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Investigations of annelids at soil monitoring sites in Northern Germany: reference ranges and time-series data

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Abstract

Soil zoological investigations at soil monitoring sites are carried out with the objective to track changes in the soil's habitat function. At 60 soil-monitoring sites in north-western Germany, earthworms and microannelids (enchytraeids, tubificids and polychaetes) are currently being used as system indicators for the soil biota. Investigations started 1992, followed by re-investigations every 5 to 10 years. Variations in abundance, biomass and species number of annelids are assessed with respect to non-directional fluctuations or directional changes, caused by natural variations of environmental factors or due to management practices. The sites are grouped according to land-use type and site condition into six different categories for which typical ranges of variation of the zoological parameters can be distinguished (reference ranges). Especially at sites that passed three investigations already, major changes become discernible. If the temporal variation goes in the same direction throughout the time series, it is considered a trend. When a value shifts substantially out of its reference range, a change of the system state is probable, especially if this applies for two or more parameters simultaneously. Three examples are given for considerable changes of the annelid community due to land-use change or natural succession. The detection of substantial changes of the community is based mainly on the species composition, but is supported by quantitative parameters using the reference ranges.

Keywords: earthworms, enchytraeids, species composition, land use, soil properties

1. Introduction

The objectives of continuous soil monitoring are to avoid degradation of soil quality and to preserve the soil's functions. Monitoring data should illustrate trends and thus enable to take precautionary measures as early as possible. Soil quality is strongly linked with the performance of soil organisms, e.g. by litter decomposition, carbon storage and bioturbation. Therefore, soil monitoring should include monitoring of soil biological parameters (Barth et al. 2000, Huber et al. 2007). Continuous monitoring of soil biological parameters produces time-series data that require interpretation and evaluation. One question to be answered generally is, whether the measured values are located within the typical range for the given parameter and site conditions, or whether they lie outside this range and thus possibly indicate anthropogenic disturbance. To detect deviations from the expected range, it is necessary to define thresholds for different site conditions. Upper and lower threshold form the reference range of what can be expected for a certain parameter at certain site conditions.

Soil monitoring sites in Germany are run by the Federal States. Although following a harmonised programme, the States are free, within certain limits, to decide on the methods and the investigated parameters. Apart from chemical, physical and microbiological parameters, earthworms are studied most often, while small annelids (enchytraeids and other terrestrial microannelids) are continuously investigated only in three Federal States out of 16, namely Schleswig-Holstein, North Rhine-Westphalia and Hamburg. In the following, we use monitoring data of both annelid groups from these three Federal States to define reference ranges for the parameters abundance, biomass (earthworms only) and species number. These reference ranges apply to soils in north-western Germany; for regions with other pedo-climatic conditions the ranges will have to be modified and expanded. Further, not all types of soil and parent material of the region were covered by the investigated sites. For example, a category 'base-rich forests with mull humus forms' unaffected by acidification is lacking among the monitoring sites of the three Federal States. Consequently no reference ranges were defined for this soil condition.

Abundance, biomass and species numbers are quantitative parameters. However, for a thorough evaluation of soil biological changes the consideration of qualitative parameters based on the species composition is necessary (Didden 2003, Römbke et al. 2005). Reference ranges for quantitative parameters were supplemented here by ranges for mean indicator values for soil moisture and soil reaction according to Graefe & Schmelz (1999) (Tab. 1). The classification of species assemblages into decomposer community types is given as additional information for the comparison of sites. It follows the approach proposed by Graefe (1993, 1998) and Beylich & Graefe (2002).

Tab. 1 Indicator values of terrestrial annelids with respect to soil moisture and soil reaction (from Graefe & Schmelz 1999).

M	**Moisture figure** – Occurrence in the gradient of soil moisture
5	fresh-site indicator, mainly in well-aerated, fresh soils
7	damp-site indicator, mainly in constantly moist or damp, but not in wet soils
9	wet-site indicator, mainly in wet, often badly aerated soils
11	aquatic species
R	**Reaction figure** – Occurrence in the gradient of soil acidity and lime content
1	indicator of extreme acidity, never in slightly acid or alkaline soils
3	acidity indicator, mainly in acid soils, but exceptionally up to the neutral range
5	indicator of moderate acidity, only occasionally in strongly acid or neutral soils
7	indicator of slightly acid to slightly alkaline conditions, never in strongly acid soils
9	basic reactions and lime indicator, always found in calcareous soils
x	indifferent or unknown behaviour, even numbers for intermediate behaviour

Investigation sites

Among the 60 permanent soil monitoring sites of the Federal States Schleswig-Holstein, Hamburg, and North Rhine-Westphalia, 55 sites were suitable for the establishment of reference values. These sites cover forest (21 sites), grassland (17 sites) and fields (17 sites). We used data collected up to the end of 2007. By that time, most of the sites had passed three investigation cycles at an interval of 5–10 years, some of them only two. Sites with special soil and/or land-use conditions as dune soils or unmanaged grassland fallow were excluded from reference-range calculations.

2. Methods

At all monitoring sites earthworms and microannelids were studied. Microannelids in this contribution comprise mainly enchytraeids, but sometimes also tubificids and polychaetes. For convenience, the terms 'enchytraeids' and 'microannelids' are used synonymously here. The parameters studied at the soil-monitoring sites are compiled in Tab. 2. Ten samples were taken at each monitoring site. Earthworm sampling was carried out by a combination of formalin extraction (0.25 m²; DIN ISO 2006a), hand-sorting, and Kempson extraction. For hand-sorting the samples were taken with a soil corer (250 cm², 20 cm depth). The hand-sorted samples were afterwards extracted with a Kempson extractor (heat extraction) to obtain very small specimens that had been overlooked during hand-sorting.

Tab. 2 Soil zoological parameters investigated at soil monitoring sites in north-western Germany.

Parameter	Indicator function
Total abundance of microannelids (ind. m^{-2}) Total biomass of earthworms (g m^{-2})	Indicators of soil faunal activity
Community structure: species composition, species number, abundance, dominance and frequency of species	Indicators of soil biodiversity
Vertical distribution of enchytraeids: total and species level	Indicator of the vertical extent and strength of biological processes
Biomass and biomass dominance of earthworm species	Ecological significance of the species
Aggregated parameters: Strategy-type and life-form spectra Average indicator values Decomposer community type	Biological indicators for the state of the system and the impact of environmental factors

Soil samples for enchytraeids were taken according to DIN ISO (2006b) with a split soil corer (diameter 3.8 cm (cropland) or 5 cm (forest, grassland)). Sampling depth was 24 cm at cropland sites and 10 cm at forest and grassland sites. Samples were divided vertically into 4 sub-samples of equal height. Soil samples were extracted over 48 h by a wet-funnel technique without heating (following Graefe 1984, as cited in Dunger & Fiedler 1989, p. 301; DIN ISO 2006b). The extracted animals were counted and identified in vivo.

Data for abiotic soil properties in the upmost mineral horizons, mainly pH and clay content, were taken from Metzger et al. (2005), Quirin & Emmerling (2004), Gröngröft et al. (2003) and Gröngröft & Karrasch (2005). Soil pH was measured in $CaCl_2$ solution (Schleswig-Holstein and Hamburg) or KCl (North Rhine-Westphalia). The pH was mostly not measured in the year when the soil fauna sampling took place.

The soil water regime can be assessed by determining a number of different parameters such as groundwater level (with varying temporal resolution), water tension or water content, or by classification figures that integrate several parameters. One of the latter is the degree of water-logging (German: 'Vernässungsgrad' **Vn**) according to Ad-hoc-AG Boden (2005). It is estimated in the field by considering hydromorphic characters of the soil profile and the humus content. The scale for Vn ranges from Vn0 (not water-logged) to Vn7 (extremely water logged). It is regularly assessed at the soil monitoring sites in Schleswig-Holstein. For the few grassland sites in North Rhine-Westphalia and Hamburg the Vn was estimated by the authors to be either lower than Vn5 (not wet) or to be equal or above Vn5 (wet) judging from absence or presence of hydromorphic horizons in the soil profile.

