Protozoa as bioindicators in agroecosystems, with emphasis on farming practices, biocides, and biodiversity

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Protozoa as bioindicators in agroecosystems, with emphasis on farming practices, biocides, and biodiversity

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Abstract

This paper emphasizes some aspects of soil protozoology related to agriculture, viz. farming systems, biocides, and biodiversity. Ecofarming slightly increases abundances and biomasses of soil protozoa and stimulates soil life in general. Agroecosystems are more sensitive to conventional farming practices than grasslands, especially under severe climatic conditions, e.g. water stress. The increased soil life under ecofarming is not associated with an increased crop yield. In contrast, yield decreases more or less distinctly. However, the yield loss is usually partially or completely compensated by the reduced operating expenses (energy input) per crop unit. Thus, ecofarming appears to be a useful practice, especially for sustaining biodiversity.

The effects of biocides on soil protozoa can be summarized as follows: (1) The general pattern of reaction of soil protozoa to biocide stress is the same as that of other organisms; (2) Many protozoan species seem to be just as sensitive to pesticides as other more commonly used test organisms; (3) Insecticides are usually more toxic than herbicides and disturb soil protozoa critically, i.e. populations often do not fully recover within 60 days; (4) Fungicides have rather varied effects but most of them very likely do not influence soil protozoa critically. There is still a great need for comprehensive, well-designed field studies on the effects of biocides on soil protozoa, especially testate amoebae.

Literature on protozoan species diversity in agroecosystems is sparse, highly scattered, and burdened with misidentifications. Our own data indicate that species richness of ciliates is usually only slightly decreased, and is sometimes even higher, in agroecosystems as compared with neighbouring natural biotopes. This contrasts with species richness of testate amoebae, which is invariably and distinctly (≥ 50%) reduced by agriculture, leaving an impoverished version of the original, much more diverse community. Testate amoebae are thus highly sensitive bioindicators in agroecosystems. © 1997 Elsevier Science B.V.

Keywords: Biocides; Biodiversity; Ciliophora; Farming system, conventional; Ecofarming; Soil protozoa; Testate amoebae

1. Introduction

This paper reviews some aspects of soil protozoology related to agriculture, viz. farming practices, biocides, and biodiversity. Recent findings and unpublished data will be emphasized since much of the older literature has been collected in reviews during the last decade (Nikolyuk, 1980; Foissner, 1987, 1991, 1994; Geltzer, 1991, 1992; Schünborn, 1992; Juma, 1993; Aescht, 1994; Darbyshire, 1994; Ekelund and Rönn, 1994; Bamforth, 1996). The heuristic background and the pros and cons of using soil protozoa and invertebrates as bioindicators have been critically reviewed by Foissner (1994) and Paoletti and Bressan (1996).

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2. Conventional farming and ecofarming

Ecofarming is a term that will be used to indicate a kind of sustainable agriculture increasingly discussed and practised worldwide. Its main philosophy is to protect and stimulate soil life in order to increase or, at least, maintain soil fertility. Ecofarming derives from biology and ecology, especially the principle of the regulation of the abundance, distribution and activity of species in space and time (Diercks, 1983; Thomas and Kevan, 1993). It is thus more comprehensive and rigid than the concept of sustainable agriculture which consists, as defined by Lehman et al. (1993), of agricultural processes that do not exhaust any irreplaceable resources which are essential to agriculture.

With regard to protozoa, investigations were performed mainly by Foissner in Austria and Zwart in the Netherlands. Both studied numerous biotic and abiotic parameters in several ecofarming variants (integrated, organic, biodynamic) in altogether 14 paired sites (conventional vs. ecological). Foissner (1992) studied testate amoebae and ciliates, Zwart heterotrophic flagellates and naked amoebae (Bloem et al., 1994). Although different methods (direct counts in soil suspensions vs. culture and MPN methods) and indicator groups were used, the results match nicely and can be summarized as follows:

