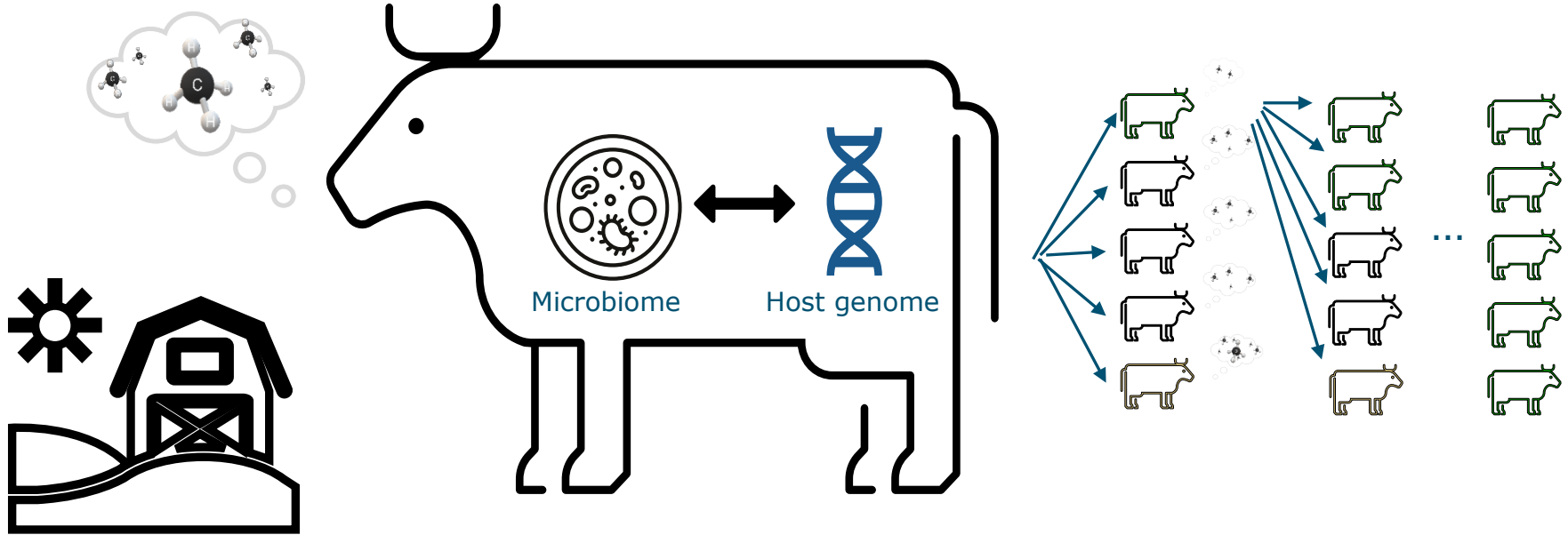


The cattle rumen microbiome and methane emissions

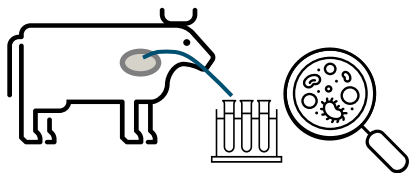
D. Flossdorf *, R. Bonifazi, B. Gredler-Grandl, H. Honerlagen, M.N. Aldridge, M. Spoelstra, A.E. van Breukelen, Y. de Haas, A.C. Bouwman



The cattle rumen microbiome and methane emissions



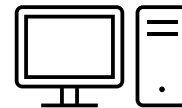
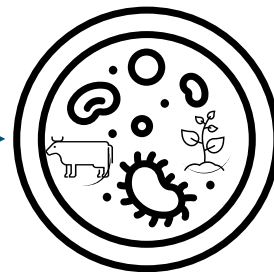
Methodology



