

## Effects of organically enriched magnesite fertilizers on the testate amoebae of a spruce forest

Erna Aesch (1) and Wilhelm Foissner (2)

(1) Biologiezentrum, Oberösterreichisches Landesmuseum, J.-W.-Klein-Str. 73, A-4040 Linz, Austria.

(2) Universität Salzburg, Institut für Zoologie, Hellbrunnerstr. 34, A-5020 Salzburg, Austria.

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### Abstract

The effects of organically enriched magnesite fertilizers on the active soil testaceans of a declining spruce forest were investigated during a 5-y-period using a completely randomized block design and a direct counting method. For revitalization 2000 kg ha<sup>-1</sup> biomag (90% magnesite and 10% dried fungal biomass; "biomag plots") and 3000 kg ha<sup>-1</sup> bactosol (dried bacterial biomass) + 2000 kg ha<sup>-1</sup> biomag ("bactosol-biomag plots") were applied once in an old and a young stand, respectively. The interpretation of the results bases on analyses of variance of all blocks and/or series. Fertilization caused a mean pH-rise of 0.9 units. None of the treatments substantially altered individual and species numbers of the testaceans, while their biomasses were significantly decreased by up to 30% in the upper layer of the bactosol-biomag plots. Likewise, species composition was changed: acidophilic species decreased, while productive acid-intolerant species increased. These shifts are very likely related, apart from the pH-rise in the treated plots, to changes in the numbers and kinds of fungi and bacteria (increased catalase and protease activities, decreased phosphatase and cellulolytic activities). The observations suggest that both fertilizers slightly enhanced decomposition and improved soil conditions. In general, testaceans and soil enzyme activities were influenced more by the bactosol-biomag than by the biomag application, very likely due to the higher amount of organic substance contained in the bactosol-biomag variant.

**Keywords:** Soil protozoa, testacea, Norway spruce forest, organic fertilizer, magnesite fertilizer, soil enzymes.

*Effets de fertilisants organiques enrichis en magnésite sur les thécamoebiens d'une forêt d'épicéas*

### Résumé

Les effets de fertilisants organiques, enrichis en magnésite, sur le peuplement de thécamoebiens du sol d'une forêt d'épicéas en dépérissement ont été étudiés pendant une période de 5 ans, à l'aide d'un protocole d'échantillonnage totalement aléatoire et d'une méthode de comptages directs. L'étude a consisté à appliquer à un moment donné deux types d'amendement, "biomag" (2000 kg ha<sup>-1</sup> d'un mélange composé de 90% de magnésite et 10% de biomasse fongique séchée) et "bactosol-biomag" (3000 kg ha<sup>-1</sup> de biomasse bactérienne séchée ("bactosol") additionnés de 2000 kg ha<sup>-1</sup> du mélange "biomag"). Les deux traitements ont été appliqués sur un site ancien et sur un site récent. Les résultats ont été comparés par des analyses de variance. Chaque traitement a entraîné une augmentation moyenne du pH de la litière de 0.9 unité. Le nombre d'individus et le nombre d'espèces de thécamoebiens n'ont pas été modifiés par l'un ou l'autre traitement. Seule la biomasse du site traité par "bactosol-biomag" a diminué significativement jusqu'à 30% dans la litière. De plus, la composition spécifique du peuplement a été modifiée: l'abondance des espèces acidophiles a diminué tandis que celle des espèces intolérantes au pH acide a augmenté. Ces modifications sont probablement liées, exceptée l'augmentation du pH, aux changements quantitatifs et qualitatifs de la microflore fongique et bactérienne (augmentation de l'activité des catalases et des protéases, diminution de l'activité des phosphatases et de l'activité cellulolytique). Les observations suggèrent que les deux fertilisants ont stimulé légèrement la décomposition et amélioré les conditions du sol. D'une manière générale, l'activité des thécamoebiens et des enzymes du sol a été davantage influencé par l'amendement du type "bactosol-biomag", probablement en raison de sa teneur élevée en matière organique.

**Mots-clés :** Protozoaires du sol, thécamoebiens, forêt de sapins de Norvège, fertilisant organique, magnésite, enzymes du sol.

## INTRODUCTION

A large woodland, the Böhmerwald in Upper Austria, shows marked signs of spruce forest decline, at least partially due to magnesium deficiency (Katzensteiner *et al.*, 1992). Similar damages, which can be attributed to various reasons, have been observed worldwide, particularly in industrialized regions. Fertilization and liming are widely considered as appropriate tools to alleviate or remove those declines associated with nutrient imbalances (e.g. Huettl, 1989). These practices, however, may also cause undesirable impacts, like mineral leaching, mobilization of heavy metals, and partial injury of the soil fauna, especially if fast soluble substances were applied (e.g. Funke, 1991). To minimize such risks private Austrian companies (Biochemie Kundl and Tiroler Magnesit) designed slow release fertilizers: Crude ( $\text{MgCO}_3$ ) and caustic [ $\text{CaMg}(\text{CO}_3)_2 + \text{MgO}$ ] magnesite were chosen to counteract magnesium deficiency and decreasing pH, while added organic material should stimulate soil life. The organic matter comprises dried biomass obtained during penicillin production, *i.e.* hyphae of *Penicillium chrysogenum* and bacteria from the sewage plant purifying the culture medium of the fungus, respectively. Consequently, all by-products of this industrial process are recycled.

In an interdisciplinary study effects of these organically enriched magnesite fertilizers were investigated on soil fauna, soil microflora (Haselwandter and Berreck, unpubl. results), soil chemistry, and wood production (Katzensteiner *et al.*, 1992, and unpubl. results). We studied the soil animals with special reference to the protozoa, since especially testate amoebae not only tolerate but even prefer acid environments and are thus numerous in spruce forests (Schönborn, 1973, 1982, 1986a, b; Foissner, 1987). Consequently, their standing crop, production, and turnover rate are high in raw humus (Meisterfeld, 1980; Schönborn, 1975, 1978, 1982, 1986a, b, 1992). Moreover, they have a rather high species richness, which is a prerequisite for a good indicator group (Weigmann, 1987), *i.e.* they can indicate a wide range of possible effects. Contrary to the ciliates, on which has already been reported (Aescht and Foissner, 1993), testaceans occur also in deeper soil layers. This allows a more detailed examination of treatment effects. Apart from two major abiotic factors, *viz.* pH and soil moisture, some enzymes as a measure of microbial activity, were also studied.

Previous investigations on soil testacea in fertilized and/or limed spruce forests are rare (Rosa, 1974; Stachurska-Hagen, 1980; Wanner, 1991) and do

not meet the statistical requirements explained, for instance, by Hurlbert (1984).

## MATERIALS AND METHODS

### Site description and experimental design

The study area is situated in the Upper Austrian part of the Böhmerwald (Bohemian Forest), close to the Czechoslovakian and Bavarian border (48°50'N, 14°00'E). The average yearly air temperature is 4.5°C; yearly precipitation is about 1000 mm. Two Norway spruce stands (*Picea abies*), both growing on abandoned pastures, were investigated: an 80-y-old "old" stand (site Bärenstein), 1000 m above sea level, and a 40-y-old "young" stand (site Pflegerwiese), 940 m above sea level. Undergrowth in the old stand consists mainly of an association of *Vaccinium*, *Oxalis*, and *Dryopteris*; the young stand almost lacks ground vegetation.

The soil type is podsol to gleyic cambisol on Eisgarner granite (Katzensteiner *et al.*, 1992). The morphology and chemical composition of the soil are different in the two stands: the humus type is moder to raw humus in the old stand and moder in the young stand; the thickness of the organic layer varies from 5-10 cm in the old and 2-7 cm in the young stand; thus mineral soil was frequently encountered in the samples of the latter. In the old stand a 12-18 cm thick A-horizon covers the loamy B-horizon, while in the young stand the A-layer measures only 2-15 cm. The bulk density, which was determined by weighing 6 air-dried replicates (25 × 25 cm) per stand and soil layer, is almost identical in the uppermost litter layer of the two stands, *viz.* 0.15 g cm<sup>-3</sup> in the old and 0.14 g cm<sup>-3</sup> in the young, but quite different at 3-9 cm soil depth, *i.e.* 0.53 and 0.88 g cm<sup>-3</sup>, respectively. The organic layer of the old stand contains 30 000 kg carbon, 1 400 kg nitrogen, 70 kg phosphorous, 100 kg potassium, 90 kg calcium, and 63 kg magnesium per hectare; the respective values in the young stand are 18 500 kg carbon, 900 kg nitrogen, 45 kg phosphorous, 95 kg potassium, 50 kg calcium, and 73 kg magnesium (Katzensteiner, pers. comm.).

