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## The role of trophic factor in microzonal distribution of soil ciliates

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### Abstract

**Purpose:** To identify the influence of the trophic factor on the pedobiont ciliates, field studies of their microzonal distribution in the forest soils of the broad-leaved forests of the Talyshian Mountains (South East Azerbaijan) were carried out.

**Material and Methods:** In total, more than 430 soil samples were collected in the forest soils. 2 plots of 1m<sup>2</sup> were marked, at a distance of 0.5 m from each other. Soil monoliths were collected, which were viewed in layers 0-10-15-20 and 25 cm. The quantitative share of ciliates was estimated by the ratio of the number of species *n* to the total number of all species *N* of the sample community. To determine pedobiont ciliates, impregnation by silver nitrate (Chatton et Lwoff, 1930) <sup>[13]</sup> and silver proteinate methods were used (Alekperov, 1992) <sup>[1]</sup>.

**Results:** In total, 72 species of pedobiont ciliates were recorded in experimental plots of forest soils. The groups of dominants, subdominants, secondary and random species and their share in the total number of pedobiont ciliates are established. The last 2 groups are the most numerous and consist of species distributed in fresh waters, which explains their presence in soils only during the period of highest humidity. The vertical quantitative distribution in the control without peptone in different soil horizons showed a high abundance and species diversity of pedobiont ciliates in the surface soil layers (0-10 cm) with a decrease with depth in monoliths with peptone additives, a high abundance, but low species diversity, was observed in these layers.

**Conclusions:** 1. The necessity of studies of ciliates in nature is substantiated. 2. The material should be sufficient for the correct statistical analysis and expressed quantitatively. 3. It is necessary to study a large number of samples with a wide spread over the site.

**Keywords:** Ciliates, microdistribution, forest soils, food, Azerbaijan

### Introduction

Currently when researching complex ecosystems, be they soil-based or aquatic, specialist ecologists mainly use two approaches: Reductionism - e.g. detailing the analysis of smaller and smaller components of the system, in order to recognize patterns characteristic to the whole, and holism, e.g. analysis first of broader, then more specific details.

It should be noted that in recent years additional methods such as experiment and modeling have had widespread use. Like in many other cases, the optimum would be a "middle ground", using both main approaches equally.

Based on the opinions of leading specialists, reliable results can currently only be achieved with methods satisfying three basic requirements.

Firstly, material collected for analysis must be enough for a proper statistical analysis, and must be quantified.

The second highly important requirement is for the analyzed material to be a product of field research rather than laboratory.

The last crucial requirement for reliable results on the effects of different factors is a large enough count of probes and an equal spread of them in the researched area. The latter is explained by the highly unequal, mosaic spread of free-living protozoa, including the ciliates in the environment.

The research of Burkovsky, 1984, Alekperov, 1997 and Sadikhova, 2006 establishes that, in both soil and aquatic biocoenoses, a deciding factor in spacial and temporal spread of free-living ciliates is the trophic factor. Every small fragment of dead organic matter in soil, of both plant and animal origin, immediately accumulates first many species of bacteria. After that arrive the primary consumers, part of which are the free-living ciliates inhabiting the soil. Around these sources of food, the number of protozoans, and the small soil-inhabiting invertebrates (oligochetes, tardigrades and so on) that prey on them, the numbers and biomass

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of pedobionts of different taxa are rather high. However, often even a distance of 2-5 cm is enough for diversity, numbers and biomass of different groups to drop off significantly.

Based on the above, to find the effects of the trophic factor on ciliate pedobionts, we conducted field studies of their microzonal spread in forest soils of broadleaf forests of the Talysh mountains (South-east Azerbaijan).

