Assessing metabolism and interspecies interactions in microbial communities via metaproteomics

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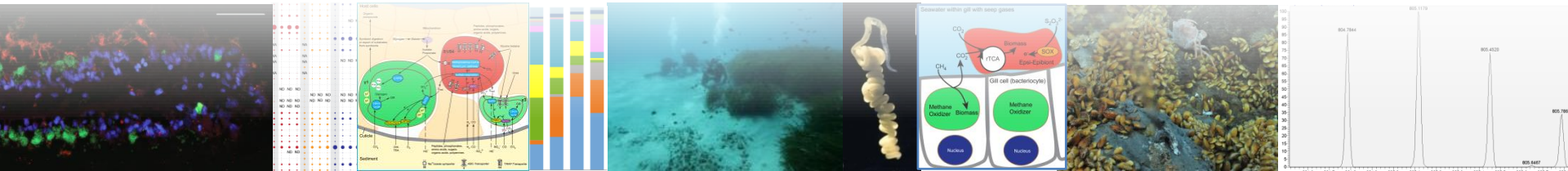
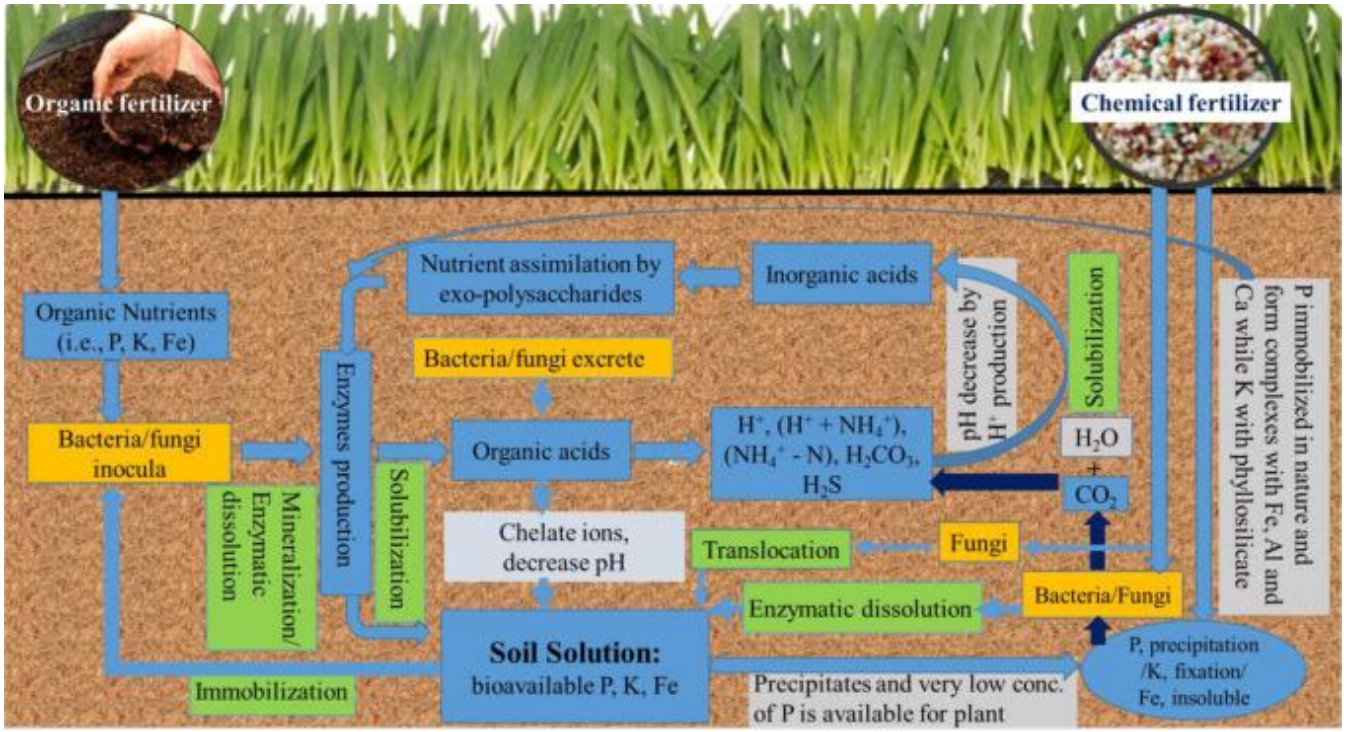


Central questions in microbial ecology



Rashid *et al.* (2016) Microbial Research 183:26-41

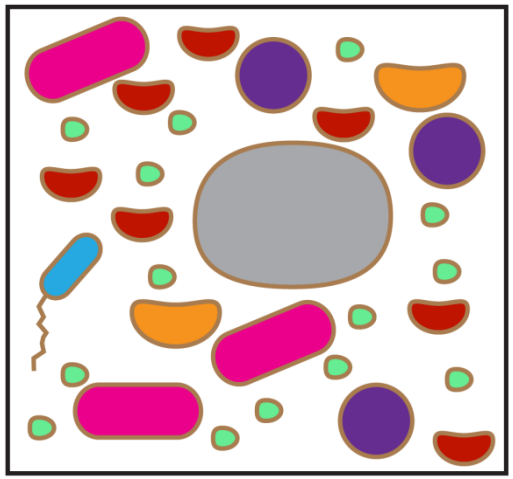
Who is there and how many?

