ENUMERATING ACTIVE SOIL CILIATES BY DIRECT COUNTING

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INTRODUCTION

Ciliates cannot be directly extracted from soil. Therefore, enumeration involving various culture techniques have been suggested (2, 7, 8). However, these methods can only estimate the abundance of active + cystic cells. Thus, direct methods, i. e. inspection of soil suspensions, must be used for counting active ciliates (1, 4). Various direct methods are known: Couteaux & Palka (3) use millipore filters and Griffiths & Ritz (5) density gradient centrifugation with subsequent fluorescent staining. However, these techniques require fixation and staining of cells which is complicated and limits identification of species. We thus recommend to count the living cells. The mean efficiency of the procedure described below is 72% (6; Fig. 3). Bamforth (1) combines direct counting with a culture technique to estimate the numbers of active and cystic ciliates.

PROTOCOL

1. Take 10 portions of fresh (wet) soil, about 0.01 g each (in total 0.1 g), with tweezers from different sites of the sample and collect them in a small vessel.
   Remarks: No systematic studies about distribution of ciliates in soil are known. Usually, 10-20 soil cores are collected from the area studied and thoroughly mixed to a bulk sample. Ciliates must be counted on the day of sampling due to their ability to encyst rapidly.
2. Add 1-3 ml diluted soil extract and mix thoroughly with a glass-stick to obtain a fine-grained suspension.
   Remarks: The diluted soil extract used prevents delicate ciliates from bursting. Dilution depends mainly on soil type. Soils with a high clay content or with high numbers of ciliates need a higher dilution than humic or weakly populated soils.
3. Place suspension dropwise (about 0.1 ml) on grease-free slide and examine without coverslip under a compound microscope at X40 magnification (objective 4:1, ocular X10). Ciliates are rather easily recognized due to their mobility.
   Remarks: Preparations should be investigated without coverslip because species identification sometimes requires that cells are isolated with a micropipette. Isolated specimens can be stored in a moist chamber (e. g., a covered petri dish with damp filter paper covering its bottom) for later identification. However, it is recommended to get acquainted with the respective species inventory beforehand to restrict time-consuming identification during enumeration. Stable voucher specimens of ciliates are obtained by the methods described in chapter C of this book.

   There is a strong positive correlation between body size and percentage of recovery (Fig. 3). 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.
4. Repeat steps 1-3 until at least 0.4 g fresh (wet) soil is examined.
   Remarks: Dividing the total sample into 0.1 g portions reduces the risk of excystment. An experienced worker needs 2-4 hours for the microscopical examination (counting)

of 0.4 g soil. Experiments showed that 0.4 g soil wet mass is usually sufficient to obtain a representative estimation of the individual abundance and species composition (Fig. 1, 2). In soils with moderately high numbers of active ciliates (> 50 individuals per g wet mass) about 85% of the species, which are found in 1 g, occur in the 0.4 g subsample. This seems to be an acceptable compromise between working time and winning of additional information. For soils with few ciliates, a sample mass of 0.4 g is obviously too small. A rather complete species inventory can be obtained with the "non-flooded petri dish method" (see this protocol).

Note: Evolved soils usually contain few (< 50 individuals per g wet mass of soil) active ciliates due to the inhibitory effects of ciliatostasis (see [4] for detailed information).

REAGENTS

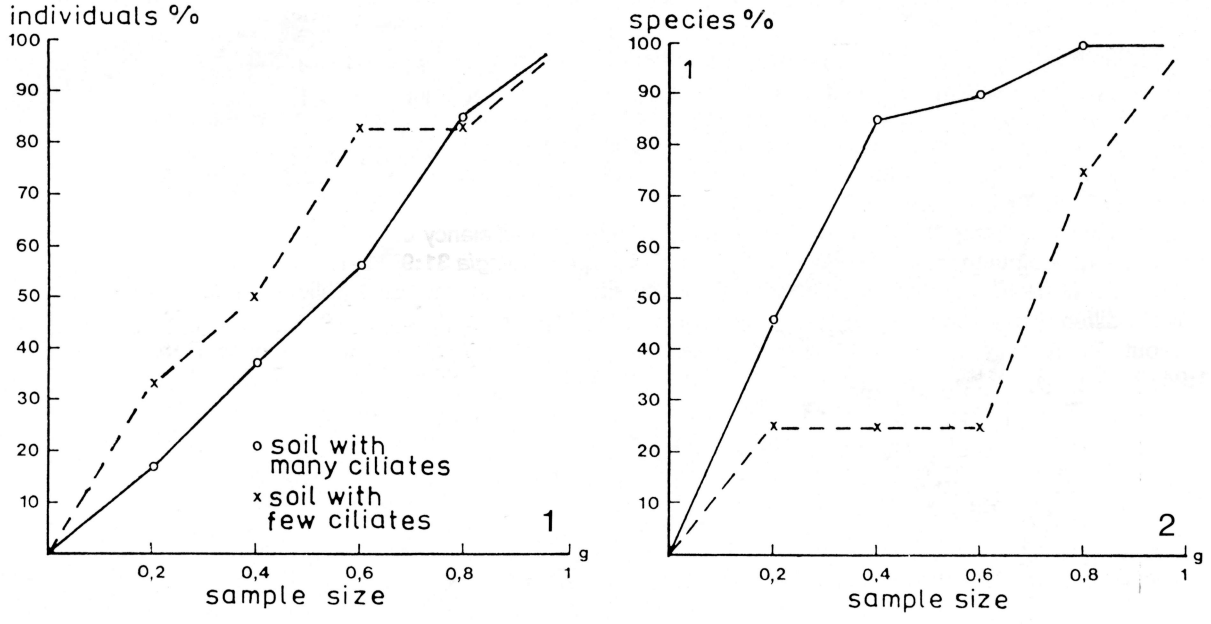
a) Soil extract (easily colonized by bacteria or fungi; check before use and eventually filter and autoclave again)
   300 g soil from sample site
   ad 1000 ml distilled water
   Boil for 10 minutes, decant, filter and autoclave
b) Diluted soil extract (prepare before use)
   1 part soil extract (see above)
   5 parts distilled water
   Adjust to pH of soil investigated with HCl or NaOH

