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Spatial Separation of Terrestrial Ciliates and Testaceans (*Protozoa*): a Contribution to Soil Ciliatostasis

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Synopsis. The vertical distribution of soil ciliates and testaceans was investigated in a spruce forest, in a meadow and at some alpine and lowland sites. A direct and an indirect (culture) method were used to count the organisms. Active ciliates are abundant in the litter (L) but low numbers occur in the F and H layer of the spruce forest. Testaceans, in contrast, reach peak numbers in the F and H horizon which causes a distinct spatial separation from the ciliates. No or very few active ciliates occur in the meadow and the other sites which have no litter layer. Cultures of air-dried and remoistened soil samples, however, nearly always yield high numbers. These findings can be explained with the concept of ciliatostasis and support the view that certain chemical substances which are present in older soils are mainly responsible for the inhibition of ciliate excystment and growth in most evolved natural soils. The annulment of ciliatostasis in cultures by drying and remoistening of the soil is perhaps achieved through the inactivation or disappearance (e.g., by evaporation) of these substances. The increased bacterial food supply may also be of some importance. However, under field conditions food is probably not the key factor, because food organisms (e.g., bacteria, fungi) are surely abundant in the upper layers of sites where no or only few active ciliates are found as in meadows and arable land. Likewise, pore space can be excluded.

With a direct counting method terrestrial ciliates and testaceans have virtually never been investigated together at the same site with the exception of works by Foissner (1981, 1985), Foissner and Adam (1981), Foissner et al. (1985, 1988) and his group (Berger et al. 1985,

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1986, Lüftenegger et al. 1986 a). Bamforth (e.g., 1971, 1984) studied both communities in cultures, but discrepancies between direct counts and culture methods make it difficult to draw conclusions on the field situation (Foissner 1987). Besides, hardly any results concerning an exact spatial distribution of ciliates and testaceans are known, because either very few active ciliates have been found in the soil or the investigated strata were too thick to get clear results. Therefore we studied the ciliates and testaceans of a spruce forest and a meadow in 0-1, 1-3 and 3-9 cm depth.

An additional reason for this investigation was to collect data on soil ciliatostasis. Ciliatostasis is a phenomenon of most evolved natural and cultivated soils and severely restricts excystment and growth of ciliates compared to their behavior under the same conditions of e.g., temperature, moisture, pH etc. in laboratory cultures (Foissner 1987). Currently, not much is known about the causes. Foissner (1987) suggested that unknown chemical substances, present mainly in evolved soils, could be responsible. The ecological implication may be to maintain an equilibrium between soil organisms as similar phenomena, termed soil microbiostasis, are already known for actinomycetes, fungi and bacteria (Ko and Chow 1976).

In his review Foissner (1987) founded the hypothesis in part on data from the literature and on unpublished observations, most of which are reported here.

Materials and Methods

Site Descriptions

Site 1: Spruce forest in Oberhaag near Aigen (Upper Austria), 860 m NN, 0-1 cm soil depth is the L layer and consists mainly of fresh needles (about 1 year old). 1-3 cm is a more compact layer of older and slightly decomposed needles (F layer). 3-9 cm is raw humus (H layer). Each zone was investigated at least 12 times for ciliates and 4 times for testaceans in Oct. and Nov. 1985 in the course of a precipitation experiment.

Sites 2-4: Meadows and arable land near Salzburg city, about 430 m NN. Detailed site descriptions in Foissner et al. (1988). At site 2 (meadow) 3 replicates of 1 sample date (Dec. 1985) were investigated. The 0-1 and 1-3 cm layers consist mainly of plant roots and plant residues, while 3-9 cm is mineral soil. At site 3 (meadow) and 4 (arable land) 8 samples were taken between Oct. 1983 and 1986. Type of humus: mull.

Sites 5-7: Grossglockner area, Hohe Tauern (Austrian Central Alps), investigated between June and Oct. 1978. Detailed site descriptions in Foissner (1981). At site 5, an alpine pasture in 1900 m NN, 5 samples were studied. At

sites 6 (alpine mat) and 7 (alpine pasture, heavily fertilized by waste water), about 2300 m NN, only 1 sample each was investigated. Type of humus: mull-like moder.

