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Species diversity and spatial distribution of enchytraeid communities in forest soils: effects of habitat characteristics and heavy metal contamination

Paweł Kapusta, Łukasz Sobczyk, Anna Rożen, January Weiner*

Department of Ecosystem Studies, Institute of Environmental Sciences, Jagiellonian University, ul. Gronostajowa 3, 30-387 Kraków, Poland

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Abstract

Species diversity of enchytraeid communities was determined in soils of the Niepołomice Forest (southern Poland) in relation to habitat characteristics (forest type, soil chemistry: pH, organic matter content, moisture, mineral elements Ca, K, Na, N–NH_4, N–NO_3, N_{total}) and xenobiotics (Zn, Pb, Cu, Cd) and humus respiration rate. Altogether 30 enchytraeid species were identified, forming assemblages of 7–29 species, 2.6×10^3 to 132×10^3 individuals (0.01–3.75 g dry mass) per m^2. The dominating species in pine forest was *Cognettia sphagnetorum*. These communities were ordinated using multivariate statistics and compared with similarly ordinated forest/soil habitats. The ordination of habitats according to soil chemistry matched exactly the forest habitat typology based on vegetation, whereas the pattern of similarities between enchytraeid communities was less clearly connected with abiotic and/or biotic (vegetational) multidimensional habitat characteristics. In general, the distribution of enchytraeids in the Niepołomice Forest reflects habitat characteristics but was not influenced by heavy metal content in soil.

Keywords: Enchytraeidae; Multivariate analysis; Niepołomice Forest

1. Introduction

Community diversity and ecosystem functions of biota can be studied by analyzing their variation in natural situations, or experimentally in artificial systems with controlled factors (Fraser and Keddy, 1997; Sala et al., 2000). Both approaches pose serious difficulties. The second approval is flawed because of the unavoidable oversimplification of the systems studied; on the other hand, the more realistically the first approach reflects the natural richness of biota and of their mutual interactions, the less likely it is to produce a conclusive explanation for the observed patterns. One of the difficulties consists in the natural variation of habitats due to environmental gradients, patchiness of the substrate, and diverse interacting effects that make it difficult to distinguish between particular factors of influence. In studies aimed at estimating the effect of anthropogenic impacts (such as pollution) upon the structure and ecosystem function of biotic communities, comparisons are made between areas differently affected by human pressure, assuming that other factors are equal, or at least that the variation in natural environmental factors is independent of the variation in anthropogenic effects. While the first assumption is

* Corresponding author. Tel.: +48-12-269-09-80.
E-mail address: weiner@eko.uj.edu.pl (J. Weiner).

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difficult to confirm, the second can be valid only in a unique spatial situation.

We believe that the Niepołomice Forest (southern Poland) is an ecosystem particularly well suited for such comparative studies (Kleczkowski, 1981; Grodziński et al., 1984; Weiner et al., 1997). The Niepołomice Forest is a lowland forest complex situated in the Vistula River Valley, approximately 20 km east of the urban and industrial area of Cracow (50°07′N, 20°23′E, elevation 184–212 m a.s.l.; Fig. 1a). It consists of two major sections: an extensive mixed pine forest of the *Pino-Quercetum* type on podzolic and seasonally wet sandy soils in the south (88.7 km^2), and smaller, oak-hornbeam *Tilio-Carpinetum* stands on alluvial soils in the north (19.8 km^2; Fig. 1b). The Niepołomice Forest represents a variety of habitats with two distinct, decoupled gradients: a latitudinal (approximately N–S) gradient of soil moisture/fertility conditions, and a longitudinal (approximately W–E) gradient of pollution. In the 1950s the forest was exposed to enormous loads of industrial dust and gases from a large steelworks built only about 15 km W of the forest (Fig. 1a). The pollution increased until the end of the 1980s, due to the extensive development of the metallurgical industry, and decreased dramatically later on when production declined and technological improvements were made (Weiner et al., 1997; Weiner, 1999). Spatial gradients of heavy metal pollution at its peak have been documented repeatedly (Grodziński et al.,

