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Soil Protozoa as Bioindicators in Ecosystems under Human Influence

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Introduction

Protozoa are increasingly used as bioindicators in soil ecosystems. Some 150 earlier papers on this subject have been reviewed by Viswanath and Pillai (1968) and Foissner (1987a, b, 1991). The present review summarizes references mainly after 1985. Other applied aspects, such as pest management and bioindication in natural ecosystems by soil protozoa are extensively discussed in Old and Chakraborty (1986), Foissner (1987a, 1987b, 1991), Klein (1988), Lousier and Bamforth (1990) and in other chapters of this book.

Heuristic Background

Bioindicators are, in a broad ecological sense, organisms that can be used for the detection and quantitative characterization of a certain environmental factor or of a complex of environmental factors; a narrower definition confines bioindicators to human influences (Bick, 1982). Several unique features favour the use of heterotrophic soil protozoa as bioindicators (Foissner, 1987b):

1. Protozoa are an essential component of soil ecosystems, because of their large standing crop and production. Changes in their dynamics and community structure very probably influence the rate and kind of soil formation and soil fertility.
2. Protozoa, with their rapid growth and delicate external membranes, can

react more quickly to environmental changes than any other eukaryotic organism and can thus serve as an early warning system.
3. The eukaryotic genome of the protozoa is similar to that of the metazoa. Their reactions to environmental changes can thus be related to higher organisms more convincingly than those of prokaryotes.
4. Protozoa inhabit and are particularly abundant in those soil ecosystems that almost or entirely lack higher organisms due to extreme environmental conditions, e.g. alpine regions above the timberline, Arctic and Antarctic biotopes.
5. Protozoa are not readily dislodged in soil (Kuikman *et al.*, 1990). Many (but not all!) are ubiquitous and are useful in comparing results from different regions. Differences in patterns of distribution are almost entirely restricted to passive vertical displacement; thus, the difficult problem, especially with the epigaeon, of horizontal migration does not affect the investigations.

There are, however, several factors that have apparently restricted the use of soil protozoa and even metazoa as bioindicators (Aescht and Foissner, 1992b):

1. The immense number of species; more than 1000 may occur in a square metre of forest soil. Many specialists are needed for identification and each species has specific requirements that are often incompletely known.
2. Enumeration of soil organisms is difficult and time-consuming.
3. Animals need other organisms for food. Thus, the constellation of factors is more complicated than in plants and bioindication often remains unspecific, i.e. different factors induce similar reactions.
4. Most soil organisms are inconspicuous and invisible to the naked eye, making them unattractive to many potential investigators.

Anthropogenic stress commonly leads to changes in ecosystems that are regressive and are best described as 'impoverishment'. Most perturbations are chronic and increase the abundance of opportunistic species. Such simplified communities are not capable of the same responses to stress as more complex communities. This may be either a result of fewer redundant species in the species pool capable of exploiting changing conditions, or of biological differences in the taxa found in early successional communities as compared to those taxa found in later successional and more mature stages. The causes of these differences, the underlying biology of which is poorly known, may be the inability of communities to disperse propagules to new habitats, to respond to toxic chemicals and to exclude invaders in the case of simple communities. Continual erosion of biological diversity may result in the loss of key species that regulate numbers of other taxa and allocation of resources to biomass (Cairns and Pratt, 1990).

Methodological Tools and Problems

The successful use of protozoa and other organisms as bioindicators depends on several methodological prerequisites. Unfortunately, some basic requirements are often neglected and must thus be discussed in some detail. In fact, methodological inconveniences will be frequently mentioned and have influenced the entire review.

Experimental design and statistics

Unfortunately, many soil protozoological studies lack a clear experimental design and appropriate statistics. Both are, however, essential for a correct interpretation of the data. In my experience, randomized blocks with at least four (better six or more) replications should be used whenever possible (e.g. Petz and Foissner, 1989b). This experimental design provides a firm basis for parametric and non-parametric statistical tests, such as analysis of variance and linear regression (cf. Köhler *et al.*, 1984).

Estimation of species richness and individual abundance

Natural and anthropogenic factors are often assessed by studying species richness and species composition. This presupposes that species are correctly identified. Unfortunately, misidentifications are frequent and this may explain many of the conflicting results in soil protozoology (Foissner, 1987a, 1991). Furthermore, the list of species should be comprehensive whenever possible. This is not easy with soil protozoa, because most cannot be extracted directly from the soil. In many studies, enumeration has involved various culture techniques (see Foissner, 1987a, for detailed description of methods and difficulties).

Population densities are widely used in applied soil protozoology. Foissner (1987a) discussed the problems relating to the commonly used counting methods and recommended direct counts rather than the culture techniques, e.g. Singh (1946), because the latter cannot reliably estimate the abundances of the *active* protozoa. In his opinion, the culture techniques yield, at best, a rough estimation of the abundances of the active *and* cystic protozoa (but see Perey, 1925). Unfortunately, the evidence presented in 1987 is still being widely ignored. I have thus included new results of a comparative study showing convincingly that culture methods greatly overestimate the abundance of the active protozoa (Table 7.1). They do show, however, the often surprisingly high numbers of cystic protozoa (up to 50,000 ciliate cysts in 1 g of soil; Table 7.1). My hypothesis that most

Table 7.1. Abundance of active ciliates g^{-1} dry mass of soil in the upper 0–5 cm layer of some grasslands (from Berthold and Foissner, 1993).

Sample	Direct counts in soil suspensions[a]	Culture method[b]	
		Active	Active + cystic
1	0	37000	46000
2	98	27000	27200
3	47	9200	9400

[a] Method of Lüftenegger *et al.* (1988). Recovery experiments showed that abundances are underestimated by about 30%.
[b] Method of Singh (1946). The numbers of active ciliates are determined by treating part of the sample overnight with 2% HCl as recommended by Singh. The number from the acid-treated portion of the sample, i.e. cysts, is subtracted from the total number of organisms to give the number of active ciliates. The estimation of *active* ciliates is therefore *indirect* and depends on the assumption that *all* cysts survive the acid treatment and that *all* cysts will excyst in the culture medium used (diluted soil extract). This assumption is obviously incorrect; most cysts did not excyst after the acid treatment and the other procedures (soil must be repeatedly washed to remove the acid) involved in this method.

soil protozoa are inactive (cystic) is supported by recent data provided by Darbyshire *et al.* (1989) and Glasbey *et al.* (1991) suggesting that few active ciliates reside inside soil aggregates of 2–5 mm diameter.

Data assessment

Commonly used indicators for environmental distress are nutrient imbalance (decrease in some nutrients and increase in others), reduced diversity of species, replacement of longer-lived by shorter-lived species (adapted to transitory novel environments), replacement of larger by smaller life forms, decline of biomass, and increase in population fluctuations of key species (Schwerdtfeger, 1975; Hill and Kevan, 1985). The evaluation and assessment of such data is a complex problem and discussion is restricted here to a few main points. Further information can be found in textbooks and the literature cited.

The faunal approach

Weigmann (1987) listed five criteria for assessing stress on ecosystems using species richness and species composition of the animals present.

1. Species richness in disturbed ecosystems should be compared with undisturbed ecosystems of the same ecotype. It is usually unsatisfactory, for example, to compare species richness and species composition before and after a forest is transformed into a meadow.

2. A faunal census should take account of whether a certain perturbation lowers or increases habitat diversity. Thus, increased species richness can indicate not only an improvement, but also a disturbance of an ecosystem.
3. Only taxa that have a high species richness in undisturbed ecosystems of the type being assessed should be used as bioindicators. Usually, only species-rich groups have sufficient ecological diversity to indicate a wide range of possible effects.
4. The choice of organisms used as bioindicators depends on the ecosystem and environmental hazard. This is an important, but often neglected point. Earthworms, for instance, are almost absent in strongly acidic spruce forests and above the timberline. In such ecosystems, other groups, e.g. the dominant acidophilic testate amoebae, should be used to assess certain influences. Similarly, the relative weighting of dominant to rare and/or stenoecious species depends on the specific problem under investigation.
5. Isovalent groups of indicator species can usually assess only a single disturbance factor. Amelioration of a moorland, for instance, can increase total species richness, but if a certain valency group completely disappears the amelioration must be assessed negatively.

Coenotic indices

Many coenotic indices have been suggested to reduce the complexity of biological communities to a simple, comprehensible number or value (Washington, 1984). However, all indices reflect only few of the basic community parameters (mostly species number and abundance) and many do not consider practical needs or the most common structure of biological coenoses. A typical example is the well-known Shannon–Wiener 'diversity index', which combines in a single value individual abundance, species richness and dominance. However, dominance is misinterpreted by this index, i.e. the diversity becomes highest in such communities where all species have the same abundance. Such coenoses hardly ever exist, because most distribution patterns fit a logarithmic series (Magurran, 1988).

Wodarz *et al.* (1992) have thus suggested a 'Weighted Coenotic Index' (WCI) that unifies in a single value the total abundance and the logarithmic dominance structure as well as species richness and ecological weightings, e.g. habitat preferences and positions of species in the *r*/*K*-continuum.

distribution component | distribution constant | abundances and weightings

$$WCI = \sum_{i=1}^{S} 10^8 \cdot \frac{p_i \cdot (1-p_i)^{S-1}}{[o_i \cdot (o_{max}-o_i)+1]} \times \frac{n_{max} \cdot \tilde{n}_i}{[(n_{max}-\tilde{n}_i) \cdot (\tilde{n}_i-n_{min})+1]} \times \frac{w_{1i} \cdot w_{2i} \cdot w_{ni}}{N \cdot S} \cdot \frac{1}{S}$$

for $p_i = S \neq 1$

where:

n_i = number of individuals in species i
$\tilde{n}_i$ = median of the n_i values
n_{max} = the highest n_i value in a sample
n_{min} = the lowest n_i value in a sample
N = the total number of individuals
o_i = 1 + ld(n_i) [ld = logarithmic dominance structure] = the octave species i belongs to
o_{max} = 1 + ld(n_{max}) = the highest octave in a sample
p_i = n_i/N = the relative abundance of species i
S = species richness, i.e. total number of species in all replicates
w_{1i} = weighting 1, e.g. degree of autochthonism in species i
w_{2i} = weighting 2, e.g. the position of species i in the *r K* continuum
w_{ni} = further weightings
10^8 = factor used to transform values to integers

Studies with simulated biocoenoses showed that ecological weighting and dominance structure are major components of the index; the ecological weighting needs to be related to the group of organisms studied and to the scope of the investigation. The WCI is a relative measure that needs a reference (control) site for a conclusive interpretation. Compared to several other diversity indices, WCI is an improvement, because of the inclusion of ecological weighting and the logarithmic dominance structure. This index was applied to published data from several field studies using protozoa (testate amoebae, ciliates) and earthworms. The results show that this index is an appropriate measure of changes and recovery processes in disturbed communities (Tables 7.2 and 7.19). The WCI is certainly no substitute for the classic methods (e.g. Schwerdtfeger, 1975; Southwood, 1978) of describing animal communities, but is very useful in summarizing data.

Scaling and persistency of disturbances

Reactions of organisms to detrimental influences have been extensively discussed by Domsch *et al.* (1983) and Beck *et al.* (1988). Doubling time and reproductive potential of the populations and sociopolitical constraints determine scaling and assessment of the reactions. Domsch *et al.* (1983) suggested that side-effects persisting for more than 60 days may be critical to populations of single-celled organisms.

Table 7.2. Weighted Coenotic Index (WCI) and Shannon–Wiener diversity (H′) of soil testacean communities. *N*, total abundance (individuals g^{-1} dry mass of soil); *S*, species number. (From Wodarz *et al.*, 1992.)