Collected **961** rumen
fluid samples



Metagenomic long-read
sequencing



Collected **genotype**, **methane**
emission and **other** phenotype data



Use data in **model**

	Cow A	Cow B	Cow C
Taxa 1	0	0	3
Taxa 2	5	10	9
Taxa 3	15	30	81

Taxa present

	Cow A	Cow B	Cow C
Gene 1	5	0	0
Gene 2	7	10	17
Gene 3	68	20	93

Genes present

Methodology



Use data in
model

	Cow A	Cow B	Cow C
Gene 1	5	0	0
Gene 2	7	10	17
Gene 3	68	20	93

Genes present

Filtered out non- methane
metabolism related reads

	Cow A	Cow B	Cow C
Taxa 1	0	0	3
Taxa 2	5	10	9
Taxa 3	15	30	81

Taxa present

Filtered out non-microbial reads
(All Eucaryotic reads except
Protozoa and Yeast)

Heritability of Genera or Genes was estimated

Genus or Genes abundance =mean+herd+lact_stage+parity+G+e

- herd as fixed effect (as fixed effect n=10)
- lactation stage (1-60,61-120,121-240,241-360,361-405)
- parity (1,2,3,4+)
- animal genetics (Gmatrix 52162 SNPs)
- Cows included (n = 902)

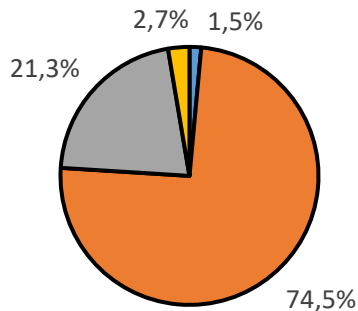
Association to methane for each Genus or Gene abundance was estimated

CH₄=mean+Genus/Gene_abund+CO₂+herd+lact_stage+parity+G+e

- mean CH₄ (5 weeks around sampling)
- Genus / Gene abundance (as covariable)
- mean CO₂ (5 weeks around sampling as covariable)
- herd (as fixed effect n=10)
- lactation stage (1-60,61-120,121-240,241-360,361-405)
- parity (1,2,3,4+)
- animal genetics (Gmatrix 52162 SNPs)
- Cows included (n = 457)

