Diversity and Functions of Protozoa in Soils

EcoFINDERS

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University of Cologne - AG Bonkowski

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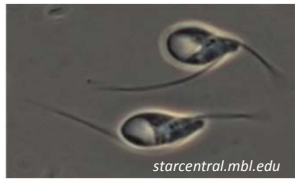
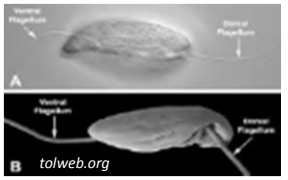
Diversity of Protozoa in Soils

- Single-celled, heterotrophic eukaryots
- Up to 100,000 individuals/gram soil
- Reproduction asexually
- Produce cysts to resist adverse conditions

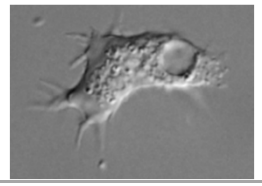
- Classically split into three morphological distinct groups
  - Flagellates (one ore more flagella)

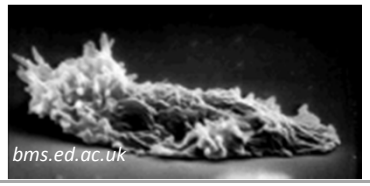




  - Ciliates (many short, hair-like cilia)

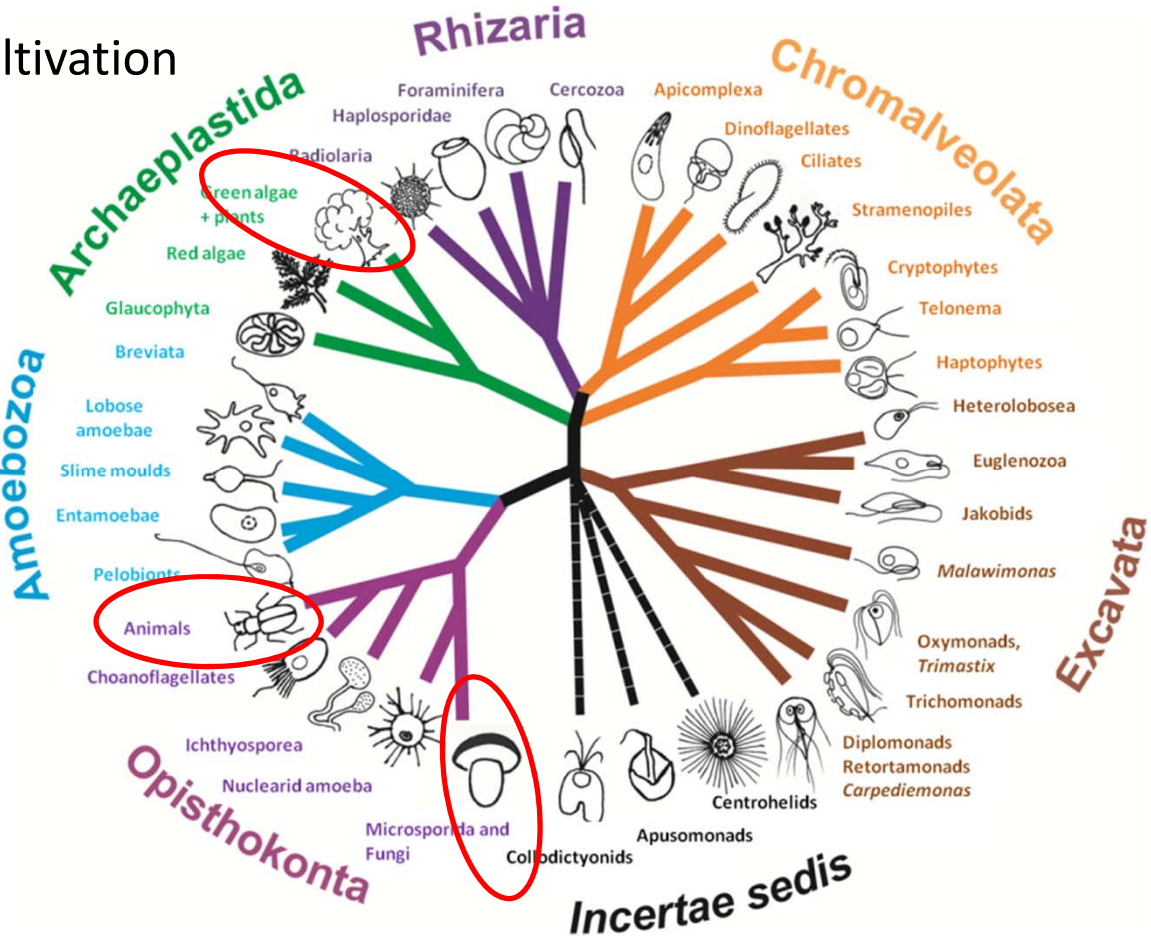
  - Amoeba (one or more pseudopodia)





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-vykum/vyzkumne-tymy/evolucni%20-%20protistologie/fylogenetika.jpg*

Diversity of Protozoa in Soils

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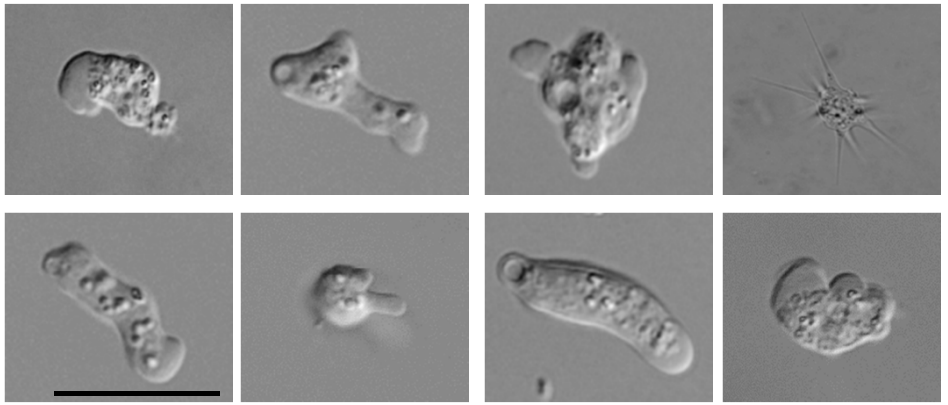
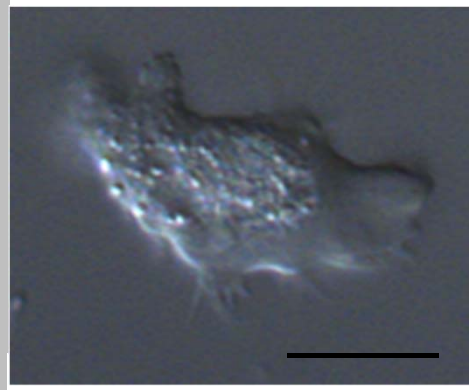
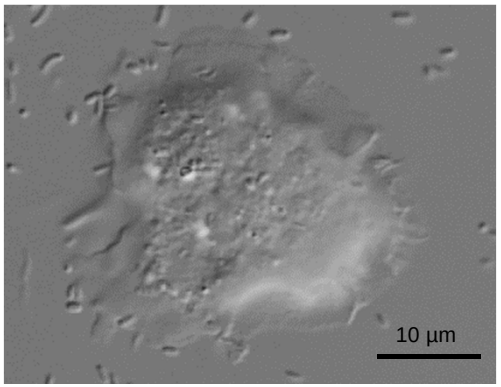
  - Difficult isolation and cultivation
  - High diversity
- Case study: amoebae
  - Absence of clear cell shape
  - ↑Variability → Hard to identify



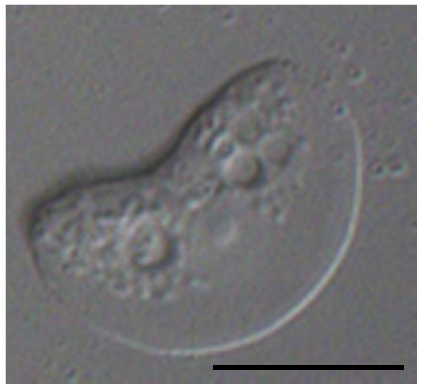
Hartmanella sp.



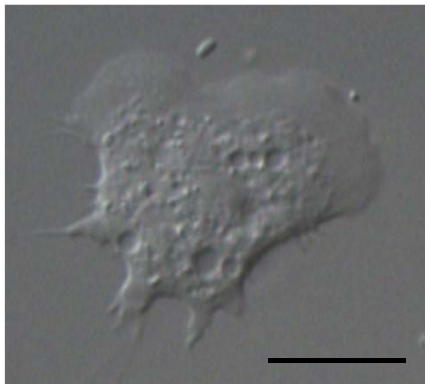
Acanthamoeba sp.



Cochliopodium sp.