1. Many of the soil zoological parameters under investigation did not differ statistically in ecofarmed and conventionally farmed fields and grasslands;
2. There were no striking differences in species composition and dominance structure of the ciliates and testate amoebae;
3. All differences that could be guaranteed with an error probability of $\alpha = 10\%$ or less invariably showed higher biological activity in the ecofarmed sites (Fig. 1, Fig. 2, Fig. 3). The soil physical and chemical investigations which ac-

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**Fig. 1.** Biomasses of testate amoebae (direct counts in diluted soil suspensions) and earthworms (formaldehyde extraction) in conventionally farmed and ecofarmed fields and grasslands in Austria. Combined data from several investigations (Foissner, 1992). Asterisks mark significant differences, i.e. error probability not exceeding 10%. DM, dry mass of soil.

**Fig. 2.** Biomasses of heterotrophic flagellates and naked amoebae (culture counts) in the top 0–10 cm layer of winter wheat fields under conventional and integrated management at the Lovinkhoeve Experimental Farm, the Netherlands. LSD$_{depth \times field} = 0.00994$ kg C ha$^{-1}$ cm$^{-1}$ depth for flagellates and 0.250 kg C ha$^{-1}$ cm$^{-1}$ depth for naked amoebae. (From Bloem et al., 1994).

**Fig. 3.** See caption for Fig. 2.
compared the zoological studies at some sites revealed that larger biological activity was correlated with a higher humus content and less soil compaction. The organic matter content was significantly higher in the ecofarmed plots, whereas soil compaction was more pronounced under conventional cultivation;

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

5. Agroecosystems are more sensitive to conventional farming than grasslands.

What is the meaning of the rather inconspicuous differences found? First, they show that conventional agriculture is often not as harmful to soil life as widely assumed. Second, we have to investigate how these differences are related to crop yield and economic performance. Unfortunately, few studies are available relating crop yield and soil life. The most comprehensive investigations were performed by Pfiffner’s group in Switzerland. They compared three production systems (biodynamic, organic, conventional) in a 7-year crop rotation. Soil life was distinctly richer in the ecofarmed variants (Pfiffner, 1990; Mäder et al., 1993; Pfiffner et al., 1993), but crop yield and yield stability were decreased by about 20% as compared with the conventionally managed system (Spiess et al., 1993; Niggli et al., 1995). However, yield loss was compensated by a 15% to 30% lower total energy input per crop unit in the ecofarmed variants (Alföldi et al., 1995). The results of the Swiss group correlate well an earlier study by Lockeretz et al. (1981), who compared organic and conventional farms in the USA. They found that the slightly lower gross production per unit of cropland on the organic farms was largely offset by comparable reductions in operating expenses, so that crop production was about equally profitable on the two types of farm, except in a year that had extremely favourable weather. The exact comparison appears to depend on growing conditions, with the organic farms doing relatively better under the abnormally poor conditions of the mid-1970s, but relatively poorer when conditions improved in 1978. Except for wheat, a minor crop in the Corn Belt for which organic farms had much poorer yields, yields of most organically raised crops generally ranged from about the same to about 10% lower than on the conventional farms. Along the same lines are very recent results by Drinkwater et al. (1995) and van Bruggen (1995), who compared 20 conventional and organic tomato agroecosystems in California. Both production systems could not be distinguished based on agronomic criteria such as fruit yield and arthropod pest damage levels. However, differences were demonstrated in many soil, plant, disease, and diversity indicators suggesting that the ecological processes determining yields and pest levels in these two management systems are distinct. In particular, nitrogen mineralization potential and microbial and parasitoid abundance and diversity were higher in the organic farms. Differences between the agroecosystems were sufficiently robust to be distinguished from environmental variation and suggest that biological processes compensated for reductions in the use of synthetic fertilizers and pesticides.

There are, however, also less favourable data available. Favretto et al. (1992), who compared mulched and tilled vineyard plots in Italy, found that green mulching significantly increased most nutrients and mesofaunal biomass, but decreased yield by an average of 80% mainly due to water competition. Along the same lines are the results by Foissner et al. (1990), who compared yield, fodder quality, and soil fauna in conventionally and ecologically farmed grassland plots in Austria. No significant differences were found for any of the parameters investigated, but labor input was higher for the ecofarmed variants than for the conventional plots.