**Materials and Methods**

In total, over 430 soil probes were collected and processed during the course of the studies. Probes were used in soils of broadleaf forests of Talysh mountains in areas untouched by human influence. 2 plots of land, each 1m<sup>2</sup> and spaced 0,5 meters from one another, were marked. On each plot, soil monoliths were collected by cutting out with glass tubes, with a diameter of 2 cm and length of 30 cm. Each soil monolith was then studied by layers at horizons of 0, -10, -15, -20 and -25 cm. Cut fragments of soil monoliths were submerged in distilled water and viewed by 1 cm<sup>2</sup> in a Bogorov chamber, where ciliate pedobiont analysis was done. Averages of ciliate numbers were then calculated and recounted at 1 dm<sup>2</sup>. To find out the role of the trophic factor in microzonal spread and localization of soil ciliates and soil biotopes, using a syringe on different soil horizons (0-10-15-20-25) we injected 1 ml of peptone solution, during the decomposition of which bacteria, flagellates and algae then began to develop in the respective horizon. Each experiment with soil monoliths was conducted in 5 series. Humidity of soil in plots used in field research was no less than 20-35%. Numeric share of species was graded via ratio of population of species n to overall number of all species of N community in probe. Results then allowed us to allocate found species of ciliate to the groups below by Tischler's scale (Tischler, 1955) [14].

1. Dominant species group -  $n/N > 5\%$
2. Subdominant species group -  $n/N > 2\%$
3. Recedent species group -  $n/N > 1\%$  (secondary)
4. Subrecedent species group -  $n/N < 1\%$  (random)

All results were processed using the computer application "Biodiversity Professional 2".

For taxonomic identification of ciliate pedobionts, we

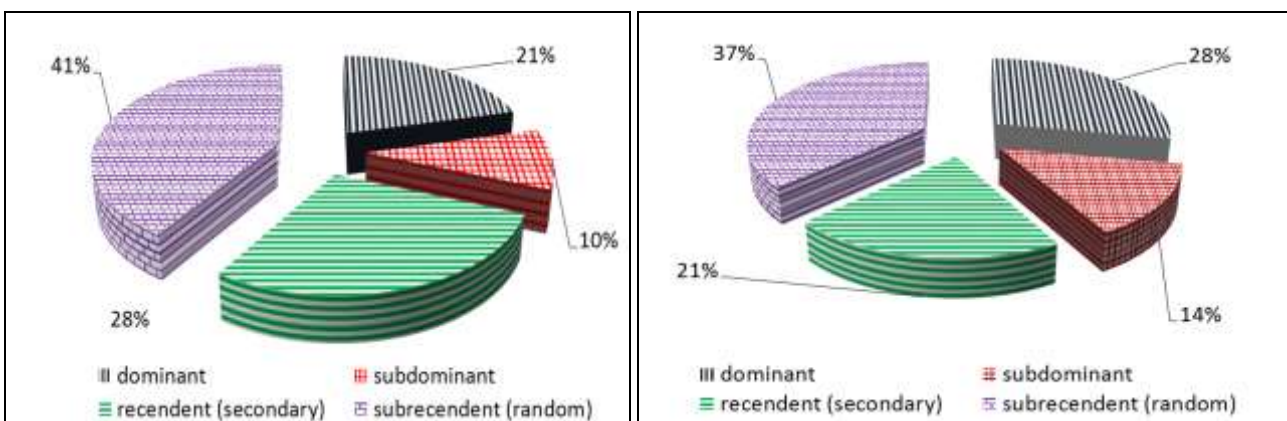
utilized methods of kinetosome nitrate (Chatton et Lwoff, 1930) [13] and silver proteinate (Aleksperov, 1992) [1] impregnation.

**Results**

Over the course of the studies, we found 72 species of ciliate pedobionts on the forest soil test sites. The analysis conducted on species occurrence has shown that, according to Tischler's scale, the dominant group contained 15-20 species, depending on the plot of land. These ciliate pedobionts made up no less than 5% of the total ciliate numbers in the probes. Notable species include *Microthorax elegans* Kahl, 1931, *M. transversus* Foissner, 1985, *Colpoda maupasi* Enriques, 1908, *C. cucullus* (Müller, 1773), *C. aspera* Kahl, 1926, *Coleps bicuspis* Noland, 1925, *Cyclidium citrullus* Cohn, 1865, *C. glaucoma* Müller, 1786 and *Uronema nigricans* (Müller, 1786).

