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Soil quality — Sampling of soil invertebrates —

Part 6: Guidance for the design of sampling programmes with soil invertebrates

Qualité du sol — Prélèvement des invertébrés du sol —

Partie 6: Lignes directrices pour la conception de programmes d'échantillonnage des invertébrés du sol

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 23611-6 was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 4, *Biological methods*.

ISO 23611 consists of the following parts, under the general title *Soil quality — Sampling of soil invertebrates*:

— *Part 1: Hand-sorting and formalin extraction of earthworms*

— *Part 2: Sampling and extraction of micro-arthopods (Collembola and Acarina)*

— *Part 3: Sampling and soil extraction of enchytraeids*

— *Part 4: Sampling extraction and identification of soil-inhabiting nematodes*

— *Part 5: Sampling and extraction of soil macro-invertebrates*

— *Part 6: Guidance for the design of sampling programmes with soil invertebrates*

Introduction

The biodiversity of soil fauna is tremendous. Soil harbours species-rich communities, which regulate ecosystem processes such as organic matter decomposition, nutrient flows or soil fertility in general, References [40], [45]. All terrestrial animal phyla can be found in soils, Reference [16]. In addition to thousands of bacterial and fungal “species“, more than 1 000 species of invertebrates in abundances of up to 1,5 million individuals can be found within a square metre of soil, References [3], [5]. This diversity can only be reliably estimated by investigation of the soil community itself, since other parameters like climate are not or only weakly correlated with species richness, Reference [24].

The composition of this community, as well as the abundance and biomass of the individual species and groups is a valuable source of information, since they integrate various abiotic and biotic effects such as soil properties and conditions, climate, competition or biogeographical influences, Reference [68]. For this reason, the evaluation of the biodiversity of soil invertebrate communities becomes more and more important for the classification and assessment of biological soil quality, Reference [51]. However, this work is only possible if data collection (i.e. sampling of the soil fauna) is carried out according to standardized methods. For this reason, a number of ISO guidelines have been prepared covering the sampling of the most important soil organism groups.

In the individual parts of ISO 23611, the practical work concerning the respective animal group is described in detail. However, (nearly) nothing is said about how to plan the use of such methods or how to evaluate the results. Despite the fact that sampling for any field study can be different depending on the individual purpose, guidance is needed for monitoring studies in a legal context. Such studies can include the following:

- site-specific risk assessment of contaminated land;
- study of potential side effects of anthropogenic impacts (e.g. the application of chemicals or the building of roads);
- the biological classification and assessment of soils in order to determine the biological quality of soils;
- long-term biogeographical monitoring in the context of nature protection or restoration, including global change [e.g. as in the long-term ecological research project (LTER)].

Spatial studies focusing on environmental and ecological questions require a carefully designed strategy for collecting data (References [31], [65]). Before identifying the optimal design, two issues have to be clarified: what is the objective of the study and what is already known about the survey area? Afterwards, one may select one of the well-known design patterns (e.g. grid sampling, random sampling, clustered sampling or random transects) or prepare a study-specific design. In any case, the field sampling design has to be practical, e.g. the volume of soil to be sampled, depending on the size and distribution of the organisms, has to be manageable (i.e. the smaller the individual animal, the smaller the size), and cost effective.

In studies focusing on soil invertebrates, it is not possible to observe the entire population. Therefore, sampling is done only at a limited number of locations. The main reason for using statistical sound sampling schemes is that such sampling guarantees scientific objectivity and avoids forms of bias such as those caused by judgement sampling. This is especially valuable if the objective is to obtain data that are representative for the whole area. At the same time, statistics-based sampling schemes ensure standardized sampling methods over time, i.e., if the same area is to be re-sampled in the future, the results will be comparable.

The rational for this guidance s on the design of field sampling methods for soil invertebrates takes into consideration the guidance provided in ISO 10381-1 describing soil sampling in general.

The design of microbiological studies is already covered by ISO 10381-6, ISO 14240-1 and ISO 14240-2.

Soil quality — Sampling of soil invertebrates —

Part 6:
Guidance for the design of sampling programmes with soil invertebrates

1 Scope

This part of ISO 23611 provides guidance for the design of field studies with soil invertebrates (e.g. for the monitoring of the quality of a soil as a habitat for organisms). Detailed information on the sampling of the most important soil organisms is provided in the other parts of this International Standard (ISO 23611-1 to ISO 23611-5).

This part of ISO 23611 is used for all terrestrial biotopes in which soil invertebrates occur. Basic information on the design of field studies in general is already laid down in ISO 10381-1. This information can vary according to the national requirements or the climatic/regional conditions of the site to be sampled.

NOTE While this part of ISO 23611 aims to be applicable globally for all terrestrial sites that are inhabited by soil invertebrates, the existing information refers mostly to temperate regions. However, the (few) studies from other (tropical and boreal) regions, as well as theoretical considerations, allow the conclusion that the principles laid down in this part of ISO 23611 are generally valid, References [4], [6], [40], [21].

This part of ISO 23611 gives information on site-specific risk assessment of contaminated land, study of potential side effects of anthropogenic impacts (e.g. the application of chemicals or the building of roads), the biological classification and assessment of soils in order to determine the biological quality of soils, and long-term biogeographical monitoring in the context of nature protection or restoration, including global change (e.g. as in long-term ecological research projects).

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 10381-1:2002, *Soil quality — Sampling — Part 1: Guidance on the design of sampling programmes*

ISO 10381-2, *Soil quality — Sampling — Part 2: Guidance on sampling techniques*

ISO 10381-3, *Soil quality — Sampling — Part 3: Guidance on safety*

ISO 10381-4, *Soil quality — Sampling — Part 4: Guidance on the procedure for investigation of natural, near-natural and cultivated sites*

ISO 10381-5, *Soil quality — Sampling — Part 5: Guidance on the procedure for the investigation of urban and industrial sites with regard to soil contamination*

ISO 10381-6, *Soil quality — Sampling — Part 6: Guidance on the collection, handling and storage of soil under aerobic conditions for the assessment of microbiological processes, biomass and diversity in the laboratory*

ISO 10390, *Soil quality — Determination of pH*

ISO 10694, *Soil quality — Determination of organic and total carbon after dry combustion (elementary analysis)*

ISO 11074, *Soil quality — Vocabulary*

ISO 11260, *Soil quality — Determination of effective cation exchange capacity and base saturation level using barium chloride solution*

ISO 11272, *Soil quality — Determination of dry bulk density*

ISO 11274, *Soil quality — Determination of the water-retention characteristic — Laboratory methods*

ISO 11277, *Soil quality — Determination of particle size distribution in mineral soil material — Method by sieving and sedimentation*

ISO 11461, *Soil quality — Determination of soil water content as a volume fraction using coring sleeves — Gravimetric method*

ISO 11465, *Soil quality — Determination of dry matter and water content on a mass basis — Gravimetric method*

ISO 11466, *Soil quality — Extraction of trace elements soluble in aqua regia*

ISO 13878, *Soil quality — Determination of total nitrogen content by dry combustion ("elemental analysis")*

ISO 14869-1, *Soil quality — Dissolution for the determination of total element content — Part 1: Dissolution with hydrofluoric and perchloric acids*

ISO 15709, *Soil quality — Soil water and the unsaturated zone — Definitions, symbols and theory*

ISO 15799, *Soil quality — Guidance on the ecotoxicological characterization of soils and soil materials*

ISO 17616, *Soil quality — Guidance on the choice and evaluation of bioassays for ecotoxicological characterization of soils and soil materials*

ISO 23611-1:2006, *Soil quality — Sampling of soil invertebrates — Part 1: Hand-sorting and formalin extraction of earthworms*

ISO 23611-2, *Soil quality — Sampling of soil invertebrates — Part 2: Sampling and extraction of micro-arthropods (Collembola and Acarina)*

ISO 23611-3, *Soil quality — Sampling of soil invertebrates — Part 3: Sampling and soil extraction of enchytraeids*

ISO 23611-4, *Soil quality — Sampling of soil invertebrates — Part 4: Sampling, extraction and identification of free-living stages of nematodes*

ISO 23611-5, *Soil quality — Sampling of soil invertebrates — Part 5: Sampling and extraction of soil macro-invertebrates*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 11074 and the following apply.

3.1 Soil biology

3.1.1
biodiversity
variability among living organisms on the earth, including the variability within and between species, and within and between ecosystems

NOTE Also often used as the number and variety of organisms found within a specified geographic region.

3.1.2
community
association of organisms, belonging to different species, families, etc. living at the same time at the same place, i.e. the living portion of an ecosystem

See Reference [42].

3.1.3
invertebrate
term embracing all organisms except the chordates and microflora

NOTE This is not a taxonomic term.

3.1.4
microfauna, mesofauna and macrofauna
way of classifying the soil fauna according to the size (length, diameter) of the individual animals

See Reference [66].

EXAMPLE Important examples of the microfauna are protozoans and nematodes, for the mesofauna collembolans, mites and enchytraeids, and for the macrofauna earthworms and snails.

3.1.5
taxocoenosis
total number of species belonging to the same higher taxonomic unit (e.g. family, order) within a community

3.2 Soil protection

3.2.1
soil quality
capacity of a specific kind of soil to function, within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and habitation

See References [16], [30].

NOTE In more recent definitions, the natural functions of soil are specifically listed: soil as a habitat for organisms, as part of natural systems (in particular nutrient cycles) and for decomposition, retention and filtration, Reference [6].

3.2.2
habitat
sum of the environment of a particular species or community (e.g. in terms of soil properties, land use, climate)

3.2.3
habitat function
ability of soils/soil materials to serve as a habitat for microorganisms, plants, and soil-living animals, and support their interactions (community or biocenosis)

3.2.4
contamination
substance(s) or agent(s) present in the soil as a result of human activity

NOTE There is no assumption in this definition that harm results from the presence of the contaminant.

3.2.5
pollutant
substances which, due to their properties, amount or concentration, cause impacts on soil functions or soil use

3.2.6
reference soil
uncontaminated soil with comparable pedological properties to the soil being studied except that it is free of contamination

3.3 Methods

3.3.1
Geographical Information Systems
GIS
in the strictest sense, a computer system capable of assembling, storing, manipulating, and displaying geographically referenced information, i.e. data identified according to their locations

NOTE Practitioners also regard the total GIS as including operating personnel and the data that go into the system (US. Geological Survey, 2006).

3.3.2
site-specific assessment
evaluation of the quality of a specific-site by using chemical, biological or other methods

3.3.3
environmental risk assessment
process of identifying and quantifying risk (probability that an effect occurs) to non-human organisms and determining the acceptability of these risks

3.3.4
soil function
property of (specific) soils, often used in legal documents

NOTE Usually natural soil functions (e.g. the soil as a habitat for organisms) and anthropogenic soil functions (e.g. soil as a substrate for crop production) are distinguished.

3.3.5
soil organism function
activity provided by individual species or, more often, by interaction of several species or the whole soil community, e.g. nitrogen fixation or organic-matter breakdown

4 Principle

4.1 General

The design of field studies for the investigation of soil invertebrates differs significantly depending on the respective aim. However, in all cases, it is necessary to take samples since the site and biological populations to be studied are usually too large to be studied in total. In addition, most soil invertebrates live hidden within the soil and/or are too small to be studied directly. The samples collected should be as representative as possible of the site to be characterized but destruction should be kept at a minimum. In addition, the occurrence of material not naturally belonging to the study site (e.g. waste or chemicals) can cause problems when taking samples in multiphase systems such as soils, which contains water, gases, mineral solids and biological material.

The study design (e.g. the position and density of sampling points, time of sampling, and the sampling method) depends mainly on the objectives of the study and on the amount and quality of information already available from the study site (e.g. historical data, personal experience). The design also depends on whether information is needed as an average value (sampling for the spatial mean, e.g. the average number of nematodes) or as a spatial distribution (e.g. sampling for a map showing nematode abundances in relation to soil properties). In addition, the sheer size and the heterogeneity of soil properties, as well as those of the organisms to be sampled shall be taken into consideration. In any case, a list of measurement end points should be compiled for the respective organism group(s) and the main limitations of the sampling method(s) shall also be known. The latter refers mainly to the high natural variability of invertebrate data. The normal statistical tests used by those who take composite samples (microflora, soil properties) or many samples (soil properties) which can be processed more or less automatically, cannot be applied here.

