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Evolutionary Implications of Soil Protozoan Succession*

Implicaciones Evolutivas en la Sucesión de los Protozooarios del Suelo

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ABSTRACT

Protozoa colonize soil, according to the sequence of (1) microflagellates, (2) naked amoebae, (3) ciliates, and finally (4) testate amoebae. Environmental factors, especially moisture and temperature, influence the extent of succession. Colonization studies of barren soils show that, after bacteria, protozoa are the first colonizers, followed by other soil fauna, and later by plants. Study of protozoan communities and their succession in these extreme soils may provide insights into the evolution of the soil fauna, and possible life on other planets.

Key words: colonization, protozoa, soil, extreme soils, succession.

RESUMEN

Los protozoarios colonizan el suelo de acuerdo a la secuencia: (1) microflagellelados, (2) amibas desnudas, (3) ciliados, y finalmente (4) amibas testacidas. Los factores ambientales, especialmente la humedad y la temperatura, influencian la extensión de la sucesión. Estudios de colonización de suelo áridos muestran que, después de las bacterias, los protozoarios son de los primeros colonizadores, seguido por otros animales del suelos, y más tarde por las plantas. Los estudios de las comunidades de protozoarios y su sucesión en suelos árjdos pueden ayudar a discernir la evolución de la fauna del suelo, y la posible vida en otros planetas.

Palabras clave: colonización, protozoarios, sucesión, suelo, suelos áridos.

Introduction

Protozoa and nematodes are important organisms in soils because their predation upon bacteria and fungi release soluble nutrients to above ground plants, upon which all terrestrial life depends. The bacteria and fungi decompose plant (and to a lesser extent animal) residues, but in unvegetated soils found at high altitudes, volcanoes, and polar deserts, bacteria and fungi decompose organic matter deposited by wind. In such soils, protozoa are the first colonizers after bacteria, and with accumulation of more organic matter and amelioration of the soil environment by vegetation, the protozoan community diversifies, following a succession that provides insight into structure of more complex soil protozoan communities elsewhere, with implication to the evolution of soil biota and possible life on other planets.

This essay traces soil protozoan succession by comparing studies of three unvegetated soils to a field colonization experiment, and reviews the researches of Smith (1996) on barren and sparsely vegetated soils, to suggest that protozoan successions can be used as bioindicators of environmental changes, and provide insights into evolutionary studies of the soil biota.

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Materials and Methods

The three unvegetated soils were collected by other scientists, and examined by me at Tulane University.

1. Surtsey, a volcanic island formed by eruption Nov. 1963, cooled by April 1964. Volcanic soil (tephra): collections from 4 sites in July 1967, from 8 sites in April 1968, by B. Maguire.

2. Dry Valleys, Victoria Land, Antarctica. Saline clay soils, several thousand years old: collections from 50 sites by D.W. Freckman and R.A. Virginia, 1991-1992. (Some preliminary results reported by Bamforth et al., 1993, 1996).

3. Kilauea Iki Volcano, Hawaii. Pumice (tephra) from 1959 eruption, forming a 4 km plain-like area at 1200 m elevation: collections from 7 sites by W.A. Eggler, 1971. (Some preliminary results reported by Bamforth and Eggler, 1973.)

A recolonization experiment was performed on two pasture and two forest sites in New Zealand. At each of the 4 sites, 3 undisturbed soil cores (27.0 cm diameter, 30 cm deep) were encased in polyvinyl chloride rings to prevent migration of soil animals, fumigated with methyl bromide, returned to their original sites, and sampled at days 0, 1, 5, 12, 26, 54 and 110. Adjacent sites were sampled as controls. The experiment is fully described by Yeates et al. (1991), and I have extracted the protozoan species data for analysis in this paper.

In all of these studies, material was placed in Petri dishes, distilled water added to saturate but not flood, and the run-off examined at 3-4 day intervals for protozoa. Additional species of amoebae were found by placing small samples of soil on agar plates streaked with bacteria, and placing soil into wells cut into bacterized agar plates, and examining at 4-5 day intervals for amoebae migrating out from the soil.