Sites 8-10: Tullnerfeld area, near Vienna, about 180 m NN. 10 samples were investigated between Aug. 1980 and Nov. 1982. Detailed site descriptions in Foissner et al. (1985). Site 8 is xerothermic (mull-like moder), site 9 is a bottomland (mull) inundated at least twice a year, and site 10 is arable land.

Methods

Soil samples were taken and prepared according to Foissner (1985) and Foissner and Pear (1985). Direct counting method: 0.005-0.2 g fresh soil are suspended in a few ml of sterile soil solution and counted directly under a microscope at 40× (ciliates) and 100× magnification (testaceans). For detailed description of these methods see Lüftenegger et al. (1988). The culture method used is very similar to that described by Buitkamp (1979): Air-dried soil is saturated with water and ciliates are counted 6 days after culture set-up. However, we used different amounts of soil, therefore the absolute numbers obtained with this method (although per g dry mass) are probably not fully comparable. Nevertheless, these values are useful for a comparison with those of the direct counts. Within a site equal amounts of soil were used with both counting methods.

Soil moisture (% of wet mass of soil), organic matter (% of dry mass of soil) and pH were determined as described by Berger et al. (1986), loss-on-ignition (% of dry mass of soil) was ascertained at 550°C, bulk density was estimated as described by Foissner (1981).

Results

Ciliates and testaceans are distinctly separated in the spruce forest (Table 1, Fig. 1). The highest numbers of active ciliates (max. 603 ind. g^{-1} dry mass [dm], $\bar{x} = 350$) occur in the uppermost 0-1 cm. Numbers decrease ($p < 0.05$) with increasing soil depth. In 7 out of 15 samples from 3-9 cm active ciliates are absent. The testaceans, in contrast, reach highest individual densities between 1-3 cm (max. 41517 ind. g^{-1} dm, $\bar{x} = 31408$) and lowest in the 0-1 cm layer. All samples contain active testaceans.

Only very few active ciliates occur in the meadows and the arable land (Table 2). However, with the culture method high numbers grow in all samples. Despite a detailed vertical investigation of site 2, no active ciliates were found in 0-1, 1-3 and 3-9 cm depth. The highest numbers of testaceans occur in the top 0-1 cm (3615 ind. g^{-1} dm). With increasing soil depth numbers decrease sharply ($p < 0.05$). The same distribution is obtained for the ciliates if investigated with the culture method (Table 2).

Table 1

Arithmetic mean ± S.D. of the abundance of testaceans and ciliates and of environmental parameters in a spruce forest[a]

Site	Soil depth (cm)	Testaceans[b] (g^{-1} dm)	Ciliates direct count (g^{-1} dm)	Soil moisture (%)	Loss-on-ignition (%)	pH	Bulk density (g cm^{-3})
1 Spruce forest	0–1	11138*[c] (±1084)	350* (±167)	54.3 (±6.7)	95.1 (±1.0)	3.9 (±0.1)	0.07
	1–3	31408* (±7427)	109* (±116)	54.3 (±8.9)	89.8 (±0.6)	3.9 (±0.0)	0.05
	3–9	17385* (±4614)	14* (±24.1)	45.2 (±6.4)	47.9 ±17.7)	3.4 (±0.2)	0.29

[a] — testaceans: n = 4; ciliates, soil moisture: n = 12–15; loss-on-ignition, pH: n = 3; bulk density: n = 1, [b] — only living individuals; dm = dry mass, [c] — different at $p < 0.05$ (*) with the H-test of Kruskal-Wallis (Köhler et al. 1984)

