

# Digital optimization of the feed-microbiome-host nexus

Phil B. Pope + many great MEMO colleagues and collaborators!

Professor

Faculty of Biosciences

Norwegian University of Life Sciences



@ThePopeLab



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The Research Council  
of Norway

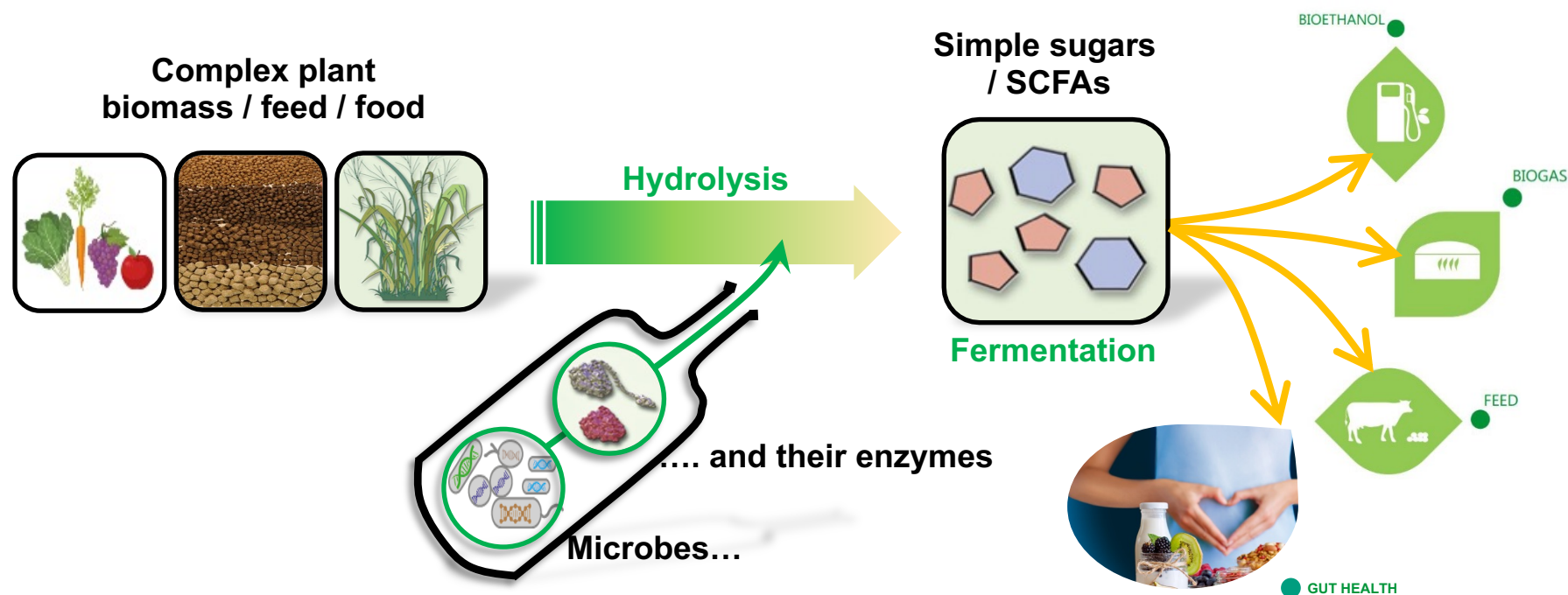


3D'omics

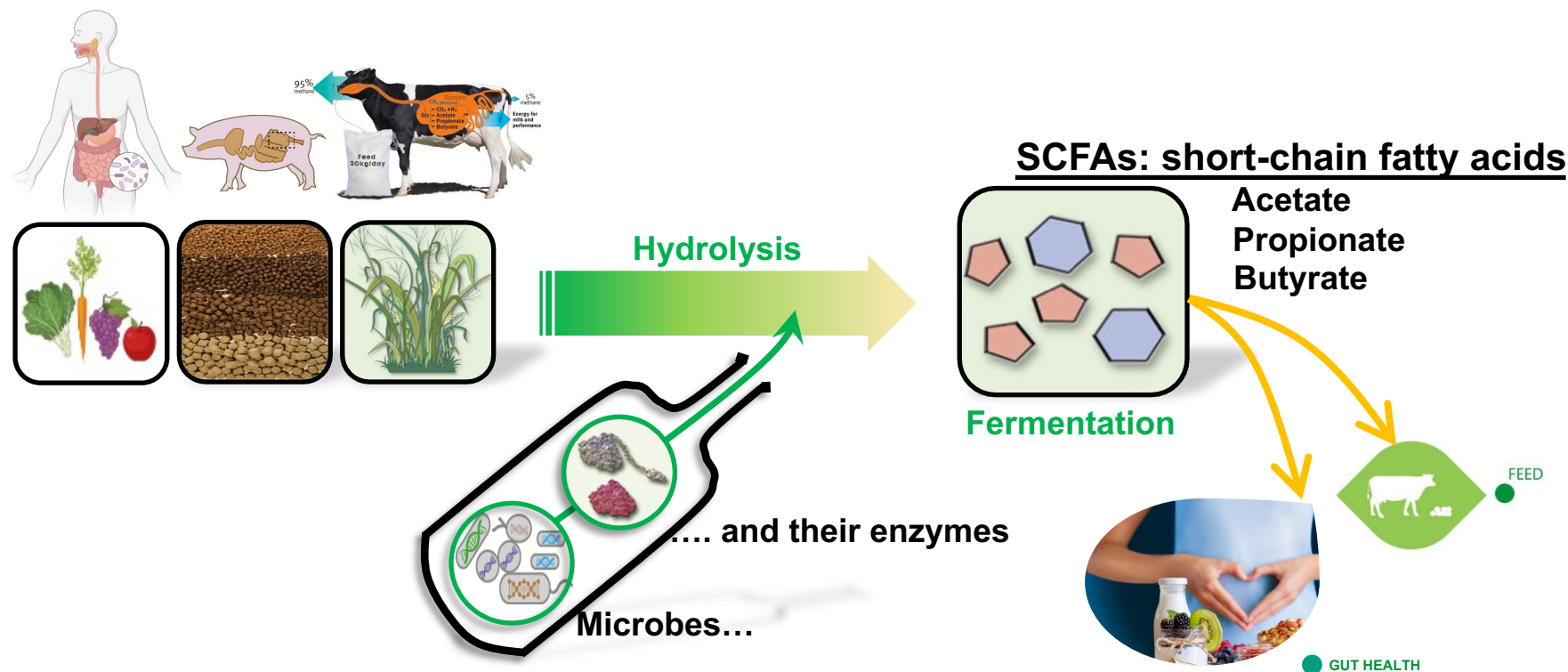


**HoloRuminant**  
Understanding microbiomes of the ruminant holobiont

# The bottleneck....



# The bottleneck....



## Energy contribution of SCFAs

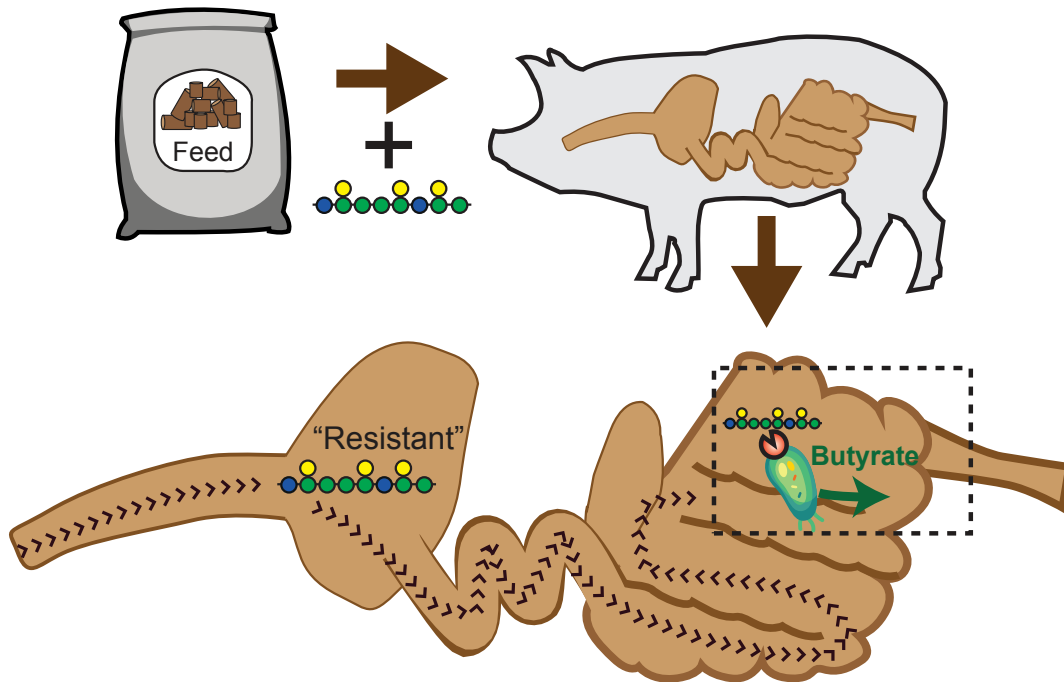
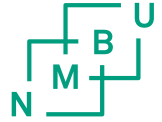
Cows: ~70%

Pigs: 30–76%

Humans: ~10%

# The “~~prebiotic~~” concept

## MDF



### Common target populations

Lactobacilli

Bifidobacteria

*Faecalibacterium* sp.

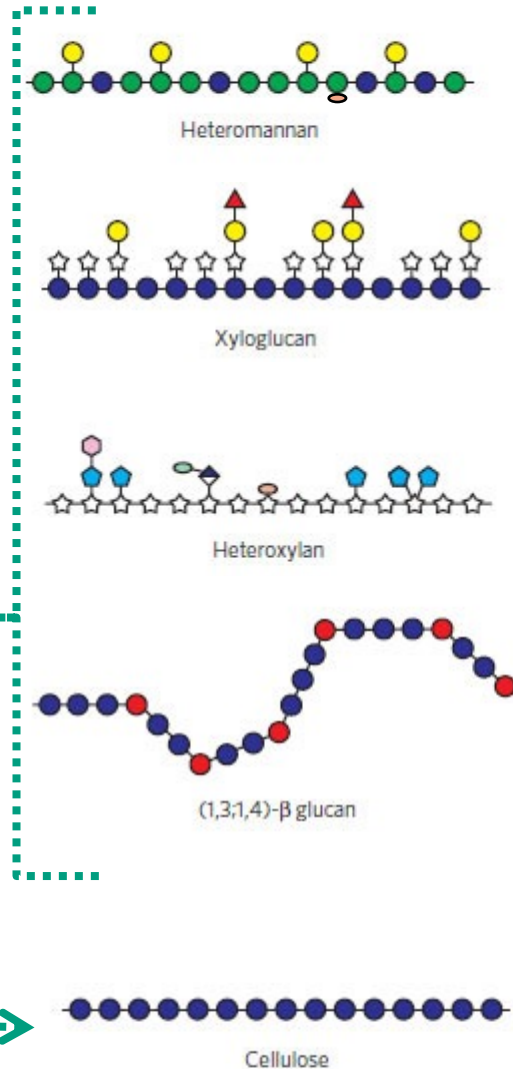
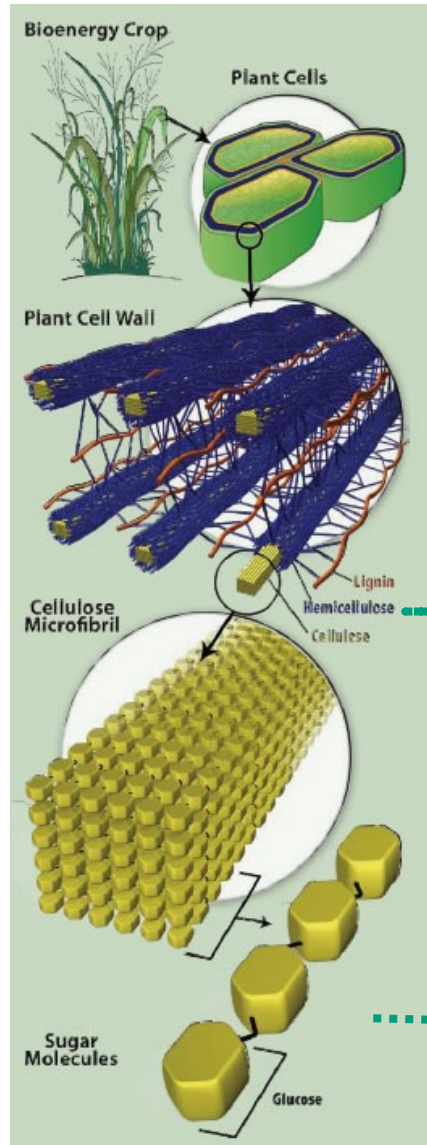
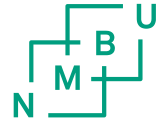
*Roseburia* sp.

**BUTYRATE-  
PRODUCERS**

- Healthy gut status
- Low disease state



# The plant cell wall



## Microbes use: CAZymes

Glycoside hydrolases (GHs)  160+ families

Carbohydrate binding modules (CBMs)  85+ families

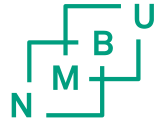
Auxilliary Activities (AAs)  15+ families

Carbohydrate esterases (CEs)  15+ families

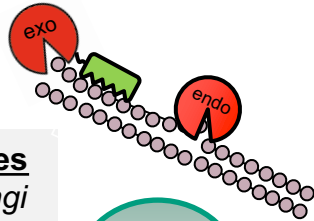
Polysaccharide lyases (PLs)



# Microbes use similar enzymes in different ways to degrade fiber



**Secreted enzymes**  
-Bacteria and fungi

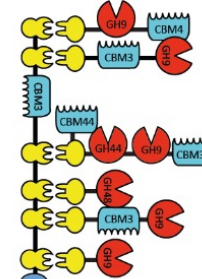


cell

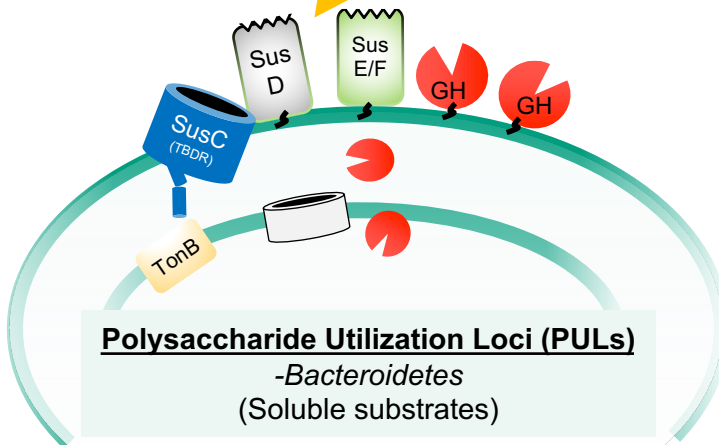


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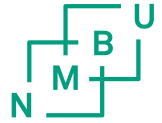
**Cellulosomes**  
-Bacteria



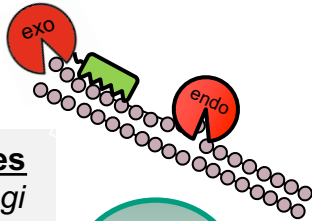
**Polysaccharide Utilization Loci (PULs)**  
-Bacteroidetes  
(Soluble substrates)



# Microbes use similar enzymes in different ways to degrade fiber



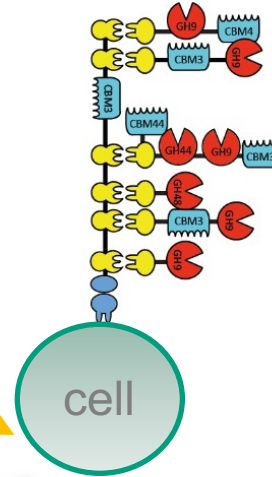
**Secreted enzymes**  
-Bacteria and fungi



cell

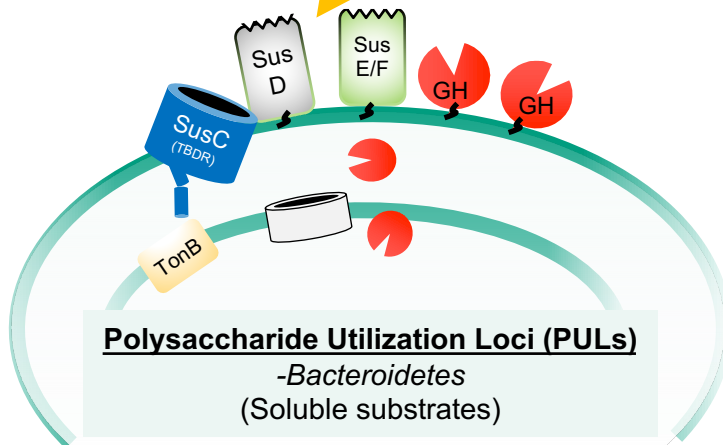


**Cellulosomes**  
-Bacteria

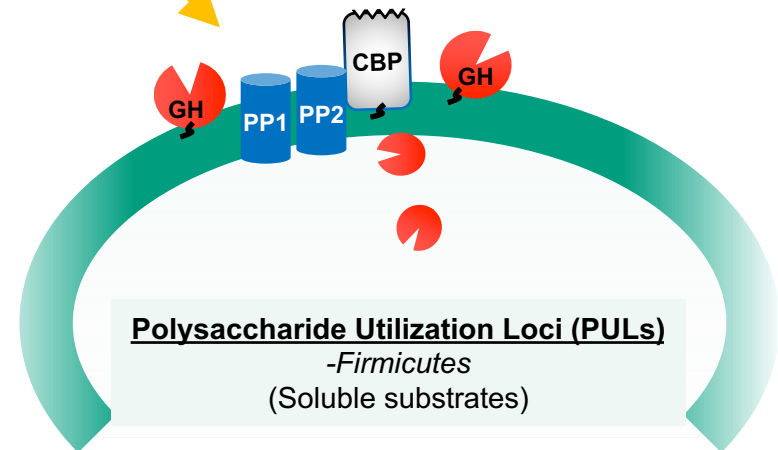


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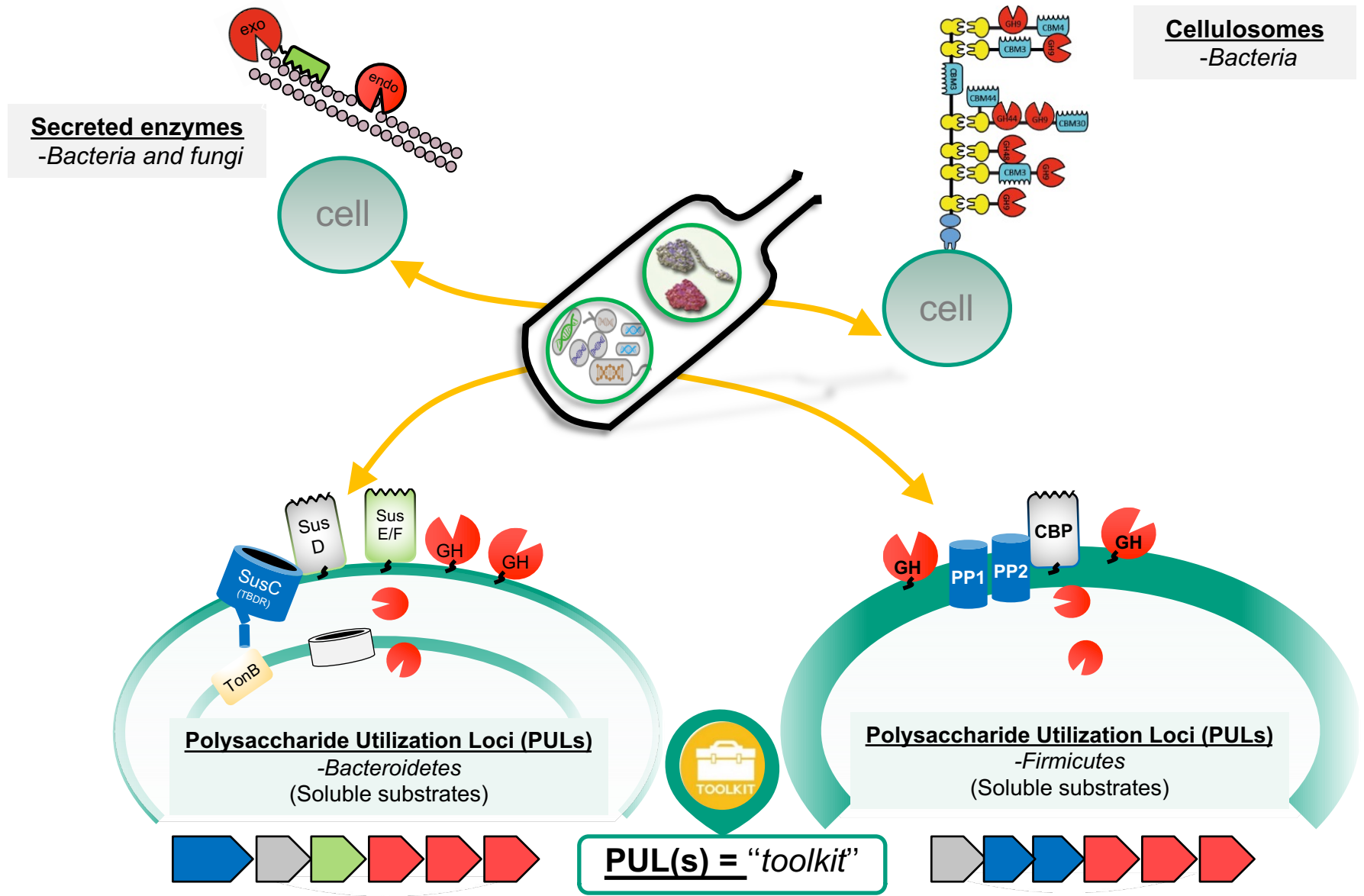
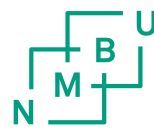
**Polysaccharide Utilization Loci (PULs)**  
-Bacteroidetes  
(Soluble substrates)



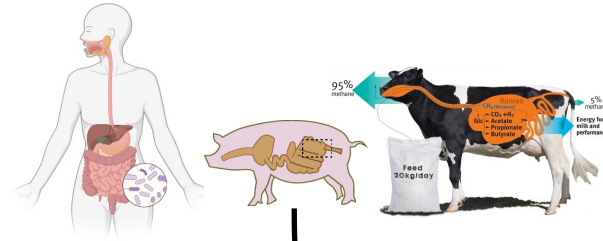
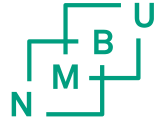
**Polysaccharide Utilization Loci (PULs)**  
-Firmicutes  
(Soluble substrates)



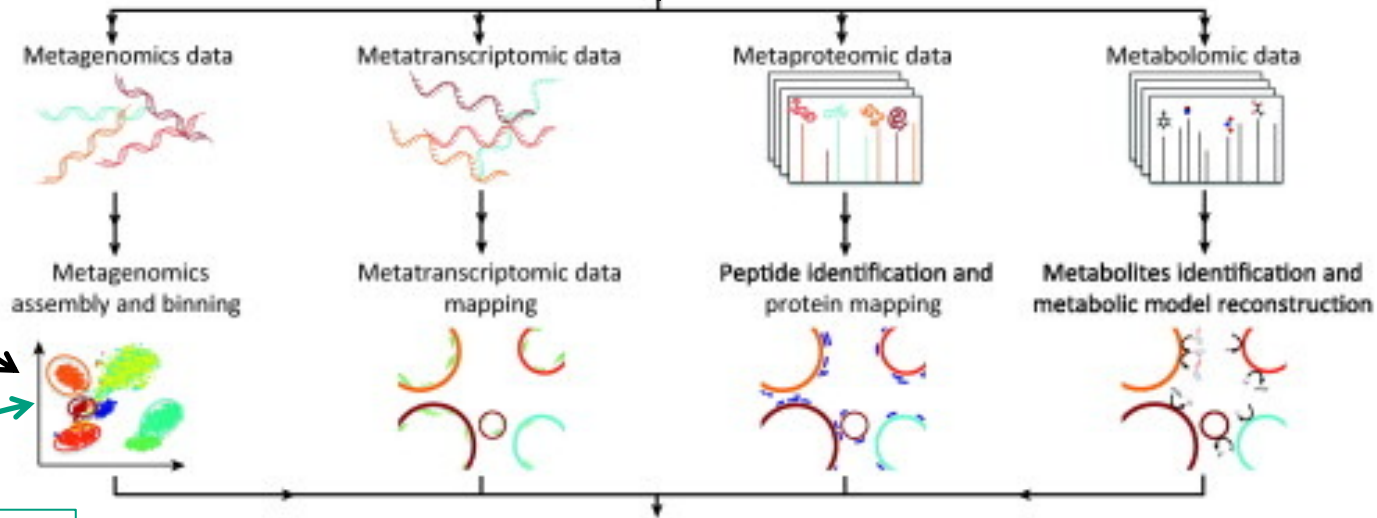
# Microbes use similar enzymes in different ways to degrade fiber



# We combine cultures and omics to overcome the cultivability bottleneck



## Metaproteomics

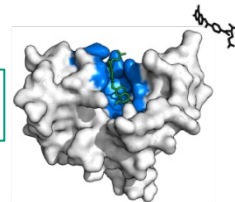


**MAGs:**  
**Metagenome**  
**Assembled**  
**Genomes**

## Enzymology



Vincent Eijsink  
NMBU





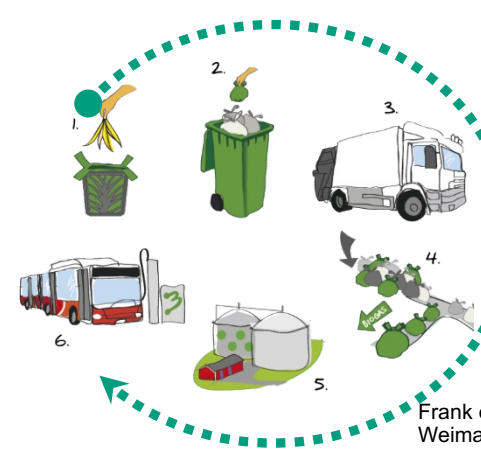
# Meta-omics on digestive ecosystems



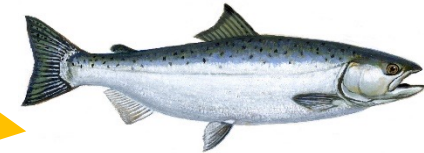
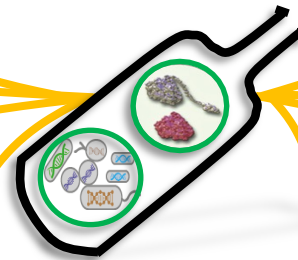
Pope et al. (2010) **PNAS** Vol 107  
 Pope et al. (2011) **ISME J.** Vol 5  
 Pope et al. (2011) **Science** Vol. 333



Naas, et al. (2014). **mBio** Vol 5  
 Arntzen et al (2017). **Environ Microbiol**, Vol 19  
 Naas et al. (2018). **Microbiome**, Vol 6  
 Hagen et al. (2021) **ISME J.**