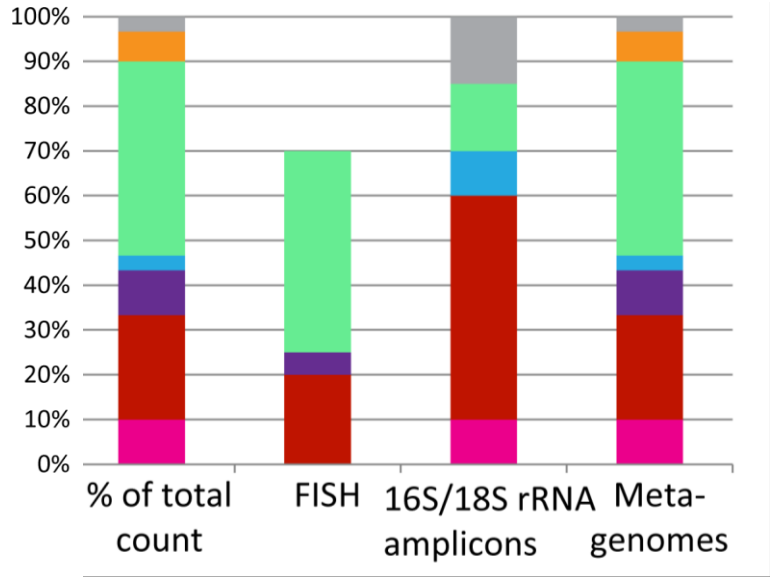
What are they doing?

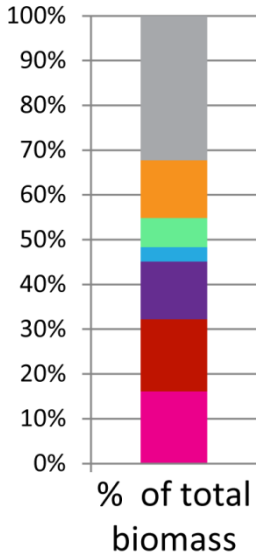
How do they interact?

Who is there and how many?

