricus badensis* (Michaelsen 1907) is an epi-anecic species endemic to the Black Forest, SW Germany. As its behavioural ecology is largely unknown, field and laboratory studies of nocturnal soil surface activities were undertaken. In mating, similarities were expected with the well-documented behaviour of the epi-anecic *L. terrestris*. A pilot study (2015) produced first observations on mating, but data were few and inconclusive. During a second field study (2019), five matings were recorded. Courtship with burrow visits was seen but otherwise the behaviour differed markedly from the *L. terrestris* model. To confirm these findings, previously unmated, laboratory-grown, mature *L. badensis*, were allowed to interact in groups of two or three. Altogether 21 matings were recorded, all following the pattern seen in the field. After the courtship, each mating involved two reciprocal bouts. First, one individual attached ventrally with some of its anterior segments to the partner's clitellar area. Thereafter, the attaching worm extended its body from its burrow to form a "loop" and rolled the partner, which led to separation after an attachment of 10-17 minutes. After an intervening period of 20-51 minutes the partners encountered each other again, and the short attachment was repeated, with roles reversed. Viable cocoons were later produced by both partners. The findings are perplexing and raise fundamental questions on the nature of *L. badensis* mating (on copulation mechanisms including sperm donation, reciprocity etc.). The results already show how varied this behaviour can be within a single genus and ecomorphological group of earthworms.

T4.3. Population genetic diversity and dispersal patterns of widespread megascolecidae earthworms in China revealed by mitochondrial multi-genes and snps

Jiang J., Li J., Jin Q., Yuan Z., Dong Y., Sun J., Zhao Q., Zhao W., Qiu J.

Unraveling the genetic structure of widespread earthworms is critical for taxonomy and resource conservation. This study synthesizes phylogeographic research on dominant Megascolecidae earthworm populations (genera *Amyntas* and *Metaphire*) in China using mitochondrial multi-locus analyses (COI, COII, 12S, 16S, ND1) and SNPs. Comparative results indicate that while mitochondrial multi-genes effectively resolve deep lineage divergence and historical biogeography dating back to the Pleistocene, SNPs provide superior resolution for fine-scale population structure and contemporary gene flow. A common evolutionary pattern of Mountain Refugia–Plain Expansion was identified across taxa. Specifically, the medicinal earthworm "Hu-Dilong" (*Amyntas pectiniferus*) exhibits deep cryptic divergence in Tianmu mountains and a recent star-like expansion into the Yangtze Delta plains. Similarly, another medicinal earthworm "Guang-Dilong" (*Amyntas aspergillum*) reveals high genetic diversity driven by topographic isolation, supporting a West-to-East dispersal history. Furthermore, studies on *Amyntas triastriatus* and *Metaphire remanens* demonstrate that parthenogenesis and glacial cycles significantly accelerate lineage differentiation. These findings suggest that geographic isolation and reproductive strategies are primary drivers of speciation. We advocate integrating genomic data to resolve cryptic species complexes, ensuring the accurate identification and sustainable utilization of earthworm resources.

T4.4. Comparison of DNA collection methods for earthworm DNA barcoding

Amirhosseini K., Müller M., Potthoff M., Gailing O.

Science-society interactions advance earthworm biodiversity research by enhancing geographical and taxonomic coverage in monitoring campaigns. However, lethal and destructive tissue sampling—as employed in earthworm DNA barcoding—risks public disengagement by posing emotional barriers that conflict with the participants' compassion for animals. Here, we compared the performance of a commercially available swabbing procedure with two standard tissue-based DNA collection methods in generating cytochrome c oxidase I barcodes from 40 earthworm specimens. Our results showed comparably high PCR amplification success rates among the methods (97.5-100%) with some variability in sequencing success (82.5-87.5% for the tissue-based methods and 66.7% for the swabs). While swabs had lower success rates overall, they produced the greatest proportion of high-quality sequences among successfully sequenced barcodes (80.8%), compared to the tissue-based methods (68.6-75.8%). Additionally, the methods showed a high level of internal consistency (>99% average intra-specimen pairwise sequence percentage identity), with same-sample barcodes receiving consistent homology-based taxonomic assignments with average similarity percentage above 99%. Overall, there was a high degree of agreement between the different collection methods, and swabbing represents a robust, emotionally-adaptive alternative to tissue-derived samples for species identification. However, the utilization of swabs in higher-resolution applications (e.g., reference library construction) requires further optimization (e.g., with respect to swabbing duration, collection solution, swab type, etc.) to improve overall barcode recovery rates. Standardizing a non-lethal approach in public-contributed earthworm barcoding projects could strengthen public-professional partnerships, enable more data collection from the field, make more information accessible from the lab, and ultimately expand knowledge of earthworm ecology.

T4.5. Biogeographic and taxonomic patterns of earthworms in India

Yadav S.

India is among the world's richest regions for earthworm biodiversity, a status attributable to its complex geological history, wide climatic gradients ranging from temperate to tropical, and high habitat heterogeneity. The country harbours valid records of 488 earthworm species distributed across nine families and 72 genera. Despite this exceptional diversity, molecular data supporting species-level identification and phylogenetic resolution remain highly fragmented. In this study, we compiled and critically analyzed a comprehensive database of Indian earthworms across major families to evaluate taxonomic coverage, endemism, and the current status of molecular sequence availability, with particular emphasis on mitochondrial cytochrome c oxidase subunit I (COI) barcodes. Our analysis reveals pronounced taxonomic imbalances among families, with Megascolecidae, Acanthodrilidae, and Moniligastridae accounting for the majority of recorded genera and species, many of which are native or endemic. This database-driven assessment provides a strategic baseline for prioritizing taxa for future sequencing, resolving cryptic diversity, and strengthening molecular systematics and conservation planning for Indian earthworm biodiversity.

Session 5: Earthworms Outside the Box & Emerging Methods

T5.1. Earthworm farming for circular food systems

Sonntag E., Parodi A., Rahmann G., Groenigen J.W., Vidal A.

While the role of earthworms as recyclers of organic matter is well studied and widely utilized in vermicomposting, their potential as an alternative source of high-quality protein remains less recognized. Based on several studies from a PhD research project, this presentation explores how integrating earthworm and mushroom farming with maize production can enhance food security and soil health in resource-limited farming systems of sub-Saharan Africa.

Maize stover, a common agricultural by-product often burned in the field, was used to sequentially cultivate oyster mushrooms and farm earthworms. The studies assessed (1) the nutritional value of farmed earthworms, (2) mushroom yield potential from maize stover, (3) earthworm protein production using spent mushroom substrate, and (4) the effect of the resulting organic amendments on soil carbon dynamics.

A systematic review of 142 studies highlighted the high nutritional quality of farmed earthworms, averaging 62% protein (dry matter) with balanced amino acid profiles comparable to other edible invertebrates. Experiments using maize stover from Ugandan smallholder systems showed that mushroom cultivation could add about 78 kg of protein per hectare annually, with an additional 16 kg of earthworm protein derived from spent mushroom substrate. Soil incubation trials indicated that these circular practices maintain soil carbon stocks compared with traditional crop residue management practices.

Overall, this research emphasizes the dual potential of earthworms—as recyclers and as food—within circular food systems. By converting crop residues into protein and organic fertilizer, integrated vermiculture–mushroom systems offer land-efficient strategies to address protein scarcity and soil degradation in sub-Saharan Africa.

T5.2. Earthworm characteristics that inspire and are mimicked by human engineers

Damoff G.A.

Earthworm characteristics have inspired and been mimicked by mechanical, electrical, chemical, and software engineers in recent decades. More than 200 publications were surveyed from dozens of peer reviewed journals and proceedings that demonstrate a broad interest among engineers in earthworm anatomy, physiology, and kinematics. The majority of the papers focus on biomimicry to develop numerous types and sizes of earthworm-like robots for medical, agricultural, marine and subsurface planetary exploration, as well as inspection/monitoring and search/rescue operations. Retrograde peristalsis (RP) is an obvious earthworm character trait amply studied, with many publications focused on detailed analysis of RP applied to robotics that include the integument, metamerism and associated microgrooves, setae, musculature, innervation, mucus, the hydrostatic skeleton, and the prostomium. Other earthworm characteristics examined in great detail by engineers include dorsal pores, electro-osmotic surface flow, medicinal substances, enzymes, light-sensitive rod cells, and the gut. In addition to robotics, examples of engineered products are agricultural implements, bionic fertilization, tunneling, health care products, fabrics, computational methods, and development of sensors/actuators. Only a handful of publications included in this survey identified earthworm species studied, and most focused on well-known lumbricids. Earthworm ecologists aware of nuanced attribute diversity among the thousands of species within earthworm families could significantly expand the exploration of bioinspiration and biomimicry through collaboration with engineers in this integrated field of study.

T5.3. Quantifying burrow network dynamics of endogeic and anecic earthworms in the field

ter Borg R.N., Larsbo M., Capowiez Y., Benard P., Taylor A., Iseskog D., Keller T.

Earthworms play a key role in soil processes, yet quantitative estimates of their burrowing activity under field conditions remain scarce. Here, we present a method to quantify field earthworm burrowing rates using repeated X-ray scanning of soil columns.

Perforated cylinders (10 cm diameter, 20 cm height) were fabricated using 3D printing and filled with soil. Each cylinder contained 244 holes (11 mm diameter) to permit earthworm entry and exit. The cylinders were buried for several months at a grassland site in Uppsala (Sweden) and periodically removed for X-ray scanning before being immediately reinstalled. In-situ sensors continuously recorded soil moisture and temperature. Earthworm burrow networks were extracted from the X-ray images to quantify burrow characteristics (volume, diameter and direction), assess burrow persistence, and calculate burrowing rates. Burrows created by endogeic and anecic earthworms were further distinguished based on diameter.

Repeated removal and X-ray scanning of soil columns at 1-2 months intervals enabled quantification of earthworm burrowing rates and assessment of burrow persistence. The estimated rates suggest that complete burrowing of the topsoil would require approximately 40 years. The data further indicate that anecic earthworm burrows accounted for about 40% of total burrow volume and that these burrows were more persistent than burrows created by endogeic earthworms. Preliminary analyses suggest that soil moisture exerted a stronger influence on burrowing rates than soil temperature. Overall, the approach provides novel insights into the links between earthworm activity and soil structure dynamics.

T5.4. A meta-analysis reveals earthworms as mutualists rather than predators of soil microorganisms

Blouin M., Robin A., Amans L., Reverchon F., Barois I., Lavelle P.

Microorganisms constitute the largest biomass on Earth after plants, and earthworms are one of the main components of animal biomass. Both are critical drivers of soil functions and ecosystem services. Studies report either positive or negative effects of earthworms on soil microbial communities, leading to contrasting views on whether microorganisms serve as prey or mutualists for earthworms. This meta-analysis aimed to settle this debate, and examines how biotic and abiotic factors affect earthworm impacts on microbial abundance and diversity. Based on a selective search, we kept 169 observations, showing that earthworms increased bacterial abundance by 16.5 % and fungal abundance by 31.4 %. Bacterial species richness rose by 8.5 % in the presence of earthworms, but fungal richness was not significantly affected. Epigeic and anecic earthworms had more notable effects than endogeics. Plant presence in experimental designs strongly amplified earthworms' effects on bacterial (+30 %) and fungal (+97 %) abundances. The largest earthworm-induced effects were observed in carbon- and nitrogen-rich soils and at low pH, while the addition of organic matter reduced these effects. Comparing microbial abundance in earthworm casts to control units without earthworms revealed effect sizes two- to three-fold greater (for bacteria and fungi, respectively) than when using the soil surrounding earthworm casts as a control. Our meta-analysis demonstrates that, despite ingesting some microorganisms, earthworms have a net positive effect on their abundance, positioning them as mutualists rather than predators of bacteria and fungi. We discuss the raw mechanisms hidden behind this net effect.

T5.5. DNA metabarcoding uncovers earthworm consumption by the invasive flatworm *Obama nungara*

Ventura M., Noël S., Fourcade Y., Dupont L., Roy V.

Invasive predators can strongly affect soil invertebrate communities, but their impacts depend on diet breadth, prey availability, and environmental context. *Obama nungara* (Platyhelminthes: Geoplanidae), an invasive terrestrial flatworm originating from South America, was first detected in Europe in 2008 and later in mainland France in 2013. It has since been reported from several European countries and colonized more than three-quarters of mainland France. Experimental studies showed that it consumes various soil macrofauna species, including gastropods and earthworms (PLATWORM project, presentation by Dupont et al.). However, to assess potential impacts on soil communities, it is necessary to characterize its diet using data collected in invaded habitats. We analyzed the gut contents of 254 *O. nungara* collected from ten sites in France, using a DNA metabarcoding approach focused on earthworm prey. Diet results were then compared with local prey communities thoroughly characterized within the project, providing a robust reference database. A total of 32 earthworm species were recorded in the field and 34 were detected in gut contents, including 27 shared species, representing all available ecological and functional groups. *Lumbricus castaneus* (epigeic), *Lumbricus friendi* (epi-anecic) and *Aporrectodea caliginosa* L3 (endogeic) were the most consumed species, and diet composition reflected local prey availability, indicating opportunistic predation driven by encounter rates. Despite the frequent consumption of all ecological categories, epigeic taxa were overrepresented, highlighting the role of accessibility in shaping feeding patterns. This flexible, generalist feeding strategy likely contributes to *O. nungara*'s invasion success while exerting diffuse impacts on soil macrofauna, particularly surface-active earthworms.

T5.6. A quartz quandary: assessing whether worms weather silica in soils

Wackett A.A., Harrison E.J., Brocard G.Y., Yoo K., Looker N., Willenbring J.K.

Silicate weathering regulates atmospheric CO₂ over geologic timescales, yet quartz—the most recalcitrant silicate in Earth’s continental crust—is widely assumed to resist chemical dissolution and primarily weather mechanically. Here we challenge this paradigm by tracing quartz grains through the critical zone in Puerto Rico’s El Yunque rainforest, a tropical montane ecosystem with the fastest recorded silicate weathering rates on Earth but conflicting evidence for quartz weathering. We integrate grain-size analyses of 75 quartz isolates with 10Be-derived particle residence times from 25 soil depth profiles to show that quartz weathers minimally during exhumation through saprolite and subsoil but undergoes rapid volumetric loss in topsoils. This depth-dependent weathering reflects pronounced fining of the coarse tail of the quartz size distribution, with D90 values decreasing from 0.97 mm in saprolite to 0.50 mm in topsoil, accompanied by shifts in grain span and sphericity. Mean D50 values also fine from saprolite (0.36 mm) to topsoil (0.27 mm) but increase at the transition to less bioturbated Bw horizons. Previous laboratory experiments, exceptionally large earthworm biomasses, and minimal fining in less worm-worked soils below knickpoints collectively implicate earthworms as key agents of quartz weathering. Additional geochemical and mineralogical data indicate this process remains invisible to traditional weathering proxies, despite our estimates that worm-induced quartz weathering contributes 1–22% of long-term Si fluxes from soils developing in relict topography. These findings reconcile longstanding discrepancies regarding silica weathering in El Yunque and highlight the key role of soil fauna in modulating silicate weathering and carbon–climate feedbacks.

T5.7. Modelling earthworm–soil interactions using a coupled discrete element and mass–spring approach

Páthy L., Keller T., Lamandé M., Tamás K.

Earthworms are integral to the formation and maintenance of soil structure; however, the mechanical processes governing earthworm–soil interactions remain poorly understood. This knowledge gap is partly attributable to the inherent challenges associated with measuring processes occurring within soil. In this study, we introduce a modelling framework designed to simulate earthworm locomotion through soil. Specifically, a mass–spring model of earthworm movement was developed and calibrated using a genetic algorithm, and subsequently coupled with the discrete element method to represent earthworm–soil interactions. Model performance was evaluated by calculating the energetic cost of burrowing and comparing the results with values reported in the literature. The framework was then applied to investigate the effects of soil moisture content and earthworm size on burrowing energy requirements. The simulations predicted increased energy expenditure in drier soils and for larger earthworms, in agreement with existing studies. In addition, the influence of earthworm mucus was explored by varying soil–worm friction coefficients and burrow bond strength parameters. Overall, the proposed modelling framework provides a novel tool for improving mechanistic understanding of earthworm–soil interactions and for identifying key knowledge and data gaps. Future research should focus on refining the earthworm representation, conducting targeted experiments to generate additional validation data, and enhancing computational efficiency.

Poster presentations

Session 1: Soil Functioning & Biogeography

P1.1. A comprehensive survey of earthworm populations in arable soils of Germany

Förster A., Tilk J., Weinfurtner K., Emmerling C.

Land-use intensity has detrimental effects on soil fauna distribution, including earthworm communities. These ecosystem engineers are highly sensitive to environmental variables, underscoring the need for distribution monitoring to sustain soil health and productivity, especially of arable land.

Earthworm sampling included 51 locations and 4 land-use types (agriculture, pasture, orchard and vineyards) across 2 years. The monitoring additionally considered soil and environmental parameters like soil organic matter, pH, water holding capacity and total precipitation of the previous year.

The results included 19 earthworm species from 5 life forms (epigeic, epi-anecic, endo-anecic, endogeic and intermediate). Some species were widespread (e.g. *L. terrestris*, *A. caliginosa*,) while others only appeared on specific sites (e.g. *M. minuscula*, *A. cupulifera*). The species and α -diversity gradient was in accordance with surface coverage: orchard > pasture > agricultural land > vineyard. Epigeic species preferred pasture over vineyard soils, while endogeic species were promoted in pasture and orchards. Results of β -diversity showed a gradient from Northeast to Southwest Germany, with highest values in the Southwest. Species number, abundance and biomass were enhanced in soils which received organic waste application. The Canonical Correspondence Analysis revealed a correlation between water holding capacity and agricultural land. pH was lowest for orchard and vineyard soils.

This comprehensive survey of the earthworm community across Germany revealed high correlation between species distribution, abundance and biomass of earthworms and land-use types. It may serve as an overview for soil health management and as a basis for evaluating biodiversity patterns across a land-use intensity gradient in Germany.

P1.2. Earthworm gut microbiota as a source of biologically fixed nitrogen

Álvarez-Jiménez M., Reyes Ceron A.C., Reverchon F., de los Santos Villalobos S., Blouin M., Guevara-Avendaño E., Cortazar Murillo E.M., Rogel Hernandez M.A., Martinez Romero E., Zamora-Briseño J.A., Barois I.

Earthworms are known to enhance soil fertility, yet the microbial mechanisms underlying their contribution to nitrogen (N) dynamics remain underexplored, particularly in N fixation. Using 16S rRNA gene metabarcoding data, we performed functional predictions of N fixation pathways in bacterial communities from soil, *P. corethrurus* foregut, hindgut, and casts. Predicted abundances of nitrogenase-related genes were markedly enriched in the gut compartments, particularly in the hindgut. These predictions were supported by molecular detection of the *nifH* gene and by the successful isolation of diazotrophic bacteria using N-free media. Nitrogenase activity was further confirmed through acetylene reduction assays conducted both in vitro on isolated strains and in vivo in adult earthworms, demonstrating active N fixation. Core microbiota analyses revealed that several bacterial genera commonly associated with diazotrophy were consistently present in the hindgut and foregut, like. *Paenibacillus*. Together, these results demonstrate that *P. corethrurus* hosts active N-fixing microbial communities, suggesting that earthworm-microbe symbioses contribute directly to soil N inputs and may represent a widespread yet underexplored mechanism in terrestrial ecosystems.

P1.3. Vegetation structure effects on earthworm horizontal distribution depend on their size: evidence from a grazed temperate grassland

Ligrone A., Jorge-Escudero G., Martínez B., Piñeiro G.

Terrestrial earthworm populations show an aggregated horizontal distribution and, although this pattern is often assumed to stem from spatial heterogeneity in resources and soil environmental conditions, our understanding of how these interactions operate remains limited. One of the most relevant factors is vegetation structure and physiognomy, which in grazed temperate grasslands typically takes the form of tussocks or shrubs interspersed with short grasses. Understanding how this structure interacts with earthworms is key to explaining population dynamics, improving sampling designs, and developing more sustainable grazing systems. In this preliminary analysis, we found evidence that vegetation structure in temperate Río de la Plata grasslands differentially affects earthworm distribution across size classes. Specifically, at a microsite scale tussock vegetation promoted aggregation of larger individuals ($p < 0.01$), but not of smaller ones. Conversely, at paddock scale greater plant biomass (i.e., forage availability) was associated with higher abundance of smaller individuals ($p < 0.01$), but not of larger ones. This suggests the presence of size-dependent mechanisms related to the spatial distribution of vegetation (e.g., mobility towards “resource islands” or favourable conditions such as higher soil moisture) and higher recruitment in grasslands with greater primary productivity. This interaction is not commonly tested in field studies, and even less so in this region. Our observations provide a potential contribution to understanding small-scale distribution patterns in these organisms, strengthening sampling design in these ecosystems, and highlighting the value of environmental heterogeneity for biodiversity conservation in grazed grasslands.

P1.4. Earthworms increase carbon incorporation into soil from plant shoots or rhizodeposition depending on feeding habits and plant traits

Ligrone A., Berenstecher P., Jorge-Escudero G., Piñeiro G.

Earthworms influence ecosystem structure and functioning, particularly the dynamics of soil organic carbon (SOC). Despite their relevance, much remains to be understood about the mechanisms behind these effects, particularly the influence of factors such as feeding habits, the quality and location of plant residues, and different soil fractions (particulate organic matter, POM; and mineral-associated organic matter, MAOM). Our objective was to assess the effect of two earthworms with different feeding habits on soil carbon dynamics. Using isotopic labelling, we tracked the dynamics of SOC and plant carbon in POM and MAOM in microcosms over 40 days, with specimens of *Aporrectodea caliginosa* (geophage) or *Lumbricus friendi* (saprophage) and plant residues of contrasting quality, i.e. *Vicia villosa* (legume, high quality) or *Avena strigosa* (grass, low quality). Earthworm activity almost doubled the incorporation of carbon into the soil, primarily to MAOM. In the presence of high-quality biomass, the geophagous earthworm showed greater carbon incorporation efficiency (five times greater effect per earthworm biomass) and reduced SOC loss. With low-quality biomass, only the saprophagous earthworm increased carbon incorporation. We also observed that the increase in carbon incorporation originating from roots depended solely on rhizodeposition, a finding with important ecological, agronomical and methodological implications. This work highlights the relevance of the interaction between earthworms and plant functional traits in their effects on SOC dynamics.

P1.5. Shaping the regenerative mechanisms in the soil by earthworms

Kliszcz A.

Earthworms as the main soil ecosystem engineers consume profound amounts of soil bacteria along with the soil and excrete a different qualitative and quantitative microbial population structure in their casts. The soil bacteria they excrete also belong to the group of PGPB (Plant Growth Promoting Bacteria) and are beneficial to plants. In many cases they are multiplied after passing through the earthworms' digestive tracts. On the other hand, the beneficial role of PGPB in various crops and ornamentals has been confirmed, but little is known about the influence of specified earthworm species in shaping the regenerative mechanisms in the agricultural soil in the species-specific relation with soil microorganisms and plants. The regenerative mechanisms confirmed so far in the soil thanks to earthworms will be presented.

P1.6. From orchards to Montado: earthworm community responses to land-use intensification in Portugal

Bartz M.L.C., Leitão R., Nascimento E., Mendes S., Dornellas L.F., Bento M., Durán J., Cunha L.K., Costa J., Sousa, J.P.

This study investigated how different land-use types and management intensities influence earthworm abundance and species richness in the Beira Baixa region, Portugal. Eight land-use systems were evaluated along an intensification gradient, including almond orchards (intensive and super-intensive), olive groves (traditional without soil disturbance, traditional with soil disturbance, and intensive), and pastures (irrigated pasture, Montado with controlled grazing, and Montado with uncontrolled grazing). Earthworms were sampled using the TSBF method during four sampling campaigns (September and December 2022; March and June 2023). At each site, three grids separated by at least 500 m were sampled, with nine sampling points per grid. In total, 864 samples were collected and 1,845 earthworm individuals. Abundance varied markedly among land-use systems, with higher densities observed in intensively managed almond orchards and Montado systems with controlled grazing, while lower abundances were found in irrigated pastures and systems with higher soil disturbance. A total of 15 earthworm species and morphospecies were identified, belonging to the families Lumbricidae, Acanthodrilidae, and Ocnerodrilidae. Species richness was lowest in irrigated pastures and Montado with uncontrolled grazing, whereas higher richness was observed in traditional olive groves without soil mobilization and Montado systems with controlled grazing. These results indicate that land-use intensification and management practices strongly influence earthworm communities in Mediterranean agroecosystems. Ongoing analyses will further explore species composition patterns in relation to soil properties and site management history, reinforcing the role of earthworms as indicators of soil ecological quality.

P1.7. Earthworms in Eucalyptus and natural regenerating forests in two Brazilian biodiversity hotspots

Bosello F., Demetrio W., Peña D., Dudas R., Cooper M., Brown G.G., Mathieu J.

Earthworms are soil ecosystem engineers providing essential ecosystem services and may play an important role in forest regeneration and forestry production. We evaluated earthworm communities in Eucalyptus sp. plantations and regenerating natural vegetation under two soil types differing in clay content and fertility in two Brazilian biodiversity hotspots. Study plots located in and near the Forestry Science Experimental Station of the University of São Paulo (Itatinga, São Paulo) occur in an ecotone between the Atlantic Rainforest and Cerrado biomes. Earthworms were sampled using a modified ISO 23611-5 TSBF handsorting method. Five 25×25 cm monoliths were collected along a 40-m transect and excavated to 10-cm depth after surface litter removal. Overall, 497 individuals+137 cocoons were recovered, from seven species: two new native worms (*Glossoscolex* sp. nov. and *Fimoscolex* sp. nov.), and five exotic/peregrine (*Pontoscolex corethrurus*, *Amyntas gracilis*, *Amyntas corticis*, *Dichogaster saliens*, *Dichogaster affinis*). At the plot scale, earthworm abundance ranged from 10-323 ind. m⁻² and biomass from 0.15-57.54 g m⁻², with *P. corethrurus* overwhelmingly dominant. Clayey Atlantic Forest soils had higher abundance (143±92 ind. m⁻²) and biomass (17.52±18.33 g m⁻²) than sandier Cerrado soils (66±60 ind. m⁻², 9.66±11.82 g m⁻²). Earthworm communities differed among land-uses: Eucalyptus monocultures (141 ind. m⁻²) and regenerating forests had higher abundance (ca. 102 ind. m⁻²) than native vegetation (42 ind. m⁻²). Overall, our results highlight the combined influence of soil texture and land-use history on earthworm communities in tropical forest landscapes.

