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With 32 Figures

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22 Microfauna

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The microfauna consists of eukaryotic, single-celled protozoans (naked and testate amoebae, flagellates, ciliates) and multicellular organisms (rotifers, tardigrades, nematodes) too small to be studied and identified without the help of a microscope.

Protozoans and nematodes are equivalent to the meso- and macrofauna with respect to matter and energy turnover (Fig. 21; Foissner 1987, Meisterfeld 1989).

Testaceans, ciliates, and nematodes are the most abundant – in terms of both individuals and species – and above all the metabolically most active of soil animals. They consume considerable amounts of bacteria, fungi and debris. Protozoans and nematodes require 10^3–10^5 and $2 \cdot 10^4$–$7 \cdot 10^5$ bacteria for each cell division and daily metabolism, respectively (Anderson 1988). Via the food chain they enhance N-mineralization and N-uptake of plants (Wasilewka 1979; Anderson 1988). Estimates of the annual C-input consumed by protozoans range from 10–22% (Foissner 1987; Meisterfeld 1989). Lousier and Parkinson (1984) presume 6% exclusively for the testaceans of a birch forest soil. For nematodes, the proportion is estimated at 0.5–2% by Persson et al. (1980) and Sohlenius (1980).

On average, 70% of the animal respiration comes from the Protozoa, i.e. much more than their corresponding contribution to the standing crop biomass (Fig. 21). The reason for this is that the smaller an organism is, the more food is needed to fulfil its energy requirements. In extreme ecosystems of the world, like high mountains and polar regions, well over 50% of the animal energy turnover is achieved by the microfauna; in earthworm-rich soils of temperate climates this proportion is more like 10–30% (Foissner 1987). In beech and spruce forest soils, the energy turnover of testaceans alone equals that of arthropods or oligochaetes plus nematodes. In mull with a sparse humus layer, it corresponds to that of nematodes (Decker 1989; Meisterfeld 1989).

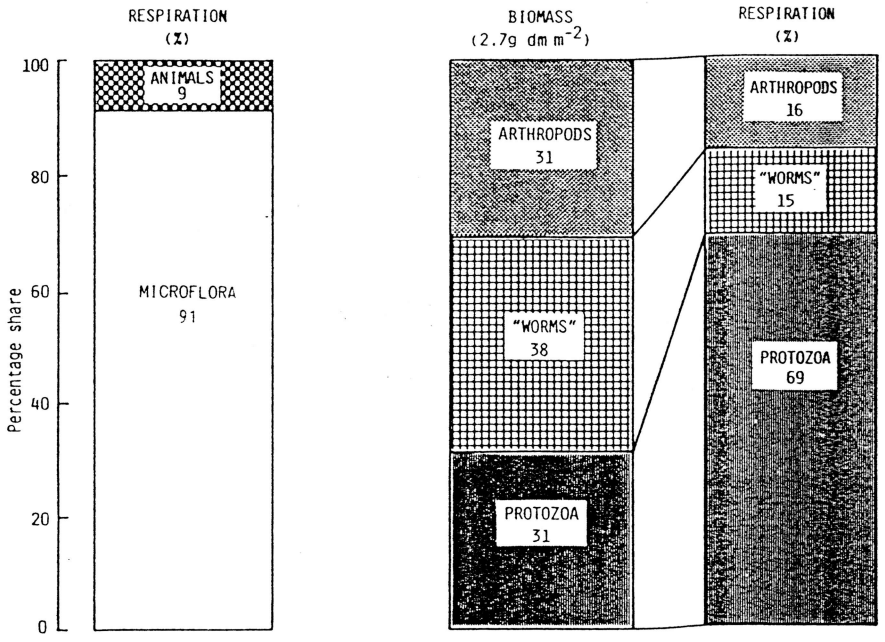

Fig. 21. Biomass and respiration of soil organisms (Foissner 1987). The graph shows average values of 14 ecosystem studies from various sites of the world. Arthropods: Coleoptera, Diptera etc.; worms: nematodes, rotifers, enchytraeids, earthworms; protozoa: naked amoebae, testate amoebae, flagellates, ciliates; *dm* Dry mass of soil

Compared to other soil animals, members of the microfauna are easily dispersed and thus at least potentially ubiquitous. Microfaunal groups, moreover, occur with large individual numbers even in biotopes that are almost or completely devoid of larger organisms due to extreme environmental conditions, e.g. above the timberline and in polar regions.

The microfauna is not readily dislodged in soil, and many (but not all!) species are cosmopolitan, which facilitates comparison of results from different regions. Since displacement is almost entirely vertical, the problem of horizontal migration, which is difficult to handle with other animals, especially with the epigaeon, does not affect the investigation.

Protozoans can react more quickly to environmental changes than any other eukaryotic organism due to their delicate external membranes directly exposed to the environment and their more rapid growth rates. Provided appropriate environmental conditions, the

generation time of soil protozoa amounts to a few hours or days, that of nematodes (from egg to egg) 1–2 months (Anderson 1988; Decker 1989). Investigations on soil protozoa are thus also appropriate to document short-term effects. Numerous investigations show that microfaunal groups are valuable bioindicators; some 200 papers on this subject have been critically reviewed by Foissner (1987, 1994).

