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PROTOZOA

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Introduction

Protozoa are unicellular, heterotrophic, eukaryotic organisms comprising four organization types: amebae, flagellates, ciliates, and parasitic sporozoans. About 1600 species, of which some are restricted to certain geographic regions, are known to live in terrestrial habitats; however, at least the same number is still undiscovered. Small body size and the ability to produce protective resting cysts are the main adaptations of protozoa to the peculiarities of the soil

environment. Many soil protozoa feed, more or less selectively, on bacteria, while others are omnivorous or highly specialized fungal feeders. Protozoa (active and cystic) inhabit the soil in great numbers, that is, some 10 000–1 000 000 individuals per gram dry mass, and produce many generations annually. They significantly enhance the flow of nutrients and growth of plants and earthworms. Accordingly, they are important soil inhabitants, and studies on their dynamics and community structures thus provide a powerful means for assessing and monitoring changes in biotic and abiotic soil conditions. Unfortunately, methodological and taxonomical problems still limit the use of protozoa as bioindicators in terrestrial environments.

This article covers the diversity, ecology, and bioindictive value of soil protozoa. The knowledge on autotrophic soil protists, which mainly live on the soil surface because they depend on light, is still in its infancy. However, they play an important role, especially as a symbiotic partner of lichens in the crust soils of extreme regions, such as desert and high mountain areas.

A Brief History of Soil Protozoology

Although there were predecessors, such as Ehrenberg, Goodey, and Russel and Hutchinson, soil protozoology as a science was established by the Austrian–Hungarian naturalist H.R. Francé and the English microbiologist D.W. Cutler in the 1920s. Their monographs showed that unicellular organisms inhabitate the soil in great number and diversity, and thus contribute significantly to soil fertility and sustainability. Francé's booklet saw many editions, the latest in the 1960s.

This first bloom of soil protozoology followed a long period where a few specialists added significantly to the knowledge available, mainly J.F. Darbyshire in the UK, L. Bonnet in France, J.D. Stout in New Zealand, S.S. Bamforth in the USA, and V.F. Nikolyuk in Russia. It was only in the last two decades of the twentieth century that soil protozoology was rejuvenated by the studies and reviews of W. Schönborn in Germany, F. Ekelund and R. Rønn in Denmark, W. Foissner in Austria, and J.F. Darbyshire, who edited the first book devoted entirely to the biology and ecology of soil protozoa. Today, the field is well established but still a domain of a limited number of specialists, while general soil ecologists have problems to incorporate the new knowledge appropriately, in contrast to marine and limnetic plankton researchers, where the 'microbial loop,' that is, the intimate connection of dissolved organic matter, bacteria, and protists, greatly changed views and research strategies.

Diversity and Biology

Diversity and Geographic Distribution

About 23 000 free-living, extant protozoa are known. Of these, roughly 1600 were originally described or later reported from terrestrial habitats (Table 1). For a long time, the soil protozoan community was considered as an ubiquitous part of limnetic species and some even suggested that nearly the same species of protozoa occur in soil, sewage, and activated sludge. However, more detailed research disproved these findings and showed that they were based on misidentification of species. Today it is widely accepted that

Table 1 Known and proposed diversity of soil protozoa

<i>Taxa</i>	<i>Known species</i>	<i>Proposed species</i>
Naked amebae ^a	60	600
Testate amebae	300	500
Flagellates	270	600
Ciliates	1000	2000
Microsporidia and sporozoans	3	?

^aWithout several hundreds of slime molds.

the main part (>70%) of the soil protozoan species evolved in and are restricted to terrestrial environments. Refined morphological methods and gene sequence data indicate that a considerable portion of soil and freshwater protists have a restricted geographical distribution (Figures 1 and 2), disproving the old hypothesis that, in microorganisms, 'everything is everywhere, the environment selects.' This matter, however, is still under discussion.

Table 1 shows the known and proposed soil protozoan diversity. While numbers of testate amebae and ciliates are supported by detailed data, those of the other groups are very speculative. Likewise, little is known of protozoan parasites. But even the known soil protozoan diversity is high and a major problem in basic and applied soil biology because comprehensive identification literature is lacking and few taxonomic specialists are available. The decreasing interest of the public and science in classical taxonomy, will soon dramatically increase our ignorance. Although molecular methods likely can solve some of the problems, nobody will be available to add names to the sequences. Thus, great efforts are necessary to educate young scientists in classical alpha-taxonomy.

Morphological and Physiological Adaptations

The soil is a very special environment for protozoa. Three factors are most important for their existence and activity: (1) the structure of the porous space; (2) the astatic water supply; and (3) intraspecific and interspecific interactions. These factors caused two basic adaptations: small body size and the universal capability to produce protective resting cysts. These and some other important adaptations will be briefly described.

Morphological adaptations Mean body length, mean body width, and mean biomass are significantly smaller in soil ciliates and testate amebae than in ciliates and testaceans from fresh water. The small size, and volume, is achieved either by an overall reduction in size or by reduction of body width. Many soil ciliates are thigmotactic creepers with a reduced ciliature and a flattened and/or worm-like

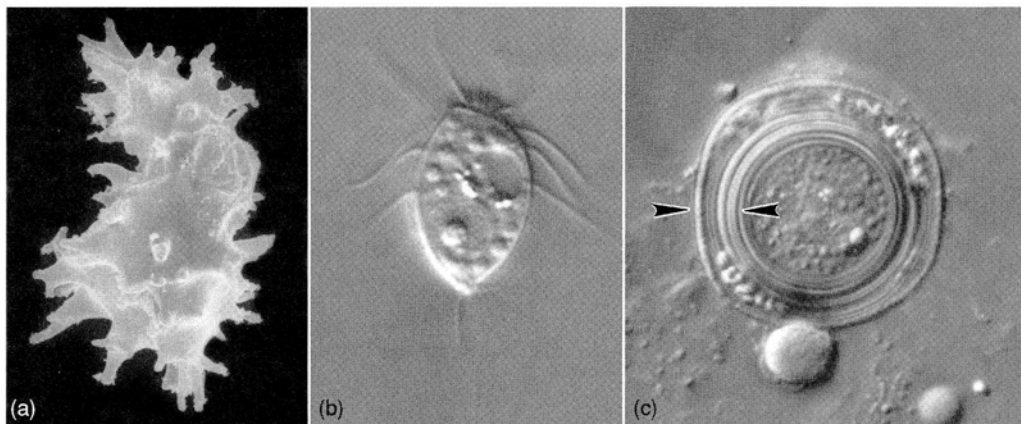


Figure 1 Small-sized and cystic soil protozoa. (a) A naked ameba about $30\text{ }\mu\text{m}$ long with many tiny pseudopodia which can exploit even minute soil pores for food. (b) A $15\text{ }\mu\text{m}$ long soil flagellate, *Hemimastix amphikineta*, which only occurs in southern terrestrial environments, that is, in Gondwana. Note the two rows of flagella which make the organism look like a ciliate. (c) Resting cyst (diameter about $35\text{ }\mu\text{m}$) of the ciliate *Exocolpoda augustini*. This ciliate occurs mainly in hot and dry soils, e.g., the Namib desert, and thus has a very thick cyst wall (arrowheads) composed of many membranes. The organism can remain viable in this condition for years.

