
**Soil quality — Method for testing
effects of soil contaminants on the
feeding activity of soil dwelling
organisms — Bait-lamina test**

*Qualité du sol — Méthode pour tester les effets des contaminants
du sol sur l'activité alimentaire des organismes vivant dans le sol —
Test Bait-lamina*



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ISO copyright office
Ch. de Blandonnet 8 • CP 401
CH-1214 Vernier, Geneva, Switzerland
Tel. +41 22 749 01 11
Fax +41 22 749 09 47
copyright@iso.org
www.iso.org

Contents

	Page
Foreword.....	iv
Introduction.....	v
1 Scope.....	1
2 Normative references.....	1
3 Terms and definitions.....	1
4 Principle.....	2
5 Reagents and material.....	3
5.1 Bait material.....	3
5.2 Reagents.....	3
6 Apparatus (equipment).....	3
7 Procedure.....	3
7.1 General.....	3
7.2 Preparation of the bait-lamina strips.....	4
7.3 Experimental design and test performance.....	5
7.4 Examination of the bait-lamina strips.....	7
8 Expression of results.....	8
9 Validity of the test.....	8
10 Statistical analysis.....	8
11 Test report.....	8
Annex A (informative) Use of the bait-lamina method for monitoring the biological quality of soils.....	10
Annex B (informative) Examples are taken from one study performed in the Brazilian Amazon forest in which the removal of the litter layer was used as stressor ^[30]	11
Bibliography.....	14

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.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT), see the following URL: [Foreword — Supplementary information](#).

The committee responsible for this document is ISO/TC 190, *Soil quality*, Subcommittee SC 4, *Biological methods*.

Introduction

Ecotoxicological test systems are applied to obtain information about the effects of contaminants in soil and are proposed to complement conventional chemical analysis [38]. In addition to laboratory tests with individual species from important organism groups (e.g. earthworms, enchytraeids, or collembolans), functional tests can be used to assess the activity of the whole soil organism community *in situ* in the field and, thus, the habitat function of soil. They are potentially useful for the evaluation of the effects of chemicals on the soil [1][11][12][26][28], as well as for the long-term monitoring of the biological quality of soils (see Annex A) [16][18][34]. Data for these purposes are gained by standardised methods since they can form the basis for far-reaching decisions (e.g. whether a given site should be remediated or not). In fact, the lack of such standardised methods is one of the most important reasons why functional methods have been so far relatively rarely used for the assessment of contaminated soils or for soil monitoring purposes.

The bait-lamina test is an approach which is used to measure the feeding activity of soil organisms *in situ* [23][35][36].

The important advantage of the bait-lamina method is its simplicity. Minimal training, special skill, or equipment is necessary. In contrast to the measurement of other functional parameters, like organic matter breakdown determined in a litterbag test [22][25][27], the bait-lamina method does not disturb the soil substrate, needs only short exposure periods (few days up to few weeks), and is rapidly evaluated.

In temperate regions, the applicability and usefulness of the bait-lamina test for the comparison and assessment of the influence of land use or chemicals on the feeding activity of soil communities has been demonstrated several times [2][14][28][31]. In addition, bait-lamina tests have also been applied in the tropics [13][15][30].

In addition to measures such as microbial respiration or litter decomposition, feeding activity is one of several functional parameters applicable for the assessment of the biological status of soils [7][13]. Recently, the bait-lamina test was used successfully in several case studies at which an environmental risk assessment for polluted soils was performed [8][21]. As a result of these works, the bait-lamina test was included into the tool box for site-specific risk assessment [e.g. the TRIAD approach (see ISO 19204)]. In fact, it became part of the recommended battery of tests for the ecological risk assessment of soils in Great Britain [9]. In addition, in the context of a European soil monitoring programme, the method was recommended for this purpose as well by the members of an EU Working Group [4].

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Soil quality — Method for testing effects of soil contaminants on the feeding activity of soil dwelling organisms — Bait-lamina test

1 Scope

This International Standard specifies a technique for determining the effects of anthropogenic impacts (e.g. substances) in the context of the prevailing environmental conditions on the feeding activity of soil organisms in the field. In addition, the use of this method for monitoring the biological quality of soil is described (see [Annex A](#)). The breakdown of organic matter by soil invertebrates and microorganisms is a crucial process that determines important soil functions such as nutrient availability for plants and the maintenance of soil fertility. In addition, decomposing plant litter provides habitats and food for a wide range of organisms, thus supporting biodiversity and ecosystem services [\[33\]](#)[\[34\]](#).

