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Symposium 5

Protozoa of Soil

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Summary

This paper reports on a symposium organized by W. Foissner (chair and report) and M. Wanner (co-chair) on the occasion of the Second European Congress of Protistology in Clermont-Ferrand, 21-27 July 1995. The symposium emphasized aspects of applied soil protozoology and treated the following subjects in some detail: integrating soil protozoology with general soil and environmental sciences, soil protozoan diversity, bioindication and autecology, soil protozoology in Russia. A guide to recent reviews and books on soil protozoa is included.

Introduction

Soil protozoology is rejuvenating after a long period of stagnation between 1930 and 1970. This is reflected not only by several important recent reviews [1, 6-8, 13, 14, 16-18] but also by the Second European Congress of Protistology, which highlighted the importance of soil protozoa by dedicating two symposia to the subject (see report by E. Piccini in this volume).

Research during the past 20 years provided convincing evidence that protozoa play an important role in the energy and nutrient flux of soil ecosystems [6-8, 18]. In spite of this, protozoa are still insufficiently recognized by general ecologists and soil scientists. Thus, John F. Darbyshire, the grand old man of soil protozoology, was invited to lecture on possible ways out of the isolation. A theme inherent also in the contributions by Erna Aescht, Ralf Meisterfeld, and Manfred Wanner, who presented aspects of applied soil protozoology, namely bioindication, an increasingly important field. However, bioindication requires, as the word implies, a profund knowledge of species and community structures. Unfortunately, soil protozoan diversity is still very insufficiently known. This was highlighted by Wilhelm Foissner in his

lecture on soil ciliates from the Etosha National Park: 53 out of 153 species found in 12 samples were new to science.

Finally, Julius Geltzer was invited introduce us to the vast Eastern European literature on soil protozoa, which has been widely neglected, probably because few colleagues can cope with the Russian language. Thus, our Russian colleagues should be encouraged to publish in English or at least to furnish their papers with extensive English summaries.

Integrating soil protozoology with general soil and environmental sciences

Dr John Darbyshire (Macauly Land Use Research Institute, Scotland) presented a storm of ideas as to how soil protozoology could be more closely related to environmental sciences in general and soil science in particular. He emphasized the need for soil protozoologists to promote their discipline more rigorously and to improve the situation themselves by integrating their studies more closely with the rest of soil ecology at the levels of ecotones, ecosystems and landscapes. John Darbyshire's paper was read by H. G. Smith (Coventry University, England) because John was unable to attend the congress.

In order to examine ways in which soil protozoology might be integrated with wider aspects of soil ecology we need to be aware of the complex and heterogeneous nature of the soil ecosystem and also that the species composition and the population fluctuations of the protozoan fauna are still imperfectly known.

Estimates of soil protozoan populations may possibly be improved by a two-phase partitioning method developed by Smith & Stribley [19]. Air-dried soil is added to an aqueous mixture of dextran and polyethylene glycol, microorganisms in the organic phase being removed by pipette.

It may be rewarding to correlate changes in soil solution and atmosphere with protozoan populations as these may suggest hitherto unsuspected interactions which merit further investigation. Suitable methods for sampling soil solution and atmosphere already exist: Low-speed centrifugation on filtration can provide sufficient volumes of solution for analysis by inductively-coupled plasma atomic emission spectrometry. Developments in gas chromatography enable the determination of the gas composition of soil atmosphere withdrawn by syringe [11].

There remains the problem of how to deal with soil heterogeneity. Geostatistics offer a potential means of studying the spatial relationships between protozoan populations and either soil microbial processes or plant distribution. This has been used to link microbial biomass and carbon mineralization with the dispersion of sage-bush plants in semi-arid ecosystems [20], to relate collembolan and microbial populations to carbon content in cultivated soils [10], and to study the distribution of soil invertebrates and litter accumulation under tropical bull-oak plantations.

