Supervision

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Research units involved	EMMAH, INRAE - Avignon Université
Title of the doctoral project	**Tracing the Invisible: PFAS Impacts on Earthworms and Agroecosystem Functions**
Keywords	Soil, PFAS, bioturbation, earthworms, ecosystem services
Thematic-area	Agroecology, soil ecology, modeling, soil ecotoxicology

Summary

Per- and polyfluoroalkyl substances (PFAS) are a large class of xenobiotics that are persistent and bioaccumulative in the environment. Some of these molecules are known to impair the health of living organisms. PFAS enter agricultural fields through the use of plant protecting products, organic waste products, contaminated irrigation water, atmospheric deposits. The consequences of PFAS for the soil fauna, and in particular earthworms (key organisms involved in several soil functions) suffer from a chronic limited attention. This doctoral project aims at evaluating the sublethal chronic toxicity of PFAS and its consequences for terrestrial oligochaeta (earthworms) from the sub-individual to the individual, population and ecosystem levels. It will combine field and mesocosm experiments to in fine parameterize a model of population dynamics in soils and shed light on the cellular processes affected by PFAS, a first step towards the determination of adverse outcome pathways.

Description of the project

Scientific and socio-economic stakes

Healthy soils produce food, biomass and store carbon. They protect groundwater by filtering and storing nutrients and contaminants, and provide habitat for the soil biota. Over 30% of European soils are considered as degraded (Panagos et al., 2022, Vidal et al. 2025). In agricultural soils, some of these degradations can be mitigated by recycling organic waste products (OWP) from various sources. These nutrient-rich materials increase the soil organic matter (OM) content, which improves or restores several soil properties while contributing to achieving circular economy objectives. However, OWP also contain various contaminants including per- and polyfluoroalkyl substances (PFAS).

PFAS are a broad class of xenobiotic substances containing at least one perfluorinated methyl (-CF3) or methylene (-CF2-) group. PFAS have been increasingly used for over 80 years. To date, the structure of nearly 15000 PFAS molecules has been documented (Comptox, 2022). Most of these are, or can decompose into, molecules that are persistent in the environment and bioaccumulative. Some of them are known to impair the health of living organisms, including humans (Lyu et al. 2022, Delor et al. 2023). OWP are not the only source of PFAS in agricultural soils. Recent evidence suggests that PFAS can reach the soil through (i) irrigation with contaminated water, (ii) wet and dry atmospheric deposition, (iii) the use of pesticides belonging to the PFAS class (Cousins et al. 2022, Korsiarski et al. 2024, Joerss et al. 2024).

Among soil organisms, earthworms fulfil various services in agrosystems: they increase the availability of nutriments, enhance the stability of the soil structure, participate in organic matter cycles, and are preys for numerous other living organisms (Blouin et al., 2013; Vidal et al. 2023). They can also be used as bioindicators of soil quality, land use and management, and soil contamination (Vidal et al., 2025).

The consequences of PFAS on earthworm health and activities suffer from a chronic limited attention. An ongoing meta-analysis realized in the framework of the ADEME funded IPANEMA project highlighted that PFAS bioaccumulation values in earthworms were reported in only 21 research articles. Surprisingly, all but two of these articles focused on the earthworm *Eisenia fetida*, a compost worm that is known to be less sensitive to pesticides than other earthworm species (Pelosi et al. 2013) and that does not dwell in natural soils.

Only a few research considered endpoints other than bioaccumulation, such as effects on mortality and growth. In most cases, these studies considered PFAS concentrations well above those found in agricultural fields. And none of them studied the impact of sublethal concentrations of PFAS on earthworm activities: creation of burrows and vertical transport of soil particles. Interestingly, preliminary experiments in our team highlighted lower burrowing rates in PFAS-contaminated soils. The impacts of bioturbation activities on PFAS fate in the soil have not been considered either: wide and vertical macropores like those created by certain earthworm species act as preferential flow pathways for water and thus PFAS (Jarvis 2007), facilitating the rapid transfer of these xenobiotics towards groundwaters as suggested by recent knowledge gained during the IPANEMA project. Conversely, the upward transfer of soil constituents by earthworms may contribute to maintain high concentrations of PFAS in the root zone as observed for other persistent and strongly sorbing contaminants: radionuclides and PCBs (Jarvis et al. 2010, Cousins et al. 1999).

