Earthworms

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DESCRIPTION OF THE INDICATOR

Name of the indicator: Earthworms, density and biodiversity of earthworm communities.

Ecological role of the organism under test: Earthworms, also called lumbricians, are invertebrate soil organisms known to all. Composed of a succession of rings, they are part of the annelid branch. Earthworms are considered to be soil engineers because of their important role in the functioning of ecosystems. They affect the decay and mixing of organic matter, soil structuration and the hydraulic soil functioning. Consequences of such bioturbations imply, among others, reduced erosion, stimulation of microbial activity, increase in plant production and reduced pollution risks.
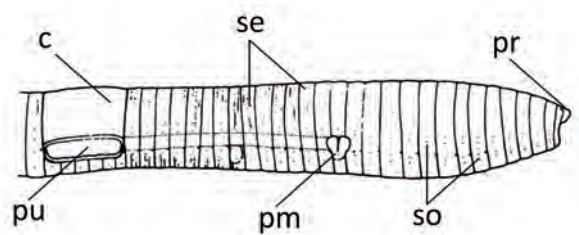

Figure 1: clitellum (c), male pore (pm), prostomium (pr), puberculum (pu), segments (se), bristles (so)

About a hundred species were indexed in Metropolitan France and classified into 3 groups called **ecological categories** according to Bouché (1972).

1 **Epigeics:** Species of small size (1-5 cm) and bright colours (red, brown). They live on the surface (within the 1st cm) and in dead organic matter (forest litter, manure, compost, green waste, purification plant sludge…). They tend not to make burrows.

Role: Participate in the fractionation of dead organic matter (diet: saprophagous)

2 **Anecics:** Very large species, from 10 to 110 cm. Their colours vary from red to brown, usually with a colour gradient from the head to the tail end. They live in the entire soil profile (burrows can reach 5m long). They make permanent vertical to sub-vertical burrows open on the surface, which allow water and air infiltration. They leave their casts on the soil surface (earthworm casts mixed with organic matter = "middens"), improving surface roughness, which limits erosion.

Role: Degradation of dead organic matter, burial and mixing of organic matter with ingested soil (diet: saprogeophagous), creation of tubular macroporosity (burrow network).

Notes: There are two main genera of anecics:
- red heads: easy to find earthworms (*Lumbricus genus*). Very reactive to temperature and humidity changes, their activity stops when soil conditions are unfavorable, but starts again when they are favourable. Their burrow network does not have many ramifications.
- black heads: these earthworms (*Aporrectodea genus*) systematically stop all their activities between June and August and build a branching burrow network.

3 **Endogeics:** Species of variable size between 1 and 20 cm. They are very pale colours or unpigmented (grey, pink or green). They essentially live within the first thirty centimeters of soil. They dig temporary horizontal to sub-horizontal burrows and fill their burrow with their casts.

Role: Creation of a "granular structure", which influences water retention and infiltration in soil (diet: geophagous), evolution of organic matter.

Biologie du lombricien

Earthworm biology
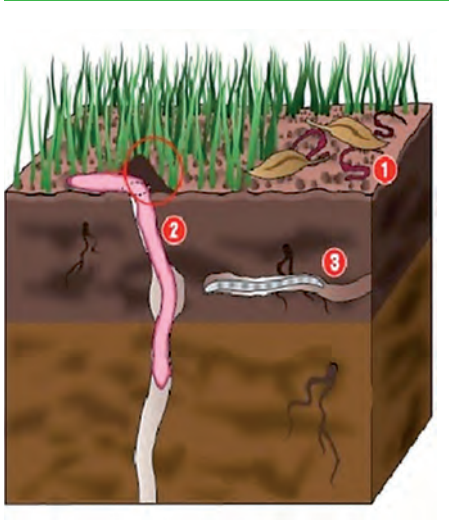
- individuals show continuous growth until the adult stage (no metamorphosis): juvenile worm becomes sub-adult, then adult,
- the adult stage is characterised by the apparition of visible male pores and prominent clitellum (the famous ring),
- most species sexually reproduce and are protandrous hermaphrodites (worms are male before becoming female), but they require reproduction.
- earthworms produce cocoons containing from 1 to 7 embryos,
- epigeous produce about a hundred cocoons a year while anecics produce a dozen,
- earthworms are mostly active at the end of winter - beginning of spring and in autumn and they are inactive in soils that are not wet enough (frozen soil in winter or dry soil in summer),
- lifespan varies from 3 months (epigeics) to 5-8 years (endogeics,
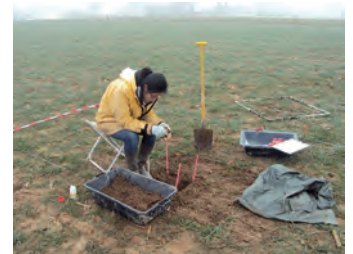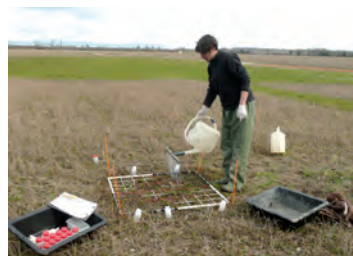
Type of indicator: Bioindicators of effect, they report the state and uses of the soil ecosystem. They meet the 4 rules of definition of a good indicator, i.e. relevance, reliability, sensitivity and reproducibility.