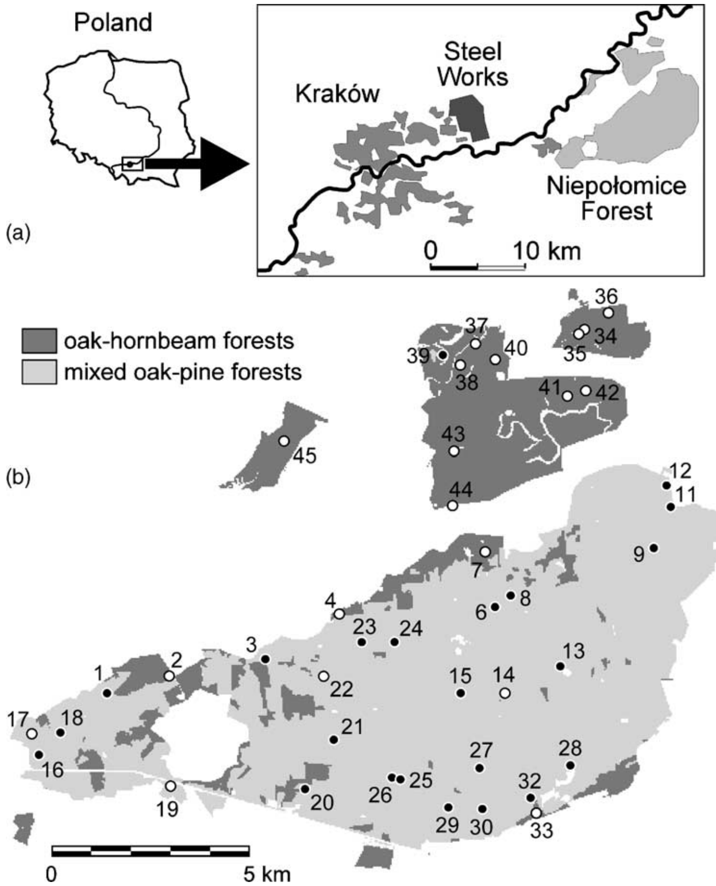

Fig. 1. The Niepołomice Forest: (a) location of the study area in the vicinity of the Kraków urban agglomeration and metallurgical plants; (b) study plots (numbers) in oak-hornbeam and mixed pine forests (generalized map); open symbols, oak-hornbeam stands; closed symbols, mixed pine stands.

1984; Weiner et al., 1997; Godzik and Szarek, 1993; Szarek-Łukaszewska et al., 2002). Recent studies address the effects of pollution varying in time and space upon species diversity and ecosystem functions of various biota in the Niepołomice Forest (Szarek-Łukaszewska et al., 2002; Laskowski et al., 2003).

This study analyses the independent effects of habitat properties and heavy metal pollution upon enchytraeid communities—important biotic elements of the litter-soil ecosystem—using multivariate analysis.

2. Materials and methods

2.1. Study area and sampling plots

The northern complex of the Niepołomice Forest is confined to a flood plain terrace of the Vistula River and is characterized by high ground water levels. The southern part, located on a higher terrace, has much less moist, seasonally changing conditions. The soils in the southern part are podzols and cambisols on sands, and in the northern part are gleysols and cambisols on clays, with an admixture of histosols (Fig. 2). These differences in habitat conditions are reflected in the prevalence of mixed oak-pine (*Pino-Quercetum*) with mor humus and mesic oak-hornbeam (*Tilio-Carpinetum*) stands with moder humus in the southern part, while moist oak-hornbeam forests with mull humus predominate in the northern part (Gruszczyk, 1981; Grodziński et al., 1984; Różański, unpublished data).

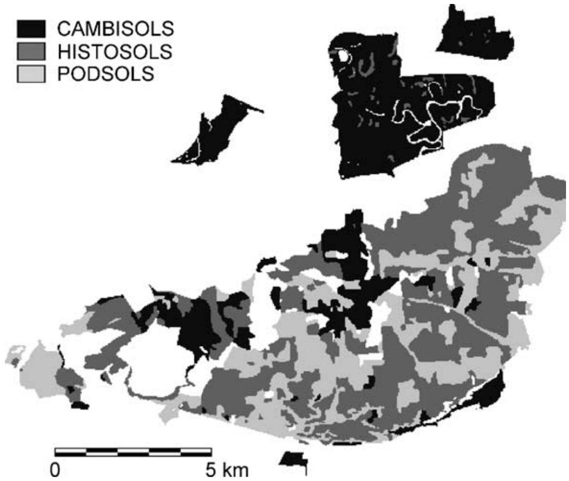

Fig. 2. Generalized map of soils in the Niepołomice Forest.

Within the area, 45 study plots were established so as to cover the maximum expected variation in pollution and to represent the major forest types: mixed oak-pine and oak-hornbeam forests (Fig. 1b). The study sites were subgrouped according to local plant associations into seven categories (Table 1). For various reasons, only 39–42 plots could be successfully sampled and/or analyzed with respect to particular variables.

2.2. Sampling and extraction of enchytraeids

Enchytraeidae were collected twice in the Niepołomice Forest in early November. In 1998 the samples were taken from 21 plots: 8 in mixed oak-pine and 13 in oak-hornbeam stands. In 1999 the animals were

Table 1
Niepołomice Forest habitat typology according to forest vegetation (Gruszczyk, 1981, Różański, unpublished data; Różański, 2003)