The revitalization experiments were planned and conducted by the Institut für Forstökologie, Universität für Bodenkultur, Vienna (for details see Katzensteiner *et al.*, 1992). Two types of fertilizers were designed by Austrian companies: (i) Biomag® (Tiroler Magnesit AG, Hochfilzen) consists of 80% crude magnesite ( $\text{MgCO}_3$ ), 10% caustic magnesite

[CaMg(CO<sub>3</sub>)<sub>2</sub> + MgO] + 10% biosol®; biosol is dried fungal biomass of *Penicillium chrysogenum* produced by Biochemie GmbH, Kundl and is more expensive than bactosol; (ii) Bactosol® (Biochemie GmbH, Kundl) is made of dried bacterial biomass from the sewage plant purifying the culture medium of *P. chrysogenum*. Both fertilizers are free of viable fungi and bacteria and do not contain antibiotics or toxic substances, which has been confirmed by expert evidences. The composition and nutrient equivalents of the fertilizers are given by Aesch and Foissner (1993).

For each stand (i) 2000 kg ha<sup>-1</sup> biomag and (ii) 3000 kg ha<sup>-1</sup> bactosol + 2000 kg ha<sup>-1</sup> biomag were applied once in granular form by hand in June 1987. Treatments were assigned in a randomized block design to 100 m<sup>2</sup> plots with 11 replications (*i.e.* a total of 33 independent 100 m<sup>2</sup> plots) in the old stand and 9 replications (*i.e.* a total of 27 separated 100 m<sup>2</sup> plots) in the young stand (Katzensteiner *et al.*, 1992).

### Sampling and counting procedures

In our study each block contained three 100 m<sup>2</sup> plots (two fertilized, one untreated). Three to six blocks (replicates) were investigated on consecutive days. The sampling dates, the months elapsed since fertilization, and the number of investigated blocks and series are summarized in Table 1.

**Table 1.** – Sampling dates, months elapsed since fertilizer application (3.6.1987 old stand; 4.6-6.6.1987 young stand), and number of blocks (replicates) per treatment, stand, and soil depth.

Series	Sampling dates	Months elapsed	Number of blocks investigated			
			Old stand (0-3 cm)	Young stand (0-3 cm)	Old stand (3-9 cm)	Young stand (3-9 cm)
1	13.07.-25.07.1987	<2	6 <sup>a</sup>	4 <sup>a</sup>	–	–
2	13.10.-24.10.1987	4	6 <sup>a</sup>	6 <sup>a</sup>	–	–
3	11.05.-22.05.1988	12	6 <sup>a</sup>	6 <sup>b</sup>	3 <sup>b</sup>	3 <sup>b</sup>
4	04.10.-15.10.1988	16	6 <sup>b</sup>	6 <sup>b</sup>	–	–
5	11.10.-23.10.1989	29	6 <sup>b</sup>	6 <sup>b</sup>	6 <sup>b</sup>	4 <sup>b</sup>
6	22.10.-02.11.1990	41	4 <sup>b</sup>	4 <sup>b</sup>	–	–
7	02.05.-13.05.1991	47	4 <sup>b</sup>	4 <sup>b</sup>	4 <sup>b</sup>	4 <sup>b</sup>
8	07.07.1992	61	–	–	4 <sup>b</sup>	4 <sup>b</sup>
Sum of blocks			38	36	17	15

<sup>a</sup> 0.0025 g fresh soil was investigated (see method section).

<sup>b</sup> 0.005 g fresh soil was investigated (see method section).

Eight subsamples per plot (*i.e.* 100 m<sup>2</sup>) were taken at random; each subsample comprised an area measuring about 3 × 3 cm collected with a spatula (0-3 cm litter layer) or a steel corer (Ø 3 cm; 3-9 cm soil depth). The subsamples were thoroughly mixed in the laboratory and pooled. Direct counting according to Aesch and Foissner (1992a) was used. Biomass for species was calculated as described in Foissner

(1985). Altogether 318 samples were investigated (3 treatments × 2 stands × 1 or 2 soil depths × number of replicates × number of series; Tab. 1).

At the beginning of the study full (active) and empty (dead) tests (cells) were counted to differentiate the humus type (Foissner, 1985); later only full tests in higher amounts of soil were enumerated to get a more complete species inventory of active testate amoebae (Tab. 1).

### Abiotic factors and soil enzymes

Soil moisture and pH (glass electrode; 0.01 M CaCl<sub>2</sub>) were determined from oven dried litter (50 g about 10 hours at 105°C). Alkaline phosphatase, catalase, cellulolytic ("cellulase") and protease activities were measured from air-dried (about 6 weeks) pooled samples using standard procedures (Hoffmann, 1968; Beck, 1971; Hofmann, 1979; Speier *et al.*, 1980). Enzymes were investigated only in the uppermost litter layer (0-3 cm) because of financial shortage.

### Statistics

The data were examined with a two-way analysis of variance (block and treatment factor). Where necessary, values were square-root or log *t* (*x*+1) transformed to meet the assumptions of this procedure [normality was checked with the Kolmogorov-Smirnov test and homogeneity of variances with the Cochran test (Sachs, 1984)]. If a treatment effect was suggested, pairwise comparisons between the control and each treatment using least significant differences (LSD) were undertaken. The Spearman rank correlation coefficient was calculated according to Sachs (1984). The weighted average ordination technique as described by Tolonen *et al.* (1992) was used to determine the distribution of certain species along environmental gradients.

The Morisita (1959) index and several other indices (Jaccard's, Renkonen's, Bray and Curtis') were used to measure species and abundance similarity. For brevity, only results obtained by the more sharply discriminating Morisita index are shown. Clusters were constructed by the unweighted pair-group method with arithmetic means (UPGMA). The ratio of testaceans with filose and lobose pseudopodia (F/L index) and the weighted coenotic index (WCI) were calculated according to Lüftenegger *et al.* (1985) and Wodarz *et al.* (1992), respectively. Both indices were used as quantitative general estimates of treatment effects. The WCI is a single value that unifies total abundance, dominance structure, species richness, and ecological weightings (habitat and pH preference and position of species in the *r*/*K* continuum). Low index values indicate undisturbed or improved soil conditions.

## RESULTS

318 samples were investigated during a period of five years. To tabulate merely the arithmetic means of all sample series would need extensive space not available. Thus, only mean values of all blocks are documented. However, interesting short-time effects are at least briefly mentioned.

### Abiotic factors and soil enzymes

In the uppermost organic layer (0-3 cm) fertilization increased the pH highly significantly from about 3 to 4.3 in the first year; only in the first two years bactosol-biomag caused a significantly higher pH-rise than biomag fertilization. Five years after fertilization the pH was on average still 0.5-0.7 units higher in the treated plots; the range of 3.0-4.2, however, demonstrates prevailing extreme conditions in the spruce litter. The mean pH values of all blocks significantly differ from the control plots by about 0.9 units (Tab. 2). In the deeper soil layer (3-9 cm) a significant increase of pH in the fertilized plots became visible only two and five years after fertilization in the old and young stand, respectively. The delayed reaction in the young stand is apparently caused by the lack of a distinct humus layer, *i.e.* the litter lies directly on the loamy mineral horizon. The mean pH-values of all blocks differ by 0.3 (old stand) and 0.1 units (young stand; Tab. 3).

Effects of fertilizers on the soil moisture were recognizable only during the first three months after fertilization; obviously the fertilizer granulate retained water to a greater extent than the spruce litter.

