Estimated abundance and diversity of heterotrophic protists in South African biocrusts

Biological soil crusts (biocrusts) occur widely in the uppermost millimeters of the soil in arid and semi-arid systems. Worldwide they cover large terrestrial areas and play a major role in the global terrestrial carbon and nitrogen cycles. However, knowledge of the microbial decomposer foodwebs within biocrusts is particularly scarce. Heterotrophic protists in soil are predominantly bacterivores, and because of their high biomass compared with other soil fauna and fast turnover rates, protists are considered an important factor for soil nutrient cycling and energy fluxes. Thus, knowledge of their biodiversity, abundance and functional roles is important to understand soil ecosystem functions. We investigated the diversity and abundance of heterotrophic soil protists in different types of biocrusts from the Succulent Karoo, South Africa. With an overall diversity of 23 distinct morphotypes, soil protist biodiversity was shown to be high. The most abundant groups were Spumella-like chrysomonads, gliding bodonids, glissomonads and heteroloboseans. Protist abundance was highly variable among samples. The abundance and diversity did not differ significantly among different types of biocrusts, indicating that microscale differences, but not macroscopic soil crust builders (e.g. cyanobacteria, lichens and bryophytes), have a major impact on the protist community.

Introduction

Biological soil crusts (biocrusts) are microscopic ecosystems. They comprise primary producers such as cyanobacteria, algae, lichens, and bryophytes together with decomposers such as fungi, bacteria and archaea. The microscopic consumers in biocrust foodwebs are adapted to arid soil conditions and taxonomically diverse, including protists, nematodes, rotifers, tardigrades, and microarthropods in variable proportions. Biocrusts grow within the uppermost millimeters of the soil in arid and semi-arid regions throughout the world, where they fulfill several highly relevant ecosystem services. They limit soil erosion by both wind and water, influence water runoff, infiltration and retention within the uppermost soil layer, and fertilise impoverished desert soils. On a global scale, biocrusts cover over 10% of the terrestrial surface area, also influencing global nutrient cycling and climate processes. Biocrusts form one subgroup within cryptogamic covers, with the latter also comprising cryptogamic communities on rock and epiphytically on plants. These cryptogamic covers have been estimated to account for 7% of the net primary production fulfilled by plants, and fix about 49 Tg N per year, corresponding to about half the maximum value estimated for the total terrestrial biological nitrogen fixation.

Detailed information is available on the diversity and species composition of the primary producers in biocrusts. According to the dominant photoautotrophic organism group (i.e. the dominant soil crust builder), they have been coarsely defined as cyanobacteria-dominated, lichen-dominated and bryophyte-dominated soil crusts. However, data on the microbial decomposer foodwebs, especially heterotrophic protists, are scarce. Nematodes, tardigrades, rotifers, mites, collembolans, heterotrophic protists, and even larger arthropods and molluscs have been observed to utilise biocrusts as a habitat. However, their diversity, frequency, geographical distribution and feeding behaviour have been investigated in only a few local studies.

Protists – with their high abundance, turnover and diversity – have been increasingly studied by soil ecologists in recent years. Based on their general morphology and means of locomotion, soil-inhabiting protists comprise amoebae, flagellates and ciliates. Most heterotrophic protists in soil are bacterivores. As a result of their high numbers and turnover rates, protists are considered to play a major ecological role in soil foodwebs by the release of nutrients from consumed microbial biomass.

Heterotrophic protists have been determined in arid soils in a number of habitats among different continents, namely southwestern USA, the Negev Desert of Israel, and Australia, China and Antarctica. However, data on biocrusts from Africa are lacking. Based on 73 soil samples from the Etoha region and the Namib Desert of Namibia, Foisser et al. identified 365 ciliate species, of which 35% had been undescribed, including a new order and suborder, three new families, and 34 new genera and subgenera of soil ciliates. These findings suggest that biocrusts in South Africa can harbour a substantial undiscovered diversity of protists.