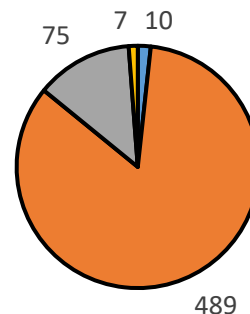
Microbiome dataset

Total genera found



■ Archaea ■ Bacteria ■ Eukaryota ■ Viruses

Core microbiome composition

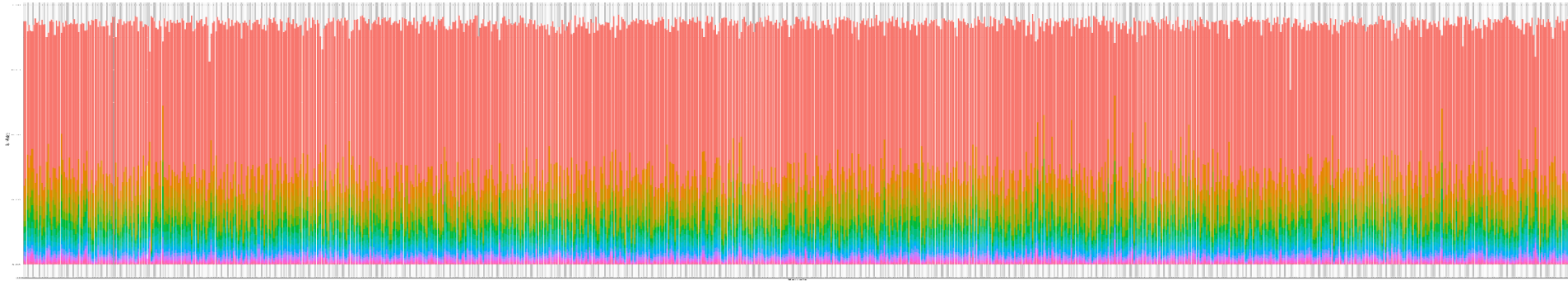


■ Archaea ■ Bacteria ■ Eukaryota ■ Viruses

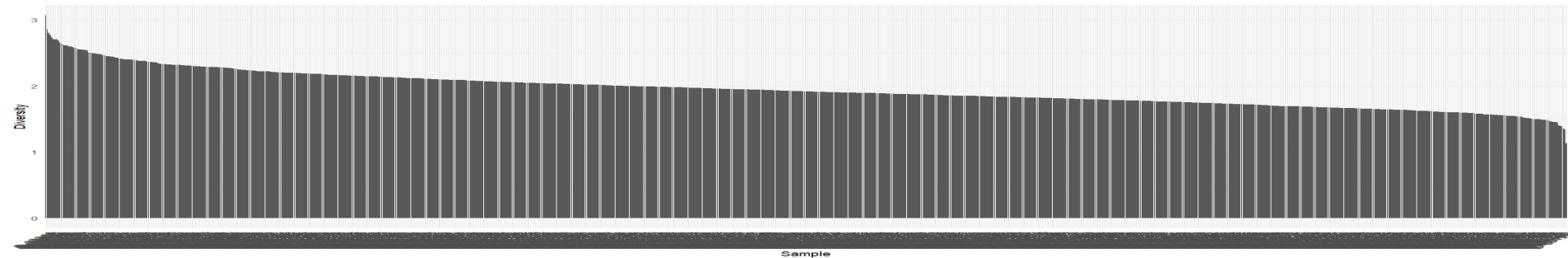
	Total microbiome, found across all samples				Core microbiome, found in at least 20% of samples		
Kingdoms	Genera found	Genera percent	Reads	Read percent	Genera found	Genera in percent	Percent of total genera found
Archaea	56	1.5%	1993123	0.8%	10	1.7%	18%
Bacteria	2854	74.6%	231586290	98.1%	485	84.1%	17%
Eukaryota	816	21.3%	1776062	0.8%	75	13.0%	9%
Viruses	102	2.7%	760453	0.3%	7	1.2%	7%
Sum	3828	100%	236115928	100%	577	100%	15%