Frank et al. (2016) **mSystems**  
 Weimann et al. (2016) **mSystems**  
 Hagen et al. (2017) **AEM**  
 Kunath/Delogu et al. (2018) **ISME J.**  
 Delogu et al. (2020) **Nature Communications**  
 Jonassen et al. (2022) **ISME J.**



Rudi et al. (2017). **AEM** Vol 84  
 Minniti et al. (2017). **Front Microbiol** Vol 8  
 Minniti et al. (2019). **Genes** Vol 10



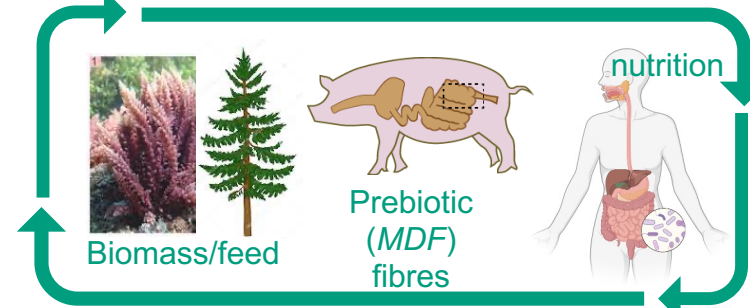
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 Mackenzie et al. (2015) **AEM** Vol 81  
 Salgado-Flores et al. (2016) **Micro. Gen.**  
 Solden et al. (2018) **Nature Microbiology**



Liu et al. (2018) **ISME J.**



Taillefer et al. (2018) **mSystems**  
 Emerson et al. (2018) **Nature Microbiology**



Omu et al. (2015). **Microbiome**  
 La Rosa et al. (2018). **Nature Communications**  
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 Ostrowski, et al. (2022) **Nature Microbiology**



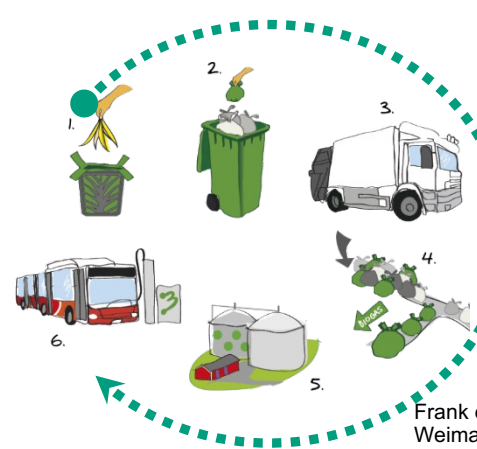
# Meta-omics on digestive ecosystems



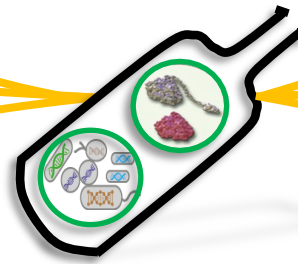
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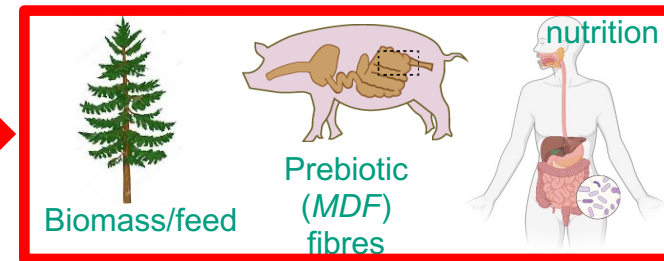
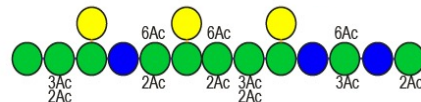
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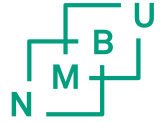
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 Ostrowski, et al. (2022) **Nature Microbiology**



# Mannan degraders in the gut are selfish

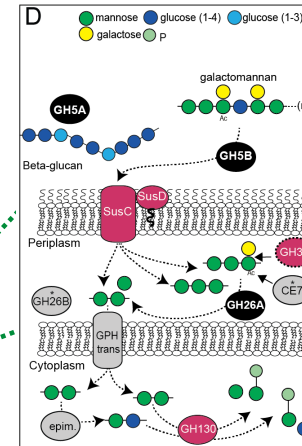
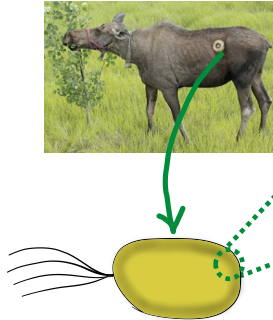


ARTICLES  
<https://doi.org/10.1038/s41564-018-0225-4>  
nature microbiology  
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## Interspecies cross-feeding orchestrates carbon degradation in the rumen ecosystem

Lindsey M. Solden<sup>1</sup>, Adrian E. Naas<sup>2</sup>, Simon Roux<sup>1</sup>, Rebecca A. Daly<sup>1</sup>, William B. Collins<sup>3</sup>, Carrie D. Nicora<sup>4</sup>, Sam O. Purvine<sup>4</sup>, David W. Hoyt<sup>4</sup>, Julia Schückel<sup>5</sup>, Bodil Jørgensen<sup>5</sup>, William Willats<sup>6</sup>, Donald E. Spalinger<sup>7</sup>, Jeffrey L. Firkins<sup>8</sup>, Mary S. Lipton<sup>4</sup>, Matthew B. Sullivan<sup>1,9</sup>, Phillip B. Pope<sup>1,2\*</sup> and Kelly C. Wrighton<sup>1\*</sup>

Solden et al. (2018) **Nature Microbiology**



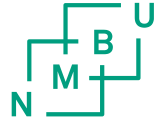
Adrian Naas  
NMBU



Lindsey Solden Kelly Wrighton  
Ohio State University



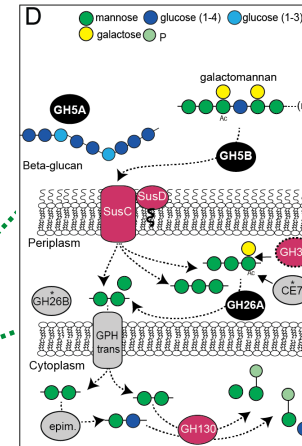
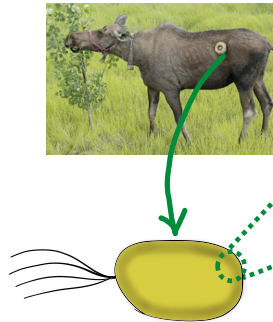
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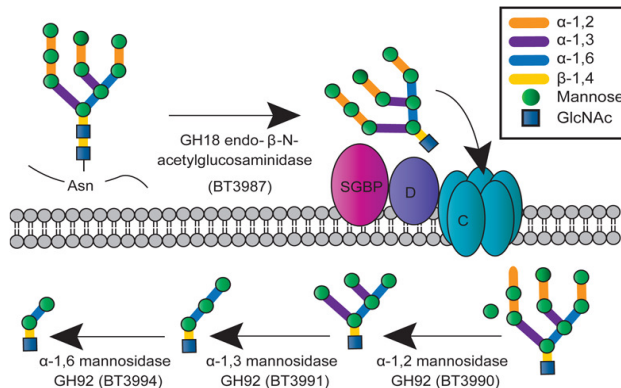


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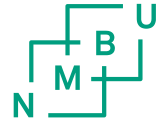
## Human gut Bacteroidetes can utilize yeast mannan through a selfish mechanism

Fiona Cuskin, Elisabeth C. Lowe [...] Harry J. Gilbert

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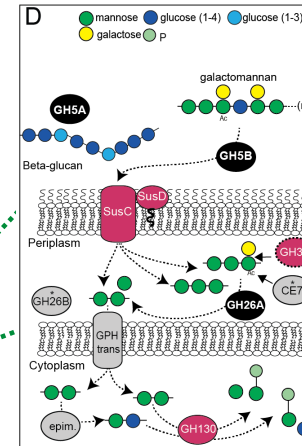
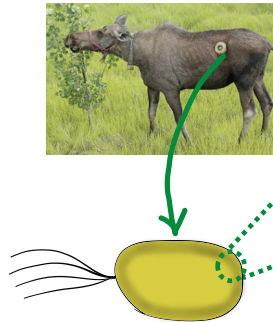
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NMBU

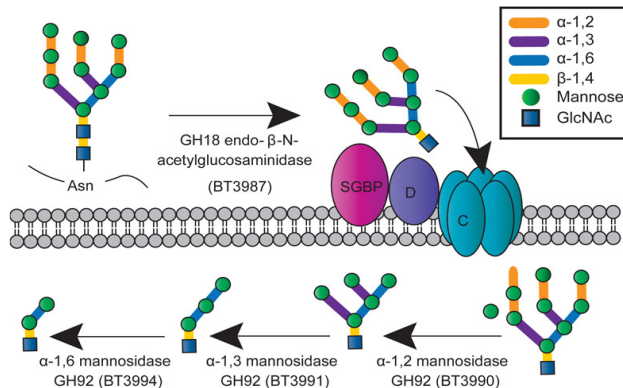


Lindsey Solden  
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## Human gut Bacteroidetes can utilize yeast mannan through a selfish mechanism

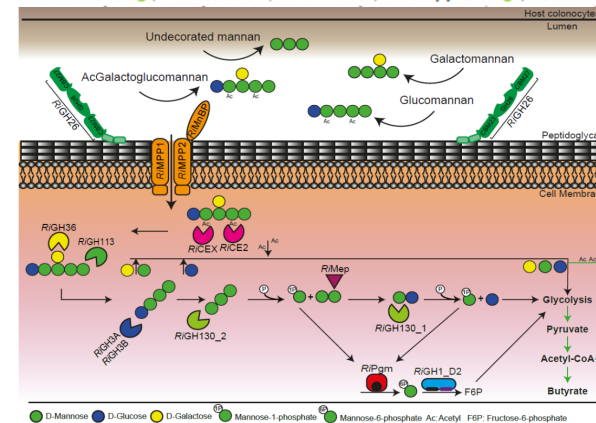
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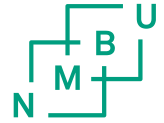
## The human gut Firmicute *Roseburia intestinalis* is a primary degrader of dietary β-mannans

Sabina Leanti La Rosa<sup>1</sup>, Maria Louise Leth<sup>2</sup>, Leszek Michalak<sup>1</sup>, Morten Ejby Hansen<sup>2</sup>, Nicholas A. Pudlo<sup>3</sup>

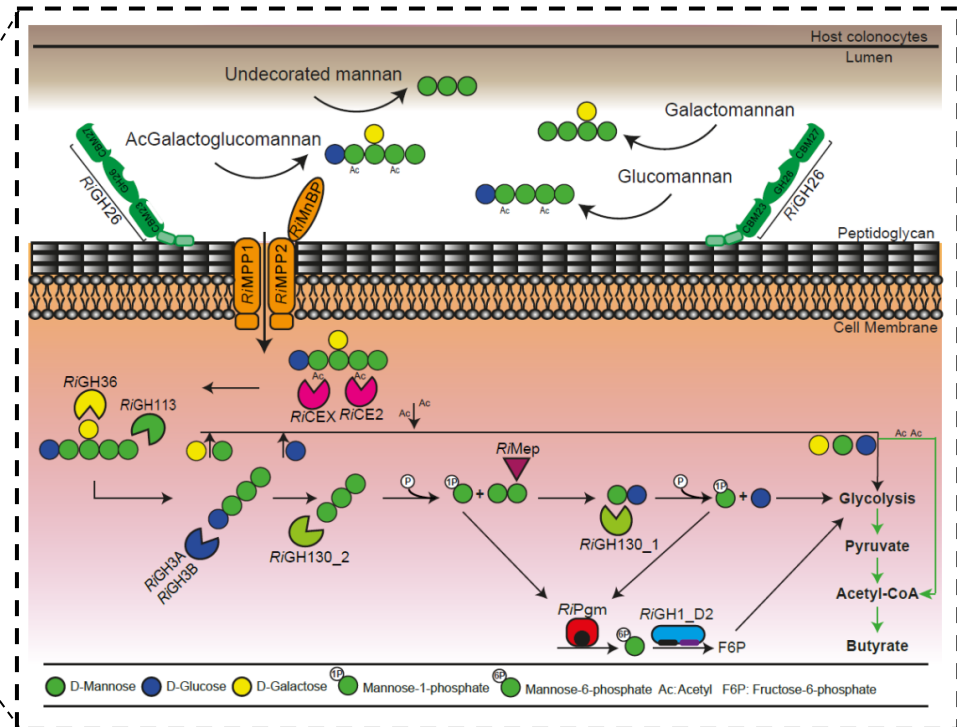
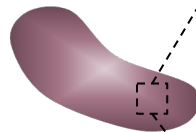


Sabina La Rosa  
NMBU

# Roseburia intestinalis is a primary degrader of mannan



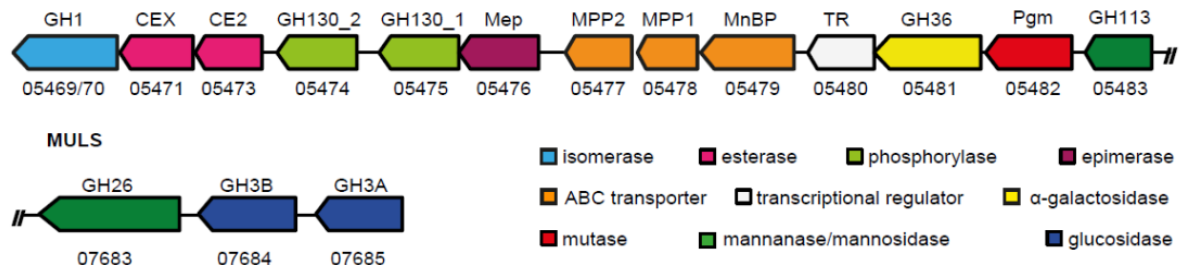
1. Needs GHs
2. Needs CEs
3. It is “selfish”
4. It produces butyrate
5. Associated with improved gut health and low intestinal disease states



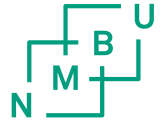
## Mannan PUL

a.k.a.

“Mannan toolkit”

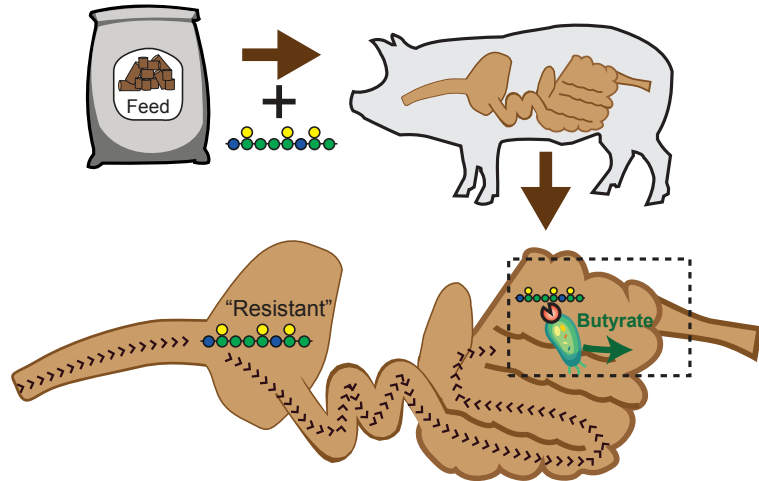
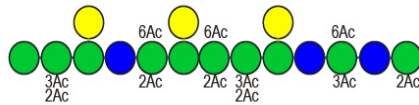


# Can we take advantage of the unique features of mannan and its degraders?



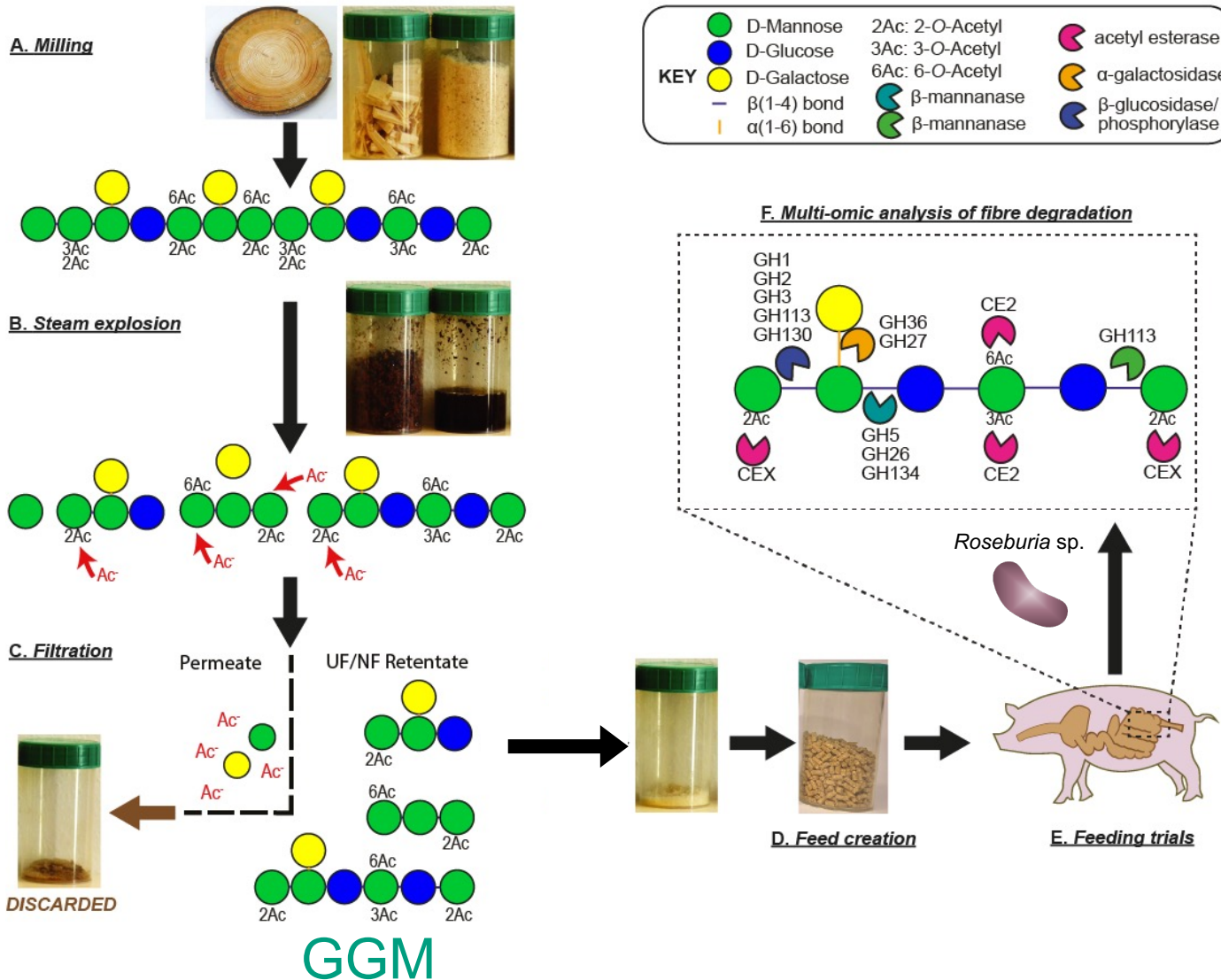
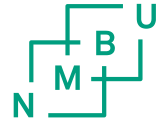
Bjørge Westereng  
NMBU

## Mannan as a prebiotic?



1. The (few) mannan degraders characterized thus far seem to be “*selfish*” (*i.e. selective*)
2. Mannan substrates requires a particular set of CAZymes (often GHs, CEs and CBMs) that are not widely found in gut commensals (*i.e. selective*)
3. Several famous butyrate-producing commensal bacteria are believed to degrade mannan (*i.e. Roseburia sp.*)

# Mannan from wood.....and into feed?



Bjørge Westereng  
NMBU

Leszek Michalak  
NMBU

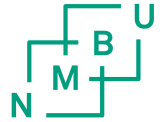
J. Chris Gaby  
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Sabina La Rosa  
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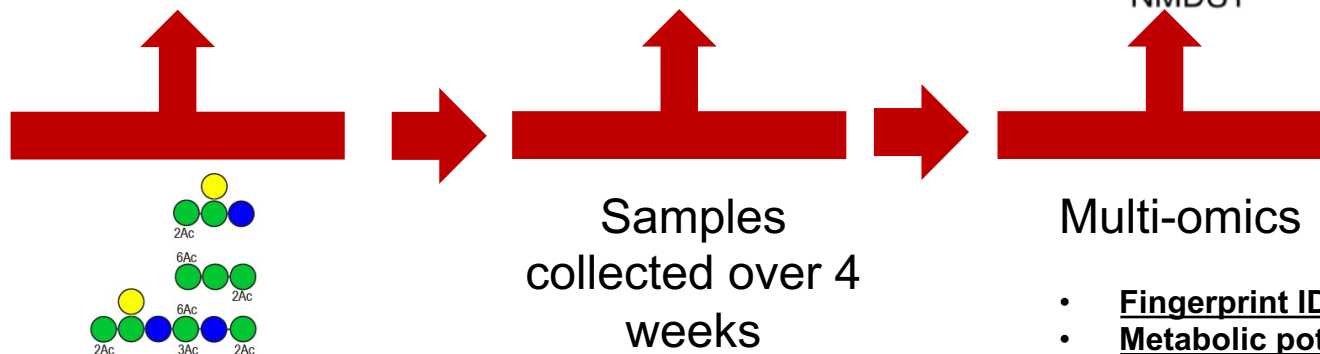
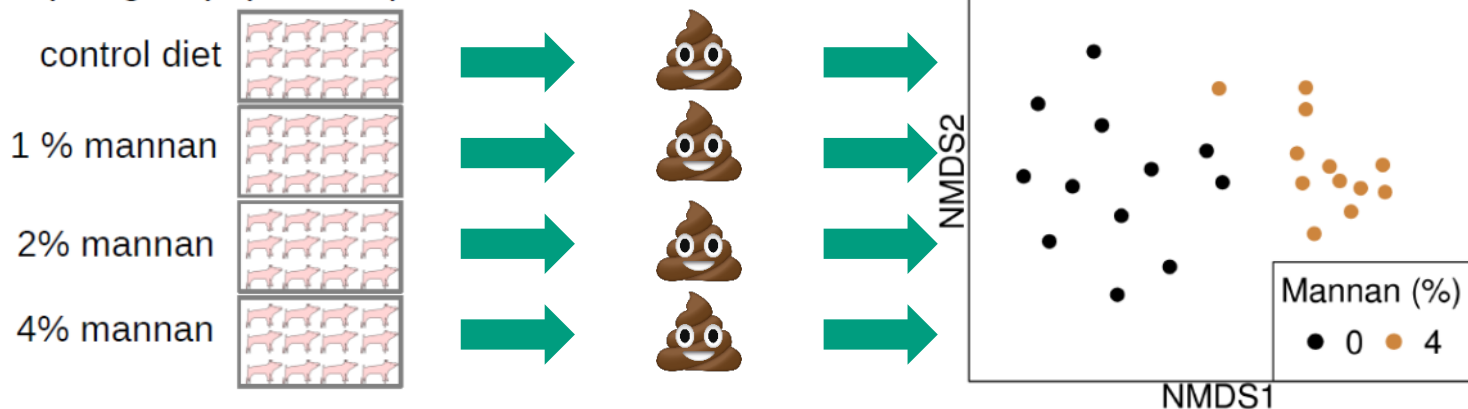
Leidy Lagos  
NMBU



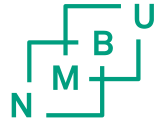
# Dietary mannan inclusion causes a dose response in the gut microbiome



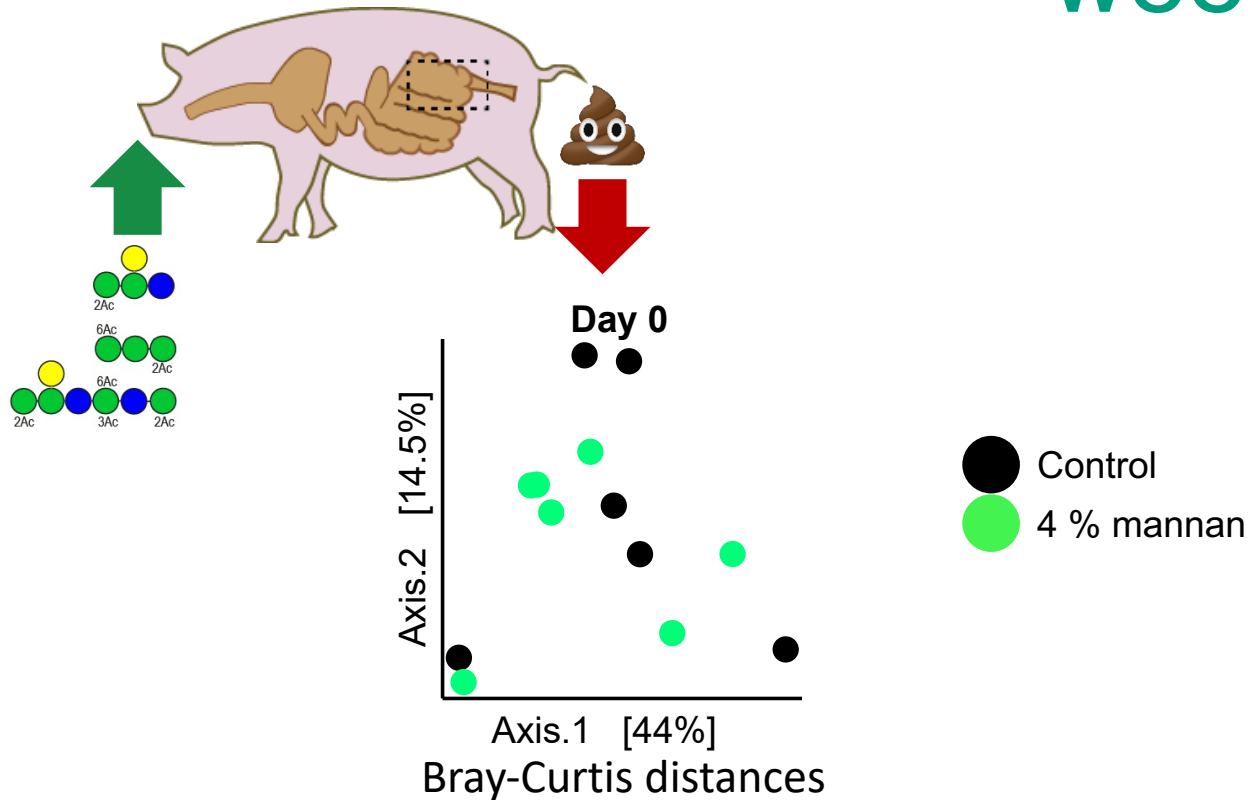
12 piglets per group (48 total)



