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Breeding for changes in enteric methane emissions: Lessons from Sheep

Suzanne Rowe, Arjan Jonker, Cesar Pinares, Melanie Hess, Larissa Zetouni, Tricia Johnson, Rudiger Brauning, Peter Janssen & John McEwan

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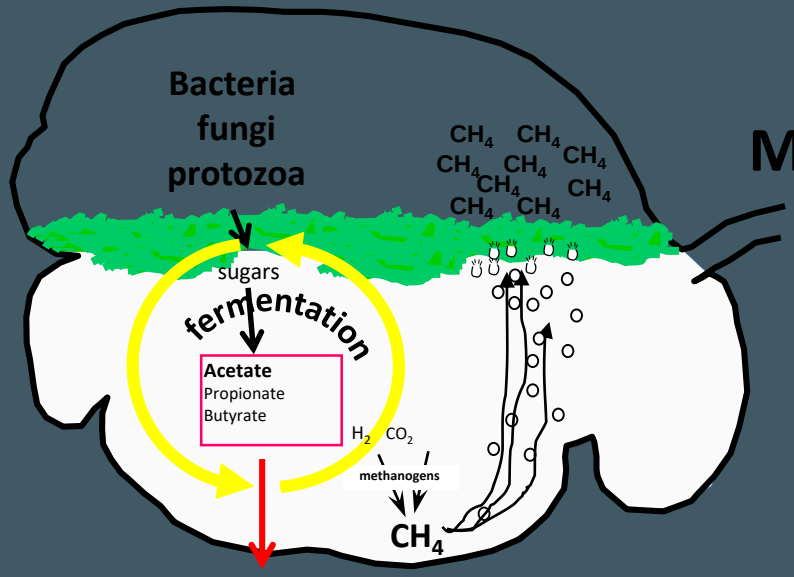
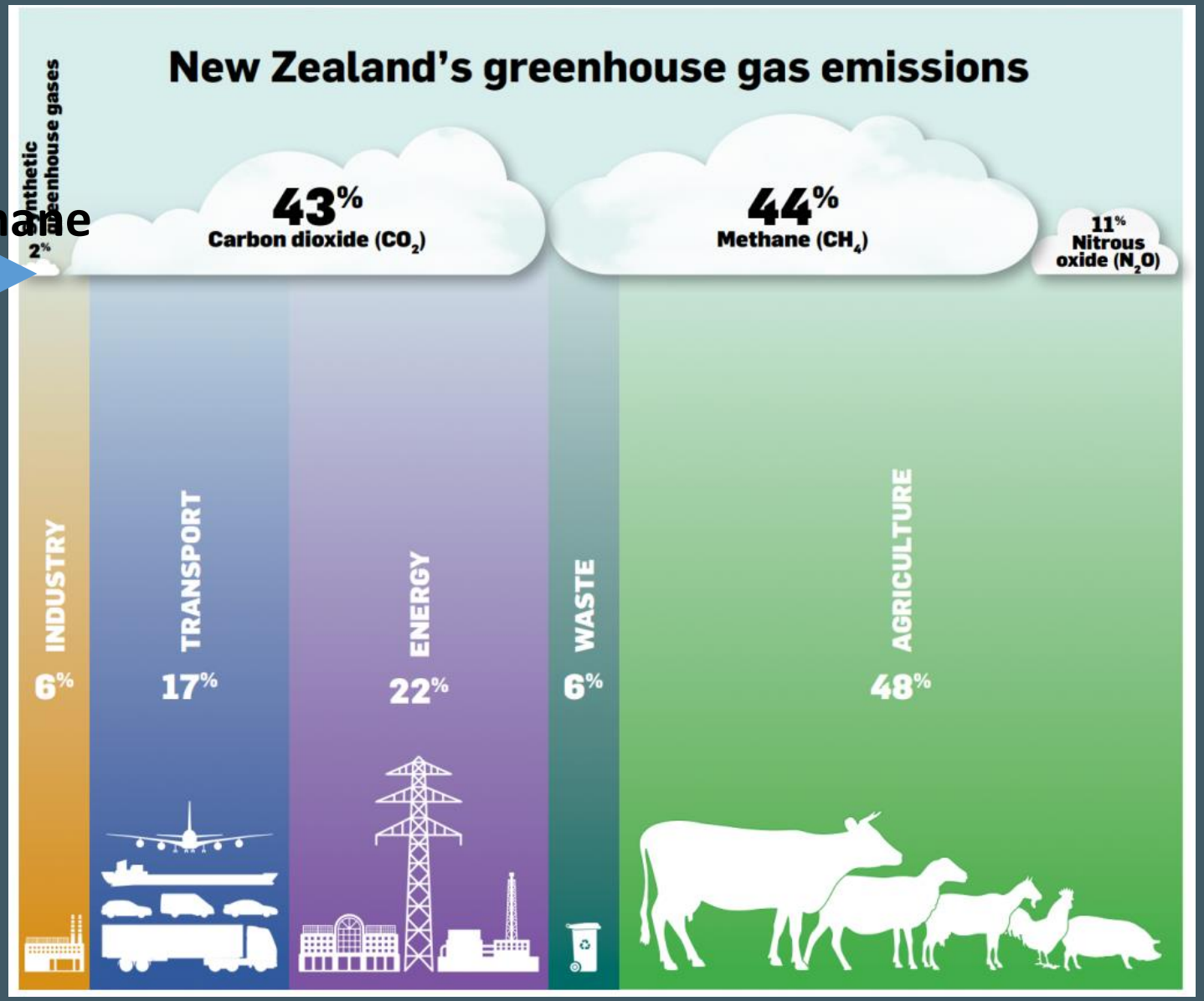
PGgRc PASTORAL GREENHOUSE GAS RESEARCH CONSORTIUM
NEW ZEALAND AGRICULTURAL GREENHOUSE GAS Research Centre



Methane



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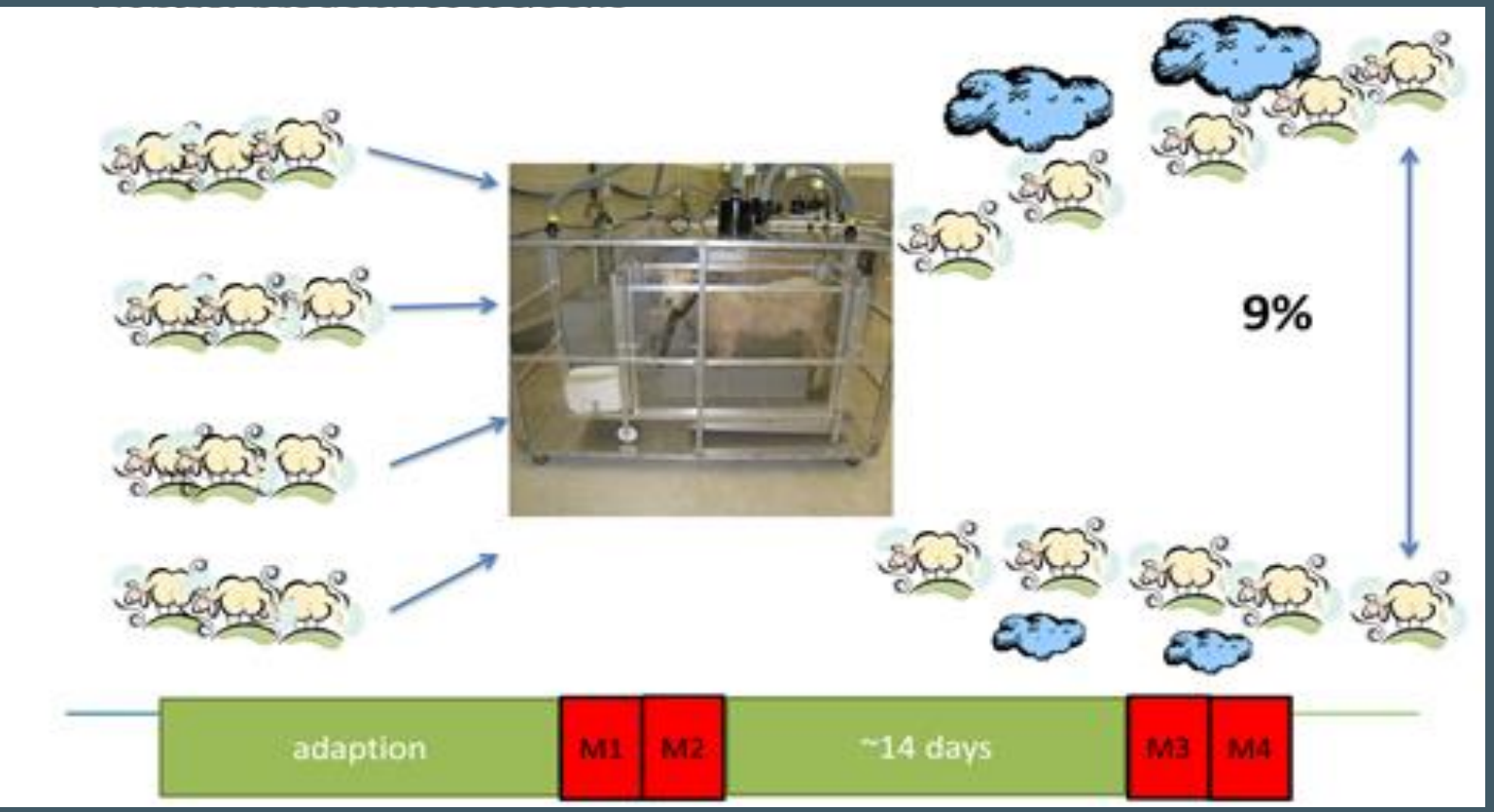
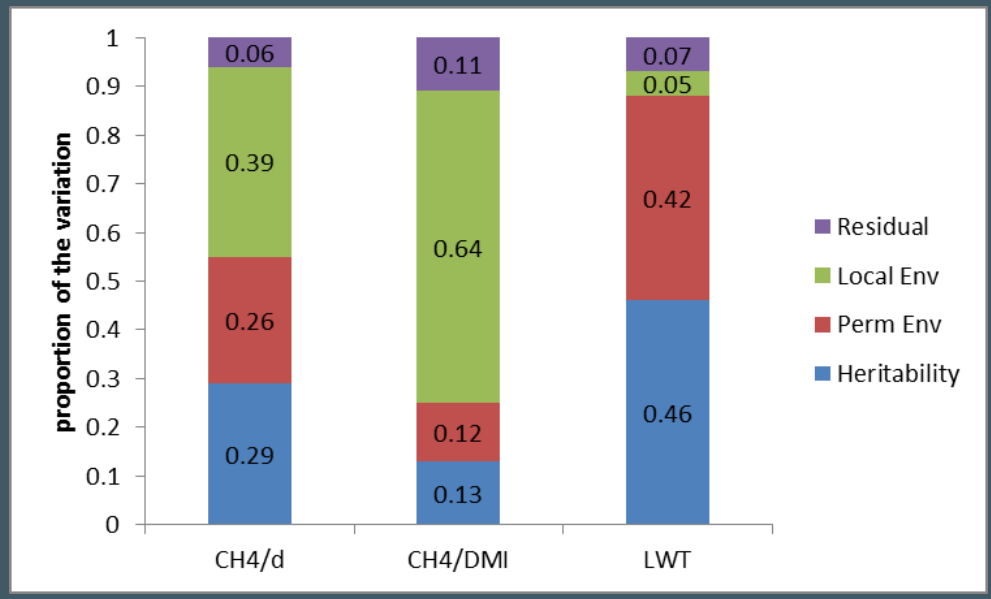
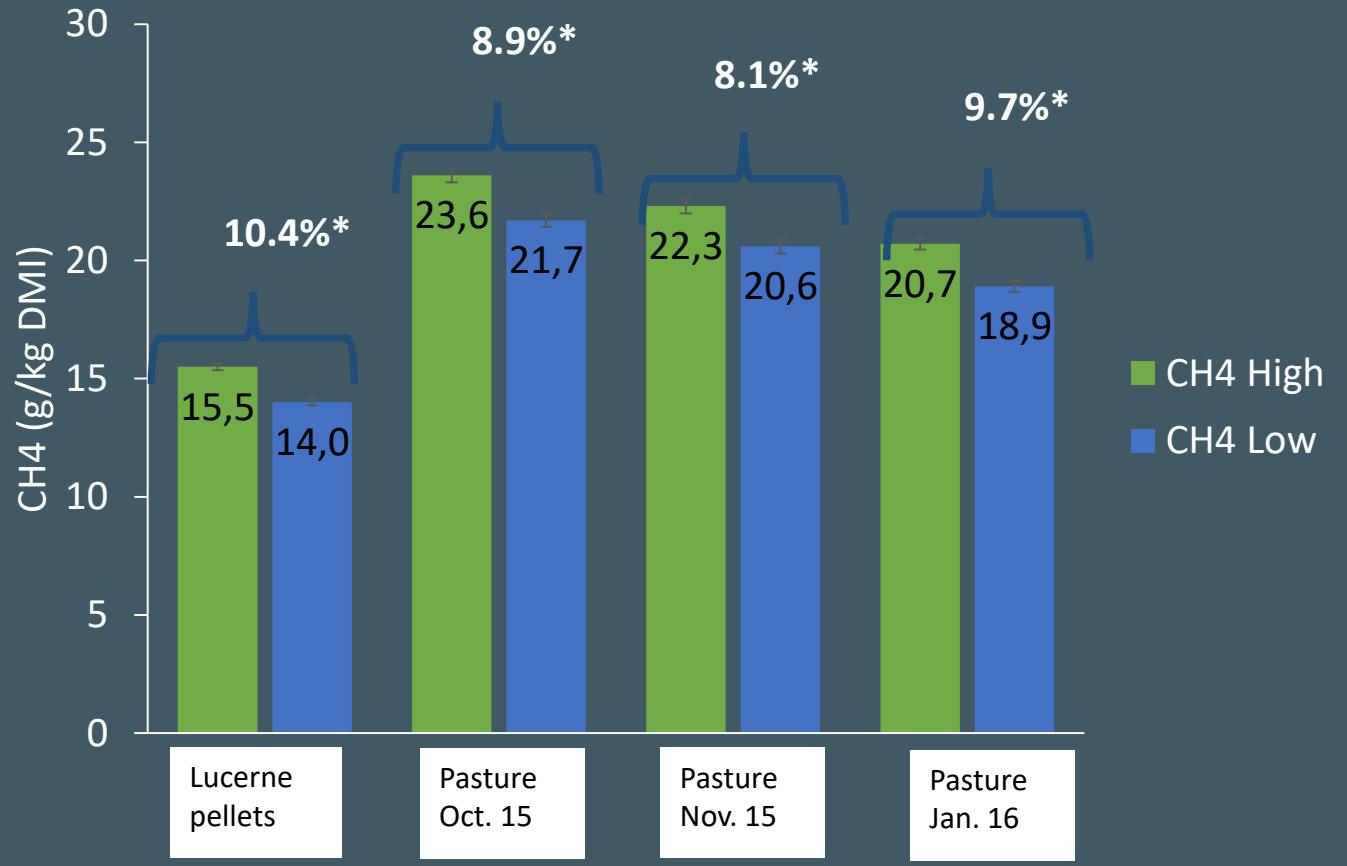
Methane Selection Lines





