

# Development of DNA metabarcoding tools for rapid assessment of soil faunal diversity

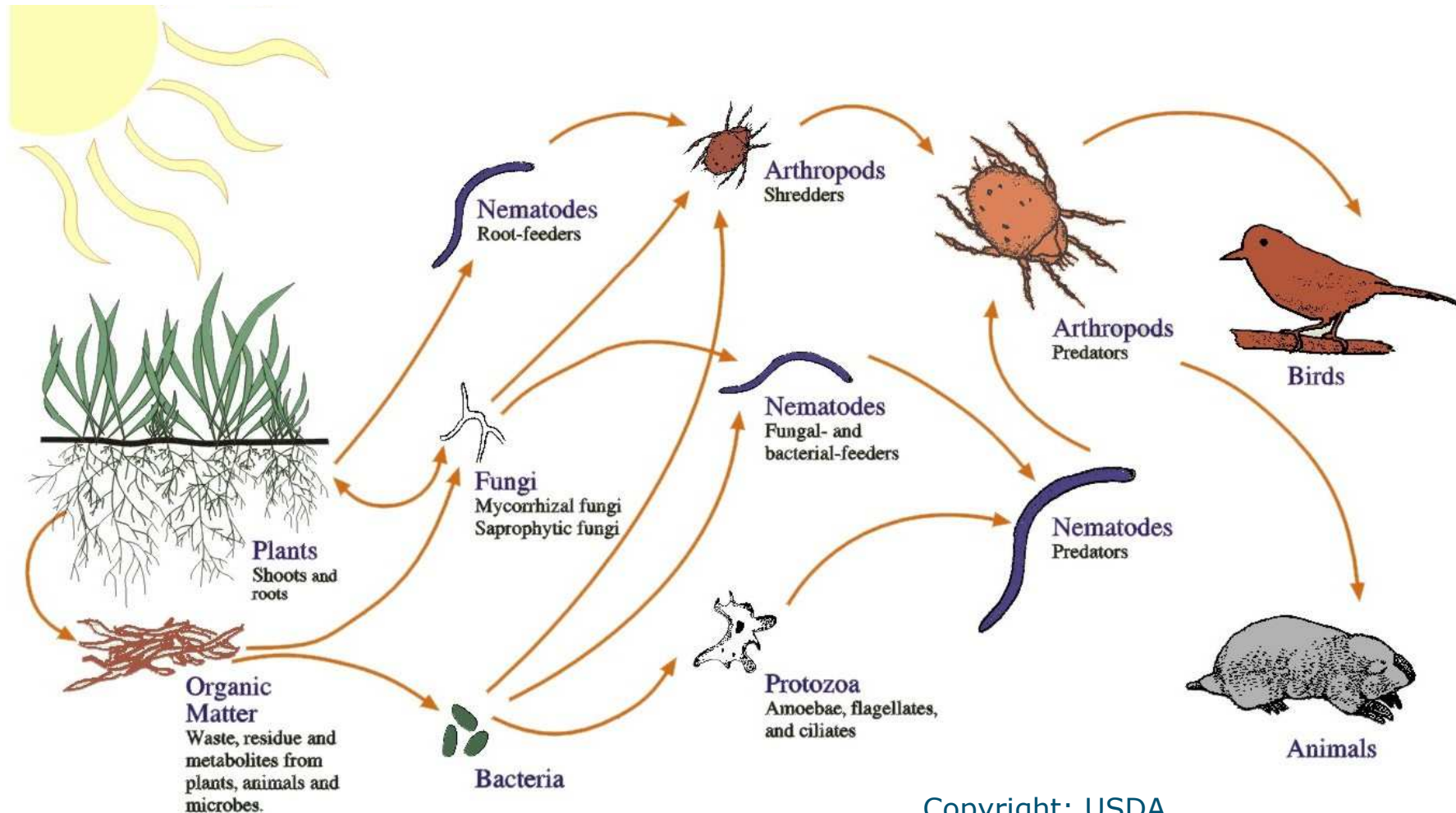
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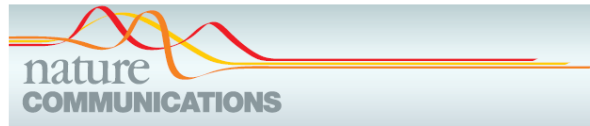
# Understanding functions of soil biodiversity

- Role in provision of ecosystem services
- Human impact and use as ecological indicators



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# Spatiotemporal patterns in soil communities



ARTICLE

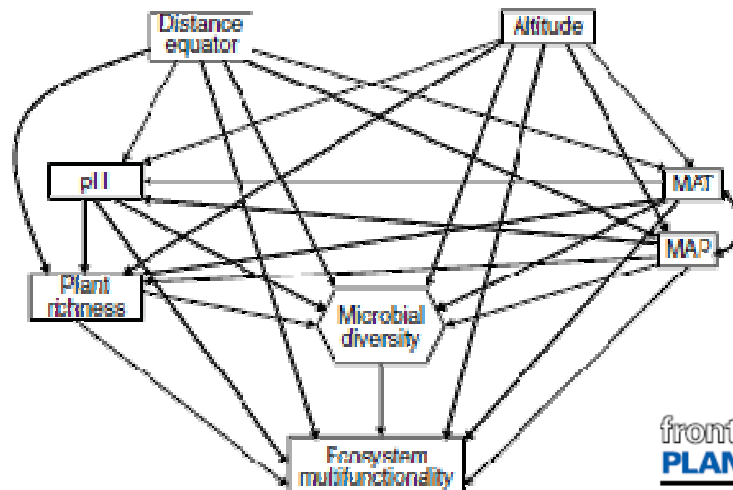
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OPEN

## Microbial diversity drives multifunctionality in terrestrial ecosystems

Manuel Delgado-Baquerizo<sup>1</sup>, Fernando T. Maestre<sup>2</sup>, Peter B. Reich<sup>1,3</sup>, Thomas C. Jeffries<sup>1</sup>, Juan J. Gaitan<sup>4</sup>, Daniel Encinar<sup>2</sup>, Miguel Berdugo<sup>2</sup>, Colin D. Campbell<sup>5</sup> & Brajesh K. Singh<sup>1,6</sup>



## The diversity and biogeography of soil bacterial communities

Noah Fierer<sup>\*†</sup> and Robert B. Jackson<sup>\*†</sup>

<sup>\*</sup>Department of Biology and <sup>†</sup>Nicholas School of the Environment and Earth Sciences,



FEMS Microbiology Ecology 39 (2002) 183–191

## Microbial diversity along a transect of agronomic zones

A.M. Ibekwe<sup>a,b,\*</sup>, A.C. Kennedy<sup>a</sup>, P.S. Frohne<sup>a</sup>, S.K. Papiernik<sup>b</sup>, C.-H. Yang<sup>c</sup>, D.E. Crowley<sup>c</sup>

