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RESEARCH ARTICLE

# Balancing sampling effort and representativeness for assessing species composition of Enchytraeidae in different habitats

**Nicole Scheunemann[1,*], Anneke Beylich[2] & Rüdiger M. Schmelz[3]**

[1] Senckenberg Museum of Natural History Görlitz, Senckenberg – Leibniz-Institution for Biodiversity and Earth System Research, Görlitz, Germany
[2] IFAB Institut für Angewandte Bodenbiologie GmbH, Hamburg, Germany
[3] Independent researcher, Enchylab, Martorell, Spain
* Corresponding author, email: nicole.scheunemann@senckenberg.de; ORCID: https://orcid.org/0000-0003-1845-6236

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## Abstract

Soil biodiversity has gained increased attention in science as well as policy during the last years, and programs for monitoring soil animal biodiversity have been approved. Enchytraeidae are often part of these programs, however, their identification to species level is time consuming and experts are rare. Probably, reducing the number of samples taken can increase efficiency of investigating enchytraeid communities, thereby reducing effort not only for morphological identification by taxon experts but also possibly for molecular approaches. However, the trade-off between effort and quality of the received data needs to be addressed carefully. To test the potential for reduction in sample number without losing important information, we used an existing high-effort dataset of enchytraeid communities from German grasslands, forests, and arable fields. We applied species rarefaction and estimates of expected biodiversity given the existing dataset to find out if the number of soil cores taken, or the number of sampling events, can safely be reduced without losing important information on indicator species. Our results show that, while missing out only very sporadic species, sampling effort can be reduced from the initial 20 soil cores by 55 % in coniferous forests, 45 % in broadleaved forests, 40 % in grasslands, and 30 % in arable fields. Despite the fact that species accumulation curves were not significantly affected, a reduction of sampling frequency from two (spring and fall) to only one (either spring or fall) resulted in the consistent reduction of the number of indicator species across different habitats. If sampling effort needs to be restricted, we therefore recommend a reduction in total number of soil cores rather than a reduction of sampling times.

## Keywords

Soil fauna | rarefaction | singletons | seasonality | Edaphobase

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# 1 Introduction

Soils provide essential ecosystem functions like decomposition of organic material and carbon and nutrient cycling, plant growth, filtration, and water storage (Bardgett 2005). In addition, soils serve as habitat for the majority of terrestrial organisms, at least during a certain period of their life (Anthony et al. 2023). Soil fauna is mainly involved in supporting decomposition processes e.g., by pre-digestion of organic material and regulation of microbial activity (Petersen & Luxton 1982; Filser et al. 2016). Diversity of soil fauna therefore strongly contributes to soil ecosystem functions, a fact that recently led to the formation of national and international regulations to monitor and increase soil biodiversity. On the European scale, the European Soil Monitoring Law (European Parliament 2025) expects monitoring of soil biodiversity from EU member states, while in Germany, for example, efforts for a national soil biodiversity monitoring program are ongoing (Ballasus et al. 2026).

Enchytraeidae (Oligochaeta, Annelida) are vermiform soil animals whose limited burrowing abilities mainly affect soil microstructure (Conti & Mulder 2022). They play an important role in decomposition by consumption of organic matter, thereby increasing carbon and nutrient availability for the soil food web as well as for plant growth (Serbource et al. 2025; Bonkowski & Roy 2012; Liiri et al. 2002, Briones et al. 2004). Their prominent role in performing ecosystem functions and wide distribution across habitats make Enchytraeidae an important part of soil biodiversity monitoring in Germany, both at the official level and different expert-driven initiatives (e.g., BioDive4Soil project; https://biodiv.rwth-aachen.de/projekte/biodive4soil/). Enchytraeid species composition has been used to evaluate agricultural management (Pelosi & Römbke 2016), soil nutrient content (Degtyarev et al. 2019), restoration of polluted sites (Józefowska et al. 2020), and allowed for distinction of different habitat types (Cuartero et al. 2025). Indicator values have been established for most of the common species, describing their specific behavior towards factors such as soil pH, moisture and humus type (Graefe & Schmelz 1999). Moreover, species composition of Enchytraeidae provides information on habitat type and ecosystem functions of a given study site (Beylich & Graefe 2002; Cuartero et al. 2025), and using species' functional traits is a promising approach for evaluating effects of Enchytraeidae on ecosystems and ecosystem functions (Vandewalle et al. 2010). Korobushkin et al. (2024) identified three different feeding guilds of Enchytraeidae, highlighting the importance of enchytraeid diversity and species composition for ecosystem functions, and therefore for monitoring of ecosystem functions.

However, monitoring of soil biodiversity by morphological species identification of soil animals - in particular of Enchytraeidae - requires expertise and is time consuming, while molecular methods still suffer from an incomplete species coverage of the respective genome libraries, and are either non-quantitative (meta barcoding) or comparatively expensive (Sanger sequencing) (Oliverio et al. 2018; Remelli et al. 2026). Reducing the workload of the available experts or lab facilities while maintaining the quality of results is therefore a pressing issue for soil biodiversity monitoring. But not only standard monitoring could benefit from an increase in efficiency. Scientists working on soil animal communities need an estimate of the sampling effort that is required to obtain a representative picture of the local species assemblages.

A possible way to increase efficiency of investigating soil animal communities might lie in the reduction of the number of soil cores taken. The reduction needs to meet a level that ensures the detection of all relevant species that occur at a particular study site, while avoiding taking surplus soil cores with low additional information regarding species assemblages and dominances (Saito et al. 2015, Sgarbi et al. 2020). For (qualitative) eDNA samples, Cuartero et al. (2025) showed that the number of different amplicon sequence variants detected within the analyzed sequences saturated, indicating that their sampling effort was "sufficient to detect a comprehensive diversity" (Cuartero et al. 2025). But saturation also indicated that a reduction in total number of samples would probably not have affected their results. In addition, in quantitative datasets, species found as a single individual i.e., singletons, are supposed to have minor impact on the community ecology of the respective habitat, and therefore they are often omitted in statistical analysis (e.g., Creamer et al. 2016). In general, natural species communities are affected by a high number of very sporadic species, which is relevant for, e.g., estimating the potential biodiversity of a given study site, but not for ecological purposes, such as evaluating energy fluxes or biodiversity monitoring.

To test the potential for reduction in the number of soil cores taken per study site for standard monitoring, we used a comprehensive dataset of enchytraeid communities based on different sampling sites and habitats in Germany (Toschki et al. 2021). In this study, communities of Enchytraeidae had been investigated at 36 sampling sites across Germany, covering the habitats of coniferous forests, broadleaved forests, grasslands and agricultural fields. Each site had been sampled twice, in spring and fall respectively, and 10 soil cores had been taken per sampling date. We applied statistics on the existing data to test the following hypotheses:

First, the initial sampling effort of 20 soil cores is sufficient to cover the entire species spectrum of

Enchytraeidae at a given site. Second, the number of soil cores can be reduced to different degrees, depending on the habitat type, if very sporadic species (defined here as singletons at a given site in the original dataset) are excluded. Third, season does not affect the results, i.e., a single sampling of a given site (spring or fall in the present case), instead of two, is sufficient.