First results – rumen microbiome analysis

Core Microbiome genus distribution top 15

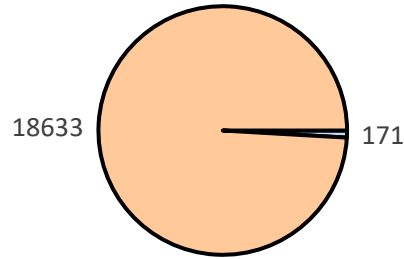


Shannon diversity index



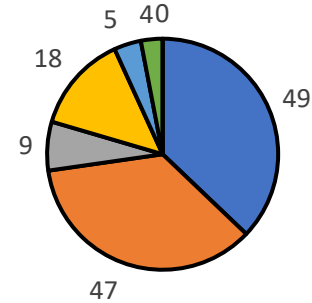
Methane gene dataset

Genes found in metagenomic data



Methane related
 Other genes

Genes related to methane metabolism

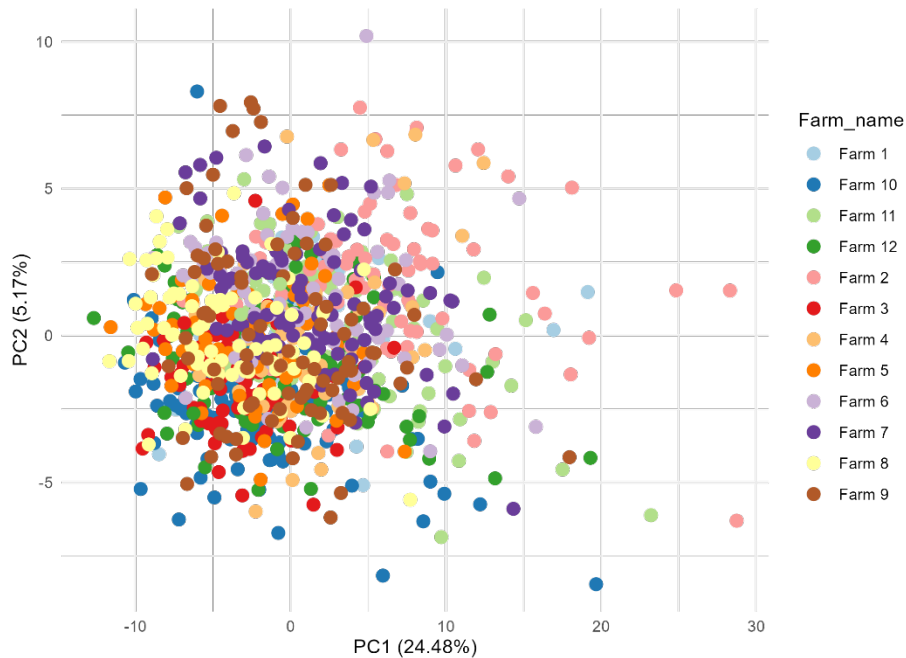


EC 1: Oxidoreductases
 EC 2: Transferases
 EC 3: Hydrolases
 EC 4: Lyases
 EC 5: Isomerases
 EC 6: Ligases
 EC 7: Translocases

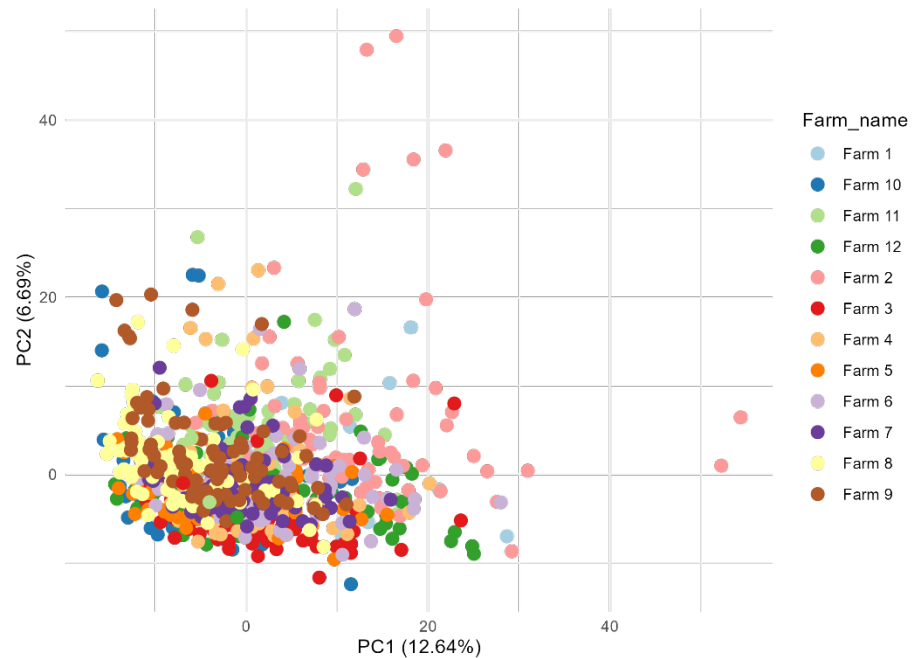
	Methane related	Other genes	Total	Percent methane related	Core methane genes (present in at least 20% of cows)
Number of genes found	171	18633	18804	1%	132
Number of reads	7178950	699724726	706903676	1%	7177527

