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*Regular research paper*

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## RESPONSE OF ENCHYTRAEID COMMUNITY (OLIGOCHAETA, ENCHYTRAEIDAE) TO MANIPULATION OF MICROBIAL BIOMASS

**ABSTRACT:** The paper describes a field experiment with the application of two biocides: oxytetracycline that reduced bacteria and captan that reduced soil fungi. The purpose of the experiment was to investigate the response of enchytraeid species to the changes of the abundance and activity of the microbial community as part of their food resources. The following variables were recorded: the biomass of microorganisms by the PLFA method, the rate of cellulose decomposition and the numbers and species composition of enchytraeids. No relationship was found between enchytraeids and abundance of microorganisms, but numbers of animals were correlated with changes in soil cellulolytic activity. Both biocides, even the non toxic oxytetracycline, reduced the diversity ( $H'$ ) of enchytraeid community by increasing the proportion of dominant species. It is suggested that biocides reduced the functional diversity of microorganisms, and this factor had an effect on the enchytraeid populations. The animals responded to the treatment and their community became predominated by the species probably with a broad food spectrum.

**KEY WORDS:** enchytraeids, microbial activity, oxytetracycline, captan, diversity, abundance

### 1. INTRODUCTION

Enchytraeids are important and abundant soil inhabitants, especially in peatlands and coniferous forests, where they account for over one-third of the biomass of soil invertebrates (Coulson and Whittaker 1978, Petersen 1982). Their ecology and especially their food preferences are poorly known. They are considered to be saprophages (Brockmayer *et al.* 1990, Didden 1993, Reichert *et al.* 1996); it means that in addition to detritus, their diet also includes microorganisms. According to Didden (1993), the microorganisms can made up even 80% of their food. It is not known, however, whether enchytraeids are selective with respect to this part of their diet and to what extent. Some species prefer bacteria (Krištůfek *et al.* 1995) or, more often, fungi (Latter and Howson 1978, Hedlund and Augustsson 1995). Literature data on their preferences are based on laboratory cultures or on observations in the sites of their occurrence. Both of these approaches, although useful, are not decisive. The most interesting but rarely used method is the modification of the abundance or quality of the supposed food sources in the field and observation of

changes occurring afterwards; this method was applied by Wolters (1988).

The aim of the present experiment was the manipulation of the quality of food sources and to monitor the reaction of enchytraeid species. The biocides used for this purpose do not change the content of organic matter, minerals and physical properties (moisture, pH) of the soil, but they modify the community of microorganisms decomposing the organic matter. It was assumed that these treatments will eliminate bacterivorous or fungivorous enchytraeids.

## 2. MATERIAL AND METHODS

The experiment with biocides was performed in sandy loam soils, poor in organic matter. It was conducted in a pine forest on acid soil (pH in water 4.3,  $C_{org}$  1.36%) and in a fallow land on soil with admixture of clay (pH = 5.7,  $C_{org}$  1.51%). Three experimental study plots of the area 5 m<sup>2</sup> each were established in both habitats: control (C), oxytetracycline (OX) and captan (CA) treated. They were big enough to take samples at random. The homogeneity of plots was tested by their moisture: 12 samples were taken from each plot for analyses of soil moisture by dry weight method. In the forest, the weight of dry soil varied from 80 to 91%, in the fallow – from 83 to 91%, but no significant differences between plots were found (ANOVA  $F = 3.9$ ,  $P > 0.05$ ).

All plots, including control plots denoted by C, were treated with glucose as water solution (23 g l<sup>-1</sup>) at a rate of 4.5 l per m<sup>2</sup> (based on Anderson and Domsch 1973). This dose was given to prevent microorganisms starvation. Plots denoted by OX were treated with the antibiotic oxytetracycline (15 g m<sup>-2</sup>), plots denoted by CA with the fungicide captan (17.5 g m<sup>-2</sup>). These concentrations of biocides were proposed and applied by Beare *et al.* (1992).

The biocides were applied three times in June–October period. Every six weeks biomass of microorganisms estimated with the phospholipids fatty acid method (PLFA) and numbers and species composition of enchytraeids were recorded. The rate of cellulose decomposition was estimated two times in above period.