## 2 Materials and Methods

For the present study, no sampling or identification work was conducted. Instead, existing data from Toschki et al. (2021) were newly compiled and statistical analyses were performed. In the following we give a brief description of the project that generated the data. For a complete overview, see Toschki et al. (2021), or find the dataset at https://portal.edaphobase.org/ (Schmelz 2016a-d). The "Edaphobase Länder Studies" were conducted at 36 study sites located in four federal states (Länder): Bavaria (3 grasslands, 6 agricultural fields), Saxony (3 coniferous forests, 3 broadleaved forests, 3 agricultural fields), Saxony-Anhalt (9 grasslands) and North Rhine-Westphalia (3 coniferous forests, 6 broadleaved forests) (Fig. 1). Soil cores were taken in 2014 (Saxony, North Rhine-Westphalia) or 2015 (Saxony-Anhalt, Bavaria). In spring and fall of the respective year, 10 soil cores per site were taken (5.6 cm diameter = 24.6 cm² surface, 10 cm depth). The Enchytraeidae were extracted by wet extraction after O'Connor (1967) with heating of the soil core to approx. 40 °C over four hours, and identified alive using a light microscope and the key of Schmelz & Collado (2010). Litter layer and mineral soil had been sampled separately, but in the present analysis abundances were summed-up for both layers. The objective of Toschki et al. (2021) was to fill data gaps in Edaphobase focusing on poorly represented habitat types, and to identify indicator species for these habitat types.

We used R statistical software (version 4.5.1; R foundation for statistical computing, 2025) to calculate species pools using the function estimateR() in the R package *vegan* (Oksanen et al. 2020) for individual sampling sites. This was done including all 20 soil cores

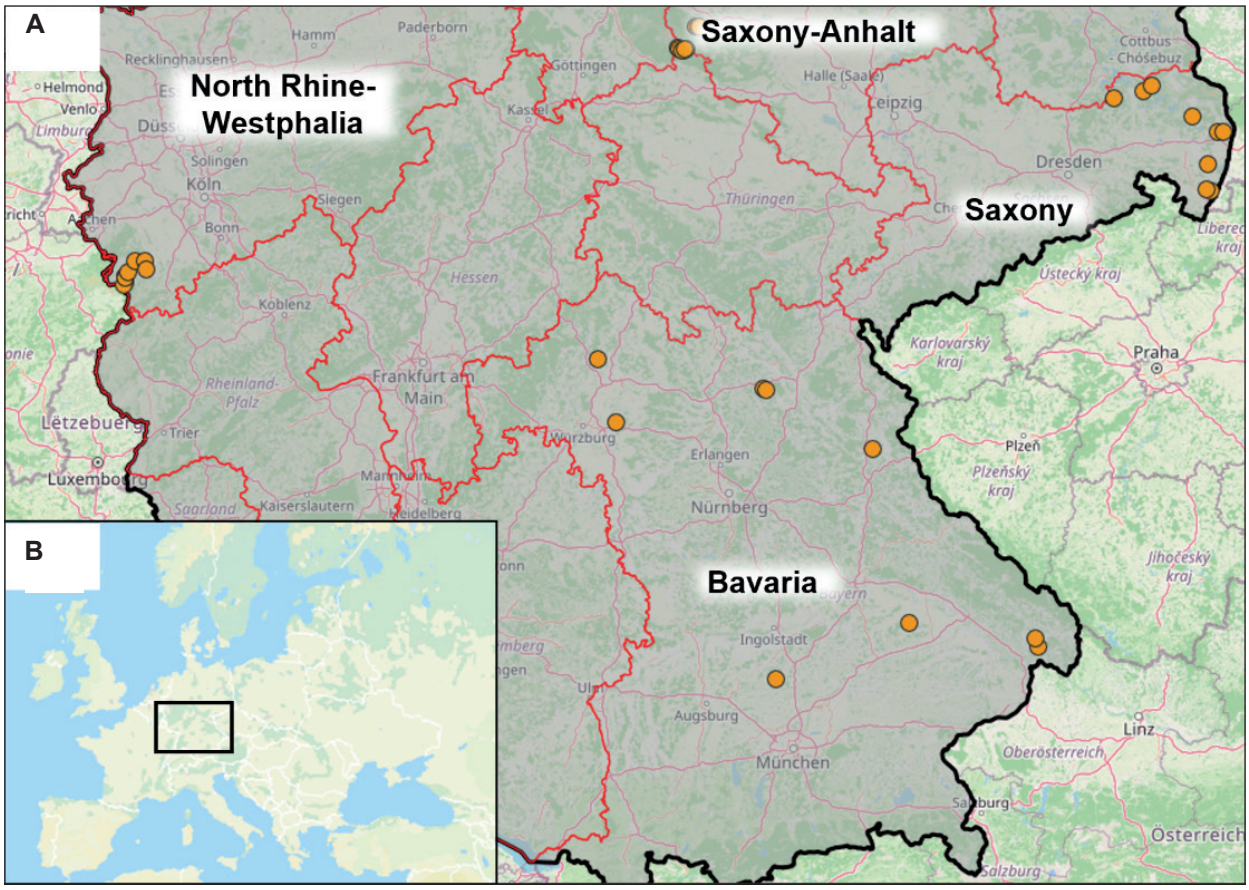



**Figure 1.** Location of sampling points for the original study of Toschki et al. (2021), depicted in orange points. In each of the federal states of Bavaria, Saxony, Saxony-Anhalt and North Rhine-Westphalia 9 sampling sites were chosen, however, the number of points in the map does not match the number of sampling sites due to spatial overlap. (**A**) The map shows the southern half of Germany with federal states borders marked in red. (**B**) The small map shows the location of the map section within Europe. Maps from www.openstreetmap.org, built at https://portal.edaphobase.org/.

first, and, in addition, with only spring or fall soil cores, respectively.

Species accumulation curves including all 20 soil cores per site were built using specaccum() with "rarefaction" option and 100 permutations, and plot() for building the graphs. For estimation of the expected number of species, the Chao1 estimator was chosen for its dependence on actual abundances (instead of presence/absence) and conservatism (i.e., rather low estimate of non-observed species). Species accumulation curves were fitted using fitspeccacum() on the initial rarefaction curve with "lomolino" background model and "coleman" method. By using params<-coef() and params["Asym"] the maximum species number (i.e., the asymptote) per site was estimated from that model. The number needed to find all these species was estimated using the reversed logarithmic equation of the species accumulation curve with the asymptote value as y, and solving the respective equation for x. All these steps were done for the dataset containing all observed species, and again after removal of very sporadic species per site.

The comparison of single-season sampling (spring n = 10, fall n = 10) vs. sampling in both seasons (n = 20) was preceded by a reduction of the number of soil cores included in the respective calculation. In a 20-step *for* loop using slice_sample(site_data, n=10), ten out of the 20 soil cores were picked randomly and saved in a list[[i]] twenty times. For every randomly picked data set of ten soil cores from that list, the species accumulation curve was calculated using poolaccum() with 100 permutations and a minimum sample size of 1. Species accumulation data, i.e., mean number of species (S) found in a given number of samples (N) from the respective data set, were saved. From these, mean and standard deviation were calculated for every N across the 20 randomly picked data sets to receive mean species accumulation curves from ten soil cores picked randomly from spring and fall, which in the following was compared to species accumulation curves that derived from ten soil cores from a single season only. In the resulting graphs, curves with non-overlapping standard deviations were considered significantly different.

The ratio of species found in both seasons and in only one season was calculated using the *ggVennDiagram* (Gao & Dusa 2026) implement in the *ggplot2* (Wickham 2016) package. Species were assigned to the corresponding season using colSums(N[ ]) and sum(N>0) for the respective rows per study site separately. Species names were extracted using list(names(N)). The lists for spring and fall soil cores for the respective site were depicted in Venn diagrams using ggVennDiagram(), and means and SDs were calculated from individual sites per habitat.

We used linear models lm() to compare species numbers between habitats to provide additional information for the reader.