PCA – Farms

PCA of methane metabolism related gene data

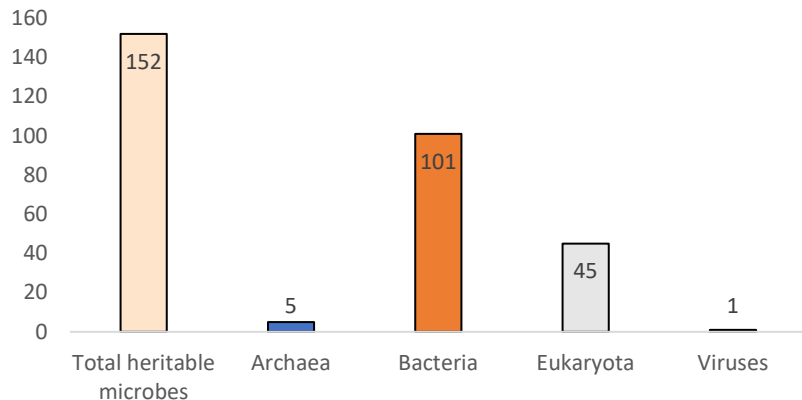


PCA of microbiome abundance data



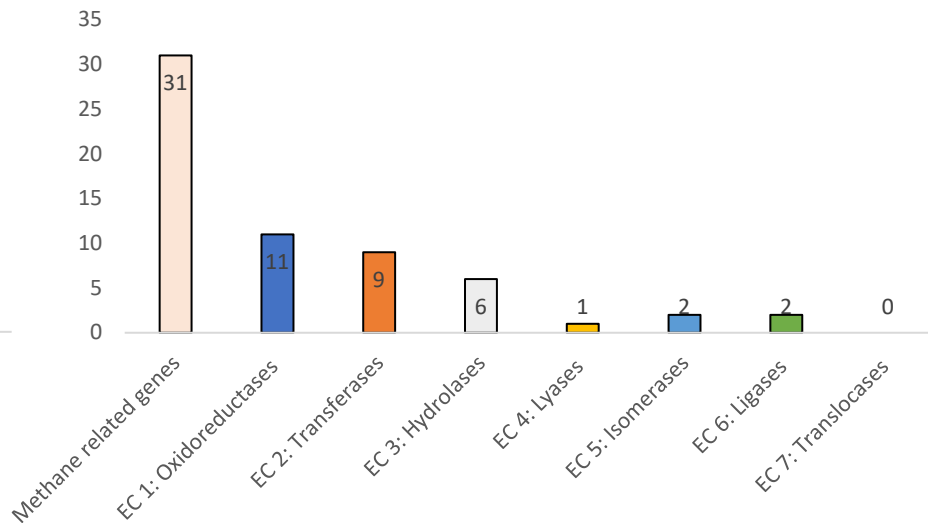
Heritability

Heritable genera found in core microbiome



152 / 577 Genera

Core heritable methane metabolism genes

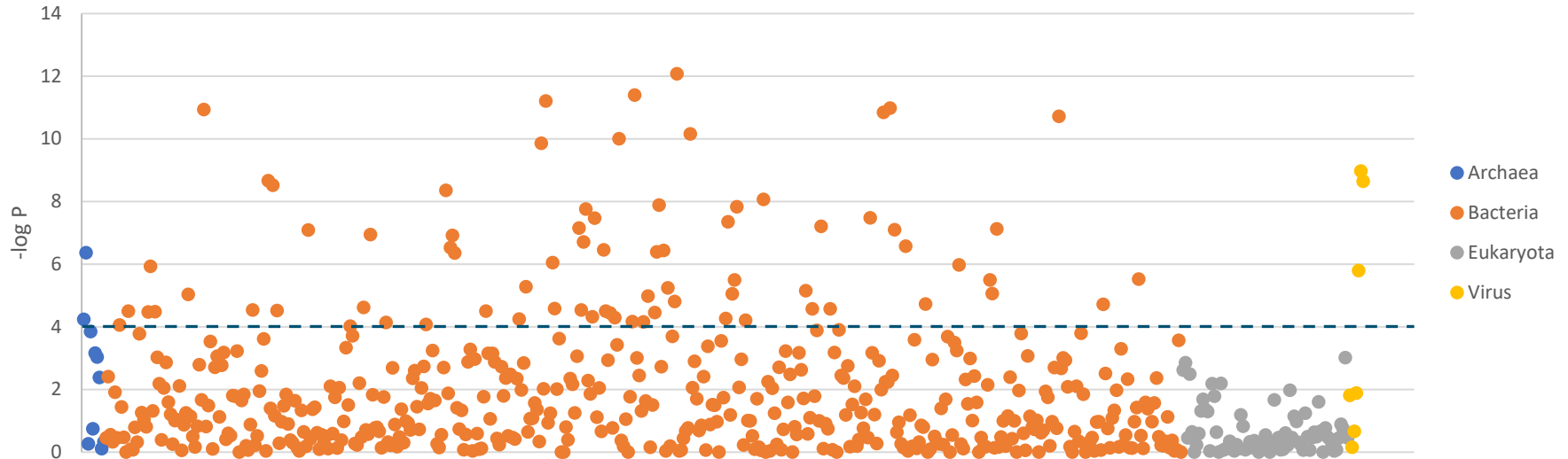


31 / 131 Methane metabolism genes

Significantly heritable [$h^2 - (SE * 1.96) > 0$]

