

Diversity and Functions of Protozoa in Soils

EcoFINDERS



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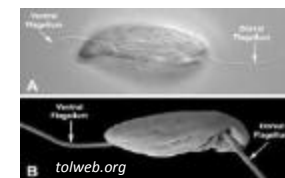
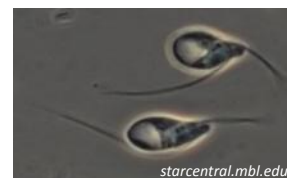
05 October 2011

Diversity of Protozoa in Soils



- Single-celled, heterotrophic eukaryots
- Up to 100,000 individuals/gram soil
- Reproduction asexually
- Produce cysts to resist adverse conditions
- Classically split into three morphological distinct groups

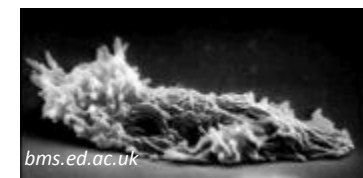
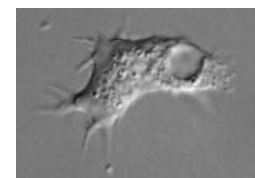
- Flagellates (one or more flagella)



- Ciliates (many short, hair-like cilia)



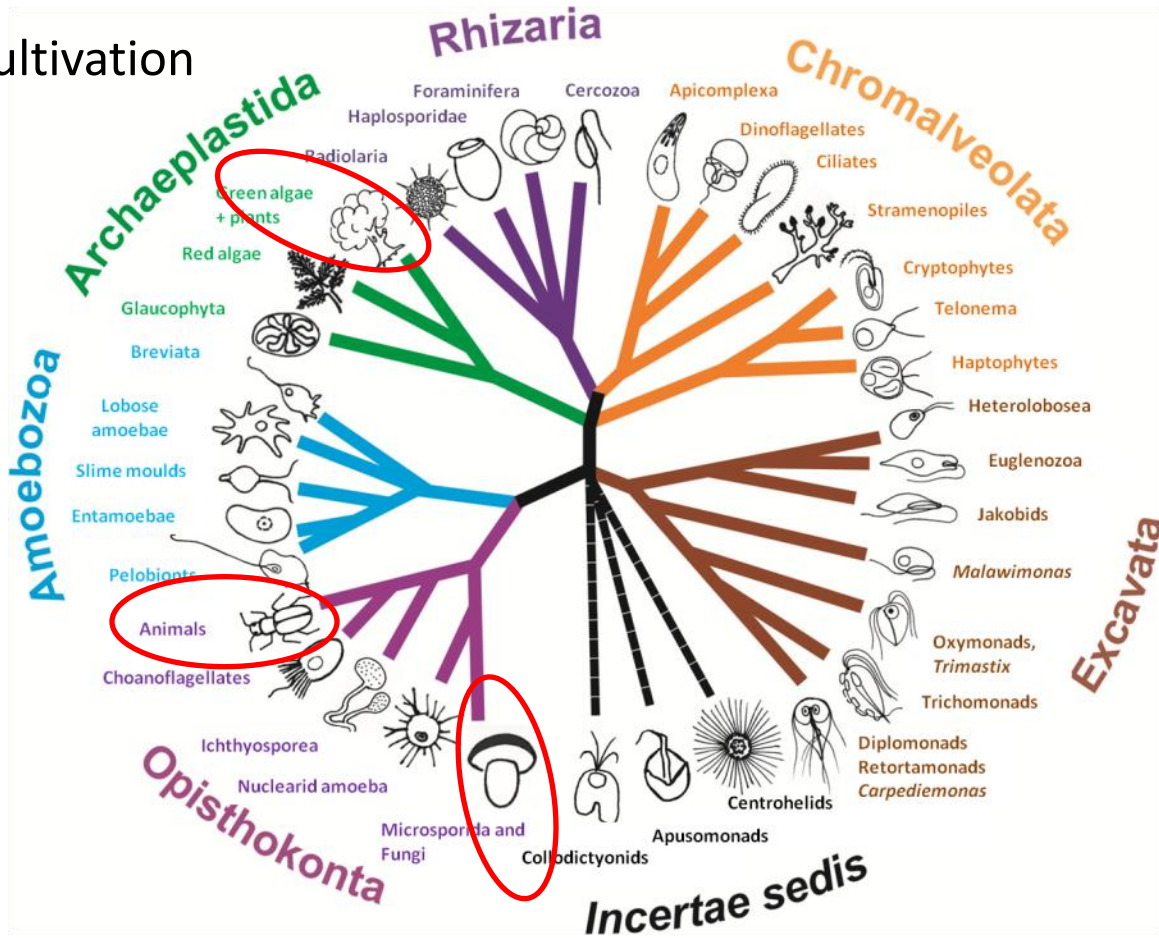
- Amoeba (one or more pseudopodia)