Standardized quantitative methods are available for testate amoebae, ciliates, and nematodes (Aescht and Foissner 1992a,b; Decker 1989). The investigation of active and free-living individuals is usually needed in ecological works. Standardized guidelines are available for root-parasitizing nematodes (Decker 1989).

For counting individuals and species of the microfauna, culture or sieving techniques are frequently used; however, few of these have been adequately tested. Our own investigations (Berthold and Foissner 1992) and data from the literature (e.g. Berthold and Palzenberger 1994) show that these methods work very selectively and thus cannot be reliably used to estimate the abundances of active individuals. Hence, direct counting, i.e. in suspended fresh soil, of the active microfauna is recommended. This method yields reliable results, as indicated by the rather high mean recovery rates (Fig. 22).

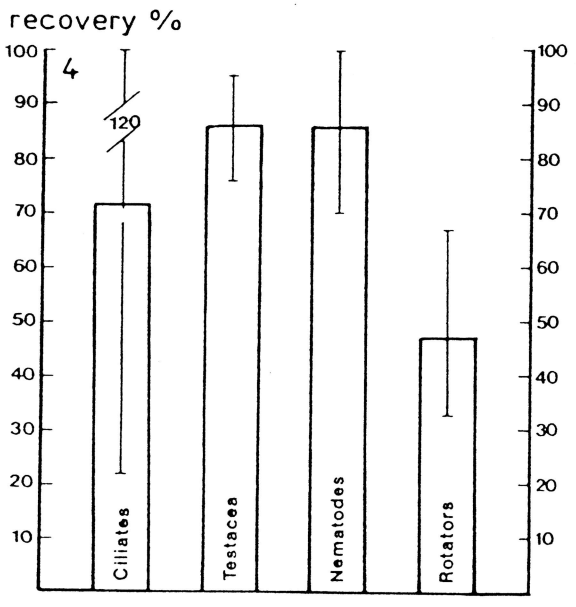

Fig. 22. Percentage of recovery of ciliates (n = 32), testaceans (n = 9), nematodes (n = 7) and rotifers (n = 4). Counting was performed in suspended soils. (Lüftenegger et al. 1988)

Table 4. Conversion factors (from Foissner et al. 1992).

Transformation factor	to	Conversion factor
Biovolume	wet mass (wm)	$1\,\mu m^3 = 1\,pg\,wm$
Biovolume	dry mass (dm)	$1\,\mu m^3 = 0.15\,pg\,dm$
Biovolume	organic carbon	$1\,\mu m^3 = 0.11\,pg\,C$
Wet mass	dry mass	$dm = 0.15 \cdot wm$
Dry mass	dry mass (ash-free)	$dm_a = 0.1 \cdot dm$
Dry mass	organic carbon	$C = 0.5 \cdot dm$
Dry mass	nitrogen	$N = 0.04-0.07 \cdot dm$
Dry mass (ash-free)	joule	$1\,mg = 17-20\,J$
Organic carbon	joule	$1\,mg = 46\,J$

For naked amoebae, however, direct counts are unfortunately inappropriate. At present, they cannot be enumerated reliably, thus they are not treated in this chapter.

Frequently used conversion factors are summarized in Table 4.

22.1 Soil Sampling

Principle

A representative number of soil subsamples (cores) is taken from the site to be studied and pooled into a bulk sample.

Materials and Equipment

In addition to basic laboratory equipment:

- Soil sampling tool (soil corer, auger, spade, plunging cylinder etc.)
- Plastic bags
- Cooler

Sampling Date

Protozoa and nematodes can be found in every season. However, maximum individual numbers frequently occur in spring and/or autumn. This does not necessarily apply to species number or single groups and biotope types; testacean maxima, for instance, may correlate with ciliate minima (Petersen and Luxton 1982; Foissner 1987). Samples should, therefore, be taken at least four times in the course of a year (spring, summer, autumn, winter); if this is impracticable, take samples in autumn.

Statistical analyses on the number of cores required and the sample size for representative recording of the microfauna are scarce. Most investigators take 10–20 subsamples (cores) from the area studied (raster or transect), and mix them to a bulk sample to obtain a representative sample of the site (Petersen and Luxton 1982; Foissner 1987; Bamforth 1991). Three parallel samples are sufficient to study nematodes if an error of 10% is tolerated (Balogh 1958). For scientific investigations, a randomized block design with at least four (better six or more) replications is recommended.

The vertical distribution of the microfauna is determined less by soil moisture than by food supply. The highest individual numbers are thus generally found in the organic layer and in the well rooted horizon (0–10 cm soil depth); with increasing depth the number of animals decreases sharply (Balogh 1958; Foissner 1987). For a uniform area, it is recommended to take samples at the following soil depths:

- Arable land: 5–15 cm
- Grassland: 0–5 cm and 5–10 cm
- Forest: organic layer; if strongly zonated separate O_L, O_F, O_H horizon.

