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Abstract

This paper emphasizes some general aspects of soil protozoa as indicators of environmental quality—pros and cons, methods, and diversity. Protozoa are at the base of the heterotrophic eukaryotic food web and an essential component in marine, freshwater, and soil ecosystems because they consume a significant portion (usually >50%) of the bacterial productivity, enhancing nutrient cycles and energy flows to the benefit of microorganisms, plants and animals. Accordingly, studies of their dynamics and community structures provide a powerful means for assessing and monitoring changes in the biotic and abiotic environment. This is exemplified by some representative studies focusing on soil oxygen regime, differentiation of humus types, pesticides, global warming, forest decline, movement of protozoan pathogens in soil, and soil protozoan bioassays. Usually, protozoa are not replaceable by higher animals (meso- and macrofauna) as indicator organisms because they have unique physiological properties: they consume more food and have a higher respiration rate per mass unit, have shorter generation and life times, and reproduce much faster. Direct counting methods should be given preference over dilution culture techniques, which are beset with uncontrollable deficiencies. Thus, ciliates and, especially, testate amoebae, whose abundance and diversity can be reliably estimated in simple soil suspensions, should be preferred in environmental studies. About 1600 protozoan species are known to live in terrestrial habitats. However, data from studies of ciliates suggest that this is only a minor portion (20–30%) of the species actually present, most of which are still undescribed. Overcoming the methodological and taxonomic problems are urgent needs which, at present, limit the use of protozoa as bioindicators in terrestrial environments. Furthermore, species monographs are required to compile the taxonomic, faunistic, and ecological information available.

Keywords: Biodiversity; Bioindicators; Methods; Soil protozoa

1. Introduction

Heterotrophic protozoa have been ignored by general ecologists for a long time. Although the reason for this neglect is difficult to pinpoint, it was probably due to lack of expertise or because protozoa are more difficult to handle than larger metazoans. The situation changed rather abruptly about fifteen years ago, when marine biologists and later also freshwater limnologists recognized the importance of protozoa in the pelagic food chains and created the concept of the 'microbial loop', which suggests that a considerable fraction of the reduced carbon is mineralized before it reaches large plankton organisms such as copepods and fish (Fenchel, 1987; Berninger et al., 1991; Schönborn, 1992a; Gaedke and Straile, 1994). It is now clear that most of the bacterial production is consumed by small protozoa (mainly heterotrophic nanoflagellates, HNF), which in turn serve as food for larger protozoa (mainly ciliates) and small metazoa. This also applies

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for streambed sediments, in which 80 to 183% of the annual bacterial productivity is consumed by protozoa (Bott and Kaplan, 1990) and protozoan production amounts to up to 30% of that of the macrozoobenthos (Schönborn, 1992b).

The exciting results obtained by marine and freshwater ecologists also rejuvenated research on soil protozoa (Foissner, 1987a; Darbyshire, 1994; Ekelund and Rønn, 1994). Although the exact contribution of protozoa to the soil energy flux is still under discussion, it is clear that they enhance flows of nutrients in soil to the benefit of microorganisms, plants and animals. Estimates from several independent food web studies confirm that protozoa, on average, are responsible for about 70% of the soil animal respiration (Foissner, 1987a) and for 14–66% and 20–40% of the C and N mineralization, respectively (Ekelund and Rønn, 1994; Griffiths, 1994). Accordingly, decomposition is delayed by at least 33–39% (8–18 months) if soil animals are excluded from the decomposer cycle (Beck, 1989). Last but not least, protozoa play a significant role in earthworm nutrition (Bonkowski and Schaefer, 1996) and may stimulate bacterial antagonism, which in turn could provide the basis for a novel approach to the biological control of plant diseases (Pussard et al., 1994).

This very brief overview shows protozoa as an essential component in all major ecosystems. Thus, their dynamics and community structures should be powerful indicators of changes in the biotic and abiotic environment. In fact, protozoa have been widely used as indicators in various freshwater ecosystems since the turn of the century (for reviews, see Curds, 1992; Foissner et al., 1994). In soil, protozoa are still rarely used as indicators, although reviews of the literature have catalogued about 200 pertinent papers demonstrating the usefulness of protozoa to indicate changes in the soil environment (Foissner, 1987a, 1987b, 1994, 1997a; Aescht and Foissner, 1991; Foissner and Wanner, 1995). Thus, the present review should stimulate applied soil ecologists to exploit more widely and successfully the indicative potential of soil protozoa.

2. Protozoa as indicators of soil quality: pros and cons

Bioindicators are, in a broad ecological sense, organisms that can be used for detection and qualitative and/or quantitative characterization of a certain environmental factor or of a complex of environmental factors; a narrower definition confines bioindicators to human influences (Bick, 1982). Of course, indication is dependent on the overall biology of the organisms, especially on some basic physiological properties. As compared to the meso- and macrofauna, protozoa and small metazoans (i) consume more food per mass unit, (ii) have a higher respiration rate per mass unit, (iii) have shorter generation and (iv) life times, and (v) reproduce faster (Sommer, 1994). Thus, the various groups of organisms have different indicative properties, i.e., are not, or usually are not, equivalent and replaceable. Accordingly, a well-designed bioindication study should contain at least one group each of the micro-, meso- and macrofauna. For instance, a comparative study on the effects of conventional and organic farming on soil animals revealed that testate amoebae were significantly reduced in the conventional plots, while earthworms remained unchanged (Foissner, 1992).

Furthermore, indication must be 'directed', i.e., the indicator group(s) must be chosen according to the peculiarities of the habitat and the goal of the study, and not, as frequently recognizable, according to the facilities and expertise available. If, for instance, the effects of a certain fertilizer on the soil fauna of a spruce forest are to be investigated, then it will be crucial that protozoa, especially testate amoebae, are included in the set of bioindicators because they are highly abundant in such habitats, from which other potential bioindicators such as most earthworms are excluded due to the high acidity (Aescht and Foissner, 1995a).

The pros and cons of using heterotrophic soil protozoa as bioindicators can be summarized as follows (Aescht and Foissner, 1991; Foissner, 1994):

1. Protozoa are an essential component of soil ecosystems, because of their large standing crop and production (see Table 2). Changes in their dynamics and community structures 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 and excellent tool in bioassays. Results are

obtained within 24 h, faster than with any other eukaryotic test system (see Chapter 5.3).
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 the prokaryotes.
4. Morphologic, ecologic, and genetic differentiation among globally distributed protozoan species is low, at least in the few soil ciliates and testate amoebae studied so far (Bowers and Pratt, 1995; Bowers et al., 1997a, 1997b; Wanner et al., 1997; Xu et al., 1997), suggesting that many indicator species can be used worldwide.
5. 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 (Foissner, 1987a).
6. Protozoa are not readily dislodged in soil (Kuikman et al., 1990). Many (but not all) are ubiquitous and are useful for comparing results from different regions. Differences in patterns of distribution are almost entirely restricted to passive vertical displacement; thus, the difficult problem of horizontal migration, especially with the epigaeon, does not affect the investigations.