Diversity of Protozoa in Soils



- Little known due to
 - Small size
 - Difficult isolation and cultivation
 - High diversity
- Case study: amoebae

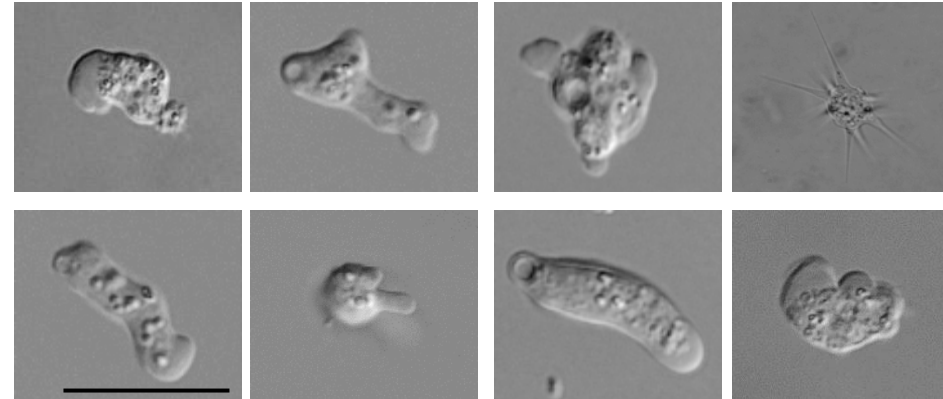


Modified from <http://www.natur.cuni.cz/biologie/veda-a-vyukum/vyzkumne-tymy/evolucni%20-%20protistologie/fylogenetika.jpg>

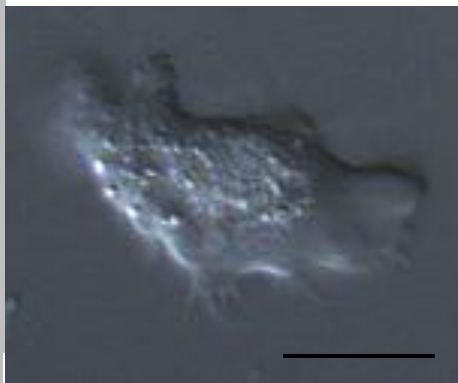
Diversity of Protozoa in Soils



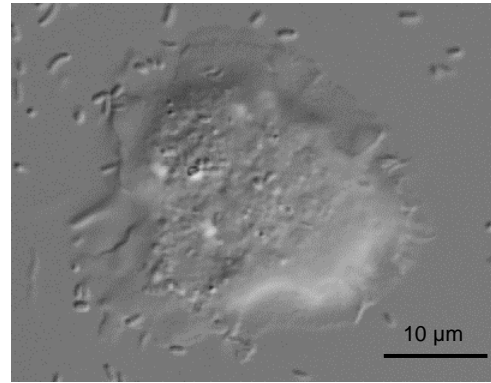
- Little known due to
 - Small size
 - Difficult isolation and cultivation
 - High diversity
- Case study: amoebae
 - Absence of clear cell shape
 - ↑Variability → Hard to identify



Hartmanella sp.



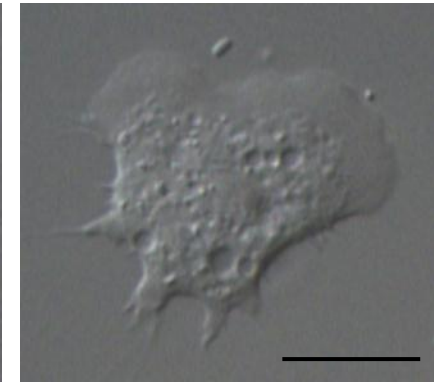
Acanthamoeba sp.



Cochliopodium sp.



Vanella sp.