A third potentially fruitful field of work is protozoa-bacteria-plant pathogen relations.

Levrat et al. [15] have shown that cell-free exudates of *Acanthamoeba castellani* can stimulate *Pseudomonas putida* to suppress the growth and sporulation of the *Fusarium* wilt fungal pathogen. There is also evidence that fungal metabolites may stimulate the growth of mycophagous giant amoebae. If the chemical identity and mode of action of microbial extracellular metabolites can be determined, then many new roles for soil protozoa may yet be discovered.

Soil protozoan diversity

Dr Wilhelm Foissner (Salzburg University, Austria) hijacked the audience to one of the nicest places of the world, the Etosha National Park in Namibia, Southwest Africa, where he started a project on taxonomy of tropical soil ciliates. His contribution, amended with many beautiful slides from soil ciliates of the Etosha Pan, showed our profund ignorance of soil protozoan diversity, especially in the tropics and subtropics, where a single sample may contain up to 80 ciliate species [9]. Dr Foissner complained that too many people are speaking and publishing about "biodiversity" and too few doing the "hard work", i. e. determining and eventually describing new species. Often, biodiversity has been misused by ecologists to aquire money for pure ecological research, e. g. for studying energy fluxes etc. at high taxa level. This is by no means biodiversity! Biodiversity needs species and individuals which are the centres of evolution; higher taxa, such as genera, orders, and functional groups are (helpful) artificial constructs.

Table 1. Number of soil ciliate species in a transect of the Etosha National Park, Namibia

Biotope	pH	Number of ciliate species[1]
Pan (saline desert)	9.7-8.7	9 - 21
Pan margin, *Suaeda* zone	8.6-8.4	43 - 57
Thorn bush savanna (1 km distant from pan margin)	7.7	28
distant from pan margin)	7.7	37

[1] *Obtained with the non-flooded petri dish method as described in [8].*

Dr Foissner investigated 12 soil samples for ciliates from the centre and periphery of the Etosha Pan. The pan soil is a very special mixture of salt, clay, and lime having a pH range of about 8.0-9.7; the air-dried mixture is like a stone, but quickly doubles its volume and becomes a fluffy pancake when it is rewetted. Most of the soil is covered with a more or less distinct layer of filamentous cyanobacteria. 153 ciliate species were found, 53 (!) of which were new to science. Most belonged to one of the following groups: hypotrichs (43 species), colpodids (35), gymnostomatids (33), nassulids (15).

The high number and frequency of nassulid ciliates, usually occurring sparsely in soil, was obviously related to the commonness of cyanobacteria, their preferred food. A transect from the pan to the surrounding savanna showed that the salt shrub (Suaeda) region had the highest species richness and that the number of species sharply decreased above pH 8.6 (Tab. 1). Refined ecological research on these special ciliate communities is urgently needed but difficult to realize because of the high number of new, as yet undescribed species.

Bioindication and autecology

Dr Erna Aescht (Biology Center of the Upper Austrian Natural History Museum) gave a general lecture on the potentialities and limits of soil protozoa as bioindicators in environmental field studies. She summarized pertinent previous studies [1] and very recent data, mainly by Berthold & Palzenberger [4].

Research in most groups of soil protozoa is hampered by methodological problems and the lack of taxonomic guides for species. Dr Aescht emphasized that the widely used cul-

ture (dilution) techniques cannot reliably discriminate active and cystic protozoa. Furthermore, they are time-consuming and are often statistically inadequate. Thus, direct counts in diluted soil suspensions should be preferred. Direct counting provides data on active individuals and species, which are prerequisites for using protozoa as bioindicators in the soil environment. Unfortunately, no reliable direct methods are known for counting naked amoebae and small, amoeboid flagellates which adhere to the soil particles. Thus, practical bioindication work is at present almost entirely restricted to ciliates and testate amoebae, for which standardized and properly tested direct counting methods have been published [2, 3]. Ciliates must be counted on the day of sampling due to their ability to encyst and excyst rapidly, while testacean collections can be conserved and stored for years. Ciliates and testate amoebae are equivalent indicator groups in raw soils, e. g. the litter layer. In evolved natural and cultivated soils testate amoebae are much more important than ciliates (and, probably, naked amoebae and flagellates, too), whose activity is strongly suppressed by microbiostatic effects [8]. The data available show that total individual and species numbers and/or functional groups are often insufficient in revealing treatment effects.

