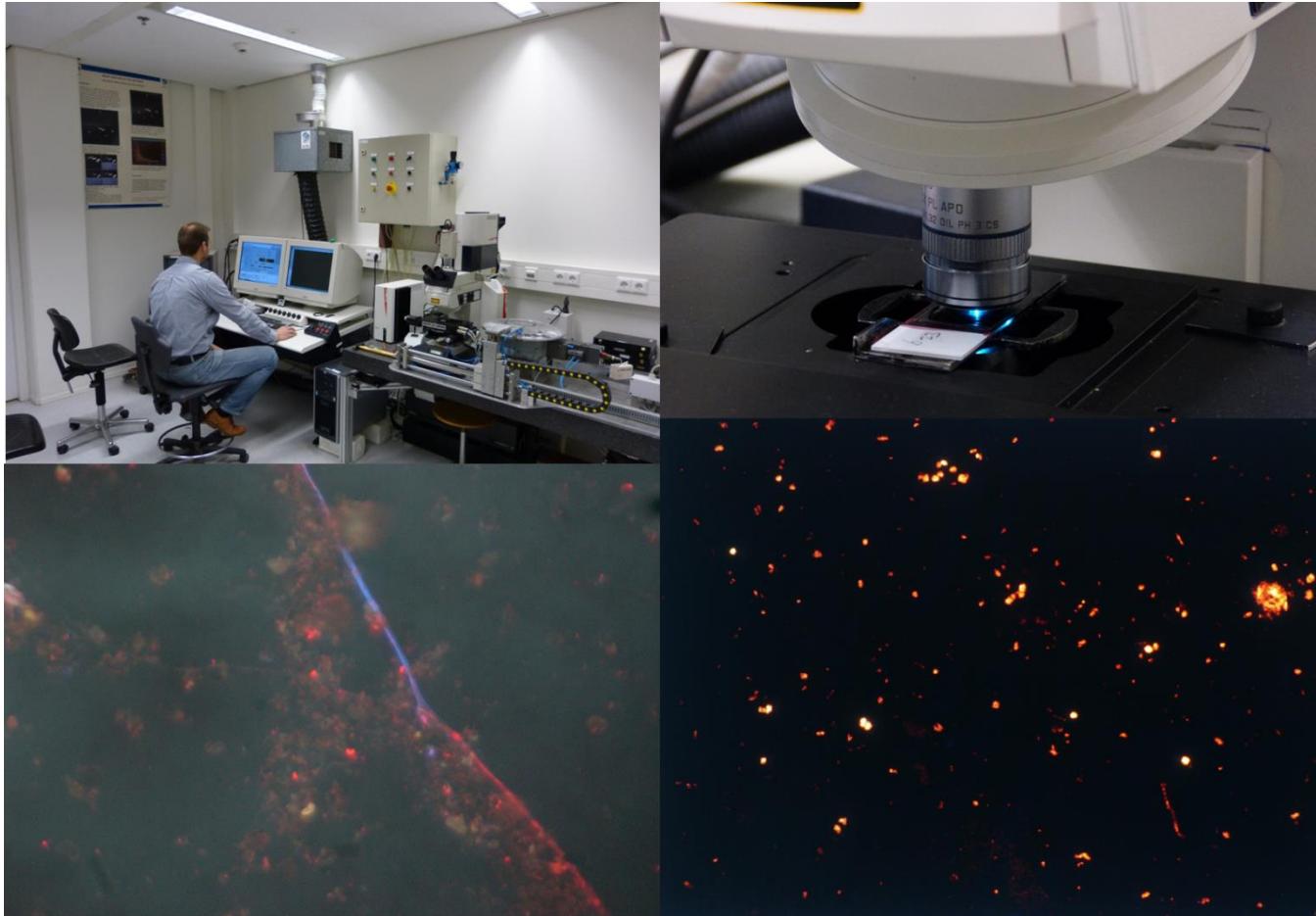


# Micro-organismen

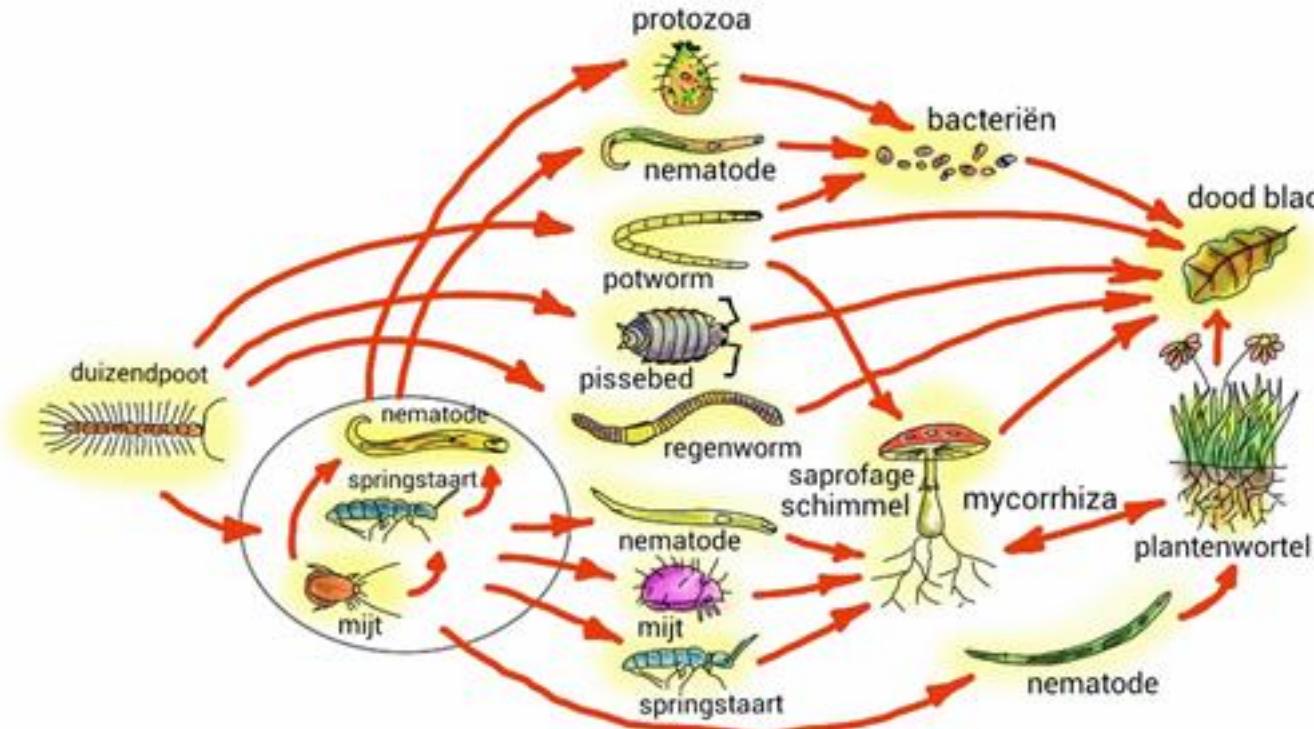
## Effecten van kalk- en organische stofgehalte in de duinen

Jaap Bloem, An Vos en Rolf Kemmers

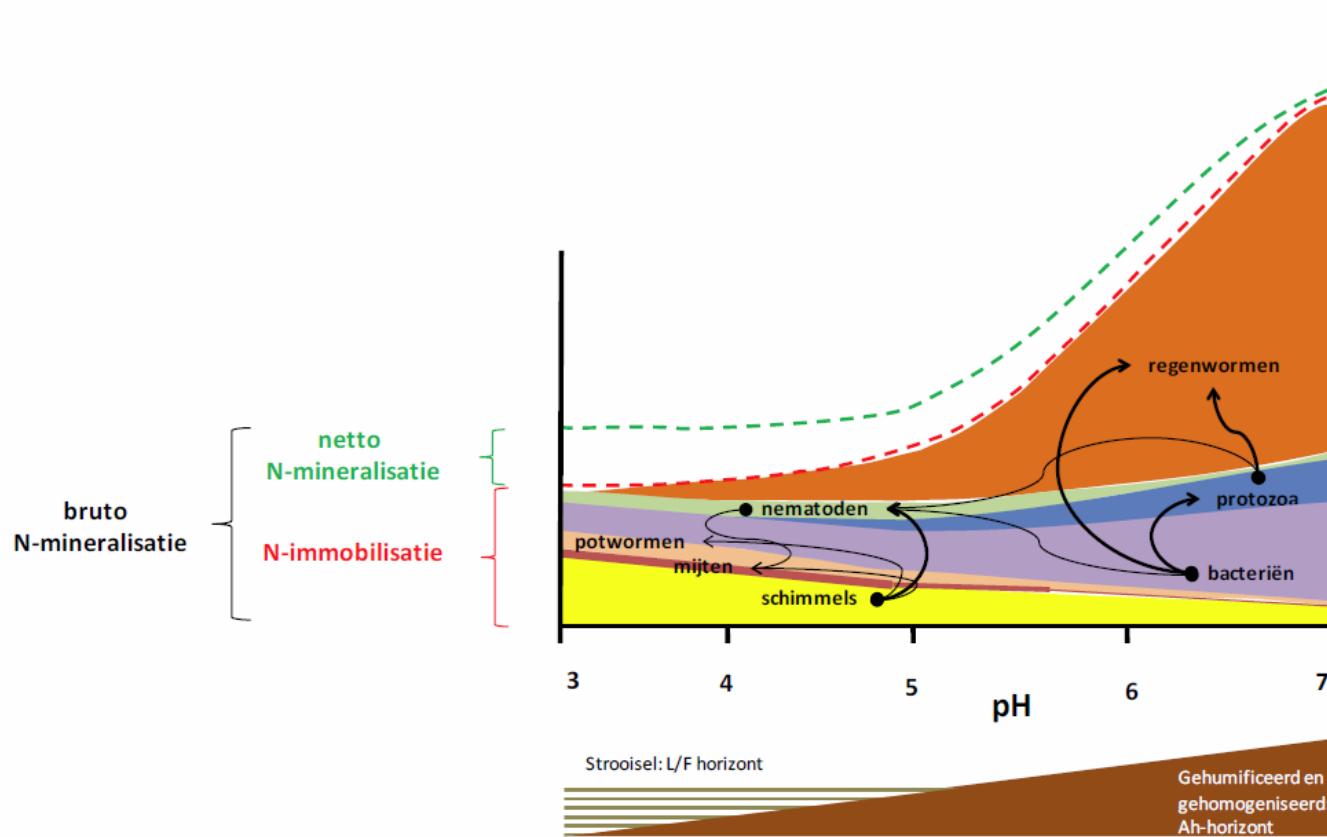


# Bodemvoedselweb: bacteriën, schimmels en fauna

Figuur Ron de Goede, WU Bodemkwaliteit



## Verstoring en verzuring remt bacteriën sterker dan schimmels minder stikstof vastlegging, meer beschikbaar voor planten