# Temporal effects of a mannan diet

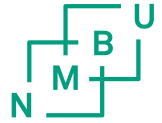


week 0

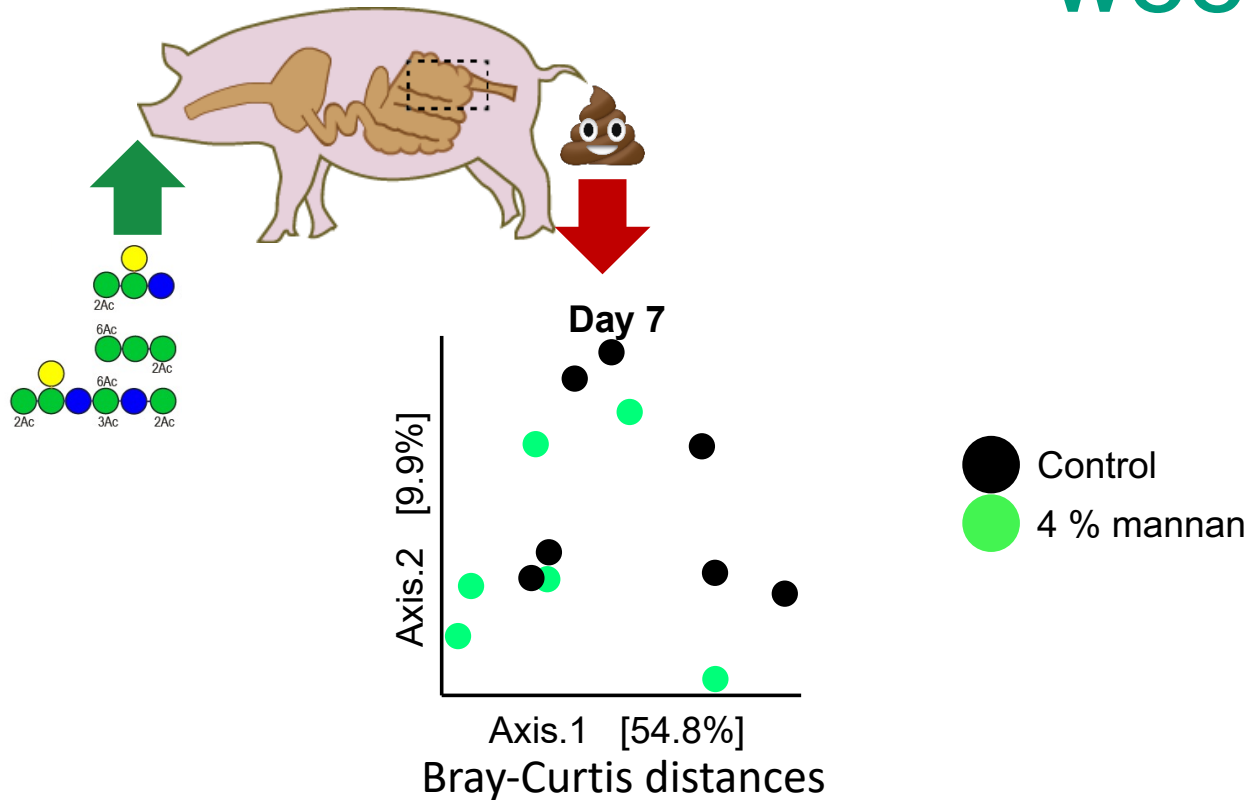


J. Chris Gaby  
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# Temporal effects of a mannan diet



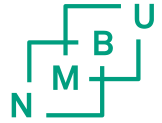
week 1



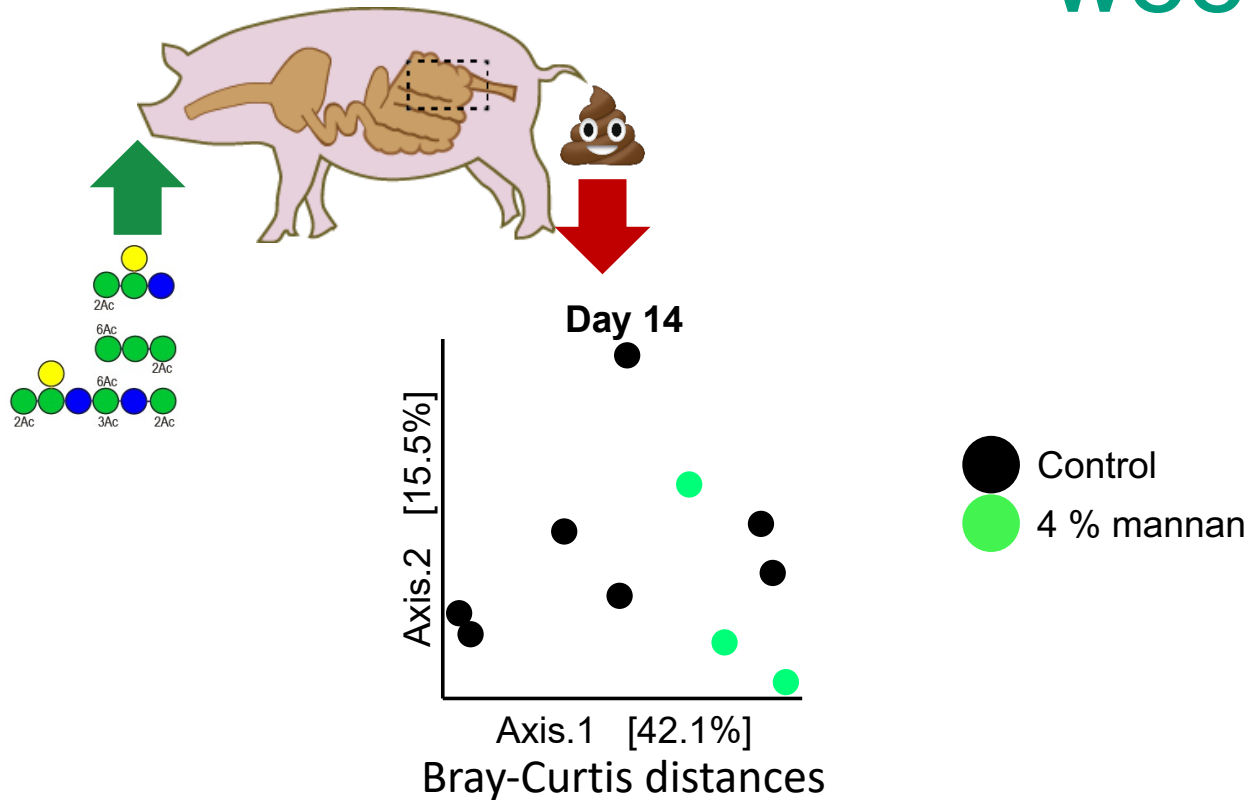
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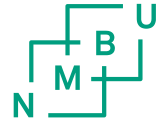


## week 2

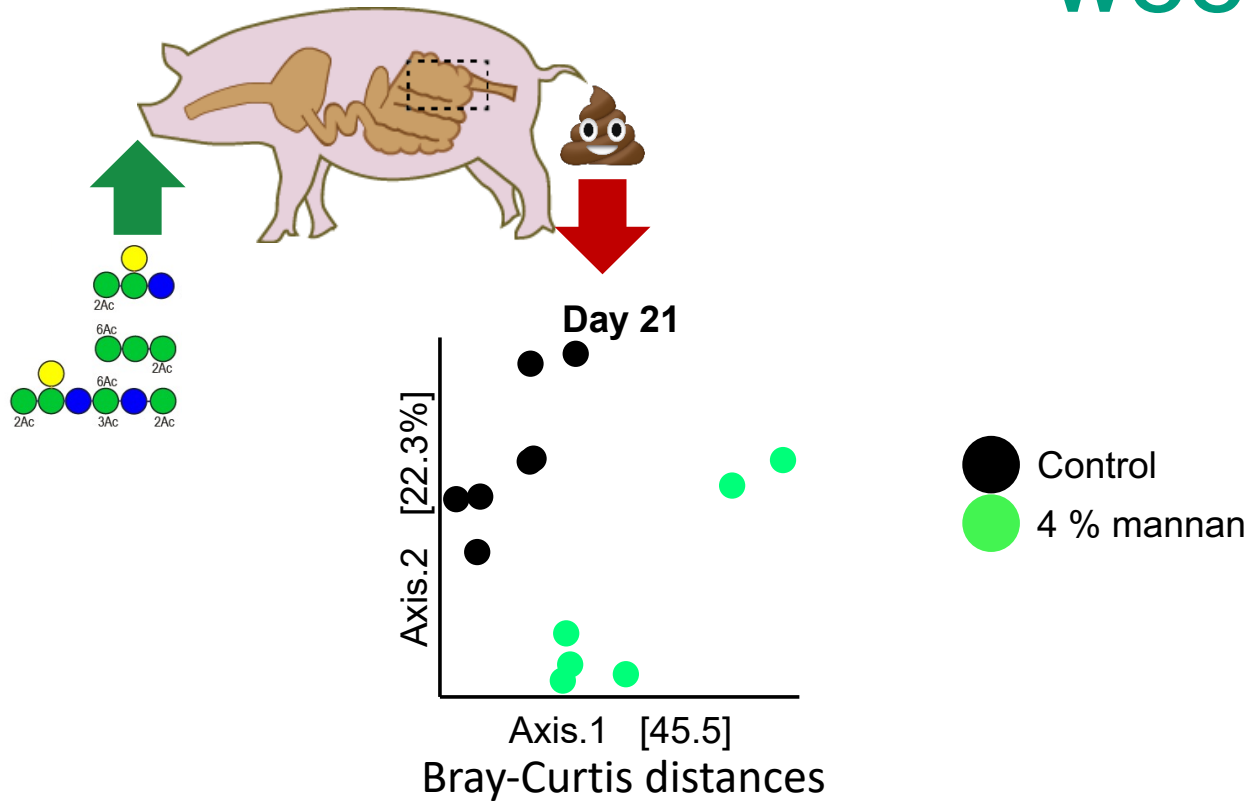


J. Chris Gaby  
NMBU

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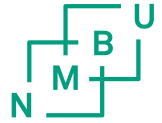


## week 3

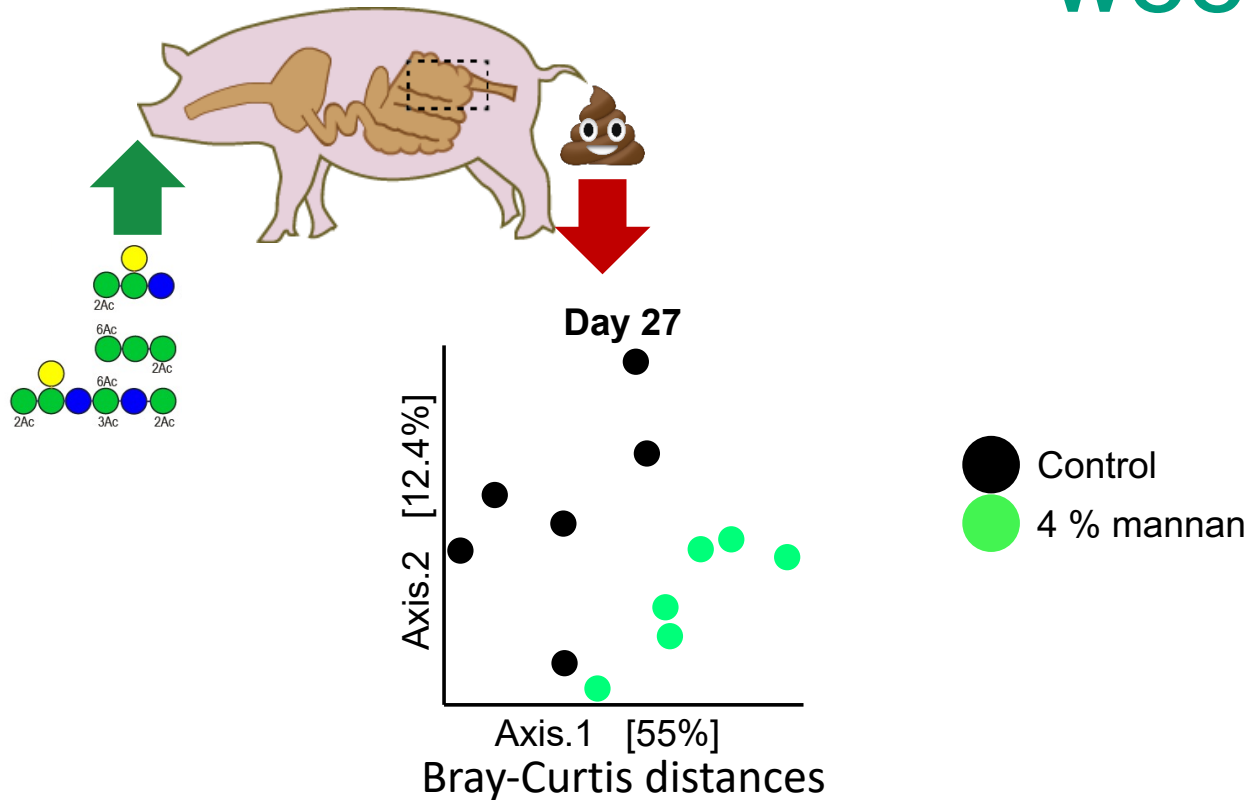


J. Chris Gaby  
NMBU

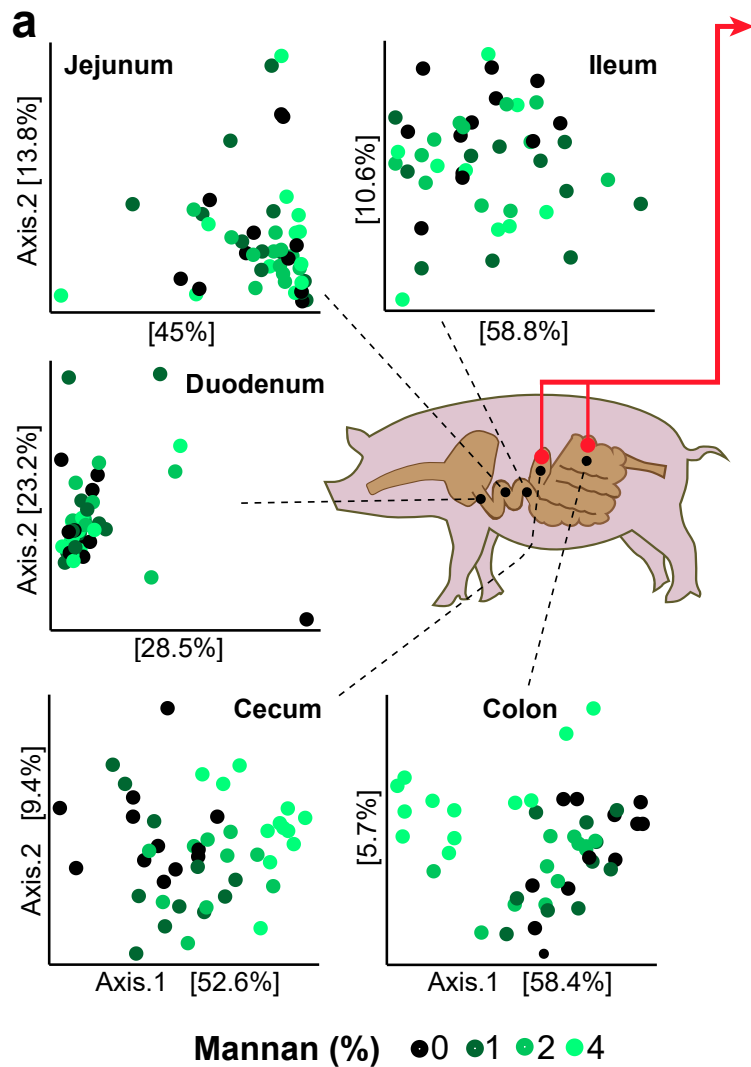
# Temporal effects of a mannan diet



week 4

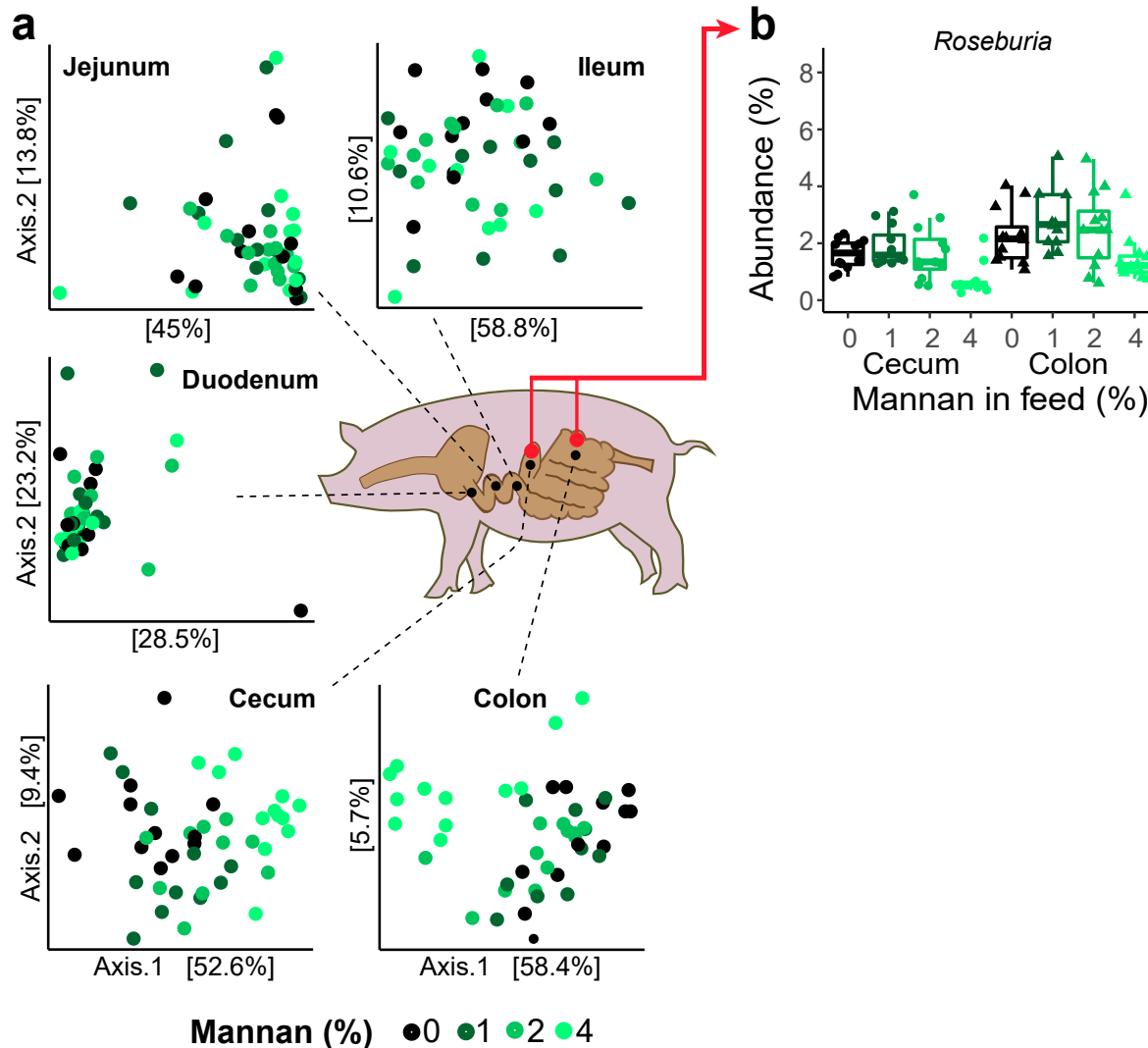


J. Chris Gaby  
NMBU

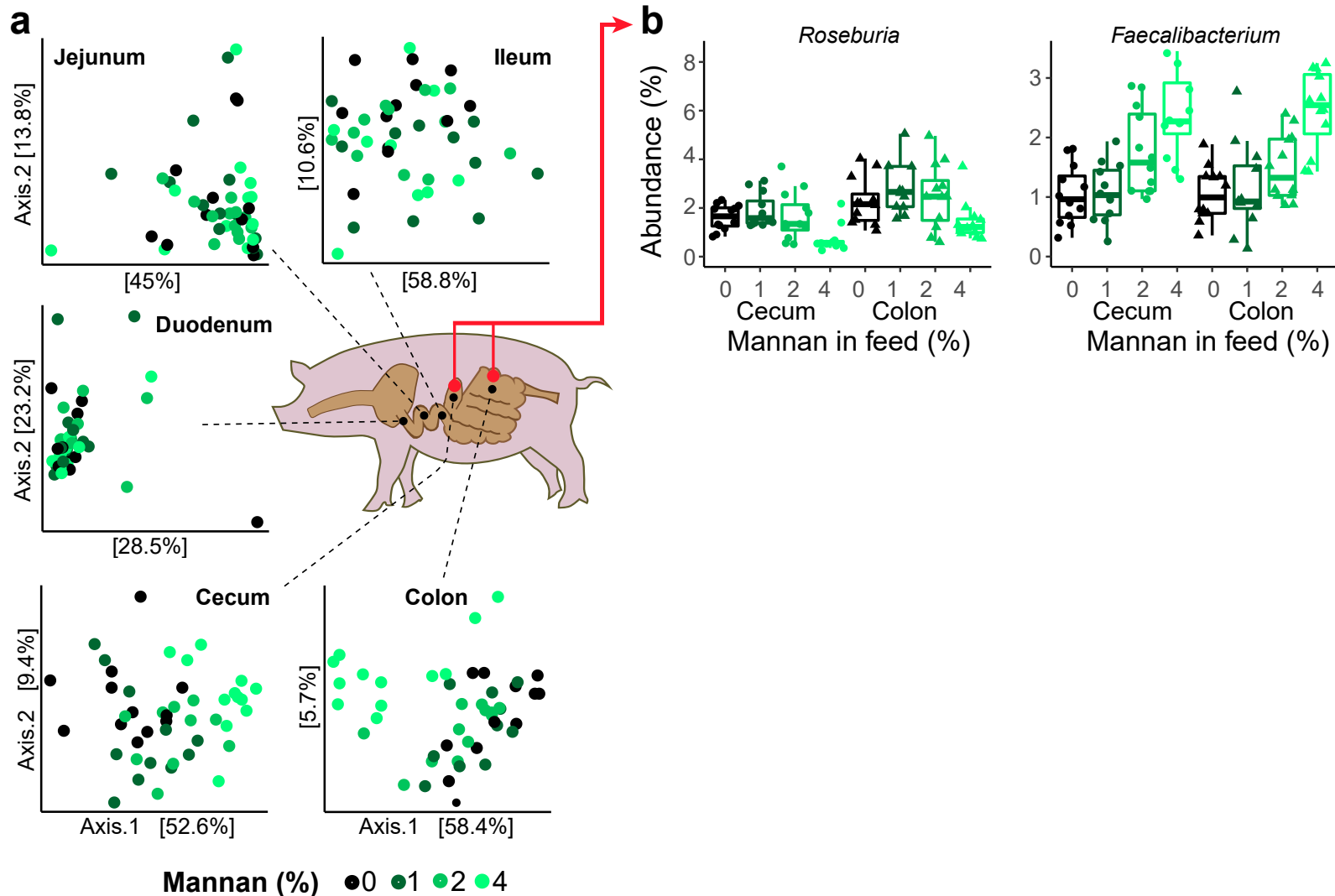


Mannan is degraded  
in the distal gut

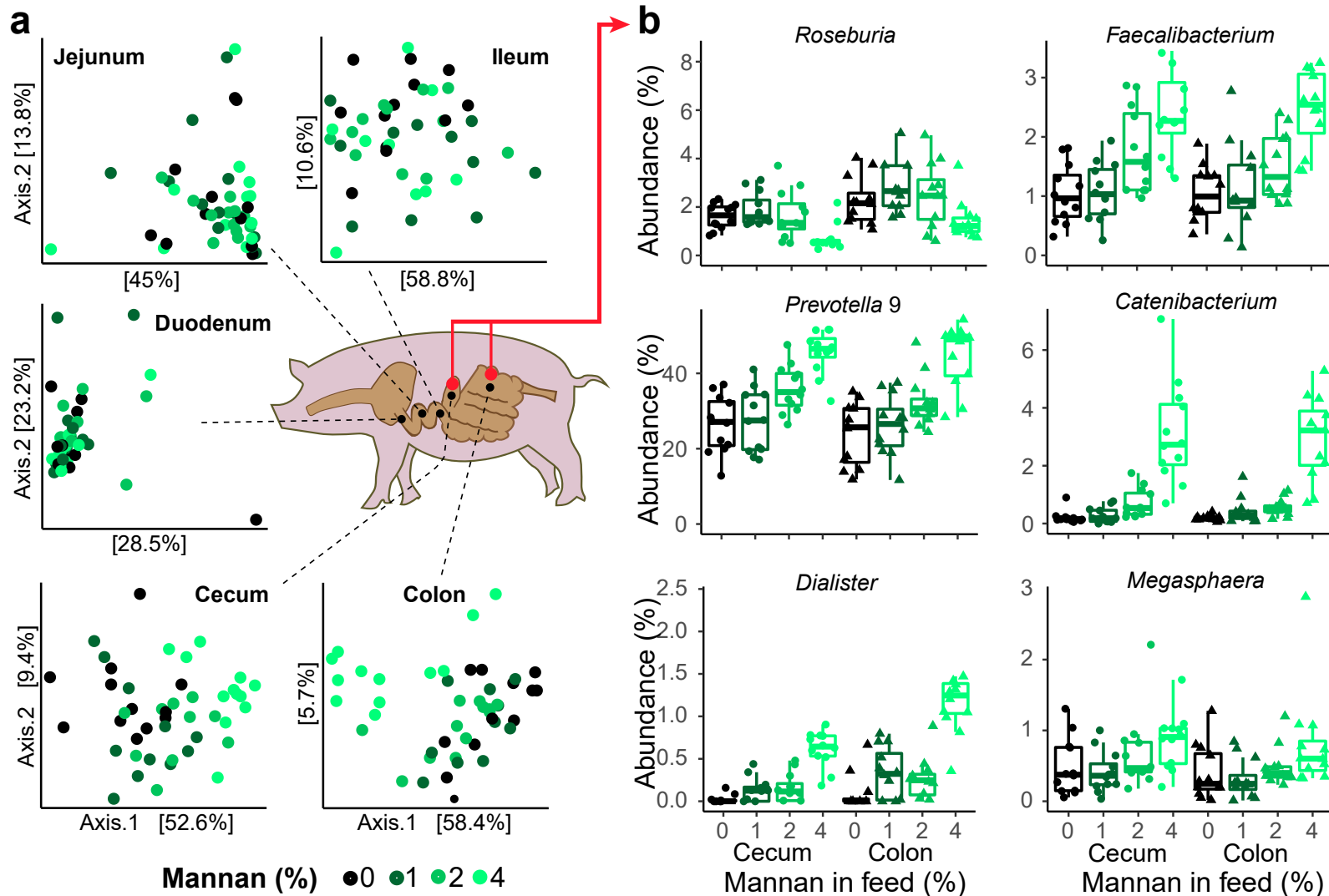
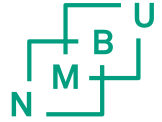
# The amount of mannan added seemingly has an effect on key commensals