For the definition of reference values we established six categories (Tab. 4). The sites were first separated according to land-use into forest, grassland and cropland (field). For further categorisation within each land-use type the zoological data were checked for significant correlations with pH or clay content. The parameter with the stronger correlation was then applied to define two categories within each land-use type. At grassland sites we used the degree of water-logging (Vn) to define the two categories, because correlations of zoological data with pH or clay content were almost absent. Since Vn is based on estimations, in some cases no check for significance was carried out. The threshold value separating each category within a land-use type was partly determined by the data structure and partly by expert knowledge (see results). The two categories of the same land-use type were than tested for significant differences of the zoological parameters. Descriptive statistics were calculated with MS Excel, tests for significance of correlations or variation were calculated with SPSS 15.0. As not all data were normally distributed and datasets for some categories were rather small, non-parametric tests were chosen for correlation analysis (Spearman) and tests for significance of differences (Mann-Whitney U-test). As reference range we define the range between minimum and maximum of the available data for one category (site type). The reference ranges are visualised by box-whisker-plots, where the box represents the central 50 % of the data within the distance between the quartiles (25th and 75th percentiles) and the whiskers represent minimum and maximum. The boxplot representation allows a quick estimation of the position of a site to be evaluated within the range (low / high / central), if desired. Outliers are excluded on the basis of box plot analysis. It defines outliers as values lying more than one and a half times the box's length outside the box.

3. Results

For the forest sites correlation analysis showed significant correlation of pH with species numbers of both animal groups and with earthworm biomass (Tab. 3). Correlation with clay content was much weaker and only significant for enchytraeid species number. Soil pH of the investigated forest sites ranges from 2.5 to 4.1. Species numbers of both annelid groups and earthworm biomass increase with increasing pH (Fig. 1). Enchytraeid abundance is negatively correlated with pH. Within forest sites, two groups are distinguishable: (1) Sites with less than

10 enchytraeid species and frequently only 1 earthworm species or no earthworms at all, and (2) sites with more than 10 enchytraeid species and earthworms always present, generally with 2 or more species. The threshold between these groups is at pH 3.4. Deviations from this pattern (Fig. 1) are partly due to differences in the pH of the upmost mineral horizon, which was used for data analysis, and the pH of the organic layer, which is the main habitat of the soil fauna. The two categories differ significantly in species numbers (T-test, $p < 0.01$). Earthworm abundance and biomass is also significantly higher at forest sites with pH ≥ 3.4. Therefore we define two reference ranges for forest sites depending on pH values (Tab. 4).

Tab. 3 Correlation coefficients for correlations between zoological parameters under different land use and soil acidity or clay content. *: significant at 0.05 % level, **: significant at 0.01 % level.

			pH	Clay content (%)
Forest	Microannelids	abundance	–0.264	0.252
		species number	0.530 **	0.314 *
	Earthworms	abundance	0.245	0.033
		biomass	0.473 **	0.123
		species number	0.471 **	0.15
Field	Microannelids	abundance	–0.069	0.073
		species number	0.075	0.145
	Earthworms	abundance	0.533 **	0.543 **
		biomass	0.457 **	0.532 **
		species number	0.534 **	0.731 **
Grassland	Microannelids	abundance	–0.262	0.015
		species number	–0.293	–0.232
	Earthworms	abundance	–0.053	0.461 *
		biomass	–0.215	–0.202
		species number	0.1	0.308

Soil pH of the cropland sites ranges from 4.2 to > 6, clay content from 2.5 % to 23.5 %. Within this range, neither soil acidity nor clay content seems a decisive factor for microannelids as the correlations were not significant (Tab. 3, Fig. 1). Earthworm abundance, biomass and species number are positively and significantly correlated with soil acidity and soil clay content (Tab. 3), the correlation with clay content being a little stronger. As the pH is correlated with and partly dependent on the clay content, the latter was considered the predominantly influencing factor at cropland sites. To differentiate reference ranges in dependence of clay content, another parameter is taken into account: the occurrence of anecic earthworm species. These species were rarely found in agricultural fields with clay contents below 8 %, but always, when the clay content was ≥ 8 % (Fig. 2). With respect to soil texture the chosen threshold forms also the borderline between 'sand' or 'silty sand' on the one hand and 'loamy sand', 'sandy loam' or 'silty loam' on the other, according to the German Guidelines for Soil Mapping (Ad-hoc-AG Boden 2005). Two reference ranges for field sites were defined depending on the clay content (Tab. 4). Species number, abundance and biomass of earthworms are significantly higher in soils with ≥ 8 % clay.

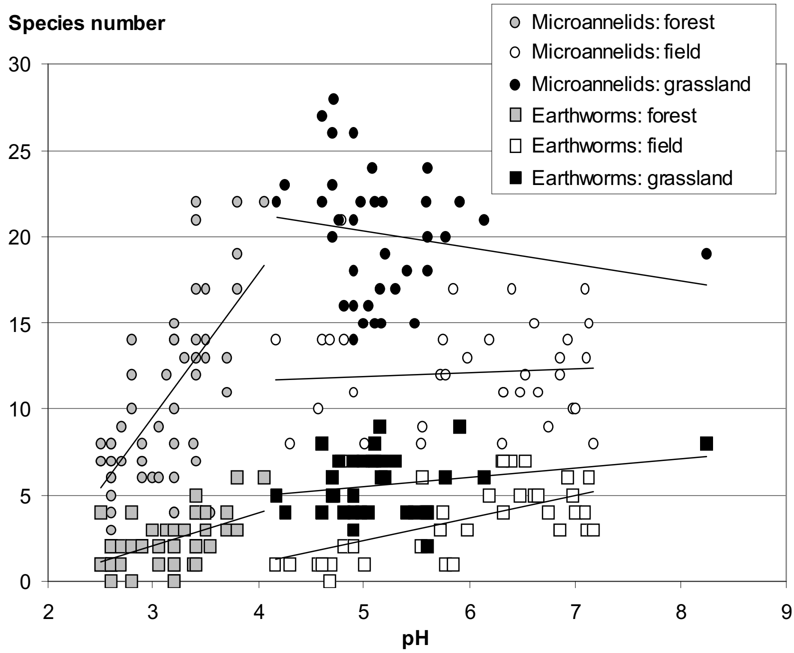

Fig. 1 Correlation of species numbers of microannelids and earthworms with pH below 4.2 (forest sites) and pH ≥ 4.2 (field and grassland). Significance of correlation see Tab. 3.

Soil pH of the grassland sites ranges from 4.2 to > 6, clay content from 2.5 % to 40 %. No significant correlation with the pH value was found neither for earthworms nor enchytraeids (biomass, abundance, species number). The texture of grassland soils was not correlated with enchytraeid parameters. A positive correlation was found for clay content and earthworm abundance. As this was not sufficient for defining reference ranges, the soil water regime was considered for further differentiation. The aim was to separate 'wet' soils from the rest. This was achieved using the Vn as estimation for water-logged conditions and separating wet grasslands (≥ Vn5) from the others (< Vn5). Species numbers and abundances are not significantly different for these two groups, but the species composition differs considerably. Characteristic for the wet grassland sites is the occurrence of wetness indicators according to Graefe & Schmelz (1999) and the absence of big anecic earthworm species. Thus earthworm biomass is significantly lower at the wet grassland sites. Two reference ranges for grassland sites was defined depending on the Vn (Tab. 4).

Tab. 4 Reference ranges for abundance, biomass and species number of terrestrial annelids. Significance of differences tested for each two categories belonging to the same land-use type. ×: mean; n: number of investigations; Med: median; sd: standard deviation; n.s.: not significant; **: significant with $p < 0.01$. Microannelid abundances of minimum, maximum and median were rounded to the nearest thousand. Vn: degree of water logging.