Microbes associated with methane emissions

- 80 / 577 Genera associated to methane emissions

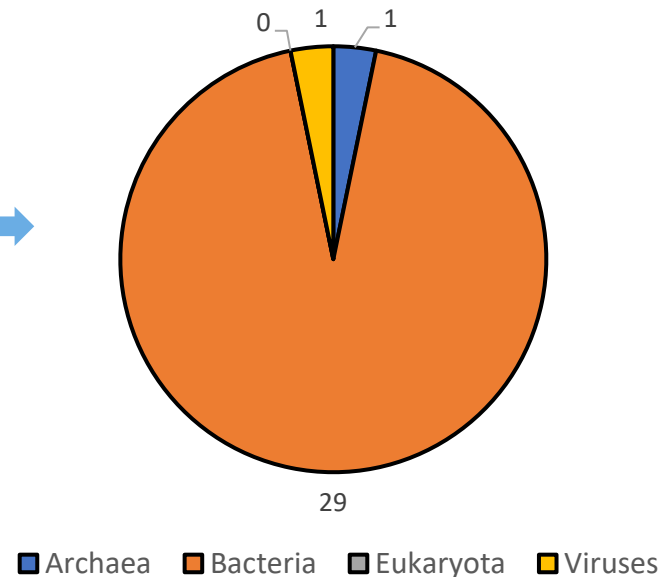


Microbes associated with methane emissions that are also heritable

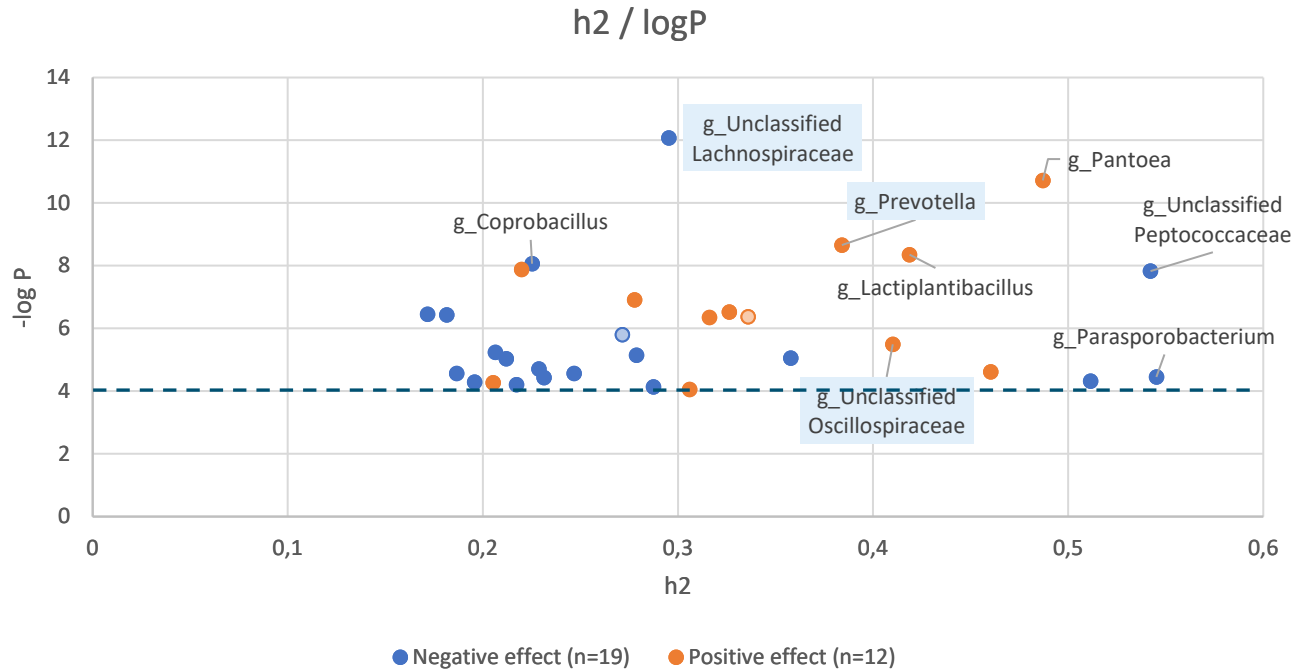
- 68 / 80 $h^2 > 0$
- 48 / 80 $h^2 - SE > 0$
- **31 / 80** **$h^2 - (SE * 1.96) > 0$**