Abbreviation	Symbol	Forest type (variant)	Phytosociological classification	Plots no.
Mixed pine forests				
o-ps	(△)	Oak-pine forest (swamp)	(*Pino-Quercetum molinietosum*)	8, 12, 15, 26, 27, 30
o-pw	(△)	Oak-pine forest (wet)	(*Pino-Quercetum molinietosum*)	6, 13, 20, 24, 28
o-pf	(▵)	Oak-pine forest (fresh)	(*Pino-Quercetum typicum*)	9, 11, 18, 21, 23, 25, 29, 32
Deciduous (oak-hornbeam) forests				
o-hw	(●)	Oak-hornbeam forest (wet)	*Tilio-Carpinetum stachyetosum*	39, 44, 45
o-hf	(•)	Oak-hornbeam forest (fresh)	*Tilio-Carpinetum typicum*	4, 17, 34, 35, 36, 37, 38, 40, 41, 42, 45
m-dw	(▲)	Mixed deciduous forest (wet)	*Tilio-Carpinetum brizoides*	2, 3, 7, 14, 33
m-df	(▴)	Mixed deciduous forest (fresh)	*Pino-Quercetum/Tilio-Carpinetum*	1, 16, 19, 22

collected from 42 plots during two consecutive days. Twenty soil samples (16.6 cm^2 and 10 cm deep) were taken using a split soil corer at 1 m intervals along 20 m transects in each plot. The transects were marked out southwards from the central point of each plot. The worms were extracted from soil using wet funnels (O'Connor, 1971) for 5 h and preserved in 4% formaldehyde. Two samples from each plot were taken for species determination. To that end, semi-solid microscopic slides were made in Amman's lactophenol. The worms were identified using Kasprzak's (1986) key. In all other samples the animals were counted, dried for 24 h at 55 °C in a vacuum oven and weighed.

2.3. Soil chemistry

At each plot, samples of litter and topsoil to a depth of 10 cm were taken for chemical analyses. All samples were analysed for pH_{H_2O} (digital pH-meter, Nester Instrument), organic matter content (550 °C, 12 h) and chemical elements. For chemical analysis, the samples were ground to powder in an agate planetary mill, dried at 105 °C for 12 h and digested in super-pure nitric acid digested samples were analyzed for Ca, Na and K by emission flame spectrometry (Jenway Ltd., model PFP 7), for Zn and Cu by flame atomic absorption spectrometry (AAS; Perkin-Elmer AAnalyst 800), and for Cd and Pb by graphite furnace AAS (AAnalyst 800). Analytical precision was checked against certified standard material and most samples fell within ±10% of the certified value. The Chinese Soil 4 (PROMOCHEM GmbH, Germany, No. GBW07404) was used as a standard reference material.

To estimate the concentrations of available (soluble) fraction of metals, samples were air-dried at room temperature and thereafter extracted in deionized water at pH 4.5. The concentrations of metals were analyzed using atomic absorption spectrometry with a graphite furnace.

Total C and total N were determined in five replicate mixed samples per plot, using a Perkin-Elmer CHN-analyzer.

For the measurements of humus respiration rates samples of ca. 0.07 m^2 of the soil organic layer ($A_{0L} + A_{0F} + A_{0H}$) were collected from the forest floor in November 1998 (Laskowski et al., 2003) (five sampling points at each plot, located 5 m from each other, along a transect traced from the center of the plot). Each sample was passed through a sieve (1 cm mesh), transported to the laboratory and thoroughly mixed. Before the incubation, dry weight was measured by drying five subsamples at 105 °C for 12 h. Samples containing 5.00 g (on a dry weight basis) of humus were placed in airtight glass jars (ca. 300 cm^3 volume) and incubated at 17 °C. The respiration rates were measured for approximately 12 h by CO_2 absorption in 0.2 N NaOH. The excess NaOH was titrated with 0.1 N HCl, using a digital Jencons burette with 0.01 ml precision. The incubation time was recorded to the nearest minute. Details of the analytical procedures are described elsewhere (Laskowski et al., 2003; Rożen et al., 2004). Average values and standard deviations are presented in Table 2.

2.4. Statistical analysis

Most analyses were done using the package STATISTICA, Ver. 5 (StatSoft Inc., Tulsa, OK, USA). Variables deviating from a normal distribution (Kolmogorov–Smirnov test with Lilliefors correction) were transformed using natural logarithms. Density and dry mass in various forests and soil types were compared with ANOVA except for cases of unequal variance (Levene's test), where non-parametric tests (Kolmogorov–Smirnov, Kruskal–Wallis) were applied.

Between-stand similarities of species composition were identified using cluster analysis with two methods of agglomeration: UPGMA and Ward's (Manly, 1989; Hair et al., 1992). The coefficients of Bray-Curtis, Jaccard, Morisita as well as Euclidean distance and Pearson's correlation coefficient were used as measures of distance; the last two were used in analyses including H' and Fisher's α species diversity coefficients (Rosenzweig, 1995), as well as density and dry mass. Euclidean distance and Pearson's correlation coefficient were applied in cluster analysis of stands grouped according to environmental conditions (Manly, 1989; Hair et al., 1992).