body (Figure 3b). These features and several physiological peculiarities produced a soil ciliate community of unique structure, characterized by a high proportion of colpodids (Figure 3a and 3b) and hypotrichs and a strong underrepresentation of sessile peritrichs and suctorians compared with fresh water ciliate communities. The soil testacean community is characterized by having a high proportion of species with reduced pseudostome size (opening of the shell), a flattened ventral side, and a globular shape (Figure 2). Soil naked amebae and flagellates have similar adaptations as ciliates and testate amebae (Figure 1a and 1b): most are very small and the amebae, for instance, can exploit with their tiny and flexible pseudopodia micropores which have a diameter of only $1\text{ }\mu\text{m}$.

Physiological adaptations Few physiological adaptations have been investigated in detail; it is probable that several are still to be discovered. Some physiological adaptations cause conspicuous morphological specializations.

Food and feeding Many soil protozoa, especially most naked amebae and flagellates, entirely or partially feed on bacteria, skimming off the dense bacterial standing crop provided by many soils. However, under natural conditions soil protozoa cannot significantly reduce bacterial abundance, and the absolute amount of organic matter consumed by the testacean community is small – 234 mg m^{-2} during a 3-month period.

In soil ciliates, 39% feed mainly on bacteria, 34% are mainly predaceous, and 20% are omnivorous. Some are strictly mycophagous and the most characteristic species of the soil ciliate community. They

have reduced the classical ciliate mouth almost completely and evolved a minute feeding tube producing discrete holes in the hyphae and spores of fungi and yeasts (Figure 3b–e). This type of feeding has been termed ‘perforation lysis’ and is also found in several naked amebae. There is evidence from pot experiments that such activities can reduce the inoculum level of plant pathogenic fungi in soils and can reduce the severity of take-all disease of wheat by the fungus *Gaeumannomyces graminis* var. *tritici*. Some flagellates are also strictly mycophagous, but take up the fungi by an ordinary cytostome. Many soil testaceans seem to feed on humus particles and/or fungal hyphae and spores, but other materials such as algae, protozoa, and bacteria are also ingested; some are polyphagous. A Gondwanan species, *Apodera vas*, can even feed on nematodes (Figure 2b). In some other species, *Diffugia lucida* and *Schoenbornia humicola*, a remarkable specialization has been described: they collect and store humus particles around the pseudostome during optimal periods and transport them into the cytoplasm during suboptimal environmental conditions (Figure 2c).

r/K-selection Field data and laboratory experiments show that colpodid ciliates are more *r*- than *K*-selected. Thus, they are particularly diverse and frequent in unpredictable and extreme habitats, such as leaf surfaces, lichens, and alpine and desert soils. In contrast, polyhymenophoran ciliates (mainly hypotrichs) are more *K*-selected and thus particularly diverse and frequent in predictable habitats (Figure 4). The ratio of these ciliate groups proved to be a sensitive indicator for biotope stability and can thus be used in studies on human-influenced soils.