# 3 Results

## 3.1 Observed species richness

Total species richness differed significantly between habitats: Coniferous forests had the significantly lowest species richness per site (11.0 ± 2.4; mean ± SD, also hereafter), while grasslands showed the significantly highest number of species per site (24.4 ± 8.0) with 25 soil cores being necessary to find all species expected by the Chao1 estimator. In broadleaved forests and arable fields, the situation was intermediate with species numbers observed in 20 soil cores as well as species numbers expected by Chao1 ranging in between those of coniferous forests and grasslands (Tab. 1). In all four habitats, taking additional soil cores would probably have increased observed species richness by finding additional very sporadic species (i.e., singletons).

The number of species observed per sampling site varied stronger within the group of grasslands and arable fields than within forest sites (although no significance has been evaluated). In addition, numbers of species per soil core were only half as high in agricultural fields (4.6 ± 3.4) as compared to grasslands (9.3 ± 3.8), but with a comparable variability (Tab. 1). In all sampling sites, singletons had been recorded with the initial effort of 20 soil cores. However, rarefaction curves were not saturated for agricultural and grassland sites (Fig. 2; Supplementary material Figs S1-5).

## 3.2 Number of soil cores required for all except very sporadic species

In coniferous forests, the effort for finding all species except very sporadic species was 6.8 ± 2.3 soil cores – rounded up to 9 (from 6.8 + 2.3 = 9.1) soil cores (Tab. 1, Fig. 2). This reflects a reduction of sampling effort by 55 % as compared to the initial 20 soil cores. In all other habitats, 20 soil cores were not enough to observe all expected species including very sporadic species, since Chao1 estimator showed slightly higher numbers of species for the respective sites (Tab. 1). Still, restricting the sampling effort to species except very sporadic species would reduce the sampling effort to 9.6 ± 1.7 = 12 soil cores (rounded up). i.e., by 40 % in grasslands. Similarly, 8.8 ± 2.1 = 11 soil cores were required in broadleaved forest, with a possible reduction by 45 %. Arable fields showed the lowest species number per soil core, but a significantly higher number of soil cores was required for finding all species (except very sporadic species) in arable fields: 10.4 ± 3.5 = 14, which corresponds to a potential reduction by 30 % (Tab. 1, Fig. 2).

### 3.3 Seasonality and number of sampling events

When including all species (i.e., including very sporadic species found with the sampling effort of 20 soil cores), in grasslands only 64 % of species occurred in both seasons (Fig. 3). In total numbers this corresponds to about four species per individual season, which would have been missed if only one sampling event had been conducted. Similarly, in broadleaved forests 65 % of species were present in both seasons, corresponding to five and three species that were restricted to only one season. In coniferous forests, 79 % of species were observed in both seasons, with only about one species being restricted to either spring or fall. In arable fields, half of the species were present in both seasons, and about 25 % of species would have been missed if sampling had been conducted in fall or spring only, corresponding to about three to four species for each season. However, the high SD shows that there were strong differences among sampling sites within the respective habitats (Fig. 3).

Among the non-singleton species present at the respective sampling sites, 17.7 ± 5.4 % occurred in only one season, either in spring or in fall. This corresponds to 4.4 ± 2.4 species in grasslands, 3.6 ± 2.9 species in arable fields, 1.2 ± 0.7 species in coniferous forests, and 3.3 ± 2.2 species in broadleaved forests (data not shown). These species often occurred in low densities, but in some cases species with comparatively high densities and indicator value were among them (Tab. 2). For example, *Fridericia galba*, considered as an indicator of open land in Toschki et al. (2021), only occurred in fall soil cores in grasslands as well as arable fields.

Species accumulation curves derived from spring soil cores only, fall soil cores only, or from ten randomly picked soil cores from both seasons, did not differ significantly (Fig. 4) when summarized for different habitats, i.e., the curves based on soil cores from spring or fall only were located within the standard deviation of the ten randomly picked soil cores' means. This was the case for datasets including very sporadic species as well as data sets after the removal of very sporadic species. However, for single sampling sites, differences between accumulation curves for spring only, fall only, and ten randomly picked soil cores, were found (Fig. S2-5, supplementary material).

## 4 Discussion

### 4.1 Observed biodiversity

Our first hypothesis, stating that 20 soil cores were sufficient to find all species in a given study site, was only partly confirmed. Only in coniferous forest sites all expected species were found with a sampling effort of 20 soil cores. However, the species number per site expected by the (conservative) Chao1 estimator was only slightly higher than the observed number of species in all habitats. Nonetheless, our results show that in arable fields and broadleaved forests the number of soil cores would have to be much higher (35 and 28, respectively)

**Table 1.** Results of species numbers and sampling effort estimations for the study of Toschki et al. (2021), separately for arable fields, broadleaved forests, coniferous forests, and grasslands. Mean values ± SD. a) species numbers observed in 20 soil cores in the original study, and number of soil cores taken until the respective species number is observed, i.e., saturation of the rarefaction curve. b) Maximum species number per site expected by Chao1 estimator. c) Number of singleton species observed per site. d) Number of non-singleton species observed per site, and number of soil cores taken until the respective species number is observed. e) Number of species observed per soil core in the original dataset (including singletons).

| | **Arable fields** (9 sites) | | **Broadleaved forests** (9 sites) | | **Coniferous forests** (6 sites) | | **Grasslands** (12 sites) | |
|---|---|---|---|---|---|---|---|---|
| | **Species No.** | **No. of soil cores needed** | **Species No.** | **No. of soil cores needed** | **Species No.** | **No. of soil cores needed** | **Species No.** | **No. of soil cores needed** |
| **Species observed in 20 soil cores** | 13.7 ± 5.8 | 14.3 ± 5.0 | 18.2 ± 4.6 | 13.8 ± 4.4 | 11.0 ± 2.4 | 7.8 ± 2.7 | 24.4 ± 8.0 | 14.6 ± 3.2 |
| **Species expected** | 15.2 ± 7.0 | 35.3 ± 36.6 | 19.2 ± 5.9 | 27.7 ± 28.9 | 11.0 ± 2.4 | 7.8 ± 2.7 | 24.9 ± 8.0 | 18.4 ± 6.9 |
| **Singletons per site** | 2.2 ± 2.1 | | 1.7 ± 1.3 | | 0.3 ± 0.5 | | 1.7 ± 0.9 | |
| **Species observed except singletons** | 11.4 ± 4.9 | 10.4 ± 3.5 | 16.6 ± 3.7 | 8.8 ± 2.1 | 10.7 ± 2.5 | 6.8 ± 2.3 | 22.4 ± 7.7 | 9.6 ± 1.7 |
| **Species per soil core** | 4.6 ± 3.4 | | 6.5 ± 3.3 | | 4.9 ± 1.7 | | 9.3 ± 3.8 | |