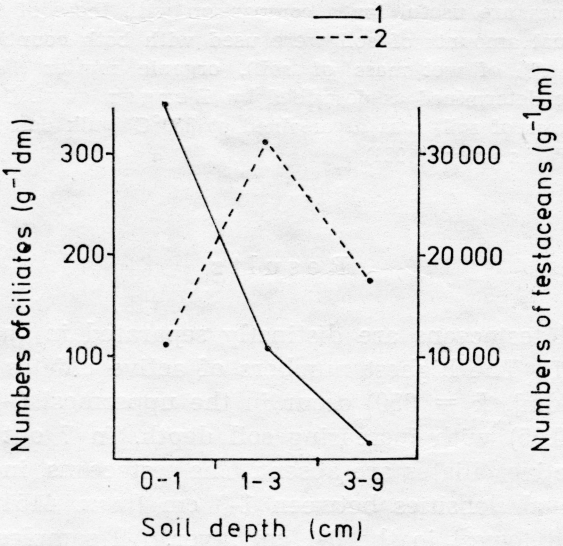

Fig. 1. Spatial separation of active ciliates (*1*) and testaceans (*2*) in a spruce forest

The striking difference between direct counting and the culture method is also demonstrated by an investigation of 3 alpine localities (sites 5-7, Table 3). No active ciliates occur in sites 6 and 7 but relatively high numbers are present in 0-2 cm of site 5 ($\bar{x}$ = 224 ind. g^{-1} dm). The culture method provides excessively high values, especially for the 0-2 cm layers, at all 3 sites. Testaceans prefer the 2-4 cm depth both at sites 6 and 7.

Table 2

Arithmetic mean±S.D. of the abundance of testaceans and ciliates and of environmental parameters in meadows and an arable land[a]

Site	Soil depth (cm)	Testaceans[b] (g^{-1} dm)	Ciliates (g^{-1} dm) direct count	Ciliates (g^{-1} dm) culture method	Soil moisture (%)	Organic matter (%)	pH	Bulk density ($g\ cm^{-3}$)
2 Meadow	0–1	3615*[c] (±1975)	0	3112* (±697)	41.0 (±1.4)	ND[d]	ND	ND
	1–3	1436* (±329)	0	1810* (±653)	40.0 ±1.0)	ND	ND	ND
	3–9	587* (±218)	0	1241* (±912)	37.8 (±0.3)	ND	ND	ND
3 Meadow	0–5	948 (±380)	2 (±5.3)	2235 (±3588)	31.4 (±3.9)	6.7 (±4.0)	5.9 (±0.6)	0.93
4 Arable land	5–15	528 (±233)	1 (±2.5)	405 (±173)	33.2 (±1.6)	5.2 (±0.6)	7.0 (±0.4)	0.91

[a] – site 2: n = 3; sites 3, 4: n = 8; organic matter, pH: n = 5; bulk density: n = 1, [b] – only living individuals; dm = dry mass, [c] – different at $p < 0.05$ (*) with the H-test of Kruskal-Wallis (Köhler et al. 1984), d – not determined in each layer; in 0–5 cm: organic matter 7.0, pH 7.1, bulk density 0.78 g cm^{-3}

Generally, moderate to low numbers are found with the direct and the culture technique in the Tullnerfeld localities (sites 8-10, Table 3). Only site 8 yields considerable numbers in the cultures which are different at $p < 0.001$ from those of the direct counts. Similarly, these values differ in the 0-5 cm layer of site 10 ($p < 0.1$), whereas in the remaining strata of sites 9 and 10 no pronounced differences exist ($p > 0.1$).

Discussion

The most surprising results are the sharp decrease in the abundance of the active ciliates between litter and slightly decomposed litter[2], and the nearly total lack of active ciliates in the uppermost humus horizon of the spruce forest (Table 1, Fig. 1) as well as in all strata of meadows and arable land (Tables 2, 3). On the contrary, testaceans favor the F and H layer of the forest (Table 1, Fig. 1) and the 2-4 cm horizons of sites 6 and 7 (Table 3). Lousier and Parkinson (1984) reported a very similar testacean distribution in a deciduous forest. Schönborn (1986) found a corresponding distribution of active ciliates in two coniferous forests. Brunberg-Nielsen (1968) reported up to 32550

[2] We observed this dramatic decline also in a single sample of a beech forest. In the 0-2 cm layer (leaves) 3326 active ciliates g^{-1} dm were recorded and none between 2-4 cm (F/H layer); testaceans: 0-2 cm 17196 g^{-1} dm, 2-4 cm 2645 g^{-1} dm.