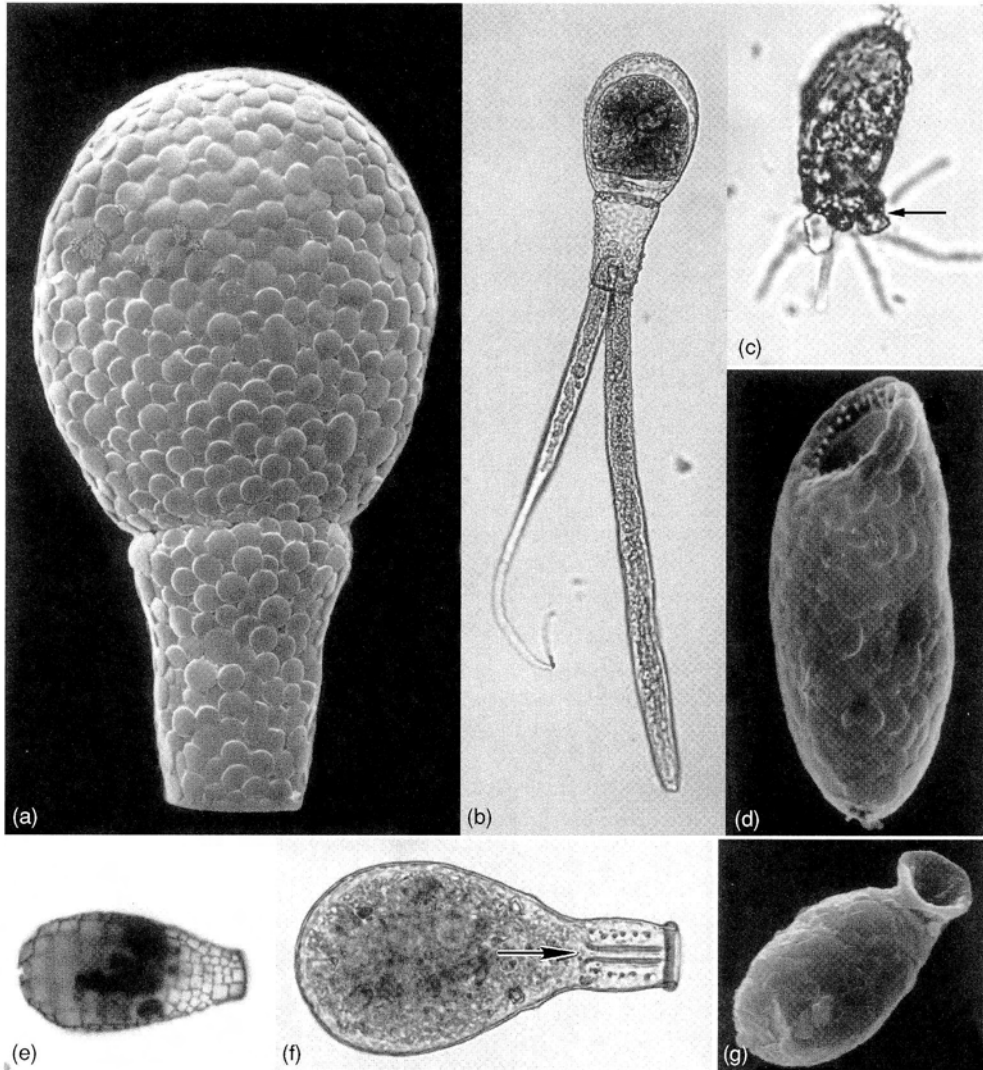


Figure 2 Soil testate amoebae. (a, b) *Apodera vas* is about 170 μm long and only occurs in the southern hemisphere, i.e., in Gondwana. It can feed on nematodes (b) and build its shell of platelets taken from the shell of another prey organism, the testate amoeba *Trinema lineare* (d). (c) *Difflugia lucida* is about 60 μm long and produces food-bundles (arrow) around the shell opening under optimal environmental conditions. These food accumulations are ingested and digested when conditions are less favorable. (d) *Trinema lineare* is about 35 μm long and one of the most common soil testate amoebae globally. Its shell is made of silicium platelets, which *A. vas* uses for building its own shell (a). (e) *Quadrullella* makes the test of quadrangular silicium platelets. (f) *Nebela certesi* is about 150 μm long and only occurs in the southern hemisphere (Gondwana). Note the oral tube (arrow) surrounded by minute teeth. (g) *Edaphonobiotus campasoides*, a soil testate amoeba about 35 μm long has, as yet, been found only in central Europe (Laurasia). Its shell is highly similar to that of some marine sand testaceans.

Testate amoebae are, in general, on the r side within the r/K -continuum, when compared with ciliates, naked amoebae, and flagellates. However, a more fine-scaled rating suggests that autochthones are more K -selected than allochthones.

Cysts All soil protozoa survive detrimental environmental conditions in a dormant stage, termed 'protective resting cyst.' When encysting, the cell rounds up, loses water, and secretes a thick wall (Figure 1c). In encysted condition, protozoa can withstand, for

instance, decades of drought and food depletion, liquid nitrogen, and strong acids. When conditions become favorable again, the cell excysts and commences feeding and reproduction. The receptors indicating favorable conditions to the encysted cell are still not known. Likewise, few reliable data are available on the factors stimulating excystment, but substances secreted by potential food organisms and CO_2 might be important. A slight increase in the CO_2 level to 0.5–0.8% enhanced excystment of soil amoebae, but a further increase inhibited it.

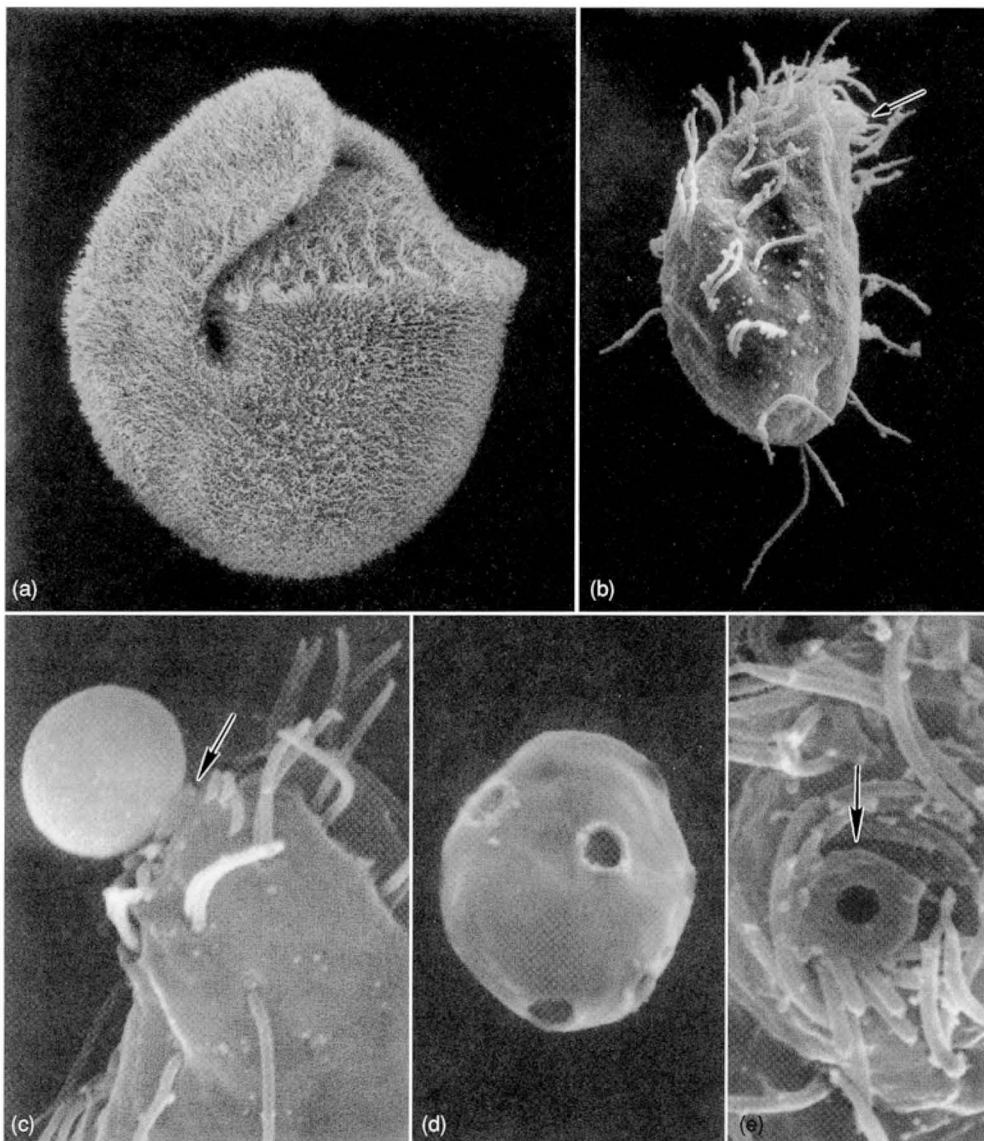


Figure 3 (a) *Bresslauides discoideus* is a colpodid ciliate up to 600 μm large and likely restricted to the northern hemisphere. It is a predator with a large mouth and one of the largest ciliates occurring in soil. (b–e) *Pseudoplatyophrya nana* is a minute species about 25 μm long that feeds only on yeasts and fungi. It feeds by perforation lysis, that is, has a minute oral tube (arrows) that enzymatically produces small holes in the yeast's cell wall (c, d).