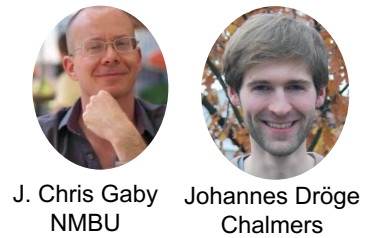
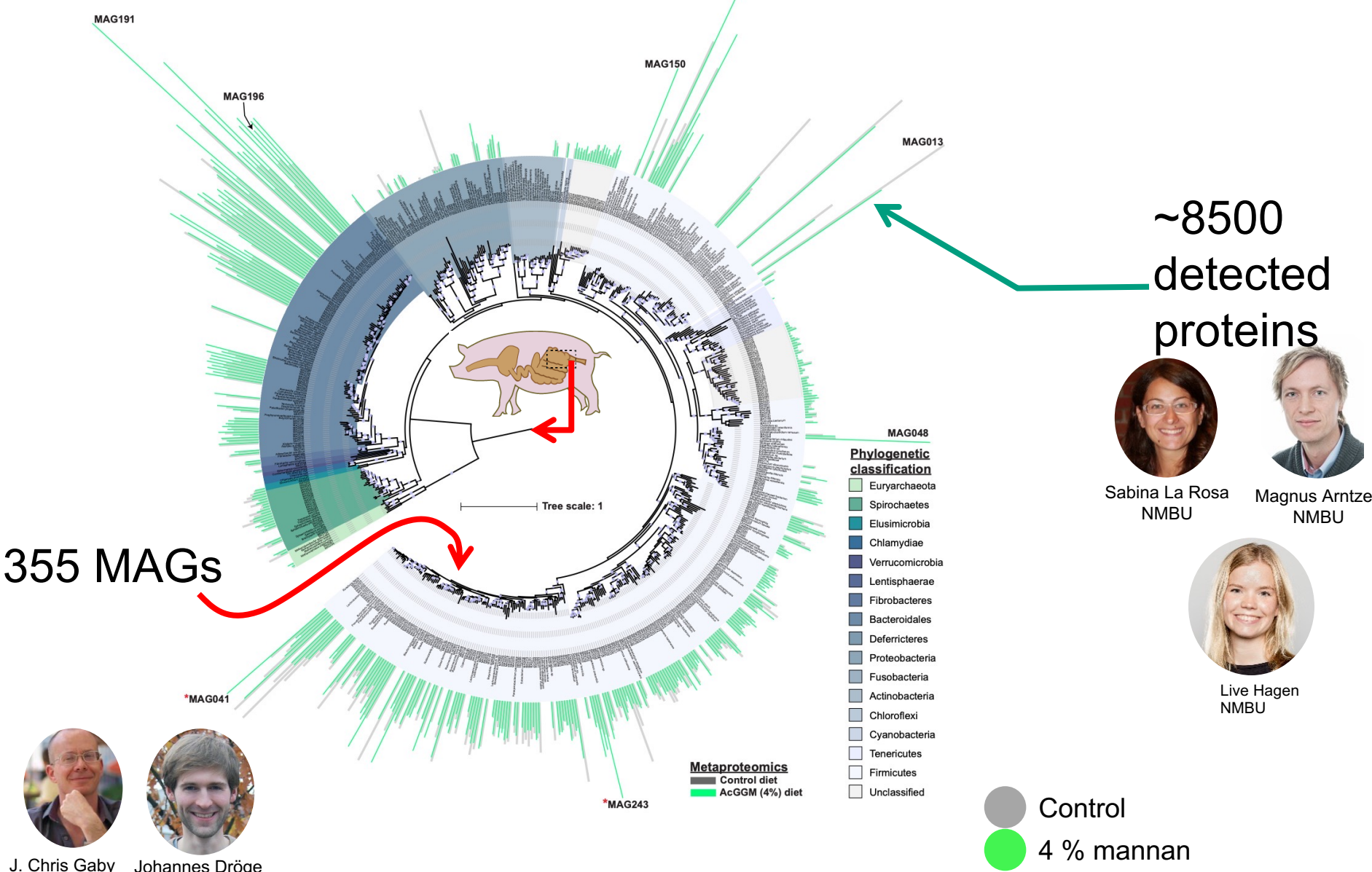
# The amount of mannan added seemingly has an effect on key commensals



# The amount of mannan added seemingly has an effect on key commensals

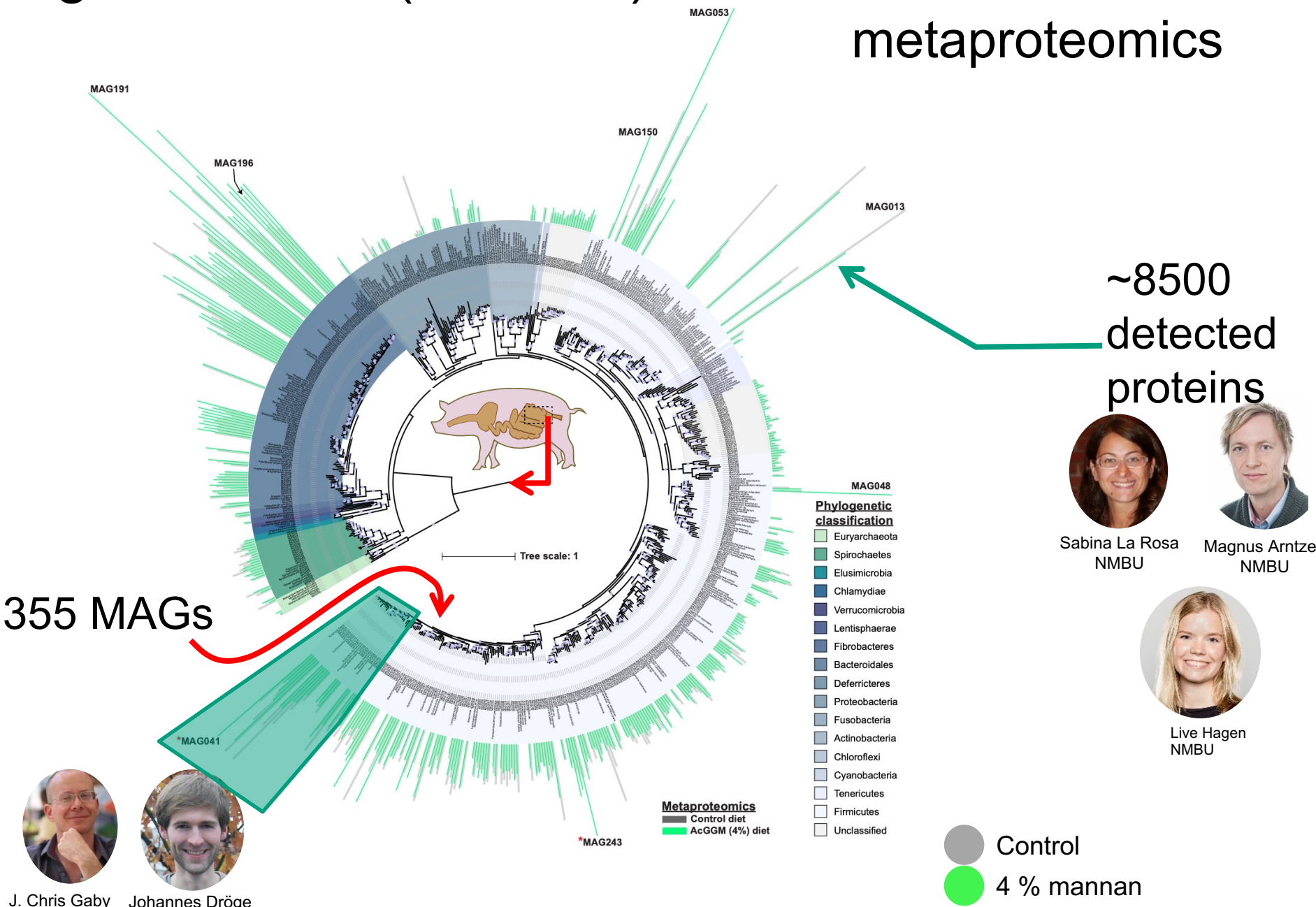


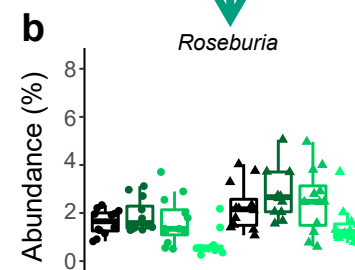
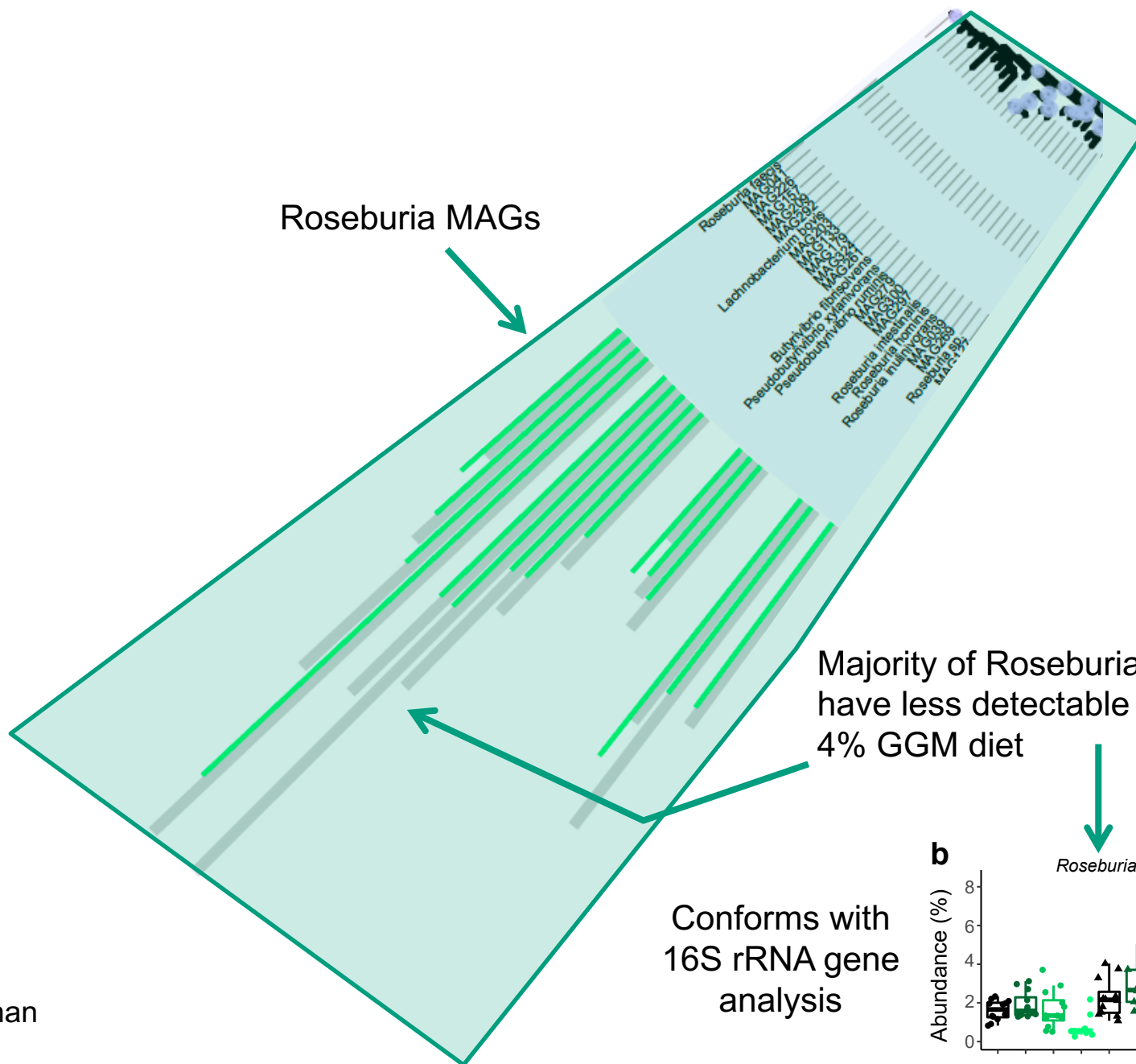
# Pigs eat wood (mannan)..... Genome-centric metaproteomics

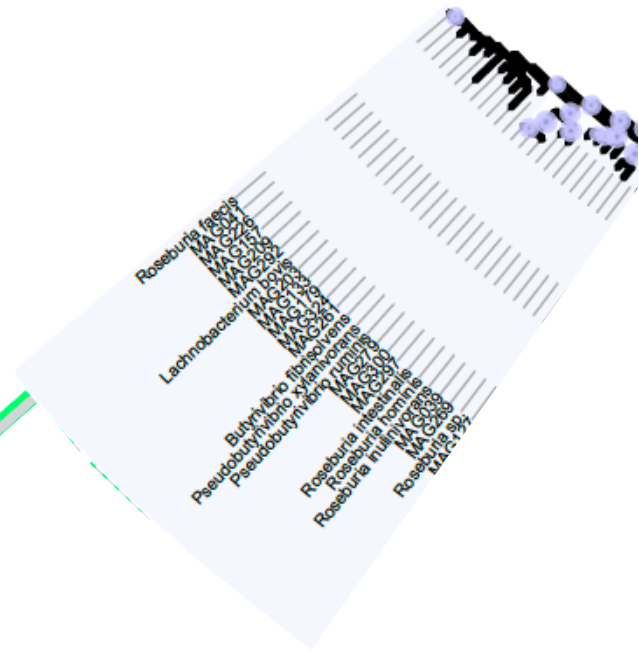




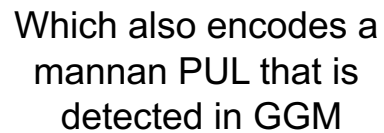
# Pigs eat wood (mannan)..... Genome-centric metaproteomics



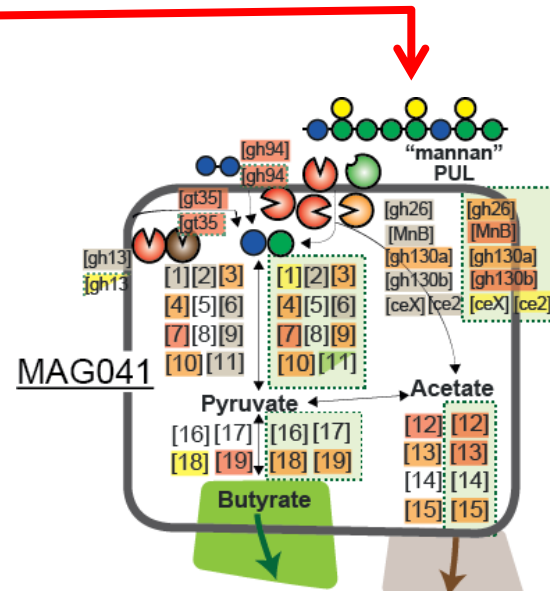


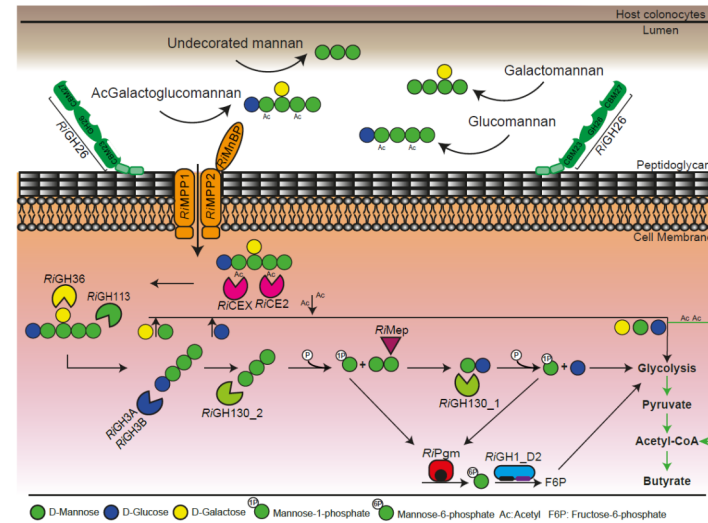
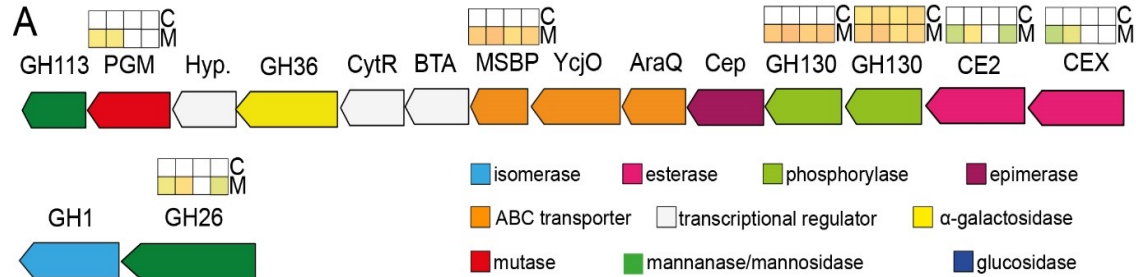


**\*MAG041**



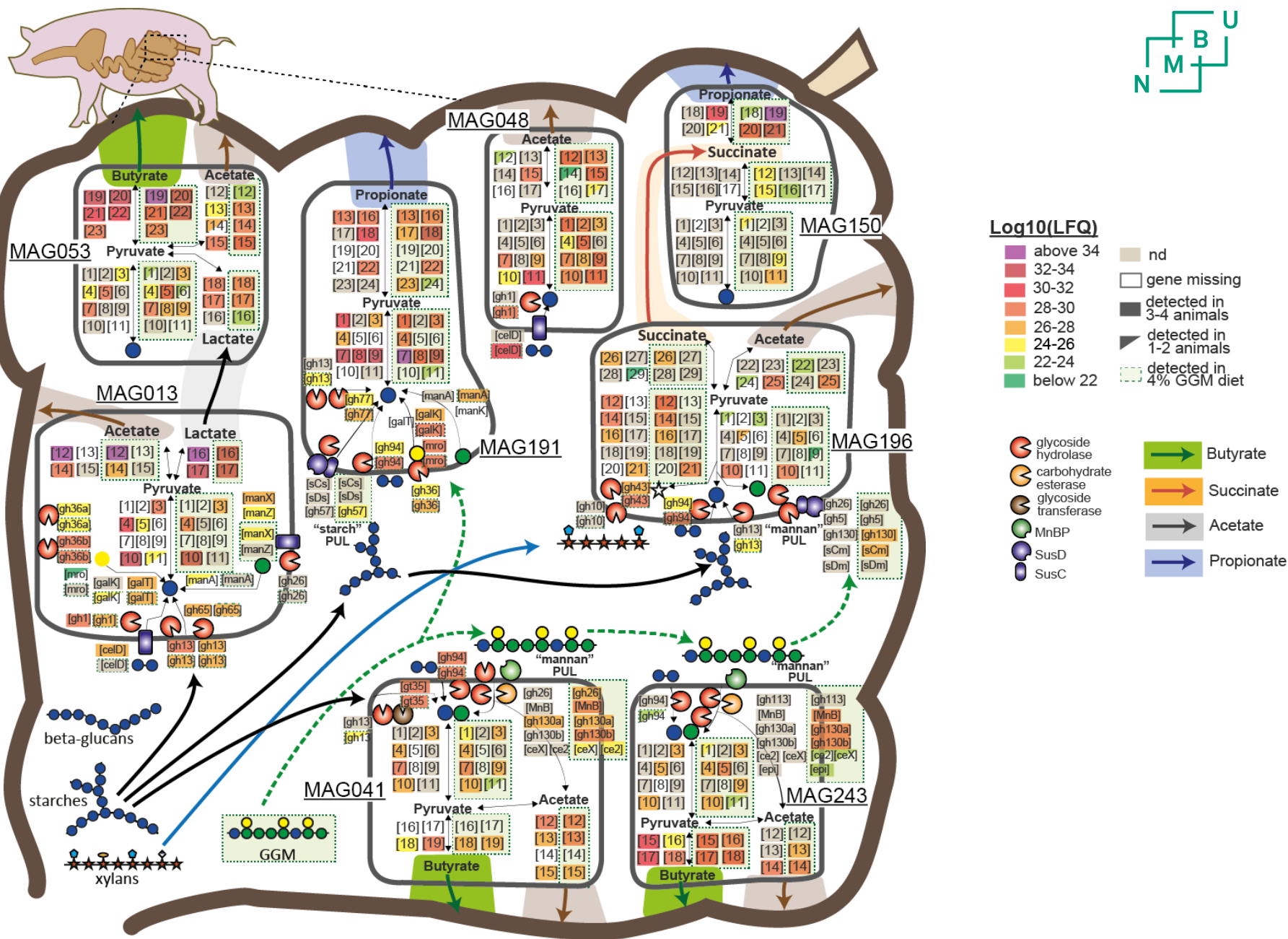
Control  
4 % mannan







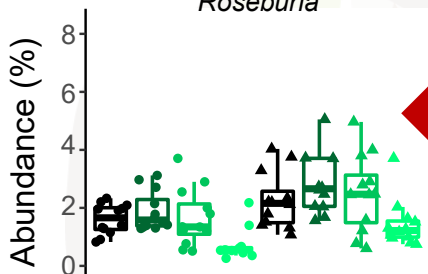




# 16S rRNA gene analysis

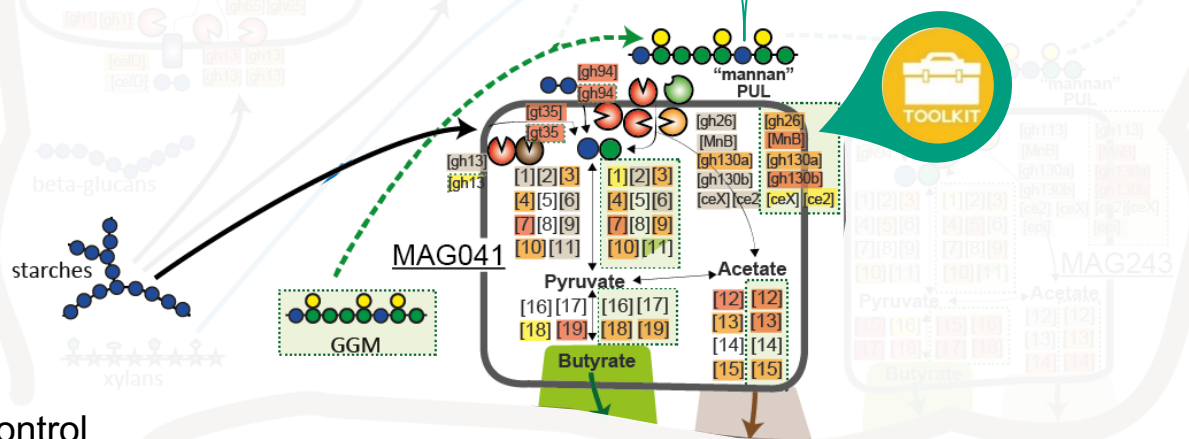
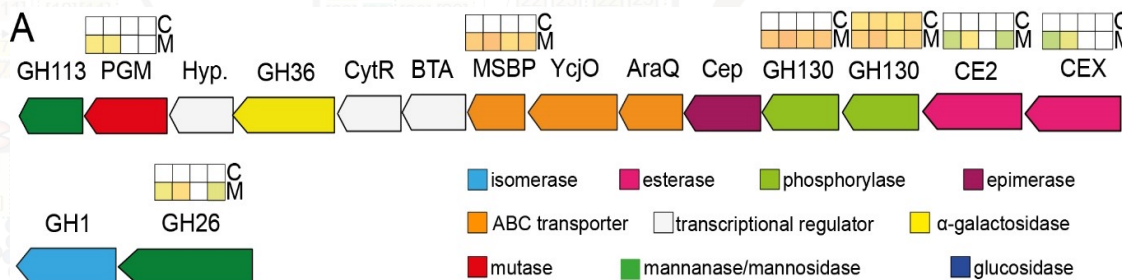
# Meta-omic analysis

*Roseburia*



Mannan-degrading *Roseburia* populations respond positively to the 4% GGM diet

MAG\_41



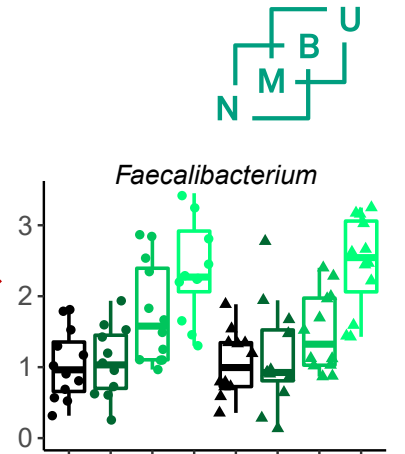
Control

4 % mannan

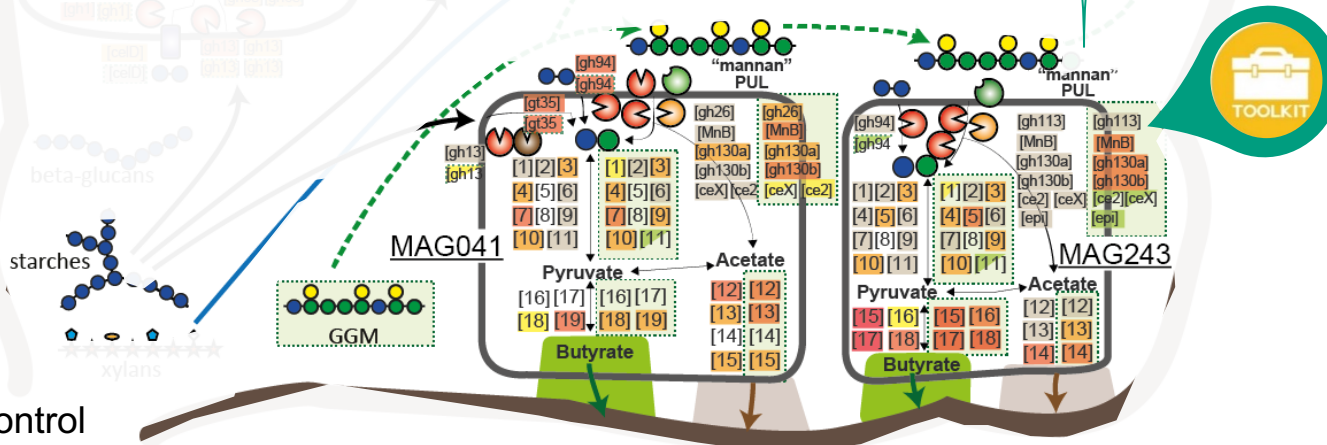
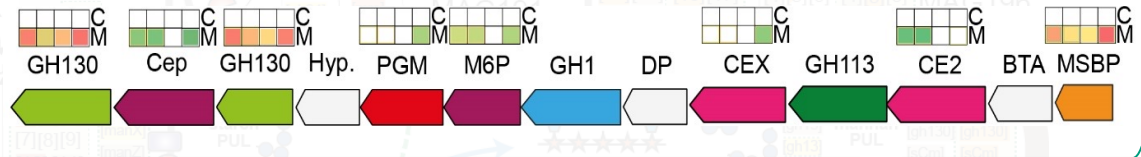
## Meta-omic analysis