Fertilization usually increased the catalase and protease activity and decreased the phosphatase and cellulolytic activity; these tendencies were statistically significant only in half of the samples (Tab. 2). Five years after fertilization significant changes in enzyme activities were, however, still measured in the bactosol-biomag treatment of the old (increased catalase) and young stand (decreased phosphatase) and the biomag treatment of the old (increased protease) and young stand (decreased cellulase; not tabulated).

### Testaceans

#### Abundance, biomass and species number

In the untreated spruce litter of the old stand abundances of active testaceans range from 2430 to 44252 and 6215 to 20449 individuals per g dry mass at 0-3 cm and 3-9 cm soil depth, respectively; the corresponding values in the young stand are 10841 to 52182 and 1692 to 19723. These extreme values (not tabulated) indicate a rather unequal horizontal distribution and considerable temporal fluctuations. In a single case (c 10/89), for instance, 380 active individuals (mostly *Corythion dubium*) were counted in 5 mg fresh soil. The total mean individual number of all blocks in the 0-3 cm layer of the old stand is only about two thirds of the young one, whereas the mean biomass is higher due to some large species, such as *Trigonopyxis arcuata* (Tab. 2). At 3-9 cm soil depth the total mean abundance of the old stand is twice as high as in the young one; the mean biomass even three times (Tab. 3). These relations tend to disappear if values are transformed to square meters. The species

Table 2. - Effects of fertilizers on abiotic factors, enzymes, and testaceans in the litter layer (0-3 cm) of an old and a young spruce forest stand. <sup>a</sup>

Parameters	n	Old stand			Young stand		
		Control	Biomag	Bactosol + biomag	Control	Biomag	Bactosol + biomag
Soil moisture (% of dm) <sup>b</sup>	38/36 <sup>d</sup>	59.8	59.5	62.1	58.9	60.0	60.5
pH (CaCl <sub>2</sub> ) <sup>b</sup>	38/36 <sup>d</sup>	2.7	3.6 ***	3.9 ***	3.0	3.8 ***	3.9 ***
Catalase (mg O <sub>2</sub> g <sup>-1</sup> dm.3 min <sup>-1</sup> ) <sup>b</sup>	6	22.2	26.1 *	26.6 *	21.9	22.9	25.1 *
Phosphatase (mg phenol g <sup>-1</sup> dm.3 h <sup>-1</sup> ) <sup>b</sup>	6	1.8	1.4 *	1.3 **	1.3	1.1	1.1
Protease (μg tyrosine g <sup>-1</sup> dm.2 h <sup>-1</sup> ) <sup>b</sup>	6	1.3	1.3	1.6	1.2	1.5 *	1.4 *
Cellulase (mg glucose g <sup>-1</sup> dm.23 h <sup>-1</sup> ) <sup>b</sup>	6	39.5	35.2 *	33.7 **	36.5	36.0	30.4
Individuals (g <sup>-1</sup> dm) <sup>c</sup>	38/36 <sup>d</sup>	21830	26455	23280	30804	26527	29112
Biomass (μg g <sup>-1</sup> dm) <sup>c</sup>	38/36 <sup>d</sup>	586	510	456 *	478	412	326 *
Total number of species	7	21	20	20	20	21	19
F/L index (abundance)	7	71	69	67	82	72	78
F/L index (species number)	7	46	50	46	61	58	63
Weighted coenotic index	7	3.1	3.7	3.3	0.9	2.3	1.3

<sup>a</sup> Arithmetic means of all blocks or series sampled (=n) per treatment during the investigation period are shown and compared by a two-way analysis of variance and by least significant difference.

<sup>b</sup> Values may be inconsistent with those in Table 3 of Aesch and Foissner (1993) due to different sampling dates.

<sup>c</sup> Multiply with 4154 and 4452 to get values per square meter in the old and young stand, respectively.

<sup>d</sup> The first value refers to the old, the second to the young stand.

Abbreviations: dm = dry mass of soil; F/L = filose/lobose ratio; n = number of samples; \* = P ≤ 0.1; \* = P ≤ 0.05; \*\* = P ≤ 0.01; \*\*\* = P ≤ 0.001.

Table 3. – Effects of fertilizers on abiotic factors and testaceans at 3-9 cm soil depth of an old and a young spruce forest stand. <sup>a</sup>

Parameters	n	Old stand			Young stand		
		Control	Biomag	Bactosol + biomag	Control	Biomag	Bactosol + biomag
Soil moisture (% of dm)	17/15 <sup>c</sup>	61.3	57.8 *	61.0	45.1	45.9	46.6
pH (CaCl <sub>2</sub> )	17/15 <sup>c</sup>	2.7	3.0 ***	3.0 ***	3.3	3.4 *	3.4 *
Individuals (g <sup>-1</sup> dm) <sup>b</sup>	17/15 <sup>c</sup>	11990	11452	15277	6114	4000	5169
Biomass (μg g <sup>-1</sup> dm) <sup>b</sup>	17/15 <sup>c</sup>	284	184	246	73	74	69
Total number of species	4	13	15	13	11	10	11
F/L index (abundance)	4	52	54	52	88	84	84
F/L index (species number)	4	42	51	49	72	60	60
Weighted coenotic index	4	67.3	19.2	44.1	506.1	162.7	215.7

<sup>a</sup> Arithmetic means of all blocks or series sampled (=n) per treatment during the investigation period are shown and compared by a two-way analysis of variance and by least significant difference.

<sup>b</sup> Multiply with 31 771 and 52 620 to get values per square meter in the old and young stand, respectively.

<sup>c</sup> The first value refers to the old, the second to the young stand.

Abbreviations: dm = dry mass of soil; F/L = filose/lobose ratio; n = number of samples; \* = P ≤ 0.05; \*\*\* = P ≤ 0.001.

numbers are almost identical in both stands (Tabs. 2, 3).

The individual numbers in the uppermost litter layer (0-3 cm) differed significantly once among treatments in the old stand and twice in the young stand: compared to the control, individual numbers increased by 42% and 52% in the treated plots of the old stand (M, O). In contrast, individual numbers decreased in the young stand by 19% to 40% in the bactosol-biomag (o) and biomag plots (m), respectively (figs. 1, 2). At 3-9 cm soil depth significant fertilizer effects appeared four years (young stand) and five years (old stand) after fertilization: compared to the controls, individual numbers were decreased by 42%, 35%, and 71% in the biomag (M, m) and bactosol-biomag plots (o) (figs. 3, 4). The total mean abundances of all blocks are, however, quite similar in both stands and soil depths: the differences ranging from 4% to 35% are statistically insignificant (Tabs. 2, 3).

Statistically significant differences in testacean biomasses were observed one month and three years after fertilization in the 0-3 cm litter layer of the young stand (not tabulated). At these dates biomasses were decreased by 55% and 42% in the bactosol-biomag (o) and biomag plots (m), respectively. Similar, but statistically insignificant differences were also found in most series of the old stand and at 3-9 cm soil depth of both stands. However, the total mean biomasses of all blocks are significantly lower in the bactosol-biomag plots than in the controls in both stands (Tab. 2). This contrasts with the increased abundances in this treatment (compare both soil depths in O; Tabs. 2, 3) and results from shifts in the community structure, i.e. tiny species, such as *Cryptodiffugia oviformis*, increased, while voluminous species, like *Trigonopyxis arcua*, decreased.

The quotient of empty and full tests ranges from 9-12 in the old and is about 6 in the young stand, corresponding to mor and moder, respectively

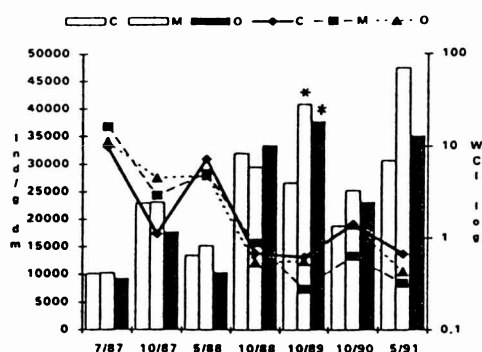
(Schönborn, 1973); this ratio was insignificantly changed during the first year after fertilization (later only active cells were counted; see method section). Similarly, no treatment effects were found in three replicates per treatment at 15-30 cm soil depth four years after fertilization. All these values are thus not shown.