DESCRIPTION OF THE SAMPLING METHOD

Sampling processes and protocol: The study of earthworm communities requires a minimum of 3 replications per plot, and identifying an area representative of the habitat. Depending on the heterogeneity of the plot, an increase of replications is desirable. Seasonal variations and daily fluctuations (temperatures and luminosity) have an influence on the quality of catch for individuals from specific species. It is important to conduct sampling during the period of activity of earthworms: at the end of winter - beginning of spring and in autumn, i.e. when soil humidity is correct. Sampling is not conductible on frozen soils or at high temperatures and under strong sunshine.

Simplified description of the measurement method:

- First step consists in clearing the surface to be sampled (ex: finely cut and export vegetation; carefully remove litter or organic bark mass)

- Earthworms are then collected using the Bouché method (1972) and ISO 23611-1 standard, adapted to the agro-pedo-climatic context (Cluzeau et al. 1999 and 2003): spraying three times a formalin solution (3 x 10 L, 0.25%, 0.25%, 0.4% concentrations) on a 1 m² area, in fifteen minutes intervals. Earthworms display escape behaviour, as a response to the irritant properties of formalin, and are collected at soil surface. Superficial scraping (1 cm depth) is conducted to catch uncollected individuals (sampling time: 1 hour). This step is completed by physical sampling: a soil block (0.25 x 0.25 x 0.20 m depth) is extracted within the m2 and manually sorted (sorting time: 15-30 minutes).

| Delimitation of the m² and clearing of the surface | 1st spraying | 2nd spraying | 3rd spraying | Superficial scraping | Extraction soil block and manual sorting |

15 mn — 30 mn — 45 mn

Sampling — Sampling — Sampling

- Caught earthworms are fixed and stored in a formalin solution (4% concentration) to preserve pigmentation of individuals or in alcohol for DNA conservation.

Pre-treatment of samples: Species are determined at the laboratory, by taking into consideration several external morphologic traits: size, colour, shape of the first segment (prostomium), distribution of bristles and position and shape of sexual characters (clitellum: ring, puberculum, male pores). Individual weighing subsequently allows us to evaluate total biomass.

Measured parameters: Output settings enable the characterisation of communities at different levels:
- global: total abundance (nb ind/m^2) and biomass (g/m^2),
- functional: abundance of ecological categories,
- taxonomic (or specific) richness (number of species), diversity and equitability (relative importance of species)

INTERPRETATION OF RESULTS

Variation range of earthworm values and frames of reference

Variation ranges of earthworm abundances and biomasses on all sites of the Bioindicateurs 2 program (excluding extreme values, first and last deciles).
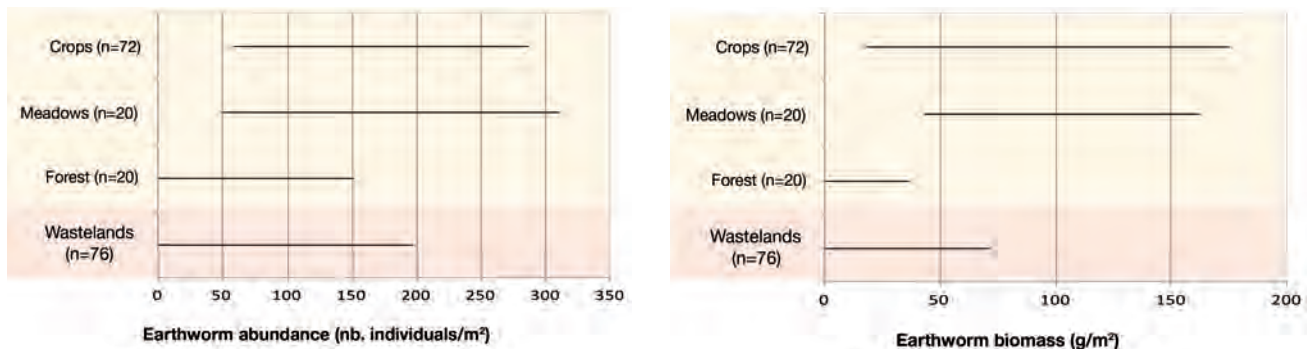

Collected data shows that variation ranges of abundances are very large; those of biomasses are narrower, especially in meadows, forests and wastelands. Such ranges reflect the variability of agricultural management in meadows and crops. Those values complement data that was collected at the regional scale in 109 sites in Brittany during the RMQS Biodiv program (Cluzeau et al., 2012) and ongoing data of the Participative Earthworm Observatory (OPVT), thus creating a first reference database.