P1.8. The Belgian Earthworm Atlas: updating a national inventory of earthworm diversity since Bouché (1978)

Buyck E., De Smedt P., Sanczuk P., Bracke J., Cottyn C., Desie E., De Vos B., Lock K., Magr L., Moereels L., Muys B., Soors J., Schelfhout S.

The first provisional atlas of Belgian earthworms published by Bouché in 1978 provided a standardized inventory of lumbricid species, and serves as a critical baseline for agronomic and ecological research. Since then, no comprehensive update of earthworm distributions in Belgium has been made, resulting in fragmented current knowledge of species occurrences and rarity.

The updated Belgian Earthworm Atlas aims to renew and expand the former baseline knowledge by compiling a standardized inventory of earthworm species presence across the entire territory of Belgium at the spatial resolution in 10 km × 10 km UTM grid squares. Following the cartographic principles of the European Invertebrate Mapping framework, data are assembled using three complementary approaches: (1) targeted field surveys in representative habitats (forests, grasslands, road verges); (2) expert-validated photographic records from citizen science platforms e.g. observation.org and (3) integration of published and unpublished research data. For species with sufficient observations, data are integrated in a distribution modelling framework to predict the habitat suitability of all recorded Earthworm species across Belgium.

We present the atlas methodology, spatial distribution of recorded species and the preliminary distribution map for Belgium. Preliminary species richness per UTM grid square ranges from 0 (likely reflecting undersampling) to 23 species. By revisiting and extending the work initiated in 1978, this atlas will provide the most recent standardized reference for Belgium and will serve as a robust baseline for earthworm ecology and diversity.

P1.9. Principal earthworm drivers in plantations vs. natural colonised forests in the UK

Dadò D., Butt K.R., Vanguelova E.

Trees and earthworms influence each other in temperate forests. Earthworms enhance litter incorporation and nutrient cycling, offering important resources for vegetation. Conversely, plants modify soil and litter properties via exudate production and leaf litter addition, creating niches for soil fauna populations. Studying variation of soil communities is particularly interesting in a forest setting through spatial changes, for instance, regarding management practices. In fact, differences in plant establishment increases niche heterogeneity and associated soil and litter parameters. However, few studies have focused on this subject in temperate regions, leaving an important gap in the description of soil communities among different forestry strategies. Here, preliminary research on the differences in earthworm communities between plantations and naturally colonised forests was conducted. Initially, five sites were selected across experimental locations in the UK, which were partitioned into three treatments (mature woodland, plantation and naturally colonised forest). Earthworm community structure and composition will be characterized across these treatments, while associated soil, litter, vegetation and climatic variables will be recorded to identify the primary environmental drivers influencing earthworm communities within these forest settings. We expect to find significant differences in earthworm communities between the plantation and the naturally colonised forest, principally explained by differences in vegetation assembly and litter/soil parameters linked to pH and nutrient contents.

P1.10 Earthworm species richness across major habitat types and biogeographical regions in Croatia

Hackenberger Kutuzović D., Đerđ T., Hackenberger Kutuzović B.

Earthworm species richness and composition were analysed across major habitat types in Croatia with the aim of establishing baseline values for soil biodiversity under different land-use contexts. Habitat types were derived from CORINE Land Cover and aggregated into agricultural land, grasslands and pastures, deciduous forests, coniferous forests and mixed forests, within three European biogeographical regions (Continental, Alpine and Mediterranean). Species richness estimates revealed clear differences among habitat types and biogeographical regions, with deciduous forests and agricultural land supporting the highest numbers of species, particularly within the Continental region. However, deciduous forests were also the most intensively sampled habitat type, which should be considered when interpreting observed richness patterns. In addition to land use, selected physico-chemical soil parameters were analysed to support the interpretation of earthworm diversity patterns. Endemic earthworm species were rarely recorded in agricultural land and were largely restricted to the Mediterranean biogeographical region, while arable sites in the Continental and Alpine regions were dominated by widespread generalist taxa. Part of the dataset is based on historical records, and therefore land-use categories derived from CORINE Land Cover may not always fully correspond to land use at the time of sampling. As an illustrative case study, results from the FUNECOSoil project are presented, where three different land-use types were sampled at the same locality, revealing distinct earthworm assemblages among land uses. Presented data could provide a descriptive framework for interpreting earthworm species richness in Croatia and represent a starting point for future soil biodiversity assessments and monitoring efforts.

P1.11. Survey on earthworm species diversity in agricultural areas of Eastern China

Wu D., Jiang J., Zhang Y., Zhao H., Sun J., Liu M., Qiu J.

From 2019 to 2023, we conducted a five-year investigation on the diversity of earthworm species in the agricultural ecosystem in eastern China. The surveyed area covered approximately 75% of the agricultural ecosystem in China. The habitats investigated included farmland, plantations and grasslands around farmland. We collected earthworm specimens using the handsorting and electric method. In total, we took over 7,600 samples and over 120,000 earthworm specimens were collected. A total of over 400 earthworm species were identified, and a series geographical maps of earthworm species were drawn. The survey results showed that most earthworm species had a narrow distribution range, and only a few species were widely distributed. In the agricultural ecosystem of eastern China, the number of earthworm species was higher in low-latitude regions than in high-latitude regions, and the density of earthworm individuals also decreased from low to high latitudes.

P1.12. Earthworm density in urban and semi-urban land uses in Curitiba, Brazil's "Ecological Capital"

Dudas R., Oliveira V.M., Sautter K.D., Bartz, M.L.C., Bruz L.S.M., Marques, R., Demetrio W., Sanches C.L., Mathieu J., Cooper M., Brown G.G.

Due to its extensive green spaces, urban and state parks, Curitiba (Paraná State, Brazil) is known as Brazil's "Ecological Capital", with approximately 60 m² green area per inhabitant. We evaluated earthworm diversity and abundance related to urbanization level, with urban sites within city limits and semi-urban sites to 5 km distance from the city margin. We also assessed how land use systems influence the urbanization gradient. Abundances (ind·m⁻²) came from soil monolith handsorting. Earthworm communities were dominated by exotic species, mostly *Pontoscolex corethrurus*, *Amyntas gracilis* and *Amyntas corticis*, with higher densities in disturbed semi-urban sites. In annual crops, *P. corethrurus* and *A. gracilis* densities were 64 and 45 ind·m⁻², respectively. Pastures had mostly *P. corethrurus* (75 ind·m⁻²) and grass lawns had 48 ind·m⁻². Native Glossoscolecidae (*Fimoscolex* and *Glossoscolex* genera), including 17 potentially new species, occurred in low densities (<2 ind·m⁻²) in native and managed forests and grass lawns, but not in crops and pastures. Overall, 38 species (22 native, 16 exotic/peregrine) from six families (Benhamiidae, Glossoscolecidae, Lumbricidae, Megascolecidae, Ocnerodrilidae and Rhinodrilidae) were recorded: 29 in urban and 22 in semi-urban sites. Grass lawns and managed forests had more species in both urban (25, 11 spp.) and semi-urban areas (13 spp. in both), while annual crops and pastures had lower richness (<8 spp.). Hence, despite intense urbanization, Curitiba still harbors a taxonomically unique earthworm fauna, highlighting the importance of subtropical urban and semi-urban green areas for earthworm conservation.

P1.13. Invasion dynamics of the exotic flatworm *Obama nungara* and impact on soil ecosystem

Dupont L., Noël S., Fourcade Y., Gauchere C., Fauré A., Fallet E., Frechault S., Gigon A., Lerch T.Z., Mazuras N., Younes G., Pando A., Araujo J., Ventura M., Roy V.

Biological invasions pose an increasing threat to terrestrial ecosystems, while the impacts of invasive predators on belowground biodiversity remain poorly understood. Over the last decade, the South American land flatworm *Obama nungara* has rapidly expanded across Europe. As a generalist predator feeding mainly on earthworms and gastropods, it may strongly affect soil biodiversity and ecosystem functioning.

The PLATWORM project investigated the ecological mechanisms driving its invasion in France using field surveys, laboratory experiments, genetic analyses and ecological modelling, and assessed its impact on soil ecosystem.

Field surveys revealed that (i) populations are at different stages of invasion, ranging from early introduction to expansion, (ii) *O. nungara* thrives in humid microhabitats providing shelter, and (iii) its population dynamics is strongly influenced by local climate.

Laboratory experiments revealed notable resilience traits, including the ability to survive for months without feeding and to reproduce after prolonged isolation. Predation trials showed that *O. nungara* consumes a broad range of prey at high rates, with no detectable effect of prey type on growth. The earthworm prey consumed were characterized by metabarcoding (presentation by Ventura *et al.*).

Mesocosm studies indicated that *O. nungara* can disrupt earthworm bioturbation and the physical processes that maintain soil structure and permeability. Modelled ecosystem trajectories suggest that soil communities may remain resilient, with prey population declines being reversible—except during prolonged droughts in which only endogeic species survive.

Overall, these findings highlight the long-term ecological risks posed by invasive soil predators and provide valuable tools for improving monitoring and predicting future impacts.

P1.14. Plant species identity affects earthworm abundance and diversity in managed grasslands

Baekelmans E., Hoekstra N., Finn J., Groot J., van Eekeren N.

Multispecies swards can match the productivity of perennial ryegrass monocultures in managed grasslands under reduced levels of nitrogen fertilisation. However, the effects of multispecies leys on soil life – another important aspect of sustainable agriculture – are not well understood. In this study we quantified the effect of plant diversity on the abundance and diversity of earthworms in the soil. We established a field experiment on a sandy soil in The Netherlands with grassland communities composed of systematically varying proportions of one up to six species (two grasses, two legumes, two herbs) according to a simplex design. Swards received a nitrogen (N) application rate of $150 \text{ kg N ha}^{-1} \text{ yr}^{-1}$; additional perennial ryegrass monoculture (Lp) plots received $300 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (Lp300). We measured earthworm abundance and diversity at the end of the three-year grassland period. Artificial fertiliser increased the number of earthworms in Lp monocultures, but red clover, white clover and plantain monocultures did not differ significantly from Lp300. We found effects of plant species identity on earthworm abundance and diversity. We did not find synergistic or antagonistic effects of combining different grassland species on abundance but we did find antagonistic effects of combining herbs and legumes and two herb species on the Shannon diversity index and on endogeic earthworm abundance. This suggests that we can steer earthworm communities by carefully selecting and combining plant species.

P1.15. Earthworm diversity across an elevational gradient in Mt. Sawtooth and its neighboring highlands in the Philippines

Angeles E.J.A., Española C.P.

An earthworm diversity survey conducted in Mt. Sawtooth and nearby highlands in the Zambales Mountain Range, part of the province of Tarlac, the Philippines, documented 34 earthworm morphospecies. The exotic species *Pontoscolex corethrurus* was recorded in lower elevations, while 33 morphospecies were classified into the family Megascolecidae and were assigned to the following genera: *Pheretima*, *Pithemera*, *Pleionogaster*, *Archipheretima*, and *Polypheretima*. Based on Jaccard's index, two clusters of earthworm assemblages were identified: a cluster with low species richness and dominance of *P. corethrurus*, and a cluster with predominantly megascolecid earthworm genera. Most megascolecid earthworms were found in areas with broad canopy cover, high soil moisture, high relative humidity, but with low air and soil temperature, and soil pH. The introduction of the non-native tree species *Eucalyptus urophylla* and *Acacia mangium* in a past afforestation project may have negatively affected earthworm diversity in lower elevations, as these species can modify soil properties and destroy natural habitats, creating conditions ideal for the invasion by *P. corethrurus*. The diversity of megascolecid earthworms in higher elevations was partially attributed to the geological history of the Zambales Mountain Range, as further investigation through molecular sequencing is needed. Mt. Sawtooth has recently been designated a Protected Landscape under Republic Act No. 12237, highlighting its increasing conservation importance.

P1.16. Cover crop in pure stands or in mixtures? Earthworm preferences in the field and laboratory

Euteneuer P., März M., Flotzinger P., Butt K.R.

Cover crops are important for sustainable farming as they can prevent soil erosion and support soil biodiversity such as earthworms. Earthworms depend on protein rich food with a low C:N ratio as from cover crop residues for growth and reproduction. Mixtures of cover crop species are recommended for farmers to increase the resilience of arable systems and to prevent shortfalls of soil coverage of single crops. In a three-year field trial (2020-2022) in Austria, we tested if earthworm abundance and biomass also gain from crop mixtures or prefer pure stands. Treatments used were: *Sorghum sudanese* (SG), *Raphanus sativus* var. *longipinnatus* (RD), *Lathyrus sativus* (GP) and a mixture of these three crops (MX) and bare fallow as control (Ctrl). As the East of Austria has only 550 mm of annual precipitation, we also tested irrigation vs. rain-fed. Results showed that using cover crops and plant C:N ratio affected total earthworm abundance in autumn more under irrigated than under rain-fed conditions. In spring, abundance for GP and RD were higher than for Ctrl under both irrigation regimes, while total biomass for GP and MX were higher under rain-fed compared to Ctrl. Contrastingly, laboratory trials showed that all cover crops increased growth of *Aporrectodea caliginosa* compared to Ctrl with soil only, while mainly RD increased cocoon production for *A. caliginosa* and *Allolobophora chlorotica*. The results show clearly, that usage of cover crops support earthworms and the C:N ratio is important, but cannot fully explain the high cocoon production for RD.

P1.17. When burrowing earthworms enter unexplored domains: effects on northern forest soils and ecosystem functioning

Krab E.J., Garamszegi P., D'Hervilly C., Blume-Werry G., Clemmensen K.E., Keller T., Lindahl B.D.

The ongoing expansion of invasive earthworms into North America has disruptive effects on forest ecosystems and is estimated to substantially reduce soil carbon stocks within only a few decades. Earthworms are widespread in European forests, but Scandinavia's remote boreal forests largely lack burrowing earthworm species. Northward recolonization following glacial retreat, potentially accelerated by increased anthropogenic activities and climate warming, may promote the establishment of burrowing earthworms in northern forests, with detrimental effects on their ecosystem services. To quantify the impacts of burrowing earthworm establishment in previously earthworm-free forest soils, we used mesocosms with intact soil turfs (including tree saplings) from northern forests in a common garden experiment near Uppsala, Sweden. In these mesocosms, we introduced soil-dwelling earthworms (a mix of *Aporrectodea* and *Lumbricus* spp.) at two densities and included two control treatments (addition of dead earthworms and no earthworms). The forest soil turfs represented a fertility gradient, allowing us to test the hypothesis that burrowing earthworm establishment and its effects on ecosystem functioning (e.g. plant growth, soil fungi, and soil carbon dynamics) are mediated by forest fertility.

P1.18. Drivers of urban earthworm communities across contrasting land use and management in Rennes (France)

Fertil A., Hoeffner K., Lecuyer T., Le Callonec L., Audusseau H., Guillocheau S., Scimia J., Cluzeau D.

Soil organisms play a key role in supporting a large portion of terrestrial biodiversity and provide essential ecosystem services. Understanding the dynamics of these organisms is a growing challenge that should be placed at the core of conservation strategies. These organisms remain understudied in urban environments, although being exposed to potentially strong constraints – soil disturbance, urbanization, surface runoff, etc. – and urban managers are in high demand of knowledge to better understand how to include soil health in sustainable urban planning.

In this study, we focused on earthworms, widely recognized as relevant soil bioindicators, and comparatively easier to observe than other below-ground biodiversity. Earthworm communities were sampled thanks to the SoilRise citizen science initiative using a hand-sorting protocol in 143 plots in the city of Rennes (West of France, 50,39 km², 4582 inhabitants/km²), covering a wide diversity of soil uses and managements in the urban center and in its close periphery. We aim to describe community-level metrics in relation to (i) land use, (ii) management practices, (iii) soil properties (e.g., texture, pH, organic matter %, etc.), and (iiii) landscape metrics surrounding the plots (e.g., distance to city center, urbanization rate, density of vegetal patches, etc.). A total of 25 earthworm species were recorded, with a mean abundance of 412 earthworms per square meter, and a mean richness of 6.2 species per square meter across all sampled plots. Results from this study will be discussed in terms of recommendations to urban managers, and of consequences these urban drivers have on soil functioning.

P1.19. Quantitative assessment of earthworm community structure and soil physicochemical properties to support urban forest management

Dissanayake G.S.B.M., Butt K.R., Doick K., Vanguelova E.

Urban soil degradation and its impacts on tree growth have emerged as a critical challenge in the 21st century. Soil conditions play a fundamental role in tree growth and establishment, highlighting the need to establish biological, chemical, and physical baselines for effective soil management. Functioning as ecosystem engineers, earthworms serve as critical bioindicators; their presence, abundance, and diversity provide high-fidelity insights into the contemporary physicochemical status and functional health of the soil. A series of surveys were planned to investigate earthworm abundance and diversity, along with key soil parameters, to inform urban forestry management strategies. A survey of two urban infrastructure typologies in Leeds, UK, in spring 2025 revealed that earthworm abundance was higher in community gardens than in street tree sites. Four earthworm species were identified: *Aporrectodea longa*, *A. caliginosa*, *A. rosea*, *Allolobophora chlorotica*, and *Lumbricus* spp., with endogeic earthworms dominating all sites. Another survey was conducted in Preston (autumn 2025) in a park and a Nature Reserve to explore tree species-specific soil conditions alongside earthworm communities. A soil survey was undertaken below the three most common urban tree species: silver birch (*Betula pendula*), English oak (*Quercus robur*), and sycamore (*Acer pseudoplatanus*). Tree-species-specific soil variation across urban transect zones (structural gradient organising human habitats from rural to urban environments) is also understudied, prompting a final soil physicochemical survey that included earthworm diversity across transect zones in four English cities. This integrated approach provides new insights into soil conditions and biotic indicators that can strengthen evidence-based urban forest management.

P1.20. Earthworm functional diversity in the Americas: trait data and global change threats

Gérard S., Grüneberger M., Vanadzina K., Kukowski R., Nagahawatte K., Ganault P., Brown G.G., Cameron E.K., Phillips H.R.P.

Earthworms are well-recognized ecosystem engineers but lack conservation recognition and efforts in the context of Global Changes (GCs). Recent research initiatives have led to a good understanding of global earthworm diversity and its drivers. Still, while being notable actors of soil functioning, earthworm functional diversity has been neglected in these macro-scale analyses, and it is still not well understood how earthworm functional diversity is threatened by GCs. It may be due, in part, to the lack of earthworm trait databases outside of European fauna. In this work, we aim to identify what are the major GC threats to earthworm functional diversity and which traits are more at risk using a comprehensive trait dataset for species from the American continent sourced from literature, primarily from species description papers. We will use this trait database in combination with an updated version of sWorm, a global earthworm community database, to measure functional diversity in the Americas. GC threats will be assessed thanks to recently published maps of anthropogenic pressures. We have currently gathered ~10,000 values of traits, consisting mostly of size, reproduction and digestion anatomy, habitat, and life-history traits. We expect functionally degraded American earthworm temperate faunas to be more strongly threatened by GCs due to higher anthropic pressure intensity. Conversely, functionally richer American tropical faunas might be less subject to pressures, but with a greater diversity of functional traits at risk. Similar to patterns observed in vertebrates, we also expect larger species to be more impacted than smaller species.

P1.21. Changes in SOM functional groups under the influence of invasive endogeic earthworms

Golovanova E.

The work investigated a set of functional groups included in soil organic matter (SOM) using IR spectroscopy to assess the impact of the native earthworm *Eisenia nordenskioldi pallida* and the invasive species *E. tracta* and *Aporrectodea caliginosa*, as well as their combinations. The field experiment was conducted in mesocosms on Gleyic Chernozems over an 11-month period. It has been established that endogeic species affect a similar set of functional groups, but at different depths. Cohabitation of the species significantly influences the emergence of new functional groups. *E. n. pallida* makes the greatest contribution to the diversity of the qualitative composition of soil organic matter under cohabitation. Earthworm invasion will change the depth of minimum and maximum diversity of SOM functional groups, the proportion of stable and labile SOM, especially in the 40-50 cm layer. The data obtained can be used to predict changes in SOM under the influence of invasive species and to develop a technology for managing the qualitative composition of SOM with the help of earthworms.

P1.22. Impact of forest management practices on soil functioning and earthworm communities in Spanish National Parks

Gutiérrez López M., Piris A., Ureña-Lara C., Pérez Corona E., Herrero-Jáuregui C., Rebollo P., Andivia E.

Forests provide key ecosystem services for human well-being, but global change and increasing disturbances are compromising their functioning, particularly in mountain forests of drought prone areas. Forest management, such as the thinning and the diversification of monospecific stands, plays a key role in the adaptation of these ecosystems to climate change. However, the impact of these measures on key drivers of ecosystem service provision—such as soil functioning and the soil-dwelling invertebrate communities—remains largely unknown. These communities play a pivotal role on soil processes like nutrient retention and cycling as well as serve as bioindicators of soil function.

The objective of this study is to evaluate the effect of thinning and species mixing on soil functioning and earthworms communities in mountain forests under Mediterranean conditions. To this end, we sampled soils in forest stands with different species composition (monospecific and mixed) and subjected to different thinning intensities in three Spanish National Parks along a latitudinal gradient (Ordesa and Monte Perdido in the north, Guadarrama in the centre, and Sierra Nevada in the south of the Iberian Peninsula). At each sampling stand, earthworms were extracted using the mustard extraction method combined with hand sorting and were subsequently identified. Soil samples were collected to analyze physico-chemical parameters. The highest earthworm abundance and diversity were recorded in Ordesa and Monte Perdido and the lowest in Sierra Nevada with only one species identified. Tree species composition and thinning seem to modify the structure and composition of these communities and the soil physico-chemical parameters.

P1.23. Urban earthworm communities driven more by climate and elevation than urbanisation intensity

Phillips H.R.P., Hoeffner K., Muys B., Le Bayon R.C., Cluzeau D., Butt K.R., Maréchal J., Briones M.J.I., Decaëns T., Hedde M., Gérard S., Pérès G., Petit-Dit-Grézériat L., Pelosi C.

Soil biodiversity, particularly earthworms, plays a crucial role in ecosystem functions and services but remains largely understudied in urban environments. In this context, we conducted a literature synthesis analysis of 41 studies from urban environments (726 records selected, 1995–2024) to (1) describe patterns of earthworm communities, (2) identify the main drivers shaping urban earthworm communities, focusing on the effects of climate, degree of urbanisation, soil properties, and local land use, with the expectation that climate would be the strongest driver, and (3) assess the occurrence of exotic earthworm species. Urban earthworm communities showed considerable variation in their abundance, biomass and species richness. Despite the strong geographic bias (with 75% of records originating in Europe), our results showed that urbanisation intensity had no consistent effects on earthworm communities. Our dataset revealed that, at the global scale, elevation and annual variation in air temperature and potential evapotranspiration are the most important predictors of total earthworm abundance. For total earthworm biomass, variation in potential evapotranspiration is the key predictor. At the plot scale, we found both positive and negative correlations between earthworm community metrics, soil properties, and specific land uses, namely urban forests and grasslands. We recommend regular standardised sampling, broader geographical and temporal coverage, and the investigation of urban-specific direct and indirect stressors as crucial steps for understanding urban soil biodiversity and promoting beneficial management practices. Overall, this analysis indicates that, given the importance of temperature and water demands, mitigating climate change would be essential for maintaining earthworm communities.

P1.24. Influence of land use patterns on earthworm communities and soil ecosystem functions

Osei J., Appiahene K., Takyiwaa M., Memgmi F.D., Poku S.O.

Soil biodiversity, particularly earthworms, plays a crucial role in ecosystem processes and functions. Understanding the diversity and abundance of earthworms in various land use patterns is essential for sustainable agricultural practices. This study aimed to assess the diversity and abundance of earthworms across different land use patterns, evaluate their relationship with soil physico-chemical properties, and promote awareness among farmers regarding the importance of earthworm diversity. Earthworm sampling was conducted in five distinct land use patterns in Eastern Region of Ghana, using a hand-sorting method within quadrates ($1 \times 1 \text{ m}^2$). Soil samples were analyzed for physico-chemical properties, and statistical analyses were performed using SPSS and Past software. A total of five earthworm species were identified, with significant variations in abundance across land use patterns ($p < 0.05$). The order of abundance was Garden > Grassland > Forest > Leaf litter > Agricultural field. Factor analysis revealed a direct relationship between earthworm abundance and soil physico-chemical properties. The study demonstrated that less disturbed soils exhibited higher earthworm diversity and abundance compared to disturbed systems. It is recommended that farmers adopt non-conventional agricultural practices to enhance earthworm diversity, thereby improving soil health and ecosystem services. Increased awareness and education on the ecological benefits of earthworms should be promoted to encourage sustainable land management practices.

P1.25. Earthworm strategies for winter survival in temperate mountain forest soils

Butt K.R., Garczyńska M., Mazur-Pączka A., Pączka G., Kostecka J.

As thin-skinned and water-rich soil organisms, earthworms have evolved strategies for survival in temperate regions during adverse soil conditions that, close to the soil surface, can range from baked dry during the summer, to freezing, during winter. This investigation sought to establish winter strategies shown by earthworms in the beech-dominated forests of the Carpathian Mountains of SE Poland. Visits (2020 – 2024) to two accessible sites allowed for investigations near the Bieszczady National Park. After snow clearance, as necessary, earthworms were searched for in organic matter, such as dead branches, and then dug from sequential layers in soil pits to 1 m. Comparisons were drawn with data gathered during similar earthworm sampling in September at both sites. A total of 9 earthworm species were found representing the 3 main ecological groups. Results showed that soil temperature, differing from one visit to another, played a critical role in determining earthworm activity. When temperatures were below freezing at the surface, earthworms, as expected, tended to be found deeper in the soil but adopted several strategies with life stages sometimes behaving differently. For example, the normally deadwood-inhabiting *Eisenia lucens* was found at 60 cm, with *Aporrectodea caliginosa* aestivating at a similar depth. More systematic sampling is required, in sequential months covering the whole winter, potentially over numerous years to be able to fully describe the behaviours of the earthworm species encountered.

P1.26. Flint burial by earthworms at Darwin's home: 18 years of monitoring

Butt K.R., Callaham Jr. M.A., McLauchlin J., Pérès G., O'Rourke A., Euteneuer P.

