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# State of knowledge of earthworm communities in German soils as a basis for biological soil quality assessment

**Stephan Jänsch[1,*], Louise Steffens[1], Hubert Höfer[2], Franz Horak[2], Martina Roß-Nickoll[3], David Russell[4], Ulrich Burkhardt[4], Andreas Toschki[5] and Jörg Römbke[1,6]**

[1] ECT Oekotoxikologie GmbH, Böttgerstrasse 2–14, 65439 Flörsheim am Main, Germany
[2] State Museum of Natural History Karlsruhe, Dept. Biosciences, Erbprinzenstr. 13, 76133 Karlsruhe, Germany
[3] RWTH Aachen University, Institute for Environmental Research, Worringerweg 1, 52074 Aachen, Germany
[4] Senckenberg Museum of Natural History Görlitz, Dept. Soil Zoology, P.O. Box 300154, 02806 Görlitz, Germany
[5] gaiac Research Institute for Ecosystem Analysis and Assessment, Kackertstr. 10, 52072 Aachen, Germany
[6] Biodiversity and Climate Research Centre BiK-F, Senckenberganlage 25, 60325 Frankfurt/Main, Germany
* Corresponding author, e-mail: s-jaensch@ect.de

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## Abstract

Within a project aiming to improve the preconditions for the protection of the habitat function of soils in Germany, the database Bo-Info was established, in which soil-biological data from permanent soil monitoring sites of several German states as well as from the literature was compiled. Soil-biological data on the occurrence and abundance of earthworms were analysed with respect to their distribution and relation to site (habitat type, land use) and soil properties (pH, texture, organic matter). Reliable data for earthworms were available from 294 sites. In total, 32 species (all species known to occur in Germany) were present in the database, 10 of which were very common. Ecological preferences regarding land use, pH, soil organic matter (SOM) and texture were derived for these 10 species. The occurrence of earthworms at the species and ecological-group level is determined by land use and soil pH value and less by soil texture. A clear distinction between epigeic species on the one hand and endogeic and anecic species on the other hand was found regarding SOM. Earthworm communities of habitat types representing the four major land use types (grassland, crop sites, deciduous and coniferous forests) clearly differed. Using three examples from different land use forms, typical species could be identified at the next sub-division level of habitat types, provided a sufficient number of data was available. As a result, qualitative expectation (= reference) values (species richness and composition) are proposed for the most important habitat types (e.g. different types of crop sites, grassland and coniferous forests). Due to their ecological relevance, the use of earthworms for soil biological site classification and assessment is recommended.

**Keywords** Biogeography | habitat function | Lumbricidae | Oligochaeta | permanent soil monitoring sites | reference system


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# 1. Introduction

## 1.1. Background

Soils are an essential component of terrestrial ecosystems. They host highly diverse organism communities organized in complex food webs that strongly contribute to natural soil functions (De Ruiter et al. 1993, Ekschmitt & Griffiths 1998, Bardgett et al. 2005, Brussaard et al. 2007, Turbé et al. 2010, Mulder et al. 2011). Despite this high ecological significance the structural and functional diversity of soil organisms, and thus the biological quality of soils, is insufficiently protected thus far (van Camp et al. 2004). In Germany according to § 2 of the German Federal Soil Protection Act (BBodSchG 1998) the habitat function of soils must be protected but specifications on how to fulfil this obligation are missing in the follow-up Federal Soil Protection Ordinance (BBodSchV 1999). In some German federal states, abiotic (in particular pedological) parameters are used to assess the biological soil quality (Blossey & Lehle 1998). However, this indirect approach is not sufficient because soil biodiversity itself as well as biological soil quality can only be effectively assessed using biological parameters (Ekschmitt et al. 2003, Beylich et al. 2005). Hence, since the late 1990s several research projects have been conducted in Germany at both the state and the federal level to create the basis for a soil biological classification and assessment system (e.g., Römbke et al. 2002a), thus following-up a long tradition of biological soil assessment (e.g., Volz 1962). In parallel, similar concepts have also been developed in other countries, often taking limnological assessment approaches as an example (in particular the British RIVPACS; Wright 2000). In recent years essential contributions to a biological soil assessment were made in the Netherlands; e.g., incorporating the use of microbial parameters (Bloem et al. 2006) or defining reference sites (Rutgers et al. 2008). These authors mostly suggest a 'battery-approach' using several invertebrate groups as well as microbial parameters for the assessment of soil quality (Römbke & Breure 2005a,b). A general agreement also exists that an assessment should best be performed using previously defined reference values. Similar conceptual approaches to the definition of reference states for soil organism communities (specially for arthropods) are also presented by Roß-Nickoll et al. (2004), Toschki (2008) and in the scope of a guideline on the monitoring of effects of genetically modified organisms (GMO) on soil organisms by the Association of German Engineers (VDI; Ruf et al. 2013).

Under the responsibility of the German federal states about 800 permanent soil monitoring sites (Bodendauerbeobachtungsflächen; BDF) have been installed. The primary purpose of these is the characterization of soil conditions and their changes due to external impacts (Werner 2002). 344 BDF are located in agricultural, 146 in grassland and 247 in forest sites, the remaining are located in special habitat types. Since 1990 there is an International Organization for Standardization (ISO) guideline (ISO 2004) addressing the selection of BDF (e.g., their representativeness for land use, landscape and European climatic regions). There are also proposals on biological parameters to be investigated in BDF (Barth et al. 2000), but so far there is no generally accepted approach to this field. Usually only isolated parameters (in particular microbial respiration and diversity of lumbricid earthworms) are recorded, but not in all federal states and at irregular intervals (UBA 2007). For further details regarding the use of BDF for biological soil quality assessments see Römbke et al. (2012).

## 1.2. Present state of knowledge of European earthworms

Earthworms belong to the saprophagous soil macrofauna. About 6,000 species are known worldwide, roughly 670 of which belong to the family of Lumbricidae (Blakemore 2003). Since the beginning of studies on soil biology over 100 years ago, earthworms are considered to be the most important soil animals in many Central European habitats. This appraisal is based on their high biomass as well as their strong contribution to ecologically and agronomically important functions. These include the bioturbation of soils, the acceleration of soil organic matter (SOM) decomposition (and thus the enhancement of nutrient supply for plants) as well as the improvement of the water holding capacity of soils by generating clay-humus-complexes (Darwin 1881, Petersen & Luxton 1982, Satchell 1983, Lee 1985, Edwards & Bohlen 1997, Edwards & Shipitalo 1998). These functions are often performed by a few key species such as the ecosystem engineer *Lumbricus terrestris* in temperate regions (Lavelle et al. 1997). For many species occurring in Central Europe, comprehensive autecological, synecological and ecotoxicological data are available (Lee 1985, Briones et al. 1995, Edwards & Bohlen 1997, Edwards 1998, Jänsch et al. 2005) and there is a standard sampling guideline (ISO 2006). Earthworms are generally divided into three ecological groups (Bouché 1977): mineral dwellers (= endogeics), litter dwellers (= epigeics) and vertical burrowers (= anecics). The primary environmental factors known to determine the distribution of this organism group are pH-value, texture, soil moisture and nutrient availability (Satchell 1983, Lavelle et al. 1997). In general, earthworms are well suitable as bioindicators of soil habitat functioning (Paoletti 1999, Didden 2003, Jänsch et al. 2005, Römbke et al. 2005, Fründ et al. 2011). The taxonomic status of some genera and species is continuously under revision. Especially the latest developments in molecular taxonomy using barcoding will lead to further changes and diversification of taxa. A historic overview and a topical introduction to the classification of lumbricids is given by Csuzdi & Zicsi (2003) and Blakemore (2003).

### 1.3. Research aims

In 2009, the authors of this contribution began a project supported by the German Federal Environmental Agency which intended to improve the protection of the habitat function of soils within the scope of the German 'National Strategy for Biological Diversity', e. g. by broadening soil biological monitoring at existing BDF. The aim of this project was to improve the preconditions for the protection of the habitat function of soil as described in § 2 of the German Federal Soil Protection Act (BBodSchG 1998), in particular in two ways: first, to identify suitable biological indicators (i.e. organism groups) for the assessment of soil quality. Second, to establish reference values useful for selected habitat types in order to help evaluate whether a soil fulfils the habitat function or not. The main activity of this project was the establishment of a database, called Bo-Info, in which the existing information on certain soil invertebrates were compiled (Römbke et al. 2012). In the meantime, these data have been transferred to the Senckenberg 'Edaphobase' database (www.edaphobase.org; Burkhardt et al. 2013), also visible in the Global Biodiversity Information Facility (GBIF; www.gbif.org). Results for enchytraeids are given by Römbke et al. (2013). This contribution focuses on earthworms. In detail, the aims of this paper are:

- to describe the actual status of earthworm biodiversity in Germany;
- to compile ecological profiles and compare the 10 most common earthworm species in order to identify potential indicator species;
- to derive reference values for the earthworm community for different levels of habitat types;
- to prepare recommendations for the improvement of biological soil monitoring.