This International Standard is applicable to all soils in which soil organisms are active. The use of the bait-lamina test is independent from whether there is a litter layer or not. The sampling design of field studies in general is specified in ISO 23611-6 (see also Reference [\[20\]](#)). The design can vary according to the aim of the study as well as conditions (e.g. soil properties, contamination, etc.) of the site to be investigated.

This International Standard is not applicable for semi-terrestrial or very shallow soils. It can be difficult to use it under extreme climatic or geographical conditions (e.g. in high mountains).

2 Normative references

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

ISO 11074, *Soil quality — Vocabulary*

ISO 23611-6, *Soil quality — Sampling of soil invertebrates — Part 6: Guidance for the design of sampling programmes with soil invertebrates*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 23611-6, ISO 11074, and the following apply.

3.1

exposure time

period in which the bait-lamina strips are exposed in the field, usually in the mineral soil [\(3.2\)](#)

EXAMPLE In temperate zones: 10 d to 20 d; in tropical areas: 4 d to 8 d.

3.2

soil

topsoil with or without a *litter layer* [\(3.3\)](#)

3.3

litter layer

layer of mainly dead plant organic material present on the surface of the topsoil, which is composed of debris in different stages of decomposition

Note 1 to entry: Examples are leaves in orchards or forests or “turf” derived from permanent grass grown in meadows [5].

3.4

contaminant

substance or agent present in the *soil* (3.2) as a result of human activity

3.5

contaminated site

site with areas of high concentrations of substances hazardous to *soil* (3.2) and *soil functions* (3.7)

Note 1 to entry: In many countries, contaminated sites are registered publicly as a consequence of a specific legislation.

3.6

soil water content

mass of water evaporating from the *soil* (3.2) when dried to constant mass at 105 °C divided by the dry mass of the soil and multiplied by 100

3.7

soil functions

description of the significance of *soils* (3.2) to man and the environment

Note 1 to entry: Important soil functions in the context of this International Standard include the following:

- control of substance and energy cycles as compartment of ecosystems;
- basis for the life of plants, animals, and man.

3.8

biodiversity

variability among living organisms from all sources, and the ecological complexes of which they are part

Note 1 to entry: This includes diversity within species, between species, and of ecosystems.

3.9

ecosystem services

benefits that humans recognise as obtained from ecosystems that support, directly or indirectly, their survival and quality of life

Note 1 to entry: These include provisioning, regulating, and cultural services that directly benefit people and the supporting services needed to maintain the direct services.

4 Principle

In this functional test, the feeding activity of soil animals is measured. The term “bait lamina” refers to small perforated plastic strips that are inserted into the soil. An artificial mixture or natural organic material (= bait) is exposed to soil organisms (e.g. earthworms, Collembola, Diplopoda, Enchytraeidae) in the apertures of the strip. In principle, the loss of the bait material is assessed by counting the empty apertures of the bait-lamina strips after a certain exposure time. The number of empty apertures (i.e. areas from which the bait material has been removed) as well as their vertical distribution along the strip are evaluated, and it is assumed that this reflects the feeding activity of soil animals.

5 Reagents and material

5.1 Bait material

Organic material used as bait for soil organisms, e.g. a mixture of cellulose powder (70 %, preferably of nutrient quality), finely ground wheat bran (25 %), and activated carbon powder (5 %).

5.2 Reagents

5.2.1 Hydrochloric acid (10 %), for cleaning of bait-lamina strips at contaminated sites (see 7.2).

6 Apparatus (equipment)

6.1 Bait-lamina strips

Plastic strips (PVC, of grey colour) of 120 mm × 6 mm × 1 mm, which have a pointed tip at the lower end. In the lower part (85 mm) of each strip 16 bi-conical apertures of 1,5 mm diameter are drilled, which are 5 mm apart from each other (see also Figure 1). The apertures should be numbered, starting with number 1 (soil depth = 0,5 cm) and ending with number 16 (soil depth = 8,0 cm).

Bait-lamina strips are commercially available, either with bait-filled or with empty apertures.

6.2 Sharp tool

Tool formed like a thin screw-driver, long nail file or a barbecue skewer which is used to drill a small slit into the soil in which the individual bait-lamina strips is inserted when the soil is too dry or compact.

7 Procedure

7.1 General

When testing the effects of soil contaminants on the feeding activity of soil dwelling organisms, usually the bait strips are exposed at the potentially contaminated sites as well as at a reference site. The latter is defined as a site which in terms of soil properties, land use, or climatic conditions does not differ from the site to be assessed, except that it is not contaminated.