Category	**pH Clay Soil water (Vn)**	**Land use Humus form**	**n**	**Abundance Microannelids [ind. m⁻²]**		**Abundance Earthworms [ind. m⁻²]**		**Biomass Earthworms [g m⁻²]**		**Species number Microannelids**		**Species number Earthworms**	
				Min–Max Med	Mean sd	**Min–Max** Med	Mean sd	**Min–Max** Med	Mean sd	**Min–Max** Med	Mean sd	**Min–Max** Med	Mean sd
1	< 3.4 < 3.4 ×: 12.9% clay	Forest or heathland; moder or mor humus forms	35	**21 000 –155 000** 74 000	82 459 46 254 n.s.	**0–45** 14	23.4 33.2 **	**0–5** 1	1.7 2.1 **	**3–14** 7.0	8.1 3.1 **	**0–3** 2.0	1.7 1.1 **
2	> 3.4 – 4.2 ×: 13.0 % clay	Forest or heathland; moder or mor humus forms	20	**25 000 – 131 00** 64 000	69 975 28 829 n.s.	**2–411** 54	123.1 153.3 **	**> 0–18** 8	7.1 4.9 **	**4–22** 15.5	15.3 5.0 **	**1–6** 3.5	3.5 1.3 **
3	4.3–5.9 < 8 % Clay	Field; Mull humus forms	13	**2000 – 37 000** 20 000	23 112 25 529 n.s.	**0–83** 32	44.2 52.8 **	**0–24** 12	11.6 8.6 **	**5–17** 12.0	11.3 3.4 n.s.	**0–3** 1.0	1.3 0.75 **
4	> 5.8 > 8 % Clay	Field; Mull humus forms	23	**2000 – 50 000** 22 000	22 135 12 974 n.s.	**35–480** 133	190.7 172.9 **	**5–126** 45	63.7 51.5 **	**8–17** 12.0	12.5 3.2 n.s.	**3–7** 5.0	4.9 1.5 **
5	> 4.2 ×: 14.0 % clay Vn 0–4	Grassland; Mull humus forms	27	**9000 – 75 000** 29 000	33 885 18 737 n.s.	**91–584** 264	312.4 190.6 n.s.	**37–335** 102	130.6 75.5 **	**14–28** 21.0	20.6 3.9 n.s.	**2–9** 6.0	5.6 1.9 n.s.
6	> 4.9 organic material Vn 5–6	Wet grassland; Mull humus forms	11	**9000 – 63 000** 34 000	31 502 18 939 n.s.	**200–484** 288	323.5 131.7 n.s.	**9–114** 64	64.7 29.4 **	**15–22** 19.0	18.8 2.3 n.s.	**4–7** 5.0	5.2 1.2 n.s.

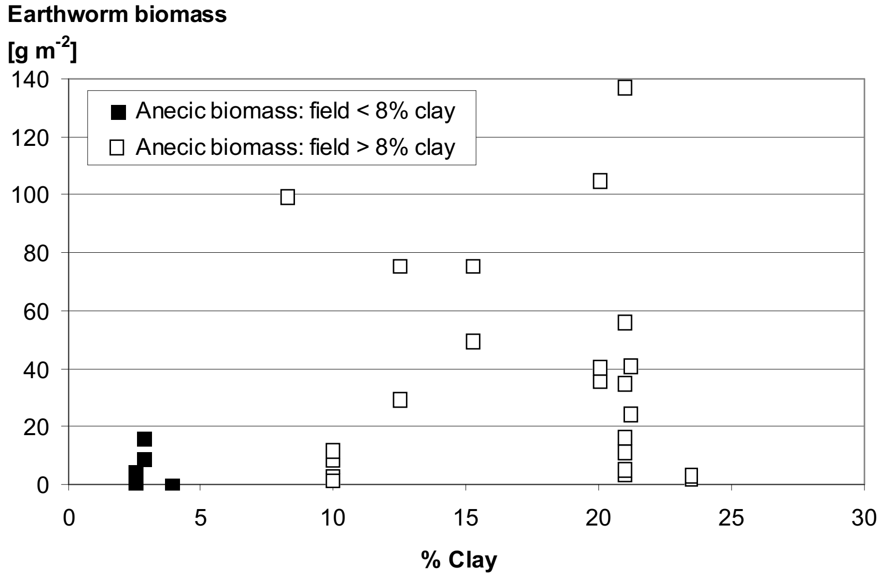

Fig. 2 Correlation of anecic earthworm biomass with clay content for field sites. n = 13 (< 8 % clay); n = 23 (≥ 8 % clay).

Reference ranges of the six categories are given in Tab. 4 and Figs 3–5. The significance of differences of the arithmetic means was checked for each parameter for the two categories of each land-use type. Enchytraeid species numbers were significantly different only between extremely and strongly acid forest sites (pH < 3.4 and 3.4–4.2, respectively). Regarding the other categories and abundance data, the ranges for microannelids overlap strongly (Figs 3, 5). Earthworm biomass always showed significant differences between the two categories of each land-use type, while earthworm abundance and species number did not differ substantially between grasslands and wet grasslands (Tab. 4, Figs 3, 4). For the detection of trends it is therefore indispensable to include qualitative parameters based on the species composition in addition to the quantitative parameters listed in Tab. 4. As species lists are hardly convenient for the definition of reference ranges, more integrating parameters are needed here. For the data interpretation of the soil monitoring sites in Northern Germany, indicator values for soil reaction and soil moisture have been used since the beginning of the investigations. These values were introduced by Graefe (1993) and are available for many common enchytraeid and earthworm species (Graefe & Schmelz 1999). Average reaction and moisture figures for the six site categories are given in Tab. 5. The agricultural sites are characterised by average reaction figures near 7, which means that the annelid community is dominated by indicators of neutral or slightly acid to slightly alkaline soil conditions. The forest sites on the other hand show average reaction figures below 5 due to the dominance of acidity indicators with low reaction figure. At forest sites and field sites with low clay content the average moisture figure is mostly near 5. At fields with higher clay content and at grasslands (site types 4 and 5) we often find some indicators of damp conditions so that the average moisture figure is slightly higher. The moisture figure of wet grasslands is considerably higher due to the high dominance of wetness indicators with moisture figures above 6.

In addition information is given in Tab. 5 on the ecological type of earthworms occurring. The virtual absence of endogeic (mineral soil dwellers) and anecic (deep-burrowing) species is characteristic of acid forest soils. Anecic species are also generally missing in wet soils and are only rarely found in sandy field soils. Epigeic species (litter dwellers) are often missing in field soils due to the absence of a litter layer.