There are, however, several factors that have apparently restricted the use of soil protozoa and soil animals in general as bioindicators:

1. There is an immense number of species, many of which have not yet been described; more than 1000 may occur in a square metre of forest soil (Schaefer, 1985). 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 (see Chapter 3).
3. Animals need other organisms for food. Thus, the constellation of factors is more complicated than in plants and indication 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.
5. There is a lack of comprehensive, easy-to-use identification literature.

3. Methodological pitfalls

A detailed discussion of some basic methodological tools and problems has been presented by Foissner (1994). Thus, here I shall concentrate on a recent study by Berthold and Palzenberger (1995), which confirms my previous, widely neglected assertion (Foissner, 1987a) that the widely used dilution culture techniques provide highly unreliable estimates of the number of active protozoa in natural soil samples; at best, such methods can provide some estimation on the number of active and cystic protozoa.

Berthold and Palzenberger (1995) performed a comparative study on the abundances of ciliates obtained with the dilution culture technique of Singh (1946) and the direct counting method by Lüftenegger et al. (1988) in three contrasting habitats—a meadow topsoil, spruce litter, and beech litter. The dilution culture method overestimated the number of active ciliates by orders of magnitude, with an average factor of 2000 (Table 1). One main problem of the dilution culture methods is that it is difficult to meet the assumptions of the MPN (most probable number) estimation model on which they are based. Furthermore, active and encysted cells cannot be separated properly with the methods available. There is thus a wide range of systematic errors causing the MPN estimates to become highly biased. But even if the basic assumptions could be met, the maximal precision of MPN estimates is far too low for a useful application within the context of soil ecology, as revealed by the confidence limits. Subjecting the data to analysis of variance showed that the three contrasting habitat types could be clearly distinguished by the ciliate numbers obtained with direct counting, whereas those obtained with the dilution culture method could not distinguish them (Table 1). The MPN estimates did not even correlate significantly with the direct counts.

The data by Berthold and Palzenberger (1995) show convincingly that active protozoa should be counted with direct methods, either in soil suspensions (Lüftenegger et al., 1988) or with the millipore filtering technique of Coûteaux (1967, 1975), which is, according to Lousier and Parkinson (1981), more efficient than the agar film technique of Heal (1964, 1971). Detailed protocols for these methods are to be found in Coûteaux (1967, 1975), Lousier and Parkinson (1981), Coûteaux and Palka (1988), Lüftenegger

Table 1
Mean numbers of active ciliates per gram dry meadow soil (0–5 cm), spruce litter (0–2 cm) and beech litter (0–2 cm) (from Berthold and Palzenberger, 1995)

Sample	Direct counts[a]		MPN simplified[b]		MPN complete[c]	
	Mean[d]	CV (%)[e]	Mean[d]	CV (%)	Mean[d]	CV (%)
Meadow ($n = 7$)	60*	80	102 000*	177	88 000*	207
Spruce ($n = 3$)	400+	57	79 000*	70	71 000*	93
Beech ($n = 3$)	8100++	13	136 000*	56	127 000*	65

[a] As described in Aescht and Foissner (1995b).
[b] Dilution culture method by Singh (1946) and most probable number (MPN) equation by Fisher (1922).
[c] Dilution culture method by Singh (1946) and refined most probable number (MPN) equation as contained in computer programs (for details, see Berthold and Palzenberger, 1995).
[d] Values followed by the same symbol are not significantly different ($P \leq 0.05$).
[e] Coefficient of variation.

et al., (1988), Schönborn (1989), and Aescht and Foissner (1995b).

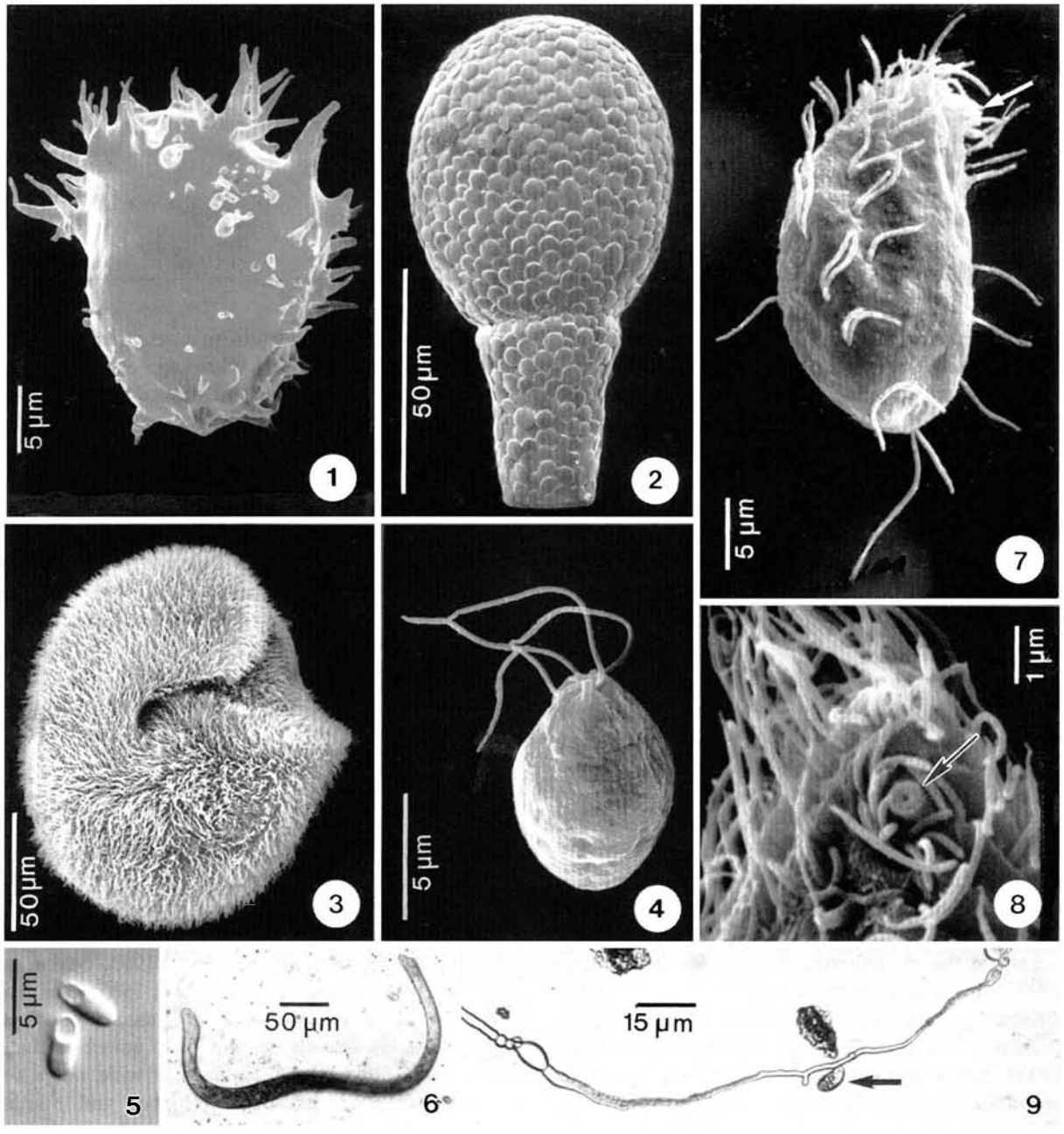
Abundances and species diversities of ciliates and testate amoebae can be reliably estimated with the direct investigation methods cited above. The abundances obtained are underestimates, on average 15% for testate amoebae and 30% for ciliates (Aescht and Foissner, 1995b). Unfortunately, direct counts are inappropriate for naked amoebae and flagellates because many of them are very small and adhere strongly to the soil particles. As a consequence, only ciliates and testate amoebae can be recommended for environmental studies at the present state of knowledge. New methods need to be developed for naked amoebae and flagellates because, as shown above, dilution culture techniques are beset with uncontrollable deficiencies and therefore fail to provide reliable data on standing crop and biodiversity. There is hope that such methods can be developed. For example, Mommertz (1997) found the density gradient method of Griffiths and Ritz (1988) useful in estimating the temporal and spatial dynamics of naked amoebae and flagellates in arable soils. Unfortunately, cells become rather distorted by the procedures involved and thus can hardly be determined to genus or species level.

