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Soil quality — Sampling of soil invertebrates —

Part 5: Sampling and extraction of soil macro-invertebrates

Qualité du sol — Prélèvement des invertébrés du sol —

Partie 5: Prélèvement et extraction des macro-invertébrés du sol

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Foreword

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This document was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 4, *Biological characterization*, in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 444, *Environmental characterization of solid matrices,* in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

This second edition cancels and replaces the first edition (ISO 23611-5:2011), which has been technically revised.

The main changes are as follows:

— Two informative Annexes were added at the end of the document. Annex B describes the procedures to be adopted when sampling macro-fauna using pitfall traps and Annex C presents a monitoring example with pitfall traps.

— The bibliographic references list was revised and updated in the entire document.

A list of all parts in the ISO 23611 series can be found on the ISO website.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

This document was prepared in response to a need to standardize sampling and extraction methods for soil macro-invertebrates globally. These methods are needed for the following purposes:

— biological classification of soils, including soil quality assessment (e.g. References [14], [28] and [37]);

— terrestrial bio-indication and long-term monitoring (e.g. References [65], [74], [75] and [76]).

Data collected using standardized methods can be evaluated more accurately as they allow more reliable comparison between sites (e.g. polluted vs non-polluted sites, changes in land-use practices).

Soils of the world host an abundance of highly diverse macro-invertebrate communities. Their biology and ecology have been widely studied. Soil invertebrates are irreplaceable actors of soil formation and conservation in natural ecosystems. Their relevance to the soil system comes from their abundance and diversity, and also from their role in key biological processes. They are sensitive indicators of soil quality and recognized actors of its fertility (e.g. References [58] and [52]). Among the wide diversity of species, adaptive strategies and size ranges represented, one specific group, also called “soil ecosystem engineers”, includes large invertebrates that determine the activities of other smaller organisms through the mechanical activities they produce in soil (e.g. References [18] and [46]).

Soil macro-invertebrates span a wide range of ecological functions in soil: decomposition of organic matter, through their own activity and by stimulating the soil's microbiological activity (e.g. References [2], [3] and [36]), predation that plays an important part in food webs (e.g. References [9], [51], [56], [59] and [63]), soil aggregation by the production of organo-mineral structures (e.g. nests, galleries, casts) that can last for days, months or years, soil bioturbation (e.g. Reference [28]), etc. These characteristics, coupled with in-depth taxonomic knowledge, have enabled their use as study organisms in several research programmes dealing with the impacts of forest practices (e.g. References [11], [36], [47], [57], [60] and [70]) or crop management practices (e.g. References [8], [19], [27], [29], [30], [33], [38], [55] and [62]). These features make them suitable organisms for use as bio-indicators of changes in soil quality, especially with respect to land-use practices and pollution (e.g. References [21], [35], [45], [48], [49], [54], [60] and [74]).

The method proposed in this document covers the sampling of all soil macro-invertebrates. However, the sampling of earthworms is already covered in ISO 23611-1. This alternative sampling method for earthworms is described in ISO 23611-1:2018, Annex C.

The method proposed in this document is a prerequisite for using macro-invertebrates as bio-indicators (e.g. to assess the quality of a soil as a habitat for organisms). The main premise of this method is rapid assessment (completing the sampling of a plot in one or two days with only basic equipment and a small number of field assistants) in order to be able to address all the taxonomic groups of soil macro-invertebrates at the same time and in the same place. The Tropical Soil Biology and Fertility (TSBF) method has evolved and some modifications have been introduced in order to use it in temperate regions.

A sampling design is specified in ISO 23611-6.

NOTE The method specified in this document is based on guidelines developed under the Tropical Soil Biology and Fertility Program (TSBF method).[1]

Soil quality — Sampling of soil invertebrates —

Part 5:
Sampling and extraction of soil macro-invertebrates

1 Scope

This document specifies a method for sampling, extracting and preserving macro-invertebrates from soils, including the litter zone.

The sampling and extraction methods in this document are applicable to almost all types of soil, with the exception of soils in extreme climatic conditions (hard, frozen or flooded soils) and matrices other than soil, e.g. tree trunks, plants or lichens.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

3.1
macro-invertebrate
soil organism whose longest dimension is greater than 10 mm

EXAMPLE These include especially the following groups: Oligochaeta, Gastropoda, Chilopoda, Diplopoda, Isopoda, Arachnida, plus various insects: Coleoptera, Orthoptera, Hymenoptera, Hemiptera, Dermaptera, Lepidoptera (larvae) and Diptera (larvae).

Note 1 to entry: See Annex A for further details.

3.2
blotted mass
mass of individuals after preservation in formalin or ethanol (when the substance used for preservation has been absorbed by the tissues)

4 Principle

Soil macro-invertebrates are collected in the field using a metallic frame to delimit the soil surface of the sampling point. Macro-invertebrates present in litter and soil are picked up separately. In temperate regions, a reagent is used to extract macro-invertebrates from soil. The sampling is completed by hand-sorting. Animals are preserved and transported to the laboratory for further identification (e.g. References [4], [5], [6], [7], [10], [12], [13], [16], [17], [22], [24], [25], [26], [31], [32], [34], [42], [43], [44], [50], [53], [64], [66], [67], [71], [72], [73] and [77]). Abundance values are usually recalculated relative to area (1 m^2).