Charles Darwin famously wrote 'The Formation of Vegetable Mould' and recorded observations of flint burial within a previously ploughed field (Great Pucklands Meadow) to a depth of 6.3 cm, over 30 years (mean rate of 0.21 cm y⁻¹). He wrote "This was certainly the work of the worms, for though castings were not frequent for several years...these gradually increased in numbers as the pasture improved". We sought to replicate this work in the same field at Down House with locally sourced flints and derive data periodically over a lengthy time. We set up replicated plots of 1 square metre in 2007 with treatments of either large or small flints, each at two densities. These were sampled 4 times over 18 years, excavating ¼ of each of the 16 plots on each visit. Number of flints visible and depth of burial was recorded each time. Results showed earthworm casting was occurring, and the individually recorded flints were buried, with depth increased over time. Small flints were covered more rapidly than large but the larger flints were buried more deeply. Overall, rate of burial was 0.4-0.48 cm y⁻¹ – more than twice that recorded by Darwin, but this slowed with time. During years 1-6; 6-10; 10-14; and 14-18, rates of burial were 0.73-0.96; 0.27-0.47; 0.13-0.36 and -0.10-0.25 cm y⁻¹, respectively. After 18 years, the flints had been buried to 7.14-8.56 cm. It is noteworthy that initial conditions were markedly different to those of Darwin, with respect to soil status and likely earthworm numbers.

P1.27. Climate scenarios shape earthworm communities in boreal agricultural lands

Konsap K., Kuu A., Escuer-Gatius J., Sutri M., Ivask M., Shanskiy M.

Earthworm community responses in boreal agricultural systems differ among climate scenarios, with moisture availability emerging as a key constraint on abundance and morphoecological groups. Earthworms are key ecosystem engineers; however, their responses to climate change remain poorly understood because of the strong interactions between climate and land use. We analysed a long-term, multi-site earthworm database from Estonia, compiling field observations collected between 1995 and 2022 across croplands and grasslands under diverse soil and management conditions. The dataset includes species identity, ecological group affiliation, abundance, biomass, and land-use information.

Recent research shows that land use strongly structures earthworm communities, whereas climate effects are primarily mediated by moisture availability (Sutri et al., 2025). We quantified baseline relationships between earthworm community metrics and climate variables using autumn samples aggregated at the field–year level. Land use was the dominant determinant of community structure, whereas climate effects were secondary but detectable.

Using CORDEX projections from the IPCC AR6 Interactive Atlas, we compared earthworm responses across different climate scenarios. The low-emission scenario (RCP2.6) was characterised by higher precipitation relative to temperature and more favourable conditions for earthworm abundance, whereas the higher-emission scenarios (RCP4.5 and RCP8.5) shifted croplands toward warmer and drier growing season conditions that constrain earthworm communities.

P1.28. When plastisphere and drilosphere meet: earthworms facilitate microbiome and nutrient turnover to accelerate biodegradation of agricultural plastic films

Huang C., Wang L., Wu W.M., Capowiez Y., Qiao Y., Hou D.

Agricultural plastic mulching films have been environmental concerns for decades. The interactions of the anthropogenic plastic niche with other soil biospheres, particularly earthworms, on the fate of plastics remain poorly understood. Here, we investigated the soil plastisphere and the decomposition of buried conventional non-biodegradable low-density polyethylene (LDPE) versus biodegradable PBTA/PLA copolymers with jumping earthworms *Amyntas cortices* in dynamic microcosms. The earthworms significantly enhanced biodegradation of both LDPE and PBAT/PLA in vitro, confirmed by mass-reduction, surface modification, and molecular weights. Specifically, earthworms participated in the microbial succession and community assembly within the plastisphere by increasing the biodiversity of bacteria and fungi, and their network complexity. The time-decay patterns in the abundance of keystone taxa for plastic degradation, including the genera *Noviherbaspirillum*, *Rhizobacter*, and *Mortierella*, were mitigated by earthworms over the incubation period. Additionally, ultrahigh-resolution mass spectrometry analysis indicated earthworms preferentially consumed recalcitrant dissolved organic matter (DOM) molecules in plastisphere soils, in turn, increased bioavailable components, serving as nutrient supplies for plastisphere microbiomes. Our findings demonstrate that earthworms enhance the decomposition of plastics in soils via cross-species interplays within the plastisphere and drilosphere, contributing to not only soil and biodiversity conditioning but also in site plastic biodegradation in agroecosystems.

P1.29. Earthworm effects on fertilizer nitrogen behavior in synthetic and organic farming systems

Appiahene K., Takyiwaa M., Memgmi F.D., Poku S.O.

The impact of earthworms on soil health and nutrient cycling has garnered significant attention, particularly regarding their influence on nitrogen (N) dynamics in agro-ecosystems. This study aims to investigate the effects of earthworms on fertilizer N uptake by plants and the subsequent reduction of N loss to the environment, comparing organic and synthetic fertilizers. A long-term field experiment was conducted, incorporating various types of fertilizers and measuring earthworm activity, soil N pools, and plant N uptake. Data were analyzed to assess the relationship between earthworm presence and fertilizer efficiency. Findings indicate that earthworms significantly enhance fertilizer N uptake by plants, particularly with organic fertilizers, which also resulted in a lower percentage of N loss to the environment. The study revealed that nearly half of the organic fertilizer remains in the soil, suggesting a sustained impact on soil N dynamics. Earthworms play a crucial role in promoting fertilizer N uptake and reducing environmental losses, highlighting their importance in sustainable agricultural practices. Future research should focus on long-term experiments that incorporate changes in the soil native N pool to further elucidate the role of earthworms in agro-ecosystem N cycling, particularly in the context of organic versus synthetic fertilizers. This will provide valuable insights for optimizing fertilizer management strategies in crop farming.

P1.30. Earthworms communities in black pine forests from Turkey: relationships with tree age and *Formica rufa* ants

Lago M.C.F., Çakir M., Briones M.J.I.

The influence of forest age on earthworm populations is not fully understood. Here, we investigated earthworm abundance, biomass and community structure in *Pinus nigra* forests of varying age in Eldivan, Turkey. In addition, because red wood ants (RWA), belonging to the *Formica rufa* species can create suitable habitats for earthworms, their presence at the vicinity of the sampling points was also recorded. Soil chemical parameters (C, N, moisture, pH, and conductivity) were also analyzed. Results showed low earthworm diversity at these forests, with only two species being recorded: *Aporrectodea rosea* and *Lumbricus* spp. Earthworm abundance and biomass were highest in the oldest forest stands, compared to the younger ones. Significant negative relationships were observed between total earthworm abundance and soil conductivity ($r = -0.60$), while epigeic earthworms correlated with soil pH ($r = 0.64$), and endogeic earthworms with soil moisture ($r = 0.69$). The presence of *Formica rufa* populations in the studied forests contributed to the low earthworm abundance registered, and less oligochaetes were found when these ants were present.

These findings suggest that forest age, ant mounds and soil conditions significantly influence earthworm communities, emphasizing the need to consider both biotic and abiotic factors in earthworm assessments.

P1.31. Earthworm ecological groups shape microbial communities and enhance phosphorus cycling in soil casts

Lenin Apuri L., Hayden H.L., Ghaderi R., Hu H.W., He J.Z.

Earthworms are key regulators of soil nutrient dynamics, yet the ecological group-specific effects on microbial communities and phosphorus (P) availability remain poorly understood. This study investigated how anecic (*Lumbricus terrestris*), epigeic (*Lumbricus rubellus*), and endogeic (*Aporrectodea caliginosa*) earthworms, individually and in combination, influence microbial assemblages and P cycling in manure enriched soils over 56 days. Amplicon sequencing of 16S rRNA and ITS regions characterized bacterial and fungal communities in earthworm casts, while soil P fractions, dissolved organic carbon (DOC), and phosphatase activities were quantified to assess nutrient dynamics.

Earthworm presence enhanced soil P, with epigeic earthworms rapidly mobilizing P, likely via selective feeding and microbial stimulation, and endogeic earthworms sustaining P release over time. Mixed-species treatments produced lower P concentrations than individual groups, suggesting that interactions among ecological groups may limit P mobilization rather than promote additive effects. Casts were enriched with bacterial taxa such as *Bacillus*, *Paenibacillus*, and *Streptomyces*, and fungal taxa including *Chaetomium* and *Lasiosphaeriaceae*, indicating selective gut-mediated processes. Bacterial alpha diversity remained stable, whereas fungal richness declined, reflecting stronger selective pressures on fungi. DOC was elevated in casts from epigeic, endogeic, and mixed treatments, correlating with higher alkaline phosphatase activity and enhanced mobilization of organic and mineral-bound P.

These findings highlight that earthworm ecological group identity and diversity strongly shape cast microbial communities and the magnitude and timing of P availability. By modulating microbial composition and enzyme activity, earthworms can enhance soil P turnover, offering strategies for sustainable nutrient management in agroecosystems.

P1.32. Earthworm community at French national scale - Results from national network RMQS Biodiversity

Delourme L., Guillocheau S., Cluzeau D., Pouzenc S., Jolivet C., Beaucher E., Busnel J.M., Carteaux L., De Feudis C., Guegan G., Hoeffner K., Laverdure T., Le Callonec L., Lerner L., Lohat M.H., Roucaute M., Pérès G.

The Soil Quality Monitoring Network -Biodiversity (RMQS-Biodiversity), established in 2021, aims to assess soil biodiversity in France (microorganisms, nematodes, microarthropods, macrofauna including earthworms).

Between 2024 and 2025, 141 sites were sampled across metropolitan France (30 grasslands, 50 arable crop sites, 39 forests, 5 fallows, and 5 vineyards). Earthworms were sampled using hand sorting (soil block: 25 cm × 25 cm × 20 cm) combined with mustard application (500 mL mustard solution). At each site, six replicates were realised. The species analysis was reinforced by adding a complementary qualitative sampling protocol ("prospection"), based on an active search for adult earthworms (spade for 30 minutes), in order to collect earthworms species who could missed by hand-sorting and mustard.

In total 12429 earthworms were collected. The results confirmed the significant impact of land use on earthworm communities, in terms of abundance, biomass, and species composition : grasslands appeared to be the most favorable habitats (mean abundance 349 individuals/m², associated to mean biomass 88 g/m² which correspond to a mean 885 kg/ha, and mean species richness of 7 species), in contrast to arable croplands (mean abundance 218 individuals/m², mean biomass 417 kg/ha, and mean species richness of 5.1 species); forests represented the least favorable habitat (abundance 93 individuals/m², biomass 178 kg/ha, and mean species richness of 4.6 species).

All these results will be presented and discussed to illustrate the value of the RMQS-Biodiversity program for the long-term monitoring of earthworm communities and for assessing the impact of land use at the national scale.

P1.33. From soil to canopy: the role of soil fauna and structure in drought-resilience in pear trees

Magr L., Vancampenhout K., Ceusters J., Bylemans D., Delsemme L., Desie E.

Extreme climatic events, particularly droughts, increasingly threaten orchard productivity in temperate regions. In Flanders, many orchards are established on silt-rich soils that are highly susceptible to compaction and structural degradation, reducing water infiltration, rooting depth, and drought resilience. Earthworms act as key ecosystem engineers in such systems, potentially mitigating compaction effects by improving soil structure, porosity, and nutrient cycling.

We conducted a full factorial mesocosm experiment to disentangle the interactive effects of soil compaction, earthworm inoculation, and drought on young pear trees (*Pyrus communis*). Soil compaction was applied at the start, combined with earthworm inoculation and repeated drought events of varying severity. Plant responses were monitored over two growing seasons, with the first season completed. Measurements focused on plant physiological stress responses, including leaf relative water content, carbohydrate dynamics (starch, glucose, fructose, sucrose), leaf pigment concentrations, and chlorophyll fluorescence as a proxy for photosynthetic performance.

We hypothesize that (i) plant stress responses scale with drought severity and duration, with photosynthetic parameters responding more rapidly than biomass-related traits; (ii) well-structured soils buffer drought stress, while compaction exacerbates physiological stress; and (iii) earthworm activity alleviates drought impacts by improving soil physical conditions and nutrient availability, particularly in compacted soils. By explicitly linking earthworm-mediated soil processes to plant physiological drought responses in a perennial orchard system, this study aims to clarify the role of soil fauna in enhancing drought resilience under increasing climate extremes.

P1.34. Unravelling the effects of ageing and soil characteristics on earthworm cast properties in tropical volcanic soils of Bengkulu, Indonesia

Kamil M.I., Jouquet P., Bottinelli N., Huot H., Rumpel C.

The complexity in understanding the properties of earthworm casts have been a huge challenge, especially in the field conditions with changes in environment is habitually. While several studies have focused on non-volcanic soils, acknowledging this process in volcanic soils remains unclear. Thus, this study aimed to determine the influence of casts aging and soil properties on earthworm cast characteristics. Massive earthworm casts (height can reach 47 cm) with 3 different ages signature (fresh, intermediate, aged) and topsoil aggregates were collected from 27 locations in the foot-slope of Mount Daun, a volcanic region of Bengkulu, Indonesia. In addition, we dug 5 profiles across the study site to align the casts properties with the soil mineral properties. Mid-infrared spectroscopy was used to determine the spectral signatures and humic index of the samples along with total carbon – nitrogen, pH, electrical conductivity. The aggregate stability and hydrophobicity were also determined for the physical properties of casts and bulk soils. The study provides a framework of how earthworm activities influence and modifying soil properties in volcanic soils. The study contributes to the process of casts evolution and spatial variability in tropical soil ecosystems.

P1.35. Earthworm communities of the island forest of Central Kazakhstan

Shashkov M., Petrunin-Sukharev A., Ivanova N.

In Central Kazakhstan, suitable habitats for earthworms are settlements and sparse forest patches enclosed by vast areas of dry grasslands, which are unsuitable for them. Larger forest areas are associated with local mountains. The Karkaraly Mountains have become a territory for research of the regional earthworm diversity.

A total of 22 forest sites of various vegetation were investigated for earthworms, 8 of them were in the Kendara Valley within the mountain range with protection status and 14 outside of the mountains. Earthworms were collected by soil sample hand-sorting during field seasons 2023–25. At least four 25×25 cm samples in area and 20 cm in depth were collected on each site.

Eight species were recorded, of which two were found in both territories of the mountain valley and near settlements: *Dendrobaena octaedra* (Savigny, 1826) and *Lumbricus rubellus* Hoffmeister, 1843. *Aporrectodea caliginosa* (Savigny, 1826) and *Eisenia pallida* (Malevic, 1956) were recorded only outside of mountains; *Aporrectodea rosea* (Savigny, 1826), *Bimastos rubidus* (Savigny, 1826), *Eisenia nordenskioldi* (Eisen, 1878), and *Octolasion lacteum* (Örley, 1881) only within the mountainous area.

The community inside the valley can be described as more diverse but less abundant in terms of density and biomass in comparison with the community on the outer part of the mountain range. At the same time, five species of them are considered invasive, besides *Eisenia* and *Bimastos*, and the main driver of such diversity is human agricultural activity, which we assumed to be responsible for the introduction and spread of invasive species.

P1.36. History, ecological impacts, and adaptation of an introduced earthworm in Ireland and the UK

McHugh D., Marchán D.F., Gerard S., Melody C., Martínez Navarro A., Novo M., Schmidt O.

Proselodrilus amplisetosus (Lumbricidae) is a small endogeic species with a very limited distribution in southern France. Given the low dispersal of the genus, it is surprising that *P. amplisetosus* has recently been reported ~ 1000 km from its endemic range: at sites within 70 km of each other near Dublin, Ireland, and at two sites within 15 km of each other southwest of London, UK. Previous survey work at multiple locations across Ireland and the UK had not otherwise revealed the occurrence of the species. To understand the history and consequences of this species, we undertook quantitative sampling in its endemic and new range; integrated stable isotope ratio analyses; and analyzed phylogeographic patterns using DNA barcodes. Our results show a wide distribution of the species across southern France, while more limited occurrences around estate houses and gardens in Ireland, albeit at very high abundances locally (up to ~ 300/m²); this indicates human-mediated transport through plants or landscaping materials. Stable isotope ratio analysis of *P. amplisetosus* and co-occurring earthworms indicate that it is the most extreme mineral soil feeder, indicating that it may be filling an otherwise vacant niche. DNA barcode analyses show that populations in Ireland and UK include the most common haplotypes from the two main haplogroups in France as well as unique haplotypes to each introduced site, indicating two separate introductions long enough ago to allow unique haplotypes to emerge. Ultimately, we will interpret our results in the context of the well-documented human history of the introduction sites.

P1.37. Into the Woods! Investigating earthworm diversity in the forests of Austria

Mittmannsgruber M., Gruber E., Wiedenegger E., Monoshyn D., Slawiczek C., Zsak K., Baumgartner C., Lepusch S., Bradley O., Püspök J., Lapin K., Zaller J.G.

Although the importance of earthworms as ecosystem engineers has long been recognized, they are still under-researched in forest ecosystems. However, forests provide important habitats for many earthworm species rarely found in open land, and knowledge regarding their distribution and ecology is especially scarce. Austria is a country dominated by forests, with almost 50% of its land surface covered by them. Yet, an overwhelming majority of earthworm studies has focused on open agricultural land so far. To address this knowledge gap, we have (i) sampled earthworms in the riparian forests of the Donau-Auen National Park along the Danube river, and (ii) established a survey of earthworms on 120 sites in various forest types across Austria, starting in 2026. Results from the National Park (i) showed 16 different species in riparian forests, some of which occurred predominantly in deadwood, i.e. *Bimastos rubidus*. Aside from peregrine species, typical forest species were also found, including the rare *Dendrobaena steineri*, which has been found again for the first time since its original description in 1994! Provisional results (ii) on earthworm distributions across forest management intensities and on the efficacy of traditional morphological identification versus environmental DNA (eDNA) will be presented. Aside from soil, deadwood will also be sampled. Finally, all of this will allow us to gain a more comprehensive understanding of earthworm diversity and the factors influencing it in Austrian forest ecosystems.

P1.38. Interactions between earthworm species enhance litter-derived carbon stabilization in the mineral soil

de Jong P., Schleppi P., Koestel J., Dötterl S., Hagedorn F.

Earthworms may act as double-edged swords for soil organic matter. While they may enhance mineralization via increased microbial activity, they can also elevate organic matter stabilization. This study examined their role in beech-dominated limestone forests. Endogeic and anecic earthworm species were kept in mesocosms with soil from two beech-dominated forests in the Swiss Jura mountains. Highly-labelled beech litter (^{13}C , ^{15}N , ^2H) was applied. Carbon fluxes were repeatedly measured. After 12 months, bulk soil, casts, and soil fractions were analyzed for isotope enrichment. All treatments showed similar total CO_2 respiration. Likewise, total soil organic carbon stocks did not differ. Earthworm effects became apparent in litter decomposition. Less litter-derived CO_2 was released with the anecic earthworm present. In the mineral soil we found 27% litter-derived C with only the anecic species present but up to 40% when both species were present. A spatial sampling within the mesocosm showed contrastingly distributed hotspots of litter-derived C enrichment depending on species composition. The anecic species transported litter-derived C only into close proximity of its burrow, while the endogeic species spread it throughout the whole mineral soil column. Our findings indicate that earthworms did not enhance overall mineralization but affected litter-derived C stabilization. When both species were present, more litter-C was incorporated into mineral soil than by a single species, suggesting enhanced organic matter stabilization on mineral surfaces.

P1.39. Cocoon distribution and earthworm survival strategies in hot summer soils

Euteneuer P., Sturm L., Stuhler J., Butt K.R.

Earthworms have various strategies to survive in unfavourable conditions such as dry soils and high temperatures. It is known that some retreat to deeper soil layers in summer to avoid heat stress and others take refuge through aestivation. In addition, earthworm cocoons can lose up to 50% of their water content and still be viable. However, detailed investigations on how earthworms behave under dry continental climate conditions are scarce. To address this, we searched for earthworms and cocoons in a long-term soil tillage trial in the north-east of Austria where annual precipitation is only 550 mm. In July 2024 and 2025, twelve soil pits of maximum 2×2×1.8 m (w×l×d) were excavated within ploughed and no-till treatments. Subsamples of 10×10×10 cm were extracted down the soil profile (n=796) and searched for cocoons by wet sieving. For ploughed, 12 and 3 endogeic cocoons m⁻² were at 0-20 cm and 60 cm, respectively, and 9 cocoons m⁻² were detected for *Lumbricus terrestris* at 0-30 cm. For no-till, a similar distribution was seen with endogeic cocoons mainly between 0-30 cm (90 cocoons m⁻²) and at a maximum of 60-70 cm. Cocoons of *L. terrestris* started at 10 cm, peaked at both 40 and 100 cm with each having 25 cocoons m⁻² with this driven by a 15% sand content. Living specimens were also extracted, and interestingly we found juvenile *L. terrestris* in aestivation at a maximum burrow length of 70 cm. Adult *L. terrestris* were found at depth and were active.

P1.40. Species distribution modelling of Iberian earthworms: current patterns and future projections

Piris A., Phillips H.R.P., Díaz Cosín D., Trigo Aza D., Gutiérrez López M., de Sosa I., Martínez Navarro A., Marchán D.F., Novo M.

Known as ecosystem engineers, earthworms are among the most ecologically important soil animal groups, yet they remain largely overlooked in conservation efforts. Because many species are constrained by environmental and edaphic conditions, such as temperature and moisture, understanding their distributions and ecological preferences is a key step for implementing effective conservation strategies, prioritising threatened taxa, and anticipating climate change-driven impacts on soil ecosystems.

With nearly 200 described species, the Iberian Peninsula represents a major biodiversity hotspot for earthworms, hosting numerous endemic genera and species. In this study, we modelled current and potential future distributions of ~55-60 Iberian taxa with >10 occurrence records. Additionally, we explored other models of more uncommon taxa (~130 species, <10 occurrences). We identified the most relevant climatic, soil and land-use predictors shaping earthworm distributions, comparing modeling methodologies across spatial scales (cosmopolitan vs. endemic species) and different occurrence datasets.

Occurrences were compiled from bibliographic sources, public repositories and recent field surveys (especially from previously underexplored areas, such as the southern and eastern Iberian Peninsula). Environmental, edaphic, and land-use variables were obtained from different databases (e.g. WorldClim, SoilGrids) and complemented with empirical measurements. Using a suite of Species Distribution Modelling (SDM) algorithms, we generated suitability maps for present conditions and for mid-century climate change projections (CMIP6, 2040-2060).

Our results highlight the taxa and regions most vulnerable to warming and moisture shifts and identify areas of potential conservation priority for maintaining earthworm diversity and the soil ecosystem services they support.

P1.41. Earthworms and agricultural soils – lessons from Norwegian research

Pommeresche R., Rittl T.F., Løes A.-K.

Earthworms are crucial in decomposition of organic matter (OM), in bioturbation, nutrient cycling, biodiversity and carbon storage in soil. The role of earthworms has been explored in several projects studying soil health in Norwegian agricultural soils.

Common grey worm (*Aporrectodea caliginosa*), rosy worm (*A. rosea*) and red worm (*Lumbricus rubellus*) are the most abundant species found. Across several projects, results demonstrate that crop rotation, fertilization, and residue management affect earthworms. Numbers of worms differed between 30 -350 individuals m⁻². In one perennial ley system we found 6 species and on average 925 ind. m⁻². Among 6 crop rotation systems, earthworm abundance was higher in systems that included ley and cattle manure. Earthworms also responded positive several years after using composted solid cattle manure compared with NPK fertilization or no fertilizer. In a long-term comparison of digested and nondigested slurry, only minor differences in earthworm abundance were observed, but the day after application more worms died with high-rate application of both slurry and digestate vs. low. In arable systems, some cover crop species increased the numbers of earthworm, while others did not.

Earthworms utilize different sources of OM, and their activities move microorganisms, plant nutrients and carbon around in the soil. An estimate of 221 tonne of topsoil per hectare passes through a typical Norwegian earthworm population per year. Earthworm casts extracted from buried litterbags contained much higher levels of carbon, nitrogen and extractable nutrients than surrounding bulk soil. All this highlights earthworms' importance for soil health and carbon storage.

P1.42. Land use-driven responses of enchytraeid communities in Atlantic Forest and Cerrado soils

Ramos G.A., Medeiros G., Durães N., Brown G.G., Niva C.C., Niemeyer J.C., Bartz M.L.C.

We evaluated the structure of enchytraeid communities across Land Use Systems (LUS) in the Atlantic Forest (AF) and Cerrado (CE) biomes in Brazil, and their relationships with soil physical and chemical attributes. Overall, 60 sites were sampled in Native Vegetation (NV), No-Tillage System (NTS), No-Tillage (NT), and Pasture (PA). Samplings were performed in August 2023 and February to May 2024, using nine points per site. Enchytraeids were extracted via hot wet method, identified alive to genus level and analysed through density, richness, and ecological indices. In the AF, densities ranged from 5,362 to 10,168 ind m^{-2} , with no significant differences among LUS. However, genus composition differed markedly, with a higher proportion of native genera in NV and a dominance of cosmopolitan genera in agricultural systems. *Guaranidrillus* was identified as an indicator for NV, whereas *Hemienchytraeus* was for NT. In the CE, densities were considerably lower (38–792 individuals m^{-2}) and similar across LUS. Nevertheless, Pielou's index was significantly higher in NTS, where *Hemienchytraeus* was also identified as an indicator, and cosmopolitan genera predominated across systems. Multivariate analyses showed that, in the AF, agricultural systems were associated with soil pH and bulk density, whereas NV with soil moisture and N content. In the CE, NV correlated with Ca/Mg ratio and sand content, while NTS with N. Overall, even in the absence of differences in conventional ecological indices, genus composition and multivariate analyses revealed consistent responses of enchytraeid communities to land use changes, highlighting their potential as bioindicators of soil health.

P1.43. Impact of regenerative management practices on earthworm populations in pasture soils

Schon N.L., Laurenson G., Young S., Aalders L., Sawicka E., Mackay A.D.

There has been increasing interest in the ability of regenerative management practices to improve soil health outcomes, including stimulating soil biology. A series of trials applying regenerative practices (e.g. sowing diverse pastures and reducing nitrogen application) have been established on dairy farms across different regions of New Zealand and earthworms monitored.

In 2021, prior to the trials commencing on ten dairy farms, earthworm populations in late winter were greater at sites receiving summer irrigation (average $450 \pm 68 \text{ m}^{-2}$) in comparison to rain fed sites (average $210 \pm 22 \text{ m}^{-2}$), but were lowest in those under a winter forage crop (average $<120 \pm 29 \text{ m}^{-2}$). Following pasture renewal there was a decline in earthworm abundance. These results align with changes in measured soil carbon concentration. Earthworms showed near-complete recovery in the two years following pasture renewal, although earthworm abundance continued to increase three years following a winter forage crop.