Table 3

Arithmetic mean±S.D. of the abundance of testaceans and ciliates and of environmental parameters in Grossglockner (5–7) and Tullnerfeld (8–10) localities[a]

Site	Soil depth (cm)	Testaceans[b] (g^{-1} dm)	Ciliates (g^{-1} dm)		Soil moisture (%)	Organic matter (%)	pH	Bulk density (g cm^{-3})
			direct count	culture method				
5 Alpine pasture	0–2	2457 (±1052)	224 (±186)	11654 (±9252)	54.7 (±8.5)	in 0–10 cm		0.40
	2–10	435 (±313)	6 (±8.2)	1573 (±1211)	33.6 (±5.3)	5.5	5.8	0.88
6 Alpine mat	0–2	3050	0	21700	53	in 0–8 cm		in 0–5 cm
	2–4	4100	0	950	45	10.0 in 0–12 cm		0.50
	4–8	570	0	300	41		4.7	in 5–10 cm
	8–12	30	0	0	27	4.9		0.82
7 Eutrophic alpine pasture	0–2	3400	0	14000	75	ND[c]	ND	in 0–5 cm
	2–4	3900	0	6500	62	ND	ND	0.26
	4–8	550	0	50	51	ND	ND	in 5–10 cm
	8–12	630	0	30	45	ND	ND	0.67
8 Xerothermic site[d]	0–5	725 (±416)	154 (±186)	720** (±443)	25.1 (±9.0)	5.6	7.5 (±0.4)	0.61
	5–10	167 (±123)	44 (±72)	603** (±674)	19.0 (±3.9)	3.6	7.6 (±0.4)	0.90
9 Bottomland[d]	0–5	1556 (±643)	51 (±43)	35 (±39)	40.7 (±7.9)	6.6	7.5 (±0.3)	0.68
	5–10	859 (±342)	16 (±20)	34 (±34)	33.5 (±2.5)	6.9	7.6 (±0.3)	0.81
10 Arable land[d]	0–5	156 (±106)	56 (±127)	37* (±42)	15.2 (±6.5)	2.6	7.4 (±0.4)	ND
	5–10	214 (±251)	18 (± 28)	23 (±30)	17.8 (±8.3)	2.8	7.6 (±0.4)	1.16

a – site 5: active ciliates, testaceans n = 5; culture method, soil moisture n = 4; organic matter, pH, bulk density n = 1. Sites 6, 7: n = 1. Sites 8–10: n = 10; organic matter, bulk density n = 1, b – only living individuals; dm = dry mass, c – not determined, d – different at $p < 0.001$ (**) and $p < 0.1$ (*) from the direct count with the U-test of Mann–Whitney (Köhler et al. 1984), no difference at $p < 0.1$ in site 9 and in 5–10 cm of site 10 as well as in testacean abundance between 0–10 cm of site 10

active ciliates g^{-1} dm in the L layer of a beech forest and somewhat less in the F horizon. These observations indicate a distinct spatial separation — at least in forests — between ciliates which prefer the L layer and testaceans which favor the F and H horizons.

How can this spatial separation be explained? *A priori*, one would expect a reverse vertical distribution because the testate amoebae have better adaptations than ciliates to resist desiccation which certainly occurs frequently in litter. At first glance, the quicker division and cystation capacities of the ciliates, which allow a more immediate response

to changed environmental conditions as compared to the testaceans, seem to account for the separation. However, how can one explain, then, that hardly any active ciliates are present in the humus layer of the spruce forest and in all strata of meadows and various other sites where a high testacean abundance indicates good living conditions; and last but not least, why do so many ciliates appear in cultures from these habitats (Tables 1-3)?

