

RuminOmics Regional Workshop

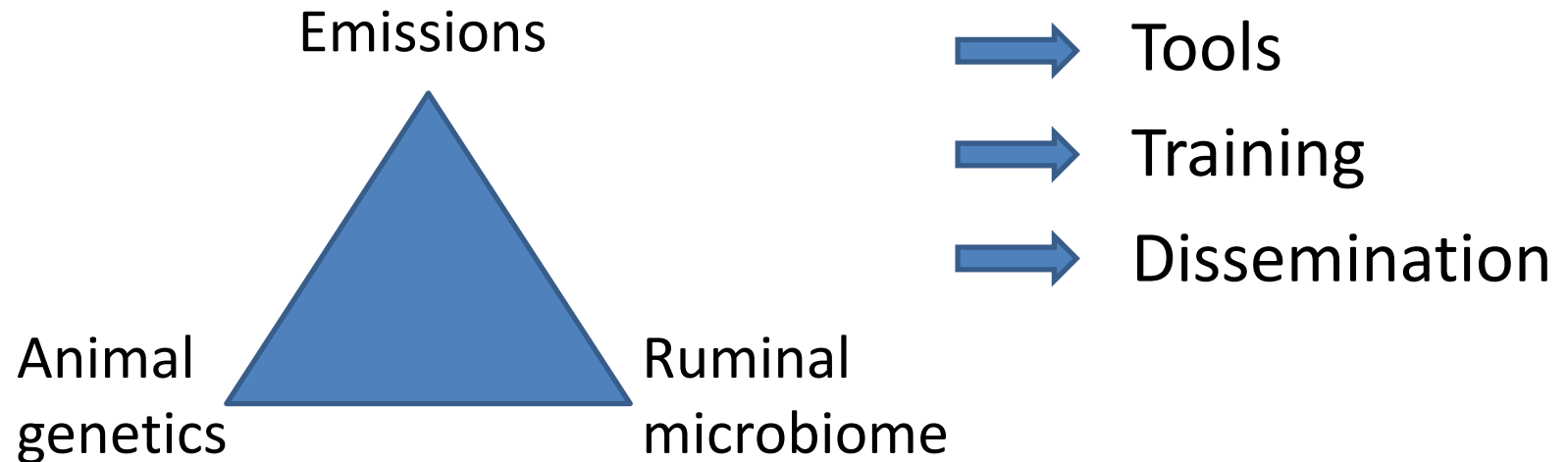
Improving efficiency and reducing environmental impact



Project outputs

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

RuminOmics - Aims of project



- **What role does nutrition play in governing emissions and the rumen microbiome?**
- **Does host animal genetics determine the rumen microbiome?**
- **If so, is this a heritable trait?**
- **Can we use this new knowledge to inform on more sustainable production systems?**

Project outputs and impact

- **New knowledge integrating nutrition, rumen ecology and host animal genetics**
- **Scientific publications**
- **Database connecting animal genotype and phenotype for 1000 dairy cows**
- **Development of tools for a range of end users**

Publications and knowledge transfer

- **8 Peer reviewed papers**
- **8 Abstracts at conference proceedings**
- **4 Regional workshops**
- **Joint workshops e.g. Annual EAAP conference**

Microbial genomics

Eubacterium ovis sp. nov., a butyrate producing bacterium from the rumen. Rosero Alpala et al., Microbiology.

Identification of xylanase genes of glycosyl hydrolase family 10 from strains of Pseudobutyrvibrio xylanivorans. Grillia et al., PlosOne

Tools and techniques

Effect of DNA extraction and sample preservation method on rumen bacterial community profile. Fliegerova et al., 2014 *Anaerobe* 29:80.

Estimation of dry matter intake by n-alkanes in dairy cows: effect of dosing technique and faecal collection time. Bani et al., 2014 *Anim. Prod. Sci.*

A comparison of ruminal or reticular digesta sampling as an alternative to sampling from the omasal canal of lactating dairy cows. Fatehi et al., 2015 *J. Dairy Sci.* 98:3274.

Nutrition and emissions

Evaluation of between-animal variation in milk urea and rumen ammonia nitrogen concentrations and the association with nitrogen utilization, urinary nitrogen excretion and diet digestibility in lactating cows. Huhtanen et al., 2015. J. Dairy Sci 98:3182.