Sites and treatments (designated according to the original papers)	Testaceans[a]			
	N	*S*	WCI	H′
Agricultural soils[b]				
Organically farmed	884	28	33	2.896
Conventionally farmed	528	25	111	2.898
Vineyard soils[c]				
Minimal (reference site)	270	13	2285	1.279
Conventional	156	12	46123	1.335
Biodynamic	239	14	18730	1.552
Organic-biological	347	16	2181	1.557
Semi-biological	748	21	162	1.706
Soil compaction experiment[d]				
Control (chamber effect)	1816	11	36	1.489
Soil compaction 10%	1709	10	248	1.924
Soil compaction 30%	906	8	973	1.802
Soil compaction 50%	151	5	263719	1.519

[a] Decreasing WCI values indicate improving soil conditions.
[b] Original data from Foissner (1992) and unpublished.
[c, d] Original data from Lüftenegger and Foissner (1989a) and Berger *et al.* (1985) respectively.
The WCI differentiates the treatments more clearly than the Shannon–Wiener index. Furthermore, the detrimental effect of, for example, a 50% soil compaction is much better expressed by the WCI than by H′.

Soil Protozoa as Bioindicators in Ecosystems under Human Influence

Effects of irrigation

Data from field and laboratory studies of the effects of irrigation on abundance and species composition of soil protozoa are contradictory (Foissner, 1987a). Two recent field studies have also produced conflicting results. Szabó (1986) stated that the total numbers of protozoa and the abundances of the ciliates were strongly related to the water content in a Hungarian chernozem soil under maize cultivation. Flagellates and naked amoebae were active at water contents as low as 11–18% vol, whereas ciliates usually encysted if soil moisture was less than 13% vol. Unfortunately, Szabó (1986) omitted to mention either the method used for counting the protozoa (probably a culture method) or whether the counts relate only to active or to

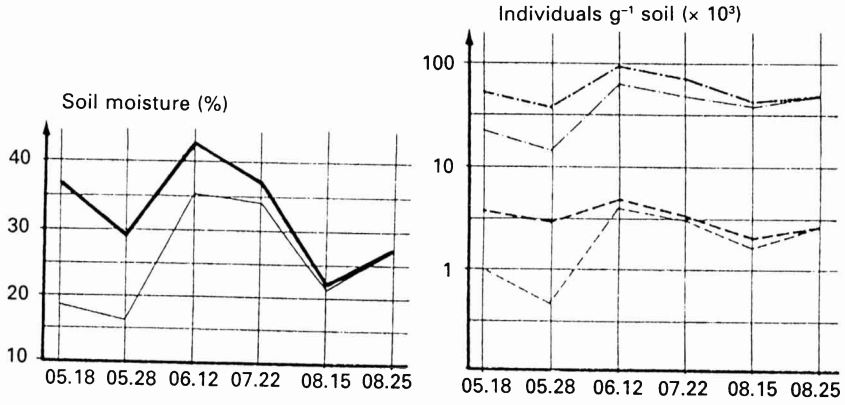

Fig. 7.1. Effects of irrigation on the moisture content and the protozoan numbers in a chernozem soil from May to August. —— irrigated, —— control, — · — · total number of protozoa in irrigated soil, — · — · number of ciliates in irrigated soil, — — — total number of protozoa in control soil, — — — number of ciliates in control soil. (From Szabó, 1986.)

both active and cystic individuals. Furthermore, the data were not evaluated statistically. At first glance, Fig. 7.1 seems to support Szabó's (1986) conclusions. A closer inspection shows, however, that the protozoa retained a much higher abundance in the irrigated soil than in the non-irrigated control, although the water contents of the treatments were very similar during the last three periods of investigation.

Petz and Foissner (1989a) observed a marked decrease ($P \leqslant 0.05$) in the abundance of the active ciliates in the litter layer of an irrigated spruce forest stand (Table 7.3); species richness, however, increased in both ciliates and testate amoebae, indicating that certain species need higher soil moisture contents. Testacean numbers did not increase significantly in response to irrigation. The nematode numbers increased by about 45% in the 0–3 cm layer of the same irrigated plots. Lousier (1974a,b), however, found significant positive correlations between soil moisture and number of total and active testaceans in a very dry aspen woodland after irrigation ($r = 0.873$ and 0.804, respectively). Wanner and Funke (1989) and Wanner (1991), like Petz and Foissner (1989a), did not find a significant correlation between soil moisture and testacean numbers (Tables 7.3 and 7.5).

In Antarctic biotopes, Smith (1973, 1985) found significant positive correlations between the flagellate *Cercobodo vibrans*, the testate amoeba *Corythion dubium* and the moisture contents of both bare fellfield fines and *Andreaea* moss cushions; correlation coefficients with maximum soil temperature were smaller. Smith (1985) therefore suggested that moisture

Table 7.3. Effects of irrigation on the microfauna of a spruce forest soil[a] (from Petz and Foissner, 1989a).

Parameters	Soil depth (cm)	Irrigated[b]	Control	*n*	Statistics
Soil moisture (% wet mass of air-dried soil)	0–3	50.0* ± 9.9	41.3† ± 12.6	15	ANOVA ($P \leqslant 0.05$)
	3–9	48.8* ± 4.3	44.7* ± 5.9	8	ANOVA ($0.2 \geqslant P > 0.1$)
Ciliates					
abundance	0–3	311* ± 141	489† ± 258	15	U-test ($0.05 < P \leqslant 0.1$)
	3–9	10* ± 10	14* ± 24	8	U-test ($P \geqslant 0.2$)
species number	0–3	12.8* ± 4.5	8.4† ± 3.3	15	ANOVA ($P \leqslant 0.005$)
	3–9	2.4* ± 2.1	1.6* ± 1.9	8	U-test ($P \geqslant 0.2$)
Testaceans					
abundance					
living	0–3	22203* ± 7040	17908* ± 3175	4	ANOVA ($P \geqslant 0.2$)
empty tests	0–3	361720* ± 17204	319081* ± 58390	4	ANOVA ($P \geqslant 0.2$)
species number					
living	0–3	11.5* ± 1.3	9.8† ± 1.0	4	ANOVA ($0.05 < P \leqslant 0.1$)
total	0–3	18.0* ± 0	19.8* ± 2.2	4	U-test ($P \geqslant 0.2$)
Nematodes					
abundance	0–3	1197* ± 349	824† ± 349	15	ANOVA ($P \leqslant 0.01$)
	3–9	308* ± 80	391* ± 173	8	ANOVA ($P \geqslant 0.2$)
Rotifers					
abundance	0–3	227* ± 88	181* ± 122	15	ANOVA ($P \geqslant 0.2$)
	3–9	38* ± 25	31* ± 18	8	U-test ($P \geqslant 0.2$)

[a] Abundances (individuals g^{-1} dry mass of soil; arithmetic mean ± standard deviation) were estimated with the direct counting method of Lüftenegger *et al.* (1988). Values followed by the same symbol are not significantly different.
[b] Irrigated plot (15 m^2) received 25 l m^{-2} water every fourth day.

rather than temperature is the principal environmental factor limiting the rate of development of biotic communities in Antarctic fellfields. Other studies, however, did not find any correlation between flagellate abundance and soil moisture (for review see Foissner, 1987a).

The data available (Foissner, 1987a, and this review) suggest that soil protozoa do not depend as much on moisture as was widely assumed and often concluded from microcosm experiments (e.g. Kuikman *et al.*, 1991). It seems likely that moderate, artificial irrigation often does not markedly influence soil protozoa. Irrigation or reclamation probably increases individual abundance and species richness only in very dry (e.g. deserts and semideserts) or very moist biotopes. Many protozoa are obviously active in rather dry soils and can live in very thin films of water. In the experiment of Petz and Foissner (1989a), the litter of the control plot appeared virtually dry; most of the water present was a constituent of the needles. Foster and Dormaar (1991) showed in an ultrastructural study that amoebae can probably exploit micropores having a diameter of only 1 μm.

Effects of organic and mineral fertilizers

Most of the extensive literature available relating to the effects of soil fertilizers on protozoa is reviewed in Foissner (1987a). The few, more recent papers discussed below support the general conclusion that most fertilizers increase the numbers of protozoan cells and change the dominance structure of the soil protozoa.

Paustian *et al.* (1990) studied the effects of nitrogen amendments on the carbon and nitrogen budgets and the soil fauna in four Swedish agroecosystems with annual and perennial crops (Fig. 7.2). The variation in the N input (1–39 g N m^{-2} $year^{-1}$) and cropping system influenced primary production (260–790 g C m^{-2} $year^{-1}$) and the input of organic material to the soil (150–270 g C m^{-2} $year^{-1}$). This was reflected in variations of total soil animal biomass (1.6–5.1 g C m^{-1}) and in variations in the abundance of the nematodes and the micro- and macroarthropods. In contrast, total bacteria, fungi, flagellates and amoebae varied quite independently of the organic matter input (Sohlenius, 1990; Fig. 7.2a-c). Protozoa showed few differences between treatments and most amoebae occurred in the unfertilized plot (Fig. 7.2b). The average number of amoebae was significantly higher ($P \leq 0.05$) in GL (meadow fescue ley receiving 120+80 kg N ha^{-1} $year^{-1}$) than in B120 (barley receiving 120 kg N ha^{-1} $year^{1}$). Schnürer *et al.* (1986) speculated that the difference in the input of organic matter was too small to produce pronounced effects, whereas Sohlenius (1990) suggested that the rather constant microbial biomass was a result of an adjustment in the grazing pressure of microbial-feeding animals to the level of microbial production. However, methodological shortcomings of the culture method,

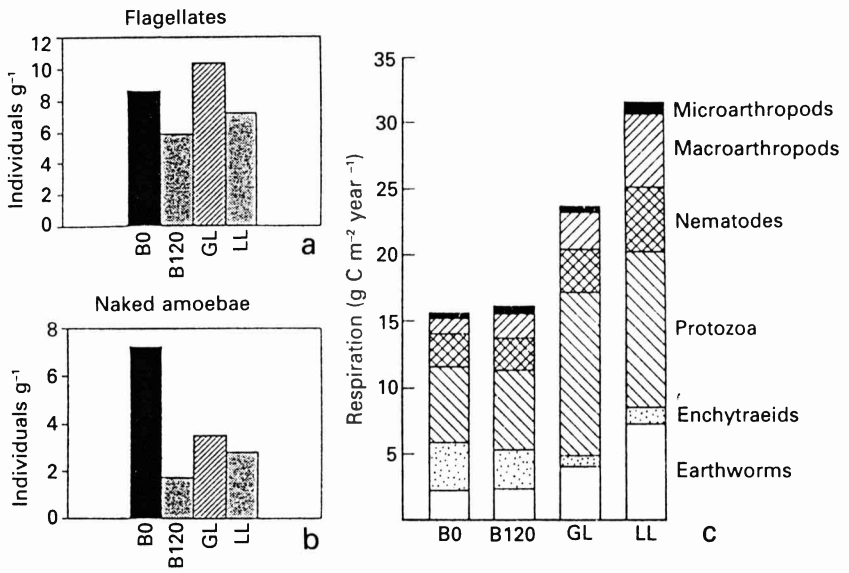

Fig. 7.2a – c. Mean numbers of flagellates and naked amoebae (g^{-1} dry mass of soil), and mean annual rates of respiration for the total fauna (herbage and soil) in barley without N fertilizer (BO), barley with 120 kg N ha^{-1} $year^{-1}$ (B120), grass ley with 200 kg N ha^{-1} (GL), and lucerne (LL). A randomized block design with four replicates was used. Total (active + cystic) abundances of flagellates and naked amoebae were estimated by a culture method in 20 samplings over three years. (From Paustian *et al.*, 1990, and Sohlenius, 1990.)

where cystic and active protozoan cells were not distinguished, could also have contributed to this rather unexpected result.

Gupta and Germida (1988) investigated the effects of five years of repeated application of elemental S fertilizer on the soil protozoa in S-deficient soils in western Canada. The application of S^0 fertilizer reduced the microbial biomass and its activity in soil. Soils treated with 44 kg S^0 ha^{-1} $year^{-1}$ for five years exhibited a 30–71% decline in protozoa feeding on bacteria and more than a 84% decline in the population of mycophagous amoebae. This decline in protozoan populations accompanied changes in microbial biomass, especially in the case of mycophagous amoebae and fungal biomass (Fig. 7.3). The adverse effect of repeated S^0 applications on microbial biomass and predatory protozoa was persistent. Since nutrient transformations (e.g. mineralization) in soil are influenced by microbial interactions, these results suggest reduced nutrient turnover via microbial predation in S^0-treated soils.

