69th Annual Meeting of the European Federation of Animal Science

Dubrovnik, Croatia, 27th to 31st August 2018

Multivariate and network analysis identified microbial biomarkers linked to methane emission

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Outline

I. Motivation

- The ruminant holobiont

II. Description of µFicient project

- Phenotyping and sampling
- Initial results:
  - Structural and functional analysis
  - Microbial biomarkers linked to CH_4y (CH_4/DMI)
  - Microbial network analysis

III. Future directions

Rumen symbionts

PROVIDE

- **Energy**
  VFA can provide up to 80% of the energy needs
- **Protein**
  microbes convert NPN into high quality protein
- **Vitamins**
  synthesis of B-complex and K vitamins
- ….

PRODUCE

- Toxic products
- **Methane**

~14.5% of total global GHG emissions

Source: Bulletin 422/2007, International Dairy Federation (2007)

Phenotyping and sampling

2016

n=65

Sep

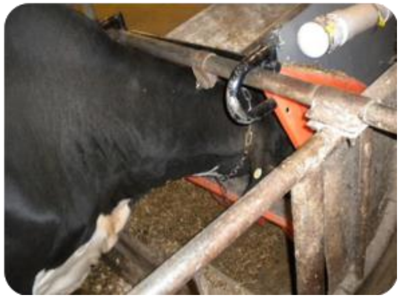
Nov

Jan

n=68

Mar

2017



GreenFeed

Calan gate syst

Methane emission	Digestibility
Body weight	Residual feed intake
Milk production	Average daily gain

Analytic workflow

From raw sequencing to OTU

- OTU picking
- Taxonomic assignment
- Phylogenetic tree

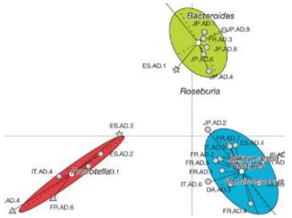
Schloss et al., 2009

silva high quality ribosomal RNA databases

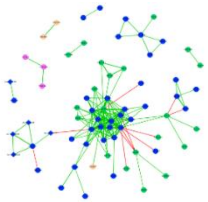
Quast et al., 2013

Ruminal ecosystem

- Diversity index (alpha, beta, richness)
- Ruminotype like-cluster
- Functional analysis (whole-metagenome)
- Microbial network inference



Arumugam et al., 2011



Ramayo-Caldas et al., 2016

Multivariate Analysis

- sPLS-DA (X=OTU, Y= CH_4y classification)
- sPLS-DA (X=OTU, Y= Ruminotype cluster)
- sPLS (regression mode: X=OTU, Y= CH_4y

mixOmics

Rohart et al., 2017

ade4

Dray et al., 2007

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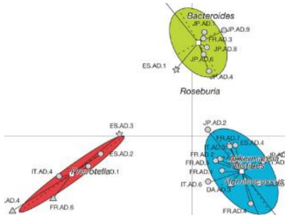
- Diversity index (alpha, beta, richness)
- **Ruminotype like-cluster**
- Functional analysis
- Microbial network inference



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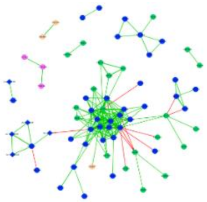

Ramayo-Caldas et al., 2016

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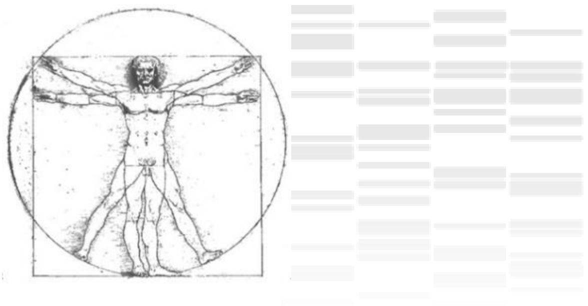
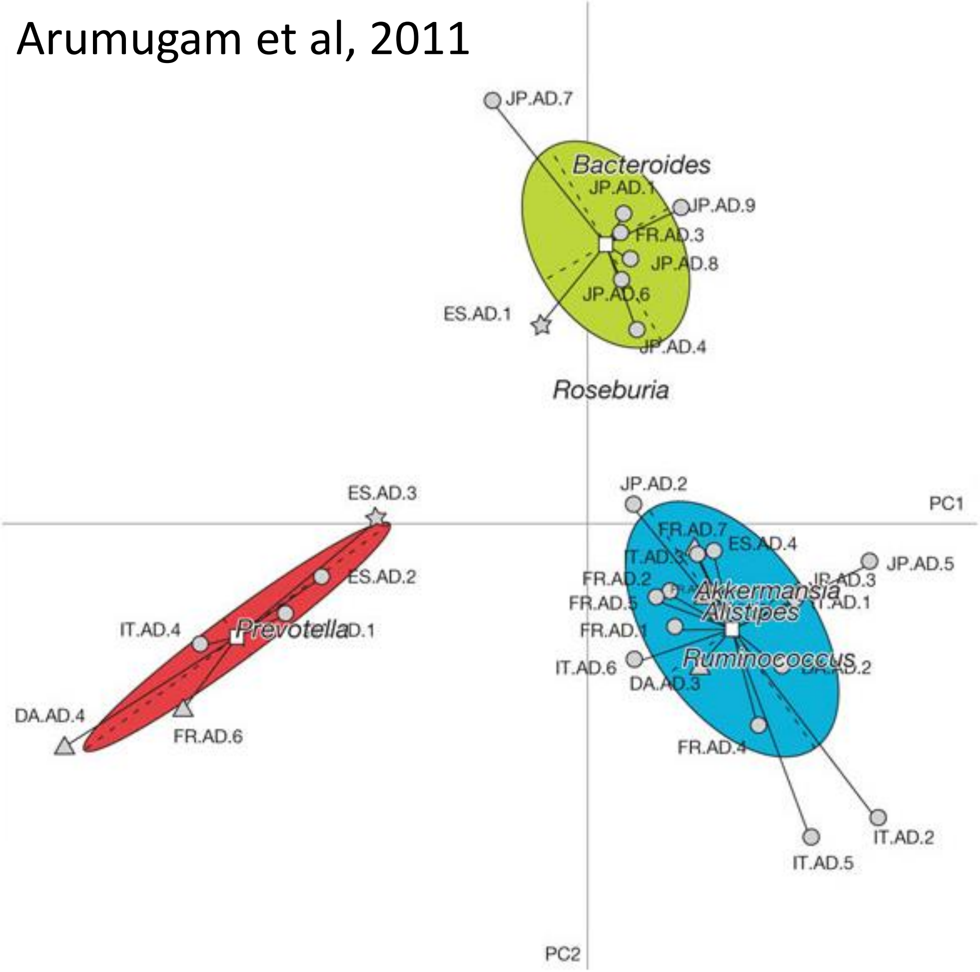