frontiers in  
**PLANT SCIENCE**

HYPOTHESIS AND THEORY ARTICLE

published: 10 December 2013  
doi: 10.3389/fpls.2013.00500



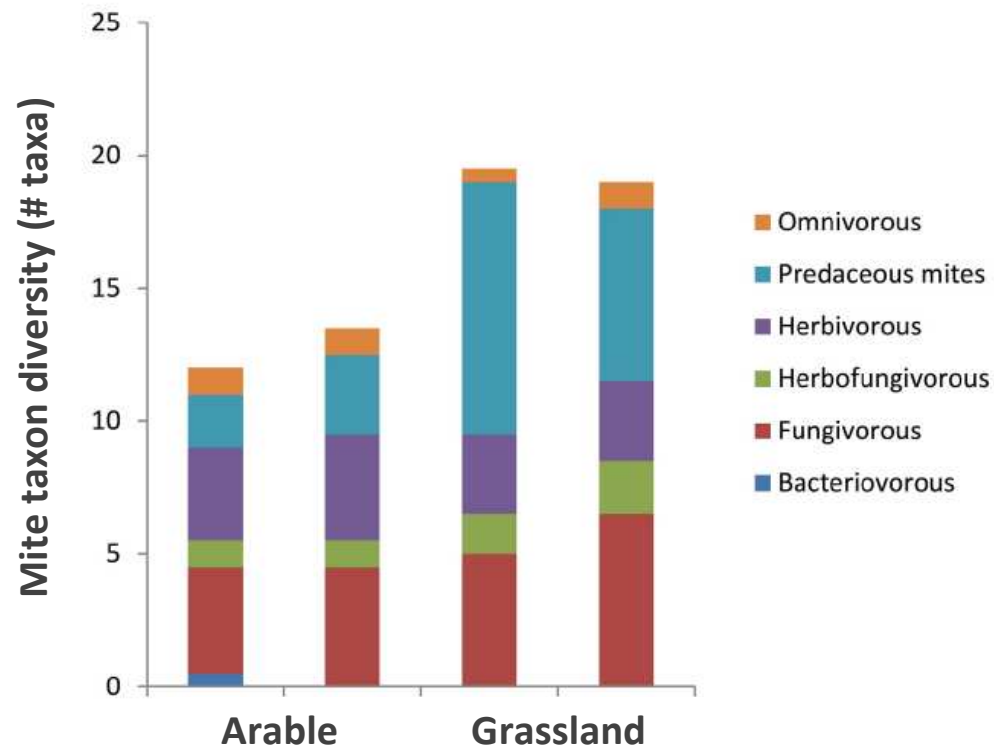
## Plant species distributions along environmental gradients: do belowground interactions with fungi matter?

Loïc Pellissier<sup>1\*\*\*</sup>, Eric Pinto-Figueroa<sup>1‡</sup>, Hélène Niculita-Hirzel<sup>2</sup>, Mari Moora<sup>3</sup>, Lucas Villard<sup>1</sup>, Jérôme Goudet<sup>1</sup>, Nicolas Guex<sup>4</sup>, Marco Pagni<sup>4</sup>, Ioannis Xenarios<sup>1</sup>, Ian Sanders<sup>4</sup> and Antoine Guisan<sup>1,5</sup>

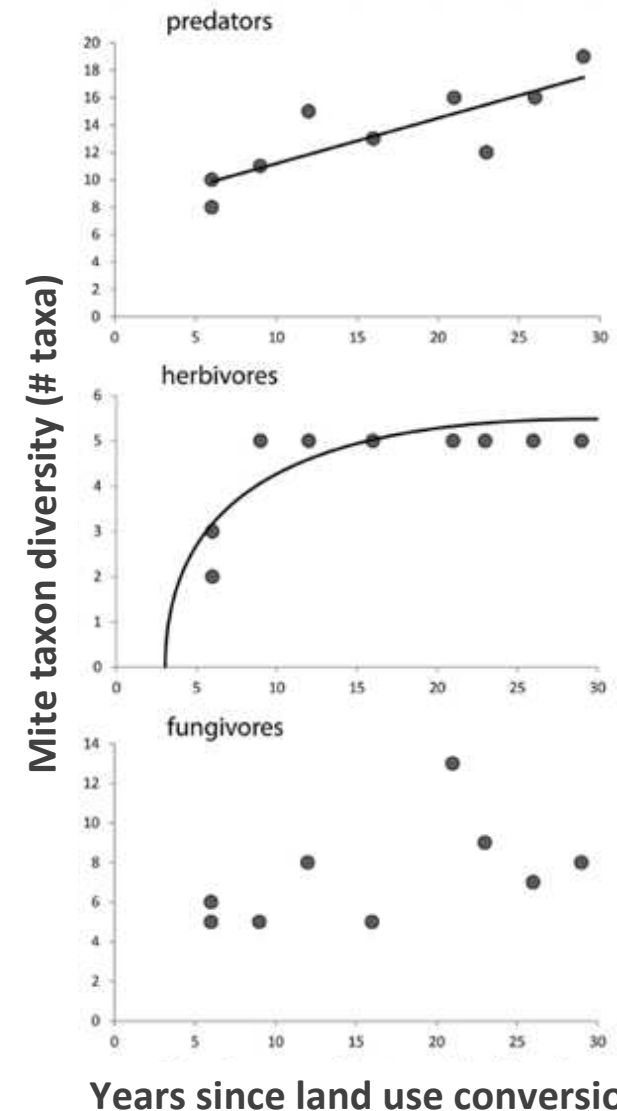


# Spatiotemporal patterns in soil communities

Data for soil fauna often restricted to limited sets of locations / time points



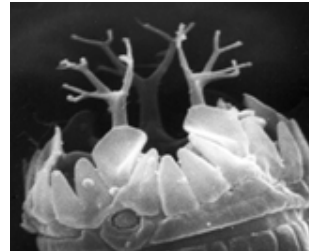
De Groot *et al.* 2016,  
Frontiers in Env. Science



# Characterizing soil fauna

Still based largely on morphological identification:

- Time consuming
- Unique characters lacking e.g. in juveniles
- Depends on highly skilled taxonomists
- Bias due to soil extraction and cultivation