4. Notes on the biology of soil protozoa related to their use as bioindicators

Traditionally, soil protozoa are classified as naked amoebae (Fig. 1), testate amoebae (Fig. 2), flagellates (Fig. 4), ciliates (Figs. 3, 6, 7, 10–12), or sporozoans (Fig. 5). General morphology and ecology of the various groups of protists are described in textbooks (e.g., Fenchel, 1987 and Hausmann and Hülsmann, 1996) and the book edited by Darbyshire (1994). Compilations on representative abundances in various soils and habitats are provided by Heal (1971), Foissner (1987a) and Cowling (1994). Here, I shall highlight, very briefly, some peculiarities related to the use of protozoans as bioindicators.

4.1. Naked amoebae

About 60 species have been recorded to occur in soil (Page, 1976). It is very likely that this is only a small portion of the species actually present (see above). The 'naked amoebae' are not a monophyletic assemblage but belong to several phyla having different ecological properties (Cavalier-Smith, 1997). Many soil protozoologists consider the naked amoebae as the most important group of soil protozoa due to their often great abundance; numbers between 2000 and 2 000 000 g^{-1} dry mass of soil have been reported. However, all estimates are from dilution culture methods and thus highly questionable, especially regarding the active portion (Berthold and Palzenberger, 1995); possibly, most are in an inactive (cystic) condition. Unfortunately, most soil naked amoebae are very small (<30 μm) and attached to soil particles. Thus, they cannot be counted in simple soil suspensions to obtain information about the active and cystic proportion. Certainly, some or many are active for at least some time. The common soil naked amoebae feed mainly and partially selectively on bacteria. With their tiny and flexible pseudopodia they can exploit micropores having a diameter of only 1 μm (Foster and Dormaar,



Figs. 1–9. Main types of soil protozoa in the scanning electron microscope (Figs. 14, 7, 8) and light microscope (Figs. 5, 6, 9). 1: *Mayorella* sp., a naked amoeba with cone-shaped pseudopodia mainly in the anterior body half; 2: *Nebela vas*, a Gondwanan testate amoeba. The shell is made of circular platelets originating from its prey, another testate amoeba, *Trinema lineare*; 3: *Bresslauides discoideus*, a large colpodid ciliate possibly restricted to terrestrial habitats of Laurasia; 4: *Polytomella* sp., a common heterotrophic soil flagellate; 5: *Ciliatosporidium platyophryae*, a microsporidian parasite affecting the soil ciliate *Platyophrya terricola*; 6: *Circinella arenicola*, a long and slender soil ciliate easily confused with small nematodes; 7–9: *Pseudoplatyophrya nana* (7, 9) and *Grossglockneria acuta* (8) are colpodid ciliates which evolved a minute, highly complicated feeding tube (arrows in Figs. 7, 8) to penetrate the cell wall of yeasts and fungi (Fig. 9, arrow). They cannot feed on other items.

Table 2
Comparison of population parameters for soil testaceans from different sites and biotopes (from Foissner, 1987a)

Parameter	Moss under beech forest (moder)	Ash-maple forest (mull)	Alpine rendzina (moder)	Alpine mat (moder)	Aspen woodland (mor)	Beech forest (mull)
Annual mean density ($\times 10^6$ m^{-2})	1.7	33.6	39.6	20.1	261	84
Annual mean biomass (mg m^{-2})	15.5	1033	2209	1165	723	1715
Number of generations (per year)	16.0	12.5	n.d.	8	90	n.d.
Mortality rate (% per day)	3.0	8.5	n.d.*	n.d.	10.8	n.d.
Production numbers ($\times 10^6$ m^{-2})	145	940	29	118	90 930	358 000
Production biomass (g m^{-2})	0.11	25.9	1.4	5.4	206	73
Biomass turnover (P_B/B)	8.1	43.9	0.7	5	285	43

* n.d. = not determined.

1991). However, there are also many mycophagous naked amoebae. Some of them feed by perforation lysis, producing discrete holes, 0.2 to 6 μm in diameter, in the fungal cell wall, like the mycophagous ciliates (Old and Chakraborty, 1986; Foissner, 1987a). There is evidence from pot experiments that such activities can reduce inoculum levels of plant pathogenic fungi in soils and can reduce the severity of take-all disease of wheat by *Gaeumannomyces graminis* var. *tritici* (Old and Chakraborty, 1986).

4.2. Testate amoebae

This group comprises amoeboid organisms with a shell (test) either produced by the cell itself (often composed of silicium scales = idiosomes), or composed of foreign particles (e.g., sand grains = xenosomes) agglutinated to a membranous sheath. We now know that the testate amoebae are at least biphyletic: the idiosome-bearing, filose taxa (e.g., *Euglypha*) belong to the phylum Rhizopoda, whereas the xenosome-bearing, lobose forms (e.g., *Difflugia*) belong to the phylum Amoebozoa (Cavalier-Smith, 1997). This is in accordance with the often distinctly different autecology of filose and lobose testaceans (Foissner, 1987a; Schönborn, 1992a; Wodarz et al., 1992). About 300 species and many varieties have been found in terrestrial environments (Bonnet, 1964; Chardez and Lambert, 1981; Foissner, 1987a), some of which have a restricted Laurasian or Gondwanan distribution, e.g., *Nebela (Apodera) vas* (Fig. 2). Standing crop numbers are moderately high, with 100–1000 individuals per gram dry mass in mineral soils, 1000–10 000 in meadow topsoils and grasslands, and 10 000–100 000 in leaf litter. However, as many testate amoebae species are rather voluminous, their standing crop and production biomass often surpasses that of all other protozoans (Cowling, 1994; Table 2). Thus, their omission in some recent ecosystem studies is an unfortunate mistake (e.g., Bloem et al., 1994).

In my opinion, testate amoebae are very useful indicator organisms in a wide range of terrestrial biotopes, not only because they are more easily counted and identified than the other soil protozoans, but also because of their high biomass and considerable abundance. Furthermore, they have a considerable species and lifestyle diversity and a distinct and deep vertical distribution. Accordingly, they can indicate a wide range of biotic and abiotic variables, so much the more in that rather detailed autecological data are available from most of the common species.