Increasing pasture diversity had little effect on earthworm numbers, as did reducing nitrogen fertiliser application, with limited changes also seen in pasture growth and composition. In the latest sampling in 2025, earthworm populations remained higher at sites receiving summer irrigation ($>550 \pm 44 \text{ m}^{-2}$) in comparison to rain fed sites ($>265 \pm 21 \text{ m}^{-2}$), while pastures previously in a winter forage crop had increased in abundance to $>550 \pm 49 \text{ m}^{-2}$. We explore whether patterns observed in earthworm abundance and diversity are consistent with other soil biological measures (e.g. microbial respiration, microbial diversity and nematode populations). Further, we investigate factors impacting earthworm populations across sites and regenerative trials.

P1.44. Earthworm biodiversity across alpine pastures: patterns and environmental controls along an altitudinal gradient

Semeraro S., Joye O., Rasmann S., Le Bayon. R.C.

Mountain ecosystems are among the most climate-sensitive environments on Earth, yet their belowground biodiversity remains critically understudied. Earthworms, as key ecosystem engineers, strongly influence soil structure, nutrient cycling, and carbon regulation. Despite their ecological importance, little is known about how their communities respond to environmental variation along altitudinal gradients, particularly in alpine pastures where climate change is progressing rapidly.

This study investigates the drivers shaping earthworm community composition along a 600-meter altitudinal gradient (1400–2000+ m) in the Swiss Prealps. At four elevations, earthworm communities were sampled using standardized soil volumes to quantify ecological groups, species diversity, abundance, and biomass. In parallel, we measured soil physicochemical properties, microclimatic variables, vegetation composition, and root density through combined field and laboratory analyses.

We hypothesize that earthworm community structure is primarily determined by soil characteristics, while altitude-related climatic factors and vegetation composition exert secondary influences. By integrating these environmental variables, our study aims to disentangle the relative contributions of soil, climate, and vegetation to earthworm diversity and spatial distribution in mountain pastures.

Understanding how earthworm communities vary across environmental gradients is essential for predicting their responses to ongoing climatic shifts. More broadly, incorporating mountain soils and their biota into conservation and monitoring programs is crucial for filling major knowledge gaps and improving our understanding of ecosystem functioning and climate feedbacks in alpine regions.

P1.45. Earthworm mediated vermicomposting as a source of bioactive Actinomycetes for sustainable agriculture

Uma T., Srinivas C., Kale R.D.

Vermicompost is widely recognized as a nutrient-rich organic amendment harboring diverse and metabolically active microbial communities, particularly actinomycetes, which play a vital role in sustainable agriculture. In the present study, vermicompost was prepared using five different organic substrates, and its physicochemical properties were analyzed. Actinomycetes were isolated from the vermicompost samples and screened for antimicrobial activity against selected bacterial and fungal plant pathogens of agricultural importance. Most isolates exhibited antifungal activity than antibacterial effects. Among the isolated actinomycetes, the isolate identified as *Streptomyces* sp. exhibited the highest inhibitory potential against the major pathogens evaluated, with its taxonomic identity confirmed via 16S rRNA gene sequencing. Morphological characterization through scanning electron microscopy revealed distinct growth phases and sporulation patterns. Furthermore, GC-MS analysis of the crude ethyl acetate extract detected several bioactive compounds associated with antimicrobial properties.

The functional efficacy of *Streptomyces* sp. was further evaluated through seed germination assays and pot culture experiments using *Abelmoschus esculentus* as a model crop. Inoculation with the selected isolate significantly improved seed germination, plant growth and yield parameters, while effectively suppressing *Fusarium oxysporum* under pot conditions. Enhanced plant performance may be attributed to improved nutrient availability, disease suppression, and the plant growth promotion. This study highlights the potential of earthworm mediated vermicompost as a valuable source of bioactive actinomycetes, bridging laboratory-based microbial screening with field-level agricultural applications and supporting sustainable crop production systems.

P1.46. Interactions among earthworms ecotypes affect soil physical properties, carbon dynamics and plant physiology

Lerch T.Z., Araujo J.H.R., Capowiez Y, Hartmann C., Dupont L.

Earthworms play a key role in regulating multiple soil ecosystem services. Natural earthworm communities usually consist of several ecotypes; however, the specific contribution of each ecotype and the interactions among ecotypes in shaping soil functioning remain poorly understood. The objective of this study was to test the hypothesis that increasing earthworm ecotype diversity enhances soil processes and plant growth. We further hypothesized that these effects depend on soil texture. To test these hypotheses, we conducted a controlled laboratory experiment using 5-L mesocosms filled with three grassland soils differing in texture, ranging from sandy to loamy. All mesocosms were covered with a layer of green waste compost. In each soil type, eight combinations of three earthworm ecotypes were established: the anecic *Lumbricus terrestris*, the epigeic *Lumbricus castaneus*, and the endogeic *Allolobophora chlorotica*. The experiment ran for approximately 14 months, during which CO₂ emissions and soil water-holding capacity were monitored. Subsequently, *Lolium perenne* was grown for six months. At the end of the experiment, soil porosity and aggregation were analyzed using X-ray tomography followed by dry sieving. Our results show that the assemblage of all three ecotypes produced the strongest effects on compost mineralization and soil hydric properties, with responses varying according to soil texture. Analyses of soil physical structure revealed that interactions among ecotypes led to significant changes in soil porosity and aggregation patterns. Ongoing analyses of plant biomass will determine whether these modifications of soil properties translate into changes in plant growth and physiology.

P1.47. Long-term recovery of earthworm communities in post-fire temperate forests: tree species effects on soil biological functioning in Podzols

Tran T.H.V., Woś B, Pietrzykowski M., Józefowska A.1

Understanding the long-term trajectories of belowground recovery is critical for developing effective post-fire forest management strategies. We investigated earthworm community assembly thirty years after a catastrophic wildfire in Poland, comparing regenerating stands of four tree species (*Pinus sylvestris*, *Larix decidua*, *Betula pendula*, *Quercus robur*) with adjacent undisturbed mature forests. Results demonstrated that earthworm density successfully recovered to reference levels ($p > 0.05$), driven by the high resilience of the acid-tolerant epigeic species *Dendrobaena octaedra*. However, the magnitude of this recovery was strongly modulated by tree species identity. Deciduous stands (Birch and Oak) supported substantially higher earthworm densities compared to coniferous stands (Pine and Larch). Notably, the community remained exclusively epigeic across all treatments, indicating that edaphic constraints of the sandy Podzol soils (low pH), rather than fire legacy, limit the colonization of endogeic and anecic groups. From an applied perspective, our findings suggest that while earthworm numerical recovery can occur passively over three decades, the selection of tree species during reforestation plays a pivotal role. Incorporating broadleaved species like Silver birch and Pedunculate oak into post-fire planting schemes is recommended to maximize belowground biomass and enhance litter-dwelling decomposer communities in restoring temperate forests.

P1.48. Linking functional differences between soil faunal and microbial communities: earthworm gut microbiomes are ecological group-specific

Liu W.T., Yang S.H., Chang C.H.

Earthworms affect soil microbial communities through gut passage. Different earthworm ecological groups have different burrowing behaviors and rely on different food resources, and are expected to have distinct gut microbiomes. However, previously studies focusing on earthworm gut microbiomes usually investigated only one species for each ecological group. Thus, their conclusions claiming characteristics of gut microbiomes in different ecological groups are confounded by interspecific differences. We address this knowledge gap by investigating earthworm communities living in four secondary broadleaf forests in the subtropics. We use carbon and nitrogen stable isotopes to categorize nine earthworm species into four ecological groups, and analyze the bacterial communities from the surrounding soils and the gut soils. We hypothesize that (H1) earthworms have unique gut microbiomes considerably different from those in the soil and (H2) different earthworm ecological groups have their own characteristic gut microbiomes differing from other ecological groups. Microbiomes are significantly different between the surrounding soil and the earthworm gut soil and between the foregut and the hindgut. Only $21.5 \pm 2.5\%$ of earthworm gut amplicon sequence variants are also found in the surrounding soil, supporting our H2. Different earthworm ecological groups have distinct microbiomes. Principal coordinates analysis of earthworm gut soil microbiomes shows a clear trend consistent with ecological groups, spanning from mesohumic endogeic earthworms to polyhumic endogeic and epi-endogeic species, and then to anecic earthworms, supporting our H2. We conclude that earthworms have unique gut microbiomes and highlight that earthworm gut microbiomes are ecological group-specific and reflect dietary preferences of different ecological groups.

P1.49. Influence of organic matter types on earthworm activity and burrowing patterns in arable soils

Heinze W.M., Keller T.

Earthworms play a key role in regulating soil ecosystem services by burrowing through the soil, altering its structure, and making nutrients more available to plants. Conversely, earthworms are affected by soil properties often causing them to be absent in soils where they could have the greatest beneficial effect on soil structure and nutrient availability. The amendment of arable soils with organic matter (OM) is widely used as a tool to improve soil properties. However, it remains unclear how different types of OM affect earthworm burrowing activities and spatial burrowing patterns. We will present an experimental set-up for studying how different types of organic matter alter bioturbation behaviours of earthworms and its implications for soil property changes. Endogeic (burrower) earthworms will be introduced into soil columns amended with different types of OM commonly applied to arable soils (straw, manure, compost, cover crops). The combined effect of earthworms and OM additions on soil structure will then be monitored over time by using non-destructive imaging techniques. Effects on nutrient availability will be monitored by destructive sampling at each timepoint. We expect that earthworm burrowing activity and its impact on soil pore structure establishment will vary with OM type due to direct effects on earthworms and indirect effects on soil properties. The results will support identifying targeted measures to accelerate earthworm burrowing and its beneficial effects on soil restoration.

P1.50. Exotic earthworms in Brazil and their invasion into protected areas: a call for applied research

Demetrio W., Brown G.G., Bartz M., Dudas R., Ferreira T., Niva C., Rosseau G., Hernández L., Nadolny H., Cunha L., Pereira J., Santos A., Römbke J., James S., Decaëns T., Mathieu J., Cooper M.

Brazilian Conservation Units (CUs) include national, state and municipal parks, and private conservation areas that serve as refuges for native species, preserving habitats from pressures of human activity like deforestation, urbanization, and pollution. They also provide essential ecosystem services, but despite their significance, they face numerous challenges, including the invasion of exotic species. Earthworms are among the invasive species of concern in Brazilian CUs. These invertebrates are usually considered beneficial in agricultural fields where they improve soil fertility and increase plant yield, but when introduced into ecosystems where they do not naturally occur, can have detrimental effects. While Brazil has >280 described native earthworm species, introduced exotic species (58 spp. known) may pose significant threats to native ecosystems. We performed an extensive bibliographic review to synthesize exotic earthworm species occurrence in CUs and used non-published data from many Brazilian colleagues. Exotic earthworms occurred in 68 CUs in Brazil, most of them (59%) in the Atlantic Forest biome, followed by the Amazon (27%) and Cerrado (8%) biomes. In total, 26 exotic earthworm species, belonging to six families (*Eudrilidae*, *Glossoscolecidae*, *Lumbricidae*, *Megascolecidae*, *Ocnerodrilidae* and *Rhinodrilidae*) were found in Brazilian CUs. The most frequent was *Pontoscolex corethrurus*, a widespread tropical geophagous species found in 48 CUs, representing 65% of the total, followed by *Amyntas gracilis* (16 CUs, 22% of total). Considering the ecology and bioturbation impacts of these species, harmful scenarios for soil functioning in native ecosystems of these CUs are possible, highlighting the urgent need for further applied research on this topic.

P1.51. Earthworms and *Bacillus* spp. synergistically improve Tobacco growth and regulate soil microbial community

Xiao Y., Zhang M., Zhong H., Tibihenda C., Jusselme M., Li X., Chao Q., Mao R., Zhang C.

The coexistence of multiple beneficial organisms is fundamental of soil- plant system health. To date, however robust evidence for synergistic effects of soil macrofauna (e.g. earthworm) and microorganisms (e.g. *Bacillus* spp.) were limited, particularly in tobacco yields, a globally important cash crop. This hinders a mechanistic understanding of how these soil organisms jointly regulate key ecosystem function. In this study, we conducted a tobacco pot experiment to evaluate the individual and interactive effects of earthworms (*Amyntas aspergillum*) and *Bacillus* spp. on soil chemical properties, microbial community diversities and plant growth and physiological traits. Four treatments were shown as follow: control (CK), earthworm inoculation alone (E), *Bacillus* spp. inoculation alone (B), and co-inoculation of earthworms and *Bacillus* spp. (EB). The results showed that earthworm activity, either alone or in combination with *Bacillus* spp., significantly increased tobacco leaf number, maximum leaf length, plant height, maximum leaf area, fresh aboveground biomass, and leaf perimeter. It also enhanced chlorophyll synthesis and increased the contents of peroxidase and free proline in tobacco leaves. Both individual and combined applications of earthworms and *Bacillus* spp. reduced soil pH and soluble organic carbon content. However, *Bacillus* spp. inoculation alone and its combination with earthworms increased soil total nitrogen content. Furthermore, the combined inoculation of earthworms and *Bacillus* spp. significantly enhanced soil β -glucosidase activity. Earthworm inoculation alone and its combination with *Bacillus* spp. significantly altered the β -diversity of soil bacteria. *Bacillus* spp. inoculation alone enriched Acidobacteriota and Gemmatimonadota; earthworm inoculation alone enriched Actinobacteria, Verrucomicrobiota, and *Clonostachys rosea*; while the combined inoculation enriched Bacteroidetes, Flavobacterium, Chaetomiaceae, and Archaeorhizomycetes. In addition, both individual and combined effects of earthworms and *Bacillus* spp. significantly reduced pathogenic fungi such as *Fusarium*. Co-inertia analysis revealed that earthworms were the primary driver promoting tobacco growth, enhancing photosynthesis, and enriching beneficial soil bacteria. In conclusion, this study demonstrates that earthworms act as the core driver for promoting healthy tobacco growth and improving soil microbial community structure, and they interact synergistically with *Bacillus* spp. to jointly construct a favorable ecological environment for tobacco-growing soil.

P1.52. European giants: admire them for what they are, protect them for what they do

Marchán D.F., Cardín M.L., Kutuzovic D.H., Capowiez Y.

While less known than their tropical counterparts, there are several representatives of the European genera *Scherotheca*, *Octodrilus*, *Boucheona* and others that reach giant size compared to the temperate earthworm fauna. These earthworms show a strong impact in soils and restricted ranges overlapping highly anthropized areas. Thus, they are potentially important ecosystem services providers and relevant targets for conservation efforts. A better understanding of their evolution and ecology (including burrowing, casting and feeding behaviors) are necessary to inform integrated management of their ecosystem services and conservation. Three examples of these animals are *Scherotheca thibauti*, *Octodrilus rucneri* and *Boucheona arrufati*. *Scherotheca thibauti* inhabits Atlantic beech forests, while *Octodrilus rucneri* and *Boucheona arrufati* are found in insular and continental garrigues. Their unique evolutionary history includes simultaneously diverging from their closest ancestors at XX under different paleogeographic settings, and sharing more than forty genes that could be involved in their large body size. The three species have been found to belong to the intense tunneler functional group, but with remarkable differences such as burrow volume, burrow diameter and bioturbated volume. Regardless, all three show a stronger effect on variables associated with ecosystem services than their mid-sized relatives. Together, these results showcase how these three representatives are important targets for species conservation not only because of their intrinsic value but due to the potential application of their ecosystem services. The project BIGWORM is an ongoing effort to extend this integrative approach to other giant European earthworms.

Session 2: Ecotoxicology & Stress Ecology

P2.1. Trophic behaviour of *A. longa* Ude and *L. terrestris* L. earthworms in relation to two lichen litters in North America

Kliszcz A., Mitchell M., Legge K.

During heavy rains, earthworms emerge to the soil surface. Rainfall lasting several weeks in the western (Pacific) part of British Columbia also causes lichen thalli to shed from trees, contributing significantly to the litter beneath the trees. Because many lichen species contain secondary lichen metabolites in their thalli, most of which are non-nutritional or toxic to living organisms, a research question arose about the trophic mechanism of *A. longa* Ude and *L. terrestris* L. earthworms in relation to the two most common lichens in the study area.

The results of the laboratory experiment (one-choice arena) indicate that *A. longa* earthworms process the litter of the lichens *Evernia prunastri* and *Platismatia glauca* (casts present), in contrast to *L. terrestris* L. earthworms (casts absent). Exposure of both earthworm species to *P. glauca* lichen litter (70% and 100%) resulted in 100% mortality within the first 24h of feeding. Lower concentrations of lichen biomass in the food substrate (16.49% for *E. prunastri* and 17.43% for *P. glauca*) resulted in a deterioration in the earthworms' condition (final stimuli assessment equal to 2 points on a 5-point scale), but they were still able to penetrate and consume these litters during the first 48h of exposure.

The amounts of processed lichen food substrate in this study are marginal compared to the control object (soil). Further research on the newly proven trophic mechanism of earthworms in relation to other lichen species is needed to better understand the complexity of the ecosystem and the earthworm's function within it.

P2.2. Bioaccumulation of chlordecone in earthworms from banana fields: effect of ecological categories, soil type and characteristics

Bataille L., Chédru E., Coulis M., Christophe S., Gelas A., Saint-Hilaire M., Pelosi C.

Chlordecone (CLD, $C_{10}Cl_{10}O_2H_2$) is an insecticide used worldwide and listed as a persistent organic pollutant. Due to its strong persistence, CLD still contaminates ecosystems, particularly soils where it can remain for several decades or centuries depending on the soil type. Andosol has fractal clays that trap CLD, whereas it is less sequestered in nitisol. Studies on soil organism contamination by CLD are limited but recent findings showed CLD biomagnification in soil macrofauna, with the highest contamination in geophagous earthworms. However, the impact of soil type on earthworm bioaccumulation have never been investigated. We addressed this question and we investigated how inter- and intra- field variability in soil CLD concentration affected bioaccumulation process in different ecological groups of earthworms.

For that, soils, endogeic and epigeic earthworms were collected in 24 banana fields in Martinique to measure CLD concentration and metabolites after a QuEChERS extraction method and analysis by LC-MS/MS in soils and earthworms.

Endogeic earthworms bioaccumulated CLD as tissue concentrations were 12.5 and 16.1 times higher than soil concentrations on andosol and nitisol respectively. Epigeic earthworms were significantly less contaminated than endogeic earthworms with tissue concentration being 1.30 and 1.75 times higher than soil on andosol and nitisol respectively. This highlights that earthworm ecological categories and soil type both significantly influenced the bioavailability and bioaccumulation of CLD. Ongoing chemical analyses will allow to assess the effect of local soil properties and contamination heterogeneity on earthworm bioaccumulation. We assume that intra-field variability of soil CLD concentration will locally impact earthworm internal concentration.

P2.3. When order matters: sequence-dependent pesticide toxicity in *Enchytraeus crypticus*

Eagles E., Short S., Rubner-Petersen F., Bart S., Sleep D., Lahive E., Cedergreen N., Spurgeon D.

Synergistic toxicity (when chemical mixtures cause greater-than-additive harm) is a major concern in mixture risk assessment because it increases toxicity to non-target species. In pesticides, synergism often occurs when one compound inhibits detoxification enzymes, such as cytochrome P450s (CYP450s), that are required to metabolise a second chemical. However, pesticides are frequently applied sequentially, creating opportunities to mitigate harmful synergistic interactions through application order and timing.

Here, we test how sequential exposure alters mixture toxicity. Using acute aquatic assays with the soil annelid *Enchytraeus crypticus*, we investigated sequential pulse exposures to the insecticide cypermethrin in combination with chlorothalonil, azoxystrobin, or prochloraz, separated by a recovery period. When cypermethrin was applied first, incomplete toxicokinetic elimination led to elevated internal concentrations and substantial carry-over toxicity during the second pulse, resulting in partial co-exposure. As expected, cypermethrin-prochloraz exposure produced synergistic toxicity when cypermethrin preceded prochloraz. Strikingly, reversing the order resulted in antagonism.

Transcriptomic analysis revealed that both cypermethrin and prochloraz induce a CYP450 gene (*Cyp18a1_like_1*), with prochloraz causing particularly large and sustained induction (>200-fold). These data suggest that prochloraz pre-exposure primes a xenometabolic state that enhances cypermethrin detoxification, reducing internal concentrations and toxicity and overcoming prochloraz-mediated CYP450 inhibition once sufficient induction is established.

Our results demonstrate that pesticide order and timing can fundamentally alter mixture toxicity via detoxification pathway induction. Exploring sequential exposure dynamics using high-throughput exposure systems that combine toxicity, internal concentration data and gene expression responses into risk assessment could help design application strategies that reduce harm to critical non-target species.

P2.4. Earthworms as indicators of soil health. The case of the EU-H2020 MINAGRIS project

Huerta-Lwanga E.

In agricultural systems, the recognition that microplastic pollution jeopardizes soil health prevails. Investigations have proven that fossil based and persistent microplastics produce changes on soil life diversity and soil physicochemical conditions. In the EU H2020 MINAGRIS project (micro and nanoplastics assessment in agricultural systems) we assessed through laboratory experiments, how microplastics impacted on epigeic, endogeic and anecic earthworm species' fitness (biomass, density), as well on microplastic content in casts and the formation of burrows. Using different soil types, three types of microplastics and three microplastic concentrations, with or without an organic stressor (eg veterinary drug or pesticide). Likewise, at field conditions, we conducted the assessment of the biomass, density, and diversity of earthworms, after 2 years of experimentation with microplastics and organic stressors. Preliminary results indicated that certified biodegradable plastic residues with the lowest concentration (0.01%w/w) enhanced the biomass of endogeic earthworms, while the same type of plastic with the highest concentration (0.1%) produced loss of biomass on the same type of earthworms. Non-certified fossil based biodegradable plastic seemed to negatively impact the growth of epigeic earthworms, independently of the concentrations evaluated. Persistent conventional fossil-based plastic enhanced the mortality of anecic earthworms in combination with the fungicide pyraclostrobin. At field conditions, results are less evident, the spatial heterogeneity of organic matter and nitrogen plays a role on how earthworms respond to the impact of microplastics. In this research, more statistical analysis will be performed for revealing more clearly the results collected.

P2.5. Contrasting effects of polystyrene and biodegradable PLA microplastics on cadmium-induced toxicity in earthworms

Shang G., Zhai J., Xu G., Sun Z., Wang X.

Microplastics (MPs) are plastic fragments with particle sizes less than 5 mm, ubiquitously distributed in terrestrial environments. However, the negative effects of MPs, such as joint-pollution with heavy metals on soil fauna remain controversial. This study investigated survival rate, growth, reproduction, avoidance behavior, histology, biochemical assays, comet assay, qPCR, Cd content, and IBR index. We found that six types of traditional MPs (PC, PP, PVC, LDPE, PET and PS, and PLA (a biodegradable microplastics) had no adverse effects on earthworm growth, survival and reproduction. Moreover, we found that earthworms exhibit an avoidance behavior towards PLA. Both PS and PLA can exacerbate Cd pollution, leading to loose circular muscle layer, DNA damage in coelomocytes, and impaired antioxidant system due to increased reactive oxygen species (ROS). mRNA level of HSP70 increased under joint-pollution of both PS and Cd or PLA and Cd compared to Cd treatment alone. MPs enhanced Cd accumulation in earthworms in Cd-contaminated soil. Notably, the Integrated Biomarkers Response index revealed that the toxicity of joint PLA and Cd was greater than the joint effect of PS and Cd, which might violate the original intention of biodegradable plastics having non-toxic influence on the soil fauna. Our findings provide new insights into the ecotoxicological effects of MPs, the joint ecotoxicological effects of MPs and Cd on earthworms, and the ecological risks of MPs to soil fauna.

P2.6. Sustainable agriculture on the Moon and Mars: an ecotoxicological perspective

Harris V., Bouwman H., Maboeta M.S.

Humans aspire to establish sustainable life on the Moon and Mars, but this requires overcoming the challenges of extraterrestrial agriculture. Current closed-loop food systems rely heavily on terrestrial input, which is expensive and resource-intensive for long-term missions. Therefore, there is a push to utilise in-situ resources, particularly native regolith. However, both Lunar and Martian regolith lack organic matter and may contain toxic elements. This study aimed to use established ecotoxicological models to understand these toxic challenges and find ways to transform regolith into a sustainable, life supporting substrate. Various organisms were exposed to Lunar Highlands (LHS-1) and Martian Global (MGS-1) regolith simulants at varying concentrations, simulating different levels of terrestrial soil addition. A series of bioassays assessed acute toxicity: *Daphnia magna*, *Heterocypris incongruens*, and *Pseudokirchneriella subcapitata* were used to evaluate mortality and growth inhibition. *Sinapis alba*, *Lepidium sativum*, and *Sorghum saccharatum* were tested for germination and root development. *Eisenia andrei* were assessed for reproduction, growth, mortality, and avoidance. Results indicate MGS-1 is highly toxic at concentrations of >80%. While LHS-1 is comparatively less toxic, it still shows notable effects, particularly on earthworm growth, reproduction, mortality, and avoidance. Different organisms showed varying sensitivities to the substrates. The current hypothesis suggests the safe upper limit for using LHS-1 will be significantly higher than for MGS-1, but neither simulant will be usable without some terrestrial input. Ongoing work is needed to refine these thresholds for practical insights into transforming extraterrestrial regolith into viable life supporting substrates for sustained space habitation.

P2.7. Impact of equine deworming agents on soil fauna

Chavanne M., Roelfstra L., Le Bayon R.C.

Horses in Switzerland are deeply integrated into culture and agriculture, with around 112'000 of them used for breeding, sport, and leisure. The permanent meadows and pastures for horses cover 70% of usable agricultural area, resulting in a density of 10 ind/km². In this context, deworming agents are widely used to kill intestinal parasites, including anthelmintics such as fenbendazole, ivermectin, pyrantel, praziquantel, and moxidectin. All these products end up in manure, but their effects on non-target soil fauna are still poorly documented in the equine context. Our research investigates this impact on earthworm communities using a combined field and laboratory approach. Earthworm sampling was conducted on four plots in the Swiss Plateau (2 with treated horses, 2 with untreated horses) using the hand-sorting method. Microcosms under controlled conditions tested the effect of four classes of anthelmintics on *Eisenia fetida* and *Lumbricus terrestris*. The field study did not reveal any significant differences in abundance, biomass or diversity between treated and untreated plots. However, the specific composition differed significantly, and we observed an overrepresentation of *Allolobophora chlorotica* in meadows where horses received deworming treatment. In the laboratory, fenbendazole (pure form) induced significant mass loss in *Eisenia fetida*, accompanied by stress symptoms. These results highlight the need for further research on the environmental persistence and long-term effects of equine dewormers on earthworms and soil fauna.