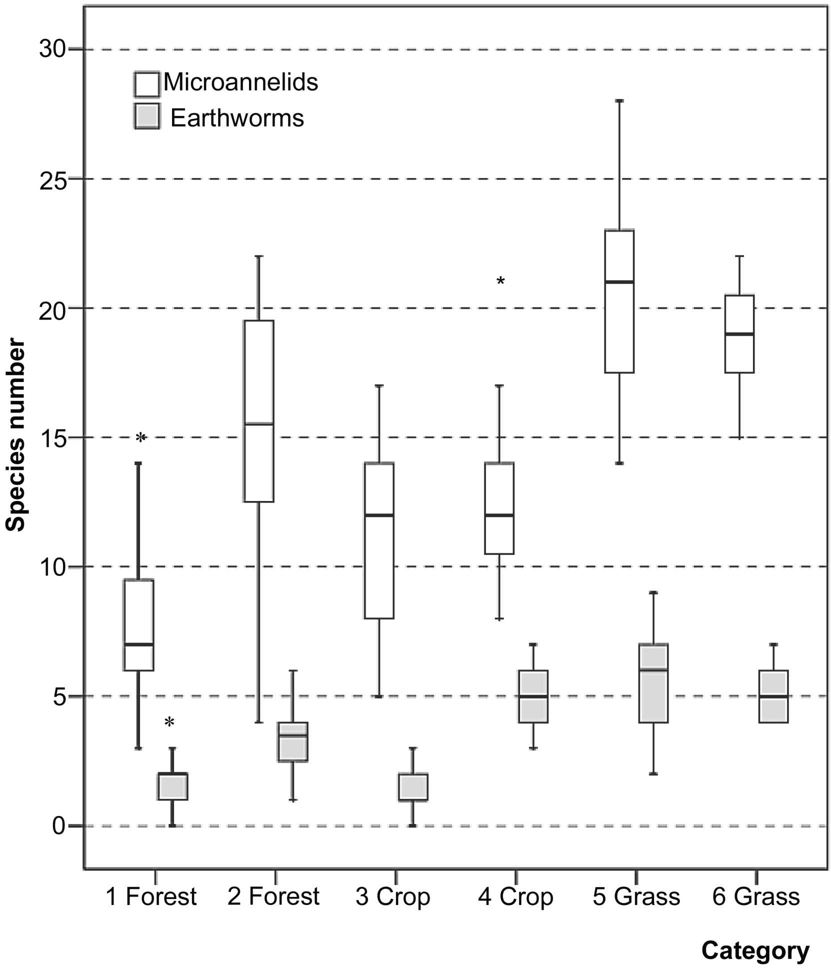

Fig. 3 Boxplots representing the reference ranges for species number of the six categories defined in Tab. 4. Boxes cover data between 25 %- and 75 %- percentiles; whiskers extend to minimum / maximum values; stars outside whiskers: outliers and extremes; bar in the box: median.

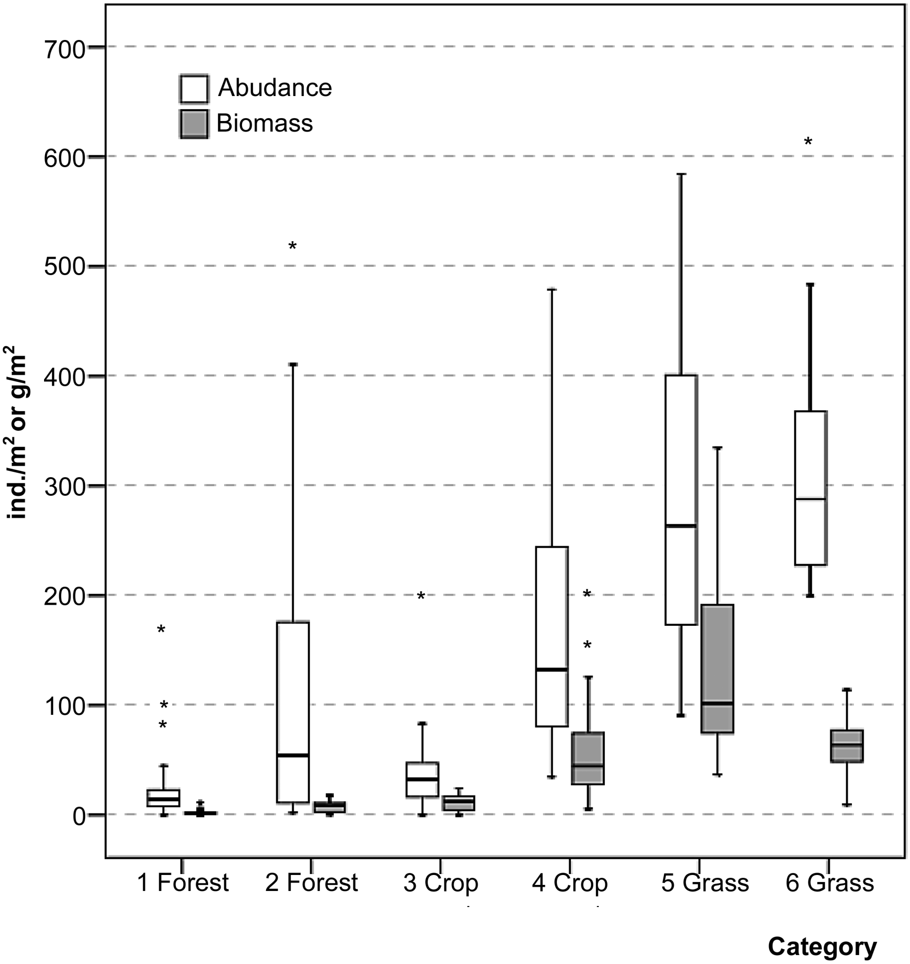

Fig. 4 Boxplots representing the reference ranges for abundance and earthworm biomass of the six categories defined in Tab. 4. Boxes cover data between 25 %- and 75 %-percentiles; whiskers extend to minimum / maximum values; stars outside whiskers: outliers and extremes; bar in the box: median. Extreme values not shown for category 4 (783 ind. m^{-2}) and category 5 (886 ind. m^{-2}) due to scale proportion.

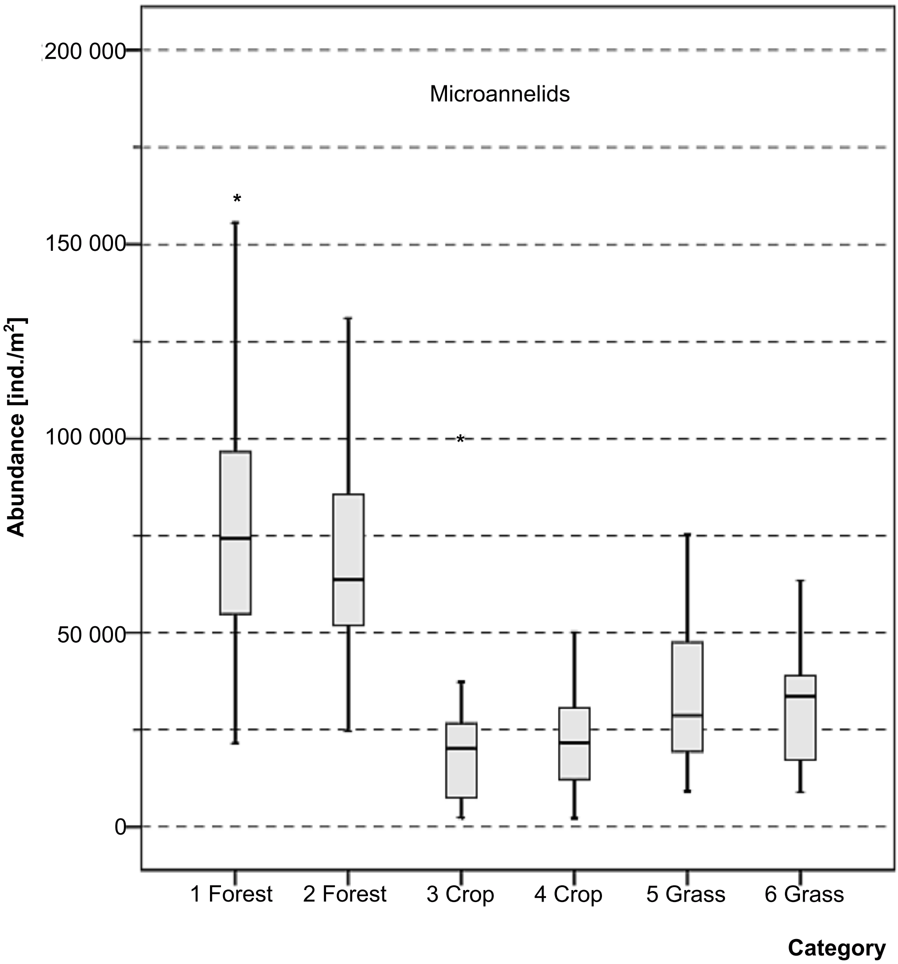

Fig. 5 Boxplots representing the reference ranges for microannelid abundance of the six categories defined in Tab. 4. Boxes cover data between 25 %- and 75 %-percentiles; whiskers extend to minimum / maximum values; stars outside whiskers: outliers and extremes; bar in the box: median. Two extreme values not shown for category 1 (> 212 000 Ind. m^{-2}) due to scale proportion.