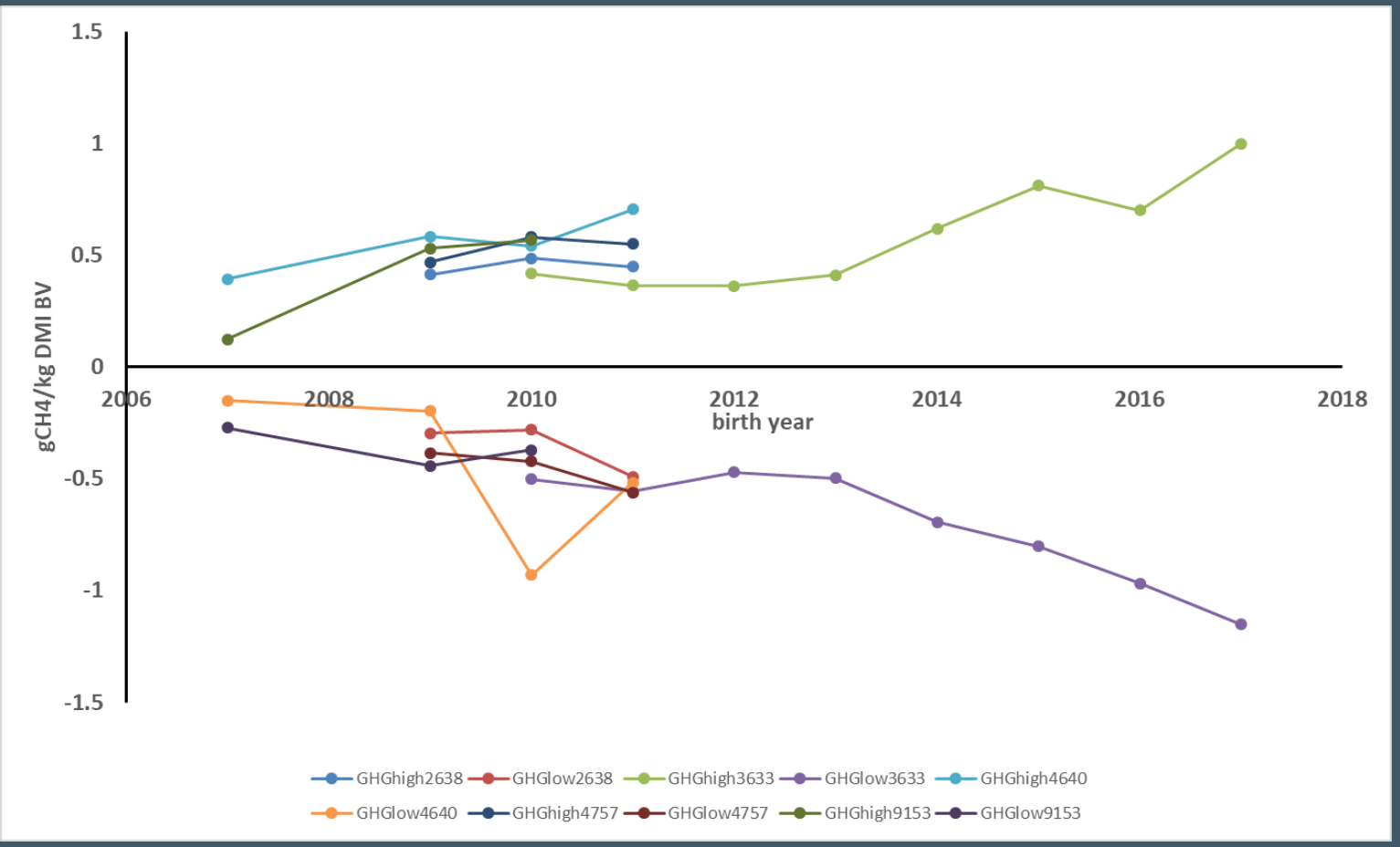
Respiration chamber - cut pasture

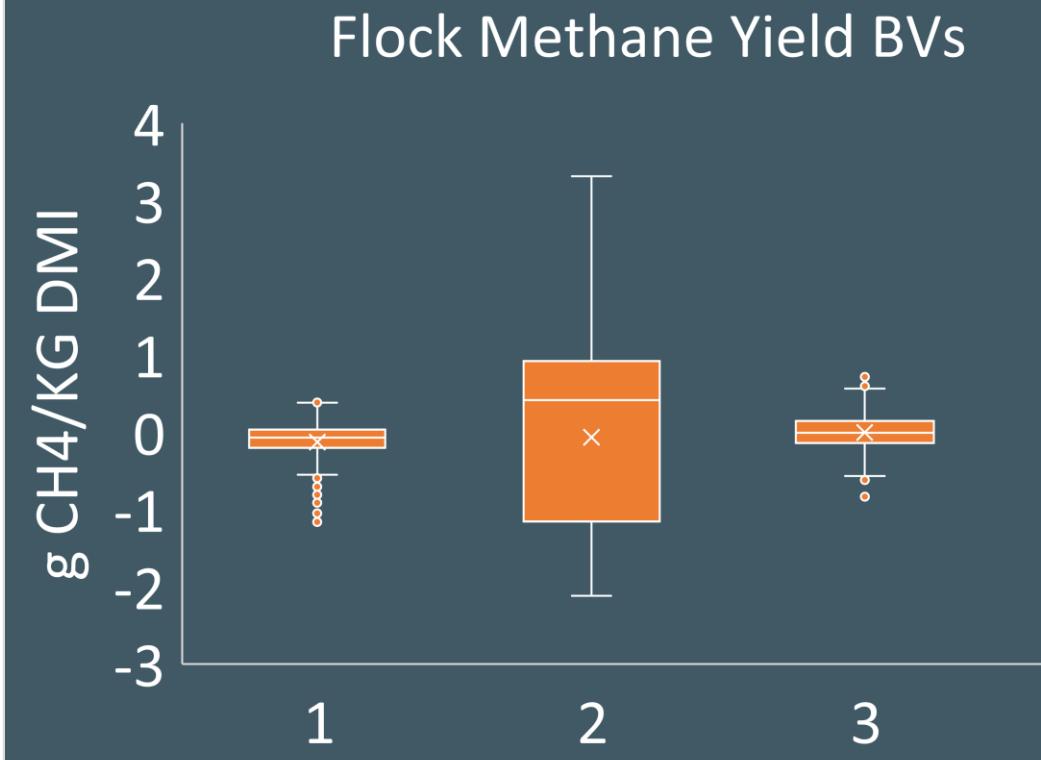
CH_4 yield from 50 low and 46 high CH_4 sheep repeated on cut pasture following the standard alfalfa pellet trial