Some consideration should also be given to the degree of detail and precision that is required and also the manner in which the results are to be expressed (e.g. maximum and minimum values in a table, graphical presentations or maps). Appropriate statistical methods for the evaluation of area-related data (including the use of GIS methods) shall be identified as well. It can often be necessary to carry out an exploratory sampling programme before the final study design can be defined in detail. The main points on which decisions shall be made are listed in 4.2, reflecting the logical order of how to proceed a study.

NOTE This clause was written in close consideration with ISO 10381-1.

4.2 Question to be answered when planning a field study

The objective of a study can be established by the following questions:

— Why is such a study going to be performed?

— What information is necessary to answer the questions asked and how can this information be clearly presented?

— Which approach is used for the interpretation of the results?

— How can the study outcome be tailored to the needs of the study sponsor (or stakeholder)?

The preliminary information can be defined by the following questions:

— What is already known about present and historical (especially land-use, management) site and soil characteristics?

— What information is missing? Can it be made available?

— Who is to be contacted for certain (e.g. historical) sources?

— Are there any legal problems such as entering the sites?

— Shall other than biological parameters be measured at the same site and time, i.e. are (negative) interactions of the various sampling programmes to be expected?

— Has the site been visited already?

The strategy of a study can be developed by the following questions:

— How are the delineations in time and space of the area(s) to be investigated determined?

— Which organism groups and measurement end points are appropriate to reach the study objective?

— Which sampling patterns, sampling points, sampling times, depths of sampling should be used?

— Can methods specified in International Standards be employed for all activities?

The decision on sampling and analysis can be made by answering the following questions:

— Can the sampling be done according to the respective International Standard or is there any deviation?

— How is the communication with the personnel responsible for sample presentation and analysis coordinated?

— Which statistical evaluation methods are being employed?

— Does sampling correspond to later data analyses?

— Is it possible to address the right taxonomic level when studying the biological material?

— How is the documentation organized?

The following questions on safety should be answered:

— Are all necessary safety precautions at that site considered?

— Is information concerning landowners, local authorities etc. secured?

— Are the requirements of ISO 10381-3, covering guidance on safety in sampling programmes, as well as those safety issues listed in other parts of this International Standard (ISO 23611-1 to ISO 23611-5 fulfilled?

The following questions on the sampling report should be answered:

— Is there any deviation from the basic content of a study report as specified in this part of ISO 23611?

— Is additional information required?

— How is it ensured that any later deviation from this part of ISO 23611 or the study plan is documented and distributed?

Answers to these questions are given in Clauses 5 to 8.

5 Objectives of sampling

5.1 General

Biological soil investigations address a number of different questions related to the status of invertebrates living in or on the soil (including many different species belonging to different trophic, taxonomic, physiological or functional groups and size classes), often after or under some kind of anthropogenic impact. In the case of ecotoxicological questions, usually laboratory tests are used to study the effects of the impact (e.g. chemicals added to the soil) on invertebrates and thus on the soil quality in general. Such methods are presented in ISO 15799, while the assessment of the test results is given in ISO 17616. Further guidance on sampling, collection, handling and preparation of contaminated soil for biological (i.e. ecotoxicological) testing has currently been prepared by Reference [21]. This is particularly important for the identification and characterization of field reference soils which are necessary for the determination of biological reference values. Examples are provided in Annex A (case studies).

5.2 General remarks

As stated in the Introduction, the principal objectives of sampling soil invertebrates can be distinguished as follows:

— the performance of the site-specific characterization and assessment of contaminated land;

— the study of potential side effects of anthropogenic impacts (e.g. the application of chemicals or the building of roads);

— the biological classification and assessment of soils in order to determine the biological quality of soils;

— long-term biogeographical monitoring in the context of nature protection or restoration, including global change (e.g. as in the long-term ecological research project (LTER)).

To a different degree, all four objectives include the determination of biological reference (or base-line) values, meaning that it shall be clarified which community of soil organisms occurs in a specific soil assuming that there is no anthropogenic impact. Since this precondition is, in many if not all soils, not fulfilled any more, such a “normal” state shall be defined, e.g. by sampling of reference soils. These soils have been selected based on criteria like being representative for certain regions or land-use forms or lack of contamination, Reference [14].

The use of the soil and site are of varying importance depending on the primary objective of an investigation. The results obtained from sampling can indicate a need for further investigation, e.g. detected contamination can indicate a need for identification and assessment of potential hazards and risks. However, assessment of such hazards or risks is not covered by this part of ISO 23611. In addition, capture-recapture methods – while often used in ecology for terrestrial above-ground invertebrates (e.g. spiders, Reference [26]) are rarely used in general monitoring schemes and thus will not be covered in this part of ISO 23611.

Often soil invertebrates are a part of an entire monitoring effort that includes other biological (mainly microbial), as well as pedological, climatic and possibly also agricultural parameters. If such monitoring programmes are performed at regular intervals, permanent sampling sites shall be set up. In such a case, additional efforts are mandatory in order to secure an effective exchange of information. Sampling is usually carried out within the main rooting zone (rarely at greater depths since most soil invertebrates live within the uppermost 30 cm of the soil). Soil horizons or layers may or may not be separately sampled (samples shall be labelled accordingly).

To adequately support legal or regulatory action, particular attention should be paid to all aspects of quality assurance. The guidance given in ISO 10381-5 is particularly relevant. After clarifying the most important pre-conditions, the four groups of main objectives as given above are briefly presented in the following subclauses. However, it should be kept in mind that, in reality, one specific study can fit into more than one of these groups.

5.3 Pre-conditions

Before designing a field study with soil invertebrates, it is highly recommended to characterize the respective area pedologically, Reference [43]. Depending on the principal objectives, it is usually necessary to determine for the body of soil or part thereof

— the nature, concentrations and distribution of naturally occurring substances,

— the nature, concentrations and distribution of contaminants,

— the physical and chemical properties and variations,

— the anthropogenic impact at that site, in particular the land use (including vegetation cover).

It is often necessary to take into account changes in the above-mentioned variables with time and space (vertically, horizontally), caused by either natural (e.g. climatic) or anthropogenic activities.

In addition, pH, particle size distribution, C/N ratio, organic matter and organic carbon content, total nitrogen, cation exchange capacity and water holding capacity of the soil should be measured in accordance with ISO 10390, ISO 10694, ISO 11260, ISO 11272, ISO 11274, ISO 11277, ISO 13878, ISO 11461, ISO 11465, ISO 15709, ISO 17616.

5.4 The performance of the site-specific assessment of contaminated land

When land is contaminated with chemicals and other substances that are potentially acting as pollutants to the environment, it can be necessary to carry out an investigation as a part of a hazard and/or risk assessment. This includes to determine the nature and extent of contamination, to identify hazards associated with the contamination, to identify potential targets and routes of exposure, and to evaluate the environmental risks relating to the current and future use of the site and neighbouring land. A sampling programme for risk assessment can also comply with legal or regulatory requirements and careful attention to sample integrity is recommended. An extensive overview of the benefits and limitations of biological parameters as a component of contaminated land assessment is given in Reference [21].

5.5 The study of potential side effects of anthropogenic impacts

Sampling can be required following an anthropogenic effect such as the input of undesirable material (mainly chemicals) which can be from a point source or from a diffuse source. Another example can be the building of roads. The study design needs again to be developed on a site-specific basis. Sampling can also be required to establish base-line conditions prior to an activity, which might affect the composition or quality of soil.

NOTE Such base-line sampling can also be performed as part of a biological soil classification and assessment (see 5.4).

5.6 The biological classification and assessment of soils in order to determine the biological quality of soils

This is typically carried out at (irregular) time intervals to determine the biological quality of a soil for a particular purpose (e.g. as part of a large-scale screening programme or in the context of a local planning activity). While it has rarely been done so far in terrestrial habitats (except with plants), the information gained here can be used for the preparation of biological soil maps, Reference [8].

NOTE The study of the biological soil quality can also be used for the determination of “base-line conditions” in the context of the assessment of anthropogenic impacts (see 5.3) or of long-term changes such as global warming (5.7).

5.7 Biogeographical monitoring in nature protection or restauration

Finally, the information gained in sampling programmes extends the knowledge on the biogeography of soil organisms, which is necessary in the context of nature protection and conservation, in particular concerning long-term changes like global warming. So far, only few soil invertebrates (mainly beetles or other insects which in their larval stage live in the soil) have been put on the Red List of endangered species. Also there is little proof that such species have been eradicated in modern times. However, in both cases, this fact is mainly caused by the poor level of knowledge on these species; many species can have died out without notice. Sampling programmes can also determine whether soil-biological assemblages (site-specifically) expected in a region become established during nature restoration or after remediation measures (control of success).

6 Samples and sampling points

6.1 General

The selection, location and preparation of the sampling points depend on the objectives of the investigation, the preliminary information available and the on-site conditions. Soil properties, the occurrence of organisms and contamination vary continuously in space; the values at locations close together are more similar than those farther apart and this spatial dependence can be described by the use of geostatistics, Reference [43]. Geostatistics are used in the development of sampling strategies and are also used to analyse the data generated from the soil sampled, Reference [41]. In this clause which closely follows the terminology used in ISO 10381-1:2002, Annex C, several (standard) options and issues to be considered are given.

6.2 Sampling patterns

Sampling patterns are based on the estimation of the distribution of the soil invertebrates in the area to be sampled. Several distribution patterns can be distinguished (of course with intermediate types):

— no specific distribution (i.e. random),

— homogenous distribution (probably very rare),

— clumped distribution,

— distribution varying according to an underlying gradient (linear or concentric).

Sampling design should be adjusted to the (theoretically expected) distribution pattern or observable local conditions which make some patterns more probable. If the area to be sampled shows differences in important properties, such as land use, soil conditions, geomorphology, vegetation patterns, the site should be subdivided according to these differences and separate samples should be taken from “homogenous” sub-areas (stratified sampling).

In agricultural or forestry sampling, a small number of convenient sampling patterns are established in order to obtain information from larger areas. Examples of such patterns are briefly described in the following (for details see ISO 10381-4):

— Systematic patterns (irregular sampling):

  — Assuming a relatively homogenous distribution, such sampling can be performed using patterns resembling an “N”, “S”, “W” or “X”. In particular the diagonal sampling in form of an “X” is popular, but one shall be aware that a serious bias towards the central area is obvious in this case. Traversing the area in a “zig-zag pattern” is another way of applying a non-systematic pattern.

— For the purpose of permanently monitored areas, the diagonal X pattern was modified in a way that an area of about 1 000 m^2 is divided into four squares of 250 m^2 each. In each of these four squares, 18 samples are taken following an X pattern. By rotating the X, the area can be sampled eight times.

— Circular grids:

— This rarely used pattern is performed when studying the influence of a regional emitting source (e.g. precipitation from industrial plants). Sampling is carried out at the section of concentric circles and the lines of the eight main points of the compass.

— Systematic sampling (regular grids):

— Samples are taken in the centre of a number of squares covering the entire area of interest (sampling is also possible at the intersection of grid lines). Grid dimensions depend on how much detail is required.

— Random sampling:

— Selection of sampling points by using a suitable randomization programme is easy, but has the disadvantage of irregular coverage and makes interpolation between sampling points difficult. In order to minimize this problem, sometimes a stratified randomized sampling is performed. Hereby, the entire area is divided into a number of grid cells and a given number of randomly distributed sampling points are chosen in each square. Finally, an unaligned random sampling on a regular grid, meaning that only one of the two coordinates of each sampling point in the regular grid is chosen at random.

— Systematic sampling on a non-rectangular grid:

— In the case of an equilateral triangular grid, each grid point is neighboured by three grid points at a unique distance.

6.3 Selecting and identifying the sampling location

The selection of sampling locations depends upon the study objectives, preliminary information, and on-site conditions, Reference [43]. Examples of on-site conditions that need to be considered when designing a sampling strategy include local topography, climatic conditions, vegetation cover (especially trees), soil type and/or soil physicochemical characteristics and, if appropriate, the location of a contaminant source (point or non-point) or the direction of contamination, Reference [21].

Identification of sampling points is not always necessary. However, where samples are taken at pre-defined points, their accurate location and identification is important for three reasons:

— to enable actual sampling locations to be revisited if necessary (note that invertebrate sampling is usually destructive; i.e. exact repetition is not possible);

— to avoid sample disturbance when taking further samples;

— to enable accurate plotting of data in relation to site features (e.g. soil properties or the concentration of contaminants);

— to prepare maps or for modelling studies.