The first results of the Bioindicateurs 2 program show the interest of using i) earthworm abundances and biomasses to characterise the biological state of soil, ii) ecological categories and traits in the functional characterisation of contaminated and agricultural sites, informing us of potential ecosystem services provided by soils (Pérès et al., 2011).

Example of application on an "Yvetot" site of the Bioindicateurs II program (manager ESITPA)

Modality	Description
GC:	Field crop, cultivation since 1993
SI:	**restored meadow** = 2nd year of restoration meadow established after 6 years of crop
SII:	**restored meadow** = 2nd year of restoration meadow established after 4 years of crop
SIII:	**temporary meadow** = 2nd year of crop after 5 years of meadow
SIV:	**temporary meadow** = 5th year of meadow after 2 years of crop
PP:	**permanent meadow**, planted in 1968
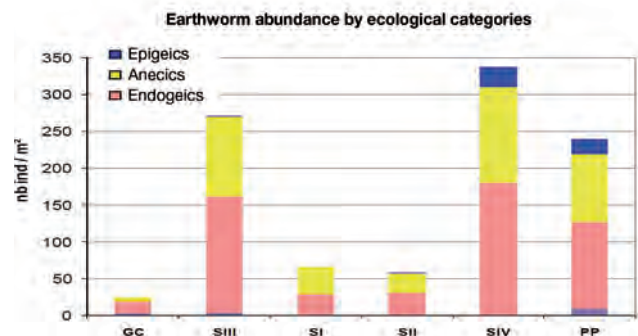
Permanent (PP) and long-term (SIV) meadows offer the best conditions for the development of earthworms (very high abundances and biomasses, strong presence of epigeics and anecics). Conversely, field crop (GC) has negative effects on earthworm communities due to annual tillage and the export of organic matter. Two years after the conversion of a cropping system into meadows (SI and SII), global and anecic abundances increase (factor 3), showing the quickness of benefits from incorporating a meadow in a rotation. Furthermore, a 2 year-old crop with a long meadow past (SIII) presents a high abundance: 2 years of tillage have not yet affected negatively communities.

The response of different earthworm ecological categories is an important tool of information because it will condition the functioning of soil (infiltration, nutrient dynamics). Earthworms and ecological categories reveal the restrictions/benefits of past and present crops and meadows, and can thus be used as rotation management tool.

INTERESTS AND LIMITS OF THE INDICATOR

+ Quick response of the indicator after modification of its habitat.
+ Strong ties to other biological parameters (example: microbiology).
+ Sampling method used for many years (existence of references).
+ Determination of user-friendly ecological categories (→ ecological and functional role).
- Time frame and conditions of habitat to be respected for samplings.
- Potential collector effect for manual sorting (especially for small individuals).
- Expertise required for specific and sub-specific determination.

FROM RESEARCH TO TRANSFER OF KNOWLEDGE

The OPVT (Participative Earthworms Observatory) is a simplified tool for the evaluation of soil biodiversity using earthworm. http://ecobiosoil.univ-rennes1.fr/OPVT_accueil.php OPVT

Its main goal is to collect, based on a simplified sampling method, national references in terms of earthworm abundance (nb earthworms/m2), under different pedo-climatic conditions and for varied soil uses. The OPVT is open to a large public such as agricultors, schools, gardeners and natural or anthropised habitat managers. In the long run, such observers will be able to compare their data with references collected under similar conditions, and gear their uses towards a better preservation of soil biodiversity.Up to now, more than 2000 persons (farmers, farmer advisors, gardeners) have implemented their data in the OPVT data base.

Benefits of the OPVT

1- The OPVT is an instructive tool that offers a simple, unrestrictive and accessible-to-all sampling method.

2- The OPVT ensures the instantaneous observation of earthworms and their classification on the field, according to the three ecological categories previously described: epigeics, anecics (red head and black head) and endogeics. These ecological categories are easy to identify with the naked eye, through size and colour criteria described on a field factsheet (available on the OPVT website).

3- The OPVT is a progressive tool. In order to adapt to requirements of different types of publics involved, from the least to the most interested, 5 levels of involvement have been defined.

- Levels 1 and 2 are made for the wider public and require, as a consequence, little time, little equipment and collected data is mostly qualitative. Earthworms are extracted by hand sorting soil block (20 cm * 20 cm * 25 cm) (level 1) or by using a solution of mustard water on a 50*50 cm square (level 2)

- Level 3, detailed hereafter, is intended for a public more aware of issues related to the ecological or sustainable management of soils, such as agricultors and technical institutes, organisers of catchment basins, or agricultural schools teachers: after the preparation of the 1 m2 sampling area (delimitation, clearing of vegetation), apply homogeneously a solution of mustard water (300 g of fine and strong mustard, i.e 2 small jars, diluted in 10 L of water); worms are caught in 15 minutes. Repeat the procedure. Determine the worms using the provided identity card at the ecological category level. Count the number of individuals per group and report results on the field factsheet.

- Levels 4 and 5 are intended for networks and programs interested in investing in the determination of the most common species (level 4) or all species (level 5) present on the sampled plot.

For more informations concerning the earthworm sampling, and OPVT :
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