RuminOmics Regional Workshop

Improving efficiency and reducing environmental impact

Tools for rapid analysis of animal phenotypes and the rumen microbiome

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2013 THE AWARDS AWARD WINNER

BBSRC

RuminOmics: Connecting the animal genome, the intestinal microbiome and nutrition to enhance the efficiency of ruminant digestion and to mitigate the environmental impacts of ruminant livestock production

Project legacy: Identification of proxies and tools for large scale phenotyping for genomic selection, optimised nutrition and better management on-farm

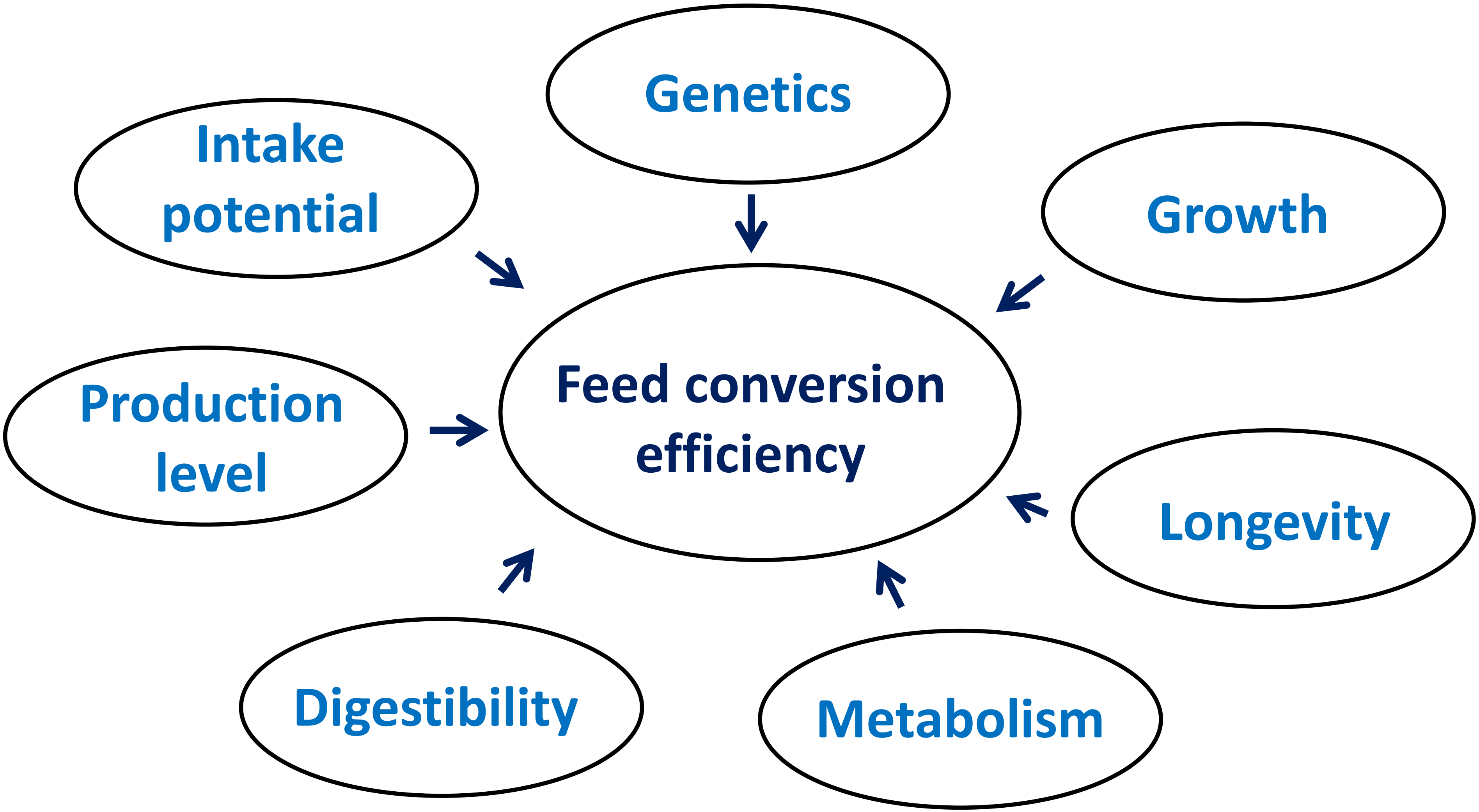
Challenges to ruminant livestock production

- Economic
- Environmental
- Societal

Scientific and technical solutions required

- Increase efficiency
- Lower emissions
- Improve product quality

Technical challenge: Phenotyping of complex traits



Advances in technologies

Technologies

- Sequencing/high density chips
- New "precision" devices for monitoring on farm
- FTIR and MS/MS: high throughput analysis

Tools

- Characterise microbial populations
- More extensive phenotypes
- Genomic selection

New phenotypes in dairy cattle

- Large scale phenotyping: number of animals, number of traits and scales from molecule to whole animal

- Genetic selection requires phenotyping of thousands of animals that remains a major constraint

- Development of high-throughput methodologies are required for application on large populations according to standardized definitions and methods

Boichard and Brochard, 2012

New traits and phenotypes in cattle

Rationale

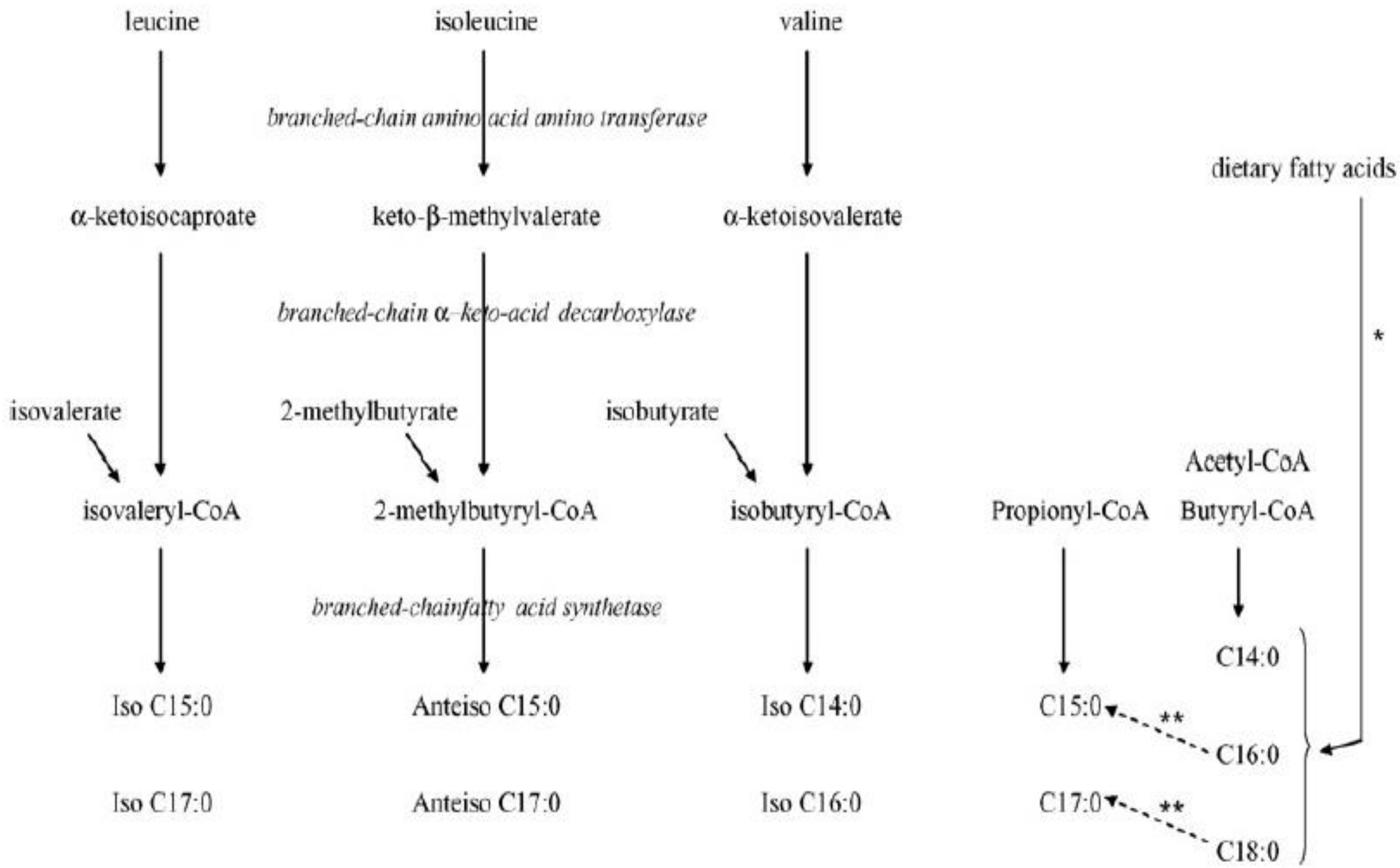
- Balance between effort of data recording and benefit
- Cost effective alternatives to difficult or expensive to measure traits

Diverse range of potential biomarkers

- **Breath**
  - Gas concentrations
- **Rumen**
  - qPCR 16S gene/Archaeol/pH
- **Milk**
  - Fat + Protein/SCC/Fatty acid composition/
    Electrical conductivity/Lactoferrin/Minerals
- **Plasma**
  - NEFA/BHB/Urea/Insulin/Acute phase proteins
- **Faeces**
  - Archaeol

New phenotypes of rumen function

Odd and branched chain fatty acid synthesis in ruminal bacteria



Vlaeminck et al., 2006

Proportions of odd and branched chain fatty acids in bacterial membranes differ between species