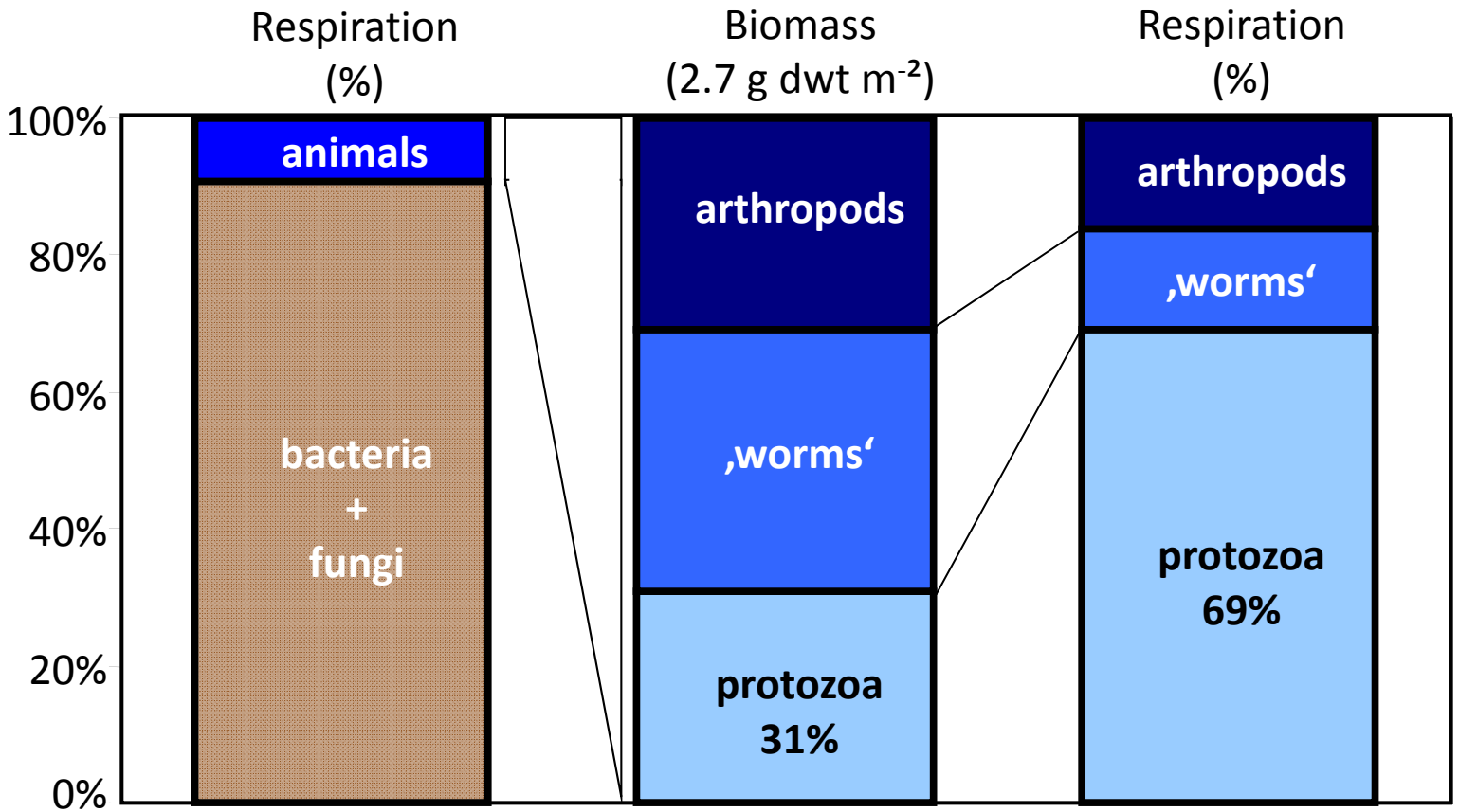
Vanella sp.



Flamella sp.

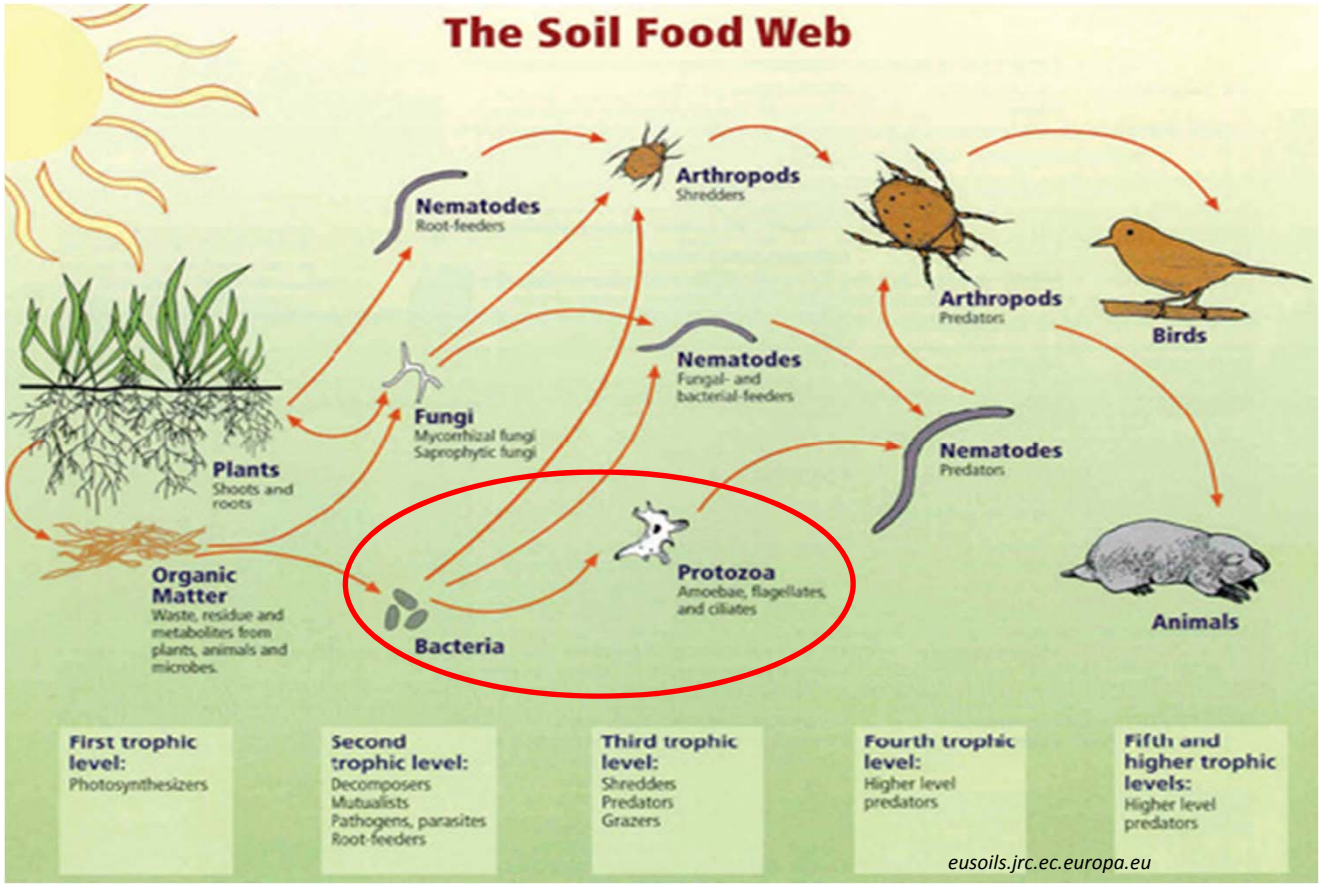
Importance of Soil Protozoa

- 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**



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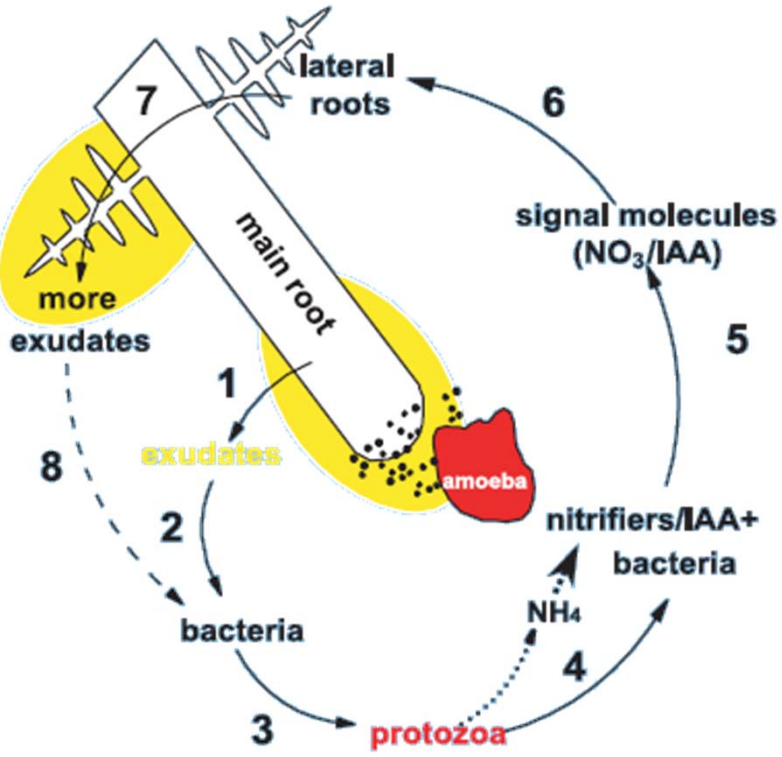
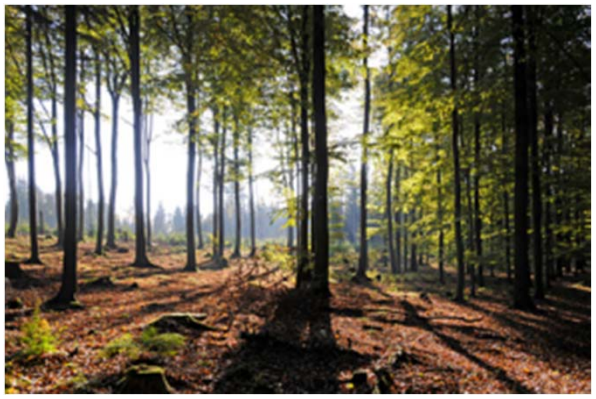


Modified from Bonkowski (2004)