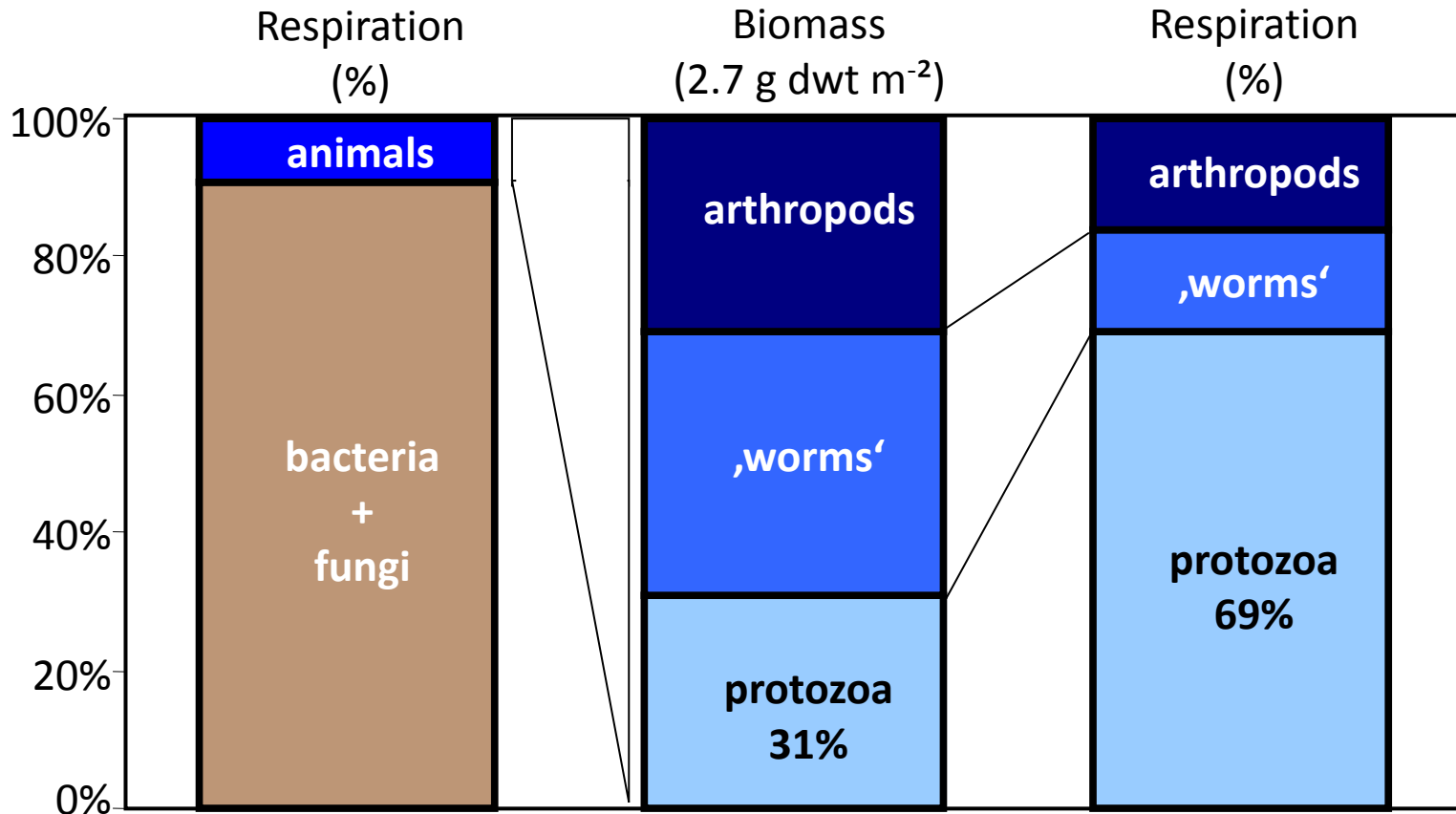


Flamella sp.