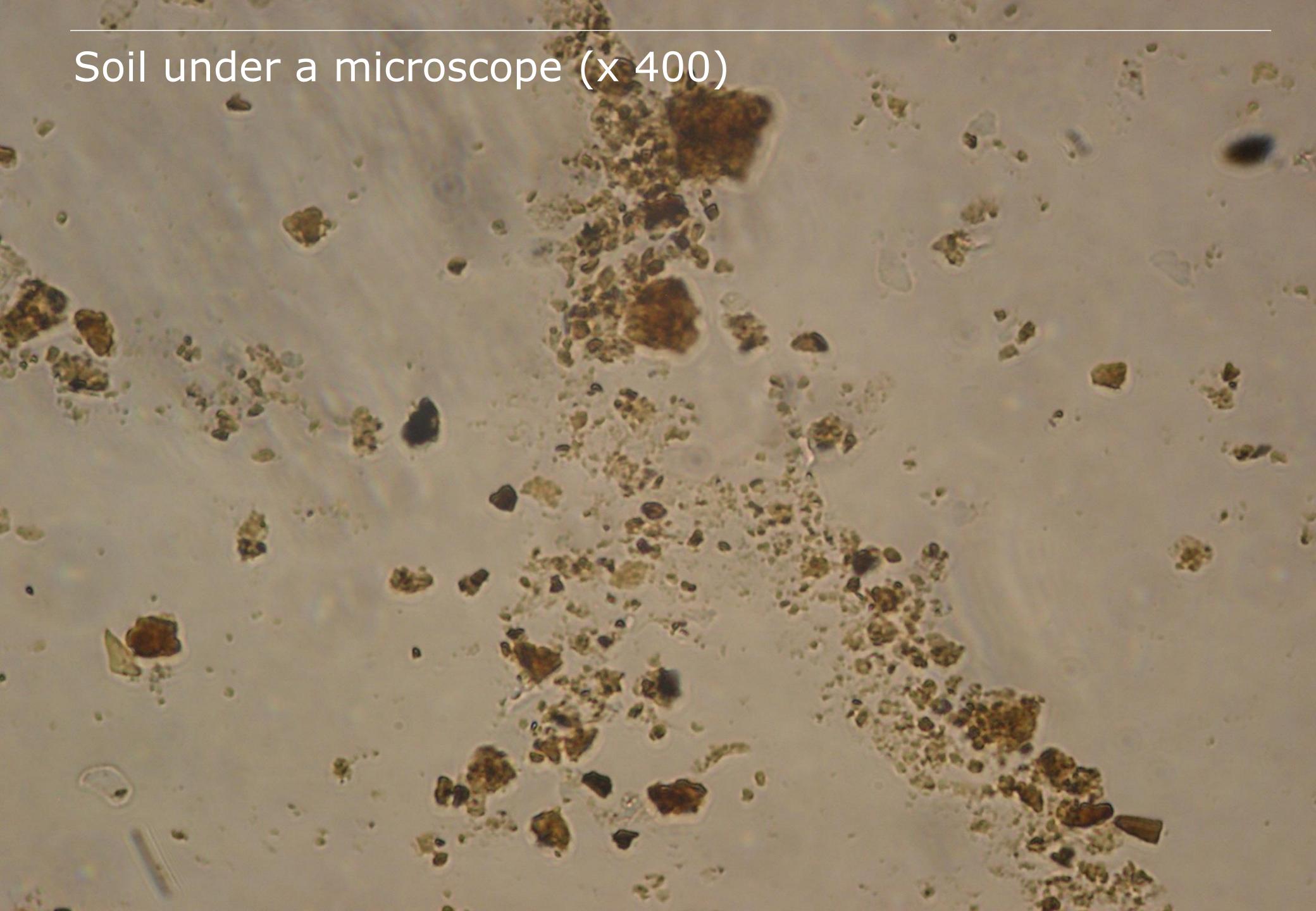


Kemmers, R. 2012. Zijn bodemorganismen van belang voor herstel van verzuurde bossen? De Levende Natuur 113, 24-28.

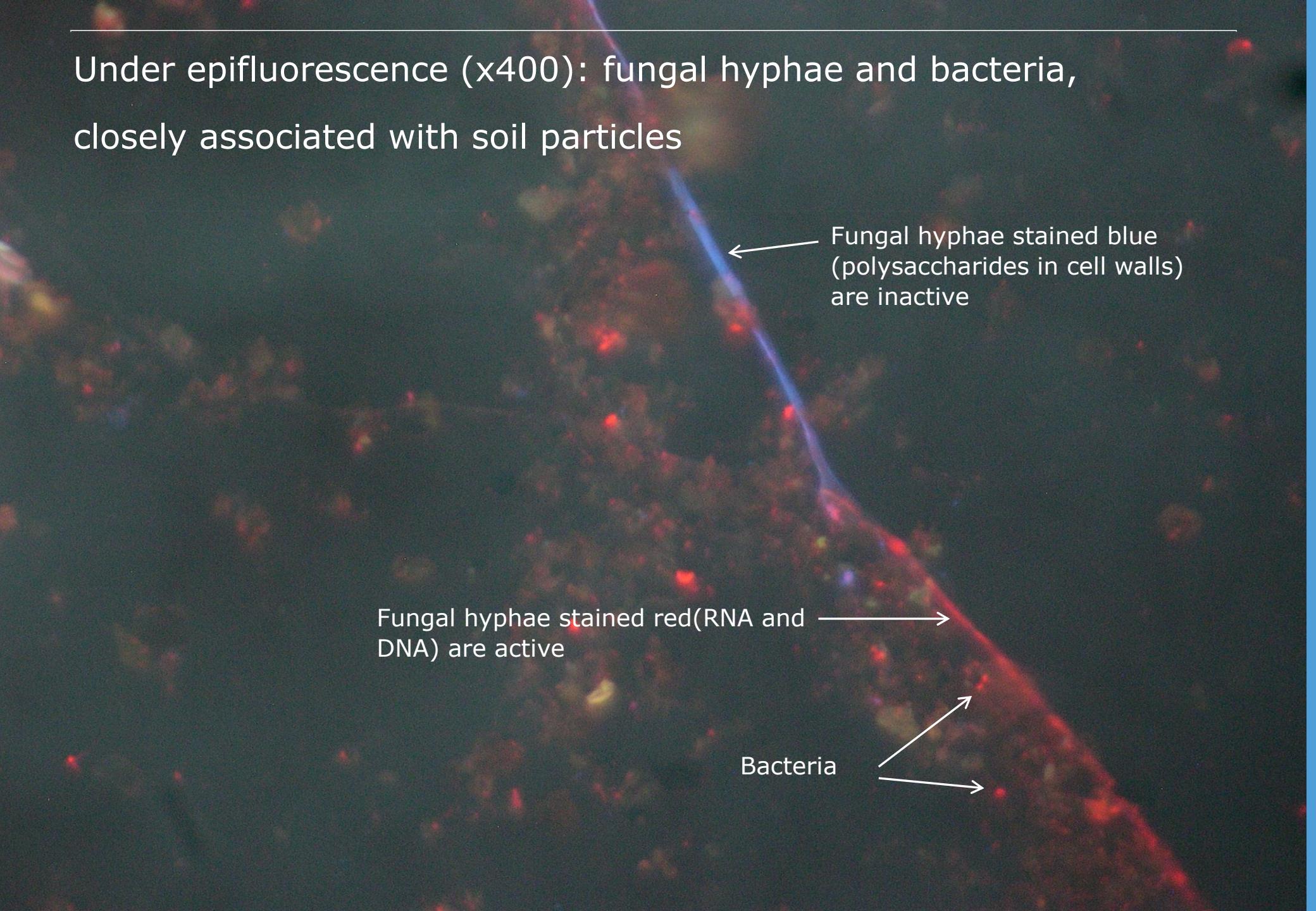
Kemmers, R.H.; Brinkman, E.P.; Bloem, J.; Faber, J.H.; Putten, van der W.H. 2011. Is bodembiodiversiteit van belang voor herstel van beekdalvegetaties? De Levende Natuur, 112, 4 - 9.

Kooijman, A.M., Kooijman-Schouten, M.M., Martinez-Hernandez, G.B. Alternative strategies to sustain N-fertility in acid and calcareous beech forests: Low microbial N-demand versus high biological activity (2008) Basic and Applied Ecology, 9, 410-421.