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



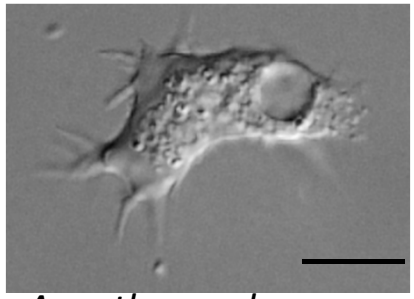
leben.audena.de

belecker-chronik.de

maehdreschen.de8

Identification of Soil Amoeba

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



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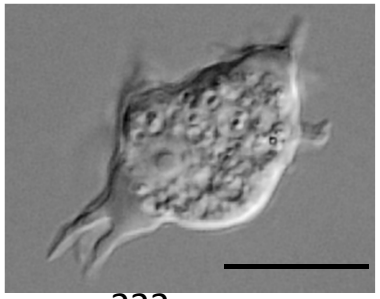
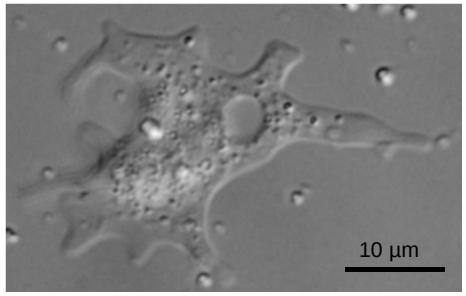


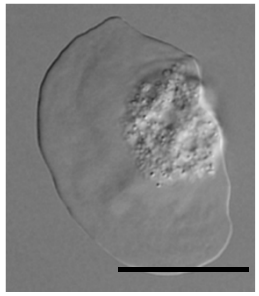
??? sp.

Hartmanella sp.

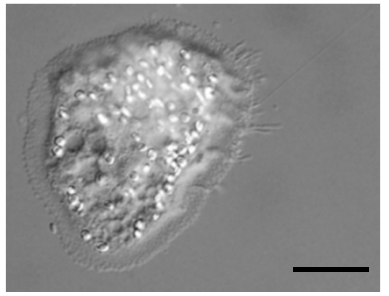


Korotnevella sp.

Cochliopodium sp.



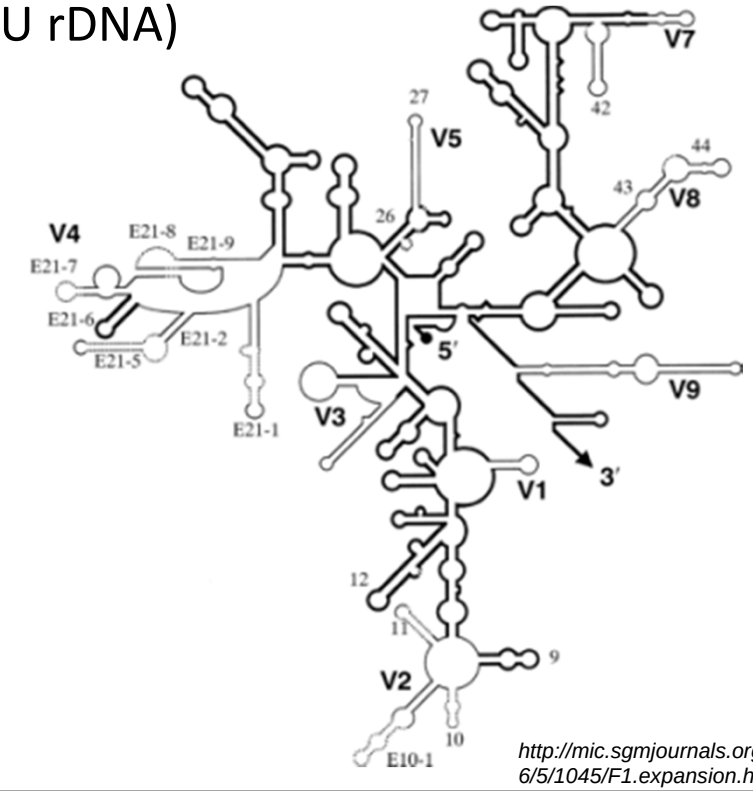
Vannella sp.



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/146/5/1045/F1.expansion.html

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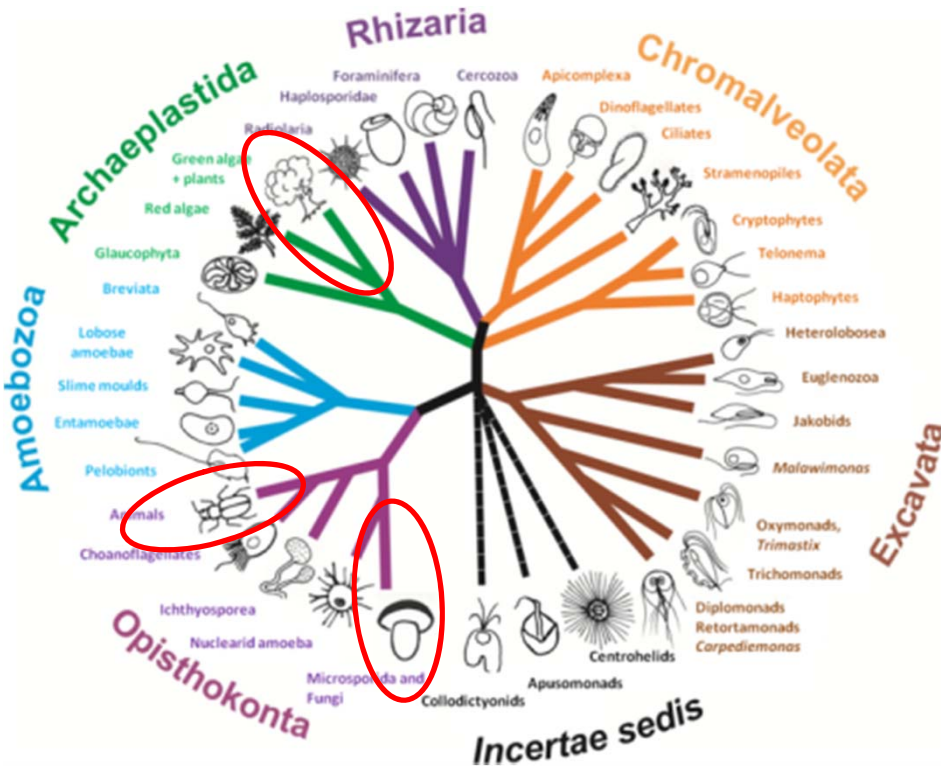
- BUT: No barcodes for protozoa!
  - Very diverse
    - →Eukaryotic primers do not work



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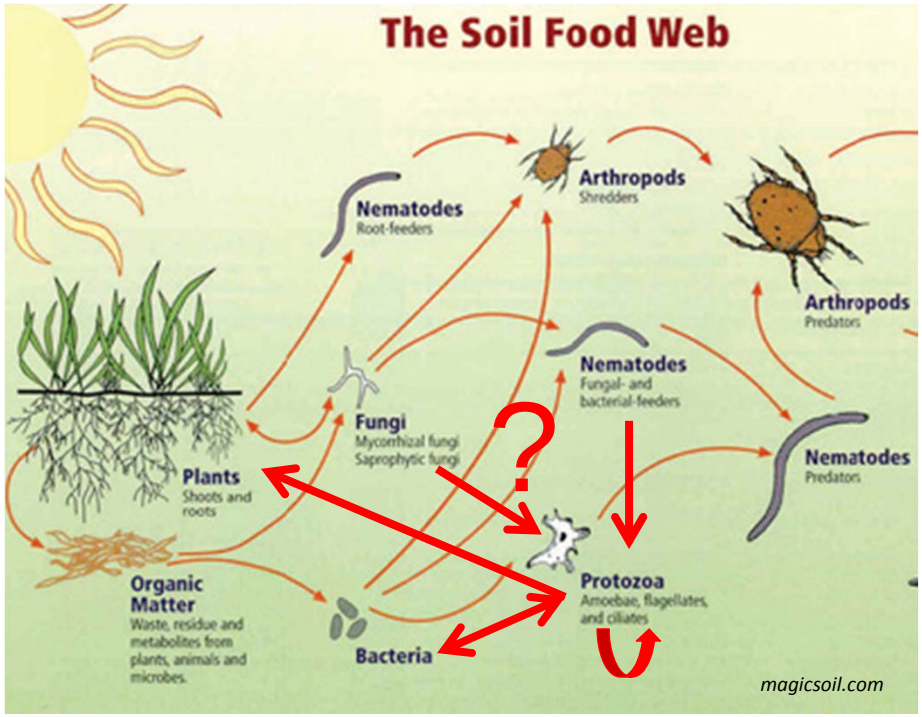
- **Group specific primers needed**
  - **Used for comparison of European soils via high-throughput sequencing**