### Community structure

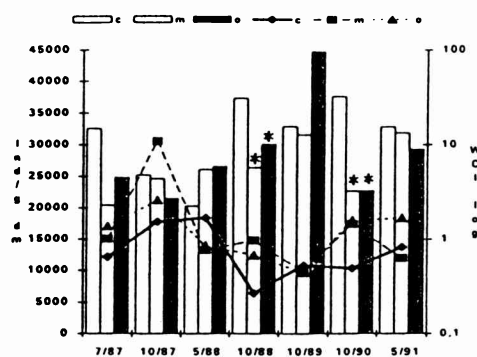
Altogether 56 species and varieties were found (Tab. 4). In terms of abundance and frequency, the control plots of the 0-3 cm litter layer are dominated by *Corythion dubium* and *Trinema lineare*, tiny bacterial feeders which constitute more than 60% of the total individuals in both sites (Tab. 4). In the remaining portion, rather pronounced differences occur between the two stands: *Hyalosphenia subflava*, *Phryganella acropodia*, *Schoenbornia humicola*, and *Trigonopyxis arcua* are more abundant in the old than in the young stand, while the contrary applies to *Centropyxis aerophila sphagnicola*, *Cryptodiffugia oviformis*, and *Trinema* spp. (Tab. 4). In the control plots of the 3-9 cm layer of the old stand *Schoenbornia humicola* and *S. viscidula* are more abundant than the aerophilic *Corythion dubium*, which dominates the upper layer; in the young stand *Trinema complanatum* and *T. lineare* are most abundant. *Hyalosphenia subflava*, *Nebela militaris*, *Phryganella acropodia*, and *Trigonopyxis arcua*, which are quite numerous in the old stand, were never found in the control plots of the young stand (Tab. 4).

Fertilization moderately changed the community structure: in both treatments *Corythion dubium*, *Euglypha strigosa*, *Nebela tincta*, *Schoenbornia humicola*, and *Trigonopyxis arcua* usually showed decreased numbers and dominances, while those of *Cryptodiffugia oviformis*, *Phryganella acropodia*, *Plagiopyxis declivis*, *Schoenbornia viscidula*, and *Trinema* spp., particularly *T. lineare* and *T. complanatum*, often

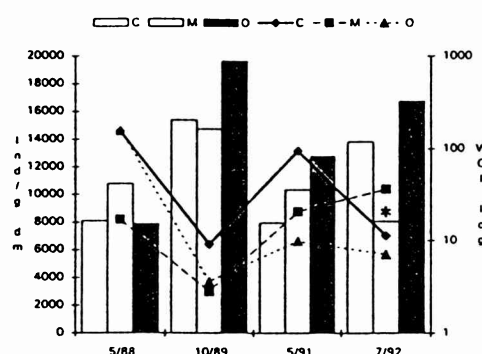
1 Old stand (0-3 cm)



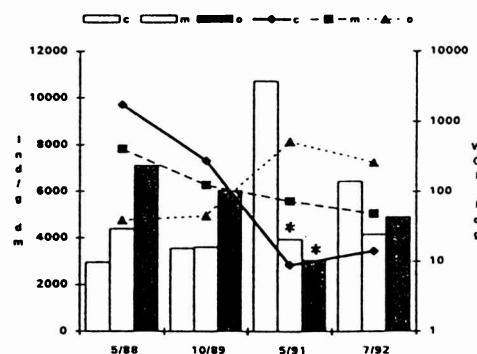
2 Young stand (0-3 cm)



3 Old stand (3-9 cm)



4 Young stand (3-9 cm)



Figures 1-4. – Abundances (left axis, columns) and weighted coenotic index (WCI; right axis, lines) of active testate amoebae at 0-3 cm and 3-9 cm soil depth of an old and a young spruce forest stand. An asterisk indicates a significant difference to the control. C, c = control; dm = dry mass of soil; Ind = individual number; log = logarithmic scale; M, m = "mineral" biomag treatment; O, o = "organic" bactosol-biomag treatment.

increased (Tab. 5; note also changes in frequency). However, *Corythion dubium* and *Trinema* spp. are still predominant in all plots, but *Cryptodifflugia oviformis* additionally became eudominant (> 10%) in the treated plots. Some species showed inverse responses, e.g. *Assulina* and *Centropyxis* spp. increased in the treated plots of the old stand, but decreased in the young one (Tab. 5). These changes are frequently statistically significant, particularly in the uppermost litter layer of the old stand, and are also recognizable in the similarity values of the Morisita index (figs. 5-8). In the old stand fertilizer effects obviously still persist five years after fertilization (figs. 5, 6).

The high F/L indices evidently show that filose testaceans dominate in the acid spruce litter regarding individuals and species, while the lobose and reticulolobose species occur sparsely (Tabs. 2, 3). The lobose testacean abundance ratio is slightly increased in the treated plots of both stands. A higher number of lobose species was only found in the deeper soil layer of the young stand, which may be related to the soil type (Tab. 3).

The weighted coenotic index (WCI) values slightly increased in the 0-3 cm litter layer of the treated plots

(Tab. 2); these changes are already recognizable during the first four months after fertilization (figs. 1, 2). This results from the increased dominances of *Trinema* spp., which are more *r*-selected and euryoecious than the acidophilic and aerophilic *Corythion dubium* [cp. Aescht and Foissner (1992b) and Wodarz *et al.* (1992) regarding the ecological weightings]. In the deeper soil layer (3-9 cm) individual and species numbers are comparatively low resulting in much higher WCI values than in the upper layer (Tab. 3). The strongly decreased mean index values in the fertilized plots, particularly in the biomag treatment, may be accounted to increased numbers of acid-intolerant and/or intermediate species, such as *Cryptodifflugia oviformis*, *Phryganella acropodia*, and *Plagiopyxis declivis*.

#### Correlations of community parameters with abiotic factors and soil enzymes

The total abundances, biomasses, and species numbers of the active testaceans and the abundances of the relevant species show correlations with nearly

**Table 4.** – Active testacean species and their mean individual dominances (%) for number of replicates see *Tab. 1*) at 0-3 cm and 3-9 cm soil depth of an old and a young spruce forest stand.