An appropriate explanation, at least partially, could be offered by ciliatostasis (see introduction). If one looks at the presented results and data from the literature, the findings reported here fit exactly into this concept: (1) High numbers of active ciliates in litters and a sharp decrease with increasing humification and therefore more evolved soil (Table 1, footnote 2). (2) Absence or low numbers of active ciliates in all strata of meadows and arable land (Tables 2, 3), where no litter layer exists, only evolved soil. Site 5 is an unusual exception if compared with other similar sites (e.g., Foissner 1985, Berger et al. 1985). (3) Nullification of ciliatostasis in the laboratory by drying and remoistening of the soil (Tables 2-4) and by addition of glucose to soil (Foissner

Table 4

Arithmetic mean±S.D. of the abundance of ciliates in fresh and washed soil and in cultures

Site	Soil depth (cm)	Ciliates (g^{-1} dry mass)		
		fresh soil	washed soil	culture
Cushion plant	0–5	11	30*[a]	1570
site n = 8		(±14.1)	(±31)	(±1910)
Alpine mat	0–10	2	49**	134
n = 10		(±3.2)	(±42)	(±90)

a – different at $0.1 < p < 0.2$ (*) and $p < 0.005$ (**) from the fresh soil and at $p < 0.01$ (**) from the culture with the U-test of Mann–Whitney (Köhler et al. 1984)

1987). These procedures enrich the substrate with energy-containing nutrients and cause an abundant growth of food organisms (e.g., bacteria, fungi) indicating that food could be an important factor in overcoming ciliatostasis. This is supported by fertilization experiments on a ski slope after top soil removal (Lüftenegger et al. 1986 a). However, the above mentioned points 1 and 2 argue against such an explanation because bacteria and fungi are surely abundant at sites where active ciliates are rarely encountered, like in the upper soil layers of meadows and in the humus layer of the forest. Besides, Foissner (1985) reported an adverse pattern of ciliate abundance and dehydrogenase activity and

no connection with catalase activity. That means that food, though certainly an important factor, is very probably not the main reason for the lack of active ciliates in certain soils. Lüftenegger et al. (1986 b) showed that top soil removal was necessary for the nullification of ciliatostasis in the ski slope. A certain portion of the high abundance obtained with the culture method results from multiplication of ciliates during the 6 days of incubation. However, it is known from investigations with Singh's (1946) dilution method that a high amount of cystic (inactive) protozoa exists in many soils.

Under field conditions, the crucial point seems to be the age of the soil, as Foissner (1987) already suggested. This exactly corresponds with the present observations that in non-evolved soil "litter" much higher numbers of active ciliates exist than in the more evolved F and H layers. These older horizons must contain a factor restricting excystment and growth of ciliates. A study by Foissner (1981) provides experimental indication of this. Small chambers with washed soil were buried at the original sites. After about 16 days they were recovered and ciliates were counted. The washed soil, although very probably containing less food, yielded more active ciliates than the fresh soil but numbers in cultures were still higher (Table 4). It is conceivable that a (chemical) factor responsible for ciliatostasis was diluted or partly washed out. Perhaps such a restricting substance is inactivated or lost (e.g., by evaporation) in laboratory cultures because rewetting of the soil hardly causes a dilution.

The influence of the age of the soil is stressed by data from site 9 and 10 (Table 3). Both the inundation of the bottomland and the tillage of the arable land produces a layer of "young" soil. Thus, as expected, the differences between direct and culture counts are comparatively low.

The age of the soil is probably not the only factor which is responsible for ciliatostasis. There are hints that ciliatostasis requires the presence of living microorganisms (Foissner 1987). But very likely, this and other parameters, e.g., soil moisture, organic matter, pH or soil density are of minor importance as shown by our data (Tables 1-3). Among these factors, especially soil density (or rather pore space) is believed to limit ciliate occurrence in the soil (Darbyshire 1976, Alabouvette et al. 1981). But field experiments by Berger et al. (1985) and our results (Tables 1-3) suggest that soil density is not crucial for the occurrence of active ciliates. Even in strata with very low bulk density, which proves plenty of larger pores (e.g., 3-9 cm of site 1 or 0-2 and 2-4 cm of site 7, Tables 1, 3), no or only very few active ciliates are encountered.

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