	Fermentation products[d]	*Anteiso* C13:0	*Anteiso* C15:0	*Anteiso* C17:0	*Iso* C13:0	*Iso* C15:0	*Iso* C17:0	*Iso* C14:0	*Iso* C16:0	C13:0	C15:0	C17:0	C17:1
R. albus[a]	A	-	9.4	1.3	-	-	0.7	20.6	11.0	-	10.3	1.4	-
B. fibrisolvens[a]	A, B, F	6.4	16.2	8.6	6.8	10.4	5.7	10.8	11.1	2.9	7.8	4.3	3.5
R. flavefaciens[a]	A, S	-	2.3	2.9	-	35.7	5.2	2.5	7.3	0.1	3.2	0.5	-
S. amylolytica[b]	A, P												
N6		-	-	-	-	52.6	10.8	1.6	5.3	1.6	5.0	-	-
B24		-	-	-	-	0.1	0.3	-	0.6	1.4	3.3	1.3	0.6
Prevotella[b,c]	A, S	1.2	36.7	4.2	3.0	14.7	2.3	3.3	3.0	1.2	12.1	2.1	-
L. multiparus[b,c]	A, L, F	-	4.0	2.6	-	1.1	1.1	1.2	1.8	0.3	2.9	0.8	0.1
S. dextrinosolvens[c]	A, S	0.8	3.6	1.0	-	0.1	-	0.6	1.5	0.5	4.0	0.7	-
R. amylophilus[b]	A, S, F	-	1.1	-	-	-	-	-	-	0.5	1.1	0.3	0.1
F. succinogenes[a]	A, S	3.9	7.7	1.2	-	0.1	0.2	3.6	3.4	9.0	30.2	2.1	-
S. bovis[b]	L	-	0.9	-	-	-	-	0.4	0.2	0.6	1.7	1.2	0.2
M. elsdenii[c]	A, P, B	-	2.8	-	0.1	0.2	0.2	1.5	0.5	1.5	6.0	4.5	3.0
E. ruminantium[b]	B, L, F												
B1C23		-	-	-	-	17.7	1.4	-	-	5.4	49.0	1.5	-
GA195		-	30.1	1.7	-	0.4	0.2	6.1	3.7	0.4	6.5	0.4	-
S. ruminantium[b]	A, P, L	-	0.1	-	-	0.2	-	0.3	0.1	1.3	6.0	2.9	2.6

[a] Bacteria fermenting cellulose and hemicellulose.

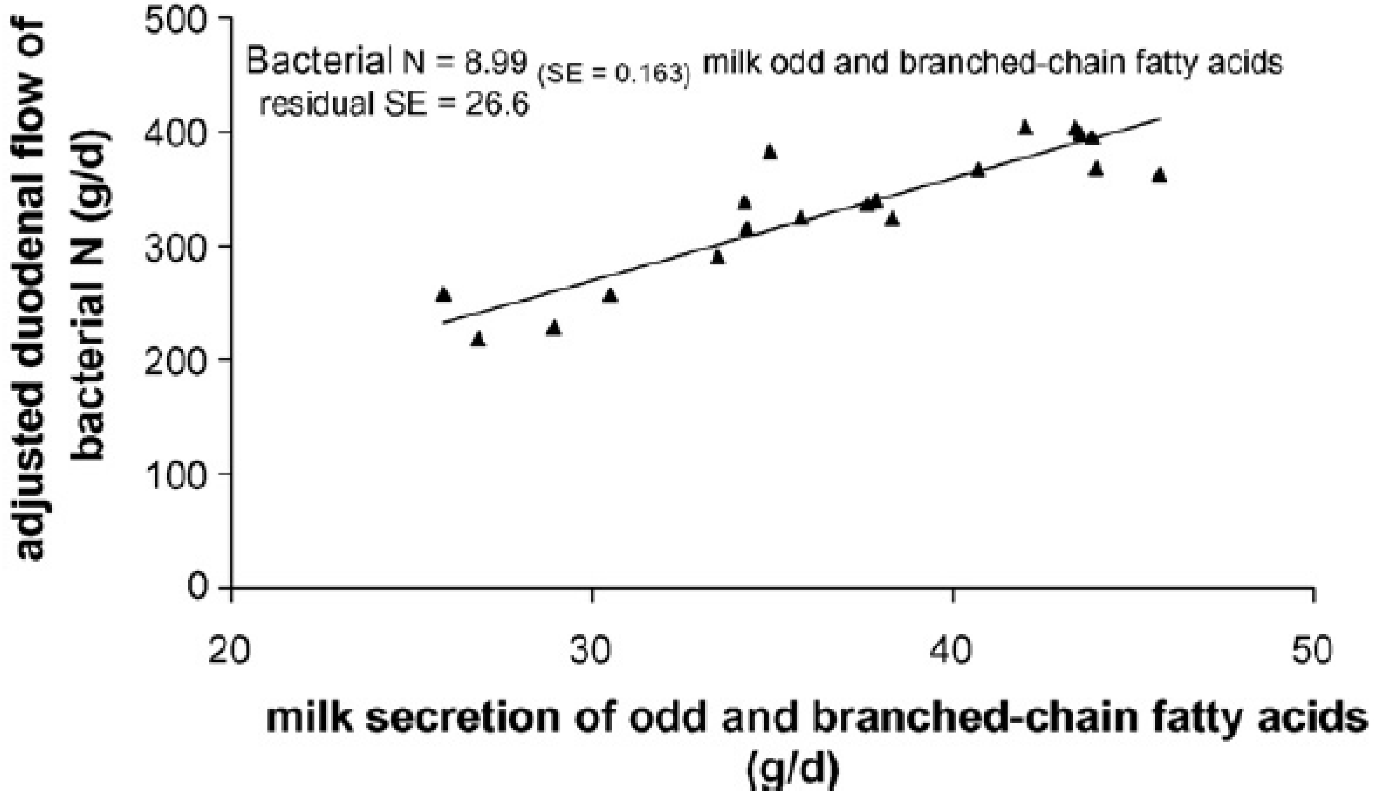
[b] Bacteria fermenting starch.

[c] Bacteria fermenting sugar and pectin.

[d] A: acetate; S: succinate; B: butyrate; F: formate; P: propionate; L: lactate.

Fievez et al., 2012

Secretion of OBCFA in milk as a biomarker of microbial N at the duodenum



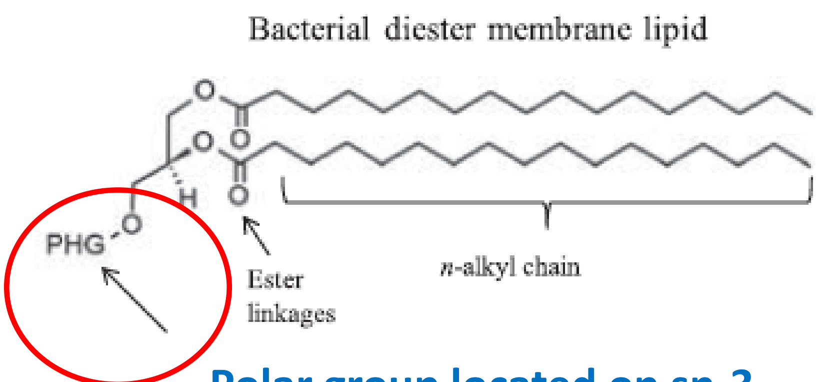
Vlaeminck et al., 2006

Milk fatty acid composition as a biomarker of methane production

Reference	Number of observations (number of studies)	Method for measuring/ estimating methane production	Treatments	Percentage of variance in methane production explained by best combination of milk fatty acids	Individual milk fatty acids related to methane production: Positive relationship	Negative relationship
Chilliard *et al.* (2009)	32 (1)	SF_6 tracer technique	Maize/grass silage and concentrates with different linseed products	95 (within study)	4:0 6:0 8:0 9:0 10:0 10:1 11:0 12:0 12:1 14:0 15:0 17:0 20:4	18:1 *trans*-16 + *cis*-14 18:2 *cis*-9, *trans*-13 16:1 *trans*-11 18:1 *trans*-12 18:1 *cis*-13 18:1 *trans*-13 + 14 18:1 *trans*-6,7,8 18:1 *cis*-15 + *trans*-17 18:2 *trans*-11, *cis*-15 18:1 *cis*-9 18:1 *cis*-10 18:1 *trans*-10
Mohammed *et al.* (2011)	16 (1)	Respiration chambers	Barley silage-based TMR with different crushed oilseeds	83 (within study)	8:0 Iso-16:0*	17:1 *cis*-9 18:1 *cis*-11 18:1 *cis*-13 18:1 *trans*-6,7,8 18:2 Iso-17:0/16:1 *trans*-6,7,8 18:2 *cis*-9, *trans*-13/*trans*-8, *cis*-12 18:3
Dijkstra *et al.* (2011)	50 (10)	Respiration chambers	TMR based on grass and maize silages with a range of supplements (fumarate, diallyldisulphide, yucca powder, fatty acids, linseed products)	73 (within study)	Iso-14:0 Iso-15:0 Anteiso-7:0	17:1 *cis*-9 18:1 *trans*-10 + 11 18:1 *cis*-11
Casto Montoya *et al.* (2011) (only considered odd- and branched-chain fatty acids)	224 (13)	Calculation based on volatile fatty acid proportions	Wide range of forages and forage/concentrate ratios	66 (cross-validation)	Iso-14:0 Iso-15:0 Iso-16:0	15:0 17:0 + 17:1 *cis*-9