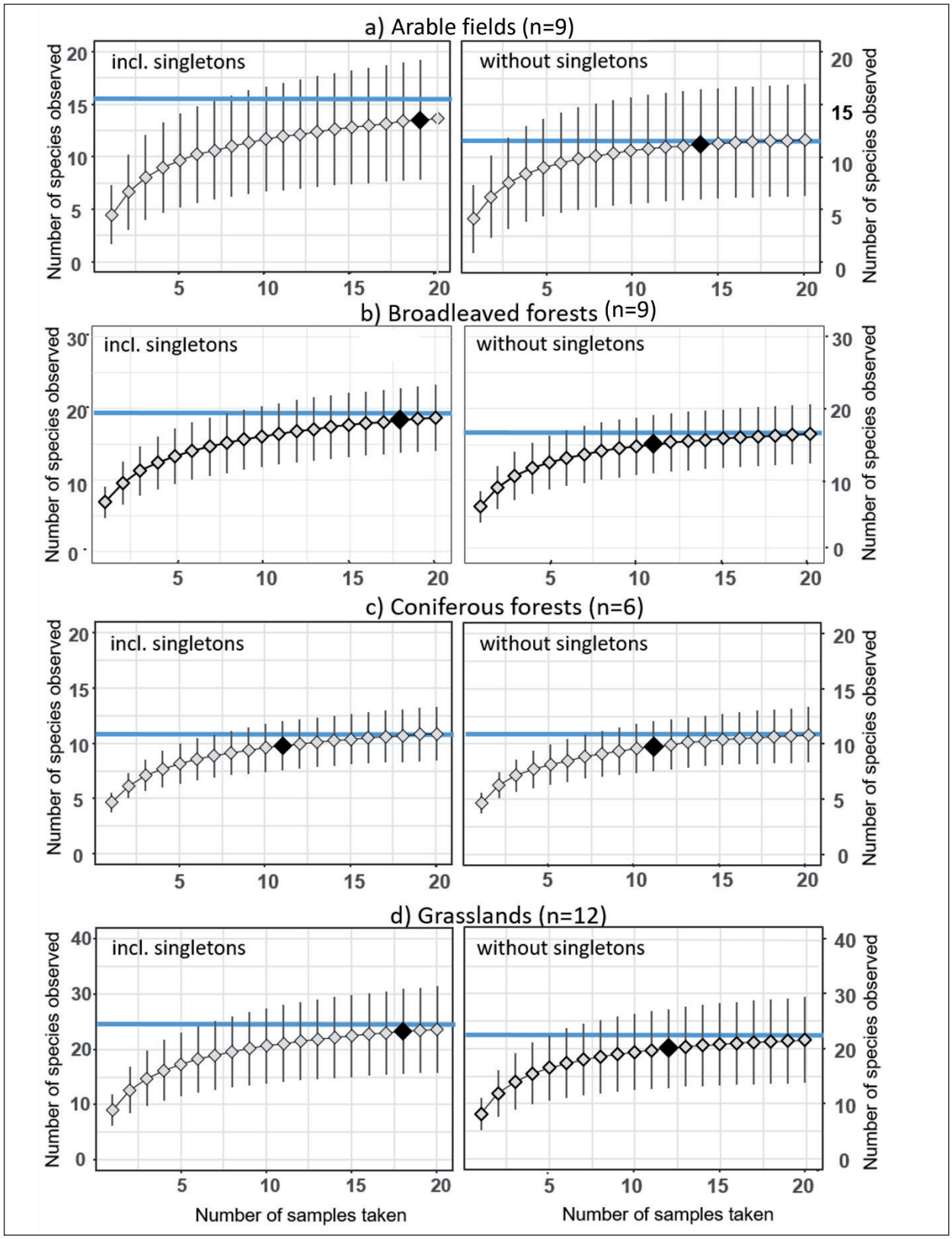



**Figure 2.** Species accumulation curves for different habitats: a) Arable fields. b) Broadleaved forest sites. c) Coniferous forest sites. d) Grassland sites. Graphs on the left side represent raw data including very sporadic species, right graphs represent data after removal of very sporadic species. Curves show means (rhombs) of the respective sites plus SD (vertical lines). The large black rhomb indicates the number of soil cores necessary to obtain all species present in the respective sites, i.e., the number of soil cores where the number of species (rounded up) did not increase further. Horizontal blue lines represent the mean expected number of species per site. Please note the different y-axis scales.

if a complete species inventory was the goal. In all investigated habitats except coniferous forests, the expected number of species was strongly influenced by the number of very sporadic species, i.e., species that were observed as one single individual at a given site. We found on average two singleton species per sampling site, which corresponded to 15% of species in arable fields, and approx. 10% in broadleaved forests and grasslands. On the contrary, when singletons were deleted from the dataset, the slope of rarefaction curves became zero at a soil core number below 20 (see below).

In general, the observed enchytraeid species richness was within the range of species richness found in other studies for the respective habitat types in Central

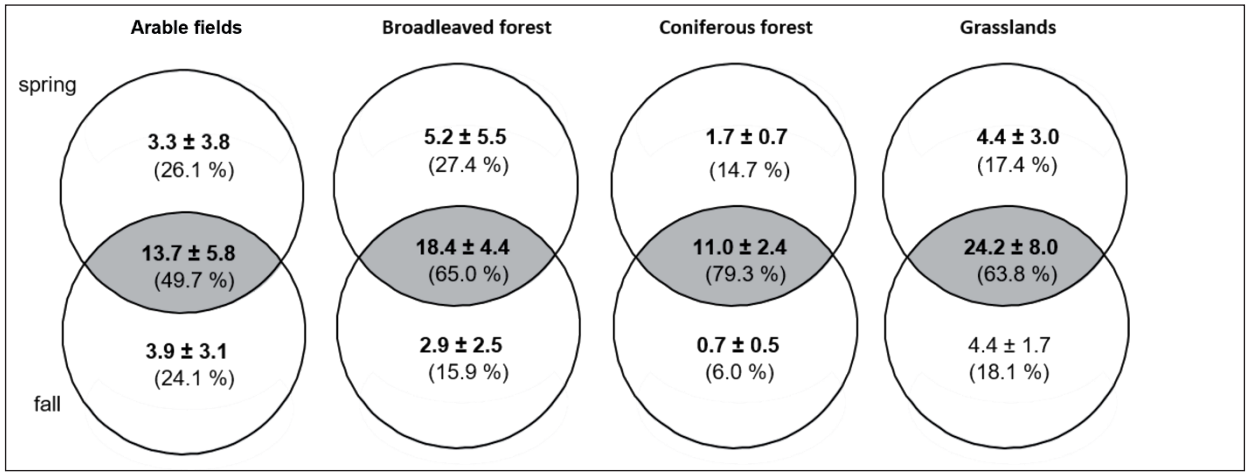



**Figure 3.** Venn diagrams of mean numbers of species (± SD), singletons included, that only occurred either in spring or fall (upper and lower circle, respectively) or in both seasons, shown for different habitats.

**Table 2.** Species that were given indicative value, but only occurred in one season in the study of Toschki et al. (2021). In case of a single sampling event, these species could potentially be overlooked. Species that occurred in only one season in an individual site but in the other season in another site of the same habitat, are not listed here.

| | Arable fields | Broadleaved forest | Coniferous forest | Grassland |
|---|---|---|---|---|
| **Spring** | *Fridericia galba* | *Enchytraeus norvegicus*<br>*Cognettia sphagnetorum (s.l.)**<br>*Fridericia dura*<br>*Fridericia monochaeta*<br>*Marionina clavata* | *Achaeta camerani*<br>*Fridericia striata* | *Achaeta affinis*<br>*Enchytronia minor***<br>*Fridericia galba*<br>*Fridericia lenta*<br>*Marionina communis*<br>*Oconnorella cambrensis* |
| **Fall** | *Fridericia bisetosa* | *Achaeta camerani*<br>*Fridericia striata*<br>*Mesenchytraeus flavus* | *Achaeta bifollicula* | *Achaeta parva*<br>*Achaeta florens***<br>*Enchytronia baloghi*<br>*Fridericia bisetosa*<br>*Fridericia cristeri*<br>*Fridericia maculatiformis*<br>*Fridericia nemoralis*<br>*Henlea ventriculosa* |

* *Cognettia sphagnetorum* includes *C. sphagnetorum* sensu stricto (Martinsson et al. 2015) and *C. pseudosphagnetorum* (Martinsson, Rota & Erséus, 2015). Both species are morphologically indiscriminate.

** *Enchytronia minor* and *Achaeta florens* as *Enchytronia* sp. (MINO) and *Achaeta* sp. (BUUS), respectively, in Toschki et al. (2021).