Enchytraeids were counted 6 weeks after the application of biocides (just before next application) at: 1 June, 15 July and 1 September in the forest and 1 July, 15 August and 1 October in the fallow. On each plot 15 separate samples were taken of 10 cm<sup>2</sup> in area and 15 cm deep divided into three 5 cm layers. These samples were extracted by O'Connor (1955) wet funnel method. At the end of the experiment (1 September in forest and 1 October in fallow) also the abundance and species composition of enchytraeids were determined in the habitat surrounding the plots, denoted by S. The numbers of enchytraeids on these plots (deprived of glucose) were the same (in the fallow) or lower (3.8 times in the forest) in comparison with the control plots C; the difference is statistically significant ( $P < 0.05$ ).

To assess the biomass of microorganisms, mixed samples from 10 cores (each 2 cm<sup>2</sup> in area and 10 cm deep) were used. The samples were taken 10 days after the application of biocides.

The PLFA method consists in the estimation of specific phospholipid contents in organic matter of soil. The phospholipids enter into composition of cytoplasmatic membrane of live microorganisms and are in constant proportion to volume of their cells. The PLFA method (Frostegard *et al.* 1991) relies on the isolation of lipids with the solution of chloroform, methanol and citrate buffer, and their fractionation to separate phospholipids. After methanolysis and methylation of phospholipids, methylene esters of fatty acids were isolated and identified by using gas chromatography/mass spectrometry. To calculate the biomass of bacteria the content of following acids was used: i 15:0, a 15:0, 15:0, i 16:0, 16:1 $\omega$ 7 t, i 17:0, 17:0, cy 17:0, 18:1 $\omega$ 7, and cy 19:0. Fungal biomass was estimated based on the acid 18:2 $\omega$ 6 (Frostegard and Baath 1996). Data were calculated in nmol per gram of soil organic matter.

Cellulose decomposition was estimated using the filter paper method. The weight of 10 cellulose paper 10 cm diameter buried in 0–10 cm layer of soil was measured once a month.

A 24-h mortality test of animals (LD<sub>50</sub>) was performed in a water culture. Enchytraeids of unknown species were kept at 11°C

in petri-dishes (5 cm in diameter) with five concentrations of the solution of captan or oxytetracycline. Three dishes, each containing 10 enchytraeids, were used in two repetitions for each of the five dilutions of biocides.

Oxytetracycline concentrations used in the field experiment were not lethal to the animals in the aquatic culture.  $LD_{50}$  of captan in the laboratory aquatic culture was 0.012 of the concentration used in the field i.e.  $48 \text{ mg l}^{-1}$ . It should be added that a parallel laboratory soil culture of *Cognetia sphagnetorum* (Vejd) treated with the same doses of captan as in the field experiment (Nowak and Piotrowska-Seget in press) did not confirm the results of the  $LD_{50}$  test (Table 1): the animals mortality did not increase or the mortality was rapidly compensated by the reproduction. Thus the same response was expected in the experiment.

The program Statistica was used for statistical analysis of the results (t-test, ANOVA, correlations, cluster analysis). Relative numbers, Euclidean distances and average linkage clustering were used in cluster analysis.

### 3. RESULTS

#### 3.1. Efficiency of biocides

The rate of microbial reduction by biocides showed significant differences between plots (ANOVA  $F = 3.57$ ,  $P < 0.05$ ) (Fig. 1). It has been found that oxytetracycline (OX) was a weak bactericide under the experimental conditions. It decreased 19% of the bacterial biomass in the fallow (from 11 to 24% in the successive samples) and 24%

(17–33%) in the forest – in comparison with control (Table 2). In the upper 0–5 cm soil layer oxytetracycline reduced 21% and 30% of bacterial biomass respectively (Table 2). At the same time, the increase of fungal biomass was not significant (106% of the control bio-