McCartney et al., 2013

Structural differences in membrane lipid of rumen archaea and bacteria

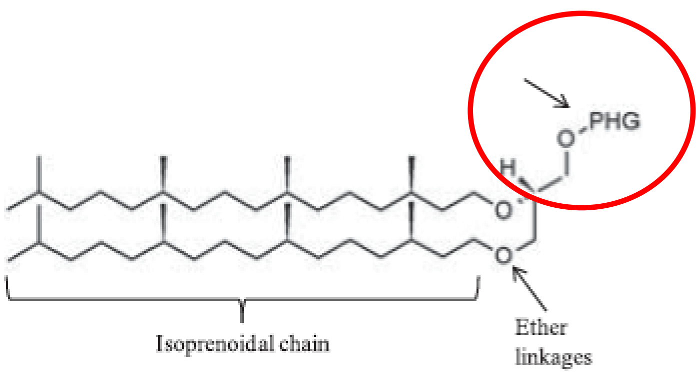


Bacteria

Polar group located on sn-3

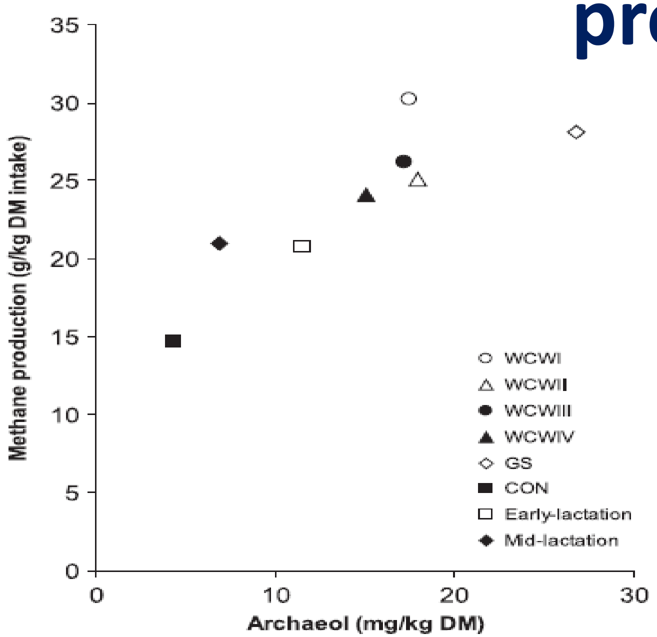
Polar group located on sn-1

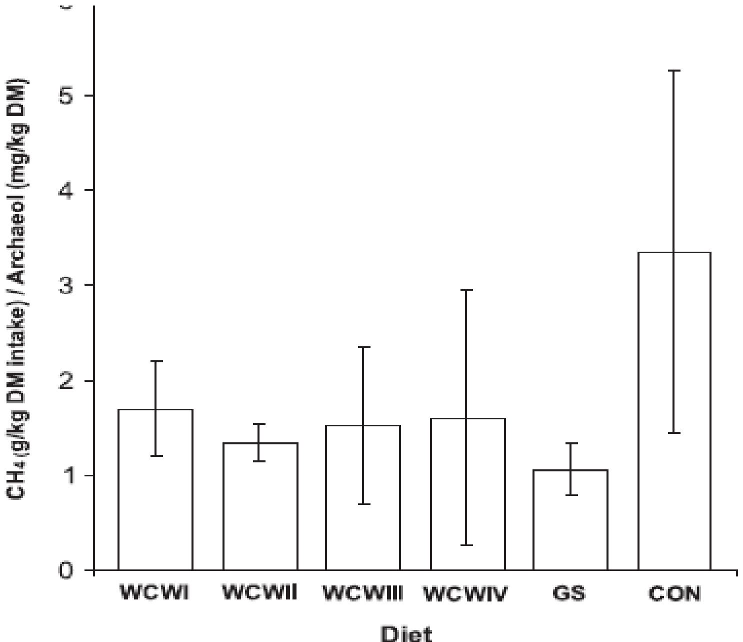
Archaea



Archaeol

Faecal archaeol as a biomarker of methane production





- Easy sample collection and processing
- Close relationship for treatment means
- Considerable variation between animals
- Poor predictor of rumen methanogenesis-selective retention in the rumen

McCartney et al., 2013

Phenotypes of rumen function

- Methane production
- Nutrient digestibility
- Rumen fermentation

Development of new tools avoiding traditional constraints in hard to measure phenotypes

- qPCR of 16S and 18S genes in ruminal digesta
- Metagenomics

Archaeal abundance in *post-mortem* ruminal digesta may help predict methane emissions from beef cattle

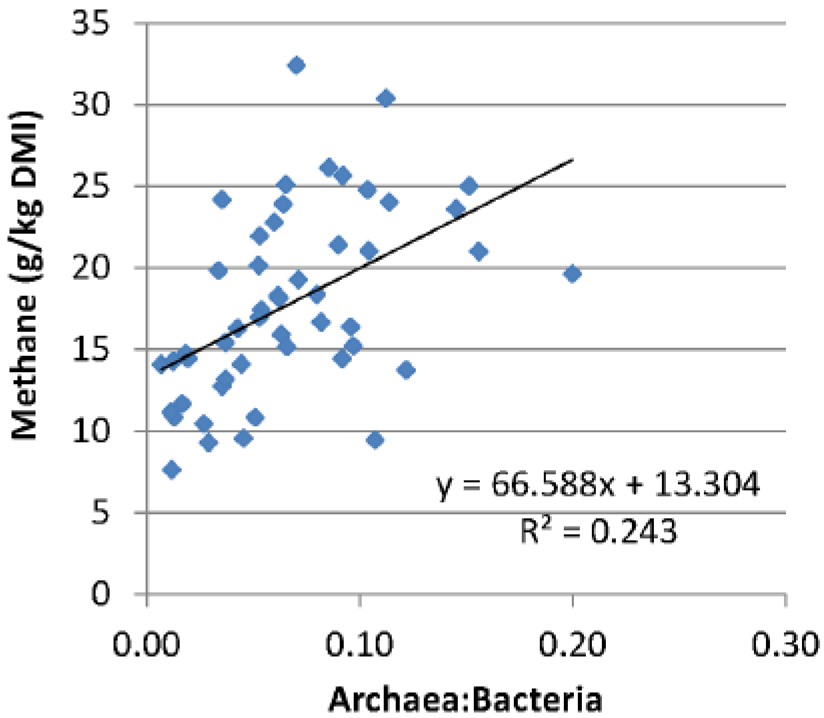


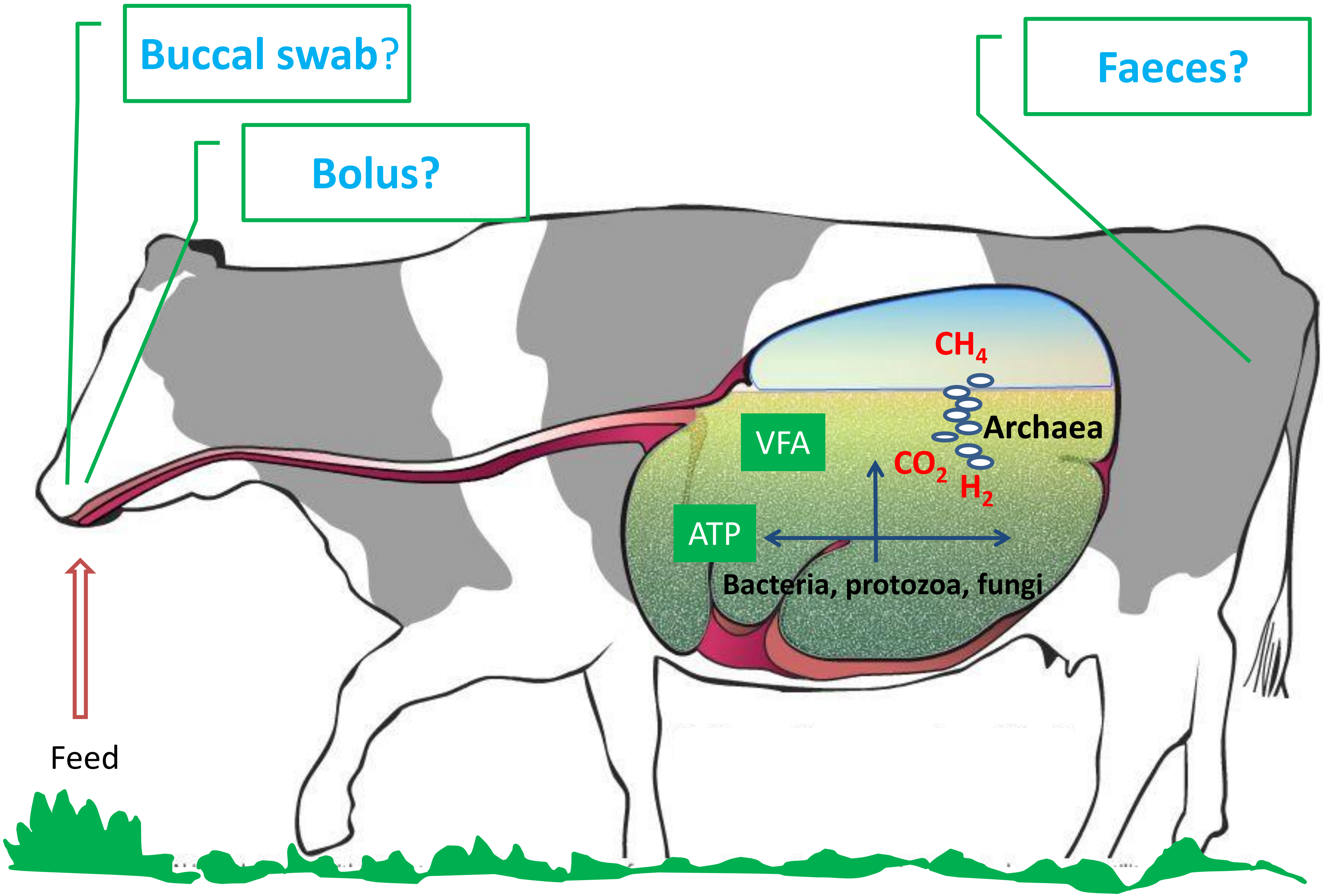
Figure 7 | Methane emissions and the archaea : bacteria ratio (A : B) in ruminal digesta samples taken from live animals immediately after exiting the respiration chamber.

Wallace et al., 2014

Development of Proxies and Phenotyping tools:

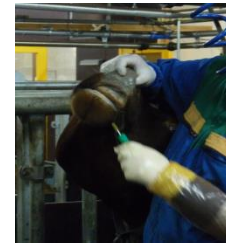
Lessons learnt in the RuminOmics project

Can we find an alternative to sampling rumen contents?