4.3. Flagellates

About 260 species have been recorded from soils worldwide, most were first or subsequently found in limnetic or coprozooic biotopes (Foissner, 1991). However, new species are described rather steadily from soil (Foissner and Foissner, 1993; Verhagen et al., 1994; Ekelund and Patterson, 1997), indicating that we possibly know only a small proportion of the species actually present. Like amoebae, flagellates are polyphyletic, showing a wide range of morphologies and ecologies (Hughes and Smith, 1989; Cavalier-Smith, 1997). Many soil protozoologists consider the flagellates, together with the naked amoebae, as the most important group of soil protozoa due to their often great abundances and short generation times (usually <5 h under laboratory conditions); numbers between 0 and 10^6 g^{-1} of soil have been reported. However, as with the naked amoebae, most counts rely on culture techniques and MPN esti-

Table 3
Diversity of soil ciliates in Europe, Africa, Australia, and Antarctica (from Foissner, 1997b)

Characteristics	Europe[a] (99 samples)	Africa (92 samples)	Australia (157 samples)	Antarctica (90 samples)
Total number of species[b]	345	507	361	95
Species/sample (mean)	26	35	23	4
Undescribed species	185	240	154	14
Undescribed species (%)	54	47	43	15
Undescribed species/sample (mean)	1.9[c]	2.6	1.0	0.2
Undescribed species/sample (%)	7.2	7.5	4.4	3.9

[a] For comparison only because, in about half the samples, the investigation method and data collection are not directly comparable with those used in the other regions.
[b] Each sample collective was considered as a unique entity. Thus, a new species found, for instance, in both Africa and Australia, was classified as 'undescribed' in each of the data sets.
[c] Sample size (n) = 52.

mates which do not give an accurate indication of the active cells present (see Chapter 3). The few direct counts available indicate that most are in an inactive encysted condition (Foissner, 1991).

Ecologically, flagellates have much in common with the naked amoebae: most feed on bacteria, are small (< 20 μm), and have an amoeboid flexibility that allows them to inhabit even very small soil pores which cannot be exploited by larger protozoans, such as most testate amoebae and ciliates. For example, Hattori (1988) isolated flagellates from 80% of the 1–2 mm sized soil aggregates examined.

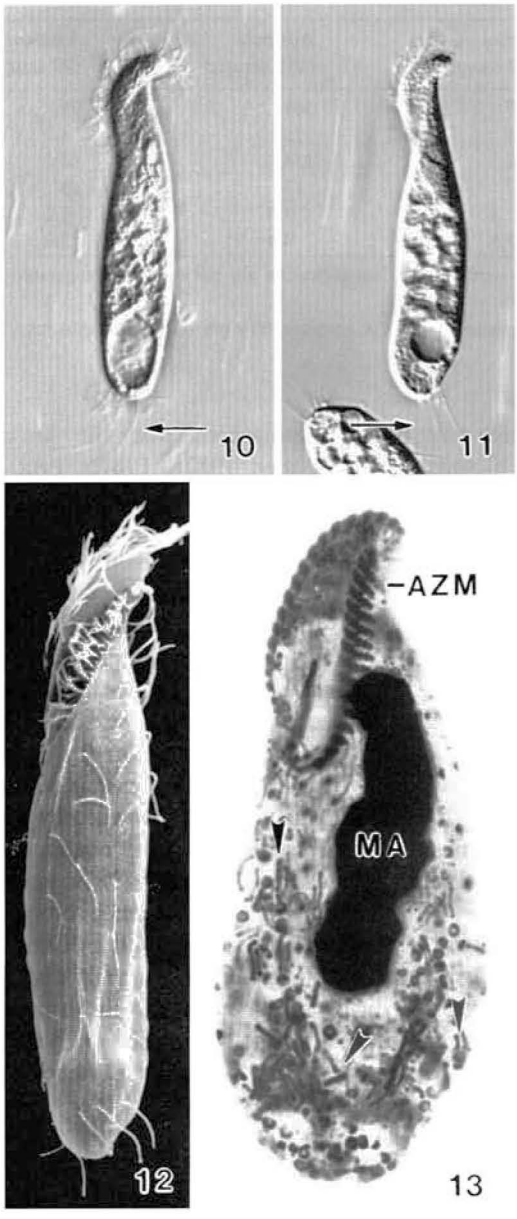
4.4. Ciliates

The ciliated protozoa comprise unicellular, heterokaryotic organisms having a macronucleus and a micronucleus of distinctly different size and function within the same cytoplasm. The macronucleus, which is usually highly polyploid, divides amitotically during asexual reproduction and controls mainly somatic functions (e.g. RNA synthesis). The diploid micronucleus is active mainly during sexual reproduction (conjugation). Diversity of ciliates is high in terrestrial habitats, that is, at least 2000 species, 70% of which have not yet been described (Foissner, 1997b, 1998; Table 3). Most soil ciliates feed on bacteria (39%) or are predaceous (34%) or omnivorous (20%). Some, however, are strictly mycophagous and highly characteristic for terrestrial habitats (Figs. 7–9); a few (mainly metopids) are anaerobic, providing a simple tool to assess the soil oxygen regime (see Chapter 5).

Soil ciliates have a particular vertical distribution, which must be taken into account when they are used as bioindicators (Foissner, 1987a, 1987b; Petz and Foissner, 1988). In contrast to the testate amoebae, appreciable numbers of active ciliates occur only in the uppermost litter layer, where abundances of up to 10 000 individuals per gram dry mass of litter are reached (Table 1). In the humus horizon and in mineral soils active ciliates are rare, although many cysts are present. Thus, habitats such as meadow topsoils and arable lands contain very few active ciliates, usually less than 100 individuals per gram dry mass of soil (Foissner, 1987a; Table 1). The reasons for these distinct distributions are not yet known; obviously, excystment is suppressed under field conditions in evolved soils ('ciliatostasis', Foissner, 1987a; Petz and Foissner, 1988). Thus, the use of ciliates as bioindicators is usually confined to habitats with a distinct litter layer, e.g. forests (Chapter 5) or heavily disturbed soils, where ciliates are an excellent tool to monitor recovery of soil health following major soil pollution or disturbance (e.g., topsoil removal; Lüftenegger et al., 1986; Foissner, 1994).

4.5. Sporozoans

This group, which contains only parasitic organisms, has rarely been used as bioindicators. There are, however, at least three studies available (reviewed in Foissner, 1994), which suggest that they are very useful for this purpose. Pižl (1985) found a significantly increased infection of earthworms by monocystid gregarines when the earthworms were exposed to an herbicide, zeazin 50, for 26 weeks. The same



Figs. 10–13. *Metopus hasei*, a common ciliate in insufficiently aerated soils in vivo (10, 11), in the scanning electron microscope (12), and after silver impregnation (13). 10–12: *M. hasei* is about 100 μm long and has caudal cilia (arrows), which were not preserved in the SEM; 13: Metopid ciliates lack mitochondria and have many rod-shaped methanogenic bacteria in the cytoplasm (arrowheads). AZM: adoral zone of membranelles, MA: macronucleus.

was observed in roadside soils polluted by heavy metals (Pižl and Sterzyńska, 1991). Purrini (1985) reported a dramatically increased infection of soil invertebrates with parasitic protozoa (gregarines, coccidids, microsporids) in regions with high SO_2 deposits. Certainly, these are quite interesting data, not only from the bioindicative point of view, but also because they indicate that protozoa may play a significant role in regulating densities of soil invertebrates. In fact, sporozoans also parasitize soil ciliates, albeit rarely (Fig. 5; Foissner and Foissner, 1995).