NOTE The use of the bait-lamina test for monitoring the biological status of soils is described in Annex A.

The test should be performed at times of the year where soil organisms are active (i.e. when unfavourable environmental conditions such as low soil water content and/or extremely high temperatures do not drive them into diapause). In detail, a soil temperature of 5 °C to 15 °C is recommended [8]. The soil water content should be sufficient for invertebrate activity (i.e. >20 %). Environmental conditions such as climate or soil water content are expected to strongly influence the results [24], therefore the method should preferably be applied for comparing the biological activity between closely situated plots (e.g. contaminated sites versus reference areas). When performing this test in the field, the site should be characterized (e.g. concerning climate such as precipitation, temperature, and land use). However, such a characterization is not covered by this International Standard.

In addition, pH (ISO 10390), particle size distribution (ISO 11277) dry bulk density (ISO 11272), organic carbon content (ISO 10694), nitrogen content (ISO 13878) actual soil moisture (ISO 11461 or ISO 11465), and water holding capacity (ISO 11268-2) shall be measured. These parameters should be determined in the same soil layer (i.e. the uppermost 8 cm of the mineral soil) or, if applicable, separately in the uppermost mineral soil layer, the organic horizons, and the litter layer, in which the bait-lamina strips are exposed. These parameters have to be determined once at the beginning of the test (exception: soil moisture, this should be measured also at the end of the test).

If the study is performed at a contaminated site, it is recommended to determine the identity and concentration of the main contaminants.

7.2 Preparation of the bait-lamina strips

In the lower part (85 mm) of each bait-lamina strip 16 bi-conical apertures of 1,5 mm diameter are drilled at 5 mm intervals (see also [Figure 1](#)). These apertures are the place where the bait is filled-in. Their bi-conical form secures the stability of the bait-material inside the apertures. Different mixtures can be used as bait-material, but the following mixture is recommended: cellulose (70 %), finely ground wheat bran (25 %), and activated carbon (5 %). The latter component's black colour facilitates the differentiation between the bait material and soil particles when assessing the feeding activity at the end of the test. It is very important to grind the bran as fine and homogenous as possible. Therefore, it is recommended to sieve the grounded bran (mesh size: 0,25 mm). By doing so, not only the homogeneity of the bait-material but also its stability in the apertures of the bait-lamina strips is improved.

The three components of the bait material shall be well mixed before water is added. The amount of water cannot be given in detail but it should be enough in order to create a paste. Bait material shall be sufficiently moist to hold together and fill the apertures of the plastic strip. Too much water can lead to cracking upon drying causing the bait to fall out of apertures. Both, too dry and too wet baits, are not stable. This work shall be performed by hand (see [Figure 2](#)). After air-drying of the strips, they shall be visually inspected. All baits which show holes, small fissures, or ruptures shall be treated with the paste of bait-material again until optically all baits are intact. The whole procedure does secure that the bait-material remains intact inside the apertures of the plastic strips, in particular when they are inserted into the soil. Prepared strips may be stored under dry conditions in plastic bags for up to six months. Before starting a test, their integrity (no mould, holes, fissures, or ruptures in the baits) shall be checked.

Dimensions in millimetres

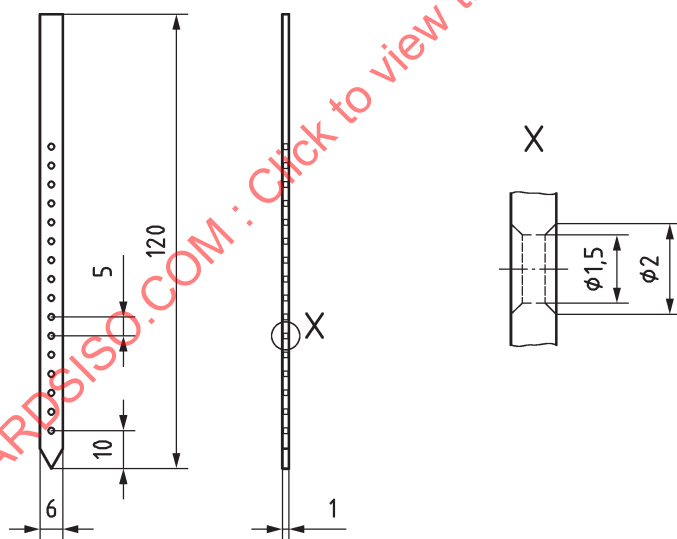


Figure 1 — Schematic view of individual bait-lamina strips^[24]



Figure 2 — Three individual materials (left: activated carbon; above: finely ground wheat bran; below: cellulose) and the mixture of the three components at right: the bait