Tirjaková (1991) investigated the effects of some agricultural fertilizers

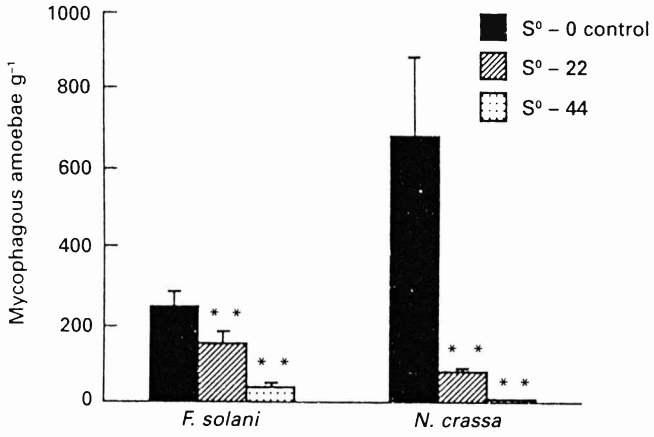

Fig. 7.3. Effect of 5 years of elemental S fertilizer application on the mycophagous soil amoebae feeding on *Fusarium solani* or *Neurospora crassa*. S⁰ applied at 22 or 44 kg ha⁻¹ yr⁻¹. Triplicate samples from brome grass–alfalfa pasture sites were analysed. Amoebae were counted with Singh's ring technique (1946) using a most probable number method. ** Significant at $P \leq 0.001$. (From Gupta and Germida, 1988.)

(NPK, urea, ammonium–calcium nitrate, ammonium sulfate) on ciliates in soil microcosms. The numbers of species decreased with increasing fertilizer concentration (2, 10, 20, 50 g l^{-1}); the type of fertilizer was of minor importance. Most species occurred rather frequently up to a concentration of 10 g l^{-1}; 20 g l^{-1} were toxic for most species. Only seven species (compared to 71 species found in the control) survived 50 g l^{-1}, viz. *Colpoda aspera, C. steinii, Enchelys gasterosteus* (probably a misidentification as this species very likely does not occur in soils), *Hemisincirra gellerti, Homalogastra setosa, Leptopharynx costatus* and *Platyophrya spumacola.* Of the four fertilizers tested, NPK was the least toxic.

Aescht and Foissner (1991, 1992) investigated protozoa (testate amoebae, ciliates), small metazoa (nematodes, rotifers) and soil enzymes (catalase, cellulase) in a reforested fertilized site at the alpine timberline (Fig. 7.4). None of the treatments caused a significant decrease of the biological parameters investigated in comparison with untreated controls. Soil life was more or less stimulated depending on the quantity and organic content of the fertilizers; 180–270 g organic material per seedling were found to be most effective. Dried bacterial biomass increased the pH by about 0.5 units, the catalase activity by about 70%, and the number of ciliates and nematodes by 150–400%. Biomass and species number of ciliates were likewise

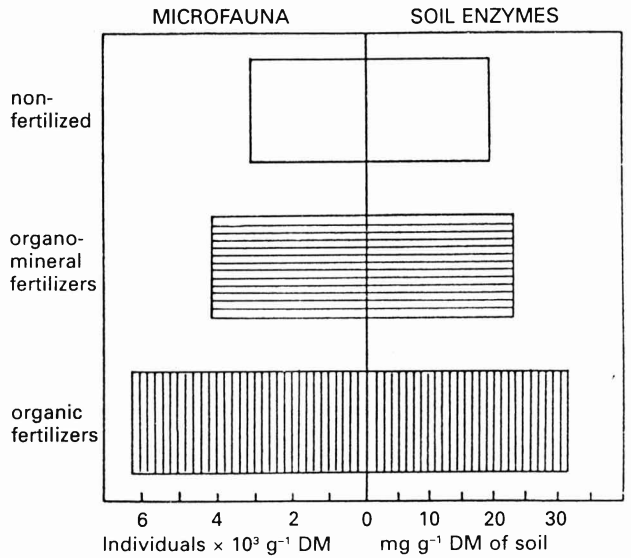

Fig. 7.4. Mean numbers of soil animals (testate amoebae, ciliates, nematodes, rotifers) and enzymatic activites (catalase, cellulase) in a reforested and fertilized site near the alpine timberline. A randomized block design with six replicates for four years was used. Mineral and organic fertilizers were applied separately (90 g NPK; 90, 180, 300, 450 g dried bacterial biomass per spruce seedling, respectively) and in combination with magnesite (90 g NPK + 300 g Mg; 90, 180, 300 g bacterial biomass + 300 g Mg each, respectively; 30 g dried fungal biomass + 270 g Mg). These fertilizers were applied in a granular form within a 10 cm radius around each seedling. Testate amoebae, nematodes and rotifers were counted with the direct method of Lüftenegger *et al.* (1988). The 'potential' abundance of the ciliates was estimated with a culture method. (From Aescht and Foissner, 1991, 1992.)

increased. Organo-mineral fertilizers caused a pH rise of up to two units and also stimulated soil life; but efficiency decreased markedly if the organic content was less than 180 g per seedling. The lowest biological activities were observed in the control and the soil fertilized with NPK. Testaceans, rotifers and cellulolytic activities were not significantly affected by the treatments. Pooled evaluation of the data (organic versus organo-mineral treatments) and community analyses show that the organic fertilizers caused a more pronounced increase in soil life and changes in the community structure than the mineral combinations (Fig. 7.5). Two years after refertilization, the differences between treatments and unfertilized controls had diminished.

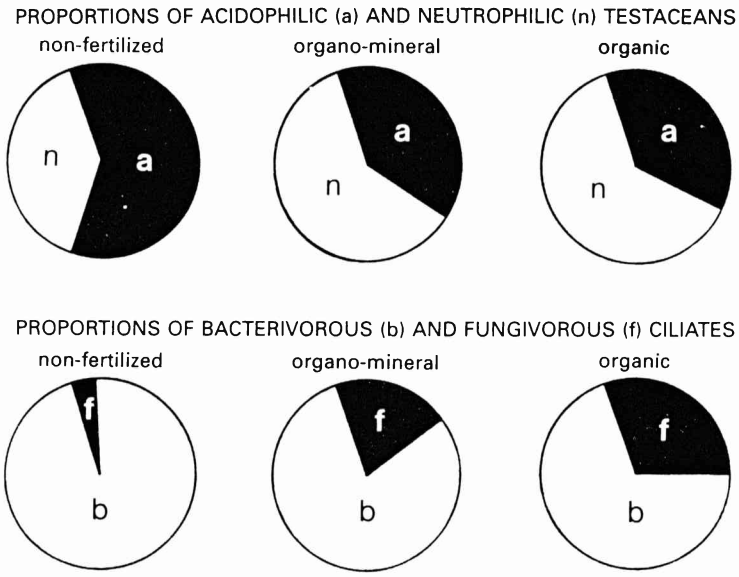

Fig. 7.5. Proportions of indicator species in a reforested and fertilized site near the alpine timberline. See Fig. 7.4 for methods. (From Aescht and Foissner, 1991, 1992.)

Effects of forest liming and fertilizers

A dramatic forest decline is evident in regions exposed to heavy air-pollution. The decline is usually associated with a significant decrease in soil pH and/or essential plant nutrients. Thus, liming and fertilizers are widely used to overcome the problem. However, many forest ecologists and soil zoologists are disquieted by such treatments, as they might induce unexpected long-term changes (Funke, 1987). As regards soil protozoa, the data reviewed in Foissner (1987a) indicate that liming and fertilizers increase the individual and species numbers of the ciliates and testate amoebae in very acidic (pH $\leq$ 4) forest soils. Ciliates react particularly quickly to changes in the physico-chemical and biological properties of their environment. Liming increases the number of species and individuals, but fertilizers decrease both. If liming is combined with fertilizers, the positive and negative effects seem to counteract each other (Lehle and Funke, 1989; Funke and Roth-Holzapfel, 1991). More detailed investigations using randomized blocks showed, however, that treatment effects are temporary and not very pronounced (Rosa, 1974; Wanner and Funke, 1989; Wanner, 1991; Aescht and Foissner, unpublished; Tables 7.4 and 7.5). Certainly, the effects depend on the amount and kind of substances applied and may be masked by the detrimental effects of heavy metals, organics (polycyclic aromatic hydrocarbons), etc. usually deposited together with the acidifying NOx and S compounds.

Table 7.4. Average individual number of protozoans in fertilized spruce forest plots.

Treatments	Flagellates	Ciliates	Testaceans	Total
Control[a]	5323	1650	1534	8507
NPKCa[b]	7569	3051	1319	11939
N[c]	7031	2414	1723	11168
Old stand[d]				
Control	9553	583	32026	42162
Biomag[e]	5403	361	38734	44498
Bactosol + Biomag[f]	9634	453	34660	44747
Young stand[d]				
Control	1696	286	39944	41926
Biomag[e]	2263	229	34063	36555
Bactosol + Biomag[f]	3830[g]	221	36531	40582

[a] Data from Rosa (1974) and compiled from tabulated values. A randomized block design with five replicates was used; each block was sampled 8–9 times during a period of one year. A rather dubious culture method was used: 5 g humus were added to 10 ml distilled water. After 24 hours the individuals present were counted and the number g^{-1} humus calculated from two replicates.
[b] Fertilized with 200 kg N, 100 kg P, 100 kg K and 300 kg lime ha^{-1}.
[c] Fertilized with 100–150 kg N ha^{-1}.
[d] Data from Aescht and Foissner (1993 and unpublished). A randomized block design with four (testaceans) to six (ciliates and flagellates) replicates was used; blocks were sampled 7–8 times during a period of five years, except for the flagellates which were sampled once, viz. two years after fertilization. Counts were made with the direct method as described by Lüftenegger *et al.* (1988) and are given as cells g^{-1} dry mass of soil.
[e] Fertilized with 1800 kg magnesite and 200 kg dried fungal biomass ha^{-1}.
[f] Fertilized with 3000 kg dried bacterial biomass, 1800 kg magnesite and 200 kg dried fungal biomass ha^{-1}.
[g] Significant difference from control (ANOVA).

Rauenbusch (1987) observed a decrease in numbers and an increased rate of malformed euglyphid testaceans in very acidic forest soils; detailed evidence is, however, not provided.

Applications of slow-acting pH-regulators and fertilizers seem unlikely to disturb soil fauna greatly and might be recommended prudently, if the vitality of the trees is increased and the groundwater does not become eutrophic from leachates.

Table 7.5. Effects of liming, fertilization, irrigation, acidification and pesticides on the testate amoebae in spruce forest stands near Ulm, Germany (compiled from Wanner, 1991).

Sites, investigation periods and treatments[a]	n^b	pH (KCl)	Testaceans[c] Individuals	Biomass	Taxa
Site A (June 1984–Oct. 1987)					
Control	9	3.1*	9091*	0.111*	18.1*
Limed + fertilized	9	4.5†	6926*	0.068*	15.1†
Limed + fertilized + irrigated	9	3.2‡	8021*	0.083*	14.2‡
Site B (April 1984–Oct. 1987)					
Control	10	2.9*	7017*	0.073*	15.0*
Limed	10	3.5†	10137†	0.122†	15.8*
Site C (Nov. 1986–May 1988)					
Control	4	3.5	15800	0.194	19.2
NaCl	4	3.5	12741	0.142	18.0
H_2SO_4	4	3.0	13215	0.098	16.2
Lindane	4	3.5	15165	0.176	17.2
Ripcord	4	3.5	10423	0.110	16.7

[a] Limed + fertilized: 20 kg 95% $CaCO_3$ 100 m^{-2} + 5 kg $5Ca(NO_3)_2NH_4NO_3$ 100 m^{-2}.
Irrigated: to –70 cm water holding capacity.
Limed: 20 kg 95% $CaCO_3$ 100 m^{-2}.
NaCl: 2.25 kg m^{-2}.
H_2SO_4: 10 l m^{-2} (5%).
Lindane: 120 g ha^{-1}.
Ripcord: 10 g m^{-2}.
[b] Number of samples investigated.
[c] Numbers (direct counts in aqueous soil suspensions) are given as active individuals g^{-1} dry mass of soil. Biomasses: mg g^{-1} dry mass of soil. Values followed by the same symbol are not significantly different ($P \geq 0.05$).