Obviously, results are at variance and strongly depend on investigation and farming methods, region, climate, and crops. Seen on a very rough scale, ecofarming appears as a promising practice, especially for sustaining biodiversity. As Lee and Pankhurst (1992) concluded, at the present state of knowledge it seems wise to prefer management technologies that conserve the biodiversity of communities because these may provide the greatest benefits for the long term sustainability of the soil resource.

3. Effects of biocides on soil protozoa

Literature on the effects of biocides on soil protozoa is comparatively extensive, but well-designed
field studies are rare. Most of the commonly used biocides, e.g. atrazine, thiram, lindane, have been tested in at least one study. However, the testate amoebae have been largely ignored, regardless of their high ecological significance and the reliable methods available for their abundance estimation. This is particularly frustrating in the light of recent studies which demonstrate more and more that a significant portion of the pesticides and their metabolites is bound to the humus fraction, a main source of food for testate amoebae (Khan, 1982; Schönborn, 1965; Scheunert, 1992). Furthermore, most studies did not consider effects at species level and used culture methods for abundance estimation, which poses many problems (Berthold and Palzenberger, 1995). Future research should thus concentrate on field studies, particularly testate amoebae, the most important indicator group. Direct counts, e.g. in diluted soil suspensions, should be preferred whenever this is feasible.

Foissner (1987, 1994) reached the following conclusions from the data available:
1. The general pattern of reaction of soil protozoa to biocide stress is the same as that of other organisms;
2. Many protozoan species seem to be just as sensi-

Fig. 4. Survival of *Rhizobium phaseoli* and total (active + cystic) protozoan numbers in nonsterile soil microcosms without and with antimicrobial drugs. Values are means from three replicates each. Singh's (1946) culture method was used for protozoan enumeration. Cycloheximide was mixed with soil to a final concentration of 0.5 mg g⁻¹ air-dried soil. Concentration of thiram was not provided. (From Chao and Alexander, 1981).

Fig. 5. The effect of adding cycloheximide and Triton X-100 (0.5 and 1.0 mg g⁻¹ soil, respectively) (○) or distilled water (●) on amoebal and flagellate numbers in soil microcosms. Each point is the mean of three replicate bottles incubated at 15°C; bar represents 1 SE. A modified Singh (1946) culture method was used for protozoan enumeration. (From Griffiths, 1989).
tive 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. Based on the recent literature, reviewed below, and some older data, mentioned in Foissner (1987, 1994), these conclusions may be extended:
5. Fungicides have rather varied effects but most of them very likely do not disturb soil protozoa critically.

The following paragraphs discuss literature published in the years 1993 and 1994 as well as some papers not contained in my previous reviews (Foissner, 1987, 1994).

Chao and Alexander (1981) investigated the effects of the fungicides cycloheximide and thiram on protozoa in laboratory microcosms. *Rhizobium phaseoli* was more distinctly reduced by protozoa in the absence of the biocides, suggesting that protozoa were inhibited although they reached and maintained a rather high level (Fig. 4). Examination of the cycloheximide-treated soil revealed that the diversity of protozoa had declined abruptly, and only one morphological type was detected, a ciliate. However, this could have been caused by methodological problems because culture methods are often and for inexplicable reasons very selective.

Griffiths (1989) studied the effects of a combined dose of cycloheximide (fungicide) and Triton X-100 (a general eukaryotic inhibitor) on soil protozoa in laboratory microcosms. Both protozoan populations (Fig. 5) and nitrification were significantly reduced.

Todorov and Golemansky (1992) studied the effects of three fungicides (Fundasol, Fuzamicin, Lavendotricin) on three common, large freshwater and soil protists, viz. *Blepharisma japonicum*, *Amoeba proteus*, and the testate amoeba *Arcella vulgaris* in liquid patch cultures. All species died within a few hours in 1% solutions of the biocides. Lavendotricin and Fuzamicin were more toxic than Fundasol, which even stimulated growth of *Arcella* at low concentrations (Fig. 6d). All species showed distinct growth inhibition, encysted or died at biocide concentrations ≥ 0.01% (Fig. 6a–d).