Importance of Soil Protozoa



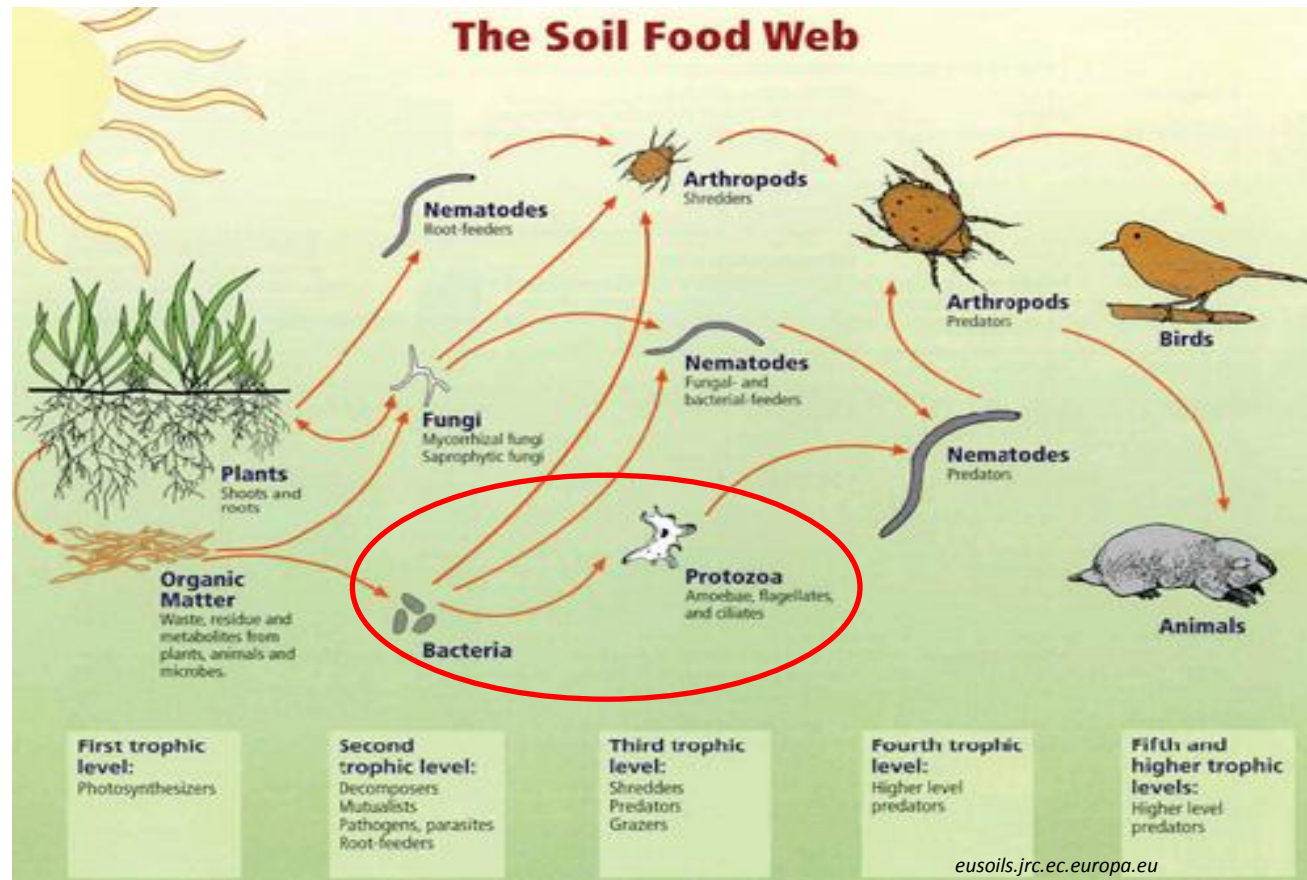
- Respiration and biomass of soil organisms
 - A comparison of 14 ecosystem studies (Foissner 1996)



Importance of Soil Protozoa



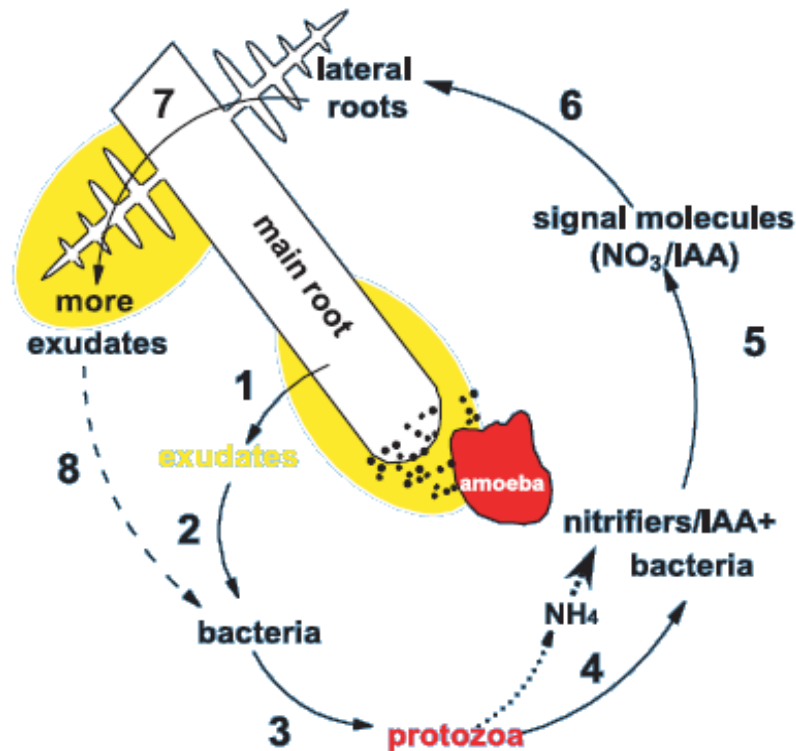
- Grazers of bacteria
 - Control bacterial energy channel



Importance of Soil Protozoa



- Grazers of bacteria
 - Control **bacterial energy channel**
 - Feed selectively on bacteria → Positive feed-back on plants



Modified from Bonkowski (2004)

Goals within EcoFINDERS



- Lab experiments confirm functional importance, BUT no knowledge on dominant taxa and diversity in soil
 - Cultivate and identify protozoan species from sampling sites
 - Find genetic barcodes for soil protozoa
 - Use those to compare different European sites via high-throughput sequencing