The second group of subdominants, meaning ciliate pedobionts constituting up to 2% of total numbers, only had 7-10 species assigned. Ones that should be noted are *Oxytricha tenella* Song et Wilbert, 1989 and *Birojimia terricola* Berger et Foissner, 1989, three species of the genus *Platyophrya* (*P. vorax* Kahl, 1926, *P. spumacola* Kahl, 1927, *P. sphagni* (Penard, 1922), and also *Grossglockneria acuta* Foissner, 1980. It should, however, be noted that this division of ciliate pedobionts into groups is conditional, due to external factors being able to make the same species be assigned to the dominant group on one plot, but to the subdominant group on another. Such highly aggregated distribution of ciliate pedobionts confirms the conclusions made earlier on their aquatic counterparts that the spread of free-living ciliates in the environment is defined by external factors within several centimeters, and is noticeably patchy in nature.

The recedent (secondary) species group was assigned 15-20 species of ciliate pedobionts, which made up no more than 1% of total numbers. Notable species include *Phacodinium muscorum* Prowazek, 1900, *Euplotes harpa* Stein, 1859, *E. balteatus* Dujardin, 1842, *Aspidisca fusca* Kahl, 1928, *Lagynophrya mutans* Kahl, 1927, *Enchelys pectinata* Kahl, 1930, *Lacrymaria olor* (Müller, 1786), *L. clavarioides* Aleksperov, 1984, *Protospathidium terricola* Foissner, 1998 etc.



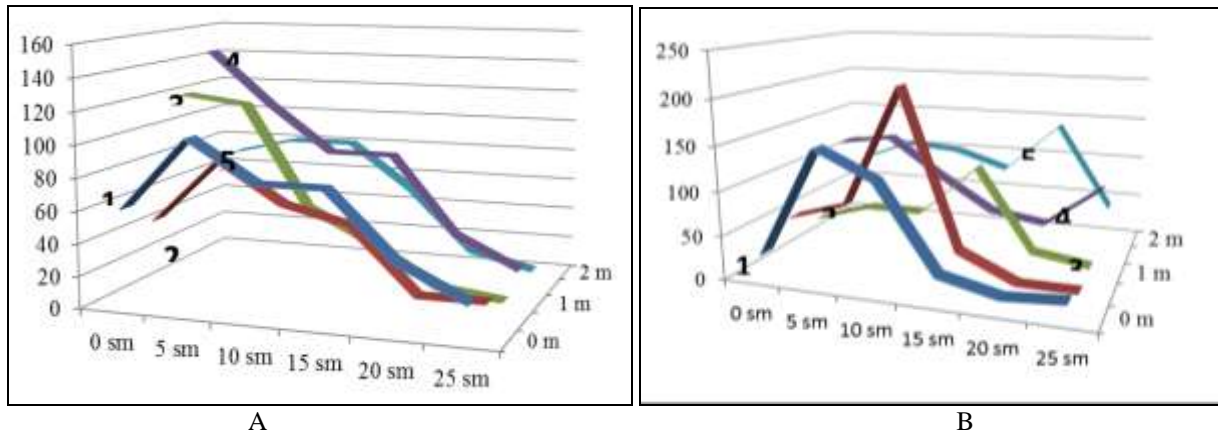
**Fig 1:** The quantitative ratio of soil ciliates species (n / N) in their total species diversity in forest soils

The last, most numerous group of subrecedent (random) species, numerically constituting under 1% of total ciliate pedobiont numbers, consists of 25-30 species. It should be noted that ciliates from this group were usually documented

in periods of highest forest soil hydration by precipitation, meaning mostly spring and autumn. Interestingly, in both the recedent and subrecedent group practically all documented species of ciliates are usual freshwater

residents. The presence of these facultative species may be explained by the conditions in soil biotopes approaching conditions freshwater ciliates usually reside in due to high humidity (especially in spring and autumn), causing the appearance of many freshwater species.

To find out the effects of the trophic factor on the spread of ciliate pedobionts in the soil, we conducted field research on soil monoliths as detailed in the methods segment. Их обобщенные результаты представлены на рис.2 А и Б.



**Fig 2:** The total number (ind/dm<sup>2</sup>) of ciliates in soil monoliths (0-25 cm) taken every 0.5 m in forest soil without addition (A), and with the addition of peptone (B).