The results of Todorov and Golemansky (1992) were not confirmed in a field trial performed by Miteva (1992). She found that Fundasol was slightly more toxic than Lavendotricin. However, data were obtained with a selective culture method not distinguishing between active and cystic protozoa. Few differences could be guaranted statistically and num-

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**Fig. 6.** The effect of various concentrations of Lavendotricin (a–c) and Fundasol (d) on protozoan numbers in liquid patch cultures. Each point is the mean of two replicates incubated at room temperature. Cells were counted alive with a dissecting microscope. a: *Blepharisma japonicum*; b: *Amoeba proteus*; c, d: *Arcella vulgaris*. 1: control; 2: 0.001%, 3: 0.01%, 4: 0.1% biocide concentrations. (From Todorov and Golemansky, 1992).
bers varied inconsistently, i.e. higher abundances sometimes occurred in the treated plots. Differences did not become more pronounced when fungicides were combined with an insecticide, Agria-1060. No differences were found in species richness and composition of the naked amoebae.

Ekelund et al. (1994) studied the effects of two insecticides (dimethoate, pirimicarb) and a fungicide (fenpropimorph) on total (active + cystic) soil protozoa in soil microcosms and on three selected species (Cercomonas sp., a flagellate; Acanthamoeba sp., a naked amoeba; Colpoda sp., a ciliate) in liquid patch cultures. The biocides had a negative impact on the colonization of the sterilized soil microcosms by a natural population of soil protozoa. Fenpropimorph had an effect at the lowest concentration applied, i.e. 25 mg l\(^{-1}\). However, treatment effects became insignificant for all biocides tested after 20 days. In single species cultures, Cercomonas sp. was the organism most sensitive to fenpropimorph and pirimicarb, while Colpoda sp. was the most sensitive to dimethoate (Table 1). The growth of Colpoda was apparently stimulated by very low concentrations of fenpropimorph or pirimicarb (Table 1). Acanthamoeba was in all cases less sensitive than the other two organisms.

Ingham et al. (1986) studied the response of protozoa to five biocides, viz. streptomycin (bactericide; 3 mg g\(^{-1}\) soil), captan (fungicide; 12 \(\mu\)g a.i. g\(^{-1}\) soil), PCNB (fungicide; 100 \(\mu\)g g\(^{-1}\) soil), carbofuran (insecticide-nematicide; 2.5 \(\mu\)g a.i. g\(^{-1}\) soil), and cygon (acaricide; 0.8 mg a.i. 4 lb gal\(^{-1}\)) in a semi-arid grassland soil in the USA. The substances were applied once in situ to soil in cylinders containing predominantly blue grama grass (Bouteloua gracilis). Abundances of active and cystic protozoa were estimated with the culture method of Singh (1946) monthly between April and October.

In streptomycin treatments, cystic flagellates were greater in July and August, while totals were reduced in May. Total amoebal numbers in the soil depth of 0–5 cm were initially increased in April but then recovered. In the 5–10 cm depth of the streptomycin treatment, fluctuations in amoebal populations and the controls were asynchronous. Streptomycin did not change ciliate responses.