## 2. Material and Methods

### 2.1. Data basis

Data on the occurrence of earthworms in Germany including information on site and soil properties were collected in the above-mentioned Bo-Info database. Four site and soil properties (major land use type, pH-value, soil texture and SOM content) which potentially influence the distribution of earthworms were classified into four to five categories each (Römbke et al. 2002a). Abiotic data measurement usually followed current German Institute for Standardisation (DIN) or ISO guidelines (Barth et al. 2000), especially from BDF, but in several cases the respective method was not indicated in the literature. When stated, soil properties were determined in the uppermost mineral soil layer (A-horizon, 5–30 cm), for pH sometimes additionally in the litter layer. Soil pH was usually measured in $CaCl_2$ solution. Thus, some uncertainty due to possibly differing methods needs to be accepted when allocating sites to the classes of abiotic factors. 547 sites (including 97 BDF) were covered, yielding about 14,000 datasets, 4,000 of these from BDF. The latter were contributed by the federal states of Brandenburg, Hamburg, North Rhine-Westphalia, Schleswig-Holstein and Thuringia. Earthworm data from the BDF of Bavaria (primarily crop and grassland sites; cf. Bauchhenss 1997) and Saxony-Anhalt (Tischer 2007) were not yet available for evaluation, while for the remaining federal states without lumbricid data it is assumed that no such data exists. The geographical distribution of the sites with available earthworm data is depicted in Fig. 1. As already mentioned data from BDFs is restricted to five German federal states which become most obvious with the distribution of crop sites, i.e. the different habitat types were not evenly sampled across Germany. With the exception of Bavaria and Saxony-Anhalt, 'blind spots' may be attributed to the lack of sampling on BDF of these federal states, but also to the lack of research projects in those regions. Thus, the overall sampling density is still unsatisfactory even for this relatively well-studied organism group.

After data compilation a reliability check was performed. In analogy to the classification system of Klimisch et al. (1997), data were classified as either (I) reliable, (II) reliable with restrictions, or (III) not reliable, using the following criteria:

I. Reliable: data from BDF or peer-reviewed publications; earthworm sampling by hand-sorting in combination with formol or electro (octet) extraction; site georeferenced; sampling date specified; comprehensive abiotic site characterization;

II. Reliable with restrictions: data from non peer-reviewed publications, reports or museum collections; earthworm sampling only by either hand-sorting or formol extraction; site georeferenced; sampling date specified; gaps in abiotic site characterization;

III. Not reliable: earthworm sampling only by electro (octet) extraction; site not georeferenced; sampling date not specified (e.g. mean values for several years); documentation not convincing for an expert judgment.

Subsequently, data from 294 sites were classified as being suitable for further assessment (i.e. belonging to Class I or II).

## 2.2. Reference system

In order to facilitate the use of biocoenotical data at the landscape level, a standard frame of reference is needed. Without this no comparable assessments of biodiversity and the factors influencing a biocoenosis are possible due to the heterogeneity of the landscape. In order to operationalize the assessment of biodiversity, a site-specific reference system was developed. Sites were classified into habitat types, based on soil and site parameters, and for each habitat type a list of earthworm species expected to occur or to be absent was established. These presence/absence lists of species serve as reference values for the sites to be evaluated. Significant deviations from these reference values are considered as an indication for an impacted habitat function (Fig. 2).

In order to develop reference values, i.e. to link the soil and site parameters and the occurrence of soil organisms, the landscape had to be classified into a limited number of ‘site categories’. For this, the habitat classification concept, compiled in the ‘German Red Data Book on endangered habitats’, was used (Riecken et al. 2006, 2009). It comprises 44 basic (first level) types with approximately 1,000 hierarchically derived sub-types. This concept is already accepted by the German authorities and has been used in the areas of the European Habitats Directive (EU 1992), nature conservation management, GMO authorization and prospectively also pesticide registration. From the habitat-type list, 21 basic types with 525 sub-types were identified as being relevant for the classification of soil organisms including earthworms.

Most sites with data on the occurrence of lumbricids could be allocated to only four of these 21 basic habitat types, representing the four major land use types (Table 1).

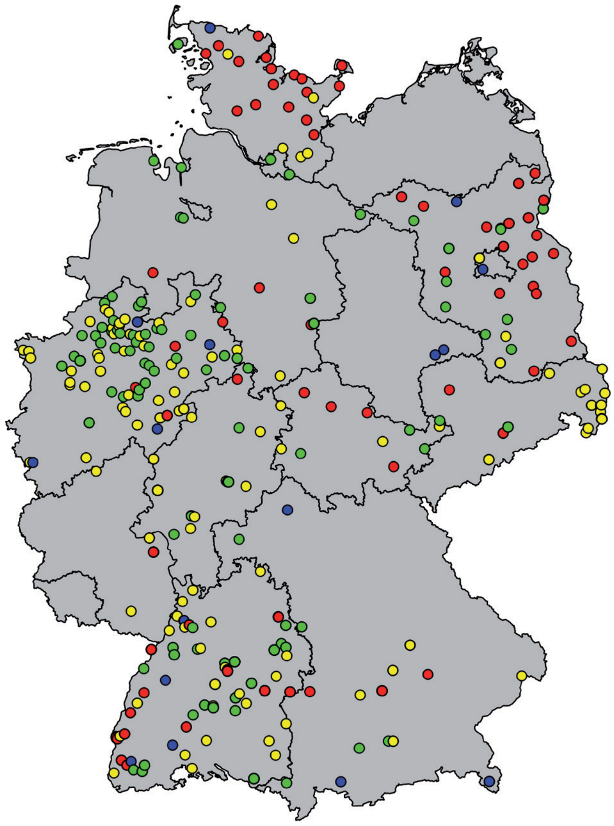

**Figure 1**. Sites with data on earthworm occurrence depending on habitat type. **Red** – crop sites, **green** – grassland, **yellow** – deciduous forest, **blue** – coniferous forest.

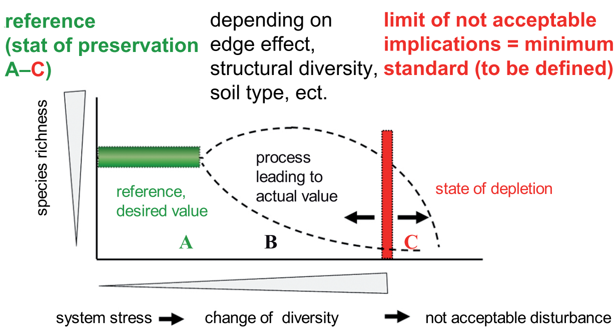


**Figure 2**. Principle of threshold values in regard to a system of reference values: **A**, **B** and **C** correspond to different states of preservation related to increasing system stress (e.g. European Habitats Directive, EU 1992).

## 2.3. Evaluation strategy

### 2.3.1. Ecological profiles of single species

Based on the classification of abiotic site and soil properties, ecological profiles were created for the 10 most common species. Earthworm taxonomy was based on the key of Sims & Gerard (1999) that includes all species relevant to Central Europe. The relative

**Table 1**. Habitat types, derived from the German Red Data Book on endangered habitats (Riecken et al. 2006, 2009), used in this study for the establishment of a reference system to evaluate the biological state of the soil. **Bold** – types at first hierarchical level. **Normal** – types at second hierarchical level.

| Habitat type number | Description |
|---|---|
| **33** | **Arable and fallow land (in the following abbreviated 'arable land')** |
| 33.01 | Farmed and fallow land on shallow skeletic calcareous soil |
| 33.02 | Farmed and fallow land on shallow skeletic silicaceous residual soil |
| 33.03 | Farmed and fallow land on sandy soil |
| 33.04 | Farmed and fallow land on loess, loam or clay soil |
| 33.05 | Farmed and fallow land on peaty or half-bog soil |
| **34** | **Natural dry grasslands and grasslands of dry to humid sites (in the following abbreviated 'grassland')** |
| 34.01 | Xeric grassland |
| 34.02 | Semi-dry grassland |
| 34.03 | Steppic grassland (subcontinental, on deep soil) |
| 34.04 | Dry sandy grassland |
| 34.05 | Heavy-metal grassland |
| 34.06 | Mat-grass swards |
| 34.07 | Species-rich grassland on moist sites |
| 34.08 | Species-poor intensive grassland on moist sites |
| 34.09 | Trampled grass and park lawns |
| **43** | **Deciduous and mixed woodlands and forest plantations (deciduous share >50 %) (in the following abbreviated 'deciduous forest')** |
| 43.01 | Birch bog woodland |
| 43.02 | Carr woodland |
| 43.03 | Swamp forest (on minerogenic soil) |
| 43.04 | Alluvial forest |
| 43.05 | Tidal alluvial forest |
| 43.06 | Ravine, boulder-field and scree forests |
| 43.07 | Deciduous and mixed forest on damp to moist sites |
| 43.08 | Deciduous (mixed) forest on dry or warm dry sites |
| 43.09 | Deciduous (mixed) plantations with native tree species |
| 43.10 | Deciduous (mixed) plantations with introduced tree species (including subspontaneous colonisations) |
| **44** | **Coniferous (mixed) woodlands and forest plantations (in the following abbreviated 'coniferous forest')** |
| 44.01 | Bog woodland (coniferous) |
| 44.02 | Natural and near-natural dry to intermittently damp pine forest |
| 44.03 | Spruce/fir (mixed) forest and spruce (mixed) forest |
| 44.04 | Coniferous (mixed) plantations with native tree species |
| 44.05 | Coniferous (mixed) plantations with introduced tree species (including subspontaneous colonisations) |

frequency of occurrence (%) for a given species within the classified site factors was evaluated. This way the preferences or tolerances regarding these factors of each species as well as their overall frequency could be assessed without introducing skewed results due to observational bias. Differences in occurrence between different factor classes were analysed using the Chi²-test with Bonferroni correction indicating whether a given factor had a statistically significant influence on the distribution of a species within the site- or soil-property categories. Thus, a total of 40 analyses were performed (4 factors for 10 species). Due to the high number of resulting figures, it is not possible to present the ecological profiles of all 10 species here in detail and these are included as supplementary data provided online at www.soil-organisms.org. No statistical analysis was conducted for the occurrence of species regarding the second level of habitat types as the number of available sites was too small (often n < 10).