The possible association between enchytraeid community structure and soil characteristics was tested by canonical correlation analysis (CCA), preceded by factor analysis (FA) to obtain uncorrelated variables. The factors were extracted by principal components analysis; then the factors with eigenvalues >1 were

Table 2
Average values and standard deviations of topsoil property variables (0–10 cm, including organic and mineral layer)

	Oak-hornbeam forest ($n = 22$[a])		Mixed oak-pine forest ($n = 19$)	
	Mean	S.D.	Mean	S.D.
Organic matter: OM (%)	48.38	14.32	77.79	10.16
Total C (% dry matter)	26.46	5.73	39.51	4.92
Total H (% dry matter)	3.48	0.74	4.84	0.63
Total N (% dry matter)	1.54	0.29	1.88	0.25
Total S (% dry matter)	0.06	0.02	0.11	0.03
Ratio C/N	17.20	2.05	21.11	1.36
Water (%)	55.38	7.86	65.44	8.57
pH_{H_2O}	4.74	0.41	4.54	0.44
Total Ca (mg kg^{-1} dry matter)	618.0	283.8	640.0	344.0
Total K (mg kg^{-1} dry matter)	3089.4	1780.1	1026.9	148.3
Total Na (mg kg^{-1} dry matter)	289.0	71.5	230.6	53.1
Mineralization N–NH_4 (mg kg^{-1} per day)	23.04	9.49	18.13	6.37
Mineralization N–NO_3 (mg kg^{-1} per day)	12.22	6.75	3.13	2.89
Mineralization N (mg kg^{-1} per day)	35.24	13.81	21.26	7.88
N–NH_4 (mg kg^{-1} organic matter)	108.4	65.6	77.1	26.6
N–NO_3 (mg kg^{-1} organic matter)	130.0	87.9	31.0	20.4
Respiration (mmol CO_2 g^{-1} OM per day)	103.3	28.1	78.60	18.04
Available Cd (mg kg^{-1})	0.019	0.011	0.011	0.006
Available Cu (mg kg^{-1})	0.30	0.11	0.36	0.12
Available Pb (mg kg^{-1})	0.17	0.14	0.42	0.09
Available Zn (mg kg^{-1})	5.57	2.01	3.47	1.45
Total Cd (mg kg^{-1})	2.24	0.68	2.34	0.52
Total Cu (mg kg^{-1})	17.0	5.3	19.2	5.4
Total Pb (mg kg^{-1})	131.0	52.0	127.7	60.0
Total Zn (mg kg^{-1})	209.9	66.8	181.2	52.4

[a] For heavy metal contents $n = 21$.

varimax-rotated. CCA was done two ways: using raw variables of greatest factor loadings, and using factor scores. Altogether, 39 stands were used in multivariate analysis. One stand was excluded because not all species could be determined, and another two because the data on environmental variables were incomplete.

3. Results

3.1. Species composition

Altogether, 30 species of Enchytraeidae belonging to seven genera were found in the Niepołomice Forest (Table 3). *Cognettia* was the most common genus, occurring mainly in the oak-pine forests and mixed deciduous forests comprising the southern forest complex. The localities in oak-pine forests had the lowest species diversity (lowest H' and Fisher's α indices; Table 3) and were dominated by one species, *Cognettia sphagnetorum* (up to 90% of the individuals in a sample). The genus *Enchytraeus* also was common in the whole Niepołomice Forest, in some places reaching densites comparable with *Cognettia*. However, in most cases it was impossible to determine species within the genus because the majority of individuals were immature. In northern complexes, comprised of oak-hornbeam forests, quite often *Fridericia* sp. and *Achaeta* sp. were recorded.

3.2. Abundance and biomass of Enchytraeidae

The density of individuals in 1998 was significantly higher in oak-pine forests than in deciduous stands ($F = 34.59$, $P < 0.0001$; Table 4). Due to the small number of plots studied in that year ($n = 21$) we did not apply any more detailed classification of forest stands and soil types. In 1999 no significant differences in the density and biomass of enchytraeids

Table 3
Species composition of enchytraeid assemblages (percentage of individuals identified) and diversity indices in various habitats of the Niepołomice Forest