5 Reagents

5.1 Ethanol, (70 % volume fraction).

5.2 Formalin (formaldehyde solution), 4 % (volume fraction).

Both 70 % ethanol and 4 % formalin should be available for the preservation of specimens (4 % formalin is more suitable for taxa with soft body parts, which can be transferred to ethanol after about 4 d fixation).

5.3 Formalin, 0,2 % (volume fraction), prepared by diluting 25 ml of formalin (39 %) in 5 l of water, for soil macro-invertebrate extraction.

6 Apparatus

Use standard laboratory equipment and the following.

6.1 Petri dishes.

6.2 Stereo-microscope.

6.3 Plastic vials.

6.4 Entomological forceps.

6.5 Pencil, notebook, water-resistant marker, labels.

6.6 Tape measures.

6.7 Knife (cut glass).

6.8 Spade.

6.9 Plastic-weave produce sacks, for spreading on the ground.

6.10 Precision balance.

6.11 Large flat plastic trays (500 mm × 400 mm × 100 mm), for sorting the soil and litter.

6.12 Trowel.

6.13 Small plastic trays.

6.14 Fine forceps (or entomological forceps), pipette, fine paint brushes.

6.15 Sample vials, in various sizes with secure alcohol-tight caps (plastic throw away or plastic/glass reusable vials).

6.16 Indian-ink pen (waterproof).

6.17 Stiff card for labels, ranging compass.

6.18 Large strong plastic bags (sealable).

6.19 Table and plastic chairs, for sorting.

6.20 Cover, for protection from heavy rain.

6.21 Chemical protection gloves, suitable for working with formalin.

6.22 Metallic frame, preferably 250 mm × 250 mm.

Sample frame (250 mm × 250 mm × 50 mm) made of stainless steel and with sharpened edges to delimit the sampling point where animals are sampled from the litter layer and soil.

6.23 Watering can.

6.24 Pair of scissors, to cut vegetation inside the frame.

6.25 Field balances.

7 Field procedure

7.1 General

Sampling should take place when accessible biodiversity is thought to be largest. In temperate regions, it corresponds to spring or autumn; and in the tropics, it should take place towards the end of the rainy season.

When sampling soil invertebrates, the site should be physico-chemically characterized. In particular, pH, particle size distribution, C/N ratio, organic carbon content and water-holding capacity should be measured using ISO 10390, ISO 10694, ISO 11274, ISO 11277, ISO 11461, ISO 11465. Natural minerals present in the site soil should also be described.

7.2 Collecting macro-invertebrates from the litter zone

At each sampling point (= monolith) (previously defined according to sampling design rules), a litter sample is collected using a metallic frame (6.22). The metallic frame is pressed into the litter by hand. The litter inside the frame is removed and checked manually in the field using a large tray (6.11). Litter invertebrates are preserved in 4 % formalin (5.2).

7.3 Collecting macro-invertebrates from soil

7.3.1 General

In temperate countries, the extraction of soil macro-invertebrates is carried out in two steps (see 7.3.2.1 and 7.3.2.2), while in tropical countries only the second step shall be performed (see 7.3.3). In both cases, extraction of macro-invertebrates may be complemented by the use of pitfall traps (see Annexes B and C for further details).

7.3.2 Temperate regions

7.3.2.1 Formalin extraction

The soil surface delimited by the metallic frame (6.22) is sprayed with 0,2 % formalin (5.3) using a watering can (6.23). Two applications of 1,5 l of formalin are performed at intervals of about 10 min. Soil invertebrates coming up to the surface are collected and preserved in vials (6.3) containing formalin (5.2).

7.3.2.2 Hand-sorting of "passive" macro-invertebrates

At the end of the formalin extraction, the metallic frame (6.22) is removed and the upper 150 mm of soil is excavated within the frame area (250 mm × 250 mm). The excavated soil is placed in a plastic bag (6.18) that can be closed with a cover to prevent animals from escaping from the soil sample.

Appropriate sub-samples of soil are taken from the container and spread on a large tray (6.11). Macro-invertebrates are collected and preserved in vials (6.3) with formalin (5.2). When hand-sorting is finished, the excavated soil is replaced to avoid creating holes on the sampling site.

7.3.3 Tropical regions

In tropical countries, soil macro-invertebrates are sampled using a 250 mm × 250 mm × 300 mm deep soil monolith. The monolith is isolated by cutting with a spade (6.8) a few centimetres outside the quadrate (metallic frame) and then digging a 20 mm wide by 300 mm deep trench around it. This facilitates cutting of the sample into horizontal strata and collecting animals escaping from the block.

The delimited block is divided into three layers, 0 mm to 100 mm, 100 mm to 200 mm and 200 mm to 300 mm; and the soil and litter material is hand-sorted in trays (6.11). Since formalin is not applied in tropical regions, the sampling depth should be doubled in order to be sure to collect endogeic and anecic species of earthworms.

For social insects, special measures should be considered that take account of their high abundance and marked patchiness; a nest can contain millions of individuals, of which none are sampled by a short transect, and the contribution of the species concerned to a macrofaunal assemblage can thus be completely missed. On the other hand, a highly populated nest sampled directly by a monolith can lead to a large overestimation of the overall numerical or biomass density. In general, the TSBF transect should be placed to avoid direct contact with termite and ant nests. For discussions, see References [35] and [36]. The protocol for a 100 m × 2 m transect designed to assess termite biodiversity (and feeding group representation) is given in Reference [48]. In suitable circumstances, this protocol can also be deployed in parallel with the TSBF transect.