These overall parameters frequently obscure the fact that some indicator species decrease or, respectively, increase. Bioindication thus needs to be done at species level. However, this is time consuming and Dr Aescht thus suggested restricting identification to dominant (> 2%) species, at least in applied environmental studies.

Dr Aescht's conclusions were impressively supported by an unpublished study of Dr Ralf Meisterfeld's group (Aachen University, Germany) on the recolonization of heavily disturbed forest soils. His data showed convincingly that testate amoebae are more sensitive indicators and far better suited to monitoring soil development than microbial activity and abundances of flagellates and naked amoebae (Figs. 1-3). Unfortunately, ciliates were not studied.

As a result of open cast mining of brown coal, heaps of overburden (area up to 10 km2, height up to 200 m) were deposited. To allow reafforestation, the almost sterile sandy mound was covered with artificial soil made up of 20 % loess and 80 % sand and more than 10 million trees (mainly beech and oak) were planted afterwards. A small experimental area was amended with humus from a primary forest to stimulate succession. To monitor the development of soil biota, soil respiration, microbial biomass, flagellates as well as naked and testate amoebae were studied. During a sampling period in 1984 almost all these parameters declined significantly from primary forest over forest humus on the mound to the artificial soil (Figs. 1, 3). In 1992 the study was repeated with somewhat different results (Figs. 2, 3). Only the organic horizon of the primary forest had a significantly higher respiration than the soils on the heap. Microbial biomass followed a similar trend with only small differences between the Ah horizon of the primary forest and the top soil on the heap. During summer, microbial biomass estimates for the soils on the heap were higher. Flagellates and naked amoebae of the soils on the heap had similar or higher abundances than those of the Ah horizon of the primary forest. Testate amoebae showed a different trend. Abundances in the forest humus on the heap were only 7 % of that of the Ah horizon of the reference forest, and the artificial soils had even less individuals. Species numbers on the heap were only 25 % of those of the primary forest (Figs. 2, 3). Furthermore, most forest litter and humus-specific species (e. g. *Centropyxis* spp., *Cyclopyxis* spp., *Plagiopyxis* spp., *Trigonopyxis* spp., *Nebela* spp.) were lost during the first year of succession. Only a few ubiquists remained, viz. *Trinema* spp., small *Euglypha* spp., and *Phryganella acropodia*.

As a result of this study, the amendment of artificial soils with forest humus does not seem to be the appropriate strategy. This technique is very expensive and its effects are quite transient. It was not possible to establish a typical community of humus and litter testate amoebae. Although abundances and species numbers are still higher on the treated plots, differences become smaller and will probably disappear within a few years. Community develo