Structure of bacterial gut ecosystem

Arumugam et al, 2011



Wang et al. 2015

Moeller et al. 2012

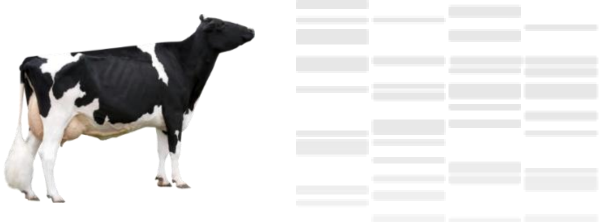
Mach et al. 2014

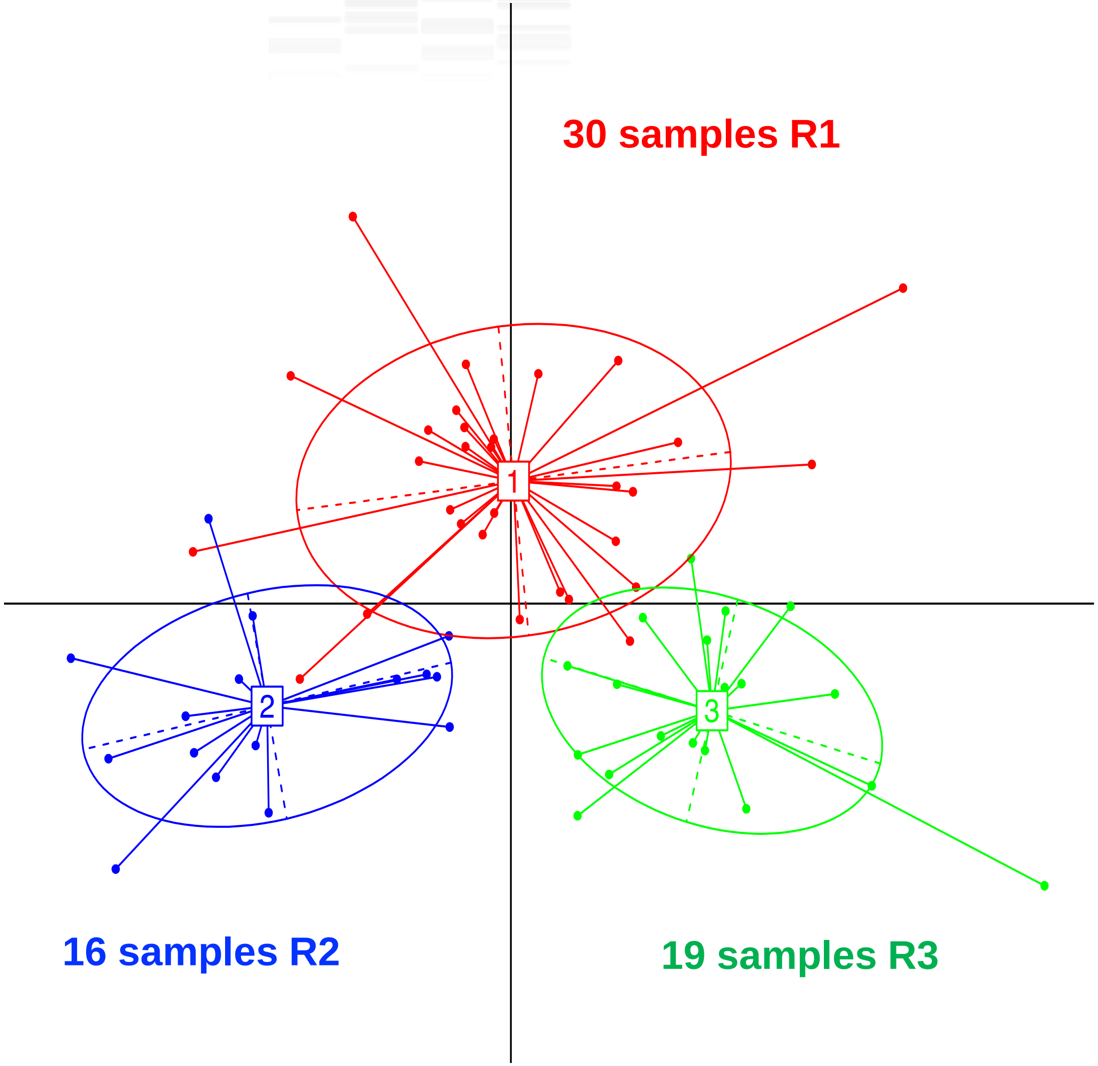
Ramayo-Caldas et al. 2018

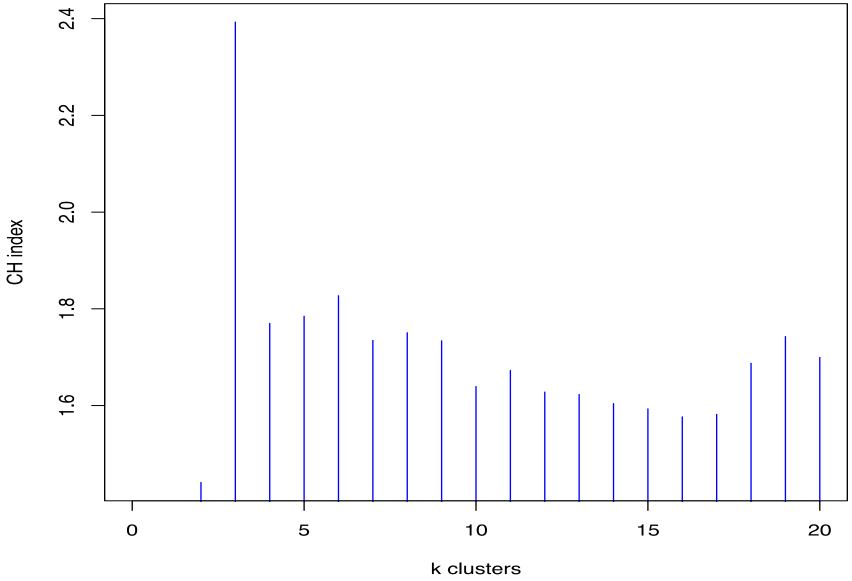