NOTE Besides the general characterization of the site, it is useful to determine the actual moisture of the soil to be sampled.

8 Laboratory procedure

8.1 Treatment of collected samples

In the laboratory, samples are cleaned with either distilled or tap water in a Petri dish with the help of a brush or placing the organisms on a 0,5 mm to 1 mm sieve under the tap. Afterwards, the animals are placed in new vials (6.15) with ethanol (70 % volume fraction) (5.1). Organisms with soft body parts are kept in formalin for at least 4 d, or forever if possible.

For taxonomic identification, specimens are placed on petri dishes (6.1) and observed under the stereo-microscope (6.2). A practical way to identify macro-invertebrates is to group them into orders first. Each order is then identified into families and each family into species using taxonomy keys (examples of taxonomy keys are the References [4], [5], [6], [7], [10], [12], [13], [17], [22], [24], [25], [26], [31], [32], [34], [42], [43], [44], [53], [66], [67], [72], [73] and [77]).

Ideally, taxonomic determination should be based on the species level. If identification of species levels fails due to time constraints, taxonomic expertise or missing taxonomic keys, e.g. mainly in tropical regions, sorting to genus (and some higher taxonomic units) represents a good compromise between the morphospecies and ordinal level approaches, especially as this allows most specimens to be assigned to a functional group.

WARNING — Appropriate precautions (i.e. gloves, mask) should be taken when dealing with formalin to avoid danger from inhalation or skin exposure. According to the Material Safety Data Sheet for formaldehyde 37 % solution published by producing companies, the compound is a skin sensitizer

and is considered to be carcinogenic (humans: limited evidence; animals: sufficient evidence). It is legally notified in industrialized countries for scientific use.

8.2 Preservation of specimens

From any mixed soil sample of macrofauna, the following steps should be followed in order to obtain standardized preserved specimens.

a) If the animal has no soft body parts, the organisms should be preserved in 70 % ethanol (commercial ethanol should be diluted).

b) If the animal has soft body parts, the organism should be fixed in 4 % formalin and should, if possible, be preserved in the same solution. Alternatively, 80 % ethanol may be used (if the organism has been fixed during at least 4 d with 4 % formalin).

c) In all cases, samples should be stored separately in different vials, according to the smallest unit of analysis (i.e. a monolith if the data are compared at that level).

d) Every vial should be labelled without using code numbers and should at least be written using permanent ink, like Indian or Chinese ink, and using sturdy paper like goatskin parchment. Every label should contain the following information:

   — country;

   — region;

   — locality;

   — collector's name;

   — date of collection.

e) For storing specimens:

   — use vials (or glass tubes) that are not degraded by the ethanol or formalin, with screw caps;

   — monitor levels of ethanol and formalin in order to keep them constant;

   — store vials away from direct sunlight;

   — change the preserving solution of each vial once every five years.

8.3 Biomass determination

Determination of biomass is performed using the preserved material. The animal's surface should be gently dried with filter paper, then weighed using a precision balance (0,001 g).

It is virtually impossible to keep invertebrates alive after their capture in order to measure fresh masses. In most cases, invertebrates are conserved in 70 % (volume fraction) ethanol or 4 % (volume fraction) formalin. The latter is recommended for earthworms that should at least be fixed in formalin before being kept in 70 % ethanol. Preservation always involves a decrease in mass, as body water is extracted by osmotic forces. The amount lost can vary between 15 % and 40 %, depending on the water content of the animal and its physiological state. Since most studies only aim to compare different sites and/or situations, mass loss is not likely to distort the result. If accurate fresh mass data are necessary, it is easy to keep an aliquot of each group and compare the mass, alive and fixed, a few days after fixation.

9 Assessment of results

The following measurement end points can be used for the bioclassification of a soil, including bio-indication or biomonitoring (e.g. anthropogenic stress-like chemicals or land-use changes):

— abundance (number of individuals per area);

— biomass;

— number of species or other taxonomically or ecologically defined groups;

— diversity indices (alpha, beta and gamma diversity).

Firstly, the number of individuals (total number by species or group) is counted and expressed as individuals per sample. Secondly, the total abundance of individuals is multiplied by a factor (16) to obtain the number of individuals per square metre.

Fresh mass measured in the field is the ideal way to calculate biomass. Failing this, the use of blotted mass, after preservation, is acceptable. Other methods are reported in the literature, for example fresh mass after blotting, dry mass at 60 °C overnight, drying to constant mass at higher temperatures, degutted fresh mass, degutted dry mass, fresh mass multiplied by a constant (for assumed water content) and head width (referenced to a calibration curve). However, these have less biological meaning than fresh mass.

10 Test report

The test report shall include at least the following information:

a) a reference to this document, i.e. ISO 23611-5:2024;

b) a full description of the study design and procedures;

c) characterization of the study site (especially soil properties);

d) sampling method;

e) description of the sampling conditions, including date and duration and time of the day of sampling in the field and weather parameters like air temperature and humidity, rain or snow, etc.;

f) details of the extraction procedure of the biological material;

g) values recalculated to 1 m^2 or another standard size, if necessary;

h) a summary of the results obtained;

i) a discussion of the results;

j) all information, including all measured raw data and all problems which have occurred or developed during all phases of the study.