In captan treatments total flagellate numbers were initially depressed but returned to control levels.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dimethoate</th>
<th>Pirimicarb</th>
<th>Fenpropimorph</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cercomonas sp.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EC(_{50})</td>
<td>690</td>
<td>94</td>
<td>2.2</td>
</tr>
<tr>
<td>NOEC</td>
<td>&lt;160 (b)</td>
<td>&lt;120 (b)</td>
<td>&lt;1.5 (b)</td>
</tr>
<tr>
<td>LC(_{100})</td>
<td>&gt;930 (c)</td>
<td>820</td>
<td>8.0</td>
</tr>
<tr>
<td>EC(_{10}) (hormesis)</td>
<td>n.o. (d)</td>
<td>n.o. (d)</td>
<td>n.o. (d)</td>
</tr>
<tr>
<td>Acanthamoeba sp.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EC(_{50})</td>
<td>510</td>
<td>1500</td>
<td>13</td>
</tr>
<tr>
<td>NOEC</td>
<td>80</td>
<td>120–1000</td>
<td>4.5</td>
</tr>
<tr>
<td>LC(_{100})</td>
<td>&gt;930 (c)</td>
<td>2000</td>
<td>&gt;25 (c)</td>
</tr>
<tr>
<td>EC(_{10}) (hormesis)</td>
<td>n.o. (d)</td>
<td>120</td>
<td>n.o. (d)</td>
</tr>
<tr>
<td>Colpoda sp.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EC(_{50})</td>
<td>300</td>
<td>830</td>
<td>9.2</td>
</tr>
<tr>
<td>NOEC</td>
<td>110</td>
<td>780</td>
<td>8.0</td>
</tr>
<tr>
<td>LC(_{100})</td>
<td>&gt;930 (c)</td>
<td>1100</td>
<td>11</td>
</tr>
<tr>
<td>EC(_{10}) (hormesis)</td>
<td>26</td>
<td>40</td>
<td>0.33</td>
</tr>
</tbody>
</table>

\(a\) EC\(_{50}\): the lowest concentration causing 50% population reduction compared with the control after four days of growth; NOEC: the lowest concentration causing no population decrease compared with the control; LC\(_{100}\): the lowest concentration causing 100% death, and EC\(_{10}\) (hormesis): the lowest concentration causing a 10% increase in individual numbers on Day 4 as compared with the control. \(b\) NOEC could not be calculated since an effect was detectable even for the lowest concentrations tested. \(c\) LC\(_{100}\) could not be calculated since the organisms survived even at the highest concentrations tested. \(d\) No stimulation (hormesis) observed.

Cystic flagellate numbers were reduced significantly in June and August so that more of the flagellates were active. Total amoebal numbers were reduced until June in the 0–5 cm depth, but then regained control levels. Only an initial reduction of amoebal numbers in the lower depth and no effect on cystic amoebae was observed. Ciliate numbers were not affected by captan.

In general, PCNB initially increased (0–5 cm) or reduced (5–10 cm) all protozoa, followed by a return to control levels. Cystic flagellate numbers were greater in June and August so that fewer active flagellates were present on these two dates than in controls. Total amoebae in the 5–10 cm depth tended to fluctuate inversely to the control. Cystic amoebal numbers tended to be lower, although not always significantly, indicating that more amoebae were active in this treatment. Ciliate numbers also tended to fluctuate close to control numbers.
Carbofuran did not affect flagellate numbers, either total or cystic, increased total amoebal numbers in April in the 0–5 cm depth but decreased them in the 5–10 cm depth, then fluctuated inversely to the control, generally ending lower than the control. In the 0–5 cm, total ciliate numbers were divergent from controls on two dates only, tenfold lower, 32 instead of 560 (controls), in May, and almost 100-fold higher, 2800 as opposed to 32 in controls, in August.

Flagellates, both total and cystic, were not affected by cygon. In both soil depths, total amoebal numbers fluctuated inversely to the controls. Cystic amoebal numbers were similar to controls, indicating that in May and June the amoebae were probably all encysted. In the lower soil depth, cygon generally reduced total amoebal numbers, so that the population was probably all encysted here also. Ciliates were generally not affected by cygon.

Ingham et al. (1991) applied streptomycin, a bactericide, and captan (25 µg g⁻¹ soil), a fungicide, to a non-tillage agroecosystem in Georgia, USA. No significant treatment effects were noted for flagellates, naked amoebae, and ciliates, either in the litter layer or in the 0–5 cm soil zone. However, the plots were analyzed only on days 7 and 14 after the biocide application and protozoan abundances were estimated with a simplified, selective culture method whose statistical soundness is doubtful (pseudo-replication; Berthold and Palzenberger, 1995).