P2.8. Earthworm community responses to soil texture and management in Flemish vineyards

Magr L., Vancampenhout K., Ceusters J., Bylemans D., Boddez N., Desie E.

Viticulture in Belgium has expanded rapidly over the past two decades, driven by climate-induced shifts in suitability toward higher latitudes. However, vineyard management practices are transferred from established wine regions and remain poorly adapted to soil and climatic conditions of Flanders. This raises concerns about soil degradation, reduced biological functioning, and the long-term resilience of vineyards under increasing climatic variability. While soil biota, particularly earthworms, are recognized as key drivers of soil structure and ecosystem functioning, their responses to management in vineyards remain poorly understood.

We conducted the first integrated, region-wide assessment of soil health in Flemish vineyards. The study encompassed 40 vineyard parcels across 20 wine domains, where paired parcels were selected to capture contrasts in management and site conditions. Soil physical, chemical, and biological indicators were assessed, including earthworm communities and soil microarthropods. Vineyard management intensity was quantified through structured surveys, while microclimatic conditions were continuously monitored using in situ loggers (TOMST-TMS4) from April to September 2024.

Preliminary results indicate a dominant role of soil texture, with earthworm abundance decreasing in soils with higher proportions of coarse particles. Management intensity showed comparatively weaker effects on earthworm densities but was associated with changes in soil physical properties, including reduced aggregate stability and increased bulk density.

This integrative framework provides a baseline for evaluating how soil properties, management practices, and microclimate interact to shape soil biological functioning in Flanders. Establishing such context-specific baselines is essential to support adaptive vineyard management strategies that safeguard vineyard resilience under future climate extremes.

P2.9. *Eisenia* is the suitable indicator to predict the risk of reproductive toxicity to earthworms

Menke U., Ernst G., Roeben V., Marx M.T.

Earthworms are considered important ecosystem engineers in many soil habitats including agricultural soils. Already since the early 1970s testing of earthworms was performed, initially starting with field sampled individuals. Although absent in agricultural fields, the compost worm *Eisenia fetida/andrei* became the standard species for acute toxicity (OECD 207, 1984) as well as for reproduction testing guidelines (OECD 222, 2004, 2016; ISO 11268-2, 1998, 2012, 2023). In addition, a composed soil equilibrated by carbonate to a pH of 6.0 (+/- 0.5) composed of sand (70%), kaolin (20%) and peat (5-10%) became the standard test substrate (so called OECD artificial soil, later also for other soil invertebrates). The suitability of *Eisenia* as representative indicator test species combined with its testing in OECD artificial soil were questioned by several research groups within the last decades.

Here we discuss the principles and relevant validity criteria for an indicator species combined with the characteristics of a universal artificial soil for lab testing. In addition, we demonstrate protectiveness in the risk assessment when using *Eisenia* Tier 1 reproduction lab test results in combination with the European safety factor of 5 by comparison to field test results for a high number of chemical substances. Furthermore, TK-TD population modeling is discussed as a future tool to extrapolate from lab to field and better connect effect and exposure by including the ecology of different earthworm groups.

P2.10. Impacts of climate change-related flooding on earthworm populations

Pile B., Berenbrink M., Daly K., Hodson M.E., Klaar M., Zhu Q.

Climate change-related flooding affects earthworm populations, with increasing frequency and duration threatening earthworm survival as soils become anoxic when flooded.

We carried out field surveys at frequently and rarely flooded sites for earthworm abundance and species distributions. We have used the common UK species *Lumbricus terrestris*, *Allolobophora chlorotica*, *Aporrectodea caliginosa*, *Octolasion cyaneum*, and *Dendrobaena veneta* in laboratory experiments.

To determine moisture preferences we carried out choice chamber experiments, with a gradient of moistures in standard soils. All species had similar, but soil-specific, preferences, choosing moist, but not waterlogged conditions.

Survival experiments exposed earthworms to restricted oxygen, simulating flood conditions. All species survived for a similar duration of approximately 22 hours at $\sim 0.25 \text{ mg l}^{-1}$ dissolved oxygen. However, our measurements indicate *A. chlorotica* has more oxygen-carrying haemoglobin ($0.22 \text{ vs } 0.125 \text{ } \mu\text{mol Hb g}^{-1}$), that is more efficient at binding oxygen than *L. terrestris* ($4.18 \text{ vs } 11.47 \text{ mmHg P50}$), suggesting *A. chlorotica* may be better adapted to survive flood conditions.

We are monitoring hatching success of cocoons submerged in oxygenated and deoxygenated water (2 mg l^{-1} vs 0.25 mg l^{-1} dissolved oxygen). Cocoons of *A. chlorotica* and *D. veneta* in low oxygen suffered lower hatching success and longer hatching time than those with unrestricted oxygen, with a greater impact on *D. veneta*.

The above evidence indicates increasing flooding could cause changes in earthworm population structure and size, with cocoons being a key component for the survival of earthworm populations after flood events.

P2.11. Meta-analysis of dose- and time-dependent metabolic pathway perturbations in earthworms exposed to agrochemicals

Namdev P.K., Yadav S.

Agrochemicals represent a major threat to soil ecosystems by interfering with critical biochemical processes in soil-dwelling organisms, particularly earthworms, which function as key ecosystem engineers and sensitive bioindicators. While traditional ecotoxicological methods provide valuable organismal endpoints, metabolomics offers a systems level approach capable of detecting early, sublethal biochemical perturbations. This meta-analysis synthesizes findings from eligible studies published up to December 2024 to quantify metabolomic disruptions in earthworms exposed to various agrochemicals. Meta-regression revealed a significant inverse dose-response relationship ($\beta = -0.45$, 95% CI: -0.62 to -0.28, $p < 0.001$), indicating intensified metabolic suppression at higher toxicant concentrations, while temporal analysis demonstrated progressive disruption with increased exposure duration ($\beta = 0.023$, 95% CI: 0.011–0.035, $p < 0.001$), suggesting cumulative toxic effects. Toxicant class-specific responses varied substantially, with neonicotinoids eliciting pronounced metabolic upregulation ($\log_2FC$: 2.48) and organophosphates inducing strong metabolic suppression ($\log_2FC$: -2.95). Pathway-level analysis revealed a consistent pattern of upregulated energy and amino acid metabolism alongside downregulated carbohydrate and Tricarboxylic Acid (TCA) cycle pathways, indicating a stress-induced metabolic shift. Species-specific differences were also evident, with *Eudrilus eugeniae* displaying greater sensitivity ($\log_2FC$: -4.2) compared to the model species *Eisenia fetida*. Despite substantial inter-study heterogeneity ($I^2 = 96\%$), coherent and recurring patterns of metabolic disruption were evident, strengthening the case for metabolomics as a robust tool in environmental monitoring. The study emphasizes the need for standardized analytical approaches to improve cross-study comparability and highlight the capacity of metabolomics to deliver mechanistic insights critical for ecotoxicological risk assessment.

P2.12. Long-term assessment of the effects of various organic inputs on earthworm communities in an experimental field trial

Roucaute M., Cluzeau D., Lerner L., Guillocheau S., Morvan T., Gaillard F., Leroy P., Carteaux L., Hotte H., Aubry A., Hoeffner K., Pérès G.

Organic amendments offer alternatives to mineral fertilization: they reduce dependance on importations by relying on local sources and they also provide food resources for soil organisms such as earthworms. The diversification of organic amendment sources is of special interest to meet farmers needs in a given territory. Biogas industries, which are more and more developed in France, can provide some of those organic amendments via their digestates from methanization process. In order to assess the benefits and/or limits of such organic resources compared to mineral fertilization or other organic sources (e.g. manure, slurry), a long term experimental site (LTE- EFELE) was implemented by INRAE at Le Rheu, Brittany, France. It started in 2010 and is expected to continue until 2030. Organized in three statistical blocks, this LTE allows comparison of eight organic input treatments with mineral fertilization. Among the soil parameters monitored in this LTE, earthworms took a special place as they are ecosystem engineers, playing a major role in soil physical proprieties and organic matter cycles. They were sampled in 2012, 2016, 2021 and 2025. In this study, we present the first results on earthworm community dynamics. We compare the effects of the different fertilization scenario on earthworm abundance, biomass, ecological structure and species structure (richness, composition, evenness). We question whether digestates are more or less beneficial for earthworm communities compared to other organic inputs and mineral fertilization.

P2.13. Effects of selenium and earthworm on the bioremediation of ryegrass to cadmium contaminated soil

Yue S., Wang R., Zhang Y., Xu J.

Cadmium (Cd) contamination in soil represents a substantial threat to ecosystems and human health. Phytoremediation utilising ryegrass (*Lolium perenne* L.) is a promising strategy; however, its efficiency is frequently constrained by limitations pertaining to the plant's biomass and tolerance. The present study investigates the synergistic effects of selenium (Se, 1 µg/g) and earthworms (E) on ryegrass growth, Cd accumulation, and antioxidant defence mechanisms in Cd-contaminated soil (30 µg/g, Cd30). The findings of this study demonstrated that the combined application of Se and earthworms (Cd30+Se1+E) most effectively promoted ryegrass growth, increasing shoot fresh and dry weight by 60.24% and 60.00%, respectively, compared to the Cd30 treatment. While Se (Cd30+Se1) significantly increased Cd translocation to the shoot, the combined treatment with earthworms redirected Cd accumulation to the roots, resulting in a higher root bioaccumulation factor (BAF_{root-Cd}) and a lower biotranslocation factor (BTFCd). Consequently, the Cd30+Se1+E treatment achieved the highest total Cd phytoextraction (89.06 µg/plant) and soil Cd removal rate (0.59%/plant). Antioxidant enzyme analysis indicated that Se alleviated Cd-induced oxidative stress by enhancing catalase (CAT) and peroxidase (POD) activities and soluble protein content. In conclusion, the Se-earthworm-ryegrass system presents a highly effective and synergistic strategy for the phytoremediation of Cd-contaminated soil, simultaneously enhancing plant biomass, modulating Cd distribution, and strengthening plant defence systems. These findings establish a novel bio-strategy for efficient Cd remediation while maintaining soil health and reducing ecological risks.

P2.14. Biochar - friend or foe to earthworms?

Marhan S.

Biochar, the product of the pyrolysis of organic material, has been debated for some time as a means of increasing carbon storage in soils. As well as enhancing the stable soil carbon pool, it is proposed that several other positive features result from applying biochar to agricultural soils, such as increasing water and nutrient retention, enhancing soil pH and reducing greenhouse gas emissions. However, there is controversy over whether biochar promotes or inhibits earthworms. The reduction in soil acidity and the increase in water retention following biochar application are thought to benefit earthworms. Conversely, the rough structure of biochar particles, the presence of potentially harmful substances, or the dilution of soil resources by recalcitrant biochar may harm earthworms directly or depress earthworm population development. This presentation will summarise what is already known about the effects of biochar on earthworms, based on literature and our own studies. The possible mechanisms by which biochar affects earthworms will be discussed separately for short- and long-term effects, as determined by microcosm and mesocosm studies and field experiments.

P2.15. Size matters: effects of chitosan-coated silver nanoparticles on the earthworm *Eisenia andrei*

Topa N., Kulczyńska J., Kyzioł A., Wilczek G., Homa J.

The increasing release of silver nanoparticles (AgNPs) into terrestrial ecosystems raises concerns about their potential long-term impact on soil invertebrates. Earthworms, key contributors to soil functioning, are widely used as bioindicators in ecotoxicological studies.

This study investigated the impact of chitosan-coated silver nanoparticles (CS-AgNPs) on stress- and immune-related responses in the earthworm *Eisenia andrei*, with particular emphasis on nanoparticle size and exposure duration.

Earthworms were exposed to CS-AgNPs of two sizes (6 nm and 33 nm) in short-term filter paper tests (3 days) and long-term soil experiments (7 and 28 days). Analyses included nanoparticle accumulation in tissues, organism-level toxicity, coelomocyte viability, antioxidant enzyme activity, and expression of genes associated with oxidative stress and immunity.

CS-AgNPs accumulated in earthworm tissues in a size- and time-dependent manner, with significantly higher accumulation of 6 nm particles, peaking after 28 days of exposure. Despite this bioaccumulation, no toxic effects were observed at the organismal level or in coelomocyte viability across all treatments. Molecular and biochemical analyses revealed only subtle changes of stress responses. Preliminary results suggest that antioxidant enzyme activity increased after 28 days of exposure, particularly in earthworms exposed to 33 nm CS-AgNPs, while changes in gene expression remained limited.

In conclusion, although CS-AgNPs accumulated in a size-dependent manner in *Eisenia andrei*, their effects were limited to subtle, delayed stress responses, with no detectable toxicity at higher levels of biological organization.

P2.16. Nanoparticle-based modulation of wound healing: insights from *Eisenia andrei*

Topa N., Kulczyńska J., Kyzioł A., Wilczek G., Homa J.

Due to their widespread application, silver nanoparticles (AgNPs) are increasingly entering terrestrial habitats, yet their implications for soil fauna remain poorly understood. Although many studies have examined AgNP-induced toxicity, the impact of AgNPs on the wound healing and regeneration processes is not well understood. Earthworms are commonly used as model organisms in soil ecotoxicology due to their high regenerative capacity, which makes them well-suited to investigating stress responses beyond standard toxicity endpoints.

In this study, we examined the effects of chitosan-coated silver nanoparticles (CS-AgNPs) on regeneration-related responses in the earthworm *Eisenia andrei*. Earthworms were exposed to CS-AgNP-contaminated soil for 7 and 28 days, followed by amputation of the posterior five body segments. Stress- and regeneration-related responses were evaluated after 5 days post amputation through gene expression analyses in coelomocytes and selected tissues, complemented by histological and immunohistochemical evaluation of regenerating segments.

Preliminary results indicate no significant inhibition of regeneration at the morphological level. However, exposure to CS-AgNPs was associated with changes in the expression of genes involved in intracellular signalling pathways, with the most prominent modulation observed in the expression of *pkc1*. These findings suggest that CS-AgNPs may influence the molecular signalling mechanisms involved in wound healing without causing overt impairment of regenerative processes.

Overall, this study emphasises the importance of regeneration-based and molecular endpoints in identifying stress-related responses caused by nanomaterials in soil organisms.

P2.17. Microplastics in earthworms: linking (sub)individual responses to population-level effects through meta-analysis

Truchet D.M., Peres G., Wiegand C.

Meta-analysis is a powerful approach for synthesizing large datasets in ecotoxicology and identifying general patterns of biological responses. This framework is particularly relevant for assessing emerging contaminants of concern (ECCs), which often lack specific toxicological targets. Among ECCs, microplastics (MPs) are of special concern due to their low degradability and increasing environmental loads, resulting in variable biological effects. In this study, we applied a meta-analytical approach to evaluate MP effects on earthworms, integrating responses from sub-individual (biochemical and physiological) to population-level endpoints (reproduction). Peer-reviewed studies published between 2013 and 2025 were systematically reviewed, and data were extracted on MPs characteristics, exposure duration, and biological responses. Effect sizes (Hedges' g) were calculated, where positive values indicate stimulation or increased responses and negatives indicate inhibition or adverse effects. The results revealed a significantly positive global effect size ($g > 0.6$; $p < 0.01$), indicating that MP exposure had significant biological responses. PE showed the highest number of positive responses, likely reflecting both its predominance in the literature and the induction of stress responses. Virgin plastics exhibited neutral responses, whereas environmental and aged MPs produced positive responses. Short-term assays associated with positive effect sizes, while chronic exposures tended to yield negative values. Mortality and oxidative stress responses showed positive effect sizes, whereas reproductive endpoints were negative. CAT, MDA, and SOD exhibited positive responses, while AChE showed negative effect sizes. This study highlights the value of meta-analysis for elucidating the global toxicological effects of plastics and provides a transferable framework applicable to other ECCs.

P2.18. Earthworm bioturbation as a driver of microplastic fibre transport in soil

Heinze W.M., Lahive E., Leicht K., Mitrano D.M., Cornelis G.

Microplastic (MP) contamination of soils is a growing concern. Synthetic textiles release MP fibres throughout their lifecycle, which can accumulate in soils when urban biosolids are applied for nutrient recycling. Understanding the fate and transport behaviour of MP fibres in soils is therefore essential for assessing their long-term distribution in and the potential exposure of the soil system. Fibres are often considered less mobile than other MP shapes due to their elongated shape. The potential role of earthworms in the transport of MP fibres is, however, often overlooked. This study investigated whether relatively long MP fibres are transported by earthworms and whether MP fibre length is decisive. The deep-burrowing earthworm *Lumbricus terrestris* was introduced to repacked soil columns spiked with metal-doped polyethylene terephthalate (PET) fibres (0.750 mm median length) in the top layer. Earthworm bioturbation led to successive transport of MP fibres shown by increasing MP fibre mass concentrations in the deeper soil with time. MP fibre length decreased with depth, but effect magnitudes of this depth-dependency were small. Earthworms were also added to a separate set of soil columns that were mixed homogeneously with MP fibres. After two weeks of exposure, earthworms were extracted and their casts collected during depuration. Even relatively large MP fibres that are otherwise often expected to be immobile in soil, i.e. up to 4-5 mm, were susceptible to transport by earthworms through ingestion and egestion. Our results underscore the importance of including earthworm bioturbation when assessing MP mobility in soils.

P2.19. Response and defense mechanisms of the earthworms *Eisenia fetida* to natural saline soil stress

Yan Y., Zhai J., Wang L., Wang X.

Salinization of soil is a serious global environmental issue, particularly in agricultural lands. Saline farmland not only endangers grain production but also affects the survival of soil fauna. Earthworms, as soil ecosystem engineers, play a crucial role in maintaining soil health and enhancing global agricultural production. However, the response of earthworms to natural saline soil stress remains poorly understood. To explore this, we investigated the effects of natural saline soil from Dongying City, Shandong Province, China, on the growth, survival, reproduction, antioxidation, and defense-related gene expression of the earthworm *Eisenia fetida*. Our findings demonstrate that the growth rate, survival rate, and cocoon production of *E. fetida* decrease under exposure to natural saline soil in a dose-dependent manner. Elevated levels of DNA damage in coelomocytes and increased reactive oxygen species (ROS) were observed. Additionally, antioxidant enzymes, such as superoxide dismutase (SOD) and catalase (CAT), increased under stress. The mRNA levels of Cyp450 and Hsp70 also rose in response to saline soil exposure. Furthermore, the activity of Na⁺/K⁺-ATPase and the expression of the osmotic sensor gene *wnk-1* were elevated. In conclusion, our findings indicate that natural saline soil induces antioxidant and osmotic stress in earthworms *E. fetida*, highlighting the detrimental effects and defense mechanisms of soil fauna under such conditions.

P2.20. Divergent efficacy and mechanisms of attapulgite versus biochar for heavy metal stabilization during sewage sludge vermicomposting

Zhong C., Li N., Du Q., Wang S., Bi Y., Sun Z.

The resource potential of municipal sludge is severely constrained by the high content and bioavailability of heavy metals. To address this limitation, attapulgite and biochar were applied as passivators during sewage sludge vermicomposting. Results showed that attapulgite outperformed biochar in immobilizing a broad spectrum of heavy metals, including Cr, Mn, Ni, Cu, Zn, and As. Microbial analysis further indicates that in the attapulgite, earthworm and functional microbiota system, attapulgite, by regulating the microenvironment (pH=6.46, EC=8.66 mS/cm), works in synergy with earthworm activities. This not only enriches *Glutamicibacter* with heavy metal tolerance (e.g., *arsA*, *arsB*, *copZ*) and passivation capacity, but more importantly, enhances the overall heavy metal passivation function of the system. This microbial-driven process converted acid-soluble and reducible fractions into residual fraction, as notably evidenced by the significant increases in residual Cu (6.75%), As (8.70%), and Cr (12.96%). This study shows that attapulgite can effectively enhance the passivation of heavy metals and regulate microbial communities in sewage sludge vermicomposting, which provides an innovative and practical strategy for sludge resource utilization.

P2.21. From bulk soil to bio-aggregates: how Pb contamination alters microhabitat physicochemical and structural traits via ecosystem engineer-driven aggregation

Zhong H., Li M., Jia L., Zhang M., Defarge C., Bruand A., Motelica-Heino M., Zhang C.

Soil ecosystem engineers strongly regulate nutrient cycling and soil physical structure through bioaggregate formation. However, the heavy-metal stress alters this process remains unclear. Here, we tested how lead (Pb) contamination modifies the formation, pore and particle-size distribution, physicochemical properties, near-infrared (NIR) spectral signatures, and Pb speciation of bio-aggregates generated by plant roots (*Brassica campestris*) and earthworms (*Amyntas gracilis*). An Acrisol was spiked with PbCl₂ (0, 100, 400, and 1000 mg kg⁻¹) and incubated for 40 days under four treatments: plant-only, earthworm-only, and combined plant-earthworm treatment, with an unbioturbed control. All indicators were evaluated on both bulk soil and isolated bio-aggregates. Aggregate mass was primarily determined by biotreatment type: earthworm-only > combined treatment > plant-only; Pb concentration had limited influence on total aggregate mass. Total Pb concentration was consistently higher in bulk soil than in bio-aggregates (11.2-57.5%), and increasing Pb shifted speciation toward greater proportions of weak acid-extractable and reducible fractions with a decline in residual Pb. Multivariate patterns of aggregate properties and NIR spectral signatures were primarily explained by biotreatment and Pb × biotreatment interactions rather than Pb alone. At 1000 mg kg⁻¹ Pb, pore and particle-size differences between bulk soil and aggregates progressively diminished. Correlation and random-forest analyses further identified dissolved organic carbon and clay as key responsive indicators linking Pb speciation to aggregate properties. Overall, ecosystem-engineer-driven aggregation can decouple Pb accumulation from bulk soil and reshape microhabitat filters under contamination, highlighting bio-aggregates as sensitive units for assessing soil functional resilience to heavy metals.

P2.22. Impact of pesticides on earthworm populations and soil health

Memgmi F.D., Asare S., Takyiwaa M., Poku S.O., Appiahene K., Osei J.

Agricultural expansion and the indiscriminate use of pesticides have led to significant soil ecosystem damage, affecting biodiversity and soil fertility. Earthworms, crucial for maintaining soil health, are particularly vulnerable to these chemicals, which can cause morphological, behavioral, and physiological changes. This study reviews existing literature on the effects of various pesticides on earthworm populations, focusing on their toxicity, bioaccumulation, and the resulting impact on soil health. Data were collected from multiple sources, including peer-reviewed articles and reports on pesticide usage and its ecological consequences. Findings indicate that pesticides significantly reduce earthworm populations, leading to adverse effects on soil fertility and biogeochemical cycles. The review highlights that most pesticides are non-specific, harming beneficial organisms and contributing to soil pollution. The detrimental effects of pesticides on earthworms underscore the need for sustainable agricultural practices. The bioaccumulation of these chemicals poses risks not only to soil organisms but also to higher trophic levels, including humans. To mitigate the impact of pesticides, it is recommended that farmers adopt organic farming practices, utilize biodegradable alternatives, and receive education on the ecological importance of earthworms. Implementing these strategies can enhance soil health and promote biodiversity, ultimately leading to more sustainable agricultural systems

P2.23. Earthworms modulate the effectiveness of biochar and nanohydroxyapatite in cadmium immobilization in soil aggregates and vegetable uptake

Tibihenda C., Zhang M., Zhong H., Jia L., Ahmad M., Xiao Y., Motelica-Heino M., Lu Y., Zhang C.

Earthworms, as soil engineers, can influence the distribution of Cd within the soil-plant system. However, the interactive effects of earthworms, biochar, and nanohydroxyapatite amendments on Cd transformation across aggregate size fractions, as well as their influence on vegetable uptake, remain significant challenges. This study examined the effects of two earthworm species, *Amyntas aspergillum* and *Metaphire guillelmi*, on Cd uptake in vegetables and related to soil biochemical properties across different aggregate sizes during remediation with biochar-nanohydroxyapatite. A 60-day pot experiment, *Brassica campestris* was grown in Cd-contaminated soil across 12 treatments (n=3) with each amendment applied at 3% dosage.

Results indicated that while amendments altered aggregate size, earthworm activity significantly increased the proportion of large macroaggregates (LMA) and microaggregates (MA). Specifically, *A. aspergillum* promoted LMA formation, whereas *M. guillelmi* favored MA development. The efficiency of Cd immobilization followed the order: nanohydroxyapatite > biochar-nanohydroxyapatite > biochar > non-amended soil. Notably, earthworm inoculation further decreased exchangeable Cd and increased residual Cd concentration, with a more pronounced effect observed for *M. guillelmi*. Principal component analysis (PCA) showed that earthworms mainly influenced plant growth and regulated Cd accumulation, while amendments had a greater effect on Cd uptake in vegetables. Proteobacteria and Ascomycota are the dominant phyla. While biochar-based amendments enhanced microbial network complexity and inter-species interactions, earthworm activity simplified community composition, promoting a more specialized core microbiome. Redundancy Analysis (RDA) confirmed that LMA is the essential fraction for biochemical processes and Cd transformation, supporting diverse microbial communities. Core genera like *Aeromonas* and *Flavobacterium* were closely associated with Cd immobilization driven by earthworm activity, whereas *Olpidium* mainly responded to amendment addition. This study bridges a gap by exploring the interactions among ecosystem engineers (earthworms), amendments (biochar-nHAP), and soil structure (aggregates), thereby providing a strategy for optimizing Cd stabilization in contaminated soils.

P2.24. Individual and combined effects of microplastics and *Aporrectodea caliginosa* on soil structure formation

Weghorst M., Van Schaik L., Baartman J.

Microplastics (MPs) are ubiquitous in soils worldwide, with concentrations varying widely across ecosystems. MPs can influence soil structure, affecting properties such as aggregate stability, water retention and hydraulic conductivity. Earthworms, as ecosystem engineers, play a critical role in soil structure formation by contributing to aggregate formation, modifying pore structure, and enhancing organic matter decomposition. Research shows that MPs can significantly alter earthworm behavior, reproduction, and survival, potentially impacting soil structure indirectly.