Annex A
(informative)

Background information

Soil macro-invertebrates can also be defined as organisms belonging to taxa of which over 90 % of specimens are visible to the naked eye. Soil macro-invertebrates comprise the following groups: Oligochaeta (Annelida), Gastropoda (snails and slugs), Coleoptera (larvae and adults), Isoptera, Diplopoda, Chilopoda, Hymenoptera, Arachnida, Dyctioptera, Orthoptera, Hemiptera, Dermaptera, Isopoda, Lepidoptera (larvae) and Diptera (larvae). It specifically excludes groups with a relatively small number of specimens visible to the naked eye such as Nematoda (e.g. Mermithidae), Enchytraeidae, Collembola, Acarina, Symphyla, Pauropoda and Diplura. Core taxonomic units should be adopted as standard units for macrofaunal sampling. The choice of 17 main taxa was made during the IBOY Workshop using the MDB (Macrofauna Data Base) containing information about 32 countries and almost 1 000 sampled sites. The 17 taxa, Oligochaeta (order Opisthopora), Coleoptera (larvae and adults), Isoptera, Diplopoda, Chilopoda, Formicidae, Gastropoda, Aranaea, Blattoidea, Orthoptera, Dermaptera, Isopoda, Hemiptera, Lepidoptera larvae, Diptera (larvae and adults) and residues (insects and non-insects), correspond to the most important soil macro-invertebrates in terms of abundance and biomass.

The choice of a 250 mm × 250 mm × 300 mm monolith size is based on extensive, although largely empirical, experience. First used by Zajonc (1956), it has been proposed as a standard for the Tropical Soil Biology and Fertility Program.[1][54] This monolith size is the same for both tropical and temperate soils. The aim was to propose a method that was not excessively time-consuming, but which can provide an accurate assessment of the composition and structure of soil macro-invertebrate communities. The method has been extremely successful and has become a standard used in several hundred sites. Although studies aimed at specific groups, especially termites, earthworms or ants, prefer different sample sizes, the size proposed represents a very good compromise that allows a reasonable number of replicates to be made and the representation of most orders in one single sample. Larger samples are excessively time-consuming and do not allow enough replicates to be made. In most cases, a group of four well-trained persons can sort out 10 samples a day. Unpublished field studies have shown that 15 to 20 samples are necessary in a single site to reduce variance to a reasonably low proportion of mean (< 20 %). However, a comparison of sites with different plant cover or soils and/or which have been subjected to different management options exposes significant differences using as little as five samples, provided adapted statistical treatment (often multivariate analyses) is used.

Annex B
(informative)

Sampling soil macro-fauna using pitfall traps

Assessing the diversity and activity of surface-dwelling fauna can be of paramount importance when interpreting TSBF (Tropical Soil Biology and Fertility) collected data in the scope of soil ecosystem functions and services, when trying to unearth patterns and possible interactions at a larger spatial scale in the complex soil-based networks on terrestrial ecosystems or when aiming at assessing the status of soil biodiversity. A common practice that may complement TSBF method, and that allows estimating soil epigeic macro-fauna diversity and activity in ecological studies, resorts to the use of pitfall traps. This method can be used to obtain quantitative estimates of epigeic fauna relative abundances. The principle behind these traps is that organisms living in soil or having a preference for soil habitat are likely to accidentally fall in open traps set at ground-level while moving on the soil surface. Once set on field, these traps can capture any organisms passing by, at a rate that should be proportional to their activity, thus allowing for an estimate of this parameter as well.

Depending on the average size of targeted organisms, different sizes of pitfall traps can be used; for a survey on soil epigeic macro-fauna in general, plastic cups, with an opening of 8 cm to 10 cm in diameter and 9 cm to 10 cm in height can be used. The pitfall should have a nested-cups design, comprising one cup to be inserted on a pre-dug hole in soil, so that their top (opening) is lined with the bottom of the leaf litter or the soil level, and an inner cup to collect the organisms. The nested-cup design makes the installation of the pitfalls and its collection from the field easiest and reduces the amount of soil and debris that can fall into the pitfall.[20,40] Although bait can be used to amplify the capture rate or to target specific organisms, pitfall traps in ecological studies, aimed at addressing the overall diversity, should be passive traps, so the cups set in a field are usually only filled to half capacity with a preserving solution. A good preserving solution should kill quickly (to prevent escape of organisms and predation), preserve the internal and external organs of trapped organisms and minimize evaporation. The type and dilution of the preserving solution depends on the objectives of the study; but when DNA isolation for further analysis is planned, non-diluted propylene glycol or 96 % ethanol should be used. Propylene glycol is colourless, hygroscopic, resistant to evaporation and non-toxic, and has a low surface tension, when compared to ethylene glycol, which is also widely used.[40,41] Ethanol can be an alternative when trapping time and weather conditions are not prone to high evaporation rates.[61] As an alternative, ethylene glycol (car anti freezing agent) diluted at 50 % with water can also be used. Although some studies have reported ethylene glycol at 50 % as a suitable sampling solution for DNA isolation,[39] the former solutions are usually adopted when a DNA analysis is intended. To prevent pitfall traps from flooding or being filled with falling litter and also to prevent larger non-target organisms (e.g. small mammals and reptiles) from entering the trap, the inner cup surface may be covered with a stone or other natural materials, always leaving enough space between each element to allow easy movement of the epigeic fauna. A roof of plastic with the same diameter of the inner cup can also be used and placed at a height of approximately 3 cm from the opening of the trap.[20] Studies proved that pitfall roofs or rain guards have no negative effects on pitfall catches;[20,23] however, depending on the area the roof, it can catch the attention of free-roaming animals, leading to the destruction of the pitfall. The number of traps to be set in a field and the frequency of sample collection depend on the focus of the study, this being a versatile method that can be applied to several sampling design methods. However, when considering each pitfall trap as an independent experimental unit, only one pitfall should be placed per square meter. For a general survey in temperate climate, pitfall traps should be left for a period of 7 to 14 days in the field (different time intervals may be considered according to different climate zones and when using baited pitfall traps). Sample collection can be done by either removing the trap from the soil and using a lid on the cup to keep its content secured or by percolating the trap content over a disposable absorbent cloth (with the help of a sieve) or a 50 µm net, that should then be tied and placed in a labelled cup with 96 % ethanol for storage to ensure good DNA preservation.[41]

