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Protozoan Grazing of Bacteria in Soil—Impact and Importance

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Abstract. Interactions between bacteria and protozoa in soil were studied over 2-week periods in the field and in a pot experiment. Under natural conditions the total biological activity was temporarily synchronized by a large rainfall, and in the laboratory by the addition of water to dried-out soil, with or without plants. In the field, peaks in numbers and biomass of bacteria appeared after the rain, and a peak of naked amoebae quickly followed. Of the three investigated groups—flagellates, ciliates, and amoebae—only populations of the latter were large enough and fluctuated in a way that indicated a role as bacterial regulators. The bacterial increase was transient, and the amoebae alone were calculated to be able to cause 60% of the bacterial decrease. The same development of bacteria and protozoa was observed in the pot experiment: in the presence of roots, amoebic numbers increased 20 times and became 5 times higher than in the unplanted soil. In the planted pots, the amoebic increase was large enough to cause the whole bacterial decrease observed; but in the unplanted soil, consumption by the amoebae caused only one-third of the bacterial decrease.

Introduction

The role of protozoa in the soil is at present unclear [29], but evidence for their central position is now accumulating. Calculations made by Stout and Heal [30] for an arable field soil indicated that protozoa consumed 150–900 g of bacteria m^{-2} $year^{-1}$, which was equal to a production of 15–85 times standing crop. In 1973 Stout [28] suggested that predation of bacteria by protozoa acted as an important mechanism in nutrient release. The possible role of protozoa in nutrient cycling was later demonstrated in microcosm experiments, where the presence of bacterial-consuming protozoa resulted in higher mineralization [14] and higher nitrogen uptake by plants [15].

For an evaluation of the importance of protozoa in the field, the sizes and fluctuations of their populations need to be described, especially in relation to their food source and their predators. Few field data are available, however. The study of protozoa is likely to have been hampered by their characteristics. These animals lack a proper cell wall and are liable to burst through a change in pH or salt concentration or through mechanical abuse. Their small size (5–30 μm) and variable shape make them difficult to recognize in soil, where they occur in numbers 10^4–10^5 times lower than those of bacteria. This makes direct counting impossible since the magnification needed would give only one animal in 1 field out of 10; this leaves only indirect methods for their estimation. The

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small size of protozoa also makes them difficult to handle by conventional zoological methods. At the same time, microbiologists have largely ignored these animals as falling outside their field.

A problem in attempting to describe protozoan-bacterial relations in soil is that it is impossible to follow the behavior of a single group of organisms. When good conditons for biological activities prevail, cyclic growth of bacteria, protozoa, and their predators must occur at the same time, although in different microniches separated in space. Even a small soil sample contains many microsites, where bacterial growth alternates with predation and death within millimeters. Under such conditions, the average observable microbial biomass is low and constant [22], although there is a large flux through the populations. Only conditions that strongly affect the total biological activity in the soil—such as rewetting after dry conditions, or a large momentary input of energy or nutrients— temporarily synchronize activities in large parts of the soil. Such behavior was demonstrated by Jensen and Ball [21] in a chemostat with natural lake water, where, by adding sugar at 7-day intervals, they induced peaks of bacteria followed by protozoan peaks; more frequent additions were found to disrupt the cyclic pattern.

In the field Cutler and Crump [7] found bacterial peaks a couple of days after a rainfall, and their observations were later confirmed by Campbell and Biederbeck [3] and Clarholm and Rosswall [4]. In the humus layer of a pine forest soil the latter observed a transient bacterial increase, lasting only 4–6 days after the rainfall despite favorable moisture conditions. The bacterial decrease could have been the result of autolysis; however, this is not likely in view of the short time elapsing after the increase. A massive simultaneous attack by bacteriophages is also unlikely in the heterogeneous soil environment, as compared with an aquatic situation, where this has been shown to occur [24]. This leaves predation as the most probable cause of the bacterial decline.

To decrease the bacterial numbers drastically in a short time requires a high grazing pressure, which implies large numbers of active predators with high growth rates. Protozoa meet these requirements, since they occur in great numbers in soil [10, 11], have rapid growth rates [6, 8], and also have the ability to decrease bacterial numbers drastically [18, 19]. Their connection with rainfall, and thus indirectly with increases of bacteria, can be concluded from the observations made by Elliott and Coleman [13], who found a peak of protozoa—of which 95% were naked amoebae—in a shortgrass prairie 7 days after irrigation.