Take samples with a steel corer (3–5 cm in diameter; e.g. from Eikelkamp, Giesbeck, Netherlands), for voluminous substrates (e.g. litter) use a spatula or a spoon. Do not remove the litter from the mineral horizon; cut only living plants at ground level.

Take at least three additional samples of known volume to obtain the bulk density for transforming abundance and biomass values to square metres. Determine bulk density according to standard procedures (e.g. ÖNORM L1068)

Use well-closed plastic bags for transport; if necessary cool them (see Sect. 22.2, "Sample Preparation"). Pool the subsamples only shortly before treatment in the laboratory (bulk sample) to avoid changes in the soil fauna.

22.2 Sample Preparation

Principle

Field-moist soil subsamples are mixed to obtain a homogeneous bulk sample.

Materials and Equipment

In addition to basic laboratory equipment:

- Bucket

Procedure

Hand-crumble soil subsamples into a bucket, and mix thoroughly. Remove stones and coarse roots. Take about 500 g of the sample and proceed as follows:

a) Weigh 100 g of soil in a box of known weight, air-dry for 14 days or oven-dry at 105 °C (Blum et al. 1989), and reweigh to determine the dry mass.
b) Weigh 0.5–2 g of soil in a storage vessel, and stain to count testaceans.
c) Weigh 0.4 g of soil for counting ciliates; this has to be done on the day of sampling.
d) Weigh 100 g (1 g in direct counts) of soil for counting nematodes; this should be done on the day of sampling.
e) Air-dry the rest, and use it to investigate the species inventory of the ciliates (non-flooded Petri dish method).

Storage

Samples have to be processed within 48 (preferably 24) h because of the short generation times of the soil microfauna and their ability to excyst and encyst. During this time, samples should be stored at site-specific temperature and moisture (therefore, take samples from plastic bags only shortly before processing).

Note

- Since soil must not dry (even to a minor extent), sieving is usually impossible, especially with wet soils. Therefore, 10–20 subsamples should be taken from the thoroughly hand-mixed bulk sample (see "Procedure"). However, statistical analyses on this method are not available to date.

22.3 Testate Amoebae (Testacea)

Individuals and species are counted in suspended soil. Active and dead (tests = shells) specimens are distinguished by staining.

In addition to basic laboratory equipment:

- 5-ml graduated pipette cut off at 4-ml mark
- Micropipettes and glass-rods with a mounted eyelash (for preparation, see Foissner et al. 1991)
- Moist chamber (a Petri dish with damp filter paper covering the bottom)
- Microscope with oil immersion and (if possible) interference contrast

- Phenolic aniline blue for fixation and staining
  Mix 5% aqueous phenol solution (C_6H_5OH), 1% aniline blue solution ($C_{32}H_{25}N_3Na_2O_9S_3$), and glacial acetic acid (= conc. acetic acid; $C_2H_4O_2$) in a ratio of 15:1:4, and filter. Use prepared solution (1000 ml) undiluted; it may be stored.
- Albumen-glycerol
  Mix 10 ml of egg albumen and 10 ml of conc. glycerol (commercial product available, for instance, by Merck).
- Xylol
- Synthetic neutral mounting medium (e.g. Euparal, Eukitt) to make stable preparations (see textbooks on histological techniques)

Individual Number (Abundance)
Put a certain amount of field-moist soil, i.e. 1–2 g of arable land or grassland and 0.5 g of forest litter, in a centrifuge tube (see "Notes") by taking 10–20 pinches from different sites of the bulk sample with tweezers. Add about 7 ml of phenolic aniline blue to the sample, shake it thoroughly by hand, and stain suspension for at least 10 h.

Before counting rinse content of storage vessel with distilled water into a 100-ml graduated cylinder, and adjust the volume to 100 ml

with distilled water. Close cylinder with parafilm, and mix thoroughly by shaking at least ten times.

Take a 1-ml subsample (corresponding to 0.01–0.02 g of arable land or grassland and 0.005 g of forest field-moist soil, respectively) from suspension using the cut off pipette (see "Materials and Equipment") to prevent selective sampling of small soil particles. This step must be done quickly to minimize sedimentation.

Add 0–2 ml of distilled water depending on individual number and clay content, and place suspension dropwise (0.1–0.3 ml) on a grease-free slide. Do not use a coverslip to allow handling with a mounted eyelash and a micropipette.

Count under a compound microscope and at a magnification of at least 100x (objective 10:1, ocular 10x). Distinguish individuals and species according to empty (unstained) and full (light to dark blue-stained cytoplasm and nucleus) tests. Record resting cysts, precystic and parasitized stages separately or include them into full tests.

Repeat this procedure nine times with arable and grassland soils, i.e. take 1 ml from the original 100 ml suspension nine times; thus at least 0.1 g soil wet mass is examined. As much field-moist soil should be investigated as is needed to record at least 15–30 (arable land) and 50–70 (grassland, forest) full tests.

Species Inventory

Record testacean species when counting the individuals. Since the frequency of individual species, however, varies depending on their requirements and the season, at least four samples taken in the course of a year or investigations over several years are required for a reasonably accurate species inventory.