Nutrition and emissions

Nutrition, emissions and the rumen microbiome in lactating cows

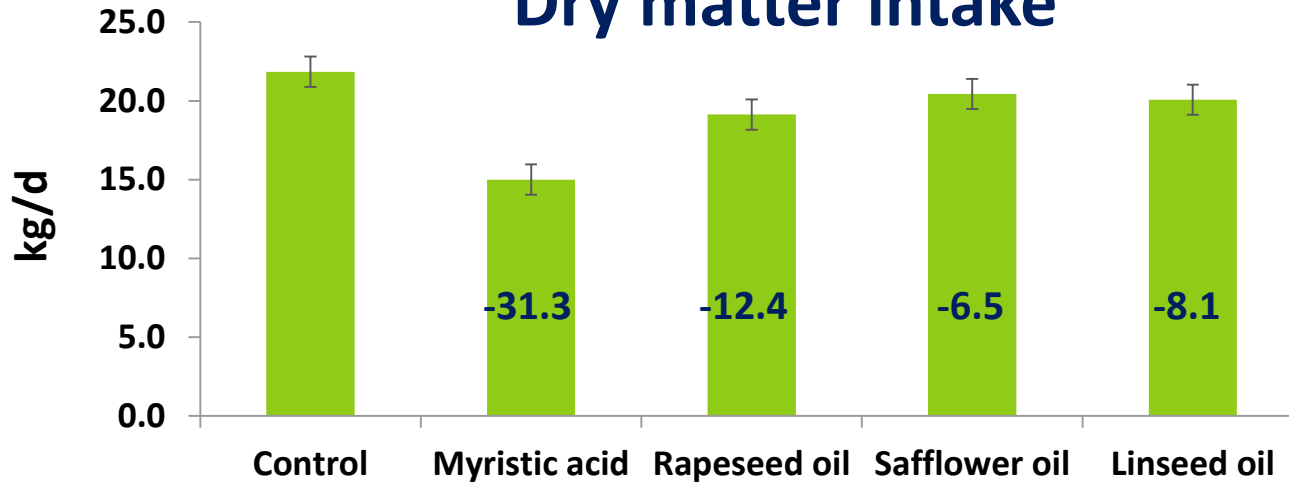
Series of dedicated experiments

- **Dietary fat**
Effect of replacing concentrate ingredients with myristic acid or plant oils
- **Carbohydrate source**
Effect of replacing grass silage with barley
- **Dietary protein content**
Effect of replacing barley with rapeseed

Dietary fat on emissions and the rumen microbiome in lactating cows

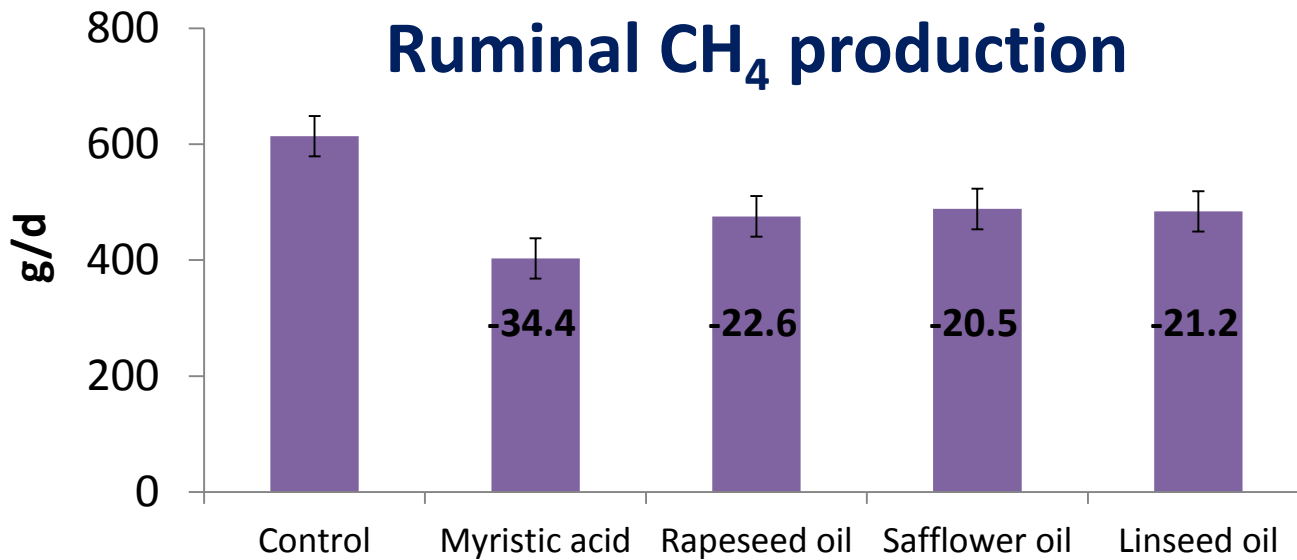
- 5 x 5 Latin square study with 5 cows
- Control (no added fat) or 50 g/kg diet DM of following supplements: myristic acid, rapeseed oil, safflower oil and linseed oil
- 2 d adaptation period, followed by 21 d supplementation and 5 d washout
- Milk production, gas emissions (SF_6), rumen fermentation, rumen microbiome, milk FA