We investigated the heterotrophic protist community of biocrusts in the Succulent Karoo, South Africa. Besides determination of the diversity and abundance of amoeboid and flagellated protists, we evaluated whether they are affected by the identity of the major primary producers in primary producers in biocrusts that are dominated by cyanobacteria, chlorolichens and bryophytes.

Material and methods

The study site was situated in the Succulent Karoo biome, South Africa, in the vicinity of the village Soebatsfontein, about 60 km south of Springbok. The Succulent Karoo biome is a unique dryland system hosting a biodiversity hotspot with an extraordinarily high plant diversity and a unique flora of succulent plants. Samples were collected next to the BIOTA observatory in Soebatsfontein (observatory number 522 at 30.19°S, 17.54°E, altitude 392 m). The hilly region comprises soils of sandy texture, some granite inselbergs and a dense pattern of fossil termite mounds. The semi-arid climate of the region is characterised by mild winter and hot summer conditions, with
air temperatures below 2 °C in July and sometimes above 44 °C in February. The study site is located in the winter rainfall area, with a mean of 129 mm rainfall which falls mainly during the cool winter months (July to August) and a second smaller peak in autumn (April to May). The study area is densely covered by diverse communities of biocrusts, reaching an overall surface coverage above 25% in regions without inselbergs and roads.

**Biocrust communities and sampling**

Biocrust communities within southern Africa have been divided into seven main biocrust types, of which we investigated three. First, we analysed well-established cyanobacteria-dominated biocrusts, which are characterised by a more-or-less uniform dark surface colouration caused by cyanobacteria growing close to the surface. When being removed, the biocrust forms relatively large flakes of up to 3.9 mm thick. Dominating cyanobacterial genera are Nostoc, Phormidium, Syctonema, Microcoleus and Leptolyngbya, with the latter two already occurring in early, initially formed biocrusts. As a second type we investigated chlorolichen-dominated biocrusts, which can only form on stabilised surfaces, normally developing from well-established cyanobacteria-dominated biocrusts. The dominating chlorlichen species was Psora decipiens, which has a particularly wide geographical distribution and occurs frequently within the study area. Bryophyte-dominated biocrusts were the third type we analysed. This biocrust type is a late-successional stage that develops from previously well-established biocrusts, and because bryophytes require somewhat more water than the other biocrust components, they frequently occur in the shade or vicinity of small shrubs. The dominating moss species in these samples was Ceratodon purpureus, a small species with an almost global distribution.

The three different types of biocrusts were collected in 10-cm Petri dishes, with five replicates each. For each sample, the lower lid of the Petri dish was pressed approximately 1 cm deep into the soil, then a trowel was pushed below the lid and together with the sample was lifted from the surrounding soil. The Petri dish was carefully turned around, surplus soil was removed, and the dish was covered with the upper lid, which was subsequently sealed with parafilm and taping band. As the biocrusts were completely dry during sampling, no additional drying of the samples was necessary. The samples were transported to Germany and stored at 4 °C in the dark for 6 weeks.

**Microbial determination of taxonomic units**

Protist abundance and community composition were assessed by a liquid aliquot method according to Butler and Rogers. Briefly, 1 g of a homogenised surface soil (uppermost 2 mm) was suspended in 350 ml of sterile distilled water and shaken for 20 min. For incubation, the suspension was diluted by a factor of 4, and 20 µL of the suspension was added to 180 µL of wheat grass medium (WG). The WG was made by adding 0.15% dried wheat grass powder (Weizengras, Sanatur GmbH, D-78224 Singen) to PJ medium, with five replicates each. For each sample, the lower lid of the Petri dish was pressed approximately 1 cm deep into the soil, then a trowel was pushed below the lid and together with the sample was lifted from the surrounding soil. The Petri dish was carefully turned around, surplus soil was removed, and the dish was covered with the upper lid, which was subsequently sealed with parafilm and taping band. As the biocrusts were completely dry during sampling, no additional drying of the samples was necessary. The samples were transported to Germany and stored at 4 °C in the dark for 6 weeks. The three different types of biocrusts were collected in 10-cm Petri dishes, with five replicates each. For each sample, the lower lid of the Petri dish was pressed approximately 1 cm deep into the soil, then a trowel was pushed below the lid and together with the sample was lifted from the surrounding soil. The Petri dish was carefully turned around, surplus soil was removed, and the dish was covered with the upper lid, which was subsequently sealed with parafilm and taping band. As the biocrusts were completely dry during sampling, no additional drying of the samples was necessary. The samples were transported to Germany and stored at 4 °C in the dark for 6 weeks.