Similar methodological weaknesses are inherent in another study (Colinas et al., 1994), investigating the population responses of target and non-target forest soil organisms to a set of biocides, viz. oxytetracycline-penicilline (combined; bactericide), captan (fungicide), fumagillin (protozoacide), and dimethoate-carbofuran (combined; nematicide and insecticide). The substances were administered to soil mesocosms (9 liter) at manufacturer-recommended rates and treatment effects were analyzed only once, 32 h after biocide application. All biocides reduced protozoa, especially fumagillin (Fig. 7), and affected populations of non-target organisms.

Miteva (1985) investigated the effects of the herbicide dual in strawberry plantations. The biocide was applied as recommended by the manufacturer and treatment effects were analysed five times between Days 13 and 135. Protozoan abundances were estimated with a modified Singh (1946) method.

Naked amoebae and ciliates, but not flagellates, were slightly decreased by the biocide. No significant differences could be found in total protozoan numbers and species composition of naked amoebae. However, there was a constant trend in that numbers of amoebae, flagellates, and ciliates were slightly decreased in the treated plots. Thus, taking the five sample occasions as independent variables, a significant difference (P < 0.1, at least) in total protozoan numbers would result.

Parker et al. (1985) studied the effects of oxamyl (nematicide and insecticide; 7 µg g⁻¹ soil) and chlordane (insecticide; 25 µg g⁻¹ soil) on litter (creosote bush, Larrea tridentata) and soil (Typic Haplud) protozoa in laboratory microcosms. Protozoan abundances were estimated with Singh’s (1946) culture method 5 and 30 days after administering the substances. Oxamyl caused a decrease of soil protozoa from 24.300 × 10⁴ g⁻¹ soil (control) to 5.440 × 10⁴ g⁻¹ soil between Days 5 and 30, but had no effect on litter protozoa. Chlordane affected neither litter nor soil protozoa.

Wanner (1994) and Wanner et al. (1994) performed many experiments on the influence of lime (CaCO₃: 1 mg per 2 ml culture medium), a mineral fertilizer (0.25 mg 5 Ca(NO₃)₂·NH₄NO₃ per 2 ml), and two insecticides (riforcd 10: cypermethrin, a synthetic pyrethroid; spruzit: an emulsion of 4% pyrethrine and 16% piperonyl butoxide) on a common soil and freshwater testate amoebae, Cyclopyxis kahl i, in liquid patch cultures. Insecticides were administered in two concentrations: 1 µg or 5 µg per 2 ml culture medium and 0.05 µl or 0.2 µl.
μl spruzit emulsion per 2 ml culture medium. These experiments supplemented and extended an earlier field trial showing that ripcord and a combined application of lime and fertilizer reduced the abundance of testate amoebae by about 30% (Wanner, 1991; reviewed in Foissner, 1994).

Spruzit (0.2 μl) extended the lag phase significantly ($P < 0.05$), despite the fact that active pseudopodia emerged from *C. kahli* immediately after addition of the biocide (Fig. 8). Lower doses of spruzit (0.05 μl) and both concentrations of ripcord did not cause marked effects ($P > 0.05$). Likewise, lime, fertilizer, or lime and fertilizer did not improve culture growth, but both insecticides increased generation time significantly. Pesticides applied at 17°C caused larger shells of *C. kahli* as compared with the control (Fig. 9). However, this effect diminished at higher temperature (21°C). No significant effects were observed after administration of the lower biocide concentrations, but increased doses (0.25 μl ripcord, 2 μl spruzit) inhibited growth completely.

In summary, these experiments showed that recommended application doses of ripcord and spruzit had significant effects on growth and shell size of *C. kahli*. However, both were usually influenced to a greater extent by food and temperature than by the insecticides. As a rule, mixtures of the biocides with fertilizer or lime showed the same effects as pure insecticide treatment. No consistent treatment adaptations were observed either in culture growth or shell size.