Microbial community

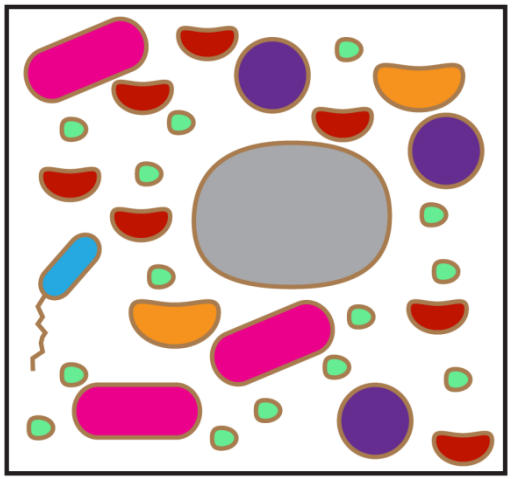


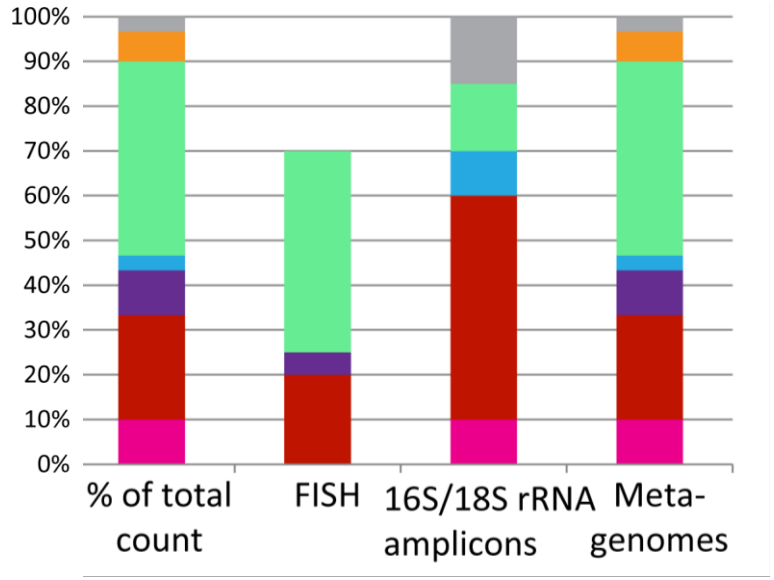


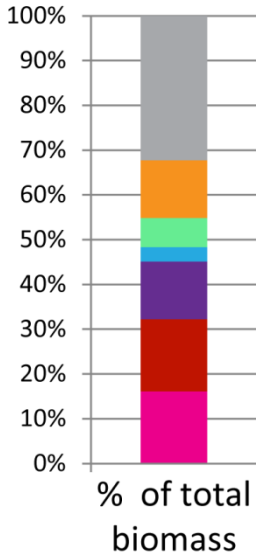


Who is there and how many?

Microbial community







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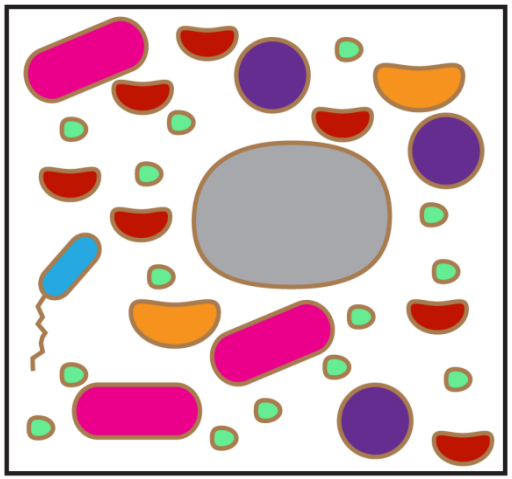
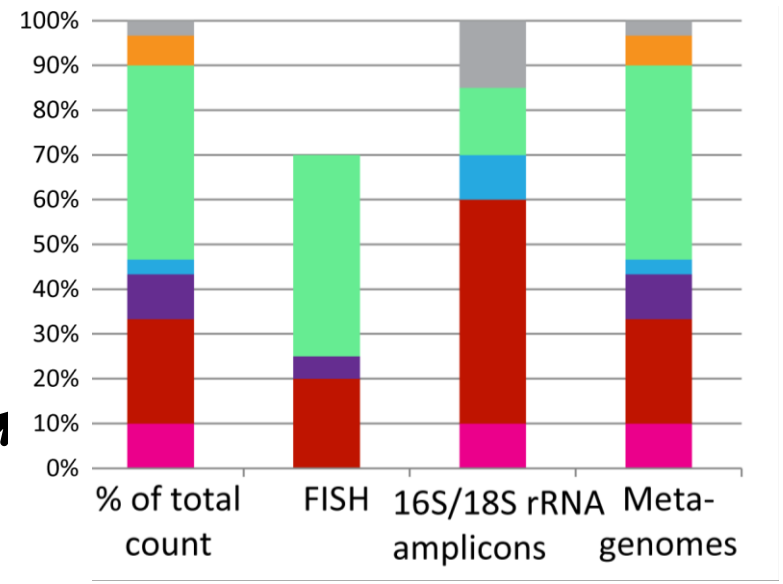
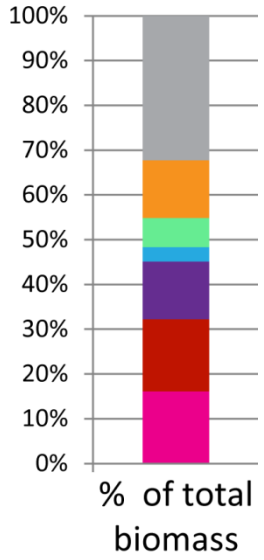
Computational tools for 16S/18S rRNA and metagenomes

Who is there and how many?

Microbial community







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Computational tools for 16S/18S rRNA and metagenomes

Assessing species biomass contributions in microbial communities via metaproteomics