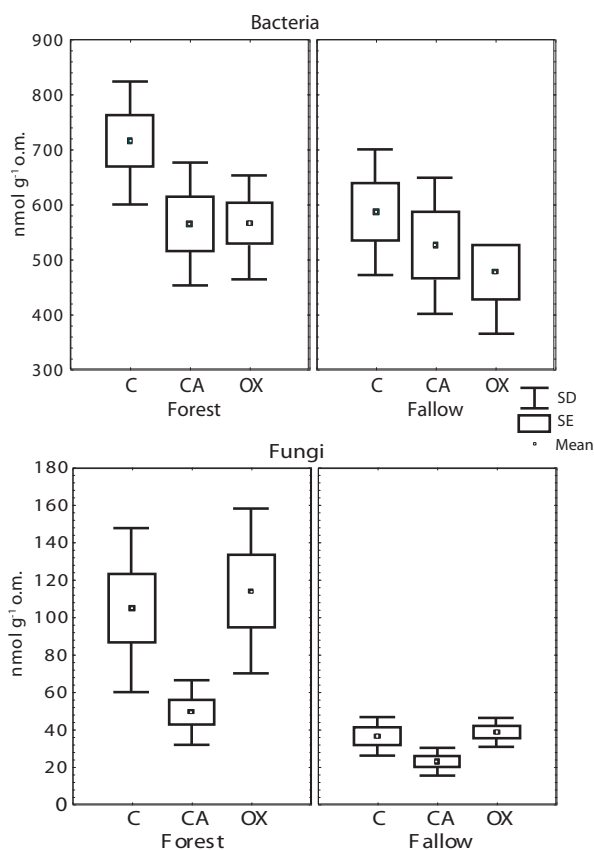


Fig.1. Biomass of microorganisms ( $\text{nmol g}^{-1} \text{ om}$  in soil) in experimental plots. C – control, CA – captan, OX – oxytetracycline treatment. All plots were simultaneously treated with glucose solution (see Methods).

Table 1. Numbers (per 50 g d.w. of soil  $\pm$  SE) of *Cognetia sphagnetorum* in microcosms (see Nowak and Piotrowska-Seget in press) treated with water C, and solution of oxytetracycline OX, captan CA, first – I and second – II culture

Treatment	I weeks of culture				II weeks of culture			
	0	6	12	18	0	4	9	14
C	25	$40 \pm 6$	$35 \pm 8$	$27 \pm 5$	25	$27 \pm 1$	$64 \pm 7$	$68 \pm 11$
OX	25	$53 \pm 8$	$11 \pm 2$	$13 \pm 9$	25	$38 \pm 6$	$83 \pm 12$	$64 \pm 7$
CA	25	$43 \pm 6$	$46 \pm 47$	–	25	$22 \pm 6$	$28 \pm 5$	$14 \pm 4$

Table 2. Effect of captan (CA) and oxytetracycline (OX) addition on biomass of soil microorganisms (PLFA method) and cellulose decomposition in successive sampling dates in two habitats. C – control. All plots were simultaneously treated with glucose solution, see Methods. I, II, III – sampling occasions see Fig. 2, a – 0–5 cm ↓, b – 5–10 cm ↓ soil layer.

	Forest			Fallow		
	biomass (nmol g <sup>-1</sup> o.m.) bacteria	biomass (nmol g <sup>-1</sup> o.m.) fungi	cellulose decomposition (mg g <sup>-1</sup> day <sup>-1</sup> )	biomass (nmol g <sup>-1</sup> o.m.) bacteria	biomass (nmol g <sup>-1</sup> o.m.) fungi	cellulose decomposition (mg g <sup>-1</sup> day <sup>-1</sup> )
Ia	845	169	—	745	47	—
Ib	601	46		683	40	
C IIa	784	148	17.5 ± 2.2	564	33	23.1 ± 0.7
IIb	567	84		436	22	
IIIa	784	88	14.6 ± 2.5	664	53	19.9 ± 0.8
IIIb	676	94		426	24	
Ia	788	62	—	689	31	—
Ib	567	35		732	28	
CA IIa	613	77	*3.6 ± 3.0	467	19	*19.7 ± 4.4
IIb	488	34		404	12	
IIIa	613	51	*1.9 ± 0.8	587	31	17.6 ± 1.6
IIIb	503	37		354	16	
Ia	738	189	—	710	53	—
Ib	523	55		521	42	
OX IIa	473	153	*15.2 ± 2.5	412	35	22.8 ± 0.7
IIb	515	92		387	27	
IIIa	473	103	*9.5 ± 1.6	445	43	*17.8 ± 2.3
IIIb	515	90		387	32	

\*statistically significant differences as compared with the control C at  $P < 0.05$ .

mass in the fallow and 108% in the forest). Captan (CA) had a stronger effect on microbial biomass (Fig. 1). It reduced not only fungal biomass (37% in the fallow and 53% in the forest as compared with the control) but also the biomass of bacteria (8% in the fallow and 16% in the forest) (Table 2). Thus, in both habitats oxytetracycline-treated plots differed from the control with respect to bacterial biomass, and captan-treated plots differed in fungal biomass (Fig. 1). The bacteria to fungi biomass ratio for the fallow was 16 in the control C, 12 – in plot treated with bactericide OX, and 24 – in plot treated with fungicide CA. In the forest, the respective values were 7, 5, and 12.