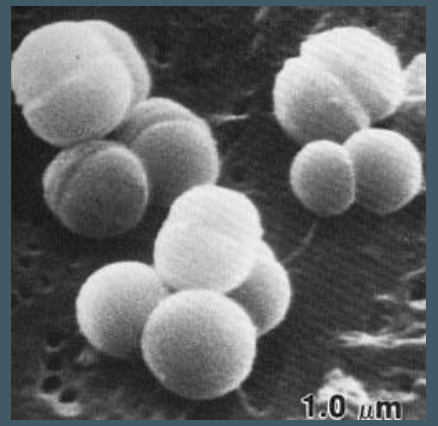


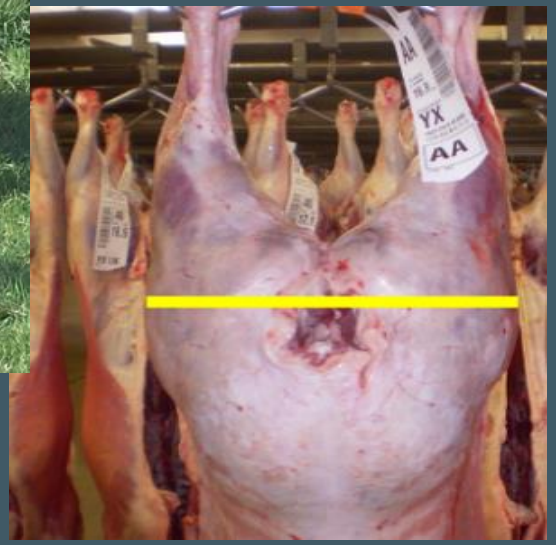
*P < 0.001

Methane Selection Lines

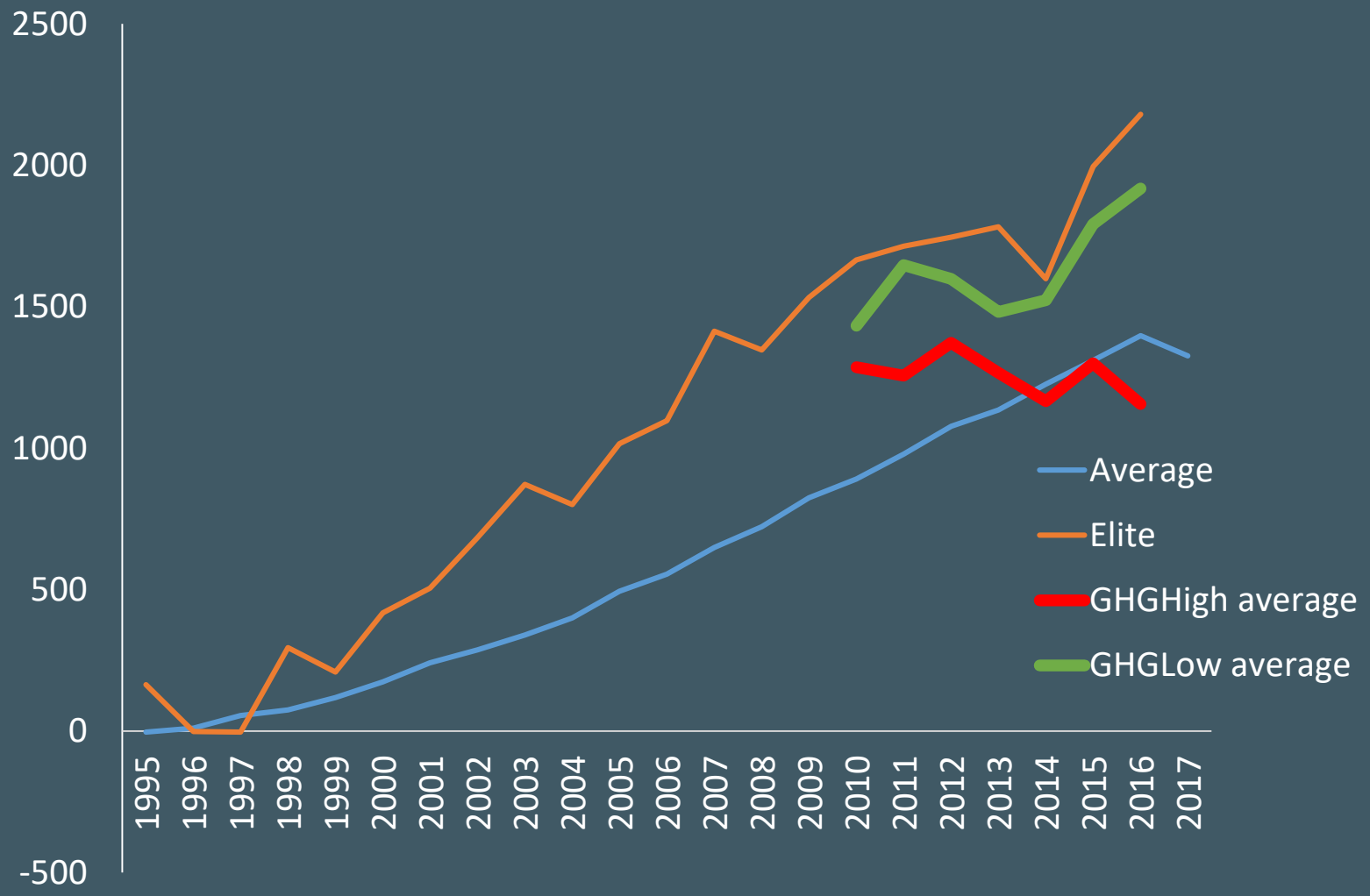


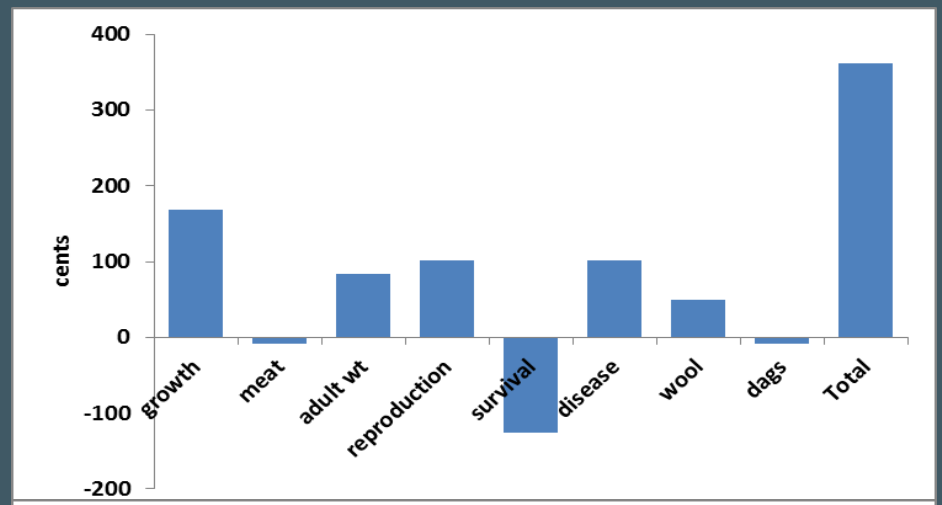


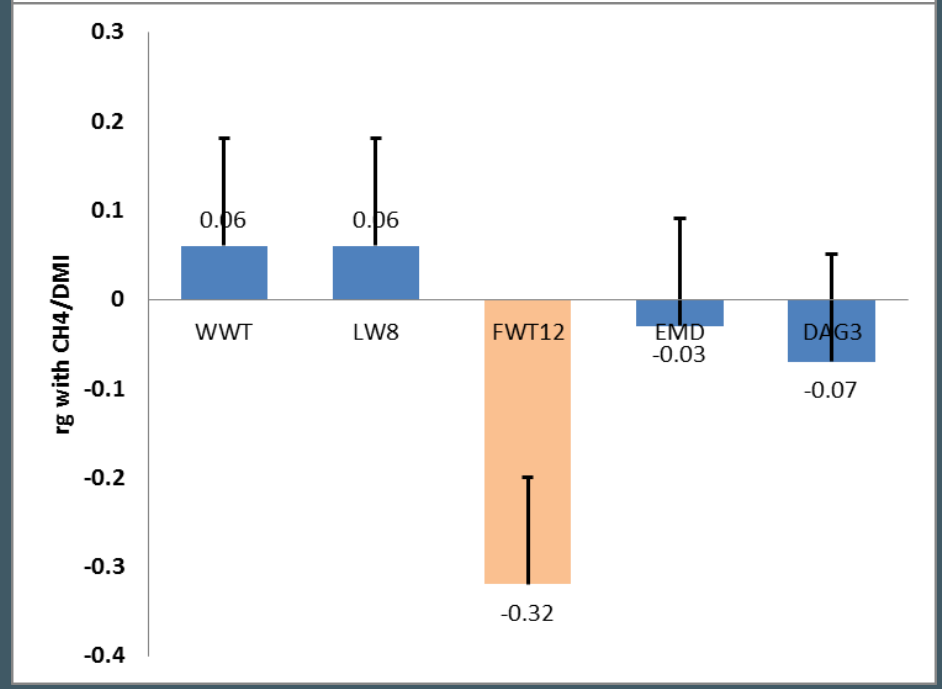

1.0 µm


YX
AA

Maternal Worth Index







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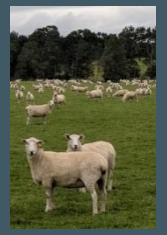
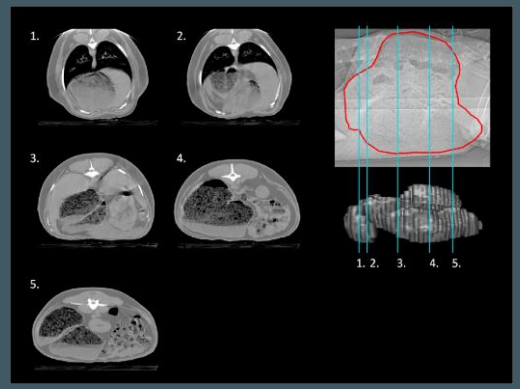
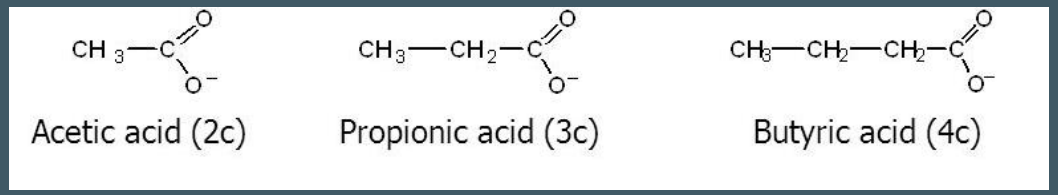
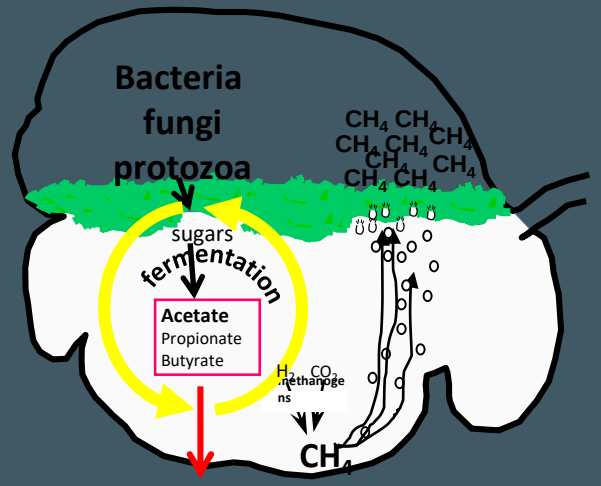