Tapio et al., Submitted

Sampling



Sample processing

DNA extraction

Yu and Morrison 2004

Amplicon library preparation

16S, 18S rRNA, ITS1

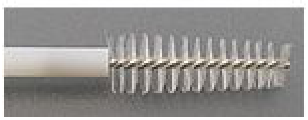
Sequencing

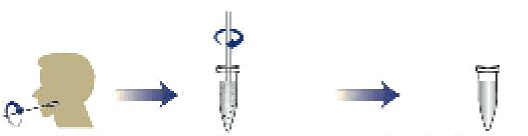
Illumina (MiSeq)

Sequencing data analysis

OBITools, Qiime, R

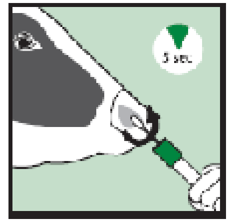
BuccalAmp™ DNA Extraction Kit from Epicentre



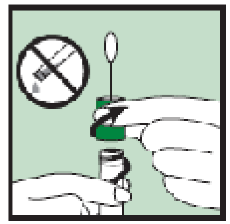


Dry swabs – up to 1 week at RT
up to 6 month at -20 C

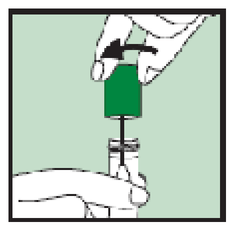
Performagene Livestock (PG-100) from DNA genotek



Holding the tube, rub the swab inside the animal's nostril for up to 5 seconds.



Hold the tube upright and unscrew the cap from the tube.



Turning the cap upside down, place the swab in the tube and close tightly.

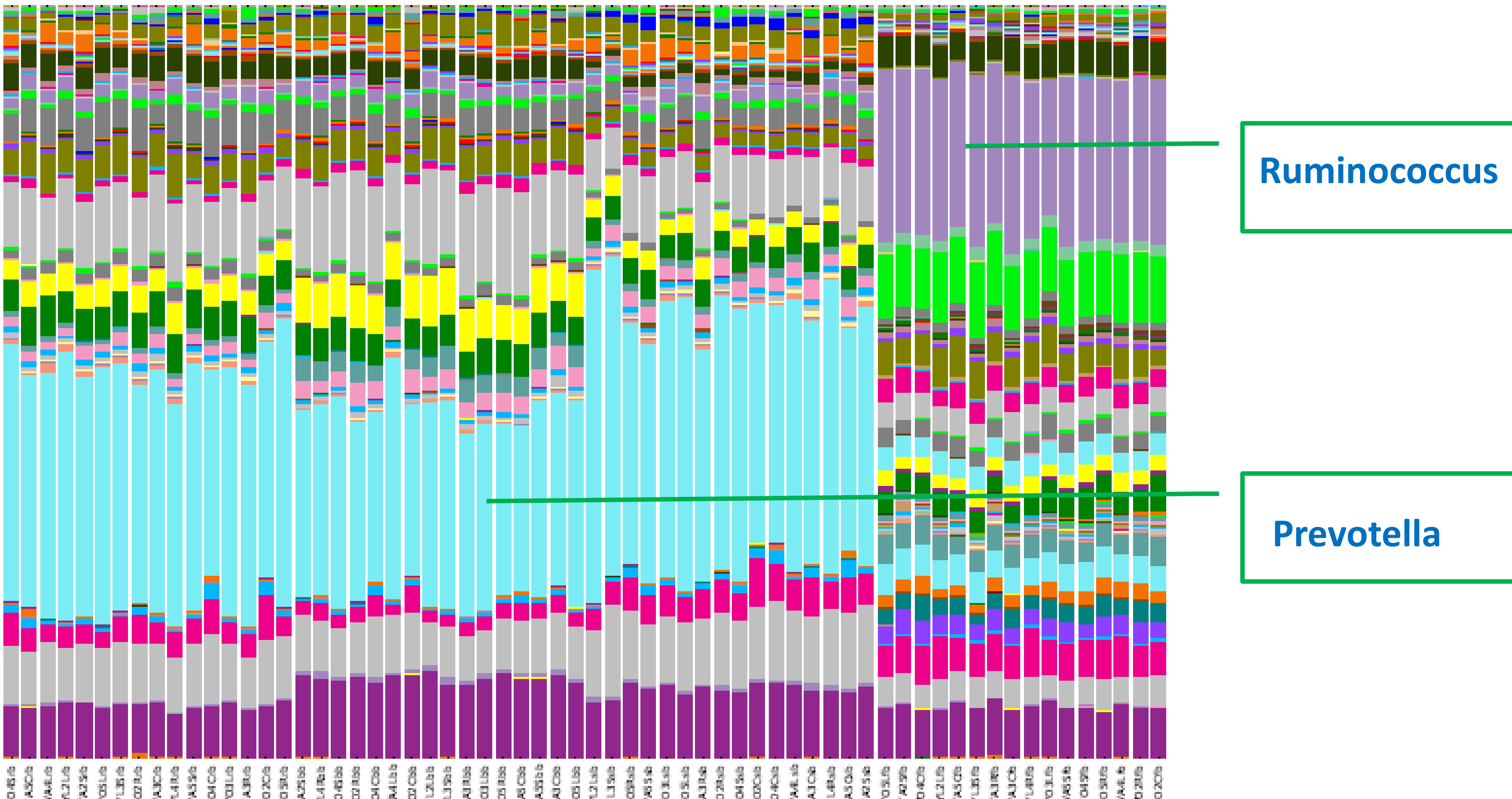
Swabs in buffer – up to 12 month at RT

Performagene Livestock In PBS – glycerol buffer

Yu and Morrison (2004)

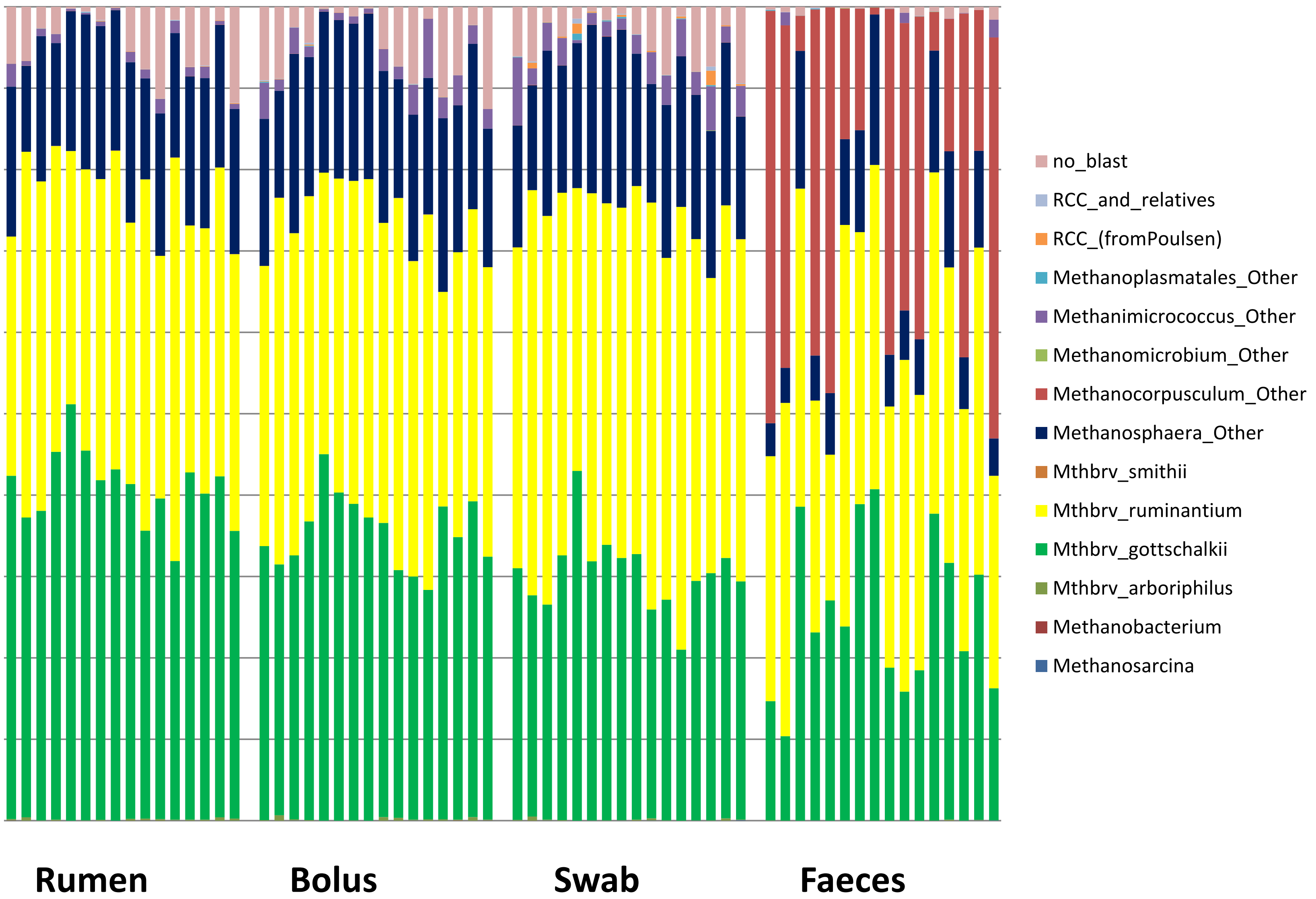
Swabs in buffer frozen on dry ice right after sampling