This study aims to unravel the individual and combined effects of MPs and earthworms on soil structure formation through an incubation experiment using the earthworm species *Aporrectodea caliginosa*. Soil columns were incubated under controlled laboratory conditions for periods of 2, 12, and 22 weeks. Key measurements include aggregate stability (wet sieving), water retention characteristics, and saturated conductivity. The findings aim to clarify how exposure of soils to MPs and earthworms influences soil structure formation.

P2.25. Impact of polyurethane microplastics on earthworm health and soil ecological functioning

Garima S., Shweta Y.

Escalating plastic production and poor waste management have resulted in the accumulation of microplastics in soils and other ecosystems, threatening soil biota, and human health. This study investigated the effects of polyurethane (PU) microplastics on *Eudrilus eugeniae* (African nightcrawler), a model earthworm species widely used in soil health assessment and vermicomposting. Earthworms were exposed to increasing concentrations of PU microplastics (0, 200, 400, 600, and 800 mg kg⁻¹) for 56 days under controlled laboratory conditions. A clear concentration-dependent reduction in body weight was observed, although no significant mortality occurred across treatments. Ingestion was identified as the primary exposure route, indicating an inability of *E. eugeniae* to discriminate PU microplastics from soil particles.

Histopathological examination of the gastrointestinal tract revealed tissue-level alterations associated with PU microplastic exposure. Significant changes were also detected in oxidative stress biomarkers, including catalase (CAT), superoxide dismutase (SOD), lipid peroxidation (LPO), and reduced glutathione (GSH), along with altered acetylcholinesterase (AChE) activity, indicating oxidative stress and neurotoxicity. Collectively, these findings demonstrate that *E. eugeniae* readily ingests PU microplastics and exhibits measurable physiological and biochemical stress responses, supporting its suitability as a sensitive bioindicator for assessing microplastic-induced stress in soil ecosystems.

Session 3: Earthworms & Society

P3.1. Vermitechnology-based recycling of fruit and vegetable waste using *Perionyx excavates*

Ansari A.A., Huntley S.

Organic waste is a serious concern across the globe contributed by human activity that can be managed by efficient process like vermicomposting which can reduce the waste that is dumped in landfills into useful product vermicompost. The research was carried out to assess the efficiency of the composting earthworm *Perionyx excavatus* in converting different combinations of organic waste into vermicompost. This research was conducted to explore the following objectives (i) rate of compost production (ii) productivity of composting units and (iii) physico-chemical characteristics of compost produced. Organic waste (fruit and vegetable waste) were collected and subjected to process of vermicomposting by using the following treatments: FW [Fruit waste (500 g) + Cow Dung (500 g)], VW [Vegetable waste (500 g) + Cow Dung (500 g)], FVW [Fruit waste (500 g) + Vegetable waste (500 g) + Cow Dung (500 g)]. The results of the three organic waste treatments belonging to the experimental group showed that the treatment with Vegetable waste generated the largest quantity of vermicompost at the end of the 38 day followed by the treatment with Fruit waste when compared to combination of Fruit + Vegetable waste. The rate of production is significantly higher in experimental groups. The nitrogen content was higher in control group (2.4 %) than experimental group (1.8 %). C: N ratio was greater in control group (9.6) when compared to experimental group (9.1). Phosphate content was 0.02 % in both experimental and control groups. Potassium content was 0.78 % and 0.27 % in experimental and control groups respectively. There was significant difference in experimental and control groups in terms of NPK. The nutrient status was within the acceptable range.

P3.2. Finding Memo: public engagement and dissemination of knowledge about earthworms through a memo game

Euteneuer, P., Epperlein, J., Krück, S.

How should we try to disseminate research findings about earthworms and their hidden life under our feet to a broader audience of all ages? We believe that memo games can overcome gaps between researchers and a non-academic audience. Memo games are usually played among children or between adults and children by matching pairs of cards with an attempt to remember where the cards were last seen. We have designed a memo game concerning earthworms and their life, with 36 motifs including photographs of ten common species such as *Aporrectodea caliginosa*, *Lumbricus terrestris* and *L. rubellus*, sketches and photographs of ecosystem services and agricultural management practices. In an accompanying booklet we explain the content of each motif such as the life cycle of earthworms, life stage, habitat requirements and benefits for sustainable agriculture. The information provided is for farmers, stakeholders and interested adults and we expect that adults can explain the content of this booklet to children to learn more about the value of earthworms for soil fertility and how to support them. In a first batch, we will produce 500 sets of the game and provide the booklet in German, with additional translations available online. If you wish to participate by translating the information into your native language or produce the memo in your country, please contact us.

P3.3. Deployment of a citizen science approach to study earthworm communities at the regional scale

Fertil A., Hoeffner K., Guillocheau S., Delourme L., Guégan G., Scimia J., Cluzeau D.

Rising awareness of the importance of soil biodiversity conservation has led to an increasing demand from society for monitoring tools, and earthworms are considered as flagship species and widely recognized as valuable bioindicators of soil health. However, current knowledge of earthworm communities in urban environment and their response to land use and management remains insufficient to provide precise and robust diagnostics.

To address this gap, the University of Rennes deployed in 2015 a citizen science approach to assess soil biological quality through earthworm communities in the Île-de-France region (around Paris, 12,011 km², 1,017 inhabitants/km²). A methodology was developed to engage a large number of non-expert participants through (i) presenting this approach to existing networks (e.g., local authorities, gardening and environmental associations, networks of natural area managers, agricultural organizations), (ii) on-site and remote training of volunteers on earthworm sampling, and (iii) systematic sharing and discussion of results during feedback meetings. Between 2016 and 2024, this approach led to the sampling of 488 plots across 87 municipalities in Île-de-France, involving over 180 participants from 70 different organizations. Most participants came from local authorities and gardening associations, while private citizens, schools, and natural area managers were less represented. Participants' dynamics show a clear evolution throughout the years, with some stakeholders being driving forces in the sampling effort. In total, 45 species were recorded, with a mean abundance of 293 earthworms per square meter across all sampled plots. Effects of land use, land management and urbanization metrics on earthworm communities will be presented and discussed.

P3.4. Linking earthworm diversity and abundance to soil characteristics under different land-use patterns

Memgmi F.D., Takyiwaa M., Poku S.O., Appiahene K., Osei J.

Earthworms play a crucial role as biological indicators of soil health and fertility, significantly impacting agricultural productivity. Their activities enhance nutrient content, improve water retention, and foster microbial activity in the soil. This study aims to explore the role of earthworm biodiversity in soil fertility and crop productivity, assess the impact of vermitechnology on reducing chemical pesticide use, and identify factors influencing earthworm activity. A comprehensive review of existing literature was conducted, focusing on the ecological functions of earthworms, their contribution to organic waste decomposition, and the benefits of vermicomposting. Research findings from various studies were synthesized to evaluate the effectiveness of earthworms in enhancing soil health. The findings indicate that earthworms significantly improve soil structure and fertility, leading to increased crop yields. Vermicomposting emerged as a cost-effective and sustainable method for organic waste management, reducing reliance on chemical fertilizers and pesticides. The study underscores the importance of earthworm biodiversity in promoting sustainable agricultural practices. Their role in enhancing soil health is vital for achieving long-term agricultural productivity. Further research is recommended to identify the most suitable earthworm species for specific geographical climates and organic waste types. Additionally, promoting vermitechnology and organic farming practices can mitigate the adverse effects of chemical inputs on human and soil health, contributing to a more sustainable agricultural future.

P3.5. Potential of vermicomposting agricultural biogas plant digestate using *Dendrobena veneta* (Rosa 1893) – adding to a circular economy

Garczyńska M., Dziuba M., Filipowicz P., Mazur-Pączka A., Pączka G., Łuka P., Butt K.R., Kostecka J.

The study assessed potential of using the digestate obtained from anaerobic fermentation as an addition to vermicomposting systems. Three treatments enabled the analysis of earthworm population dynamics and the assessment of vermicompost nutrient properties. These treatments were garden soil (S); garden soil with added digestate (SD); and garden soil with added digestate and horse manure (SDM). The experiment was conducted in an air-conditioned chamber (20 ± 5 °C, 70% relative humidity). As expected, significant differences in vermicomposting rate were observed depending on the substrate used ($SDM > SD > S$). Vermicomposting rate was correlated with the size of developing *Dendrobaena veneta* population. At experimental end, earthworm numbers were: S - 18 ± 9 ; SD - 46 ± 15 , and SDM - 117 ± 38 ind. $\cdot$ pot $^{-1}$ ($p < 0.05$). Similarly, final biomasses were as follows: S - 0.8 ± 0.1 ; SD; 2.9 ± 0.4 ; and SDM; 3.8 ± 0.7 g $\cdot$ pot $^{-1}$ ($p < 0.05$). Therefore, addition of digestate (and horse manure) was more conducive to the development of earthworm populations than horticultural soil alone. SD and SDM vermicomposts were characterized by higher nitrogen (N-NO₃), potassium (K), calcium (Ca), and magnesium (Mg) compared to the initial substrate biomass. The average characteristics of vermicomposts were for, N-NO₃: SD - 1850 ± 64 mg dm³, SDM - 1989 ± 79 mg dm³; K: SD - 2420 ± 56 mg dm³, SDM - 3620 ± 59 mg dm³; Ca - SD 2380 ± 49 mg dm³, SDM 2498 ± 68 mg dm³; Mg – S - 296 ± 43 mg dm³, SD - 780 ± 54 mg dm³, SDM - 870 ± 88 mg dm³. The results confirm the great potential of vermicomposting using digestate in a circular economy model for sustainable agriculture.

P3.6. Vermicomposting of waste from vegetable processing

Garczyńska M., Waberska S., Mazur-Pączka A., Pączka G., Szura R., Butt K.R., Kostecka J.

Waste used came from a fruit and vegetable plant in the Podkarpackie Province, where 3,000 tons of raw materials are processed annually. The experiment used a mixture of beet, carrot, potato, and cucumber residues, mixed in a 1:1:1:1 weight ratio as the test material (VM), and evaluated vermicomposting with *Dendrobaena veneta*. Five treatments were set up at $20\pm5^{\circ}\text{C}$, and relative humidity 70%, with combinations of VM mixed with cellulose (C): VM-(100%); VM75-(VM75%+C25%); VM50-(VM50%+C50%); VM25-(VM25%+C75%); C-(VM0%+C100%). Dynamics of earthworm populations were recorded and fertilizing properties of vermicomposts assessed. Significant differences in vermicomposting rate were observed. Final earthworm counts were: VM – 150 ± 34 , VM75 – 160 ± 38 , VM50 – 520 ± 67 , VM25 – 660 ± 96 and C – 140 ± 26 ind.·pot⁻¹, with final biomass totals of 24 ± 11 ; 31 ± 9 ; 54 ± 14 ; 65 ± 16 and 30 ± 13 g·pot⁻¹, respectively. VM25 also had the most favorable effect on *D. veneta* reproduction (920 ± 65 cocoons unit⁻¹) compared, for example, with C (220 ± 31 cocoons unit⁻¹). VM25 and VM50 also yielded the most favorable vermicompost composition, with significantly higher nitrogen (N-NO₃), phosphorus, potassium, and magnesium levels than the initial substrates. The average characteristics of vermicompost from these substrates were as follows: N-NO₃ (VMC25; 1640 ± 37 mg dm³, VM50; 2122 ± 45 mg dm³); P (VM25 350 ± 57 mg dm³, VM50 460 ± 66 mg dm³), K (VM25 957 ± 56 mg dm³, VM50 1340 ± 66 mg dm³); Mg (VMC25 157 ± 34 mg dm³, VMC 50 188 ± 42 mg dm³). Results confirm vermicomposting to be a practical, circular-economy-compliant method for managing organic waste, including agri-food waste, thereby promoting its rational use and reducing its negative environmental impact.

P3.7. Harnessing multi-species earthworm combinations to enhance phytoextraction through reshaping rhizosphere microecosystem

Jia L., Li X., Xu W., Zhong H., Zhang M., Tibihenda C., Wu L., Liu C., Motelica-Heino M., Zhang C.

The combination of phytoextraction with earthworm-assisted bioremediation represents a promising strategy for ecological regulation and natural recovery of metal-contaminated soils. However, it remains unclear whether functional complementarity among earthworm species, and between earthworms and the hyperaccumulator, drives rhizosphere microecosystem remodeling and thereby enhances phytoextraction efficiency beyond additive effects, particularly relative to single-species application? The underlying mechanisms are still poorly understood. In this study, we systematically evaluated the individual and combined bioaugmentation effects of two Asian earthworm species, epigeic species *Amyntas corticis* and anecic species *Amyntas aspergillum*, on the 90-day phytoextraction efficiency of *Sedum plumbizincicola* in contaminated soils with different Cd/Zn pollution levels. We evaluated plant growth and metal uptake, soil metal speciation and solution-phase dynamics, and microbial community structure in both bulk and rhizosphere soils. Earthworm presence significantly increased the aboveground and roots biomass of *S. plumbizincicola* by 0.17 to 4.64-fold, and enhanced total Cd and Zn uptake by up to 540% and 207%, respectively. Although combined earthworm species showed similar or slightly lower effects compared to individual species, they improved interspecific compatibility and markedly enhanced earthworm survival under metal stress. Earthworm activity enhanced soil Cd and Zn bioavailability and mobility, promoting their continuous transfer from soil solid-phase into soil solution and subsequently into plant tissues. Importantly, earthworm activity substantially altered soil microbial biomass, diversity, and composition, particularly in the rhizosphere, enriching taxa with primary functions in organic matter decomposition, nutrient cycling, and metal resistance. Structural equation modeling indicated that earthworms indirectly influenced plant metal uptake by influencing soil properties and microbial communities. Moreover, the key microbial predictors for Cd and Zn accumulation exhibited contamination-level dependency, highlighting the adaptive role of microbiota in metal phytoextraction. Overall, inoculating multiple earthworm functional groups significantly improve earthworm survival under metal stress. The synergy of the plant and earthworm forms a systemic approach based on the regulation of functional microorganisms, aiming to directionally control the rhizosphere microecosystem and thereby promote the natural ecological restoration of metal-contaminated soil.

P3.8. How reliable is citizen science for earthworm monitoring in a soil health context?

Hoeffner K., Bergerot B., Butt K.R., Gérard S., Pelosi C., Briones M.J.I., Decaëns T., Delaveau N., Guillocheau S., Hedde M., Hotte H., Le Bayon R.C., Muys B., Phillips H.R.P., Poupelin M., Pérès G., Cluzeau D.

Citizen science offers a promising approach to boost earthworm monitoring efforts, both from a naturalist (assessment of biodiversity and conservation status) and an agro-ecological scope (soil health assessment through quantification of abundance or biomass of functional groups). Yet concerns remain about the reliability of data collected by non-specialists. This citizen science study assessed the accuracy of earthworm data collected within the French national '500 ENI' agricultural soil health monitoring network, where primarily farmer advisors annually sample and count earthworms and classify them into four morphotype categories (i.e., epigeics, endogeics, red head anecics and black head anecics). Based on 48,946 individuals collected over 7 years from 950 plots, we calculated two indicators: misclassification rate (MR; % of incorrectly classified individuals in a morphotype) and undetected rate (UR; % of unnoticed individuals in a morphotype). On average, MR was 28% and UR 32%, with substantial variation among morphotypes: endogeics were most reliably classified, while epigeics and anecics showed higher error rates. Analyses revealed that practical experience - measured by the cumulated number of plots and individuals sampled - improves reliability more than years of participation. Earthworm community characteristics such as higher abundance, greater proportion of adults, and higher morphotype diversity also enhanced classification accuracy. These findings highlight the need for targeted training, improved identification resources, and strategic sampling during favorable periods to minimize errors. Despite limitations citizen science can provide valuable data for understanding soil health in agroecosystems, provided that quality control and adapted protocols are implemented. This study also provides insights into the potential correction of citizen-generated data for scientific use.

P3.9. Capacity building on earthworms in a researcher-student-citizen chain of participatory science

Nicolai A., Fertil A., Potthoff M., Euteneuer P., Józefowska A., Schmidt O., Sturm L., Cluzeau D., Hervé M.E.T.

SoilRise is a European participatory research project seeking to (1) increase knowledge about the distribution of earthworm communities, (2) compare taxonomy and genetic information on specific taxa, and (3) raising awareness on earthworms. It relies on a participatory chain linking citizens in urban and rural areas, mentors who are trained students leading field sampling, and researchers. We analyzed how participatory processes developed in each partner region of the project, to rationalize the approaches and the methods used. During the project, we used methods such as participative observation, semi-directive interviews and questionnaires to collect data among all actors of the participatory chain. We focused on both quantitative indicators that allow to compare participatory processes and on quantitative data that offer a more comprehensive overview of the experience lived by the actors involved in the project. Our results highlight that, despite a common and shared objective and research design, each team set the process differently. Four participatory strategies emerged with various focus on (1) intensive data collection, (2) students' education, (3) public environmental education, and (4) collaboration with key-stakeholders. Each local strategy was highly linked to the specific educational context and the researchers' local networks. Our results suggest that participatory science designs require a certain degree of adaptation to local contexts when applied at large scale while responding as a whole to the project objectives.

P3.10. Between tweet and science – the online image of the earthworm

Pacanowski P, Józefowska A., Czernecka Borchowiec N., Feluch K., Sturm L., Schmidt O., Clune S., Clemençon R., Suarez H., Juch J., Surminski R., Potthoff, M.

The era of printed encyclopaedias and newspapers is rapidly giving way to a fully digital information landscape. In this flood of online content, factual knowledge about earthworms—key ecosystem engineers—can be obscured by misinformation, “fake news,” and even hate speech that exploits the term “earthworm.” This study reconstructs the digital portrait of the earthworm that has emerged on the internet in Poland and selected European countries (Austria, Germany, France, and Ireland) during 2025.

Our aim is to assess how scientific knowledge about earthworms permeates the public consciousness through social media platforms (Facebook, X) and online news portals. We will retrieve 100 relevant entries per country using Google (e.g. site:.il “earthworm” after:2024 12 31 before:2026 01 01) and complement these data with posts from X and Facebook. Each item will be evaluated by experts for tone (negative, neutral, positive), factual accuracy (fact vs. myth), relevance (direct reference to earthworms or merely metaphorical use), thematic focus (agriculture, biodiversity, composting, angling, etc.), and authorship (news agency vs. personal commentary).

By mapping the prevalence of accurate versus distorted information, the study will identify the most effective communication channels and content strategies for scientists aiming to improve public understanding of earthworms and their ecological importance.

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P3.11. Effects of vermicompost produced from organic kitchen waste on selected characteristics of *Allium cepa* L. 'Exhibition'

Pączka G., Mazur-Pączka A., Hajduk E., Garczyńska M., Szura R., Kostecka J., Pakla M., Salach T., Butt K.R.

One of the most commonly used organic substrates in the construction of growing media is vermicompost. As a product of earthworm activity, this substrate is characterized by a high content of plant nutrients in readily available forms. The effect of vermicompost produced from selected kitchen waste using *Eisenia fetida* (Sav.), as a soil amendment for onion (*Allium cepa* L.) cultivation, was studied under field conditions using a raised bed growing system. For research purposes, four experimental treatments were established. These were: SL – agricultural soil; V10 – 10% vermicompost (V) + 90% SL; V25 – 25% V + 75% SL; and V50 – 50% V + 50% SL. The soil used in the SL control group came from the Podkarpackie Agricultural Advisory Center in Boduchów (southeastern Poland), where the field experiments were conducted in 2024-2025. Vermicompost had been produced annually at the Faculty of Technology and Life Sciences, University of Rzeszów and characterized: Organic Carbon 40.1%; Total Kjeldahl N 1.6%; Available P 1400 mg kg⁻¹; Available K 4770 mg kg⁻¹; Ca 46551 mg kg⁻¹; Mg 1638 mg kg⁻¹; Fe 2480 mg kg⁻¹; C/N ratio 25.1; pH in H₂O 6.32; Electrical Conductivity 6570 µS cm⁻¹. Results showed that substrates with either a 10 or 25% addition of vermicompost had the most beneficial effects on selected plant parameters (biomass, height, diameter, and bulb circumference) and their selected physicochemical characteristics. In comparative terms, V10 = V25 > V50 > SL.

P3.12. Earthworm ecological differentiation across sites: contrasting signals from stable isotopes and infrared spectroscopy

Pham Q.V., Rumpel C., Lam D.H., Zi Y., Bottinelli N.

Stable isotopes ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) are widely used to study earthworm feeding ecology, but tissue isotope signatures can be strongly site-specific, often limiting comparisons to within-site patterns. Here we tested whether diffuse reflectance infrared Fourier-transform spectroscopy (DRIFT) provides complementary information less sensitive to site conditions. We sampled 26 earthworm species across 17 sites in northern Vietnam and measured $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in tissues after removal of the digestive tract and in corresponding soils, together with diffuse reflectance infrared Fourier-transform spectroscopy (DRIFT) spectra on the same tissues. Soil-normalized isotope offsets (Δ = earthworm – soil) remained strongly structured by site (24% of $\Delta^{13}\text{C}$ and 40% of $\Delta^{15}\text{N}$ variation explained by site) and showed only weak relationships with ecological categories. DRIFT spectra showed consistent lipid-, protein- and carbohydrate-related regions. DRIFT profiles were significantly structured by ecological category (PERMANOVA $R^2 = 0.28$), whereas site identity explained only a small and non-significant fraction of spectral variation ($R^2 = 0.06$). Species similarity matrices derived from DRIFT and from soil-normalized isotopes were not correlated (Mantel $r = 0.07$). Overall, DRIFT emerges as a useful tool for investigating ecological differentiation of earthworms across contrasting sites.

P3.13. Comparative biotransformation of organic substrates by detritivorous isopods and earthworms: implications for nutrient cycling and waste management

Quintanilla M., Martínez Navarro A., Trigo D., Gutiérrez López M.

Detritivorous macroinvertebrates are key drivers of organic matter decomposition and nutrient cycling, yet their functional performance differs among taxa and substrates. While earthworms are widely used in composting systems, the role of terrestrial isopods remains poorly explored. This study compares the biotransformation of two contrasting substrates—sheep manure and *Populus nigra* forest humus—by the earthworm *Dendrobaena veneta* and the isopod *Porcellio laevis*. Microcosm experiments were conducted using equivalent earthworm and isopod biomass, assessing survival, biomass dynamics, and changes in physicochemical properties of both substrates (pH, moisture, NH_4^+ , total C and N, C/N ratio, and Ca^{2+}) over 44 days.

Marked species- and substrate-dependent responses were observed for both biotic and abiotic variables. *D. veneta* showed lower mortality in both substrates and maintained or slightly reduced their weight in sheep manure, whereas surviving individuals of *P. laevis* increased their biomass in both substrates despite a mortality rate over 50%. Overall, substrate composition was more strongly altered in forest humus than in sheep manure. In humus, earthworms increased moisture content and C/N ratio, whereas isopods increased ammonium concentrations; both species increased total carbon and nitrogen and reduced pH relative to the control untreated substrate. In sheep manure, earthworms increased moisture content, and both species reduced pH compared to the control substrate.

These results highlight the importance of substrate-specific decomposer effects when evaluating detritivore-mediated organic matter transformation. These contrasting functional roles suggest that decomposer selection should be based on substrate type and management objectives, rather than assuming functional equivalence among detritivorous taxa.

P3.14. Citizen science highlights earthworms (Annelida: Clitellata) as prey for mammals in Taiwan

Shen H.P., Chang S.W., Ho Y.C., Chih W.J., Yu P.W., Chang C.H.

Earthworms play a key role in the terrestrial food chains. However, there is still scanty knowledge of the identity of earthworms consumed by their predators. This hinders our understanding of predator-prey interactions on the predation of earthworms. Since 2015, we have been processing carcasses of mammals sent by participants of the Taiwan Roadkill Observation Network (TaiRON), and more than a thousand carcasses have been dissected to date. It was found that earthworms occurred in the stomach contents of seven mammalian species. Among them, earthworms are an important source of food for insular mole, Kano's mole, ferret badger, and Indian civet, while Siberian weasel, masked palm civet and crab-eating mongoose consumed earthworms sometimes or only occasionally. Furthermore, the preference for the exotic *Pontoscolex corethrurus*, the most dominant earthworm species at elevations below 1000 m in Taiwan, differed considerably among these animals. Frequency of occurrence of this species reached 20.3% in the stomach contents of the insular mole, while that was only 1.3% for badgers and none of the other animals preyed on this species. In other words, these animals prefer native or peregrine earthworm species of Asian origin except for the insular mole. The contribution of citizen science volunteers is crucial to these findings which deepen our understanding of earthworms and their role in ecosystems.

P3.15. Tillage depth – a possible driver for earthworm diversity in arable land

Sturm L., Stuhler J., Zaller J. G., Butt K.R., Euteneuer P.

Earthworms play a key role in the soil ecosystem, but in agroecosystems soil tillage can reduce their numbers. However, the specific effects of soil tillage depth and frequency on earthworm communities have not yet been sufficiently studied. Therefore, the following research questions arise: (I) What are the impacts of soil tillage depth and frequency compared to machinery type such as plough, cultivator, disc harrow? (II) What are the impacts of precipitation, soil type and texture in this context? To answer these very broad questions, the SoilRise project (2024-2027) is using a citizen science approach to collect on-farm data such as earthworm parameters, soil properties and management practices. Preliminary results from Austria, indicate that tillage depth (0-10; 10-20; 20-30 cm) reduced overall abundance of earthworms by 27-28% with each 10 cm step. In total, 16 species were found on the sampled arable fields with *Aporrectodea caliginosa*, *A. rosea*, and *Allolobophora chlorotica* the most abundant species, found at 68, 66, and 24% of n=127 sites, respectively. The overall mean ($\pm$ standard deviation) abundance (m^{-2}) and biomass (g m^{-2}) were 158 ± 193 and 33.1 ± 38.2 , respectively. Interaction of tillage depth $\times$ precipitation showed that earthworm abundance was more dependent on shallow tillage (0-10 cm) in annual precipitation categories < 570 and $570\text{-}900$ mm than > 900 mm. However, the results suggest that tillage depth has a higher impact on earthworms than tillage machinery type, and this should be considered in future research.

P3.16. Influence of surrounding land-use on earthworm communities in arable fields

Sturm L., Stuhler J., Zaller J.G., Butt K.R., Euteneuer P.