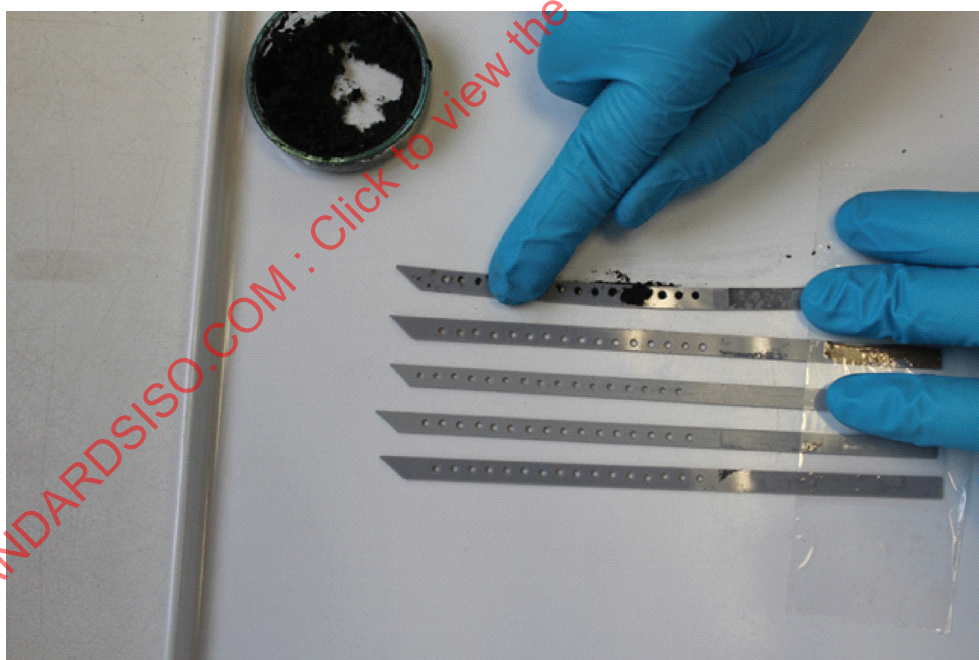


Figure 3 — Filling of bait-material into the bait-lamina strips

7.3 Experimental design and test performance

The prepared bait-lamina strips are exposed in the soil for an appropriate period of time, depending mainly on the climate, the soil properties, and the composition and abundance of the soil organism community at the test site. On average, the exposure time lasts 10 d to 20 d in temperate zones (e.g. References [8] and [12]) and 4 d to 8 d (partly 14 d) in tropical areas, e.g. Reference [30]. However, there are experiences that these numbers can differ considerably: e.g. at coniferous forest sites in Germany, with an acid soil with little macro-fauna, an exposure time of 49 d was used [29], and since

only 13 % were consumed after this period, an even longer time would have been better. In a European ring test performed at various grasslands, exposure times of 56 d and 112 d (including summer) were suitable [12] while in a Polish forest bait-lamina were exposed for 33 d and 42 [32].

Because of these differences, it is recommended to determine the exposure time in a screening or preliminary test, exposing a few bait-lamina strips (e.g. 5 to 10) for different periods of time in order to identify the duration in which at least 30 % but preferably not all bait slits are pierced [11]. For example, at a tropical forest site the strips were exposed for 2 d, 4 d, 8 d, and 12 d. Based on the results of this screening test, the optimal exposure duration was set to 7 d [30].

At the beginning of the test, the strips are inserted vertically by hand in the soil in a way that the uppermost aperture is just beneath the soil surface. The depth of the insertion of the strips shall be determined carefully, since it is often not really clear where the soil surface actually starts [e.g. due to a litter layer or extended roots (turf)]. The main criterion is that each bait aperture is accessible to soil organisms and that the way of exposure of all strips is similar. In dry soils as well as in those with high clay content, the insertion by hand is often not possible. In such cases, a metallic tool shall be used [e.g. a thin screw-driver, nail file, or barbecue skewer (see 6.2)]. However, it shall be ensured that the slits made with this tool are not larger than the thickness of the plastic strip itself. The strips should always be in close contact with the surrounding soil.

Finally, the location of the strips shall be marked since due to their colour and small size individual bait-lamina strips are often difficult to find, especially after long exposure periods (see Figure 4).