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

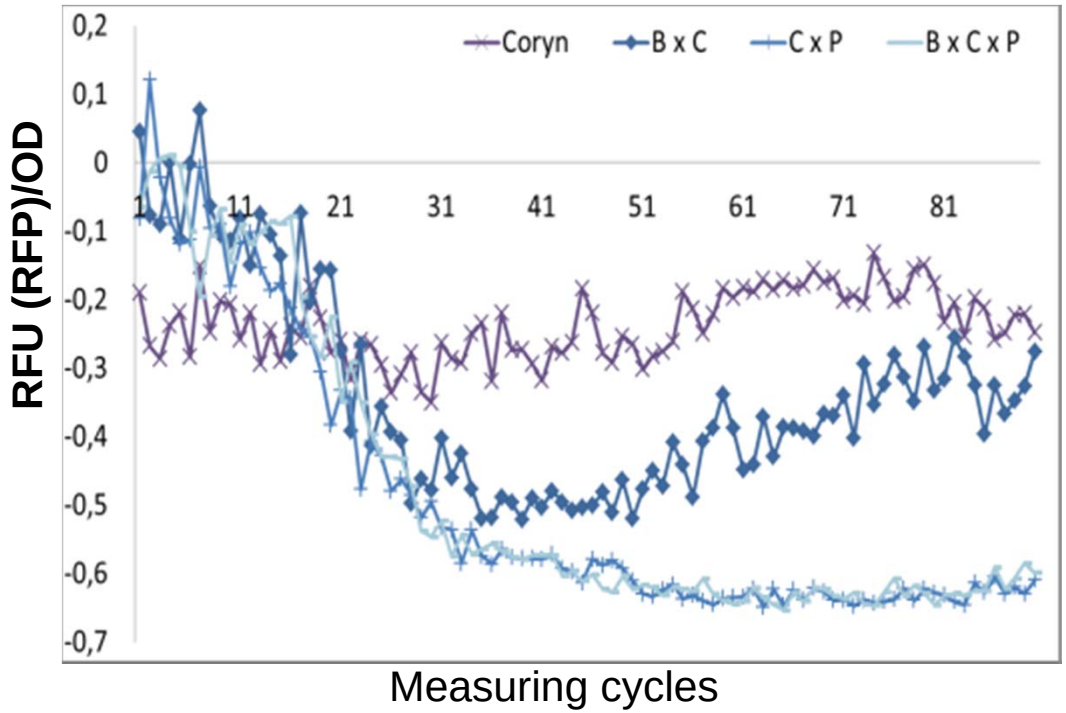
Interaction Study - Hypotheses

- 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



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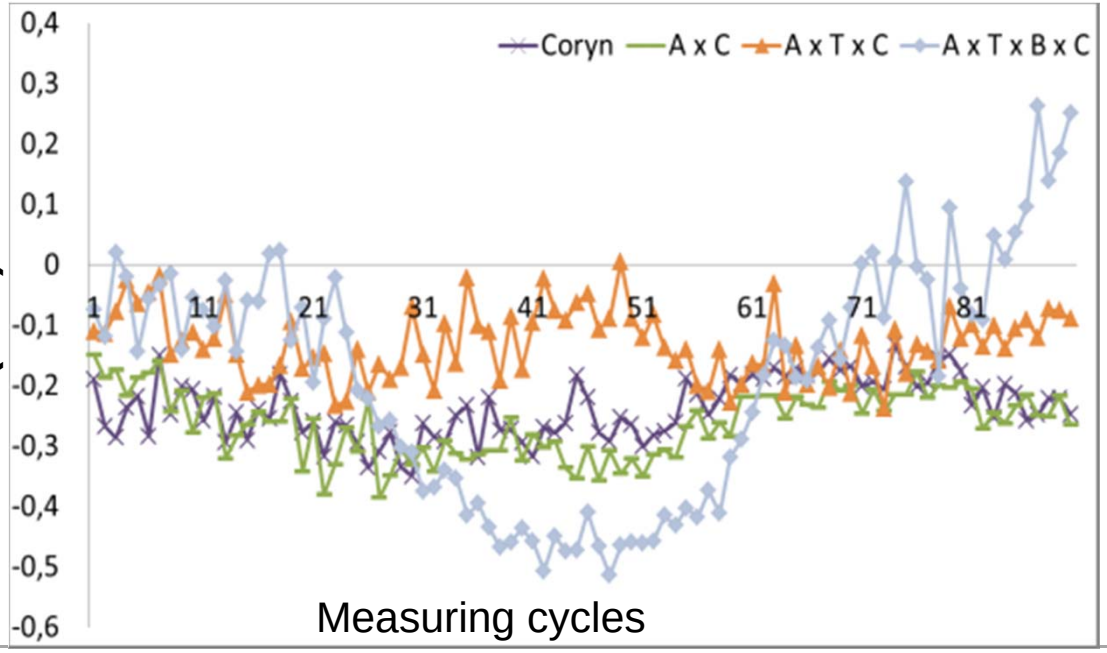

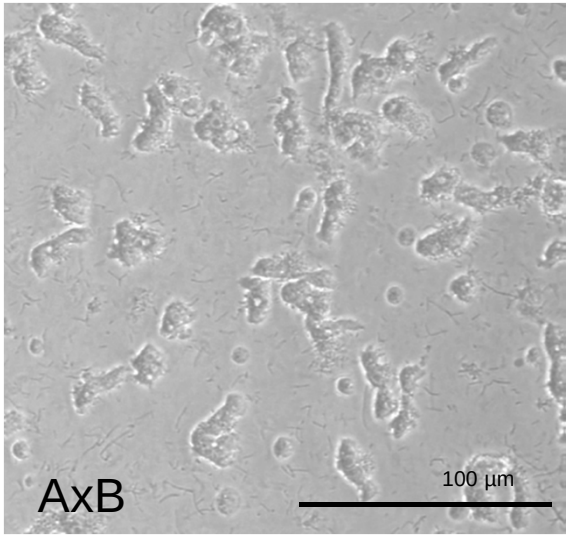

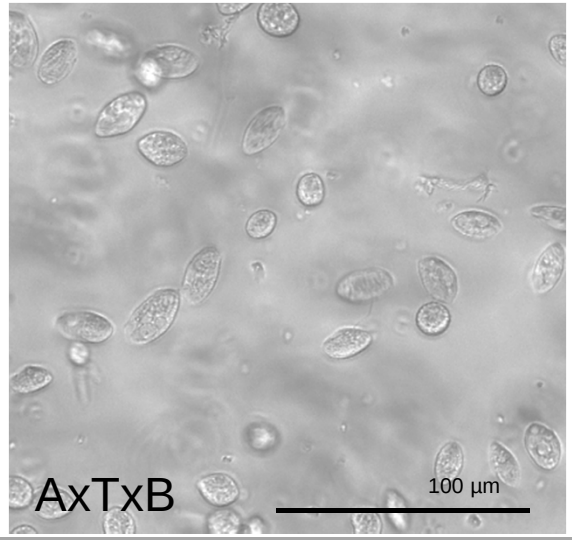

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- **Diversity of bacteria AND protozoan determines microbial community**

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

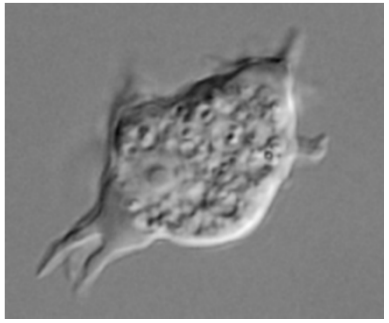
  → Do soil protozoa serve as trojan horses for (pathogenic) bacteria?
- …

Acknowledgements

- Philippe Lemanceau

- Michael Bonkowski
- Jan Weinert

- Anne Winding



Thank you for your attention!

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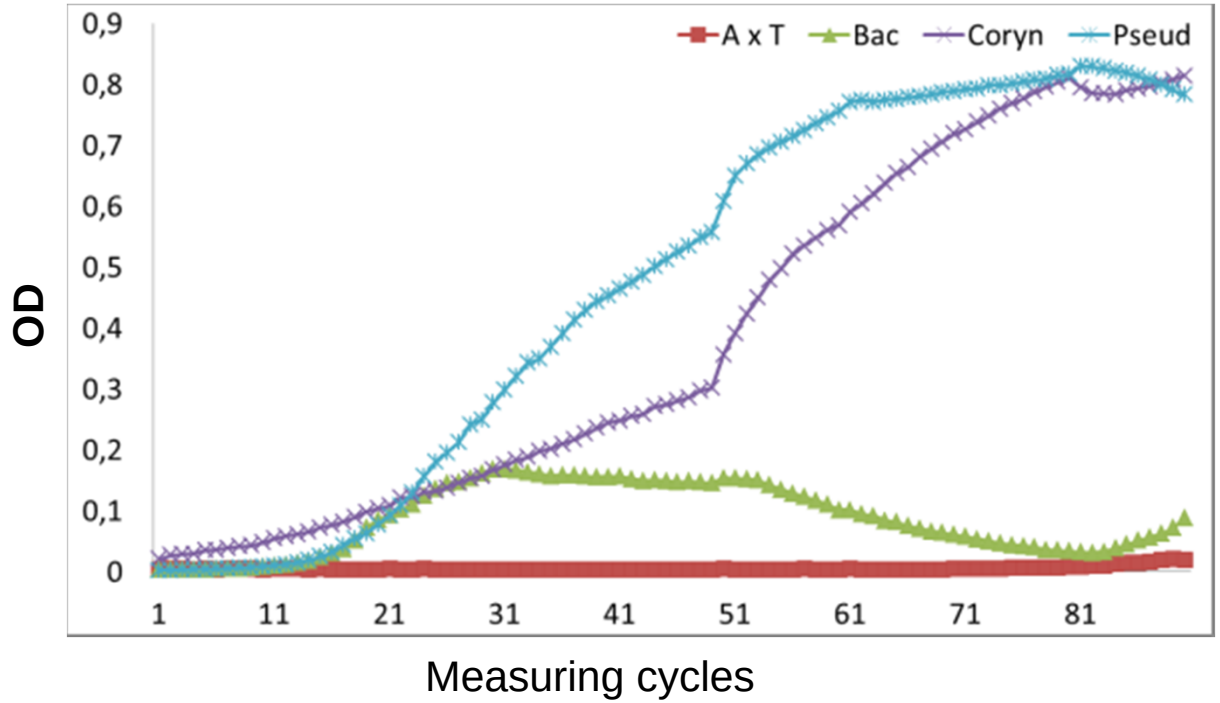