The present study was designed to detect fluctuations in, and possible interactions between, the bacterial and the protozoan populations in the humus soil where bacterial fluctuation had previously been observed [4]. A series of enumerations of both groups was therefore carried out in the field after a rainfall. Since both bacteria and protozoa reach their highest numbers in the rhizosphere [10, 11], a pot experiment with and without plants was set up to clarify the importance of the roots, especially as producers of readily available energy. The experiment was run with wheat in arable soil, since it is difficult to grow pine seedlings in humus in the laboratory [1]. If the bacterial decreases observed were caused by protozoan predation, then this relationship would be found in all kinds of rhizospheres, and maybe even more markedly in connection with an annual plant, with only a short period of growth.

Materials and Methods

The field study was carried out through observations of bacteria and protozoa in the humus layer [78.6% loss on ignition, 0.73% N, pH (H_2O) 3.5–4.0] of a 120–130-year-old stand of Scots pine (*Pinus silvestris* L.). The

stand is situated on a sandy sediment soil located at Ivantjärnsheden, central Sweden (60°49'N, 16°30'E), site Ih Va of the Swedish Coniferous Forest Project, which is more fully described in Clarholm and Rosswall [4]. The soil profile is an iron podsol with a weakly developed bleached horizon.

Samples were taken 9 times over a 17-day period (Fig. 1). Three replicate soil cores (35.3 cm^2) were sampled each time and processed separately. Samples for the estimations of bacteria (2.5 g fresh weight) and protozoans (5.0 g) and for gravimetric determinations of the water content were withdrawn after mixing the whole humus layer. Bacterial numbers and size distributions were estimated by direct microscopy in fluorescent light after staining with acridine orange, and amounts of bacterial biomass were calculated using the size classes described in Clarholm and Rosswall [4].

Protozoa were enumerated by a most probable number method using two-fold dilutions in microtiter plates [12]. In the field observations, 1/10 TSB (Tryptone Soya Broth: Oxoid) in modified Neff's amoeba saline [25] was used as dilutant, and the bacterial flora inoculated with the soil suspension grew up and served as food source for the protozoa.

The use of the natural microbial flora as a food source had its disadvantages, as fungi often grew in the wells and inhibited the bacterial and protozoan growth. In the pot experiment the food source in the MPN estimations therefore consisted of isolated soil bacteria cultivated on 1/10 TSB, centrifuged and redispersed in modified Neff's amoeba saline. The different protozoan groups were recorded in the microtiter plates as soon as possible, since more information is obtained if the animals are not encysted through lack of food. Ciliates were recorded after 3 days, flagellates after 3 and 5 days, and naked amoebae after 7 and 10 days.

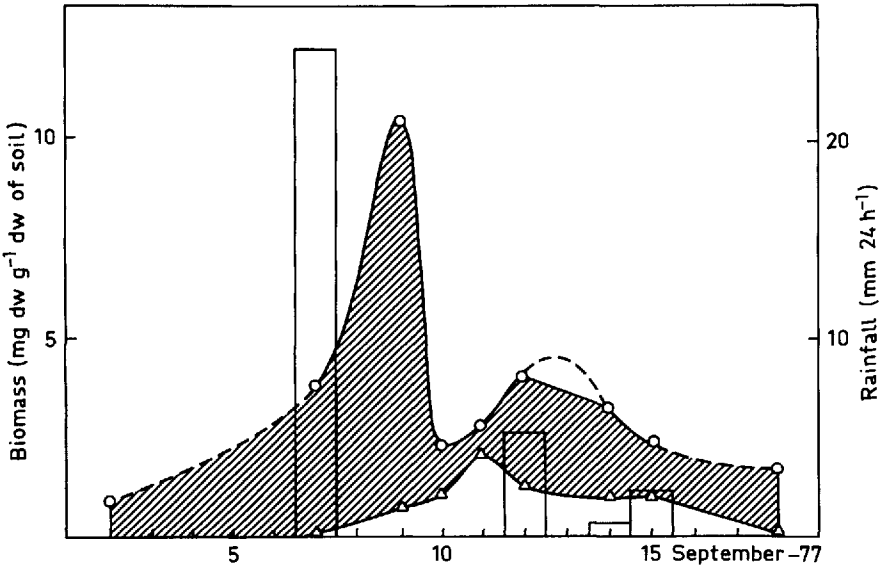
In the pot experiment, 300 g air-dried, milled topsoil of a sandy loam [6.6% loss on ignition, 0.25%N, pH (H_2O) 6.7] from the study area of the Ecology of Arable Land project was planted with 2-day-old wheat seedlings germinated on filter paper, 14 plants per pot. A second series, without plants, was also prepared and treated in the same way. Seventeen days after planting, the soils were dried as much as possible without allowing the plants to wilt. The day before the first sampling, 3 pots from each treatment were incubated overnight in gas-tight plastic bags in a dark room, and CO_2 evolution from the whole system was measured by gas chromatography. Neon served as volume determinant and internal standard. On day 0, all pots except the sampled ones were watered with fresh tap water to simulate a rainfall and kept moist ($\approx$ field capacity) throughout the experimental period by additional waterings (indicated by arrows in Fig. 2). CO_2 evolution from the pots was measured the night before they were sampled. At the times indicated in Fig. 2, bacteria, protozoa, and water contents were determined as stated above. At sampling, the root mass from the planted pots, with adhering soil, was lifted out of the pot, mixed, and subsampled; subsamples from the unplanted soil were withdrawn after mixing the entire pot contents.