Soil under a microscope (x 400)



Under epifluorescence (x400): fungal hyphae and bacteria,  
closely associated with soil particles



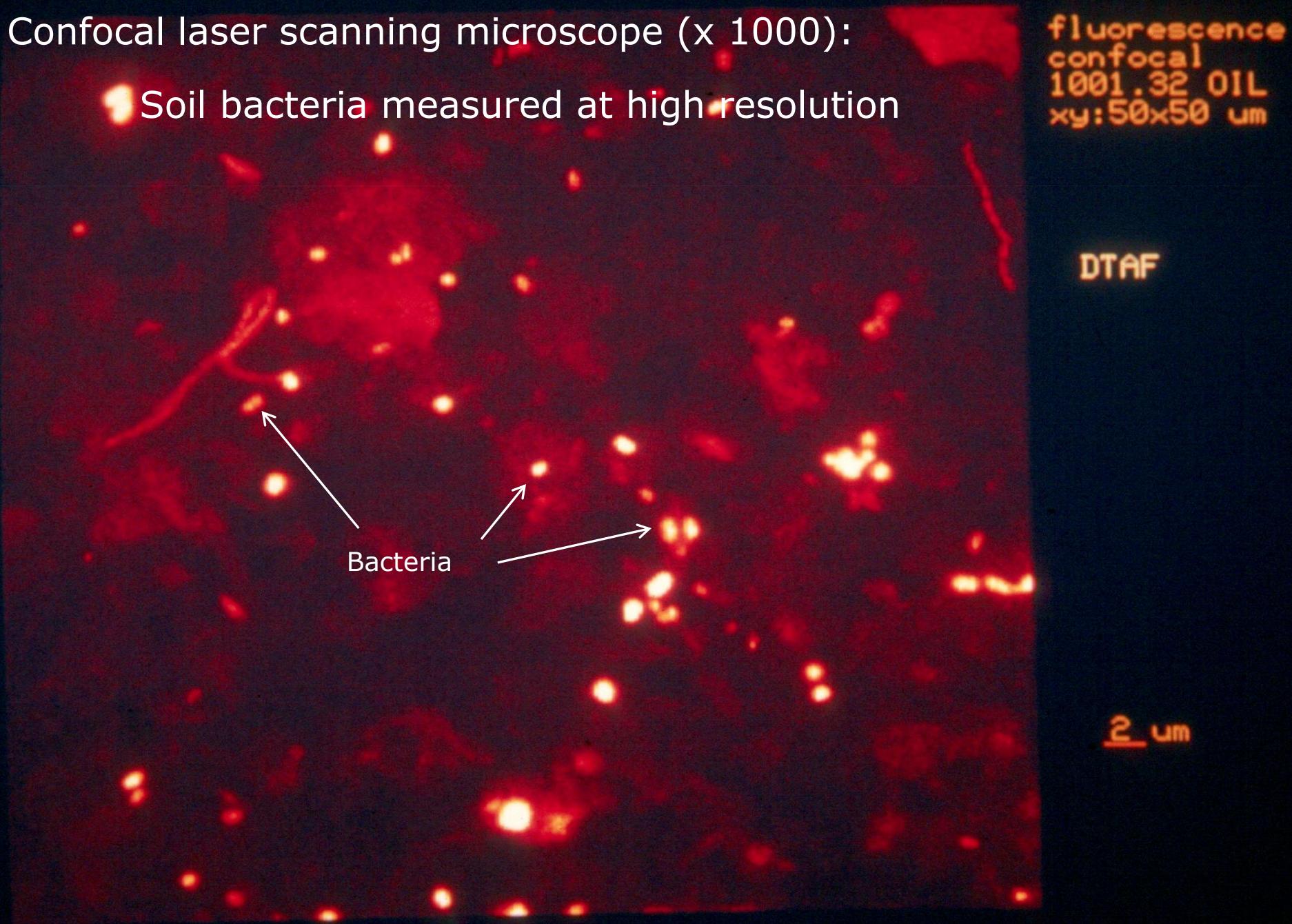
Fungal hyphae stained blue  
(polysaccharides in cell walls)  
are inactive

Fungal hyphae stained red(RNA and  
DNA) are active

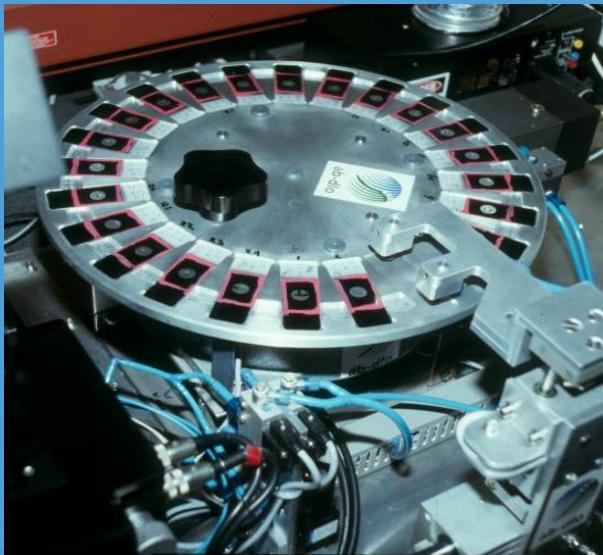
Bacteria

Confocal laser scanning microscope (x 1000):

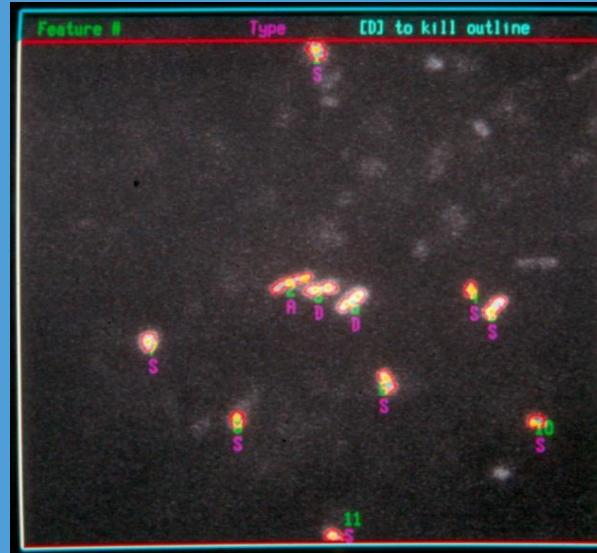
1 Soil bacteria measured at high resolution



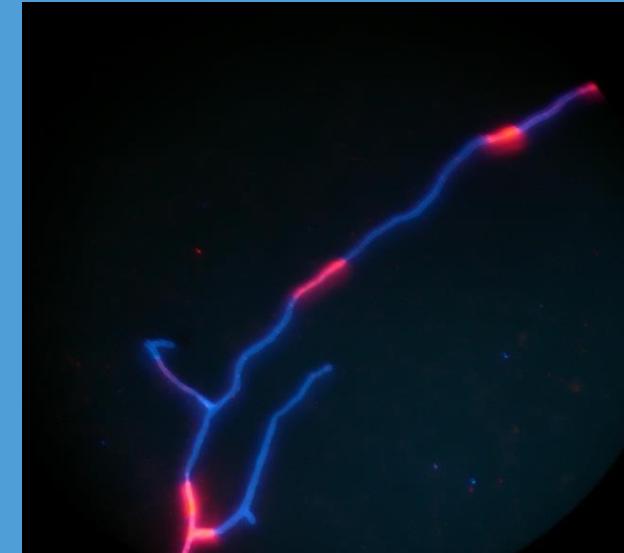
# Biomass of bacteria and fungi measured by direct microscopy



Automatic scanning of  
soil smears



Bacteria measured by  
automatic image analysis



Fungal hyphae, active  
(red) and inactive (blue)

Bloem et al., 1995, Applied and Environmental Microbiology 61, 926-936.  
Bloem, J. and A. Vos. 2004. In "Molecular Microbial Ecology Manual", 2nd edition

# Bacterial activity

Bacterial growth rate: incorporation rate of  $^3\text{H}$ -thymidine and  $^{14}\text{C}$ -leucine into bacterial DNA and proteins during a short incubation (1h).  
Very sensitive.



# N mineralization and mineralizable N

## Aerobic incubation



net N mineralization



Difference is  
proxy for  
N immobilization

Anaerobic -----> part of microbial biomass killed



higher N mineralization

# Microbial community structure measured by PLFA

- Phospholipid fatty acids (PLFA) are membrane components of all living cells
- Fingerprint of microbial community structure (about 30 PLFAs)
- Biomarkers: fungi, total bacteria, major groups of bacteria (actinomycetes, Gram +, Gram -,...)

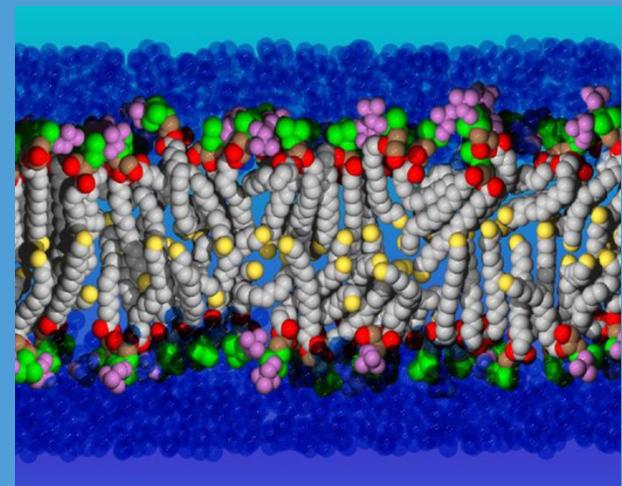
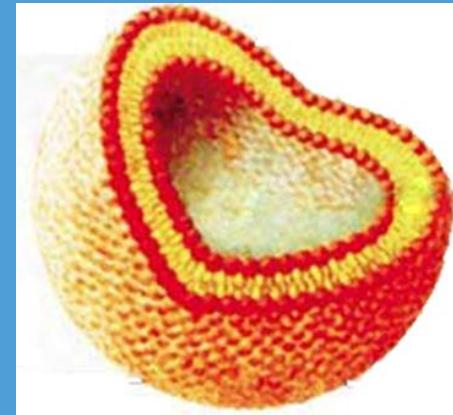


Image courtesy of Wikimedia Commons under the  
GNU Free Documentation License

## Microbial biomass

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- Direct microscopy (may be combined with automatic image analysis): number and body size (biomass) of different functional groups i.e. fungi, bacteria, protozoa....; biomass calculated from biovolume
- Chloroform fumigation extraction (CFE): soil is fumigated with chloroform, increase in extractable organic carbon (and nitrogen) is a measure of total microbial biomass (C and N)
- Amounts of PLFA also used as proxy for biomass