CALCULATION

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

$$I\ g^{-1}\ dm = \frac{lwm}{wm \cdot dm} \qquad I\ m^{-2} = \frac{lwm}{wm \cdot dm} \cdot b \cdot d \cdot 10^4$$

b bulk density in g cm^{-3}
d depth (cm) of soil layer sampled (e. g., 5 cm)
dm dry mass of soil expressed from 0.0 to 1.0 (e. g., 0.4 if soil contained 60 % water)
I individual number (abundance)
lwm total individual number counted in wet mass (wm) of soil
wm wet (fresh) mass (in gram) of soil examined (e. g., 0.005 g forest litter)
10^4 factor to relate bulk density to 1 m^2 (= 10000 cm^2)



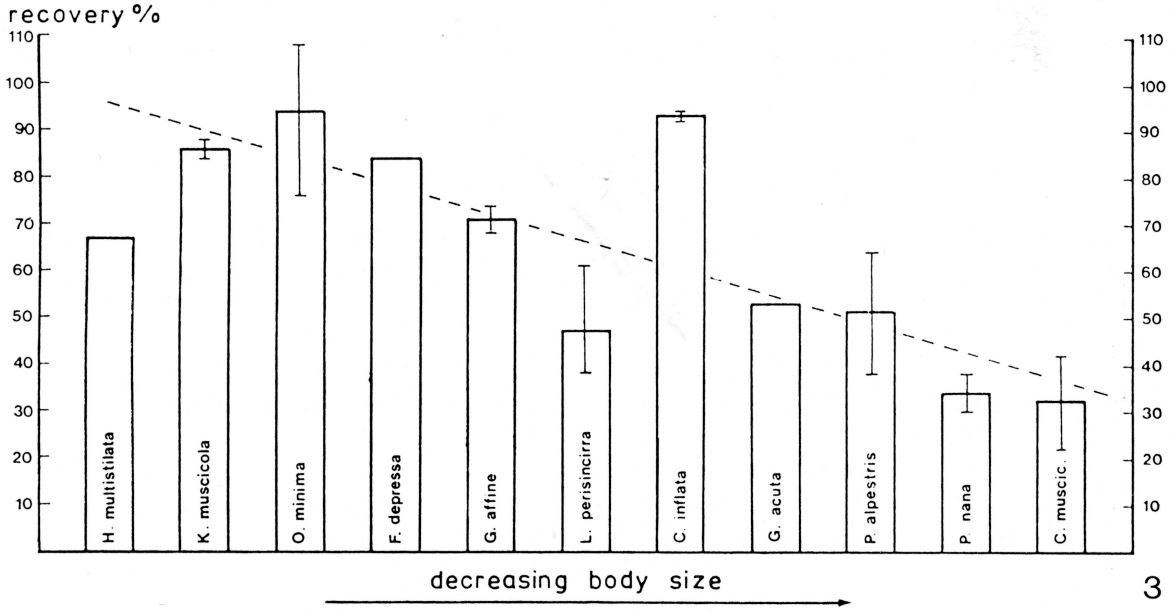


Fig. 1, 2. Test for optimal sample size for ciliate counting (from [6]). **1.** The individuals found in 1 g fresh soil of a levelled ski slope represent 100%. The 1 g soil sample was counted by subsampling of 0.2 g (5 x 0.2 g). The curves are almost straight, indicating a homogeneous distribution of the organisms. **2.** The same as in Fig. 1 was done for the species number (see remarks at step 4 of the protocol for further explanation).

Fig. 3. Recovery rates of ciliate species. Single species experiments with sterilized soils (from [6]). Correlation (r_s = 0.7) between body size and percentage of recovery is indicated by broken line.

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