Figure 4 — Bait-lamina strips at a forest site, showing how deeply they were inserted and their marking by a band (height of the litter layer: 2 cm to 3 cm)

The question on how many strips have to be used at one site depends very much on the objective of the study as well as the practical circumstances. Most often, the following approach is used, which has originally been proposed by References [8], [35], [36]. It is usually used for the comparison of sites which differ only in one factor (e.g. contamination, management): At each site, at least one plot [size: at least 10 m × 10 m, often much larger (up to 1 ha)], in which three to five subplots of bait-lamina are delimited, each consisting of a group of 16 strips, inserted in a 4 × 4 grid within an area of about 30 cm × 30 cm. Following recommendations by Reference [20], the statistical power is higher by decreasing the number of strips per group (e.g. from 16 to eight), keeping the number of groups of bait-lamina strips per plot similar (e.g. five) and increasing the number of plots (at least three). Such a design has recently been used in the EU project EcoFINDERS when comparing two different agricultural measures at farm sites.

The number of bait-lamina strips and/or of subplots of bait-lamina strips can be increased considerably, meaning that their sampling is performed at several points in time (e.g. once per week). However, this design has not been used in reality yet.

7.4 Examination of the bait-lamina strips

At the end of the exposure period, bait-lamina strips are carefully retrieved from the soil by hand and cleaned from soil or organic particles. Afterwards (still in the field), they are visually assessed by holding the strips against the light and the empty apertures are counted (see [Figure 5](#)). Number and place, i.e. between No 1 (soil depth = 0,5 cm) and No 16 (soil depth = 8,0 cm) are recorded immediately in a form. An aperture is counted as “pierced” if at least half of the bait has been taken. Measurement parameters are the total number and the vertical distribution of empty apertures:

- the total number of “pierced” bait (i.e. apertures from which at least half of the organic bait material has been removed) is taken as a measure of the feeding activity, often given in per cent of the total of filled apertures for a group of strips exposed together (e.g. per subplot or plot);
- the vertical distribution of the feeding activity, i.e. the number of “pierced” baits at a given soil depth, e.g. per cm depth, or, more often, per upper (0,5 cm to 4,0 cm soil depth) versus lower (4,5 cm to 8,0 cm) soil layers.

NOTE 1 Either in the field or in the laboratory the visual assessment can be facilitated by using a portable or bench-top light box for examining the strips. However, experience shows that this is not essential.

NOTE 2 The number of pierced baits is five, four, and seven (from top to bottom).

Utilized bait-lamina strips can be reused if refilled after soaking and cleaning in water. If used at contaminated sites, cleaning in hydrochloric acid (10 %), followed by two runs in water, is recommended. In case of doubt, used strips should be disposed.



**Figure 5 — Visual examination of an individual bait-lamina strip at the end of a test
(Photograph: H. Hoefer)**

8 Expression of results

In the bait-lamina test, the main end point is the feeding activity. Results of the bait-lamina test are usually expressed as follows:

- description of the mean feeding activity ($\pm$ standard deviation) as percentage of pierced baits, e.g. on reference versus impacted plots (typically in a barplot);
- visualization of the amount of feeding in the various depths, i.e. between the soil surface and the soil in a depth of 8 cm.

Examples are given in [Annex B](#).

9 Validity of the test

In contrast to other field studies, e.g. the earthworm field test [37], information on the “normal” operative range (NOR) of the feeding activity is still limited, since it depends on various abiotic (e.g. soil properties, climate) and biotic (e.g. the composition of the soil organism community) factors. In the meantime, the following criterion is proposed in order to consider a bait-lamina test as valid: at least 30 % of the baits are pierced at one of the 16 depth levels of the reference plots.

10 Statistical analysis

The main end point is the feeding activity. It can be assessed either as total rate or as depth profile measured at a potentially contaminated site in comparison to a reference site. The GLM (General Linear Model) analysis for binomial distribution is the preferable approach for data evaluation. However, depending on the design used, different statistical options are possible. For example, effects of management activities on total feeding activity at one site can be compared with a reference site by using an ANOVA. Effects of management on the vertical distribution of feeding activity and its interactions with the respective site can be tested in a two-way ANOVA using the mean portion of empty apertures over all groups per plot as dependent variable, plot and site condition (reference or potentially contaminated) as categorical variables and depth (hole 1 near surface to hole 16 in approximately 8,5 cm depth) as covariate, e.g. Reference [30]. The quantification of this measurement end point may be interpreted as follows: feeding activity is adversely affected when the total feeding activity or its depth distribution in treatment plots is statistically different from the activity in the reference plots.

