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## **Identification, functional roles and ecosystem services of protozoa in soil**

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### **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). Despite small sizes of protozoa in soil (5-200  $\mu\text{m}$ ), their high abundance and fast turnover make them one of the key regulators of bacterial biomass and nutrient cycling.

Even though they occupy important functional roles, we still have only a vague idea on the identity of the dominant protist taxa in soil. One major reason for the general ignorance of protists in environmental studies is methodological difficulties in quantifying small protists in the opaque soil environment, their uneven distribution and the lack of taxonomic expertise. However, recent developments in high-throughput sequencing and in the cultivation of so-called uncultivable protists now allow closing the methodological gap on this functionally important trophic link in the soil food web.

Within the EU-project EcoFINDERS we aim at designing DNA-based barcodes for dominant protozoan taxa in soil. Protozoan diversity will be compared between five long-term observatories across Europe using high-throughput sequencing.

Cultivation of amoebae from Dutch and Sardinian grassland soils to improve phylogenetic information of this rarely studied assembly of organisms indicates an enormous diversity. Based on culture isolates, we constructed phylogenetic trees based on two genes (18s rDNA and cytochrome oxidase 1) to decipher deep-relationships among protozoa and to identify genetic barcodes targeting individual taxa for pyrosequencing, which we are currently conducting.

Ecological studies investigating protozoan grazing of bacteria has been shown to be a major structuring force for bacterial diversity in the plant rhizosphere. Therefore we suggest that protozoa may provide an important ecosystem service by removing pathogenic microorganisms from soils. Laboratory experiments with selected dominant protozoan taxa will be performed to investigate the effects of protozoan predation on different pathogenic and beneficial soil microorganisms.

Preliminary results indicate that bacterial spores are resistant to predation i.e. germinate and grow inside food vacuoles, while vegetative bacteria are killed by protozoan predation. This will be tested along with the dependency of morphology and feeding behaviour of protozoa on bacterial predation and related to soil ecosystem services.