Mannan-degrading  
*Faecalibacterium* sp respond  
positively to the 4% GGM diet

MAG\_243



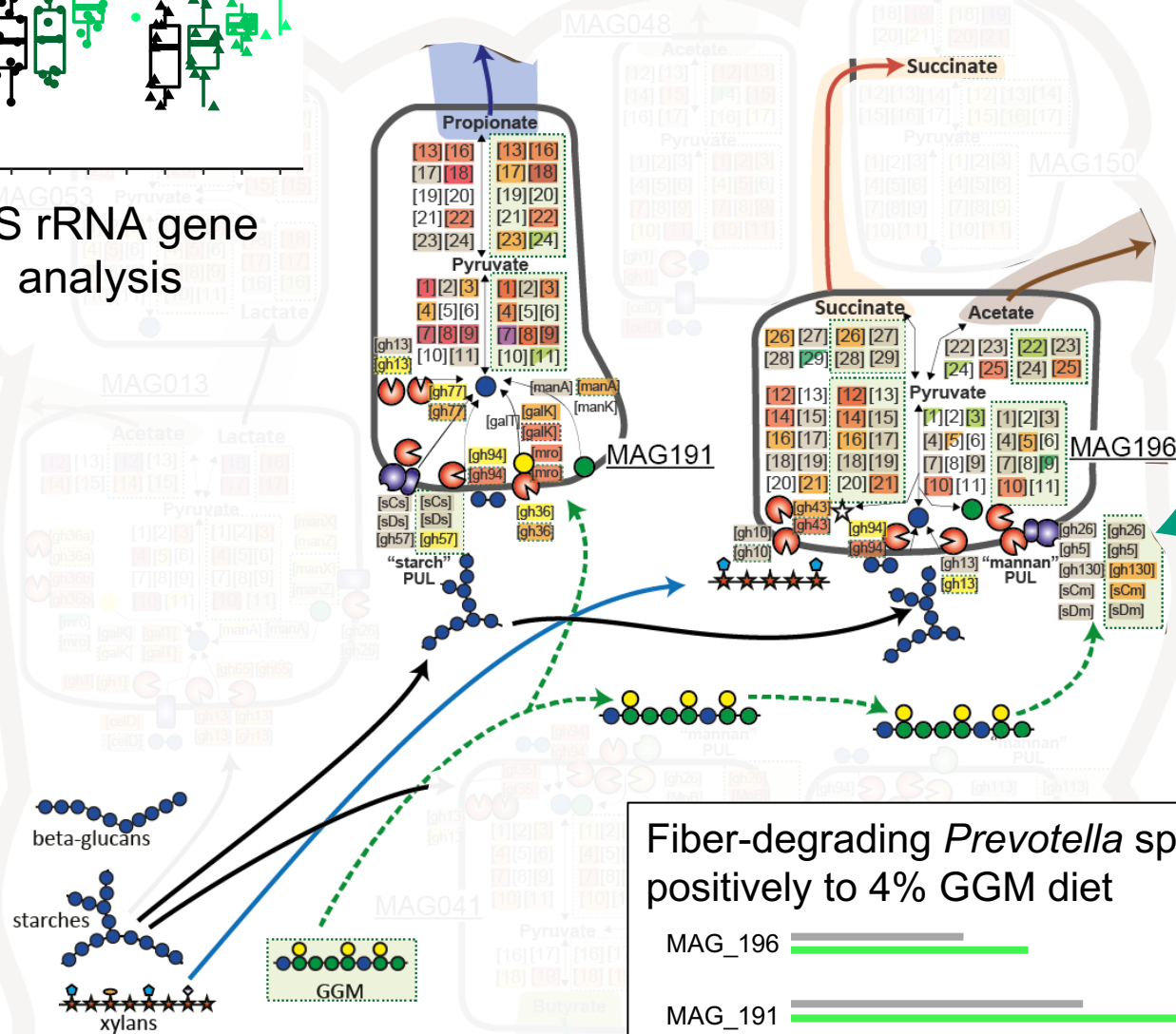
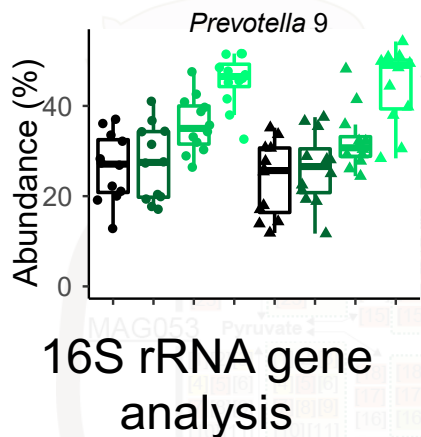
16S rRNA gene  
analysis



Control

4 % mannan





Fiber-degrading *Prevotella* sp. respond positively to 4% GGM diet

MAG 196 

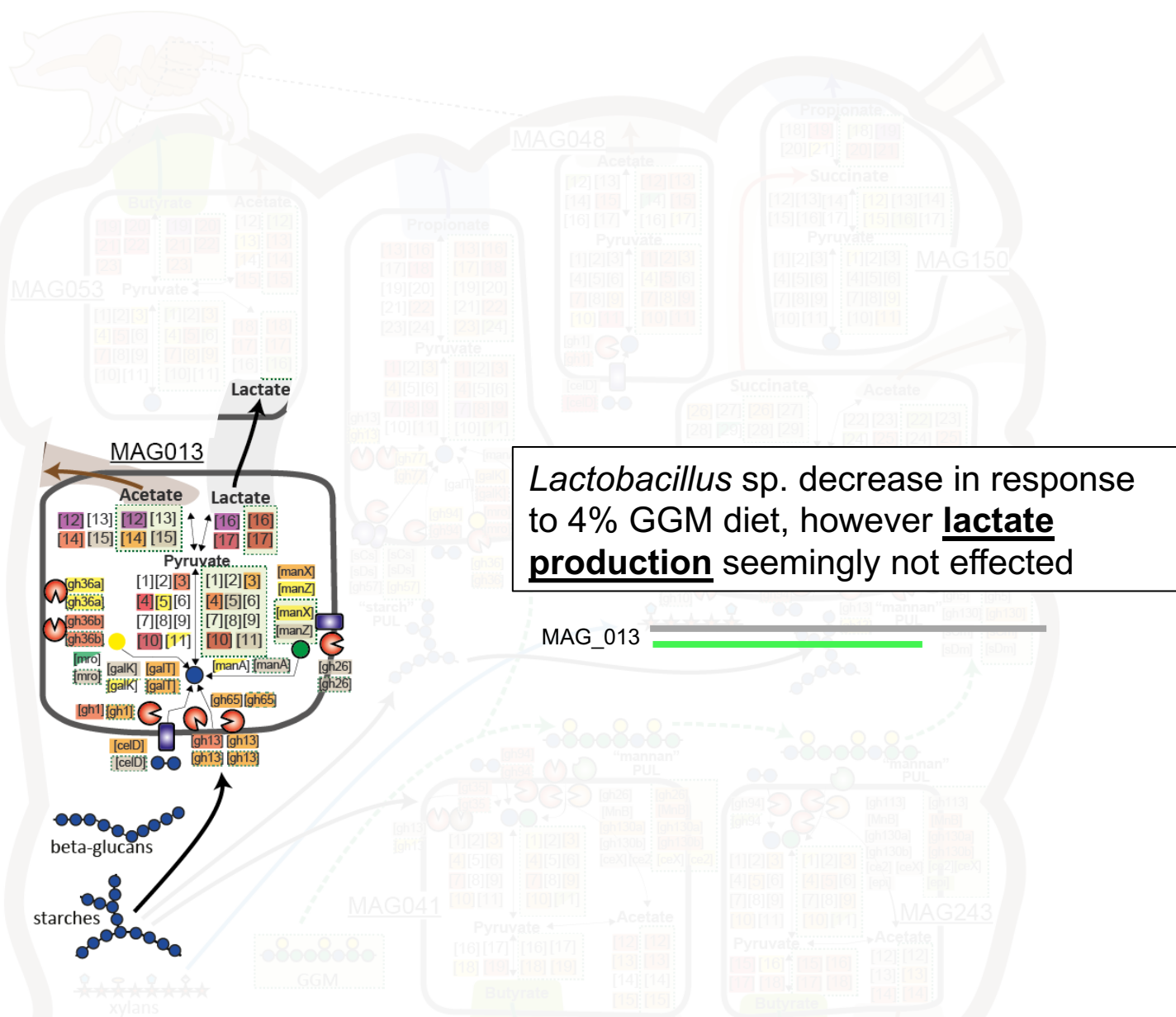
MAG\_191 

# Meta-omic analysis

Michalak et al. (2020) **Nature Communications**

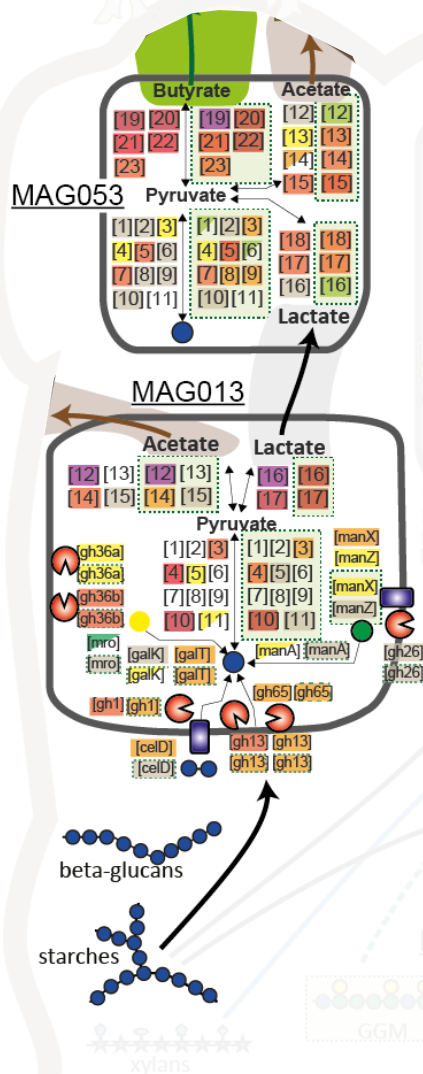
Control

4 % mannan



Meta-omic analysis

- Control
- 4 % mannan



*Megasphaera* sp. (**lactate** **utilizers**)  
increase

MAG\_53



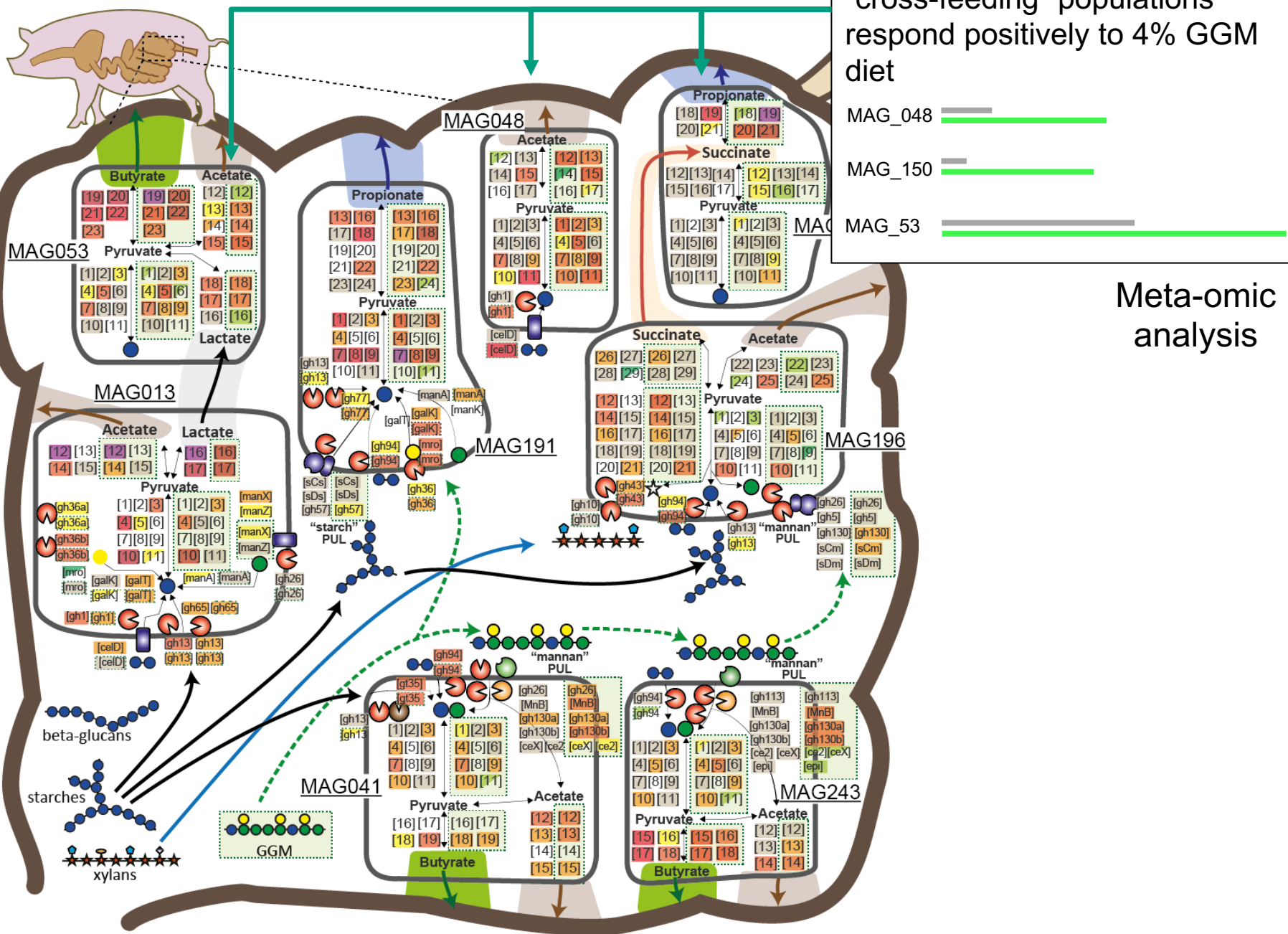
Lactobacillus sp. decrease in response  
to 4% GGM diet, however **lactate**  
**production** seemingly not effected

MAG\_013

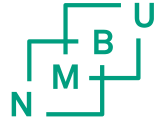
Meta-omic  
analysis

Control

4 % mannan

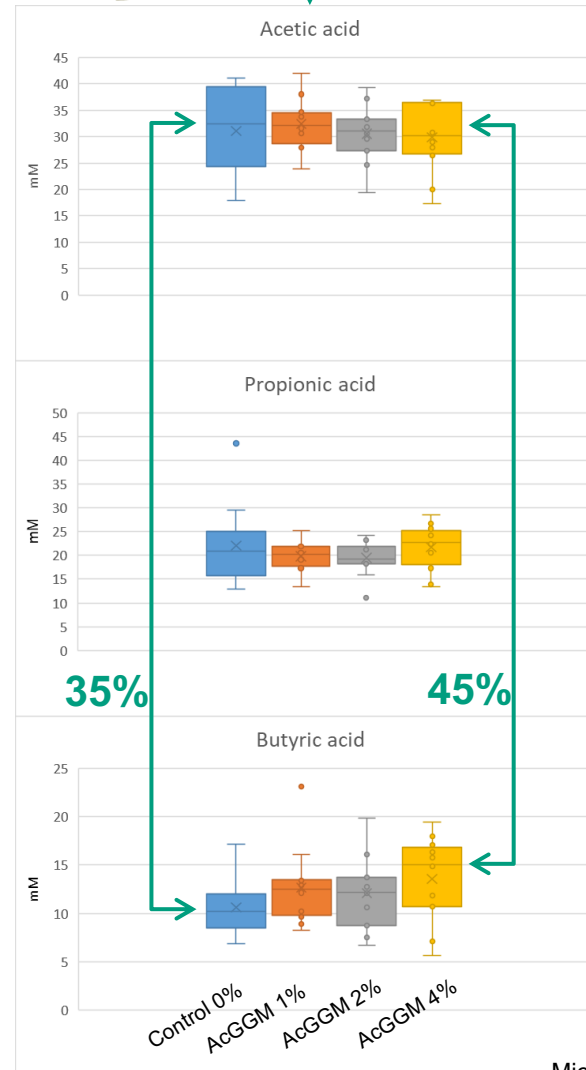
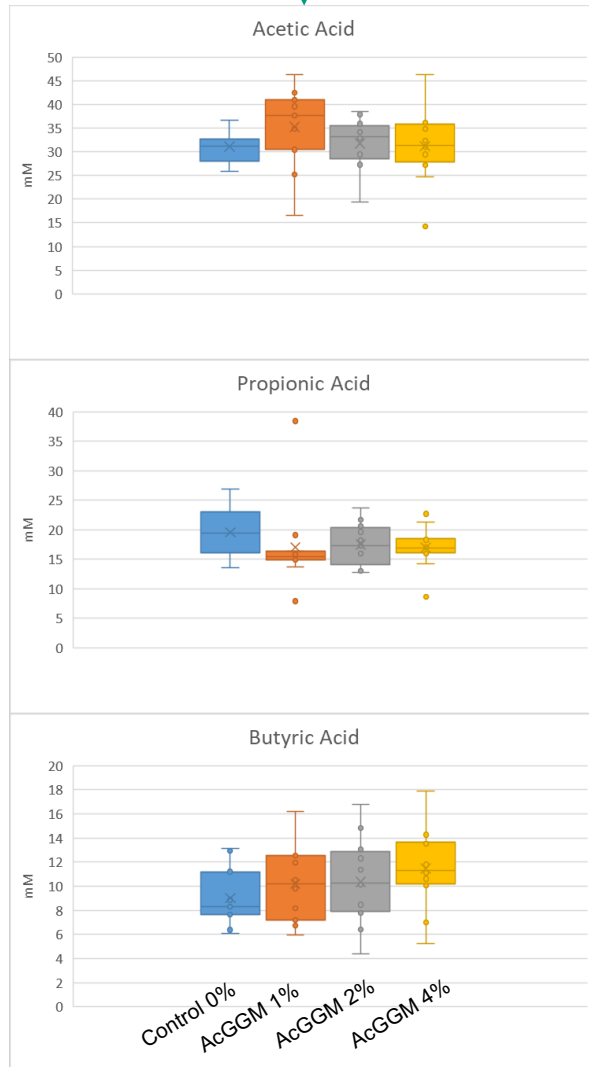


# What effect does AcGGM have on metabolites?



Cecum

Colon



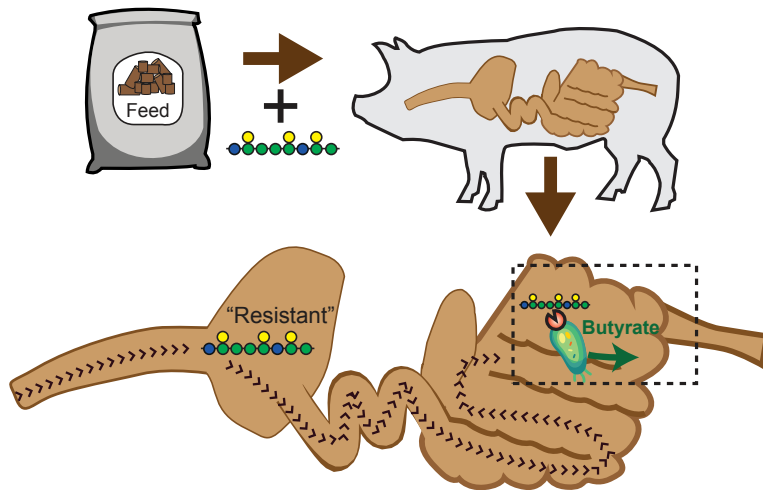
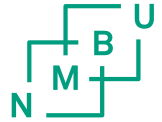
## Acetate:propionate:butyrate (ratio)

Control = 100 : 72 : 35

AcGGM (4%) = 100 : 74 : 45

*p*-value = 0.004

# So what does this all tell us?

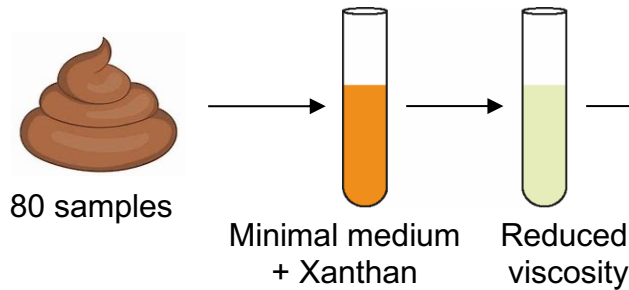
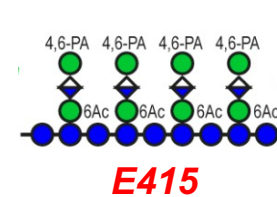


Mannan as a prebiotic?

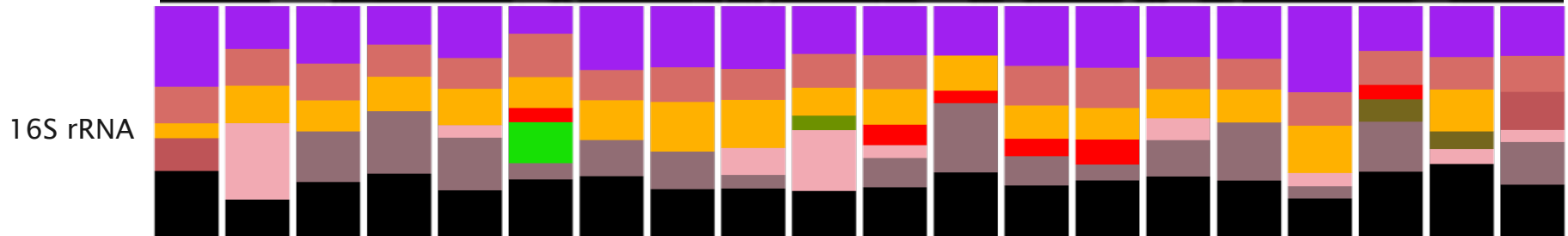
1. Understand your microbes and their tools
2. Understand your **substrate**
3. Even **if you can manage to** “tap your specific microbe on the head”, there will be a “**butterfly effect**”. To understand this requires “*more of everything*”



# We eat MDFs every day



80 individuals were sampled we identified 40 positive cultures,



Eric C. Martens  
UMich

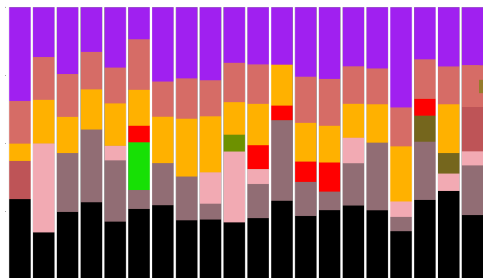


Matthew Ostrowski  
UMich

**Gro UM!**

Generating a Repository of Organisms by  
Untargeted culturing of Microbiomes

## 16S rRNA



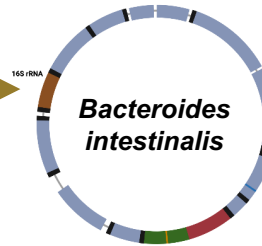
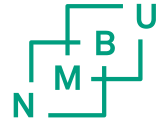
Nanopore  
MetaG

16S rRNA

**Ruminococcaceae**  
**UCG13**

## MetaGenomics

Circular HQ-MAGs  
from metagenomes  
(with 16S rRNA  
operon!!!)



***Bacteroides***  
***intestinalis***



Live Hagen  
NMBU

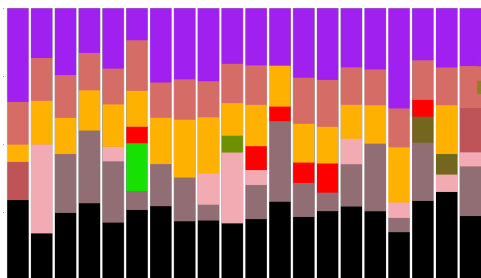


Sabina La Rosa  
NMBU



Matthew  
Ostrowski

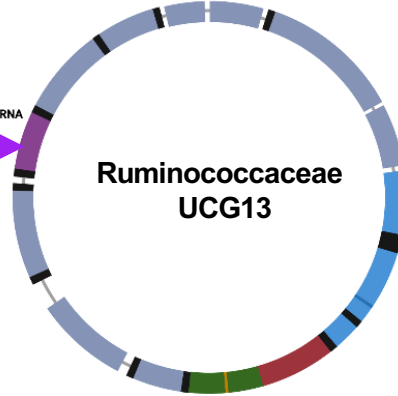
## 16S rRNA



Nanopore  
MetaG

16S rRNA

**Ruminococcaceae  
UCG13**



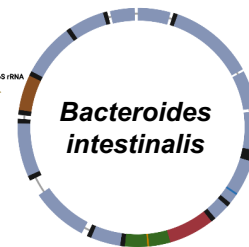
## MetaGenomics

Circular HQ-MAGs  
from metagenomes  
(with 16S rRNA  
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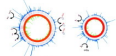
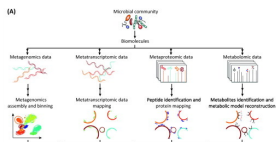


Live Hagen  
NMBU

***Bacteroides  
intestinalis***

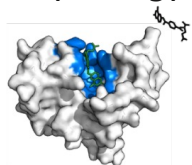


## Meta-omics



+

## Enzymology

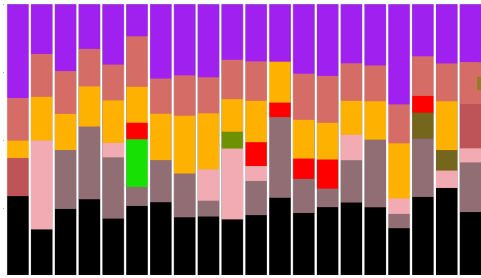


Sabina La Rosa  
NMBU



Matthew  
Ostrowski

## 16S rRNA



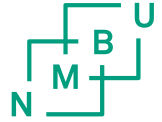
Nanopore  
MetaG

16S rRNA

**Ruminococcaceae  
UCG13**

## MetaGenomics

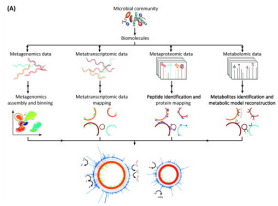
Circular HQ-MAGs  
from metagenomes  
(with 16S rRNA  
operon!!!)