Kleiner *et al*. (2017) Nature Communications 8, 1558

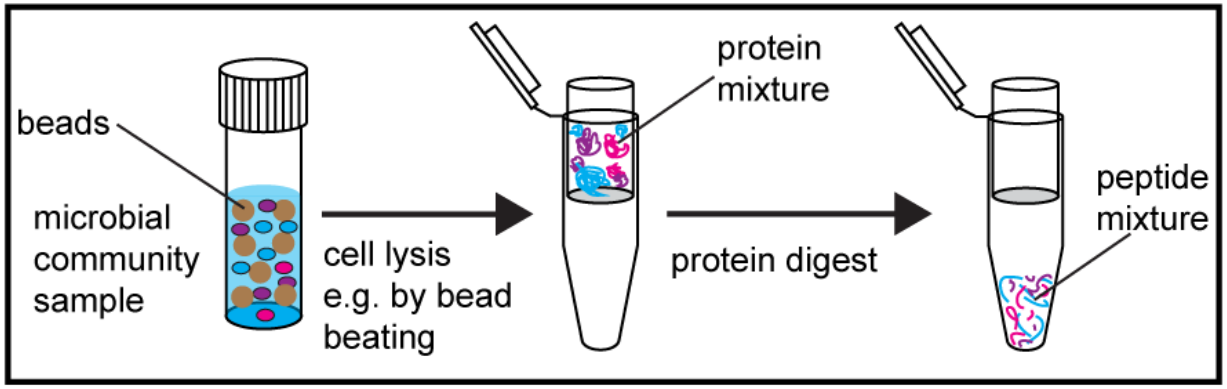
What are they doing and how do they interact?

Metaproteomics:
The large-scale characterization of the entire protein complement of environmental microbiota at a given point in time

How does metaproteomics work?

Sample collection

Sample preparation



Starting material needed:

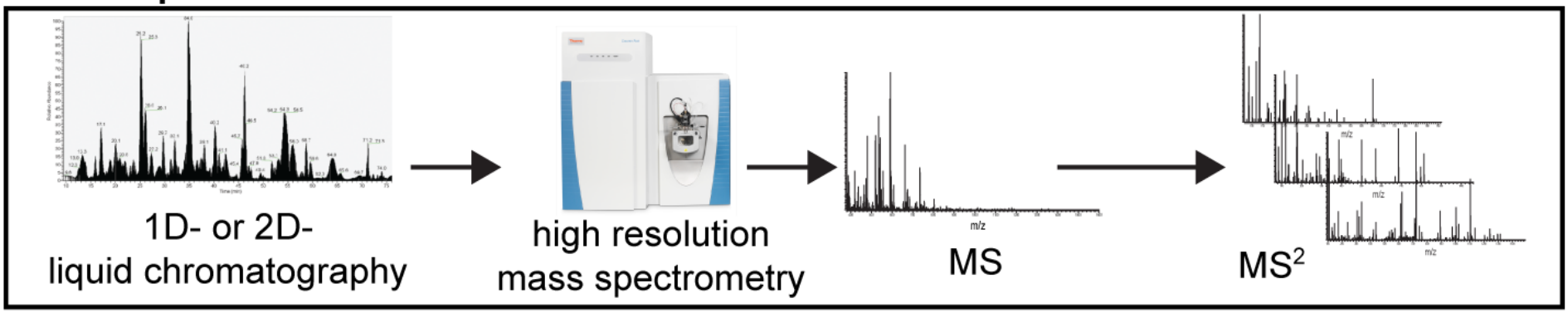
>0.5 mg wet weight

>5x10e7 cells

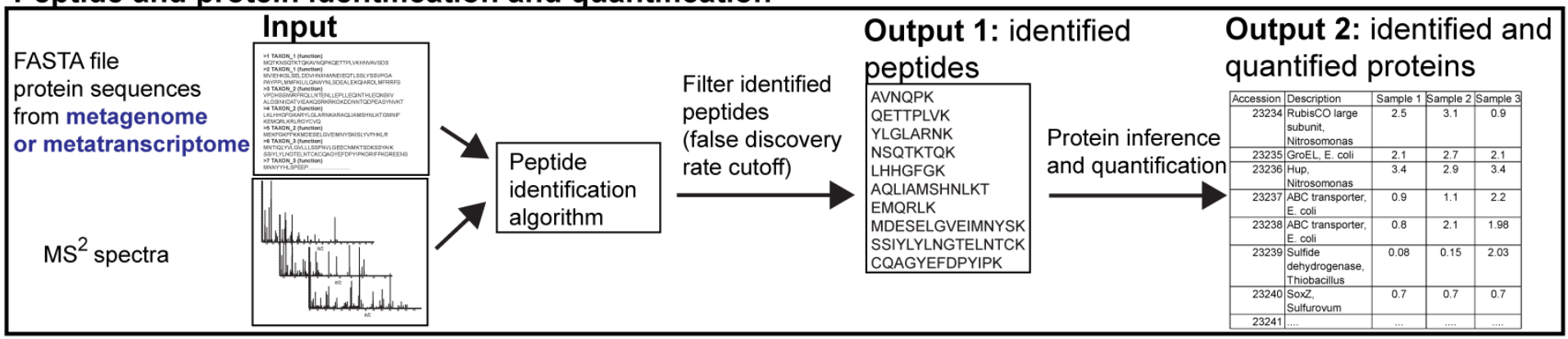
More is always better

How does metaproteomics work?