Effects of soil management

Most investigations relating to the effects of cultivation, crop rotation and organic farming on soil protozoa were reviewed in detail by Foissner (1987a). The few more recent studies that have been published agree with the previous conclusions that cultivation and organic farming tend to increase the abundance and species richness of soil protozoa compared with virgin lands and fallows (Mordkovich, 1986; Tirjaková, 1988; Foissner, 1992). Tirjaková (1988) investigated 147 samples from 22 agricultural soils in Czechoslovakia and found a characteristic community of ciliates: *Colpoda inflata, C. steinii, Cyrtolophosis elongata; Gonostomum affine, Histriculus*

muscorum, Homalogastra setosa, Leptopharynx costatus; the proportion of rare species was high.

Foissner *et al.* (1986, 1990), Foissner (1987c) and Lüftenegger and Foissner (1989a) studied some ecofarmed and conventionally farmed fields and grasslands in Austria with special reference to the protozoa. The results obtained from the evaluation of a total of 13 paired sites (ecofarmed vs. conventionally farmed) have been summarized by Foissner (1992):

1. Many of the soil zoological parameters under investigation did not differ statistically in ecofarmed and conventionally farmed fields and grasslands.
2. There were no striking differences in species composition and dominance structure of the ciliates and testate amoebae.
3. All differences that can be guaranteed with an error probability of α = 10% or less invariably show higher biological activity in the ecofarmed plots (Fig. 7.6). The soil physical and chemical investigations which accompanied the zoological studies of some sites revealed larger biological activity is correlated with the larger humus content and smaller soil compaction. The organic matter content is significantly larger in the

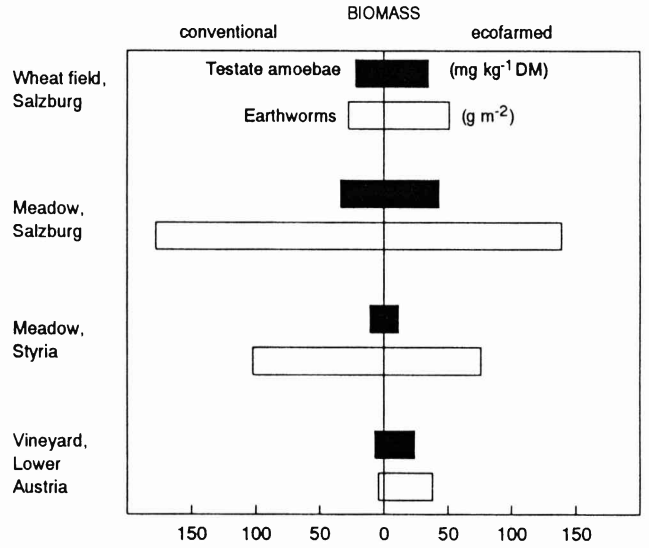

Fig. 7.6. Biomass of testate amoebae (direct counts) and earthworms (formaldehyde extraction) in conventionally farmed and ecofarmed fields and grasslands in Austria. Combined data from several investigations. DM, dry mass of soil. (From Aescht and Foissner, 1991.)

ecofarmed plots, whereas soil compaction is more pronounced under conventional cultivation.

4. Conventional agriculture has a more detrimental effect on soil fauna in semiarid regions without animal husbandry than in Atlantic regions with mixed farming.

These and other results from the literature show that generalizations like 'conventional farming destroys life in the soil' or 'ecofarming stimulates soil life' are only partially supported by the available data. A far more comprehensive analysis including climate, soil type and farm management is required. However, the detrimental effects caused by conventional farming already discernible on soil fauna call for serious consideration and ought to stimulate the development of more compatible agricultural technology and intensified soil biological research. Future research should include studies on productivity of soil animals under various management systems, the analysis of single factors (e.g. the special admixtures used in biodynamic farming) to elucidate causative mechanisms, and studies on the relationship between soil animals, crop production and sustained yield.

Zoologists are frequently perplexed by practical questions posed by farmers. For example, 'What do I stand to gain by having say about 10% more soil animals in my fields? Will my yield also go up by 10% or will I get a more sustainable yield?' Unfortunately, soil zoologists cannot provide satisfactory answers to these important questions, because of insufficient data related to crop yield. There is an urgent need for integrating soil zoological studies with plant agronomy and agricultural economics. Only when we can provide conventional farmers with conclusive evidence of the economic importance of soil animals, may we expect to convince them of the benefits of ecofarming.

Effects of biocides

Biocides, including pesticides and herbicides, play a major and increasingly controversial role in modern agriculture. Foissner (1987a) reached four conclusions from the data available:

1. The general pattern of reaction of soil protozoa to biocide stress is the same as that of other organisms.
2. Many protozoan species seem to be just as sensitive to pesticides as other more commonly used test organisms.
3. Insecticides are usually more toxic than herbicides.
4. Insecticides disturb soil protozoa critically, i.e. populations often do not fully recover within 60 days.

These conclusions have been substantiated and extended by other studies. These are reviewed in some detail in this chapter since there is a strong

possibility of replacing vertebrates by protozoa as test models for pesticides (Pons and Pussard, 1980). The problems of adaptation to biocide stress are, however, also evident in protozoa (Raederstorff and Rohmer, 1987a, 1987b). The systemic fungicides tridemorph and fenpropimorph decreased the growth rate of the soil amoeba, *Acanthamoeba polyphaga*, at a 40 μM concentration. After several weekly subcultures, the growth rate returned to normal and the inhibitor concentration stabilized at 80 μM. After four to five extra subcultures, the growth rate was again identical to that in untreated control cultures. The amoebae could tolerate even higher concentrations of inhibitors (up to 160 μM) without apparent damage. Their sterol biosynthesis was, however, greatly influenced. Not only was there a much larger amount of sterols in the fungicide-exposed cells, but these were also synthesized via a different metabolic pathway from those found in the control cells. These results are confirmed and extended by a recent paper of Groliere *et al.* (1992), indicating that *Tetrahymena pyriformis* GL can convert a dithiocarbamate fungicide, thiram, into metabolites toxic to this ciliate.

Another new approach to the problem is taken in the paper by Pižl (1985). He found a significantly increased infection of earthworms by monocystid gregarines when the earthworms were exposed to a herbicide, zeazin 50, for 26 weeks (Table 7.6).

Schreiber and Brink (1989) devised an *in vitro* toxicity test for pesticides using soil and freshwater protozoa as test organisms. They observed quite variable sensitivities, and the conventional soil application rates used by farmers for chlorex, MCPA, and benlate were toxic to some of the organisms (Table 7.7).

There is still a great need for well-designed field studies on the effects of biocides on soil protozoa, particularly on testate amoebae (Foissner, 1987a). The only such studies available are those by Petz and Foissner

Table 7.6. The effect of the herbicide zeazin 50 on the incidence of infection of earthworms by monocystid gregarines (from Pižl, 1985).

	% incidence of infection[b]			
Species[a]	Control without cysts	Control with cysts	5 kg ha^{-1} zeazin 50	40 kg ha^{-1} zeazin 50
Lumbricus castaneus +	0	64.0	83.3**	100.0**
Lumbricus terrestris +	0	56.0	84.0**	96.0**
Octolasium lacteum ++	0	36.0	52.0*	75.0*

[a] Symbols: + earthworms infected by *Monocystis agilis*, ++ earthworms infected by *Aplocystis herculea*.
[b] χ^2-test: *$P \leq 0.05$; ** $P \leq 0.01$.

Table 7.7. Conventional agricultural concentrations (CC) applied to arable land, and 9-hour lethal concentrations for six pesticides and the protozoans *Colpoda cucullus* (*C. c.*), *Blepharisma undulans* (*B. u.*) and *Oikomonas termo* (*O. t.*). (From Schreiber and Brink, 1989.)

Pesticide	CC (ppm)	9-h LC_{50}[a] (ppm)		
		C. c.	*B. u.*	*O. t.*
Chlorex	1000	320	360	40000
MCPA	3	100	1	2
Benlate	1	4	0.7	10
Dichlorprop	2	>>100	>>100	>>100
Matrigon	0.5	>>100	>100	4
Sumicidin	2	>>100	>100	6

[a] Concentrations at which 50% (LC_{50}) of protozoan population died after 9-hour incubation.

(1989b), Wanner and Funke (1989) and Wanner (1991). Petz and Foissner (1989b) investigated the effects of a fungicide, mancozeb and an insecticide, lindane, on the active microfauna of a spruce forest soil using a completely randomized block design and a direct counting method (Table 7.8). The effects were evaluated 1, 7, 15, 40, 65 and 90 days after application of a standard or high (× 10) dose (0.096 g m^{-2} and 6 g m^{-2} active ingredient, respectively). Mancozeb, even at the higher dose, had no pronounced acute or long-term effects on absolute numbers of the taxa investigated. The number of ciliate species decreased one day after treatment with the standard dose ($0.05 < P \leq 0.1$), but soon recovered (Fig. 7.7). However, the community structure of ciliate species was still slightly altered after 90 days. Mycophagous ciliates were distinctly reduced in the first weeks after application of the fungicide (Table 7.9). Testaceans were not reduced before day 15 with the higher dose or before day 40 with the standard dose ($0.05 < P \leq 0.1$). A standard dose of lindane caused acute toxicity in ciliates and rotifers ($P \leq 0.05$), although the latter soon recovered. The number and community structure of ciliate species were still distinctly altered after 90 days ($0.05 < P \leq 0.1$), indicating the crucial influence of lindane. Testaceans were reduced only on day 15 and nematodes only on day 40 ($0.05 < P \leq 0.1$). At the high dose of lindane, severe long-term effects occurred in soil moisture, total rotifers ($P \leq 0.05$), total nematodes ($0.05 < P \leq 0.1$) and in the structure of the ciliate community. Some species were encouraged by lindane after 90 days, e.g. *Colpoda inflata*, *C. steinii* and *Pseudoplatyophrya nana*, possibly due to reduced competition and their *r*-selected survival strategy, whereas *Avestina ludwigi*, very dominant in the control plots, became extinct (Table 7.8). Generally, there were marked differences

Table 7.8. Percentage of the dominant species of active ciliates and testaceans 1 and 90 days after treatment with mancozeb and lindane at normal (0.096 g m^{-2} and 6 g m^{-2} active ingredient, respectively) and high doses (0.96 g m^{-2} and 60 g m^{-2} active ingredient, respectively). (From Petz and Foissner, 1989b.)

Species	Day	Control	Mancozeb		Lindane	
			1 ×	10 ×	1 ×	10 ×
Ciliates						
Avestina ludwigi	1	23.5	44.3**	37.4	15.2**	0.0**
(Aescht & Foissner)	90	41.3	45.8^{+}	42.9	26.7^{++}	0.0**
Platyophrya	1	19.4	12.7	21.7	12.1	0.0**
spumacola (Kahl)	90	13.1	9.8	13.6	22.4^{+}	4.4**
Pseudoplatyophrya	1	18.5	10.0*	6.0	15.2	0.0**
nana (Kahl)	90	10.8	13.6	17.7	24.2**	35.8**
Colpoda steinii	1	4.5	1.4	4.0	6.1	χ[a]
(Maupas)	90	0.0	0.0	0.7	2.5^{++}	44.8**
Colpoda inflata	1	0.1	1.0	0.5	0.0	0.0
(Stokes)	90	0.8	0.0	0.7	0.6^{+}	9.3**
Testaceans						
Corythion dubium	1	48.1	74.6*	63.3	46.0	51.3
(Taranek)	90	47.9	52.2	48.0	39.7	32.7
Trinema lineare	1	11.3	7.5	0.0	18.7	12.2
(Penard)	90	9.6	17.5	17.8	18.3**	15.7
Schoenbornia	1	0.0	0.0	2.8	3.0	8.7
humicola (Schönborn)	90	9.6	9.7	6.4	6.9	17.0

[a] High value, not representative because only two individuals were found to be active.
* $0.05 < P \leq 0.1$; ** $P \leq 0.05$; differences from control.
$^{+}$ $0.05 < P \leq 0.1$; $^{++}$ $P \leq 0.05$; differences from high dose.

between the effects of the standard and the high dose of lindane, but not with mancozeb. Ciliates showed very pronounced changes after the pesticide applications, whereas testaceans were more resistant (Table 7.10).