4. Discussion of reference ranges

In the investigated forest soils the decisive abiotic factor is soil acidity. Species numbers of annelids and earthworm biomass increase with increasing pH as only few species tolerate very low pH values. Enchytraeid abundance shows the opposite behaviour: At low pH values a few acid tolerant species occur with high abundance, a fact generally known from acid forest soils in Northern Europe (Abrahamsen 1972, Huhta 1984, Graefe et al. 2001). The decomposer community type Achaeto-Cognettietum is assigned to both forest categories, as the characteristic species are the same, e.g. *Cognettia sphagnetorum* (Vejdovský, 1978), *Achaeta aberrans* Nielsen & Christensen 1961, *A. brevivasa* Graefe, 1980, *A. camerani* (Cognetti, 1899) (Graefe & Beylich 2003). Due to slightly higher pH-values other species indicating moderately acid soils occur additionally in category 2, contributing to a more species-rich version of the same community. The species numbers of earthworms that can be expected according to Römbke et al. (2005) in strongly acid forests correspond with our findings. Rutgers et al. (2008) published references for biological soil quality in the Netherlands. For the category 'mixed woodland on sand' with a mean pH($_{KCl}$) value of 3.2, they specify much lower enchytraeid densities (mean: 15 050 ind. m^{-2}) and lower species numbers than in this study. Reasons for deviations from the reference ranges can be different methods applied or deviating pedo-climatic conditions. Deviations from the reference ranges are also probable to occur after liming or under urban influence (urban forests; e.g. Figs 3 and 4: outlier for earthworm species number and abundance of category 1). Further it has to be taken into account that the pH data used for the correlations are topsoil data from the topmost mineral horizon. Deeper horizons can have substantially higher pH values. In this case we found anecic earthworms at low numbers even at sites with strongly acid topsoil (category 2 according to Tab. 4). Casting of anecic worms near the soil surface may then locally improve the conditions for enchytraeids and other soil organisms.

Earthworms seem to respond strongly to the clay content of agricultural field soils. As the texture affects a number of other soil properties, this is presumably an indirect effect. A loamy soil with higher water holding capacity might be habitable for more species than an easily desiccating sandy soil. On the other hand, loamy and clayey soils are more often subject to water-logging as a consequence of compaction due to heavy machinery traffic. The absence of anecic earthworm species in most of the sandy fields can be seen in connection with the detrimental effect of tillage on the permanent vertical burrows of these species. Possibly these effects are less pronounced in loamy soils with a more stable soil structure, so that anecic species occur regularly in fields with clay contents above 8 %. Consequently we find two decomposer community types: Fridericio-Enchytraeetum, with high dominance of *Fridericia*- and *Enchytraeus*-species and lack of anecic earthworms at the sandy field sites, and Fridericio-Lumbricetum with anecic earthworms, mostly *Lumbricus terrestris* Linnaeus, 1758, occurring at the loamy field sites. The lack of anecic earthworms in sandy cropland soils is in accordance with the expectation values found by Römbke et al. (2005). However, the adverse tillage effect is strongly dependent on tillage methods and frequency, which were not considered in detail for this study (see Langmaack et al. 1996, 1999). At soil monitoring sites of sandy cropland (clay content < 5 %) in the federal state of Brandenburg, Germany, Krück et al. (2006) found the anecic species *Lumbricus terrestris* only when the organic matter content was ≥ 3 %. This shows that under favourable conditions (reduced tillage, high humus content, high pH) the Fridericio-Lumbricetum can also be found at sandy field sites. Different tillage practices may well be the reason for higher mean abundances of annelids on sandy fields in other regions (Rutgers et al. 2008). The correlation of earthworm parameters with soil acidity in fields is probably at least partly indirect due to the correlation of pH with clay content (r^2=0.47). The maximum clay content of category 4 is 24 % (silty loam); clay soils are not represented. Thus the reference range for category 4 might not be apt for clayey field soils.

Tab. 5 Decomposer community types according to Graefe (1993), mean indicator values for soil reaction and soil moisture (Graefe & Schmelz 1999) and occurrence of ecological types of earthworms (Bouché 1972) for the six categories given in Tab. 4. Med: median; mR’: average reaction figure; mM’: average moisture figure; +: generally present, (+): not neccessarily present; Vn: degree of water logging.

Category	**pH Clay Soil water (Vn)**	**Land use**	**Decomposer community type**	**mR’**	**mM’**	**Ecological types of earthworms**		
				Min-Max Med	Min-Max Med	**epigeic**	endogeic	anecic
1	< 3.4	Forest or heathland; moder or mor humus forms	Achaeto-Cognettietum	**1.7–4.7** **2.6**	5.0–5.3 5.0	+		
2	> 3.4–4.2	Forest or heathland; moder or mor humus forms	Achaeto-Cognettietum	**2.0–5.4** **4.1**	5.0–5.3 5.0	+		
3	4.3–5.9 < 8 % Clay	Field; Mull humus forms	Fridericio-Enchytraeetum (Fridericio-Lumbricetum)	**5.5–7.0** **6.8**	5.0–5.1 5.0		+	(+)
4	> 5.8 > 8 % Clay	Field; Mull humus forms	Fridericio-Lumbricetum	**6.9–7.0** **7.0**	5.0–6.8 5.9	(+)	+	+
5	> 4.2 Vn 0-4	Grassland; Mull humus forms	Fridericio-Lumbricetum	**6.1–7.0** **6.9**	5.0–6.5 5.4	+	+	+
6	> 4.9 Vn 5-6	Wet grassland; Mull humus forms	Octolasietum tyrtaei	**6.1–7.0** **6.8**	6.8–8.9 7.4	+	+	

Grassland shows highest earthworm abundance and biomass as well as enchytraeid species number of all site types. Wet grassland sites are characterised by the lack of anecic earthworm species, which evidently do not tolerate waterlogged or regularly flooded soils (Graefe & Beylich 1999, Beylich & Graefe 2002, Plum & Filser 2005, Römbke et al. 2005). However a definite threshold of soil moisture for the occurrence of anecic species has not been deduced so far. Other factors, as soil temperature, season of elevated ground water or organic matter input play also a decisive role and impede the definition of such a threshold. Terrestrial annelids are also influenced by grassland management (intensity of grazing / mowing, fertiliser, trampling). This could be the reason for earthworm abundance and biomass exceeding the reference ranges for grassland in Tab. 4 (e.g. for wet grassland Keplin & Broll 2002).

The comparison of loamy fields and grasslands (categories 4 and 5) shows significant differences for most quantitative parameters given in Tab. 4 (except earthworm species number). Species numbers and abundance tend to be lower in cropland in consequence of adverse conditions caused by e.g. tillage, compaction, pesticide application and litter removal.

However, the mean indicator values do not differ substantially and both types are assigned to the decomposer community Fridericio-Lumbricetum, because the characteristic species are present in both cases (*Lumbricus terrestris*, *Fridericia*-species).

The soils of the wet grassland sites are mainly drained Histosols. Among the grassland sites on mineral soils, a further differentiation according to the clay content may be possible. Rutgers et al. (2008) found that abundance and diversity of annelids differ considerably between sand and clay on dairy farms in the Netherlands. The relation between clay content and earthworm biomass is not linear (Beylich & Graefe 2007); biomass is highest in loamy grassland and lower in sandy and clayey grasslands. For the latter two the number of investigated sites was too low to define seperate reference ranges.

Time-series examples

The following three examples are meant to demonstrate the interpretation of temporal variation using reference ranges, taking the information of Tabs 4 & 5 into consideration. Data from three samplings at intervals of several years can show different degrees of variation. Basic information of the site characteristics is given in Tab. 6.