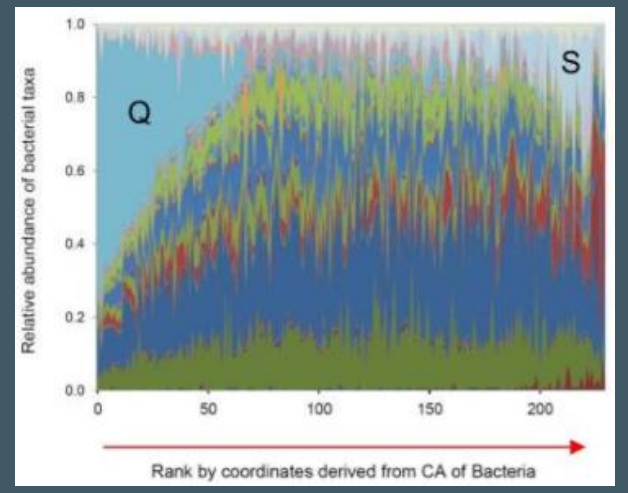
Low v High Methane Sheep

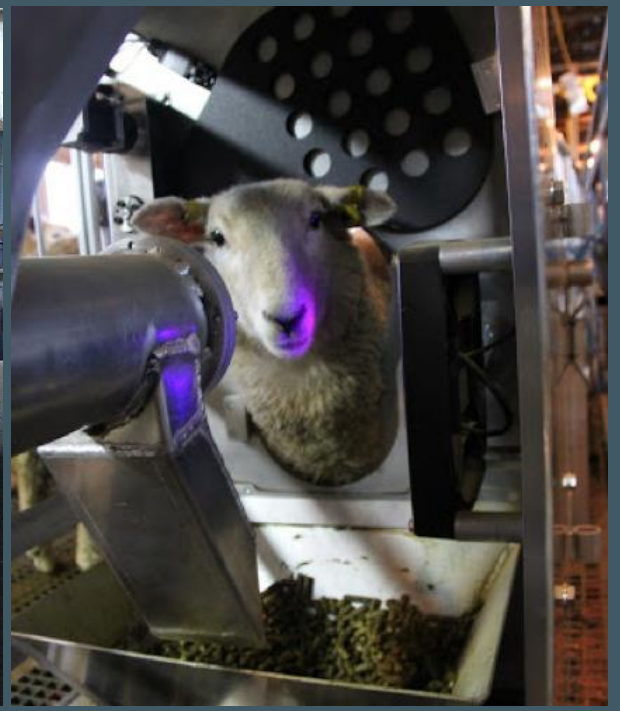
- ~11% less methane per kg feed
- 20% smaller rumens
- Different energy profile
- Different microbial fermentation



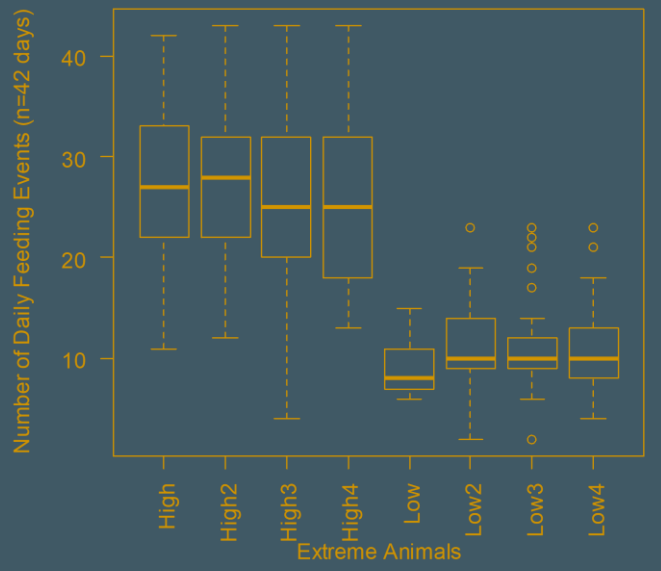




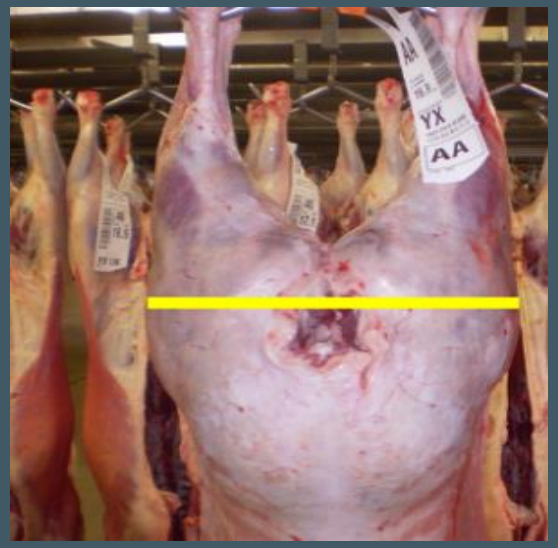




	Methane	Methane Yield
	r_g	r_g
RFI	-0.28 ± 0.16	**-0.41 ± 0.15**
MEIntake	**0.33 ± 0.17**	**-0.24 ± 0.09**
GROWTH_RATE	**0.34 ± 0.10**	0.10 ± 0.16
Av_Intake_Feed	**0.41 ± 0.13**	**0.21 ± 0.08**
Fat_Start	-0.001 ± 0.13	**0.27 ± 0.10**
Change_C	-0.27 ± 0.23	-0.26 ± 0.13
Fat_End	-0.04 ± 0.15	0.06 ± 0.08
Eating_Events	**-0.28 ± 0.08**	**-0.38 ± 0.15**
Feeding_Rate	**0.55 ± 0.16**	**0.27 ± 0.11**