Small parts of the organism's body can be preserved separately for DNA extraction (e.g. legs), leaving the rest of the material available for morphological analysis. The latter should be preserved in 70 % ethanol for long-term storage. Processing of collected samples in laboratory may be similar to that of the TSBF method.

Annex C
(informative)

Monitoring example with pitfall traps[68]

C.1 Study aim

This study was conducted to evaluate the spatial distances between local communities and the intensity of habitat heterogeneity at a given spatial scale. This evaluation aimed to assess, at different scales, the relative importance of spatial and environmental factors that shape edaphic (Collembola) and epigeous (Carabidae) communities.

Although pitfall trap method has been proposed as a sampling method for macro-invertebrates, this method was selected for the study as it also covers specific epigeic mesofauna groups (such as Collembola).

C.2 Study area

Sampling was carried out in a typical agro-forest mosaic - a Mediterranean cork-oak field (*Quercus suber* L.) - located in the consolidated alluvial plain of the Tagus River, in "Companhia das Lezírias" (Alcochete, approximately 20 km east of Lisbon), Portugal (approximately 388 530 N, 088 520 W). The past land-use history and management shaped the Cork-oak agro-forest systems at the landscape scale. Sampling sites comprised four non-identical landscape windows (LW, 1 km² each), chosen along a land-use management gradient, from unmanaged woodland (LW1) to areas subjected to traditional management practices such as forestry (LW2, LW3 and LW4) and pastures (LW3 and LW4). Thereby, while LW1 and LW2 were dominated by closed cork-oak woodlands (less managed forests), in LW3 and LW4 open woodlands and pasture lands predominated (highly managed forests) (Table C.1, see Reference [69] for more details). The characterization of the landscape windows (LW1, LW2, LW3 and LW4) chosen for this assessment is presented in Table C.1. This table also shows the mean values of environmental variables (and SD when applicable) related to the microhabitat of the plot, the management of the patch and the landscape structure within each LW. The indicators used in forestry, pastoral and agricultural variables served as the basis for measuring management intensity, while landscape variables were recorded in agreement with FRAGSTATs' Class metrics for each mainland-use type (Forest – F, Pastures/Grassland – G).

Table C.1 — Landscape windows characterization (LW1, LW2, LW3 and LW4)

		LW1	LW2	LW3	LW4
Main properties	Ecosystem type	Natural woodland	Managed woodland	Agro-forest	Agro-forest
	Management type	None	Logging	Logging/Grazing	Logging/Grazing
	Management intensity	None	Low	Low	Medium
Nº of sampling plots per land-use type	Closed woodland	8	9	3	4
	Open woods	3	3	8	6
	Grass/Shrub/Pastures	5	3	5	6
Microhabitat based on % of vegetation cover ("µhab")	Stone				1,45 (4,10)
	Litter	50,93 (28,16)	38,42 (25,48)	32,68 (28,80)	28,39 (24,20)
	Lichen	7,27 (12,85)	0,49 (1,59)	0,21 (0,54)	1,35 (2,93)
	Moss	3,38 (9,31)	0,70 (0,96)	0,18 (0,29)	0,72 (1,51)
	Herb	40,40 (31,91)	24,92 (20,09)	66,23 (21,77)	77,58 (14,65)
	Low shrub	18,67 (15,56)	22,71 (18,59)	6,80 (11,28)	2,88 (5,53)
	Tall shrub	13,25 (11,28)	18,09 (16,42)	9,83 (12,81)	9,45 (18,73)
	Tree	30,57 (25,79)	16,14 (17,04)	24,11 (27,35)	17,12 (20,15)
Management variables ("man")	Area Cut[a] (km^2)	0,896 9 (0,285 5)	0,917 9 (0,210 9)	0,871 4 (0,326 8)	0,328 1 (0,229 5)
	Area Cork[b] (km^2)	0,896 9 (0,285 5)	1	0,70 (0,254 2)	0,328 1 (0,229 5)
	Time Cork[c] (years)	3	2	3	2
	Grass[d] (km^2)			0,071 4 (0,099 4)	0,353 1 (0,387 9)
	Density[e] (n/km^2)			6 (3)	13 (8)
	Grazing[f] (days)			51,43 (21,79)	39,38 (20,40)
	Ninp[g] (kg/km^2)				250 (447)
	Pinp[h] (kg/km^2)				325 (581)
Landscape metrics ("lan")	F_Area[i] (%)	81,08	95,17	90,03	70,27
	F_NP[j] (nº)	3	2	2	1
	F_AreaMN[k] (km^2)	0,270 3	0,475 9	0,450 2	0,702 7
	F_Gyrate[l] (m)	145,3	237,7	195,3	380,3
	F_Shape[m]	1,34	1,35	1,31	2,29
	F_Circle[n]	0,58	0,53	0,55	0,52
	F_Contig[o]	0,70	0,98	0,65	0,98
	F_Prox[p]	38,13			
	G_Area[i] (%)	16,31	3,95	9,84	29,57
	G_NP[j] (nº)	1	1	1	5
	G_AreaMN[k] (km^2)	0,163 1	0,039 5	0,098 4	0,059 1

