

SoildiverAgro

Soil biodiversity enhancement in European agroecosystems to promote their stability and resilience by external inputs reduction and crop performance increase

D5.1- HANDBOOK ON WP5 SETTING UP, PROTOCOLS FOR SAMPLING, SAMPLE PROCEDURE AND ANALYSIS

Universidade de Vigo



D5.1. HANDBOOK ON WP5 SETTING UP, PROTOCOLS FOR SAMPLING, SAMPLE PROCEDURE AND ANALYSIS

Summary

The WP5 aims to determine the relationship that exists between soil biodiversity and other variables such as the crop production or emission of greenhouse gases. The first step in carrying out this work package is to establish a series of standardized procedures for experimental set up, sampling, processing of these samples, data collection and characterization, and data analysis.

This deliverable presents, in the form of an appendix, a handbook of methods to carry out all the analyzes that will allow establishing the soil and crops characteristics of the different pedoclimatic regions. Therefore, the protocols agreed by the project members are included. The aspects considered in this handbook of methods are:

- Experimental design
- Crop growth and quality properties. This part includes the development of the crops, the yield, the incidence of diseases, and the nutritional evaluation of cereals, legumes and vegetables. Types of sampling of the soils (oriented to determine dry bulk density, biodiversity or physicochemical characteristics).
- Earthworm sampling.
- Physical characterization of the soil including determination of dry bulk density, coarse fragments, water holding capacity, humidity, soil hydraulic conductivity, particle size distribution and aggregates.
- Chemical characterization of the soil including determination of pH, salinity, and content of organic matter, organic and inorganic carbon, total and inorganic nitrogen, available phosphorus, potassium, calcium, magnesium, boron, effective exchange capacity, cations (iron, manganese, copper, zinc and molybdenum), and pesticides.
- Biological analyzes that include biodiversity measures (of worms, nematodes and microorganisms), using techniques such as DNA extraction and purification, or fatty acid analysis.
- Soil greenhouse gas emission.

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1 Introduction

Taking into account that, through this WP (WP5) it is intended to determine the capacity of edaphic biodiversity to solve current economic, environmental and agronomic problems, it is essential to establish a reference framework to carry out the pertinent experimental design, sampling and analyzes. Thus, through this book **"HANDBOOK ON WP5 SETTING UP, PROTOCOLS FOR SAMPLING, SAMPLE PROCEDURE AND ANALYSIS"** included in **Annex I**, the members of the SoildiverAgro project tried to standardize the methods that will be used to design field experiments, follow crop performance, and sample/characterize the soils and crops of 15 case studies in 6 different pedoclimatic regions throughout Europe. The methods included in the handbook cover techniques for experimental design, follow crop performance (growth, yield, pest incidence, and nutritional quality of vegetables, among others), soil sampling and determination of chemical properties (pH, salinity, organic matter, available cations, etc.), physical (bulk density, texture, water holding capacity, among others) and biological (nematodes, earthworms, microorganisms, etc). Moreover, method for soil greenhouse gases emission analysis is also included.



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2 Annex 1: Handbook



HANDBOOK ON CASE STUDIES SET UP, PROTOCOLS FOR SAMPLING, SAMPLE PROCEDURE AND ANALYSIS

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5.1. EARTHWORM DIVERSITY

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PRINCIPLE AND APPLICATION

Earthworm populations are usually characterized according to different criteria: i) developmental stage to quantify the proportion of adults and juveniles; ii) ecological groups to describe the feeding and burrowing behaviour; iii) species identification to determine species richness.

This protocol describes how:

- to identify earthworm species
- to assign each specimen to one of the established ecological groups (Bouché, 1977): epigeic, anecic and endogeic,
- to distinguish developmental stages: adult, subadult and juvenile
- to separate incomplete specimens and fragments from all others
- to determine total abundance (individuals m⁻²) and biomass (g m⁻²)

PROCEDURE

Wear disposable protective gloves and use plastic forceps when handling earthworms. The specimens from each replicate sample should be placed into a plastic container filled with cold tap water. Before the species identification under the stereo microscope and weighing, each specimen should be cautiously dried on tissue paper.

Developmental stage

Earthworms are first separated according to their developmental stage by looking at their sexual maturity:

- Adults have a fully developed clitellum (glandular thickened section of the body wall in the middle or towards the anterior end of the body)
- Subadults do not have a developed clitellum but tubercula pubertatis (glandular thickenings on the ventral surface) are present
- Juvenile worms do not show any visible signs of clitellum nor tubercula pubertatis.

Figure 5.1.1. shows some of the morphological characters for identifying earthworms to species level, and includes the position of the clitellum and the tubercula pubertatis, two distinct characteristics of fully adult specimens.

Ecological groups

Most earthworm individuals (adults and sub-adults but often also juveniles) can be assigned to their ecological groups according to their dorsal pigmentation:

- Epigeic species are heavily pigmented small earthworms.
- Anecic species are large earthworms and moderate to heavily pigmented on their front end (until some segments behind the clitellum);
- Endogeic species are unpigmented or nearly unpigmented; in some species, the epidermis is translucence showing through inner organs



EQUIPMENT AND MATERIAL

- Disposable protective gloves
- Tissue paper or paper towels
- Plastic forceps
- Flat plastic containers (vol.: ca. 1 L) with cold fresh tap water
- Plastic cups with cold fresh tap water
- Stereo microscope (magnitude: ca. 10x to 50x)
- Analytical balance (precision: 0.01 g)
- Identification keys
- Data sheets



REMARKS

All fragments of earthworms in the sample must be included for determining total earthworm biomass. However, only those fragments that include the head and as best also the clitellum can be identified to the species level and if this is the case, they can be used for total abundance and diversity estimates. Any other fragments are listed as “unidentified fragments”.