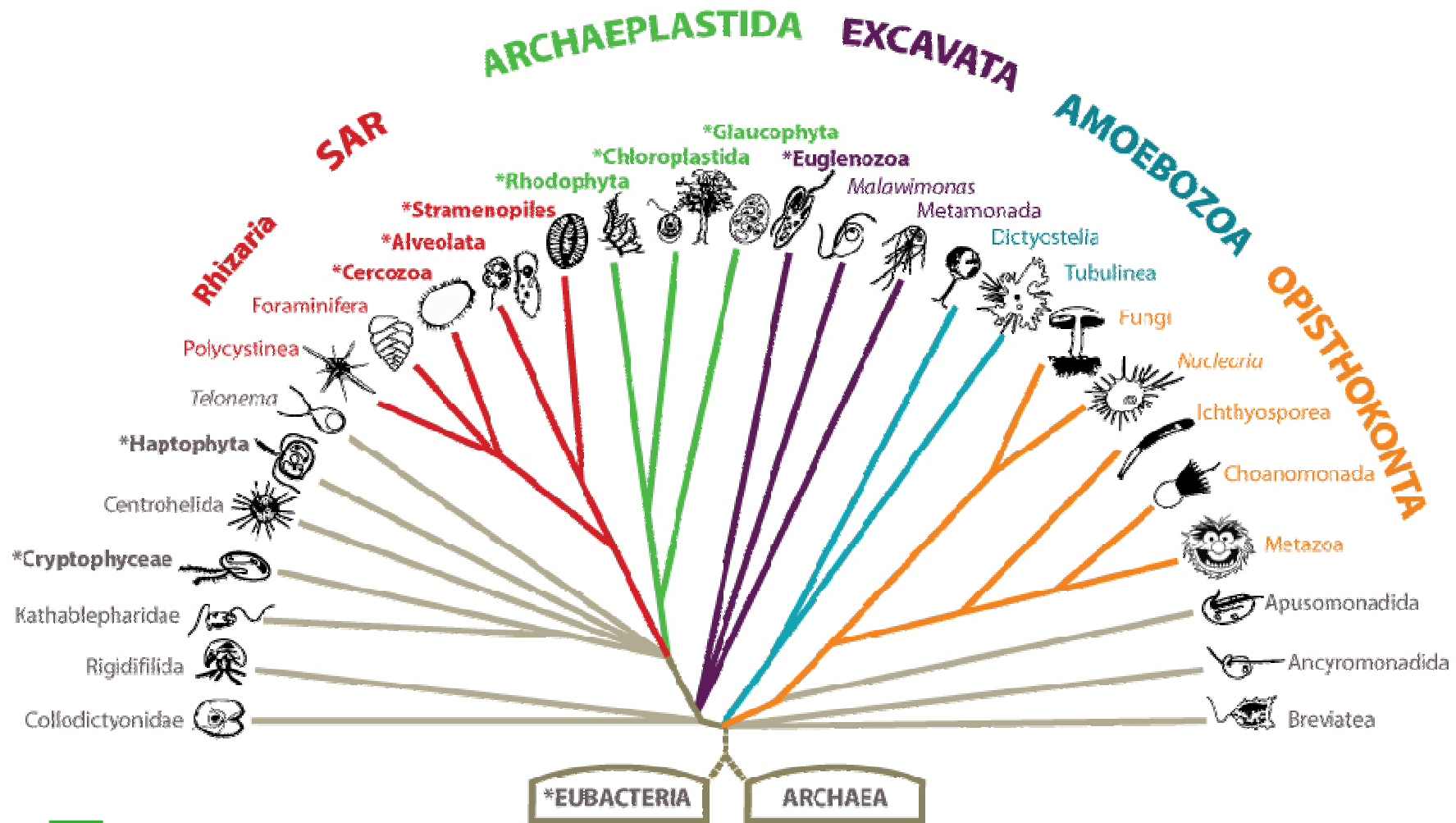


➤ **Molecular alternatives promise higher throughput & resolution**



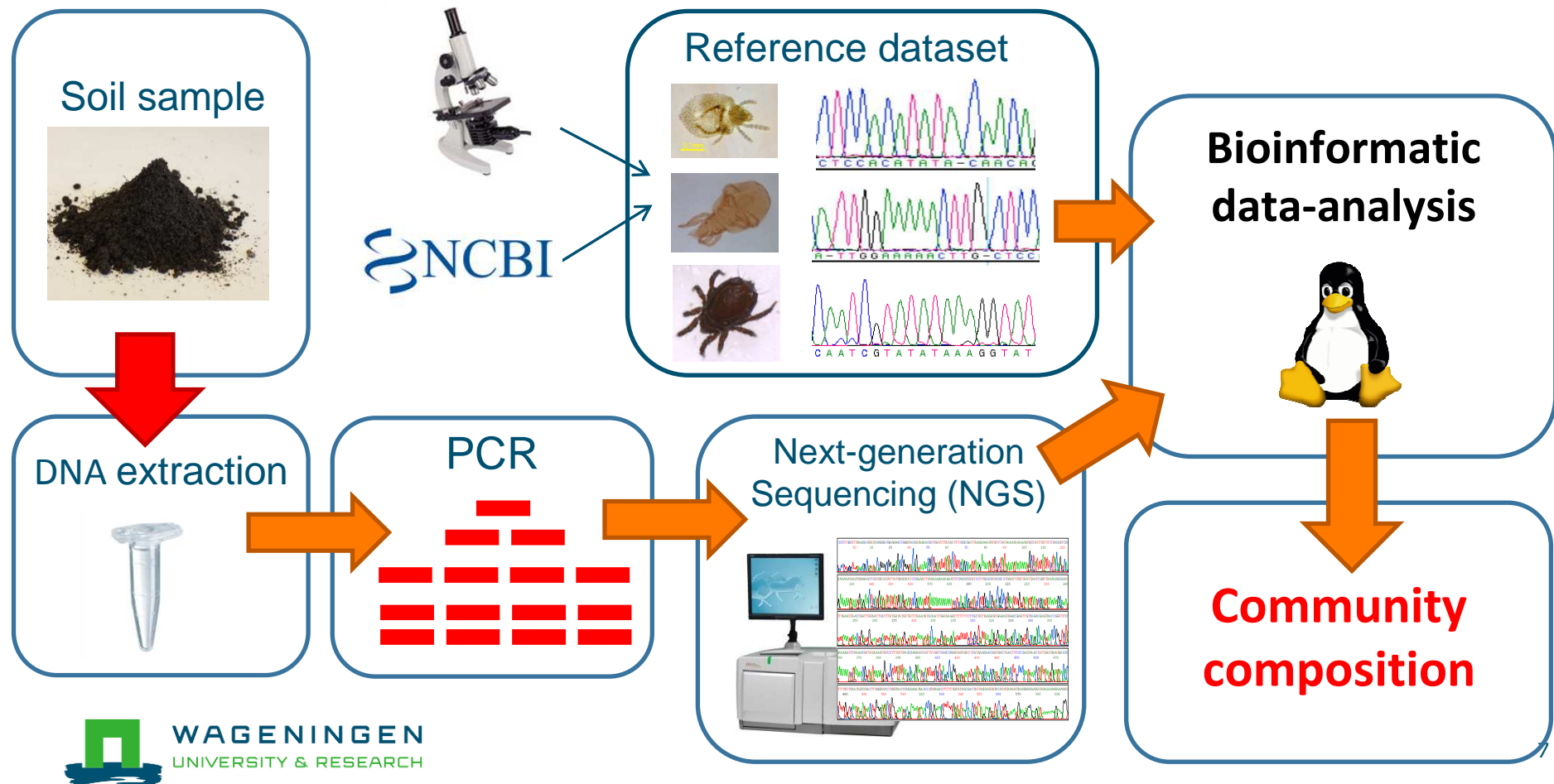
# Soil fauna in perspective

Huge diversity of other soil eukaryotes, many of which are poorly understood

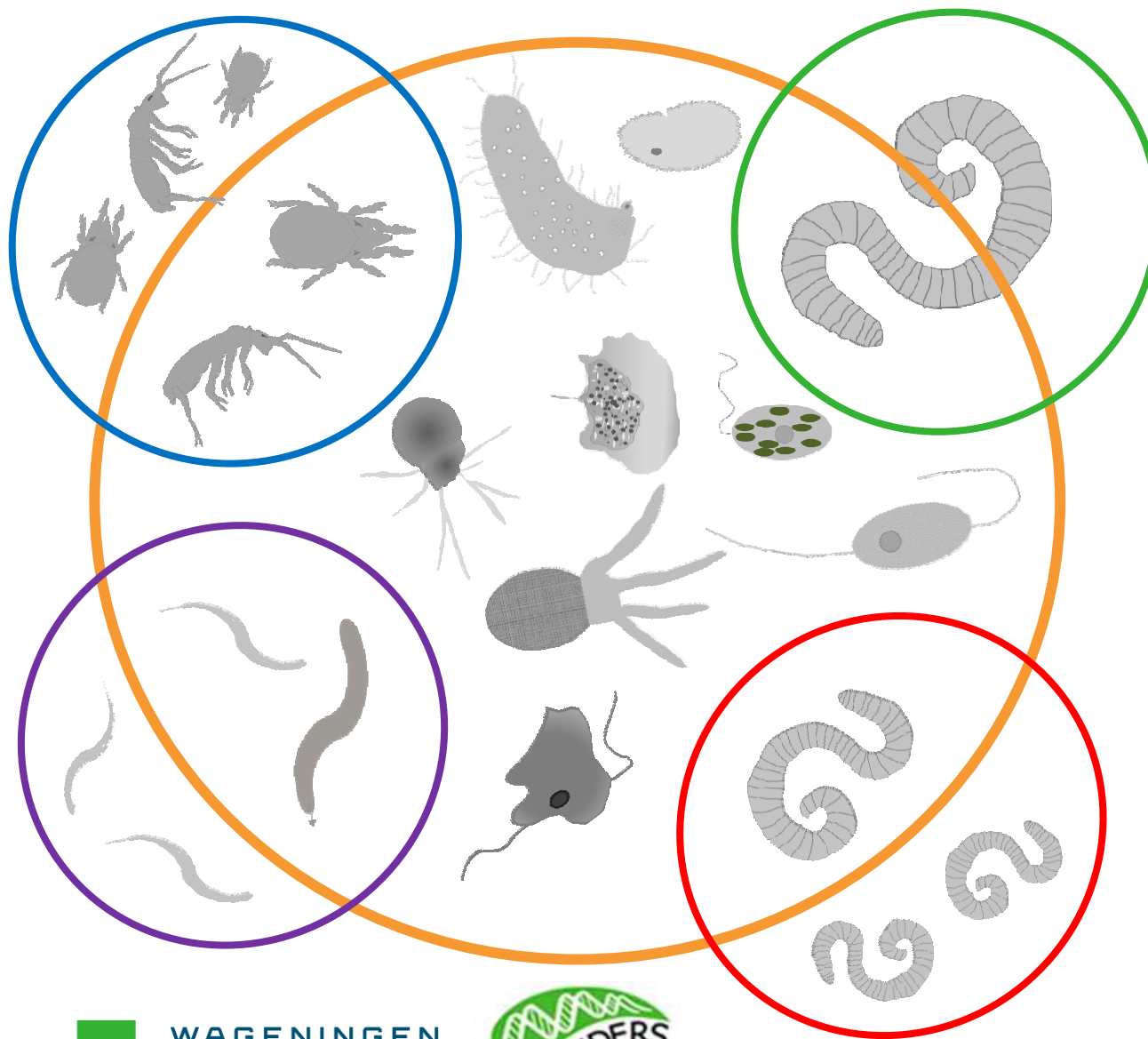