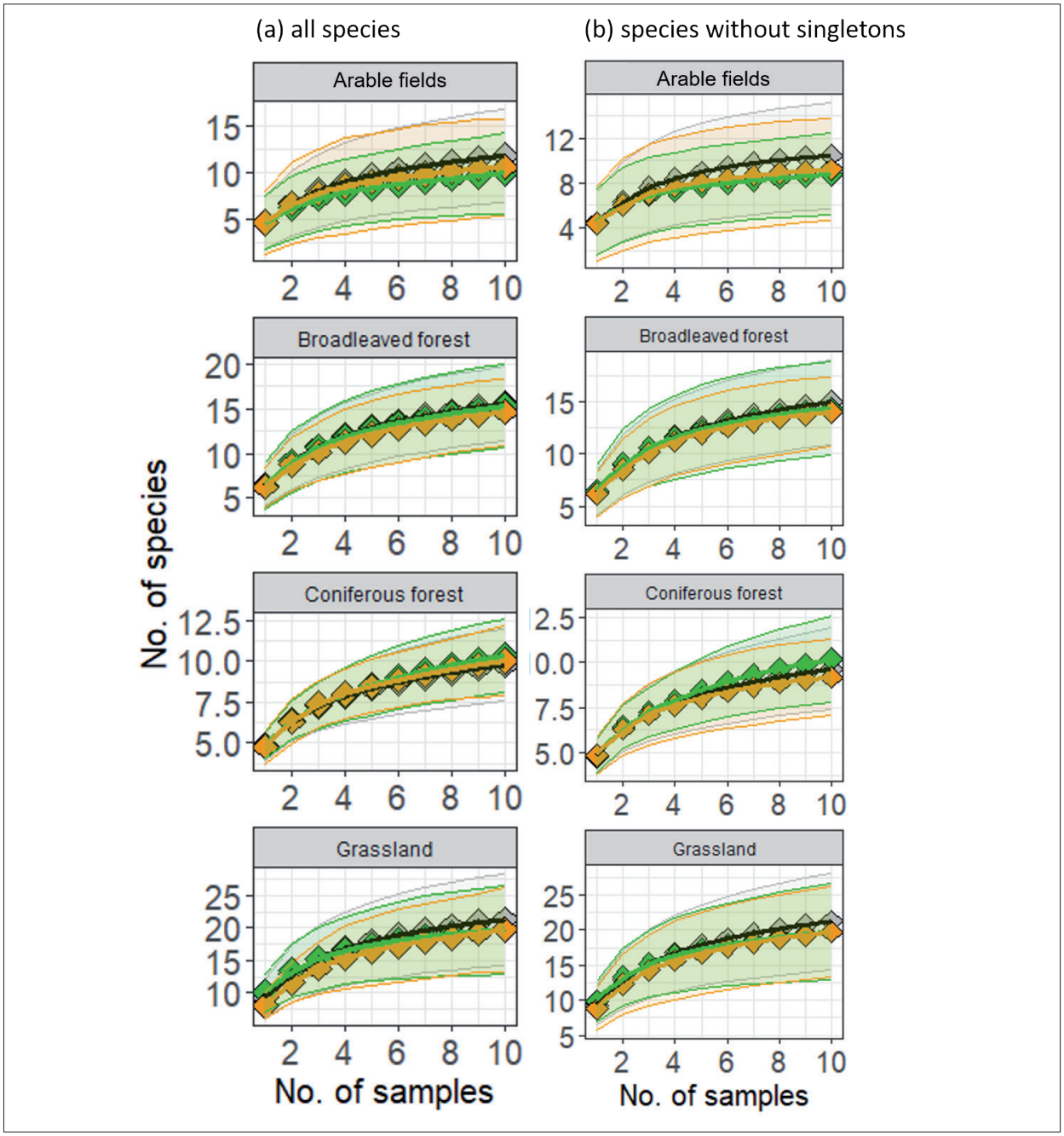



**Figure 4.** Comparison of species accumulation curves between soil cores taken from spring (green), fall (orange), or ten randomly picked soil cores from both seasons (grey; all with ± SD).

European climate (Römbke et al. 2013). The high total species number in grassland sites combined with an intermediate number of singletons resulted in the fact that more than 20 soil cores would have been needed to find all expected species. On the other hand, only twelve soil cores would have been needed to find all species except very sporadic species, which is similar to deciduous forests. But, in contrast to deciduous forests, enchytraeid species assemblages were rather homogeneous in grasslands as indicated by the high number of species per soil core.

Coniferous forests, on the contrary, showed the lowest total species richness, with low variability between sites as well as between soil cores. About half the species present at the respective sites were found in each soil core, and very sporadic species were nearly absent, resulting in the low number of nine soil cores required to represent the entire enchytraeid community. Interestingly, in the present data set, spruce forests showed a slightly higher species richness than pine forests (Fig. S1, supplementary material), while homogeneity of the species composition seemed to be slightly higher in spruce than pine when

comparing the species accumulation curves' saturation. The enchytraeid species composition of coniferous forests in central Europe has been described as species poor and homogeneous before (Schlaghamerský 2002; Lundkvist 1981), with *Cognettia sphagnetorum* s.l. (most often *C. chlorophila* in recent publications) being the dominant species in coniferous forests with mor or moder humus type, low soil pH and high accumulation of needle litter.

Communities of Enchytraeidae in broadleaved forests were intermediate between grasslands and coniferous forests in terms of total species richness, species richness per soil cores as well as presence of very sporadic species. Still, with (non-significantly) lower species richness the number of very sporadic species was similar to grasslands, indicating a less homogeneous distribution of species in broadleaved forests as compared to grasslands. Therefore, the number of soil cores required to find all species except singletons (11) was similar to that in grasslands despite the lower species richness. The high variability of enchytraeid communities between broadleaved forests has also been observed and explained by variability in microhabitats earlier by Schlaghamerský (2010).

Surprisingly, arable fields had a rather heterogeneous enchytraeid species assemblage (despite their relatively low species numbers) that was more similar to coniferous forests than to deciduous forests or grasslands (but not differing significantly from either). Due to this heterogeneity, the soil core number required to find all species was similar to that of broadleaved forests and grasslands, while for all species except very sporadic species, the highest number of soil cores was necessary (i.e., 14). The original publication by Toschki et al. (2021) emphasized that agricultural fields can be very species rich, in particular on sandy soil. Probably, most species arriving in agricultural fields by dispersal were not found as singletons but in low numbers between 1 and 5. In the present dataset, the observed species number of Enchytraeidae in agricultural fields varied between five and 19, which is actually a four-fold increase between the lowest and highest diversity. Schlaghamerský & Kobetičová (2005) found similar diversity in an arable field, but highest species richness in abandoned fields. Van Vliet et al. (1997) explained the high variability of enchytraeid densities in agricultural fields by tillage practice. They emphasize that deep tillage can translocate Enchytraeidae to deeper soil layers which has not been covered by sampling at shallow soil depth (like in the present dataset). The steep slope of the species accumulation curves in arable fields, however, strengthens the view of dispersal as the main driving factor of enchytraeid species richness in agricultural fields. Land use intensity, on the contrary, seemed to be not connected to species richness, since there were intensively as well as extensively managed sites among the most species rich sampling sites. Jänsch et al. (2005) emphasized that only very general distinctions between land use types i.e., between agriculture and grassland, can be made using enchytraeid species richness alone.

## 4.2 Sample number reduction

Our analyses of the dataset of Toschki et al. (2021) confirm that by reducing the number of soil cores no ecologically relevant species would have been missed (hypothesis 2 confirmed). Applying this approach to other habitats or regions might therefore be possible as well and might be considered in biodiversity monitoring or ecological studies. Using only the minimal required number of soil cores will of course result in missing a number of species in the respective sites. Therefore, the approach will not be useful in cases where a complete species inventory of sites is targeted. Further, if enchytraeid abundances are highly variable between soil cores, it might be beneficial to take a higher number of soil cores to increase credibility of results (Gotelli & Colwell 2001).