Overall, there is a critical need for research studies to **characterize the effects of PFAS on earthworms,** which are key organisms in maintaining and restoring ecosystem services related to soil, as well as the **role of earthworms on PFAS mobility in soils**, and to predict with adequate models these complex biological phenomena. For that, we need to understand and model the cascade of consequences of earthworm exposure to low doses of PFAS from the sub-individual level (damage to DNA or protein productions) to ecosystem functioning (mobility of PFAS in soils). This doctoral project will therefore address these issues at different levels of biological organization.

Research questions and hypotheses

The main assumption that will be evaluated in this work is that sublethal concentrations of per- and polyfluoroalkyl substances (PFAS) present in agricultural soils will have consequences at different biological levels: sub-individual (DNA, primary metabolites), individual (growth, survival), population (reproduction), and ecosystem functioning (burrowing and bioturbation, i.e., the transport of soil particles carried out by the soil fauna, essentially invertebrates, including earthworms).

An assumption related to the previous one is that biomarkers at the sub-individual level may be used as early-warnings of effects observed at higher levels (i.e., population level or higher, e.g., behavior and effects on the system functioning).

Another assumption is that it is possible to define a contamination level deemed safe for earthworm communities and the functions they fulfill in agroecosystems. This would help setting guidelines in terms of fluxes of PFAS in agrosystems. To do so, this study will emphasize on the consequences of soil PFAS exposure on two distinct earthworm species:

- *Lumbricus terrestris*, an anecic (i.e. earthworms that form continuous vertical macroporosity in soils) species;
- *Aporrectodea caliginosa*, an endogeic (i.e earthworms that form complex horizontal macroporosity in deeper soil horizons) species.

The inclusion of these two species, commonly found in temperate agricultural soils, will provide a realistic view of the bioporosity resulting from earthworm bioturbation.

The two studied earthworm species, according to their ecological category (endogeic and anecic) will have different exposure (*A. caliginosa* eat more soil than *L. terrestris* which targets fresher OM) and thus will be differently affected by PFAS.

With an interdisciplinary research program based on early discussions between physicists, biologists, ecotoxicologists and modelers, models can be calibrated and coupled to predict the effects of PFAS on earthworm-related ecosystem functions.

Research program (describe actions, calendar, tools and methodologies).

The research program will (i) build on results gathered during the ADEME-funded IPANEMA project (2021-2025) and (ii) reinforce and expand the tasks planned in the FluorAgro project (February 2026 – 2030) funded by ANR (see section “Other information” bellow).

The PhD student research program will combine **field and mesocosm experiments** including **metabolomics** to assess the ecotoxicological effects of PFAS on **soil oligochaetes** from **sub-individual** to the **population** level and finally their consequences on **ecosystem functioning**.

1/ Field work will rely on the Qualiagro and PROspective long-term observatories that have received organic waste products (OWP) of different origins, some of them containing important PFAS loads (Munoz et al. 2021) that transferred to soils (Michaud et al. 2025). We will select the OWP treatment leading to the two highest soil PFAS concentrations and the unamended controls. In the four replicates corresponding to these treatments and in the control plots, we will determine at 2 timepoints (i.e., two seasons of activity for earthworms, namely fall and spring), the abundance, biomass and diversity of earthworms and assess PFAS bioaccumulation in two earthworm species that have different habitat and behavior (one endogeic and one anecic species).

2/ In a second step, the PhD student will incubate for a minimum of 2 months (the duration will be precisely determined based on literature) **naive juvenile and adult earthworms**, of the endogeic and anecic species found in the field, in mesocosms filled with the two most contaminated amended soils and non-amended soils from Qualiagro and PRO'spective (6 replicates per treatment). At the end of the incubation, she/he will: (i) evaluate life history traits: survival, growth and reproduction by counting the number of cocoons in the mesocoms, (ii) measure PFAS bioaccumulation in earthworms, (iii) assess the physiological status of the earthworms by measuring enzymatic activities involved in chemical detoxification (carboxylesterases; glutathion-S-transferase; glucose-6-phosphate deshydrogenase) and energy reserves (glycogen, lipids, proteins), (iv) perform a metabolomic study at the Metaboscope platform (Avignon University) and (iv) analyze DNA breakage rates and oxidative DNA damage using the modified comet assay test. These data will shed light on the affected cellular processes, a first step towards the determination of adverse outcome pathways.