# The principle of DNA metabarcoding

- *DNA-barcode*: fragment of DNA of which the code differs among taxa
- Up to 200 samples analysed in one run



# A tiered approach for high taxonomic resolution



## **Tier 1**

Eukaryotes  
incl. protists  
(18S)

## **Tier 2**

Microarthropods  
(CO1)

Enchytraeids  
(H3)

Nematodes  
(18S)

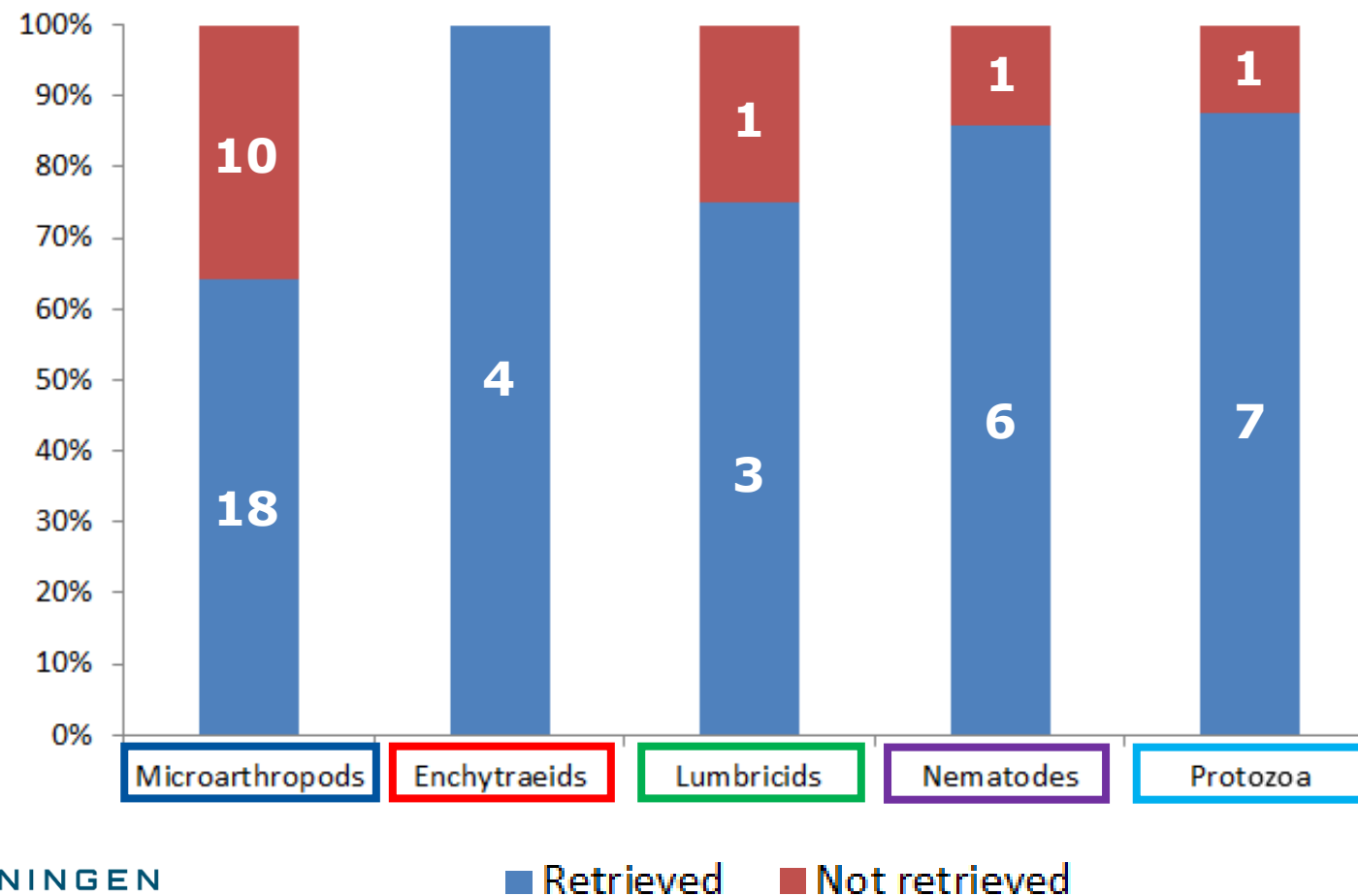
Lumbricids  
(CO1)