In order to compare the preferences or tolerances of the 10 most common species evaluated, they were depicted in one diagram per ecological factor. The data basis was the relative frequency of each species regarding sites for each factor class (supplementary data). These were stacked and normalized to 100%, thus representing the theoretical overall distribution of a species while assuming an even number of sampled sites for each factor class (Formula 1). From the proportion of each factor class, the preference or tolerance of each species regarding the factors can be estimated and allows a comparison of the ecological profiles of all species. Thus, ecologically similar species can be grouped. However, it must be kept in mind that in this data representation, the information on the absolute frequency of occurrence for a species is lost.

Formula 1: $$Z_i = \frac{x_i/y_i}{\sum_{i=1}^{n} x_i/y_i} * 100$$

With: $Z_i$ = Relative proportion of species records from sites belonging to factor class i
$x_i$ = Absolute number of species, records in sites belonging to factor class i
$y_i$ = Absolute number of sampled sites belonging to factor class i

### 2.3.2. Derivation of reference values

Using the relative frequency of individual species (i.e. presence/absence data), those species (and thus ultimately communities) were identified that can be expected to occur or to be absent at sites belonging to specific habitat types, which means that the resulting community is unaffected by contamination or other forms of anthropogenic stress other than the land use itself (see also Chapter 2.2). Due to the heterogeneous data basis results from sampling campaigns had to be integrated that strongly differed regarding sample size, sample number and number of sampling dates. Hence, it could not be assumed that there was an equal chance of subdominant and recedent taxa to be represented between different sampling campaigns. For this reason, reference values were only defined for the 10 most common species that are either expected to be present or absent at a certain habitat type. As a criterion for being a typical species for a specific habitat type, an occurrence at more than 50% of all sites belonging to that habitat type was used. Expecting absence of species from a specific habitat type was based on 0% occurrence at the analysed sites that belong to this habitat type. This was first performed for the basic habitat types representing the four major land use types. The results of the statistical evaluation regarding differences in occurrence between the land-use types from the ecological profiles of the species (see chapter 2.3.1.) were also considered here. Additionally, the average of species number and abundance at sites belonging to one habitat type were calculated. For the calculation of the average abundance only animals determined to species level are included, i.e. most juvenile specimens are missing. In a second step, this exercise was repeated for the second level of habitat types given a sufficient availability of data.

Principal component analysis (PCA) was performed for the four major habitat types using Canoco for Windows Version 4.56 (Ter Braak & Šmilauer 2009) in order to determine whether complete biocoenoses can differentiate between habitat types and to validate the derived reference values. In contrast to the reference values that were based only on species occurrence, the mean abundance per site of all earthworm taxa determined to species level (28 species) was used, disregarding species occurring at only one site (singletons) and omitting data considered as not reliable (see chapter 2.1). As supplementary ecological information, data for pH value, SOM content, C/N ratio and texture (as percentages of sand, silt and clay) were included in the plots but did not contribute to the site ordination. Only species with a contribution of >15% to the ordination were displayed. For crop sites having the best data availability and offering three second-level habitat types for comparison, a PCA was also performed based on the abundance on those 13 species occurring at these sites. Here, texture was not included in the diagram as this was only available for few sites. Also, for habitat type 33.01 no data on C/N ratio and SOM were present, which needs to be kept in mind when interpreting the plot.

## 2.4. Case study: comparison between contaminated and reference sites

In order to evaluate the relevance of the reference values derived from the Bo-Info database regarding the soil quality assessment of specific sites, the earthworm community of two contaminated grassland sites were compared to these values. The sites were located in Gorleben and Nordenham (Lower Saxony, Germany). Their soil properties were similar, so they were therefore allocated to the same soil factor classes: pH 5.5 and 5.3, SOM content 16.5 and 11.5 % and silty clay texture. For Gorleben a lead content of 320 mg/kg, a copper content of 360 mg/kg and dioxin at about 280 ng/kg were measured. For Nordenham the corresponding values were 340 mg/kg (lead), 150 mg/kg (copper) and 45 ng/kg (dioxin). The heavy metal pollution thus clearly exceeded the precautionary values of the German Federal Soil Protection Ordinance (BBodSchV 1999).

# 3. Results and discussion

## 3.1. Species number and their distribution according to soil and site properties

The number of sites with reliable biological data and data for those factors most frequently present in the database are displayed in Fig. 3: basic habitat type, corresponding to the four major land-use types, pH-value, soil texture and SOM content. 226 of the 294 sites belonged to one of the four basic habitat types. The pH value, texture and SOM content were measured at 181, 169 and 122 sites, respectively. The majority of sites are crop sites (86), followed by deciduous forests (65), grassland (48) and coniferous forests (27), the last of which are clearly underrepresented. This may be attributed to the fact that this forest type is mainly found in Germany on acidic soils where earthworms are naturally less diverse and abundant. This is confirmed by the low number of sites with a pH-value less than 4.5 (22) and less than 3.6 (27), while the other three classes are more and equally frequent (42–45 sites). For soil texture the number of sites with earthworm data is highest for loam (61) and sand sites (54), followed by silt (34) and clay (20). Finally, sites with an SOM content of 4.1–8.0 % are fewest (22), while the remaining three SOM classes are evenly distributed (31–35 sites).

A total of 32 valid lumbricid species was recorded from German soils. In the following, the 10 species with the best data availability are presented (Table 2). There is one species each of the genera *Allolobophora*, *Dendrobaena*, *Dendrodrilus* and *Octolasion*, and three species each of *Aporrectodea* and *Lumbricus*. Regarding the ecological groups these species represent two anecic, four endogeic and four epigeic species. The overall frequency of occurrence varies strongly: *A. longa* was least frequently found (24 sites) and *A. caliginosa* most frequently (148 sites), followed by *L. rubellus* (115 sites). The individual ecological profiles for these species regarding geographical distribution and the factors habitat type, pH-value, texture and SOM content can be found in the supplementary data.

## 3.2. Preference in relation to habitat type

The occurrence of individual earthworm species differs strongly among the four major habitat types (Fig. 4). Three groups of species can be identified:

- species occurring mostly at crop and grassland sites (together >70 %): *A. chlorotica*, *A. caliginosa*, *A. longa* and *L. terrestris*, i.e. two anecic and

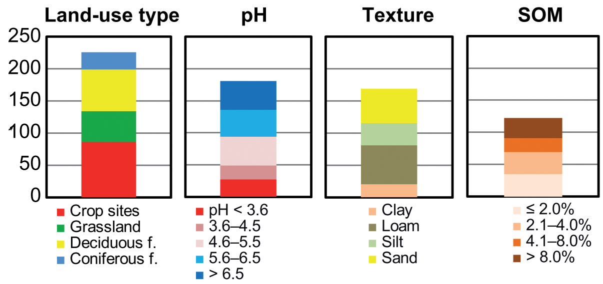


**Figure 3**. Number of sites with earthworm data and data for those factors most frequently present in the database: habitat types, pH-value, soil texture and soil organic matter (SOM) content.