Live Hagen  
NMBU

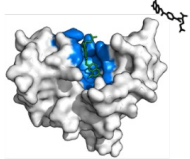
**Bacteroides  
intestinalis**

## Meta-omics



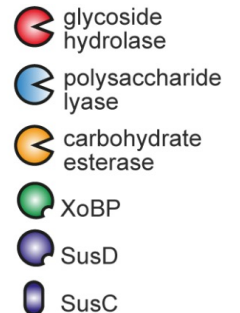
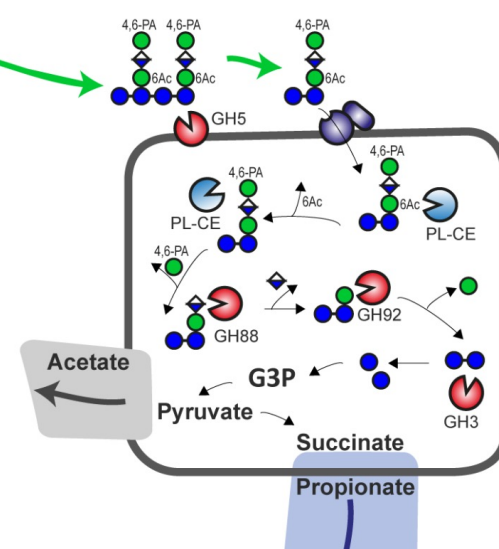
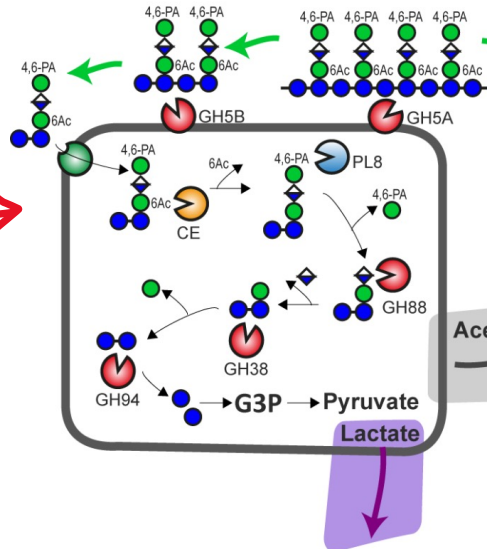
## Cross-feeding between

## Enzymology



**Ruminococcaceae UCG13**

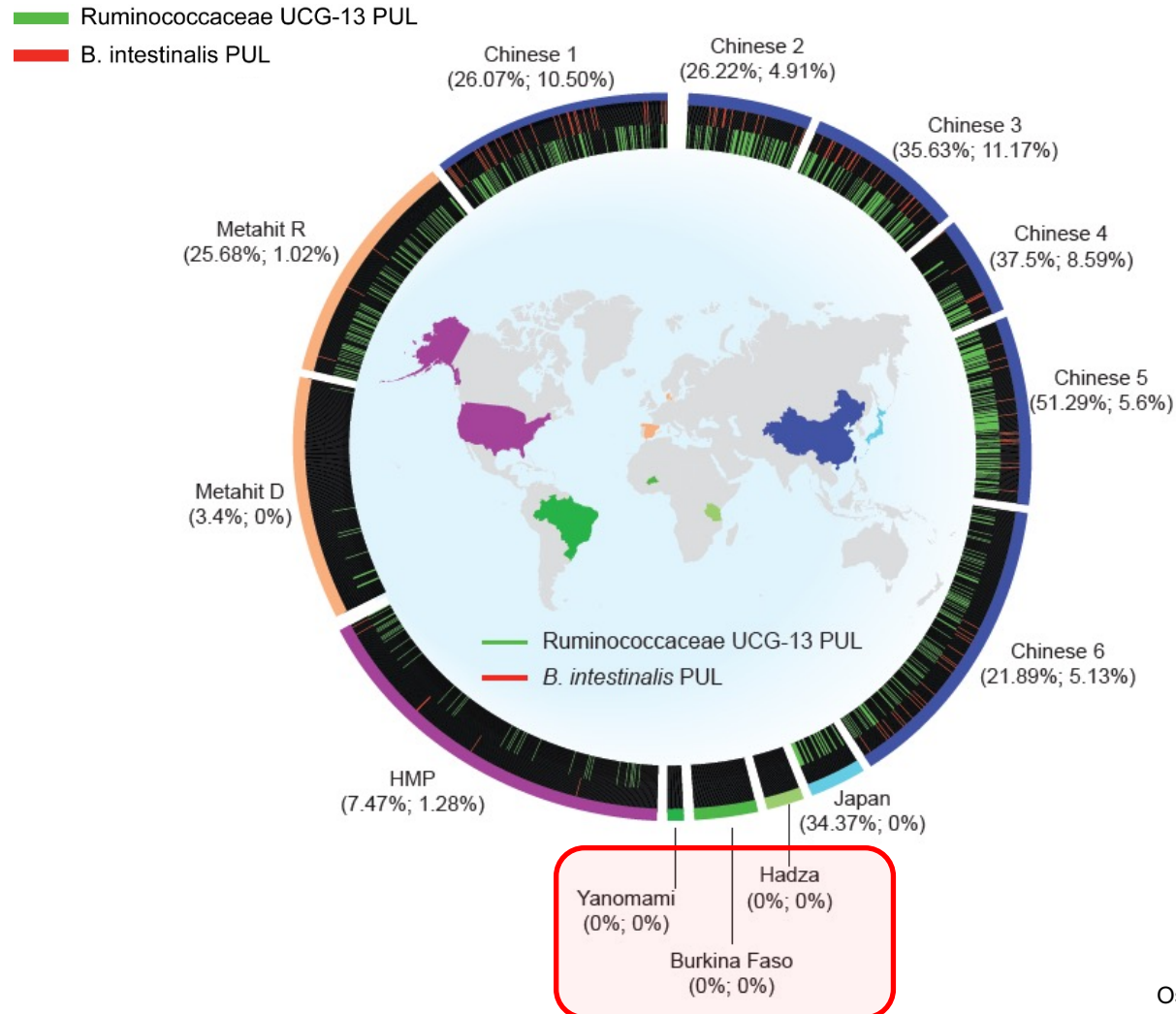
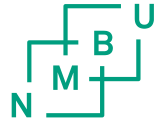
**Bacteroides intestinalis**



Sabina La Rosa  
NMBU

Matthew  
Ostrowski

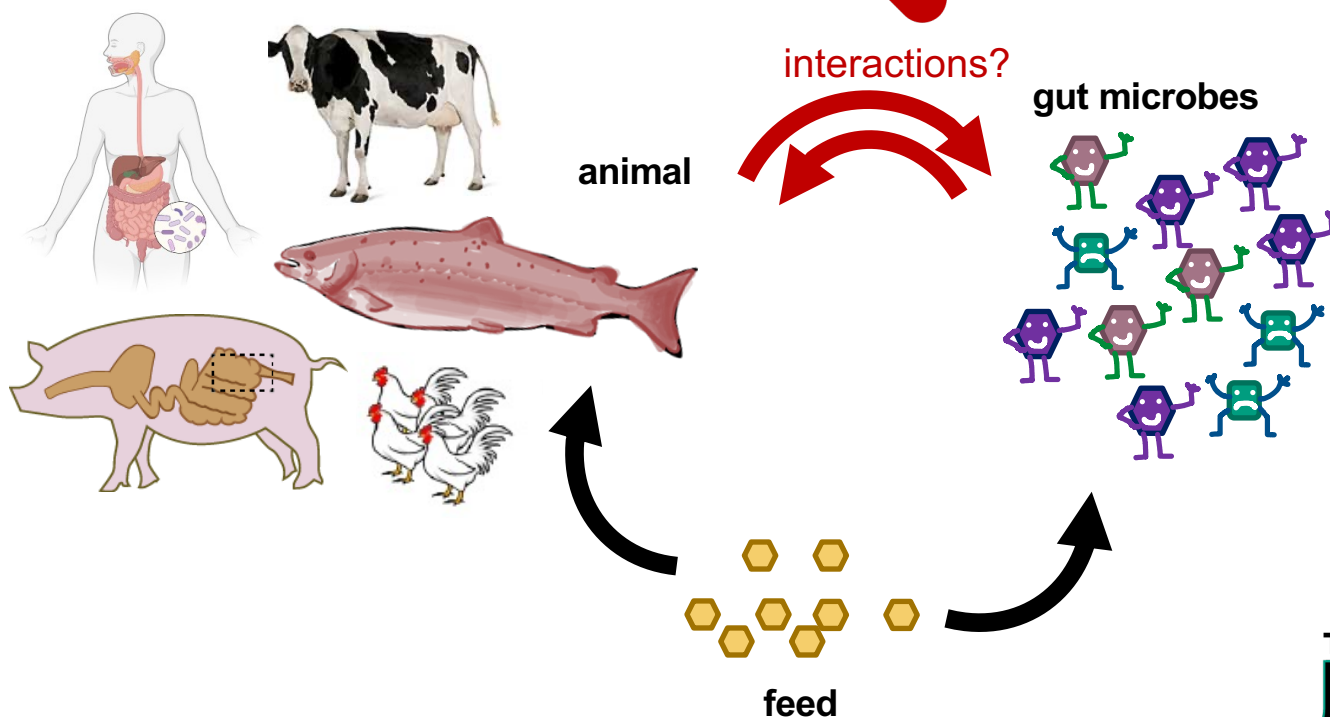
# Xanthan utilization loci are widespread in modern microbiomes



# So, where are we going from here?



- interactions that effect host metabolism
- does host genetics matter?

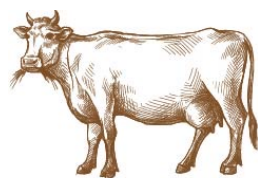




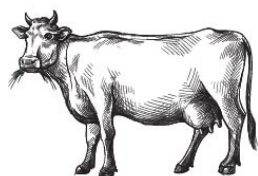


# SuPAcow:

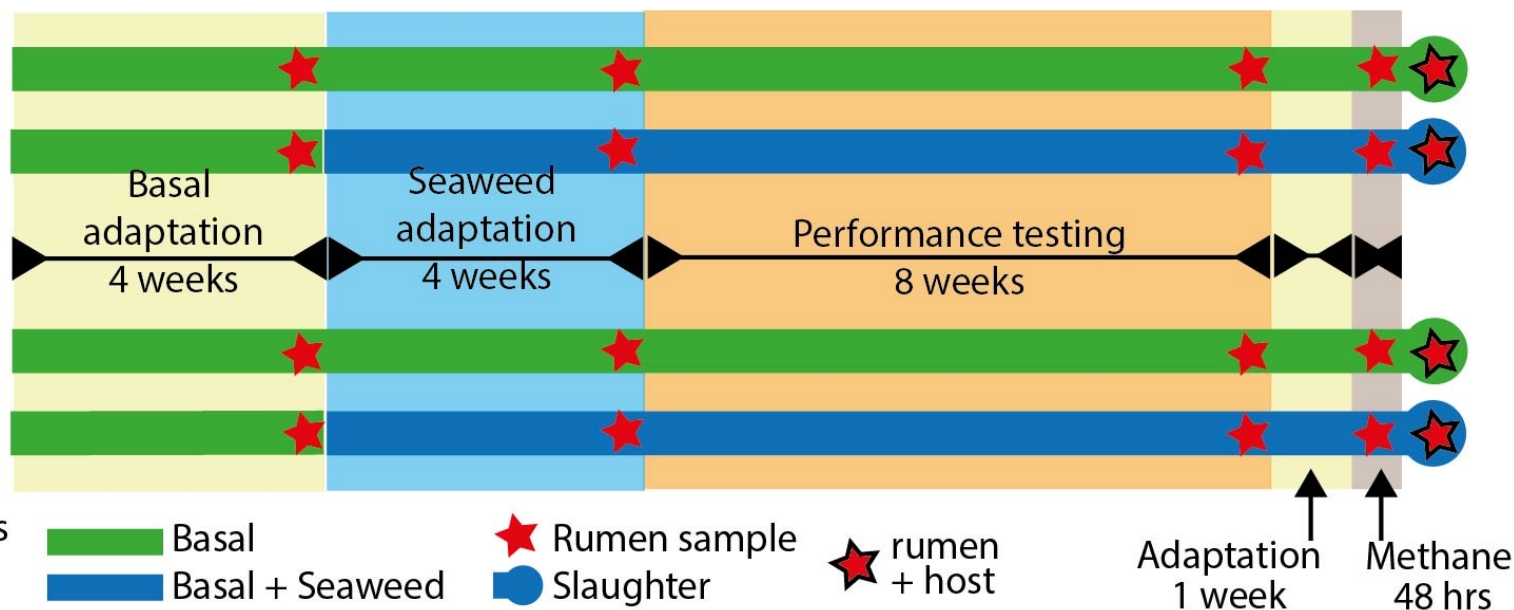
## The feed-microbiome-host axis in cows



Luig



Limousin x  
Aberdeen Angus



Thea Os  
Andersen

Ianina (Yanna)  
Altshuler

Torgeir Hvidsten

Rainer Roehe

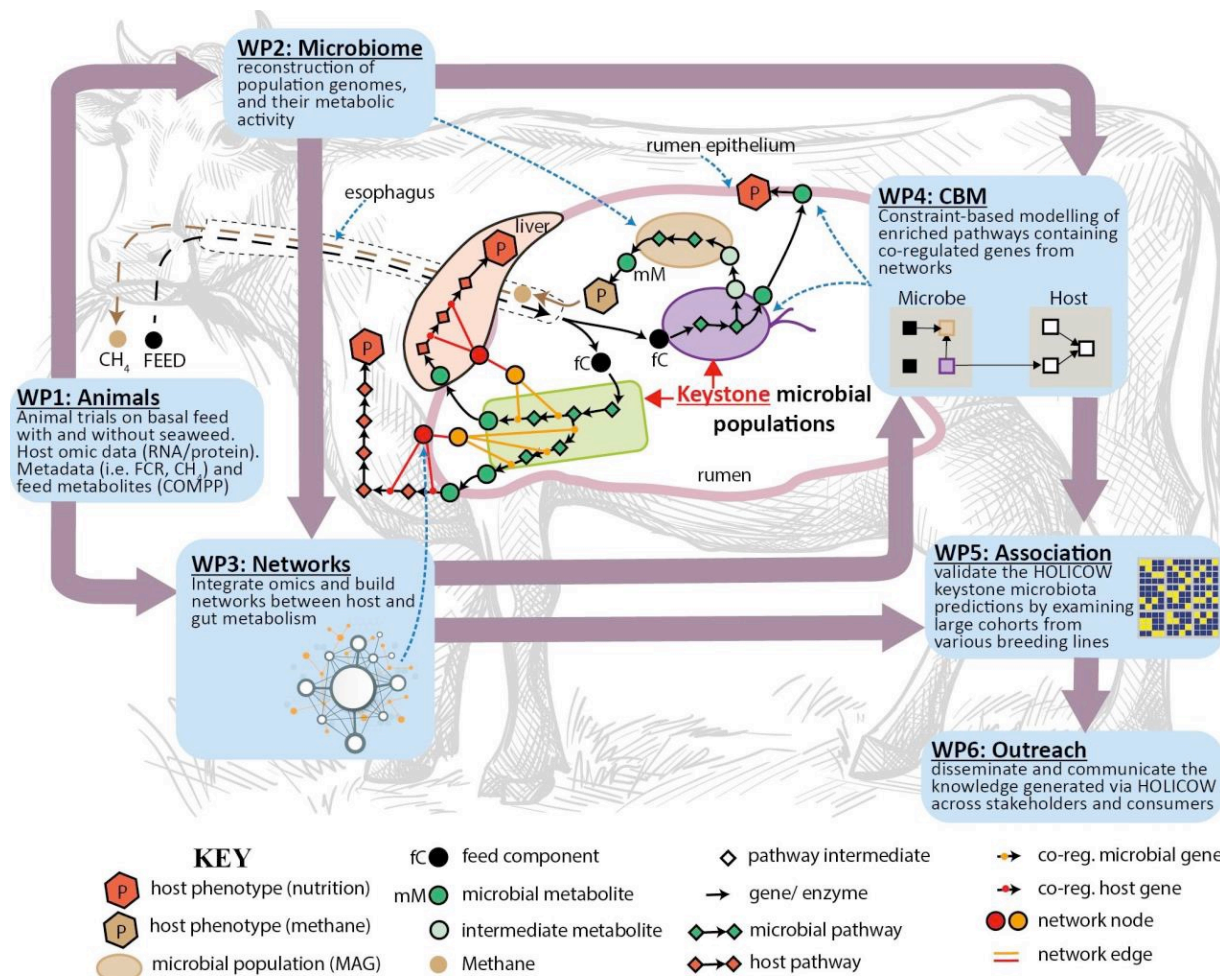
Carl Kobel



novo  
nordisk  
fonden



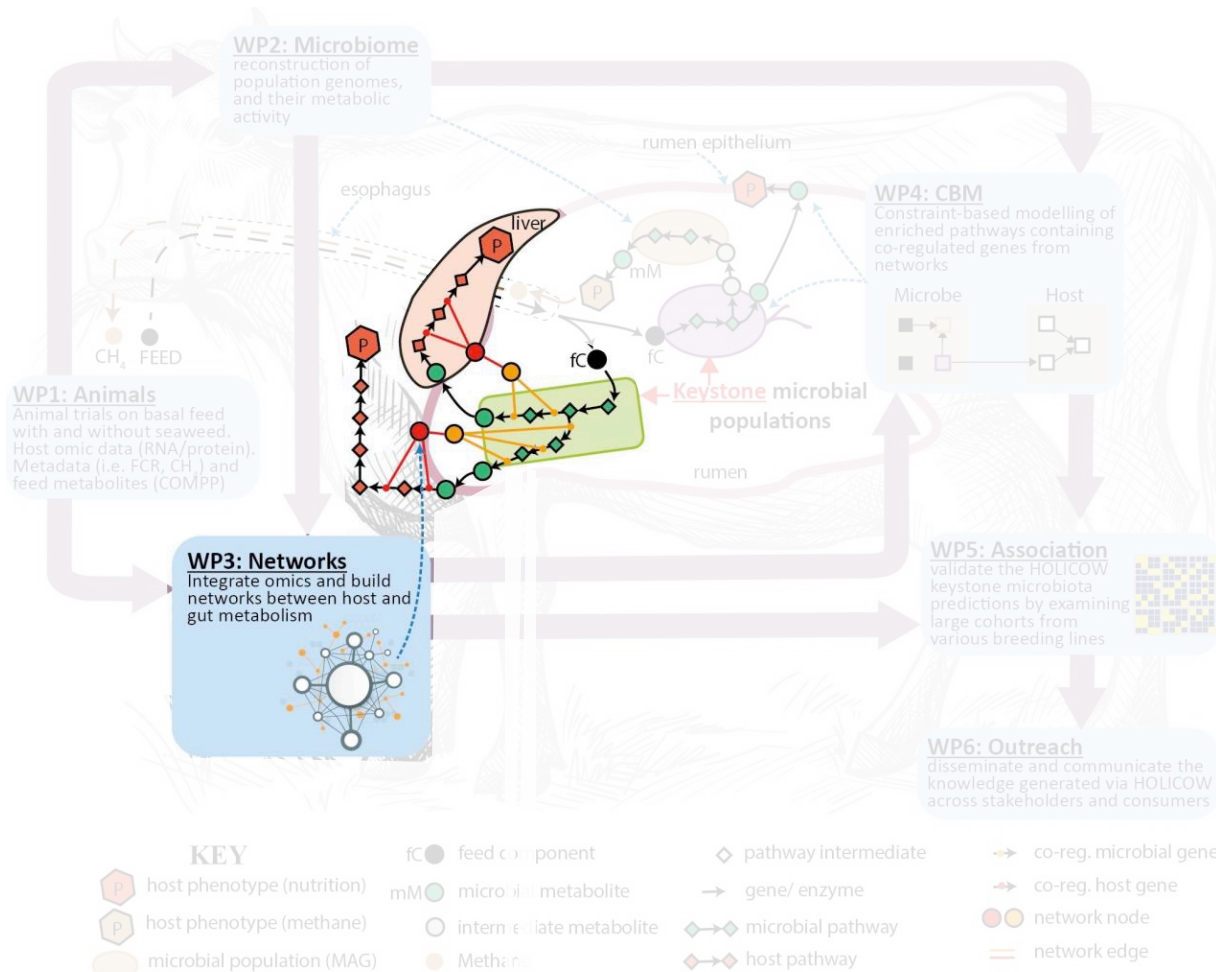
# SuPAcow: The feed-microbiome-host axis in cows





# SuPAcow:

## The feed-microbiome-host axis in cows



novo  
nordisk  
fonden



# Protein Network Analysis

Do proteins expression levels interact across species?

*keystone protein groups  
detected in at least 12 of  
32 of (control) animals*

Ianina (Yanna)  
Altshuler



Rainer Roehe

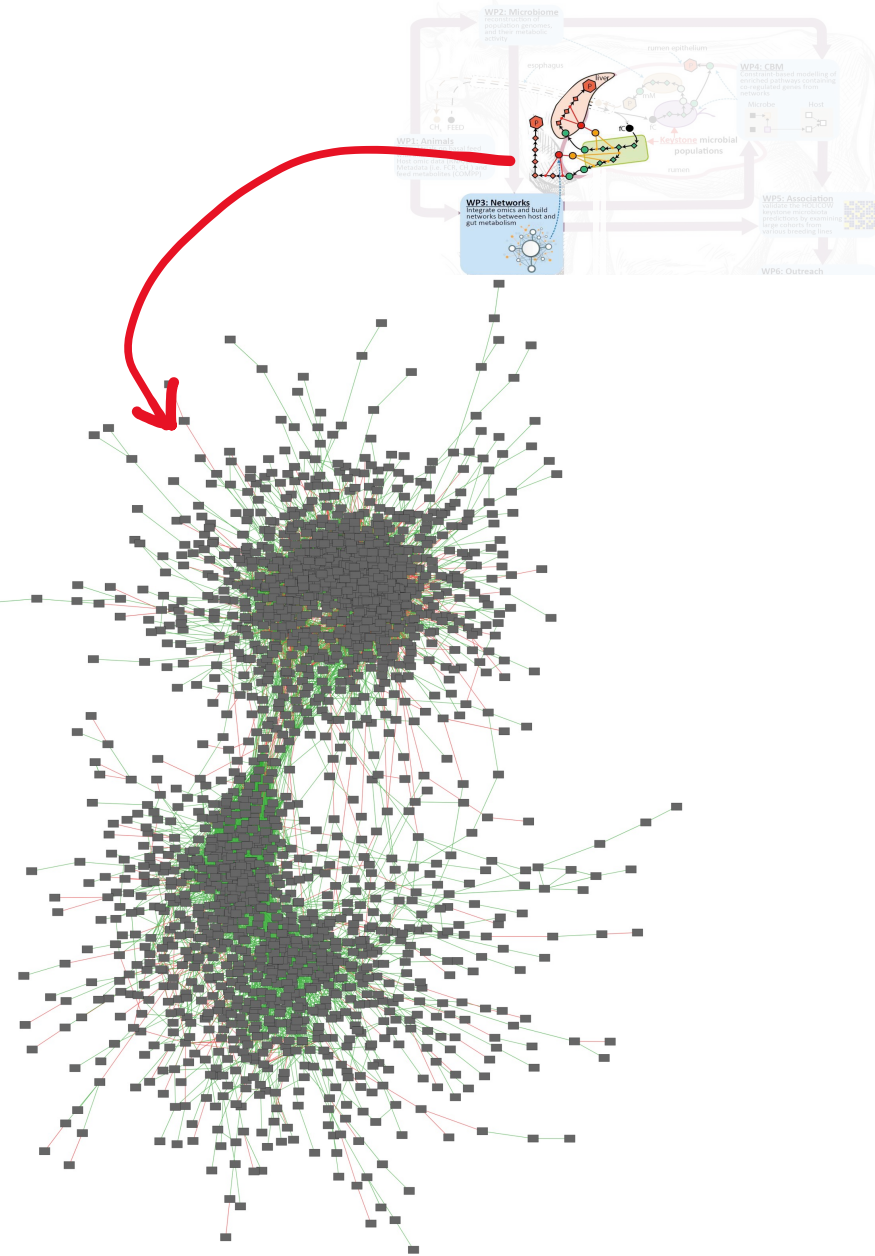


Torgeir Hvidsten



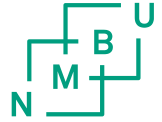
novo  
nordisk  
fonden

Altshuler, et al. (2022) In preperation

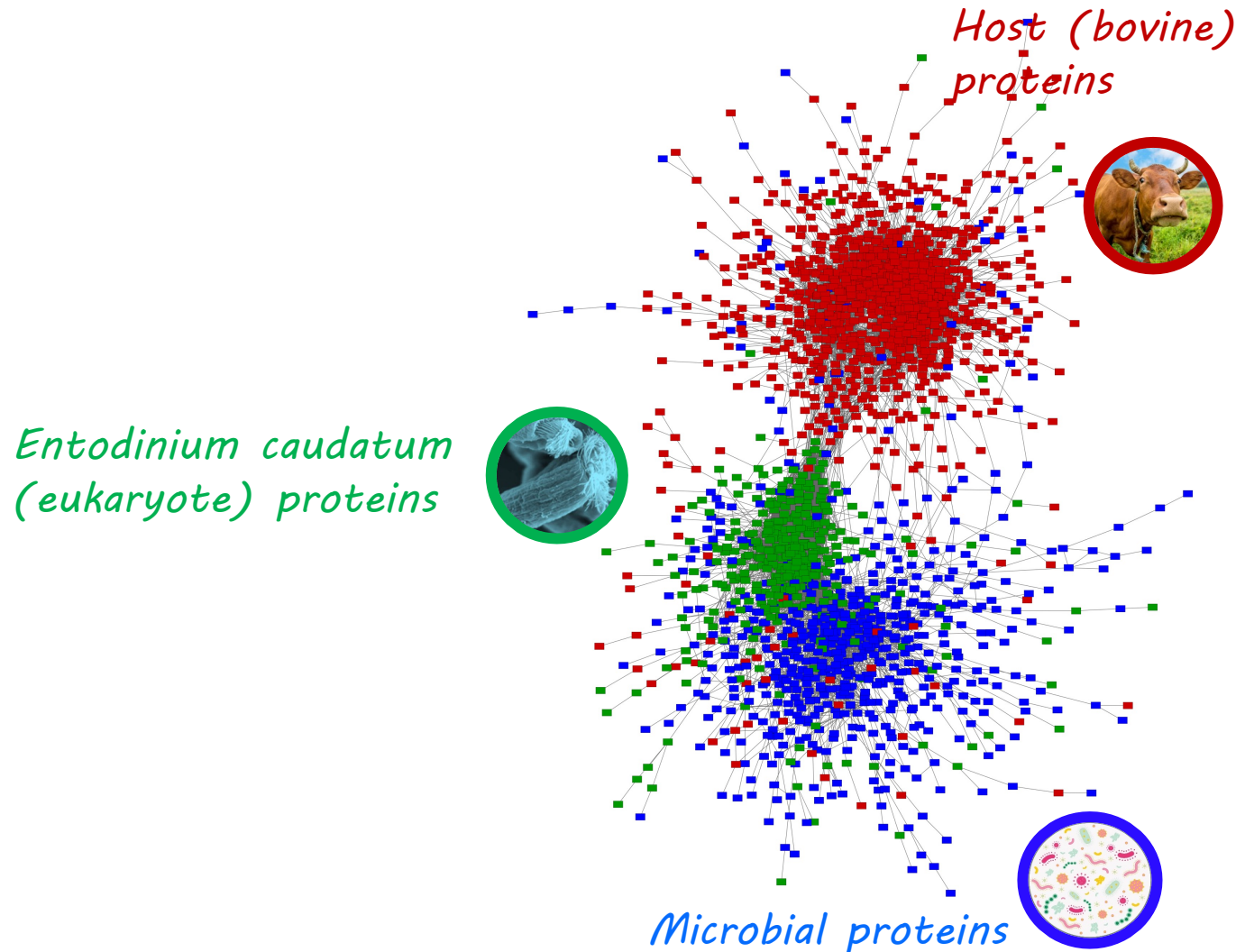


Correlation > |0.65| +ve and -ve  
p-val < 0.001

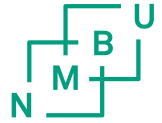
# Protein Network Analysis



Do proteins expression levels interact across species?