Data acquisition: LC-MS/MS



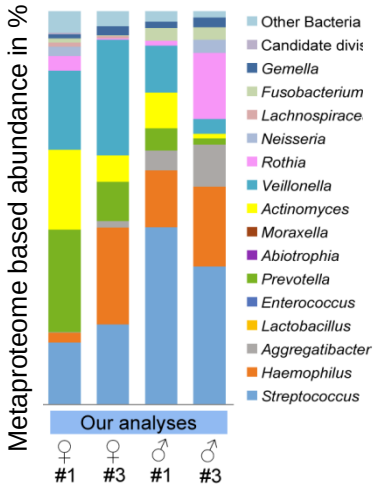
Peptide and protein identification and quantification



Accession	Description	Sample 1	Sample 2	Sample 3
23234	RubisCO large subunit, Nitrosomonas	2.5	3.1	0.9
23235	GroEL, E. coli	2.1	2.7	2.1
23236	Hup, Nitrosomonas	3.4	2.9	3.4
23237	ABC transporter, E. coli	0.9	1.1	2.2
23238	ABC transporter, E. coli	0.8	2.1	1.98
23239	Sulfide dehydrogenase, Thiobacillus	0.08	0.15	2.03
23240	SoxZ, Sulfurovum	0.7	0.7	0.7
23241	…	…	….	….

What questions can we address?

Who is there and how many?

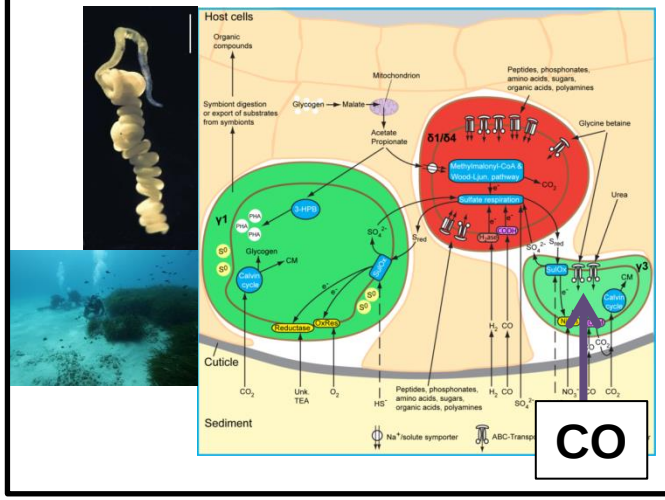


Metaproteomics for assessing species biomass contributions in microbial communities

e.g. Saliva microbiome

Kleiner *et al.* (2017) Nature Communications 8, 1558

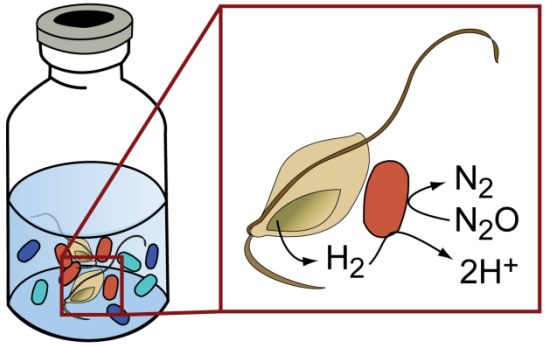
What are the nutrient and energy sources?



A marine bacteria-animal symbiosis that uses **carbon monoxide** as an energy source

Kleiner *et al.* 2012 PNAS 109:E1173-E1182

How do microbial species interact?



A mutualistic bacteria-protist symbiosis based on hydrogen transfer

Hamann *et al*. 2016 Nature 534: 254–258

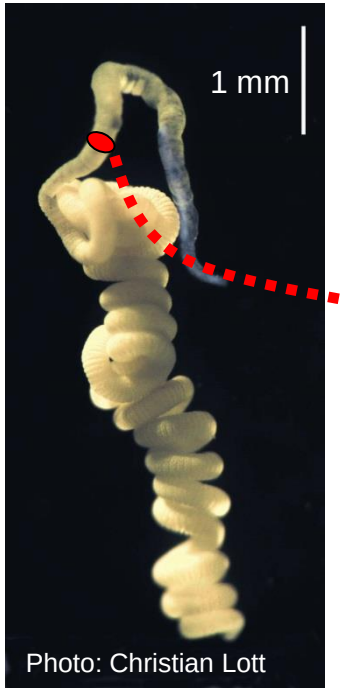
What carbon sources are used by individual species in microbial communities?