Dry matter intake



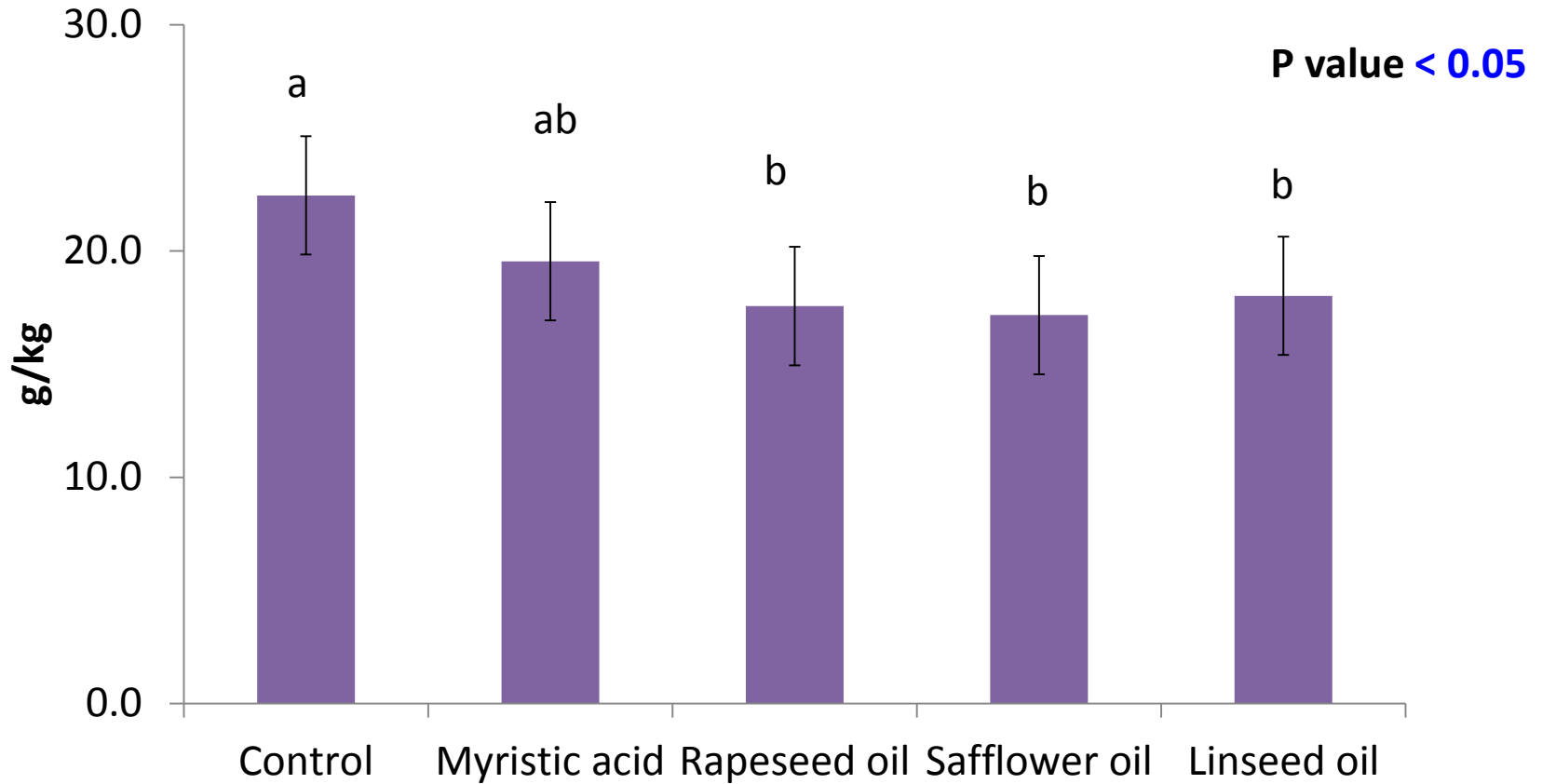
P value < 0.01

Ruminal CH₄ production



P value < 0.05

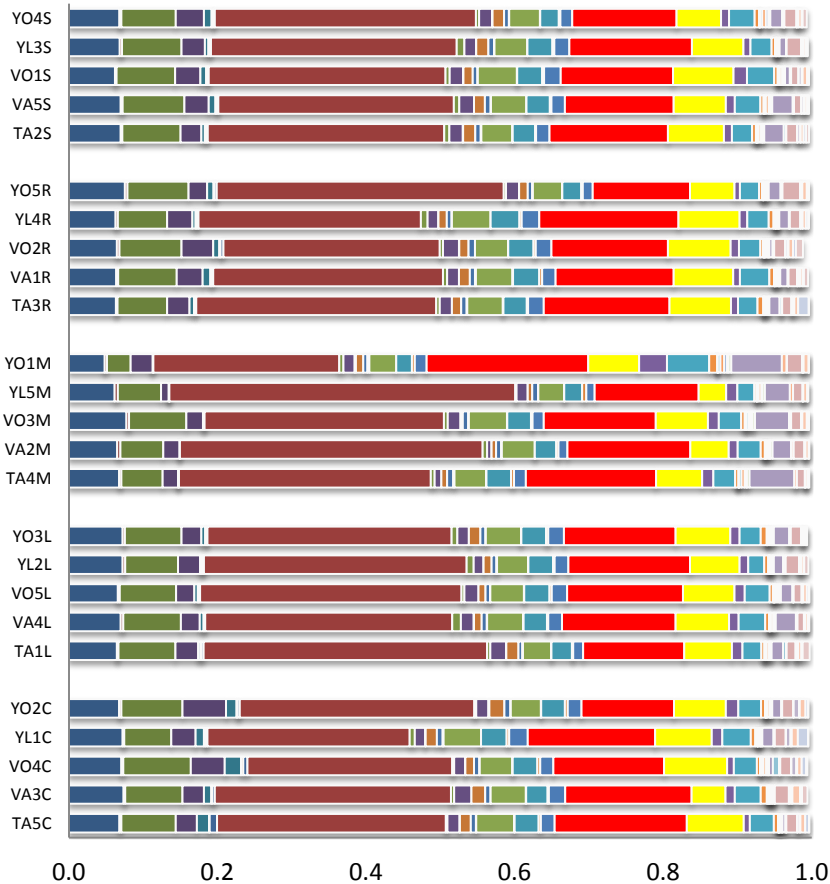
Methane/kg milk



Compared to control (%) -13.0 -21.8 -23.6 -19.8

Distribution of rumen bacteria (OTUs were assigned using the Greengenes 12_10 database)