**Table 2**. Earthworm species most common in Germany that were ecologically characterized with number of sites records and ecological group.

| Species | Number of sites with reliable records | Ecological group |
|---|---|---|
| *Allolobophora chlorotica* (Savigny, 1826) | 44 | endogeic |
| *Aporrectodea caliginosa* (Savigny, 1826) | 148 | endogeic |
| *Aporrectodea longa* (Ude, 1885) | 24 | anecic |
| *Aporrectodea rosea* (Savigny, 1826) | 104 | endogeic |
| *Dendrobaena octaedra* (Savigny, 1826) | 76 | epigeic |
| *Dendrodrilus rubidus* (Savigny, 1826) | 48 | epigeic |
| *Lumbricus castaneus* (Savigny, 1826) | 36 | epigeic |
| *Lumbricus rubellus* (Hoffmeister, 1843) | 115 | epigeic |
| *Lumbricus terrestris* Linnaeus, 1758 | 99 | anecic |
| *Octolasion tyrtaeum* (Savigny, 1826) | 59 | endogeic |

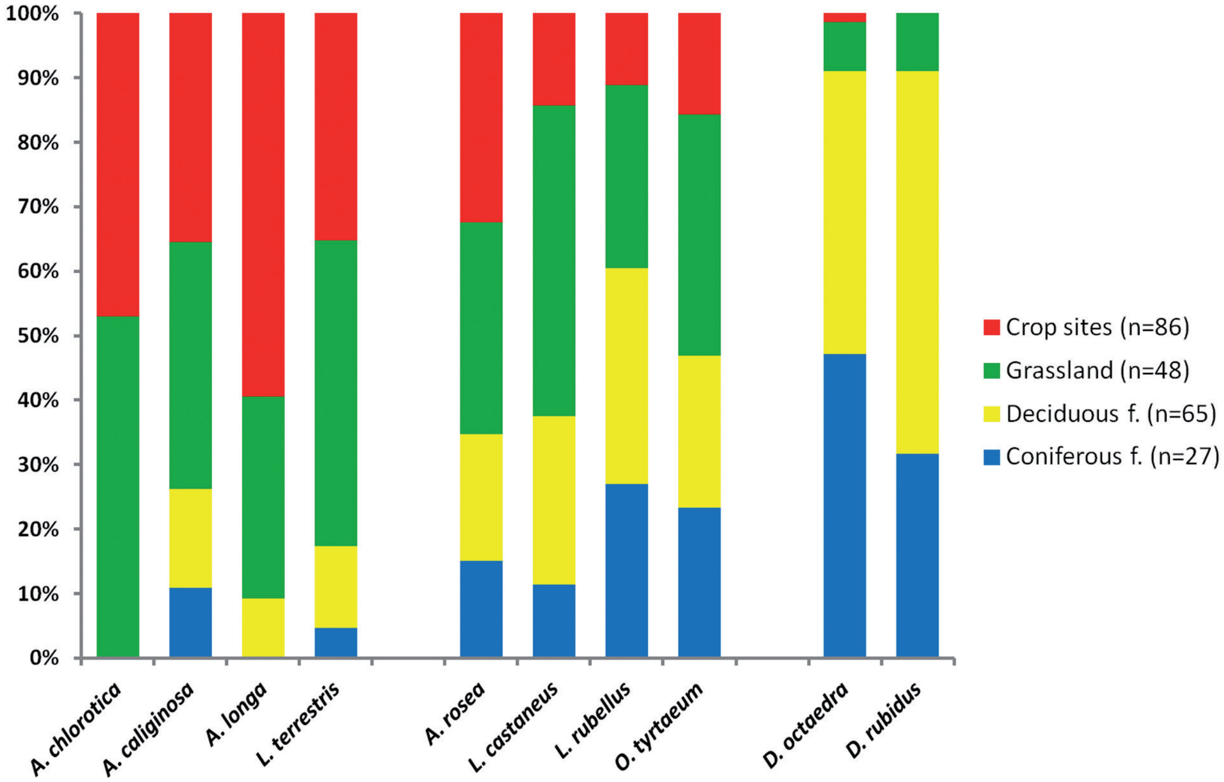


**Figure 4**. Preference of 10 lumbricid species in regard to the four major habitat types.

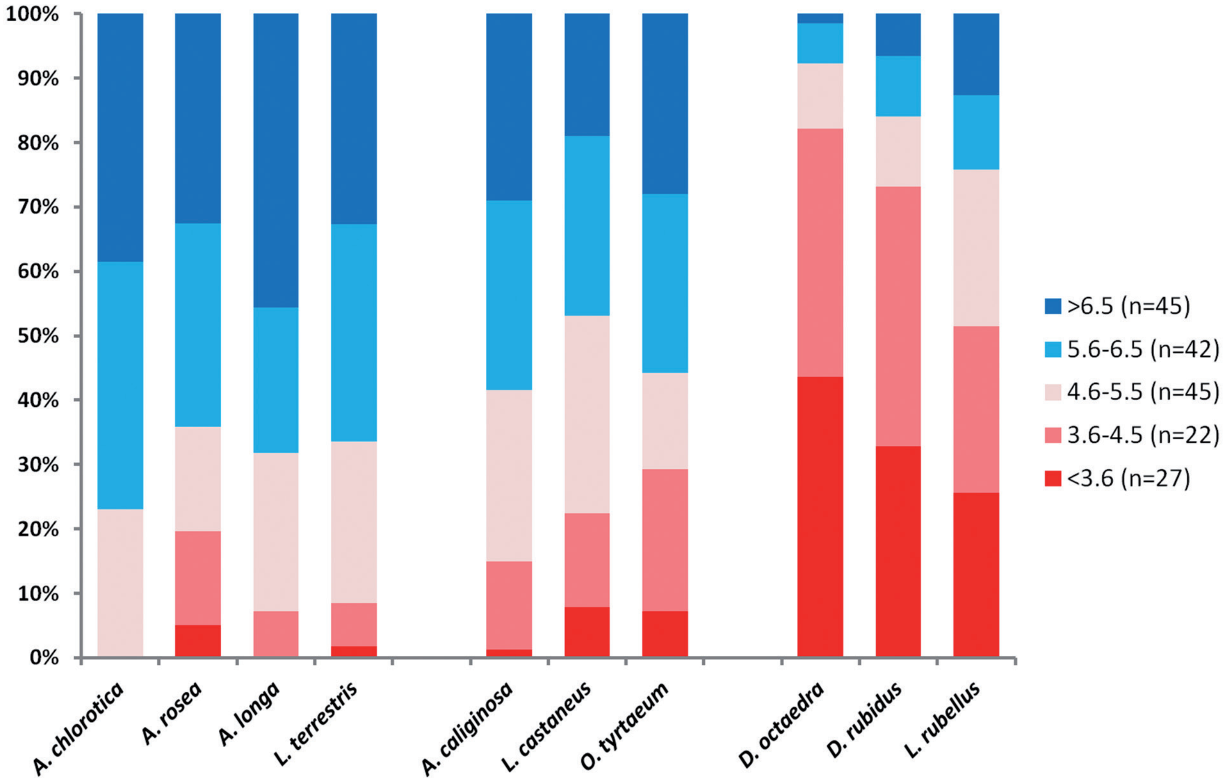


**Figure 5**. Preference of 10 lumbricid species in regard to five classes of soil pH-value.

two endogeic species. A preference for crop and grassland is most pronounced for the species *A. chlorotica*, a species known to prefer moist habitats (Graff, 1953), that does not occur at forest sites at all;

- species without a clear preference: *A. rosea*, *L. castaneus*, *L. rubellus* and *O. tyrtaeum*; thus, two endogeic and two epigeic species belong to this group;
- species occurring mostly at forest sites (>70 %): these are the epigeic species *D. octaedra* and *D. rubidus*, which are known to be acido-tolerant (Satchell 1955, Sims & Gerard 1999).

This pattern is most likely caused by soil properties such as pH-value (see below) and tolerance (or not) to soil management practices.

The results for crop sites and grassland are in good agreement with the investigations of Bauchhenss (1997), who defined typical species groups based on investigations of 116 crop sites and grassland BDF sites in Bavaria (not included in the Bo-Info database). In addition to the species given above, he also regards *O. tyrtaeum* to be typical for crop sites and grassland and also *L. castaneus* for the latter habitat type.

## 3.3. Preference in relation to soil pH-value

As mentioned above, the occurrence of the 10 earthworm species depended strongly on the pH-value of the soil (Fig. 5). The grouping of the species in regard to their pattern of occurrence is almost the same as for the four major habitat types described above: species occurring at pH ≥5.6 are also found at agricultural sites whose soils have often (especially at crop sites) been adjusted to this range through anthropogenic measures such as liming. Species tolerant towards the range of all pH classes also occur at all habitat types. The epigeic species (with the exception of *L. castaneus*) are highly acido-tolerant, and in particular *D. octaedra* is very rarely found at sites with a pH-value >5.5.

## 3.4. Preference in relation to SOM content

Regarding the occurrence of the 10 earthworm species in relation to SOM content, three groups of species can be identified (Fig. 6):

- species that are rather found in sites with lower SOM content (≤4.0%): *A. chlorotica*, *A. caliginosa*, *A.longa*, *A. rosea* and *L. terrestris*; i.e., most endogeic and both anecic species belong to this group.