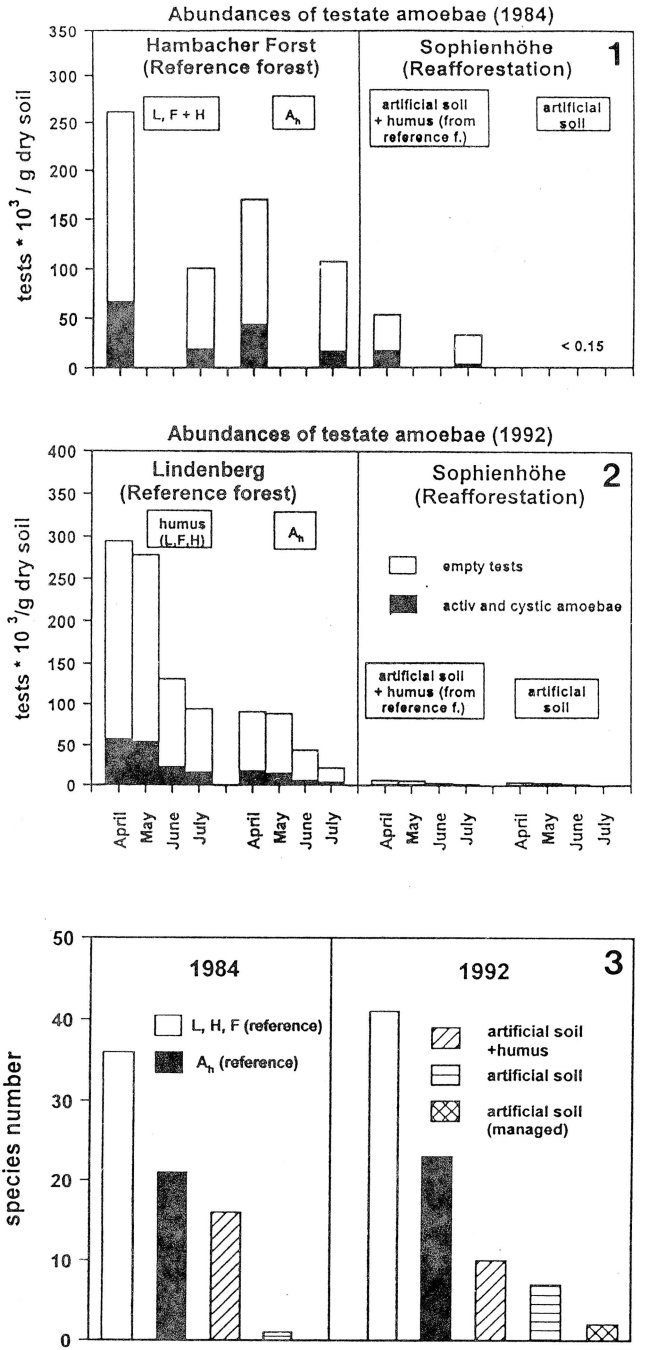

Figure explanations

Figs. 1-3. *Abundance and species richness of testate amoebae in natural and heavily disturbed forest soils.*

pment in this stage of succession does not depend on immigration but on availability of suitable microhabitats. Typical forest species can obviously only survive and establish larger populations in evolved, humus rich zonal soils having a distinct litter layer. In young stands annual litter input is too low and the rate of decay too high to allow the accumulation of a sufficiently thick litter and fermentation layer. As a consequence, adverse abiotic factors like desiccation have an immediate effect on the testacean community. The mineral soil below 5 cm was still almost sterile, compact and had a low pore volume and could thus not serve as a refuge for medium and large-sized protozoan species.

Finally, Dr Wanner (Aachen University, Germany) enlarged on the practical aspects discussed by Dr Aescht and Dr Meisterfeld. He presented a very fine piece of work on the ecology and taxonomy of a common soil testate amoebae, *Cyclopyxis kahli*. The results presented were based on 500 000 (!) morphometrically analyzed shells and included both published [21] and unpublished data. Dr Wanner emphasized that for ecological (e. g. bioindication) and taxonomical purposes the proportion of environmentally dependent variability to the total must be known.