There is evidence that cysts are adapted to the overall environmental conditions in which the trophic cells live. Cysts formed, for instance, in arid environments can survive desiccation for decades, while those produced in rainforests survive drought for only a few weeks or months. The same is true for temperature. In experimental microcosms, diversity of soil ciliates and reproduction are highest at or near the mean annual temperature of the habitat, that is, around 20°C for soils from the region of Bonn, Germany, and 25–30°C for those from an African savannah. These data show the existence of many local physiological races adapted to the specific conditions of the habitat.

There are further cyst variations in soil protozoa. Testate amebae can produce a so-called 'precystic stage' for bridging short unfavorable periods, an adaptation to the often quickly changing soil environment. When forming this stage, the organism produces a thin membrane (epiphragm) closing the shell opening (pseudostome), and rounds up, but does not form a thick cyst wall. During excystment, the epiphragm is lysed or digested. Another spectacular cyst is produced by the epiphytic, colpodid ciliate genus *Sorogena*. Under favorable conditions, it multiplies rapidly, aggregates, encysts, and produces noncellular stalked aerial sori which look highly similar to those

of cellular slime moulds. The sorogenesis requires alternate light and dark conditions, just as in the pink slime mold *Acrasia rosea*.

Anabiosis Anabiosis (cryptobiosis, anhydrobiosis) is the property of organisms to resist desiccation without marked morphological changes, such as the production of a special cyst or membrane. It is common in some groups of soil- and moss-inhabiting animals, such as rotifers, tardigrades, and nematodes, but is very restricted among soil protozoa, where it likely occurs in a few naked amebae and in a suctorian ciliate, as I myself have observed.

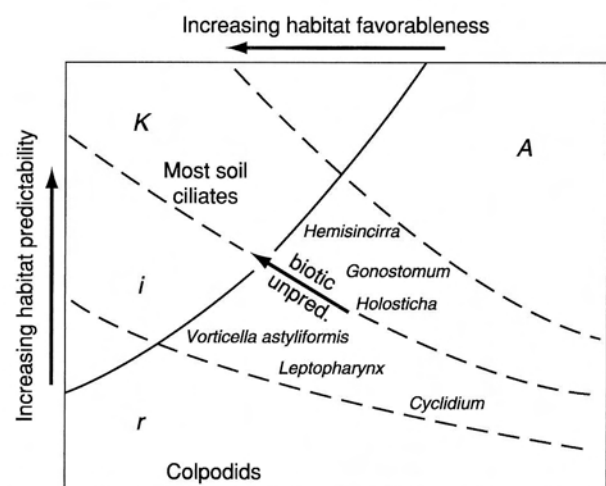


Figure 4 Ordination of soil ciliate taxa on a two-dimensional template. Colonizing *r*-organisms, colpodids, characterize ephemeral habitats of low resources (lower left to lower right of diagram). A few intermediate *K*-selected species (e.g., *Cyclidium* and *Holosticha*) can adapt to harsh conditions. Species diversity increases towards the upper left corner of the diagram where the abiotic factors of the habitat improve, enabling more biotically unpredictable interactions due to competition and predation. *A*, adversity selection; *i*, intermediates. Reproduced with permission from Bamforth SS (2001) Proportions of active ciliate taxa in soils. *Biology and Fertility of Soils* 33: 197–203.

Number and Biomass

In soil, protozoa are present as active (trophic) and inactive (encysted, dormant) cells. It is likely that many soil protozoan species are encysted most of their life. Thus, the encysted portion of the total number is usually much larger than the active. There is currently no reliable method available for separating active and dormant specimens. Furthermore, most counts rely on culture techniques followed by a most probable number (MPN) calculation of protozoan abundance. However, only few of the many protozoan species present excyst and grow to detectable numbers under the limited conditions of such cultures. All these methodological shortcomings mean that many of the quantitative data available in the literature are doubtful, especially as concerns the active and thus ecologically relevant portion. Only numbers obtained by direct microscopic counts in soil suspensions are reliable (Table 2). Unfortunately, naked amebae and flagellates can hardly be counted with direct methods because they strongly adhere to the soil particles and thus cannot be seen. Accordingly, all numbers on active naked amebae and flagellates are highly questionable.

In spite of these problems, it is clear that protozoa inhabit soil in great numbers, that is, some 10 000–1 000 000 individuals per gram dry mass (Tables 2–4, Figure 5). Biomass tends to correlate with abundances, and values between 0.1 g and 5 g m⁻² are frequent. Naked amebae and flagellates (active plus cystic) usually show numbers between 2000 and 1 000 000 g⁻¹ dry mass of soil. Standing crop numbers of active testate amebae are moderately high i.e. 100–1000 individuals per gram dry mass in arable land and mineral soils, 1000–10 000 in meadow and grassland topsoils, and 10 000–100 000 in forest leaf litter and upper humus horizons. However, as many testate amebae are rather voluminous, their standing crop and production biomass often surpasses that

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

Sample	Direct counts ^a		MPN simplified ^b		MPN complete ^c	
	Mean ^d	CV (%) ^e	Mean ^d	CV (%) ^e	Mean ^d	CV (%) ^e
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

^aMicroscopic counts in soil suspensions.

^bDilution culture method by Singh and most probable number (MPN) equation by Fischer.

^cDilution culture method by Singh and refined MPN equation as available in computer programs.

^dValues followed by the same symbol are not significantly different ($P \leq 0.05$).

^eCoefficient of variation.