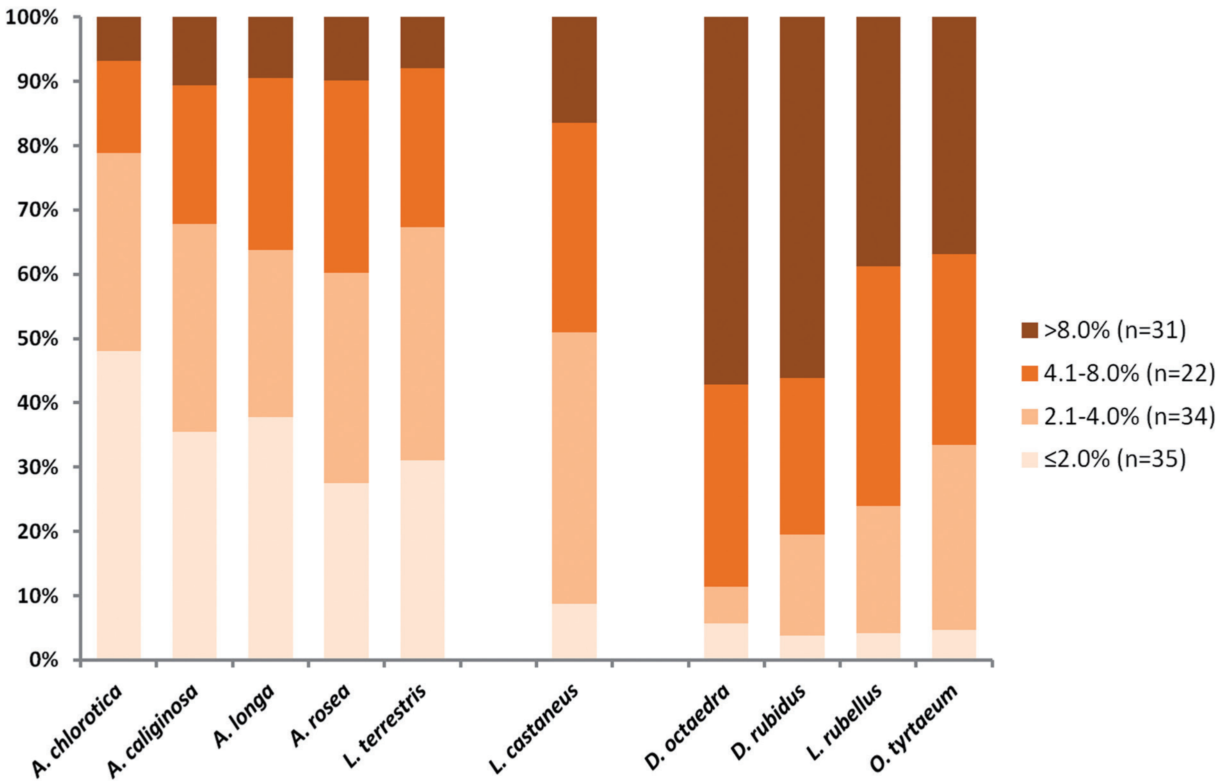


**Figure 6**. Preference of 10 lumbricid species in regard to four classes of soil organic matter content.

- species with a clear preference for sites with high SOM content (>4.0 %): *D. octaedra*, *D. rubidus*, *L. rubellus* and *O. tyrtaeum*; thus, with the exception of *L. castaneus*, all epigeic species belong to this group. Additionally, the endogeic species *O. tyrtaeum* appears in this group, while being seemingly indifferent regarding habitat types and pH-value.
- the epigeic species *L. castaneus* is the only species forming an additional group by occurring more or less equally at sites with low and high SOM content.

## 3.5. Preference in relation to soil texture

Regarding the occurrence of the 10 lumbricid species in relation to soil texture, no grouping seems to be applicable: while there are obvious differences between single species (e.g., *O. tyrtaeum* and *A. rosea* are rarely found at sandy sites and *A. longa* and *D. octaedra* rarely at clayey sites), there seems to be more a continuum than a strict differentiation between species. Additionally, no connection to ecological groups seems to exist, and species such as *A. caliginosa*, *L. rubellus* and *L. terrestris* are equally frequent at sites of all texture classes (supplementary data).

## 3.6. Reference values

One aim of this contribution is the derivation of qualitative reference values (presence or absence of certain species) for earthworms in relation to different habitat types. This system of reference values should be able to differentiate between the four major habitat types in order to demonstrate that different site and soil properties will lead to specific earthworm communities. If this is the case, it can be expected that anthropogenically induced contamination or other changes in these properties will become visible in the earthworm community structure. In a first (arbitrary) approximation, we assume that a species occurring at >50 % of all sites of a habitat type may be considered typical for an unimpacted site belonging to this habitat type (Tab. 3). The validation of this threshold value is beyond the scope of this contribution, and further assessment criteria need to be defined for a practical application of these reference values for evaluating a specific site. A 50 % probability of occurrence for a single species obviously cannot be applied as a sole assessment criterion as the absence of this species at a specific site is within the same probability range (50 %). Hence, we propose a weight-of-evidence approach to deal with the statistic uncertainty of individual species' occurrence, integrating the presence/absence probabilities for various species. For example, if more than one species expected to occur with 50 % probability is missing, this could be interpreted as an impacted soil habitat function. Additionally, occurrence of one or more species expected to be absent at a certain habitat type can also be a sign

**Table 3**. Species composition (relative frequency), average species number and mean total abundance of adults, separated according to the four land-use forms/habitat types at the 1st hierarchical level, using the information from the Bo-Info data base. **Cro** – Crop sites, **Gra** – Grassland sites, **Dec** – Deciduous forest sites, **Con** – Coniferous forest sites. Typical species (= those with a frequency of more than 50 % of all sites) given in bold. Asterisks indicate a statistically significant influence of habitat type on species distribution at p < 0.05 (*), 0.01 (**) and 0.001 (***). **SD** – standard deviation, **CV** – coefficient of variation.

| Species | Cro (33) (n = 86) | Gra (34) (n = 48) | Dec (43) (n = 65) | Con (44) (n = 27) | Chi²-Test Bonf.-corr. |
|---|---|---|---|---|---|
| *A. chlorotica* | 31.4 % | 35.4 % | 0.0 % | 0.0 % | *** |
| *A. caliginosa* | **84.9 %** | **91.7 %** | 36.9 % | 25.9 % | *** |
| *A. longa* | 19.8 % | 10.4 % | 3.1 % | 0.0 % | - |
| *A. rosea* | **55.8 %** | **56.3 %** | 33.8 % | 25.9 % | - |
| *D. octaedra* | 2.3 % | 12.5 % | **72.3 %** | **77.8 %** | *** |
| *D. rubidus* | 0.0 % | 8.3 % | **55.4 %** | 29.6 % | *** |
| *L. castaneus* | 9.3 % | 31.3 % | 16.9 % | 7.4 % | - |
| *L. rubellus* | 24.4 % | **62.5 %** | **73.8 %** | **59.3 %** | *** |
| *L. terrestris* | **55.8 %** | **75.0 %** | 20.0 % | 7.4 % | *** |
| *O. tyrtaeum* | 17.4 % | 41.7 % | 26.2 % | 25.9 % | - |
| Mean Ind./m² ± SD | 49.3 ± 86.2 CV: 175 % | 75.6 ± 92.6 CV: 122 % | 36.6 ± 68.4 CV: 187 % | 18.3 ± 24.5 CV: 134 % | |
| Mean species no./site ± SD | 3.3 ± 1.9 CV: 58 % | 5.0 ± 2.3 CV: 46 % | 3.9 ± 2.3 CV: 59 % | 2.9 ± 2.3 CV: 79 % | |

of disturbance. Finally, some sort of weighting could be applied, so that absence of a species with a 90% probability of occurrence is considered more important than absence of a species with 50% probability.

The reference system as proposed here is upgradeable for quantitative values, e.g. earthworm abundance. However, precise quantitative reference values are difficult to establish due to their high spatial and temporal variability. For this reason, as a first approximation we provide only mean total adult earthworm abundance for comparison between habitat types.

The highest mean abundance of adults is found at grassland sites followed by crop sites, deciduous and coniferous forests. The differences between sites can be very high as indicated by the coefficient of variation for all habitat types. The mean number of species is practically not affected by the absence of juveniles that can rarely be determined to species level and is in the expected order of magnitude (Beylich & Graefe 2009), i.e. highest in grassland and lowest in coniferous forests. Differences between sites can be large as well, but not as great as for abundance.

Differences in species composition are most pronounced between open-land and forest sites. Typical (frequency of >50% per habitat type) or absent (frequency of 0% per habitat type) species per habitat type are (in descending order of frequency; Tab. 3):

- Crop sites: typical = *A. caliginosa*, *A. rosea*, *L. terrestris*; absent: *D. rubidus*
- Grassland: typical = *A. caliginosa*, *L. terrestris*, *L. rubellus*, *A. rosea*; absent = none
- Deciduous forest: typical = *L. rubellus*, *D. octaedra*, *D. rubidus*; absent = *A. chlorotica*
- Coniferous forest: typical = *D. octaedra*, *L. rubellus*; absent = *A. chlorotica*, *A. longa*

Considering abundance of 28 species, in the ordination from the PCA there is some separation of the four main land use forms, but also a large overlap (Fig. 7). The first axis mainly separates open-land and forest sites. The underlying abiotic factor determining the earthworm community structure appears to be pH value, likewise increasing in this direction. Grasslands become slightly separated from crop sites along the second axis. No abiotic factor explaining this pattern can be currently identified. The most homogeneous group are coniferous forests, characterized by a low pH value and high SOM content. The majority of deciduous forest sites are also grouped together with coniferous forest sites (again, mainly due to their pH value, SOM content but also sand content), but these sites strongly scatter along both the first and second axis, resulting in overlap with some grassland and isolated crop sites. Notable is the isolated position of a grassland site at the very top of the plot. This is a marshland BDF, allocated to habitat type 34.08, in the vicinity of Hamburg, characterized by a high abundance of *Eiseniella tetraedra*, an epigeic species known to occur in (semi-)limnic habitats (Graff 1953, Sims & Gerard 1999). Finally, as was to be expected, sites belonging to other habitat types are scattered over the entire plot.