Example 1: Conversion from grassland to cropland (Figs 6, 7)

The first example is a loamy grassland site (category 5) which was ploughed and converted into cropland in 2002 (category 4). At the first two investigations (1994, 2000) the data for enchytraeid abundance and species number were rather high within the reference range for category 5 (Figs 6, 7, Tab. 6). Earthworm biomass was high within the reference range (outside the box of the boxplot in Fig. 4); earthworm abundance at the first investigation even exceeded the range. The qualitative parameters were in accordance with the reference given in Tab. 5. The third investigation took place five years after the conversion into a field. Earthworm biomass and abundance differ significantly from the two former samplings (Fig. 7, $p < 0.01$). Enchytraeid abundance shows a significant decline from second to third sampling ($p < 0.05$). Species numbers are comparatively high: seven earthworm species and more than 20 enchytraeid species at all sampling dates. The enchytraeid species number is thus outside the range for category 4 and still typical of grassland sites. While the mean

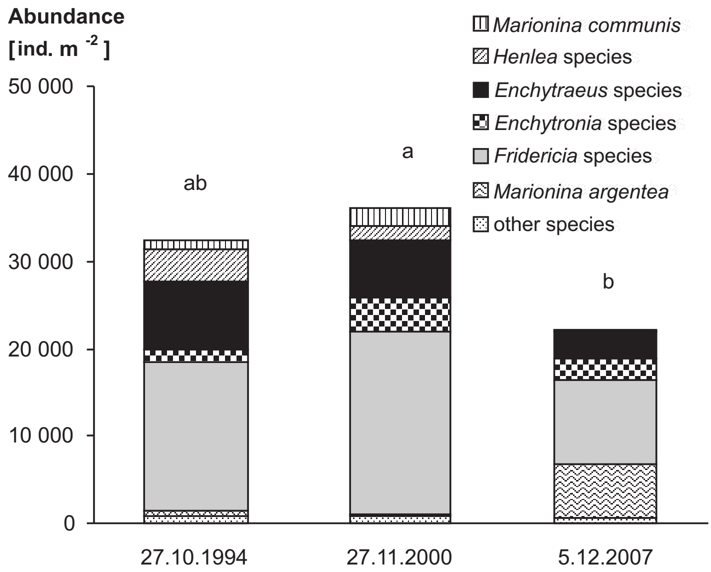

Fig. 6 Microannelid abundance for example 1 (conversion from grassland to cropland) for three sampling dates. Different letters: significant difference ($p < 0.05$).

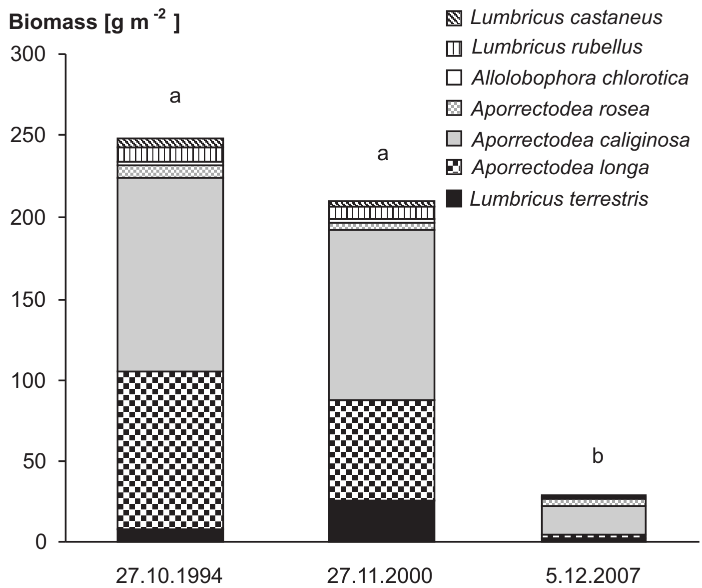

Fig. 7 Earthworm biomass for example 1 (conversion from grassland to cropland) for three sampling dates. Different letters: significant difference ($p < 0.01$).

reaction figure remained stable, the mean moisture figure rose after the land use change, mainly due to the increased dominance of *Marionina argentea* (Michaelsen, 1889). This is probably caused by water logging following soil compaction and by especially wet conditions in 2007 in comparison with 2000. Striking is the decline of deep-burrowing earthworms (*Lumbricus terrestris, Aporrectodea longa* (Ude, 1885)), which largely contributes to the decrease in total earthworm biomass and numbers. While these species were present at each of the ten sample points in 1994 and 2000, they were found at only four sample points in 2002. Likewise, two epigeic species (*Lumbricus rubellus* Hoffmeister, 1843, *Lumbricus castaneus* (Savigny, 1826)) have been reduced due to the disturbance of their preferred habitat by ploughing. The decline of earthworms as a whole, but especially of anecic and epigeic species, can be expected after land-use change from grassland to cropland. As the characteristic species of the Fridericio-Lumbricetum were still present at the third sampling, the community type was not altered by the land-use change. It can be reasoned however that a decline in species diversity is going on, indicated by the strongly reduced frequency of some species at the last sampling. Evaluation: Annelid coenosis shifting from category 5 to 4 due to land-use change, in accordance with expectations.

Tab. 6 Characteristics of the three exemplified soil monitoring sites. Occurrence of anecic earthworm species: – = absent; + = present; +++ = abundant; mR': average reaction figure; mM': average moisture figure; Vn: degree of water logging.

Example	**pH Clay Vn**	**Land use**	**mR'**	**mM'**	**Decomposer community type**	**Anecic species**
1	pH ≈ 5 21 % clay Vn3	Grassland until 2002 conversion into corn field	7.0 7.0 6.9	5.6 5.8 6.4	Fridericio-Lumbricetum	+++ → +
2	pH ≈ 7 23 % clay Vn3	Nature Reserve; grass land fallow with ongoing succession since 1979	7.0 7.0 6.9	5.0 7.7 6.7	Fridericio-Henleetum → Fridericio-Lumbricetum	–
3	pH 4.2 → 4.8 2.5 % clay < Vn5	Field	5.9 6.1 6.8	5.1 5.0 5.0	Fridericio-Enchytraeetum → Fridericio-Lumbricetum	– → +

Example 2: Succession of marshland (Figs 8, 9)

The second site given as example in Tab. 6 is a grassland fallow, unmanaged since 1979, on marsh soil near the North Sea coast. It is part of a polder (reclaimed from the sea by dyking) that was constructed in the 1970s. The site is left to natural succession, and a vegetation of tall forbs, grasses and scattered shrubs and trees has developed. The abundance of enchytraeids is within the reference range for grassland and shows no temporal trend (Fig. 8). Their species number at the third sampling date was below the reference range for grassland.

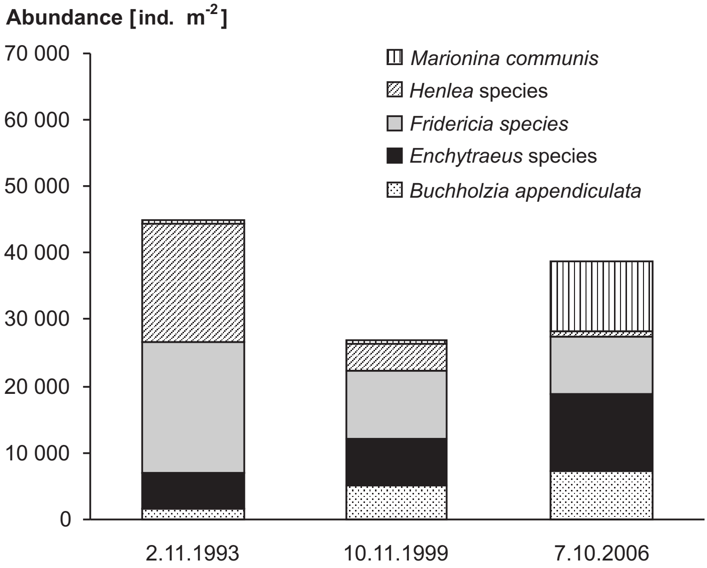

Fig. 8 Microannelid abundance for example 2 (marshland succession) for three sampling dates. Differences not significant.