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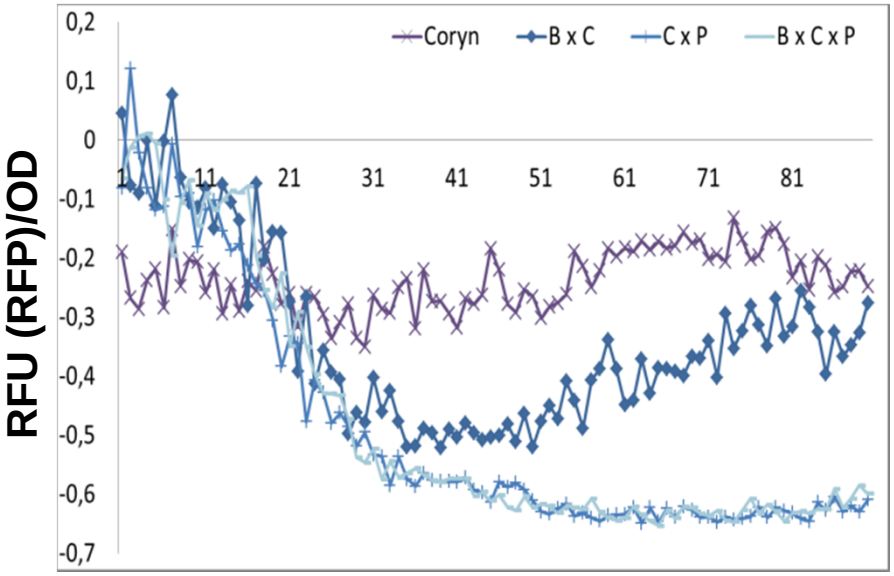
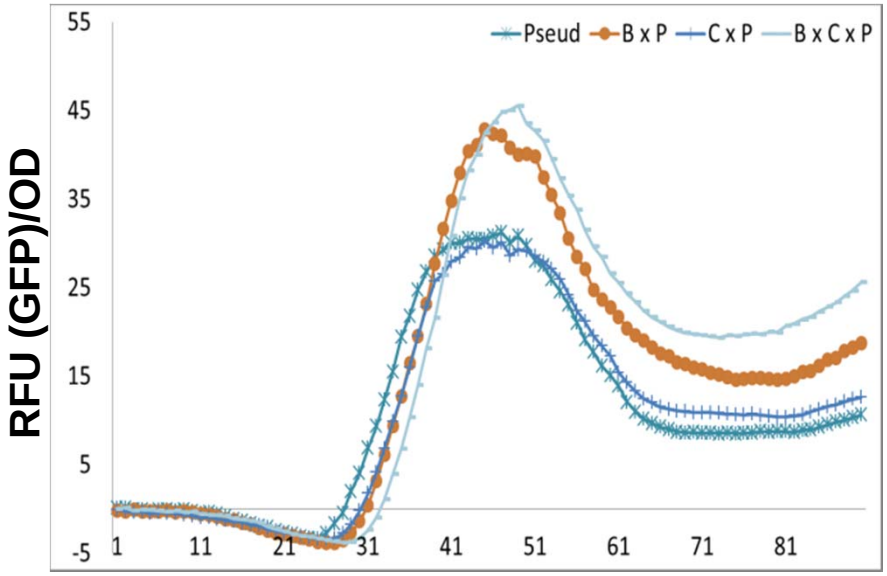
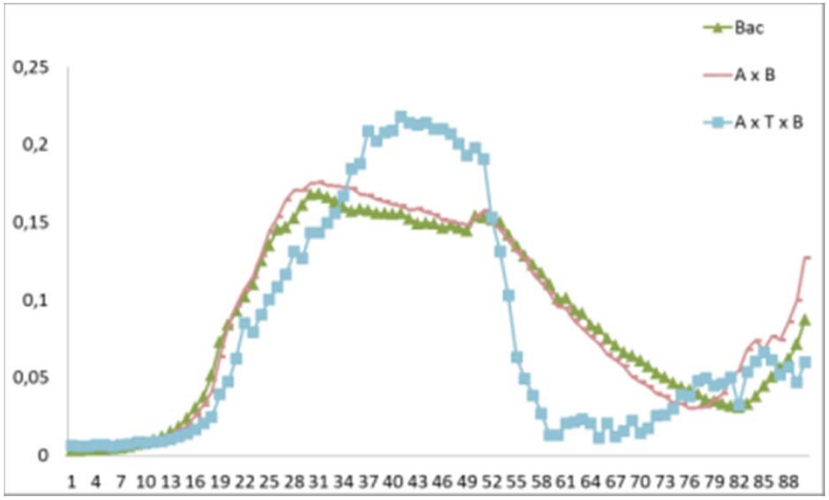
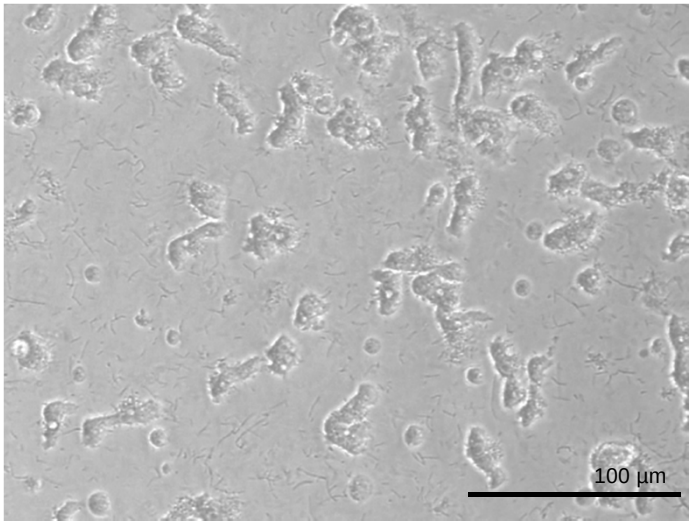