Species	Deciduous forest	Oak-pine forest	o-hf	o-hw	m-df	m-dw	o-pf	o-pw	o-ps
Achaeta sp.	5.9	2.1	4.2	5.6	6.7	9.3	4.3	–	1.4
A. bohemica	+	–	–	–	–	+	–	–	–
A. parva	+	–	+	–	–	+	–	–	–
Cognettia sp.	11.5	16.2	14.2	10.4	4.4	12.0	14.5	15.8	18.5
C. cognettii	+	+	1.1	–	–	+	+	+	+
C. glandulosa	+	+	+	–	3.1	–	–	–	+
C. sphagnetorum	28.6	57.3	17.8	19.2	52.8	38.8	47.9	57.9	67.9
Enchytraeus sp.	+	–	+	–	+	–	–	–	–
E. sp. *juv.*	25.2	20.7	32.8	26.2	8.1	21.5	27.8	25.0	8.7
E. albidus	+	+	–	–	+	–	+	–	–
E. buchholzi	3.9	–	5.4	4.4	3.1	+	–	–	–
E. irregularis	+	–	–	–	–	+	–	–	–
Fridericia sp. *juv.*	12.3	–	9.9	31.6	6.7	10.5	–	–	–
F. alata	+	–	+	–	–	–	–	–	–
F. bulbosa	+	–	+	–	–	1.0	–	–	–
F. bulboides	2.1	+	2.6	–	1.3	3.0	+	–	–
F. callosa	+	–	+	–	–	–	–	–	–
F. galba	+	–	1.5	–	–	–	–	–	–
F. gracilis	+	–	+	–	–	–	–	–	–
F. hegemon	+	–	+	–	–	–	–	–	–
F. leydigi	+	–	–	–	–	+	–	–	–
F. paroniana	+	–	1.2	–	–	–	–	–	–
F. perrieri	+	–	+	–	–	–	–	–	–
F. ratzeli	+	–	+	–	–	–	–	–	–
F. reducata	+	–	–	–	–	+	–	–	–
F. striata	+	–	–	–	–	+	–	–	–
F. tubulosa	+	–	+	1.5	–	–	–	–	–
Henlea sp.	+	+	–	+	–	–	+	–	–
H. nasuta	+	–	+	–	–	–	–	–	–
H. reticulosa	+	–	+	–	–	–	–	–	–
H. perpusilla	1.2	–	+	–	6.3	–	–	–	–
H. similis	+	–	–	–	+	–	–	–	–
Marionina sp.	2.0	+	1.9	–	6.3	–	–	+	–
Mar. argenta	+	–	+	+	–	+	–	–	–
Mesenchytraeus sp.	+	2.6	1.1	–	–	–	3.9	+	3.0
Mes. armatus	+	–	+	–	–	+	–	–	–
Mes. glandulosus	+	–	+	–	–	–	–	–	–
Mes. sanguineus	–	+	–	–	–	–	–	+	–
Number of items	37	12	29	9	11	17	9	7	7
Shannon's H'	1.26	0.81	1.50	1.02	1.01	1.24	0.80	0.80	0.82
Fisher's α	2.28	0.90	2.45	1.29	3.60	1.43	1.04	0.85	0.77

Forest type abbreviations as in Table 1; (+) denotes species share <1%.

were detected between the two forest categories. In oak-pine and deciduous forests, density reached 34,400 and 22,300 ind. m^{-2} (Table 4), and dry mass 0.70 and 0.72 g m^{-2}, respectively. No significant differences were found between the 21 plots which had also been studied in 1998.

Between-site differences were statistically significant when a more detailed subdivision of forest vegetation into seven classes was applied (Table 1): $F = 5.84$, $P < 0.0003$ for density and $F = 0.92$, $P < 0.001$ for biomass (Table 4). The density was higher in swampy oak-pine forests (o-ps; Tukey test,

Table 4
Enchytraeid density (ind. $\times$ m^{-2} $\times$ 10^3) and biomass (g dry mass $\times$ m^{-2}) in various forest types and soil categories in the Niepołomice Forest

	n	Density		Biomass	
		Mean	S.D.	Mean	S.D.
1998					
Deciduous forests	13	13.8	6.00	–	–
Oak-pine forests	8	34.9	11.60	–	–
1999					
Deciduous forests	23	22.3	17.90	0.72	0.568
Oak-pine forests	19	34.4	33.35	0.70	0.946
o-hf	11	17.9 a	9.43	0.82 a	0.571
o-hw	3	22.6 ab	14.97	1.05 ab	0.728
m-df	4	15.8 a	10.44	0.19 ab	0.093
m-dw	5	37.4 ab	31.08	0.71 ab	0.541
o-pf	8	11.2 a	5.74	0.11 b	0.087
o-pw	5	36.4 ab	29.62	0.66 ab	0.750
o-ps	6	63.7 b	36.91	1.53 a	1.175
Podzols	13	16.6 a	17.25	0.21 a	0.294
Cambisols	16	24.2 ab	19.83	0.91 b	0.543
Histosols	18	54.4 b	39.24	1.28 b	1.210

Groups in column with a common letter do not differ significantly ($P > 0.05$). Abbreviations as in Table 1.

HSD, Table 4) than in fresh variants (o-pf; $P < 0.001$), mixed deciduous forests (m-df; $P < 0.05$) or fresh oak-hornbeam forests (o-hf; $P < 0.05$), whereas enchytraeid biomass (Table 4) was lower in fresh (o-pf) than in swampy oak-pine forests (o-ps; $P < 0.001$) and fresh oak-hornbeam forests (o-hf; $P < 0.005$).

We also analyzed the differences between sites according to generalized soil type categories: (1) podsols (PS); (2) histiosols (peat and peat-gleyey soils, HS); and (3) cambisols (brown and gley soils, CS). We excluded plots 17, 19, 20, 22 and 39, whose soil types could not be attributed to one of the above general categories. In HS soils the density and biomass of enchytraeids were higher that in podsols ($P < 0.005$), whereas in brown (CS) soils only the dry mass was higher than in podsols ($P < 0.0005$; Table 4).

3.3. Cluster analysis of enchytraeid communities and soil properties

The dendrograms obtained by clustering sites according to soil parameters were quite independent of the different similarity indices and agglomeration methods used. Cluster analysis divided all sites into two distinct categories, one comprised of oak-pine forests and the other of deciduous forest stands. The second cluster was subdivided into oak-hornbeam and mixed deciduous forest stands (Fig. 3a). This clustering corresponded almost perfectly with the independent forest site classification based on vegetation composition (Różański, unpublished data; Różański, 2003). Only a few stands (sampling plots 1, 3, 16, 22) attributed to deciduous forests fell within the cluster of oak-pine forests, probably due to the relatively high admixture of Scots pine at these particular locations.