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Table 3 Effects of irrigation on the microfauna of a spruce forest soil

Parameters ^a	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 ($P \leq 0.05$)
	3–9	48.8* ± 4.3	44.7* ± 5.9	8	ANOVA ($0.2 \geq P > 0.1$)
<i>Ciliates</i>					
Abundance	0–3	311* ± 141	489 [†] ± 258	15	U-test ($0.05 < P \leq 0.1$)
	3–9	10* ± 10	14* ± 24	8	U-test ($P \geq 0.2$)
Species number	0–3	12.8* ± 4.5	8.4 [†] ± 3.3	15	ANOVA ($P \leq 0.005$)
	3–9	2.4* ± 2.1	1.6* ± 1.9	8	U-test ($P \geq 0.2$)
<i>Testate amoebae</i>					
Abundance					
Living	0–3	22 203* ± 7040	17 908* ± 3175	4	ANOVA ($P \geq 0.2$)
Empty tests	0–3	361 720* ± 17 204	319 081* ± 58 390	4	ANOVA ($P \geq 0.2$)
Species number					
Living	0–3	11.5* ± 1.3	9.8 [†] ± 1.0	4	ANOVA ($0.05 < P \leq 0.1$)
Total	0–3	18.0* ± 0	19.8* ± 2.2	4	U-test ($P \geq 0.2$)
<i>Nematodes</i>					
Abundance	0–3	1197* ± 349	824 [†] ± 349	15	ANOVA ($P \leq 0.01$)
	3–9	308* ± 80	391* ± 173	8	ANOVA ($P \geq 0.2$)
<i>Rotifers</i>					
Abundance	0–3	227* ± 88	181* ± 122	15	ANOVA ($P \geq 0.2$)
	3–9	38* ± 25	31* ± 18	8	U-test ($P \geq 0.2$)

^aAbundances (individuals g⁻¹ dry mass of soil; arithmetic mean ± standard deviation) were estimated with a direct counting method in soil suspensions. Values followed by the same symbol are not significantly different.

^bIrrigated plot (15 m²) received 25 l m⁻² water every fourth day.

ANOVA, analysis of variance.

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Table 4 Comparison of community parameters for soil testaceans from different sites of the world

Parameters	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 (year ⁻¹)	16.0	12.5	ND	8	90	ND
Mortality rate (% day ⁻¹)	3.0	8.5	ND	ND	10.8	ND
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

ND, not determined.

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of all other protozoans (Table 4). Thus, their omission in many recent studies is an unfortunate mistake. Abundances of active ciliates are low in evolved soils (humus and mineral horizons), usually <100 cells g⁻¹ (Tables 2 and 3). However, up to 10 000 individuals per gram dry mass can be reached in developing soils and leaf litter (Table 2), showing that ciliates are important during the early stages of soil formation.

Vertical and Horizontal Distribution

Like as many soil organisms, protozoa are usually most abundant and diverse in the upper 0–10 (30) cm

soil layer, where food is concentrated in the form of leaf litter and the rhizosphere. However, there is a distinct, not yet fully understood, niche separation of ciliates and testate amoebae. While active ciliates occur mainly in the uppermost (fresh) leaf litter layer, where abundances of up to 10 000 individuals per gram dry mass are reached, testate amoebae peak underneath, that is, in the evolving humus horizon ('fermentation layer'; Table 3). Thus, habitats such as meadow topsoils and arable lands contain very few active ciliates, usually <100 g⁻¹ dry mass, although a lot of viable resting cysts are present. The inability to excyst (germ) in evolved soils has been termed ciliatostasis,

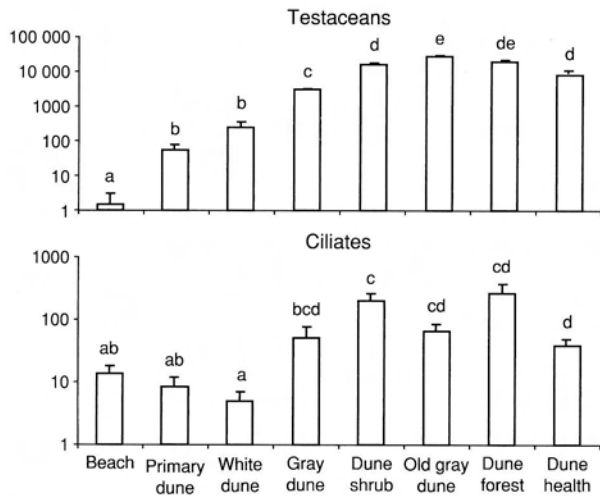


Figure 5 Abundance (individuals g⁻¹ dry mass of soil) of active testate amebae and ciliates in dune soils along a series of soil (dune) development. Significant differences ($P < 0.05$) are indicated by different letters. Reproduced from Verhoeven R (2002) The structure of the microtrophic system in a development series of dune soils. *Pedobiologia* 46: 75–89.

in analogy to fungistasis. The reasons for ciliatostasis are not yet clear, but enrichment of certain detrimental substances during humification, density-dependent processes, and general food depletion are discussed.

Little is known about the horizontal distribution of soil protozoa. However, a very clumped distribution is evident from several faunistic and 'hot spot' investigations. Recent data suggest that spatial organization of protozoan communities occurs at scales below 10 cm.

The 'Water Paradox'

Protozoa are aquatic organisms, and free water is thus indispensable for their life. Accordingly, it appears reasonable to assume that water is the key factor for soil protozoan abundance and diversity. Surprisingly, carefully designed field and laboratory studies do not support this assumption, but over the years have provided many conflicting results. Two examples from recent studies should demonstrate the 'water paradox.'

In one study, aspen woodland plots were irrigated during the seasonal dry period every 3 days with 3.78 l m⁻² and testate amebae were sampled on three occasions. The general effect of adding water to the soil was to increase significantly the number of active testaceans and to decrease the number of encysted specimens. Numbers of active testaceans and of total living testaceans (active plus encysted) showed a significant, positive correlation with soil moisture content. At higher soil moisture levels, the larger (>60 µm) species present tended to have a larger proportion of living individuals as cysts than

did the smaller forms which numerically dominated the population. Generation times were shorter and secondary production higher in the watered plots.

In the other study, spruce forest plots were irrigated during the seasonal dry period every 4 days with 25 l m⁻² (Table 3). This caused a marked decrease ($P \leq 0.05$) in the abundance of the active ciliates in the litter layer; species richness, in contrast, increased in both ciliates and testate amebae, while testacean abundance did not increase significantly. The nematode numbers increased dramatically by about 45%, while rotifer abundance did not change significantly.

As yet, there is no convincing explanation for these and many other contrasting results. However, most soil protozoologists agree on the basic assumption that water is a key factor for protozoan life, not only because they basically depend on water but also due to several morphological peculiarities most meaningfully explained as adaptations to the astatic water regime of the soil environment in general. I suggest, very briefly, two hypotheses for explaining the 'water paradox':

1. Water correlates with other, not yet recognized factors, especially the presence or growth of appropriate food organisms. This hypothesis is supported by the observation that laboratory microcosms, where food has been tested to be edible for the protozoans under investigation, show more consistent results, that is, increasing protozoan abundance and biomass with increasing soil moisture.