Both sketch maps and photographs (including a scale and a direction marker) should be prepared in the field. Sampling locations should be determined with an appropriate degree of accuracy. The use of GPS (Global Positioning System) is highly recommended. The location of sampling points should be marked before sampling begins, using poles or markers of colour sprays.

6.4 Preparation of the sampling site

Depending on the objective of the investigation, a sampling pattern is chosen at the design stage and is then applied in the field. Afterwards, preparation of the site includes, for example: establishment of safety measures or installation of markers for the exact sampling points. This work becomes very time consuming if it is not possible to take a sample at the planned location due to a variety of reasons (e.g. trees, rocks, or access difficulties). Contingency plans for dealing with such situations should be made in advance (ad hoc decisions in the field can lead to a bias). The action taken depends on the circumstances: the point may be ignored, or a nearby substitute location (e.g. within 10 % of grid spacing away from the original location) can be chosen. In every case where a sampling point shall be re-located, this and the reason for relocation shall be clearly indicated in the report.

6.5 Further general advice on sampling performance

Details of the sampling performance are given in ISO 23611-1 to ISO 23611-5. However, some general advice can be given in the following:

Mountain regions or hilly areas with pronounced slopes require special consideration before starting sampling. No general recommendation can be given on the depths at which samples should be taken. This depends on the objectives of the study and the respective organism groups to be sampled. The same is true for the timing and frequency of sampling. In addition, the sample quantity varies considerably according to the method used (approximately 100 g to 5 kg, see Reference [21] for a general overview). In most sampling guidelines for agricultural (including microbial) investigations, composite samples are recommended, while for the study of soil invertebrates, single samples are usually taken. Other information relevant to conduct the sampling (e.g. sample containers, transport and storage of samples and preservation of animals) are given in ISO 23611-1 to ISO 23611-5 and, in particular for contaminated soils, in Reference [21]. In any case, each sample shall be clearly and unmistakably marked and their location in the field noted. Preferably, labelling should be done both within and outside of the containers.

Finally, if the sampling programme is performed for legal purposes, all raw data gained should be collected in accordance with local quality assurance/quality control programmes (see Clause 10), meaning, for example, that in order to facilitate data documentation, specific forms (e.g. chain-of-custody forms used during transport of samples from the field to the laboratory) are used.

7 Practical considerations for the biological sampling of soils

7.1 General

Specific attention is drawn to the requirements for sampling personnel and to the safety precautions necessary in various situations (see ISO 10381-3).

7.2 Formal preparations

All important information on the sampling programme should be laid down in a sampling plan which provides specific guidance for the methods and strategies for data and sample collection. The sampling plan should – at least – contain a description of the study objective, a characterization of the site, a description of the experimental design, the sampling procedure, and the end points to be measured. Already at this stage, besides personnel experienced in soil ecology, experts from other areas, like site managers, statisticians or soil scientists, should be consulted.

In any case, it is recommended to prepare a sampling check list, as well as a field observation notes check list, Reference [21]. The former contains the sampling plan, as well as detailed information about the site location, the sampling locations, the sampling devices and procedures, documentation material and devices, packaging and storage material and general field equipment including health and safety equipment. The latter includes prepared forms etc. on soil sampling, sample handling, field measurements, on-site observations and storage and transportation forms.

7.3 Requirements on sampling personnel and safety precautions

The design of the sampling programme needs to take into account the sampling experience of the personnel and their ability to contribute to the design of the sampling programme relative to the investigation needs (see ISO 10381-1). Sampling should preferably be carried out by an experienced scientist or another appropriately qualified person. The sampler should have a knowledge of the applied techniques and tools (see ISO 10381-2). Sampling depends on team work. Responsibilities should be made clear at all stages of the sampling campaign, both in the field and at the office. Staff working on the site should have detailed knowledge about necessary safety precautions, particularly when sampling contaminated sites (see ISO 10381-3).

7.4 Preliminary survey

7.4.1 General

A preliminary survey should be carried out prior to any sampling programme, although the effort devoted to it depends on the objective of the investigation. It should always comprise a desk-top study (see 7.4.2) and a site visit. In addition, a limited amount of sampling may be carried out (see 7.4.3). The principal objectives of the preliminary study are to gain knowledge about the present condition of the site, and of past activities on the site and adjacent land which can have affected it in order to enable the sampling programme to be designed to be both technically effective and cost effective. In addition, measures shall be identified that protect the health and safety of the investigating personnel and of the environment.

Other information relevant to conduct the sampling programme may also be gathered (e.g. means of access, availability of power). It shall also be ensured that all necessary permits for carrying out the preliminary survey (e.g. for site access) have been obtained. Such information is of particular relevance when investigations for risk assessment shall be carried out.

7.4.2 Desk-top study

This step includes collection of relevant information of the site, e.g. references to the location, infrastructure, utilization or historical information. Possible sources of this information are publications, maps, aerial photographs and satellite imagery from, for example, land surveyor's offices, geological surveys, industrial inspection boards, mining companies, regional archives, or agriculture and forestry authorities. Particularly important is information on the physical and chemical properties and the possible spatial distribution of the soil parameter(s) relevant for the investigation. In addition, ecological information (such as geological, hydrogeological, botanical, and pedological classification of the site) shall be collected. In some cases it can even be possible to classify the site to a certain ecoregion or ecozone.

7.4.3 Visiting the site

A visit of the site should preferably be done in conjunction with the desk-top study. Depending on the local situation and the objectives of the study, an experienced person should be chosen for this task. Such a visit gives a first impression about the correlation between existing maps and site reality and provides much additional information in a comparatively short time. Samples are not often taken during preliminary surveys. If they are, it is usually for obtaining an overview of the type of soil in order to choose the right equipment for later activities (see also ISO 10381-4 to ISO 10381-6). For example, screening soil samples by means of a Pürckhauer corer[1)] can be taken to become acquainted with the soil profile and the heterogeneity of soil properties. An inventory of plant indicator species as part of a vegetation survey can also be helpful. The output of the preliminary survey can include a first or additional map of the site, as well as a compilation of all available information in the form of a report.

Maps can be prepared by following either of two methods:

— The sampling grid is plotted onto an existing map. The sampling units are coloured according to the result of the respective sampling.

— Cartography programmes can be used that interpolate between adjacent sampling points. It is important to ascertain that the topography of the survey region and the density of sampling units allow the use of such algorithms.

1) Pürckhauer corer is an example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

7.5 Main study

As already mentioned, the aims of sampling soil invertebrates can be very different, leading to a high number and diversity of design options. Since it is by far not possible to cover these options here, in Clause 8 representative examples together with references are given for the most often used purposes.

As stated already in Clause 4, soil organism communities are characterized by high (but differing according to the individual group) variability of populations in time and space. While options for handling the spatial variability have already been discussed in Clause 6, addressing variability in time is difficult on a general level. Mainly it depends on the objective of the study (meaning that in many cases just one sampling is possible or necessary) but as a general rule it can be stated that – if possible – at least two samplings in different years should be performed.

From an assessment point of view, again different designs and statistical methods shall be applied depending on the respective objective. However, some very general recommendations can be given:

Site-specific risk assessment of contaminated land:

— e.g. after single events like an oil-spill: BACI-Design (before – after - control – impact), followed by ANOVA (one way analysis of variance) or GLM (general linear model) or, in the case of a point source of impact, gradient design followed by regression analysis or CCA (canonical correspondence analysis)

Study of potential side effects of anthropogenic impacts (e.g. the application of chemicals or the building of roads):

— e.g. usually block or factorial design, followed by ANOVA (one way analysis of variance) or GLM (general linear model)

The biological classification and assessment of soils in order to determine the biological quality of soils or long-term biogeographical monitoring in the context of nature protection or restoration:

— e.g. representative sampling with determination of means and error estimation.

8 Design options for sampling soil invertebrates

8.1 Introduction

Soil invertebrates cover a wide spectrum of life-form and life-history types, which inhabit a multitude of soil habitats and niches that themselves can be spatio-temporally variable, Reference [40]. For this reason, no one sampling method can assess the entire soil fauna and different standardized methods are necessary for evaluating the various taxonomic, functional or life-history soil-faunal groups, Reference [20].

The assessment of soil fauna often draws upon their significance as reaction- or impact-indicators, whereby changes in life-history patterns or the abundances of single species or entire communities indicate changes in soil biology, chemistry or physics, Reference [59]. This can take place through direct indication, which often utilizes single test or monitoring species to assess the impact of known contaminants, or through indirect indication, whereby single species or entire communities are used to indicate habitat conditions or their changes, References [10], [60], [11], [13]. Especially, indirect indication usually finds an application in environmental soil protection, as well as in the biological classification and assessment of soils. In this regard, entire communities of a higher-level taxon (multi-species assemblage or taxocoenosis) or guilds (functionally defined multi-taxon groups) are commonly assessed due to the integrative ability of different species to respond at different degrees to a similar impact or differentially to different habitat factors, References [32], [69], [44], [30]. This increases the amount of information available and allows an integrative evaluation, but also increases the complexity of assessment.

For a community-level biological assessment or characterization of soils, the investigated taxon should ideally fulfil important prerequisites (References [18], [19], [29]):

— efficient and cost-effective registration (collection) of the individuals of the taxon through standardized methods,

— medium to high densities and species richness,

— unambiguous ascertainability of active stages,

— unambiguous reference to a closely defined habitat due to low radius of activity,

— rapid response ability to environmental changes due to an advantageous voltinism (one to many generations per year),

— good characterization of life forms, nutritional demands, autecology, etc.,

— sensitivity to habitat changes, disturbance and contaminants.

Furthermore, for community-level use of soil fauna as reaction indicators in environmental soil-protection or biological classification and assessment of soils, field sampling shall allow the collected data to incorporate certain requirements:

— To allow a thorough biological soil assessment, the species assemblages (taxocoenosis or functional group) shall be sampled as representatively as possible, avoiding omission of important species.

— Since the most abundant soil mesofauna species are often eurytopic and euryoecous species, Reference [74], thus not allowing sufficient indicative site differentiation, not only these species, but especially also secondary or corollary species, shall be included in the sampled communities (for the discussion of specific problems when using mesofauna, see Reference [37]).

— The spatial distribution of the individual samples within a plot shall take into account the patchy distribution of the communities and species (see below).

— Despite the above requirements, sampling shall remain cost effective.

Sampling is complicated by the strongly aggregated occurrence of the soil mesofauna, causing a large spatial and temporal heterogeneity and thus non-normal distribution including many gaps and patches, References [35], [67], [22], [25], [2]. For example, on average of 25 to 35 soil cores (each 76 cm^2) are necessary to obtain representative data on the taxonomic diversity of large soil invertebrates of Russian forest sites, Reference [58]. However, there are indications that in some regions no such detailed numbers can be given yet: for example, when sampling micro-arthropod communities in Amazonian savannahs, it seems that as more plots were sampled, more species were recorded, Reference [57]. This heterogeneous horizontal distribution of soil organisms creates high demands on the sampling design to achieve the above requirements, mostly in terms of sampling intensity (number of samples). Most studies involve a compromise between sampling intensity (data precision) and cost effectiveness. Differences in sampling design, however, can cause methods-based discrepancies within the data, thereby limiting the value of temporal (within site) or spatial (between site) comparisons. Thus, the use of standardized methods for data collection is as important as that for data analysis or evaluation methods.

8.2 Description of possible sampling strategies

Two main categories of sampling strategies can be distinguished: deterministic and probabilistic ones (Table 1). In deterministic (often called judgmental) sampling, sample locations are selected based on expert knowledge of the site or on professional judgment. With probabilistic sampling strategies, sampling locations are selected by applying statistical theory and the application of random chance to location selection. Judgmental sampling strategies can be less expensive and more efficient then probabilistic strategies, however they depend heavily on expert knowledge, there is no way to measure the precision of the data, and the data cannot be interpreted statistically. In contrast, probabilistic sampling strategies are more difficult to implement (often requiring the assistance of a statistician), but, when used, the uncertainty in the data can be measured and quantitative conclusions can be made.

When using the examples in Table 1 one shall be aware that the proposed strategies can be used on different scales, i.e. at least on the plot scale (e.g. 10 m × 10 m), on the site scale (e.g. a hectare) or on the landscape (e.g. several square km). In addition, some of the listed strategies can be used in combination (see some examples at the end of Table 1).