Kittelmann et al. 2014

Danielsson et al. 2017

Structure of ruminal ecosystem







Cluster stability

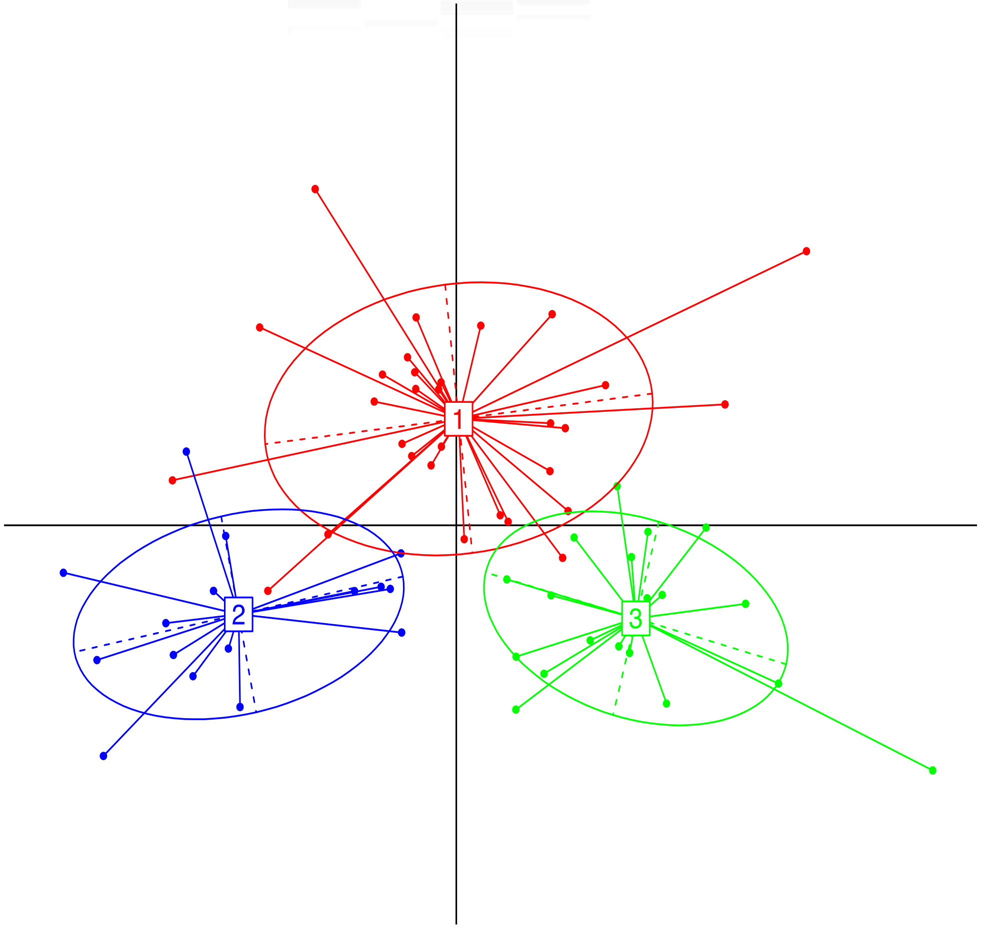
Clusterwise Jaccard 100 bootstrap

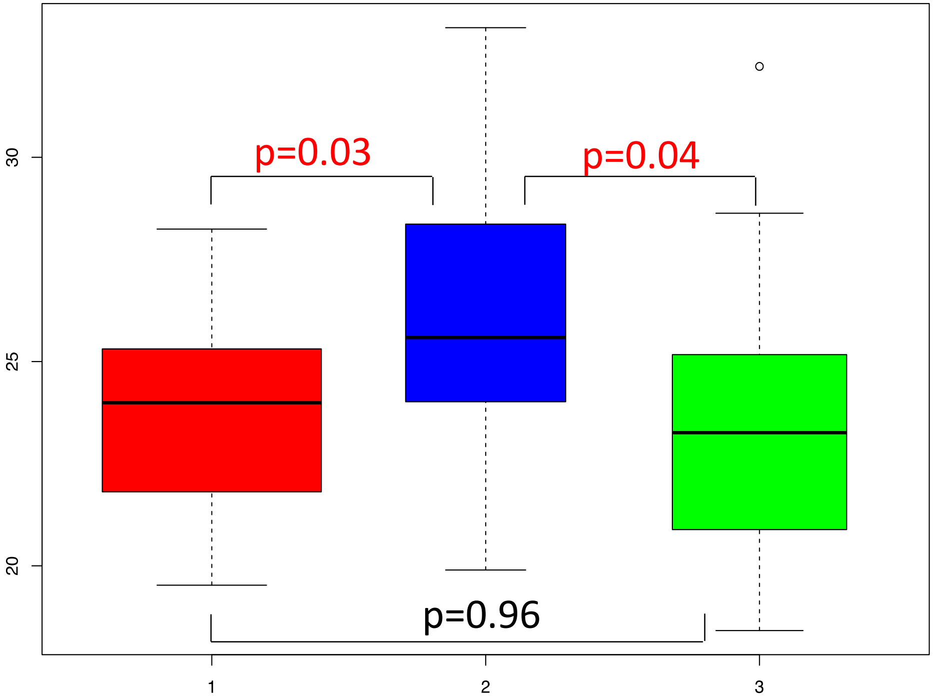
K2 = (0.61, 0.44)