Species <sup>a</sup>	0-3 cm						3-9 cm					
	C	M	O	c	m	o	C	M	O	c	m	o
<i>Arcella arenaria compressa</i>	–	+	–	+	+	+	–	+	–	–	+	–
<i>Assulina muscorum</i>	+	+	+	+	+	+	–	–	+	–	+	–
<i>Assulina seminulum</i>	+	–	+	+	+	–	–	+	–	–	–	–
<i>Centropyxis aerophila</i>	–	+	+	+	+	+	+	–	–	–	–	+
<i>Centropyxis aerophila sphagnicola</i>	+	+	+	2.1	+	+	–	–	–	+	+	1.0
<i>Centropyxis laevigata</i>	–	+	–	–	–	–	–	–	–	–	–	–
<i>Centropyxis orbicularis</i>	+	+	–	–	–	–	–	–	–	–	–	–
<i>Centropyxis sylvatica</i>	+	+	+	+	+	+	–	–	–	–	–	–
<i>Corythion asperulum</i>	+	1.1	+	1.8	+	+	–	–	–	–	–	–
<i>Corythion dubium</i>	40.0	25.4	20.1	40.6	27.4	26.6	7.5	3.0	4.2	11.8	10.6	7.3
<i>Cryptodiffugia compressa</i>	–	–	–	–	+	–	–	–	+	–	–	–
<i>Cryptodiffugia oviformis</i>	4.3	14.0	12.8	6.9	16.1	14.2	–	7.6	6.4	2.8	3.8	2.9
<i>Cyclopyxis eurystoma</i>	+	+	+	–	+	+	+	–	–	–	–	–
<i>Diffugia lucida</i>	+	–	+	–	–	–	–	–	–	–	–	–
<i>Diffugia</i> sp.	+	+	+	–	–	–	–	–	–	–	–	–
<i>Edaphonobiotus campascoloides</i>	+	+	–	–	+	–	+	+	+	–	–	–
<i>Euglypha ciliata</i>	+	–	–	+	–	–	–	+	–	–	–	–
<i>Euglypha compressa</i>	1.1	+	+	+	+	+	–	–	+	–	–	–
<i>Euglypha compressa glabra</i>	+	–	–	+	+	+	+	–	–	+	–	–
<i>Euglypha denticulata</i>	+	+	+	+	+	+	–	+	–	–	–	–
<i>Euglypha laevis</i>	+	+	+	+	+	+	+	–	–	–	–	–
<i>Euglypha rotunda</i>	2.4	2.5	2.5	2.3	1.2	1.8	2.4	3.4	3.0	5.6	3.9	8.8
<i>Euglypha rotunda minor</i>	–	+	+	+	+	+	–	–	–	–	–	–
<i>Euglypha strigosa</i>	3.5	2.4	1.1	2.8	1.7	1.0	–	+	–	1.3	1.9	–
<i>Euglypha strigosa glabra</i>	–	–	–	–	+	–	+	–	–	–	+	–
<i>Euglypha</i> sp.	–	–	–	+	+	+	–	–	–	–	–	–
<i>Heleopera petricola</i>	+	+	+	+	+	–	+	+	–	–	–	–
<i>Heleopera petricola humicola</i>	+	+	–	–	–	–	–	–	–	–	–	–
<i>Heleopera</i> sp.	+	–	–	–	–	–	–	–	–	–	–	–
<i>Heleopera sylvatica</i>	+	–	+	+	–	–	–	–	+	–	–	–
<i>Hyalosphenia subflava</i>	1.4	+	1.2	+	–	–	1.8	+	1.2	–	–	–
<i>Nebela collaris</i>	+	+	+	+	+	–	+	–	–	–	–	–
<i>Nebela lageniformis</i>	–	–	–	–	+	–	–	–	–	–	–	–
<i>Nebela militaris</i>	2.4	+	+	+	–	–	1.3	+	+	–	–	–
<i>Nebela tinctoria parvula</i>	4.3	1.7	2.1	3.0	1.3	1.0	+	+	–	+	1.3	–
<i>Nebela tubulata</i>	–	–	–	–	–	–	+	–	–	–	–	–
<i>Phryganella acropodia</i>	2.7	3.9	4.5	+	1.1	+	4.5	5.2	9.3	–	1.3	–
<i>Phryganella paradoxa</i>	–	+	+	–	–	–	–	+	–	–	–	+
<i>Plagiopyxis callida</i>	+	–	–	–	+	+	–	+	–	+	1.3	–
<i>Plagiopyxis declivis</i>	+	2.3	1.6	+	1.1	+	+	+	1.1	+	4.8	1.6
<i>Plagiopyxis minuta</i>	+	–	+	–	–	–	–	–	–	–	+	–
<i>Pseudodiffugia fascicularis</i>	+	+	+	+	–	+	+	+	+	–	–	–
<i>Pseudodiffugia gracilis terricola</i>	+	+	+	+	+	–	–	–	+	–	–	–
<i>Pseudodiffugia</i> sp.	–	–	–	+	–	–	–	–	–	–	–	–
<i>Schoenbornia humicola</i>	6.4	4.0	4.4	1.5	1.6	1.2	23.7	18.7	16.6	3.7	+	2.4
<i>Schoenbornia viscicola</i>	1.8	3.2	3.5	1.7	2.0	1.9	13.0	9.2	10.5	3.7	1.3	4.0
<i>Tracheleuglypha dentata</i>	–	1.4	1.7	+	+	+	–	+	–	–	–	+
<i>Trachelocorythion pulchellum</i>	+	1.5	+	+	+	2.1	+	2.1	1.2	2.3	2.5	4.1
<i>Trigonopyxis arcuata</i>	1.5	+	+	+	+	+	2.2	1.0	1.0	–	–	+
<i>Trigonopyxis minuta</i>	–	+	–	–	–	–	–	–	–	–	–	–
<i>Trinema complanatum</i>	4.7	7.9	7.7	6.4	7.5	9.5	7.5	7.9	6.1	12.5	10.7	12.9
<i>Trinema enchelys</i>	+	+	1.1	1.1	1.5	1.9	–	–	+	1.5	1.9	3.4
<i>Trinema lineare</i>	16.1	21.8	28.3	20.0	25.9	29.0	29.8	34.2	37.1	48.2	50.0	48.7
<i>Trinema penardi</i>	+	+	+	+	1.1	1.3	–	–	–	+	+	–
<i>Valkanovia delicatula</i>	–	–	+	–	–	–	–	–	–	+	–	–
<i>Valkanovia elegans</i>	+	+	+	+	+	+	1.0	1.5	–	2.2	–	+

<sup>a</sup> Authors of species can be found in Aesch and Foissner (1989).

Abbreviations: C=control, old stand; c=control, young stand; M="mineral" biomag treatment, old stand; m="mineral" biomag treatment, young stand; n=number of blocks; O="organic" bactosol-biomag treatment, old stand; o="organic" bactosol-biomag treatment, young stand. + = dominance &lt; 1%; – = not found.

**Table 5.** – Abundance, individual dominance, and frequency of relevant testacean species (in alphabetical order) in the litter layer (0–3 cm) of an old and a young spruce forest stand.<sup>a</sup>

Species		Old stand			Young stand		
		Control	Biomag	Bactosol + biomag	Control	Biomag	Bactosol + biomag
<i>Assulina</i> spp.	Ind.	55	168	180	359	187 *	264
	ID%	0.3	0.6	0.8	1.1	0.7	0.9
	BD%	0.0	0.4	0.4	0.6	0.5	0.6
	F%	7.9	18.4	15.8	33.3	19.4	38.9
<i>Centropyxis</i> spp.	Ind.	96	311	212	771	272	309
	ID%	0.4	1.2	0.9	2.3	1.0	1.0
	BD%	1.0	3.5	2.6	6.9	3.2	4.6
	F%	10.5	28.9	26.3	33.3	33.3	47.2
<i>Corythion dubium</i>	Ind.	8724	6726 *	4857 ***	13575	7798 **	8100 **
	ID%	40.0	25.4	20.1	40.6	27.4	26.6
	BD%	7.5	6.7	5.3	14.2	9.5	12.6
	F%	97.4	100.0	100.0	97.2	100.0	97.2
<i>Cryptodiffugia oviformis</i>	Ind.	935	3698 **	3097 *	2305	4587 *	4337 *
	ID%	4.3	14.0	12.8	6.9	16.1	14.2
	BD%	0.2	1.0	0.9	0.6	1.5	1.8
	F%	50.0	71.1	65.8	86.1	97.2	88.9
<i>Euglypha</i> spp.	Ind.	1681	1608	1262	1975	1283 **	1244 **
	ID%	7.7	6.1	5.2	5.9	4.5	4.1
	BD%	11.6	12.0	8.8	16.7	11.9	12.3
	F%	81.6	78.9	71.1	94.4	80.6	77.8
<i>Nebela</i> spp.	Ind.	1628	734 **	756 **	1134	387 **	280 **
	ID%	7.5	2.8	3.1	3.4	1.4	0.9
	BD%	26.6	16.3	17.3	28.4	11.7	10.4
	F%	86.8	57.9	50.0	80.6	44.4	41.7
<i>Phryganella acropodia</i>	Ind.	587	1028 *	1052 *	115	320 *	222
	ID%	2.7	3.9	4.4	0.3	1.1	0.7
	BD%	3.1	6.1	7.0	0.8	2.4	2.1
	F%	44.7	63.2	68.4	13.9	38.9	27.8
<i>Plagiopyxis declivis</i>	Ind.	186	613 **	379	175	326	213
	ID%	0.8	2.3	1.6	0.5	1.1	0.7
	BD%	2.9	11.4	7.9	3.4	7.5	6.1
	F%	21.1	44.7	39.5	27.8	33.3	25.0
<i>Schoenbornia humicola</i>	Ind.	1397	1063	1071	506	442	376
	ID%	6.4	4.0	4.4	1.5	1.6	1.2
	BD%	2.4	2.2	2.4	1.1	1.0	1.2
	F%	68.4	63.2	68.4	55.6	41.7	30.6
<i>Schoenbornia viscidula</i>	Ind.	389	838 *	855 *	564	561	582
	ID%	1.8	3.2	3.5	1.7	2.0	1.9
	BD%	0.2	0.6	0.7	0.4	0.5	0.6
	F%	34.2	52.6	42.1	36.1	38.9	41.7
<i>Tracheleuglypha dentata</i>	Ind.	0	357 *	415 *	88	98	129
	ID%	0	1.4	1.7	0.3	0.3	0.4
	BD%	0	1.0	1.1	0.2	0.2	0.6
	F%	0	23.7	26.3	13.9	16.7	19.4
<i>Trigonopyxis arcuata</i>	Ind.	330	192	166	71	166	13
	ID%	1.5	0.7	0.7	0.2	0.6	0.0
	BD%	29.0	18.4	18.6	7.5	20.6	2.1
	F%	42.1	28.9	21.1	11.1	16.7	2.8
<i>Trinema</i> spp.	Ind.	4635	8263 **	9041 **	8663	9535	12035 **
	ID%	21.2	31.2	37.4	25.9	33.5	39.5
	BD%	7.0	16.7	18.6	17.8	25.7	42.6
	F%	92.1	100.0	97.4	100.0	100.0	100.0