When choosing the adequate strategy (either deterministic or probabilistic), the selected sampling points shall be identified and marked *in situ* to facilitate field work using GPS (Global Positioning System) or similar methods. In fact, statistical methods shall be used on any scale, i.e. for the selection of sampling points on a sample plot but also for the identification of sampling sites. Once the results of sampling are obtained, they should be evaluated regarding their representativity for the plot, site or landscape under investigation using geostatistical methods (for further advice see Reference [21] where this issue is discussed in detail).

Note that the different sampling strategies described in Table 1 were originally compiled for one of the four main categories of sampling invertebrates in the field, i.e. the performance of site-specific assessments of contaminated land. However, with slight adaptations, these strategies are valid for any kind of such studies. Thus, the toxicity of chemicals is often used as an example, but in fact any anthropogenic stressor or environmental factor such as soil pH or climate gradient can be used instead.

Table 1 — Summary of the most commonly used sampling strategies and selection criteria for their use with different study objectives (adapted from Reference [21]). Examples for (almost) all strategies are given in Annex A (see reference under “Comments” in Table 1.

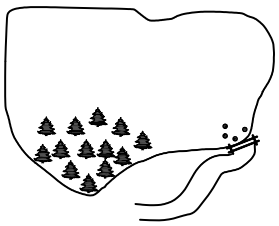
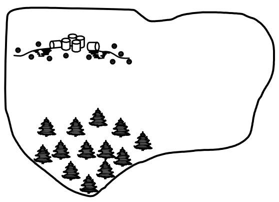
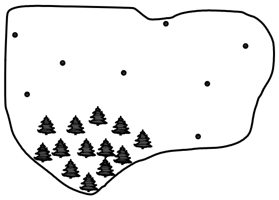
Strategy	**Description**	**Selection criteria**	**Study objective(s)**	**Comments** **(see Annex A)**
Deterministic				
Ad hoc sampling	Selection of locations has no predetermined pattern 	Access to site difficult; limited time	Preliminary investigation; limited access or time	Should be conducted by an experienced investigator; not recommended No specific case study
Judgmental	Selection of locations based on professional judgment 	Constrained budget or schedule; reliable site information exists; site relatively small; small samples to collect	Site screening; emergency sampling; hotspot sampling	Level of sampling uncertainty cannot be measured; statistical inferences about data limited A.2.1 (plot scale), A.3.2 (plot level)
Probabilistic				
Simple random sampling	Sampling locations selected so that each sample has the same chance of being taken from any given location 	Site is relatively uniform	Site screening; e.g. average toxicity at a site; compare site to reference; remediation evaluation	Simplest but judged as less efficient than all other strategies; basis for many other strategies; protects against bias; sample locations may not be spread evenly over site No case study

Table 1 *(continued)*

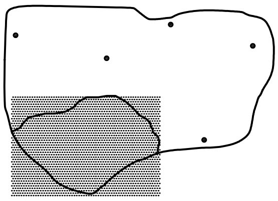
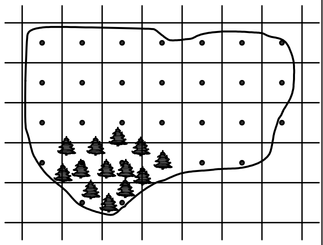
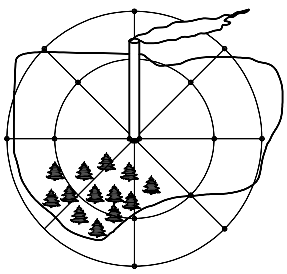
Strategy	**Description**	**Selection criteria**	**Study objective(s)**	**Comments** **(see Annex A)**
Stratified sampling	Samples collected from within selected, discrete areas (strata) on site (e.g. soil type, topography, vegetative cover). Samples can be collected within strata randomly or systematically 	Strata on site are well defined; variability within strata expected to be lower than variability among strata	Average toxicity at a site; e.g. sample along concentration gradient (concentrations can be strata); compare site to reference; determine influence of soil/habitat type on toxicity; remediation evaluation	Judged more efficient and precise than the random sampling; samples more representative; samples provide more information especially if soil variables are correlated with toxicity and/or bioavailability; information can be obtained on risk to ecoreceptors according to habitat A.3.1.1, A.3.1.2, A.3.2, A.4.2, A.4.3, A.5.1
Systematic grid sampling	Samples collected in a regular grid pattern over entire site; starting location and grid orientation randomly chosen; grids can be square, triangular, circular etc.  	No information about site; identifying toxicity on site; identify pattern/extent of suspected toxicity on site; map of soil toxicity is end goal	Site screening; for example, identify toxicity hotspots; generate map of soil toxicity; average extent of a stress factor (e.g. compaction) at a site; compare site to reference; remediation evaluation; long-term monitoring	Practical and convenient; precise; uniform coverage; best strategy for use of geostatistics like kriging to develop toxicity maps A.4.2, A.4.4, A.5.2 (Figure A.11)

Table 1 *(continued)*

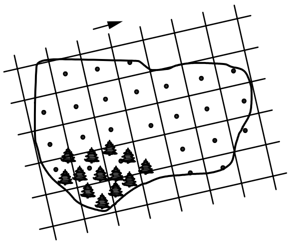
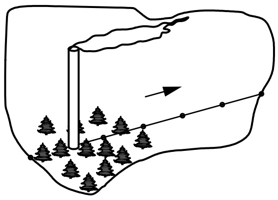
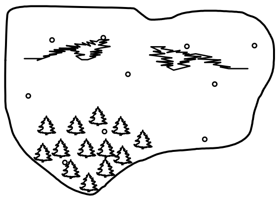
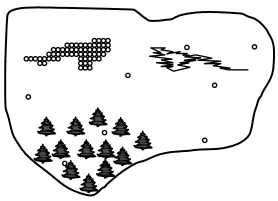
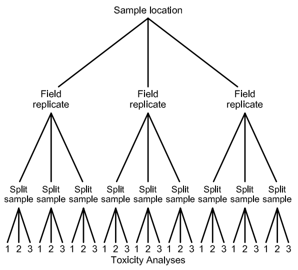
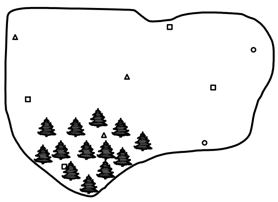
Strategy	Description	Selection criteria	Study objective(s)	Comments (see Annex A)
Systematic transect sampling	Samples collected in a regular pattern with one grid axis, e.g. parallel to contaminant plume axis 	Prior information about the site; e.g. sampling along contaminant plume desired	Sample along concentration gradient; compare site to reference; generate map of toxicity; long-term monitoring	Practical and convenient; precise; good for regression-design evaluations; can use geostatistics to develop toxicity maps A.2.1 (landscape)
Transect sampling	Samples collected in a regular pattern, e.g. parallel to the contaminant plume axis 	Prior information about the site; e.g. sampling along contaminant plume is desired	Sample e.g. along a concentration gradient; compare site to reference; long-term monitoring	Practical and convenient; cost-effective; good for regression-design evaluations; A.2.1
Cluster sampling	Probabilistically choose initial samples; then collect adjacent samples following predetermined selection rules  	Little prior information about site	Hotspot sampling; site screening	Can apply geostatistics to data; can be expensive if definitive toxicity tests are used and/ or if test turnaround time is slow A.4.1

Table 1 *(continued)*

Strategy	**Description**	**Selection criteria**	**Study objective(s)**	**Comments** **(see Annex A)**
Nested sampling	Multiple sample taken at each location (field replicates); each field replicate subdivided (split samples); each split sample tested as one lab sample; each lab sample subdivided into laboratory replicates 	Used if want to identify sources of variability (e.g. location, sample replication, preparation, testing)	Can be used with any other sampling strategy or study objective	Provides information on the components of variance in a study; laboratory replicates for toxicity testing can be taken from any level depending on the study objectives and budget A.2.2, A.3.1.1, A.3.1.2 (Figures A.3 and A.4), A.5.2
Probabilistic + Deterministic				
Ranked set sampling	Randomly chosen samples ranked into different sets according to professional judgment and subsample from sets Ranked set 	Prior information about site; cost of analyses is high	For example, average toxicity at a site; compare site to reference; identify toxicity hotspots; sample along concentration gradient	More precise than random sampling with same samples; cost of ranking samples in field can be high No case study
	△ set 1 ○ set 2 □ set 3			

8.3 Recommendations from the European programme ENVASSO (Environmental Assessment of Soil for Monitoring)

The EU project ENVASSO aimed to design a single, integrated and operational set of EU-wide criteria and indicators to provide the basis for a harmonised comprehensive soil and land information system for monitoring in Europe (References [1], [34]). For the purpose of long-term monitoring of soil biodiversity the following recommendations were proposed by a large working group consisting of experts experienced in soil biology sampling and monitoring, References [77, 78, 79, 80]. While this proposal was originally developed in the context of EU-wide monitoring, as proposed in the Draft Soil Framework Directive of the European Union, References [81, 82], the indicators proposed can also be used for other purposes, such as the study of potential side effects of anthropogenic impacts or the biological classification and assessment of soils. It can be divided into three steps. The biological indicators were selected for three different levels (Triad approach) and should always be used in combination with a detailed site and soil characterization.

a) Step 1: Site description and soil characterization according to

— ISO 23611-1:2006;

— Land management, land use and vegetation type should follow FAO 2006 classification (ftp://ftp.fao.org/agl/agll/docs/guidel_soil_descr.pdf).

— Soil type should follow WRB 2006 (ftp://ftp.fao.org/agl/agll/docs/wsrr103e.pdf), or a referred international soil classification as FAO 2006.

b) Step 2: Installation of the sampling area (surface definition, localization, replicates)

— Sampling area shall be about 100 m^2.

— If there is an existing monitoring network which assesses the site and soil characteristics (“conventional” monitoring area), in order to use the collected data (e.g. climatic, land use, physicochemical analysis) the biological sampling area should be located inside the ‘conventional’ monitoring area or, nearby the ‘conventional’ monitoring area (5 m from the conventional area at the most).

— If there is no monitoring network, complementary analyses shall be performed on a composite sample from the investigated area to explain/interpret biodiversity data (required parameters)

— Localization of the sampling area in a homogeneous area (based on pedological characteristics and soil cover).

— Record the location of the sampling area position with a differential GPS device.

— Sampling strategy: minimum of three replicates, with equal distance between subplot/replicates.

c) Step 3: Soil sampling area preparation

— Cut the vegetation or take off the soil cover as mulch without damaging the soil surface

— In the case of forest: take the litter and put it in a plastic sample bag in order to assess the fauna in the laboratory.

To interpret the biological data, several soil analyses are generally required as follows:

— pH in accordance with ISO 10390;

— Soil moisture content in accordance with ISO 11465;

— Organic carbon, total carbon in accordance with ISO 10694;

— Heavy metal analysis in accordance with ISO 14869-1, and ISO 11466;

— Texture in accordance with ISO 11277.

NOTE 1 These recommendations made for monitoring purposes can be relevant for other purposes, too.

A proposal was also made for a set of suitable indicators for monitoring soil biodiversity, Reference [78] (see Table 2). Indicators were selected both from a literature review and an inventory of national monitoring programmes. Soil biodiversity was defined as the forms of life (gene, species and, rarely, higher level) living in soils (both in terms of quantity and variety) and of related functions. To select level I indicators, three stringent criteria were applied: an indicator should

1) have a standardized sampling and/or measuring methodology,

2) be complementary to other indicators, and

3) be easy to interpret at both scientific and policy levels.

The level I indicators were chosen as representative of three different taxonomical groups and functional levels:

— abundance, biomass and species diversity of earthworms – macrofauna (see Note 2);

— abundance and species diversity of Collembola – mesofauna;

— microbial respiration.

Biodiversity (species level), as well as ecological functions of soil organisms, are covered by these groups and levels. In principle, when considering soil biodiversity, all soil organisms and the biological functions which they provide are important and should be assessed. However, for priority level I (Table 2) three indicators were selected to act as surrogate measures for overall biodiversity. Depending on the availability of resources and specific requirements, this minimum set of indicators could be extended to include priority levels II and III (Table 2), e.g. all macrofauna, nematode diversity, bacteria and fungi diversity and activity, faunal activity as biogenic structures or feeding activity. The three priority level I selected indicators are as given in Table 2.

NOTE 2 When earthworms are not supposed to be found (e.g. in acidic soils) the diversity and abundance of enchytraeids should preferably be measured.