2. Protozoa can tolerate a wide range of soil moisture; only under extreme conditions do communities react. This hypothesis is supported by the occurrence of local, physiological races and the ability of many protozoan species to live in very thin films of water covering the soil pores and soil particles. In the spruce forest experiment described above, the litter of the control plot was hand-dry; most of the water present was a constituent of the needles (Table 3).

Ecological Significance

Standing Crop, Respiration, and Annual Production

Several ecosystem studies show consistently that the microflora (bacteria and fungi) accounts for about 90% of the heterotrophic soil respiration. Only about 10% of the total comes from the 'animals,' and about 70% of the 'animal' respiration is due to the protozoa, although their contribution to the standing crop is 'only' approximately 30% (Figure 6). This is due to their small size and fast reproduction. However, in earthworm-rich soils the proportion of

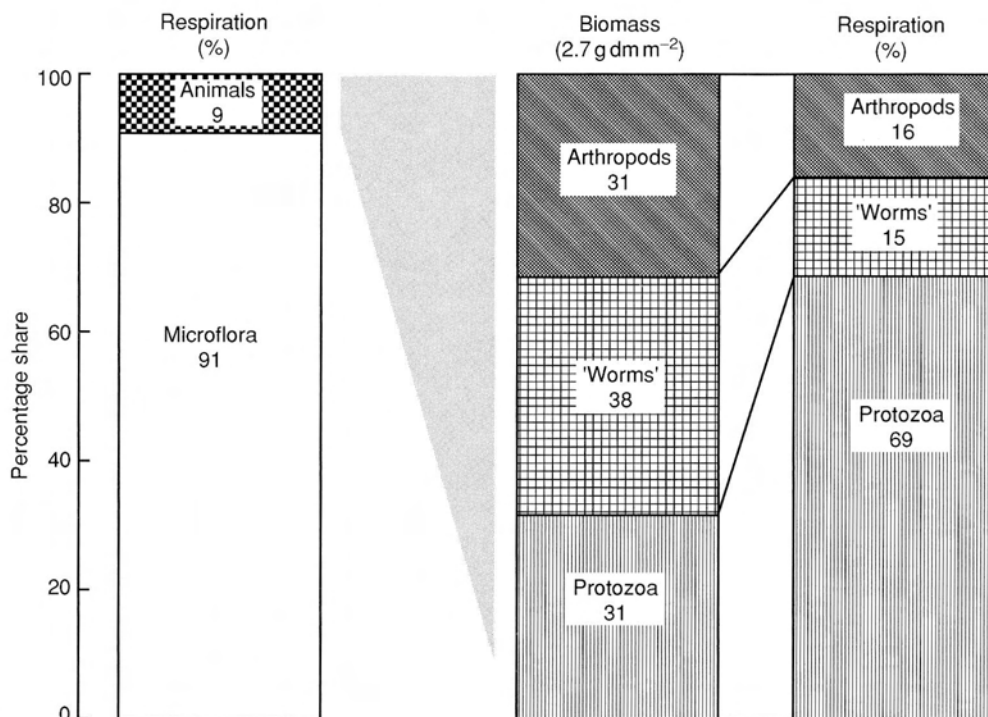


Figure 6 Contribution of protozoa to dry biomass (dm) and respiration of soil organisms. The graphs show the mean of 14 ecosystem studies from various sites of the world. Reproduced with permission from Foissner W (1987) Soil protozoa: fundamental problems, ecological significance, adaptations in ciliates and testaceans, bioindicators, and guide to the literature. *Progress in Protistology* 2: 69–212.

protozoa to the total biomass is considerably lower than stated above, while it is higher in extreme regions, where earthworms are few or lacking, for instance, in coniferous forests, deserts, and high mountains.

Field studies of production of soil protozoa are mostly restricted to testate amebae, the shells of which can be used to estimate their mortality. The annual production is in the same order of magnitude as that of the earthworms, especially when productions of naked amebae and flagellates are added (Table 4). The high contribution of the protozoa to the total annual animal production is due to the many generations produced and shows that protozoa play an important role in the energy flux of the soil ecosystem.

Mineralization and Plant Growth

Considering the fast growth of protozoa and their high annual production (Table 4), it is not surprising that they enhance flows of nutrients in soil to the benefit of plants and microorganisms. Experiments suggest that a complex 'microbial food-web' is operating rather than a simple 'microbial loop' during decomposition and growth. Convincing evidence is available that protozoa enhance plant growth significantly ($\geq 20\%$) either directly by increasing the

availability of nitrogen in soil and leaching water and/or indirectly by nonnutritional effects, such as selective grazing on microbial populations, modification in the concentrations of plant hormones in the rhizosphere, or suppression of pathogenic bacteria. This matter is still under discussion. About 40% of the ingested microbial biomass N will be excreted by protozoa, leading to a constant release of nutrients available for plant uptake. Protozoa are not only responsible for respiring about 10% of the total carbon input but also for 20–40% of net N mineralization in the field. Furthermore, protozoa enhance growth of the plant root system and thus nutrient uptake (Figure 7).

Protozoa also cause a strong decrease in phosphate leaching. Phosphate is one of the most immobile nutrient ions in soil and continuous microdisturbance by protozoa and other microfaunal organisms (e.g., nematodes) is likely particularly important for microbial phosphate availability.