Bacteria relative abundance

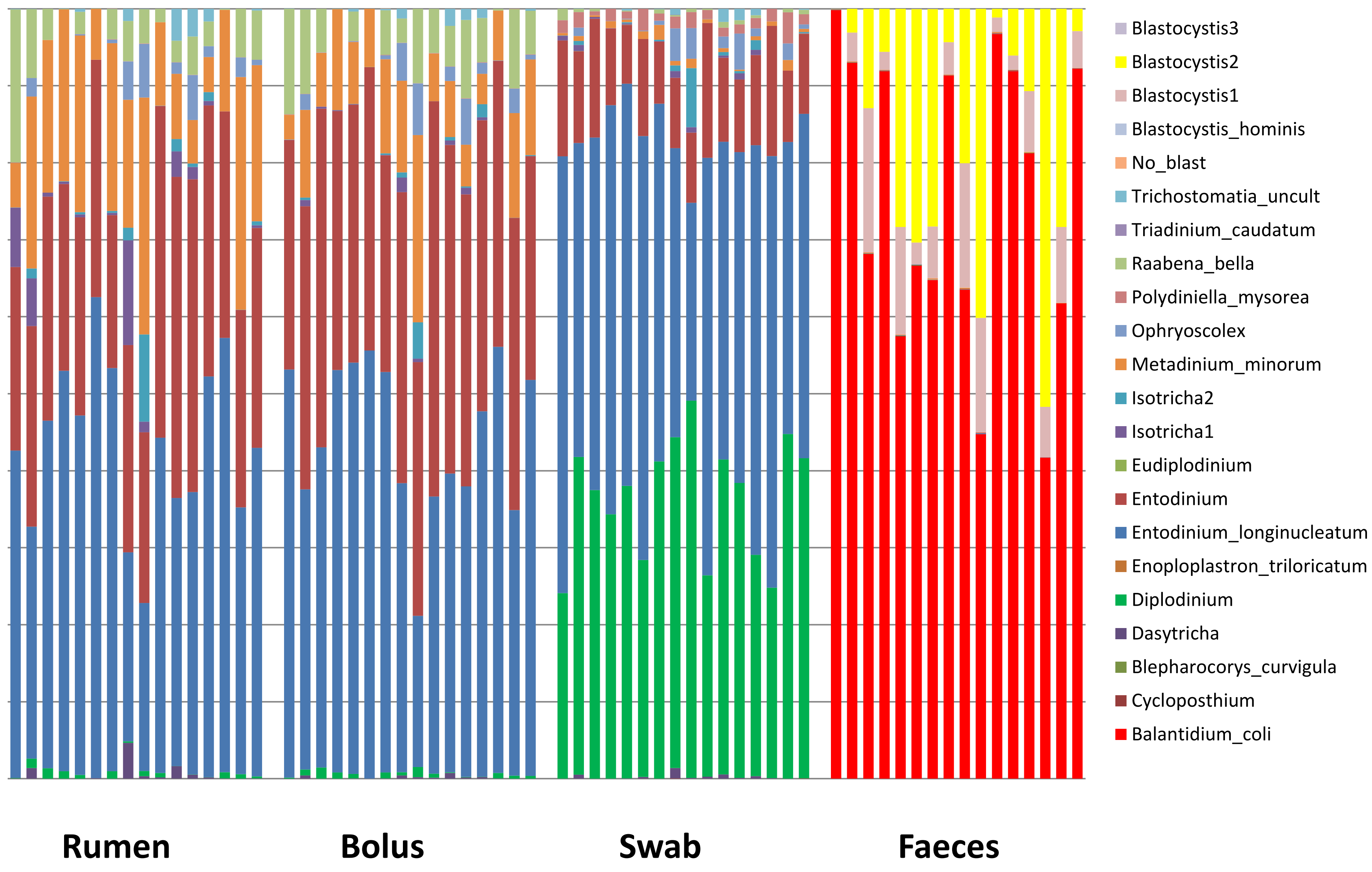


Rumen **Bolus** **Swab** **Faeces**

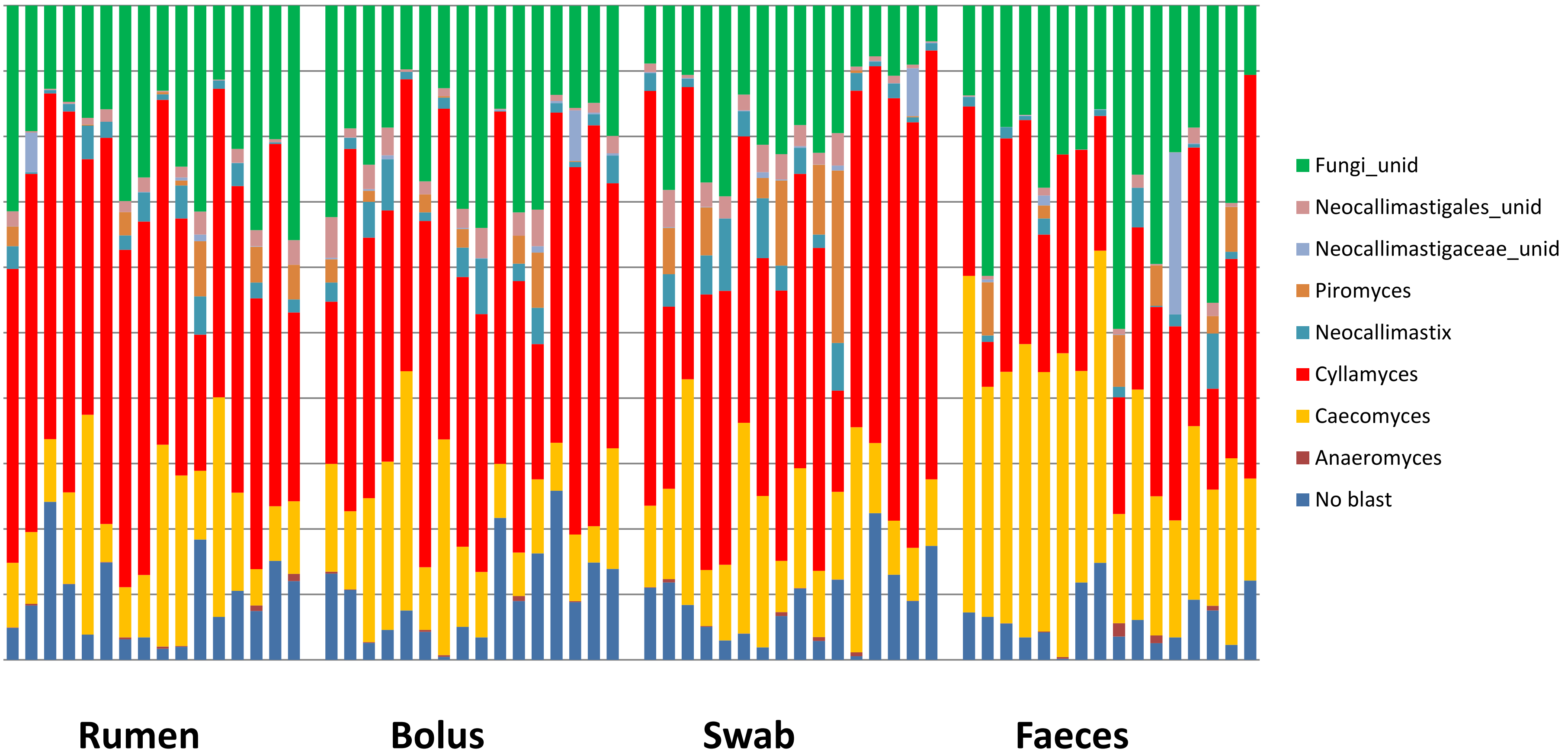
Archaea relative abundance

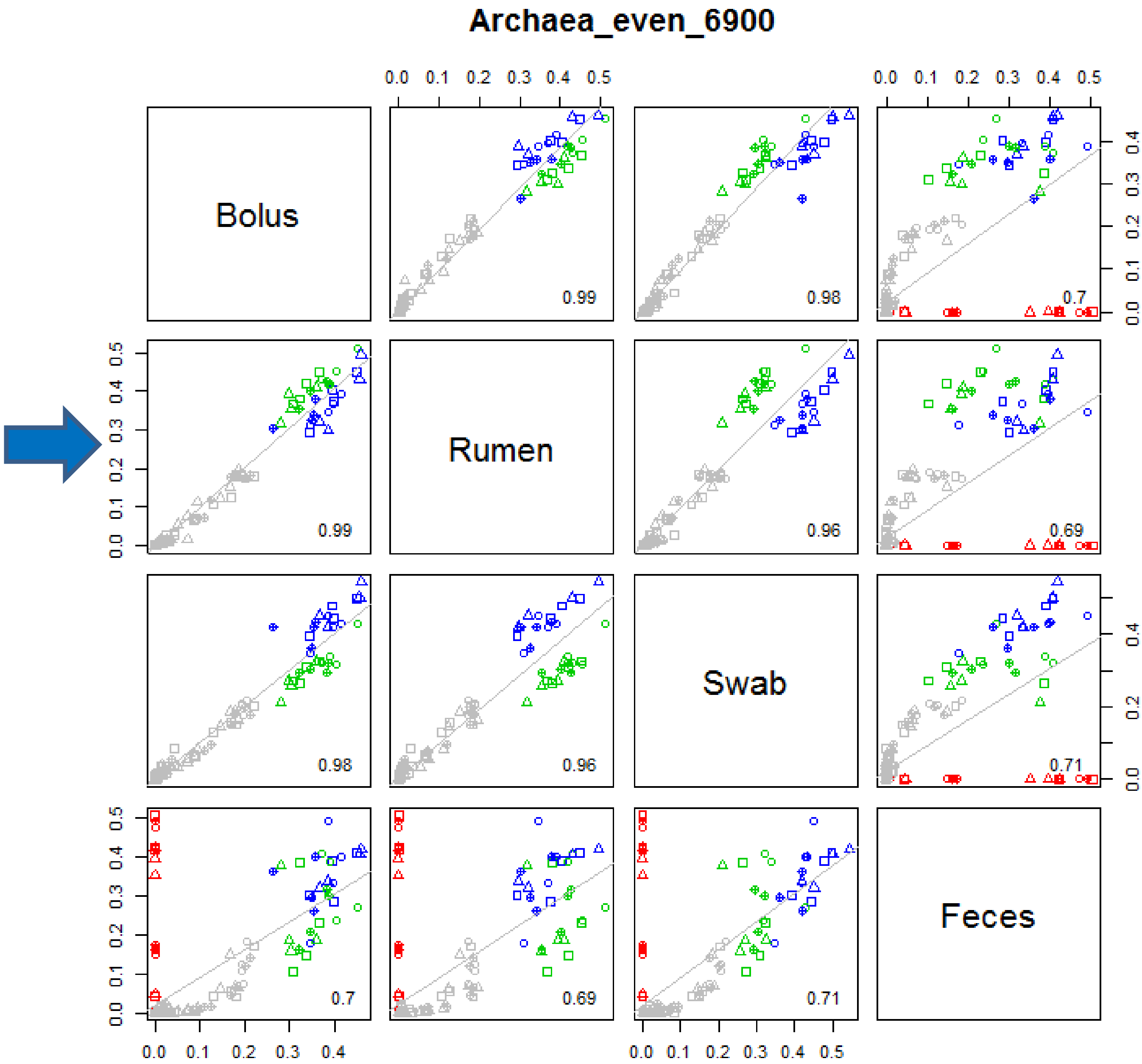


Ciliate protozoa relative abundance



Anaerobic fungi relative abundance

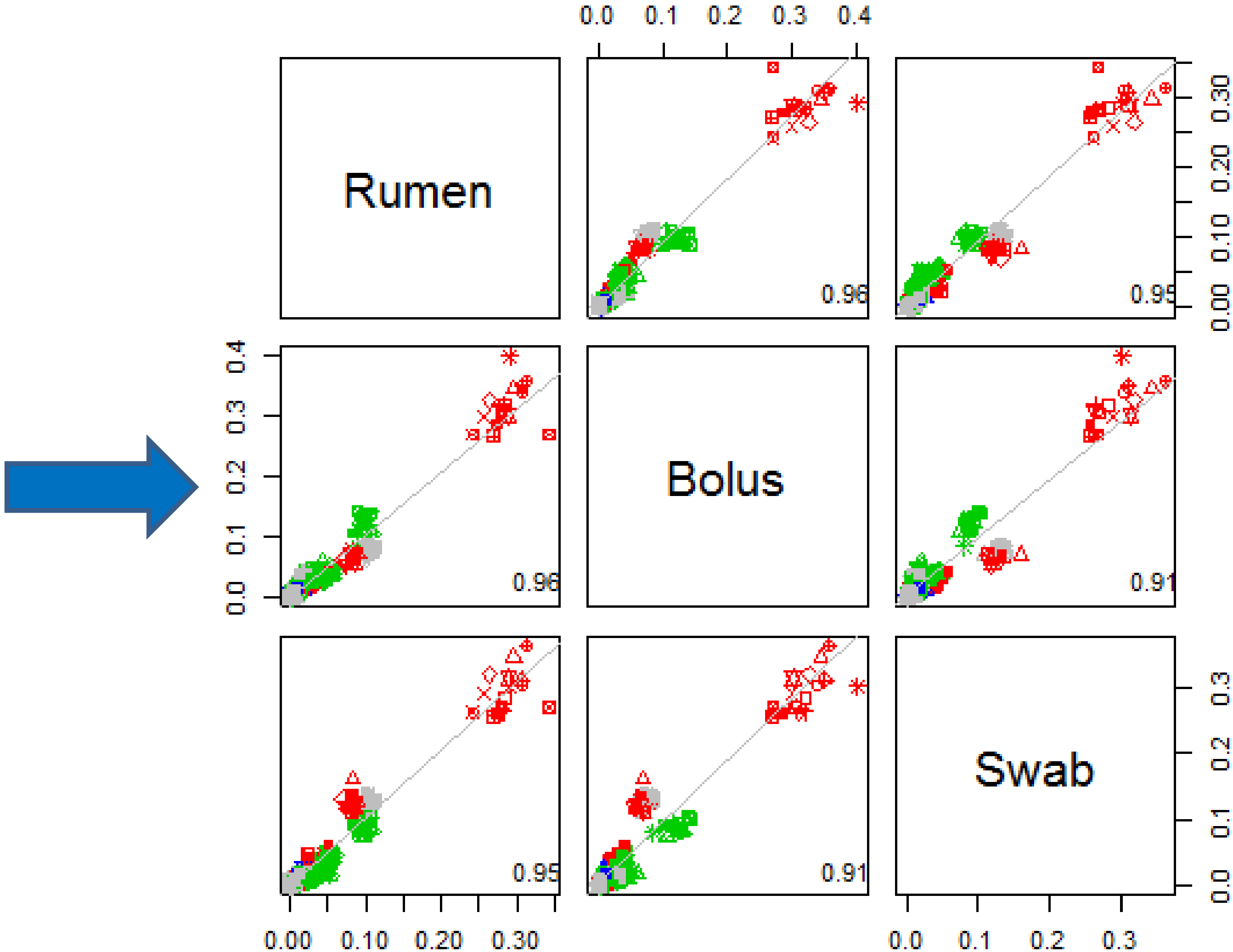




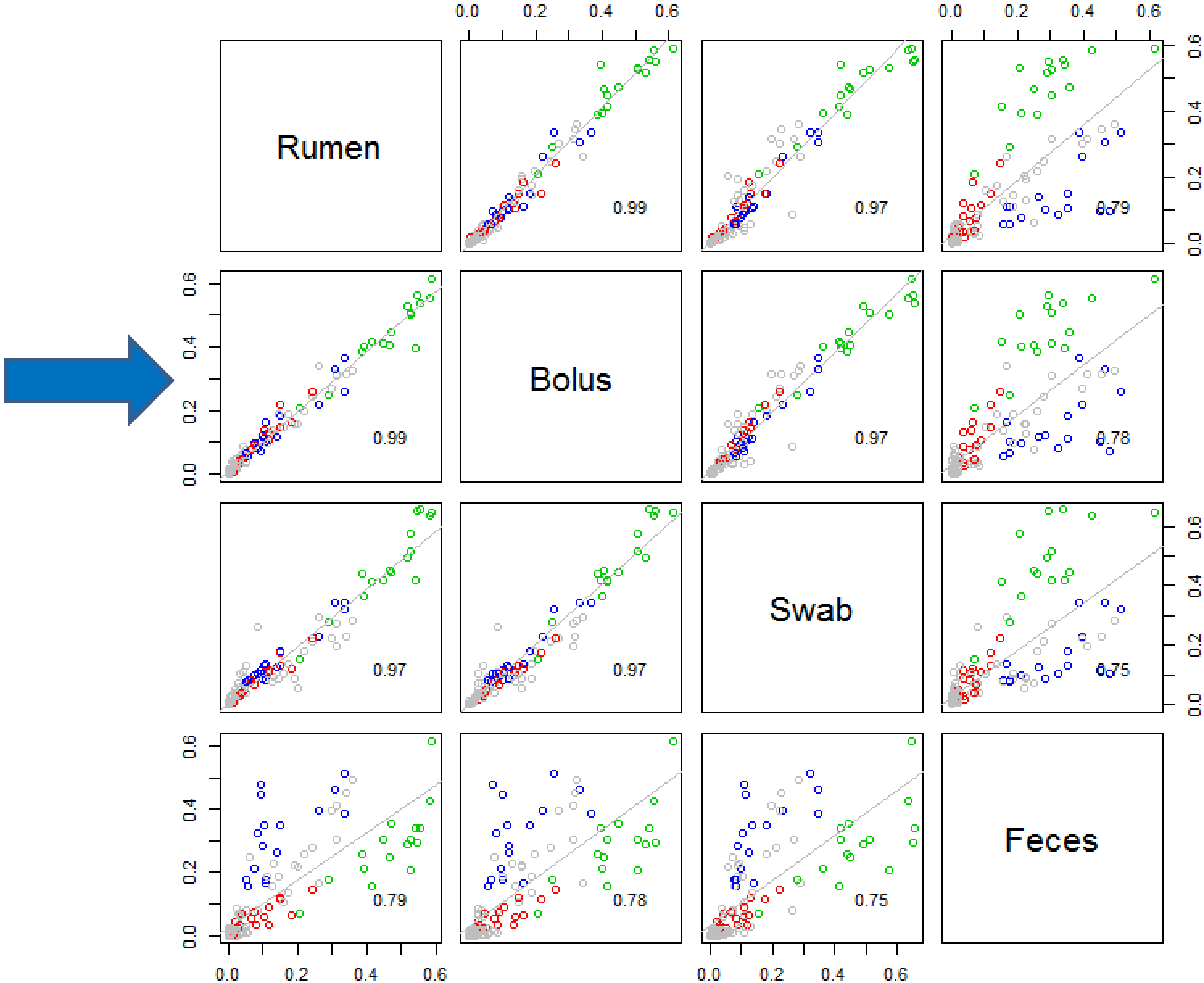
Rumen-Bolus comparison

Sample ID	Pearson correlation coefficient	CI
TA2S	0.9924	0.9755-0.9977
YO4S	0.9585	0.8707-0.9871
YL3S	0.9931	0.9775-0.9979
VA5S	0.9895	0.9663-0.9968
YL4R	0.9926	0.976-0.9977
VO2R	0.9841	0.9492-0.9951
TA3R	0.9908	0.9705-0.9972
YO5R	0.9781	0.9304-0.9932
VO4C	0.9971	0.9906-0.9991
YO2C	0.9917	0.9731-0.9974
TA5C	0.9917	0.9731-0.9974
VA3C	0.9927	0.9764-0.9978
VA4L	0.9968	0.9895-0.999
YL2L	0.9964	0.9884-0.9989
YO3L	0.9921	0.9744-0.9976
VO5L	0.9905	0.9693-0.9971