Results

In the field study a large rainfall caused a 10-fold increase in bacterial biomass with a peak value of 10 mg dw g^{-1} dw soil after 2 days (Fig. 1). The decrease was also dramatic, values having reverted to pre-rain level 2 days after the peak. An increase in biomass was observed in connection with a second rain; slightly higher numbers (not shown) were noted on September 14, but no increase in biomass was recorded on that occasion.

Naked amoebae were found to be the most abundant protozoa. During the rainfall their number was 10^5 individuals g^{-1} dw soil, and 4 days later their population had become 20 times larger (Fig. 1). The decline of the amoebae was equally rapid. The rate of decrease was somewhat slowed during September 14–15, but by September 18 the peak was over. In numbers, flagellates followed a pattern similar to that of bacteria, with a large peak on September 9 and a smaller one after the second rainfall. Ciliates were much more irregular in occurrence; they appeared with a mean number of 1,340 SD 720 (N=14) and with a maximum of 2,120 SD 860 (N=3) on September 14, five days after the bacterial peak. The moisture content of the humus was over 200% of the dry weight during the whole period of observation.

In the pot experiment, both with and without plants, the highest CO_2 evolution was recorded 2 days after the first addition of water (Fig. 2). The moisture content was then



Standard deviation for

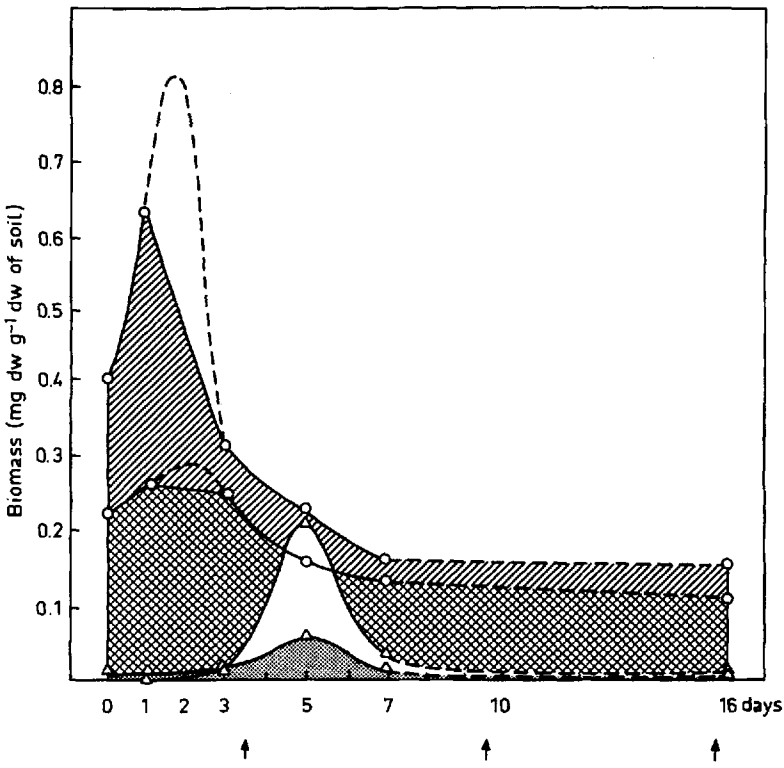
bacteria	0.29		0.69		5.0	0.55	1.2	2.6		0.54	0.53		0.21
amoebae	-		0.04		0.12	0.30	0.24	0.50		0.20	0.20		0.04