Table 2 — Priority level of indicators for decline in soil biodiversity (ENVASSO)

Key issue	**Groups of species**	**Level I** (all core points of the monitoring network)	**Level II** (all core points or selected points relevant for specific issues and availability of resources)	**Level III** (optional)
Species diversity	Macrofauna	Earthworm species	All macrofauna	
	Mesofauna	Collembola (Enchytraeidae if no earthworms)	Acarina sub-orders	
	Microfauna		Nematode (functional) diversity based on feeding habits	Protista
	Microflora		Bacterial and fungal diversity based on DNA/ PLFA extraction	
	Vascular plants			For grassland and pastures
Biological functions	Macrofauna			Macrofauna activity (e.g. biogenic structures)
	Mesofauna			Mesofauna activity based on litter bags or on bait lamina
	Microflora	Soil respiration	Bacterial and fungal activity	

9 Sampling report

The sampling report shall refer to this part of ISO 23611 and shall contain a summary of the methods and parameters used during the study and the results obtained. It shall provide the following information:

a) detailed description of the study objective used;

b) characterization of the study site (especially soil properties), including the coordinates of the sample location(s);

c) a full description of the experimental design and procedures;

d) sampling procedure;

e) all modifications or changes compared to the methods described in ISO guidelines (in particular ISO 23611-1 to ISO 23611-5);

f) description of the sampling conditions, including date and duration of sampling in the field and climatic parameters like air temperature;

g) unambiguous sample identification numbers;

h) all information, including all measured raw data and all problems that might have occurred, developed during all phases of the study;

i) discussion of the results.

NOTE In addition, chain-of-custody forms can be important when samples are required for legal purposes.

10 Quality control and quality assurance

The goal of QA/QC programmes is to identify, measure, and control the errors associated with every component of a sampling study, including planning, sampling, testing and reporting, Reference [21]. Because of the various reasons for and objectives of sampling, there can be no single set of quality control and quality assurance procedures to be followed by all organizations offering sampling services under all circumstances. However, it is strongly recommended that, as far as practicable, the guidelines set out in ISO 9000 should be followed. In particular, the preparation of a sampling plan, the inclusion of qualified personnel in planning and performing the work, as well as the detailed documentation of all steps of the study (and here especially of the field work), is of utmost importance.

Annex A
(informative)

Case studies

A.1 Preliminary considerations

In the following, case studies are presented in a tabular form (plus usually one figure). The criteria used to describe them are briefly defined here.

In the beginning, each case study is characterized by a specific title and a literature reference.

Objective	Aim of the study or basic hypothesis; if feasible, also the legal background
Preliminary information	Data relevant for designing the study and/or assessing the results; e.g. site history (land use, site management), available abiotic or biotic data
Location	Site, region or country where the study was performed for which the proposal originally has been made
Stressor	Any anthropogenic (e.g. chemicals, compaction, genetically modified organisms) or natural (e.g. soil properties such as pH, texture, organic matter content, as well as climatic properties, e.g. precipitation, temperature) factor
Design strategy	Referring to Table 1, the design strategy of the individual study is classified depending on the respective scale (e.g. plot, site or landscape level). Note that the selected design is not per se the optimum one but depends on a variety of factors, including availability of resources, time etc. For definitions for each design strategy, see Table 1
Sampling and analysis of organisms	Organism group to be sampled (if possible, reasons why selected), as well as the measurement end points (e g. abundance, species composition) and the way of evaluating the results (e.g. calculation of a diversity index)
Methods used	Includes sampling of biotic (i.e. organism groups) and abiotic (e.g. soil properties) end points; in case methods described in International Standards have not been used, information about the comparability and validity of the results gained should be given.
Remark	Any potentially relevant information not mentioned yet.

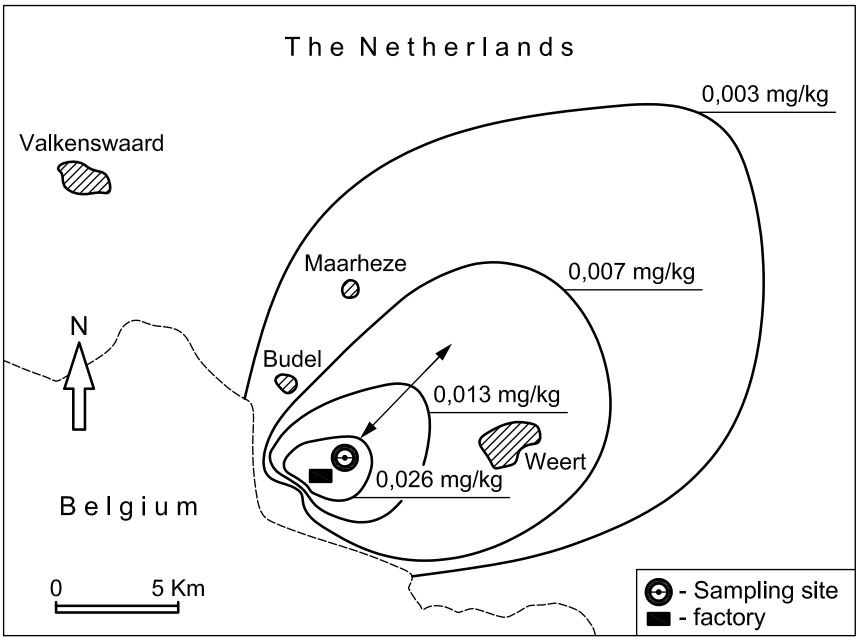
Preliminary note regarding monitoring programmes in general:

Ideally, such monitoring would be performed regularly in as many as possible sites and regions because these data are necessary to define reference values (i.e. which community is “normal” or “expectable” under certain environmental conditions (e.g. climate, vegetation, soil properties)). In theory, only sites with no anthropogenic impact are used but this is neither possible nor useful, since in most parts of the world the soil has been influenced by man in one way or another. Therefore, specific reference values are necessary for different land-use forms (at least agricultural, grassland and forest sites shall be distinguished). However, so far biological parameters have rarely been implemented in soil monitoring programmes (e.g. in France, microbial, mesozoological and macrozoological sampling was started in 2006 as part of the RMQS (soil quality monitoring network).

A.2 Site-specific characterization and assessment of contaminated land

A.2.1 Heavy metal pollution, Reference [50]

Objective	Assessment of heavy-metal emissions from a smelter (point source) on the diversity of an important soil fauna group
Preliminary information	Heterogeneous soils with a dominant metal and pH gradient. Site has partly been used as a military training ground, partly for recreational purposes. Vegetation: mainly coniferous forest (*Pinus*), but grassland plots are not rare.
Location	Zinc smelter located near Budel (The Netherlands)
Stressor	Zinc and cadmium emissions since 1892 till the 1980s (see Figure A.1).
Design strategy	Landscape: transect; plot scale: judgemental. When fixing the transect, distance to the source, the prevailing wind direction and the properties of the contaminant were to be taken into account.
Selection and analysis of organisms	Enchytraeidae (dominant mesofauna group at the study site).
Methods used	Sampling according to ISO 23611-3. Based on information gained in pilot studies, samples were taken at 30 sites (one replicate each) located on a gradient starting 1 km northeast from the former zinc smelter plant and ending 6 km downward. The prevailing wind direction and the properties of the contaminant were taken into account. Biggest problem: avoidance of confounding local factors (i.e. soil disturbance like compaction) at individual sampling sites in order to focus on the main factor of interest, i.e. metal concentrations.
Remark	Such studies were already done in the 1980s (e.g. at a brass mill in Sweden (Gusum; Reference [8]) or a comparable site in England (Avonmouth; Reference [64]). More recently, sampling of soil invertebrates at a site to be assessed is done as part of the TRIAD approach, meaning that the results of the field work are evaluated together with those from bioassays and residue analysis, Reference [33].

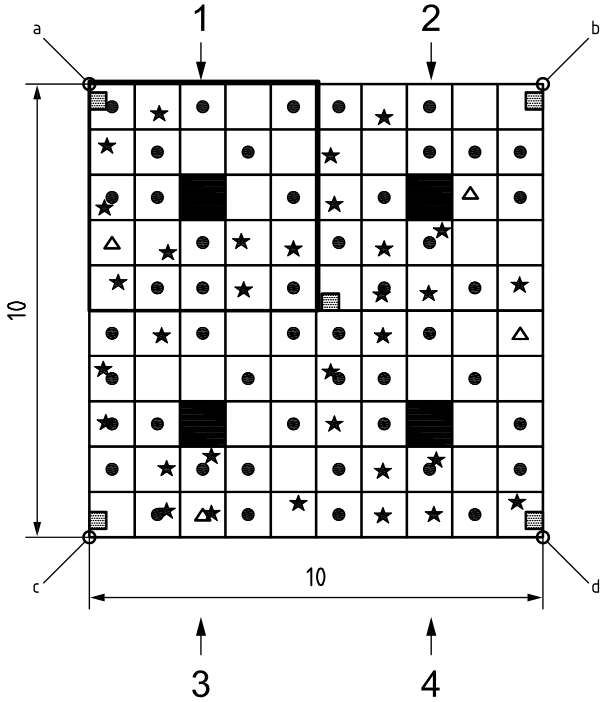


NOTE 30 enchytraeid samples were taken along a transect of 5 km (arrow). Numbers indicate the concentration of cadmium in soil depending on the distance from the plant.

Figure A.1 — Plan of the Budel site, showing the contaminated area, Reference [47].

A.2.2 Different sources of pollutions: French proposal

Objective	Validate biological indicators (bioindicators) to describe the pollution level of soil at a national level
Preliminary information	Chemical and physical environmental data (pedology, geology, etc.), field history If possible, sources of pollution, e.g. PAHs or trace elements (Pb, Cu, As)
Location	France
Stressor	Trace elements, PAHs etc.
Design strategy	Stratification according to the nature of contaminants (e.g. trace element or organics) to select the site and according to a gradient of contamination to select sampling area. Nested sampling at the plot level
Selection and analysis of organism group	Vegetation, including diversity, bioaccumulation and biomarkers Bacteria and fungi, including diversity and activity Soil fauna, including Collembola and nematodes (diversity), earthworms and total macrofauna (diversity, activity, bioaccumulation, biomarkers), snails (bioaccumulation), micromammals (bioaccumulation, biomarkers)
Sampling method used	At each plot, the sampling design (100 m^2) is organized in four adjoining replicates (see Figure A.2). Per each replicate, a soil composite sample taken from 12 soil cores (8 cm diameter, 15 cm depth) is used to measure microbial parameters, nemato-fauna and physical-chemical parameters. Collembola and mites are extracted in the laboratory from soil samples (8 cm diameter, 10 cm depth). Earthworms (1 m^2) are extracted using chemical extraction and hand sorting. Total macrofauna (25 cm^2 × 15 cm) are extracted using the TSBF method.
Remark	The biological and soil sampling methods used were designed to meet, as far as possible, ISO standards and ENVASSO recommendations.