Strong toxicity of lindane to soil protozoa has also been reported by others (for review see Foissner, 1987a). *In vitro*, tetrahymenid ciliates may survive rather high concentrations of this insecticide, i.e. 8–100 mg l^{-1} (Komala, 1978; Dive *et al.*, 1980; Wiger, 1985). However, pronounced changes in shape and distinct inhibition of the synthesis of DNA, RNA and proteins occur at much lower concentrations, i.e. 2.5 mg l^{-1} (Wiger, 1985; Mathur and Saxena, 1986, 1988).

The results of Petz and Foissner (1989b) were confirmed by Wanner and Funke (1989) and Wanner (1991), who investigated the effects of lindane and Ripcord (insecticides against bark beetles) on the testate amoebae of a strongly acidic spruce forest soil in Germany (Table 7.5). These authors

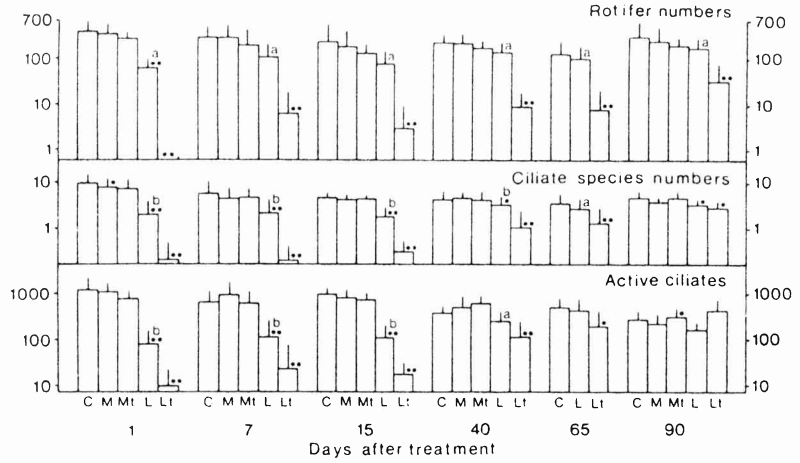

Fig. 7.7. Arithmetic means (± SD) of the abundance and species number of active ciliates and of numbers of rotifers at 0–3 mm soil depth following pesticide treatments. Control (C); standard dose: mancozeb (M), lindane (L); high dose: mancozeb (Mt), lindane (Lt). Numbers (log e) are given as individuals g^{-1} dry soil. *$0.05 < P \leq 0.1$, **$P \leq 0.05$, differences from control; a, $0.05 < P \leq 0.1$; b, $P \leq 0.05$, differences from high dose. (From Petz and Foissner, 1989b.)

used, however, a simpler experimental design than Petz and Foissner and the first samples were taken only 20 months after application of the biocides.

Rather different results were reported by Laminger and Maschler (1986), who investigated the effects of some biocides (mancozeb, parathion, paraquat) on the protozoa of a subalpine arable soil in Austria. This paper, however, is difficult to understand and very probably contains some major mistakes. Laminger and Maschler (1986) themselves suggest that their control plots were contaminated by the biocides and accordingly used an earlier investigation at another site as control plots (column 'a' in their figure 2). In my experience, it is impossible to substitute controls in this manner, because abundances and dominance structures of soil protozoa usually fluctuate so distinctly that treatment effects could be confounded.

Most of the data reviewed in the following paragraphs do not consider effects on species level and have used Singh's culture method without distinguishing between active and cystic individuals. Future research will decide whether such crude methods are of any value.

Popovici *et al.* (1977) studied the effects of the common herbicide atrazine on the soil protozoa of a maize field in Romania (Table 7.11). They found a strong, dose-dependent decline of the individual numbers, especially of the flagellates. The inhibiting effect was still present four

Table 7.9. Percentage of ecological groups of ciliates 1 and 90 days after treatment with mancozeb and lindane at normal (0.096 g m^{-2} and 6 g m^{-2} active ingredient, respectively) and high (β g m^{-2} and 60 g m^{-2} active ingredient, respectively) doses. (From Petz and Foissner, 1989b.)

			Mancozeb		Lindane	
Ecological group	Day	Control	1 ×	10 ×	1 ×	10 ×
Grossglocknerids	1	18.5	9.6**	6.7*	15.3·	0.0**
(mycophagous)	90	14.8	15.2	20.6	26.0	39.1**
Colpoda spp.	1	4.8	2.2	4.2	5.6	χ[a]
(bacteriophagous)	90	1.5	0.9	1.3	4.1··	53.6**
Other colpodids	1	40.2	55.3*	55.3	26.4··	0.0**
	90	52.8	53.4	53.5**	46.7··	4.3**
Hypotrichs	1	9.6	7.4**·	8.7	6.9·	0.0**
(omnivorous)	90	19.2	15.2	11.0	16.6··	0.8**
Remaining ciliates	1	23.6	23.9**	19.6**	38.8	χ[a]
	90	10.7	13.9	10.0**	4.1··	1.0**

[a] High value, not representative because only two individuals were found to be active.
* $0.05 < P \leq 0.1$; ** $P \leq 0.05$; differences from control.
· $0.05 < P \leq 0.1$; ·· $P \leq 0.05$; differences from high dose.

months after atrazine application. These results are at least partially supported by data of Pons and Pussard (1980), who tested six herbicides on 21 strains of naked amoebae in monoxenic cultures (Table 7.12). Siduron (up to 150 mg l^{-1}), neburon (up to 5 mg l^{-1}), diuron (up to 42 mg l^{-1}) and the synthetic phytohormone 2.4 D (up to 84 mg l^{-1}) were not toxic at the tested concentrations. Atrazine (40 mg l^{-1}), however, acted on two strains and 250 mg l^{-1} dinitroorthocresol (DNOC) inhibited growth and multiplication in all species.

Tomescu (1977) investigated the effects of the insecticide heptachlor on the protozoa of a brown earth and an alluvial–colluvial forest soil (Fig. 7.8). The substance caused a distinct decline of the individual numbers in 0–10 cm and 10–20 cm soil depth. A considerable reduction was still recognizable on three occasions in the year after application, especially in 10–20 cm and in the brown earth soil. The decline of the amoebae and ciliates was less pronounced in the upper layer of the alluvial soil, possibly due to rapid leaching of the pesticide to deeper soil layers.

Odeyemi *et al.* (1988) tested 11 agrochemicals on various groups of soil microorganisms using soil microcosms and a standard plate-count technique. Biocides were applied at recommended rates and effects were evaluated three days after application. Pentachloronitrobenzene (PCNB) and agrosan completely eliminated the protozoa in the soil, whereas

Table 7.10. Arithmetic means (± SD) of the abundance of active (A), precystic (B), and cystic (C) testaceans, of the abundance (D) and the species number (E) of viable (active + precystic + cystic) individuals (g^{-1} dry soil) and of the abundance of parasitized specimens (F) 15 and 90 days after treatment with mancozeb and lindane at normal (0.096 g m^{-2} and 6 g m^{-2} active ingredient, respectively) and high (0.96 g m^{-2} and 60 g m^{-2} active ingredient, respectively) doses. (From Petz and Foissner, 1989b.)

Days after application		Control	Mancozeb 1 ×	Mancozeb 10 ×	Lindane 1 ×	Lindane 10 ×
15	A	7833 ± 5029	8200 ± 2854	6513 ± 2817*	3817 ± 1636*	6596 ± 611*
	B	1988 ± 1828	2681 ± 2366	1638 ± 1534	2537 ± 2684	743 ± 1025
	C	1645 ± 2487	1720 ± 1696	1383 ± 597	1203 ± 570·	182 ± 446
	D	11467 ± 3593	12600 ± 3076	9534 ± 2300	7557 ± 1581**	7522 ± 5926**
	E	5.2 ± 0.8	6.5 ± 1.3	5.4 ± 0.5	5.2 ± 0.8·	3.7 ± 1.4*
	F	6342 ± 2062	6193 ± 4963	3788 ± 2361	6677 ± 3860	7465 ± 3307
90	A	5404 ± 4343	8407 ± 4639	6136 ± 646	6223 ± 2625	3898 ± 2956
	B	1725 ± 2443	2057 ± 1508	1906 ± 1576	0 ± 0	545 ± 970
	C	2569 ± 2108	3488 ± 2782	1781 ± 2294	1419 ± 664	1263 ± 796
	D	9698 ± 7398	13952 ± 4270·	9823 ± 2425	7641 ± 2873	5706 ± 4212
	E	5.4 ± 1.8	6.4 ± 2.1	4.8 ± 1.2	4.5 ± 1.3·	2.8 ± 1.2**
	F	3539 ± 1260	5160 ± 4833	3582 ± 2574	4819 ± 1730	5560 ± 2200

* $0.05 < P \leq 0.1$; ** $P \leq 0.05$; differences from control.
· $P \leq 0.05$; differences from high dose.

Table 7.11. Effects of the herbicide atrazine on protozoan populations (averages g^{-1} moist soil)[a] (from Popovici *et al.*, 1977).

Sampling			Atrazine	
Time after treatment	Organisms	Control	5 kg ha^{-1}	8 kg ha^{-1}
One month	Flagellates	16000	4000	700
	Naked amoebae	470	140	110
	Ciliates	170	120	110
Four months	Flagellates	1800	920	260
	Naked amoebae	260	120	170
	Ciliates	1400	540	210

[a] The individual numbers were estimated with a modified Singh (1946) method. Four replicate plots were sampled.

Table 7.12. Effects of the herbicides atrazine and dinitroorthocresol (DNOC) on naked amoebae in monoxenic cultures. + growth (multiplication), – no growth (from Pons and Pussard, 1980).

	Atrazine ($mg\ l^{-1}$)		DNOC ($mg\ l^{-1}$)		
Species (Strain)	40	70	10	50	250
Gephyramoeba delicatula	–	–	–	–	–
Thecamoeba similis (Pel)	+	+	–	–	–
Vannella sp. (Ru)	+	+	+	–	–
Platyamoeba placida	+	+	+	–	–
Saccamoeba sp. (Ir F1d)	Not tested		+	+	–
Thecamoeba granifera (Tr75–S2)	+	+	–	–	–
Hartmannella vermiformis	+	+	–	–	–
Vahlkampfia avara	+	+	–	–	–
Naegleria gruberi 1518/1d CCAP	+	+	–	–	–
Naegleria gruberi 113/1	–	–	–	–	–
Acanthamoeba comandoni	+	+	+	+	–
Acanthamoeba castellanii strain Neff	+	+	+	–	–
Acanthamoeba mauritaniensis	+	+	+	+	–
Acanthamoeba polyphaga	+	+	+	+	–
Acanthamoeba quina	+	+	+	+	–
Acanthamoeba rhysodes	+	+	+	–	–
Acanthamoeba divionensis	+	+	+	–	–
Acanthamoeba paradivionensis	+	+	+	–	–
Acanthamoeba triangularis	+	+	+	+	–
Acanthamoeba lenticulata	+	+	+	+	–
Acanthamoeba culbertsoni	+	+	+	–	–

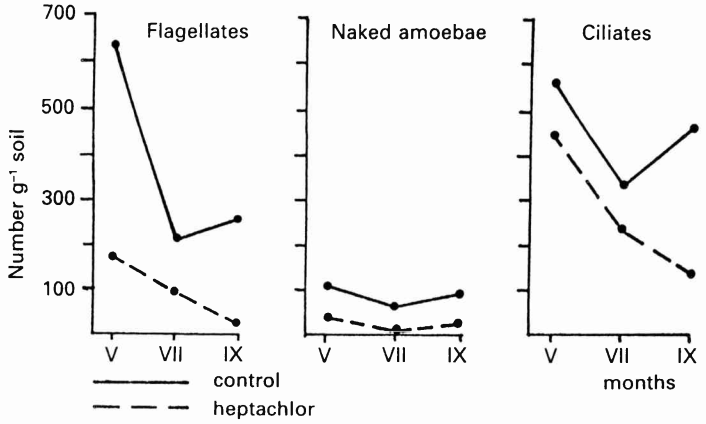

Fig. 7.8. Effects of the insecticide heptachlor (1 kg ha^{-1}) on the protozoa of a brown earth forest soil. Numbers were estimated with a modified Singh (1946) method in 10–20 cm soil depth three times in the year after application of the biocide and are the means from three plots (replicates). (From Tomescu, 1977.)

thiram and gammalin 20 reduced protozoa from 200 to 20 and 81 individuals g^{-1} soil, respectively. Benlate, brestan and vetox 85 slightly depressed the protozoan population; the herbicides (gramoxone, dacthal, preforan, dual) did not have any adverse effect. This study showed that protozoa and fungi are more susceptible to pesticides, especially fungicides and insecticides (PCNB, gammalin 20, agrosan) than bacteria and actinomycetes.