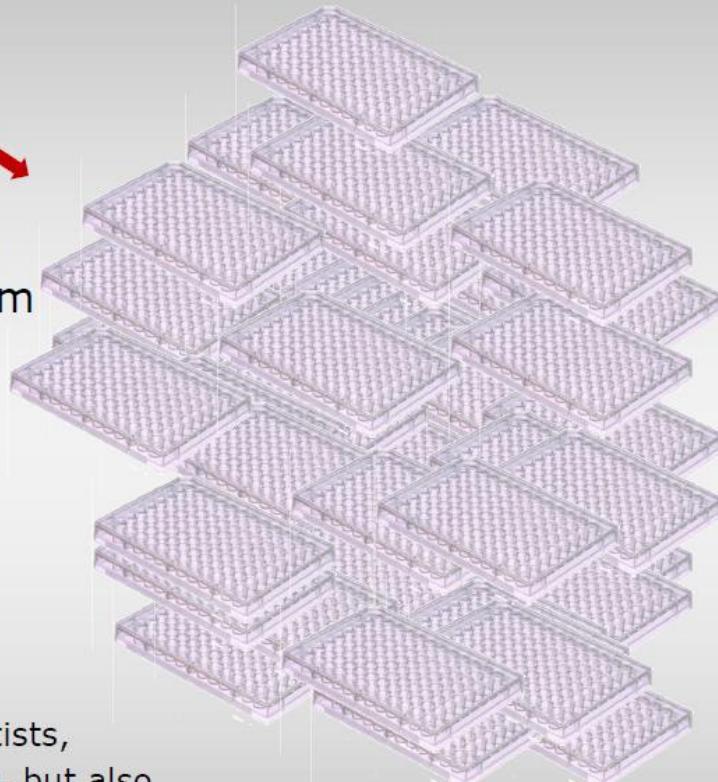
# Protozoën geteld na seriële verdunning in voedingsmedium

(next figures: Dr. Anna Maria Fiore-Donno, University of Cologne)

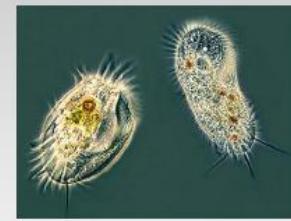


soil suspended  
in some medium

## Most Probable Number



When applied to protists,  
not only the medium, but also  
the available food influence the outcome.  
Observation possible only after some days,  
protists need some time to multiply



Ciliaten



Schaal-amoebe

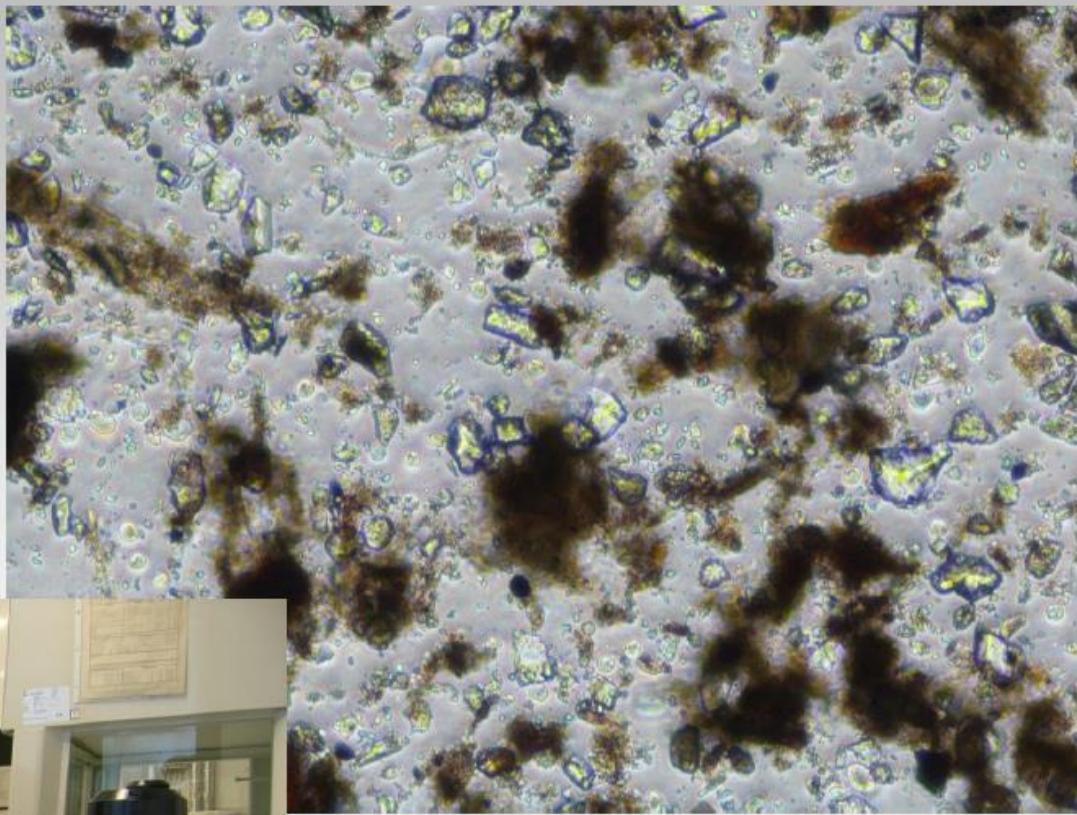
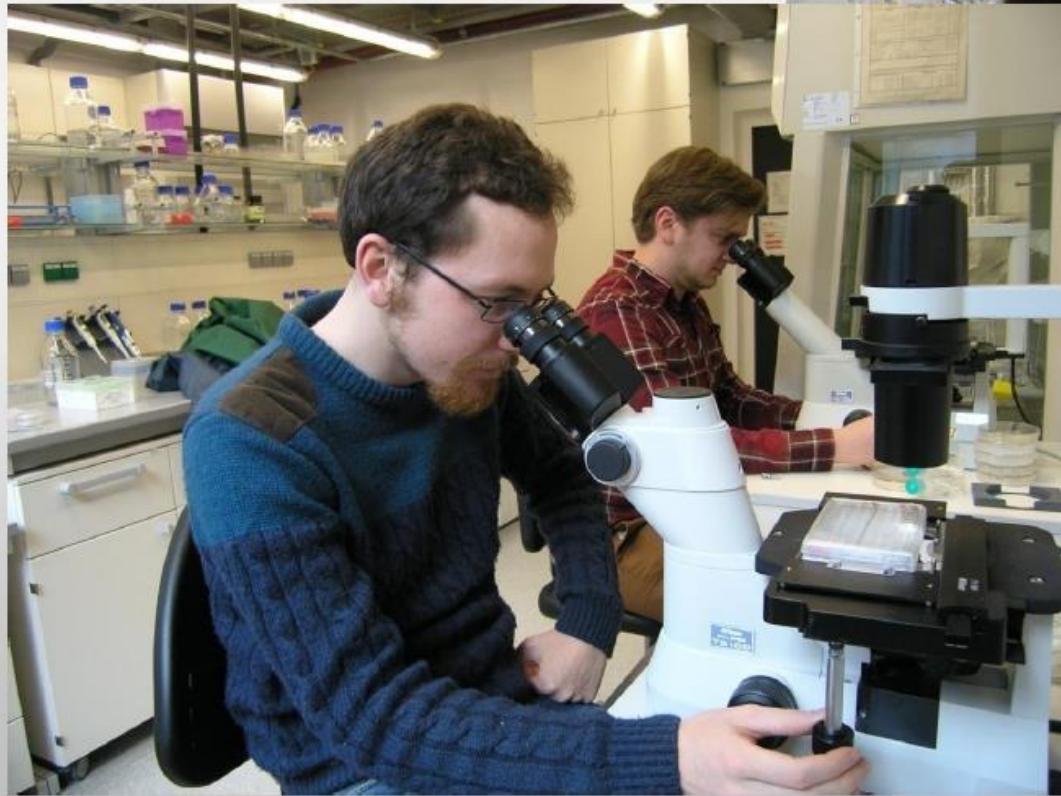