K3= (0.63, 0.64, 0.63)

most stable solution at K=3

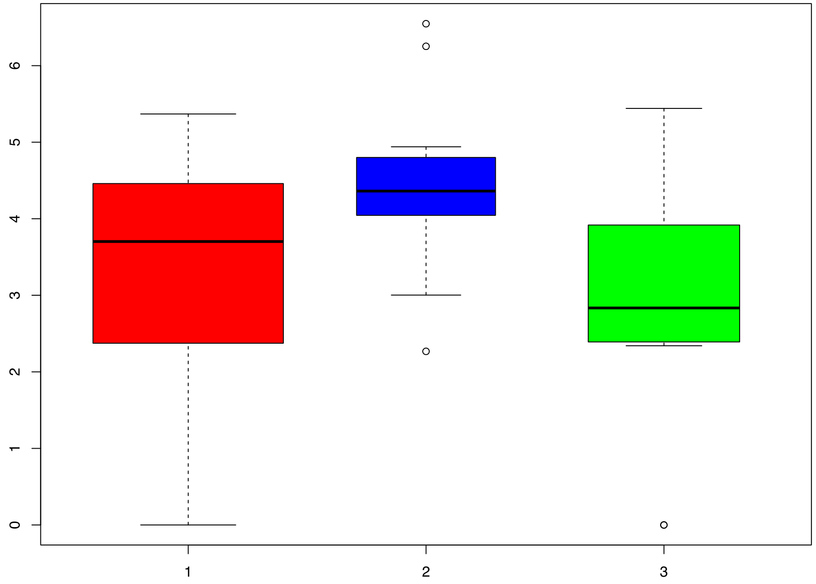
Ruminotype-like cluster is linked to CH_4y





Taxonomic composition

Ruminococcaceae

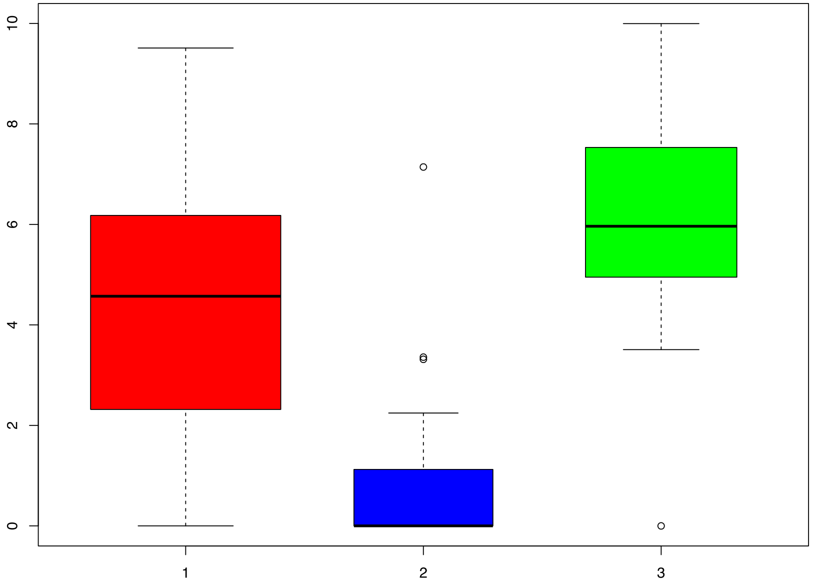


Linked to High CH_4y

Kittelmann et al. (2014)