5. Examples of using soil protozoa as bioindicators

Much of the literature regarding soil protozoa as bioindicators in natural and human-influenced ecosystems has already been extensively reviewed (Lee, 1986; Aescht and Foissner, 1991; Foissner, 1987a, 1987b, 1990, 1994, 1997a; Foissner and Wanner, 1995). A few representative examples from these compilations and some very recent data and developments will be discussed in the following paragraphs. The examples emphasize the need for species identification, i.e., investigation not only of total individual and biomass changes, if results obtained with bioindicators are to be meaningful and reproducible.

5.1. Soil protozoa as indicators in natural ecosystems

5.1.1. Oxygen regime

Certain ciliates are excellent and simple bioindicators for the soil's oxygen regime, especially for periodic or sporadic oxygen depletion, which is often difficult to detect with conventional physicochemical methods. The indication is based on metopid heterotrichs, which can live and reproduce only under microaerobic and/or anaerobic conditions because they lack typical mitochondria (for review, see Foissner et al., 1992). Metopids live in symbiosis with methanogenic bacteria (usually *Methanobacterium formicicium*), using the hydrogen gas produced in the ciliates' hydrogenosomes, which are very likely highly modified mitochondria (Finlay and Fenchel, 1991). The most common soil metopid is *Metopus hasei*, a slender, medium-sized (70–120 μm) species. The methanogenic bacteria can be visualized by silver carbonate impregnation (Figs. 10–13).

Table 4
Occurrence of metopid (anaerobic) ciliates in various soils and habitats

Sites	Habitat	Soil depth investigated (cm)	No. of samples investigated	No. of metopid species[a]	References
1	Beech forest in Salzburg, Austria	0–10	5	0	Foissner, unpublished
2	Beech forest in Lower Austria	0–10	10	0	Foissner et al. (1985)
3	Spruce forest in Upper Austria	0–3	48	0	Aescht and Foissner (1993)
4	Two bottomlands in Lower Austria	0–10	20	2	Foissner et al. (1985)
5	Two xerothermic, treeless sites within Site 4	0–10	20	0	Foissner et al. (1985)
6	Two ploughed fields near Sites 4 and 5	0–10	20	0	Foissner et al. (1985)
7	Alpine mat in Austria	0–10	8	0	Foissner (1981)
8	Eutrophified alpine mat in Austria	0–10	3	1	Foissner (1981)
9	Swamp soil near Cairns, Australia	0–10	1	6	Foissner, unpublished
10	Corn field near Site 9	0–10	1	0	Foissner, unpublished
11	Two floodplain rain forests in Manaus, Brazil	0–10	2	10	Foissner (1997c)
12	Two terra firma rain forest soils near Site 11	0–10	2	0	Foissner (1997c)
13	Municipal landfill sites in England	50–800	>10	1	Finlay and Fenchel (1991)

[a] Cultivated with the non-flooded petri dish method as described in Foissner (1987a).

Table 4 shows a selection of habitats (soils) which are known to be usually aerobic (e.g., forest litter, ploughed fields) or anaerobic for some time (e.g., flood plains). Metopids occur only in the anaerobic soils. The eutrophic (by cattle dung and urine) pasture is of special interest, showing that indication by metopids is not trivial and could be helpful in detecting oxygen deficits that are not particularly obvious. Certainly, more detailed investigations are needed, but the available field evidence looks highly suggestive.

5.1.2. *Differentiation of humus types*

The important role of the humus type in the distribution of soil animals and for soil fertility is well known. Relevant data on soil protozoa were provided by several authors and reviewed by Foissner (1987a). Unfortunately, the subject has received little attention during the past decade.

The most valuable protozoan humus indicators are several species of testate amoebae; for ciliates the data are still preliminary (Table 4). Although the indicator species do not occur exclusively in a certain type of humus, their incidence and individual numbers are mostly low outside their preferred habitat. Even subtle differences in the humus type, which frequently pose problems to soil scientists, are nicely distinguished by the testate amoebae (Tables 5 and 6). Mull and mor are clearly distinguishable also by the relation of full (alive) and empty (dead) testaceans, whose shells decompose markedly slower in mor than in mull soils: $\leq 1:2$ in mull, 1 : 2 to 1 : 10 in moder, and $\geq 1:10$ in mor (Foissner, 1987a; Schönborn, 1973).

5.2. *Soil protozoa as indicators in ecosystems under human influence*

5.2.1. *Pesticides*

Petz and Foissner (1989) 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). The effects were evaluated 1, 7, 15, 40, 65 and 90 days after application of a standard or high (10x) dose. 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. However, the community structure of ciliates was still slightly altered after 90 days. Mycophagous ciliates (e.g. *Pseudoplatyophrya nana*) were substantially reduced in the first weeks after application of the fungicide (Table 7). Testaceans were not reduced before Day 15 with the higher dose or before Day 40 with the standard dose ($0.05 < P \leq 0.1$). The 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

Table 5
Species characteristic of the testacean and ciliate communities in mull and mor soils (from Foissner, 1987a and Aescht and Foissner, 1993)

Type of humus	Characteristic species	
	Testaceans	Ciliophora
Mull	*Centropyxis plagiostoma*	*Urosomoida agilis*
	Centropyxis constricta	*Urosoma* spp.
	Centropyxis elongata	*Hemisincirra filiformis*
	Plagiopyxis minuta	*Engelmanniella mobilis*
	Geopyxella sylvicola	*Grossglockneria hyalina*
	Paraquadrula spp.	*Colpoda elliotti*
Moder and mor	*Trigonopyxis arcula*	*Frontonia depressa*
	Plagiopyxis labiata	*Bryometopus sphagni*
	Assulina spp.	*Dimacrocaryon amphileptoides*
	Corythion spp.	*Avestina ludwigi*
	Nebela spp.	

Table 6
Dominance (%) of testacean species characteristic of mull and mor/moder (cp. Table 5) in some alpine pseudogleys (from Foissner, 1987a)

Sites[a]	A	B	E	C	D
Type of humus	Mull-like moder			Moder	
C/N ratio	9.9	10.4	10.2	10.2	13.9
Plagiopyxis minuta	0.4	0.0	5.6	0.0	0.0
Centropyxis plagiostoma	0.4	0.0	2.3	0.0	0.0
Centropyxis elongata	1.8	6.0	0.9	0.4	0.0
Trigonopyxis arcula	0.0	0.0	0.0	1.1	1.6
Nebela parvula	0.0	0.0	0.0	0.0	1.9
Corythion spp.	0.0	0.0	0.5	1.4	1.3
Assulina spp.	1.3	1.7	0.0	2.2	4.5
Plagiopyxis declivis	22.2	14.8	17.9	12.7	7.2

[a] A: grazed alpine pasture; B: little grazed alpine pasture; C: isolated alder stand; D: alder stand mixed with dwarf shrubs and spruces; E: heavily eutrophic alpine pasture (*Rumicetum alpini*).