In the original publication, all observed species had been sorted in tables according to their indicator value for the respective habitats. Species were sorted into indicators (= Kennarten) which were classified as indicative for either a specific habitat and/or soil acidity. The remaining species were classified as either "frequent associate species" (= stetige Begleitarten) or "other associate species" (= sonstige Begleitarten). The first were defined by Toschki et al. (2021) as occurring in rather high densities across a number of different habitats, while the latter were identified as rare, randomly occurring species. Species from both groups of associate species were not given indicator value for describing the respective habitats by Toschki et al. (2021), and are therefore not considered important for the purpose of the present analysis.

If missing very sporadic species present at a given study site is of low concern, a reduction in soil core number is possible in all cases. Thus, all potentially relevant species would have been found with a sampling effort reduced by 30 to 55 % compared to the original study. Comparing the list of possibly missed singleton species with the lists of indicator species in the original publication, we found no overlap between both groups i.e., no indicator species (in the respective site) would have been overlooked by reducing the number of soil cores.

Interestingly, the minimum number of soil cores required is in a similar range in broadleaved forests, coniferous forests and grasslands, although far higher total species numbers in grassland sites as compared to

coniferous forest sites have been observed. On the one hand, the far higher number of species found per soil core in grasslands leads to this effect, suggesting that the (non-singleton) species are relatively homogeneously distributed in grassland. In coniferous forests, on the contrary, very sporadic species were nearly absent and the species composition was the most homogeneous one.

Surprisingly, the number of soil cores required to find all relevant species is higher in agricultural fields than in other habitats. Arable fields represent a harsh environment for all groups of soil animals, as tillage is the predominant disturbance factor (Van Vliet et al. 1997). In addition, use of pesticides, soil compaction, reduced resource availability due to low soil organic matter content, monoculture alternating with fallow periods, and increased erosion reduce habitat quality of agricultural soils for soil fauna (Yeates et al. 1999; Postma-Blaauw et al. 2010). The species inventory of soil animals in agricultural areas is therefore fundamentally limited, as only few species can establish permanent populations under these conditions. At the same time, there is a steady influx of species from surrounding, more species-rich grasslands or forests. While for a number of soil animals wind dispersal (Ojala and Huhta 2001; Ingimarsdóttir et al. 2012; Lehmitz et al. 2012) has been described, little is known about dispersal abilities of Enchytraeidae. For example, dispersal of cocoons attached to the hoofs of wild deer might be possible. Of the individuals that are moved to new habitats, only a few can successfully colonize the sites in the long term, which is also reflected in the low number of species per soil core. This leads, in this study, to a comparatively high number of soil cores required to gain a representative picture of the species assemblage in agricultural fields, in spite of an intermediate to low total number of species. However, in view of these fluctuations, repeated sampling with extended time intervals is possibly more effective than simply increasing the number of soil cores, in order to effectively assess the persistent species assemblage.

### 4.3 Number of sampling events

Our third hypothesis stated that sampling effort can be reduced by reduction of the number of sampling events, but it was not confirmed. While in the original publication, ten soil cores had been taken in spring and ten in fall, we evaluated differences between species accumulation curves at the same site at different seasons or sampling events, and investigated whether reduction to one single sampling event, either spring or fall, would change the results.

First, for the species accumulation curves we did not find evidence for a change in results depending on the season of sampling or number of sampling events, although in individual sampling sites effects of season on the shape of the accumulation curves were observed. Therefore, we cannot advise future studies to concentrate on one specific season, nor to spread their sampling campaign over two seasons, as long as the total number of soil cores is not increased. Only a general increase in sampling intensity led to an increase in the expected number of species per site. However, unfortunately our analyses cannot distinguish between the effect of simply increasing soil core number, the effect of repeated sampling (and therefore temporal differences which might be independent from season), and the effect of season itself. Further, temperature and precipitation in the days / weeks before the sampling event may affect density and probably diversity of drought sensitive enchytraeid species (Ventins & Malecis 2025).

In a second, more qualitative analysis, we looked at the species found at individual sites in only one season, and whether these were species considered indicators in the original publication. The analysis of indicator species in the publication of Toschki et al. (2021) was based on the *indicspecies* package (De Cáceres et al. 2012) which uses the existing dataset to evaluate species for their indicative value. Therefore, only species that have been observed can be designated as indicators, i.e., the indicative value of a species is context (i.e., data set) specific and cannot be transferred easily to other study sites.

It was surprising that in arable fields and forests up to 24 % of the species were found in only one of the two sampling events, while in grasslands it was only 16 % of species. However, in absolute numbers, this corresponds to only two to three species in forests and agricultural fields, but four species in grassland due to the generally higher species richness in grasslands. In the study of Toschki et al. (2021) some species that had been given indicative value only occurred in one of the two seasons. In grasslands, this was the case in *Henlea ventriculosa, Fridericia christeri*, and *F. bisetosa*, among others, which were found only in fall. On the other side, *F. lenta, F. galba*, and *Marionina communis*, among others, were collected only in spring. All of these species are characterized as indicator species for open land or grassland by Toschki et al. (2021), so the number of indicator species would have been much lower if soil cores had been taken in only one of the two sampling events. For agricultural land, only few indicator species could be defined in the original publication, with two of them — *F. galba* and *F. bisetosa* — being also observed in the same distribution pattern in grasslands i.e., being only found in fall. Overall, in open land (grassland and agriculture) there were more indicator species restricted to fall than to spring soil cores in Toschki et al (2021). However, knowledge about seasonality of enchytraeid species is very limited, and higher species

numbers might have been the result of repeated sampling in two consecutive fall or spring seasons, too. We further cannot rule out the possibility that species that had only been observed in one season would have been observed in the other season in another year, or by simply taking more soil cores at a single sampling event. Therefore, the observations of Toschki et al. (2021) do not indicate a specific season for sampling of Enchytraeidae, but highlight that in open land repeated sampling (probably independent of the season) is necessary for gaining a representative picture of the local species composition.

Based on the data from nine study sites, Toschki et al. (2021) describe *M. clavata*, *F. dura*, and *F. monochaeta*, among others, as indicator species for deciduous forests, all of which were found only in spring. Therefore, the proposed indicator species for deciduous forests would have been different ones if the sampling had only been conducted in fall. One of the few enchytraeid species that are known to exhibit distinct seasonal behavior is *S. niveus* that migrates into the litter layer as the leaves fall and retreats deeper into the mineral soil in spring (Dósza-Farkas 1973). This species, which is typical of humus-rich soil types, is therefore most reliably detected in late fall. As one of the few indicator species for coniferous forests, *Achaeta bifollicula* was detected only in fall, suggesting that fall is the preferred season for sampling in coniferous forests as well. On the other side, the most abundant indicator species for coniferous forests – *Enchytraeus norvegicus* – occurred both in spring and fall. Given the similarity of species accumulation curves for spring and fall in combination with the varying behavior of individual species, a clear recommendation for a sampling season cannot be given based on the dataset analyzed here. But like in open land, repeated sampling will probably improve completeness of the enchytraeid species composition in forest sites.

If Toschki et al. (2021) had collected soil cores in only a single sampling event, their list of indicator species would probably have differed from the present results and — with hindsight — would have been incomplete. We found hints that some species with indicator value might only be observed when sampling is conducted in repeated sampling events, most favorably in both spring and fall. Increasing the number of sampling events and time covered - i.e., several years - might result in a more comprehensive overall picture of the enchytraeid fauna of the respective area; it will also show better the dynamics in the species composition. However, this is often not possible due to resource constraints within time-limited research projects. Fortunately, biodiversity monitoring offers the opportunity not only to track temporal changes in enchytraeid species composition but also to complete the species list by consecutive sampling during the course of the monitoring program.