Ingham and Coleman (1984) and Ingham *et al.* (1986) tested several biocides and biocide combinations on soil protozoa in microcosms under laboratory conditions (Table 7.13). Chloroform and a combination of streptomycin (bactericide) and fungizone (fungicide) produced the most distinct decline of the protozoa, possibly due to direct action (chloroform) or by reduction of the food organisms (streptomycin + fungizone). Reactions to other treatments and under field conditions (Ingham *et al.*, 1989; Fig. 7.9) varied rather distinctly, possibly due to the methodological problems inherent to Singh's culture method and the effects of ciliatostasis (Foissner, 1987a, 1989); furthermore, the litter, which contains most protozoa, was removed in these experiments.

Rather varied effects of some pesticides on the soil protozoa of a forest were also observed by Laskauskaite (1982). As this paper is written in Russian, I am unable to review it adequately.

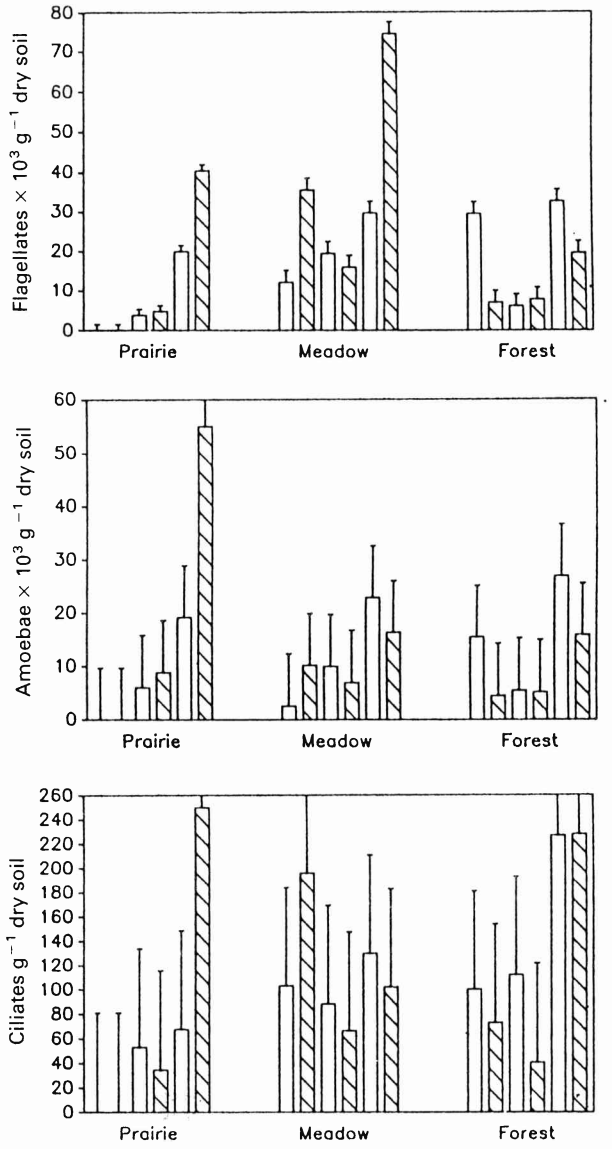

Fig. 7.9. Total (active + cystic; Singh's (1946) culture method) flagellate, amoebal and ciliate numbers in biocide (carbofuran and dimethoate; cross-hatched bars) and control (white bars) treatments in the upper soil layer (0–5 cm; litter removed) of a prairie, a meadow, and a pine forest on three sample dates. The first pair of bars within a group of three pairs represents the first sample values (about 6 months after biocide application), the second pair represents the second sample date values (about 8 months after biocide application), and the third pair represents the third sample date values (about 12 months after biocide application). (From Ingham *et al.*, 1989.)

Table 7.13. Effects of biocides on soil protozoa (from Ingham and Coleman, 1984, and Ingham *et al.*, 1986).

Biocides[a] (concentration g^{-1} soil)	Target group	Protozoa[b]		
		Naked amoebae	Flagellates	Ciliates
Streptomycin (1 mg)	Bacteria	NE	NE	DEC (1/5)
Streptomycin (3 mg)	Bacteria	NE	NE	NE
Streptomycin (3 mg)	Bacteria	INC (2/5)	INC (1/5)	DEC (2/5)
Streptomycin (30 mg)	Bacteria	DEC (2/5)	DEC (4/5)	NE
Cycloheximide (1 mg)	Fungi	VAR	NE	VAR
Fungizone (1.2 mg)	Fungi	NE	NE	DEC (1/5)
Fungizone (1.2 mg)	Fungi	DEC (2/5)	NE	DEC (2/5)
Fungizone (12 mg)	Fungi	INC (2/5)	NE	DEC (2/5)
PCNB (quintozene; 100 μg)	Fungi	NE	DEC (1/4)	NE
Captan (25 μg)	Fungi	NE	NE	NE
Cygon (0.2 mg)	Acari	NE	NE	DEC (2/5)
Diazinon (47 μg)	Insects	NE	DEC (4/4)	DEC (2/4)
Carbofuran (25 μg)	Insects + nematodes	NE	NE	NE
Chloroform (1 ml)	Protozoa + bacteria	DEC (5/5)	DEC (5/5)	DEC (5/5)
Cygon + carbofuran + chloroform (0.2 mg, 25 μg, 1 ml)	Acari + insects + protozoa	DEC (5/5)	DEC (5/5)	DEC (5/5)
Streptomycin + fungizone (3 mg, 1.2 mg)	Bacteria + fungi	DEC (3/5)	DEC (5/5)	DEC (1/5)

[a] Microcosms with 10 g air-dried soil were used and sampled on days 1, 4, 6–8, 14–15 and 22–25. Individual numbers were estimated by the culture technique of Singh (1946).
[b] DEC, decreased with respect to control ($P \leq 0.05$); INC, increased with respect to control ($P \leq 0.05$); NE, no effect of biocide, i.e. treatment not significantly different from control; VAR, significant increase at one time, decrease at another, to give variable response. Numbers in parentheses indicate number of sample dates per total number of sample dates the decrease or increase occurred.

Effects of industrial depositions and heavy metals

Tirjaková and Matis (1987) investigated the moss ciliates in polluted and relatively unpolluted regions in Czechoslovakia. A total of 58 species was found in polluted Bratislava and 105 species in relatively unpolluted Slovenia. There was some correlation between the number of species and the level of pollution in Bratislava. An increased proportion of morphological abnormalities was noticed at the very highly polluted sites; unfortunately, Tirjaková and Matis (1987) did not describe these abnormalities in detail.

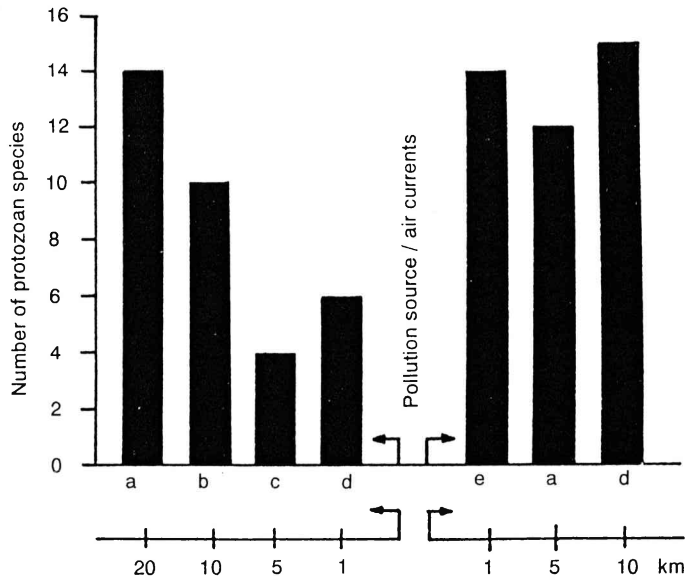

Fig. 7.10. Number of protozoan species in forest soils polluted by industrial emissions. Samples were taken at different distances from the pollution source and under different vegetation. a = *Quercus petraea*, b = *Fagus–Carpinus*, c = *Quercus robur*, d = *Fagus sylvatica*, e = *Pinus nigra*. (After Tomescu, 1987.)

Their species list also contains obvious misidentifications and the data are generally not presented in great detail. However, they look interesting and agree with those by Tomescu (1987), who investigated forest soil protozoa in a heavily polluted (SO_2, SO_3, Pb, Cd, Zn, Mn, Fe) region in Romania. The species number and diversity were correlated with both the distance from the source of aerial pollution and the prevailing wind direction (Fig. 7.10). In the most heavily polluted site only *r*-selected colpodids (*Colpoda inflata, Cyrtolophosis* sp., *Platyophrya vorax, Woodruffia rostrata*; see Foissner, 1993, for taxonomy and general ecology of colpodid ciliates) and some flagellates (*Cercobodo crassicauda, C. longicauda*) occurred. A total of only 25 protozoan species (flagellates, amoebae, ciliates) were found, indicating that the method used (agar and soil extract) was very inefficient. Furthermore, only few of the sites investigated had the same vegetation; differences could thus be confounded partially by differences in natural biotopes.

Even more doubtful are the results by Sztrantowicz (1980, 1984, 1987), who investigated the forest soil protozoa in an industrial region in Poland polluted by coal extractives and industrial emissions (dust, sulfur oxide,

Table 7.14. Infection (I) of soil invertebrates (N, number of specimens examined) with parasitic protozoa (Sporozoa) in Tanzania (7 unpolluted sites), Thailand (9 slightly polluted sites), and Germany (20 sites with high SO_2-deposits). (From Purrini, 1983.)

	Tanzania		Thailand		Germany	
Soil invertebrates	*N*	*I* (%)	*N*	*I* (%)	*N*	*I* (%)
Enchytraeidae	47	6	53	22	2600	70
Lumbricidae	72	7	66	12	1800	30
Oribatidae	981	10	318	20	5500	50
Other Acarina	199	2	88	12	no data	no data
Collembola	597	0	440	20	4500	30
Other Apterygota	193	0	89	17	no data	no data
Myriapoda	60	0	246	14	no data	no data
Other Arthropoda	175	8	135	16	2000	20

nitric oxide, heavy metals). Only 4–17 species of protozoa per site (testate and naked amoebae + flagellates + ciliates) were found, clearly indicating the incompleteness of these studies; no statistics were provided nor were active and cystic protozoa distinguished. I therefore refrain from a detailed discussion and mention only the last sentence from her 1987 paper: 'The obtained results have not revealed any helpful microbiological indicators of a degree of degradation of the environment'.

Much more convincing are the data by Purrini (1983), who made a survey on the infection of soil macro- and mesofauna with parasitic protozoa in virgin biotopes in Tanzania, in slightly polluted sites in Thailand and in heavily polluted (SO_2) sites in Germany. His results strongly suggest that SO_2-pollution dramatically increases the infection of soil invertebrates with parasitic protozoa (Table 7.14) and can be compared with the results obtained with earthworms exposed to the herbicide zeazin 50 described above (Pižl, 1985).