# Protein Network Analysis



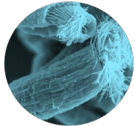
Identification of modules with WGCNA analysis

***Groups of proteins that share similar expression pattern  
that are consistent across individual cows***

## Identified 9 modules



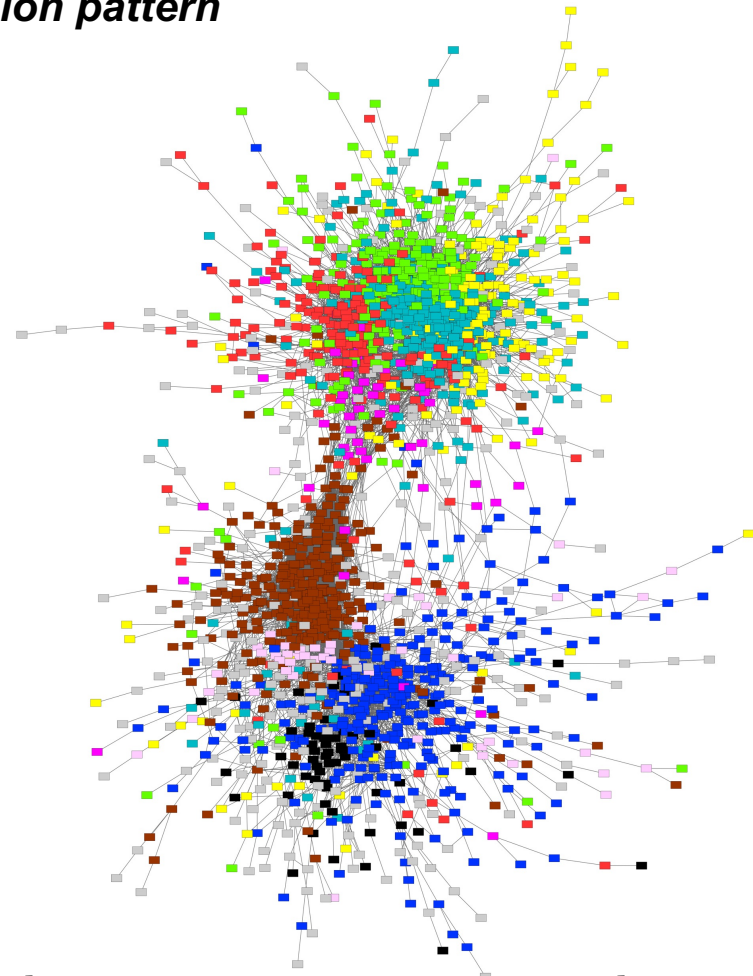
5 bovine



2 protozoan



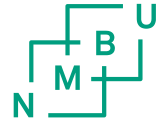
2 microbial



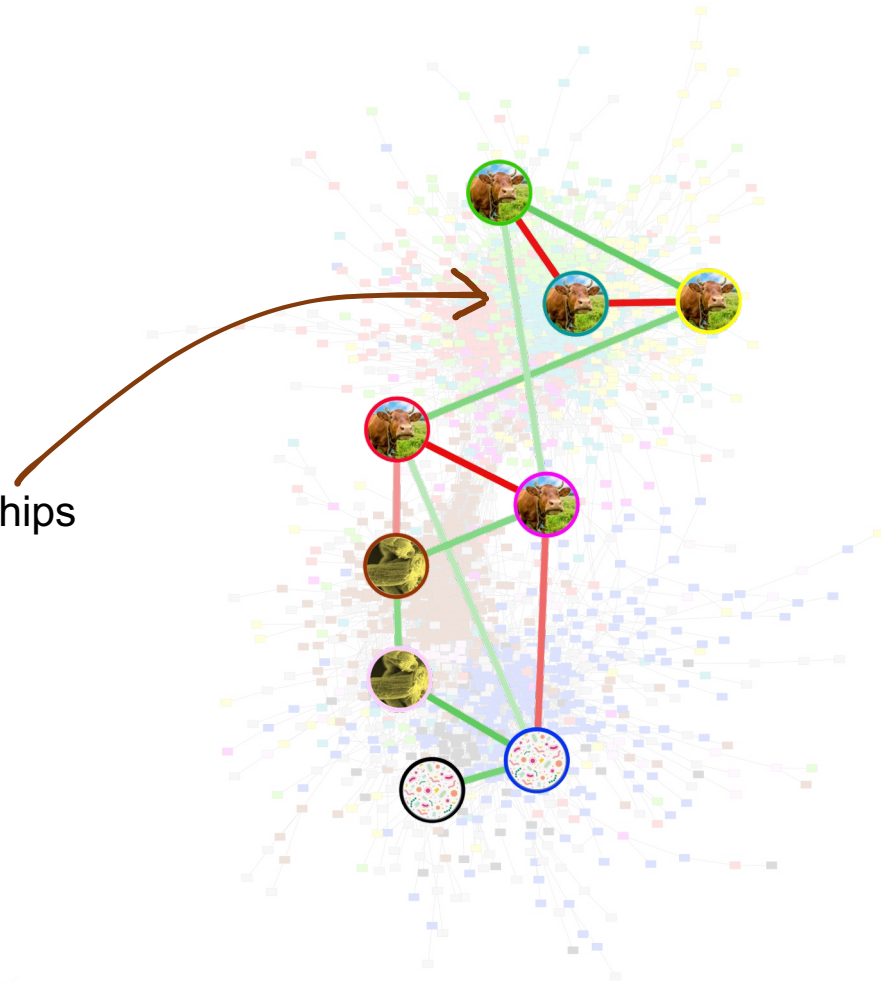
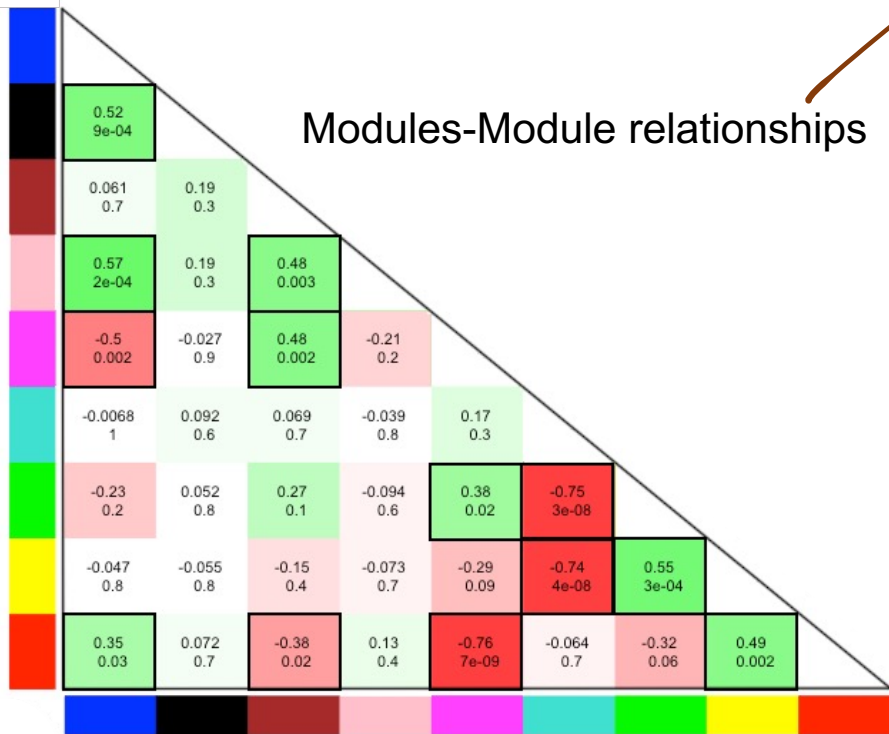
Weighted Gene Co-expression Network  
Analysis (WGCNA)



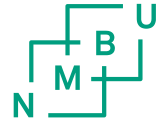
# Protein Network Analysis



Modules-Module relationships

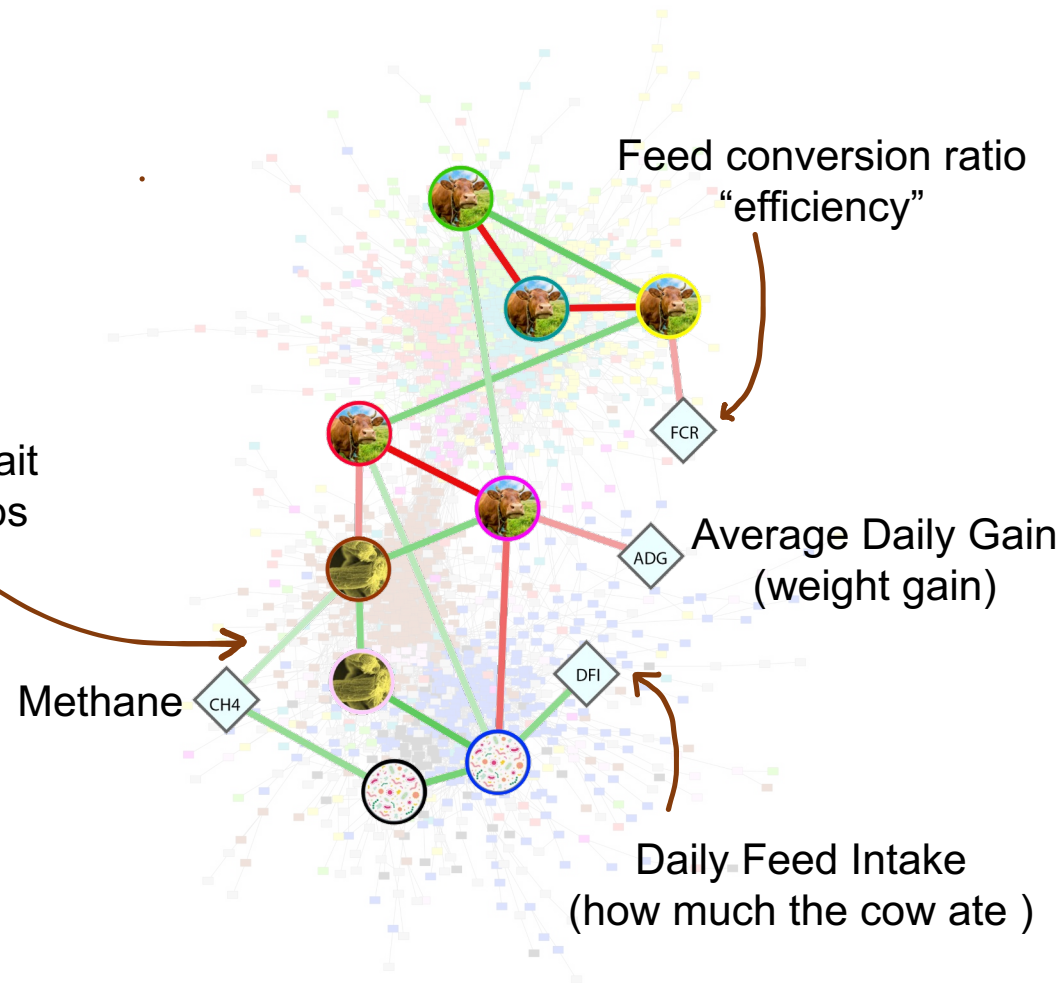


# Protein Network Analysis

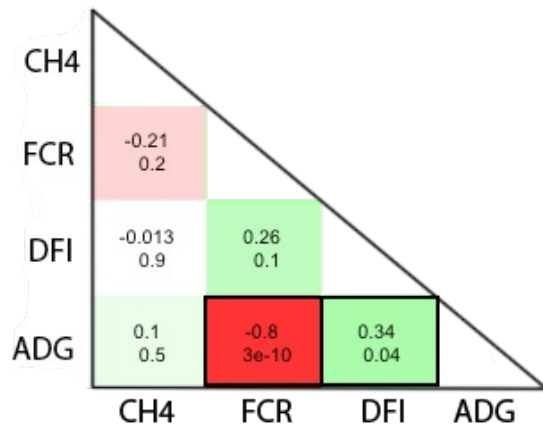
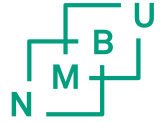


	-0.052 0.8	-0.089 0.6	0.46 0.004	0.31 0.07
	0.44 0.006	-0.07 0.7	0.18 0.3	0.14 0.4
	0.33 0.05	0.017 0.9	-0.16 0.3	-0.17 0.3
	0.21 0.2	0.044 0.8	0.079 0.6	-0.031 0.9
	0.16 0.4	0.13 0.4	-0.31 0.07	-0.35 0.04
	0.042 0.8	0.28 0.09	0.12 0.5	-0.2 0.2
	-0.017 0.9	-0.16 0.4	-0.22 0.2	-0.017 0.9
	-0.052 0.8	-0.33 0.05	-0.29 0.09	0.13 0.4
	0.09 0.6	-0.13 0.4	0.0071 1	0.14 0.4
CH4	FCR	DFI	ADG	

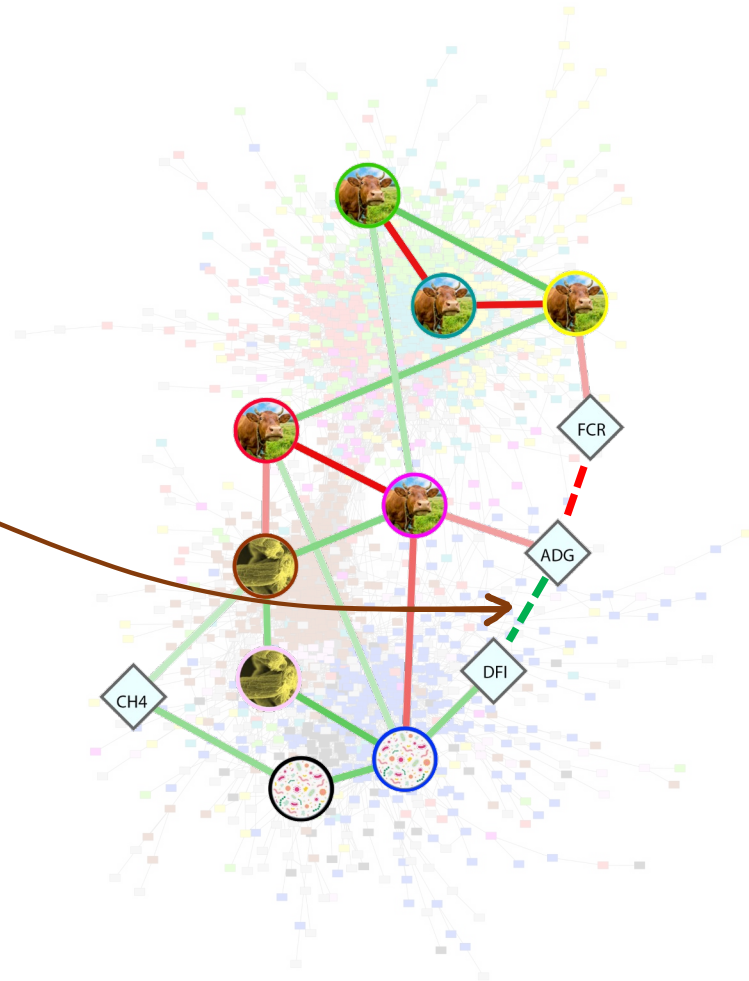
Modules-Trait relationships



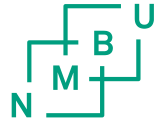
# Protein Network Analysis



Trait -Trait  
relationships



# Protein Network Analysis

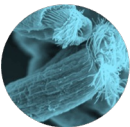


## Enrichment analysis of modules

### Enrichment of organisms



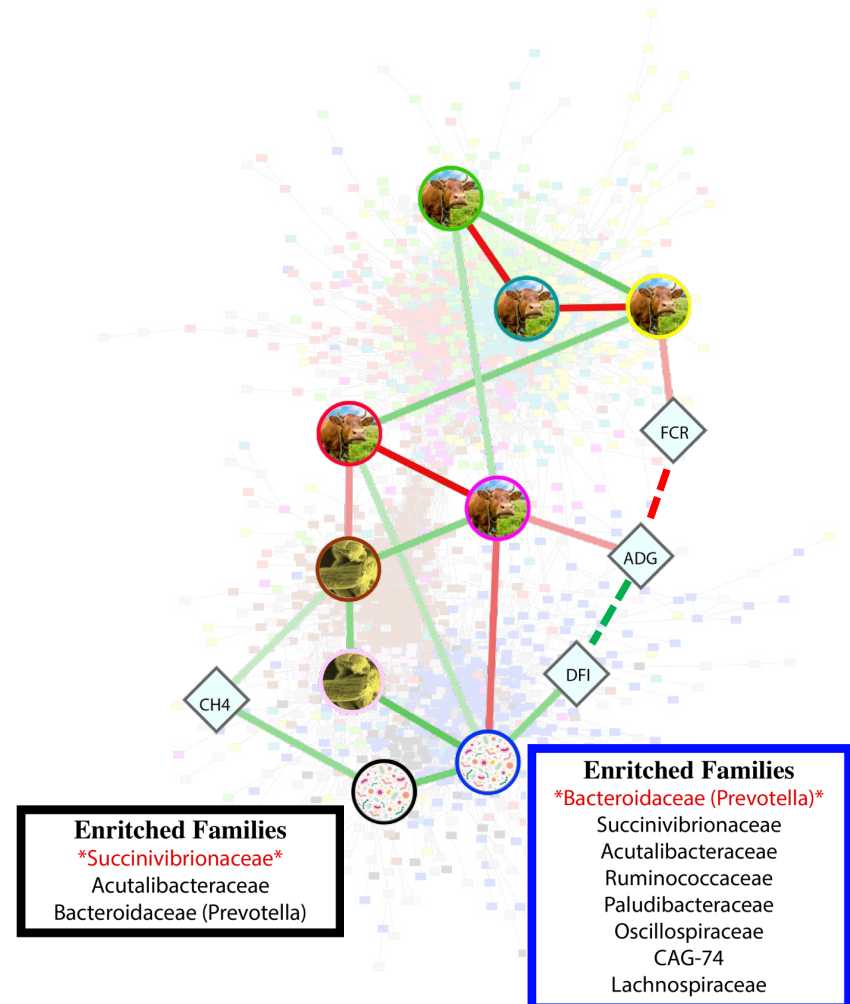
5 bovine



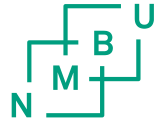
2 protozoan



2 microbial



# Protein Network Analysis

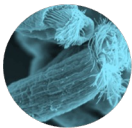


## Enrichment analysis of modules

### Enrichment of organisms



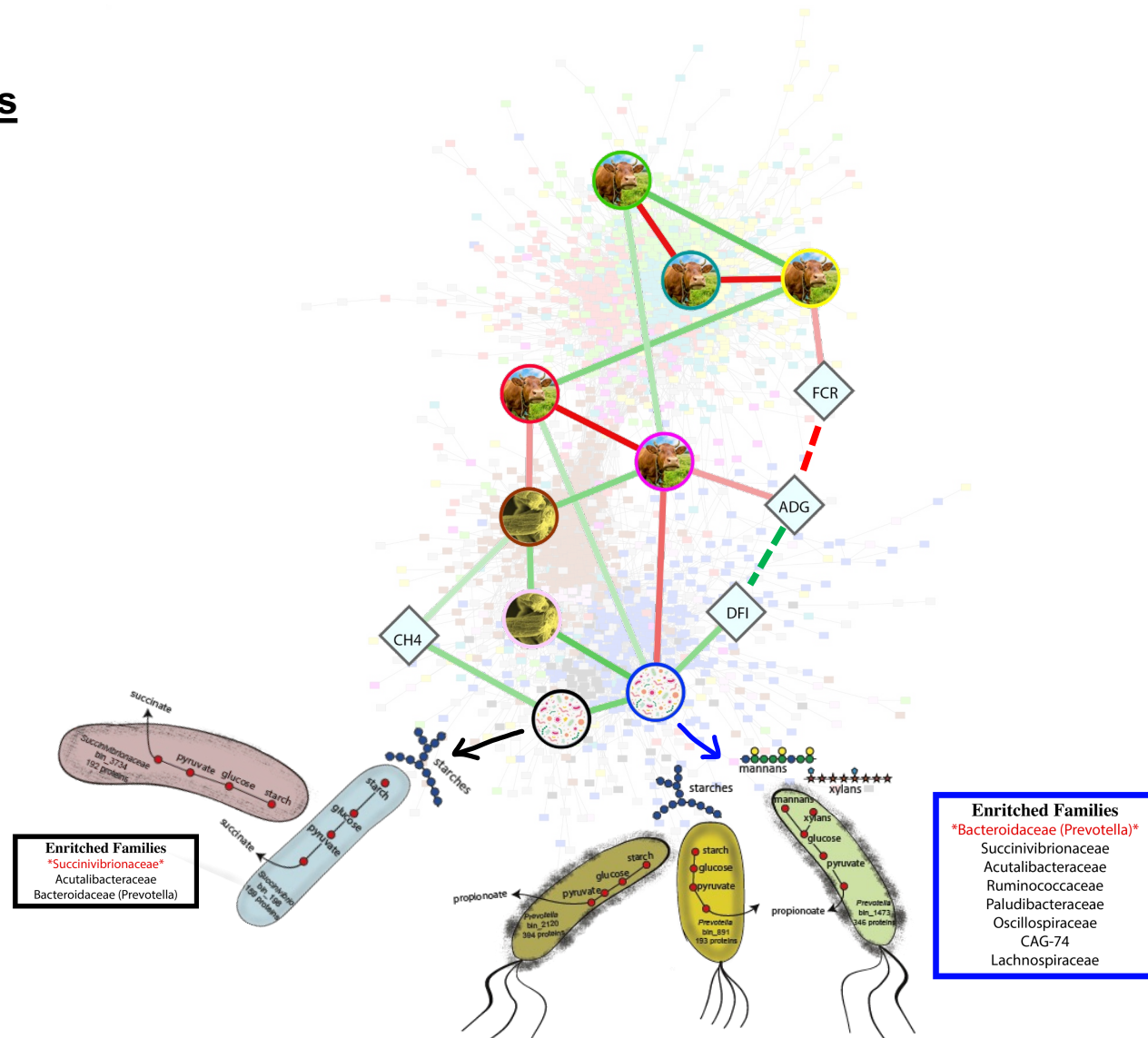
5 bovine



2 protozoan



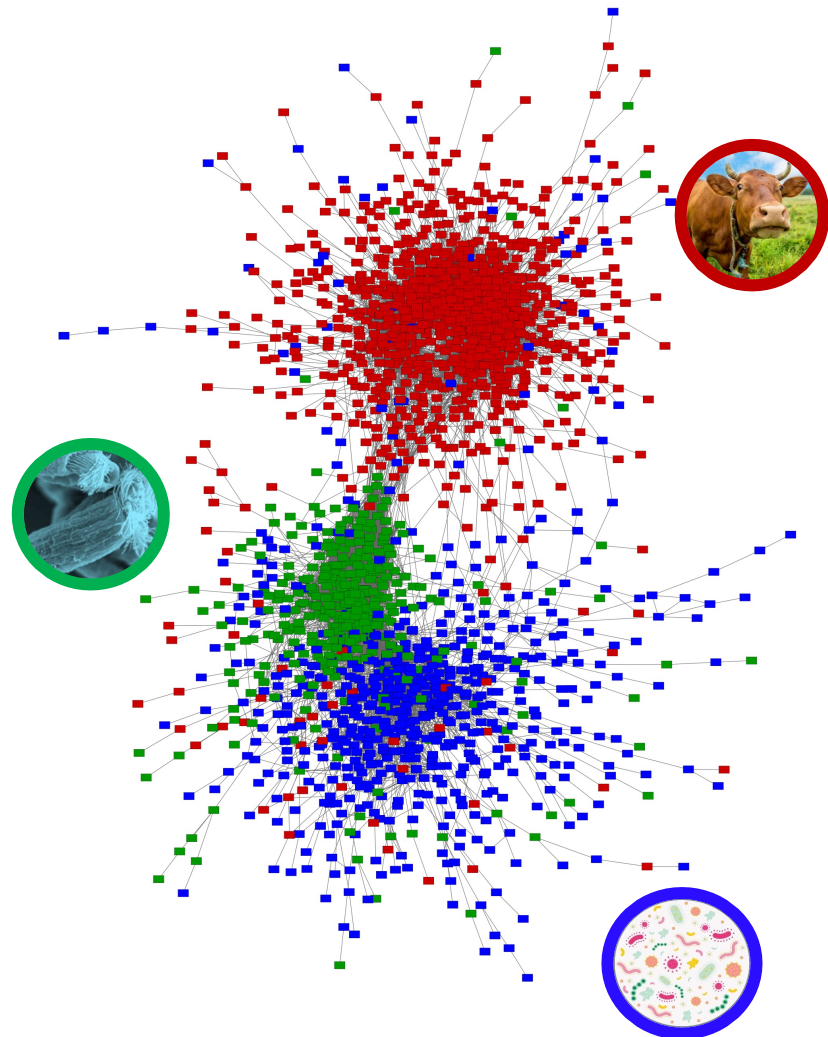
2 microbial



What are the contributions of the eukaryotes (protozoa + fungi)?