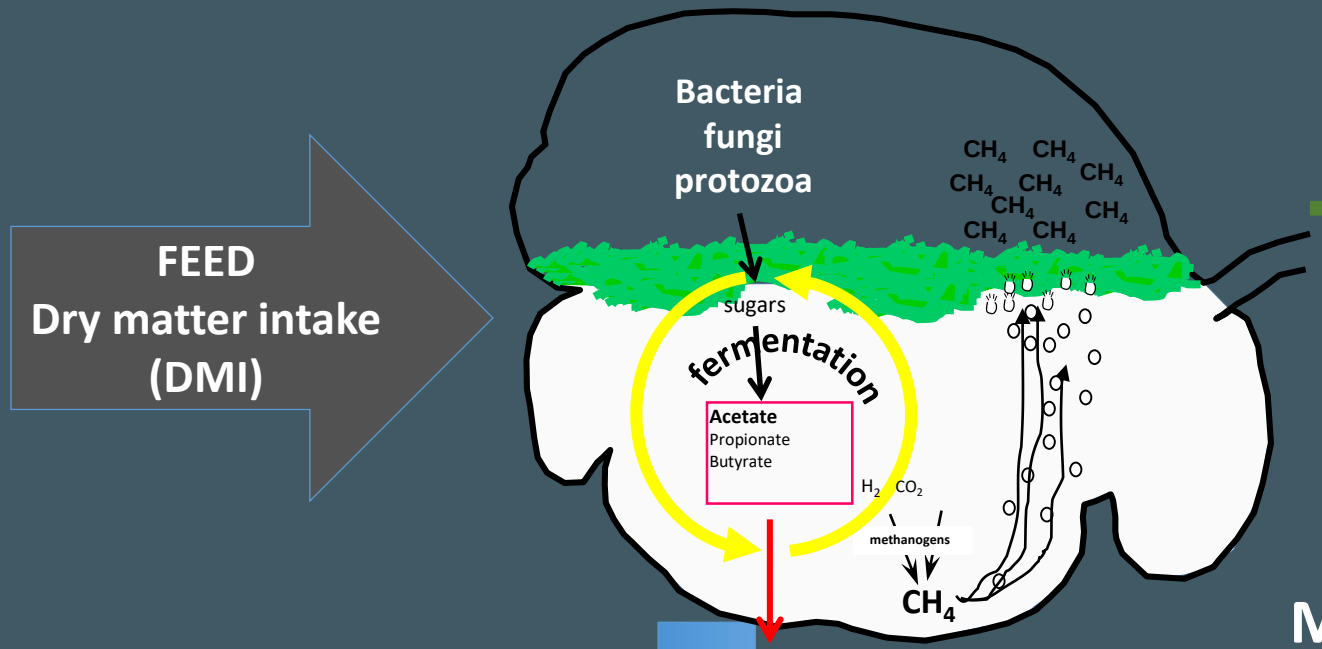


Meat – fatty acid profiles



	HighGHG	LowGHG	P value		HighGHG	LowGHG	P value
GR	16.5 ± 1.06	14.8 ± 1.28	0.0853	C18:1 t11	0.56 ± 0.03	0.62 ± 0.03	0.0046
IMF%	4.67 ± 0.39	4.84 ± 0.46	0.0673	C18:1c9	1.58 ± 0.01	1.57 ± 0.01	0.0136
SFA	3.86 ± 0.01	3.84 ± 0.01	0.0196	C18:1c11	0.27 ± 0.00	0.28 ± 0.00	0
MUFA	3.77 ± 0.01	3.76 ± 0.01	0.4728	Unknown 15	0.06 ± 0.00	0.06 ± 0.00	0.0002
PUFA	2.07 ± 0.02	2.16 ± 0.03	0	Unknown 16	0.02 ± 0.00	0.02 ± 0.00	0.1392
BC	0.84 ± 0.02	0.87 ± 0.02	0.0232	Unknown 17	0.13 ± 0.00	0.13 ± 0.00	0.0009
C10:0	0.05 ± 0.00	0.04 ± 0.00	0.0676	Unknown 18	0.05 ± 0.00	0.06 ± 0.00	0.0138
Artifact:1	0.05 ± 0.00	0.05 ± 0.00	0.8649	Unknown 19	0.04 ± 0.00	0.04 ± 0.00	0.7978
C12:0	0.03 ± 0.00	0.03 ± 0.00	0.6162	C18:2tt n6	0.12 ± 0.00	0.13 ± 0.00	0.0001
iso C14	0.01 ± 0.00	0.01 ± 0.00	0.2387	Unknown 20	0.06 ± 0.00	0.07 ± 0.00	0
C14:0	0.43 ± 0.01	0.43 ± 0.01	0.9527	Unknown 21	0.01 ± 0.00	0.01 ± 0.00	0.2926
iso C15	0.06 ± 0.00	0.06 ± 0.00	0.1388	Unknown 22	0.10 ± 0.00	0.12 ± 0.01	0
anteiso C15	0.06 ± 0.00	0.07 ± 0.00	0.0185	C18:2 n6	0.48 ± 0.01	0.52 ± 0.01	0.0001
C14:1	0.01 ± 0.00	0.01 ± 0.00	0.1821	Unknown 23	0.03 ± 0.00	0.03 ± 0.00	0.1553
C15:0	0.12 ± 0.00	0.13 ± 0.01	0.0499	Unknown 24	0.01 ± 0.00	0.01 ± 0.00	0.3738
Artifact:2	0.24 ± 0.01	0.26 ± 0.01	0.3848	Unknown 25	0.02 ± 0.00	0.02 ± 0.00	0.8705
iso C16	0.05 ± 0.00	0.05 ± 0.00	0.0235	Unknown 26	0.01 ± 0.00	0.01 ± 0.00	0.7695
Unknown 3	0.02 ± 0.00	0.02 ± 0.00	0.0578	C20:0	0.03 ± 0.00	0.02 ± 0.00	0.1941
Unknown 4	0.03 ± 0.00	0.03 ± 0.00	0.0666	C18:3 n3	0.43 ± 0.01	0.46 ± 0.01	0
C16:0	1.38 ± 0.01	1.37 ± 0.01	0.5731	9 11 CLA	0.29 ± 0.02	0.33 ± 0.02	0.001
Unknown 5	0.03 ± 0.00	0.04 ± 0.00	0.0538	Unknown 27	0.01 ± 0.00	0.02 ± 0.00	0.0723
Unknown 6	0.01 ± 0.00	0.01 ± 0.00	0.6384	Unknown 28	0.01 ± 0.00	0.01 ± 0.00	0.5364
Unknown 7	0.01 ± 0.00	0.02 ± 0.00	0.1102	Unknown 29	0.01 ± 0.00	0.01 ± 0.00	0.7318
C16:1t	0.04 ± 0.00	0.05 ± 0.01	0.0014	Unknown 30	0.07 ± 0.00	0.09 ± 0.00	0
iso C17	0.15 ± 0.00	0.16 ± 0.00	0.053	Unknown 31	0.07 ± 0.00	0.06 ± 0.00	0.0001
Unknown 8	0.13 ± 0.00	0.13 ± 0.00	0.1536	Unknown 32	0.03 ± 0.00	0.04 ± 0.00	0.173
C16:1	0.28 ± 0.01	0.29 ± 0.01	0.0099	C20:4 n6	0.19 ± 0.01	0.20 ± 0.01	0.5907
anteiso C17	0.17 ± 0.00	0.18 ± 0.01	0.0298	C22:1	0.01 ± 0.00	0.01 ± 0.00	0.3251
Unknown 9	0.02 ± 0.00	0.01 ± 0.00	0.3494	Unknown 33	0.02 ± 0.00	0.02 ± 0.01	0.3411
C17:0	0.28 ± 0.00	0.28 ± 0.00	0.2142	Unknown 34	0.01 ± 0.00	0.01 ± 0.00	0.7671
Artifact:3	0.27 ± 0.01	0.26 ± 0.01	0.5595	Unknown 35	0.01 ± 0.00	0.01 ± 0.00	0.6732
Unknown 10	0.06 ± 0.00	0.06 ± 0.00	0.2063	Unknown 36	0.02 ± 0.00	0.02 ± 0.00	0.7064
Unknown 11	0.04 ± 0.00	0.04 ± 0.00	0.2563	C20:5 n3	0.20 ± 0.01	0.21 ± 0.01	0.55
C17:1	0.14 ± 0.00	0.15 ± 0.00	0.0384	C22:2	0.01 ± 0.00	0.02 ± 0.00	0.066
Unknown 12	0.01 ± 0.00	0.02 ± 0.00	0.0622	Unknown 37	0.01 ± 0.00	0.01 ± 0.00	0.4659
Unknown 13	0.04 ± 0.00	0.04 ± 0.00	0.9629	C22:5	0.17 ± 0.01	0.17 ± 0.01	0.9921
C18:0	1.30 ± 0.01	1.28 ± 0.01	0.0023	C22:6 n3	0.08 ± 0.00	0.07 ± 0.01	0.3091
Unknown 14 C18:1t	0.05 ± 0.00	0.06 ± 0.00	0.0159				
C18:1 t9	0.08 ± 0.00	0.08 ± 0.00	0.0056				

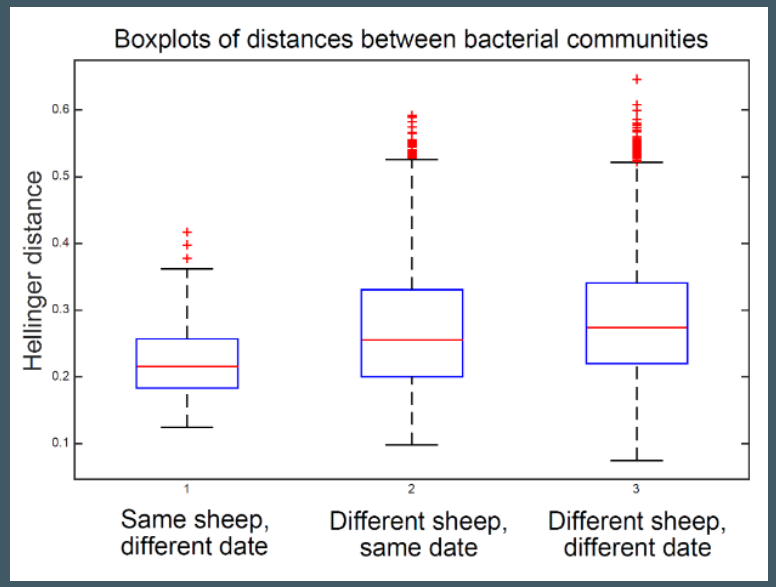
Microbial fermentation in the rumen

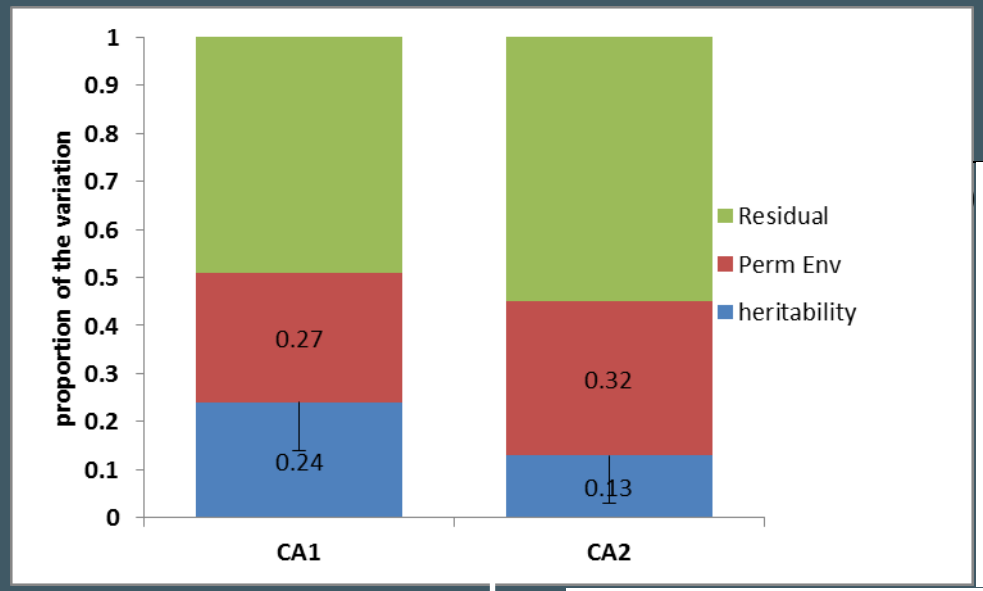


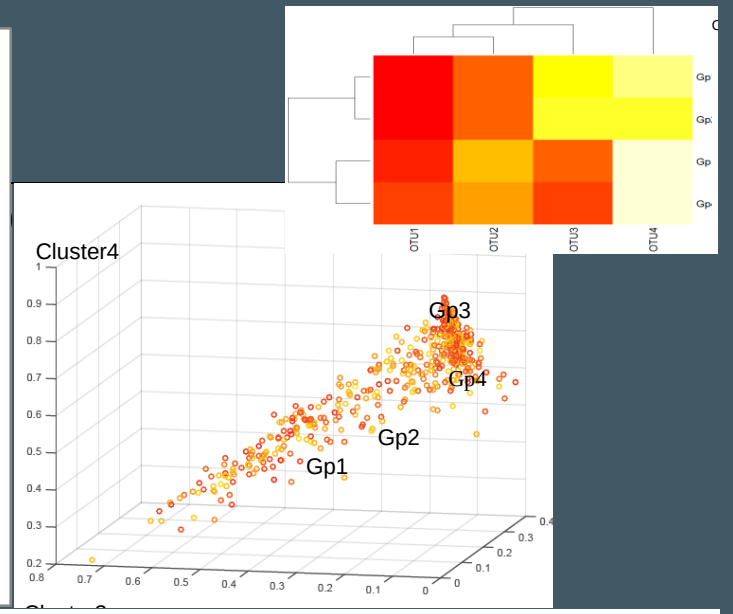
Methane per kg feed

g CH4 / kg DMI

Genetic Parameters: RMC

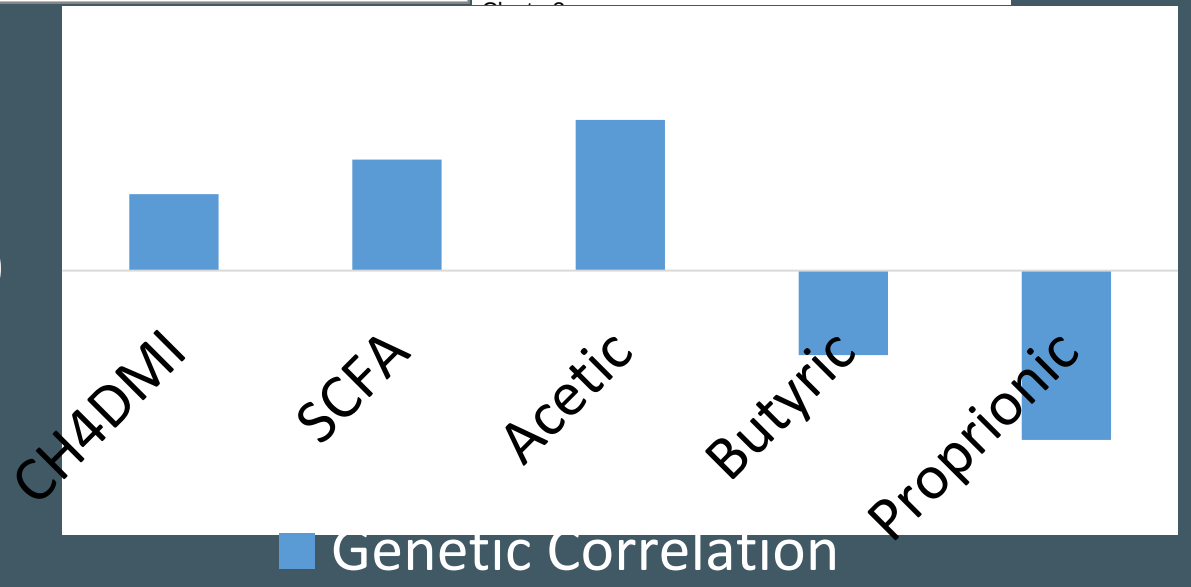






- N = 260 x 2 measures
- Most variation is in 2 dimensions (CA1, CA2)
- This variation is repeatable and heritable