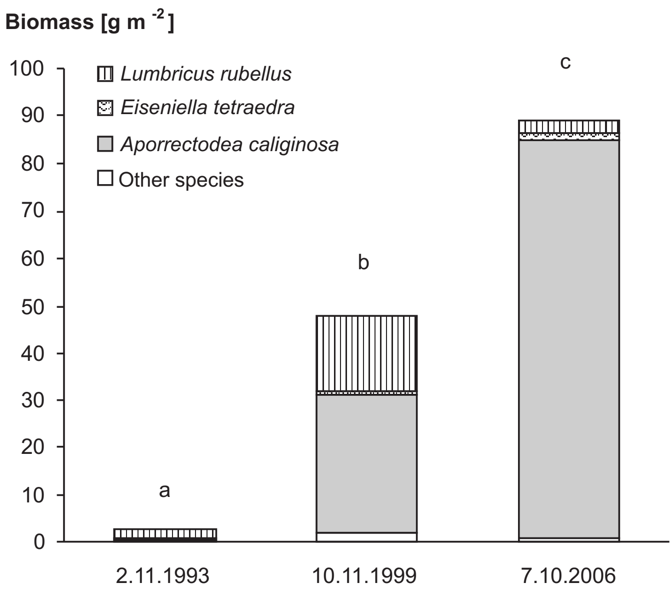

Fig. 9 Earthworm biomass for example 2 (marshland succession) for three sampling dates. Different letters: significant difference (bc: $p < 0.05$; ac, ab < 0.01).

Earthworm biomass and abundance were considerably below the reference range for grassland at the first sampling, but increased strongly and significantly in the following years (Fig. 9). The species number increased from 3 to 6. *Aporrectodea caliginosa* (Savigny, 1826) is the only endogeic earthworm species, all other species are epigeic. At the first sampling date a 2–3 cm thick holorganic horizon of partly fragmented and humified litter was observed above the silty mineralic topsoil. The holorganic horizon disappeared in the following years due to the increased activity of earthworms. Deep-burrowing earthworms are still missing at the site. Even though the wetness indicator *Eiseniella tetraedra* (Savigny, 1826) is present in 1999 and 2006, wetness is not considered here as the factor that prevents the occurrence of anecic earthworms. The site is surrounded by draining ditches and is classified as 'moderately waterlogged' (Vn3 according to Ad-hoc-AG Boden 2005) which normally allows anecic species occurrence (Tab. 5). Conspicuous is also the dominance of salt-tolerating enchytraeid species (e.g. *Henlea perpusilla* Friend, 1911, *H. ventriculosa* (d'Udekem, 1894)) and of one oligohaline species (*Fridericia parathalassia* Schmelz, 2002) at the first sampling date. Their dominance strongly decreased over time indicating eluviation of salts. This process has now, approximately 30 years after the impoldering, apparently finished. The decomposer community type first assigned as Fridericio-Henleetum (typical of early succession stages in reclaimed polders and marshlands), has subsequently changed to Fridericio-Lumbricetum. The absence of anecic earthworms indicates that this development has not yet reached its endpoint. One possible reason for the absence of anecic species might be the low age of the site and the fact that some species just 'have not arrived' yet. It is unknown whether these species occur in the surrounding field sites The distance to the old dyke with 'older' soils behind it is about 800 m. The migration rate of earthworm populations is considered to be much slower for anecic species than for epigeic species (Dunger 1991). Evaluation: Change of annelid coenosis due to succession in progress, endpoint not yet reached.

Example 3: Cropland on sand with low pH (Figs 10, 11)

While in the previous examples the earthworms showed a definite trend, either upwards or downwards, Fig. 11 shows little development of earthworm biomass as opposed to the decline in enchytraeid numbers from a relatively high level (upper reference range / upper 'whisker' of the boxplot in Fig. 5) to a moderate level (central reference range / box in Fig. 5). At first glance, the biological soil condition at this sandy cropland site seems to deteriorate. However, the quantitative parameters are all within the reference ranges and the decline in enchytraeid abundance is statistically not significant due to high variance. While the species number of enchytraeids remained constant (14), the species composition changed considerably during the investigated period: The acid tolerant *Achaeta*-species had a total dominance of > 80 % in 1992 but nearly disappeared in 2005. At the same time, the proportion of indicators of slightly acid conditions increased (genera *Enchytraeus*, *Enchytronia*, *Henlea*, and *Fridericia*). Consequently, the mean reaction figure rose from 5.9 to 6.8. No earthworms were detected at the second sampling, which was probably caused rather by adverse sampling conditions (ground frost) than by their actual absence. The appearance of *Lumbricus terrestris* on the third sampling date points at decreased acidity and improved living conditions for earthworms. In fact the soil pH($CaCl_2$) rose from 4.2 (1995) to 4.8 (2001), which is still low for field soils but obviously sufficient for substantial changes in the decomposer community. The surrounding hedgerows (German: 'Knick') may have served as a reservoir for the immigration of anecic earthworms. Evaluation: Shift of annelid coenosis due to changed management practice.

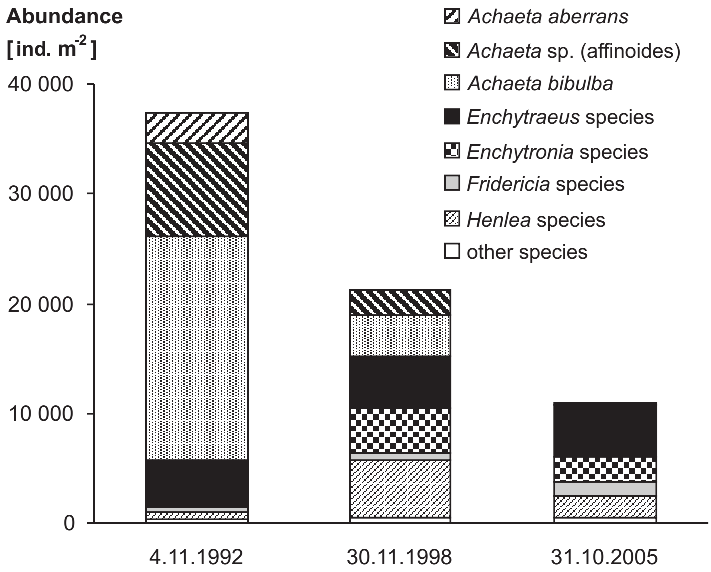

Fig. 10 Microannelid abundance for example 3 (cropland with low pH) for three sampling dates. Differences not significant.

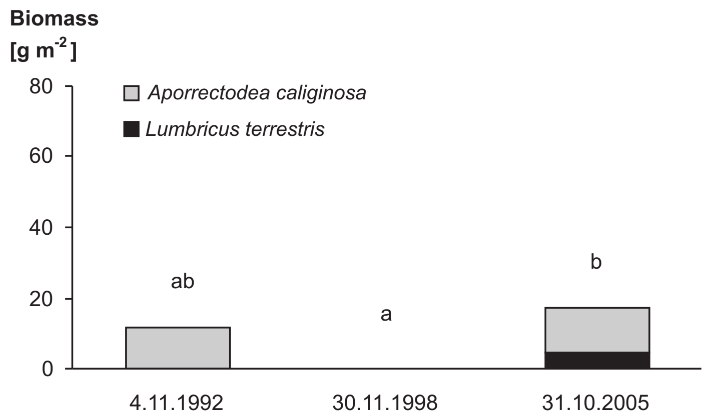

Fig. 11 Earthworm biomass for example 3 (cropland with low pH) for three sampling dates. Different letters: significant difference ($p < 0.05$).

5. Conclusions

One problem of assessing zoological data in long-term soil monitoring is to distinguish non-directional fluctuations of quantitative parameters as abundance or biomass from directional changes. If the temporal variation goes in the same direction throughout three consecutive investigations, it is considered a trend. Quantitative parameters of microannelids and earthworms do not necessarily show the same trends. Quantitative parameters support the interpretation of qualitative data based on the species composition and thus contribute to coherent conclusions.

6. Acknowledgements

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7. References

Abrahamsen, G. (1972): Ecological study of Enchytraeidae (Oligochaeta) in Norwegian coniferous forest soils. – Pedobiologia **12**: 26–82.

Ad-hoc-AG Boden (2005): Bodenkundliche Kartieranleitung. – 5. Auflage, Hannover: 438 pp.