Amoebe

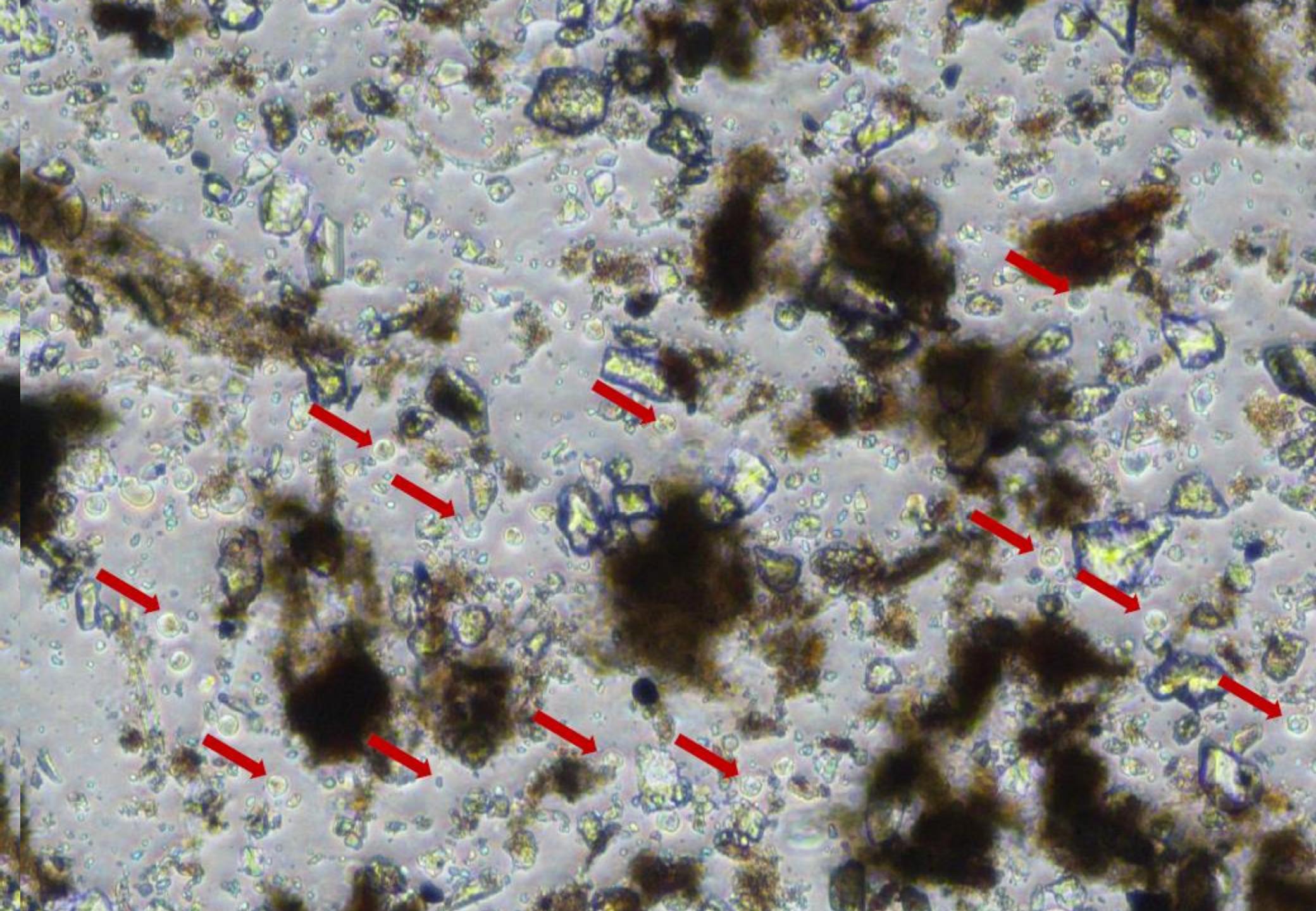


flagellaat

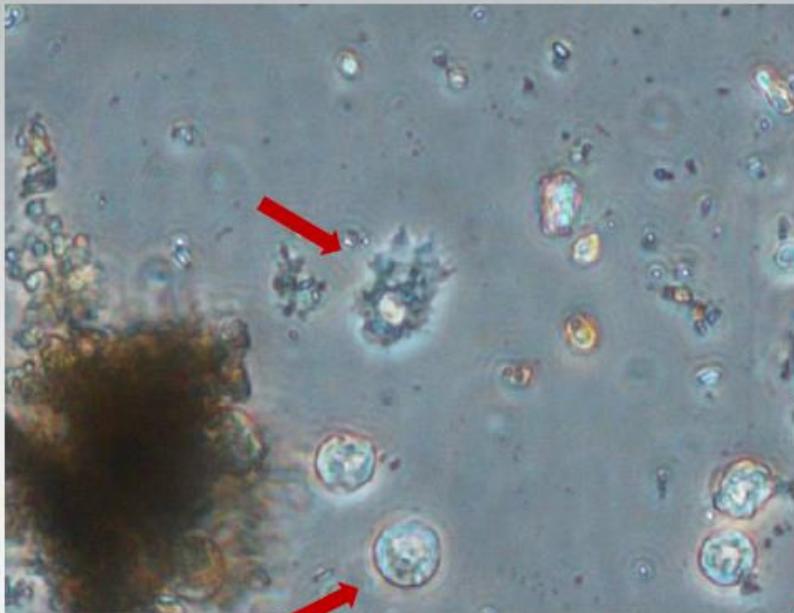
A difficult and repetitive task



... that is what they see



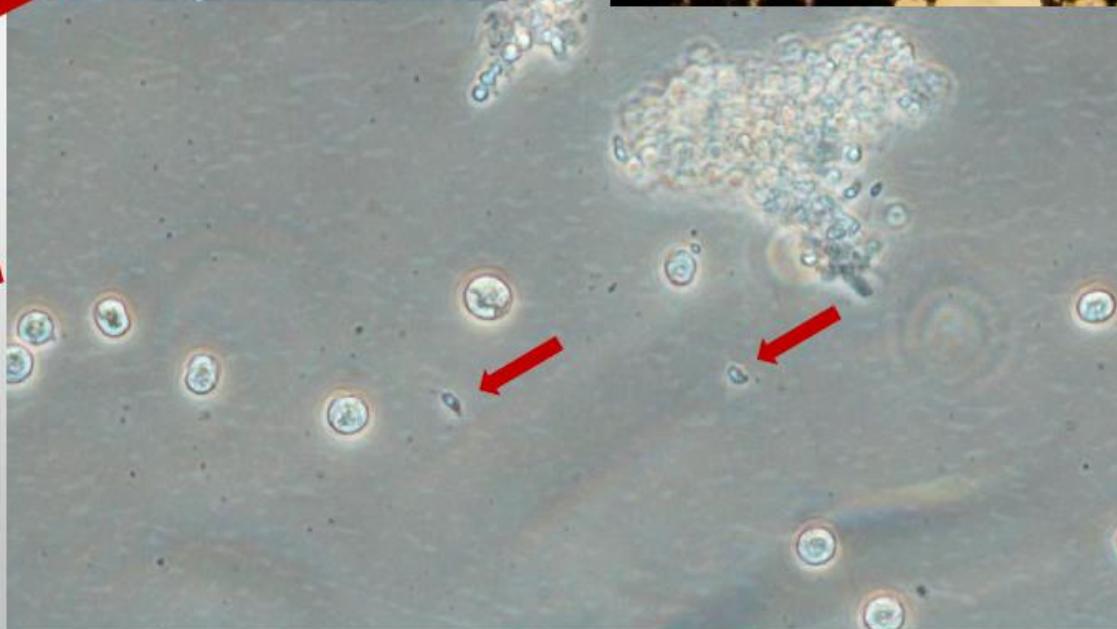
*Acanthamoeba* sp.



Testate amoeba (Arcellina)

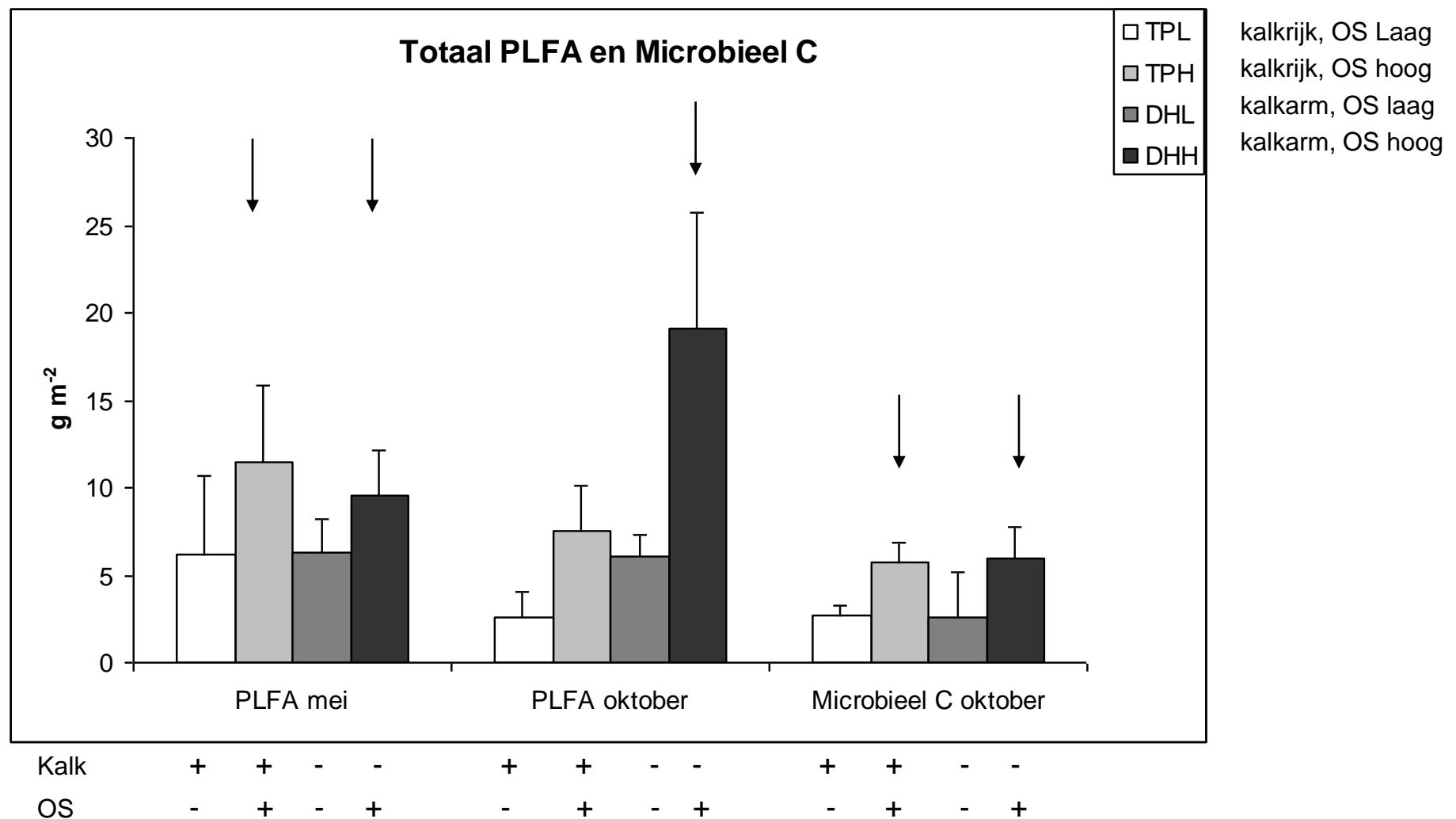


Testate  
amoebae  
(Rhizaria)