Danielsson et al. (2017)

Succinivibrionaceae

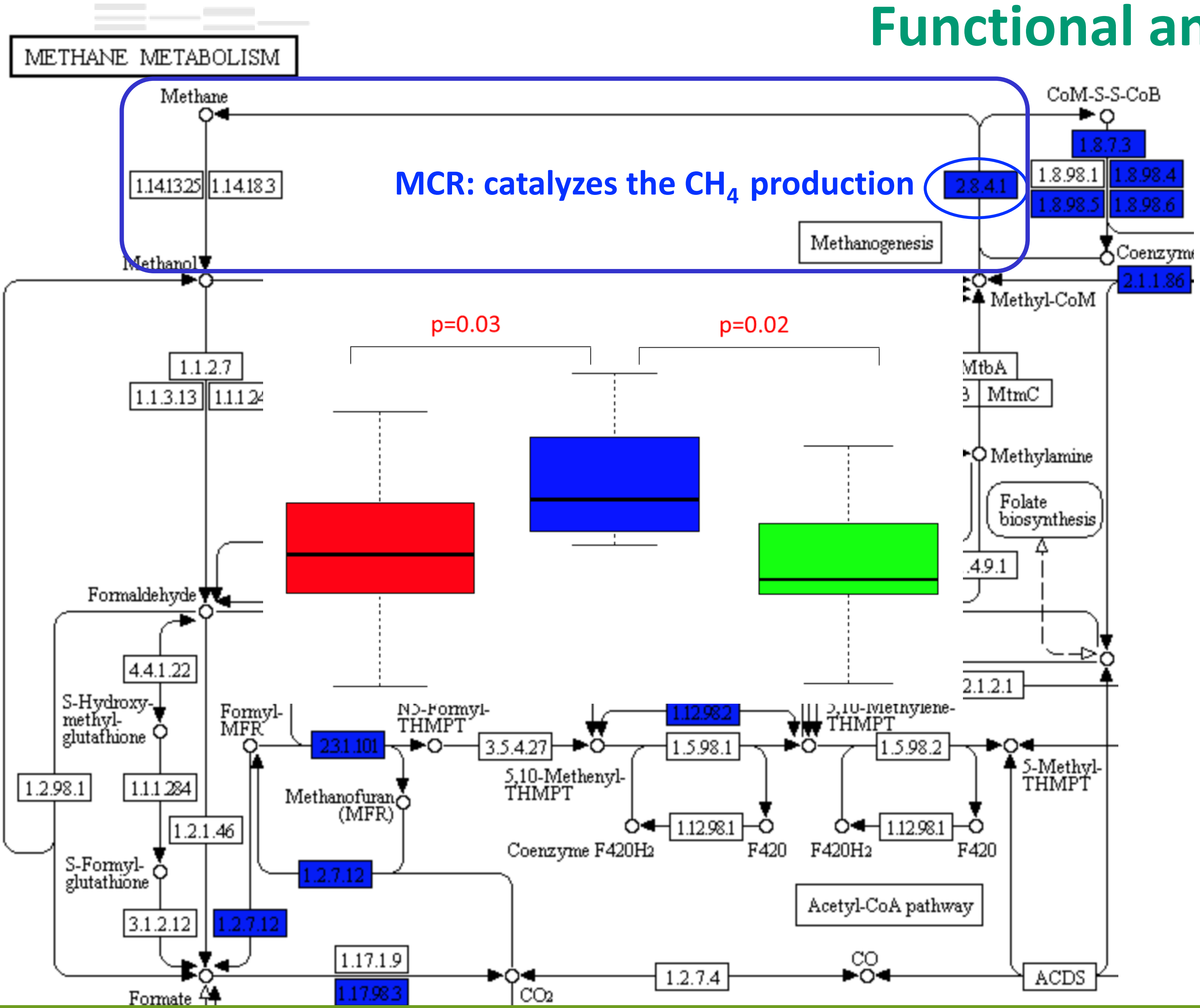


Linked to Low CH_4y

Wallace et al. (2015)

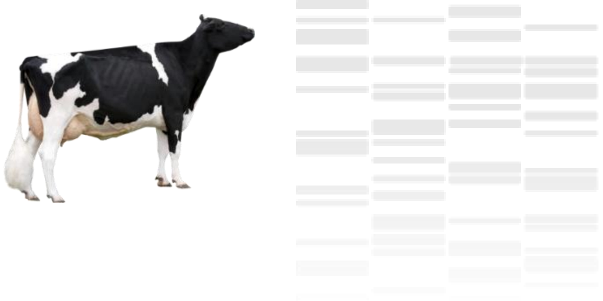
Danielsson et al. (2017)