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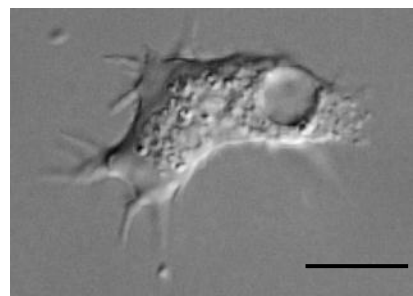
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Identification of Soil Amoeba

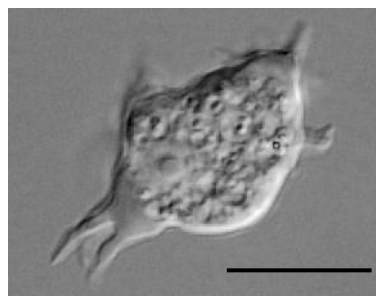


Intro
Identification
Sequencing
Interaction studies
Outlook

- Combination of morphology and phylogeny
- First clonal cultures from Sardina (Italy)



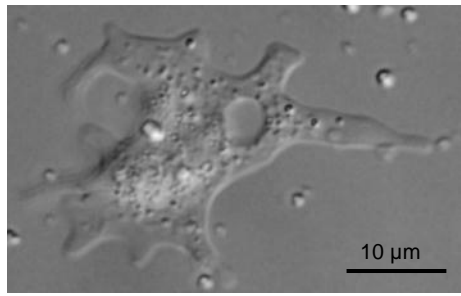
Acanthamoeba sp.



??? sp.



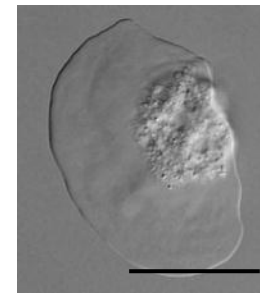
Hartmanella sp.



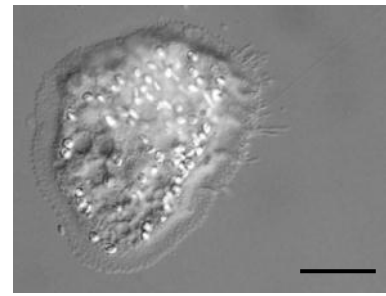
Korotnevella sp.



Cochliopodium sp.



Vannella sp.

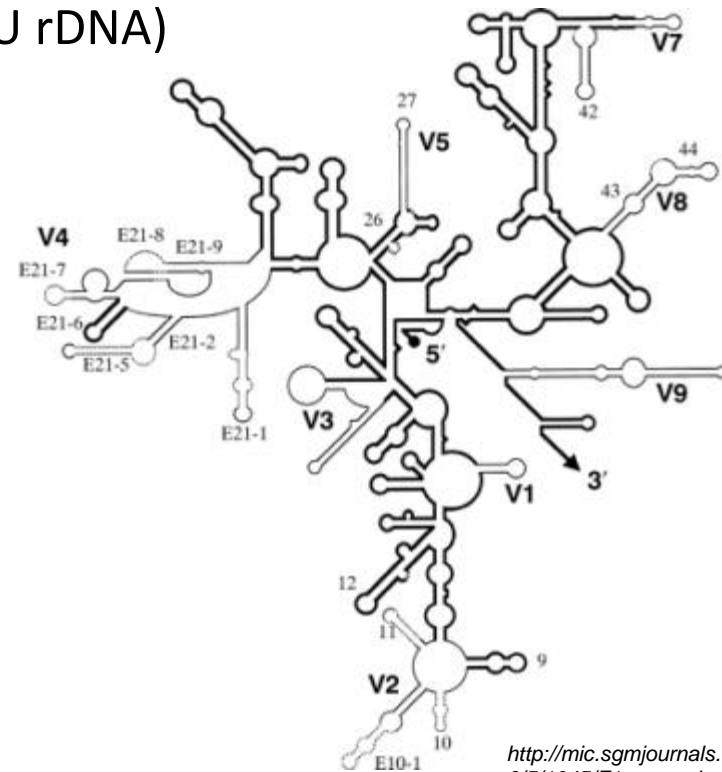


Cochliopodium sp.

Barcoding and Sequencing



- Barcoding
 - Specific sequence targeting a single taxon
- Examples
 - Cytochrome C Oxidase I (CO1)
 - Small Subunit Ribosomal DNA (SSU rDNA)