For qualitative analysis, e.g. flotation of tests by gas bubbles (Schönborn 1989) may be used; its efficiency is, however, insufficiently tested (Aescht and Foissner 1992a). Since motility of testaceans is low, species only found as empty tests are also included in the total species number.

Individual Number (Abundance)

Numbers are calculated per gram dry mass of soil and/or per square metre. Accordingly, water content and/or bulk density of the respective soil layer must be determined by standard methods (see textbooks on soil investigation).

$$\frac{I_{wm} \cdot 100}{wm \cdot \%dm} = I \cdot g^{-1}dm$$

$$\frac{I_{wm} \cdot B \cdot D \cdot 10^4 \cdot 100}{wm \cdot \%dm} = I \cdot m^{-2}$$

I_{wm}	individual number counted in soil wet mass
wm	wet (fresh) mass of soil examined (g)
I	individual number (abundance)
B	bulk density (determined e.g. after ÖNORM L1068; $g \cdot cm^{-3}$)
D	depth of soil layer sampled or analyzed (cm)
10^4	factor to relate bulk density to 1 m^2 (1 m^2 = 10^4 cm^2)
$100 \cdot \%^{-1}dm$	factor for soil dry mass

Biomass

For simplicity, calculations are according to the size of the tests. At least 10 individuals per species should be measured. The average volume obtained can be numerically equalized as weight/wet mass, because specific mass of microfauna approximates $1\,g \cdot cm^{-3}$.

$$\sum_{i=1}^{s} I_{idm} \cdot W_i \cdot 0.15 = BM \cdot g^{-1}dm$$

$$\sum_{i=1}^{s} I_i \cdot W_i \cdot 0.15 = BM \cdot m^{-2}$$

s	total number of species
I_{idm}	individual number of species i per g soil dry mass (see "Individual Number")
W_i	wet mass per species (ng)
0.15	factor to convert wet mass to dry mass (see Table 4)

BM biomass (dry mass)
dm dry mass of soil (g)
I_i individual number of species i per m^2 (see "Individual Number")

Species Number
Divide species into full and empty tests. The total species number comprises full and empty tests (see "Procedure for individual number").

Notes

- Testate amoebae are usually counted by direct microscopy of aqueous soil suspensions; various modifications are used worldwide (Couteaux 1967, 1975; Décloitre 1960; Lousier and Parkinson 1981; Foissner 1987). We recommend the method of Lüftenegger et al. (1988) because recovery experiments revealed a mean efficiency of 86% (Fig. 22).
- A sample volume of 0.1 g soil wet mass is usually sufficient, because studies on the minimal area showed that this amount provides a representative view of the testacean community.
- Samples can be stored in phenolic aniline blue for an unlimited period of time. Centrifugation tubes with screw tops are ideal for mixing and storing samples. If soil suspension becomes colourless after a few hours of staining (sometimes with calcareous soils), centrifuge sample, and replace supernatant with fresh phenolic aniline blue.
- Dilution required for counting depends mainly on soil type. Soils with a high clay content or with high numbers of testate amoebae need a higher dilution than humic or sparsely populated soils.
- Preparations should be investigated without coverslip because identification often requires that tests are turned with a mounted eyelash. Species can be isolated with a micropipette and stored in a moist chamber for later identification. However, prior acquaintance with the respective species inventory is recommended to avoid time-consuming checks during counting.
- Add 0.1 ml of albumen-glycerol to 1 ml of soil suspension if soil particles tend to aggregate on the slide (e.g. in humic soils).

- An experienced worker needs about 8 h for examination (counting) of testaceans in 0.1 g soil from arable land or grassland, and about 2–4 h for 0.005 g forest litter.
- No comprehensive literature on soil testaceans is available; however, a detailed key is in preparation and will be published by Fischer in the series *Protozoenfauna* (for specialized literature, see Aescht and Foissner 1989).
- Stable voucher specimens can be obtained with the following method: Collect tests with a micropipette, and place them onto a slide covered with a thin layer of albumen-glycerol. Dry the preparation at room temperature, then transfer slide to xylene for about 10 h, and mount in synthetic neutral mounting medium of low viscosity (e.g. Eukitt, Euparal). To avoid destruction of voluminous tests support coverslip corners with small pieces of coverslip glass.

22.4 Ciliates (Ciliophora)

Active individuals and species are counted in vivo in suspended soil. For qualitative analysis with the non-flooded Petri dish method, microbiostasis (ciliatostasis) is reduced.

In addition to basic laboratory equipment:

- Micropipettes and glass-rods with a mounted eyelash (for preparation, see Foissner et al. 1991)
- Moist chamber (Petri dish with damp filter paper covering the bottom)
- Microscope with oil immersion and (if possible) interference contrast

- Soil extract standard stock solution
  Boil 300 g of soil from sample site in 1000 ml of distilled water for 10 min. Decant, filter, and autoclave extract. Because soil extract is easily colonized by bacteria or fungi, check before use, and autoclave again if necessary.