In contrast, site clustering according to enchytraeid community structure did not produce a clear division into oak-pine and deciduous sites. If species composition only was taken into account (similarity indices of Bray-Curtis, Jaccard and Morisita) the results differed from those obtained when other variables were included (density, biomass, H' and Fisher's α diversity indices). However, clusters dominated by oak-pine or deciduous forests were still visible in most cases (Fig. 3b).

3.4. Relationships between biotic characteristics and soil properties

Of the physicochemical characteristics of the soils studied, the following variables were used for multivariate analysis: pH value, total content of C, H, S, N_{total}, K, Na and Ca, organic matter (OM), moisture, content of N–NH_4, N–NO_3 in organic matter, concentrations of xenobiotics (Pb, Cu, Zn, Cd) in available form, humus respiration rate and nitrogen mineralization rate.

Factor analysis allowed us to reduce the number of variables describing physicochemical properties of soil to six factors jointly explaining 85.9% of all variation (Table 5). From the biotic variables, two factors were derived by factor analysis, accounting for 92.2% of all variation (Table 5). In CCA only the correlation of the first pair of canonical variables appeared statistically significant ($r = 0.71$, $P < 0.001$; Table 6). The first factor, which we interpret as organic matter content in soil associated with soil moisture, is negatively correlated with community diversity; it explains about 25% of its variation. The remaining