Functional analysis



MCR: catalyzes the CH_4 production

Multivariate analysis

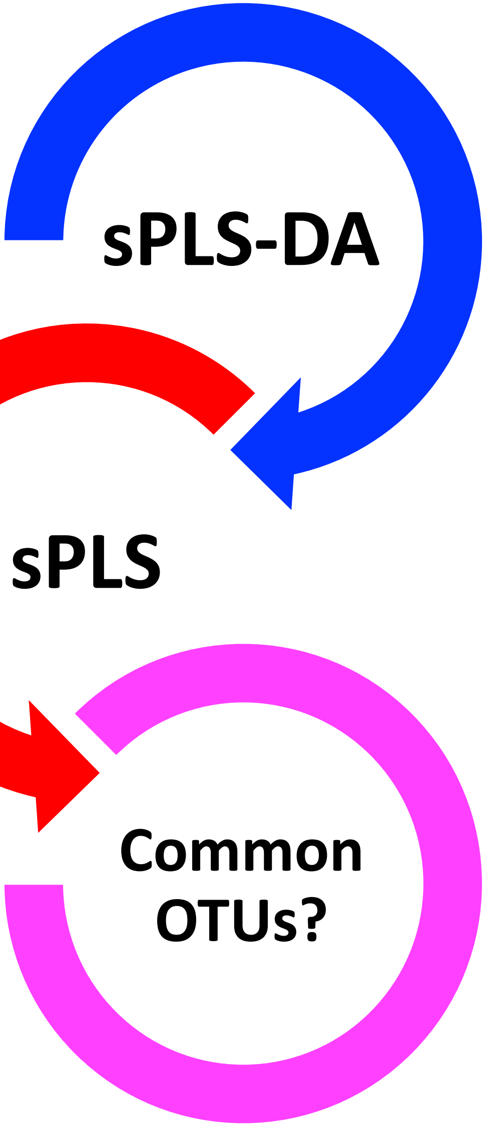


Ch4$_y$ classification

(X=OTU, Y= H$_{Ch4y,}$ L$_{Ch4y,}$ Int$_{Ch4y}$)

ruminotypes assignation

(X=OTU, Y= R1, R2, R3)



sPLS-DA

sPLS

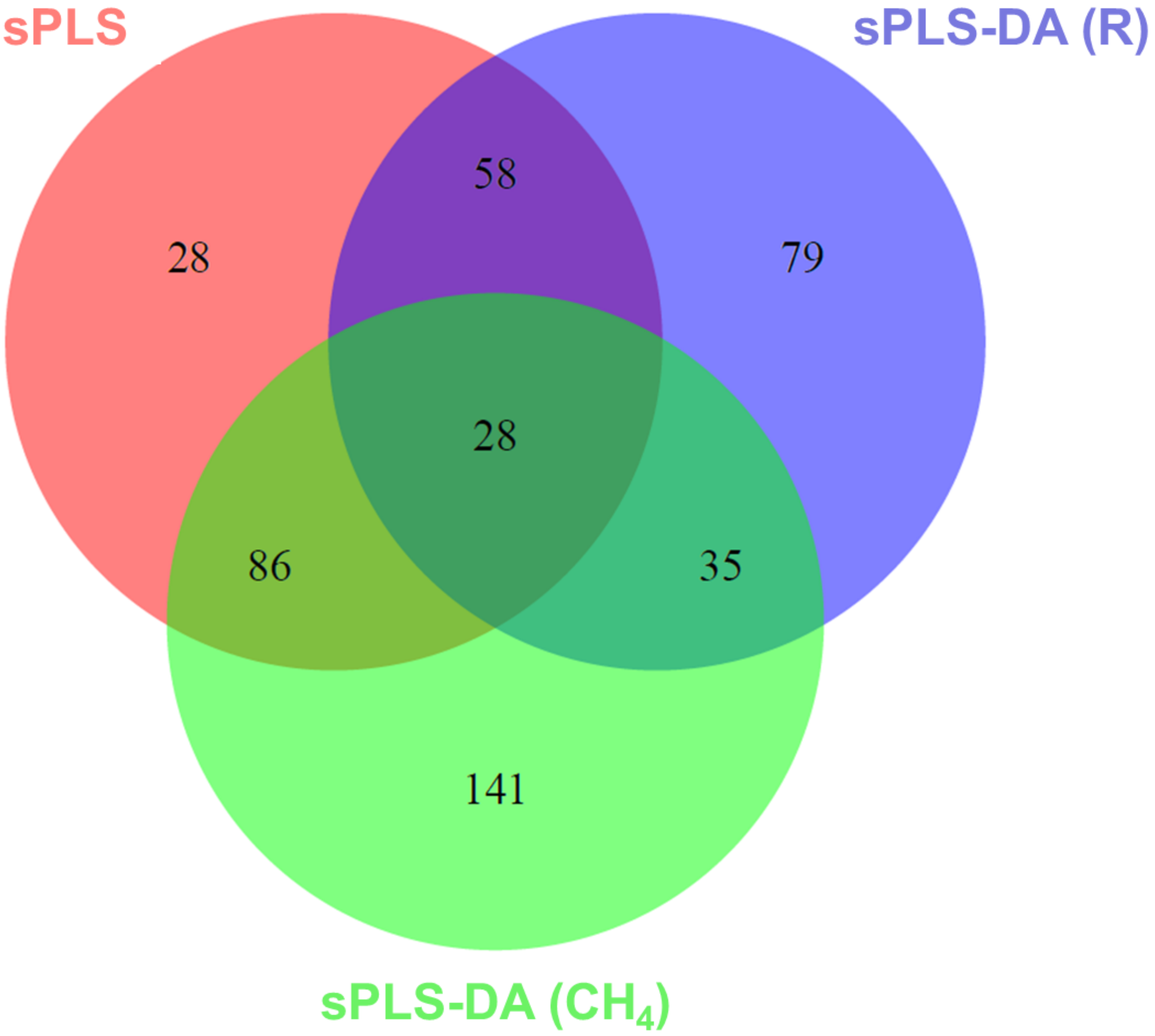
Common OTUs?