[a] Area Cut = area harvested

[b] Area Cork = area with cork production

[c] Time Cork = time since last cork removal

[d] Grass = share of permanent grassland

[e] Density = animal density

[f] Grazing = number of days with grazing herbivores

[g] Ninp = N input (amount of mineral fertilizer)

[h] Pinp = P input (amount of mineral fertilizer)

[i] Area = sum of the areas of all patches of a given patch type

[j] NP = number of patches of a certain patch type

[k] AreaMN = area-weighted mean of each class

[l] Gyrate = a measure of patch extent given by the mean distance between each cell in the patch and the patch centroid

[m] Shape = a simple measure of overall shape complexity of given patch type

[n] Circle = a simple measure of overall patch elongation

[o] Contig = the spatial connectedness, or contiguity, of cells within a grid-cell patch to provide an index of patch boundary configuration and thus patch shape

[p] Prox = the size and proximity of all patches whose edges are within a specified search radius of the focal patch

Table C.1 *(continued)*

		LW1	LW2	LW3	LW4
	G_Gyrate[l] (m)	200,1	116	119,5	88,87
	G_Shape[m]	1,59	1,68	1,24	1,32
	G_Circle[n]	0,77	0,81	0,22	0,59
	G_Contig[o]	0,98	0,95	0,98	0,95
	G_Prox[p]				3,22

[a] Area Cut = area harvested
[b] Area Cork = area with cork production
[c] Time Cork = time since last cork removal
[d] Grass = share of permanent grassland
[e] Density = animal density
[f] Grazing = number of days with grazing herbivores
[g] Ninp = N input (amount of mineral fertilizer)
[h] Pinp = P input (amount of mineral fertilizer)
[i] Area = sum of the areas of all patches of a given patch type
[j] NP = number of patches of a certain patch type
[k] AreaMN = area-weighted mean of each class
[l] Gyrate = a measure of patch extent given by the mean distance between each cell in the patch and the patch centroid
[m] Shape = a simple measure of overall shape complexity of given patch type
[n] Circle = a simple measure of overall patch elongation
[o] Contig = the spatial connectedness, or contiguity, of cells within a grid-cell patch to provide an index of patch boundary configuration and thus patch shape
[p] Prox = the size and proximity of all patches whose edges are within a specified search radius of the focal patch

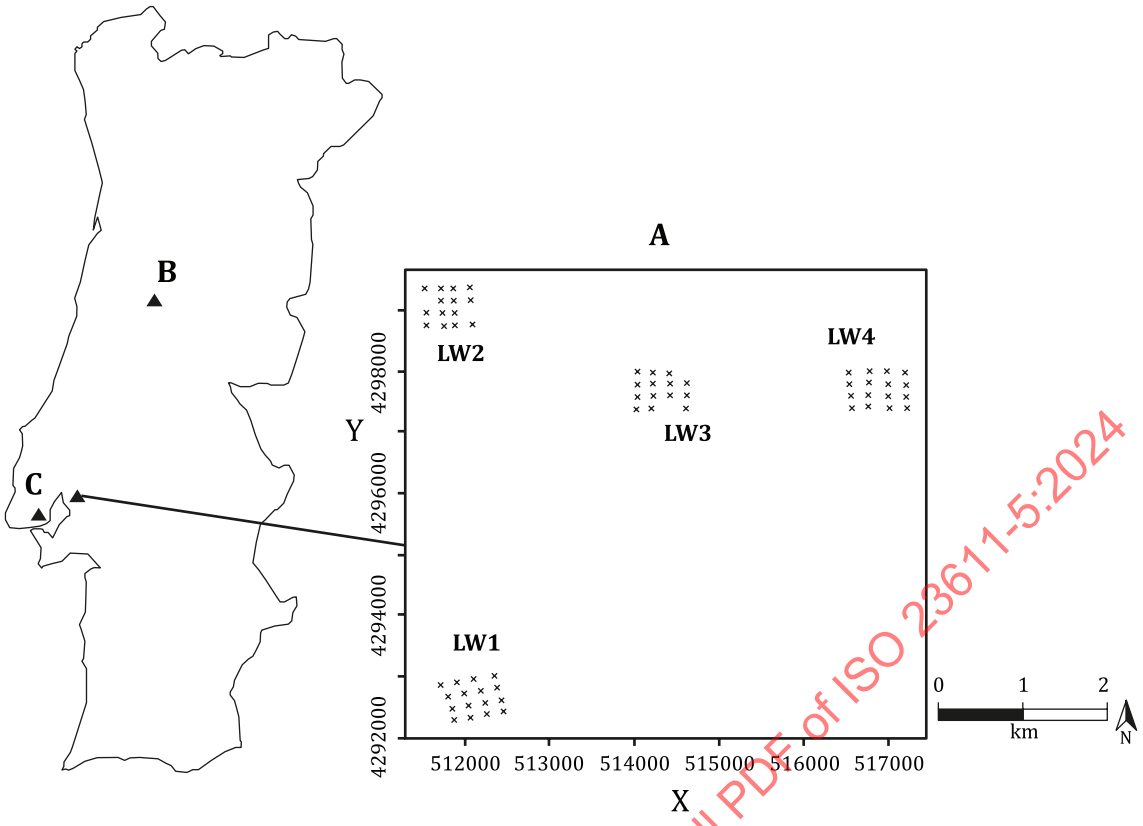
Land-use management intensity was based on indicators used in forestry (Area Cut, Area Cork, Time Cork), grazing variables [Grass, Cuts (number of cuts), Density, Grazing] and agriculture variables (Ninp, Pinp). At the LU scale, landscape variables were recorded based on FRAGSTAT's class metrics for each main land-use type (Forest – F, Pastures/Grassland – G). For each land-use type, the total class area (Area), mean patch area (AreaMN), number of class patches (NP), radius of gyration (Gyrate), shape index (Shape), related circumscribing circle (Circle), contiguity index (Contig), and proximity index (Prox) were recorded.