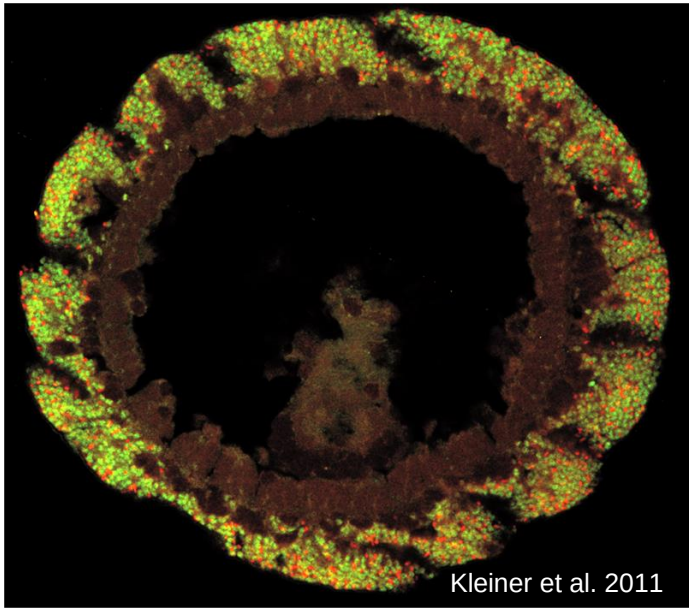
Available as preprint:
M Kleiner *et al.* (2018) bioRxiv, 245290

What are the nutrient and energy sources?

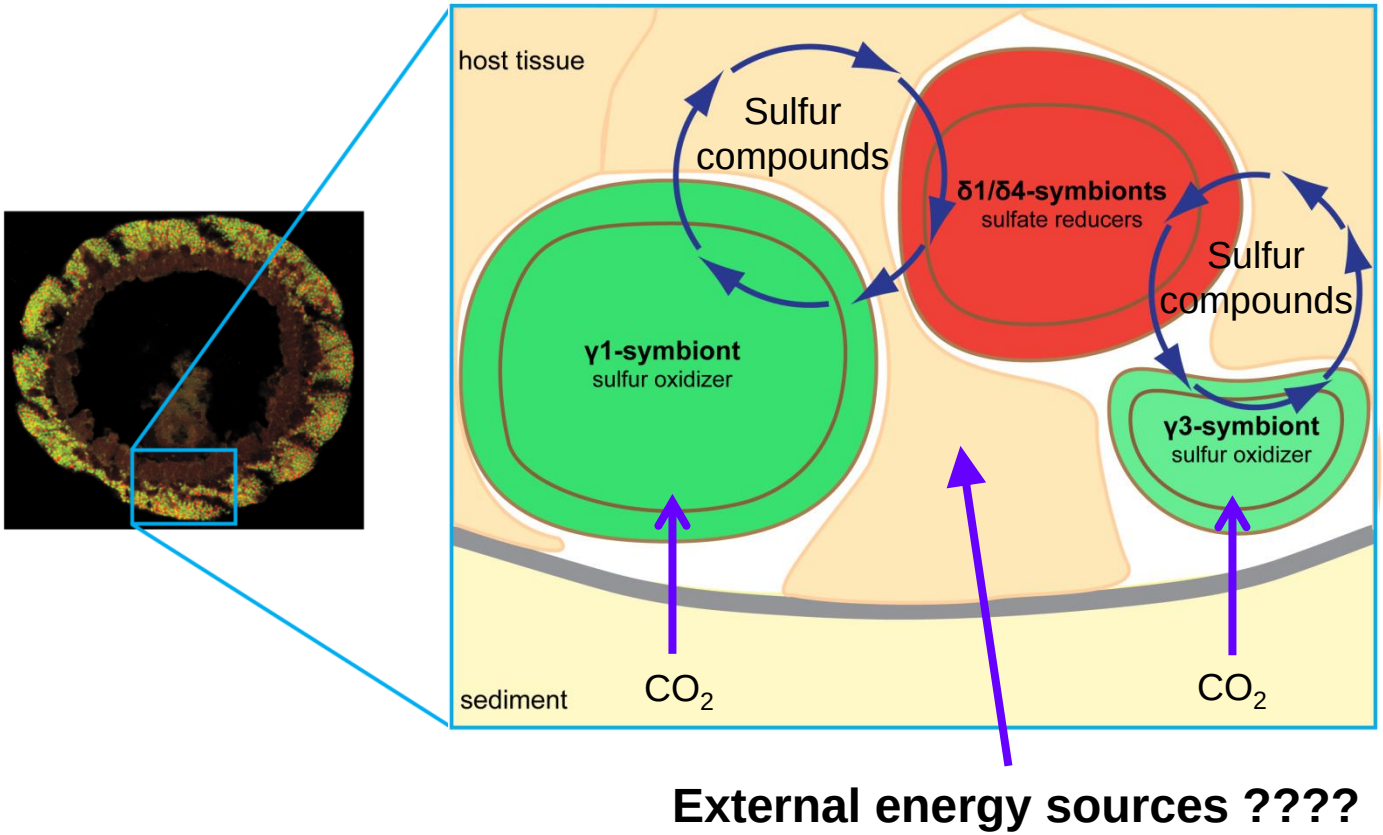
The gutless marine oligochaete *Olavius algarvensis* from Elba, Italy