Overall, our results on the importance of seasonality might be applied to temperate regions like Central Europe, while enchytraeid species assemblages in regions with less pronounced seasonal differences might show lower variability. On the other hand, seasonal changes in moisture have been shown to strongly affect enchytraeid populations, e.g., in hemiboreal (Ventins & Malecis, 2025) and tropical regions (John et al. 2021), but the available literature in particular on tropical Enchytraeidae is scarce.

## 5 Conclusion

In the present dataset, a reduction in total soil core number by 30–55 % (depending on habitat) would have been possible without losing important information on species assemblage and dominances. By reducing the number of sampling events, i.e., taking soil cores only in spring or in fall instead of both seasons, in contrast, species considered important indicators might not have been found, because some of these species were only present in one season.

For future studies, the decision about the number and time of sampling is mostly dependent on the respective study goal. A complete species inventory that includes even very sporadic species will need more than 20 soil cores in most habitats, and it needs to cover at least two sampling events, probably in different seasons. An analysis of indicator species can be done with a reduced number of soil cores, in particular in forest sites. However, restricting the sampling to one single sampling event will likely result in incomplete species inventories. Therefore, we recommend a reduction in total number of soil cores rather than the reduction of sampling events.

## Supplementary Online Materials

**Figures S1–S5** accessible at https://doi.org/10.25674/514.

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## References


Anthony, M. A., Bender, S. F., & van der Heijden, M. G. A. (2023). Enumerating soil biodiversity. *Proceedings of the National Academy of Sciences,* 120. https://doi.org/10.1073/pnas

Ballasus, H., Lachmann, C., Weiß, L., Nabel, M., Lenzen, J., Pieper, S., Glante, F., Höper, H., Fröhlich, J., Walter, , et al. (2026). *Konzept für ein bundesweites Biodiversitätsmonitoring – Grundlagen und bundesweite Ersterfassung.* https://doi.org/10.5281/zenodo.20152935

Bardgett, R. D. (2005). *The biology of soil: A community and ecosystem approach* (1st ed.). Oxford University Press.

Beylich, A., & Graefe, U. (2002). Annelid coenoses of wetlands representing different decomposer communities. In G. Broll, W. Merbach, & E.-M. Pfeiffer (Eds.), *Wetlands in Central Europe* (pp. 1–10). Springer.

Bonkowski, M., & Roy, J. (2012). Decomposer community complexity affects plant competition in a model early successional grassland community. *Soil Biology and Biochemistry,* 46, 41–48. https://doi.org/10.1016/j.soilbio.2011.11.013

Briones, M. J. I., Poskitt, J., & Ostle, N. (2004). Influence of warming and enchytraeid activities on soil CO2 and CH4 fluxes. *Soil Biology and Biochemistry,* 36, 1851–1859. https://doi.org/10.1016/j.soilbio.2004.04.039

Conti, E., & Mulder, C. (2022). Chemistry-driven Enchytraeidae assemblages acting as soil and ecosystem engineers in edaphic communities. *Ecological Indicators,* 144, 109529. https://doi.org/10.1016/j.ecolind.2022.109529

Creamer, R. E., Hannula, S. E., Leeuwen, J. P. V., Stone, D., Rutgers, M., Schmelz, R. M., Ruiter, P. C., Hendriksen, N. B., Bolger, T., Bouffaud, M.-L., et al. (2016). Ecological network analysis reveals the inter-connection between soil biodiversity and ecosystem function as affected by land use across Europe. *Applied Soil Ecology,* 97, 112–124. https://doi.org/10.1016/j.apsoil.2015.08.006

Cuartero, J., Briones, M. J. I., Rast, B. M., Stierli, B., Maurer-Troxler, C., Hug, A. S., Widmer, F., Schlaghamerský, J., & Frey, B. (2025). Earthworm and enchytraeid indicator taxa of different land-use types identified using soil DNA metabarcoding. *Applied Soil Ecology,* 206, 105891. https://doi.org/10.1016/j.apsoil.2025.105891

De Cáceres, M., Legendre, P., Wiser, S. K., & Brotons, L. (2012). Using species combinations in indicator value analyses. *Methods in Ecology and Evolution,* 3, 973–982. https://doi.org/10.1111/j.2041-210X.2012.00246.x

Degtyarev, M. I., Korobushkin, D. I., Gongalsky, K. B., & Zaitsev, A. S. (2019). Enchytraeid community (Annelida, Clitellata, Enchytraeidae) and its dependence on edaphic conditions in rice agroecosystems in Russia. *Russian Journal of Ecology,* 50, 384–390.

Dósza-Farkas, K. (1973). Saisondynamische Untersuchungen des Enchytraeiden-Besatzes im Boden eines ungarischen Quercetum petraeae cerris. *Pedobiologia,* 13, 361–367.

European Parliament and the Council of the European Union. (2025). *Directive (EU) 2025/2360 of the European Parliament and of the Council of 12 November 2025 on soil monitoring and resilience (Soil Monitoring Law).* https://eur-lex.europa.eu/eli/dir/2025/2360/oj

Filser, J., Faber, J. H., Tiunov, A. V., Brussaard, L., Frouz, J., De Deyn, G., Uvarov, A. V., Berg, M. P., Lavelle, P., Loreau, M., et al. (2016). Soil fauna: Key to new carbon models. *SOIL,* 2, 565–582. https://doi.org/10.5194/soil-2-565-2016

Gao, C., & Dusa, A. (2026). *ggVennDiagram: A 'ggplot2' implement of Venn diagram* (Version 1.5.7) [R package]. https://doi.org/10.32614/CRAN.package.ggVennDiagram

Gotelli, N. J., & Colwell, R. K. (2001). Quantifying biodiversity: Procedures and pitfalls in the measurement and comparison of species richness. *Ecology Letters,* 4, 379–391. https://doi.org/10.1046/j.1461-0248.2001.00230.x

Graefe, U., & Schmelz, R. M. (1999). Indicator values, strategy types and life forms of terrestrial Enchytraeidae and other microannelids. *Newsletter on Enchytraeidae,* 6, 59–67.

Ingimarsdóttir, M., Caruso, T., Ripa, J., Magnúsdóttir, Ó. B., Migliorini, M., & Hedlund, K. (2012). Primary assembly of soil communities: Disentangling the effect of dispersal and local environment. *Oecologia,* 170, 745–754. https://doi.org/10.1007/s00442-012-2334-8

John, K., Zaitsev, A. S., & Wolters, V. (2021). Soil fauna groups respond differentially to changes in crop rotation cycles in rice production systems. *Pedobiologia,* 84, 150703. https://doi.org/10.1016/j.pedobi.2020.150703

Jänsch, S., Römbke, J., & Didden, W. (2005). The use of enchytraeids in ecological soil classification and assessment concepts. *Ecotoxicology and Environmental Safety,* 62, 266–277. https://doi.org/10.1016/j.ecoenv.2004.10.025

Józefowska, A., Wos, B., Pietrzykowski, M., & Schlaghamerský, J. (2020). Colonisation by enchytraeids as a suitable indicator of successful biological reclamation of post-mining technosols using alders. *Applied Soil Ecology,* 145. https://doi.org/10.1016/j.apsoil.2019.06.003

Korobushkin, D. I., Guseva, P. A., Gongalsky, K. B., Saifutdinov, R. A., Zaitsev, A. S., & Degtyarev, M. I. (2024). Are there different trophic niches of enchytraeids? A stable isotopic (δ13C, δ15N) evidence. *Soil Biology and Biochemistry,* 194, 109422. https://doi.org/10.1016/j.soilbio.2024.109422

Lehmitz, R., Russell, D. J., Hohberg, K., Christian, A., & Xylander, W. E. R. (2012). Active dispersal of oribatid mites into young soils. *Applied Soil Ecology,* 55, 10–19.