Rate of cellulose decomposition in different treatments of the experiment is the another indicator of changes in microbial communities. It was higher in the fallow than in the forest (Table 2). Oxytetracycline was less

effective than captan in reducing cellulose decomposition. The cellulolytic activity in plot CA in the forest accounted for only 21 and 13% of that in the control plot C, in two sampling dates respectively. In the fallow it was 81 and 88% respectively. The differences are statistically significant ( $P < 0.05$ ). Biocides had stronger effect on the activity of microorganisms, than on their biomass.

### 3.2. Enchytraeid communities

Enchytraeid communities living in two habitats under study differed in abundance and species composition although the soil of both sites was similarly poor in organic matter. The numbers of animals in the forest was low. In all experiments it fluctuated about a level lower by half of that in the fallow (Fig. 2).



Fig. 2. Numbers of enchytraeids ( $\text{ind. } 10^3 \text{ m}^{-2}$ ) in experimental plots (C, CA, OX see Fig. 1) for three sampling occasions: A – in 0–15-cm soil layer, B – in 0–5-cm soil layer.

In June, the first application of biocides in the forest made the numbers of enchytraeids increased in 0–15 cm layer in both treatments; in plot OX by a factor of 3 and in plot CA – by a factor of 8, as compared with the control C. On the second (July) and third (September) sampling date, no significant differences were found in enchytraeid abundance between treatments (Fig. 2A). In the fallow, the treatment with oxytetracycline in July reduced enchytraeid numbers by half. In later periods no significant differences were found. Captan reduced the numbers of animals in the second (August) and third (October) experimental period (Fig. 2A).

Enchytraeid community dynamics was also analysed in the top 0–5cm soil layer,

which was subjected to most intense impact of biocides. This layer was inhabited by 36–61% of total numbers of enchytraeids in the forest and by 48–72% of enchytraeids in the fallow. In general, the results were similar to those obtained for the whole 0–15-cm layer. Differences were only found in the fallow, where on two occasions enchytraeid abundance decreased in the plots treated with oxytetracycline (Fig. 2B). At the end of study period the enchytraeid numbers ranged in the order  $C > OX > CA$ .

Numbers of animals vary independently of the treatment but they conform to variation in the rate of cellulose decomposition in the plots (Fig. 3). The correlation coefficient  $r = 0.72$  is statistically significant at

$P = 0.01$ . This relationship can be described by the formula:  $y = 2.18x + 10.82$  where  $y$ , is enchytraeids numbers in  $10^3 \text{ m}^{-2}$ ,  $x$  – cellulose decomposition in  $\text{mg g}^{-1} \text{ day}^{-1}$ . The results are better approximated by the second-order equation:  $y = 35.5 - 4.9x + 0.3x^2$  with a correlation coefficient  $r = 0.91$ . It seems that the linear regression is valid for  $>15 \text{ mg g}^{-1} \text{ day}^{-1}$  of cellulolytic activity. No correlation was found between the biomass of microorganisms and numbers of enchytraeids neither between cellulolytic activity and biomass of fungi. Fungal activity is a factor diversifying cellulose decomposition on homogenous plots with respect to their moisture content. The correlation between cellulolytic activity and biomass of fungi was only found in the data for forest plots.

The two study habitats were considered to be dry and poor in organic mater. They were inhabited by 17 enchytraeid species (Table 3), i.e. the community rather poor in species.

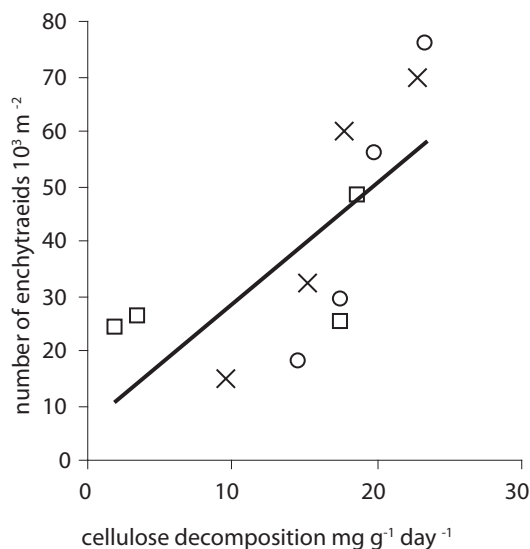


Fig. 3. Relationship between enchytraeid abundance and the rate of cellulose decomposition. Open circles – control C, squares – plots treated with captan CA, crosses – plots treated with oxytetracycline OX.