- Soil extract working standard
  Dilute soil extract standard stock solution with distilled water in a ratio of 1:4 to 1:6 and adjust to pH of the investigated soil with HCl or NaOH. Prepare about 10 ml of working standard just prior to use.
- Diluted hydrochloric acid (1 M)
- NaOH solution (1 M)

Procedure

Individual Number (Abundance)

Take 10 pinches of field-moist soil, about 0.01 g each (in total 0.1 g), from different sites of the bulk sample with tweezers, and collect them in a small vessel (2–3 cm in diameter). Add 1–3 ml of soil extract working standard depending on individual number and clay content, and mix thoroughly with the glass rod to obtain a fine-grained suspension. Place suspension dropwise (0.1–0.3 ml) on grease-free slide. Do not use a coverslip to allow handling with a mounted eyelash and a micropipette. Perform counts under a microscope at 40× magnification (objective 4:1, ocular 10x).

Repeat this procedure four times so that a total of 0.4 g fresh (wet) soil is investigated. Dividing the total sample into 0.1 g portions reduces the risk of excystment.

Species Inventory

The non-flooded Petri dish method is recommended for qualitative analysis (Foissner 1987, 1992, 1993): Put 10–50 g of the air-dried (for at least 2 weeks) bulk soil sample in a Petri dish with 10–15 cm diameter, and saturate but do not flood the sample with distilled water. Saturation is obtained if 5–20 ml soil solution drains off when the Petri dish is tilted (45°) and the soil is gently pressed with a finger. Complete saturation takes some time, the culture should thus be checked after 12 h.

Cover Petri dish with the lid but pinch a clip (or something similar) between bottom and lid to enable gas exchange.

Inspect cultures on days 2, 6, 12, 20, and 30 by taking 1–2 ml from the runoff of different sites, and record species present. Two to five samples distributed over 1 year produce 50–80% of the species found in 10 such samples investigated over 2 years.

Individual Number (Abundance)
For calculation, refer to Section 22.3 ("Testate Amoebae").

Biomass
For simplicity, calculations are based on measurements of fixed and stained individuals taking into account the shrinkage of cells during fixation and preparation; corresponding factors are given by Foissner (1985) and Dragesco and Dragesco-Kernéis (1986). Biomass values of most known species are given by Foissner (1987). The average volume value obtained can be numerically equalized as weight, because specific mass of the microfauna is close to $1\,g \cdot cm^{-3}$.

For calculation, refer to Section 22.3 ("Testate Amoebae").

Species Number
Species numbers obtained by the quantitative and qualitative (non-flooded Petri dish method) investigations are given separately. The total species number comprises all species found with both methods.

- The frequently used culture (most probable number) methods taken from microbiology (e.g. Singh 1946) are insufficient to count active ciliates (Foissner 1987; Aescht and Foissner 1992b; Berthold and Foissner 1992; Berthold and Palzenberger 1994), because these methods can at best estimate the abundance of active + cystic cells. Bamforth (1991) combines direct counting with a culture technique to estimate the numbers of active and cystic ciliates. The direct method described above is a useful technique because on average 72% of ciliates have been recovered. In counting small species, the error may be considerable because there is a strong positive correlation between body size and percentage of recovery. By using a higher magnification, estimates could probably be improved, but the working time would increase too much for practical purposes, e.g. bioindication studies. The values obtained by direct counts thus represent minimum values, the more so since minor losses are unavoidable during sample preparation (e.g. suspension).

- Couteaux and Palka (1988) describe a membrane filter technique to count active + cystic cells; Griffiths and Ritz (1988) recommend density gradient centrifugation with subsequent fluorescent staining. Since both techniques require fixation, identification of species is limited.
- Ciliates must be counted on the day of sampling due to their ability to encyst rapidly.
- A magnification of 40x is sufficient to count ciliates due to their motility.
- 0.4 g soil wet mass is usually sufficient for soils with moderately high numbers of active ciliates (>50 individuals per g field-moist soil). For soils with few ciliates, the sample mass should be increased. Undisturbed, evolved soils usually contain less than 50 active ciliates per gram soil wet mass because their growth is suppressed by microbiostasis (Foissner 1987).
- The soil extract working standard prevents delicate ciliates from bursting. Dilution required for counting depends mainly on soil type. Soils with a high clay content or with high numbers of ciliates (e.g. in litter) require more dilution than humic or sparsely populated soils.
- Single individuals may be isolated with a micropipette and stored in a moist chamber (see "Materials and Equipment") for later identifica-tion. However, prior acquaintance with the respective species inventory is recommended to avoid time-consuming identification during counting.
- An experienced worker needs 2–4 h for the examination (counting) of 0.4 g soil.
- No comprehensive identification key of soil ciliates is available. However, one of the dominant groups, the Colpodea, has been reviewed in detail (Foissner 1993). Specialized literature on other groups is given by Foissner (1987) and Foissner and Foissner (1988).
- Note that when using the non-flooded Petri dish method the sample (soil) must not be flooded. The runoff is often very rich in species and individuals and thus ideal for preparations, such as silver staining. The sample should contain plenty of litter and fine plant roots and must be spread over the bottom of the Petri dish in an at least 1 cm thick layer.