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 became more abundant 90 days after treatment with lindane, 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). Generally, there were marked differences 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.

5.2.2. *Global warming*

Treonis and Lussenhop (1997) grew *Brassica nigra* at either ambient or twice-ambient CO_2 levels within open-top chambers in the field for 4 weeks. Plant biomass, above and below ground, was unaffected by increased CO_2. Direct count bacterial density was also unchanged under increased CO_2. Flagellate density tended to increase, whereas the number of naked amoebae significantly declined under increased CO_2 (Fig. 14). Treonis and Lussenhop (1997) suggested that these changes were caused by a trophic transfer of the increased CO_2 fertilization effect through the soil food chain. Unfortunately, the protozoan counts of Treonis and Lussenhop (1997) are based on a culture method which is known to give highly ambivalent results (Berthold and Palzenberger, 1995); thus,

Table 7
Percentage of the dominant species of active ciliates and testate amoebae in spruce forest litter 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, 1989)

Species	Day	Control	Mancozeb[a,b]		Lindane[a,b]	
			1x	10x	1x	10x
Ciliates						
Avestina ludwigi Aescht and Foissner[c]	1	23.5	44.3**	37.4	15.2**	0.0**
	90	41.3	45.8+	42.9	26.7++	0.0**
Platyophrya spumacola Kahl	1	19.4	12.7	21.7	12.1	0.0**
	90	13.1	9.8	13.6	22.4+	4.4**
Pseudoplatyophrya nana (Kahl)	1	18.5	10.0*	6.0	15.2	0.0**
	90	10.8	13.6	17.7	24.2**	35.0**
Colpoda steinii Maupas	1	4.5	1.4	4.0	6.1	χ^d
	90	0.0	0.0	0.7	2.5++	44.8**
Colpoda inflata (Stokes)	1	0.1	1.0	0.5	0.0	0.0
	90	0.8	0.0	0.7	0.6+	9.3**
Testate amoebae						
Corythion dubium Taranek	1	48.1	74.6*	63.3	46.0	51.3
	90	47.9	52.2	48.0	39.7	32.7
Trinema lineare Penard	1	11.3	7.5	0.0	18.7	12.2
	90	9.6	17.5	17.8	18.3**	15.7
Schoenbornia humicola (Schönborn)	1	0.0	0.0	2.8	3.0	8.7
	90	9.6	9.7	6.4	6.9	17.0

[a] * $0.05 < P \leq 0.1$; ** $P \leq 0.05$; differences from control.
[b] + $0.05 < P \leq 0.1$; ++ $P \leq 0.05$; differences from high dose.
[c] Misidentified as *Hausmanniella discoidea* by Petz and Foissner (1989).
[d] High value, not representative because only two individuals were found to be active.

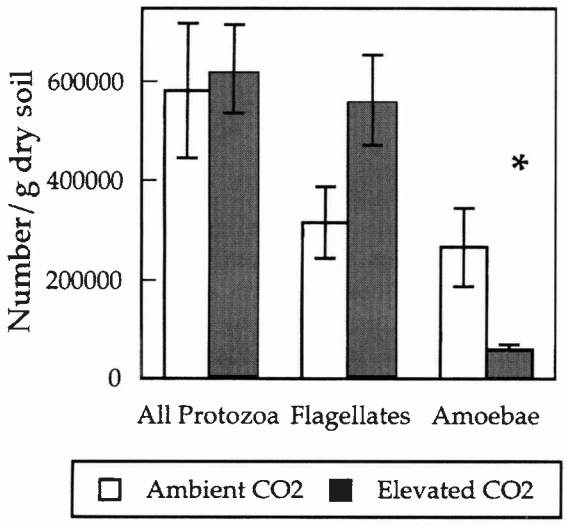


Fig. 14. Response of 'total protozoa' (flagellates + naked amoebae), flagellates, and amoebae in soils of plants grown under elevated CO_2 (from Treonis and Lussenhop, 1997). Values were obtained with a culture method and show treatment means ($n = 20$); bar lengths represent 1 unit of standard error. Asterisk (*) indicates significant difference ($P = 0.046$) to control.

the study is not definitive. However, the data at least suggest that soil protozoa could be a valuable and efficient system for exploring the effects of increased CO_2 levels and hence global warming on soil organisms. Smith (1996) in fact predicted from a faunal census and some experiments that the ciliate genus *Colpoda*, which has its southern limit of frequent and ubiquitous distribution at the sub-Antarctic/maritime Antarctic boundary, would quickly spread over the maritime Antarctic as a result of global warming.

5.2.3. *Forest decline*

The effects of slow release, organically enriched magnesite fertilizers on soil organisms of a declining spruce forest in Upper Austria were studied during a five-year period using a completely randomized block design (Aescht and Foissner, 1993, 1994, 1995a). For revitalization, 2000 kg ha^{-1} BIOMAG© (90% magnesite and 10% dried fungal biomass; 'biomag plots') and 3000 kg ha^{-1} BACTOSOL© (dried bacterial biomass) + 2000 kg ha^{-1} biomag ('bactosol-biomag plots') were applied once to an old and to a young

Table 8
Abundance, individual dominance, and frequency of important ciliate groups and species in the litter layer (0–3 cm) of a declining, ameliorated spruce forest (from Aescht and Foissner, 1995a)

Feeding type	Parameter[a]	Treatment[b]						Difference to (%; C,c = 100%)			
		C	M	O	c	m	o	M	O	m	o
Fungal feeders (total)	ID%[c]	55	58	62	62	53	58	5	13	−14	−6
Avestina ludwigi	Ind.[c]	95	97	59	47	42	30	2	−38	−11	−36
	ID%	15	25	14	20	20	14	66	−5	2	−30
	F%[c]	94	86	81	94	83	81	−9	−14	−12	−14
Rostrophryides australis	Ind.	88	38	62	38	24	24	−57	−30	−37	−37
	ID%	14	10	15	16	11	11	−30	7	−28	−31
	F%	100	92	96	88	83	90	−8	−4	−6	2
Grossglockneridae[d]	Ind.	55	34	47	17	24	38	−38	−15	41	124
	ID%	9	9	11	7	11	17	0	30	59	144
	F%	85	90	88	90	90	83	6	4	0	−8
Bacteria feeders (total)	ID%	24	22	17	14	25	25	−8	−29	79	79
Cyclidium muscicola	Ind.	126	39	43	27	23	16	−69	−66	−15	−41
	ID%	20	10	10	11	11	7	−50	−48	−4	−35
	F%	100	83	92	79	92	73	−17	−8	16	−8
Colpoda ssp.	Ind.	19	23	29	13	25	21	21	53	92	62
	ID%	3	6	7	5	12	10	93	130	119	76
	F%	38	44	58	33	58	71	17	56	75	112