Table 3. Species composition and average density (ind.  $10^3 \text{ m}^{-2}$ ) of enchytraeids in two habitats. C, CA, OX – see Table 1 ( $+ < 0.1$ ). Bold – dominant species.

Species	Forest			Fallow		
	C	CA	OX	C	CA	OX
<i>Hemifridericia parva</i> Niel. et Chr.	<b>8.8</b>	<b>21.2</b>	<b>15.8</b>	–	–	–
<i>Acheta</i> sp.*	1.5	2.7	0.8	0.8	2.6	2.6
<i>Enchytraeus buchholzi</i> Vej	1.4	–	+	8.0	9.7	8.5
<i>Enchytraeus norvegicus</i> Abrah	0.1	+	0.1	0.2	0.6	0.2
<i>Enchytronia parva</i> Niel et. Chr	0.6	1.9	0.9	<b>17.5</b>	<b>13.1</b>	<b>26.5</b>
<i>Cognetia sphagnetorum</i> (Vejd)	1.8	6.0	3.9	–	–	–
<i>Fridericia bisetosa</i> (Lev.)**	1.2	0.1	+	7.3	7.0	2.1
<i>Fridericia galba</i> (Hoffm.)**	0.9	+	–	1.1	0.3	3.3
<i>Fridericia ratzeli</i> Eis**	–	–	–	0.2	0.3	–
<i>Marionina</i> sp.	0.7	0.3	0.2	1.0	0.8	1.5
<i>Marionina argentea</i> (Mich)	–	–	–	–	–	0.1
<i>Buchholzia fallax</i> Mich.	0.5	–	0.7	14.7	3.0	1.0
<i>Mesenchytraeus gland</i> (Lev.)	0.2	–	+	–	–	–
<i>Henlea perpusilla</i> Friend	–	–	–	13.1	9.0	7.7
<i>Henlea nasuta</i> (Eis)	–	–	–	0.2	–	0.7
<i>Henlea ventriculosa</i> (d'Udek)	–	–	–	–	+	0.3
<i>Černosvitoviella atrata</i> (Bret.)	–	+	–	–	–	–
Total density	17.7	32.2	22.4	64.1	46.4	54.5
Number of identified individuals	380	444	382	520	481	415

\* mainly *A. camerani* Cog.