Heritable genera related to methane production

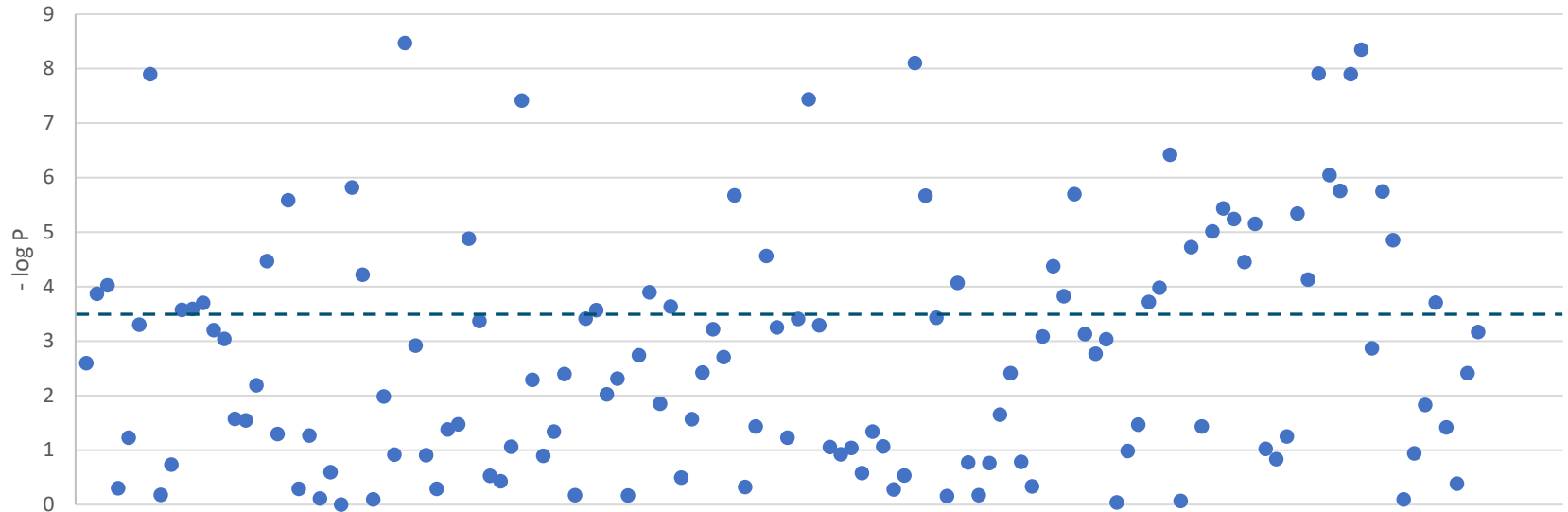


Microbes associated with methane emissions that are also heritable



Methane metabolism genes associated with methane emissions

- 47 / 131 Methane metabolism genes associated to methane emission

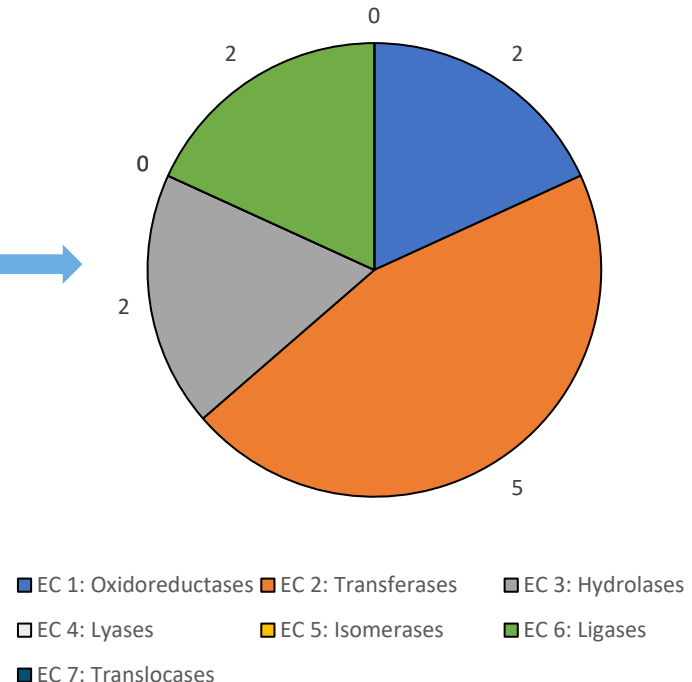


Methane metabolism genes associated with methane emissions that are also heritable