C.3 Sampling design

For LW1 and LW4 sites, a regular grid with 16 plots was carried out (4 lines of 4 plots). For LW2 and LW3 sites, the grids had 14 plots (4 lines of 4 to 3 plots) as shown in Figure C.1. Each plot was separated by 200 m. At each plot, four unbaited pitfall traps, filled with ethylene glycol 50 % diluted (to preserve the specimens), were used. The pitfall traps were placed at a distance of 5 m from each other. The samples of carabid beetles were taken during spring (May to June) and autumn (September to October). In each sampling period, pitfall traps were collected fortnightly for 45 days. Collected carabid species were identified taxonomically to species level using suitable identification keys.

C.4 Statistical analysis

The differences in community structure of the two taxonomic groups (i.e. collembolan and carabid) within and between LWs were assessed using Bray-Curtis (BC) similarity indices.[15] The significance of differences between pairs of sampling plots was estimated using the analysis of similarities (ANOSIM) with 5 000 data permutations (Bray-Curtis similarity values were log transformed for ANOSIM analysis).



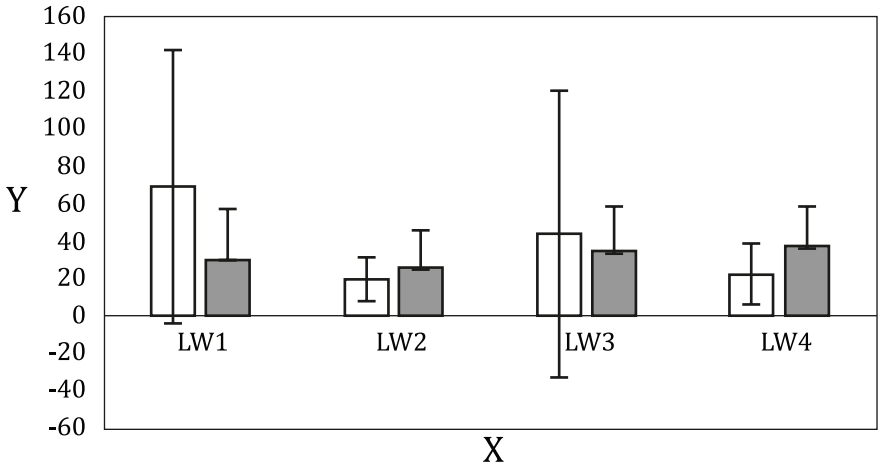
Key

Y	spatial coordinates
X	spatial coordinates
A	Companhia das Lezirias (Alcochete)
B	Coimbra
C	Lisboa
LW1	unmanaged cork-oak woodland
LW2	managed closed woodlands
LW3	managed agro-forest dominated by open woodlands
LW4	managed agro-forest dominated by open woodlands and pastures

Figure C.1 — Sampling plots (spatial coordinates) of the selected landscape windows (LW1 to LW4) along the consolidated alluvial plain of the river Tagus (Alcochete)

C.5 Results

Results can be graphically presented as in Figures C.2 and C.3.