<sup>a</sup> Arithmetic means of all blocks per treatment during the investigation period are shown and compared by a two-way analysis of variance and by least significant difference.

Abbreviations: BD = biomass dominance; Ind. = individuals g<sup>-1</sup> dry mass of soil (median); F = frequency (%); ID = individual dominance; \* = P ≤ 0.1; \* = P ≤ 0.05; \*\* = P ≤ 0.01; \*\*\* = P ≤ 0.001.



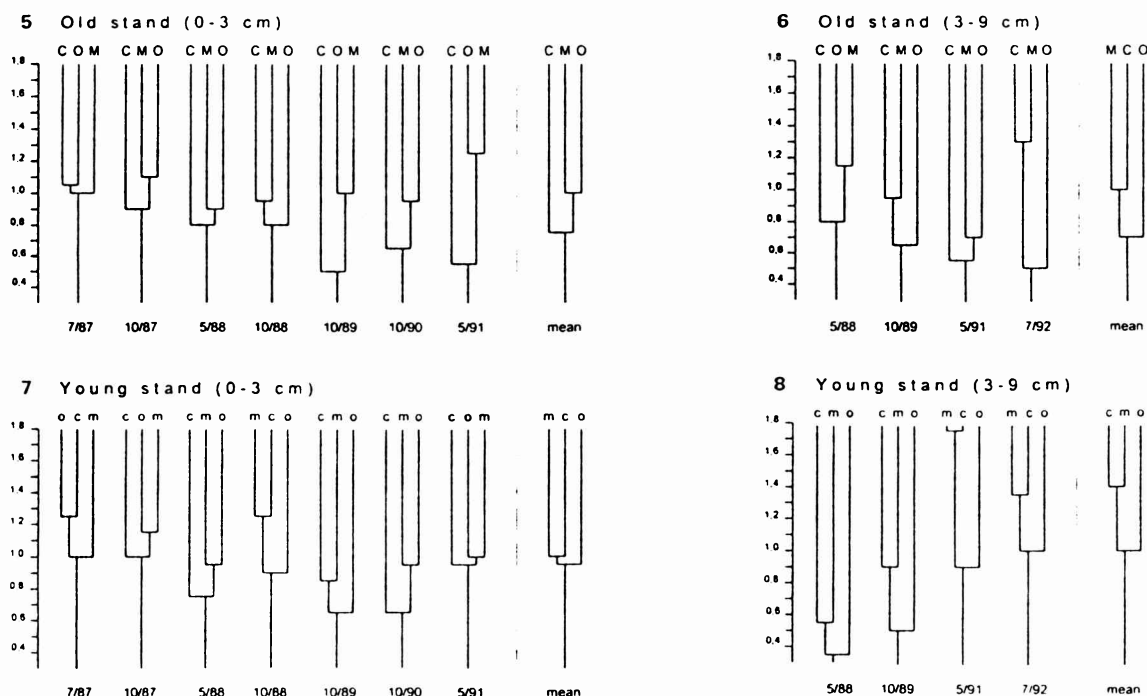
**Table 6.** – Spearman rank correlations between testaceans and some soil variables (0-3 cm). First line = old stand; second line = young stand.

Parameter	pH <i>n</i> = 21	Moisture <i>n</i> = 21	Catalase <i>n</i> = 18	Phosphatase <i>n</i> = 18	Protease <i>n</i> = 18	Cellulase <i>n</i> = 18
Total abundance	-0.1821 -0.3477 *	0.7727 *** 0.3497 *	0.6904 *** 0.4974 *	0.6832 ** 0.5578 **	0.2508 0.4200 *	-0.2358 0.2967
Total biomass	-0.5276 ** -0.5471 **	0.6532 *** 0.3555 *	0.3911 * 0.2291	0.7874 *** 0.5258 *	0.1166 -0.0299	-0.1533 0.1729
Total species number	-0.3253 * -0.2818	0.6094 ** 0.6432 **	0.5728 ** 0.4649 *	0.7079 *** 0.6775 **	0.3705 * 0.3003	0.4499 * -0.1058

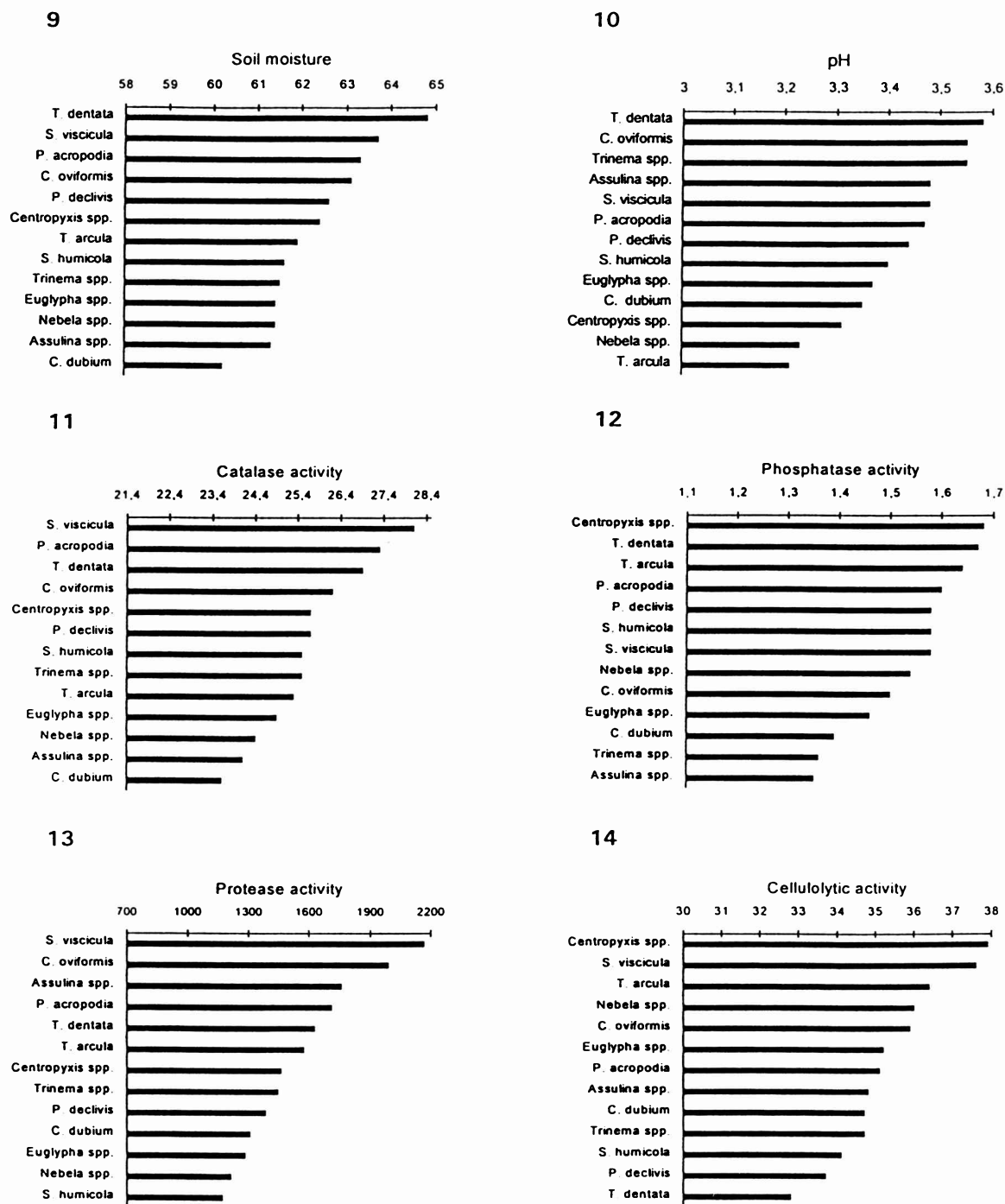
Abbreviations: *n* = number of arithmetic means of the series; \* =  $P \leq 0.1$ ; \* =  $P \leq 0.05$ ; \*\* =  $P \leq 0.01$ ; \*\*\* =  $P \leq 0.001$ .