Liiri, M., Ilmarinen, K., & Setälä, H. (2002). The significance of Cognettia sphagnetorum (Enchytraeidae) on nitrogen availability and plant growth in wood ash-treated humus soil. *Plant and Soil,* 246, 31–39. https://doi.org/10.1023/A:1021515313890

Lundkvist, H. (1981). *Enchytraeidae (Oligochaeta) in pine forest soils: Population dynamics and response to environmental changes.* Doctoral thesis. Uppsala University.

Martinsson, S., & Erséus, C. (2014). Cryptic diversity in the well-studied terrestrial worm *Cognettia sphagnetorum* (Clitellata: Enchytraeidae). *Pedobiologia,* 57(1), 27–35.

Martinsson, S., Rota, E., & Erséus, C. (2015). Revision of *Cognettia* (Clitellata, Enchytraeidae): Reestablishment of *Chamaedrilus* and description of cryptic species in the *sphagnetorum* complex. *Systematics and Biodiversity,* 13, 257–277.

Ojala, R., & Huhta, V. (2001). Dispersal of microarthropods in forest soil. *Pedobiologia,* 45, 443–450.

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P. M., et al. (2020). *vegan: Community Ecology Package*. https://cran.r-project.org/package=vegan

Oliverio, A. M., Gan, H., Wickings, K., & Fierer, N. (2018). A DNA metabarcoding approach to characterize soil arthropod communities. *Soil Biology and Biochemistry,* 125, 37–43. https://doi.org/10.1016/j.soilbio.2018.06.026

O'Connor, F. B. (1967). The Enchytraeidae. In A. Burges & R. Raw (Eds.), *Soil biology* (pp. 213–257). Academic Press.

Pelosi, C., & Römbke, J. (2016). Are Enchytraeidae (Oligochaeta, Annelida) good indicators of agricultural management practices? *Soil Biology and Biochemistry,* 100, 255–263. https://doi.org/10.1016/j.soilbio.2016.06.030

Petersen, H., & Luxton, M. (1982). A comparative analysis of soil fauna populations and their role in decomposition processes. *Oikos,* 39, 288–388.

Postma-Blaauw, M. B., De Goede, R. G. M., Bloem, J., Faber, J. H., & Brussaard, L. (2010). Soil biota community structure and abundance under agricultural intensification and extensification. *Ecology,* 91, 460–473.

R Foundation for Statistical Computing. (2025). *R version 4.5.1 (2025-06-13 ucrt): Great Square Root* [Computer software].

Remelli, S., Gruss, I., & Menta, C. (2026). Advances in soil arthropod identification: Integrating morphological, molecular, and AI-based approaches. *European Journal of Soil Biology,* 128, 103812. https://doi.org/10.1016/j.ejsobi.2026.103812

Römbke, J., Jänsch, S., Höfer, H., Horak, F., Roß-Nickoll, M., Russell, D. J., & Toschki, A. (2013). State of knowledge of enchytraeid communities on German soils as a basis for biological soil quality assessment. *Soil Organisms,* 85, 123–146.

Saito, V. S., Fonseca-Gessner, A. A., & Siqueira, T. (2015). How should ecologists define sampling effort? The potential of Procrustes analysis for studying variation in community composition. *Biotropica,* 47, 399–402. https://doi.org/10.1111/btp.12222

Schlaghamerský, J. (2002). The Enchytraeidae of spruce forest plots of different exposure and acid deposition in a German mountain range. *European Journal of Soil Biology,* 38, 305–309. https://doi.org/10.1016/S1164-5563(02)01164-0

Schlaghamerský, J. (2010). The small annelids (Annelida: Enchytraeidae, Rhyacodrilinae, Aeolosomatidae) in soils of three forests in the White Carpathians (Czech Republic). *Acta Societatis Zoologicae Bohemicae,* 74, 103–115.

Schlaghamerský, J., & Kobetičová, K. (2005). A small annelid community (Enchytraeidae, Tubificidae, Aeolosomatidae) during meadow restoration on arable land and in a nearby well-preserved meadow. *Proceedings of the Estonian Academy of Sciences: Biology and Ecology,* 54, 323–330.

Schmelz, R. M. (2016a). *Edaphobase Länderstudien Bayern Enchytraeidae* [Data set]. Edaphobase. https://portal.edaphobase.org/

Schmelz, R. M. (2016b). *Edaphobase Länderstudien NP Eifel Enchytraeidae* [Data set]. Edaphobase. https://portal.edaphobase.org/

Schmelz, R. M. (2016c). *Edaphobase Länderstudien Sachsen Enchytraeidae* [Data set]. Edaphobase. https://portal.edaphobase.org/

Schmelz, R. M. (2016d). *Edaphobase Länderstudien Sachsen-Anhalt Enchytraeidae* [Data set]. Edaphobase. https://portal.edaphobase.org/

Schmelz, R. M., & Collado, R. (2010). A guide to European terrestrial and freshwater species of Enchytraeidae. *Soil Organisms,* 82, 1–176. https://doi.org/10.1007/978-94-017-5279-4_3

Serbource, C., Sammartino, S., Cornu, S., Papillon, J., Adrien, J., & Pelosi, C. (2025). Enchytraeids: Small but important ecosystem engineers. *Geoderma,* 453, 4–10.

Sgarbi, L. F., Bini, L. M., Heino, J., Jyrkänkallio-Mikkola, J., Landeiro, V. L., Santos, E. P., Schneck, F., Siqueira, T., Soininen, J., Tolonen, K. T., & Melo, A. S. (2020). Sampling effort and information quality provided by rare and common species in estimating assemblage structure. *Ecological Indicators,* 110. https://doi.org/10.1016/j.ecolind.2019.105937

Toschki, A., Burkhardt, U., Haase, H., Höfer, H., Jänsch, S., Oellers, J., Römbke, J., Roß-Nickoll, M., Salamon, J.-A., Schmelz, R. M., Scholz-Starke, B., & Russell, D. J. (2021). Die Edaphobase-Länderstudien: Synökologische Untersuchungen von Bodenorganismen in einem Biotop- und Standortgradienten in Deutschland 2014–2018. *Peckiana,* 14, 1–367.

Van Vliet, P. C. J., Coleman, D. C., & Hendrix, P. F. (1997). Population dynamics of Enchytraeidae (Oligochaeta) in different agricultural systems. *Biology and Fertility of Soils,* 25, 123–129. https://doi.org/10.1007/s003740050291

Vandewalle, M., de Bello, F., Berg, M. P., Bolger, T., Dolédec, S., Dubs, F., Feld, C. K., Harrington, R., Harrison, P. A., Lavorel, S., et al. (2010). Functional traits as indicators of biodiversity response to land use changes across ecosystems and organisms. *Biodiversity and Conservation,* 19, 2921–2947. https://doi.org/10.1007/s10531-010-9798-9

Ventins, J., & Melecis, V. (2025). Boreal forest enchytraeids (Clitellata, Enchytraeidae) population increase due to precipitation: Long-term data from Latvia. *Applied Soil Ecology,* 206, 105882. https://doi.org/10.1016/j.apsoil.2025.105882

Wickham, H. (2016). *ggplot2: Elegant graphics for data analysis*. Springer.

Yeates, G. W., Wardle, D. A., & Watson, R. N. (1999). Responses of soil nematode populations, community structure, diversity and temporal variability to agricultural intensification over a seven-year period. *Soil Biology and Biochemistry,* 31, 1721–1733.