Key

●	soil composite sample: microbiology, nematofauna, physico-chemistry	2	REP2
△	Collembola, mites	3	REP3
■	earthworms (community)	4	REP4
▩	total macrofauna	a	GPS1
★	flora	b	GPS2
○	points	c	GPS3
1	REP1	d	GPS4

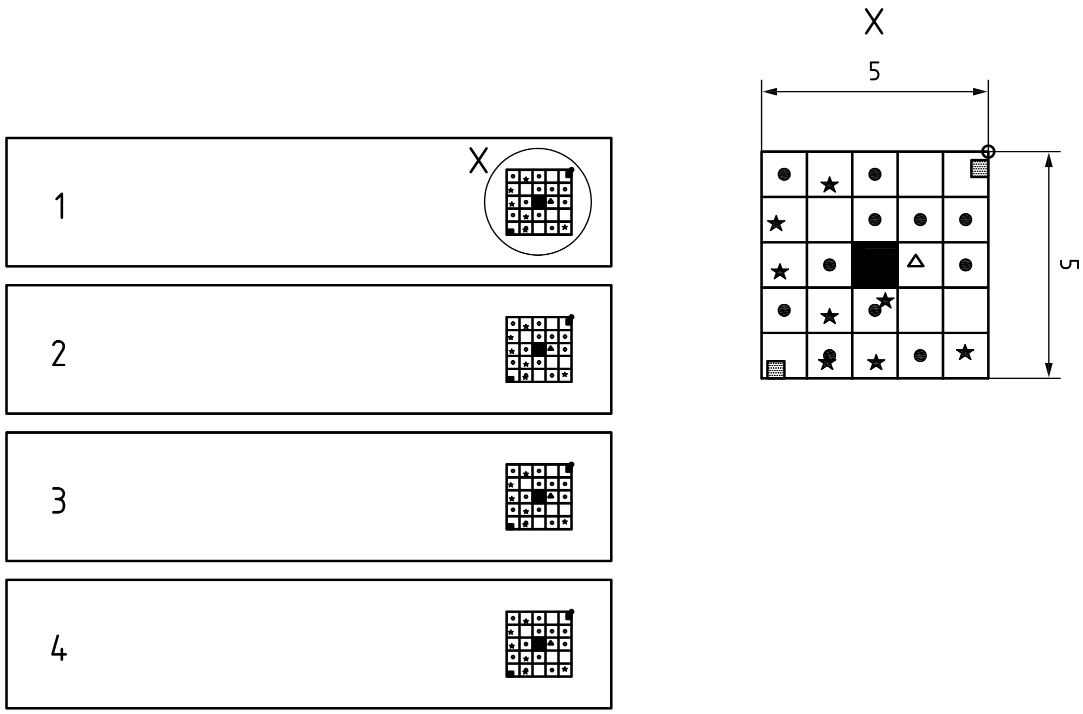
Figure A.2 — Sampling design organized in four adjoining replicates

A.3 The study of potential side effects of anthropogenic impacts

A.3.1 Impact of land-use forms

A.3.1.1 Agricultural impact

Objective	Assessment of the impact on soil organisms of different agricultural practices (e.g. tillage vs. no-tillage, organic farming vs. conventional farming, rotation vs. mono-culture vs. rotation culture/pastures, compost inputs).
Preliminary information	Field histories, including agricultural practices and land uses, soil agronomic parameters [pH, cation-exchange capacity (CEC), etc.] and contaminants (pesticides, metals, etc.)
Location	France
Stressor	Agricultural practices
Design strategy	Stratification according to the land use/agricultural practices to select the site Nested sampling at the field and plot level
Selection and analysis of organism group	Bacteria and fungi, including diversity and activity Soil fauna, including Collembola and nematodes (diversity), earthworms and total macrofauna (diversity, activity, bioaccumulation, biomarkers), snails (bioaccumulation), micromammals (bioaccumulation, biomarkers)
Sampling method used	The sampling design benefits from the field bloc design (4 blocs at minima). In each bloc, one replicate is done (see Figure A.3). Per each replicate, a soil composite sample taken from 12 soil cores (8 cm diameter, 15 cm depth) is used to measure microbial parameters, nemato-fauna and physical-chemical parameters. Collembola and mites are extracted in the laboratory from soil samples (8 cm diameter, 10 cm depth). Earthworms (1 m^2) are extracted using chemical extraction and hand sorting. Total macrofauna (25 $cm^2 \times$ 15 cm) are extracted using the Tropical Soil Biology and Fertility (TSBF) method.
Remark	The biological and soil sampling methods used were designed to meet as far as possible the ISO standards and European recommendation (ENVASSO programme).



Key

- ● soil samples (one composite sample from 12 scores) to assess microorganisms, nematofauna, physical and chemical parameters
- ▒ total macrofauna
- △ mesofauna (Collembola, mites)
- ★ plants
- ■ earthworms
- ○ points

Figure A.3 — Sampling design to assess agricultural impact benefiting from a bloc sampling design

A.3.1.2 Impact of forest management, References [61, 62]

Objective	Assessing the effects of reforestation and forest management practices on soil mesofauna
Preliminary information	Aerial photographs of the area; vegetation characterization
Location	Portugal; various forest sites
Stressor	Reforestation and different management techniques; introduction of exotic tree species
Design strategy	Nested design. In the case of different vegetation patches within the site: stratified designed
Selection and analysis of organism group	Collembola (springtails); highly diverse and functionally important mesofauna group. Species number and species composition are used as end points.
Sampling method used	ISO 23611-3. Comparison of different sites with different forest types or different forest treatments samples (see Figure A.4). Soil cores should be taken over an area large enough to be representative of the site under assessment, avoiding spatial pseudo-replication. Assuming a homogeneous vegetation cover and depending on the size of the site, an area of 100 m × 100 m can be chosen (Figure A.4). Experimental units (squares of 8 m × 8 m) were randomly selected. Our soil cores were taken in each unit, and these were spaced 2 m, avoiding spatial autocorrelation.
Remark	High amount of information available

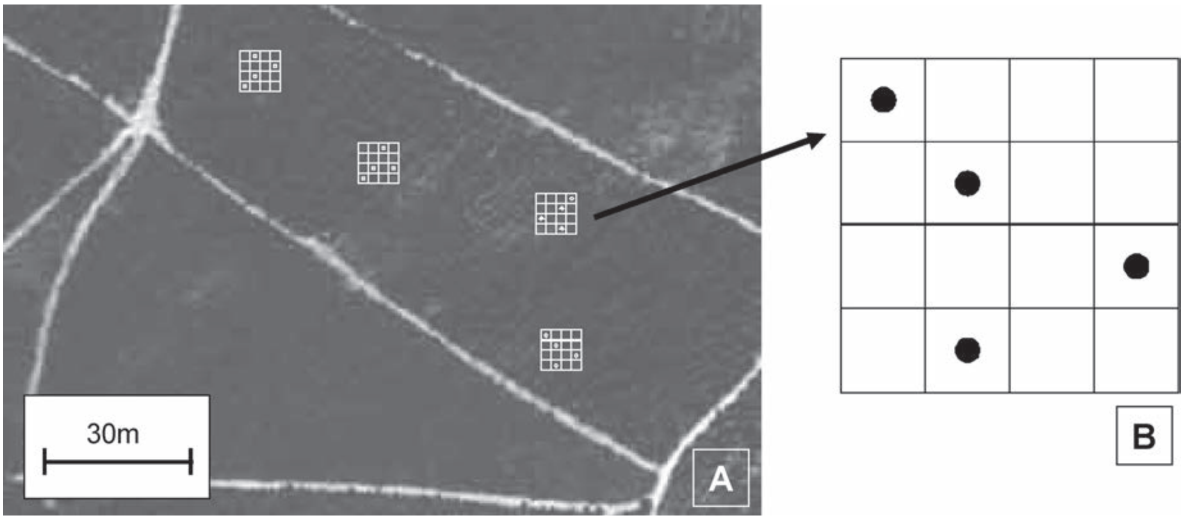


Figure A.4 — A) Homogeneous forest stand with superimposed experimental units following a nested design; B) Detail of an experimental unit with sampling points

A.3.2 Unspecific (chemical) contamination (biological quality of soil biota), Reference [48]

Objective	Determination of bioaccumulation of chemicals by earthworms in order to survey retrospectively long-term chemical changes in, e.g., terrestrial ecosystems [German Environmental Specimen Bank (ESB)].
Preliminary information	Screening shall be conducted to define the sampling sites, the target species, and the random sample number. A further objective of this pre-study is to characterize the soil properties, the variation within the pollutant concentrations, and the spatial scheme of the pollution burden (Figure A.5). Based on the screening, all sites potentially suited for anecic earthworms, with comparable land use, vegetation, and edaphic conditions, are mapped.
Location	About 50 sites considered as unpolluted located all over Germany
Stressor	Potentially, all chemicals (mainly airborne): heavy metals, PAHs, or pesticides.
Design strategy	Stratified sampling (site level), judgemental (plot level)
Selection and analysis of organism group	Anecis earthworms are representatives of the decomposer community in soil . (*Lumbricus terrestris, Aporrectodea longa*). Their high biomass facilitates the determination of even low concentrations of a wide array of chemicals.
Sampling method used	A grid (raster size e.g. 50 m × 50 m) is laid over each site, from which at least 15 sections are randomly selected as sampling points. The sampling sites for routinely conducted samplings are determined according to Figure A.6. Due to ecosystem type and size of the sampling area, the number of the sampling plots varies. One sampling plot can consist of several partial plots, which shall not be in conjunction with each other. Location and size of the sampling plot(s) shall be registered in the area-related sampling scheme. In any case, at least 10 sampling points shall be randomly selected per plot. Following the scheme in Figure A.6, generator, ampere meter, and the electrode lines are arranged on the trapping zones. Both electrode lines are stuck in a zigzag pattern into the soil at a distance of max. 30 m to each other. The zigzag pattern is recommended because it enlarges the trapping area per line, compared to a linear arrangement of the electrodes. If the values for pace voltage and/or the current are, despite a 220 V output voltage, too low to bring the earthworms to the soil surface, both electrode lines shall be put closer together. Exact statements for a suitable voltage cannot be made, because, depending on the conductivity of the soil, even in the case of low values good trapping profits can result. Collection always takes place at both lines of electrodes.
Remark	*Lumbricus terrestris* turned out to be more suitable for this purpose compared to *Aporrectodea longa*.

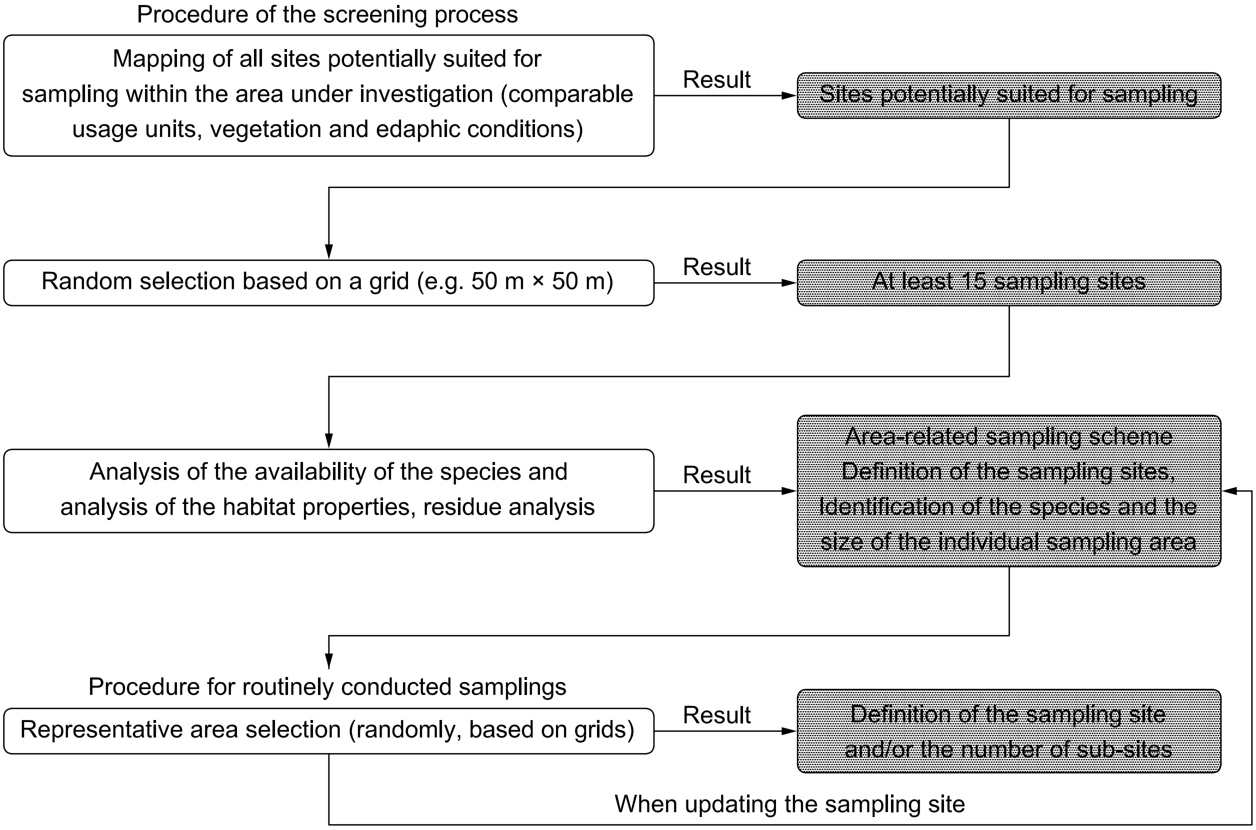
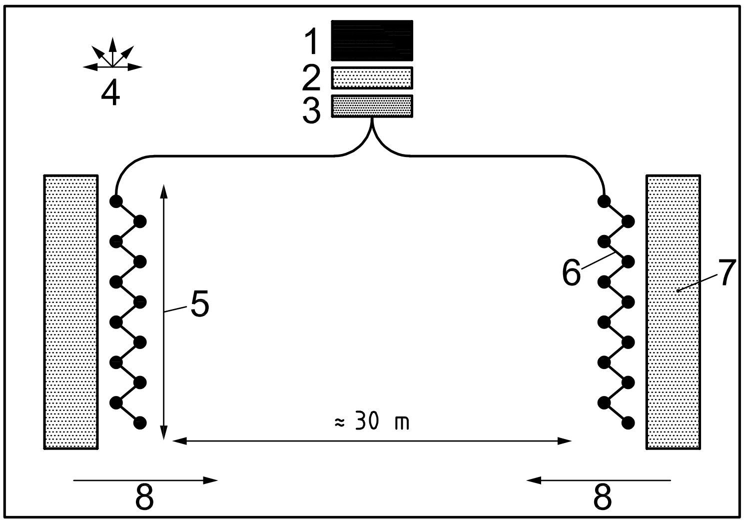