As seen in fig. 2A, studies conducted on vertical distribution of ciliate pedobionts on the land without use of peptone in soil horizons showed normal localization of most forest soil species in top soil layers. For example, in monolith 1, maximum total number of soil ciliates was observed in the 5 cm horizon (100 spec/dm<sup>2</sup>), and subsequently fell in deeper layers, with a minimum density of 20 spec/dm<sup>2</sup> in the 25 cm horizon. In monolith 2, studied in a distance of 0,5m, highest number of ciliate pedobionts was also observed in the topmost layer at 5 cm (85 spec/dm<sup>2</sup>). The subsequent drop of ciliate pedobionts with increasing depth was also observed, reaching a minimum in the 20 cm and 25 cm layers, respectively 20 and 10 spec/dm<sup>2</sup>.

Unlike in monoliths 1 and 2, highest ciliate numbers were observed both in litter, meaning on the surface, and in the 5 cm soil horizon in monoliths 3 and 4. In monolith 3, 120 spec/dm<sup>2</sup> in litter and 115 spec/dm<sup>2</sup> in the 5 cm layer, and in monolith 4, 145 spec/dm<sup>2</sup> and 110 spec/dm<sup>2</sup> respectively. A sudden drop in ciliate pedobiont numbers occurs in deeper layers of monolith 3, reaching 5 spec/dm<sup>2</sup> at 20 cm, and a complete lack of documented ciliates deeper (25 cm). This pattern was also recorded in soil monolith 4, where the deepest soil horizon's ciliate numbers were only 10 spec/dm<sup>2</sup>. Results of distribution analysis of ciliate pedobionts in soil monolith 5 showed no more than 70-80 spec/dm<sup>2</sup> respectively in the higher layers of 0-10 cm. Deeper layers were documented having a density of 50 spec/dm<sup>2</sup> in the 15 cm horizon, with a subsequent gradual drop until the minimum of 5 spec/dm<sup>2</sup> in the 25 cm layer. These results are characteristic for virgin forest soils untouched by human activity. This typical spread of overall numbers of most species in surface layers and a consistent decrease with depth is described in a number of works (Nikitina, 1988, 2000; Alekperov & Mamedova, 2013, 2015a, 2015b) [5].

The next part of the analogous field research was done by adding peptone as a food source to the different soil horizons.

As seen in data presented in fig. 2B, the above patterns change drastically when peptone is added to the soil horizons. For instance, the sum of ciliate pedobionts in the

litter (0 cm horizon) in monolith 1 was only 25 spec/dm<sup>2</sup>, however at a depth of 5 cm where peptone was added it rose sharply to 150 spec/dm<sup>2</sup>. In deeper horizons (10 cm) total number of ciliates dropped to 120 spec/dm<sup>2</sup>, and in horizons of 15 cm and deeper it drops sharply from 25 spec/dm<sup>2</sup> to 10 and 15 spec/dm<sup>2</sup> respectively for layers at 20 and 25 cm. Next, in monolith 2, peptone was injected in the 10 cm layer. As seen in fig. 2B, total ciliate pedobiont numbers in this monolith in layers 0 and 5 cm respectively were equal to 50 and 70 spec/dm<sup>2</sup>, however the 10 cm layer was recorded to have numbers over three times higher, reaching 220 spec/dm<sup>2</sup>. Deeper in horizons at 15-20 and 25 cm we recorded a sharp drop of total ciliate count, respectively up to 30 spec/dm<sup>2</sup> in the 15 cm layer, and a complete lack of ciliates in deeper horizons.

Peptone was added at a depth of 15 cm in soil monolith 3. In this case, the topmost layers (0-5-10 cm) were recorded to have total numbers of 30-50-50 spec/dm<sup>2</sup> respectively, then at 15 cm an increase to 110 spec/dm<sup>2</sup> was recorded, and the deeper layers at 20 and 25 cm dropped sharply to 15 spec/dm<sup>2</sup> and 5 spec/dm<sup>2</sup> respectively.