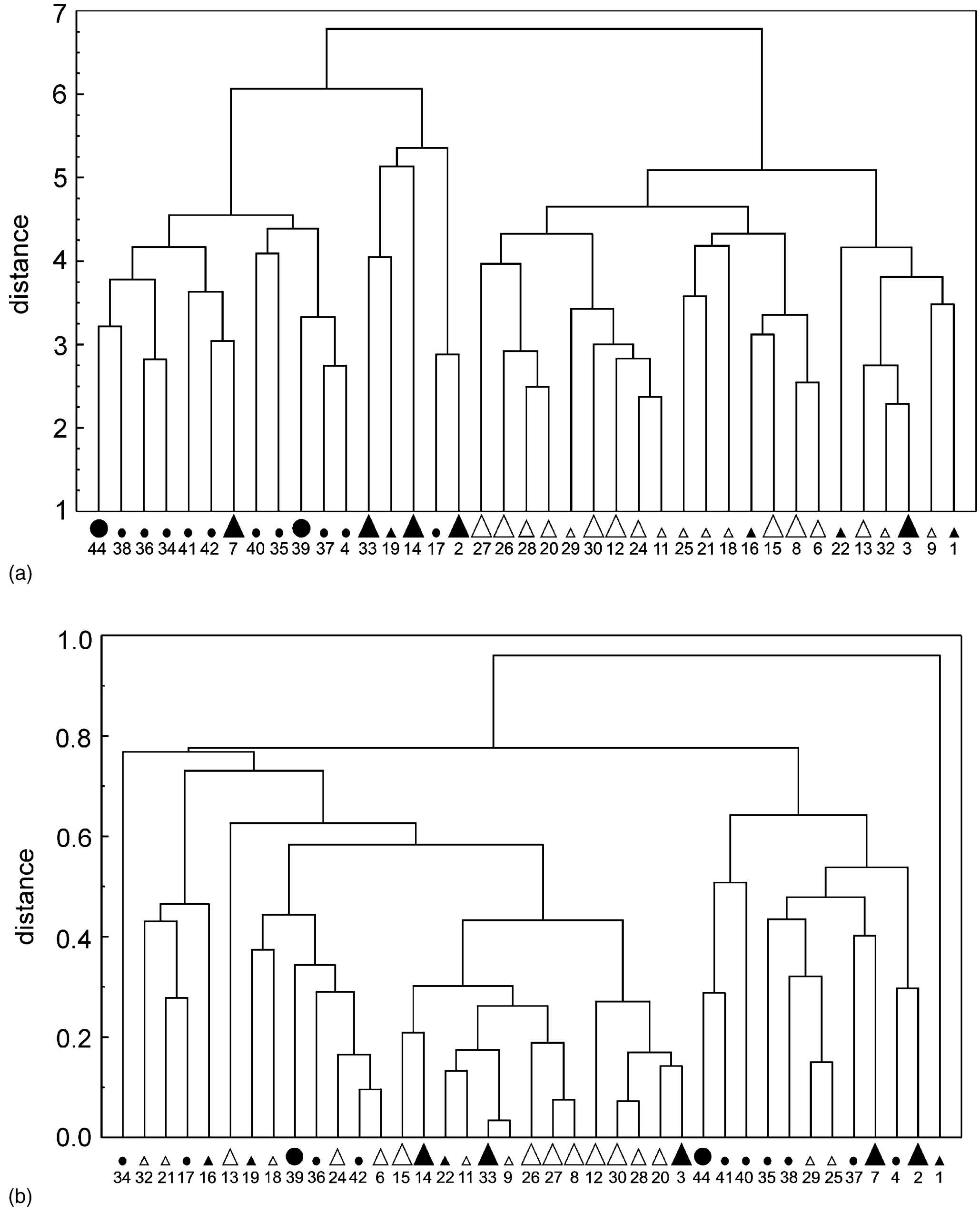

Fig. 3. Clustering of sampling plots: (a) according to soil properties (Euclidean distances, UGPMA); (b) according to characteristics of enchytraeid assemblages; species composition (Bray-Curtis index, UPGMA). Oak-pine forest (fresh) (△); oak-pine forest (wet) (△); oak-pine forest (swamp) (△); mixed deciduous forest (fresh) (▲); mixed deciduous forest (wet) (▲); deciduous forests: oak-hornbeam forest (fresh) (●); oak-hornbeam forest (fresh) (●).

Table 5
Factors (PCA) derived from soil chemistry and biotic variables

Factor no.	Variables (factor loadings in parentheses)	Variation (%) explained
Soil chemical properties		
1	N (0.92), H (0.87), C (0.85), moisture (0.82), organic matter (0.83), S (0.83)	26.7
2	K (−0.82), Na (−0.77), Pb (0.76), Cu (0.73), organic matter (0.54)	20.0
3	N-mineralization rate (0.83), respiration (0.79), NH_4 (0.63)	12.4
4	Cd (0.89), Zn (0.72)	12.0
5	Ca (0.93)	7.6
6	pH (0.88)	7.3
Biotic characters of communities		
1	Density (0.94), dry mass (0.95)	46.9
2	H' index (0.96), Fisher's α index (0.96)	45.2

Only variables with factor loadings greater than 0.5 are listed.

variables, with low loadings, do not help to explain the distribution of biotic characters.

CCA was also performed on raw variables with the highest factor loading, or after excluding some of the variables (nitrogen mineralization rates, humus respiration rate, NH_4- and NO_3-form nitrogen contents). In all such cases the results were similar: a significant effect of organic matter content and moisture upon the species diversity of enchytraeid communities.

Table 6
Canonical correspondence analysis (CCA); factor numbers as in Table 5

First set (factor no.)	U1	U2
1	**0.84**	−0.36
2	**0.42**	0.68
3	−0.15	−0.46
4	−0.05	−0.09
5	−0.23	0.40
6	−0.20	−0.18
Canonical *R*	0.71	0.51
P-value	<**0.001**	<0.10
Redundancy of second set (%)	25.4	12.9
Second set ($n = 39$)	V1	V2
1	0.26	−0.96
2	**−0.96**	−0.26

4. Discussion

4.1. Species composition and habitat characteristics

Many studies concerning the population dynamics of Enchytraeidae in relation to habitat variation have dealt only with the density and biomass of communities, or with single-species populations (Abrahamsen, 1971; Phillipson et al., 1979; Lundkvist, 1982; Huhta, 1984; Vavoulidou et al., 1999). However, abiotic parameters usually cannot explain the distribution of abundance and biomass values of these animals, because these variables depend strongly upon interactions between species (Didden, 1993). In this study we attempt to take into account species composition. Most often the papers published so far make comparisons between faunal communities of contrasting habitats (Dash and Cragg, 1972; Healy, 1980) or between successional stages (Pilipiuk, 1995; Nowak, 2001), thus covering a very broad spectrum of habitat variation.

The forest stands delineated here according to vegetation composition may be equally well defined by soil chemistry, as the cluster analysis shows. This variation in habitat characteristics is not very large, and consequently it accounts only for about 25% of the distribution pattern of enchytraeids, especially species composition. A detailed analysis of particular species' habitat requirements and of possible between-species interactions would require a much greater number of sample. Our data allow only general conclusions.

Enchytraeid diversity was negatively correlated with organic matter content in the topsoil layers. The number of species was low in the oak-pine forest characterized by acid soil and slow decomposition, and thus with a thick mor humus layer. In extreme cases the communities were almost totally dominated (99%) by a single species, *Cognettia sphagnetorum*. This finding confirms many previous reports (Abrahamsen, 1971; Górny, 1975; Lundkvist, 1982; Kasprzak, 1986; Pilipiuk, 1993, 1995). In some oak-pine stands *C. sphagnetorum* was accompanied by *Enchytraeus* sp., a species relatively numerous and common in the whole area. Similarly high densities were already recorded in other pine forests in Poland (Pilipiuk, 1993, 1995). The share of other species increased with decreasing content of organic matter in soil. Besides *Enchytraeus* sp. in mixed deciduous forests species belonging to the genera *Mesenchytraeus*, *Achaeta* and *Fridericia* also occurred quite commonly. This last genus typically avoids conifer forests and is common in oak-hornbeam stands (Górny, 1975; Kasprzak, 1986; Graefe and Schmelz, 1999), where the highest richness of species is also noted. This trend is attributed to the increased diversity of vegetation, and consequently, of soil microhabitats (Kasprzak, 1982; Pilipiuk, 1995). According to Healy (1980), vegetation significantly affects the distribution of enchytraeids when it contributes to soil formation. We suggest that in the Niepołomice Forest situation the share of Scots pine (*Pinus silvestris*) in the stand is important; the pine litter is converted to mor humus, which in turn strongly affects the chemical and structural characteristics of the soil.