Earthworm Growth

Early reports that earthworms feed on protozoa and are an essential constituent in the diet of the compost worm *Eisenia fetida* to reach sexual maturity were recently confirmed and extended by carefully designed experiments. These showed that *Aporrectodea caliginosa* actively searches for places with high

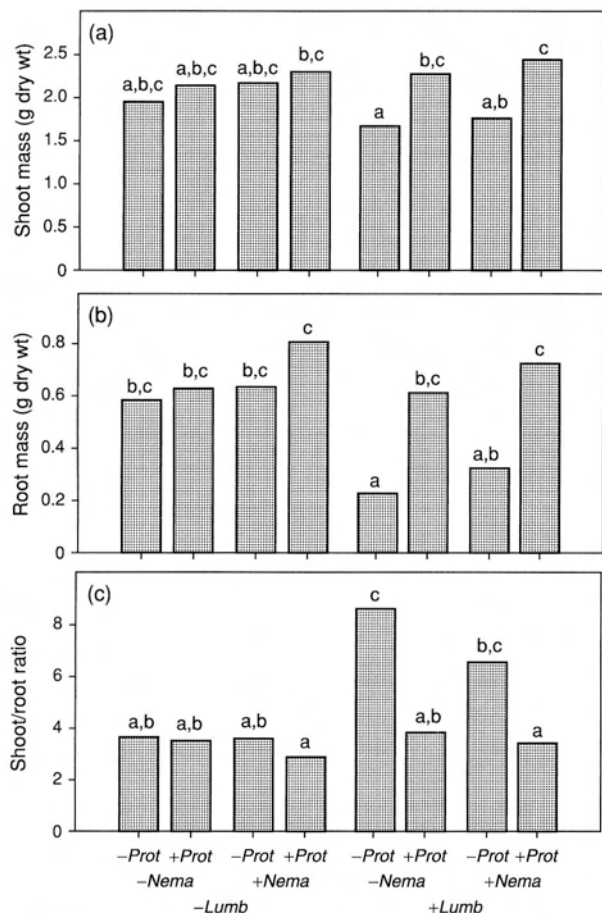


Figure 7 Effects of protozoa (-Prot without protozoa, +Prot with protozoa), nematodes (-Nema without nematodes, +Nema with nematodes) and earthworms (-Lumb without earthworms, +Lumb with earthworms) on (a) shoot biomass; (b) root biomass; and (c) shoot/root ratio of *Hordelymus europaeus*, a grass, in experimental chambers at the end of the experiment (112 days). Bars sharing the same letter are not significantly different. $P > 0.05$ (three-way ANOVA). Reproduced with permission from Alpehi J, Bonkowski M, and Scheu S (1996) Protozoa, Nematoda and Lumbricidae in the rhizosphere of *Hordelymus europaeus* (Poaceae): faunal interactions, response of microorganisms and effects on plant growth. *Oecologia* 106: 111-126.

protozoan densities and feeds on them. The weight gain of young *A. caliginosa* in soil with naked amebae was twice that in soil without amebae.

Protozoa as Bioindicators in Terrestrial Environments

There is considerable literature on the use of protozoa as bioindicators in terrestrial environments, and recently appropriate soil protozoan bioassays became available. Previous reviews summarized data obtained until 1998 and discuss general problems and methodology of bioindication. It has been shown that the full power of bioindication needs

identification of species and/or functional groups because the information content of individual numbers and biomasses is often rather limited. Here, some recent studies are reviewed to demonstrate the great potential of protozoa in the investigation of natural and human-influenced soil ecosystems.

Compared to the soil meso- and macrofauna, protozoa have several specific features making them especially useful for bioindication:

1. Protozoa are an essential component of terrestrial ecosystems, because of their large standing crop and production and their high food consumption and respiration per mass unit (Figure 6, Table 4). Thus, changes in their numbers and diversity influence the rate and kind of soil formation and soil fertility.

2. Protozoa, with their rapid growth, short life-time, 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 a few days, faster than with any other eukaryotic test system.

3. The 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 and genetic differentiation among globally distributed protozoan species is rather low, 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, especially earthworms, due to extreme environmental conditions.

6. Protozoa are not readily dislodged in soil. The difficult problem of horizontal migration, especially with the epigaeon, does not affect the investigations.

Protozoa also pose problems when used as bio-indicators; specifically, counting methods are time-consuming and species identification is troublesome due to the lack of comprehensive identification literature. There are indications that molecular methods and taxonomic reviews can partially solve these drawbacks.

Soil Protozoa as Indicators in Natural Ecosystems

The main humus types, mull, moder, and mor, can be finely discriminated by certain protozoan species, especially testate amebae. Further, some ciliates are excellent indicators for periodic or sporadic oxygen depletion, which is often difficult to recognize and

measure with physicochemical methods. Of course, protozoa can also be used to characterize certain habitats and soil development, for instance, in coastal dunes of Germany (Figure 5). This study shows, *inter alia*, that the content of organic matter is a key factor for the abundance and diversity of the microfauna, and testate amoebae are the most important group, reaching up to 50 000 individuals per gram dry mass. Further, active ciliates are comparatively abundant. These data are in accordance with niche separation of testate amoebae and ciliates and the concept of ciliatostasis reported in the section on vertical distribution.

Protozoa as Indicators in Reclaimed Open-Cast Coal Mining Areas

Topsoil removal, as occurs in open-cast mining areas and during the preparation and use of high-mountain ski slopes, heavily impoverishes protozoa and soil life in general (Figure 8). However, colonization occurs within a few months. Abundances and biomasses in 2- up to 46-year-old afforested mine soils are in the same order as in undisturbed forest soils, but typical humus-inhabiting, large-sized testacean species are lacking or occur rarely, showing that reclamation was only partially successful. Six ubiquists out of 48 taxa contributed 61–87% to the overall abundance. Generally, all test sites had a distinct testacean community, whose structure depended on age, substrate, and stocking.

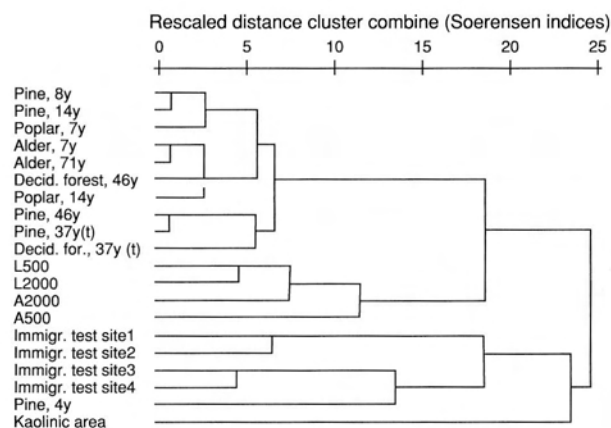


Figure 8 Cluster based on similarity (Soerensen index, UPGMA-linkage) of numbers and species of testate amoebae in various immigration test sites, minicontainers (sites L, A), and recultivated forests. The distances are rescaled to fall in the range of 1–25. Quality and development of the substrates exposed for different time intervals were more important for immigration and colonization success than the adjacent source habitats. Reproduced with permission from Wanner M and Dunger W (2001) Biological activity of soils from reclaimed open-cast coal mining areas in Upper Lusatia using testate amoebae (protists) as indicators. *Ecological Engineering* 17: 323–330.