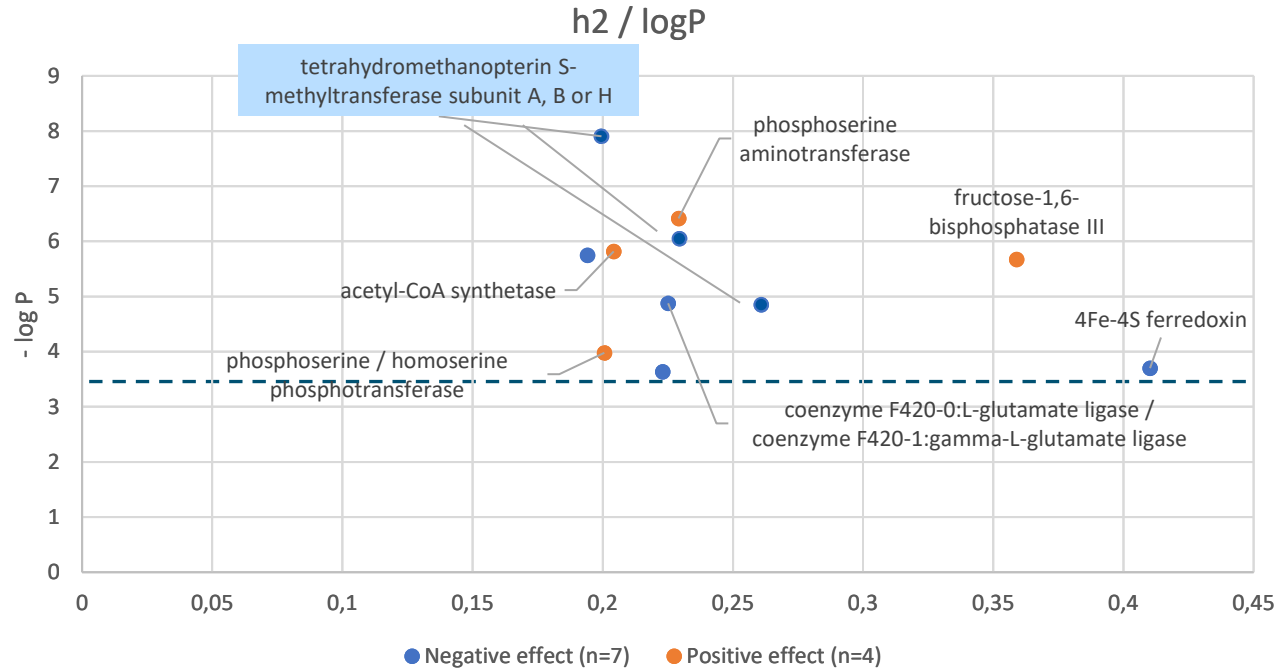
- 43 / 47 $h^2 > 0$
- 29 / 47 $h^2 - SE > 0$
- **11 / 47** **$h^2 - (SE * 1.96) > 0$**



Heritable genes related to methane production



Methane metabolism genes associated with methane emissions that are also heritable



Conclusion

- Provided potential targets for methane production related microorganisms that could potentially be controlled through breeding
 - 31 Genera were found to be significantly heritable and related to methane emissions
- Provided potential target list of genes for methane production in rumen content
 - 11 Genes were found to be significantly heritable and related to methane emissions

Questions ?

This study was supported by the **Horizon Europe** program (Re-Livestock grant agreement No.01059609) and the Knowledge and Innovation Agenda for Agriculture, Water and Food of the **Dutch Ministry of Agriculture, Fisheries, Food Security and Nature**.

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