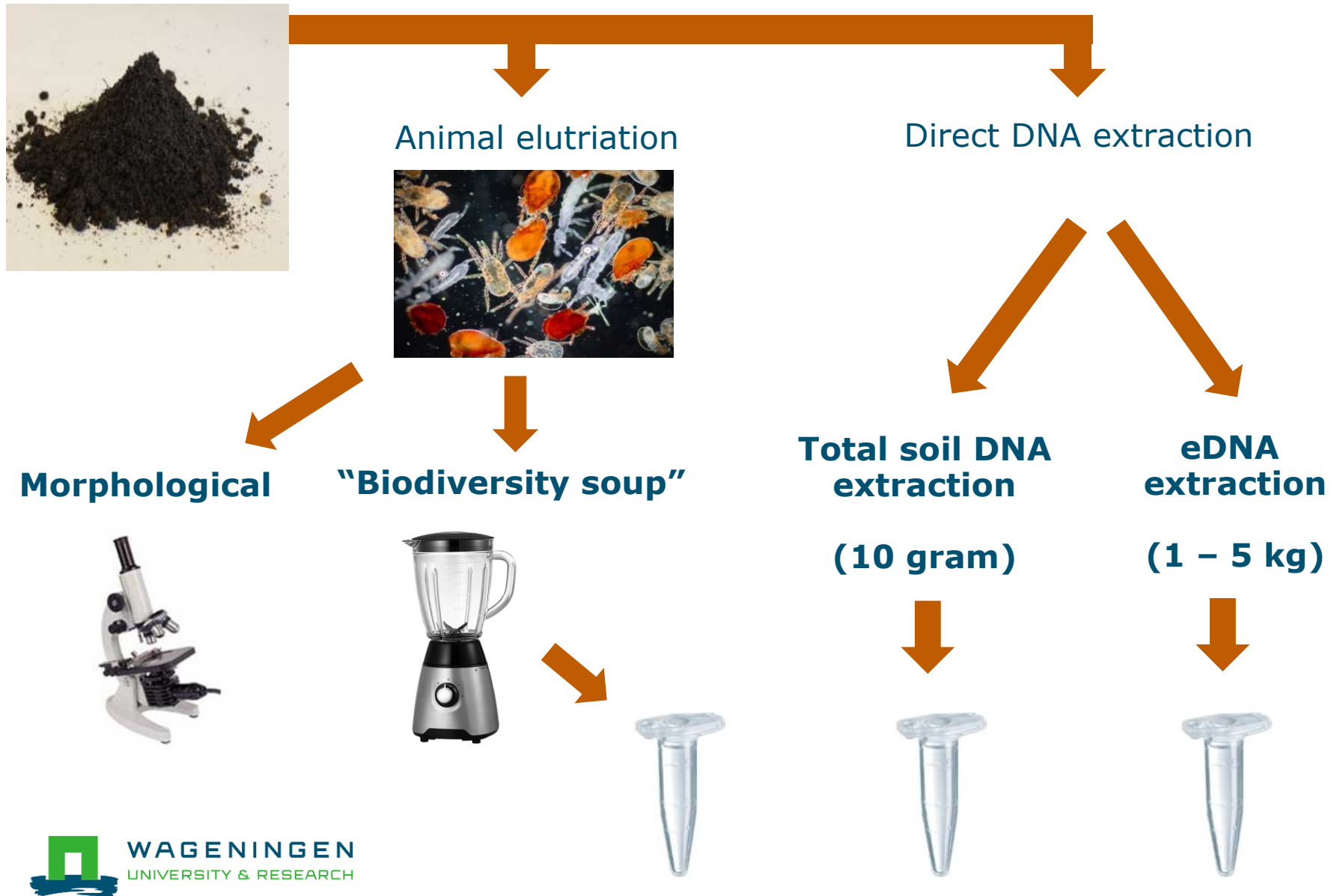
# Method validation: mock communities



- DNA extracts from artificial communities with known composition
- Majority of added taxa retrieved, but some are missing



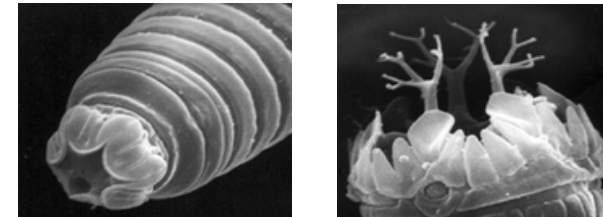
# Comparison of methods: ways to extract DNA



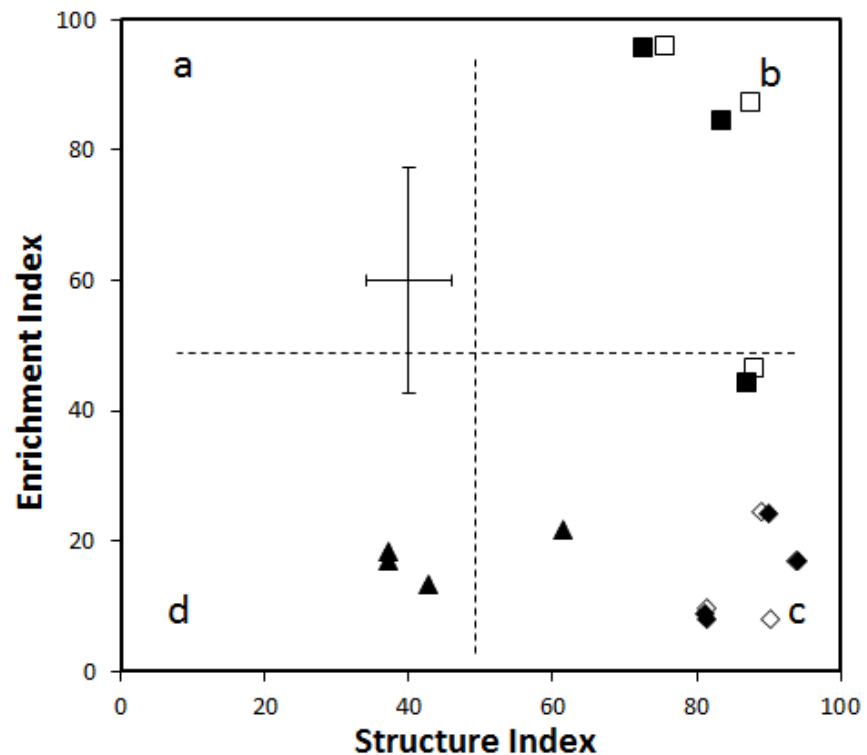
# Comparison of methods: morphology vs DNA

Nematode taxonomic and functional composition via three methods:

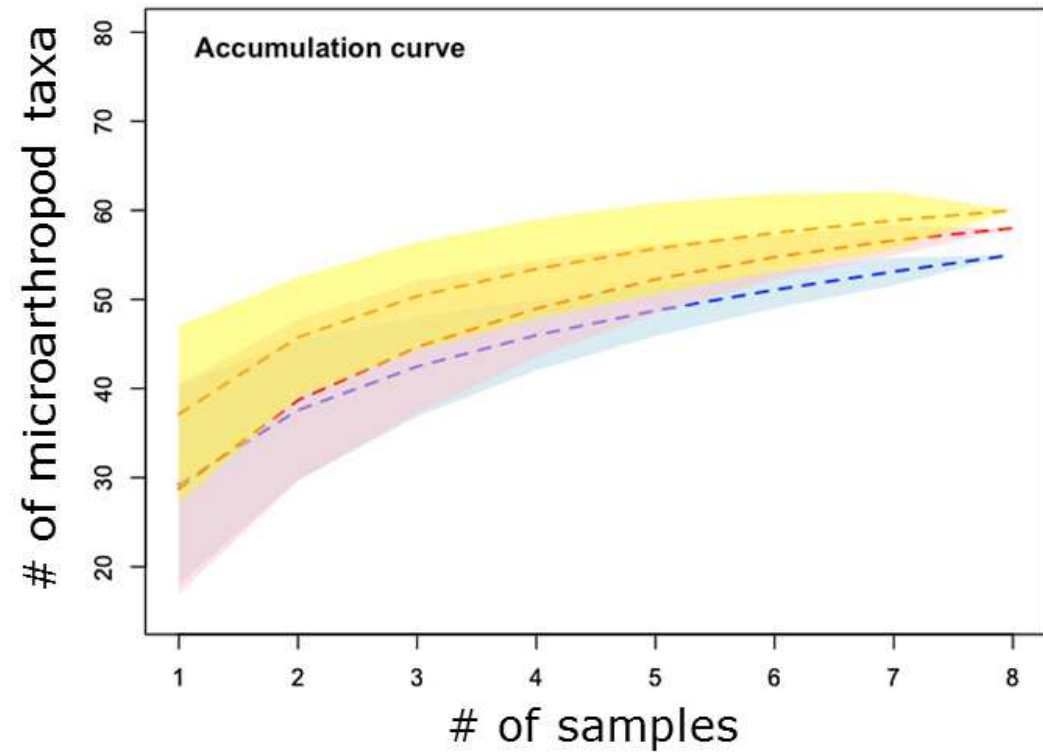
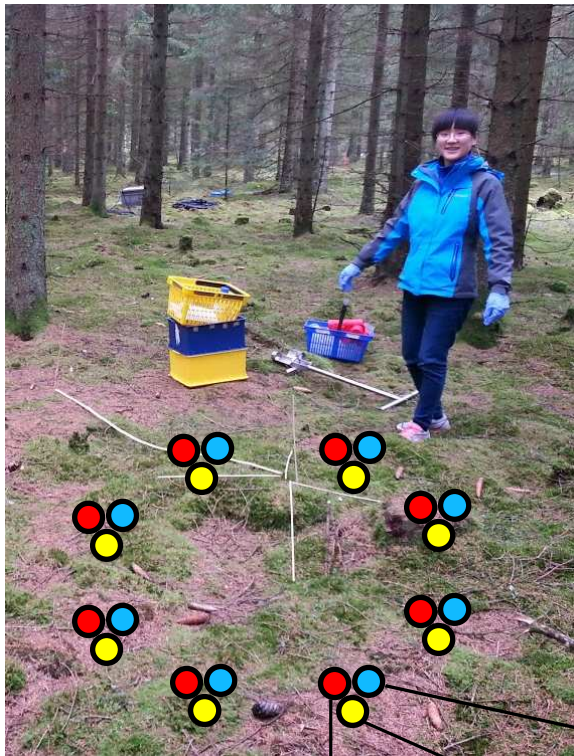
- ▲ Morphological identification
- ◆ DNA metbarcoding (diversity soup)
- DNA metbarcoding (total soil DNA)