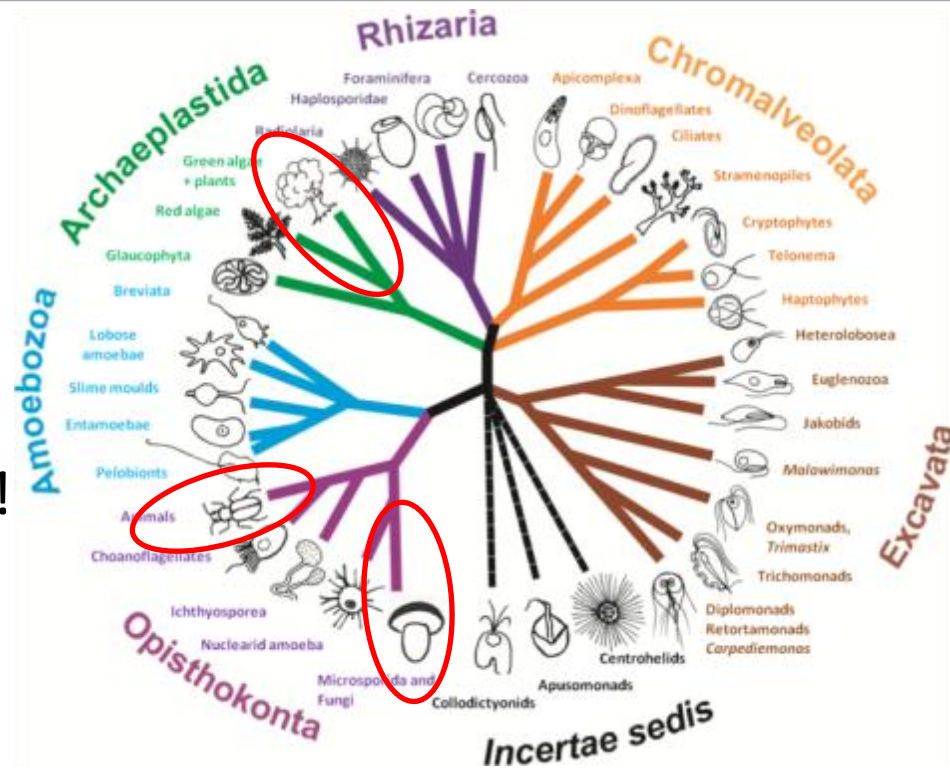


<http://mic.sgmjournals.org/content/14/6/5/1045/F1.expansion.html>

Barcoding and Sequencing



- Barcoding
 - Specific sequence targeting a single taxon
- Examples
 - Cytochrome C Oxidase I (CO1)
 - Small Subunit Ribosomal DNA (SSU rDNA)
- BUT: No barcodes for protozoa!
 - Very diverse
 - Eukaryotic primers do not work

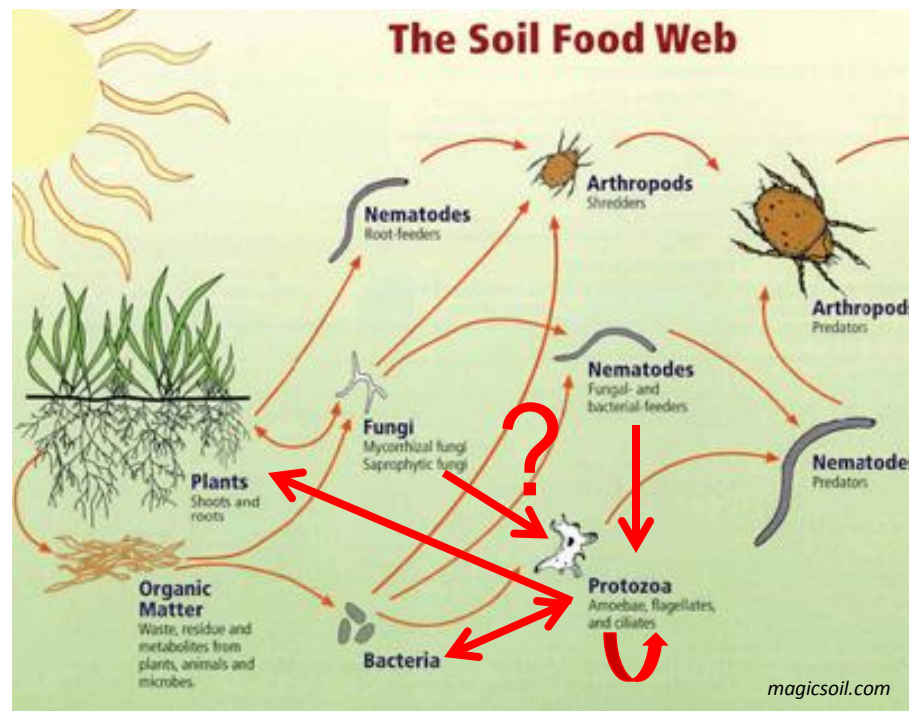


Modified from <http://www.natur.cuni.cz/biologie/veda-a-vyukum/vyzkumnetymy/evolucni%20-%20protistologie/fylogenetika.jpg>

- **Group specific primers needed**
 - Used for comparison of European soils via high-throughput sequencing

Functional Importance of Protozoa

- Evaluate interactions with other organisms
 - ecological and economical value of protozoa
- Case study
 - Interaction study between protozoa and different bacteria