\*\* young specimens identified by numbers of setae

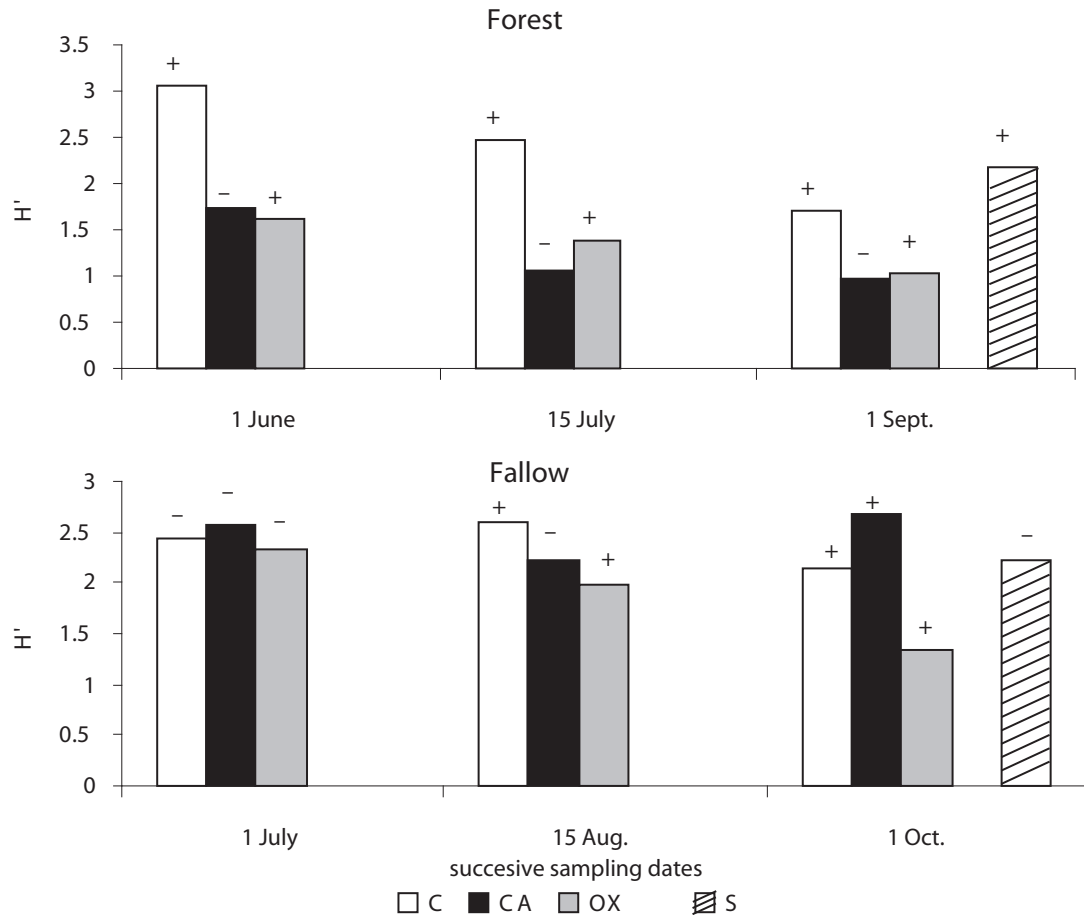


Fig. 4. Index of species diversity ( $H'$ ) of enchytraeid communities in the forest and fallow for three sampling occasions in surrounding site (S) and experimental plots (C, CA, OX see Fig.1). +, -, significant and not significant difference with right neighbour.

For instance: Ryl (1980) found 22 species in a crop field. Of these species, 8 were common to the both habitats. In the forest *Hemifridericia parva* was the dominant species, followed by *Cognetia sphagnetorum*, typical dominant species in many coniferous forests. The next more abundant species were the species of genus *Acheta* (mainly *A. camerani*). The dominance of these species varied between the three successive sampling dates. During the first period, *C. sphagnetorum* slightly predominated. The dominance of *H. parva* increased with time (from 14% in June to 66% of the total number in September). The species composition of enchytraeids in the forest surrounding the experimental plots (S) was rather similar. *Acheta* sp. were most abundant and *H. parva* and *C. sphagnetorum* were subdominants.

In the fallow land, *Enchytronia parva* was the dominant species in the control plots C, and in surrounding habitat S, followed by *Buchholzia fallax* and *Henlea perpusilla* (Table 3).

Typically, the dominants of the two habitats – *H. parva* in the forest and *E. parva* in the fallow, are not frequent in other habitats in Europe.

After the treatment with biocides, the abundant species generally continued to occur in large numbers (Table 3). But in the forest, *Enchytronia parva* increased in numbers, moving from position 8 on the species list of abundance in the control plot C, to position 4 in the captan-treated plot CA, and to position 3 in the oxytetracycline-treated plot OX. In the fallow, the position of *Enchytraeus buchholzi* also changed. On the plots CA and