	▲	◆	■
OTU		33	25
Taxon		28	23
Family	12	18	18
Shannon	4.3	4.3	4.8



# Comparison of methods: ways to extract DNA



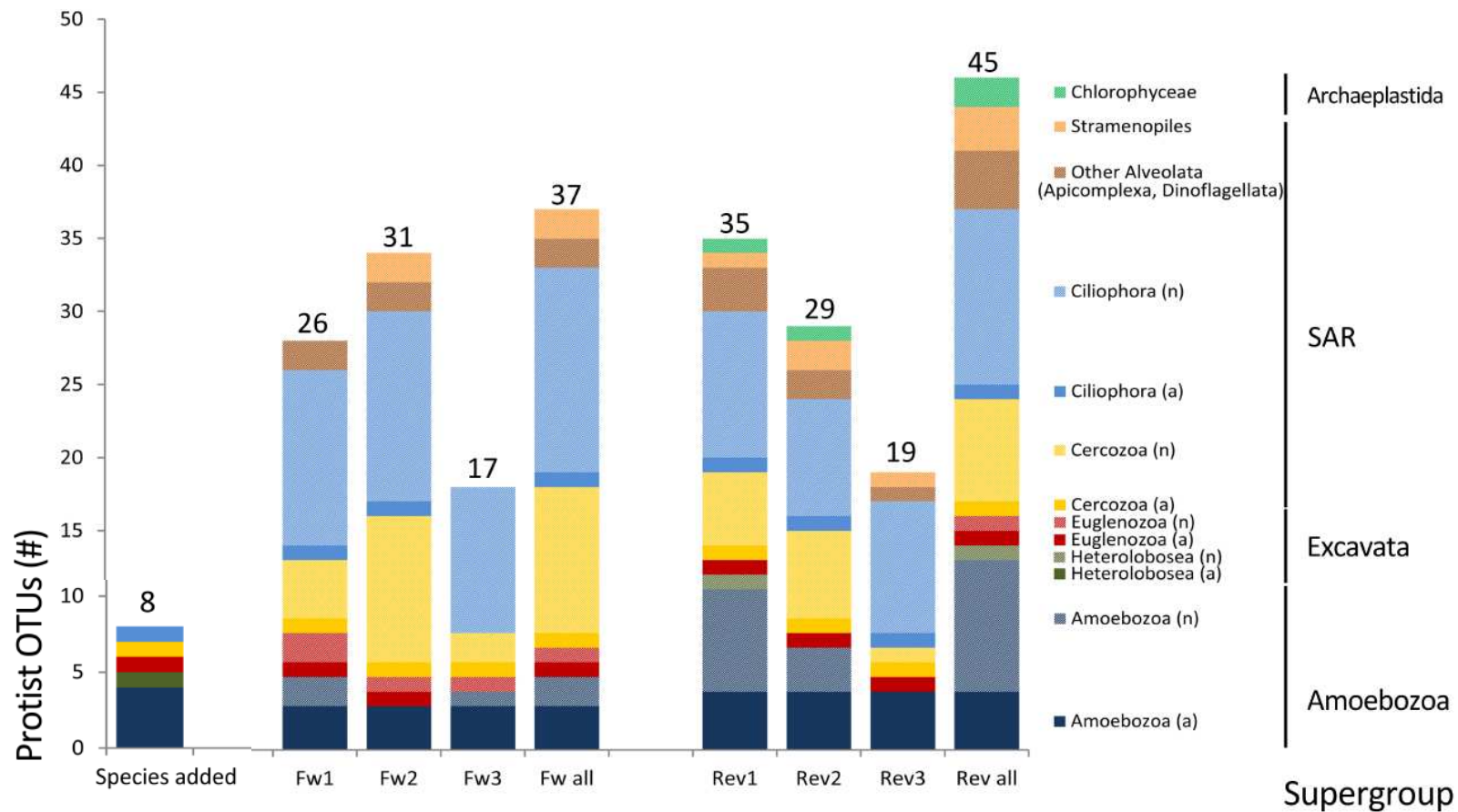
Diversity  
soup

Total  
soil extract

eDNA  
soil extract

# Protist parasites revealed

- Metabarcoding approach on a known mock community
- Immense diversity of non-added taxa