regression model

(X=OTU, Y=CH4y)

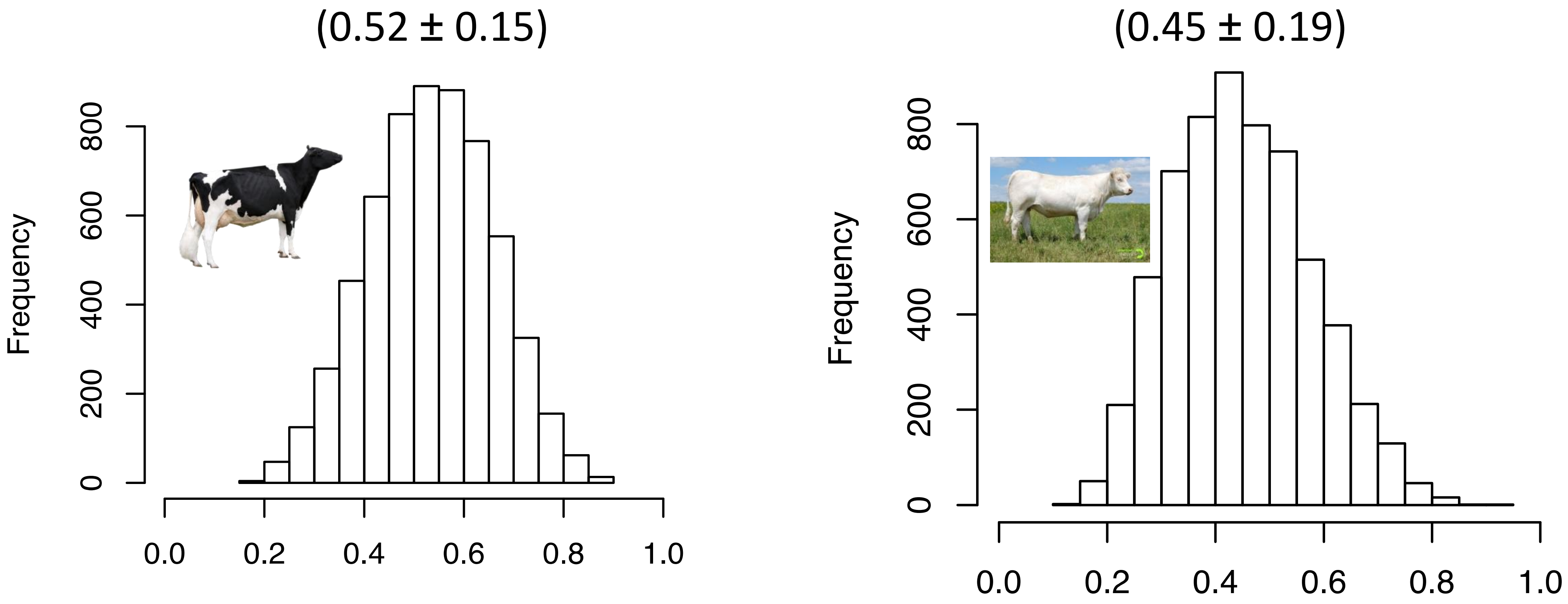
OTU jointly associated with CH4y and sample classification

Common OTUs



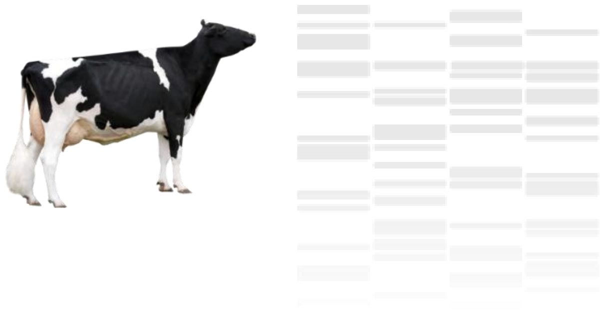
Microbial mixed model

BGLR (de los Campos and Perez, 2013)


(0.52 ± 0.15)

(0.45 ± 0.19)

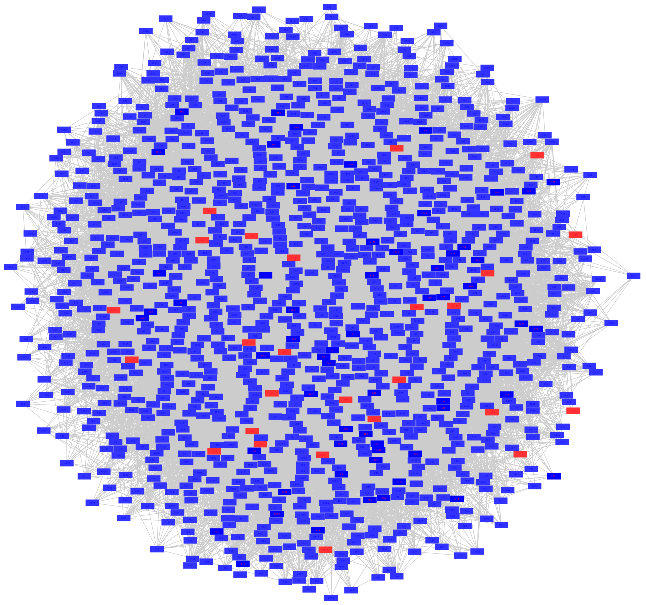
Marginal posterior distribution of CH4y microbiability