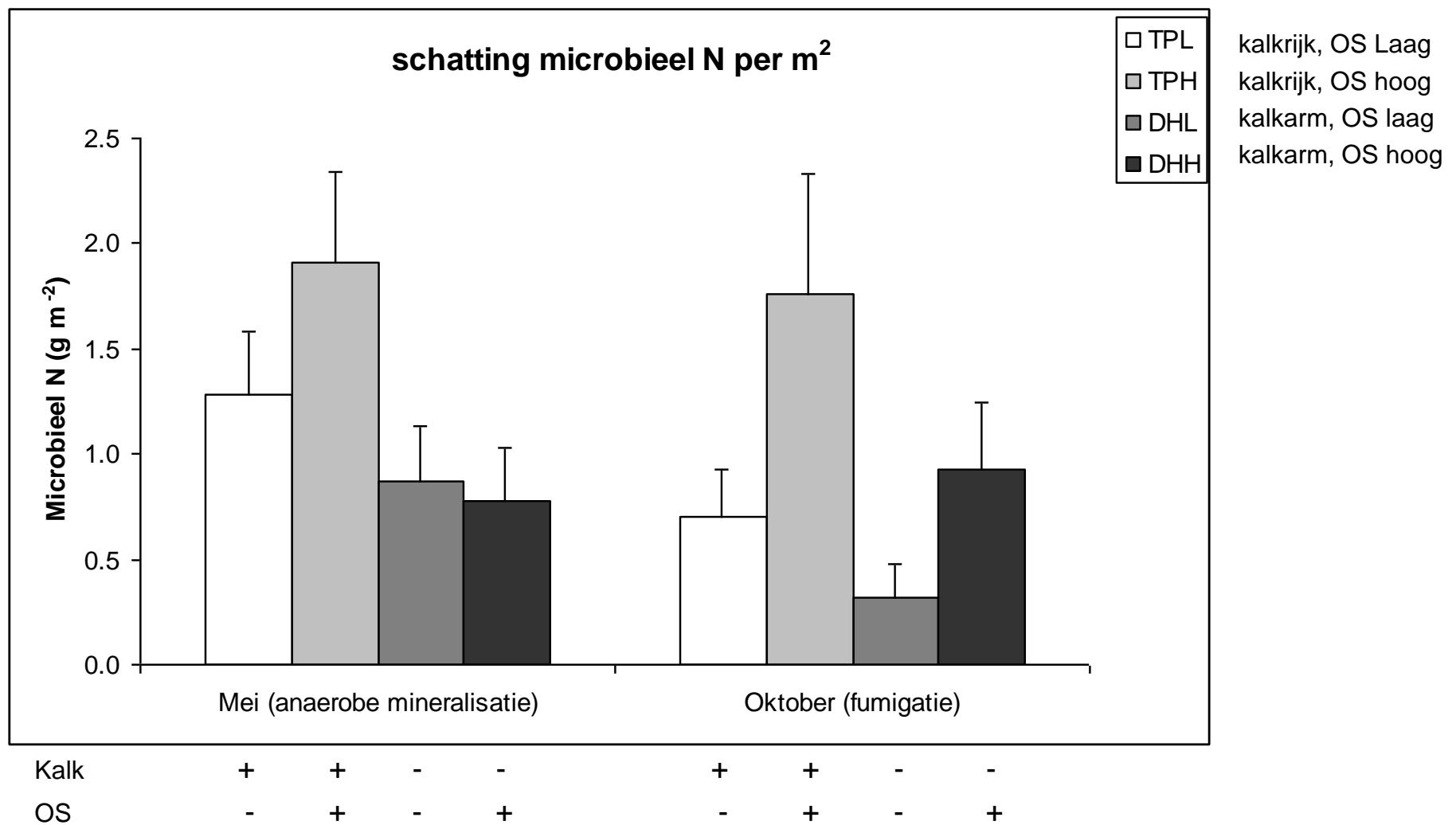
Flagellates  
(Cercozoa)

## Microbiële biomassa C en PLFAs hoger met meer organische stof, geen effect van kalk

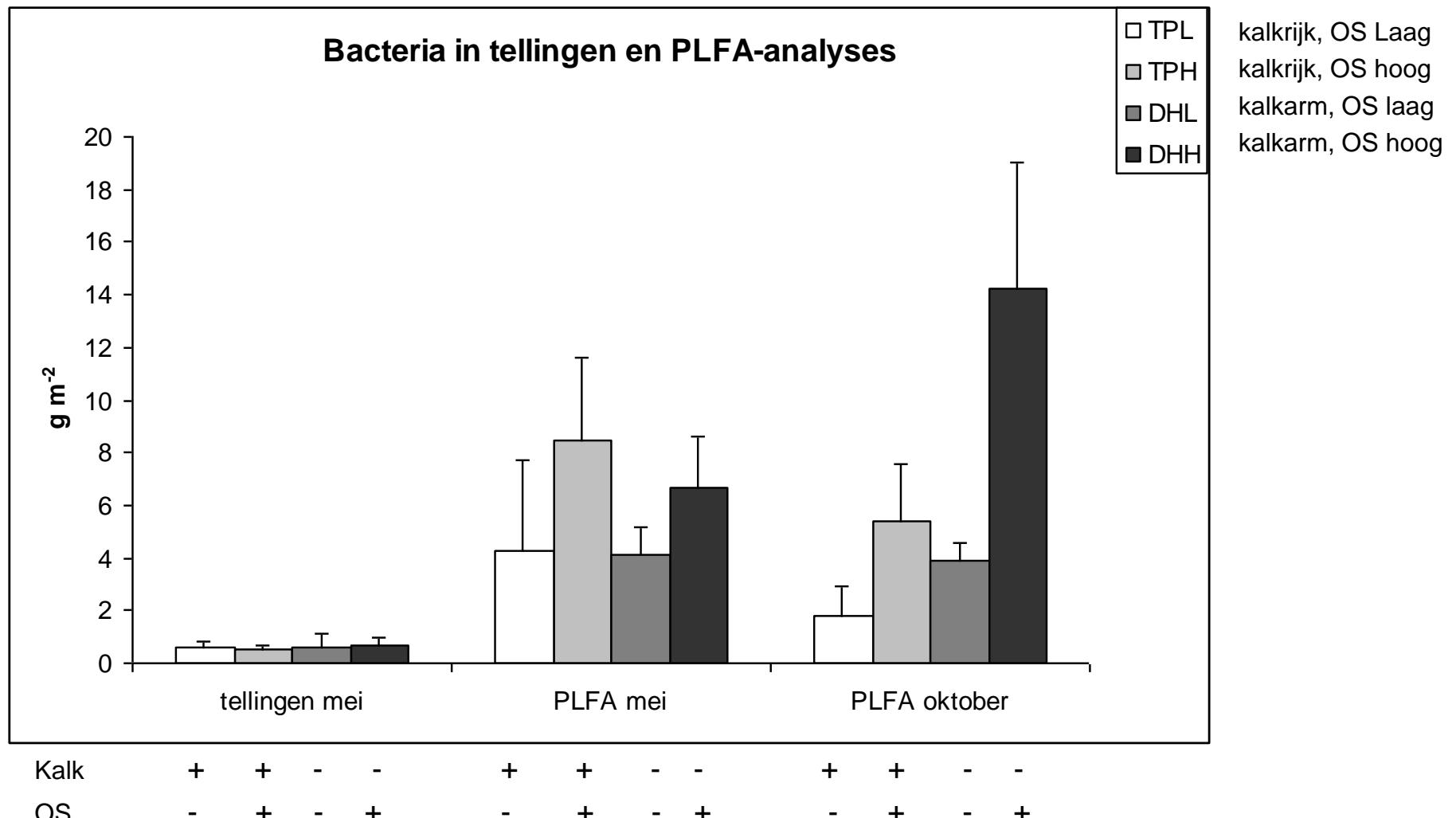


## Microbiële biomassa-N

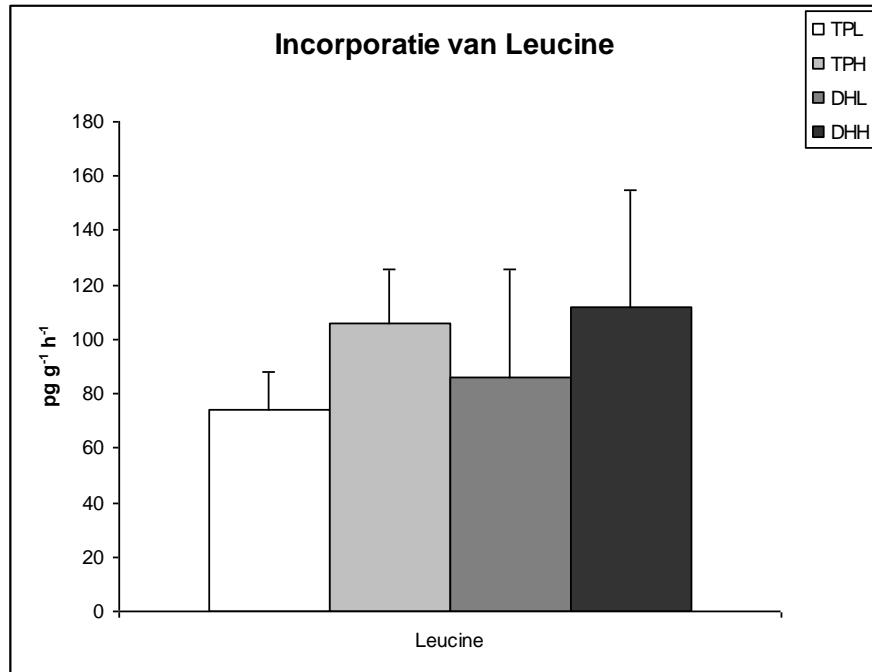
Hoger met kalk (en met organische stof in oktober)