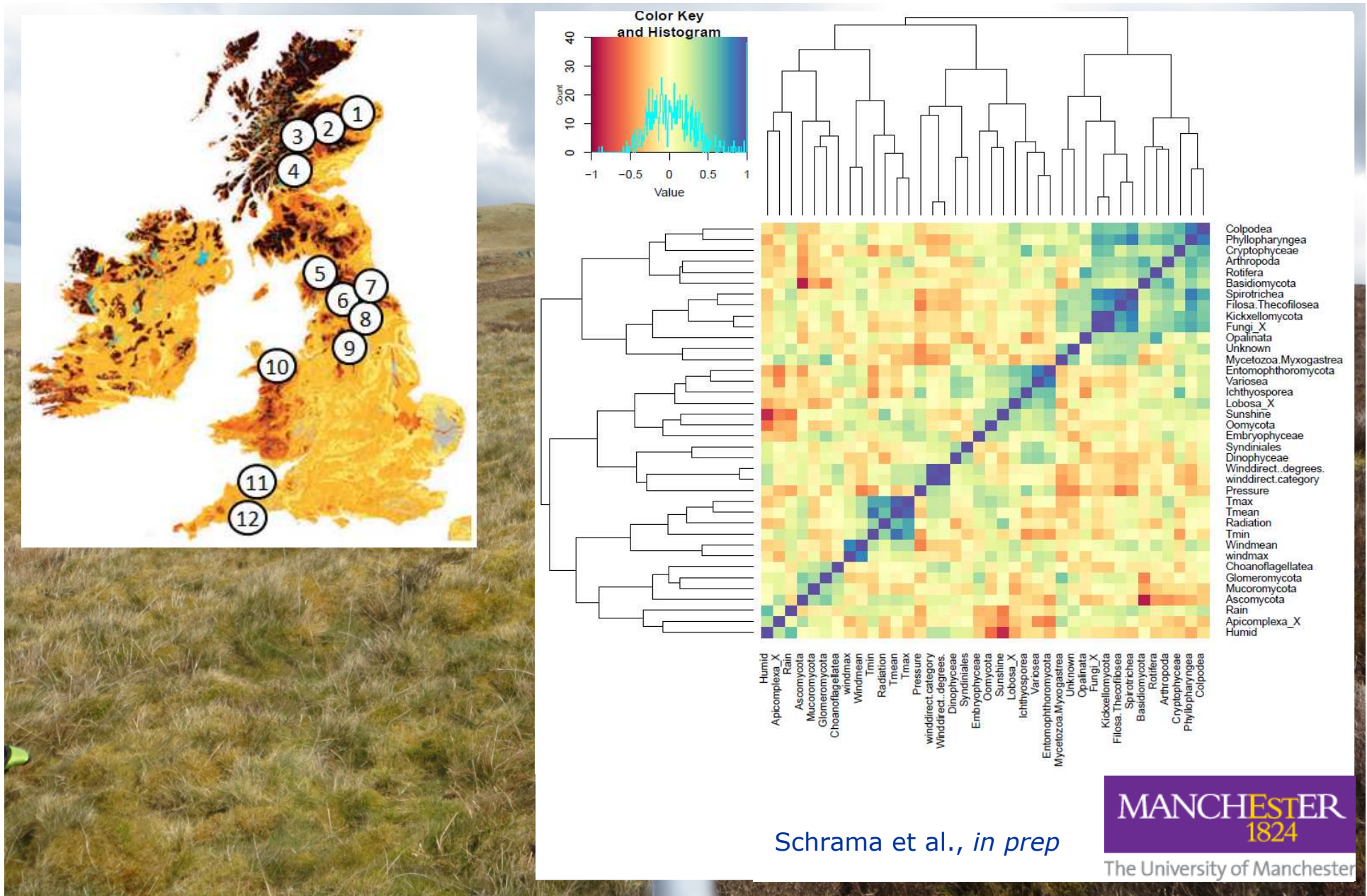
# Protist parasites revealed

- Metabarcoding approach on a known mock community
- Immense diversity of non-added taxa
- Many of which closely resemble plant or animal parasites

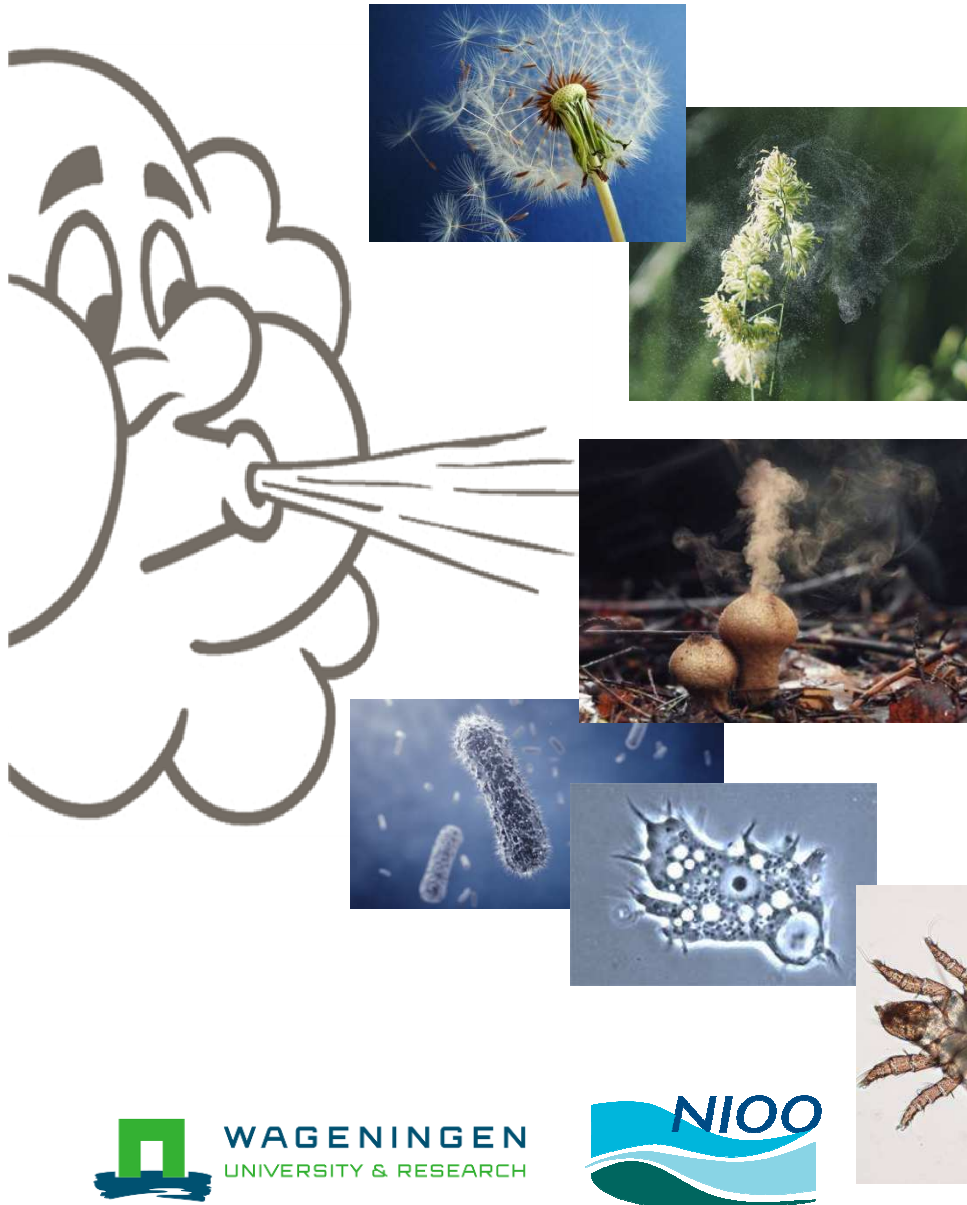
OTUId	Taxonomic groups	MI (%)	Accession #	Best blast hit	Reported host	Rel. abu. pool1 (%)	Rel. abu. pool2 (%)	Rel. abu. pool3 (%)
FwOTU24	Apicomplexa	89	AF108862	<i>Cryptosporidium felis</i>	Animals	0,21%	0,37%	0%
FwOTU56	Apicomplexa	87	JQ970325	<i>Gregarina sp.</i>	Isopod	0,09%	0,02%	0%
		98	EF024723					
revOTU40	Apicomplexa	97	AJ697751	<i>Cryptosporidium struthionis</i>	Ostrich	0%	0,04%	0%
		98	KJ761400					
revOTU45	Apicomplexa	96	FJ459749	<i>Gregarina tropica</i>	Insects	0,05%	0%	0,06%
		100	EF024723					
revOTU82	Cercozoa	100	KF111226	<i>Polymyxa graminis</i>	Plants	0%	0,04%	0%
FwOTU27	Ciliophora	95	U51554	<i>Anophyroides haemophila</i>	Lobster	0,11%	0%	0,41%
FwOTU66	Ciliophora	95	FJ858379	<i>Paranophrys marina</i>	Fish	0%	0,07%	0%
		97	JX914665					
revOTU77	Ciliophora	98	DQ512674	<i>Philasterides dicentrarchi</i>	Fish	0%	0,03%	0%
revOTU84	Ciliophora	98	AY547546	<i>Anoplophrya marylandensis</i>	Annelids and anura	0,02%	0%	0%
FwOTU40	Fungi	100	HM135172	<i>Paecilomyces hepiali</i>	Nematodes	0%	0,19%	0%
RevOTU7	Fungi	100	KJ909964	<i>Curvularia lunata</i>	Plants; humans	0,91%	0,78%	0,38%
revOTU33	Fungi	100	KC610804	<i>Cordyceps cardinalis</i>	Insects and worms	0,05%	0,04%	0%
FwOTU59	Fungi	93	NW003533857	<i>Leptosphaeria maculans</i>	Plants	0,05%	0,09%	0%
FwOTU74	Fungi	96	AB016011	<i>Syncephalis depressa</i>	Fungi	0,05%	0%	0%
		97	AB755410					
revOTU69	Fungi	100	KC708440	<i>Sphaerocreas pubescens</i>	Liverworts and hornworts	0,02%	0%	0,06%
revOTU32	Fungi	100	KF816444	<i>Athelia rolfsii</i>	Plants	0,07%	0,04%	0,06%
revOTU37	Fungi	100	EU192366	<i>Malassezia pachydermatis</i>	Animals	0,09%	0,08%	0%
FwOTU38	Other eukaryote	97	AY630452	<i>Lepinotus reticulatus</i>	Plants	0%	0,09%	0%
		99	AF139485					
FwOTU44	Other eukaryote	100	AB830755	<i>Phthirus pubis</i>	Animals and plants	0%	0,09%	0%
revOTU65	Stramenopiles	99	XR608068	<i>Phytophthora parasitica</i>	Plants	0%	0%	0,28%