4. Protozoan diversity in agroecosystems

Literature on protozoan species diversity in agroecosystems is sparse, highly scattered, burdened with misidentifications, and has never been reviewed in detail. This has several reasons: first, most taxonomists prefer taking samples from natural biotopes expecting a higher diversity of the community and more interesting species; second, many faunistic data were obtained by ecologists not trained in taxonomy; thus a huge number of misidentifications has accumulated over time, as exemplified for ciliates by Foissner (1987); third, soil zoologists and ecologists have only recently started focusing on agroecosystems; fourth, α-taxonomists and faunists, who are doing the ‘hard work’, i.e. determining and eventually describing new species, are decreasing in num-

![Fig. 8. Effects of lime and fertilizer (LM: 1 mg 95% CaCO₃ plus 0.25 mg 5 Ca(NO₃)₂·NH₄NO₃ solved in 2 ml culture medium), ripcord and fertilizer (RF: 1 μg a.i. plus 0.25 mg 5 Ca(NO₃)₂·NH₄NO₃ solved in 2 ml culture medium), ripcord and lime (RL: concentrations as above), spruzit and fertilizer (S20F: 0.2 μl emulsion plus 0.25 mg 5 Ca(NO₃)₂·NH₄NO₃ solved in 2 ml culture medium), and spruzit and lime (S20L; concentrations as above) on growth (lag-phase at 17°C and 21°C) of *Cyclopyxis kahli*, a common soil and freshwater amoeba.](image)

![Fig. 9. Effects of two insecticides, ripcord (R) and spruzit (S), on shell size of *Cyclopyxis kahli*, a common soil and freshwater testate amoeba. Two experiments are shown Experiment 1 (left half): low food level at 17°C and 21°C; Experiment 4 (right half): high food level at 21°C in illuminated (i) and pretreated (j) cultures. A randomized block design and liquid patch cultures were used. Four replicate experiments each are shown. C: control; Ci: control, illuminated cultures; R1: ripcord 0.01 μl; R5: ripcord 0.05 μl; R5i: ripcord 0.05 μl, illuminated; RS, S20: ripcord (0.05 μl) and spruzit (0.2 μl) in pretreated cultures, i.e. amoebae from an insecticide-treated culture were used as inoculum for a new insecticide-treated culture; S20: spruzit 0.2 μl. (From Wanner et al., 1994).](image)
Table 2
Species diversity of testate amoebae and ciliates in some Austrian agroecosystems as compared with neighbouring natural biotopes

<table>
<thead>
<tr>
<th>Site no.</th>
<th>Site description</th>
<th>Species number</th>
<th>No. of samples analysed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Conventionally farmed wheat field (A)</td>
<td>62</td>
<td>18</td>
</tr>
<tr>
<td>2</td>
<td>Riverside forest soil near Site 1</td>
<td>71</td>
<td>44</td>
</tr>
<tr>
<td>3</td>
<td>Xerothermic site without trees within Site 2</td>
<td>74</td>
<td>28</td>
</tr>
<tr>
<td>4</td>
<td>Conventionally farmed wheat field (B)</td>
<td>69</td>
<td>15</td>
</tr>
<tr>
<td>5</td>
<td>Riverside forest soil near Site 4</td>
<td>74</td>
<td>37</td>
</tr>
<tr>
<td>6</td>
<td>Xerothermic site without trees within Site 4</td>
<td>59</td>
<td>26</td>
</tr>
<tr>
<td>7</td>
<td>Conventionally farmed field (C)</td>
<td>74</td>
<td>27</td>
</tr>
<tr>
<td>8</td>
<td>Ecofarmed field (D) near Site 7</td>
<td>82</td>
<td>30</td>
</tr>
<tr>
<td>9</td>
<td>Conventionally farmed field (E)</td>
<td>76</td>
<td>30</td>
</tr>
<tr>
<td>10</td>
<td>Ecofarmed field (F) near Site 9</td>
<td>59</td>
<td>29</td>
</tr>
<tr>
<td>11</td>
<td>Conventionally farmed maize field (G)</td>
<td>10</td>
<td>27</td>
</tr>
<tr>
<td>12</td>
<td>Conventionally farmed maize field (H) near Site 11</td>
<td>58</td>
<td>6</td>
</tr>
<tr>
<td>13</td>
<td>Hedge near Site 11</td>
<td>77</td>
<td>18</td>
</tr>
<tr>
<td>14</td>
<td>Riverside forest soil near Site 11</td>
<td>91</td>
<td>29</td>
</tr>
</tbody>
</table>

* Sites 1–6 are in Lower Austria and described in detail in Foissner et al. (1985). Data are based on 10 sampling occasions each during two years. Sites 7–10 are in Salzburg and described in Foissner (1992). Data are based on eight sampling occasions each during 2 years. Sites 11–14 are in Upper Austria and described in Steinberger and Thaler (1994). Data are based on three sampling occasions each during three months.