Barth, N., W. Brandtner, E. Cordsen, T. Dann, K.-H. Emmerich, D. Feldhaus, B. Kleefisch, B. Schilling & J. Utermann (2000): Boden-Dauerbeobachtung – Einrichtung und Betrieb von Boden-Dauerbeobachtungsflächen. – In: Rosenkranz, D., G. Bachmann, W. König & G. Einsele (Hrsg.): Bodenschutz. Kennziffer 9152. – Erich Schmidt Verlag, Berlin: 127 pp.

Beylich, A. & U. Graefe (2002): Annelid coenoses of wetlands representing different decomposer communities. – In: Broll, G., W. Merbach & E.-M. Pfeiffer (eds): Wetlands in Central Europe. Soil organisms, soil ecological processes and trace gas emissions. – Springer Berlin: 1–10.

Beylich, A. & U. Graefe (2007): Lumbriciden in der Boden-Dauerbeobachtung: Darstellung von Referenzbereichen, Baselines und Veränderungstendenzen an Beispielen aus Norddeutschland. – In: Umweltbundesamt (ed.): Bodenbiologische Bewertung von Boden-Dauerbeobachtungsflächen (BDF) anhand von Lumbriciden. Texte **34**(07): 33–52.

Bouché, M. B. (1972): Lombriciens de France. Écologie et Systématique. – Institut National de la Recherche Agronomique, Paris: 671 pp.

Didden, W. (2003): Development and potential of a stereotype as a reference site in ecological monitoring. – In: Didden, W. & P. Van Vliet (eds): Proceedings of the 5th International Symposium on Enchytraeidae.– Newsletter on Enchytraeidae 8. Wageningen University, Wageningen: 33–40.

Dunger, W. (1991): Zur Primärsukzession humiphager Tiergruppen auf Bergbauflächen. – Zoologisches Jahrbuch Systematik **118**: 423–447.

Dunger, W. & H. J. Fiedler (1989): Methoden der Bodenbiologie. – Gustav Fischer Verlag, Stuttgart: 432 pp.

Graefe, U. (1993): Die Gliederung von Zersetzergesellschaften für die standortsökologische Ansprache. – Mitteilungen der Deutschen Bodenkundlichen Gesellschaft **69**: 95–98.

Graefe, U. (1998): Annelidenzönosen nasser Böden und ihre Einordnung in Zersetzergesellschaften. – Mitteilungen der Deutschen Bodenkundlichen Gesellschaft **88**: 109–112.

Graefe, U. & A. Beylich (1999): Bodenbiozönosen als Ausdruck standörtlicher Bedingungen in der Elbtalaue. – Auenreport Sonderband **1**: 88–95.

Graefe, U. & A. Beylich (2003): Critical values of soil acidification for annelid species and the decomposer community. – In: Didden, W. & P. Van Vliet (eds): Proceedings of the 5th International Symposium on Enchytraeidae. – Newsletter on Enchytraeidae 8. Wageningen University, Wageningen: 51–55.

Graefe, U. & R. M. Schmelz (1999): Indicator values, strategy types and life forms of terrestrial Enchytraeidae and other microannelids. – In: Schmelz, R. & K. Sühlo: Newsletter on Enchytraeidae 6. – Universitätsverlag Rasch, Osnabrück: 59–67.

Graefe, U., J. Gehrmann & I. Stempelmann (2001): Bodenzoologisches Monitoring auf EU-Level II-Dauerbeobachtungsflächen in Nordrhein-Westfalen. – Mitteilungen der Deutschen Bodenkundlichen Gesellschaft **96**: 331–332.

Gröngröft, A. & M. Karrasch (2005): Wiederholungsuntersuchung an der Boden-Dauerbeobachtungsfläche Wohldorfer Wald. – Abschlussbericht im Auftrag der Freien und Hansestadt Hamburg, Behörde für Stadtentwicklung und Umwelt, Geologisches Landesamt: 53 pp.

Gröngröft, A., P. Hoyer & L. Schubert (2003): Wiederholungsuntersuchung an der Boden-Dauerbeobachtungsfläche Amsinckpark. – Abschlussbericht im Auftrag der Freien und Hansestadt Hamburg, Behörde für Umwelt und Gesundheit, Amt für Umweltschutz: 37 pp.

Huber, S., G. Prokop, D. Arrouays, G. Banko, A. Bispo, R. Jones, M. Kibblewhite, W. Lexer, A. Möller, J. Rickson, T. Shishkov, M. Stephens, J. Van den Akker, G. Varallyay & F. Verheijen (2007): Indicators and Criteria report. – ENVASSO Project (Contract 022713) coordinated by Cranfield University, UK, for Scientific Support to Policy, European Commission 6th Framework Research Programme. – [http://www.envasso.com/Publications/ENV_D2_WP1+Annex_bk.pdf].

Huhta, V. (1984): Response of *Cognettia sphagnetorum* (Enchytraeidae) to manipulation of pH and nutrient status in coniferous forest soil. – Pedobiologia **27**: 245–260.

ISO (International Organization for Standardization) (2006a): Soil quality - Sampling of soil invertebrates - Part 1: Hand-sorting and formalin extraction of earthworms. ISO 23611-1. – ISO Geneva.

ISO (International Organization for Standardization) (2006b): Soil quality - Sampling of soil invertebrates - Part 3: Sampling and soil extraction of enchytraeids. ISO 23611-3. – ISO Geneva.

Keplin, B. & G. Broll (2002): Earthworm coenoses in wet grassland of Northwest-Germany. Effects of restoration management on a Histosol and a Gleysol. – In: Broll, G., W. Merbach & E.-M. Pfeiffer (eds): Wetlands in Central Europe. Soil organisms, soil ecological processes and trace gas emissions. – Springer Berlin: 11–34.

Krück, S., M. Joschko, R. Schultz-Sternberg, B. Kroschewski & J. Tessmann (2006): A classification scheme for earthworm populations (Lumbricidae) in cultivated agricultural soils in Brandenburg, Germany. – Journal on Plant Nutrition and Soil Science **169**: 651–660.

Langmaack, M., R. Röhrig & S. Schrader (1996): Einfluß der Bodenbearbeitung und Bodenverdichtung auf terrestrische Oligochaeten (Enchytraeidae und Lumbricidae) landwirtschaftlicher Nutzflächen. – Braunschweiger naturkundliche Schriften **5**(1): 105–123.

Langmaack, M., S. Schrader, U. Rapp-Bernhardt & K. Kotzke (1999): Quantitative analysis of earthworm burrow systems with respect to biological soil-structure regeneration after soil compaction. – Biology and Fertility of Soils **28**: 219–229.

Metzger, F., R. Haag & I. Stempelmann (2005): Bodendauerbeobachtung in NRW - Konzeption und Sachstand. Anhang des Berichts zur Bodendauerbeobachtung in NRW: Standortbeschreibungen, Karten und Kenndaten zu den Flächen. – Landesumweltamt Nordrhein-Westfalen (LUA NRW): 52 pp.

Plum, N. M. & J. Filser (2005): Floods and drought: Response of earthworms and potworms (Oligochaeta: Lumbricidae, Enchytraeidae) to hydrological extremes in wet grassland. – Pedobiologia **49**: 443–453.

Quirin, M. & C. Emmerling (2004): Gesamtgutachten zu bodenmikrobiologischen Untersuchungen an den BDF in Schleswig-Holstein im Zeitraum von 1995–2002. – Endbericht im Auftrag des Landesamtes für Natur und Umwelt Schleswig-Holstein: 146 pp.

Römbke, J., S. Jänsch & W. Didden (2005): The use of earthworms in ecological soil classification and assessment concepts. – Ecotoxicology and Environmental Safety **62**: 249–265.

Rutgers, M., C. Mulder & A. J. Schouten (2008): Soil ecosystem profiling in the Netherlands with ten references for biological soil quality. – RIVM report 607604009/2008, RIVM, Bilthoven, The Netherlands: 86 pp.

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