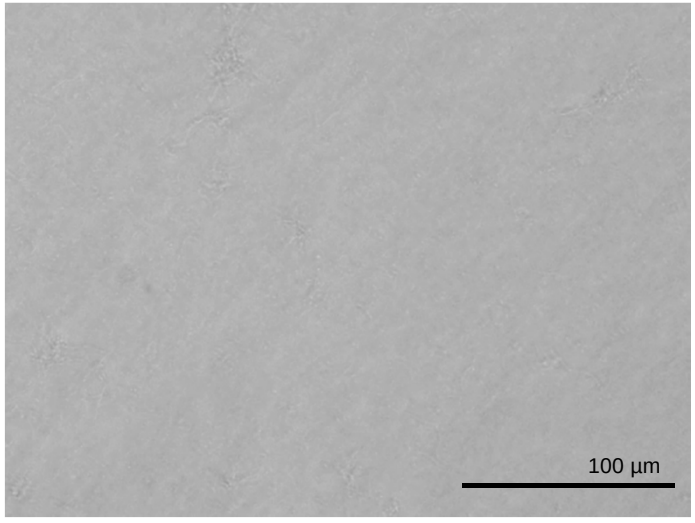


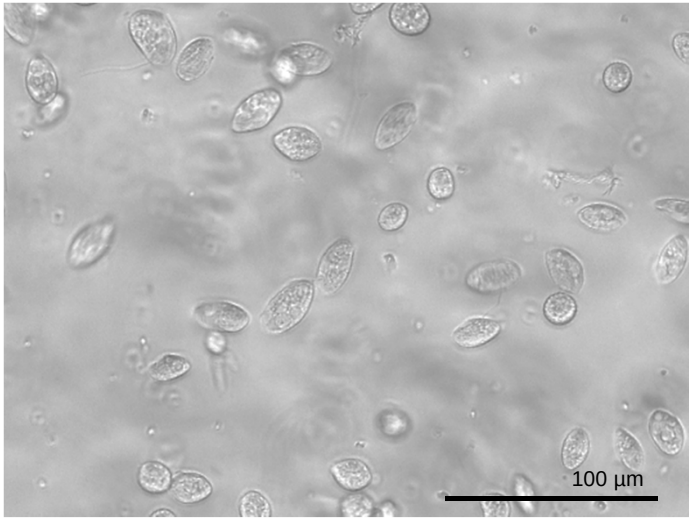
Measuring cycles

Interaction studies - Results









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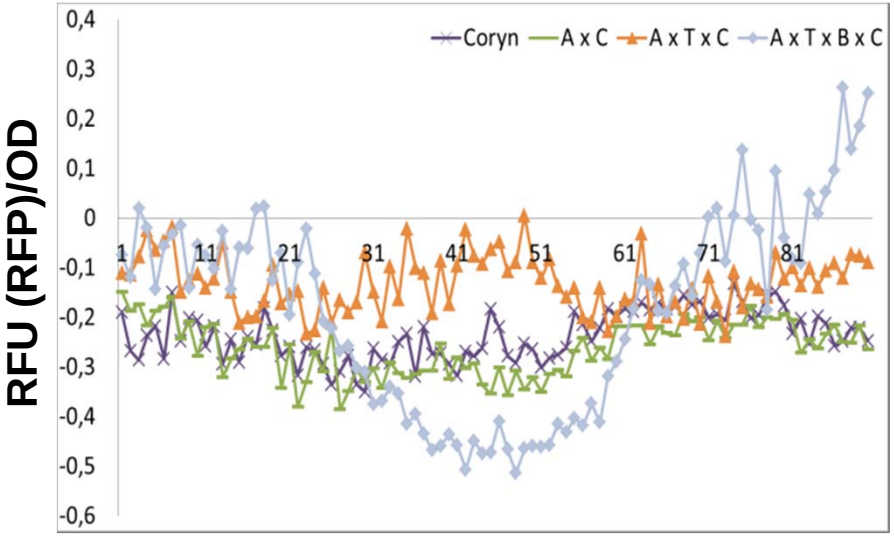
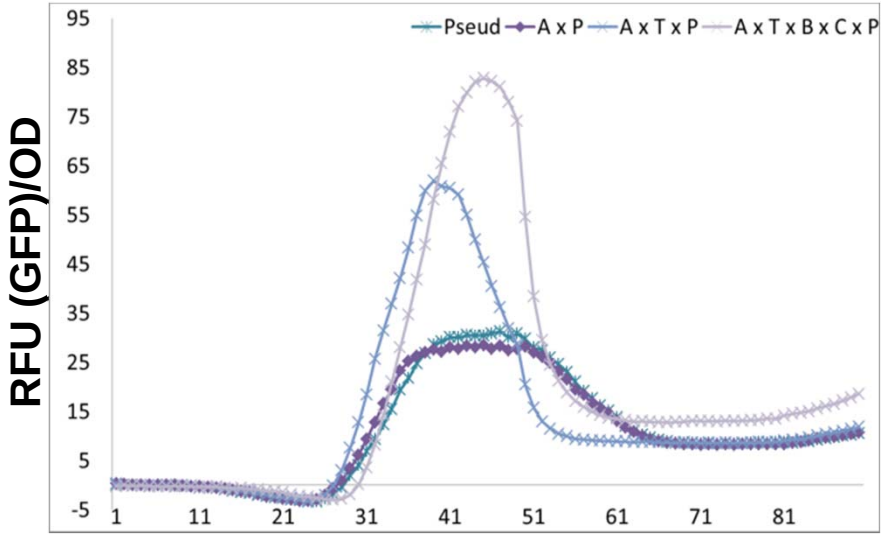
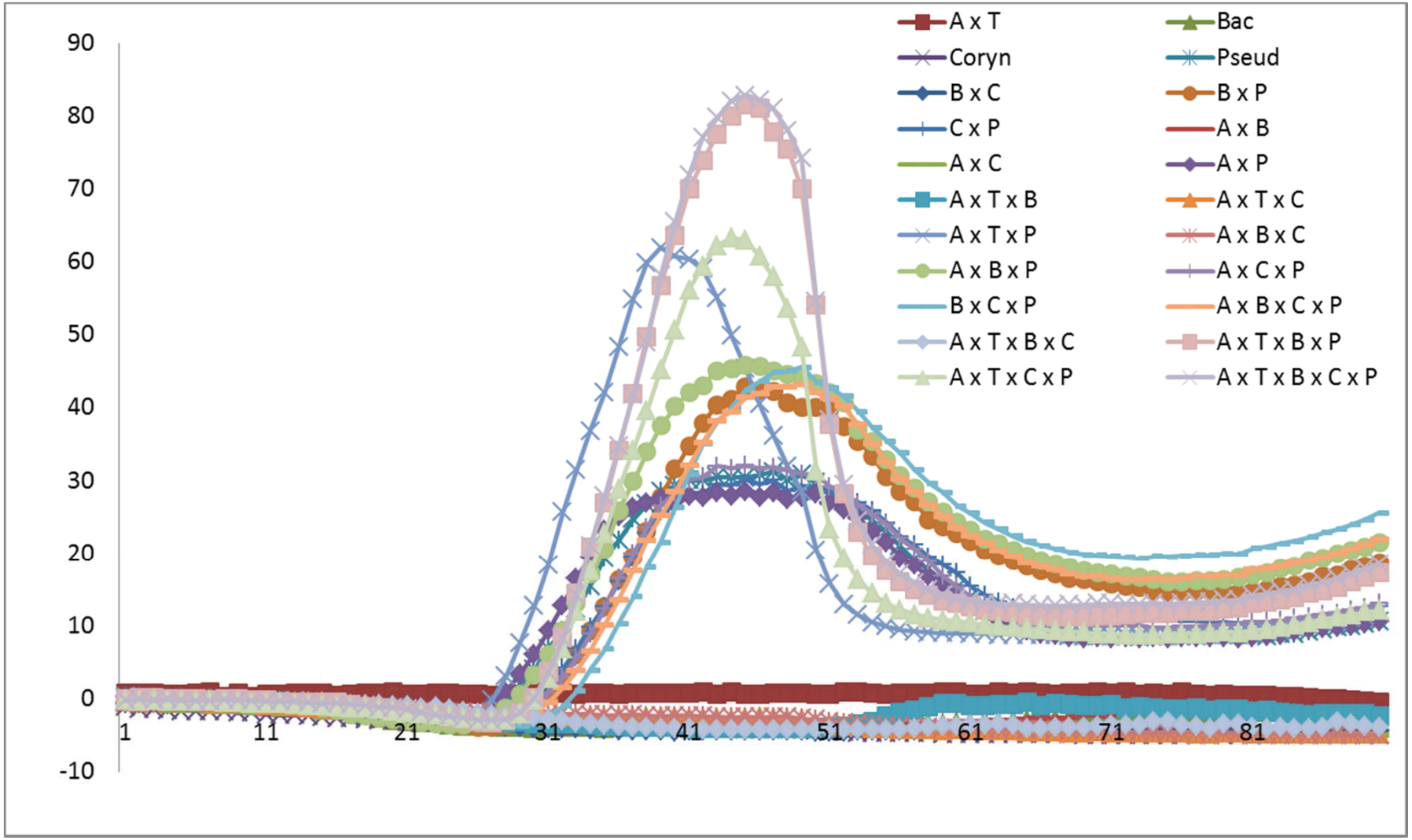