# Effects of grazing intensity on soil biodiversity



# Airborne dispersal of (soil) organisms



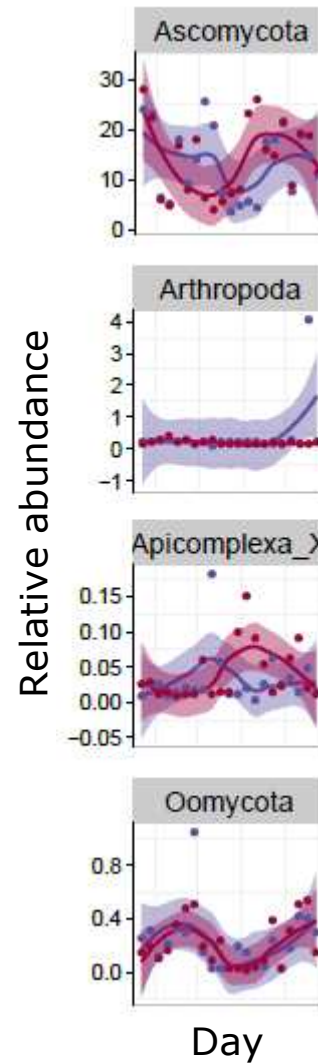
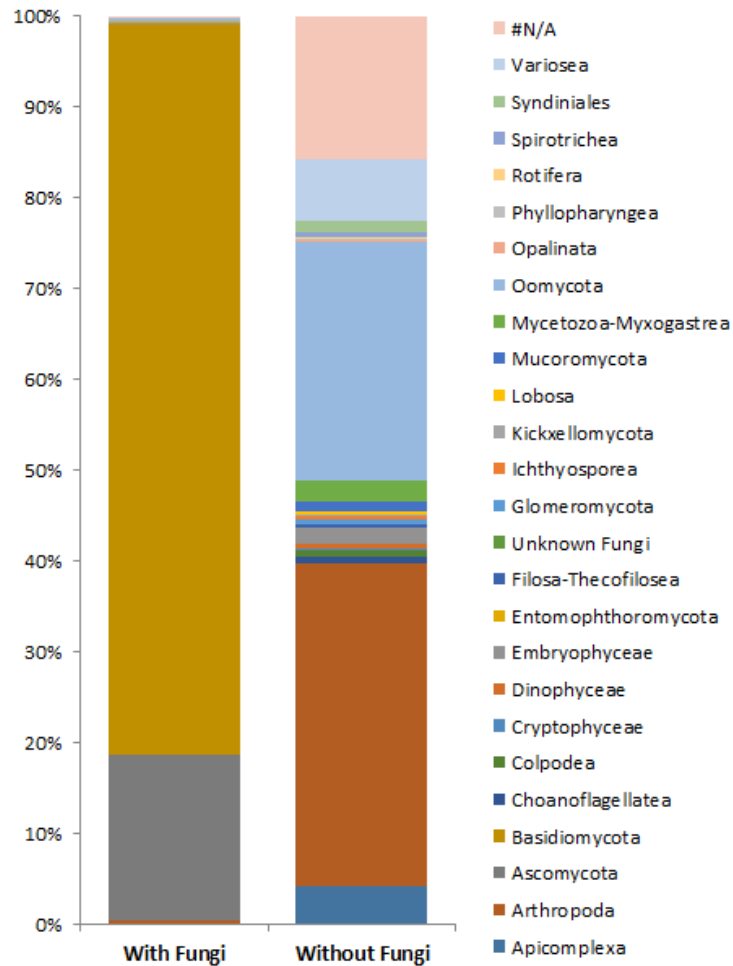
- Two sites on Wageningen Campus
- 21 days continuous sampling
- Three markers:
  - rbcL (plants)
  - 18s (fungi, protists, fauna)
  - 16s (bacteria)



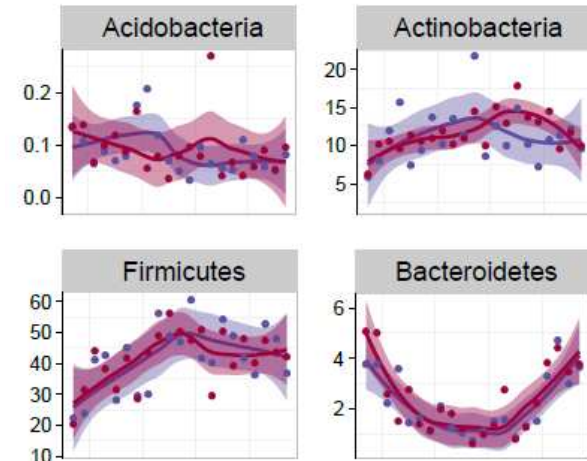


# Airborne dispersal of (soil) organisms

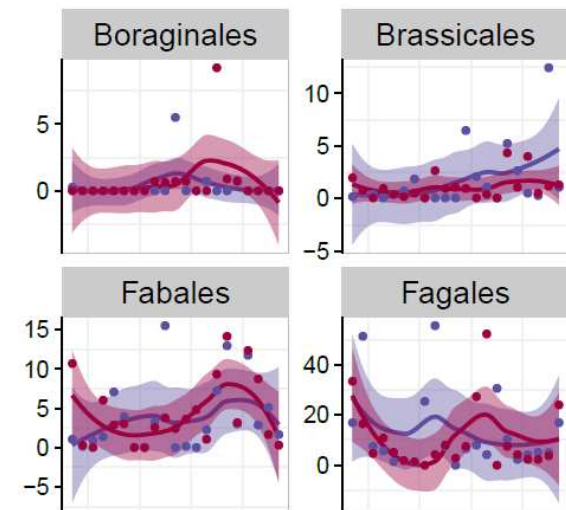
## 18S (eukaryotes)



## 16S (Bacteria)



## rbcl (Plants)



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# Take home messages

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- DNA metabarcoding tools now available for rapid and high throughput assessment of the soil faunal biodiversity
- Versatile set of markers to zoom in on specific taxonomic groups
- Observed communities will slightly differ among identification methods, but DNA-based methods may detect larger diversity
- DNA-based methods open doors to explore new study questions:
  - Variation across time, space, climates, etc
    - Effects of land management / climate change on e.g. ecosystem service provision
  - Deeper understanding of functional interactions
    - More detailed food webs
    - Host relations and dispersal of pathogens / parasites

# Thank you!

## And thanks to:

Paul Henning Krogh

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Lars Hestbjerg Hansen

Maarten Schrama

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Michael Bonkowski

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