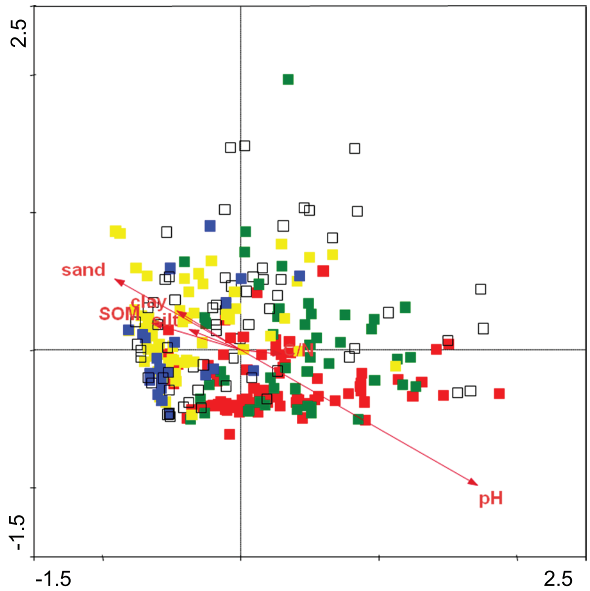


**Figure 7**. Principal component analysis based on abundance of 28 earthworm taxa determined to species level. **Red** – habitat type 33 (crop sites), **green** – 34 (grassland sites), **yellow** – 43 (deciduous forest sites), **blue** – 44 (coniferous forest sites), **white** – other habitat types. Length of gradient from detrended correspondence analysis = 3.3, first axis = 33.7%, second axis = 16.9% of variance. SOM = soil organic matter.

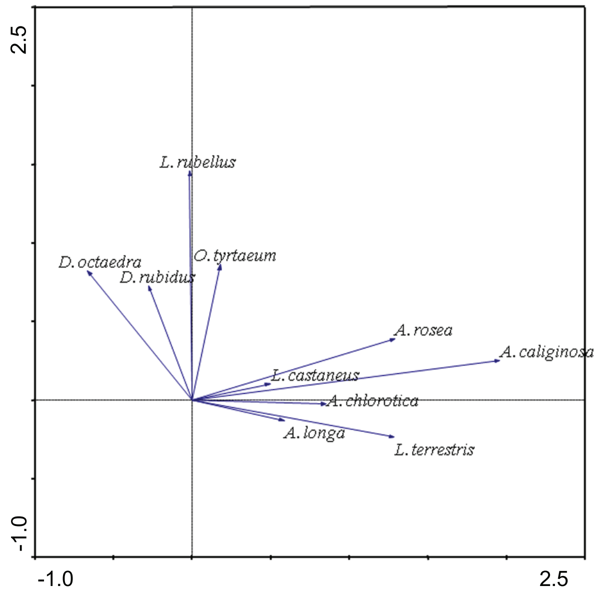


**Figure 8**. Species-biplot from principal component analysis based on abundance of 28 earthworm taxa determined to species level in Fig. 7. Only species with a contribution of >15% to the ordination are displayed.

In Fig. 8, those species with a contribution of >15 % to the ordination are displayed. These turned out to be the same 10 species used for the derivation of reference values above. This confirms that these are not only the most common species but also those that are most significant for the differentiation between habitat types. The alignment of sites along the first axis is mainly due to an increase in abundance of the endogeic species *A. caliginosa* and *A. rosea* as well as the anecic species *L. terrestris* from left to right. The allocation of sites along the second axis is mainly determined by the increasing abundance of the epigeic species *L. rubellus*, *D. octaedra* and *D. rubidus* as well as the endogeic species *O. tyrtaeum*. The abundance of the first species seems to be the main factor separating some grassland from crop sites. Coniferous forests are characterized by a generally low abundance of earthworms, especially of endogeics and anecics while some deciduous forests may also accommodate a higher abundance of all ecological groups. Thus, the pattern of reference values derived from the occurrence of species at sites of the four major land-use types is also well represented in the ordination based on all species' abundance. For individual sites or more specific habitat types, additional species may become important as illustrated by the example of *E. tetraedra* shown above. This calls for a further refinement of reference values supported by a fortified data basis through additional ecological investigations, especially at so far underrepresented habitat types.

The occurrence of species at one of the four major habitat types (= land use) is mainly dependent on ecological preferences or tolerances towards different environmental factors. Crop and grassland sites in Germany usually have a higher pH-value than deciduous and coniferous forests. On the other hand, SOM content is usually higher in forests than in open-land habitats. Thus it can be argued that, at sites with environmental properties deviating from this generalized pattern, other species may occur than those expected for the given habitat type. For instance, at a deciduous forest with neutral soil (nowadays rarely found in Germany since these sites have been largely converted to agricultural sites) an earthworm community might be found that would be more expected in a grassland site.

From these considerations it becomes clear that among earthworms there are no specific indicator species for certain habitat types as a differentiation between the major habitat types based on the species composition of the earthworm community is only partially possible. However, for each major habitat type some earthworm species can be identified whose absence or presence at a given site belonging to that habitat type could be an indication for disturbance. For example, the soil quality of a German grassland site without *A. caliginosa* or *L. terrestris* or an acidic forest without *D. rubidus* or *D. octaedra* could be impaired. Likewise, *D. rubidus* shouldn't occur at an unimpacted crop site or *A. chlorotica* at an unimpacted forest site.

Data availability allowed a partial assessment at the second hierarchical level of four major habitat types as given by Riecken et al. (2006, 2009): crop sites, deciduous and coniferous forests; grassland had only one second level habitat type with more than one site (Table 4).

For crop sites, three second-level habitat types could be distinguished (Tab. 4). On farmed and fallow land on shallow skeletic calcareous soil (habitat type 33.01), at least three endogeic species with a mean total adult abundance of 28.7 ind./m² should occur. In addition to *A. caliginosa* and *A. rosea*, *O. tyrtaeum* but not the anecic *L. terrestris* was most frequently present. *D. octaedra* and *D. rubidus* are not expected to occur at this habitat type. On farmed and fallow land on sandy soil (habitat type 33.03), only *A. caliginosa* should always occur (100 % of all 21 sites in the present data basis) with a total mean adult abundance of 18.9 ind./m² and *D. rubidus*, *L. castaneus* and *O. tyrtaeum* should be absent. On farmed and fallow land on loess, loam or clay soil (habitat type 33.04), at least four species can be expected: besides *A. caliginosa*, *A. rosea* and *L. terrestris*, the endogeic *A. chlorotica* was also frequently found at this habitat type. This habitat type thus showed the highest mean species richness and also by far the highest mean abundance of adults (93.2 ind./m²) of all crop-site types. Acido-tolerant epigeic species (in particular *D. octaedra* and *D. rubidus*) were almost totally missing here. Variability in abundance was high for all three habitat types.

For species-poor intensive grassland on moist sites (habitat type 34.08), the endogeic *A. caliginosa* and the anecic *L. terrestris* should occur with a high probability at an overall mean adult abundance of 86.3 ind./m². *L. rubellus* and *A. rosea* were not as frequent here as for grasslands as a whole, which was reflected in an overall lower mean species number. *A. longa* did not occur at any of these sites. Variability in abundance can be extremely high between sites of this habitat type (Tab. 4).

For deciduous forests, two second-level habitat types could be more closely assessed (Tab. 4). In ravine, boulder-field and scree forests (habitat type 43.06), six species with a mean total adult abundance of 31.3 ind./m² can be expected. At all six sites the epigeic *D. octaedra* was found. In addition to the three epigeic species expected for deciduous forests, three endogeic (*A. caliginosa*, *A. rosea*, *O. tyrtaeum*) and the anecic *L. terrestris* were also frequently found. This habitat type thus harboured the greatest diversity of all the second-level habitat types more closely investigated. However, *A. chlorotica*, *A. longa* and *L. castaneus* should not be found here. In deciduous and mixed forests on damp to moist sites (habitat type 43.07), only the epigeics *L. rubellus* and *D. octaedra* but not *D. rubidus* were found at more than 50 % of all sites. It is noteworthy that at these damp to moist sites, the hygrophilous endogeic *A. chlorotica* was totally missing in the present data basis. The mean total adult abundance was 21.5 ind./m², but variability was high for both habitat types.