Measuring cycles

GFP/OD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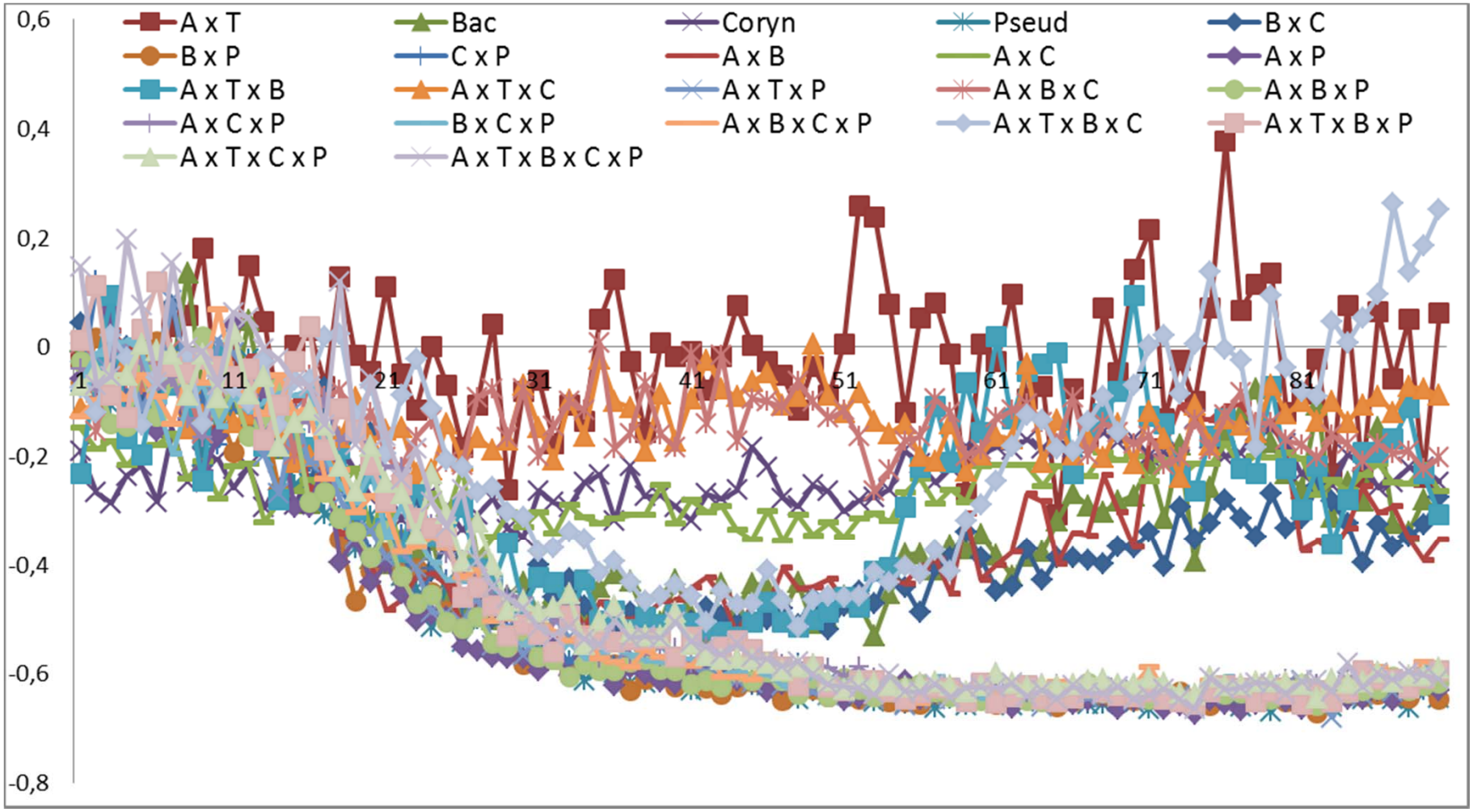
RFP/OD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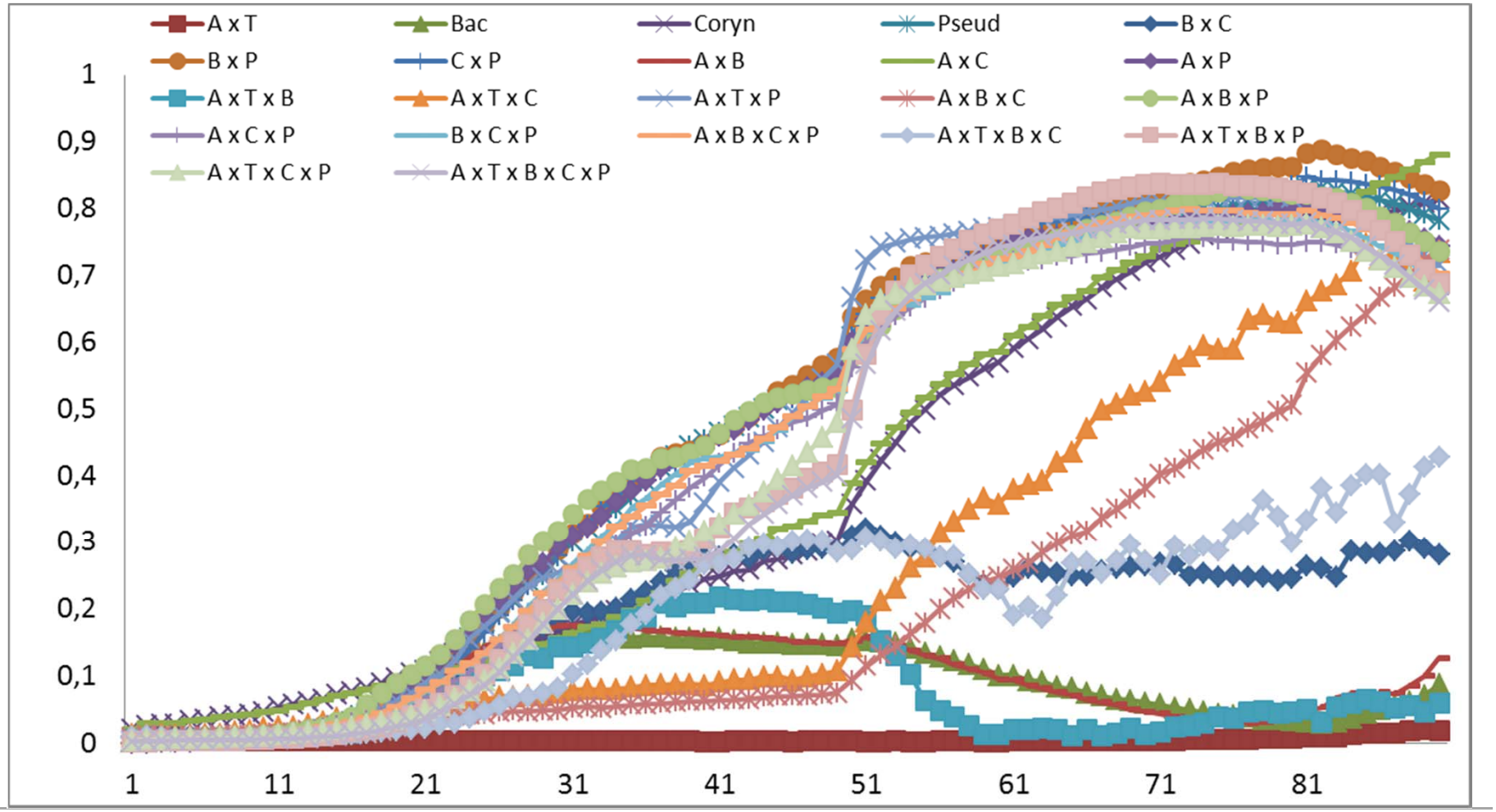
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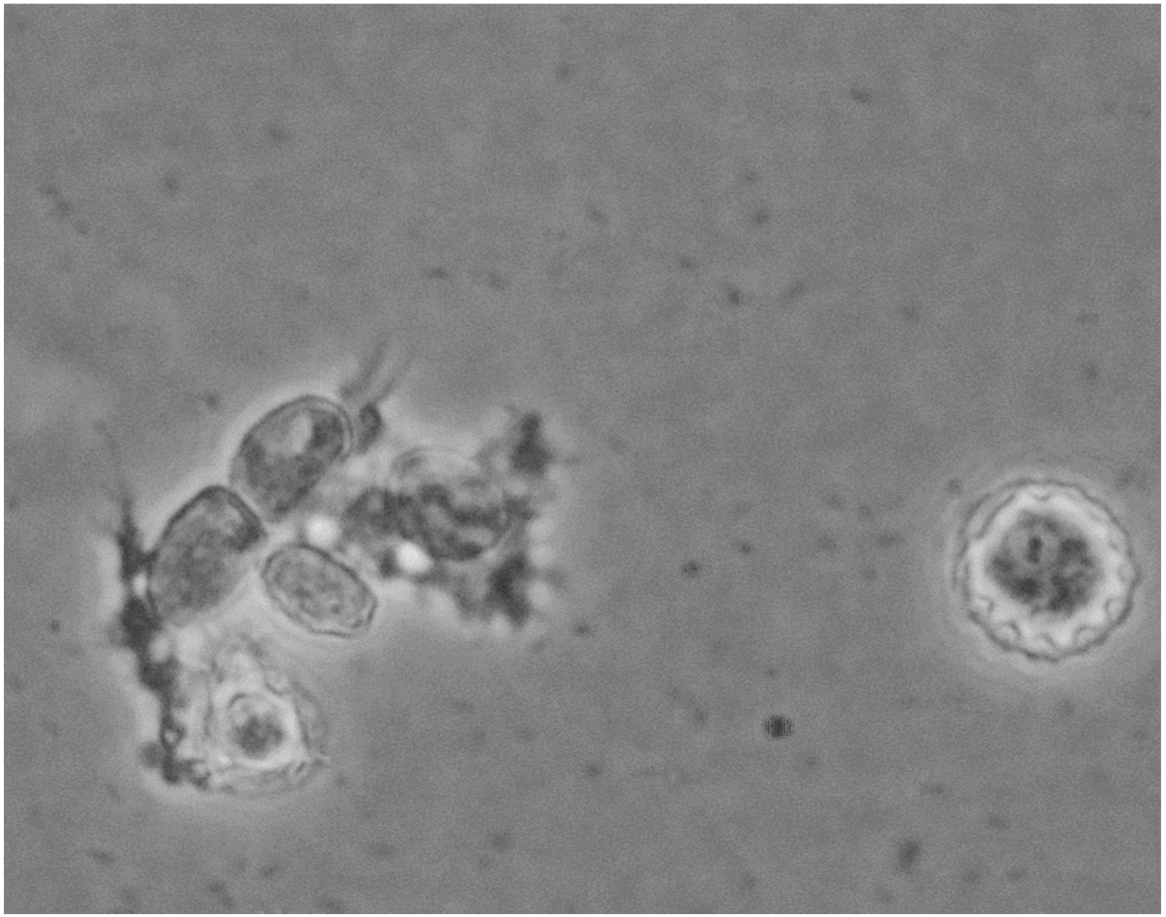
Life stage Acanthamoeba

- Trophozoit and cyst of *Acanthaamoeba castellanii*



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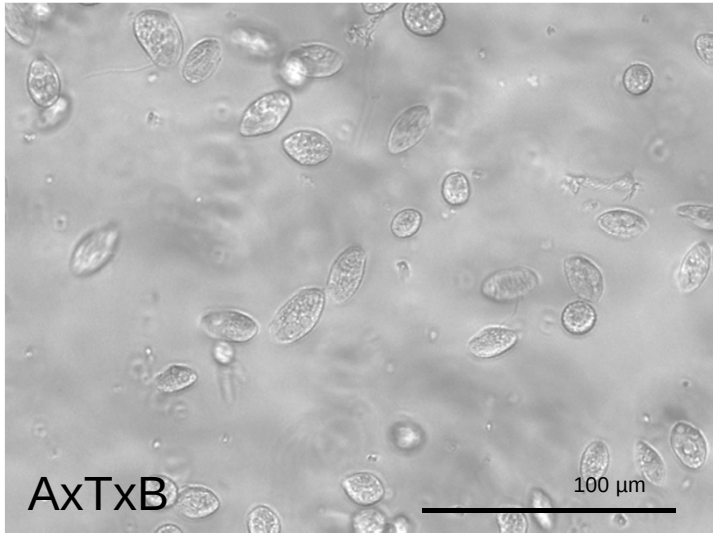