Protozoa as Indicators of Soil Decontamination

Decontamination of polluted soils is a current challenge for scientists from various fields. Few data are available as concerns the soil fauna, and only one study included protozoa (Figure 9). Immediately after decontamination, active protozoa, nematodes, and collembola were not detected in treated soil. However, protozoan cysts survived treatment (5700 ± 4200 cysts per g dry soil). Following 1 week of exposure, no active protozoa were found. From the second sampling date onwards (after 3 weeks), protozoa were observed with significantly increasing biomass until the end of the experiment 12 weeks after exposition.

Effects of Biocides

A considerable amount of literature is available on the effects of biocides on soil protozoa. Several reviews showed that: (1) the general reaction of soil protozoa to biocide stress matches 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; and (5) investigations should be performed at species and/or functional group level because the simple measure 'total protozoan abundance' is often too insensitive. This is also obvious from a recent study, where the effects of the fungicide fenpropimorph have been investigated in laboratory microcosms and field experiments. While the microcosms showed that bacterivorous protozoa were affected by fenpropimorph at concentrations

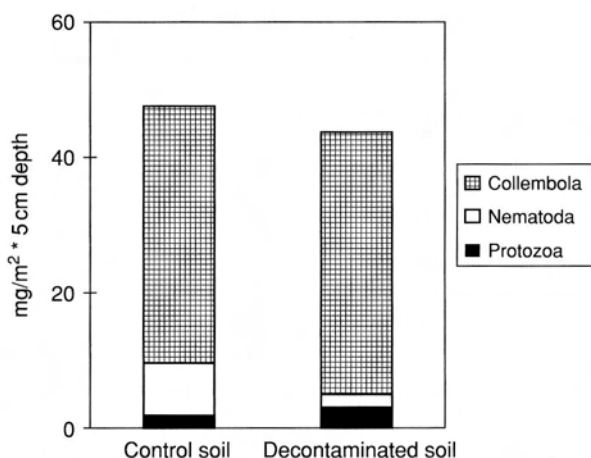


Figure 9 Biomass of Collembola, Nematoda and Protozoa in control and decontaminated soil after 12 weeks of exposure in an arable field. Reproduced with permission from Böckl M, Blay K, Fischer K, Mommertz S, and Filser J (1998) Colonisation of a copper-decontaminated soil by micro- and mesofauna. *Applied Soil Ecology* 9: 489–494.

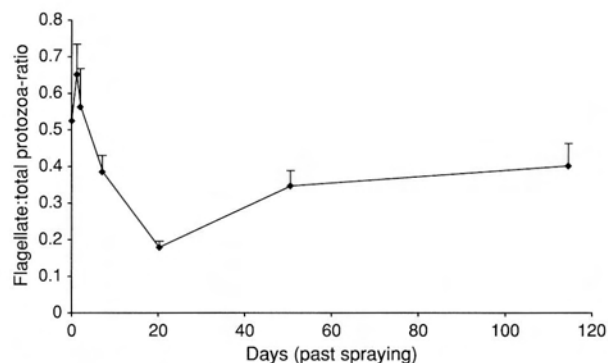


Figure 10 A significant decrease in the flagellate:total protozoan ratio was recognizable when fenpropimorphic acid, a degradation product of fenpropimorph, peaked 20 days after pesticide application. Total protozoan abundance was, however, not significantly changed. Reproduced with permission from Bjørnlund L, Ekelund F, Christensen S *et al.* (2000) Interactions between saprotrophic fungi, bacteria and protozoa on decomposing wheat roots in soil influenced by the fungicide fenpropimorph (Corbel®): a field study. *Soil Biology and Biochemistry* 32: 967–975.

lower than those expected in soil after normal field application, the field experiments showed only a slight decrease in the flagellate-to-total protozoan ratio after 20 days (Figure 10).

See also: **Bacteria:** Plant Growth-Promoting; Soil; **Fauna;** **Fungi;** **Microbial Processes:** Environmental Factors; **Mycorrhizal Fungi;** **Nematodes;** **Pollutants:** Biodegradation; **Root Exudates and Microorganisms**

Further Reading

Alpehi J, Bonkowski M, and Scheu S (1996) Protozoa, Nematoda and Lumbricidae in the rhizosphere of *Hordelymus europaeus* (Poaceae): faunal interactions,

response of microorganisms and effects on plant growth. *Oecologia* 106: 111–126.

Bamforth SS (2001) Proportions of active ciliate taxa in soils. *Biology and Fertility of Soils* 33: 197–203.

Bjørnlund L, Ekelund F, Christensen S *et al.* (2000) Interactions between saprotrophic fungi, bacteria and protozoa on decomposing wheat roots in soil influenced by the fungicide fenpropimorph (Corbel®): a field study. *Soil Biology and Biochemistry* 32: 967–975.

Böckl M, Blay K, Fischer K, Mommertz S, and Filser J (1998) Colonisation of a copper-decontaminated soil by micro- and mesofauna. *Applied Soil Ecology* 9: 489–494.

Bonkowski M and Schaefer M (1997) Interactions between earthworms and soil protozoa: a trophic component in the soil food web. *Soil Biology and Biochemistry* 29: 499–502.

Corliss JO (2000) Biodiversity, classification, and numbers of species of protists. In: Raven PH and Williams T (eds) *Nature and Human Society*, pp. 130–155. Washington, DC: National Academy Press.

Darbyshire JF (ed.) (1994) *Soil Protozoa*. Oxon: CAB International.

Foissner W (1987) Soil protozoa: fundamental problems, ecological significance, adaptations in ciliates and testaceans, bioindicators, and guide to the literature. *Progress in Protistology* 2: 69–212.

Foissner W (1994) Soil protozoa as bioindicators in ecosystems under human influence. In: Darbyshire JF (ed.) *Soil Protozoa*, pp. 147–193. Oxon: CAB International.

Foissner W (1999) Soil protozoa as bioindicators: pros and cons, methods, diversity, representative examples. *Agriculture, Ecosystems and Environment* 74: 95–112.

Verhoeven R (2002) The structure of the microtrophic system in a development series of dune soils. *Pedobiologia* 46: 75–89.

Wanner M and Dunger W (2001) Biological activity of soils from reclaimed open-cast coal mining areas in Upper Lusatia using testate amoebae (protists) as indicators. *Ecological Engineering* 17: 323–330.