Interaction Study - Setup



- Organisms
 - Bacteria
 - *Bacillus subtilis*
 - *Corynebacterium glutamicum* (RFP labeled)
 - *Pseudomonas fluorescence* (GFP labeled)
 - Protozoa
 - *Acanthamoeba castellanii*
 - *Tetrahymena pyriformis*
 - Added in a full factorial design in a 96 well plate
- Automatic plate reader constantly measuring for 3 days
 - Optical density (OD)
 - Relative fluorescence units (RFU) from GFP and RFP signals



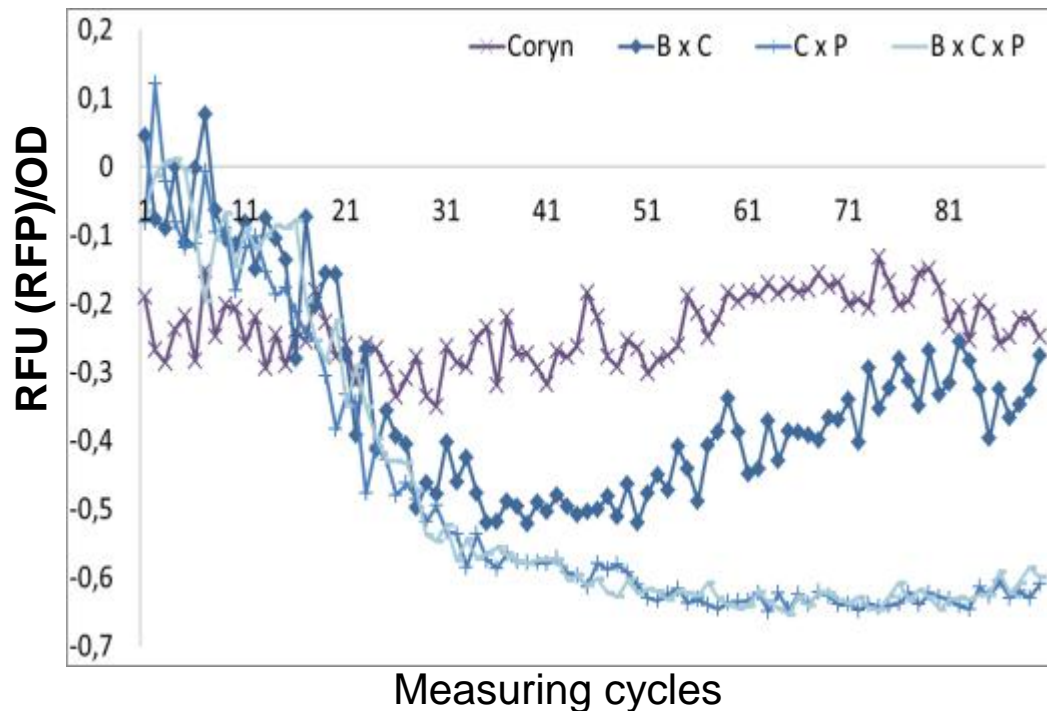
Interaction Study - Hypotheses

- Presence of *Acanthamoeba* and *Tetrahymena* → higher feeding pressure → increased reduction of bacteria
- Grazing protection by
 - *Bacillus* through filaments and spores
 - *Pseudomonas* through production of toxic compounds

Interaction Study - Results



- Bacteria
 - Growth rate: Pseudomonas > Corynebacterium > Bacillus
 - Strong intra-bacterial competition
 - Bacillus and Corynebacterium inhibited
 - Pseudomonas profits