Results

The species richness data for the three unvegetated soils and the recolonization experiment, presented in Table 1, with data from Smith's (1996) review, showed the four ecological groups followed the succession of microflagellates, then naked amoebae, ciliates, and finally testacea (testate amoebae). In the recolonization experiment, two flagellates, *Oikomonas termo* and *Pleuromonas jaculans* (*Bodo saltans*), and a small vahlkampfid amoeba survived the fumigation. The first colonists were *Heteromita globosa* and *Bodo mutabalis*, then other microflagellates. Small naked amoebae then appeared, followed by small ciliates, three colpodids (*Colpoda inflata*, *C. steini*, and *Platyophyra vorax*) and *Cyclidium muscicola* and *Leptopharynx costatus*. Other ciliates, mainly hypotrichs appeared later, followed by testacea.

The same colonization pattern was observed in the three unvegetated soils, arranged in Table 1 in order of increasing complexity. The same microflagellates in the recolonization study were the only protozoa found in the 3-4 year old tephra soils of Surtsey. Microflagellates were present at 90% of the sites in the Dry Valleys, and amoebae, mainly *Acanthamoeba* spp. and *Hartmanella vermiformis*, were present at 70% of the sites. There were 3 species of ciliates, one at each of 3 sites. A more diverse ciliate population occurred in the Kilauea Iki tephra, accompanied by seven species of filose testacea.

Discussion

Colonization of soil by protozoa follows a directional change in community composition (ecological succession), the extent of which depends upon environmental factors, such as temperature, moisture, soil type, and organic inputs. The predictability of this succession is determined by soil porosity and reproductive rate for succession of the four ecological groups, and by vagility (dispersal ability) and r/K selection for species appearing within the groups.

Soil pore size determines the spatial distribution of protozoa (Bamforth, 1985). Microflagellates and small amoebae are the most abundant soil protozoa because they occupy all pore spaces down to 8 mm diameter, where they can prey on those bacteria unavailable to larger protozoa and nematodes, and be protected from predation, and to a certain extent, from environmental stress (e.g., the 3 surviving species from methyl bromide in the recolonization experiment). Ciliates, and the more

slowly growing testacea, inhabit the larger pore spaces (with nematodes). Within each of the four ecological groups, easily dispersed (vagile) species, and r-selected, rapidly reproducing, environmentally tolerant species usually appear before intermediate and K-selected competitive species, with narrower ecological niches.

This successional pattern also determines the appearance of species in richness culture methods. The larger, and a few of the smaller protozoa appear in the non-flooded Petri dish method (Foissner, 1987), and follow the succession observed in the recolonization experiment. Supplemental methods reveal a greater diversity of the protozoa inhabiting small pore spaces. Placing coverslips, underlaid with lens paper, on top of the material in the Petri dish, induces colonization by a diversity of microflagellates within a day, due to anaerobic conditions produced by the cover slip, driving the flagellates out of small pore spaces. More amoebae appear using the culture methods described earlier: the bacteria on the agar plate induce amoebae to migrate out from the soil, and the thin water film over the agar inhibits ciliates (and nematodes) from the larger pore spaces.

Additional examples of succession have been described by Smith (1996) on maritime Antarctic islands. (Table 1.) In 1970, 13 species of microflagellates and ciliates were found on Deception Island. A volcanic eruption sterilized part of the island, but 10 months later, the first recolonizing species was found, and colonization proceeded at an estimated rate of 3.1 species per year to produce, 10 years later, 36 species, from all 4 ecological groups.

The ameliorating influence of vegetation, providing more organic matter and more favorable habitats, is evident on Signy Island, where moss-peat habitats supported twice the numbers (50) of the species of soil protozoa as the relatively barren fellfields. Elephant Island, with grass as well as moss habitats, also supported a large number (54) of species.

Table 1. Species richness of terrestrial protozoan communities.

Location	Habitat Sites	Protozoa*				Source
		F	A	C	T	
Surtsey						
1967	tephra (4)	4	0	0	0	This study
1968	tephra (8)	6	0	0	0	This study
Dry Valleys	morraine (50)	5	7	2	0	This study
Kilauea Iki, Hawaii	tephra (7)	6	5	14	7	This study
Deception I.						
1970	tephra (6)	6	0	7	0	Smith, 1996
1980	tephra + moss (6)	11	2	19	4	Smith, 1996
Signy Island						
Fellfields	tephra = moss (12)	11	0	9	4	Smith, 1996
Moss-peat	moss (6)	16	0	17	17	Smith, 1996
Elephant I.	moss + grass (23)	17	4	18	15	Smith, 1996
New Zealand	forests (2)	8	12	42	17	Yeates, *et al.* 1991
New Zealand:	forests (4) pasture					Yeates *et al.*, 1991
Recolonization exp.						
Day 0		2	1	0	0	
Day 1		6	3	4	0	
Day 5		7	3	5	0	
Day 12		7	6	7	0	
Day 26		7	6	16	0	
Day 54		9	10	43	1	
Day 110		12	15	48	4	