Microbial Network

SPCIT (Ramayo-Caldas et al. 2016)

Whole network

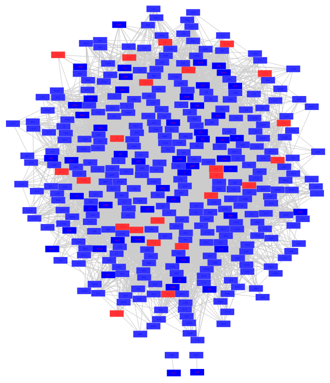


1,198 nodes (OTUs)
&
19,072 edges (interactions)

Cluster analyses

→

five modules



30% (384 OTUs)

89% (25/28)

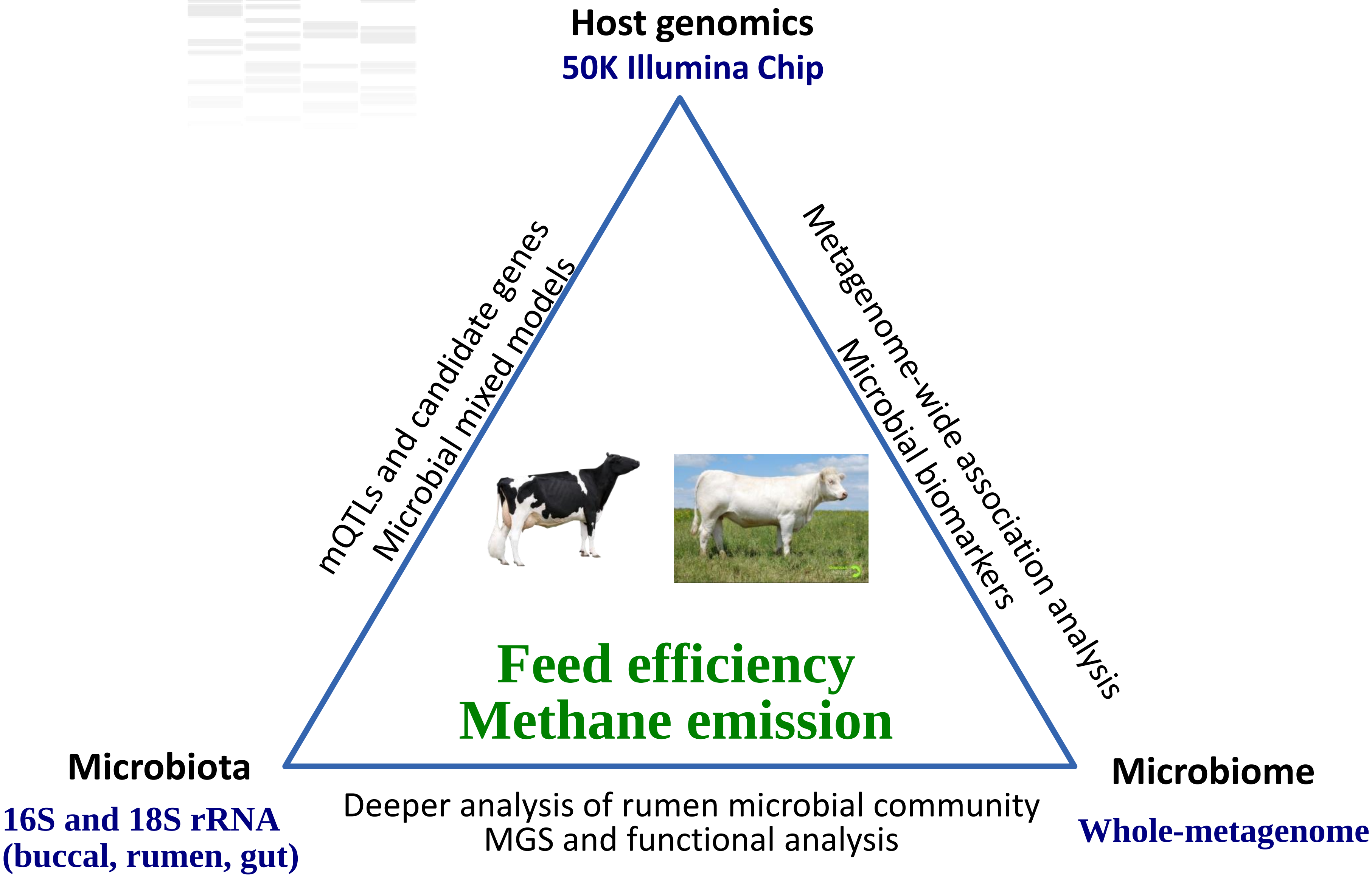
Conclusions

- The structure of the ruminal bacterial community is linked to CH4y emission.

- OTUs associated with CH4y emission predominantly belong to hydrogen-producing genera.

- These OTUs explained an important proportion of the CH4y phenotypic variance in Holstein and Charolaise breeds.

Future directions



Host genomics

50K Illumina Chip

mQTLs and candidate genes
Microbial mixed models

Metagenome-wide association analysis
Microbial biomarkers

Feed efficiency
Methane emission

Microbiota

16S and 18S rRNA
(buccal, rumen, gut)

Deeper analysis of rumen microbial community
MGS and functional analysis

Microbiome

Whole-metagenome

Nature special: Interdisciplinarity 16 Sept 2015

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MARIE CURIE ACTIONS

APIS-GENE
Investir Innover Valoriser

Thank you for your attention

Your

Matters