- Stable voucher specimens of ciliates and flagellates are obtained by silver staining methods, which are however rather time-consuming (Foissner 1991).
- Ciliate species are recorded when counting the individuals. Since the frequency of individual species varies depending on their requirements and the season or a species may be encysted, at least four samples taken in the course of 1 year or investigations over several years are required for a reasonably accurate species inventory.

22.5 Flagellates

Flagellates are, in principle, counted like ciliates. Note the following alterations:

a) Most soil flagellates are smaller than 10 µm. Thus, they have to be counted at 200–300x magnification.
b) Take 0.01 ml of the suspended soil (see Sect. 22.4, "Ciliates") with a graduated pipette, and cover the drop with a coverslip which is large enough so that the suspension can spread, but will not spill out from slide and coverslip. Examine the whole preparation.
c) Repeat steps four times as with ciliates. Add counts and calculate individuals per gram soil dry mass and square metre, respectively, according to the dilution factor.

22.6 Nematodes (Nematoda)

Individuals and species are counted either in suspended soil or after sieving and centrifugation in a density gradient.

In addition to basic laboratory equipment:

- Kitchen sieve made of stainless steel (about 12 cm in diameter)
- Measuring can made of plastic or steel (2-l capacity, about 21 cm in height, about 15 cm in diameter)
- Stainless steel analytical sieve (0.038 mm mesh width, 200 mm diameter)

- Stainless steel analytical sieve (0.03 mm mesh width, 50 mm diameter)
- Glass rod with a mounted eyelash (for preparation, see Foissner et al. 1991)
- Microscope with oil immersion and (if possible) interference contrast
- Dissecting microscope

Chemicals and Reagents

- Magnesium sulfate solution
  Dissolve 450 g of magnesium sulphate ($MgSO_4 \cdot 7\ H_2O$) in tap water, and dilute the volume to 1000 ml with tap water in a volumetric flask. The solution can be stored at 4 °C.
- Fixative (FP 4–1)
  Pipette 10 ml of formalin (HCHO; commercial concentration, about 37%) and 1 ml of propric acid in a 100-ml volumetric flask, and dilute to volume with distilled water.
- Dehydration mixture I (Seinhorst I)
  Pipette 20 ml of ethanol (96% v/v) and 1 ml of glycerol in a 100-ml volumetric flask, and dilute to volume with distilled water.
- Dehydration mixture II (Seinhorst II)
  Pipette 5 ml of glycerol in a 100-ml volumetric flask, and dilute to volume with ethanol (96% v/v).
- Bordering lacquer
  (e.g. Deckglaslack Chroma, Schmidt GmbH, D-73257 Köngen, Germany)

Procedure

Individual Number (Abundance)

Direct Counting

Direct counting (Lüftenegger et al. 1988) is performed as with ciliates, but 1 g instead of 0.4 g soil wet mass should be examined to record 100–200 individuals. If species are not identified, nematodes can also be accurately counted in suspended soil, fixed and stained with phenolic aniline blue solution (see Sect. 22.3, "Testate Amoebae").

Centrifugation Method (Jenkins 1964, modified)
Weigh 100 g of field-moist soil in a beaker by taking several pinches from different sites of the bulk sample, and rinse with a strong jet of water (about 1 l) over the kitchen sieve into the measuring can; discard the rest remaining in the sieve. Mix the sample thoroughly by tilting. Let coarse soil particles settle for about 10 s, pour the supernatant rapidly over the 0.038-mm sieve, and discard the coarse soil particles. Wash off residue in the sieve with a shower from the rear. Rinse remains on bottom of the sieve from the rear into a beaker with a wash-bottle. Centrifuge the content of the beaker for 3 min at 2200 rpm. Decant and replace with magnesium sulfate solution. Suspend pellet thoroughly, and centrifuge for 3 min at 2200 rpm. Pour supernatant over the 0.03-mm sieve and discard pellet. Rinse nematodes from the rear of the sieve into a clean beaker with as little water as possible. The labelled sample can be stored at 4 °C until investigation. Suspension should be diluted depending on individual numbers or is counted entirely under a compound microscope at 40x magnification or a dissecting microscope (if species are not identified).

Species Inventory
Record nematode species when counting the individuals. According to Balogh (1958), the species number of nematodes per site reaches its maximum already at 0.5–1 cm^2, i.e. it does not increase by extending the sample area to 5, 10 or 15 cm^2. However, since the frequency of individual species varies, depending on their requirements and the season, several samples taken in the course of a year or investigations over several years are required for a reasonably accurate species inventory (Wasilewska 1979).

Individual Number (Abundance)
For calculation, refer to Section 22.3 ("Testate Amoebae").