* F = Flagellates A = Amoebae C = Ciliates T = Testacea

The limiting effect of severe climate on protozoan succession is evident in the pauperization of the larger protozoa - ciliates and testacea - with increasing latitude in Antarctica (from 60 S to 78 S). Foissner (1996), reporting ciliates from 59 Antarctic region sites, found a mean of 9.6 species in the least severe region, Signy Island; 1.0 for the Antarctic Peninsula; and 0.4 in the Dry Valleys. Many sites, even under moss, did not contain any ciliates, and most of the few species were early successional, r-selected ubiquists.

Smith and Wilkinson (1987) found a loss of 3.3 species for every 1° C drop in mean January temperature for testacea, from 26 species in the South Orkney Islands (Signy and Elephant) to 5 species in South Victoria Land, Antarctica. In the latter region, a few ubiquitous r-selected species, especially *Corythion dubium*, predominated.

The influence of both climate severity and organic matter is dramatized by comparing the soil protozoa of the young, 12 year old Kilauea Iki tephra and the several thousand year old Antarctic Dry Valley soils (Table 1). The barren Hawaiian tephra are close to vegetated colonization centers, in a tropical climate, whereas the Dry Valley soils are isolated, with short summers, and the protozoan succession in them is arrested.

The composition of the soil protozoan population at Kilauea Iki in 1971 (Table 1) was similar to that of one warm and two cool U.S. deserts (Bamforth, 1984; Bamforth and Bennett, 1985) but in Hawaii, succession has continued to produce a community resembling the soil populations of adjacent forests, whereas in the deserts, as in the Antarctic Dry Valleys, protozoan succession is arrested by aridity, evidenced by the high proportion of r-selected ciliates. More stable conditions, e.g., in New Zealand forests (Table 1), reduce environmental stresses in the larger pore spaces of soils, to favor persisting K-strategist polyhymenophoran species of ciliates, and K-strategist taxa of testacea, such as *Bullinularia*, *Schwabia terricola*, and *Heleopera* spp.

Most soil protozoan communities lie between those of Surtsey in 1967 and of temperate forests (e.g., New Zealand). Individual species in the communities, and the stage of succession of communities themselves can serve as bioindicators of soil conditions (Foissner, 1994).

Colonization of unvegetated soils by protozoa suggests that a soil biota could have arisen before plant evolution on land. For example, in the Antarctic Dry Valleys, protozoa of small pore spaces (microflagellates and amoebae) are found in low numbers. The few ciliates share the larger pore spaces with three species of nematodes (Freckman and Virginia, 1997), hence the most extreme terrestrial environment on earth supports a low-diversity soil fauna. The dominant nematode, *Scottnema lindsayae*, is a psychrophilic endemic (Overhoff *et al.*, 1993), implying that some of the oldest soils in the world contain an ancient faunal community. In the climatically less severe maritime Antarctic regions, soils have developed further, supporting algal mats and mosses; and on Elephant Island, grass, the beginning of a vascular plant land flora. These soils also support a more diverse soil fauna (e.g. rotifers and tardigrades). Hence the soils of the Antarctic region suggest how a soil fauna could develop.

Precambrian terrestrial microbial life dates back to 800-1200 million years (Horodyski and Knauth, 1994). The terrestrial protozoa living in the top several cm of exposed soils of the Antarctic Dry Valleys and Signy Island fellfields lend support to Swan's (1992) hypothesis that a soil fauna preceded the land flora: organic matter blown from the sea onto the land, colonized by bacteria and later by fauna, providing a favorable environment for land plants to evolve. Additional studies of protozoa in extreme environments, such as Smith's (1996) description of physiological attributes of four Antarctic microflagellates, can provide insights into the biology of the first soil fauna, and perhaps to the origin of the first organisms. These implications can extend to the nature of life on other planets.

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