An interesting result was observed in total quantitative spread of ciliate pedobiont numbers in monolith 5, where peptone was added to the bottommost horizon at 25 cm. As seen in fig. 1B, maximum total count of ciliate pedobionts was recorded in topmost layers at 0 and 5 cm, respectively 115 spec/dm<sup>2</sup> and 125 spec/dm<sup>2</sup>. Because in this case peptone was injected into the deepest horizon at 25 cm, we assume that its lack of effect in increasing overall ciliate pedobiont numbers is explained by its inhibition by the depth of the soil horizon. Because at this depth only true soil ciliate species were encountered, belonging to genera such as *Colpoda*, *Microthorax*, *Platinematum*, and also some small hypotrichs, such as *Oxytricha minor*, *Birojimia terricola*, etc.

Highly interesting was also the comparison between the species diversity of ciliate pedobionts in different vertical layers. It was found that species diversity in higher soil horizons was invariably higher than in deeper layers. For instance, the overwhelming majority of soil ciliates develop and localize in the control in soil layers at 0-5 cm. Other

than that, the higher layers with comparatively larger water-filled cavities in the soil, due to the higher humidity, harbor many species of ciliates that are normally parts of freshwater communities, meaning high soil humidity in topmost layers causes the appearance of a large number of facultative species for which presence in higher layers is temporary. We also recorded another pattern. Typically soil ciliates, morphologically adapted to living in soil layers (small size, flattened body, ability to encyst quickly in unfavorable conditions and excyst as quickly in optimal environments) usually inhabit deeper horizons with smaller soil cavities and a higher soil density. Ones that should be named first and foremost are hypotrichs, representatives of the genera *Oxytricha*, *Tachysoma*, *Aspidisca*, *Fuscheria*, *Enchelys*, *Microthorax*, *Leptopharynx*, *Drepanomonas*, and most species of the genus *Colpoda*. The specified ciliates are present in forest soils throughout the entire year, giving way both in quantity and in quality to facultative species in higher layers (0-10 cm) during periods of highest soil humidity (spring and autumn). In the hotter summer period, facultative species fall out of soil communities, and true pedobionts migrate to deeper layers (15-25 cm), where humidity is high enough for their survival. As was shown, the effect of peptone on species diversity of ciliates in the injected area depends on the depth of its location in the soil. We have also conducted a study on species diversity of ciliate pedobionts both in the zone where peptone was added in monoliths, and in the other soil horizons in the researched monoliths. Results show that, when in other horizons of the monolith species diversity of soil ciliates changes on average from 12 to 28 species, in all five monoliths in horizons where peptone was added, species diversity fell noticeably, and the number of species of soil ciliates in zones where peptone was added was noticeably small, not exceeding 4-7 species in all monoliths. It should also be noted that practically all of these species of ciliate are bacteriophages and eurybionts, possessing significant ecological plasticity.

Thus, the results we arrived at during the field experimenting generally fit the patterns of microzonal spread of free-living ciliates in aquatic biotopes (Aleksperov, 1988), and Bamforth's data on the effects of external factors in areas of 3-5 cm on ciliates. (Bamforth, 1963).

Currently it is already proven that a leading role in food selection in ciliates is taken by chemoreception (Seravin *et al.* 1987; Aleksperov, 1987), with each species possessing its own set of chemical inductors, causing a food reaction to specific foods, in addition to a reaction to movement. Within the limits of their own range of food objects, a majority of ciliates are capable of consuming food of highly diverse origins. All ciliates are united by the ability to use food in large quantities, and a high regeneration speed in an abundance of food. This way, the intensity of feeding in ciliates is in direct dependence on concentration of food, in an abundance of which the division rate of a majority of species rises sharply.

In natural communities a high reaction speed of ciliate number to changes in concentration of specific food objects is due to not only the rate of reproduction, but also the process of excystation in favorable conditions.

### Conclusions

This way, summing up the above we may note several basic principles of the modern approach to ecological research of ciliates:

1. The inability to sufficiently correctly extrapolate experimental data dictates a necessity to conduct field research at the level of ciliate populations.
2. All analyzed factual material must be enough for a proper statistical analysis, and must be quantified.
3. Considering the extreme mosaic nature of distribution of free-living ciliates in the environment, to arrive at correct results, a large enough number and wide distribution of probes for analysis is necessary.

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