Sandy sediments
Poor in energy and nutrient sources



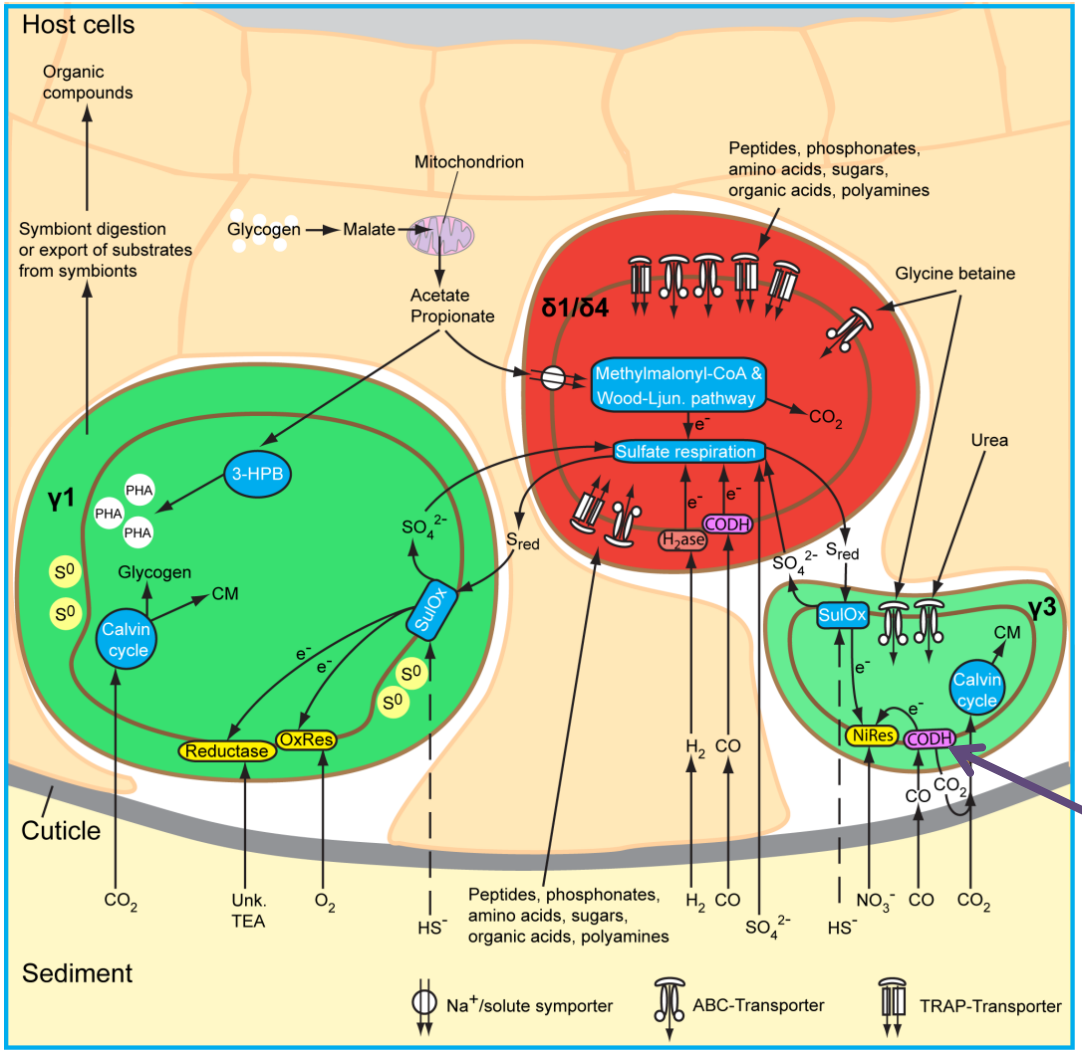


What are the nutrient and energy sources?



What are the nutrient and energy sources?