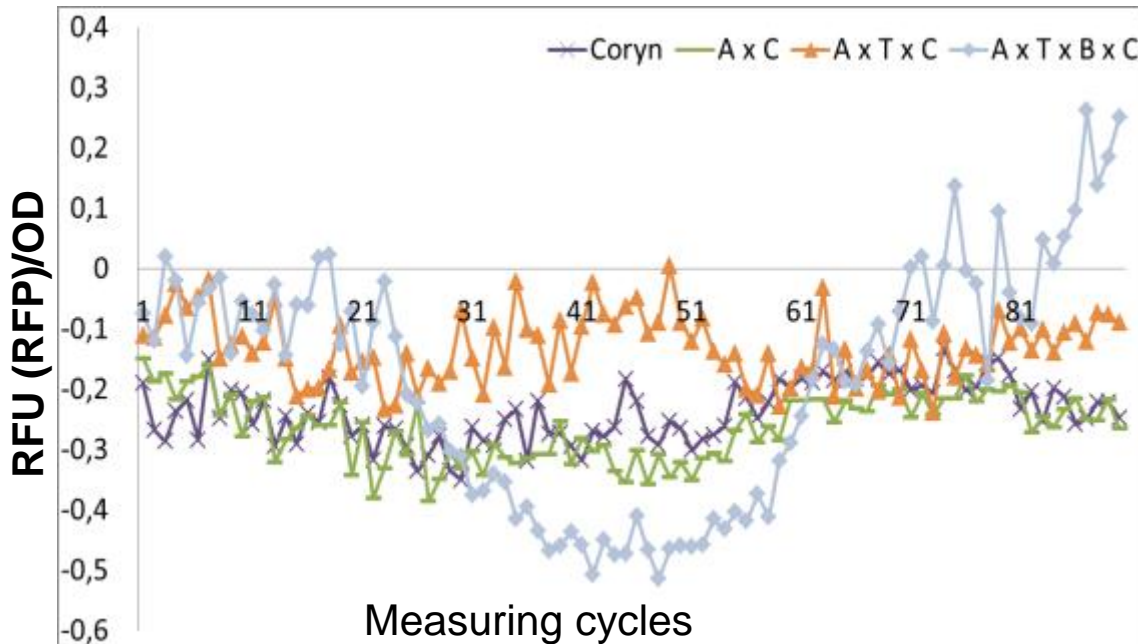
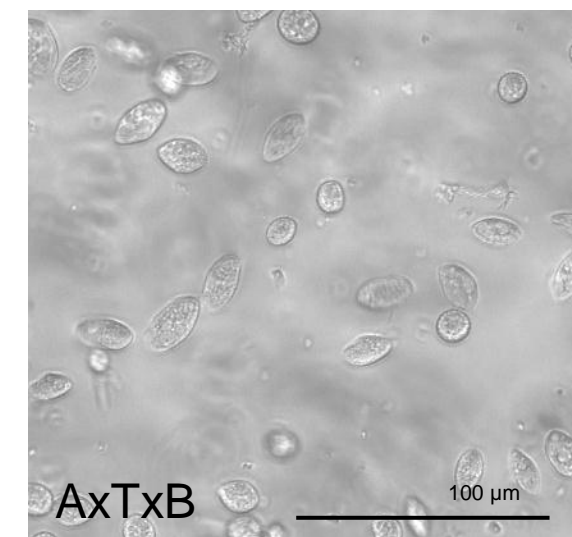
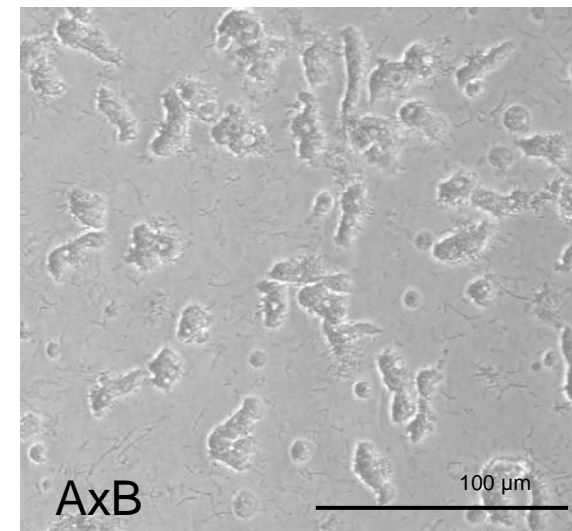


Interaction Study - Results



- Protozoa

- Food preference of protozoa
 - Bacillus > Corynebacterium > Pseudomonas
- Strong competition between protozoa
- Presence of Bacillus as prey for Tetrahymena
→ Corynebacterium and Pseudomonas benefit



Interaction Study - Discussion



- Hypotheses
 - Presence of *Acanthamoeba* and *Tetrahymena* → higher feeding pressure
→ increased reduction of bacteria
 - Grazing protection by
 - *Bacillus* through filaments and spores
 - *Pseudomonas* through production of toxic compounds
- **Diversity of bacteria AND protozoan determines microbial community**

Outlook

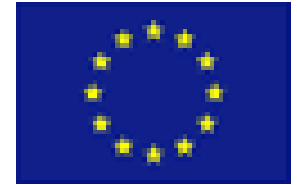


- Further experiments evaluating
 - Detailed response of protozoan numbers → Labeling protozoa
 - Effects of protozoan diversity on bacteria → Add protozoan species
- Determine influence of bacterial life stage on protozoa with spores of *Bacillus*
 - Do soil protozoa serve as trojan horses for (pathogenic) bacteria?
- ...

Acknowledgements



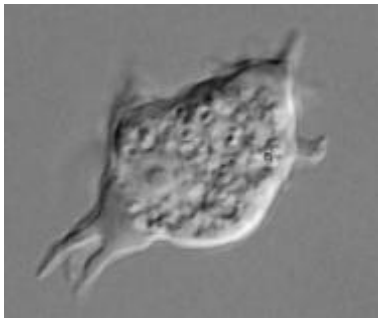
- Philippe Lemanceau



- Michael Bonkowski
- Jan Weinert



- Anne Winding



Thank you for your attention!