Rowe et al 2015



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Microbial Sequencing

- Global Production Livestock Emissions research (GPLER)
- ~3,000NZ plus 500 merino (DPI New South Wales)
- Known methane status, known feed intake
- Variation in individual, breed, diet
- Detect low methane signatures across systems/environments

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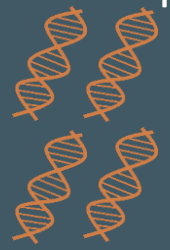
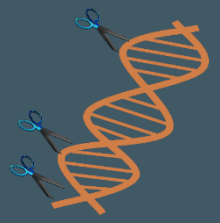
RE-RRS: Restriction Enzyme Reduced Representation Sequencing

DNA extracted from sample



Global Rumen Census freeze-dried DNA extraction method

DNA digested by restriction enzyme(s)



ApeKI or PstI

Ligation with 'barcode' oligos

To link sequences with samples

Fragment size-selection

193-318bp

Fragments sequenced

Illumina HiSeq2500

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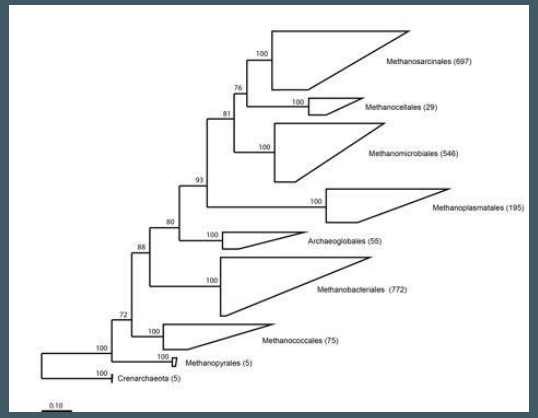
GBS Microbial Profiling

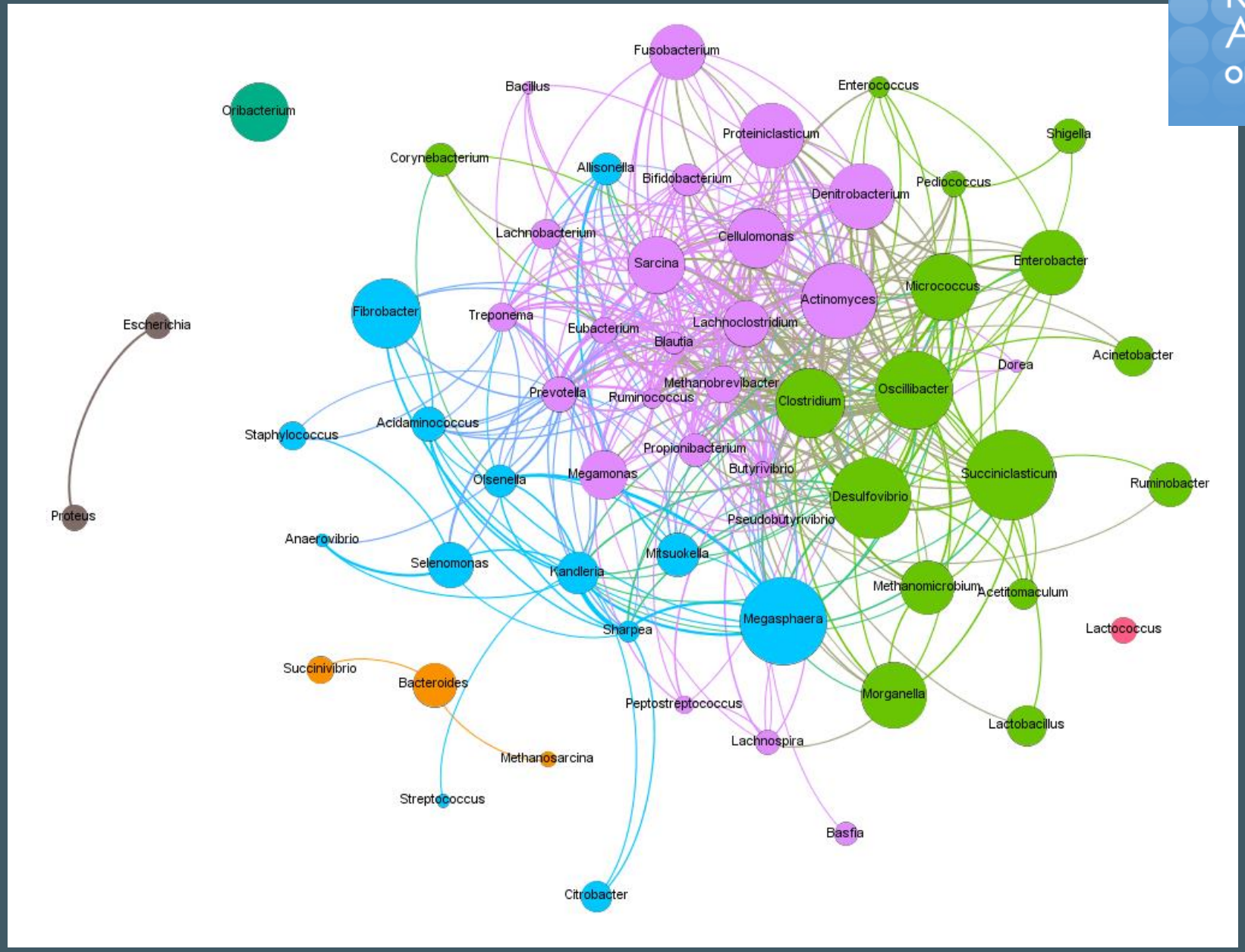
Sample

GBS

Reference-Based



Network Analysis



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Reference-Based Approach

Query sequences against a reference database (e.g. Hungate1000)
Assign sequences to taxonomies
End up with a table of counts for each taxonomic group

Method	RE	Accuracy by taxonomic level (%)						
		Kingdom	Phylum	Class	Order	Family	Genus	Species
BLASTN	ApeKI	100.0	99.8	99.7	99.5	98.2	96.1	83.6
	PstI	100.0	99.9	99.8	99.6	98.5	97.8	86.3
BLASTX	ApeKI	100.0	99.5	98.9	98.9	96.9	96.0	83.8
	PstI	100.0	99.0	98.7	98.7	96.8	96.8	86.3

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Reference-Based Results

RE	Sample	Genus Hit Rate (%)	
ApeKI	High	4.7	$p=1.4e^{-8}$
	Low	5.9	
PstI	High	6.3	$p=4.0e^{-5}$
	Low	7.3	

Samples from low-methane animals had higher hit rates

Genus	ApeKI			PstI		
	High (SD)	Low (SD)	P-value	High (SD)	Low (SD)	P-value
Fibrobacter	2.8 (1.4)	3.8 (2.2)	$3.21e^{-5}$	1.1 (0.5)	1.5 (0.8)	$2.00e^{-5}$
Kandleria	0.1 (0.1)	0.2 (0.4)	0.0014	0.4 (0.5)	0.6 (0.8)	0.0261
Olsenella	2.4 (1.2)	3.5 (2.9)	0.0009	0.6 (0.9)	0.7 (1.2)	0.1908
Sharpea	0.1 (0.4)	0.2 (0.5)	0.2017	0.1 (0.2)	0.1 (0.3)	0.4814

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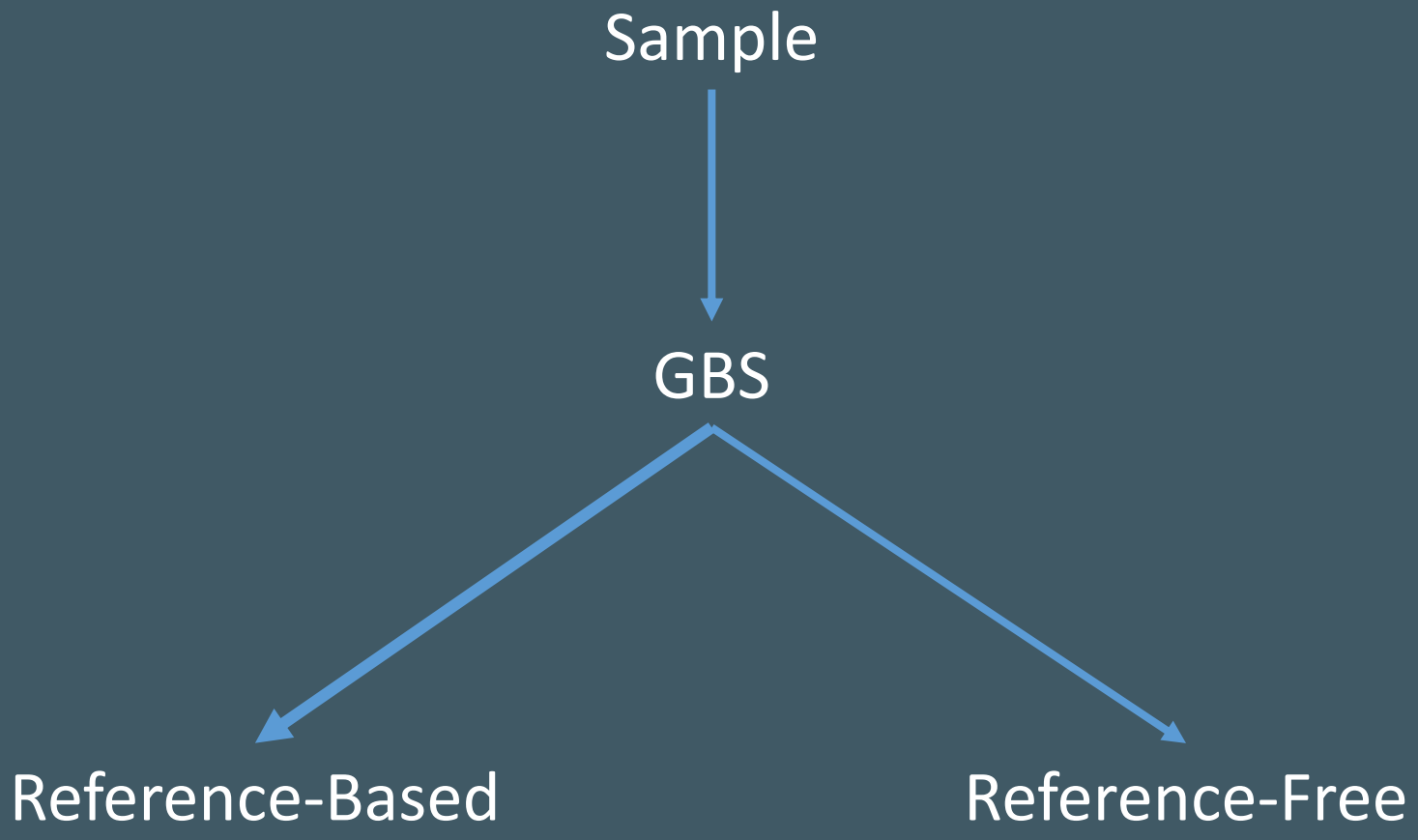
Microbiomes to predict methane