Landscapes and surrounding types of land-use can have a strong impact on soil organisms in agricultural fields, affecting species richness and diversity of, for example, earthworms. However, specific effects of the surrounding land-use structure at a smaller scale have not yet been sufficiently investigated. In our study, we tested the impact of grassland, forest or built-up area presence on earthworm abundance, biomass and community composition. To address this, earthworm data was collected using a citizen science approach as a part of the SoilRise project (2024–2027). For this particular analysis, 75 sites in arable production, from across Austria were considered. The European Strategy for the Alpine Region (EUSALP) land-use/land-cover (LULC) map was used to analyze land-use composition in a 50 m radius around each sampling point. The 65 land-use classes from the LULC map were categorized as: grassland/pastures, forest/woody vegetation, urban/built-up area, water bodies, arable land and urban green. Preliminary results showed higher species richness (25%) and Shannon diversity index (17%), in arable fields with grassland within a 50 m radius than in fields without. Similar was found for the diversity index with forests, but diversity decreased where built-up areas showed a proportion of >5% of the surrounding land. These results show that surrounding land-uses have substantial impacts on earthworm community structure in arable landscapes and that diversity is not only directly affected by field management practices, but also by surrounding habitats.

P3.17. Modern applications of earthworms (Di long) in human and pet food in Asia

Sun Z., Zhao T., Li Z., Rui J.

Since the rise of earthworm farming internationally in the 1980s, earthworms have become a major source of insect protein. Earthworms, known as Dilong in traditional Chinese medicine, have evolved from traditional Chinese medicine applications to functional foods related to human health, especially for cardiovascular prevention and the prevention and rehabilitation of chronic diseases such as hypertension and hyperglycemia in the elderly. This article reviews the various unique bioactive components of Dilong protein (earthworm extract), mainly including: 1. Enzymes of Lumbrukinase: The most famous component, which has potent thrombolytic (fibrinolytic) and anticoagulant effects. 2. Superoxide Dismutase (SOD) and Catalase (CAT): These have significant antioxidant capabilities, can scavenge free radicals, and protect cells. 3. Active proteins and peptides, such as G-90 (glycolipid protein extract): Studies have shown its multiple functions, including anti-tumor activity, wound healing promotion, and antioxidant properties. 4. Antimicrobial peptides: They exhibit inhibitory activity against bacteria, fungi, and viruses, and contribute to anti-inflammatory and analgesic effects. 5. Earthworm serine protease (ESP): It also possesses antithrombotic activity. 6. Other Ingredients · Rich in amino acids, nucleotides, and minerals. These ingredients collectively form the basis for the application of earthworm protein in pharmaceuticals and functional foods. Based on the aforementioned activities, the application of earthworms (Di Long) in pharmaceuticals and health foods was discussed, mainly including: (1) Cardiovascular and cerebrovascular health applications: prevention and adjuvant therapy for thrombotic diseases (such as cerebral infarction and secondary prevention after myocardial infarction). (2) Potential anti-tumor adjuvant applications: as a potential adjuvant therapeutic agent. Research shows that earthworm extracts (such as G-90) can inhibit cancer cell growth and metastasis. (3) Wound repair and tissue regeneration applications: promoting the healing of chronic wounds (such as diabetic foot ulcers) and skin repair. Utilizing its comprehensive abilities of anti-inflammatory, antioxidant, promoting collagen production, and angiogenesis. Patented technologies have been used to develop skin repair products. (4) Antioxidant and anti-aging applications: used in health supplements and skincare products. Utilizing its ability to scavenge free radicals and promote collagen production. There are patents specifically for "anti-aging earthworm peptides". (5) Auxiliary blood glucose regulation applications: as a food additive or adjuvant that helps maintain stable blood glucose levels. Industry status: Currently, earthworm protein peptides, lumbrukinase, and other extracts have been supplied as raw materials to pharmaceutical companies, and related health foods have also been launched. Finally, the development prospects of earthworm (Di Long) protein in functional pet foods are introduced, especially for elderly cats and dogs.

P3.18. Earthworm communities under differentiated management of urban soils: the case of the city of Lausanne (CH)

Roulin M., Coudene M., Bullinger G., Brunner P., Turberg P., Le Bayon R.C.

To tackle heat islands in densely built-up areas with high levels of human activity, urban planning needs to highlight the ecosystem services provided by soils, particularly in regulating water and heat. We assume that earthworm bioturbation activities and differentiated management of cities' grassland areas impact several soil properties essential for water infiltration and retention: bulk density, porosity, aggregates and organic carbon content. We sampled 14 sites – 7 managed as lawns and 7 as meadows – across 9 parks in the city of Lausanne. Linear models explored relationships among management, soil properties, and earthworm communities. Interviews with SPADOM (Parks and Estates Department) employees helped to learn about specific practices for differentiated management (lawns – meadows). Our results show that earthworm abundance is positively associated with soil 'habitable depth' and a lower proportion of coarse elements. The presence of a massive structure horizon constrains the progression of earthworms deep in the soil. The average biomass of earthworms was significantly higher in lawns, and this trait is positively associated with soil macroporosity. No significant relationship exists between individual biomass and soil organic carbon content or bulk density. However, future studies should also consider watering and soil moisture, depending on the management type, to test whether these factors explain the significant difference in average individual biomass. Current water infiltration experiments will provide some answers regarding the link between earthworm biomass, landscaping practices, and the water-regulation potential of soils in the city of Lausanne's parks.

P3.19. Beneath the park surface: earthworm abundance and diversity as function of soil conditions and landscape context in urban green spaces

Melis R., Desie E., Muys B., Samson R.

Parks are essential components of urban green infrastructure and provide essential ecosystem services, yet their belowground biodiversity remains poorly quantified. Earthworms are key ecosystem engineers influencing soil structure, organic matter decomposition, and nutrient cycling, but their distribution and functional diversity in urban park soils are still insufficiently understood, particularly at city scale.

This study will investigate how soil properties and urban context shape earthworm communities across public parks and green spaces in the city of Antwerp (Belgium), covering a wide range of park sizes, and management practices along an urbanisation gradient. At three locations within each park, earthworms will be sampled in open park areas. Soil physical and chemical conditions will be measured concurrently, including bulk density, penetration resistance, gravimetric soil moisture, texture, pH, organic matter content, and soil C:N ratio. These data will be complemented with park-level variables such as park size, age, vegetation structure, tree density, management regime (e.g. mowing, mulching, leaf litter retention), and indicators of human activity and surrounding urbanisation.

By integrating biological, soil, management, and landscape variables, this project aims to identify key drivers of earthworm abundance, diversity, and functional composition in urban parks. The findings are expected to support evidence-based urban green space management that explicitly accounts for soil biodiversity and ecosystem functioning.

Session 4: Earthworm Taxonomy

P4.1. The only earthworm collection in Cuba: taxonomic update, curatorial revision and research potential

Capote Alvarez C.C., Cabrera Dávila G.

Earthworms play a fundamental role in soil structure and functioning, making up-to-date knowledge of existing collections essential for studies in ecology, conservation and sustainable management. The earthworm collection of the Felipe Poey Natural History Museum (MHN) represents one of the most important historical holdings and remains, to date, the only reference collection of Oligochaeta in Cuba. However, it has not undergone a comprehensive cataloguing and taxonomic update for more than 20 years. This study involved the review of 342 specimens currently housed in the collection, based on direct examination of the material and analysis of the associated database. The primary survey focused on the recovery and standardization of label information, reorganization of the material, taxonomic revision of the specimens and correction of relevant geographic and ecological data. The main objective was to update the taxonomic and curatorial information of the collection, transforming it into a renewed and functional scientific resource. Key results include a reorganized and properly catalogued collection, an updated database, and the revision and correction of the taxonomic classification of the specimens. In the longer term, this work is expected to lead to the description of new species and to improve understanding of earthworm biodiversity across different Cuban ecosystems. Furthermore, the collection will serve as a foundation for the expansion and establishment of a new earthworm collection at the Cuban Institute of Biodiversity, supported by ongoing research and development projects at the Institution.

P4.2. A comparative study of earthworm diversity in pastures and croplands in northern Iran

Latif R., Roohi Aminjan A., Briones M.J.I .

Agricultural activities have a profound impact on earthworm populations, which are key indicators of soil health. Intensive farming practices, such as frequent tillage, monoculture systems, and the heavy use of chemical fertilizers and pesticides, often disrupt soil structure and reduce the availability of organic matter, leading to a decline in earthworm abundance and diversity. In this study, the diversity of earthworms is compared in pastures and croplands in northern Iran. Earthworms play a vital role in both ecosystems by influencing soil structure and composition. The research was conducted in 10 locations, comprising five pastures and five croplands. In each study site, three soil blocks measuring 25 × 25 × 25 cm were examined, and earthworms were collected using the hand-sorting method. The collected earthworms were then washed and identified using taxonomic keys. A total of eight earthworm species were recorded in pasture areas. In croplands, four earthworm species were identified. The relative density, frequency, and species diversity index of earthworms varied significantly between different habitats. The lowest level of diversity was observed in rice-cultivated fields, where only a single species was present (*Amyntas cortices*). In contrast, wheat and rapeseed-cultivated fields exhibited higher species richness. Agricultural practices such as soil inversion through ploughing and the application of fertilizers likely disturbed the natural habitat of earthworms, leading to their migration to adjacent areas or a decline in their local populations. The highest diversity and density of earthworms were found in grasslands, where both metrics were more than twice those observed in croplands.

P4.3. Digging in search of local biodiversity: earthworms of the genus *Microscolex*

Capurro L., Martínez-Debat C., Ligrone A., Jorge-Escudero G..

Although there are only two species of earthworms within the *Microscolex* genus reported for Uruguay, the diversity of this genus might be underestimated due to the existence of species complexes, as has been demonstrated for *M. phosphoreus*. To determine whether there is such an underestimation locally, mitochondrial COI sequences of several *Microscolex* specimens found in Uruguay were analysed together with others available in GeneBank and Bold systems, totalling 133 sequences corresponding to *M. dubius*, *M. phosphoreus* and *M. enzespergeri* as outgroup. The bioinformatic analysis included the search for homologies using BLAST, sequence alignment, and the reconstruction of phylogenetic relationships through Bayesian inference using the BEAST program. Additionally, species delimitation methods based on genetic distances (ASAP and ABGD) were applied to identify molecular operational taxonomic units (MOTUs). As hypothesized, the diversity of the genus *Microscolex* in Uruguay was greater than previously recognized. Molecular evidence suggests the existence of at least two species within the *M. dubius* complex and possibly two within *M. phosphoreus*. The detection of lineages exclusive to Uruguay reinforces the importance of regional studies to understand the true biodiversity of soil invertebrates and highlights the need to continue morphological and molecular studies on soil fauna in South America. Overall, this work emphasizes the need to continue integrating morphological and molecular approaches for the taxonomic revision of the genus *Microscolex*, as well as to expand sampling in South America, to improve knowledge about the biogeography, diversity, and ecological role of earthworms in regional ecosystems.

P4.4. Decoding earthworm resilience: genomic drivers of climatic and phenotypic adaptation in the *Allolobophora molleri* complex

Martínez Navarro A., Caproni L., Dell'Acqua M., Mager S., Taboada S., Gracia C., Trigo D., Marchán D.F., Novo M.

Adaptation to soil environments has led to simplified body plans in earthworms, often resulting in cryptic species and species complexes. The *Allolobophora molleri* complex comprises at least five green-pigmented species of mixed ploidy, differentiated primarily by a single morphological trait: the position of the clitellum. Previous phylogenetic studies supported the synonymy of this complex, presenting an unusual case of high phenotypic plasticity in a key diagnostic trait. This study investigates the interplay between genotype, phenotype, and environment along a latitudinal gradient within this group. To this end, 192 individuals from 43 populations collected across the Iberian Peninsula and northern Morocco were genotyped using double-digest RAD sequencing. Genomic diversity analyses were performed via mapping to a reference genome, applying software designed to account for polyploidy. Results revealed geographically cohesive genetic clusters at both large (Morocco vs. Iberian Peninsula) and regional scales, following a latitudinal gradient that contradicts currently described morphospecies. Genomic associations with climatic and phenotypic variation were further explored using Gradient Forest (GF), Redundancy Analysis (RDA), and Genome-Wide Association Studies (GWAS). GF and RDA predicted genomic composition across the species' distribution range based on non-collinear climatic variables, revealing three main geographic areas shaped by precipitation and temperature, while GWAS identified SNPs with additive effects on clitellum position under a Mixed Linear Model. As a pioneering investigation into the genotype-phenotype-environment interaction in earthworms, these findings uncover the molecular basis of adaptation, offering crucial insights for predicting the future resilience of these animals to climate change.

P4.5. Hidden lineages, different futures: integrative phylogeography and ecological preferences of the *Allolobophora chlorotica* species complex

Piris A., Martínez Navarro A., Tilikj N., Dupont L., Marchán D.F., Novo M.

Earthworm taxonomy often masks cryptic diversity, which can bias phylogeographic inferences and compromise climate change projections derived from Species Distribution Models (SDMs). The endogeic lumbricid *Allolobophora chlorotica* (Savigny, 1826) is a widespread European earthworm with marked colour dimorphism, and reported ecological differences between morphs. Its taxonomic uncertainty has been reported by molecular and cross-reproduction studies, suggesting that this complex includes multiple mitochondrial lineages across its range (L1-L5) and several historically recognised subspecies (e.g. *A. chlorotica postepheba*, *A. chlorotica waldensis*).

Here, we provide an integrative reassessment of the *A. chlorotica* complex, combining dense barcoding sampling from southwestern Europe (newly and previous sequences), a multi-locus dataset (COI, 12S, 16S, ND1, and the nuclear 28S and H3), morphological analyses, and SDMs under present and future climate scenarios.

Together, the molecular data support several deeply divergent and geographically structured lineages, with southeastern France as a hotspot where almost all lineages and subspecies occur. In the Iberian Peninsula, only two lineages are detected (L2 and L5). This deep molecular divergences support several candidate species-level unrecognized taxa, including *A. c. postepheba*, *A. c. waldensis*, and a Mediterranean lineage (L5). Morphological comparisons also reveal diagnostic characters for the subspecies included, while L5 shows subtle but consistent differences in body size. SDMs reveal contrasting climatic associations and different future projections between lineages, with L2 and L5 showing distinct ecological preferences and distribution shifts in future scenarios.

Our results highlight how integrative taxonomy and lineage-specific modelling together improve biodiversity assessment in soil fauna, necessary for developing adequate conservation strategies.

P4.6. Dead or alive every earthworm counts: a comparison of live and preserved earthworm identification from arable soils

Stuhler, J., Sturm, L., Euteneuer, P.

Earthworm identification methods can affect the outcome of a research study in terms of numbers of detected species, earthworm community composition and biomass. To evaluate field identification of living earthworms compared with stereomicroscopic identification of preserved earthworms (96% ethanol), we used data from the SoilRise project (2024 – 2027). First, live and preserved total biomass was measured to obtain a conversion factor from preserved to live biomass, which was 1.395. Second, living earthworms from 99 arable fields in the north-east of Austria were identified by earthworm researchers in the field (Field) with no auxiliary devices and then preserved and observed under the stereomicroscope in the laboratory (Lab). In total twelve species were detected in the Lab and ten in the Field, while only seven species were shared between both methods. Interestingly, almost twice as many earthworms were identified as *Aporrectodea caliginosa* in the Lab than in the Field, but the opposite found for *A. trapezoides*. These differences can be explained as Field identification of *A. trapezoides* also uses pigmentation, which can fade after alcohol preservation. In addition, the Field method identified 2% less adult earthworms than the Lab, possibly by mistaking *Proctodrilus tuberculatus*, for an endogeic juvenile earthworm, due to its small body size. Ecological groups showed similar values regardless of identification method. These preliminary results demonstrated that identification without a stereomicroscope can be challenging and that species may be misidentified and stay undetected. However, Lab identification can also be misled, by the loss of pigmentation during alcohol preservation.

P4.7. Earthworm species diversity and systematics in Northwest and Northeast of China

Zhang Y., Zhao H., Bi Y., Zhang H., Li J., Qin Y.

Earthworm taxonomy and systematics have been controversial and are unstable. Several molecular phylogenetic analyses of earthworms which were mostly collected from North and South America, Africa and Europe were conducted using the nuclear genes and the mitochondrial genes to shed light on the evolutionary relationship of the different earthworm families. However, some relationships have not been clearly resolved due to limited taxa and appropriate genes to use. Although there have been phylogenetic studies of earthworms in Asia, these are limited to resolving mainly on a particular group of species in certain biogeographic regions and using limited gene markers and not targeted to resolving species-complex relationships. The use of mitogenomic data have proved to be useful in resolving formerly troublesome phylogenies, clarifying the relationships within phylogenetically difficult groups where high divergence rate made other markers ineffective. Here, we will be conducting a phylogenetic analysis of earthworms in different habitats of Northern China, including the species from Northeast and Northwest of China and the adjacent regions using mitogenomes to provide insights to the taxonomy and phylogeny of earthworm groups in this region. Earthworm samples will be collected from representative sites in different habitats of Northern China (Up to now, the finding was 3 families 7 genus 17 species). Available mitogenomic data will also be obtained from GenBank. With this work, we will be able to elucidate the evolutionary processes, morphological adaptations, the patterns of migration and colonization involved in radiations of these soil-dwelling animals, especially for the Lumbricidae.

P4.8. Reproductive relationships between the two epi-anecic earthworms *Lumbricus terrestris* (L.) and *Lumbricus friendi* (Cognetti): Potential pre- and postzygotic isolating mechanisms

Butt K.R., Nuutinen V.

Lumbricus terrestris (LT) and *Lumbricus friendi* (LF) are highly similar, obligatory biparental species. LF shows a southerly distribution in Europe, but co-occurs with LT, e.g., in southern Ireland. To study their reproductive isolation, a series of experiments were undertaken. Individuals grown to maturity in isolation were put into microcosms as (a) single LT; (b) single LF; (c) paired LT; (d) paired LF; (e) paired LT and LF. Treatments (a) and (b) were to examine potential parthenogenesis, (c) and (d) for comparative reproduction and (e) for potential hybridisation. After 3 weeks, the pairs in (c), (d) and (e) were separated and individuals monitored for cocoon production alongside (a) and (b). The focus of the work was any offspring from mixed species matings (e). Parthenogenesis did not occur. Viable cocoons were produced from (c) and (d). From mixed matings (e), LT parents produced non-viable cocoons, whereas cocoons of LF parents hatched (=hybrids referred to as "LF/LT"). Mature LT and LF attained masses of c. 8 and 4 g respectively, whilst LF/LT reached > 17 g, suggesting hybrid vigour. On maturity, LF/LT were mated together and both partners produced inviable cocoons. Backcrossing of LF/LT with LT and with LF gave mixed results. In a parallel behavioural study, video records showed no sign of LF mating on the soil surface, but viable cocoons were recovered. Pairings of LF, and normally surface-mating LT, showed mating, partially below ground. This behavioural difference is possibly one mechanism maintaining reproductive isolation in sympatry.

Session 5: Earthworms Outside the Box & Emerging Methods

P5.1. Developing ecoacoustic tools to detect, identify and monitor soil biodiversity

Almeida Streveler N., Ganault P., Saint-André L., Aubert M.

In studies of soil macrofauna, sampling is most commonly performed using the TSBF method, by excavation of a soil monolith, followed by organisms' hand-sorting. While widely used, this approach presents several limitations: (1) it is invasive as individuals are removed from sampling site, (2) it disturbs soil physical properties limiting monitoring over time; (3) it is selective, favoring easily detectable taxa; and (4) it provides only sporadic observations usually limited to one or two sampling per year.

An alternative consists in recording soil macrofauna activity through acoustic monitoring, and identifying species-specific sounds signature using artificial intelligence. This non-invasive and non-destructive method enables continuous recording of soil fauna activity, allowing the study phenology across different temporal scales (hours, days, weeks). The overall objective is to develop a coupled microphone-AI system capable of continuously recording and identifying whole soil soundscapes.

To achieve this, the first step involves acoustic recordings under controlled laboratory conditions to assess the frequency bands of macrofauna generated sounds, and determine the optimal audio gain to record the sounds. A second step focuses on controlled experiments with selected macrofauna species to identify species-specific acoustic signatures supporting the design of the microphone and AI training. Finally, the developed microphones will be deployed in forest soils, to initiate in situ recording and identification of forest soil macrofauna.

P5.2. Linking soil carbon, earthworms, and microbial communities under bio-based fertilisation in grassland soils

Nobre A., Doherty M.K., Leitão R.V., Ashekuzzaman S.M., Pereira J.C., Forrester P., Cunha L.

Bio-based fertilisers are increasingly promoted as sustainable alternatives to conventional mineral fertilizers, yet their effects on soil biota and carbon dynamics remain insufficiently understood. Here, a grassland field experiment evaluated the effects of four fertiliser treatments and an unfertilized control on soil functioning by integrating earthworm community assessments, soil carbon measurements, and bacterial microbiome characterization.

Earthworms were sampled and identified using morphological and molecular approaches. Soil organic carbon (SOC) and soil organic matter (SOM) were quantified, and bacterial communities were characterized using 16S rRNA gene metabarcoding. Fertiliser application did not significantly affect total earthworm density nor short-term soil carbon stocks. However, cattle slurry and struvite supported higher earthworm species and biomass diversity. Fertiliser treatments also influenced earthworm community composition, with distinct species–treatment associations. *Allolobophora limicola* was dominant, and *Aporrectodea caliginosa* and *Allolobophora chlorotica* occurred consistently across treatments. Bacterial alpha diversity presented no consistent fertilizer-driven differences in richness, evenness, or phylogenetic diversity. Community composition showed substantial overlap among treatments, characterized by a large, shared core microbiome. Fertilised plots, particularly those receiving organic and recycled inputs, harbored additional treatment-specific ASVs and shifts in the relative abundance of fermentative and anaerobic taxa within dominant bacterial phyla (e.g., Firmicutes, Bacteroidota). A positive correlation between organic matter inputs and SOC/SOM suggests that sustained organic fertilizer application may gradually enhance soil carbon storage. Overall, the results indicate that bio-based fertilisers modulate more than one soil biotic group through compositional tuning rather than disruption, with potential long-term benefits for soil ecological functioning and resilience.

P5.3. The role of earthworms in enhanced mineral weathering: from carbon capture to sustainable fertilisation

Calogiuri T., Vidal A., Van Groenigen J.W., Wichern F., Janssens I., Verdonck T., Poetra R. P., Rieder L., Janssens I. A., Hartmann J., Neubeck A., Niron H., Singh A., Vlaeminck S. E., Van Tendeloo M., Corbett T., Servotte T., Vicca S., Hagens M.

Actively removing atmospheric carbon dioxide (CO₂) is essential to meet the Paris Agreement target of limiting global warming to 1.5°C. Enhanced Mineral Weathering (EMW) is a promising approach that accelerates natural weathering to sequester CO₂ as inorganic carbon (IC), while also impacting organic carbon (OC) and improving soil fertility. To date, many studies have focused on the abiotic controls of EMW, while the role of biota remains largely understudied. Earthworms are particularly promising in this context due to their digestive processes, burrowing, and casting activities, which may accelerate mineral weathering.

Here, we present results from a series of experiments investigating how earthworms influence carbon dynamics and plant fertilisation through EMW. In a column experiment using an artificial organo-mineral system, we found that earthworm survival and activity were mainly driven by variables controlling the structure of the system. Additionally, live and dead earthworms had contrasting effects on weathering indicators, exerting negative and positive effects, respectively. A follow-up experiment showed that live earthworms had limited effects on IC sequestration, but enhanced OC stabilisation through physico-chemical pathways, whereas dead earthworms significantly enhanced both IC sequestration and OC stabilisation via microbially mediated processes.

A subsequent greenhouse experiment demonstrated that both earthworm-processed and unprocessed organo-mineral mixtures enhanced grass growth, alleviated potassium deficiency, and promoted carbon sequestration through mineral weathering, primarily driven by plant root processes. Overall, our findings highlight the importance of considering the full life-cycle of earthworms and the relevance of their interactions with other biota in EMW.

P5.4. Autonomous behavioural monitoring in earthworms: a scalable platform for high-throughput ethological studies

Mehrotra D., He Z., Geissmann Q.

Earthworms are vital soil engineers whose burrowing, feeding, and casting behaviors maintain ecosystem health by supporting nutrient cycling and soil structure. Despite their ecological importance, the behavioural mechanisms underpinning these functions—and their sensitivity to environmental stressors such as warming, drought, and agrochemical exposure remain poorly understood. Traditional observation methods are labor-intensive, limited in temporal resolution, and prone to observer bias, constraining progress in quantitative behavioural ecology.

We present a modular, scalable platform for continuous, autonomous monitoring of earthworm behaviour. The system integrates high-resolution imaging, acoustic sensing, and automated machine-learning analysis within a flexible experimental framework that permits precise control of environmental conditions, including moisture and lighting. It captures both aboveground and underground activity, enabling long-duration, fine-resolution behavioural assays across a broad range of experimental contexts.

Pilot studies demonstrate that the platform reliably detects food-preference behaviour under simultaneous choice conditions, expressed as biased surface movement and prolonged residence near preferred resources. These movement patterns indicate non-random foraging and active resource discrimination, illustrating the system's utility for studying foraging decisions, activity timing, and movement ecology in soil-dwelling invertebrates.

By removing the need for human observers, the platform supports high-throughput behavioural studies and captures subtle dynamics often missed by manual methods. Its modular design facilitates adaptation to other invertebrate taxa, providing a flexible methodological framework for advancing experimental ethology under controlled environmental conditions.

P5.5. A novel approach for farmers to estimate active earthworm abundance

Euteneuer P., Butt K.R.

Earthworms and soil structure depend on soil tillage, and earthworm abundance usually decreases with soil tillage intensity and under dry conditions. Complex interactions of tillage, soil bulk density and soil moisture affect soil penetration resistance and earthworm abundance. We therefore hypothesised that earthworm abundance decreases with decreasing soil penetration resistance for ploughing, whilst abundance increases with decreasing soil penetration resistance for no-till, as a function of soil moisture. The hypothesis was tested with preliminary data from a long-term experiment (LTE) in the north east of Austria with chernozem soil of loamy silt texture. The LTE consists of plough (25 cm depth) and no-till (0 cm) for 18 years. We took earthworm (4 soil monoliths 20 × 20 × 20 cm) and soil resistance (15 points per plot) samples in April 2022, 2023, 2024 and October 2023. For no-till, results showed a higher soil moisture content and lower soil penetration depth than plough. Earthworm numbers responded with higher abundance with decreasing soil penetration depth for no-till, related to higher soil moisture than for ploughing. These preliminary results showed that earthworm abundance can be predicted by maximum soil penetration depth. Potentially, this is a novel approach for farmers to estimate abundance of active earthworms.