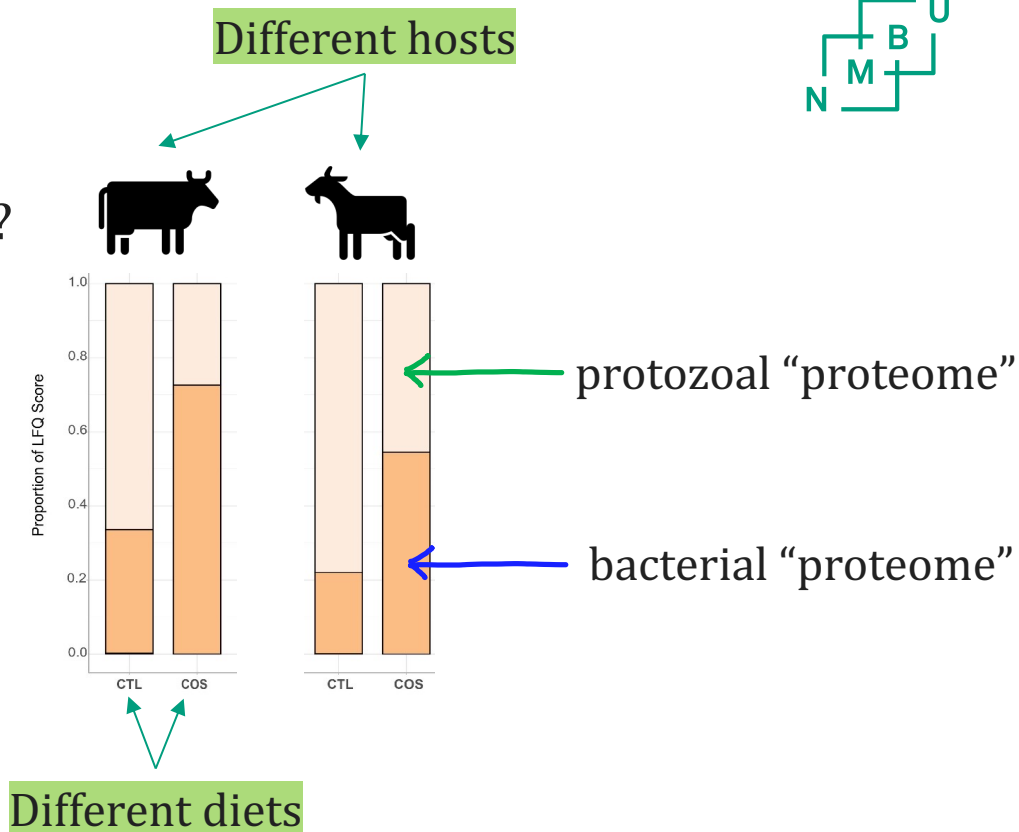
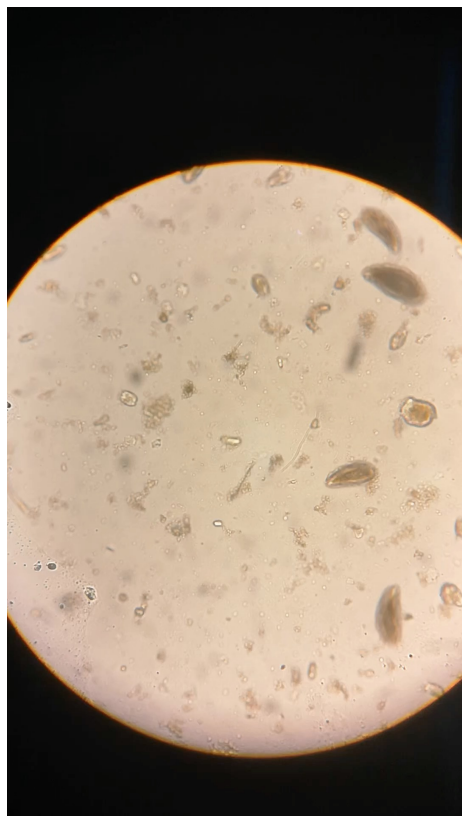
Prokaryotes	50-90%
<i>Protozoa</i>	10-50 %
<i>Fungi</i>	5-10%

% of microbial biomass in the rumen





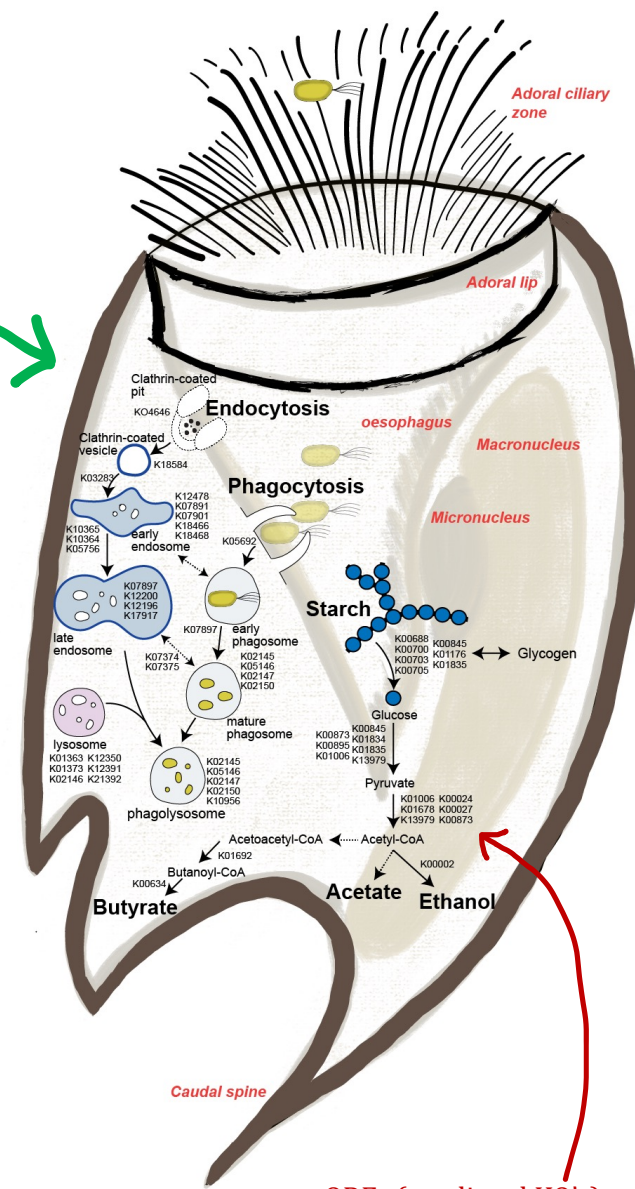
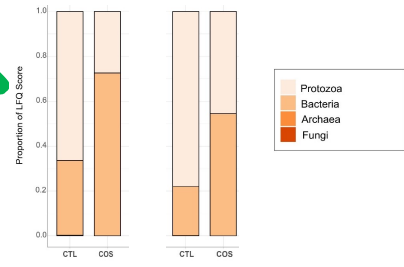
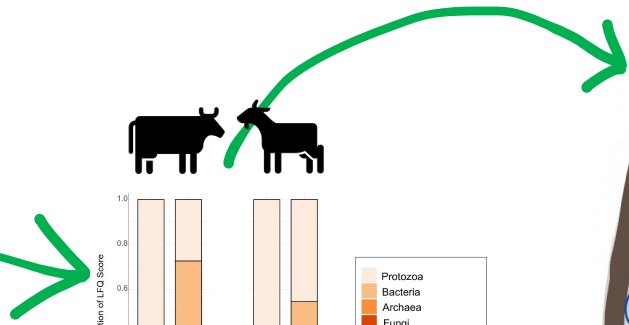
What are the contributions of the eukaryotes (protozoa + fungi)?



Thea Os Andersen



What are the contributions of the protozoa?

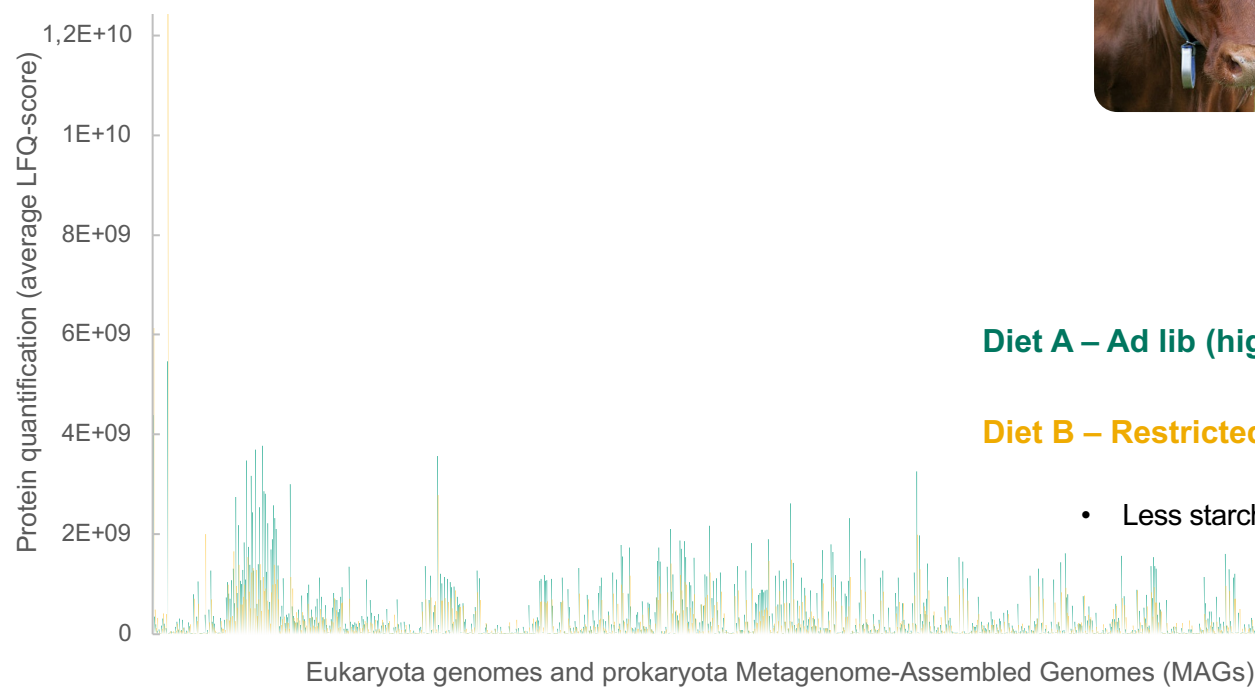
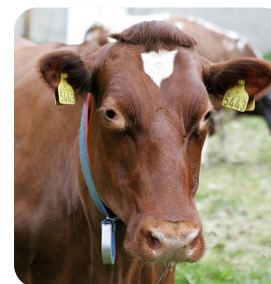


ORFs (predicted KO's) detected in metaproteome

Thea Os Andersen







**Diet A – Ad lib (high starch)**

**Diet B – Restricted**

- Less starch available

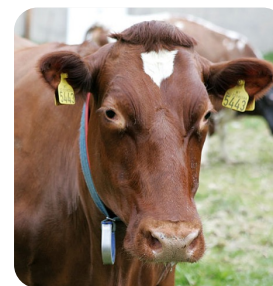
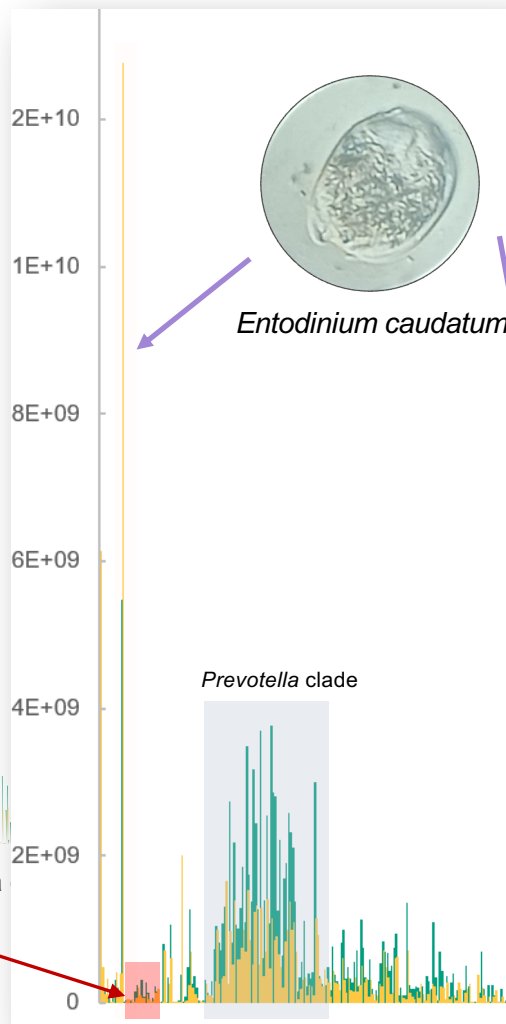


Magnus Ø. Arntzen  
NMBU



Live H. Hagen  
NMBU

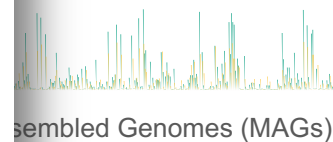
Norwegian University of Life Sciences



**Diet A – Ad lib (high starch)**

**Diet B – Restricted**

- Less starch available



Magnus Ø. Arntzen  
NMBU



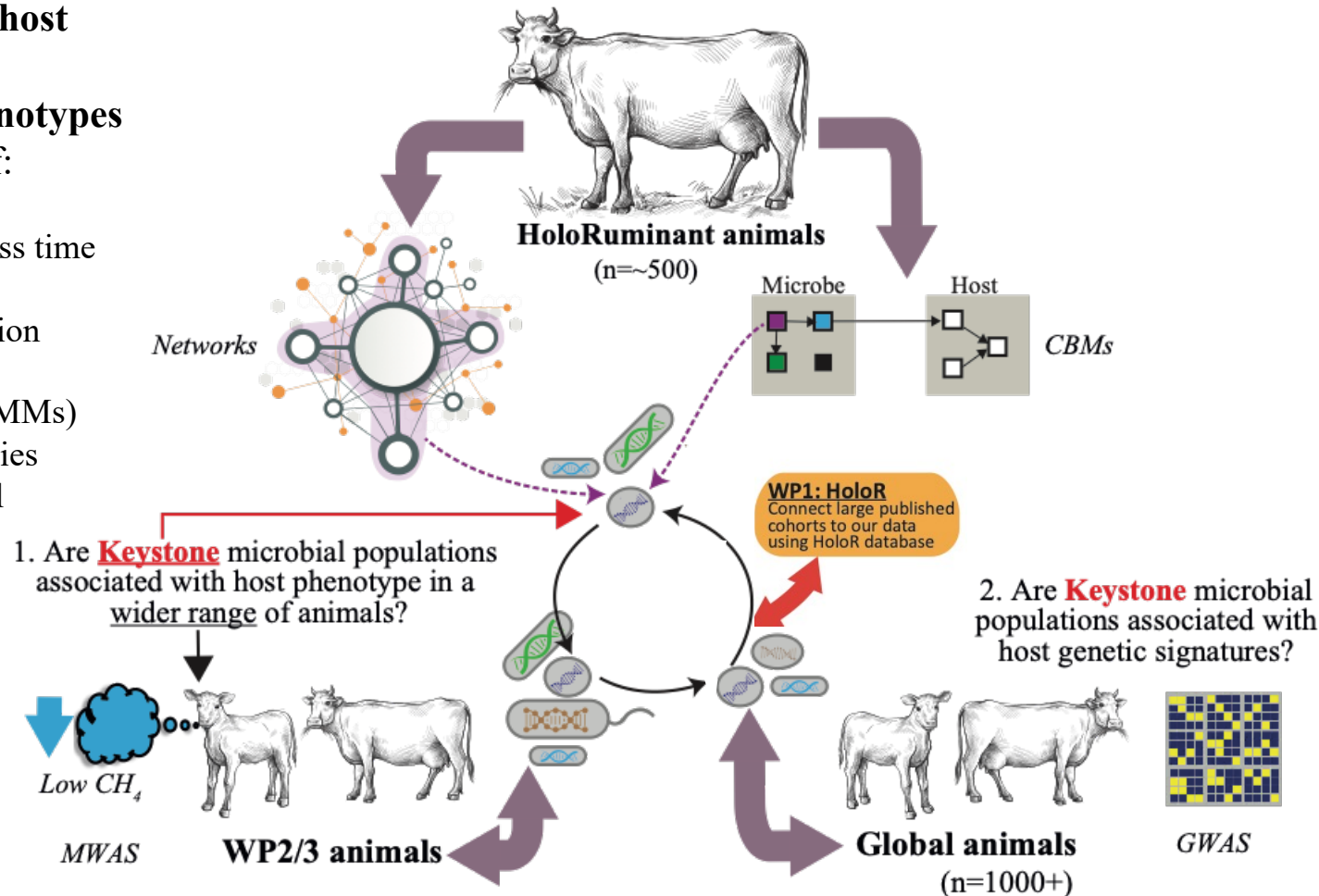
Live H. Hagen  
NMBU

Norwegian University of Life Sciences

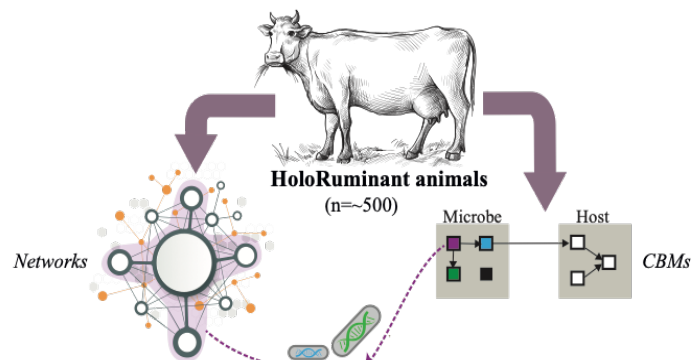


**Genetic variation in the host  
will be connected to  
microbiome-related phenotypes**  
by using a combination of:

- microbiome dynamics across time and its potential sources
- metagenome-wide association studies (MWAS)
- bayesian mixed models (BMMs)
- existing public GWAS studies performed on a global level







1. Are **Keystone** microbial populations associated with host phenotype in a wider range of animals?

2. Are **Keystone** microbial populations associated with host genetic signatures?

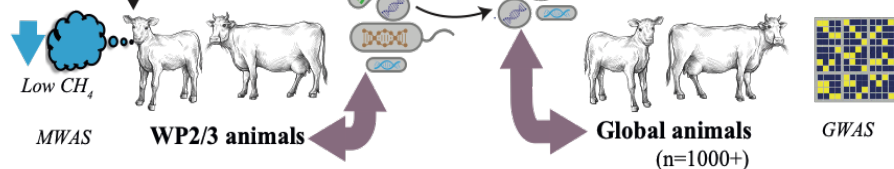
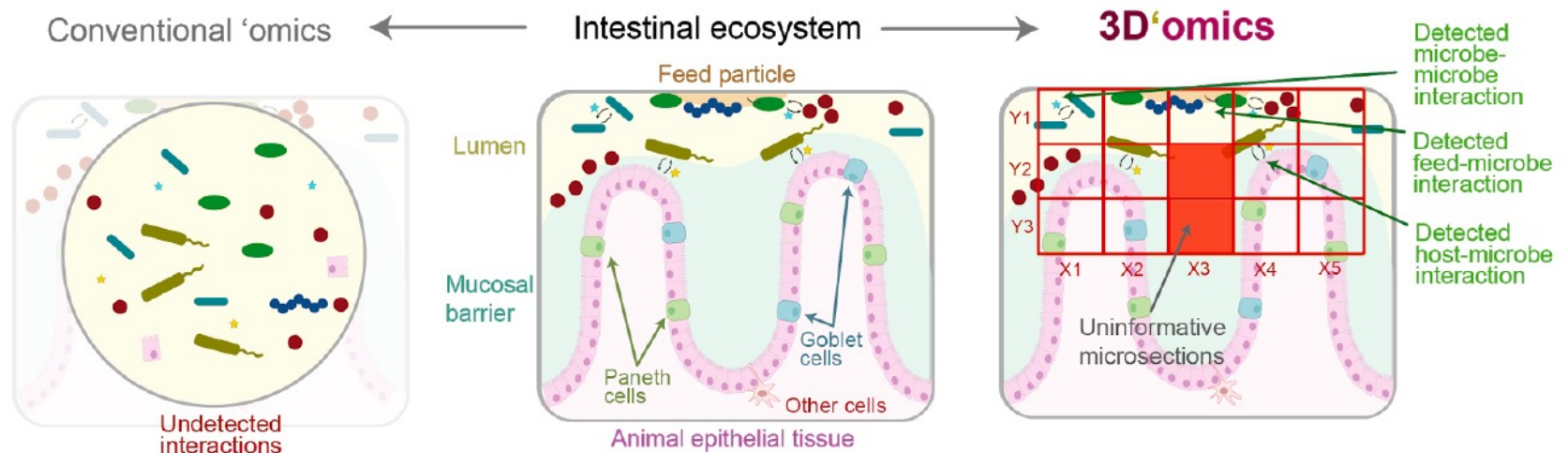


Table: List of data that will be stored in HoloR for use by WP4

Project/Origin	Breed	#animals	Genotyped	Phenotyped	MetaTaxo	Meta-omics
HoloRuminant	Holstein (calf)	500+	✓	✓	✓	✓
	Holstein-Friesian (calf)	180+	✓	✓	✓	✓
	Norwegian Red (calf)	40	✓	✓	✓	✓
	Finnish Nordic Red (calf)	40	✗	✓	✓	✗
	Charolais (calf)	40	✓	✓	✓	✓
	Jersey (calf)	30	✓	✓	✓	✓
	Normande (calf)	30	✓	✓	✓	✓
	Angus (calf)	20	✓	✓	✓	✓
	Murciano-Granadina (goats)	100	✓	✓	✓	✓
	Romane (sheep)	100	✗	✓	✓	✓
Ruminomics	Laucane (sheep)	50	✓	✓	✓	✓
	Segureña (sheep)	50	✓	✓	✓	✓
	Holstein (calf)	816	✓	✓	✓	✗
CSIRO	Finnish and Swedish Nordic Red (cows)	200	✓	✓	✓	✗
	Brahman (calf)	56	✓	✓	✓	✓
MASTER/TEA GASC	Limousin	500	✓	✓	✓	✓*
INRAE	Holstein	108	✓	✓	✓	✓*
	Charolais	61	✓	✓	✓	✗
AGR	Romney and Coopworth mix (sheep)	1100	✓	✓	✓	✗
SRUC	Aberdeen Angus	104	✓	✓	✓	✓
	Limousin	103	✓	✓	✓	✓
	Charolais	71	✓	✓	✓	✓
	Luing	85	✓	✓	✓	✓
UAL	Angus	200	✓	✓	✓	✓
	Charolais	150	✓	✓	✓	✓
	Hybrid (UAL)	500	✓	✓	✓	✓

# *“Three-dimensional holo’omic landscapes to unveil host-microbiota interactions shaping animal production”*



Antton Alberdi



Phil B. Pope



Torgeir Hvidsten

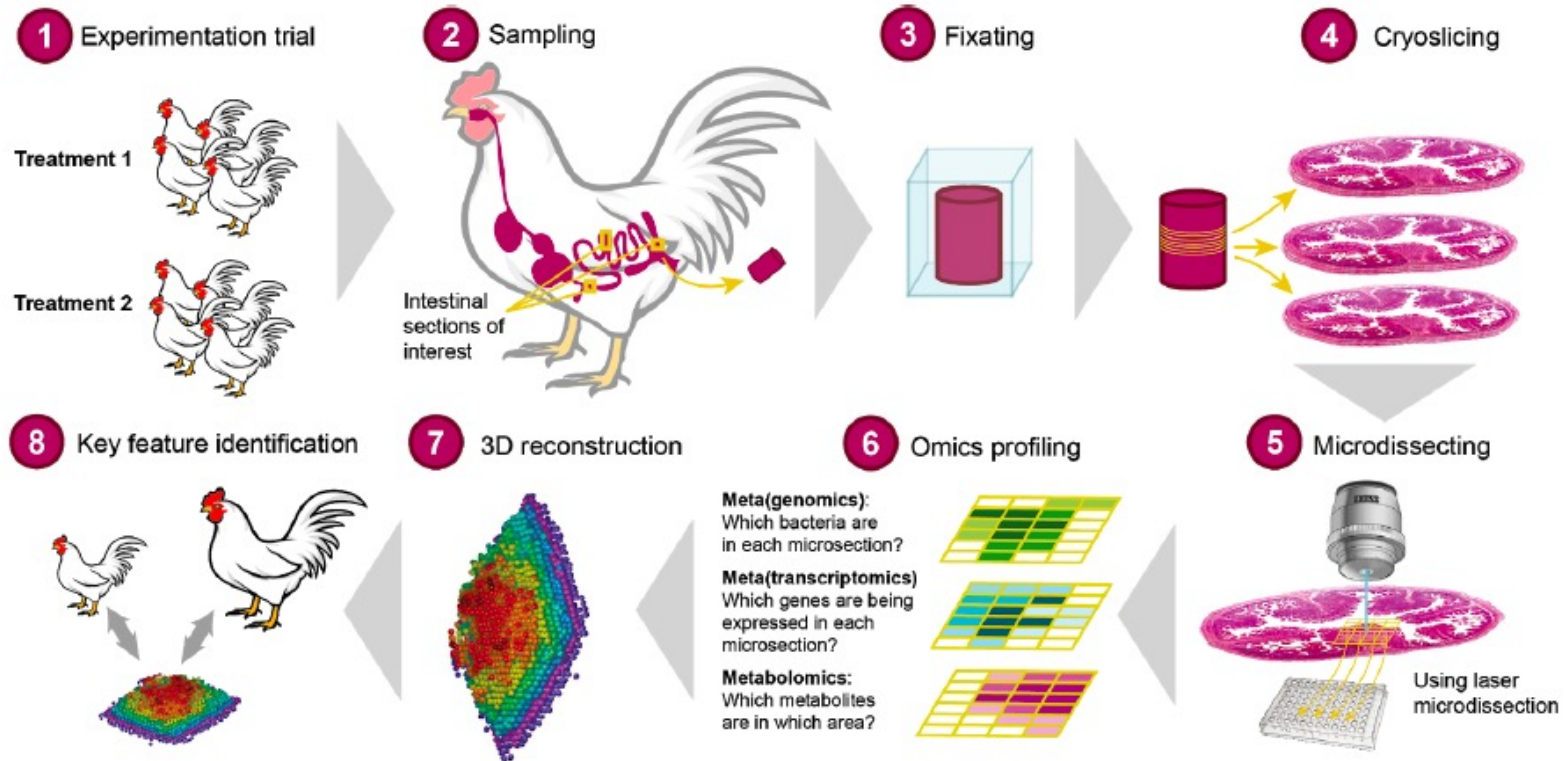


Irma Oskam



Bjørge Westereng





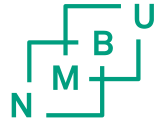




• **MEMO scientists**

- Sabina La Rosa
- Live Hagen
- Arturo Vera Ponce De Leon
- Adrian Naas
- Magnus Ø. Arntzen
- Thea Os Andersen
- Ianina Altshuler
- Alessandra Ferrillo
- Carl M. Kobel
- Valerie Schiml
- Juline Walter

# Acknowledgements



- **NMBU scientists**
  - **Vincent Eijsink**
  - Bjørge Westereng
  - **Torgeir Hvidsten**
  - **Simen R. Sandve**
  - Margareth Øverland
- **UC Davis**
  - Matthias Hess + group
- **Ohio State Univ.**
  - Matt Sullivan + group
- **Colorado State Univ.**
  - Kelly Wrighton + group
- **Univ. of Michigan**
  - Nicole Koropatkin + group
  - Eric Martens + group
- **Univ. of Copenhagen**
  - Tom Gilbert
  - Morten Limborg
  - Antton Alberdi
- **CNRS-CAZy**
  - Bernard Henrissat
  - Nico Terrapon
  - Vincent Lombard
- **Chalmers Univ.**
  - Johan Larsbrink
- **Ben Gurion Univ.**
  - Itzhik Mizrahi
- **Univ. of Newcastle**
  - William Willats
- **SRUC**
  - Rainer Roehe
- **Univ. of Luxembourg**
  - Paul Wilmes
  - Francesco Delogu
  - Benoit Kunath

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The Research Council  
of Norway



3D'omics



HoloRuminant  
Understanding microbiomes of the ruminant holobiont



@ThePopeLab