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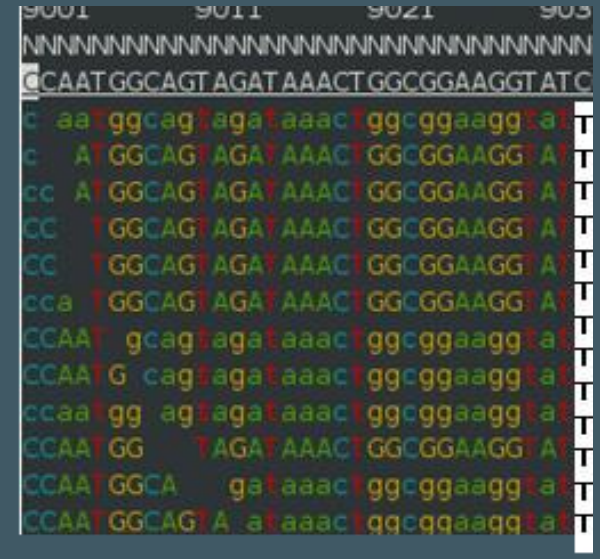
Reference-Free Approach

- Not all sequences will be unique
- Find the 65bp reads (tags) that are common
- Assign to bins/groups



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RMC profiling using GBS
with and without a reference dataset

Information	Parameter	Sheep1	Sheep2	Cattle
Sequencing	Number of Samples	236	654	186
	Samples per Lane	118	164	188
	Number of Reads/Sample	2.7M ± 680k	1.5M ± 586k	759k ± 147k
Reference-Based	Percent Assigned	6.8 ± 1.8	6.3 ± 1.2	9.3 ± 1.6
Reference-Free	Number of Tags*	503k	375k	423k
	Percent Assigned	38.1 ± 16.2	39.1 ± 3.2	64.3 ± 6.8

* Tags are 65bp reads present in at least 25% of samples

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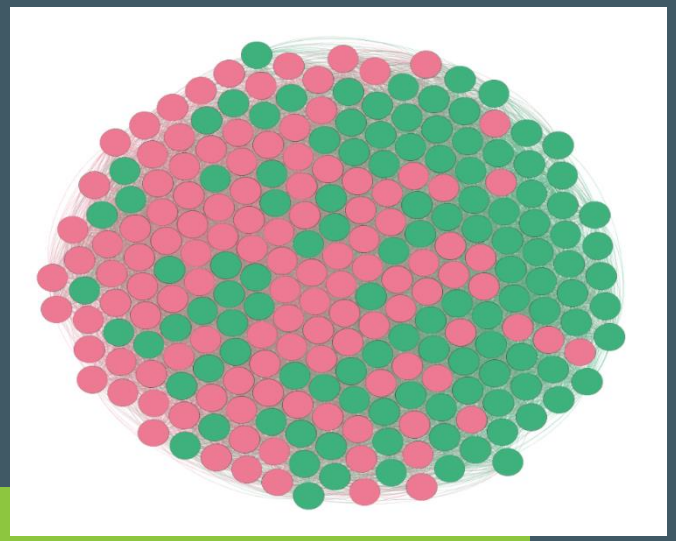
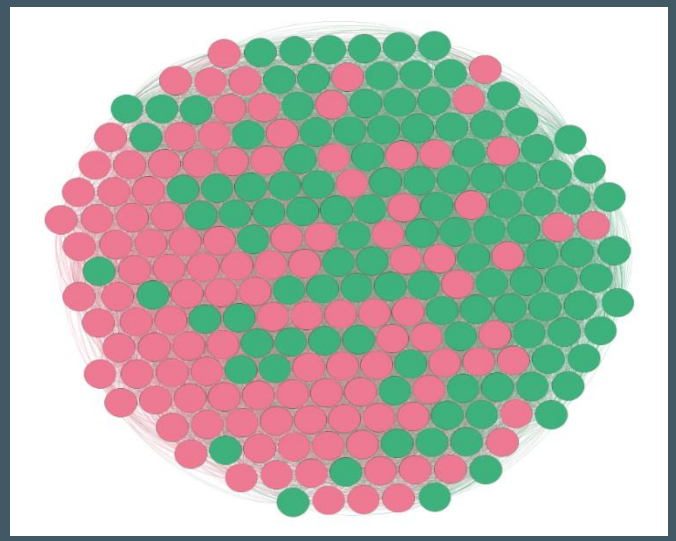
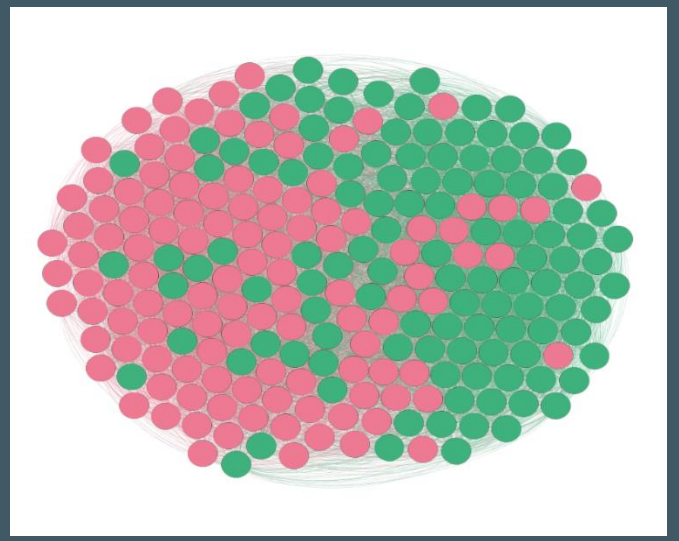
Sheep Results

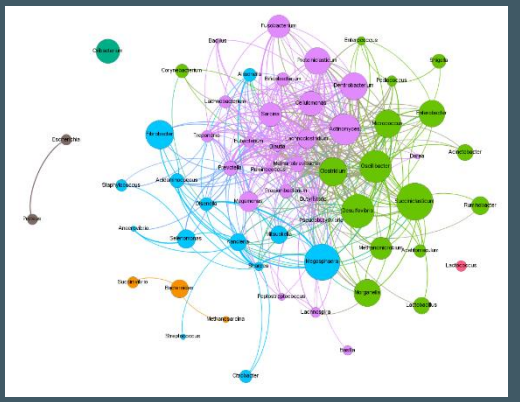
Approach	h^2	Repeatability	Correlation CH_4Yield
16S	0.26 (0.23)	0.45 (0.08)	0.65 (0.47)
Reference	0.62 (0.06)	0.62 (0.06)	0.59 (0.32)
No Reference	0.23 (0.26)	0.62 (0.06)	0.83 (0.31)