P5.6. Microbial thresholds as indicators of compost pathogen suppression: a comparison in Biocomplete- and vermicompost extracts

Garcia-Knight N., Reverchon F., Gonzalez-Amaru R., Contreras-Ramos S.M., Barois I.

Background: The Biocomplete™ method proposes universal microbial thresholds to predict compost tea quality using compound microscopy and active microbial biomass estimations. It is unclear if suppressiveness can be predicted using these biomass thresholds, or if the unique "core microbiome" associated to *Eisenia fetida/andrei*-processed vermicompost provides superior, more resilient function compared to threshold-matched Biocomplete™ thermal compost. Objective: To determine if vermicompost extract (VCE) exhibits greater and more dilution-resistant suppression of *Phytophthora infestans* compared with Biocomplete™ thermal compost extract (TCE), even when microbial thresholds are matched. Methods: A VCE and a TCE were diluted to meet key microbial thresholds (bacterial & fungal biomass $\geq 135 \mu\text{g/ml}$). Each extract was then serially diluted (10^0 to 10^{-6}). Every dilution was characterized via microscopy active biomass estimations and assessed for suppressiveness in-vitro antagonism of *Phytophthora infestans*. Expected Significance: This study tests the ecological resilience conferred by inoculum origin. Results will reveal whether standardized thresholds are sufficient predictors, or if the specific, co-evolved microbial consortia in vermicompost provides a critical functional advantage for developing reliable biological inputs.

P5.7. Refinement of the QBS-e Index: improving earthworm-based soil quality assessment in european agroecosystems and semi-natural environments

Gavinelli F., Popović F., Monticelli M., Coletta M., Fusaro S., Squartini A., La Terza A., Trakić T., Paoletti M.G., Concheri G.

Earthworms are key bioindicators for monitoring soil quality and ecosystem functioning. However, assessments often report only total abundance, occasionally distinguishing between immature and adult specimens, or restrict interpretations to broad ecological categories. To provide a more comprehensive understanding of earthworm-mediated soil functioning, the QBS-e index (Soil Biological Quality Index based on Earthworms) was developed to integrate densities and life stages across all ecological categories. Years of application have revealed several limitations, particularly weak relationships between index values and environmental variables and the lack of normalization across different environmental contexts.

More than 700 field observations conducted in Italy and Serbia, along with published datasets and outcomes, were analyzed to derive refined soil quality classes specific to habitat and management contexts. This study develops the QBS-e Index through a normalization process that accounts for habitat type and cropping system, aiming to obtain realistic and standardized values comparable across European agroecosystems and natural environments. In parallel, the Eco-Morphological Index (EMI) scores are revised based on eco-morphological features to improve consistency, given recent updates to ecological categories, and to reduce bias in the final index values.

The normalized QBS-e appears to better discriminate among habitat types and management intensities, together with key soil/pedological properties, thereby enhancing its robustness as a scientifically sound and operational tool for soil quality assessment and making it more comprehensible to stakeholders. These refinements provide a standardized earthworm-based framework to support environmentally sustainable soil management, guide improvements in agronomic practices, and promote overall biodiversity conservation in agricultural and semi-natural environments.

P5.8. TimeWorm - 40 years of global earthworm diversity changes

Grüneberger M., Gérard S., Kukowski R., Nagahawatte K, Phillips H.

Earthworms are crucial for maintaining healthy soil ecosystems. Their communities are constantly changing over time, a process which might be accelerated by global drivers such as climate change and the human impact. Studying how earthworm communities have changed over time could help us detect long-term patterns of change and make predictions for future earthworm diversity change. Similar work has focused on the local scale, but not the global level. We extracted global time series data, i.e. data from sites in which earthworm communities were sampled for at least two years. This data was identified by a quantitative review of published and unpublished time series data of earthworm communities. We screened 13,000+ articles and found ~100 to be relevant for our analyses. To identify global drivers impacting earthworm communities, we also included data on climate, habitat, land use and soil properties. We will analyze the community data for changes in abundance, biomass and species richness over time as well as their relationships with global drivers. We expect to find decreases in earthworm alpha diversity metrics which will be more pronounced for more recent time series. We predict the main drivers of earthworm community change to be land use intensification and climate change. Understanding how earthworms are responding to human impacts is vital given the ongoing nature of global changes. Large-scale analyses, such as this, are an important component of comprehending the impact of these global problems.

P5.9. Citrus waste vermicompost as a sustainable substrate for rabbit forage production

Hernández-Hernández E, Barois I., Álvarez-Jiménez M, Hernández C.

The growing demand for animal protein intensifies pressure on forage production. Hydroponic green forage has emerged as a promising alternative due to its rapid growth, digestibility, and minimal land use. However, it requires specialized knowledge and costly technologies for its implementation. This study explored whether vermicompost -produced with *Eisenia fetida/andrei* and citrus waste feedstock- could serve as a substrate for rabbit forage production without the need of advanced equipment. For that wheat seeds (200 g) were soaked for 9 hours and sown in plastic germination trays filled with vermicompost. Then, another thin layer of vermicompost was added on top, and plants were cultivated for 14 days. Germination occurred within 24 hours; plants reached a height of 19 ± 2.6 cm on day 14. No fungal or bacterial infections were observed in plants. Growth rates were found to be higher ($P < 0.001$, $n = 30$) than previous hydroponic results in the literature. Rabbits were fed with this forage being a successful palatability and nutritious testing. The proposed method is simple, replicable, and does not require technical expertise empowering people in rural areas. Although further research is needed to assess more precisely the nutritional quality of the forage and determine optimal supplementation strategies for animal growth and health. These findings suggest that vermicompost-based forage systems could give an aggregate value to citrus waste and to high-quality food production. This work has been a real statement of research from the laboratory to the active field in the studies of earthworms.

P5.10. Monitoring earthworm activity using soil sound signatures and machine learning

Huisman T., Vidal A., Munnaf A.

Earthworms play a fundamental role in soil structure, nutrient cycling and overall soil functioning. These agro-ecosystem functions are driven by their activity. However, earthworm activity remains difficult to quantify. Existing methods are labour-intensive, intrusive and provide only snapshot measurements. Because activity is dynamic and strongly influenced by soil conditions, there is a need for non-invasive techniques that allow continuous monitoring.

Soil ecoacoustics, the use of sound produced within soil systems, offers a promising alternative for real time soil system monitoring. Past acoustic approaches to monitor earthworm activity have relied on inspecific sound indices. However, when applied into field conditions, these indices turned out to be too sensitive to other sources of soil acoustics. Therefore, we set out to identify specific earthworm acoustic signals for the automated recognition of earthworm activity using a supervised machine learning pipeline.

To identify earthworm specific sounds, we recorded *A. caliginosa* earthworms for 22 hours in microcosms (11 cm diameter, 22 cm height), which we placed in a sound isolation box to reduce background noise and isolate soil acoustic signals from ambient vibrations. Earthworms were added to loamy soil (23% clay, 40% silt, 37% sand; pH 7.55; 6.6% organic matter), soil moisture was adjusted to three levels (30, 60 and 80% WHC), and earthworms were introduced at three densities (1, 2 and 4 individuals per microcosm), resulting in 36 recorded microcosms. Recordings were conducted after a seven-day acclimation period.

Preliminary results suggest that soil acoustic activity is correlated with biological activity. Distinct acoustic patterns emerge during active phases, indicating that specific sound signatures produced by earthworms may provide a viable proxy for earthworm activity.

P5.11. Worms in motion: tracing spatiotemporal patterns of earthworm bioturbation

Tekin M., Rhodes E.J., Hodson M.

Earthworms provide a wide range of ecosystem services, many of which are directly facilitated by their bioturbation capabilities. Thus, achieving accurate estimates of earthworm bioturbation is crucial to understanding their effects on terrestrial ecosystems. Developing reliable bioturbation models requires detailed, high-resolution information on earthworm behaviour and the movement of soil particles. Yet our current knowledge of earthworm bioturbation remains constrained, mainly because of methodological restrictions and limited numbers of species used in existing research. This study attempts to address these gaps by measuring and comparing bioturbation of earthworms belonging to different functional groups in a short term study using tracer particles. Luminophores (fluorescent sand), 2D terrarias, and photography were used to measure continuous bioturbation. Different coloured luminophores at different depths allow us to measure bioturbation activity at different soil horizons, and using photography as a non-destructive sampling method allow us to capture change in bioturbation over time. Luminophores enabled us to observe the vertical translocation of soil particles, and identify how ingestion and egestion redistribute soil particles based on cast colour and location. The findings illustrate how bioturbation differs between species, and how it is dependent on both depth and time. By offering detailed spatial and temporal insights into earthworm behaviour and associated soil mixing, this technique facilitates the production of more accurate and robust bioturbation models.

P5.12. Casting for earthworms: a novel methodology for the vertically stratified sampling of earthworm communities in situ

Falconer O., Schmidt O.

We know little about how natural earthworm communities are vertically structured and functionally active within soil layers. To address this, a novel method was developed facilitating vertically stratified sampling of earthworm communities in situ. Falconer's Worm-CASTER (FWC) records active earthworm compositions and activity-density-index (ADI) in situ, with 2.5-5 cm interval resolution down to 30 or 50 cm depth.

A comparison with conventional hand-sorting (HS) revealed FWC achieved its core-function whilst revealing multiple indicators of its operational sensitivity. FWC species composition did not significantly differ to HS ($p = 0.79$), however vertical stratification of earthworm species between 0 and 20 cm depths was detected (e.g., *M. muldali* sampled exclusively at 15-20 cm).

While total community composition over repeated sample sessions did not change ($p = 0.139$), FWC detected significant changes in community composition over time between depths ($p = 0.026$). Some of these were explained by temperature changes. Since it does not involve digging, FWC provided greater sample quality with significant reductions in damaged specimen compared to HS.

This novel method has wide applicability to determine active-depth ranges of individual earthworm species and their response to vegetation composition, land use and management practices, as well as environmental stressors.

P5.13. Biodiversity in forest soils: establishing a long-term monitoring of soil organisms in forest soils of south-western Germany

Ott C., Hartmann P., Karzel Z., Schwineköper M., Puhlmann H.

Earthworms are key drivers of temperate forest soil processes, fragmenting and decomposing organic material, stabilizing mineral soil, and forming macropores that improve aeration, water infiltration, and overall soil structure. Despite their ecological significance, soil fauna has received little attention in forest monitoring and conservation. The project "Biodiversity of Forest Soils: Soil Fauna" at the Forest Research Institute Baden-Württemberg (FVA) addresses this gap by implementing the first large-scale, long-term assessment of soil organisms in Baden-Württemberg's forests, focusing on earthworms, collembola, and oribatid mites. In 2024, monitoring began at 114 sites representing major forest types and environmental gradients. Earthworms are sampled in spring using a combination of electrical extraction (octet method) and hand-sorting. Steep, stony sites in the Black Forest required methodological adaptations to the DIN protocol, derived from three years of preliminary sampling. Identifying living individuals allows the inclusion of juveniles, ensuring comprehensive community assessments. Results from the pilot phase (2018–2020) indicate a generally positive relationship between soil pH and earthworm abundance, diversity and biomass. However, these patterns vary not only among species but also across ecological categories, with some (primarily epigeic) species being associated with acidic, high-altitude coniferous forests. By analysing links between earthworm communities, soil properties, forest types, and management practices, the project provides essential knowledge for sustaining soil functioning and forest resilience. Ultimately, the results will support the establishment of a permanent soil-biodiversity monitoring programme and enhance the use of earthworms and other soil organisms as bioindicators of soil health within broader forest biodiversity assessments.

P5.14. From spades to sequences: comparing approaches for earthworm monitoring in the Netherlands

Riggi L.G.A., van Hout A., Faber J., Polling M., de Groot A., Edlinger A.

Understanding below-ground biodiversity at scale requires robust and comparable monitoring methods capable of detecting ecological responses to land use and management practices. Earthworms are widely recognised as key bioindicators of soil health; however, traditional assessments based on hand-sorting and morphological identification are labour-intensive and may overlook cryptic or low-abundance taxa. Environmental DNA (eDNA) metabarcoding offers a high-throughput alternative and may improve the detection of juvenile and morphologically ambiguous species. Nevertheless, each approach has distinct strengths and limitations, and their relative performance under comparable field conditions remains poorly understood. This knowledge gap is critical for evaluating how earthworm-based indicators can be reliably scaled up for large-scale monitoring.

Here, we present results from two studies that compare soil eDNA metabarcoding and classical morphological methods for assessing earthworm biodiversity across gradients of land use and management intensity in arable fields in the Netherlands. Preliminary results reveal discrepancies in the ranges of the investigated biodiversity metrics, as well as generally weak correlations between the two approaches. Despite these differences, both approaches were able to detect land-use-related patterns. We therefore summarise and discuss the main causes of disagreement between approaches and consider their implications for large-scale monitoring, particularly in the context of the Soil Monitoring Directive.

P5.15. Meet the Megadrill – a novel solution for collecting soil monolith mesocosms

Robinson. A., Rogers J., Humphreys M., Smith B., Jurgens M., Lahive E., Johnson A.

Mesocosms are valuable for soil or earthworm researchers, bridging the gap between small-scale lab experiments and field trials. Traditionally, obtaining realistic cores is challenging. Owing to the complex 3D structure and fauna of natural soils, packed column approaches offer limited realism. Small-scale samples can be taken by hammering in PVC pipes. This approach has major limitations. In agricultural soils, the maximum diameter is limited to around 15cm and the depth to 30cm. This is insufficient to capture realistic levels of earthworm diversity. In addition, they cope badly with stones and there is significant edge compression, damaging the soil structure. Alternatively, digging round a soil column and fitting a collar is very slow and very difficult to avoid significant edge effects or a poor fit.

We have developed a digger mounted drill to take perfect soil cores 40 cm wide and 50 cm deep. Due to a combination of a sharpened "cookie cutter" and drilling flutes to remove spoil, the collected monolith is a perfect fit for the column and suffers no compression or damage to the edges. The cores are large enough to allow realistic earthworm or community based experiments. The sample is collected inside a stainless steel sleeve where it remains for the duration of the experiment allowing convenient handling. This comprises a stack of 10 x 5cm tall stainless steel rings, which can be unbolted and sectioned for accurate stratified sampling.

This tool represents a major advance in realism and practicality for large scale earthworm or soil studies.

P5.16. Using eDNA to monitor earthworms across agricultural regimes

Schneider S.A., Müller-Stöver D.S., Manzeke-Kangara M.G., Watt P.J.

Systemic soil degradation threatens global food security, with the EU soil observatory reporting critical loss of functioning in 89% of agricultural soils. Over-reliance on intensive agricultural practices has depleted soil carbon stocks, damaging structure and aggregates leading to nutrient leaching and drainage issues, ultimately reducing crop yields. Earthworms promote healthy soil functioning by contributing to carbon sequestration via organic matter decomposition, improving soil fertility and structure, and augmenting crop growth and nutrient uptake. Enhancing biological communities in the soil, particularly earthworms, is therefore vital to soil regeneration and preserving agro-ecosystem services. Environmental DNA (eDNA) metabarcoding can be used as a relatively efficient method to assess earthworm diversity. This project will use eDNA to explore the relationship between earthworm diversity and crop performance, across a range of agricultural regimes, as part of the One Crop Health project, which takes a holistic approach to agroecosystem health in an effort to reduce reliance on pesticides. This research draws on the project's network of 100 working farms across the UK and Denmark and long-term experiments. We expect systems that promote earthworms and soil biota to enhance soil health and functioning, with benefits to crop growth, nutrition, and production.

P5.17. Using qPCR to assess earthworm species and abundance: a New Zealand example

Schon N.L., Hsu P.C., Loeffen S., Devey K.

Earthworms are ecosystem engineers, modifying their environment and contributing to soil functions. Ecological groups summarize the diversity of earthworms and their impact on soil functions. Due to their important role in soil, abundant earthworm populations are recognized as indicators of soil health. However, our ability to assess earthworm populations relies on the physical hand-sorting of individuals from the soil, with subsequent identification. This project aimed to utilize molecular techniques to improve our ability to assess earthworm populations, both in terms of diversity and abundance.

Using quantitative polymerase chain reaction (qPCR) we identified and quantified the DNA of the most common New Zealand pasture earthworm, endogeic *Aporrectodea caliginosa*. The effect of soil preparation on qPCR results was examined, with extraction from air-dried soils being successful. Air-dried soil eDNA samples were compared to results collected by physical hand-sorting earthworms from >100 sites. Overall, the field calibration demonstrated a strong positive correlation between *A. caliginosa* eDNA concentration and organism abundance (R^2 of 0.67). Highest *A. caliginosa* abundances were observed under permanent pasture ($>318 \pm 27 \text{ m}^{-2}$), followed by recently cultivated pastures ($<214 \pm 26 \text{ m}^{-2}$), with the lowest abundances in cropping soils ($<107 \pm 26 \text{ m}^{-2}$).

When the same qPCR approach was applied to species (common in pasture soils) from other ecological groups, the correlation with abundance was not strong, although species were still reliably detected. This may reflect differences in the spatial and depth distribution of the other earthworm species. We assess the potential of molecular approaches to quantify earthworm species and their abundance across other land uses.

P5.18. Combination of vermicomposting lake silt with active soil formation on site for recovering barren mountains

Li S., Li Y., Jiang H., Bi Y., Sun Z.

The silt (clear bottom silt) from plateau lakes in Yunnan, China, exceeds 20 million tons per year. The current practice involves mechanically dewatering and transporting the silt to the mountains, which is costly. This study employed vermicomposting method, where lake silt is mixed with local straw and chicken manure for pretreatment to feed both earthworms, *Eisenia fetida* (surface-dwelling earthworm) and *Pheretima aspergillum* (a deep-dwelling earthworm). The combination of lake silt treatment and active soil formation was conducted in the local barren mountainous areas with karst topography in order to lay the foundation for soil improvement initiatives and the development of characteristic agriculture in the mountainous regions of southwestern China. The experimental materials for earthworm breeding designed a 1:1 mixture of lake mud (main material) and livestock manure and corn straw (auxiliary materials). 4 different animal manure (pig, sheep, chicken and cow) with corn straw were used as experimental factors, the amount of livestock manure and corn straw used per square meter for each treatment was 0.3 m³, and the inoculation amount of earthworm spawn per square meter was 1 kg. Each treatment had three plots with three replicates, with each plot being 1 m wide, 2 m long, and 0.3 m high, covering an area of 2 m², with a spacing of 1.5 m between plots. The experimental results showed that the earthworm casts from each treatment had lower Ec, pH values, and heavy metal content compared to the materials before the experiment. The fecal coliform count was all converted to negative, and the nutrient contents such as organic matter, total nitrogen, total phosphorus, total potassium, and effective viable bacterial count increased. Preliminary experiments indicate that using a mixture of lake mud, cow manure, and corn straw in a ratio of 2:1:1 for earthworm breeding results in the best reproduction and growth of earthworms, with the highest earthworm yield of 9.66 kg; the shortest time required for material conversion into earthworm casts is 61 days, and the earthworm casts have good fertilizer efficiency indicators, with the highest yield reaching 0.89 m³.

P5.19. Where do earthworms prefer to be? A laboratory study on the temperature preferences in *Aporrectodea caliginosa*

ter Borg R.N., Larsbo M., Iseskog D., Benard P., Capowiez Y., Taylor A., Keller T.

As key soil ecosystem engineers, earthworms play a central role in soil processes and ecosystem services. Their behaviour and activity are strongly influenced by soil temperature and soil moisture, making them sensitive to changes in soil conditions. Although numerous studies have examined earthworm responses to soil conditions under controlled conditions, empirical data on their temperature preferences remain limited. Here, we present an experimental setup designed to identify the soil temperature preferences of earthworms.

A two-dimensional terrarium constructed from plexiglass (100 cm x 10 cm x 1 cm) was equipped with five heat pads (SKU 41010841; 13 cm x 5.5 cm) evenly distributed along its length. Each heat pad was paired with a soil temperature sensor inside the terrarium, allowing independent regulations of soil temperature at each position and thereby generating a longitudinal temperature gradient. The entire setup was insulated and placed in a climate-controlled room with regulated temperature and humidity.

For each experiment, two to four earthworms were introduced at different positions within the 2D-terrarium, corresponding to distinct initial soil temperatures. Earthworm movement and distribution were monitored over a 72-h period to infer soil temperature preference. To approximate soil conditions typical of Sweden, the imposed temperature ranged from 5 to 15°C. Experiments are currently underway, and data analyses are ongoing. The resulting data will elucidate the preferred temperature range of *Aporrectodea caliginosa* and inform predictions of earthworm-mediated soil processes under changing climatic conditions.

P5.20. Earthworm antimicrobial peptide EWAMP-R can induce apoptosis-like death in *Escherichia coli* by targeting ClpX

Wu Y., Li Y.

Earthworms harbor a rich diversity of antimicrobial peptides (AMPs), offering a promising source of novel antibacterial agents to combat the growing threat of drug-resistant bacteria. In our previous study, we identified an earthworm antimicrobial peptide named EWAMP-R through genome analysis of *Eisenia andrei*. EWAMP-R can induce apoptosis-like cell death in *E. coli* by inducing secondary responses beyond membrane damage. To identify the intracellular target of EWAMP-R, we generated EWAMP-R resistant *E. coli* strains, which exhibited an increased minimum inhibitory concentration (MIC) from 8 µg/mL to 64 µg/mL. Whole-genome sequencing revealed a mutation in the *clpX* gene (1108C>T), resulting in an amino acid substitution (R370C) in the ClpX protein. Transcriptomic analysis showed significant upregulation of genes involved in flagellar assembly, two-component systems, and chemotaxis in the resistant strains. Notably, CRISPR/Cas9-engineered *clpX* (1108C>T) mutants exhibited similar phenotypes to the induced resistant strains, including increased MIC of EWAMP-R and "hyperflagellate phenotype". Surface plasmon resonance assays further confirmed that EWAMP-R could directly bind to the ClpX protein, supporting ClpX as a potential target. Molecular docking and dynamics simulations revealed that EWAMP-R can bind to the Walker A motif, inhibit its conformational changes and suppress its ATPase activity. This leads to impaired degradation of substrates such as LexA 1-84. As a result, accumulation of LexA 1-84 can downregulate the SOS response, ultimately inducing apoptosis-like death in *E. coli*. These findings establish ClpX as a novel target for antimicrobial peptides and highlight EWAMP-R as a promising candidate for the development of new antibacterial therapeutics.

P5.21. Research on earthworm field mesocosm from pot to plot

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In order to realize the promotion of soil functions we designed an earthworm field mesocosm on the 0.4 hector field, with comparative experiments on the earthworm bio-ridge and furrow intercropping model, with conventional cultivation as the control, the experimental results were as follows: (1) Physicochemical properties of earthworm casts in the ridge and furrow treatment: The organic matter content of earthworm casts increased by 35.7% compared to the materials before the experiment, while pH and EC decreased by 10.1% and 43.7% respectively compared to the materials before the experiment. (2) Yield and quality in the ridge and furrow treatment: The yield increased by 6.9% compared to the control, and the soluble solids, vitamin C, soluble sugars, and organic acids in tomato fruits increased by 13.1%, 59.8%, 3.2%, and 17% respectively compared to the control. (3) Fertilizer and pesticide usage: The amount of fertilizer used decreased by 24.05% compared to the control, and the amount of pesticide used decreased by 34.4% compared to the control. Considering the conversion of earthworm castings, tomato yield, and quality, the intercropping and inter-cultivation mode using earthworm biological ridges and furrows showed better results.

P5.22. Non-invasive field monitoring of earthworm surface activity via red-light imaging and deep learning

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Understanding the surface behaviors of earthworms is fundamental for revealing their ecological functions, yet direct field observation remains challenging due to their predominantly nocturnal and disturbance-sensitive activity. In this study, we developed a red-light-illuminated time-lapse camera trap (miniMon-Red) to continuously observe earthworm behaviors at the soil surface without altering their natural activity patterns under field conditions. Red illumination (650 nm) was selected because earthworms exhibit low sensitivity to longer wavelengths, enabling non-invasive imaging during nighttime when surface activity is most frequent. Using time-lapse recordings collected over multiple nights, we constructed an annotated image dataset of earthworm surface activity and developed a deep learning-based segmentation and detection framework to automatically delineate earthworm bodies and extract behavior-relevant features under low-light conditions. To further enhance subtle movements, a motion-enhancement imaging algorithm was integrated as a preprocessing step to improve segmentation accuracy and temporal consistency. The proposed framework enables automated identification of key surface behaviors, including partial emergence associated with surface foraging and exploratory probing, short-range surface locomotion, and occasional full-body emergence. Results demonstrate that the miniMon-Red system, combined with deep learning-based image analysis, provides a reliable, scalable, and non-invasive approach for quantifying earthworm surface behaviors under natural field settings.

P5.23. Tracking earthworms in space and time – is there an ongoing colonization of Scandinavian boreal forests by burrowing earthworms?

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After the ice-sheet retreat, Scandinavian ecosystems opened up for earthworm recolonization. While in southern parts earthworms arrived likely in prehistoric times and became common soil macrofauna, ecosystems at higher latitudes evolved without burrowing earthworms. Commonly this is explained by the adverse conditions that prevail in boreal forests. However, the generally slow natural dispersal rate of burrowing earthworms, and occasional observations in northern Sweden around anthropogenic sources suggest that dispersal limitation also contribute. Given the dramatic impacts earthworm colonization can have on forests, it is important to assess the likeliness of burrowing earthworm expansion in Scandinavia.

By combining molecular techniques and a large-scale forest-soil inventory, we tracked earthworm distribution across Swedish forests. We identified earthworm presence by DNA traces both in recent and in archived soil samples from the past century. From a selection of corresponding site-specific data of vegetation, soil characteristics, climate, and human influence, we determined the most important factors explaining earthworm distribution. While most spatial variation was explained by edaphic factors such as soil pH and texture, burrowing earthworm presence in forests was also strongly linked to proximity to anthropogenic sources (agricultural fields). This suggests that their distribution is to some extent dispersal limited, and that parts of the boreal biome are more susceptible of earthworm colonization than previously anticipated.