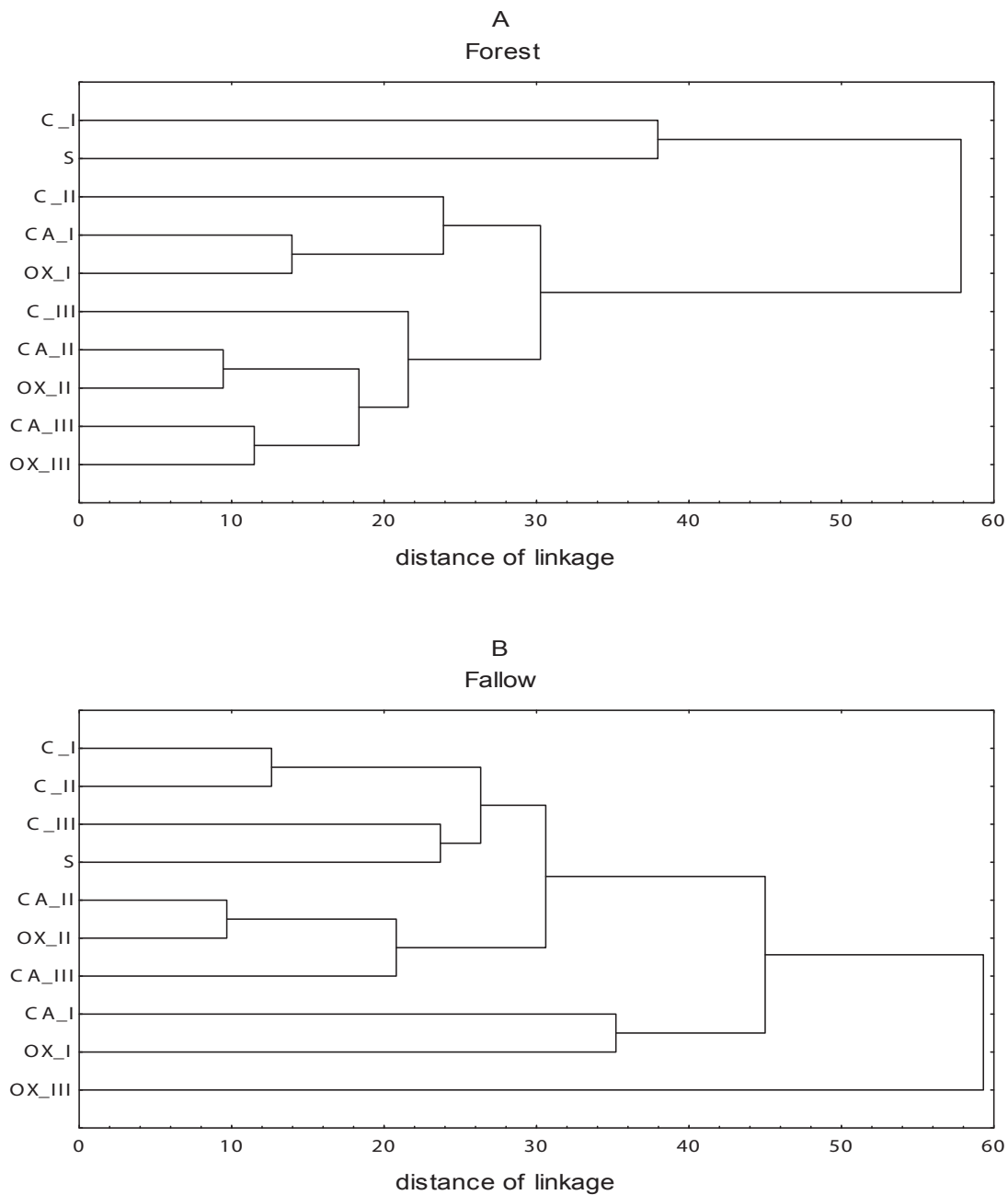


Fig. 5. Similarity in the species composition of enchytraeids (euclidean distance, average linkage clustering) between plots C, CA, OX, S (as Figs 1, 4) and different sampling occasions (I–III see Fig. 2) in forest and fallow sites.

OX it became a subdominant, whereas the abundance of the earlier subdominant *B. fallax* rapidly declined, so that it shifted to position 5 in plot CA, and to position 7 in plot OX (Table 3).

Enchytraeid communities showed differences in their dominance structure in the control plots. In the forest, it conformed to the broken stick distribution, whereas in the fallow – to the geometric distribution (Trojan 1992). Biocide treatment made both these types of distribution more sharp. However, the species richness in plots CA and OX was not changed, but the contribution of dominant species, *H. parva* in the forest and *E. parva* in the fallow, increased considerably (84 and 78% respectively in oxytetracycline plots at the end of the experiment).

As a result, the Shannon index of species diversity,  $H'$ , was changed. In the forest the  $H'$  value in control plots C at the beginning of experiment was high and steadily decreased on successive dates. The species diversity on CA and OX plots was similar and clearly lower than in control plots C in this habitat (Fig. 4). In the fallow, captan did not reduce the diversity of animals, but oxytetracycline, which did not affect numbers of animals in this habitat, reduced their diversity in August and October (Fig. 4). Like in the case of numbers, the diversity for control plots C in the fallow remained unchanged as compared with the surroundings S, whereas in the forest the diversity was significantly reduced.