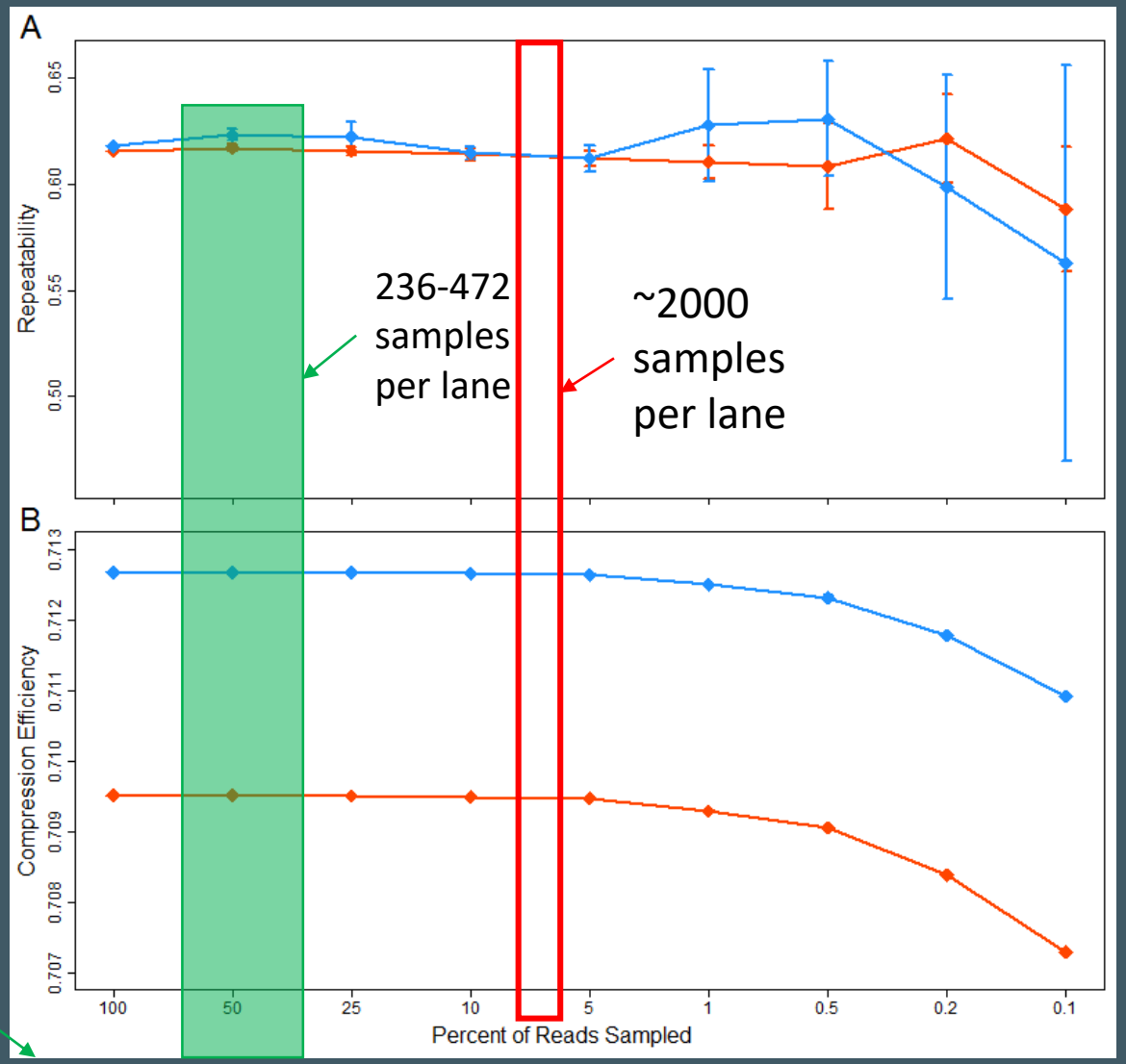




Sensitivity Analysis



ApeKI PstI



118
samples
per lane

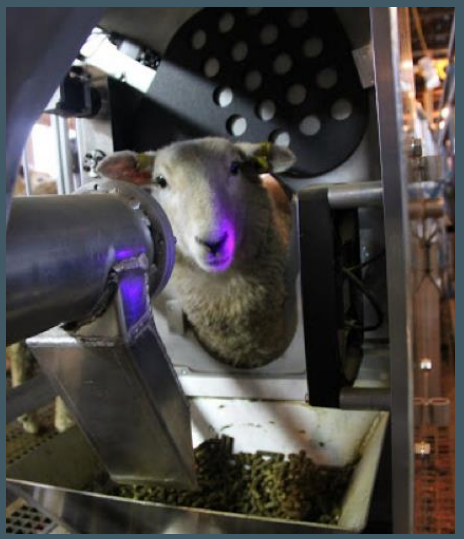
Dataset II

978 lambs across 3 flocks
Feed Intake and Methane

Flock	2014	2015	2016
A	88	130	160
B	96	96	53
C	55	149	144

Training

Validation



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Methane

Model	Var exp	Accuracy	Var exp Tr
Pedigree	0.29 ± 0.09	0.08	0.45 ± 0.15
Microbes	0.74 ± 0.14	0.29	0.69 ± 0.18
Both	0.89 ± 0.14	0.27	0.96 ± 0.19

Total Gas

Model	Var exp	Accuracy	Var exp Tr
Pedigree	0.20 ± 0.08	0.11	0.27 ± 0.13
Microbes	0.20 ± 0.14	0.03	0.25 ± 0.20
Both	0.39 ± 0.15	0.11	0.49 ± 0.21

Methane Yield

Model	Var exp	Accuracy	Var exp Tr
Pedigree	0.35 ± 0.09	0.11	0.42 ± 0.13
Microbes	0.66 ± 0.14	0.24	0.78 ± 0.18
Both	0.84 ± 0.14	0.24	0.96 ± 0.19

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beef+lamb new zealand GENETICS

New Zealand Dataset

Individuals

Flock	YOB	Lamb		Adult		Total
		Grass Diet	Pellet Diet	Grass Diet	Pellet Diet	
A	2014	88	87	49	80	88
	2015	133	146	76		146
	2016	160	159	91		161
B	2014	96	96	96		96
	2015	96	96	53		96
	2016	57	94	61		104
C	2014	55	109	62	98	110
	2015	149	149	84		150
	2016	144	144	82		148
Total		978	1080	654	178	1099

Samples

Flock	YOB	Lamb		Adult		Total
		Grass Diet	Pellet Diet	Grass Diet	Pellet Diet	
A	2014	88	88	49	80	305
	2015	133	146	76		355
	2016	160	159	91		410
B	2014	287	192	175		653
	2015	96	96	53		245
	2016	57	94	61		212
C	2014	55	109	62	98	324
	2015	149	151	84		384
	2016	145	144	82		371
Total		1170	1179	733	178	3260

Australian Dataset, n=508

Table 3: Numbers of ewes and tests, dates, birth years, and means for liveweights, feed offered (FDO) and eaten the day before testing (FIDP) for each batch

PAC0 = Portable Accumulation Chamber (PAC) test immediately off feed; PAC1 = PAC test commencing 1 hour after removing feed; RC = respiration chamber. FDO = feed offered, FIDP = feed eaten (kg) over the 24 hours to 0800 hr on the test day

	Numbers of ewes and tests				Dates	Birth	Live-weight (kg)	FDO (kg)	FIDP (kg)
Batch	Ewes	PAC0[A]	PAC1[A]	RC[A]	1st test - last test	years	mean ±s.d.	mean ±s.d.	mean ±s.d.
1	79	96	96	160	16 Apr. - 13 May 15	2012	50.1 ±4.7	1.37 ±0.10	1.36 ±0.10
2	82	96	96	176	21 May - 19 June 15	2012	45.4 ±4.0	1.27 ±0.09	1.26 ±0.10
3	29	36	36	64	27 Aug. – 17 Sep. 15	2007-12	48.9 ±5.8	1.43 ±0.12	1.42 ±0.13
4	80	84	96	168	8 Oct. – 5 Nov. 15	2013	45.8 ±5.5	1.36 ±0.12	1.35 ±0.13
5	80	84	96	168	19 Nov - 17 Dec. 15	2013	52.6 ±7.8	1.51 ±0.27	1.48 ±0.20
6	80	84	96	168	14 Jan. - 1 Feb. 16	2013	56.3 ±7.3	1.59 ±0.15	1.52 ±0.19
7	80	84	96	160	25 Feb. - 23 Mar.16	2007-8	52.0 ±5.5	1.54 ±0.12	1.43 ±0.18

[A] Includes repeat tests on some animals.

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Roll Out.....

	Production only index		Prod plus Methane index	
	Prod	Prod + CH4	Prod	Prod + CH4
Average Economic ($)	36.65	35.96	35.63	37.63
Change ($)			-1.03	+0.98
Physical (%)		+1.69		-3.69

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Next steps for sheep

GLOBAL RESEARCH ALLIANCE ON AGRICULTURAL GREENHOUSE GASES

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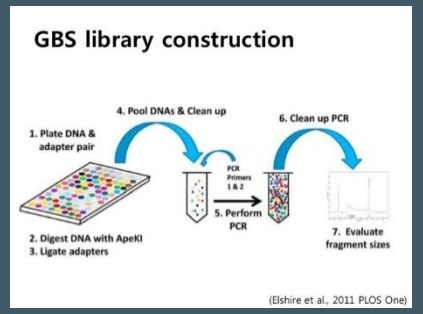
NEW ZEALAND AGRICULTURAL GREENHOUSE GAS Research Centre

- Modelling and visualisation of all microbial data
  - Develop agnostic predictors & test accuracy
  - Functional grouping information – bioinformatics
  - Compare breed, diet
- Milk fatty acid differences between the selection lines
- Genomics of physiological changes
- Additivity of strategies



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Comment on this paper

A restriction enzyme reduced representation sequencing approach for low-cost, high-throughput metagenome profiling

Melanie K. Hess, Suzanne J. Rowe, Tracey C. Van Stijn, Hannah M. Henry, Sharon M. Hickey, Rudiger Brauning, Alan F. McCulloch, Andrew S. Hess, Michelle R. Kirk, Sandra Kittelmann, Graham R. Wood, Peter H. Janssen, John C. McEwan

doi: https://doi.org/10.1101/694133

This article is a preprint and has not been peer-reviewed [what does this mean?].

Abstract | Full Text | Info/History | Metrics | Preview PDF

Abstract

Microbial community profiles have been associated with a variety of traits, including methane emissions in livestock, however, these profiles can be difficult and expensive

Acknowledgements

Financial support was provided by:

- Microbes to predict methane project funded by Global Partnerships in Livestock Emissions Research through Ministry for Primary Industries
  - SOW-AGR-GPLER-SP5
- Pastoral Greenhouse Gas Research Consortium
- New Zealand Agricultural Greenhouse Gas Research Centre
- AgResearch Curiosity fund
- MBIE funded Genomics for Production and Security Programme
- Beef and Lamb New Zealand Genetics

AgResearch (Animal) Genomics Team

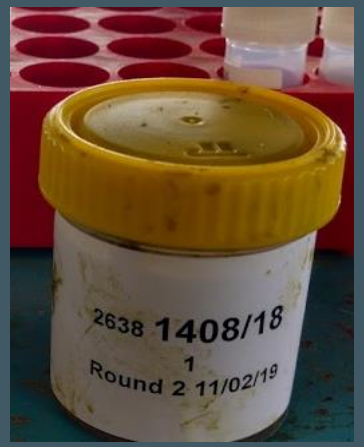
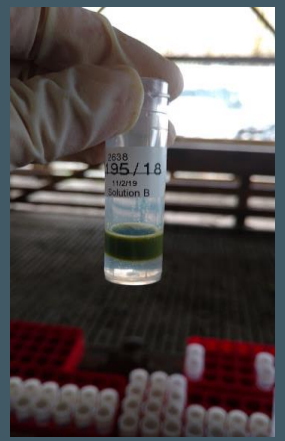
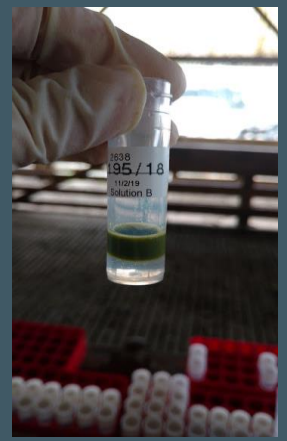
Final Stage - Analysis

Over 3500 NZ sheep rumen samples
508 Australian sheep rumen samples

- Across Diet: Pasture or Lucerne Pellet
- Across Age: Lamb or Adult
- Multiple Breeds
- Phenotypes:
  - Methane emissions
  - Feed intake
  - Production traits

Sample Collection Strategies
Low-cost, high-throughput

Rumen samples

1	2	3	4
(Kittelmann et al, 2014)	1ml rumen sample + 1ml of 2X concentration solution A	1ml rumen sample + 1ml of 2X concentration solution B	1ml rumen sample + 2mls of 100% alcohol solution C
			

Juliana Budel and John McEwan

Expand to Cattle…..GRA Flagship

GLOBAL RESEARCH ALLIANCE ON AGRICULTURAL GREENHOUSE GASES

- LEARN Post-Doc – Quantitative Genetics
- Starting with sequencing NZ dairy cattle
- Expand to additional systems and partners
- Make new sequencing methods available to all partners
- Protocols and agreement to share samples and phenotypes
- 1,000 samples fully funded
- Developing microbial and genomic predictors for differences in methane