Another group which plays an important role in the soil process is earthworms. In the Niepołomice Forest seven species of earthworms were found with a density of 65–175 ind. m^{-2} in oak-hormbeam forest (Rożen, 1982, 1988) and only 20–30 ind. m^{-2} (Rożen, unpublished data) in mixed oak-pine stands. However, because of a different sampling method and spatial scale required it was impossible to include earthworms in this study.

High humus content in soil facilitates retention of water, and thus these variables are correlated. In stands of the Niepołomice Forest where mor humus has developed, the upper soil layer does indeed maintain high moisture. Although *Cognettia sphagnetorum* can tolerate a broad range of soil moisture, it prefers habitats highly saturated with water (moisture content above 80%; Healy, 1980). Rota et al. (1998) noted a highly positive correlation between species richness and soil pH. In the Niepołomice Forest this tendency is not statistically significant; pH variation in the whole area is small, and enchytraeids are usually quite tolerant of a broad range of pH values (Healy, 1980).

In the Niepołomice Forest, the content of heavy metals in the soil reflects the impact of human activity. If the effect of this factor upon soil fauna were strong, one might expect the distribution of enchytraeids to depend not only on the habitat gradient but also on the distance from the source of pollution (Bengtsson and Rundgren, 1982; Posthuma et al., 1998). Such an effect was not detected. The current concentration of heavy metals in the soil was relatively small. Enchytraeids, particularly *Cognettia sphagnetorum*, demonstrate high resistance to these substances (Salminen and Haimi, 1999). Only lead content correlated significantly and positively with enchytraeid density, and negatively with species diversity. This, however, may be due to the high affinity of this exobiotic for organic matter. At high content of organic carbon, low pH and low potassium concentrations, lead tends to accumulate in the organic matter fraction of soil (Egli et al., 1999), so its content may correlate with community parameters parallel to organic matter content without having any direct relationship with them. Other studies confirm this. The concentration of heavy metals in enchytraeids (Rożen et al., 2004) fluctuated along with the content of available forms of these metals in soil.

4.2. Quantitative characteristics of enchytraeid communities

Generally, the densities of enchytraeids in coniferous forests are reported to be higher than in deciduous (Górny, 1975; Kasprzak, 1986). In the current study this pattern was confirmed only in the first year (1998). However, the average density of enchytraeids estimated for both oak-pine (34,000 ind. m^{-2}) and at deciduous sites (22,000 ind. m^{-2}) fell within the values reported for this part of Europe. In Poland it ranges from about 10,000 to 60,000 ind. m^{-2} in coniferous forests (Górny, 1975; Makulec, 1983; Pilipiuk, 1993, 1995), while in an oak-hornbeam forest it was estimated at 38,600 ind. m^{-2} (Makulec, 1983). In various regions of Europe, enchytraeid density may range from a couple thousand to as much as 140,000 ind. m^{-2}, regardless of the habitat type (Didden, 1993).

According to Phillipson et al. (1979) the biomass of Enchytraeidae measured in European forests may also vary in a broad range, with a minimum value recorded in Finland (1–2 g wet mass m^{-2}) and as much as 11 and 22 g wet mass m^{-2} noted in UK in coniferous and deciduous forests, respectively. In the Niepołomice Forest the biomass of Enchytraeidae (recalculated from dry mass following Persson and Lohm, 1977) averages 3.7 g wet mass m^{-2} in pine-oak, and 4.1 g wet mass m^{-2} in deciduous sites. However, these communities of practically identical biomass did differ substantially in species composition: oak-pine sites were dominated by *C. sphagnetorum*, while oak-hornbeam sites were rich in species.

On the other hand, sites of a given forest type sometimes differed in density and biomass while having a similar species composition. For example, in oak-pine forests developed on different soils, in moist organic soils (peat and peat-gley) with high content of humus the density and biomass of enchytraeids reached a maximum, while very small numbers and biomass were recorded from podzolic soils.

5. Conclusions

The clustering of study sites according to environmental variables closely agrees with the classification based on vegetation. This justifies the typology of Niepołomice Forest habitats as oak-pine forests and deciduous forest, the last group subdivided into oak-hornbeam forests and mixed deciduous forests. However, the ordination of enchytraeid communities according to their structural (species composition) and quantitative parameters (density, biomass) does not correspond exactly with the classification of forest stands. The physicochemical properties of the upper layer of the soil profile account for only 25% of the variance in enchytraeid community structure, mainly in species composition, whereas the variance in density and biomass remains unexplained. Among soil properties the most important effect apparently is exerted by the organic matter and moisture content of the top layer, correlating negatively with species diversity. Na and K contents have opposite effects. On the other hand, heavy metal contamination does not influence the enchytraeid community. The only exception is lead content, strongly associated with organic matter and therefore correlating with enchytraeid community structure in an indirect way.

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