Biomass
The mass of one nematode is calculated using Andrassy's (1956) formula by measuring at least 10 individuals per species.

$$\frac{W^2 \cdot L}{16 \cdot 10^5} = M$$

W	maximum body width (μm)
L	body length (μm)
$16 \cdot 10^5$	factor gained by experience
M	mass (μg)

The specific mass of a nematode is approximately 1.084 g·cm^{-3}, according to Andrassy (1956); other workers assume 1.02 g·cm^{-3}. Therefore, using a mass of 1 g·cm^{-3}, as with protozoa, is recommended. The percentage of nematode dry mass is reported to be 20–25% (Yeates 1979). Matter and energy equivalents are also very similar to those of Protozoa. The C:N-ratio amounts to 10:1 (Hunt et al. 1987); the ash-free energy equivalent is approximately 17.9 J (Yeates 1979).

Notes

- The methods for extracting nematodes may be classified into three groups:
  a) Dynamic procedures, e.g. Baermann funnel
  b) Mechanical procedures, e.g. flotation methods (density gradients or sieving cascades)
  c) Direct examination in suspensions of small soil samples.

  Dynamic procedures cannot be recommended for counting nematodes. Although efficiency has been stated to be about 90% (Oostenbrink 1971), test procedures are frequently dubious. Comparing results achieved with funnel and flotation methods under standardized conditions showed that the latter operates two to eight times more effectively. Direct counts again double the number found by flotation. The flotation method described above may be endorsed and is routinely used. However, our own experiments with grassland and arable soils showed that, compared to the direct counting method, 60–90% individuals less are recorded, especially small species are lost (mesh width 38 μm!).

  Direct counting of small, highly diluted soil samples is superior to all other methods with respect to accuracy of the result, the more so because it is equally appropriate to all types of substrate (Decker 1989). However, it is rarely practised because it is time-

consuming. The direct counting method proposed by Lüftenegger et al. (1988) and described above is in this respect a compromise between efficiency (on average 85%; tested in recovery experiments, Fig. 22) and time taken, since nematodes and ciliates can be counted simultaneously within about 4 h.

- For soil nematodes, Andrassy's (1984) and Bonger's (1988) identification keys are recommended. Simplified keys to the major taxa are given by Decker (1989).
- Archiving (Seinhorst 1962): Collect living specimens in a small amount of water in a watch glass, kill them by brief heating (50–60 °C), and subsequently fix with fixative (see above) for at least 24 h. Then transfer nematodes under the dissecting microscope by means of a mounted eyelash (see above) into another watch glass containing 0.5 ml dehydration mixture I. Incubate watch glass for at least 12 h at 35–40 °C in an exsiccator filled to about 1/10 of its volume with 96% ethanol to remove water. Fill the watch glass afterwards with dehydration mixture II, and allow remaining ethanol to evaporate slowly for 3–5 h at 40 °C in a half-open Petri dish. Put a drop of glycerol on a slide, transfer each nematode separately, cover with a coverslip, and seal with bordering lacquer.

Aescht E, Foissner W (1989) Stamm: Rhizopoda. Catalogus Faunae Austriae Ia

Aescht E, Foissner W (1992a) Enumerating active soil testate amoebae by direct counting. Protocols Protozool:B-6.1–B-6.4

Aescht E, Foissner W (1992b) Enumerating active soil ciliates by direct counting. Protocols Protozool:B-7.1–B-7.4

Anderson JM (1988) Spatiotemporal effects of invertebrates on soil processes. Biol Fertil Soils 6:216–227

Andrassy I (1956) Die Rauminhalts- und Gewichtsbestimmung der Fadenwürmer (Nematoden). Acta Zool 2:1–15

Andrassy I (1984) Klasse Nematoda (Ordnungen Monhysterida, Desmoscolecida, Araeolaimida, Chromadorida, Rhabditida). Akademie, Berlin

Balogh J (1958) Lebensgemeinschaft der Landtiere. Ihre Erforschung unter besonderer Berücksichtigung der zoozönologischen Arbeitsmethoden. Akademie, Berlin

Bamforth SS (1991) Enumeration of soil ciliate active forms and cysts by a direct count method. Agric Ecosyst Environ 34:209–212

Berthold A, Foissner W (1992) Singh's dilution culture method is inappropriate for estimating individual numbers of active soil ciliates (Protozoa). In: Haimi I, Pitkänen PL (eds) Soil organisms and soil health. Jyväskylä Congr Publ 3:148

Berthold A, Palzenberger M (1995) Comparison between direct counts of active soil ciliates (Protozoa) and MPN (most probable number) estimates obtained by Singh's dilution culture method. Pedobiologia 79:348–356

Blum WEH, Spiegel H, Wenzel WW (1989) Bodenzustandsinventur. Konzeption, Durchführung und Bewertung. Bundesministerium für Land- und Forstwirtschaft, Wien

Bongers T (I988) De Nematoden Van Nederland. Koninklijke Nederlandse Naturhistorische Verengiging, Utrecht

Couteaux MM (1967) Une technique d'observation des thécamoebiens du sol pour l'estimation de leur densité absolue. Revue Écol Biol Sol 4:593-596

Couteaux MM (1975) Estimation quantitative des thécamoebiens édaphiques par rapport à la surface du sol. C R hebd Séanc Acad Sci, Paris 281:739–741