11 Test report

The test report shall include the following information:

- a) a reference to this International Standard, i.e. ISO 18311:2016;
- b) a full description of the study design and procedures;
- c) characterization of the test site (especially soil properties);
- d) details of the test performance (especially a detailed description of the bait material used);
- e) description of the test conditions, including date and duration of the test in the field and climatic parameters like air temperature during the test period;
- f) in case the study was performed as part of an environmental risk assessment (ERA): number and concentrations of main contaminants;
- g) number of apertures emptied including their vertical distribution;
- h) details of the statistical analysis;

- i) all information, including all measured raw data and all problems which might have occurred, developed during the study;
- j) discussion of the results.

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Annex A (informative)

Use of the bait-lamina method for monitoring the biological quality of soils

The performance of the bait-lamina test in monitoring programs does not differ from the description in [Clauses 5](#) to [8](#). However, since there are not two different conditions (reference versus potentially contaminated plots), the design of the test (and thus also the assessment of the results) shall be modified.

Depending on the aim of the specific monitoring program, the design can differ considerably (an overview of various possibilities is given in ISO 23611-6). However, in order to facilitate the usage of this method, the design of three case studies is given.

- a) The bait-lamina test has been used for the description of the biological status of 30 forest sites, belonging to the Atlantic Coastal Rain Forest of Brazil. In detail, four forest regeneration stages were replicated three times, and these 12 sites were identified in two forest regions being 50 km apart. In addition, six agroforestry (mainly banana) sites in a third region were studied. In each forest, plots measuring 30 m × 50 m were selected [\[6\]](#). On each plot, 10 individual subplots at 3 m to 5 m apart were selected (e.g. arranged in two transects), each one consisting of a group of five bait-lamina strips (i.e. in total, 50 strips were used per plot). Each subplot had a size of 20 cm × 20 cm, with four strips at the corners and the fifth one in the middle of the square. The total feeding activity at different sites was compared statistically by using an ANOVA, followed by a Bonferroni Post-Hoc-Test in case significant differences did occur.
- b) The feeding activity of soil fauna was studied in spring and autumn of two consecutive years in three regions in southwest, central and northeast Germany at 12 to 20 plots per region, each with a size 5m × 5 m [\[3\]](#). Per plot, four to eight bait-lamina sticks were put into the soil. After 14 d, all 1 024 sticks were removed. In parallel, soil properties, climatic variables, vegetation characteristics, and soil fauna abundance (earthworms, Collembola, Enchytraeidae) were determined at these plots. The relationship between feeding activity and abiotic and biotic predictors was assessed using distance-based linear models, followed by structural equation model to test for multiple causal pathways. So far, this study seems to be the most elaborated investigation to identify those factors determining soil organism feeding activity.
- c) Reference [\[32\]](#) studied the influence of 14 tree species on the feeding rate of soil organism at two sites (each 180 m × 60 m) in a forested area of southwestern Poland. Each site was divided into 27 plots (20 m × 20 m) and in each plot 50 bait-lamina sticks were inserted into the soil. After an exposure period of 42 d (in summer) and 33 d (in autumn) all sticks were removed and assessed. Thus, 3 to 6 replicates per tree species, each representing the mean feeding rate of 50 sticks, were analysed. A GLM analysis with the Tukey post hoc test was used. In addition, an index of average depth of trophic activity at a plot was used [\[20\]](#).

In the case of using the bait-lamina test in soil monitoring, results are usually discussed in relation to soil and site properties (i.e. which biotic or abiotic factors determine the feeding activity?). An absolute assessment is only possible if regional and/or soil specific reference ranges are available. However, information on such ranges is very limited so far.

Annex B

(informative)

Examples are taken from one study performed in the Brazilian Amazon forest in which the removal of the litter layer was used as stressor^[30]