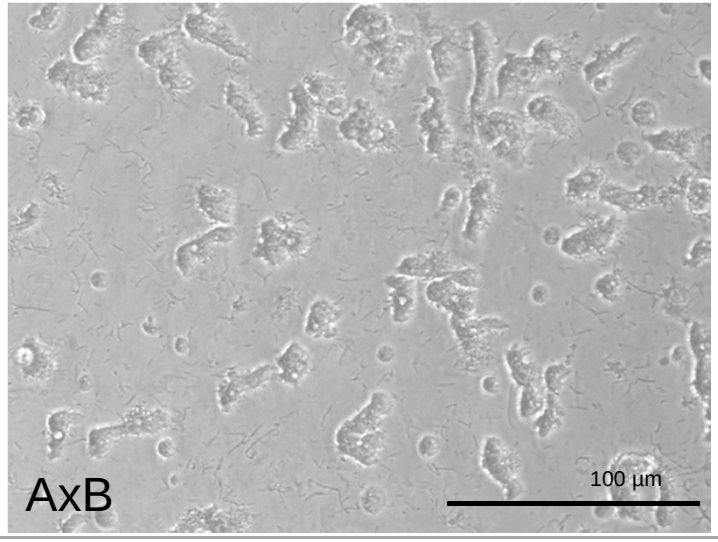

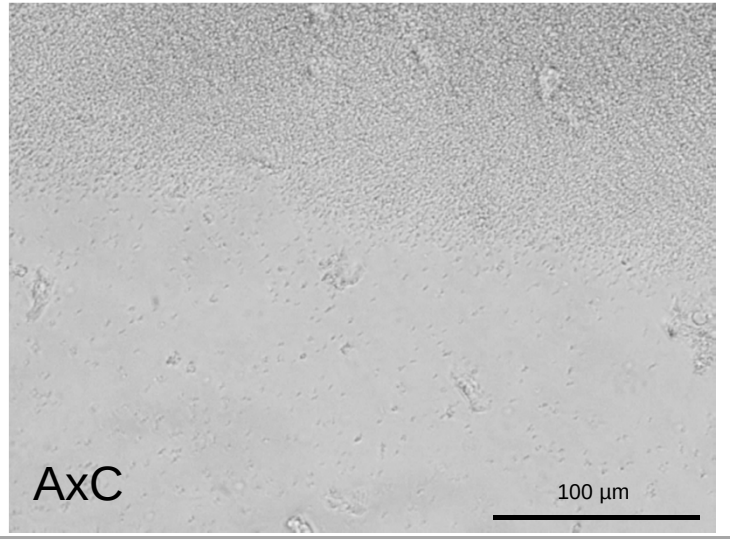

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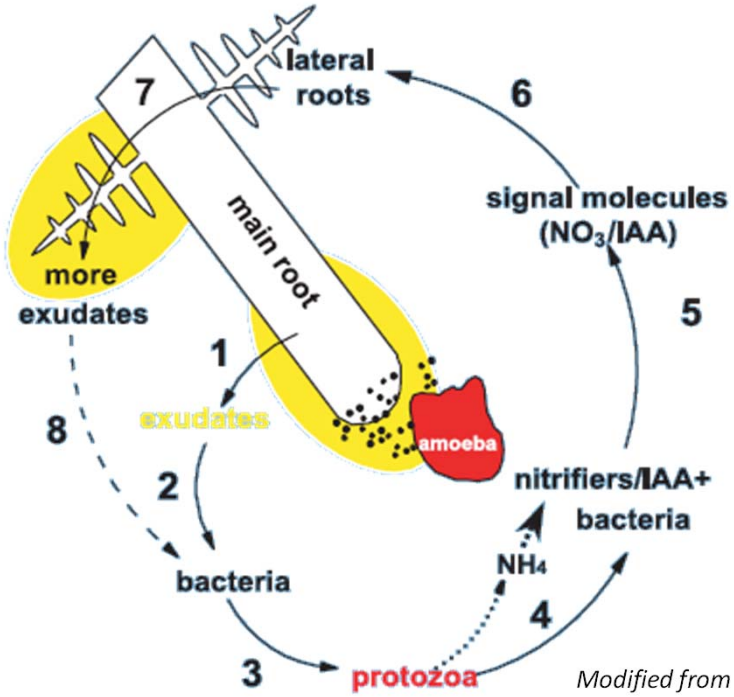
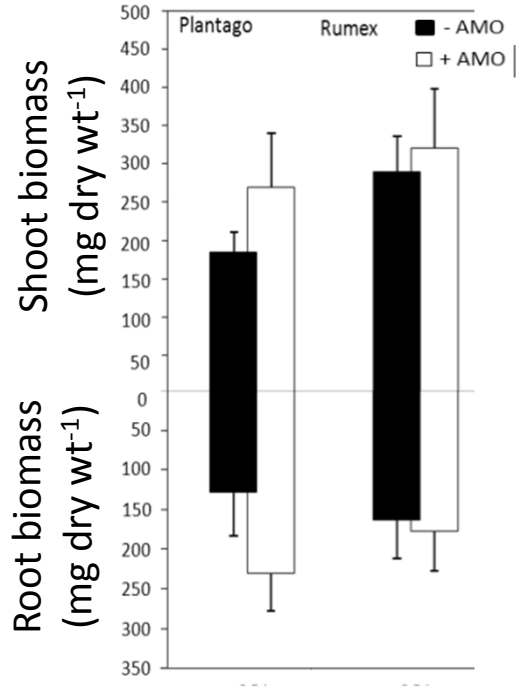
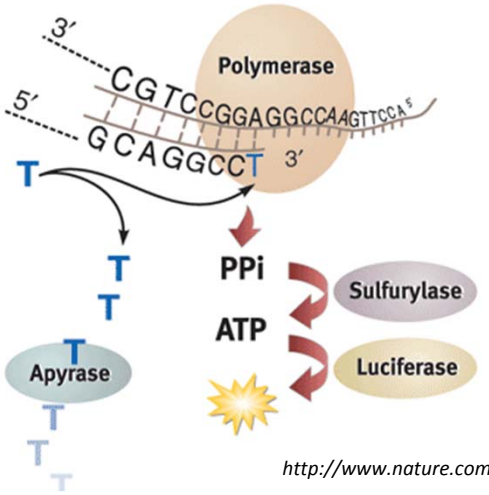


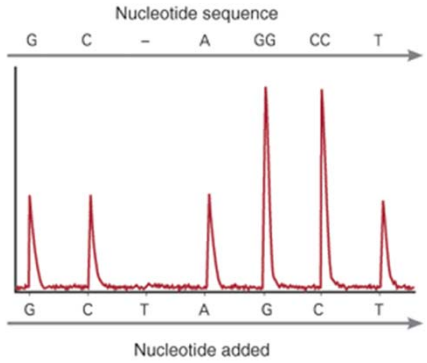
Modified from **Bonkowski (2004)**



High-Throughput Sequencing

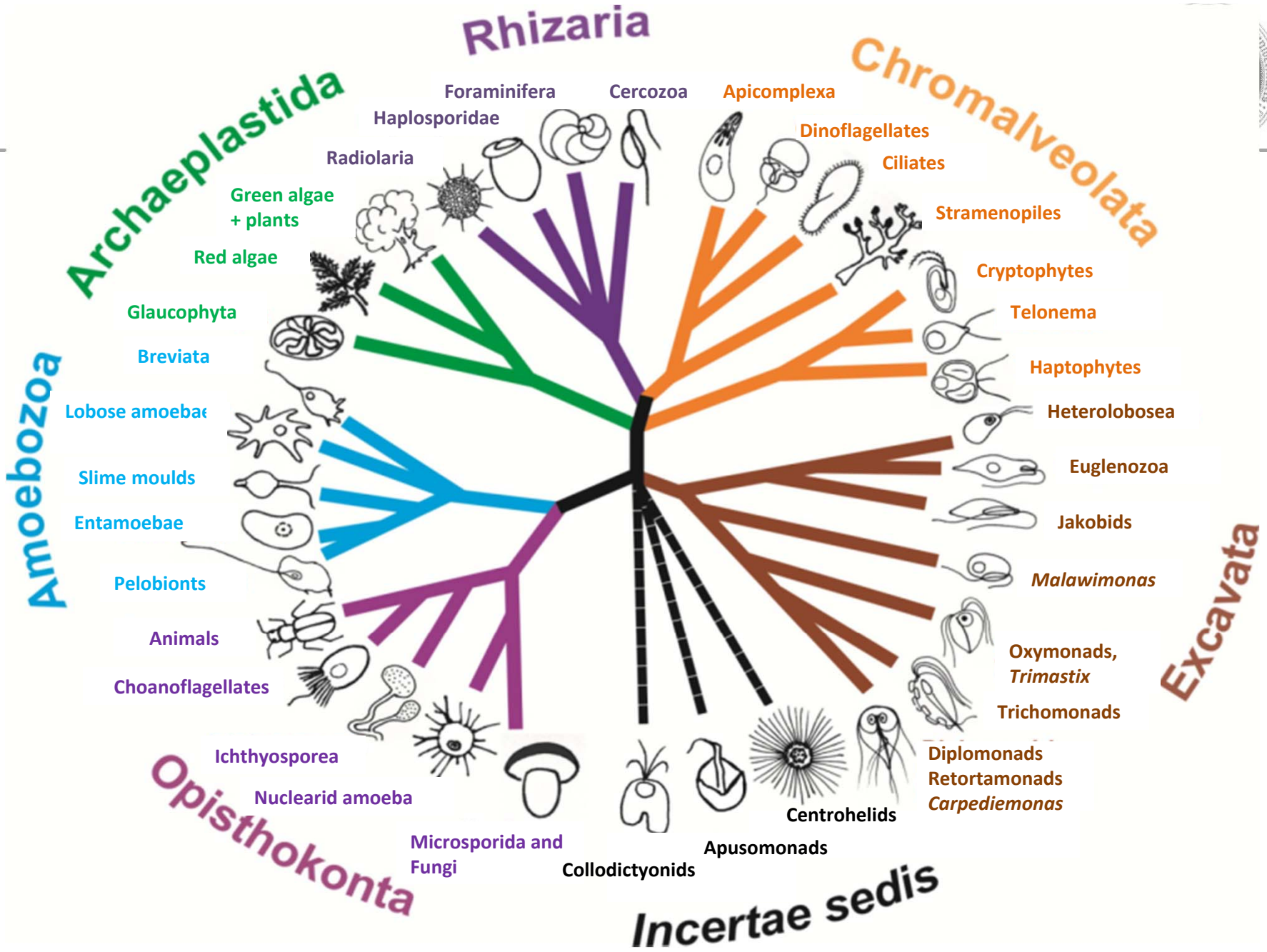
- **Pyrosequencing /454 (Roche)**





http://www.nature.com/nmeth/journal/v2/n10/images/nmeth800-F1.gif

Advantages	Disadvantages
Culture-independent	Only information: DNA sequence
Multi-parallel-sequencing >1,000,000 sequences/run	Complex bioinformatics
Almost fully automated	PCR bias
Cheap price/base	High total cost



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