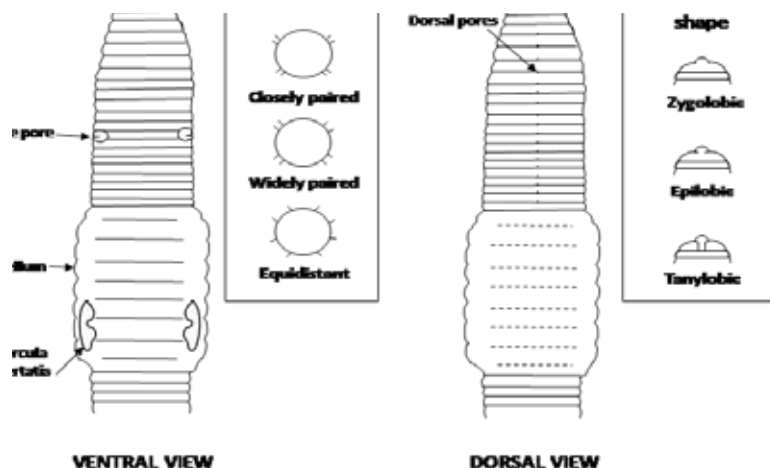


Figure 5.1.1. Some of the main morphological characters that are used in identifying developmental stage and species of earthworms

Figure 5.1.2. shows one example species, its typical pigmentation and type of burrow system for each ecological group.

Taxonomical identification

Adult earthworms can be identified to species level by using a stereo microscope (magnitude: 10x to 50x) and a taxonomic identification key. For example, the identification guide by Sims and Gerard (1999) and key to the earthworms of the UK and Ireland by Sherlock (2018) are useful for identifying the majority of the common European species. Because they do not cover completely European earthworm fauna, national or regional keys should also be used when necessary.

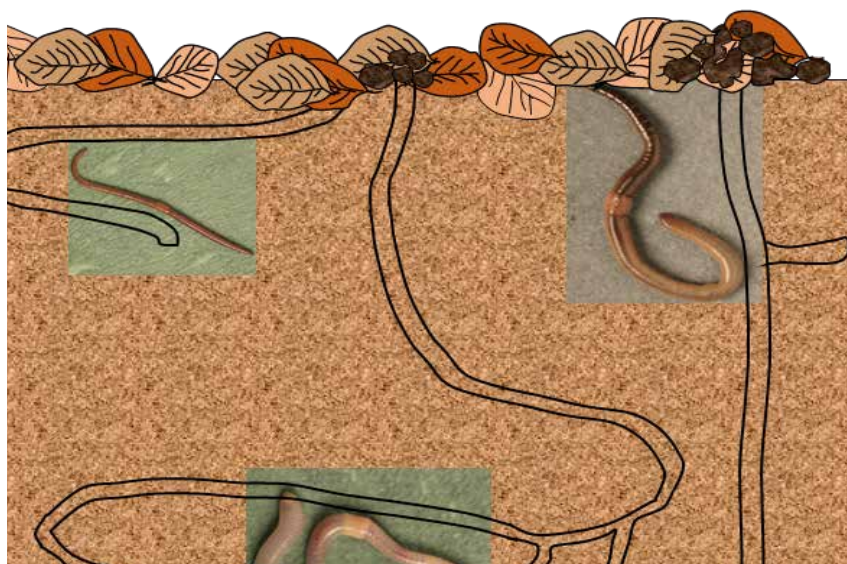


Figure 5.1.2. Earthworm ecological groups (Bouché, 1977): epigeic (*Lumbricus castaneus*; top left), anecic (*Lumbricus terrestris*; right), and endogeic (*Octolasion cyaneum*; bottom) (the size of the species not to scale). Photo credit: Guide to British earthworms (<https://www.opalexplornature.org/earthwormguide>).

The most important diagnostic characters are the position and shape of the clitellum and location of the tubercula pubertatis (see Figure 5.1.1.). Other important diagnostic characters are the shape of prostomium (in front of the first segment) and the clitellum (annular, saddle), setae pattern (distance between setae on each segment) (Fig. 5.1.1.). Subadult earthworms can be identified to species in many cases, however, juvenile earthworms can only be assigned to ecological groups (see above). If the specimen cannot be accurately identified, then annotate all available information and classify it as 'unknown'.



CALCULATIONS

Use a balance with precision of 0.01g for determination of the preserved earthworm biomass (g). Determine the biomass per species, per ecological group and in total for each sample. Express all abundance (of individual species and ecological groups) and biomass data as individuals per square meter (ind. m⁻²) and grams per square meter (g m⁻²), respectively. In case of a 50 cm x 50 cm sampling quadrat, multiply the density and mass in the sample by 4. In case of a 25 cm x 25 cm sampling quadrat, multiply by 16.

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