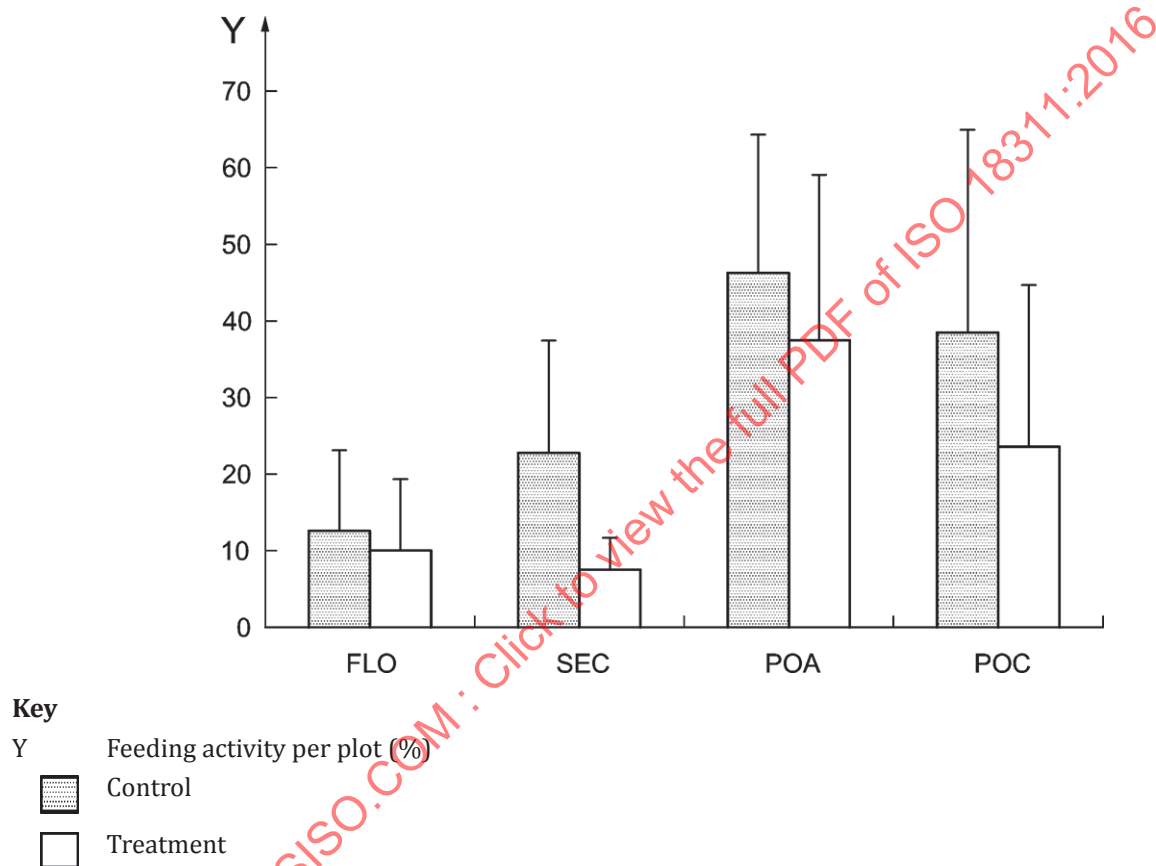
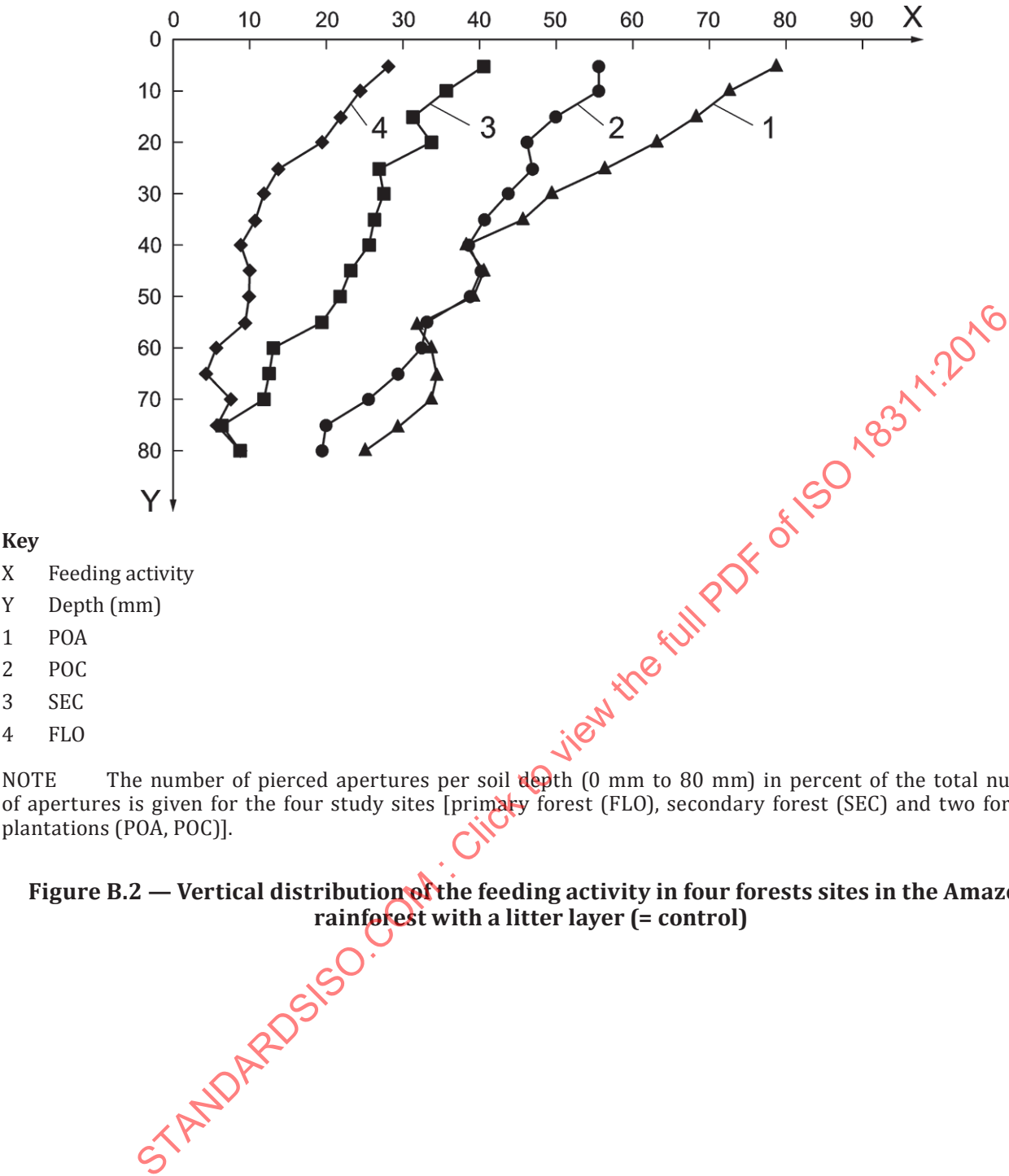
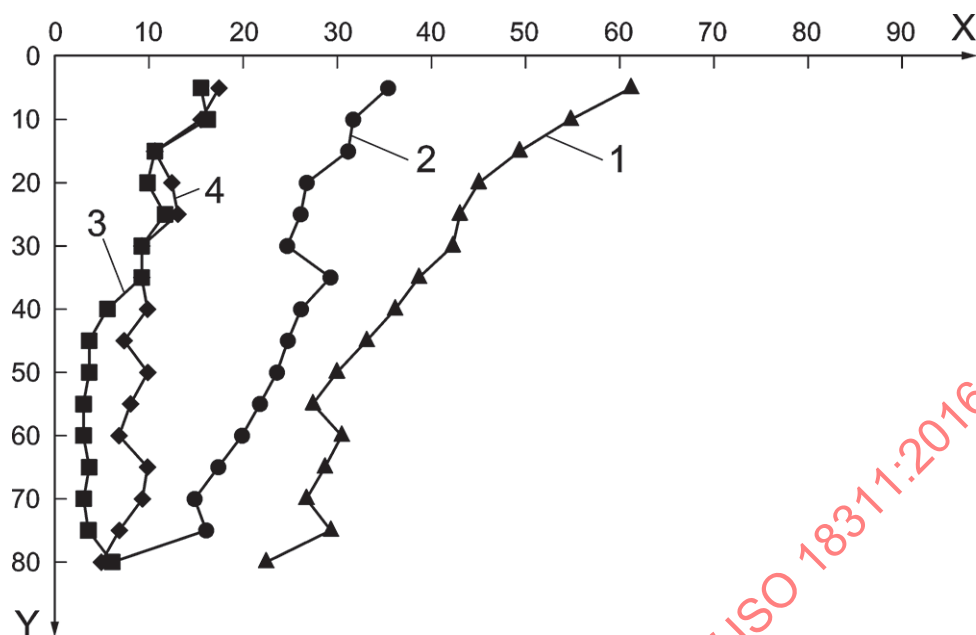


Figure B.1 — Feeding activity (mean of 10 subplots $\pm$ SD) in four forests sites in the Amazon rainforest with (= control) and without (= treatment) a litter layer: primary forest (FLO), secondary forest (SEC) and two forestry plantations (POA, POC), measured as the proportion of apertures emptied of the total number of bait-lamina apertures^[30]



**Key**

X Feeding activity

Y Depth (mm)

1 POA

2 POC

3 SEC

4 FLO

NOTE The number of pierced apertures per soil depth (0 mm to 80 mm) in percent of the total number of apertures is given for the four study sites [primary forest (FLO), secondary forest (SEC) and two forestry plantations (POA, POC)].

Figure B.3 — Vertical distribution of the feeding activity in four forests sites in the Amazon rainforest without a litter layer (= treatment)