all of the investigated abiotic factors and enzyme activities (Tabs. 6, 7). A total of 192 correlation coefficients (96 parameter pairs in both stands) were calculated, 106 of these coefficients were positively or negatively significant: 25 with soil moisture and alkaline phosphatase, respectively; 22 with catalase, 15 with protease, 12 with pH, and 7 with cellulase. Two thirds of all correlations could only be found in the old or the young stand. However, only one of the 96 correlated pairs, namely the pH with *Trinema* spp., was inconsistent, i.e. positive (+0.705;  $P \leq 0.05$ ) in the old, but negative (-0.306;  $P \leq 0.1$ ) in the young stand. Interestingly, correlations between the dominant species, particularly *Corythion dubium*, and the parameters investigated are comparatively weak (Tab. 7). Significant correlations with pH are usually negative, while those with soil moisture and enzyme activities are mostly positive.

The weighted averages provide additional information concerning the preferences of the relevant species for certain factor levels (figs. 9-14). For instance, the ranking scale based on the median of the weighted averages of soil moisture indicates that *Corythion dubium*, *Euglypha* spp., *Nebela* spp., *Schoenbornia humicola*, and *Trigonopyxis arcuata* tolerate drier conditions than the other taxa (Tab. 7, fig. 9). *Tracheleuglypha dentata* apparently needs a rather high soil moisture and phosphatase activity, but a low cellulolytic activity (figs. 9, 12, 14). *Trigonopyxis arcuata*, *Corythion* spp., *Nebela* spp., and *Euglypha* spp. prefer acidic conditions (Tab. 7, fig. 10), which is in accordance with data in the literature (Schönborn, 1973; Foissner, 1987; Aesch and Foissner, 1989). Although positive correlations with pH are nearly absent, most individuals of *Cryptodiffugia oviformis*, *Phryganella acropodia*, *Schoenbornia viscidula*, *Tracheleuglypha dentata*, and



**Figures 5-8.** – Species and abundance similarity (Morisita index) of active soil testaceans at 0-3 cm and 3-9 cm soil depth of an old and a young spruce forest stand. C, c = control; M, m = “mineral” biomass treatment; O, o = “organic” bactosol-biomass treatment.



Figures 9-14. – Distribution of testacean species along the weighted average gradient of different environmental variables. See Table 2 for the units of the parameters and Table 4 for the genus abbreviations.

*Trinema* spp. were found at higher pH values (Tab. 7, fig. 10). In contrast to the acidophilic testaceans, less acid-tolerant species show peaks at higher catalase and protease activities (figs. 11, 13). Interestingly, filose testaceans with idiosomes have their peak

abundances largely at low phosphatase levels, while lobose species with xenosomes prefer higher activities of this enzyme (fig. 12). The weakest correlations were found with the cellulolytic activity (Tab. 7; fig. 14).

**Table 7.** – Relations of relevant testacean species to some environmental variables according to Spearman rank correlations (first sign) and weighted average ordination (second sign; cp. figs. 9-14).

Parameter	Moisture	Phosphatase	pH	Catalase	Protease	Cellulase
<i>Corythion dubium</i>	0 –	+-	--	--	0 –	0 –
<i>Euglypha</i> spp.	+-	+-	--	+-	0 –	0 –
<i>Schoenbornia humicola</i>	+-	++	0 –	+-	--	--
<i>Nebela</i> spp.	+-	++	--	0 –	--	0 +
<i>Trigonopyxis arcula</i>	+-	++	--	0 –	0 –	++
<i>Schoenbornia viscidula</i>	++	++	0 +	++	++	++
<i>Cryptodiffugia oviformis</i>	++	+-	0 +	++	++	0 +
<i>Phryganella acropodia</i>	++	++	0 +	++	++	0 –
<i>Tracheleuglypha dentata</i>	++	++	++	++	+-	0 –
<i>Trinema</i> spp.	? –	+-	++	+-	0 –	--
<i>Plagiopyxis declivis</i>	+-	++	0 +	+-	0 –	0 –
<i>Assulina</i> spp.	+-	0 –	0 +	+-	++	+-
<i>Centropyxis</i> spp.	+-	++	0 –	+-	+-	++

Frames: Groups of species are recognizable according to the methods used.

Abbreviations: 0 = correlation coefficient not significant; + = significant positive correlation (first sign in columns) or > the median of the ranking scale based on the weighted averages of the parameters for the species (second sign in columns); – = significant negative correlation (first sign) or ≤ the median of the ranking scale based on the weighted averages of the parameters for the species (second sign); ? = inconsistent correlation, i.e. positive in the old, negative in the young stand.

## DISCUSSION

### Testacean communities in spruce forest litters

The individual numbers and biomasses of testaceans calculated per square meter (see footnote in Tabs. 2, 3) in our sites are at the upper end of the scale known from the literature. They are quite similar to those found by Petz and Foissner (1988) in neighbouring sites and by Volz (1967) in pine forest soils of Germany. The numbers given by Meisterfeld (1980), Schönborn (1986b), and Wanner (1991) are much lower (about one quarter), possibly partially due to differences in the soil depths sampled and humus types investigated. Meisterfeld took only few samples in May. Taking into account the considerable temporal fluctuation of testaceans (figs. 1, 2; Couteaux, 1976; Foissner, 1985; Tolonen *et al.*, 1992), his values may be underestimates.

30 to 70 testacean taxa can be expected in certain sites of spruce forests in Central Europe, depending on the species and subspecies recognized (cp. Rosa, 1957, 1974; Chardez, 1962; Schönborn, 1973, 1986b; Lousier, 1975; Couteaux, 1976; Chardez and Lambert, 1981; Rauenbusch, 1987; Petz *et al.*, 1988; Wanner, 1991). However, a total species list compiled from the papers mentioned above amounts to 146 taxa including 31 subspecies. This number is in the same order of magnitude known from other biotopes, especially of beech forests and fens and bogs (Bonnet, 1964; Chardez, 1962; Couteaux, 1976; Meisterfeld, 1977; Foissner *et al.*, 1985; Foissner and Peer, 1985).

The species composition in spruce litter agrees quite well in the papers cited above and the following species may be considered, in terms of frequency,

as characteristic: *Assulina* spp., *Corythion* spp., *Centropyxis aerophila*, *C. orbicularis*, *Cryptodiffugia oviformis*, *Euglypha ciliata*, *E. laevis*, *E. rotunda*, *E. strigosa*, *Hyalosphenia subflava*, *Nebela militaris*, *N. tincta*, *Phryganella acropodia*, *Plagiopyxis callida*, *P. declivis*, *Schoenbornia humicola*, *S. viscidula*, *Tracheleuglypha dentata*, *Trigonopyxis arcula*, and *Trinema* spp. Some of these species, such as *Centropyxis aerophila*, *Euglypha laevis*, *Phryganella acropodia*, *Trinema lineare* and *T. complanatum*, frequently occur also in other biotopes.