Key

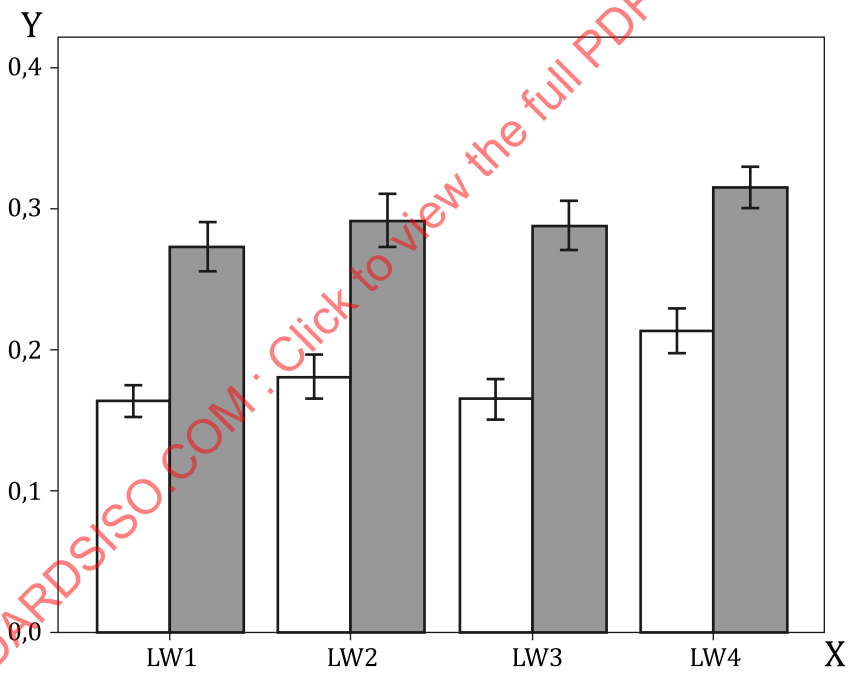
X LW

Y average N values per pitfall trap (±SD)

Collembola

Carabidae

Figure C.2 — Average (and SD) abundance values in different LWs for the two taxonomic groups



Key

X LW

Y Bray-Curtis similarities (±SD)

Collembola

Carabidae

NOTE See Reference [15] for details about Bray-Curtis similarity index.

Figure C.3 — Average (and SE) of Bray-Curtis similarity values between sampling plots within LWs for the two taxonomic groups

Bibliography

[1] Anderson, J.M. and Ingram, J.S.I. Eds., 1993. *Tropical Soil Biology and Fertility – A Handbook of Methods*. 2nd ed. CAB International, Wallingford

[2] Andren, O., Brussaard, L., Clarholm, M. Soil organism influence on ecosystem-level processes – Bypassing the ecological hierarchy? *Applied Soil Ecology*, 1999, **11**, pp. 177-188

[3] Araujo, Y., Luizao, F.J., Barros, E. Effect of earthworm addition on soil nitrogen availability, microbial biomass and litter decomposition in mesocosms. *Biol Fertil Soils*, 2004, **139**, pp. 146-152

[4] Attems, G., 1929. Myriapoda 1: Geophilomorpha. *Das Tierreich*, **52**: 388 pp.

[5] Attems, G., 1930. Myriapoda 2: Scolopendromorpha. *Das Tierreich*, **54**: 308 pp.

[6] Backeljau, T. and De Bryun, L. Notes on Arion hortensis s.l. and A. fasciatus in Denmark. *Apex.*, 1988, **4 (3)**, pp. 41-48

[7] Backeljau, T. and De Bryun, L. On the infrageneric systematics of the genus Arion Férussac, 1819 (Mollusca, Pulmonata). *Bull. Inst. Roy. Sci. Nat. Belg.*, 1990, **60**, pp. 35-68

[8] Bardgett, Richard, D., Cook, Roger. Functional aspects of soil animal diversity in agricultural grasslands. *Applied Soil Ecology*, 1998, **10 (3)**, pp. 263-276

[9] Bengtsson, J., Lundkvist, H., Saetre, P., Sohlenius, B., Solbreck, B. Effects of organic matter removal on the soil food web: Forestry practices meet ecological theory. *Applied Soil Ecology*, 1998, **9**, pp. 137-143

[10] Bernard, F., 1968. Les Fourmis (Hymenoptera Formicidae) d'Europe occidentale et septentrionale. Masson et Cie éditeurs, Paris, 411 pp.

[11] Bird, Simon, Coulson, Robert, N., Crossly, Jr., D. A. Impacts of silvicultural practices on soil and litter arthropod diversity in a Texas pine plantation. *Forest Ecology and Management*, 2000, **131 (1-3)**, pp. 65-80

[12] Bolton, B., 1994. *Identification Guide to the Ant Genera of the World*. Harvard University Press eds, 222 pp.

[13] Bouché, M.B., 1972. *Lombriciens de France. Écologie et Systématique*. Annales de Zoologie et Écologie animale. Numéro hors série. Publications INRA (72-2), 671 pp.

[14] Bouché, M.B., Al-Addan, F. Earthworms, water infiltration and soil stability: Some new assessments. *Soil Biology and Biochemistry*, 1997, **29**, pp. 441-452

[15] Bray, J.R., Curtis, J.D., 1957. An ordination of upland forest communities of southern Wisconsin. *Ecol Monogr* 27: 325-349

[16] Brölemann, H.W. and Ribaut, H., 1912. *Essai d'une monographie des Schendylina (Myriapodes, Géophilomorphes)*. Nouv. Arch. Mus. Hist. Nat., (5) IV, Paris 1912: 53-183, pp. 1-11

[17] Brölemann, H.W., 1930. Eléments d'une faune des Myriapodes de France: Chilopodes. Imprimerie Toulousaine, 405 pp.

[18] Brown, G.G., Barois, I., Lavelle, P. Regulation of soil organic matter dynamics and microbial activity in the drilosphere and the role of interactions with other edaphic functional domains. *European Journal of Soil Biology*, 2000, **36**, pp. 177-198

[19] Brown, George, G., Moreno, Ana, G., Barois, Isabelle, Fragoso, Carlos, Rojas, Patricia, Hernandez, Benito, Patron, Jose C. Soil macrofauna in SE Mexican pastures and the effect of conversion from native to introduced pastures. *Agriculture, Ecosystems & Environment*, 2004, **103 (2)**, pp. 313-327