[a] Values are means of direct counts from eight sampling campaigns between 1987 and 1991.
[b] C, c: control; M, m: BIOMAG treatment (90% magnesite and 10% dried fungal biomass, 2000 kg ha^{-1}); O, o: BACTOSOL–BIOMAG treatment (3000 kg ha^{-1} dried bacterial biomass + 2000 kg ha^{-1} biomag). Capital letters designate the old stand (>80 years), lower case letters the young stand (>40 years).
[c] F: frequency; ID: median of individual dominance; Ind.: individuals per gram soil (litter) dry mass.
[d] *Grossglockneria acuta, Nivaliella plana, Pseudoplatyophrya nana, Mycophagophrys* (formerly *Pseudoplatyophrya*) *terricola.*

stand. The treatments caused a mean pH rise of about 0.9 units. The biomasses of testaceans and the individual numbers of ciliates significantly decreased on some plots. Likewise, community structures were changed: individual numbers of acidophilic testacean species decreased and circumneutral species increased; mycophagous and bacteriovorous ciliates increased or decreased depending on species (Tables 8 and 9). Apart from the increase in pH, these changes could be related to altered numbers and kinds of food organisms such as fungi and bacteria, as indicated by an increased catalase and protease activity and decreased phosphatase and cellulolytic activity. The abundances of rotifers and proturans significantly increased in the bactosol-biomag plots of the old stand, and earthworm abundances increased with all treatments. Nematodes, mites, springtails, and enychtraeids were hardly affected. In general, soil fauna and enzyme activities were influenced to a greater extent by the bactosol-biomag than by the biomag treatment. These investigations also showed that it is essential to take into account species and their autecology, i.e., indication remains rather unspecific and ambiguous when confined to abundance and biomass changes.

5.2.4. Soil compaction

Soil compaction decreases soil fertility and increases soil erosion. It is frequently caused by the use of heavy agricultural machines and by the reduced application of humus-producing substances such as organic manures. Investigations on the effects of soil compaction on soil protozoa were performed by Berger et al. (1985) and Coûteaux (1985). The results of these studies demonstrate the inhibitory effects of even slight compaction on soil protozoa and the dramatic reduction of life in heavily compacted soils. The changes are very likely due to reduced pore space and lower moisture content. The ciliates appear to be more sensitive but less selective than the testaceans.

Berger et al. (1985) performed their investigations with special compaction chambers that compacted 500 cm^{-3} of the upper soil layer of an alpine pasture on

Table 9
Abundance, individual dominance, biomass dominance, and frequency of important testacean species in the litter layer (0–3 cm) of a declining, ameliorated spruce forest (from Aescht and Foissner, 1995a)

pH type	Parameter[a]	Treatment[b]					
		C	M	O	c	m	o
Acidtolerant species	ID%	47.5	28.2	23.2	44.0	28.8	27.5
Corythion dubium	Ind.	8724	6726+	4857***	13 575	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
Nebela 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
Circumneutral species	ID%	25.5	45.2	50.2	32.8	49.6	53.7
Cryptodifflugia oviformis	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
Trinema spp.	Ind.	4635	8263**	9041**	8663	9535	12 035*
	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

[a] Values are means of direct counts from eight sampling campaigns between 1987 and 1991. BD: biomass dominance; F: frequency; ID: median of individual dominance; Ind.: individuals per gram soil (litter) dry mass.
[b] C, c: control; M, m: BIOMAG treatment (90% magnesite and 10% dried fungal biomass, 2000 kg ha^{-1}); O, o: BACTOSOL–BIOMAG treatment (3000 kg ha^{-1} dried bacterial biomass + 2000 kg ha^{-1} biomag). Capital letters designate the old stand (>80 years), lower case letters the young stand (>40 years). Differences to control: + $P < 0.1$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.0001$.

alpine pseudogley by 10, 30 and 50%. The chambers were exposed in the field in three parallel sets for three months at the same sites. The results showed a significant decrease in the abundance of the testaceans and the nematodes and a loss of the infrequent species with increasing compaction (Fig. 15 (a) and (b)). *Centropyxis aerophila* var. *sphagnicola* was most abundant in the control and in the 10 and 30% compaction trials, whereas the smaller *Trinema lineare* dominated at 50% compaction, perhaps because of the reduced pore space. The number of species, the abundance of testate amoebae and nematodes, and the soil moisture content correlated negatively with the bulk density of the soil. Empty tests (dead individuals) increased with compaction, indicating a higher mortality and/or reduced decomposition. Active ciliates (direct counting method) were most abundant at 30% compaction. Coûteaux (1985) found the individual numbers of the ciliates significantly depressed in 4 g microcosms made of compacted forest humus (Fig. 15 (c)).

5.2.5. Movement of protozoan pathogens in soil

Livestock wastes may contain pathogenic protozoa, especially sporozoans, and it is now recognized that, following the application of livestock waste to land, there is a potential for the transfer of these microorganisms to humans after contamination of plants, soil, and subsequently, water courses. *Cryptosporidium parvum* is of particular interest because as few as 10 infective oocysts may be required to cause infection, and its transmissive oocyst is, furthermore, resistant to current methods of drinking water treatment.

Mawdsley et al. (1996) investigated the potential for transfer of *C. parvum* through soil to land drains and, subsequently, water courses following the application of livestock waste to land. They used intact soil cores mounted on a special soil tilting table apparatus in the laboratory. Following irrigation over a 21-day period, *Cryptosporidium parvum* oocysts applied to the surface of soil cores (initial inoculum concentration 1×10^8 oocysts per core) were detected, albeit in low numbers, in the leachates from clay loam and silty