Data availability for the three more frequent second-level habitat types of coniferous forest was generally low (six to eleven sites per habitat type; Tab. 4). In the natural and near-natural dry to intermittently damp pine forests (habitat type 44.02), mean species richness (1.3) and mean total abundance of adults (4.8 ind./m²) were the lowest of all second-level habitat types investigated here. Only the epigeic *D. octaedra* but not *L. rubellus* was found in more than 50 % of all sites belonging to this habitat type while *A. chlorotica*, *A. longa*, *A. rosea*, *L. castaneus* and *O. tyrtaeum* were altogether missing. *D. octaedra* was also the most frequent in spruce/fir (mixed) forests and spruce (mixed) forests (habitat type 44.03), but also *L. rubellus* and the endogeics *A. rosea* and *O. tyrtaeum* were frequently found, at a mean total adult abundance of 20.6 ind./m². This habitat type is the only one where the anecic *L. terrestris* was never found, along with *A. chlorotica*, *A. longa* and *L. castaneus* (but based on a total number of only six sites). In coniferous (mixed) plantations with native tree species (habitat type 44.04), four species with a mean total adult abundance of 28.5 ind./m² can be expected. The most frequent species were the epigeics *D. octaedra* and *L. rubellus* while *A. chlorotica* and *A. longa* never occurred. Again, variability was high for all three habitat types, the least for habitat type 44.04, the only habitat type with a CV of total abundance less than 100 %.

The data suggest that at a higher level of habitat-type differentiation, the expected values may be further refined, thus becoming reliable enough to allow a definition of threshold values for the assessment of biological soil quality. However, this depends on a broader data basis and a practical validation procedure.

Along the first axis of the PCA ordination based on the abundance of those 13 species occurring at second-level habitat type crop sites (Fig. 9), mainly the habitat types 33.01 (farmed and fallow land on shallow skeletic calcareous soil) and 33.03 (farmed and fallow land on sandy soil) were separated from habitat type 33.04 (farmed and fallow land on loess, loam or clay soil). This was mainly due to the increasing abundance of the anecic *L. terrestris* and the endogeic species *A. caliginosa* from left to right. However, sites of habitat type 33.04 could strongly differ in their earthworm community composition and abundance and thus formed a heterogeneous group. Along the second axis, habitat types 33.01 and 33.03 were well separated, mainly because of a higher abundance of *A. rosea* and *O. tyrtaeum* at 'farmed and fallow land on loess, loam or clay soil' sites. As an underlying environmental factor, the C/N value may have had an influence, but as mentioned in the methods section, interpretation is difficult due to missing data for sites from habitat type 33.01. The pH value may also have

**Table 4**. Species composition (relative frequency), average species number and mean total abundance of adults, separated according to level-2 habitat types, using the information from the Bo-Info data base. **Cro** – Crop sites, **Gra** – Grassland sites, **Dec** – Deciduous forest sites, **Con** – Coniferous forest sites. Typical species (i.e. those with a frequency of more than 50 % of all sites) given in bold. (***). **SD** – standard deviation, **CV** – coefficient of variation.

| Species | Cro (33) | | | Gra (34) | Dec (43) | | Con (44) | | |
|---|---|---|---|---|---|---|---|---|---|
| | 33.01 (n = 16) | 33.03 (n = 21) | 33.04 (n = 31) | 34.08 (n = 10) | 43.06 (n = 6) | 43.07 (n = 37) | 44.02 (n = 9) | 44.03 (n = 6) | 44.04 (n = 11) |
| *A. chlorotica* | 12.5 % | 14.3 % | **54.8 %** | 10.0 % | 0.0 % | 0.0 % | 0.0 % | 0.0 % | 0.0 % |
| *A. caliginosa* | **75.0 %** | **100.0 %** | **87.1 %** | **90.0 %** | **83.3 %** | 32.4 % | 11.1 % | 33.3 % | 27.3 % |
| *A. longa* | 6.3 % | 4.8 % | 41.9 % | 0.0 % | 0.0 % | 5.4 % | 0.0 % | 0.0 % | 0.0 % |
| *A. rosea* | **75.0 %** | 14.3 % | **87.1 %** | 40.0 % | **83.3 %** | 35.1 % | 0.0 % | **50.0 %** | 36.4 % |
| *D. octaedra* | 0.0 % | 9.5 % | 0.0 % | 10.0 % | **100.0 %** | **59.5 %** | **55.6 %** | **83.3 %** | **90.9 %** |
| *D. rubidus* | 0.0 % | 0.0 % | 0.0 % | 10.0 % | **66.7 %** | 40.5 % | 11.1 % | 16.7 % | 45.5 % |
| *L. castaneus* | 12.5 % | 0.0 % | 16.1 % | 10.0 % | 0.0 % | 18.9 % | 0.0 % | 0.0 % | 18.2 % |
| *L. rubellus* | 43.8 % | 9.5 % | 16.1 % | 30.0 % | **66.7 %** | **73.0 %** | 44.4 % | **50.0 %** | **81.8 %** |
| *L. terrestris* | 37.5 % | 33.3 % | **83.9 %** | **80.0 %** | **50.0 %** | 18.9 % | 11.1 % | 0.0 % | 9.1 % |
| *O. tyrtaeum* | **62.5 %** | 0.0 % | 12.9 % | 30.0 % | **66.7 %** | 27.0 % | 0.0 % | **50.0 %** | 36.4 % |
| Mean Ind./m² ± SD | 28.7 ± 37.2 CV: 130 % | 18.9 ± 28.1 CV: 149 % | 93.2 ± 126.1 CV: 135 % | 86.3 ± 160.0 CV: 185 % | 31.3 ± 33.5 CV: 107 % | 21.5 ± 30.8 CV: 143 % | 4.8 ± 5.9 CV: 123 % | 20.6 ± 36.9 CV: 179 % | 28.5 ± 23.9 CV: 84 % |
| Mean species no./site ± SD | 3.4 ± 1.9 CV: 56 % | 1.9 ± 1.2 CV: 63 % | 4.4 ± 1.7 CV: 39 % | 3.5 ± 1.8 CV: 51 % | 5.3 ± 2.5 CV: 47 % | 3.7 ± 2.3 CV: 62 % | 1.3 ± 1.0 CV: 77 % | 3.0 ± 2.0 CV: 67 % | 3.9 ± 2.7 CV: 69 % |

had an influence, which was on average lowest at sites belonging to habitat type 33.03 (mean pH 5.3; 33.01. = 6.8; 33.04. = 6.4). Overall, from the PCA, differences are indicated in the species composition of the different level-two habitat types, allowing more refined reference values to be identified, but the analysis is hampered by low data availability in terms of both number of sites and environmental parameters.

### 3.6. Comparison with other soil classification concepts using earthworms

Earthworms have been often used in biological soil classification and assessment concepts (e.g. Phillipson et al. 1976, Doube & Schmidt 1997, Spurgeon & Hopkin 1996, Beylich & Graefe 2002, Ehrmann et al. 2002, Beylich et al. 2005, Krück et al. 2006, Tischer 2007, 2008, Lindahl et al. 2009, EFSA 2010). However, only two of them have been regularly used, one in the Netherlands, the other in some regions of Germany. In the following, these two will briefly be presented.

The reference system of the Dutch National Institute for Public Health and the Environment (RIVM; Rutgers et al. 2008) defines reference values for biomass, abundance and diversity of various organism groups, including earthworms, at sites classified into ten categories, defined mainly by two site properties: land use (e.g. arable land) and soil type (e.g. marine clay). Based on expert knowledge and data from the 'biological indicator for soil quality' (BISQ) project (Schouten et. al 1997) and the Netherlands Soil Monitoring Network (LMB), sites were chosen for the derivation of these reference values (Rutgers et al. 2008). Unfortunately, the site categories specified for the Netherlands are not directly comparable to our results because the environmental factors used for their definition differ partly from those used here to define habitat types. However, the number of earthworm species at four common Dutch site categories (arable land, cattle or dairy farms on clay soils and sandy soils (Rutgers et al. 2008)) were similar to those at German sites (not to habitat types according to Riecken et al. 2006, 2009) with the same land use and soil texture: low numbers (about three) at arable sites on sandy soils but high (about nine) at cattle or dairy farms on clay, supporting the usefulness of diversity data for site classification. However, data on species composition of earthworm communities typical for individual Dutch site categories are not published. Therefore, for now, a detailed comparison of advantages and shortcomings of the Dutch approach and our proposal is not possible.