The dominant testaceans species are rather different in European coniferous forests: *Euglyphidion enigmaticum* (a species rarely encountered by other authors; possibly a misidentified *Schoenbornia humicola*) and *Phryganella acropodia* dominate in French forests (Couteaux, 1976), while in Southern Germany *Trinema lineare* or *Schoenbornia humicola* or *Hyalosphenia subflava* dominate (Wanner, 1991). In our forest *H. subflava* is only recedent or subrecedent, whereas *Corythion dubium* is a major component in the organic layer (Tab. 4). This agrees with results by Schönborn (1986b) from spruce forests of Thuringia; however, in his sites the second dominant species was *Assulina muscorum*, which plays only a minor role in our forest and the papers cited above. Similar differences in dominant species also occur in alpine soils of France and Austria (Bonnet, 1964; Foissner and Adam, 1981).

Obviously, the species number in various biotopes is less variable than the individual numbers and the dominant species, which even differ considerably in the same biotope type. The reasons are unknown. The testacean community of our spruce forests corresponds

to the *Corythion dubii* alliance established by Bonnet (1961) for acid soils.

### Relations to abiotic factors and soil enzymes

Numerous environmental factors have been argued to influence testacean populations significantly, e.g. soil moisture, pH, temperature, soil pore dimensions, availability of minerals for shell construction, and food density as well as quality (Bonnet, 1964; Martin, 1971; Lousier, 1974; Couteaux, 1976; Meisterfeld, 1977; Laminger, 1978; Bunescu, 1979; Foissner, 1987; Petz and Foissner, 1989b). In our study, nearly all species show positive correlations with the soil moisture, confirming that this is a key factor (Tab. 7). However, *Corythion dubium* prefers or at least tolerates comparatively dry conditions as indicated by the species ranking based on the weighted averages (fig. 9) and the data by Bonnet (1964), Schönborn (1983), and Smith (1973), who even found that *C. dubium* is limited by high moisture in moss peats and grassland soils of Signy Island, Antarctica.

Rather weak correlations were found with the pH in this and other studies (Lousier, 1975; Bunescu, 1979; Wanner, 1991). However, the weighted averages reveal a distinct separation between acidophilic species, especially *Corythion dubium*, *Nebela* spp., and *Trigonopyxis arcuata*, well known as indicators for acid humus (Schönborn, 1973; Foissner, 1985, 1987; Schönborn *et al.*, 1987), and less acid-tolerant species, i.e. *Cryptodiffugia oviformis* and *Trinema* spp. (Tab. 7, fig. 10).

Protease and cellulase are produced mainly by fungi (for references see Aescht and Foissner, 1993). Remarkably, some acidophilic species, viz. *Corythion dubium*, *Euglypha* spp., and *Schoenbornia humicola*, showed lower individual numbers at higher levels of these enzymes, while the weighted averages of *S. viscidula* and *Cryptodiffugia oviformis* showed peaks of abundance at high protease and cellulolytic activities (figs. 13, 14). Thus, these species could have affinities to different fungal species. However, correlations between testaceans and enzymes produced by fungi are usually weaker than those with alkaline phosphatase, which is produced exclusively by bacteria (Tab. 7). This indicates that bacteria are the main source of food for testate amoebae, at least for the non-predatory species. At higher phosphatase levels the number of testaceans increased to about the same extent as at higher soil moisture. Close relationships between testaceans and microbial decomposition of organic substances are also indicated by positive correlations with catalase and dehydrogenase activities (Tab. 7, Foissner, 1985). The less acid-tolerant testaceans, such as *Cryptodiffugia oviformis*, *Phryganella acropodia*, *Schoenbornia viscidula*, and *Tracheleuglypha dentata*, seem to prefer higher catalase levels than acidophilic species (fig. 11). However, Foissner (1985) observed a significant negative correlation between this

enzyme and species number, abundance, and biomass of testaceans in podsollic alpine soils, while no relationships with catalase and cellulolytic activity could be found in a high-altitude reforestation trial by Aescht and Foissner (1992b).

### Fertilizer effects

The mean individual and species numbers of testaceans from all blocks were almost identical in the controls and treatments. In both stands, however, the total mean biomasses were decreased by fertilization; in the bactusol-biomag treatment the decrease was statistically significant (Tab. 2). This results from a shift to smaller, but more productive species; thus the decreased biomasses should not be assessed negatively. It is worth to mention that testacean biomasses in the controls are 10 to 20-times higher than those of the earthworms and equal those of enchytraeids and microarthropods (unpubl. results). The biomass of the earthworms, which were investigated twice (in July and September) five years after fertilization, increased by a factor of 7 to 10 in the treated plots (unpubl. results), also indicating a slow recovery of the mesofauna depleted by the low pH. In spruce forests of Southern Germany liming with  $\text{CaCO}_3$  ( $2000 \text{ kg ha}^{-1}$ ) slightly increased (no statistics given) the individual numbers of the testaceans during the first year after application, whereas a combined application of lime and nitrogen [ $\text{Ca}(\text{NO}_3)_2\text{NH}_4\text{NO}_3$ ;  $500 \text{ kg ha}^{-1}$ ] caused an insignificant decrease; the community structure was hardly affected (Wanner, 1991). Earthworms increased transiently, whereas enchytraeids and diplopods distinctly decreased (Funke, 1991). Stachurska-Hagen (1980) reported that the numbers of rotifers, nematodes, and most testacean species, especially of *Corythion* sp. and *Phryganella acropodia*, were decreased in a young Scots pine forest in Norway which had been limed with 900 and 8000  $\text{kg CaCO}_3$  four years earlier. In Czechoslovakian spruce forests Rosa (1974) found inconspicuous changes after application of NPK and lime. However, the methods used are rather dubious and the low individual numbers indicate that Rosa (1974) overlooked most cells.

Both fertilizers moderately affected the community structure of the testaceans and ciliates (cp. Aescht and Foissner, 1993) as well as the activity of extracellular microbial enzymes (Tab. 5, figs. 5-8). The shifts in the community structure are very likely related to the pH-rise and to changes in the numbers and kinds of fungi and bacteria, i.e. an altered food spectrum, as indicated by the rather strong correlations between testaceans and some soil enzymes, the inverse changes of the dominant bacterial feeders, *Corythion dubium* and *Trinema lineare*, as well as by the remarkable increase of obligate fungivorous ciliates (Aescht and Foissner, 1993). Obviously, the acidophilic and aerophilic *C. dubium* is more stenoeicous than *T. lineare*, which could benefit, i.e. increase its number, from the

chemical and microbial changes after fertilization. These species also respond differently to pesticides (Petz and Foissner, 1989a): the fungicide mancozeb increased the dominance of *C. dubium*, while *T. lineare* was unaffected; in contrast, the insecticide lindane increased *T. lineare*, but did not affect *C. dubium*. In the present study different reactions were even observed in congeneric species: fertilization caused a slight decrease of the abundance (old stand) or frequency (young stand) of *Schoenbornia humicola*, while *S. viscidula* became more frequent and abundant in both stands (Tab. 5). This may be related to different ecological requirements: *S. viscidula* apparently prefers a higher pH, soil moisture, catalase, protease, and cellulolytic activity than *S. humicola* (figs. 9-14). These observations confirm Schönborn *et al.* (1987), who showed that *S. humicola* is an indicator species of acid humus and depressed by lime, if liming causes an excessive increase of the pH by about 2 units.

Inconspicuous differences in total abundance were found between the two treatments applied (Tabs. 2, 3). However, the biomasses and the community analyses show that testaceans were more affected by the combined bactosol-biomag application (figs. 5-8). This matches the results for active ciliates (Aescht and Foissner, 1993). Taken altogether, viz. protozoa and metazoa, the fertilizers applied caused modest changes; the effects were largely restricted to the uppermost litter layer. Thus, the application of slow-acting pH-regulators does not seem to disturb the soil fauna excessively; hence prudent forest fertilization may be recommended, if the vitality of the trees is increased and the ground water does not become eutrophic from leachates.

**Note.** Trade names and company names are included for the benefit of the reader and do not imply endorsement or preferential treatment of the products by the authors.

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