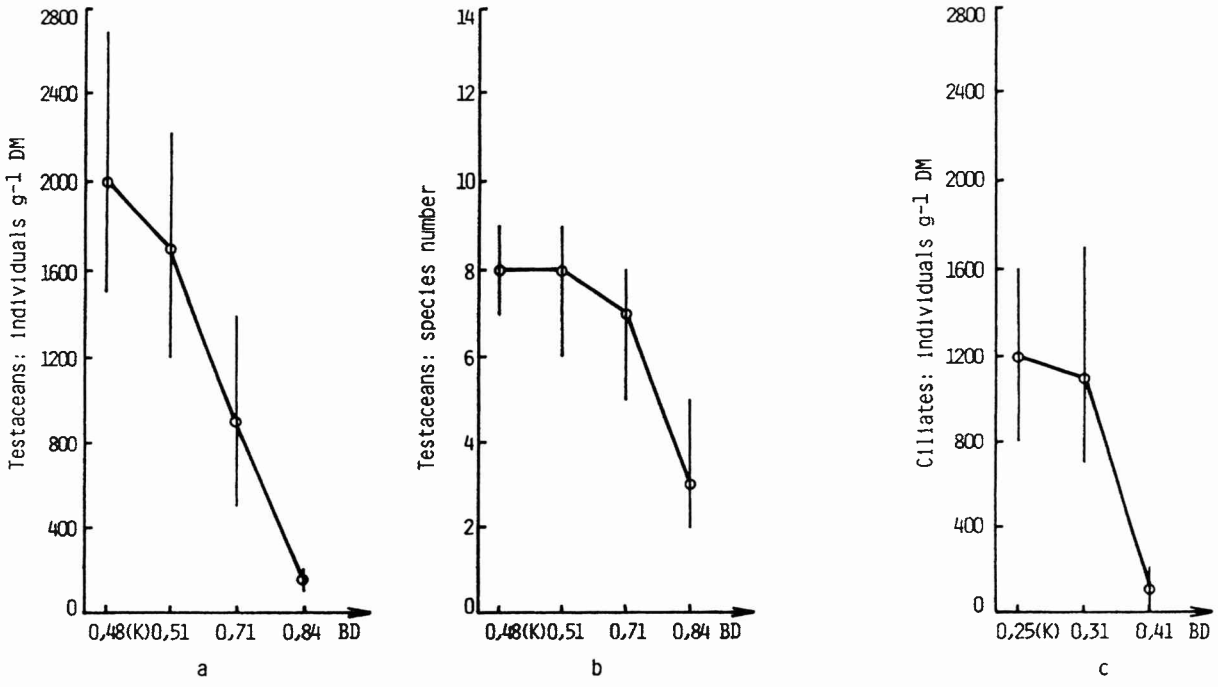


Fig. 15. Effect of soil compaction on testate amoebae and ciliates. (a), (b): Field experiment with the very humic topsoil (0–5 cm) of an alpine pasture (after data from Berger et al., 1985). Methods: randomized block design (bars: extreme values), 12 weeks compaction in chambers with 500 cm^3, direct counts as described in Foissner (1987a); (c): Laboratory experiment with the humus layer of a mixed forest (after data from Coûteaux, 1985). Methods: 9 weeks compaction of 4 g humus in compaction chambers, direct counts and calculation with a most probable number method. BD: bulk density; DM: dry mass of soil; K: control.

loam soils, but not in the leachate from a loamy sand soil. Variations in leaching patterns were recorded between replicate cores. At the end of the study, soil cores were destructively sampled to establish the location of oocysts remaining within the soil. Distribution within cores was similar in all three soil types; the majority ($72.8 \pm 5.2\%$) of oocysts were found in the top 2 cm of soil, with numbers decreasing with increasing depth to $13.2 \pm 2.8\%$, $8.39 \pm 1.4\%$, and $5.36 \pm 1.4\%$ at depths of 10, 20, and 30 cm, respectively.

5.3. Soil protozoan bioassays

Although soil protozoa have frequently been used as test organisms (for reviews, see Foissner, 1987a, 1994, 1997a) definite test systems have only recently been developed (Forge et al., 1993; Pratt et al., 1997). Both systems are very similar and measure the 24 h growth response of either *Colpoda steinii* or *C. inflata*, two very common and well-known soil ciliates (Foissner, 1993).

These test systems have been applied with heavy metals in laboratory and field trials (Forge et al., 1993; Bowers et al., 1997a, 1997b; Campbell et al., 1997; Pratt et al., 1997). The results agree well and suggest that soil ciliates are at least as sensitive to environmental hazards as more commonly used test organisms (e.g., earthworms). There is thus a strong likelihood that protozoa can replace and/or supplement invertebrates and vertebrates in some assays. However, as with other organisms, results are highly dependent on procedural differences and other, not yet fully understood variables such as pH and organic compounds. Acute toxicity values for a given metal may vary by as much as almost two orders of magnitude, emphasizing the need for strict test standardization (Bowers et al., 1997a, 1997b). Sensitivities of *Colpoda* spp. to heavy metals, as tested by Bowers et al. (1997a) and Forge et al. (1993), tended to fall in the lower range for all metals, supporting the inclusion of a ciliate test in a 'battery of tests' approach to soil toxicity assessment. Furthermore, test results are obtained within 24 h, i.e.,

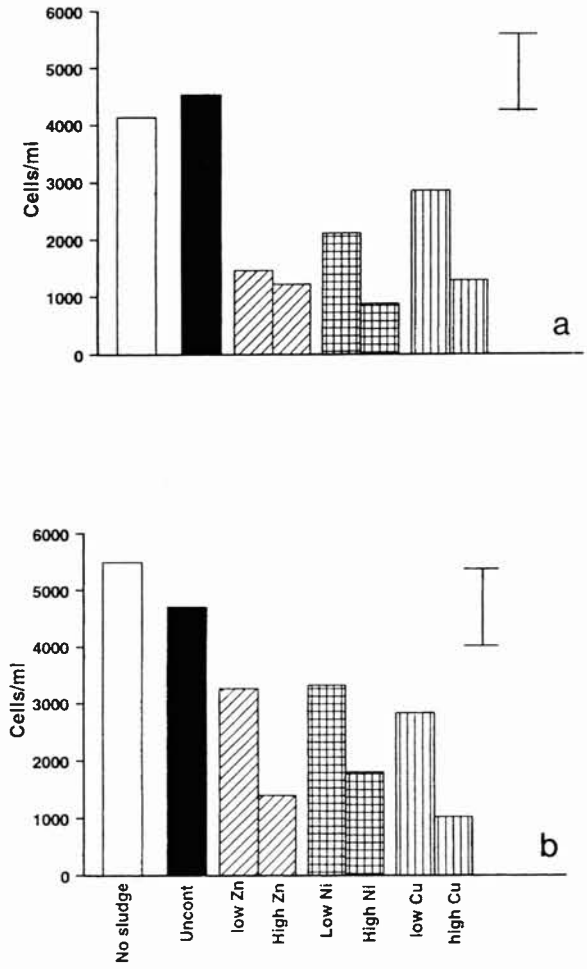


Fig. 16. Effect of sewage sludge treatment on growth of *Colpoda steinii* in soil solution extracted from (a) Lee Valley and (b) Luddington unamended control soils, soils amended with uncontaminated sludge and soils amended with sewage sludge contaminated with either high or low amounts of Zn (16 000 or 8000 mg kg^{-1}, respectively), Ni (4000 or 2000 mg kg^{-1}, respectively) or Cu (8000 or 4000 mg kg^{-1}, respectively). Error bars = $LSD_{0.05}$. The test system of Forge et al. (1993) was used (from Campbell et al., 1997).

much faster than with any multicellular system, e.g., the standard 5-day seed germination test or the *Eisenia fetida* test endorsed by the EC, which requires 7–14 days to complete.

As an example, Fig. 16 shows some of the results obtained by Campbell et al. (1997), who used the test system of Forge et al. (1993). Campbell et al. (1997) investigated the toxicity and bioavailability of heavy metals in soil solutions extracted from two long-term experiments that were amended with sewage sludge. The sludges were predominately contaminated with either Ni, Cu, or Zn. Growth of *Colpoda steinii* was strongly inhibited in all metal-amended soils compared to the equivalent unamended control soils. The EC_{50} values for relative growth were in order of decreasing toxicity Cu > Ni > Zn, which corroborates results by Madoni et al. (1992), who found Cu > Hg > Cd > Zn for activated sludge ciliates. Using the same assay to study the toxicity of metal sulphate solutions, Forge et al. (1993) found toxicity decreased in the order of Ni > Cu > Zn, which is similar to the ranking obtained by Pratt et al. (1997), who reported Cd > Cu > Zn.

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