* Data for testate amoebae were obtained by inspecting 0.1 g soil suspended in water at each sampling occasion. Species numbers of ciliates were obtained with the non-flooded petri dish method as described by Foissner (1987). Briefly, this simple method involves placing 10–50 g of air-dried moss, litter and/or soil in a petri dish (10–15 cm in diameter) and saturating but not flooding it with distilled water. Such cultures were grown at room temperature and analyzed for ciliates on Days 2, 7, 14, 21 and 28 by inspecting about 2 ml each of the run-off. The non-flooded petri dish method is not perfect, i.e. not all species present can be reactivated from the resting cysts, but probably the most efficient method available. Repeated investigations of some soils showed that 2–5 samples distributed over one year produce 50–80% of the species found in 10 samples investigated over 2 years (Foissner, 1987). Thus, the samples investigated very likely contain more species than shown in Table 2 and Table 3.

search (see Cotterill, 1995; Foissner and Wanner, 1995 and Prance, 1995 for a more detailed discussion of this controversial matter).

Table 3
Species diversity of ciliates in agroecosystems of various regions of the world as compared with neighbouring natural biotopes

<table>
<thead>
<tr>
<th>Site no.</th>
<th>Site description</th>
<th>Species number of ciliates</th>
<th>No. of samples analysed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Hop field near Mt. Fields NP, Tasmania</td>
<td>40</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>Pasture near Site 1</td>
<td>39</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>Nothofagus forest near Site 1</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>Sugar cane field near Eugenbenee, Australia</td>
<td>27</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>Swamp near Site 4</td>
<td>42</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>Xerothermic hill within Site 5</td>
<td>24</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>Rice fields in Japan</td>
<td>83</td>
<td>4</td>
</tr>
<tr>
<td>8</td>
<td>Woodlands near Site 7</td>
<td>52</td>
<td>4</td>
</tr>
<tr>
<td>9</td>
<td>Pasture, Monte Verde, Costa Rica</td>
<td>36</td>
<td>1</td>
</tr>
<tr>
<td>10</td>
<td>Rain forest (Quetral Trail) near Site 9</td>
<td>34</td>
<td>1</td>
</tr>
<tr>
<td>11</td>
<td>Wheat field, Israel</td>
<td>17</td>
<td>1</td>
</tr>
<tr>
<td>12</td>
<td>Eucalyptus forest near Site 11</td>
<td>19</td>
<td>1</td>
</tr>
</tbody>
</table>

* Same method as described in Footnote b of Table 2.
Some of our results on protozoan species richness in agroecosystems, most shown here for the first time and still rather fragmentary, are summarised in Tables 2 and 3. Some main distinctions are evident from this compilation. Ciliate species richness is usually only slightly decreased, sometimes even higher, in agroecosystems as compared to neighbouring natural biotopes (Table 2, Table 3). In contrast, species richness of testate amoebae is invariably and distinctly (≥ 50%) reduced in agroecosystems, whose testatean fauna is mainly composed of small, euryoecious species (e.g. *Trinema lineare, Euglypha rotunda, Phryganella acropodia*), i.e. it is a residue of the original, much more diverse community (Table 2). Thus, I consider testate amoebae as the most important protozoan indicator group in agroecosystems, at least as long as reliable comparative investigations become available for flagellates and naked amoebae, which very likely numerically dominate in soil (Ekelund et al., 1994). However, flagellates and naked amoebae are more difficult to determine and the estimation of their species richness is, as in ciliates, more complicated, requiring culture methods.

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