Seasonal changes in the species composition of enchytraeids followed a similar pattern when the cluster analysis was used. In the forest, the enchytraeid communities of plots CA and OX were similar in species composition and relative abundance on successive data. In the fallow land, the communities of plots CA and OX were similar in August, while the communities of plots C were similar to each other on two successive dates, July and August (Fig. 5).

When only the top soil layer was considered, the similarity in the species composition between plots, was reduced by half but the pattern remained unchanged. Plots CA and OX were most similar in both habitats (euclidean distances in the forest were 8 for the enchytraeids with total depth in all experiments and 17 for the upper layer, whereas

in the fallow – 25 and 32 respectively). Plots C and CA (22 for the total depth and 42 for upper layer) in the forest and plots C and OX in the fallow (32 and 47, respectively) were the least similar.

#### 4. DISCUSSION

Based on the results obtained by Bear *et al.* (1992), it was expected that oxytetracycline will reduce about 50% of microbial biomass and captan about 60%. That is, the effect should be stronger than that obtained in the present experiment. However, many authors observed that the efficiency of biocides vary depending on environmental conditions (Ingham *et al.* 1991, Landi *et al.* 1993, Colinas *et al.* 1994, Martinez-Toledo *et al.* 1998).

A larger microbial biomass was expected in a more fertile soil of fallow than in the forest, but this was not the case. The biomass of bacteria was higher in the forest than in the fallow by about 24%, and fungal biomass in the fallow accounted for only 35% of its biomass in the forest. In spite of this, both cellulolytic activity and enchytraeid abundance were lower in the forest than in the fallow.

Biocides caused only small changes in the total biomass of microorganisms, and with two exceptions, they did not influence on the numbers of enchytraeids. In the forest, numbers of enchytraeids increased after the first application of biocides, may be at the expense of dead microorganisms. It may be expected that successive applications would have reduce the reproduction, rather than increased mortality of microorganisms, and that the biomass of dead microorganisms was higher in the forests than in the fallow. Captan, which can be toxic to enchytraeids, reduced numbers of these animals only in the fallow. This is incomprehensible because in this richer for enchytraeids habitat their mortality should be compensated by immigration.

Biocides were only responsible for small quantitative changes in microorganisms but much larger for qualitative changes. The cellulolytic activity of biocide-treated plots decreased. This decrease is parallel to the decrease of animal numbers. Under conditions

of uniform soil moisture in the present experiment, this relationship can be interpreted as a dependence of enchytraeids on the degree of organic matter decomposition as indicated by cellulolytic activity. A relationship between the degree of organic matter decomposition and enchytraeid community was noted also by Dozsa Farkas (1982).

The species diversity of enchytraeids ( $H'$ ) clearly varied between the experiment plots. In both habitats it decreased in oxytetracycline-treated plots and in the forest (on one occasion also in the fallow) also in the captan-treated plots. Ruess *et al.* (2001) described a decrease in the diversity of nematodes treated with fungicides, but they ascribed this decline to their mortality. In their experiment, antibiotics did not give rise to changes in diversity neither to the mortality of these animals.

There is some evidence that the diversity of microorganisms was reduced after the application of biocides. It includes a decrease in the cellulolytic activity that was not proportional to changes in fungal biomass. Another indication is previously mentioned laboratory culture of *C. sphagnetorum* (Nowak and Piotrowska-Seget in press) in which two biocides inhibited organic nitrogen mineralization with  $N-NO_3$  release (control culture  $-3.8$ , captan culture  $-1.9$ , oxytetracycline culture  $-2.2$  mg  $l^{-1}$  N, ANOVA  $P < 0.001$ ). Griffiths *et al.* (2000) found that fumigation had a stronger reducing effect on the diversity of functional activity of microorganisms than on their general functions such as biomass measured in SIR (the substrate induced respiration method comparable to PLFC, Zelles 1999). A decrease in species diversity of enchytraeids in this study should be ascribed to a decrease in the diversity of microorganisms in their food.

Changes in the species diversity of enchytraeids in this study resulted from an increase in abundance and percentage contribution of dominants, without elimination of less frequent species. It seems that such species as *H. parva*, *E. parva*, *C. sphagnetorum*, and *A. camerani* are omnivorous dominants. Numbers of these species increased at expenses of decreasing numbers of species more susceptible to changes in food resources, such as *B. fallax*. It was a subdominant

species in the fallow, and based on the results of this study, it is the only species considered as the highly selective towards microorganisms.

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