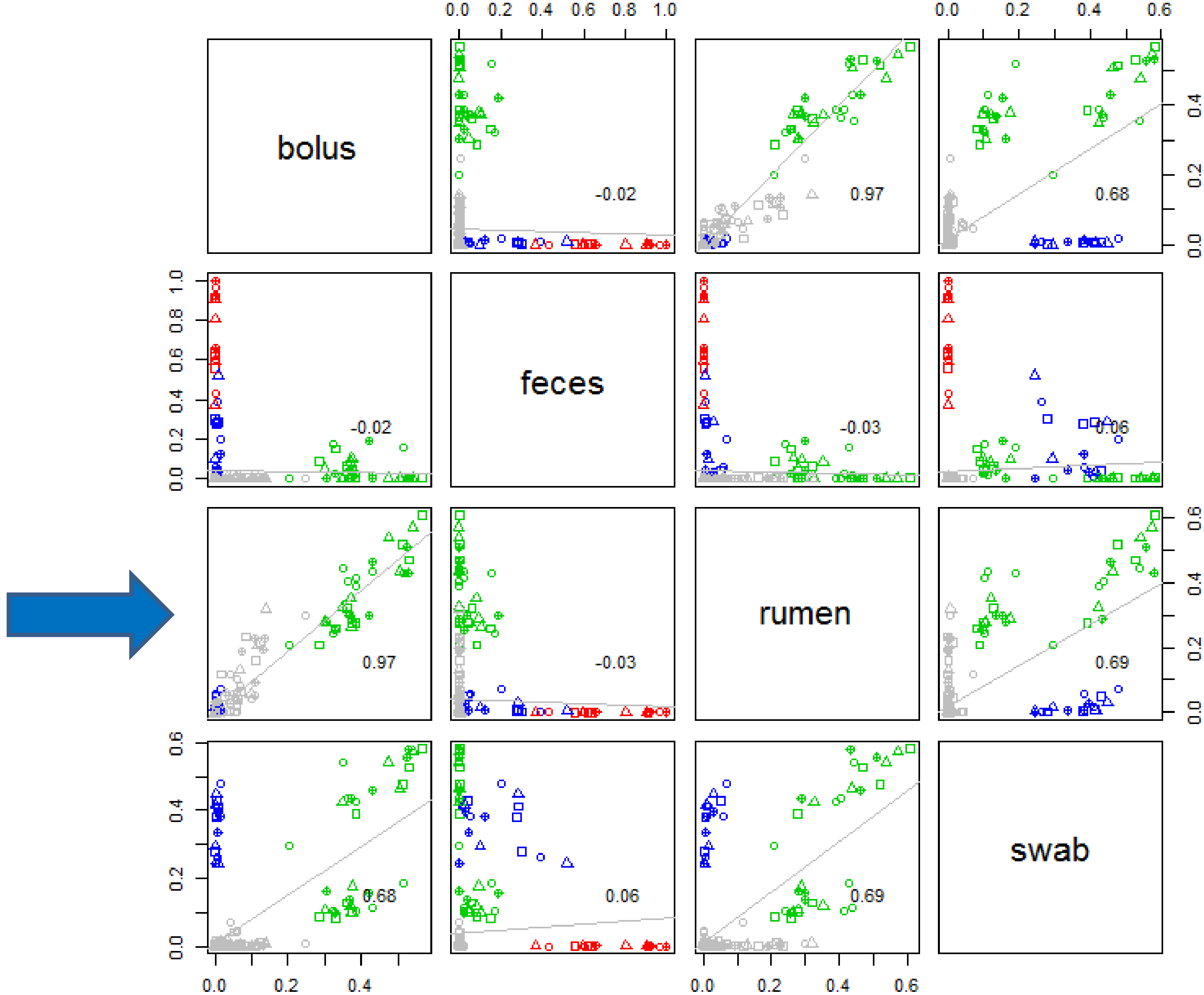
Bacteria_total_0.1%



Fungi_even1660



Ciliate_even_5700



Outcomes

- Faeces – not a viable surrogate of the rumen microbial community
- Bacteria - bolus and buccal swab
- Archaea - bolus and buccal swab
- Anaerobic fungi – bolus and buccal swab
- Ciliate protozoa - bolus

Nitrogen economy of the lactating cow

N intake

503 g/d

N Urine

37%

N Faeces

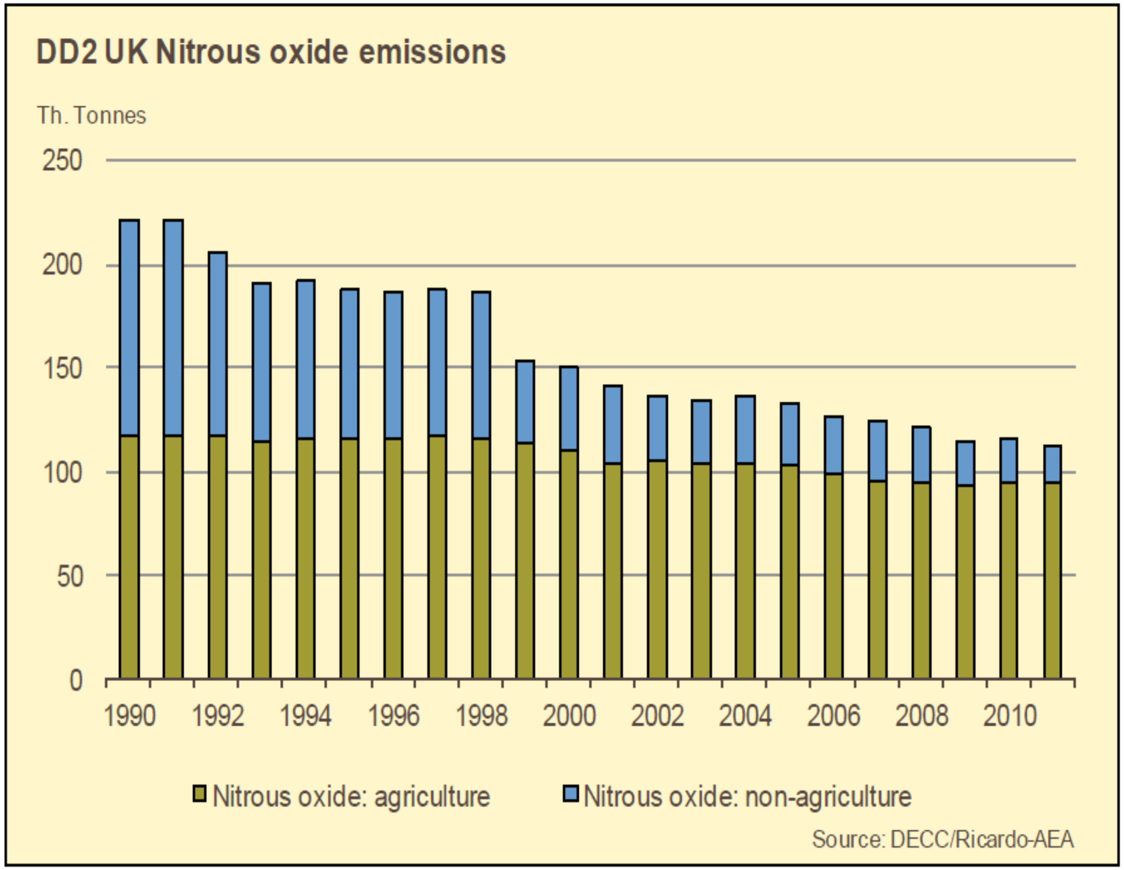
33%

N Milk

28%

Mills et al., 2009

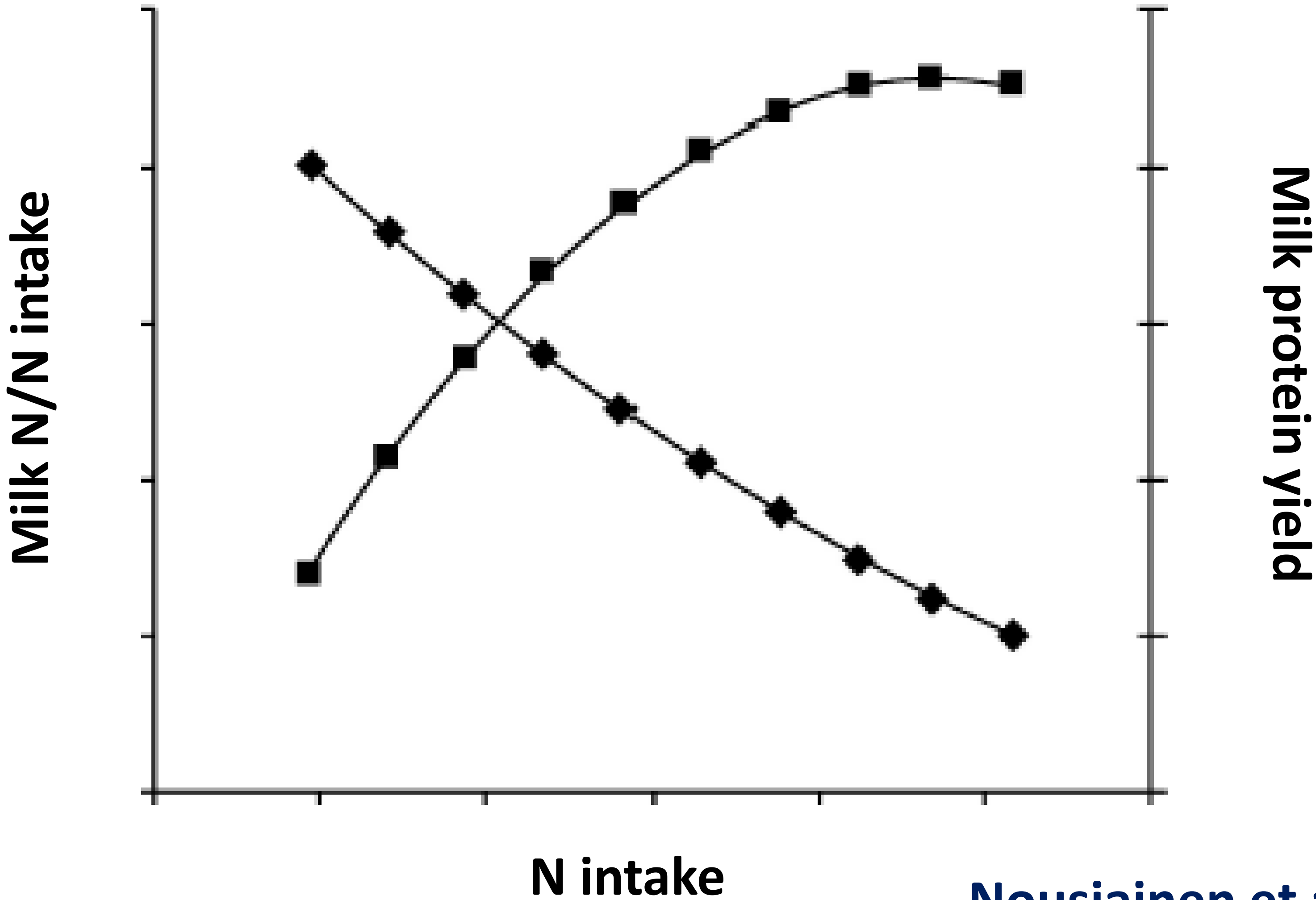
Annual nitrous oxide emissions in the UK



Total nitrous oxide emissions fell by 49% between 1990 and 2011. The largest reductions were in emissions from adipic acid production (a key raw material of polyurethanes) between 1998 and 1999. Reductions in industrial process emissions have continued to decline primarily due to decreases in the production of adipic and nitric acid.