Fig. 1. Development of bacterial (*striped*) and naked amoebic (*white*) biomass in the humus layer of a podsolized pine forest soil. The dashed line indicates a probable development not registered because of too infrequent sampling. Rainfalls are given in columns. Standard deviations are given in the table below. Three replicates were sampled at each time.

raised from suboptimal to optimal conditions. For 32–48 hr after watering, the planted pots produced CO_2 at twice the rate of days 1 and 3, while the rate increase in the unplanted pots was only 30%. On day 0 (17 days after planting) the bacterial biomass in the planted soil was 30% greater than in the unplanted soil. One day after watering (day 1), the biomass in the planted pots had increased by 100%, while the unplanted pots showed a modest increase of only 20% (Fig. 2). On day 3 decreased amounts of biomass were recorded in both treatments, and by day 5 both values were below the starting values.

On day 0 numbers of naked amoebae were approximately 10^4 g^{-1} dw in both treatments and stayed at that level throughout day 3, but drastic increases were recorded on day 5 (Fig. 2). In the unplanted series, amoebic numbers increased six-fold over the initial estimates, and in the planted series the increase was 30-fold. These high numbers were transient; 2 days later the numbers had decreased to three times the initial value in the planted soil, and to twice the initial value in the unplanted pots.

The largest number of bacterial-feeding flagellates, 14,470 SD 4,770 g^{-1} dw (N=3), was recorded on day 3 in the planted pots. There were no drastic changes over the period, and the mean value was 8,910 SD 7,390 (N=18). The corresponding values for the unplanted pots were 8,460 SD 2,760 (N=3) and 5,250 SD 3,520 (N=18).



WITH PLANTS

CO_2 evolution µl $pot^{-1}h^{-1}$ $\bar{X} \pm sd$	950 ±120	800 ±90	1610 ±110	870 ±100		670 ±70		800 ±70		480 ±40		550 ±20

Standard deviation for

bacteria	0.05	0.07	nd	0.19		0.01		0.02		nd		0.02
amoebae ×10	0.15	0.30	nd	0.40		4.35		1.04		nd		0.02

WITHOUT PLANTS

CO_2 evolution µl $pot^{-1}h^{-1}$ $\bar{X} \pm sd$	350 ±60	360 ±70	490 ±70	350 ±40		320 ±80		350 ±50		150 ±20		200 ±30

Standard deviation for

bacteria	0.07	0.07	nd	0.04		0.07		0.01		nd		0.02
amoebae ×10	0.17	–	nd	0.36		3.50		0.30		nd		0.10

Fig. 2. Development of bacterial (*striped* with plants; *checked*, without plants) and naked amoebic (*white*, with plants; *dotted*, without plants) biomass in a pot experiment with an arable soil with and without wheat plants. Watering is indicated by arrows. The dashed lines indicate probable developments not registered because of too infrequent samplings. CO_2 evolution rate the night prior to sampling and standard deviation for the biomass estimates are given in the tables below. Three replicates were sampled at each time.

Ciliates were recorded at every sampling in the soils with plants; the peak value was obtained on day 3, with 260 SD 130 (N=18). In the unplanted soils, ciliates rarely reached detection level ($\approx 50\ g^{-1}$ dw).

Discussion

The numbers of protozoa recorded in the arable soil used in the pot experiment were, for all groups, in good agreement with those reported by Darbyshire and Greaves [10, 11] for arable soil, and by Elliott and Coleman [13] for fertilized and irrigated grassland soil. For the forest soil, no comparable quantitative estimates have been found. The numbers of protozoa per unit of soil weight were almost 10 times higher in the humus than in the arable soil, but if the comparison was made on an areal basis, the values were of the same order of magnitude. The large dominance of naked amoebae in terrestrial systems as compared with aquatic ones [16, 29] may be explained by some of their properties in which they differ from the other groups of protozoans. Their sliding motion on surfaces enable them to feed on the soil particles, where most of the bacteria grow [20], and their highly flexible cells are well adapted for grazing activities within the thin water films surrounding the soil particles.