Pratt *et al.* (1988) evaluated the potential toxicity of leachates from several soils contaminated with organics (polycyclic aromatic hydrocarbons, e.g. fluoranthene, benzene) and/or heavy metals. Acidified tap water (pH 4.5) was used to elute toxic materials from soil columns. The leachates were used as complex mixtures in acute toxicity tests using *Daphnia* and in chronic toxicity tests using aquatic microcosms. Three classes of effects were observed. Three soil leachates showed acute and chronic toxicity at less than 3% leachate. Two of these soils were contaminated by substances used in wood preservation, and the third soil was contaminated with heavy metals and acid. Two soils showed moderately acute toxicity, but no chronic toxicity in microcosm tests. One of these soils was contaminated with low levels of chromium while the other soil was from a coal storage area (Fig.

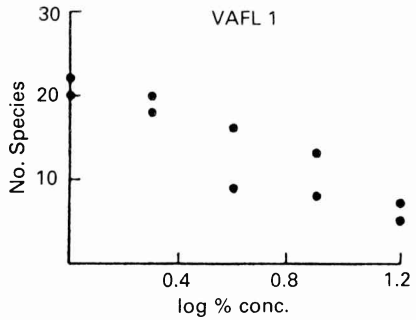

Fig. 7.11. Protozoan species survival in microcosms developed with leachates from hazardous and toxic waste sites. Control values with no leachate are plotted on the ordinate. VAFL1 = soil contaminated by heavy metals (e.g. Cu, Cd, Ni, Pb, Zn). (From Pratt *et al.*, 1988.)

7.11). The remaining two soil samples showed no toxicity in either acute or chronic toxicity tests. One of these soils was from an agricultural field used as a control while the other soil was contaminated with solvents. The failure to detect toxicity in the solvent-contaminated sample was attributed to the hydrophobicity of the toxic material. Results of these toxic screenings are in the same range as leachate toxicities estimated using other methods, although other methods use extraction materials that may interfere with some biological tests. Pratt *et al.* (1988) suggest that toxicological evaluations should be used in remedial studies of cleaned sites to ensure that toxic materials have been effectively removed from the site.

The approach by Pratt *et al.* (1988) is very useful and promising. The only problem is that they used aquatic rather than terrestrial protozoans as test organisms. In my experience, limnetic ciliates survive poorly in soils (see Table 15 in Foissner, 1987a), and soil protozoa might have a different sensitivity to test substances. For example, the soil ciliate *Oxytricha granulifera* Foissner is much more resistant to cadmium than the freshwater species, *Stylonychia lemnae* Ammermann and Schlegel (Irato *et al.*, 1991).

Very recently, Forge *et al.* (1991) used a similar method to Pratt *et al.* (1988) to study the toxicity of heavy metals with sewage sludge. Soil solutions obtained by centrifugation from experimentally contaminated soil were tested against the soil ciliate, *Colpoda steinii* Maupas (Table 7.15). No significant growth inhibition occurred with the February soil solutions. In July, however, when the soil solutions contained concentrations of zinc and nickel within the inhibitory ranges obtained in laboratory experiments with standard sulfate salt solutions, growth of *C. steinii* was significantly depressed. For all metals tested in sulfate solutions in the laboratory, growth of *C. steinii* was significantly reduced at 1.0 ppm ($P \leq 0.01$). This indicates

Table 7.15. Numbers of the ciliate *Colpoda steinii* after growth for 24 hours in soil solutions from experimental plots that received sewage sludge and heavy metals to result in metal concentrations at two times the maxima recommended in recent EC guidelines. (From Forge *et al.*, 1991, and unpublished.)

	February 1991		July 1991	
	Metal (mg l^{-1})	*C. steinii* (cells ml^{-1})[a]	Metal (mg l^{-1})	*C. steinii* (cells ml^{-1})[a]
Cadmium	0.007	118500	0.01	86000
Chromium	0.209	100600	0.194	89400
Copper	0.115	85000	0.257	89200
Lead	0.291	105000	0.106	84500
Nickel	0.287	95400	0.859	60700
Zinc	1.310	99800	2.693	65000
Control Soil	0.0	90400	0.0	97500

[a] In February, treatments and controls do not differ ($P \geq 0.05$). In July, nickel and zinc treatments differ from controls ($P \leq 0.05$, ANOVA).

that *C. steinii* is more sensitive to heavy metals than other soil organisms that have been tested, but its sensitivity is similar to that of organisms used for monitoring pollution of freshwaters, such as *Daphnia magna, Gammarus pulex* and other protozoans. This protozoan bioassay thus appears to be faster and more sensitive than the existing earthworm protocols officially approved by the EC.

Effects of road traffic

Roadsides are often polluted by heavy metal and polycyclic products from combustion engines. Grass verges along busy motorways should obviously not be used as fodder for milking cows. Only two preliminary studies are available on the protozoa of such biotopes. Lüftenegger and Foissner (1989b) studied the soil fauna in two transects at right angles to a major motorway in Austria. There was only a slight increase of heavy metals and organics (polycyclic aromatic hydrocarbons, e.g. pyrene) in the soil near the motorway and the soil fauna was richest at those sites of the transects having the highest level of total organic carbon, Pb and organics. The lowest abundances and biomasses of testate amoebae, nematodes and earthworms occurred, however, at the sites that were nearest to the motorway (Table 7.16).

Balik (1991) studied the soil testate amoebae from ten localities with different levels of road traffic pollution in Warsaw (Poland). He found a distinct loss of individuals and species near the most frequented roads (sites 1, 2, 3 in Table 7.17) as compared to natural meadows and forest soils

Table 7.16. Pedological and soil zoological parameters at two transects on meadows near a motorway (from Kasperowski and Frank, 1989, Lüftenegger and Foissner, 1989b).

	Site number[a]					
Parameters	7a	7	8	11	12	12a
Total organic carbon (%)	5.9	8.2	4.6	5.8	8.5	8.3
Nitrogen (%)	10.7	10.0	11.3	12.4	10.8	11.0
Pb (mg kg^{-1} DM)	39	30	30	21	34	29
Cd (μg kg^{-1} DM)	350	473	581	598	661	575
Chloride (mg kg^{-1} DM)	55	55	67	44	75	58
Polycyclic aromatic hydrocarbons (ppb)	181	188	160	118	198	151
Testate amoebae[b]						
Number (g^{-1} DM)	340	305	197	135	342	262
Biomass (μg g^{-1} DM)	9.7	8.9	2.5	3.0	6.6	4.3
Number of species	20	19	15	10	20	19
Nematodes[b]						
Number (g^{-1} DM)	167	190	101	95	220	211
Lumbricids[c]						
Number (m^{-2})	97	82	85	68	202	164
Biomass (g m^{-2})	32	20	16	11	60	44

[a] The motorway is between sites 8 and 11, which are both about 10 m away from this road; sites 7 and 12 are about 50 m, and sites 7a and 12a are about 100 m away from the motorway. About 11,000 vehicles per day pass these sites.
[b] Direct counts in soil suspensions diluted with water (Lüftenegger *et al.*, 1988). DM, dry mass of soil.
[c] Formaldehyde extraction.

(sites 5, 9, 10 in Table 7.17). Small and euryoecious species were dominant in the polluted sites.

Rauenbusch (1987) observed an exceptionally high number of testate amoebae in the soil near a motorway in Germany where the pine trees were completely destroyed by NaCl applied to the roads and unnaturally high moss carpets (up to 30 cm) developed. He also noted a loss of certain genera, e.g. *Trinema*. Wanner (1991) observed a slight decrease of the testate amoebae in a NaCl-treated plot (Table 7.5).

Effects of polychlorinated biphenyls

Polychlorinated biphenyls (PCBs) are rather toxic compounds and their commercial use is controlled in many countries. Food contaminated with PCB can cause the Yusho-disease (Verband der Chemischen Industrie, 1982). Steinberg *et al.* (1990) studied the effects of a commercial PCB

Table 7.17. Influence of road traffic on testate amoebae. (From Balik, 1991.)

Parameters[a]	Sites[b]									
	1	2	3	4	5	6	7	8	9	10
Number of species	8	6	6	13	28	10	9	9	21	18
Individuals cm^{-2}	6300	4200	3600	13800	55200	4500	13200	6600	40500	19200
Diversity	1.56	1.57	1.70	2.12	2.42	1.21	1.65	1.94	2.52	2.34
Evenness[c]	0.75	0.88	0.95	0.83	0.73	0.96	0.75	0.88	0.83	0.81

[a] Direct counts by the method of Lüftenegger *et al.* (1988).
[b] Sites 1, 2, 3 are near very busy roads; sites 6, 7, 8 are near busy roads; and sites 4, 5, 9, 10 are rather distant from roads.
[c] Diversity divided by ln of species number. Indicates whether the size of the diversity index is caused by an even distribution of individuals or by a high number of species. See textbooks and Wodarz *et al.* (1992) for further explanation and problems with the calculation of diversity indices.

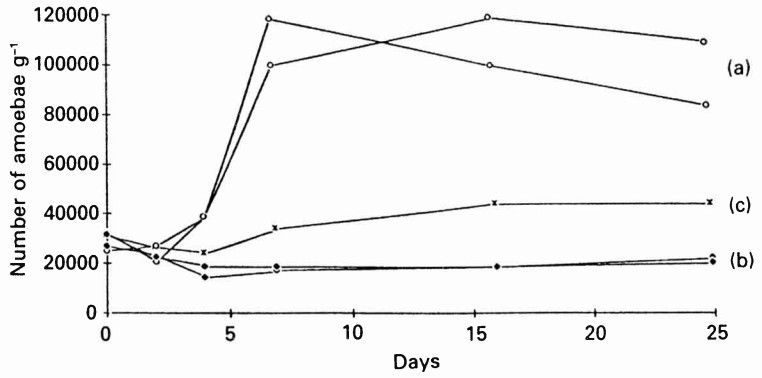

Fig. 7.12. Effect of pyralene on the growth of indigenous naked soil amoebae in microcosms (two replicates each). Numbers (active + cystic) were estimated with Singh's (1946) culture method. a = microcosms inoculated with *Azospirillum lipoferum*, b = microcosms inoculated with *A. lipoferum* and contaminated with 2500 ppm pyralene, c = control (i.e. not inoculated with bacteria and not contaminated with pyralene). (From Steinberg *et al.*, 1990.)

product, pyralene (Prodelec, France; PCBs in trichlorobenzene) on the predator/prey relationship of bacteria and naked soil amoebae. In the absence of pyralene, the inoculated bacterial population decreased from 10^7 to 10^4 cells g^{-1} dry mass of soil, i.e. was grazed by the indigenous amoebae whose number increased three-fold. In the presence of 2500 ppm pyralene, the introduced bacteria (*Azospirillum lipoferum*) survived at a higher level (3×10^6 bacteria g^{-1} dry soil) while the number of amoebae decreased slightly (Fig. 7.12). The indigenous bacterial microflora was not affected quantitatively by pyralene. Bacterial growth was inhibited and amoebae were killed in pure cultures containing 2500 ppm pyralene. Steinberg *et al.* (1990) conclude from these experiments that the active amoebae could not encyst and were killed in the contaminated microcosms.

Effects of oil pollution

The data reviewed in Foissner (1987a) indicate that crude oil does not damage the protozoan fauna of soil and that ciliates enhance the *in vitro* microbial degradation of crude petroleum; the ciliate populations in tidal sand-flats are, however, seriously disturbed by crude oil. The resistance of soil protozoa to oil stress is confirmed by a more recent study (Borisovich, 1985). The total abundance and the species richness of the protozoa were usually higher in the oil-contaminated plots than in the controls and even

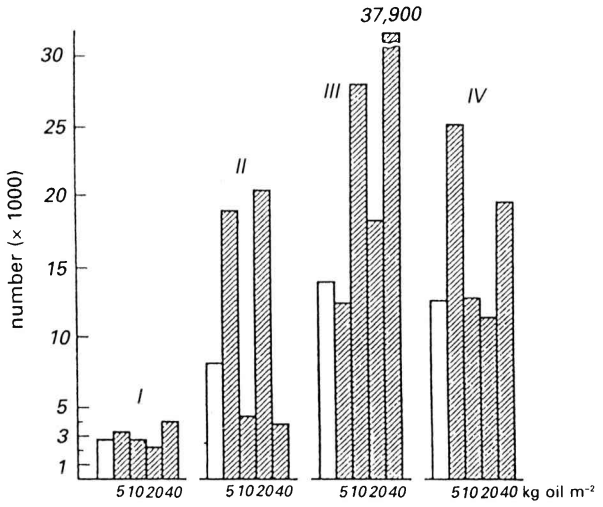

Fig. 7.13. Effects of various amounts of oil on the total number (g^{-1} dry soil) of soil protozoa. Control: unhatched; oil-contaminated: hatched. I = three days after contamination, II = two months after contamination, III = 11 months after contamination, IV = three years after contamination. (From Borisovich, 1985.)

high doses of oil had no detrimental effects (Fig. 7.13). The microbial degradation of the oil could be enhanced by fertilizing the plots with farmyard manure, urea, lime and/or superphosphate.