Figure A.5 — Flowchart for the implementation of screenings and routinely conducted samplings, Reference [48]



Key

1 generator
2 control transformer
3 ampere meter, emergency switch off
4 wind direction
5 length of electrode line: 5 m
6 electrode line in zig-zag pattern
7 insulating mat (= position of staff)
8 working direction

Figure A.6 — Arrangement of the trapping gear, Reference [42]

A.4 The biological classification and assessment of soils

A.4.1 The BBSK approach

Objective	Assessment of the habitat function of soils
Preliminary information	All sites are intensively characterized (i.e. pH-value, organic matter content, C/N ratio, soil moisture, and soil texture (usually by methods described in International Standards) as well as history, land use, etc.).
Location	Pilot study with 25 sites all over Germany (mainly forests, but also grassland and crop sites.
Stressor	The chosen sites cover a broad spectrum of soil properties, climatic regions but also potential human impacts (e.g. by air-borne pollutants), from sites close to industrial centres to more pristine rural landscapes.
Design strategy	Cluster sampling (plot level), ISO 10381-1; i.e. samples were arranged in an X manner (distance between individual samples: 5 m)
Selection and analysis of organism group	Ecologically important groups: microfauna (nematodes), mesofauna (enchytraeids, mites) and macrofauna (earthworms, ground beetles)
Sampling method used	Sampling was done according to International Standards (e.g. ISO 23611) in spring and autumn when soil organisms are active. Per site (approximately 100 m × 100 m) different numbers of samples were taken: nine for micro-arthropods, enchytraeids and nematodes (litter and soil layers were differentiated) and six for earthworms. The sampling design followed the recommendations given in International Standards. Specific points at the sites (e.g. pathways in forests) which can influence the distribution of the soil invertebrates were avoided as much as possible.
Remark	This study served as a pilot project in which methods for classifying fauna (theoretical approach: Figure A.7). Reference values were defined by expert knowledge for each taxon separately. Deviations from the reference values were classified in "-" (clear deviation between reference and actual value), "±" (some differences, but not clear), and "+" (no deviation within 30 % range).

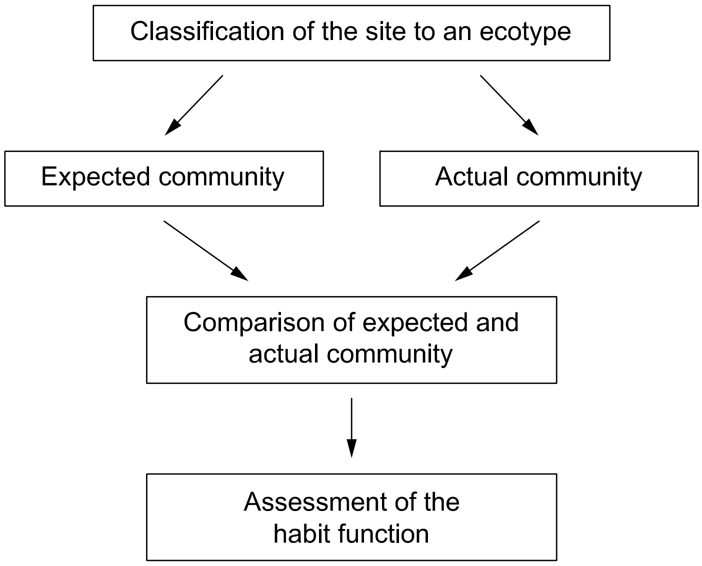


Figure A.7 — Schematic overview of the BBSK-concept

A.4.2 Sampling of microarthropods

Objective	Monitoring of microarthropod diversity at the habitat level (Forest Focus monitoring programme)
Preliminary information	The placement of the grid was based on the analysis of an aerial photograph, avoiding margin effects.

Location	Inundation forest near Karlsruhe (Germany)
Stressor	Different forest types and/or management practices
Design strategy	Grid design (in the case of the existence of different vegetation patches within the field site, a stratified sampling is advisable)
Selection and analysis of organism group	Microarthropods (especially Collembolans), because they belong quantitatively and qualitatively to the most important soil animal groups, References [72], [22], [16], are taxonomically well described and much data concerning their zoogeography and reactions to environmental factors are available (References [12], [27], [49])
Sampling method used	Samples (= soil cores) should be taken over an area large enough to be representative of the site under assessment, avoiding spatial pseudo-replication (an area of e.g. 150 m × 150 m). The distribution of experimental units within the sampling area follows a 3 × 5 grid design (Figure A.8, with sampling points separated by 30 m). 15 sampling points were chosen (10 is a minimum). At each point one sample core and one soil monolith were taken and also one pitfall trap was setup (for a period of 10 d). Collection of samples followed ISO 23611-2. Samples shall be evenly distributed within a plot (randomly or according to patterns given in ISO 10381-1; see also 6.2) in order to detect all occurring species. It is thereby necessary to ensure that all environmental gradients are equally represented and a minimum distance of 1 m is kept from major geo-morphological and biological field structures, References [38], [39]. A minimum distance of 1 m and an optimum distance of 2 m to 3 m should be kept between individual samples to avoid autocorrelation.
Remark	Field studies and meta-analyses of literature data have been carried out to elucidate appropriate field designs with respect to heterogeneous faunal distribution and data precision, References [15], [22], [23], [54]. The recommendations given in these papers are adaptable to many studies and habitat types. However, major differences occur regarding the soil depths to be sampled. Whereas in grassland and woodlands, the largest abundances and species richness occur in the upper 5 cm to 10 cm of mineral soil, arable soil depends on the plough depth. The distribution of Collembola was studied in such detail that it can be used as an example here, Reference [53].

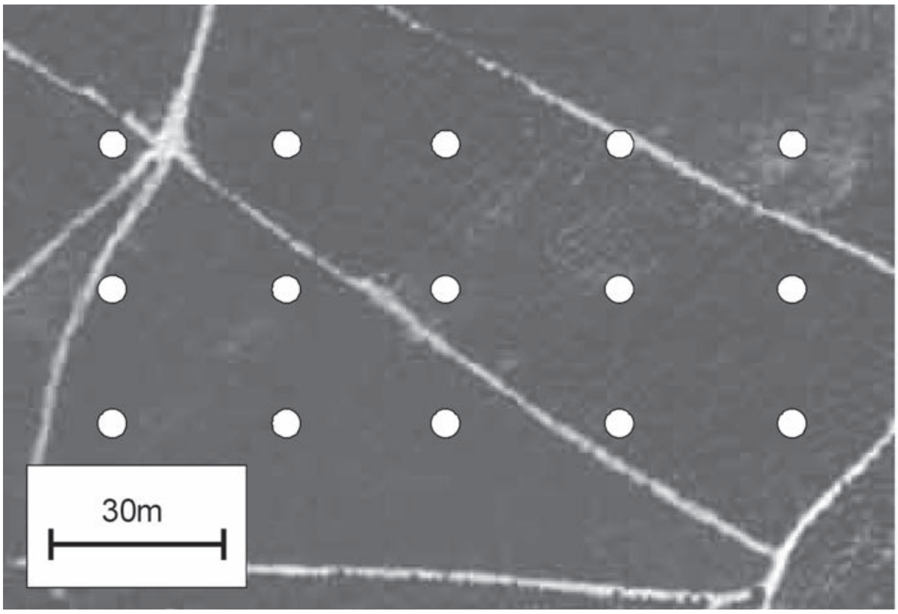
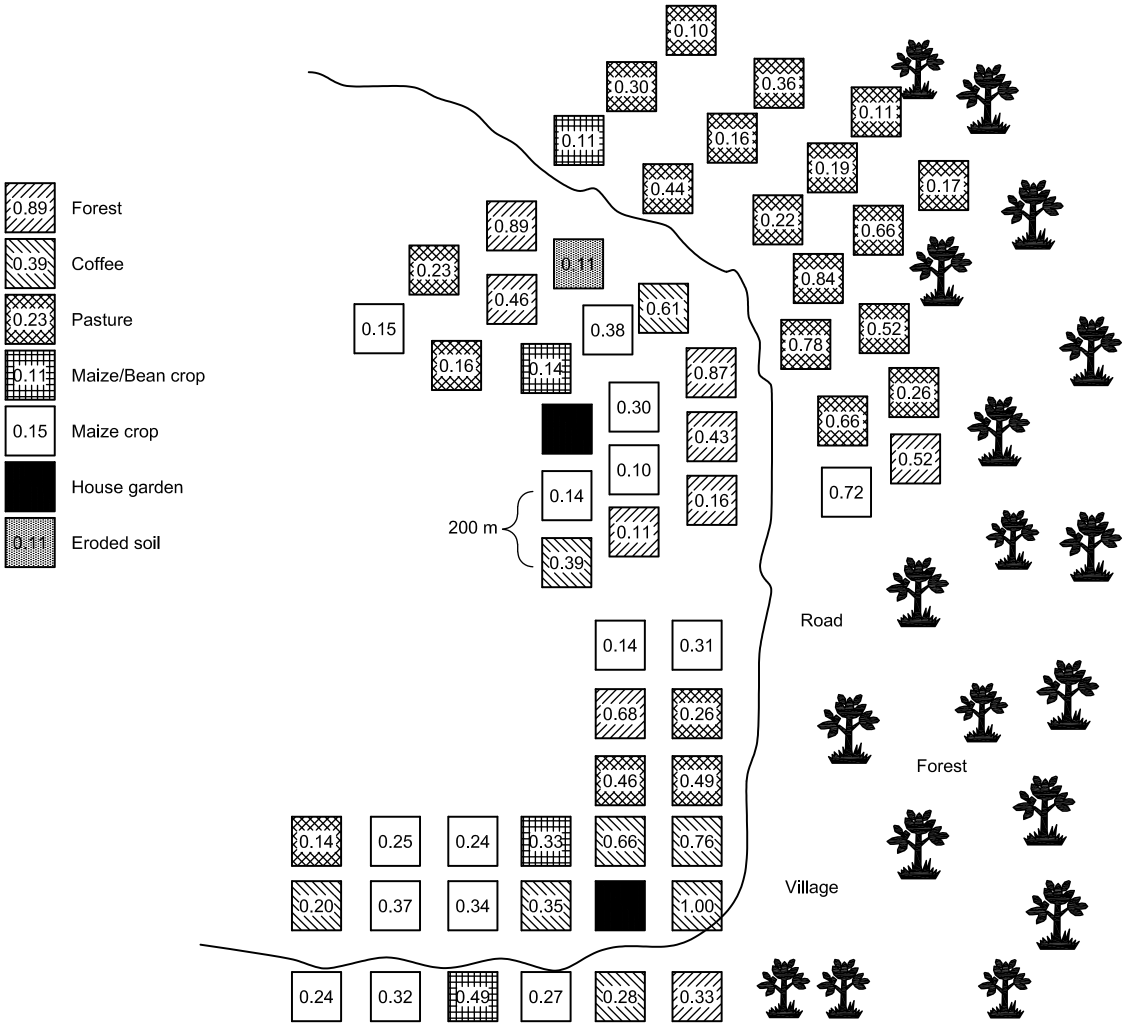


Figure A.8 — Homogeneous forest stand with superimposed sampling points on a grid design

A.4.3 Macrofauna at the landscape level — A case study in Nicaragua, References [4, 70, 71, 73]

Objective	Assess the effect of different land-use practices at the landscape level, design a general indicator reflecting the quality of soil invertebrate communities and rank these sites according to this criterion.
Preliminary information	In particular, data on agricultural measures and land management
Location	Watershed of Wibuse, central Nicaragua. Hilly area covered with a variety of land uses displayed in a mosaic array. The sampled landscape was an area of ca. 5 km^2 with a mosaic of secondary forest, coffee plantations, pastures and maize crops (Figure A.9).
Stressor	Land use, crop types and land management
Design strategy	Stratified sampling
Selection and analysis of organism group	16 taxa of macrofauna (e.g. earthworms, ants, termites, cockroaches, centipedes, diplopods), known to dominate these soils by biomass
Sampling method used	The sampling protocol was a regular grid with sampling points distant 200 m. Five samples (monolith 25 cm × 25cm × 30 cm) positioned according to the TSBF protocol (see ISO 23611-1) were taken at each point and hand sorted for soil macro-invertebrates. To assess soil macro-invertebrates at this taxonomic level, three sampling points seems to be a satisfactory number. Invertebrates were identified at the order level (16 taxa) and multivariate analysis showed a significant effect of land-use types, with a clear gradient of richness and abundance from coffee plantations and secondary forests to fallows, house yards, mixed crops, single crops and pastures, and eroded soils surrounded by monocultures (e.g. maize) at the other end. An indicator of soil macrofauna abundance and diversity was especially designed based on a multivariate approach.
Remark	Land degradation is a very serious issue in hillside ecosystems in Centro America where erosion, loss of organic matter and nutrient mining practices are still little compensated with adequate inputs (chemical fertilizers and organic inputs) and conservation practices.