Metagenomics and metaproteomics

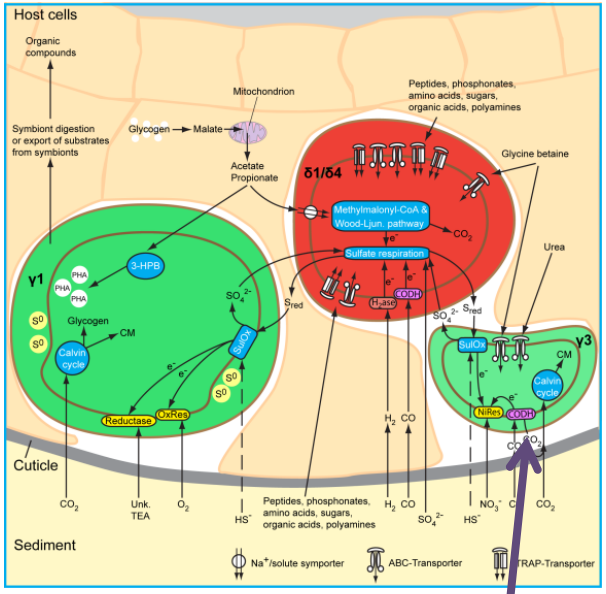


Abundant proteins for carbon monoxide use

Woyke *et al*. 2006 Nature 443:950-955

Kleiner *et al*. 2012 PNAS 109:E1173-E1182

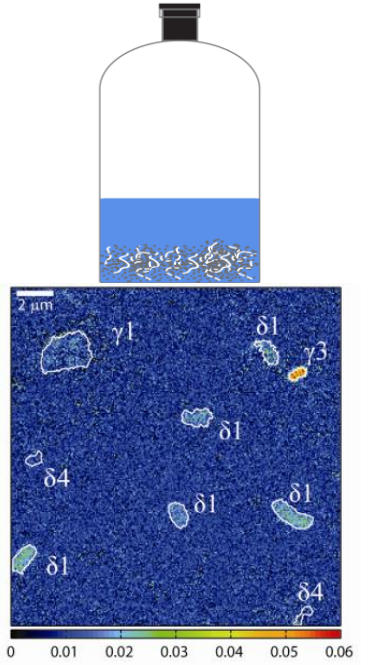
What are the nutrient and energy sources?



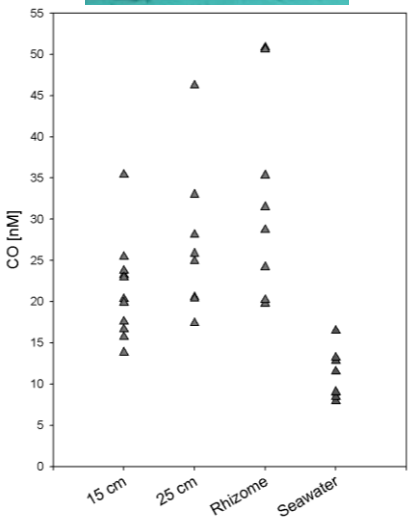
Kleiner *et al.* 2012 PNAS 109:E1173-E1182

Abundant proteins for CO use

Single cell imaging of carbon isotopes using nanoSIMS



Incubation of worms with CO leads to increased CO_2 fixation by symbionts.



Kleiner *et al.* 2015 Environ Microbiol 17:5023-5035

Technology for metaproteomics develops rapidly

2010

- 15 cm columns, 400 bar
- 2 h chromatographic separations
- ~4,000 MS^2 spectra/hour
- Car sized instruments
- pre-separation of peptides or proteins needed to get good proteome coverage
- lots of material needed

  ~20,000 worms sacrificed
- ~500 h machine time

>>> in total **2819 Proteins** identified and quantified

2016

- 50 cm columns, 1000 bar
- 8 h chromatographic separations
- ~40,000 MS^2 spectra/hour
- Benchtop instruments
- > often no pre-separation needed
- > low input material needed
- 1 worm (**~ 1 mg wet weight**)
- 5 h machine time

>>> in total **4500 Proteins** identified and quantified

2017

- 1200 bar
- 75 cm columns
- ~100,000 MS^2

What about metaproteomics for the rhizosphere and soils?

Challenges:

- Interfering substances from soil >> new sample preparation methods
- High microbial diversity >> Improved technology

NC STATE UNIVERSITY

Plant Soil Microbial Community Consortium

PSMCC sponsored project on:
"Development and evaluation of metaproteomics methods for root-associated microbes"

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Acknowledgments

Kleiner lab

Fernanda Salvato Rebecca Hughes Nick Taylor Jessie Maier

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UNC Chapel Hill Collaborators

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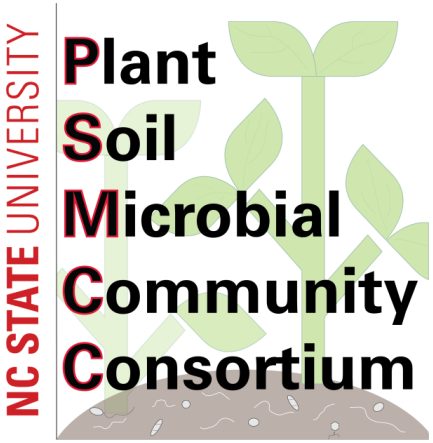
Northeast Forestry University, China

Ben Niu



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