Effects of radioactive pollution

Most protozoa are highly resistant to ionizing radiation, viz. doses of 100–300 Ci do not produce marked effects in laboratory experiments. However, some spathidid and colpodid soil ciliates are rather sensitive, showing reduced survival at 25 kR (Foissner, 1987a). Literature on radioactive pollution and soil protozoa is sparse. The following review is mainly based on the account by Krivolutsky *et al.* (1983) who summarize a Russian experiment; see also Foissner (1987a) for further information.

300 Ci did not influence *Amoeba terrestris*, but 500 Ci produced some excitation. 1–10 Ci Co^{60} γ-radiation increased the motility and division rate in the soil ciliates *Colpoda maupasi* and *C. steinii* while 50–100 Ci decreased them; 300–500 Ci was lethal for some specimens depending on the dosage. Treatment with doses of up to 100 Ci (at a rate up to 36,000 R/min) caused death of the ciliates within 3–4 days.

Table 7.18. Species number and total (active + cystic) abundance of protozoa in an experimental plot polluted with Sr^{90} (2–3 Ci m^{-2}). (From Krivolutsky *et al.*, 1983.)

	Polluted plot		Control	
Protozoa[a]	Species number	Abundance	Species number	Abundance
Flagellates	8	17250	9	165655
Naked amoebae	8	7170	15	183785
Ciliata	1	25	1	45
Total	17	24445	25	349485

[a] Singh's (1946) culture method was used.

Korganova (1973) and Krivolutsky *et al.* (1983) studied the protozoa in the upper layer (0–5 cm) of a chernozem meadow soil experimentally polluted with Sr^{90}. In spite of the above-mentioned resistance there was a rather distinct decline in the number of individuals and species in the polluted site possibly due to the enrichment of Sr^{90} in the water film surrounding the contaminated soil particles (Table 7.18).

Recolonization of heavily disturbed soils

Re-establishment of biological activity in soils that have been subjected to gross physical or chemical disturbance is desirable as soon as possible for productivity and for aesthetic reasons. The use of indicator organisms or biochemical indices have been suggested as rapid techniques for assessing the recovery of 'soil health' following a disturbance. With regards to animals, most studies have been concerned with the meso- and macrofauna. There are, however, a few protozoological studies. Most were initiated in response to Russell and Hutchinsons' hypothesis that 'sick' infertile soils were caused by protozoan grazing on bacteria. These studies were reviewed by Foissner (1987a) and showed that recolonization of partially sterilized soils (by formalin or steam) occurs in a few months. This is substantiated by a recent, well-designed study that assessed the course of recovery in biological activity in the top 5 cm of soil cores (30 cm diameter, 30 cm deep) that had been fumigated in the laboratory with methyl bromide (Bamforth and Yeates, 1991; Yeates *et al.*, 1991). The cores were returned to their original pasture and forest sites, and untreated soils at all sites served as controls. Sampling took place 0, 1, 5, 12, 26, 54, 110 and 126 days after fumigation. Fumigation almost totally eliminated protozoa; only the flagellates *Oikomonas termo* and *Pleuromonas jaculans* and a small vahlkampfid amoeba

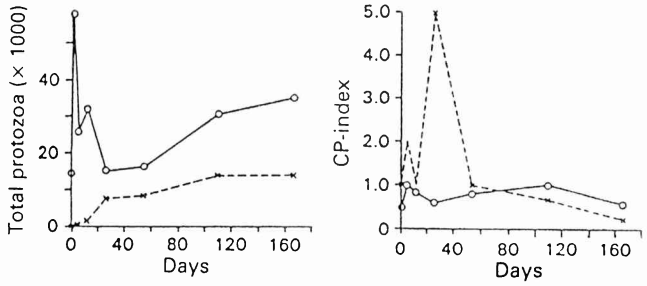

Fig. 7.14. Changes in the individual abundances (g^{-1} dry mass of soil) of protozoa and the CP-index (ratio of colpodid/polyhymenophoran ciliates) in an untreated (o——o) and a fumigated (x----x) pasture soil over 166 days. Each point is the mean of three replicates. Active + cystic naked amoebae, flagellates, and ciliates were estimated by the culture technique of Singh (1946); testate amoebae were counted in soil suspensions diluted with water. (From Yeates *et al.*, 1991.)

survived. *Bodo globosus, B. mutabilis, Colpoda inflata, C. steinii, Cryptodifflugia compressa, Difflugiella oviformis, Microcorycia flava, Platyamoeba placida, Tracheleuglypha acolla* and *Trinema lineare* were the earliest colonists. The proportion of earliest colonists to total species decreased from 100% to 50% by day 26; and the colpodid/polyhymenophoran ratio (CP-index; see Lüftenegger *et al.*, 1985) decreased below 1, suggesting a return to the original conditions (Fig. 7.14). Species richness was restored to the original by day 110.

A similar experiment was performed by Palka (1991) using, however, laboratory soil microcosms and three methods of soil sterilization (propylene oxide, autoclaving, γ-irradiation). According to this author, three groups of ciliates can be distinguished: indifferent species (*Colpoda aspera, C. inflata, C. steinii, Gonostomum affine*), semisensitive species (*Chilodonella uncinata, Holosticha adami, H. multistilata, Spathidium* sp.), and sensitive species (*Blepharisma* sp., *Drepanomonas* sp., *Urosomoida agiliformis*). These results support the suggestion of Lüftenegger *et al.* (1985) and Wodarz *et al.* (1992) that colpodids are more *r*-selected than spirotrichs.

Recolonization is slow after topsoil removal. Five to seven years after smoothing mountain ski slopes, the abundance and biomass of testaceans and nematodes were still approximately ten times lower in the centre of the ski slopes than in the neighbouring alpine pasture (for review see Foissner, 1987a). Recolonization with ciliates and nematodes can be significantly ($P \leq 0.05$) stimulated by organic fertilizers (Lüftenegger *et al.*, 1986). The ciliate numbers in the organically fertilized plots did not differ significantly ($P \geq 0.1$), but the Weighted Coenotic Index (WCI) shows that dried fungal biomass is more effective than dried bacterial biomass (Table 7.19). The

Table 7.19. Testate amoebae (upper rows) and ciliates (lower rows) in the soils of levelled, revegetated and fertilized mountain ski slopes. (From Lüftenegger *et al.*, 1986.)

Treatments[a]	*N*[b]	*S*[c]	WCI[d]	H[e]
Undisturbed alpine mat	540	23	87	2.628
	2	5	874631	1.495
Dried fungal biomass	9	2	440000000	0.693
	332	18	83	1.988
Dried bacterial biomass	1	1	calculation	useless
	225	17	237	2.153
NPK fertilizer	1	1	calculation	useless
	57	21	1614	2.525
Revegetated, unfertilized	0	0	calculation	useless
	5	9	425863	1.642

[a] Samples were collected three years after levelling, revegetation, and fertilization.
[b] *N*, individuals g^{-1} dry mass of soil; direct counts with the method of Lüftenegger *et al.* (1988). Values are arithmetic means from five sampling occasions in September 1985.
[c] Total number of species.
[d] WCI, Weighted Coenotic Index (Wodarz *et al.*, 1992). With testate amoebae, decreasing WCI values indicate improving soil conditions. With ciliates, which are inhibited by the effects of ciliatostasis in natural, evolved soils (Foissner, 1987a), decreasing WCI values usually indicate nullification of ciliatostasis and pioneer soils, respectively.
[e] Shannon–Wiener's diversity index.

marked difference between the organic and mineral fertilizers is well expressed by the Weighted Coenotic Index, whereas the Shannon-Wiener index is highest in the site fertilized with NPK. Although ciliate abundance is low, this is due to the unnatural, almost even distribution of most species (high evenness). The high value of the WCI in the revegetated, unfertilized plot is obviously caused by shortage of food. The community structure of the ciliates and the low abundance of the testaceans indicate that despite three years of recultivation the soil fauna in the levelled ski slopes was still far from natural, although there was a trend in this direction, particularly in the organically fertilized plots (Lüftenegger *et al.*, 1986).

Rosa (1956) investigated the micro-edaphon in the soil cover of slowly burning brown coal dumps. He found many species of protozoa and noted that algae colonized depleted areas as soon as the temperature had fallen below 52°C.

Overall, the results by Lüftenegger *et al.* (1986) and Yeates *et al.* (1991) suggest that protozoan and nematode populations could provide a useful medium-term ecological index of the recovery in comprehensive soil biological activity following major soil pollution or disturbance.

Summary and Conclusions

Protozoa have several unique characteristics favouring their use as bioindicators in natural ecosystems and those disturbed by man, viz., rapid growth, delicate external membranes, eukaryotic genomes, large numbers even in such ecosystems that are almost or completely devoid of higher organisms due to extreme environmental conditions (e.g. polar regions, deserts) and an almost stable and ubiquitous distribution.

This review is concerned mainly with literature after 1985 dealing with soil protozoa as bioindicators in ecosystems under human influence and the associated methodological problems. The data available show that protozoa rapidly reflect changes in their environment by changes in their population density, number of species and dominance structure.

Soil cultivation, organic farming and fertilizers usually increase the number of soil protozoa as compared with virgin lands and fallows. Prudent applications of slow-acting pH-regulators (e.g. magnesite) and natural (e.g. farmyard manure) and synthetic (e.g. dried bacterial biomass) fertilizers do not appear to disturb the soil fauna and can be recommended when the vitality and yield of crops are increased and there is no ensuing groundwater pollution.

Current evidence suggests that soil protozoa are at least as sensitive to environmental hazards (pesticides, heavy metals, etc.) as more commonly used test organisms (e.g. earthworms). There is thus a strong likelihood that protozoa can replace vertebrates in some assays. Likewise, protozoa are very rapid indicators of the recovery of biological activity in soils that have been subjected to gross physical or chemical disturbance.

Methodological problems still delay progress in soil protozoology and interfere with their use as bioindicators. The methods available for estimating the numbers of *active* soil protozoa are either rather time-consuming (direct counting in diluted suspensions) and/or unreliable (e.g. dilution culture methods). Direct examination of hundreds of fresh soil samples from various regions and under different moisture conditions showed that most protozoa are inactive (cystic) in evolved soils, except testate amoebae, which are probably the most important soil protozoans (Foissner, 1987a). Methodological problems are, however, not confined to soil protozoa (as widely assumed), but occur also in more familiar groups of soil animals, e.g. earthworms (Ehrmann and Babel, 1991).

Most studies that have used protozoa for assessing side-effects of human activities have been restricted to population densities, although there is evidence that on many occasions the community structure and species present are affected. The current inadequacy of soil protozoan systematics and lack of modern identification keys of soil protozoa seriously handicap further use of protozoa as bioindicators.

Overcoming the methodological problems is a critical requirement for such problems as teratologic testing (for a review see Schardein, 1988) and adaptation to stress (for reviews see Cairns and Niederlehner, 1989, and Nilsson, 1989). Well-designed field experiments are still rare and should help to make soil protozoology more reputable. There is, in my opinion, a genuine readiness by ecologists, governments and private companies to use protozoa in the future as rapid indicators for the re-establishment of biological activity in heavily polluted or disturbed soils and in assays for hazardous materials like pesticides and heavy metals.

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