In a first experiment with clonal batch cultures of *Cyclopyxis kahli*, the influence of lime and mineral fertilizer was tested by measuring shell size of the amoebae using an image-analyzing system [21]. The control showed the smallest mean shell diameter (82 µm), while an average of 93 µm was measured in the fertilized cultures, a significant difference of more than 10 mikrons. The shell opening was inversely affected, with the largest opening in the control group. Because the food yeast was more abundant in the treated cultures than in the control group, amoebae growth may have been influenced indirectly by food supply. Therefore a second experiment with food supply changing across different temperatures (15°C, 20°C) was established. Amoebae fed by bacteria formed significantly smaller shells than those fed by yeast across the two temperatures. Bacteria fed amoebae kept at 20°C had significantly smaller shells than those kept at 15°C, whereas no temperature effects occurred when yeast was fed. Food and temperature interacted highly significantly: temperature effects were compensated by the influence of yeast well utilized by the amoebae with the consequence that the smallest shells occurred when bacterial food was provided at the higher temperature. Inverse relations were observed concerning shell opening.

A complex set of consecutive experiments [21] allowed a detailed statistical analysis of interaction between and adaptations to several environmental factors. Significant changes in culture growth (lag time, generation time, final culture density) and shell size parameters depended primarily on food and temperature, whereas insecticides had a minor but significant influence. Lime and fertilizer had no direct effects. Interaction between all tested factors occurred frequently, but no consistent adaptation phenomena could be observed. The shell size effects were reversible within a few days. Shell size of *Cyclopyxis kahli* was significantly affected by environmental conditions, in particular food and temperature. This was also proven on other clones and taxa of testate amoebae. Therefore new possibilities in bioindication may be conceivable on the one hand, but on the other taxonomical problems may arise because separation of closely related taxa depends largely on shell size. Therefore a genetic approach, based on RAPD-PCR [22], was developed to complete conventional ecological and taxonomical results.

Nearly all analyzed cultures of testate amoebae *(Cyclopyxis kahli, C. eurystoma, Euglypha strigosa, Trinema lineare)* provided specific fingerprints. It was, however, surprising that some cultures of *Cyclopyxis kahli* showed similar, clone-specific patterns of amplified DNA-fragments with and without the amoebae, although no eukaryotic contamination was evident. Additional experiments corroborated the assumption that some external but clone specific DNA was located within the amoeba casing, and was therefore inevitably transferred with the amoebae to each new subculture. This problem was circumvented by using isolated

nuclei of testate amoebae. At present, different DNA-extraction protocols have been tested using isolated nuclei as a reference.

Soil protozoology in Russia

Dr Julius Geltzer (Moscow State University) was invited for a lecture on "Soil Protozoology in Russia". Unfortunately, he could not attend, but he provided a written version which is reported here, albeit much abbreviated. The development of soil protozoology in Russia is historically connected with agriculture and the need for its intensification, the role of protozoa being considered to be regulators of bacteria.

Some local faunistic and ecological investigations were carried out in the twenties and thirties by, e. g., Nowikoff, Losina-Losinsky, Martynov, Strelkov, and Belajeva. These studies were critically reviewed and extended by Brodsky [5] in his monograph "Soil protozoa and their relative importance on soil activity". He postulated some general principles, such as trophic activity of soil protozoa in conditions of normal humidity, dependence of protozoan abundance on physical and chemical soil properties, and influence of protozoa on nitrification.

In the sixties, Nikolyuk and his students demonstrated the stimulating effect of metabolites from soil protozoa on bacteria and higher plants and the influence of such processes on soil biodynamics. Local faunistic studies proceeded during this time, e. g., by Reinhard in Ukrainia, Lepinis in the Baltic countries [14], Ibadov in Azerbaidjan, and Mordkovich in Siberia. These and the former studies served as basis for important reviews on the ecology and taxonomy of soil protozoa [14, 16, 17].

Since the sixties, Geltzer and Korganova and their students invented methods of combined cultivation of protozoa, bacteria and plants to observe the behaviour of protozoan populations in the rhizosphere and to reveal the protistocidic activity of soil fungi and actinomycetes. Under their guidance several studies were performed, or are in progress, relating soil protozoology to general soil science and environmental problems [12, 13].

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Key word

Bioindication — *Cyclopyxis kahli* — Etosha National Park — Open cast mining — Soil protozoan diversity