Graefe and co-authors have developed the concept of decomposer communities based on investigations on BDF in Schleswig-Holstein, Hamburg and North Rhine-Westphalia (Graefe 1993, Graefe & Schmelz 1999, Beylich & Graefe 2009). The results of their research are proposals for quantitative reference values for the abundance, biomass and diversity of earthworms at sites classified into six categories based on land use, soil pH, texture and degree of water logging. Thus, these categories do not correspond to the habitat types used in this contribution, meaning that a direct quantitative comparison with our reference values is not possible. In

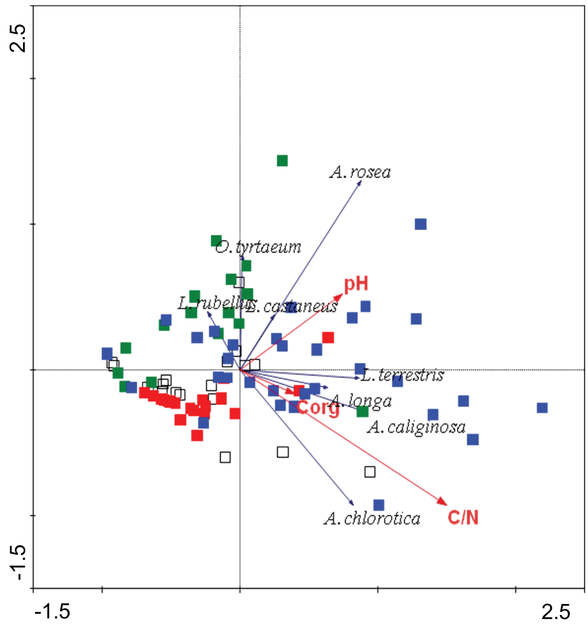


**Figure 9**. Principal component analysis discerning different crop habitat types, based on abundance of 13 earthworm taxa determined to species level. **Green** – habitat type 33.01, **red** – 33.03, **blue** – 33.04, **white** – 33 (not assignable to level two). Length of gradient from detrended correspondence analysis = 3.3, first axis = 42.5%, second axis = 13.4% of variance. Only species with a contribution of >15% to the ordination are displayed.

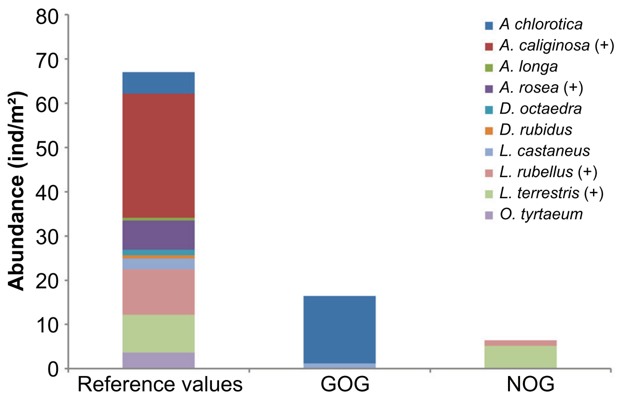


**Figure 10**. Reference values for uncontaminated grassland sites: mean abundance (ind./m²) of earthworm species (+ = occurrence at >50% of all sites; see Tab. 2) and comparison with abundance and species composition of contaminated sites Gorleben (**GOG**) and Nordenham (**NOG**) in Lower-Saxony, Germany.

addition, it must be kept in mind that most of the data used by Graefe et al. has also been included in the Bo-Info database; thus their data set and the one used here are not independent. A qualitative comparison between the decomposer communities used by Beylich & Graefe (2009) as defined by Graefe (1993) (i.e. a combination of enchytraeid and lumbricid species typical for a given site with its specific properties) with our reference values is more easily possible. For example, for forest and heathland sites, Beylich & Graefe (2009) expect the decomposer community type 'Achaeto-Cognettietum', meaning that only *D. octaedra* is expected at such sites. This is also the only species expected for all forest habitat types investigated in this contribution. For the field sites with a higher pH-value and the dryer grassland sites, a 'Fridericio-Lumbricetum' is expected (typical lumbricid species: *L. castaneus*, *L. terrestris*, *A. longa*, *A. caliginosa*, *A. rosea*, *A. limicola*, *O. cyaneum, O. tyrtaeum*). This list includes all species expected for arable field and grassland sites as defined in this contribution (as well as others). However, Beylich & Graefe (2009) do not assign a specific probability of occurrence for individual species and site categories. In summary, the proposals made in literature support the general idea of using earthworms for the assessment of the habitat function of soils.

### 3.7. Case study: comparison between contaminated and reference sites

The mean abundance of species from the reference values for grasslands (Tab. 2) were compared to the mean species abundance (also without juveniles) of the contaminated sites Gorleben and Nordenham (Lower Saxony, Germany) (Fig. 10). At the Gorleben site, only *A. chlorotica* and *A. rosea* with less than 20 ind./m² were found and in Nordenham only *L. rubellus* and *L. terrestris* with less than 10 ind./m². *A. caliginosa* and *L. rubellus*, which would usually be expected at grassland sites, were missing at both sites. This clearly deviates from the reference values for grasslands regarding total abundance, species number and composition and, thus, represents a first indication that the habitat function is impaired at these sites. The application of the reference system would have indicated a contamination of these sites without knowledge of the chemical analyses, which demonstrates the usability of this concept for the biological soil-quality assessment. However, this is just one example; further studies based on comparable sampling methods and a broad site characterization need to be performed.

### 3.8. Recommendations for improving biological soil monitoring

The results presented here for earthworms need to be utilized within a broader context. Based on the experiences made in the course of this research, the following recommendations for a comprehensive German federal soil monitoring programme can be given. For a minimum set of sites, it is recommended to use a grid, based on the distribution of existing BDFs. The sites should be evenly distributed among all federal states and should be nationally coordinated to ensure a harmonized approach. The major habitat types (crop sites, grassland, deciduous and coniferous forests), integrating four to five second-level types (Riecken et al. 2006, 2009) with 10 sites each (i.e., roughly 160 to 200 sites), should be covered and sampled within the course of five years. This sampling program may appear insufficient given the high variability between sites observed from the data, but standardization regarding both point in time and method of sampling should strongly reduce variability and strengthen data comparability. The sites should be representative regarding the soil factors in those ranges relevant for Germany: pH-value, soil texture, surface soil conditions (humus form, litter layer/mineral soil), geographical regions. Finally, site selection should allow integration into European monitoring programs.

Recommendations of parameters for a minimum soil characterization (all measurements should be performed according to available ISO-guidelines or other comparable standards; Barth et al. 2000, Römbke et al. 2002b, Turbé et al. 2010, ISO 2011): pH-value ($CaCl_2$, KCl), SOM content, cation exchange capacity, soil dry mass, texture, soil density. With respect to the biological monitoring focus, nitrogen content, C/N-ratio, water holding capacity and humus form (especially for forest sites) should also be recorded. Additionally, the following site properties should be recorded: site history (land use, prior samplings), exact geographical location (coordinates), current land-use type, climate data (at least: mean annual and monthly air temperature and precipitation; annual course of surface soil temperature), ground-water level, anthropogenic impact (concentrations of common contaminants, e.g., heavy metals, PAH, etc.); physical stress (management practice, compaction, fertilization, erosion, etc.).

Recommendations for a methodological standard for biological monitoring comprise the organism groups of Oribatida, Collembola, Lumbricidae, Enchytraeidae and the diversity of microorganisms, an expansion of the 'ENVASSO Tier 1' (Bispo et al. 2009). Sampling should be seasonally matched (spring/autumn) and performed according to available ISO-guidelines. Vertical distribution between litter and mineral soil layer should be addressed where appropriate, and sampling should be repeated with a frequency of three to five years.

The data raised in such an improved biological soil-monitoring programme can thus be utilized to fill existing data gaps regarding the occurrence of lumbricids and other soil organism taxa at different habitat types. Subsequently, the biological soil-quality assessment approach presented in this contribution can be subjected to a validation step and then be implemented for routine practical application.

## 4. Summary and conclusions

The results may be summarized as follows:

- the diversity of German earthworms was largely covered in the Bo-Info database: 32 valid species were included, 10 of which are common;
- the available data for earthworms was heterogeneously distributed geographically between German federal states and regions;
- ecological profiles regarding habitat type, pH-value, SOM content and soil texture were determined for the 10 most common species, considerably widening the ecological knowledge on several lumbricid species;
- the occurrence of earthworms was clearly determined by land use, inter-correlated with pH-value, and (less) by SOM content and soil texture;
- a differentiation of habitat types by reference values for the structure of the earthworm community is possible at the first hierarchical level (corresponding to major land-use types), but also at a further level of refinement given sufficient data availability;
- despite the long-known correlation between the occurrence of single species or whole communities with site and soil parameters, the derivation of quantitative reference values is difficult;
- filling data gaps (abiotic and biological) is still necessary despite a broad data basis (compared to other organism groups) on taxonomy, biogeography and ecology of earthworms. This is particularly true for certain regions of Germany (e.g. some federal states) as well as some habitat types like coniferous forests;
- the use of earthworms for biological soil-quality assessment is recommended despite their relatively low species number (and thus, low potential for differentiation) due to comparably good data availability and their high ecological relevance;
- recommendations for a comprehensive German federal soil monitoring programme including site selection, soil and site characterization and soil faunal parameters could be given.

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Supplementary data is provided for this paper at www.soil-organisms.org.

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