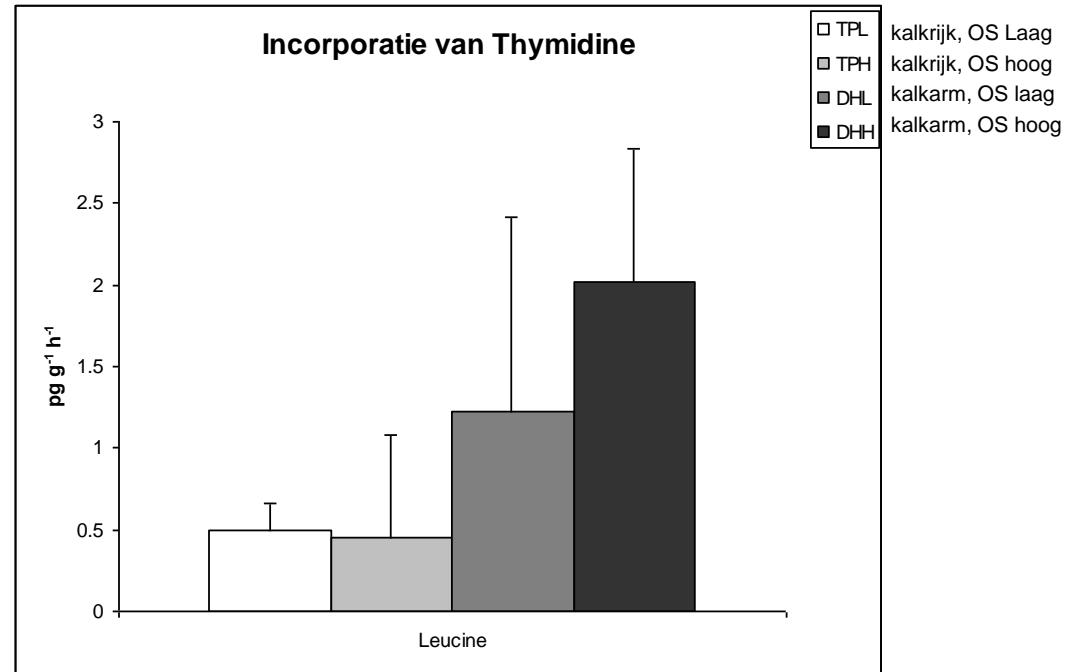
## Bacteriebiomassa: PLFA hoger met meer organische stof microscopische tellingen zeer laag



## Bacteriële productie: DNA synthese (thymidine inbouw) *hoger* in kalkarm, alle waarden erg laag



kalk	+	+	-	-
OS	-	+	-	+

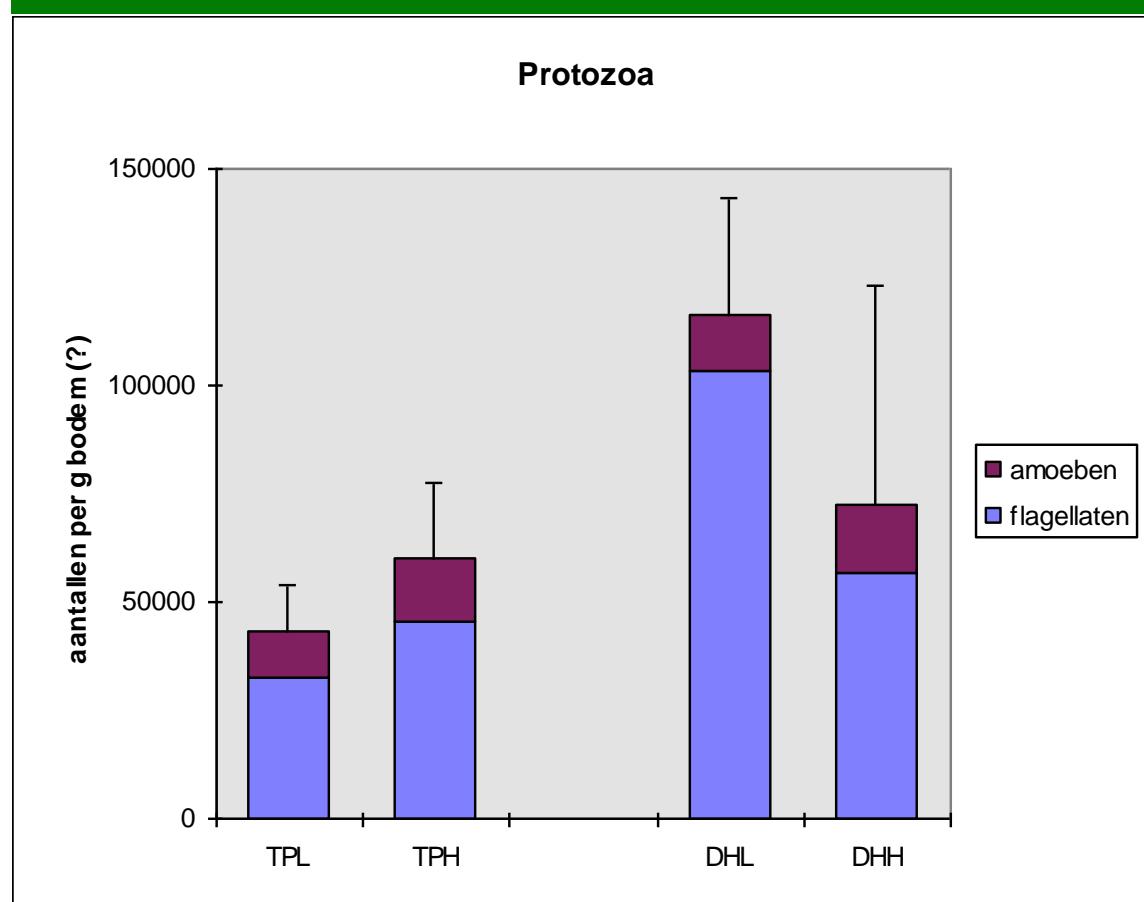


kalk	+	+	-	-
OS	-	+	-	+

TPL	kalkrijk, OS Laag
TPH	kalkrijk, OS hoog
DHL	kalkarm, OS laag
DHH	kalkarm, OS hoog