- **N_2O accounts for ca. 6% of UK anthropogenic greenhouse gas emissions**
- **About 80% of N_2O from agriculture from soils**

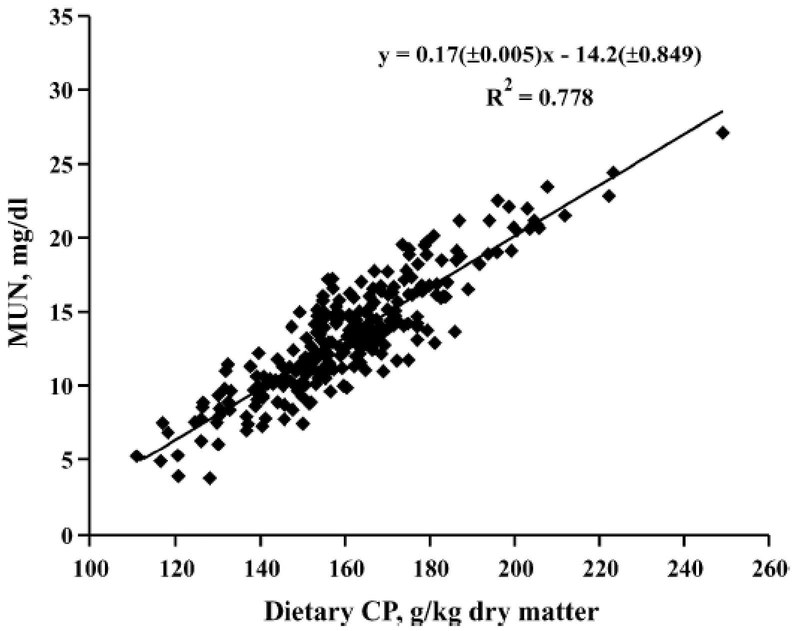
Nitrogen intake, production and nitrogen use efficiency

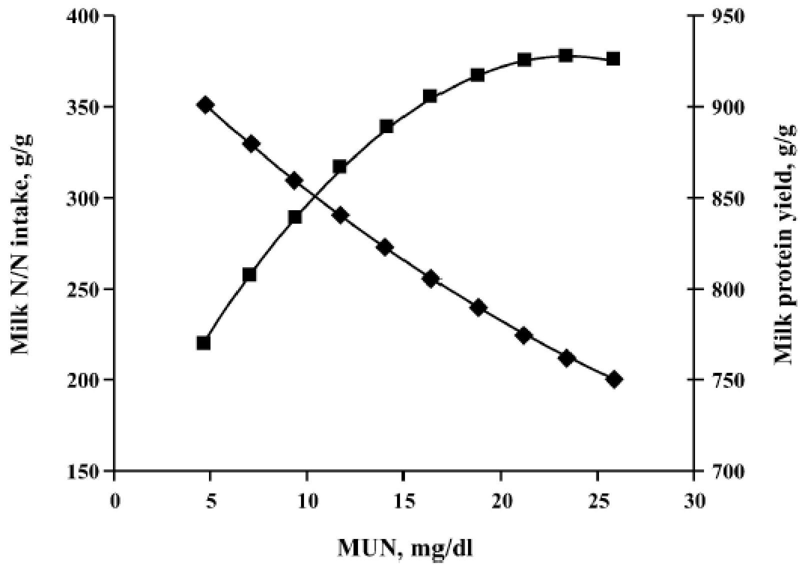


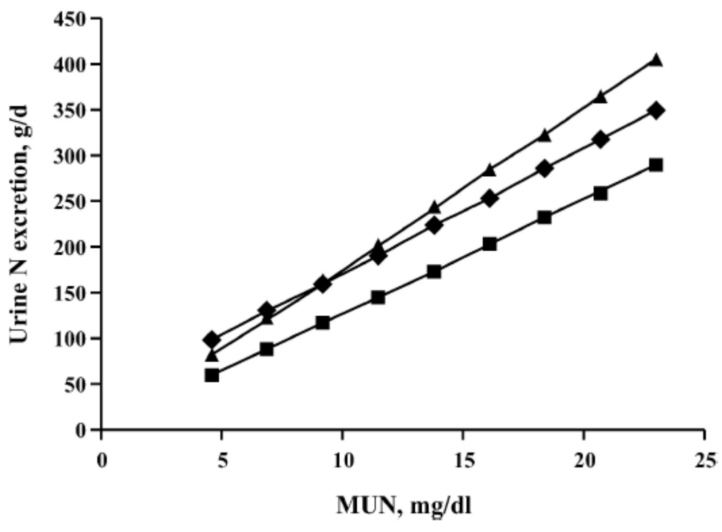
Nousiainen et al., 2004

Milk urea concentration as a phenotype of nitrogen use efficiency

Measurements of milk urea nitrogen







Meta-analysis based on 306 treatment means from 50 experiments

Nousiainen et al., 2004

Meta-analysis to understand between-animal variation in MUN and rumen ammonia N concentrations and the association with diet digestibility and N use efficiency

- 1804 cow/period observations from 21 production trials
- 450 cow/period observations from 29 metabolic studies
- Data were analyzed by mixed-model regression analysis
- Model included diet within experiment and period within experiment as random effects: effect of diet and period excluded

Huhtanen et al., 2015

Results

- Between cow variation in MUN 0.13 and 0.11 % for production and metabolic datasets
- Between cow variation in MNE 0.07 and 0.08 % for production and metabolic datasets
- Including MUN and RAN in the model accounted for more variation in MNE than milk yield alone
- Between-cow variation had a smaller influence on the relationship of MUN with urinary N excretion or MNE than when based on treatment means

Huhtanen et al., 2015

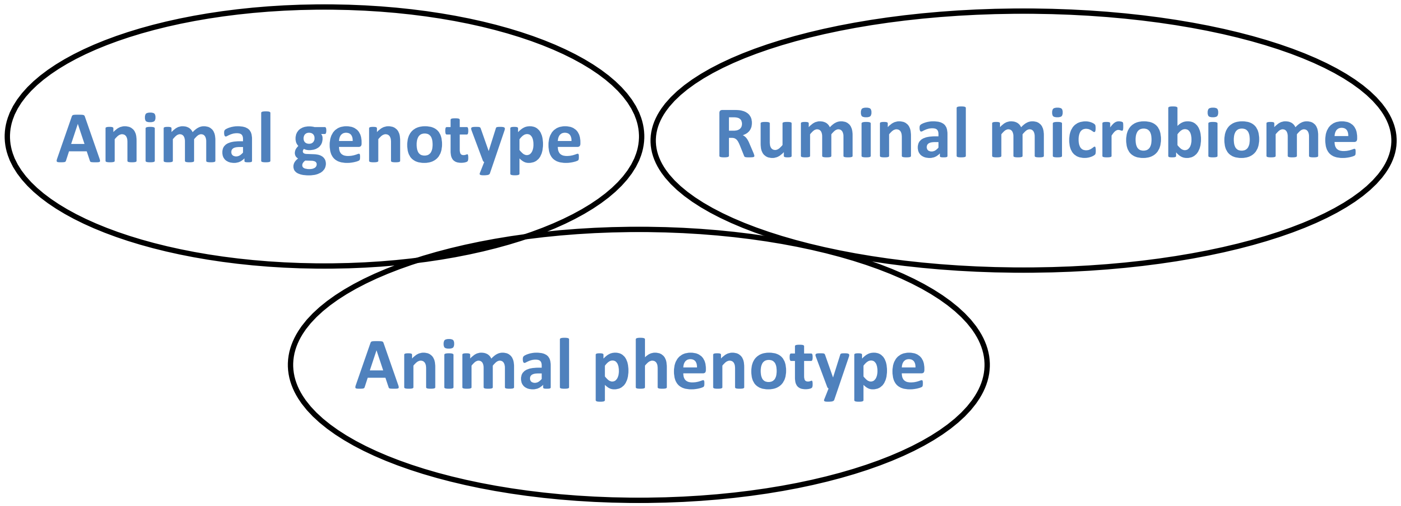
Conclusions

- Between-cow variation in MUN had a smaller effect on MNE compared with published responses of MUN to dietary crude protein content
- Closer control over diet composition relative to requirements has greater potential to improve MNE and lower UN on farm than genetic selection
- Measurements of MUN are more useful as a management tool than as a phenotype for genetic selection of more nitrogen efficient cows

Huhtanen et al., 2015

Future perspectives

RuminOmics – Large scale data



Intake, milk production, digestibility, methane output, fermentation characteristics, blood metabolome, milk fatty acid composition

Project goals

- Understanding the role of host animal genetics, rumen microbiome and diet on methane production, nitrogen emissions, feed efficiency and milk quality

Outcomes

- Generation of new large data for mining new biomarkers of rumen function, animal performance and milk fatty acid composition

Thank you for your attention