Couteaux MM, Palka L (1988) A direct counting method for soil ciliates. Soil Biol Biochem 20:7–10

Decker H (1989) Nematoden. In: Dunger W, Fiedler HJ (eds) Methoden der Bodenbiologie. Fischer, Jena, pp 290–298

Décloitre L (1960) Perfectionnements apportés à un appareil pour une technique d'isolement des microorganisms du sol, des mousses, des eaux. Int Rev Ges Hydrobiol 45:169–171

Dragesco J, Dragesco-Kernéis A (1986) Ciliés libres de l'Afrique intertropicale. Introduction à la connaissance et à l'étude des ciliés. Faune Tropicale 26:1–559

Foissner W (1985) Protozoologische Untersuchungen an Almböden im Gasteiner Tal (Zentralalpen, Österreich). III. Struktur und Dynamik der Testaceen- und Ciliatentaxozönose. Veröff Österr MaB-Programms 9:65–95

Foissner W (1987) Soil Protozoa: Fundamental problems, ecological significance, adaptations in ciliates and testaceans, bioindicators, and guide to the literature. Progr Protistol 2:69–212

Foissner W (1991) Basic light and scanning electron microscopic methods for taxonomic studies of ciliated Protozoa. Eur J Protistol 27:313–330

Foissner W (1992) Estimating the species richness of soil protozoa using the "non-flooded petri dish method". Protocols Protozool:B-10.1–B-10.2

Foissner W (1993) Colpodea (Ciliophora). Fischer, Stuttgart Jena, New York

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

Foissner W, Berger H, Kohmann F (1992) Taxonomische und ökologische Revision der Ciliaten des Saprobiensystems – Band II: Peritrichia, Heterotrichida,

Odontostomatida. Informationsberichte des Bayerischen Landesamtes für Wasserwirtschaft, Heft 5/92

Foissner W, Blatterer H, Berger H, Kohmann F (1991) Taxonomische und ökologische Revision der Ciliaten des Saprobiensystems - Band I: Cyrtophorida, Oligotrichida, Hypotrichida, Colpodea. Informationsberichte des Bayerischen Landesamtes für Wasserwirtschaft, Heft 1/91

Foissner W, Foissner I (1988) Stamm: Ciliophora. Catalogus Faunae Austriae Ic

Griffiths BS, Ritz K (1988) A technique to extract, enumerate and measure protozoa from mineral soils. Soil Biol Biochem 20:163–173

Hunt H, Coleman DC, Ingham ER, Elliott ET, Moore JC, Rose SL, Reid CPP, Morley CR (1987) The detrital food web in a shortgrass prairie. Biol Fertil Soils 3:57–68

Jenkins WR (1964) A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Dis Rep 48:692

Lousier JD, Parkinson D (1981) Evaluation of a membrane filter technique to count soil and litter testacea. Soil Biol Biochem 13:209–213

Lousier JD, Parkinson D (1984) Annual population dynamics and production ecology of Testacea (Protozoa, Rhizopoda) in an aspen woodland soil. Soil Biol Biochem 16:103–114

Lüftenegger G, Petz W, Foissner W, Adam H (1988) The efficiency of a direct counting method in estimating the numbers of microscopic soil organisms. Pedobiologia 31:95–101

Meisterfeld R (1989) Die Bedeutung der Protozoen im Kohlenstoffhaushalt eines Kalkbuchenwaldes (Zur Funktion der Fauna in einem Mullbuchenwald 3). Verh Ges Ökol 17:221–227

ÖNORM L1068 (1988) Bestimmung der Dichte von Böden (Determination of soil density). Österreichisches Normungsinstitut Wien, Heinestrasse 38, A-1021 Vienna, Austria

Oostenbrink M (1971) Comparison of techniques for population estimation of soil and plant nematodes. IBP Handb 18:72–82

Persson T, Baath E, Clarholm M, Lundvist H, Söderström BE, Sohlenius B (1980) Trophic structure, biomass dynamics and carbon metabolism of soil organisms in a Scots pine forest. Ecol Bull 32:419–459

Petersen H, Luxton M (1982) A comparative analysis of soil fauna populations and their role in decomposition processes. Oikos 39:287–288

Schönborn W (1989) Urtiere (Protozoa). In: Dunger W, Fiedler HJ (eds) Methoden der Bodenbiologie. Fischer, Jena, pp 282–290

Seinhorst JW (1962) On killing, fixation and transferring to glycerin of nematodes. Nematologia 8:29–32

Singh BN (1946) A method of estimating the number of soil protozoa, especially amoebae, based on their differential feeding on bacteria. Ann Appl Biol 33:112–120

Sohlenius B (1980) Abundance, biomass and contribution to energy flow by soil nematodes in terrestrial ecosystems. Oikos 34:186–194

Wasilewska L (1979) The structure of soil nematode communities in natural ecosystems and agrocoenoses. Pol Ecol Stud 5:97–145

Yeates GW (1979) Soil nematodes in terrestrial ecosystems. J Nematol 11:213–229