Tagungsbeitrag zu: Lebensraum Boden
Tagung Kommission III „Bodenbiologie und Bodenökologie“
der Deutschen Bodenkundlichen Gesellschaft, 20.09.-
21.09.2012 in Stuttgart-Hohenheim
Berichte der DBG (nicht begutachtete online Publikation)

http://dbges.de

Identification and functional roles of amoeboid protozoa in soil

Stefan Geisen[1], Michael Bonkowski[1]

Protozoa; Soil amoeba; Phylogeny; Ecological functioning

Abstract

Protozoa are the major consumers of bacterial production in soil, forming the base of the heterotrophic eukaryotic food web that channels the energy flow via bacteria to higher trophic levels in soil (i.e. the bacterial energy channel). Among them, amoebae and amoeboid flagellates are of major importance due to their small size, high abundance, fast turnover and ability to forage for prey in even the smallest pores with their flexible pseudopodia, making them key regulators of bacterial biomass and nutrient cycling.

Despite their functional importance we have only a vague idea on the identity of the dominant taxa of amoeboid organisms in soils. Major reasons for the general ignorance in environmental studies of these key eukaryotes are methodological difficulties in cultivation and quantification in the opaque soil environment as well as a severe lack of taxonomic expertise. However, recent developments in molecular techniques now allow closing the methodological gap on this functionally important trophic link in the soil food web.

We have designed specific primers and DNA-based barcodes for dominant taxa of amoeboid organisms, with the aim to determine their diversity across soils

[1]Zoological Institute, Department of Terrestrial Ecology, University of Cologne, Zülpicher Str.47b, 50674 Köln; *geisens@uni-koeln.de*

throughout Europe and China using high-throughput sequencing.

Cultivation of amoeboid organisms from soils on long term observatories in the Netherlands, on Sardinia and in high altitudes in China already indicated an enormous undescribed diversity. In addition to already described species, we extracted a plethora of new species and even genera from each of these soils. Morphological and molecular information retrieved from the cultures indicates deep phylogenetic relationships among many amoeboid organisms, independent of origin, and the existence of high numbers of undescribed taxa. Therefore, combining the molecular information with descriptive morphology is crucial for developing effective genetic barcodes to target broader protozoan taxa by high-throughput environmental surveys. Phylogenetic information retrieved from mass sequencing would be meaningless without knowing the respective morphotype.

In addition, information on the functional traits of protozoa is urgently needed to fully assess their significance in terrestrial ecosystems. Therefore, we set up initial experiments investigating differential grazing of amoebae. Until now, studies with few selected amoeba species have confirmed strong impacts on total bacterial biomass turnover and community composition, but it is unclear if these results can be generalized. Therefore, we designed experiments in multiwell plates to compare the grazing efficiency of different amoeboid genera with distinct morphotypes, and of *Acanthamoeba* ecotypes with similar morphology but different origin and phylogenetic relatedness. We use a diverse natural isolate of soil bacteria but added GFP-labelled *Streptomyces* sp. and RFP-labelled *Pseudomonas fluorescence* DSM50090 as biomarkers for different microbial growth strategies. *Streptomyces* is producing anti-predatory secondary metabolites whereas *Pseudomonas* is lacking them.

The different morphotypes and ecotypes of amoeba were added individually and in combination to determine the single and combined effects of each amoeba species on the bacterial community. Optical density was used as a measure for total bacterial biomass and monitored together with GFP- and RFP-signals every day for two weeks. Amoebae were enumerated microscopically every day. After two weeks, total bacterial abundance was determined via flow-cytometry, and bacterial DNA was extracted from each well to analyse the changes in the bacterial community structure via denaturing gradient gel electrophoresis.

Total abundance of bacteria generally decreased in presence of amoeba and numbers of both amoeba and bacteria showed and inverse correlation. GFP values were higher in presence of amoebae indicating a release of bacterial competition on *Streptomyces*. *Pseudomonads* were not affected by amoeba. The grazing impact of amoebae generally depended on morphotype and also on ecotype of the respective amoeba. In combined treatments, the presence of additional amoeba morphotypes and ecotypes further decreased bacterial abundance, suggesting complementarity in amoeboid grazing. Even closely related taxa showed different impacts on the bacterial communities. In conclusion, our results provide further evidence of a major influence of soil amoeba on nutrient cycling in soils.