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In the Succulent Karoo, short periods of moisture (soils are wet for roughly 45 days per year) and large temperature extremes between day and night mean that the protist community is likely to be influenced more by an accumulation of morning dew during the night than by periodic rainfall events. Small protists with a rapid life cycle will have an adaptive advantage under these conditions.

All the protists we identified were cyst-forming bacterivores. Cysts enable protists to tolerate frequent wetting–drying cycles and must be considered a major functional adaptation in desert protists. Non-encysting protists that feed on cyanobacterial filaments identified in cyanobacterial crusts of Australia are likely an exception. Cysts of some protist taxa are viable over decades and will gradually accumulate under conditions where microbial production and environmental conditions are favourable. Cultivation-based methods therefore estimate the abundance of cysts together with active stages of protists, which can be regarded as an integrated measure of past microbial production in these soils.

**Protist diversity**

The diversity of heterotrophic protists in biocrusts comprised ‘typical’ soil protists such as acanthamoebae, vermamoebae and cercozoans (especially glissomonads and cercomonads), as well as less-frequently found and likely rarer taxa such as apusomonads. A high abundance of glissomonads and heteroloboseans is commonly observed in soils, but the general lack of thaumatomonads and vannellids was quite surprising. It has recently been shown that protist diversity responds to changes in soil dryness, especially with regard to larger protists, which quickly disappear with decreasing soil moisture content. Prolonged dryness therefore might have far-reaching negative effects on some protist groups, but more detailed studies are necessary to confirm this possibility.

Knowledge on the functional roles of protists is particularly scarce. Generally, chrysomonads, bicosoecids and some bodonids are considered as interception feeders in biofilms. They create a water flow with their flagella to capture bacteria in water films. Organisms that depend on a water current for feeding may be considered more strongly moisture-dependent than (for example) amoebae that graze within biofilms. Therefore it was not surprising that bicosoecids and the mostly swimming or sessile bodonids, such as *Bodo saltans*, were missing from our samples. However, *Spumella*-like chrysomonads, which usually show high abundances in soil systems, could be confirmed for soil crusts.

Most amoebae and amoeboflagellates (Cercomonas-like, i.e. Cercomonas-like and Paracercomonas-like amoeboflagellates) can attach to particles and feed on bacteria in biofilms and in tiny soil pores, resulting in relatively high abundances in drier soil. In addition, Darby et al. found amoebae (rather than nematodes and other protists such as ciliates and flagellates) to be highly tolerant to extreme environmental conditions, including increased temperatures and altered precipitation. In line with these results, our findings also showed that amoebae and amoeboflagellates were the most abundant functional groups in our study.

The comparison of protist diversity among the three different biocrust types revealed no statistically significant differences. This finding indicates that the different macroscopic soil crust builders might have no major effect on bacterivorous protist community composition. However, data on protists in soil are too scarce for us to propose a general conclusion. To our knowledge, ours is the first study on protist communities in biocrusts on the African continent, and is one of few studies that give detailed quantitative estimates on the cultivable amoeboid and flagellated protists. Future studies need to include molecular approaches in order to estimate the non-cultivable taxa to the protist community.

**Conclusion**

This is the first detailed study on protist abundance and their morphotypes in biocrusts on the African continent. The diversity of protists was high, comprising solely cyst-forming protists. The abundance of heterotrophic
Protists in biocrusts was found to be more than 10 times higher than effects of altered temperature and precipitation on desert protococci associated with biological soil crs. J Eukaryot Microbiol. 2006;53(6):507–514. http://dx.doi.org/10.1111/j.1550-7480.2006.00314.x


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42. Weber, personal communication.