3/ Effects at the population level will be modeled by using the data determined in step 2 to parameterize the WORMDYN model (Pelosi et al., 2008) for the different conditions (PFAS levels and soil characteristics). The model classifies the earthworms in four categories (cocoons, juveniles, sub-adults, and adults) which can evolve at each time step in four different ways. Accordingly, the model requires 16 parameters. A systematic analysis of the literature will be performed to determine the parameters that will not be available from step 2. The model outputs will be compared to the time dynamics of earthworm population recorded in step 1. Overall, the model will constitute a tool allowing to determine the ecological risk for earthworms linked to the presence of PFAS in OWP. *In fine*, this will permit to evaluate whether amended soils, although they may content PFAS, are more favorable for earthworms than unamended soils.

4/ The PhD will assess the consequences of PFAS on earthworm's bioturbation activities. This will involve the preparation of 12 columns (4 columns per treatment, each 18 cm high and 14.5 cm diameter, at three concentrations: control, highest and intermediate PFAS concentration) containing repacked soil (target density 1.2g cm^{-3}). Each column will receive 5 naïve worms of *Aporrectodea caliginosa* and 2 of *Lumbricus terrestris*. The columns will be incubated during 4 weeks and imaged with an X-ray CT scanner (voxel size 300µm) weekly to determine the volume of burrow, and bioturbated zones linked to burrow backfilling, soil compaction during burrow creation and casts lining the burrow walls (Capowiez et al. 2011). Surface casts will be collected at the end of the experiment and their PFAS concentration will be measured to assess the vertical rate of PFAS transfer by bioturbation. Rainfall experiments will be conducted onto each column to assess the role of earthworm macropores on PFAS leaching.

In addition to their intrinsic interest, the model of point 3 will be coupled with a water and PFAS transfer model included in the VSoil modeling platform developed at EMMAH. Data gathered in point 4 will be used to parameterize this transfer model.

Provisional calendar

Months 1 to 10: Field study and data acquisition on life history traits and sub-individual biomarkers.

Months 10 to 20: Quantification of bioturbation in soil columns and consequences on water and soil particle transfers.

Months 20 to 30: Parameterization of the population dynamics model, and test of the model by comparing model predictions with earthworm populations determined in PRO'spective and Qualiagro long term observatories.

Months 30 to 36: Thesis manuscript redaction

In addition to the tasks already mentioned, a background task will be performed during months 1 to 20 to gather values of the parameters required in the model of population dynamics of *A. caliginosa* from the available literature (as done in Pelosi et al., 2008 for *L. terrestris*). This work will lead to the publication of a meta-analysis article.

Competences expected to be acquired by the doctoral student

Ecotoxicology, ecology, modeling, image processing, ability to work in a multidisciplinary environment.

Scientific partners, partners from the private sector / national and international

IMBE for enzymatic activities, Metaboscope platform for metabolomics

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Other information

The PhD grant is already acquired through 100% EUR Implanteus PhD contract.

The experimental program will be supported by the four-year FluorAgro project that will start in February 2026. It is funded by the French National Research Agency and led by E. Michel. It will fund PFAS and metabolomic analysis, field trips to the PRO'spective long-term observatory and a six-month internship for a master degree student. Additional financial resources (coming e.g., from paid provision of services performed by thesis supervisors) will be made available if necessary.

In addition to funding for the experimental program, the PhD student will find a stimulating scientific environment in the framework of this project, and will have the opportunity to interact with the different members of the consortium — four INRAE research units, a CIRAD laboratory and a large company, Veolia Agriculture) — during the annual meetings, and with a PhD student funded by Veolia and a post-doctoral researcher and that will respectively study and model the fate of PFAS in undisturbed soils.