NOTE Numbers in boxes indicate the local value of the Macrofauna sub indicator calculated after Reference [71].

Figure A.9 — Sampling design in the Wibuse water catchment area (Nicaragua)

A.4.4 Mesofauna sampling at landscape level, References [5, 63]

Objective	Assessment of changes in diversity patters at the landscape level
Preliminary information	Geographic location, soil and climate properties, vegetation characteristics,
Location	Different sites in Europe, e.g. France.
Stressor	Land-use changes, situated along a transect from a forest dominated landscape to an agricultural dominated landscape, passing through a mixed-use landscape
Design strategy	Grid design
Selection and analysis of organism group	In this example: Collembola, being a highly diverse and functionally important mesofauna group.
Sampling method used	Collection of samples followed ISO 23611-2. Samples were taken over an area large enough to be representative of the landscape configuration under assessment. The size of these landscape windows depends on the grain size of the landscape, i.e., coarse-grain landscapes might need larger areas. Six landscape land-use units of 1 km^2 (LUUs) were chosen. These six LUUs constitute the gradient in question, and also can be seen as a gradient of land-use intensification. At each LUU, 16 sampling points were defined over a 4 × 4 grid spaced 200 m (Figure A.10). This sampling grid embraced almost all land-use types within the landscape window. At each sampling point, one, but preferably more, soil core should be taken. To relate biodiversity descriptors with landscape structure and composition, landscape metrics at different spatial scales were obtained by image analysis.
Remark	This study was part of a pan-European study in which, following the same sampling strategy, other soil organism groups were also samples.

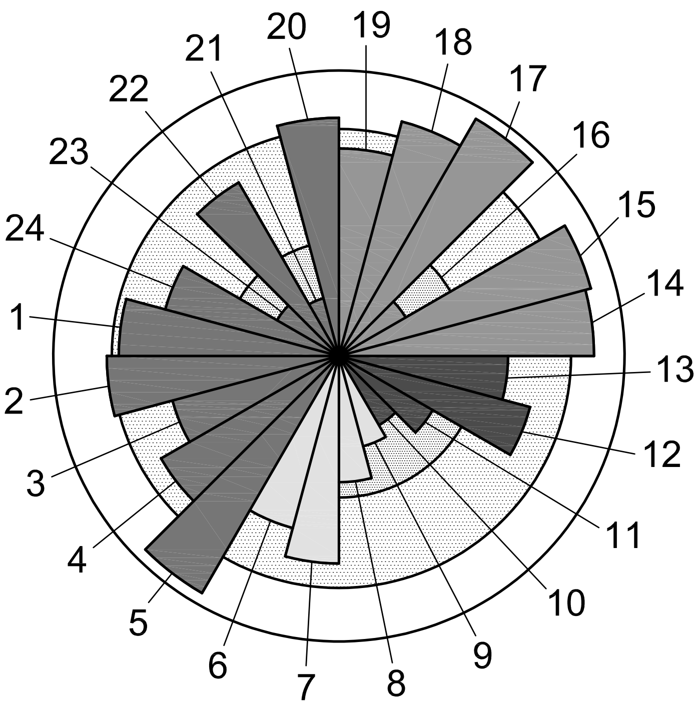
Figure A.10 — Landscape window (LUU) of 1 km^2 with superimposed sampling grid (16 points)

A.5 Long-term biogeographical monitoring

A.5.1 Dutch National soil monitoring network, References [55, 56]

Objective	Provision of a basis for an evaluation of the biological quality of Dutch soils
Preliminary information	Geographical location, land-use history (in particular management), soil and climate properties, vegetation
Location	About 300 sampling locations (= farm sites) located all over The Netherlands,
Stressor	Land use, in particular the intensity of agricultural management
Design strategy	Random stratified design comprising stringent combinations of land use and soil type

Selection and analysis of organism group	Various parameters describing the bacterial community and the main invertebrates (nematodes, collembolans, mites, enchytraeids and earthworms)
Sampling method used	All soil biological measurements are undertaken on a regular schedule (every six years) according to standard ISO methods. For example, in order to describe the nematode community on the farm level, 320 individual soil cores were taken, equally divided over the whole area, which produced a mixed sample of about 15 kg. After homogenization, samples of about 1 kg can be taken from this mixture. Larger organisms were sampled from six discrete plots evenly spread across the whole location. At each of these plots, three individual samples were taken.
Remark	Common problem for monitoring activities: How can the results of such complex programmes be communicated? Here, the information gained is resented in a radar diagram (“Amoebe”, Figure A.11), a circular histogram plot representing all indicator values, scaled against a historical, undisturbed, or desired situation, Reference [14]. The establishment of a proper reference, separately for every combination of soil type and soil use, is under way, Reference [55]. As an example, the indicator data from a biological grassland plot were used in Figure A.11 as a reference for intensively used plots. Each reference variable is scaled as 100 %. This yields a circle of 100 %-values for the reference location. The indicative parameters appeared almost all within the 100 %-circle. Apparently, biodiversity within functional groups, and the related process rates, were lower in plots under intensive management than in the conventional plots.

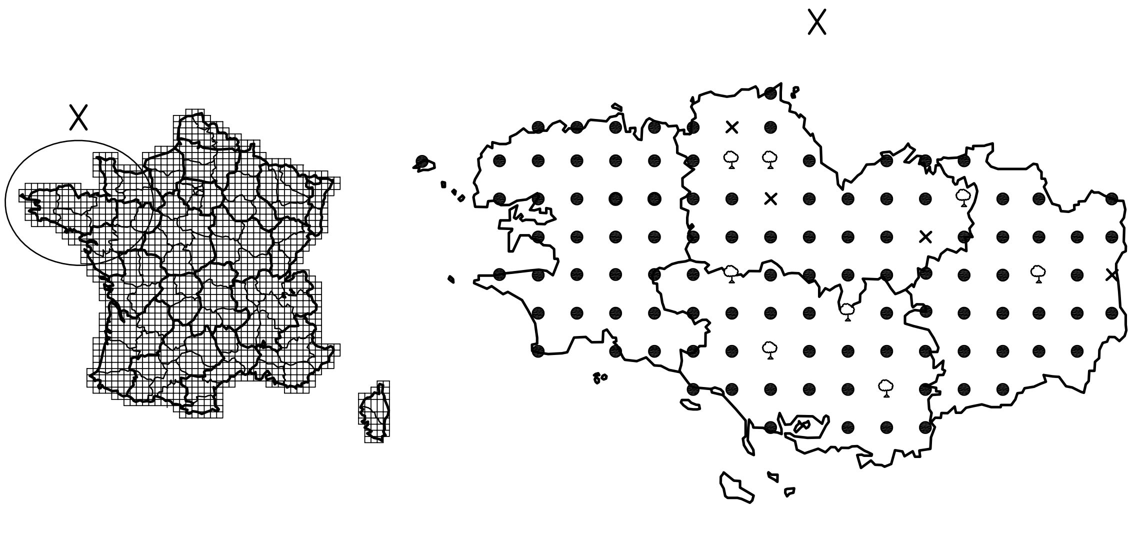


NOTE All results of the reference site (a biological farm) have been scaled as 100 % (the circle). The monitoring results of the regular farms are given by the bars.

Figure A.11 — Presentation of the monitoring results of a specific group of farms in an “Amoebe”

A.5.2 Soil biodiversity monitoring at regional scale: “RMQS BioDiv”, a French Pilot area experience

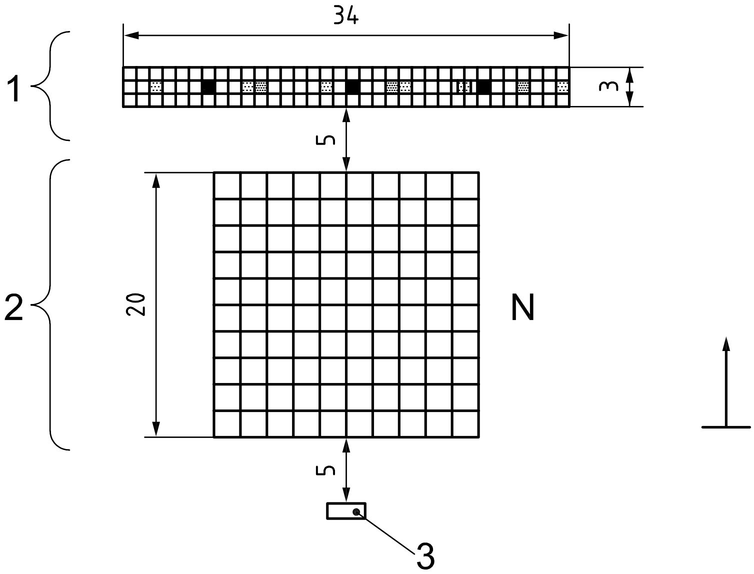
Objective	Monitor soil biodiversity (species and function) in relation to land use (mainly agricultural practices) and pedoclimatic parameters
Preliminary information	Programme connected to a larger soil monitoring network realized at national scale (Soil Quality Measurement Network - RMQS) which monitored soil parameters (chemistry, physics) and agricultural management using a regular grid (16 km × 16 km) covering the whole national territory. Field history, including agricultural practices and land uses, soil agronomic parameters (pH, CEC, etc.) and contaminants (pesticides, metals, etc.).
Location	France (regional level of Brittany)
Stressor	Land use (forest, pastures, cultures), agricultural practices (rotations, fertilisation, etc.) and environmental parameters (climate, geology, peodology, biogeography, etc.)
Design strategy	Systematic grid in Brittany (16 km × 16 km) to select the site Nested sampling at the field and plot scale
Selection and analysis of organism group	Macrofauna (earthworms and total macro-invertebrates), mesofauna (Acarina and Collembola), microorganisms (nematodes, microbial biomass, bacterial diversity), as well as functional biological parameters (soil respiration, humus index).
Sampling method used	The sampling scheme followed a systematic approach based on a 16 km × 16 km squared grid (Figure A.12). 109 sites were sampled in 2006 and 2007. At each sampling point, RMQS BioDiv area (100 m^2) is located 5 m away from the classical RMQS zone, in a homogeneous area (based on pedological characteristics and soil cover). Sampling design is previously defined in order to avoid perturbations between the different biological groups (Figure A.13): total macro-invertebrates: six replicates (25 cm × 25 cm); earthworms: 3 replicates (1 m^2); Collembola and Acarina: three replicates; nematodes and microflora: composite sample from 32 cores.
Remark	The biological and soil sampling methods used were designed to meet, as far as possible, the ISO standards and European recommendation (ENVASSO programme).



Key

- ● BioDiv sites
- ⚲ Forest sites
- × Unrealised sites

Figure A.12 — Systematic sampling design (16 km × 16 km grid)



Key

▒	Total macrofauna	1	RQMS Biodiv sampling zone
■	Earthworms	2	Classical RQMS device – RQMS composite sampling (physico-chemical analysis)
▒	Mesofauna	3	Pedological pit
□	Microfauna and microflora		

Figure A.13 — Classical RMQS and RMQS BioDiv sampling zone with location of the different biological group samples

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