The only group of protozoa that increased their numbers enough to decrease the bacteria were the amoeba. For a quantitative estimate of their impact, the increase in amoebic biomass must be calculated. The naked amoebae are a heterogeneous group; with many species; and depending on the nutrient situation, even the same species can vary in size at the time of division [6, 8]. For two typical soil amoebae, *Hartmannella* and *Achanthamoeba*, 0.8×10^{-9} g dw [2] and 1.2×10^{-9} g dw [5] per animal, respectively, have been reported; and 1.0×10^{-9} g dw, together with a 40% growth efficieny [5], was used in the present calcualtions. In the forest, 4.6 mg dw of bacteria would then be needed to produce the increase of 18.3×10^5 naked amoebae recorded, and this should be compared with an observed bacterial decrease of 8.0 mg. In the pots with plants in arable soil, 0.53 mg would have been consumed, and the recorded decrease was 0.50 mg. In the unplanted pots, the bacterial decrease was 0.45 mg, and 0.14 mg was needed to account for the observed increase in protozoans. The comparatively low bacterial peak values recorded in this experiment were most probably due to missed recordings on day 2, since a high production could be inferred from the CO_2 evolution rate (Fig. 2).

No large peaks of either bacteria or protozoa could be observed later in the pot experiment because in a continuously wet soil grazing takes place simultaneously at all levels; bacteria are eaten by protozoa, which are eaten by, e.g., nematodes as soon as they are produced. This is also why the second bacterial peak observed in the field is smaller and the protozoan peak can be seen only as a delay in the decrease. Low amounts of bacterial biomass were found in the planted pots on day 3, before the amoebic biomass increase was registered. The fact that the biomass was calculated from numbers and based on average size—without taking into account any increase of the cell before the division—may, at least partially, explain this discrepancy.

Grazing by flagellates is probably of less importance for the bacterial decrease, since many species are saprozoic, using dissolved nutrients. Umorin [31] estimated the consumption by bacterial-feeding soil flagellates to be only 0.2% of the bacterial production. Ciliates, which are filter feeders, have a high capacity for ingesting bacteria

in water [9], but in soil their larger size restricts their active feeding to periods with a very high water content, which probably diminishes their overall importance. Bacterial-feeding nematodes at the forest site have been calculated to consume 2.1 g C m^{-2} $year^{-1}$ [26], which is only 2% of the bacterial production [4]. Also, their generation times are generally too long to permit a rapid increase in the grazing pressure.

Naked amoebae thus stand out as the largest single group of bacterial consumers in the soils investigated, and a substantial part of the observed decreases in bacterial numbers following peaks induced by increases in moisture seem to have been caused by their grazing. The calculated grazing impact is a minimum figure, since the number of amoebae killed in the soil preparations preceding the MPN determinations is not known. Previous work also supports their large grazing capacity. After direct observation of the surface of a wheat root, Geltzer [17] reported: "In the immediate vicinity of roots and on their very surface a large number of amoebae appeared. They multiplied readily, exterminating nearly all the bacteria in a short time."

To understand the importance of protozoan grazing, one must see the feeding activities of these animals in relation to the rest of the ecosystem. In unfertilized soils, inorganic nitrogen levels are always low, and the nitrogen for plant uptake must be released through decomposition of dead organic matter by microorganisms. In the rhizospere, where normal energy limitations of bacteria in root-free soil [27] are temporarily lifted by the production of readily available carbon by the roots, nitrogen is the most limiting nutrient for bacterial growth. Most of the nitrogen released by decomposition in the rhizosphere is therefore first incorporated into microbial biomass. At the same time the most important factor governing protozoan growth is an ample food supply [23]. An increase in bacteria therefore leads to an increase in protozoa. Protozoans and bacteria have about the same nitrogen content, and consequently 60% of the bacterial nitrogen is excreted by the protozoans as ammonia close to the roots. Their grazing thus speeds up the release to the water phase of nitrogen and other inorganic nutrients from the bacterial cells.

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