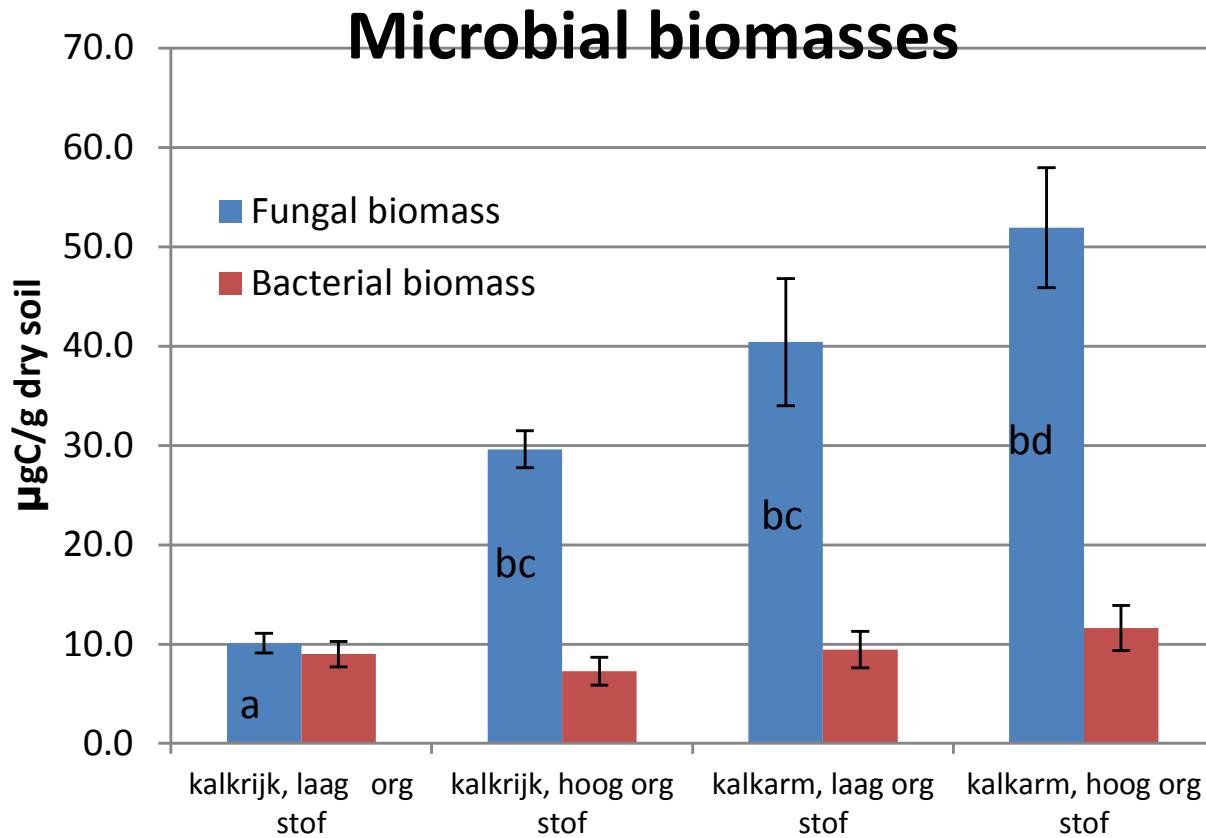
## Protozoën

meer flagellaten in kalkarme bodem, komt overeen met bacterieproductie



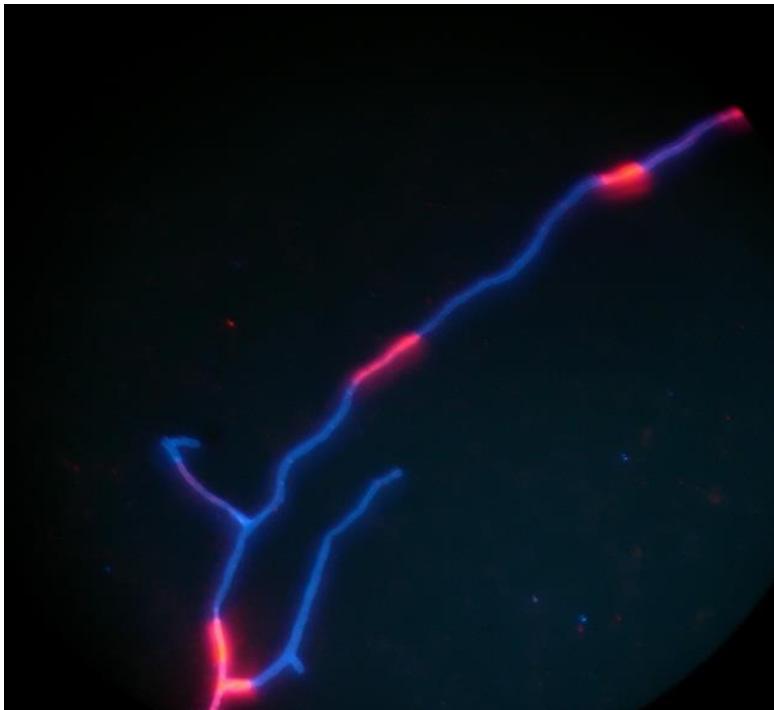
kalk	+	+	-	-
OS	-	+	-	+

## Biomassa van schimmels en bacteriën (microscopische metingen) schimmel-gedomineerde bodems



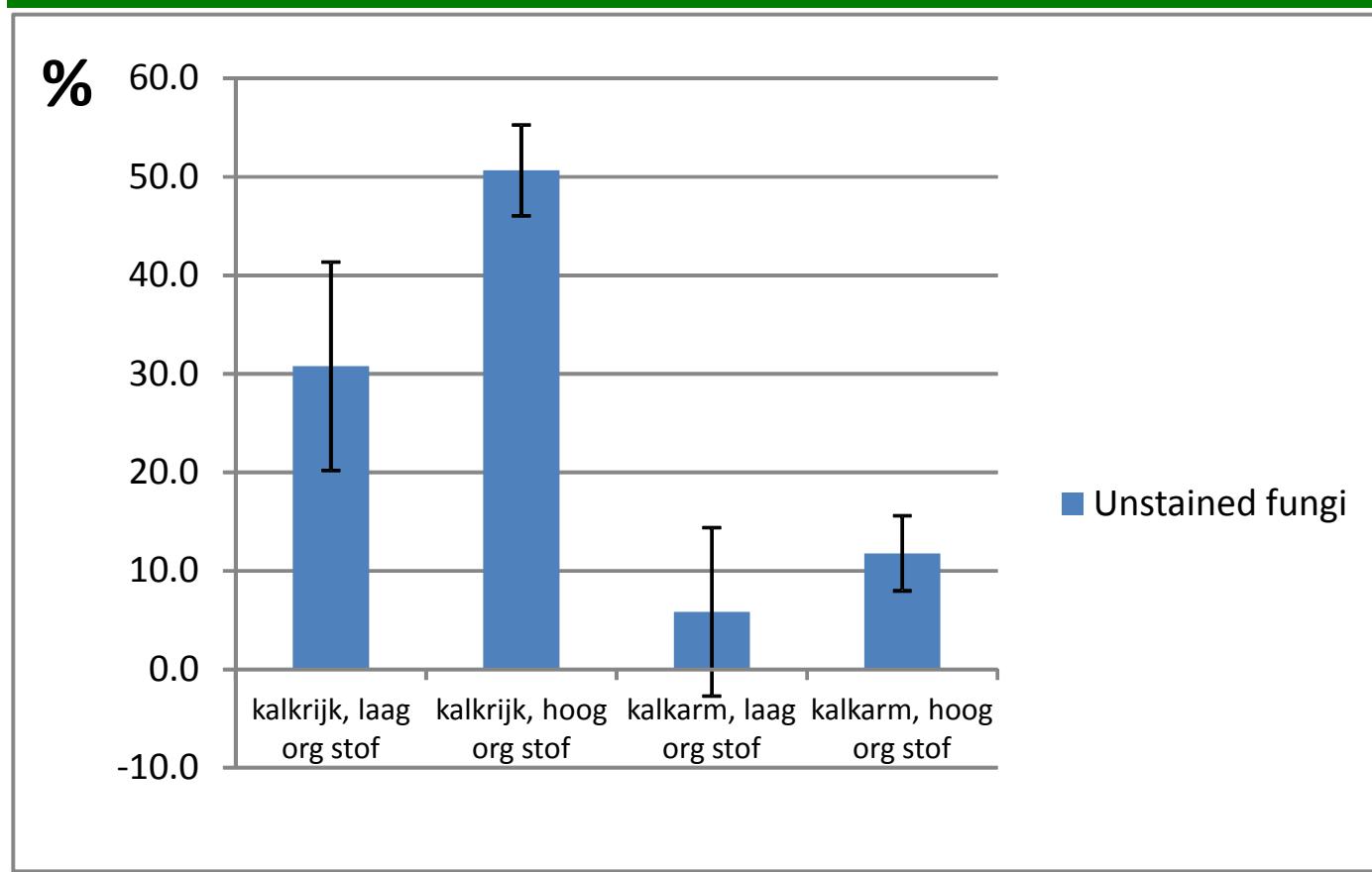
## Gekleurde (hyaline) en ongekleurde (melanine) schimmels

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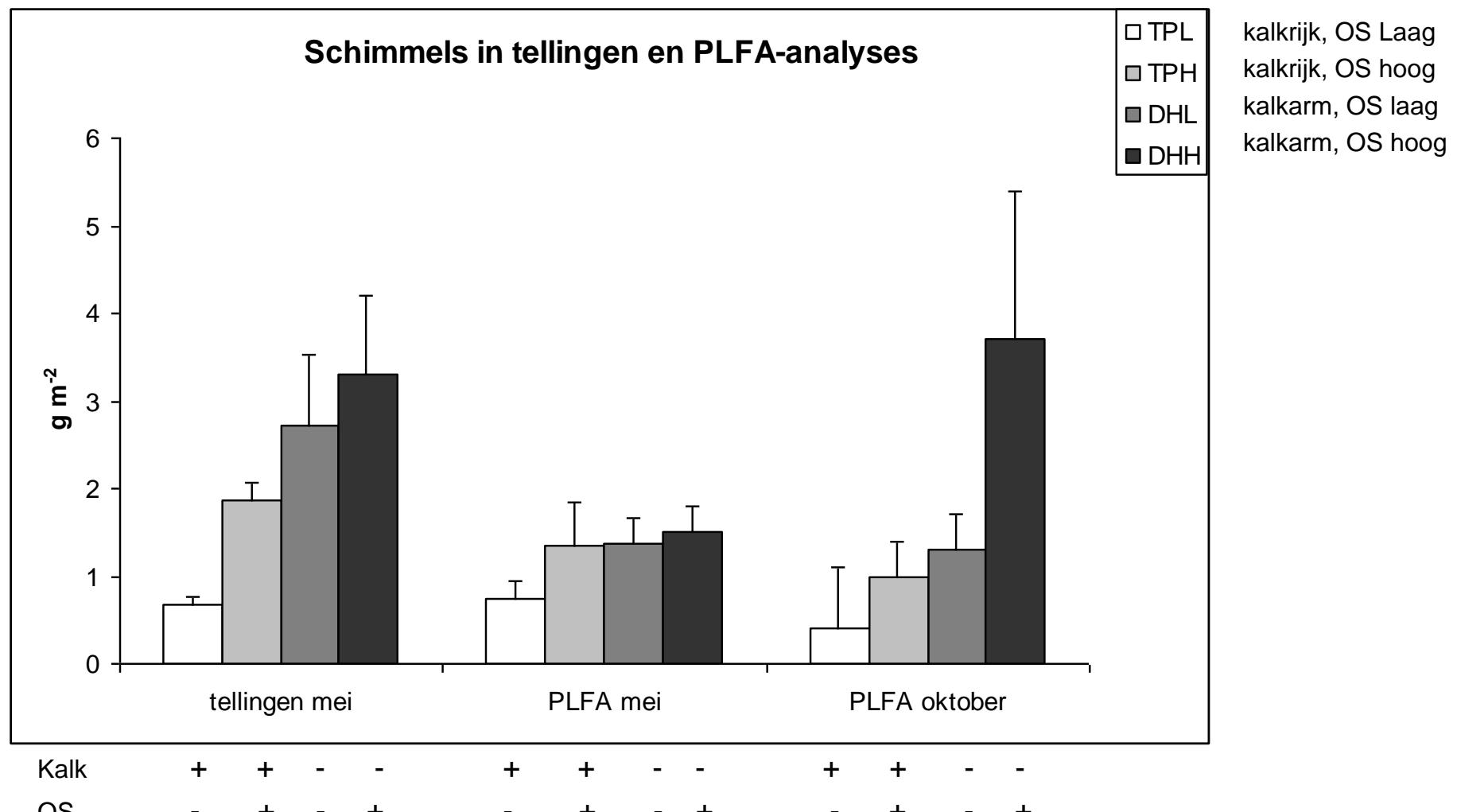
Melanine is donker pigment in celwanden  
bescherming tegen stress: straling, hitte, droogte, antagonisten, infecties en grazers  
complexe ringstructuren, moeilijk afbreekbaar, vertraagt afbraak necromassa, speelt rol bij humusvorming

## Kalkrijke bodem meer (niet gekleurde) melanine schimmels



# Schimmelbiomassa

Hoger in kalkarme bodem, neemt toe met organische stof



# Conclusies

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- Microbiële biomassa neemt toe met organische stof
- Kalk geen effect op biomassa C
- Kalkrijke bodem
  - meer biomassa N
  - meer mineraliseerbare N
  - groter aandeel melanine schimmels
- Kalkarme bodem
  - meer schimmels
  - hogere bacteriële groeisnelheid (celproductie)
  - meer flagellaten (bacterivore protozoën)
- Microbiële biomassa sterk gedomineerd door schimmels, bacterie activiteit laag:
  - wijst op weinig N vastlegging via bacteriële voedselweb.
  - mogelijk wel N vastlegging via schimmels (celwanden en residuen)