Bacteria

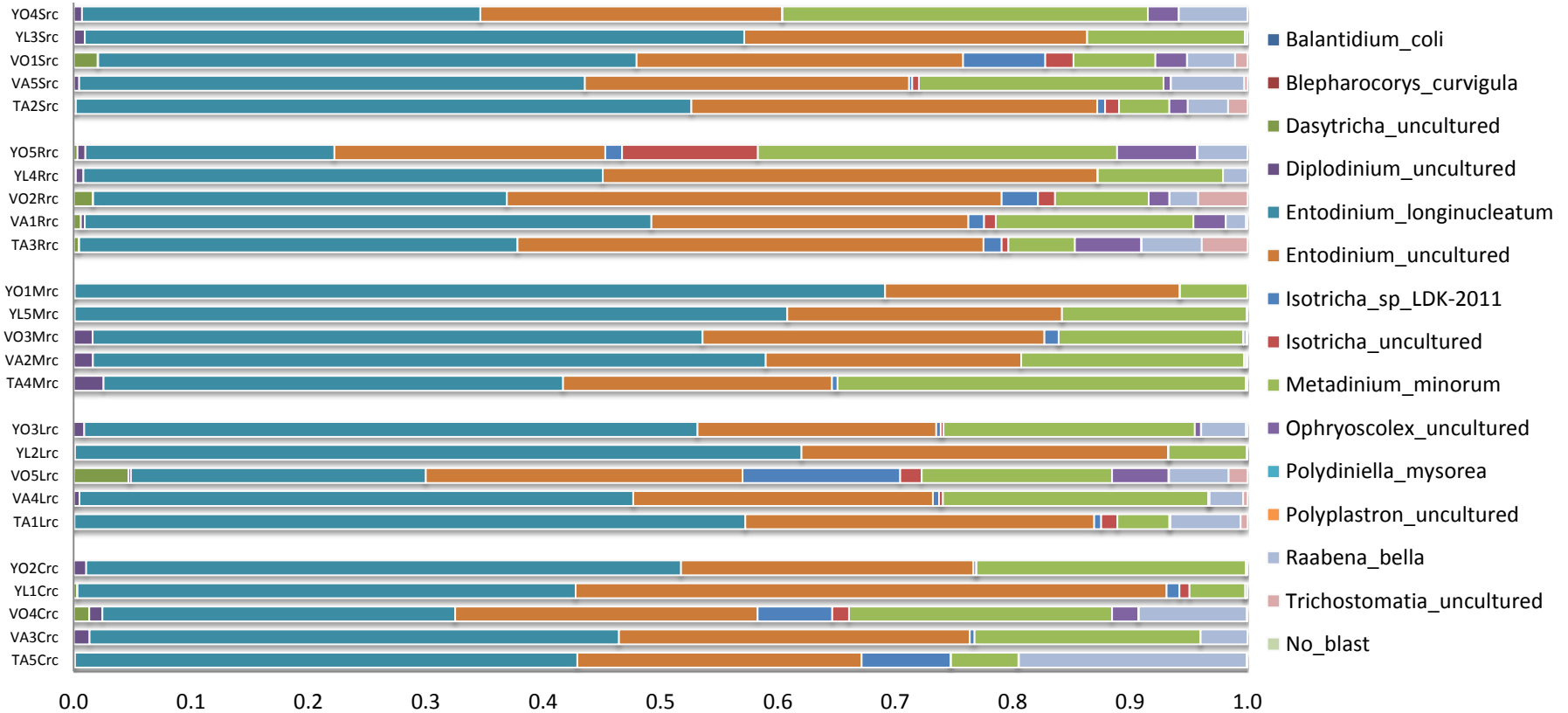


B10	p_Bacteroidetes	}	B
B12	o_Bacteroidales_1		
B13	o_Bacteroidales_2		
B14	o_Bacteroidales_f_BS11		
B15	f_Bacteroidaceae		
B16	f_Porphyrimonadaceae		
B18	o_Bacteroidales_f_S24-7		
B28	f_Fibrobacteraceae		
B34	f_Lactobacillaceae		
B62	p_Proteobacteria		
B65	c_Alphaproteobacteria_o_RF32		
B70	f_Alcaligenaceae	}	P
B79	c_Gammaproteobacteria		
B81	f_Succinivibrionaceae		
B89	p_Spirochaetes_o_PL-11B10		
B99	p_TM7_f_F16		
B104	f_Anaeroplasmataceae		

Distribution of rumen ciliate protozoa

(OTUs were assigned using SILVA 18S database (Quast et al.,2013))

Ciliate protozoa



Emissions and fat supplements

- Fat supplements can be used to decrease methane but depending on source may compromise animal performance
- Changes were associated with altered rumen microbial communities and the abundance of some less common taxa
- Analysis of the rumen archaea population suggests that myristic acid decreases methanogenesis by a mechanism that differs to plant oils.
- Across all treatments no clear association between ruminal methane output with the relative abundances of bacteria, ciliate protozoa or anaerobic fungi

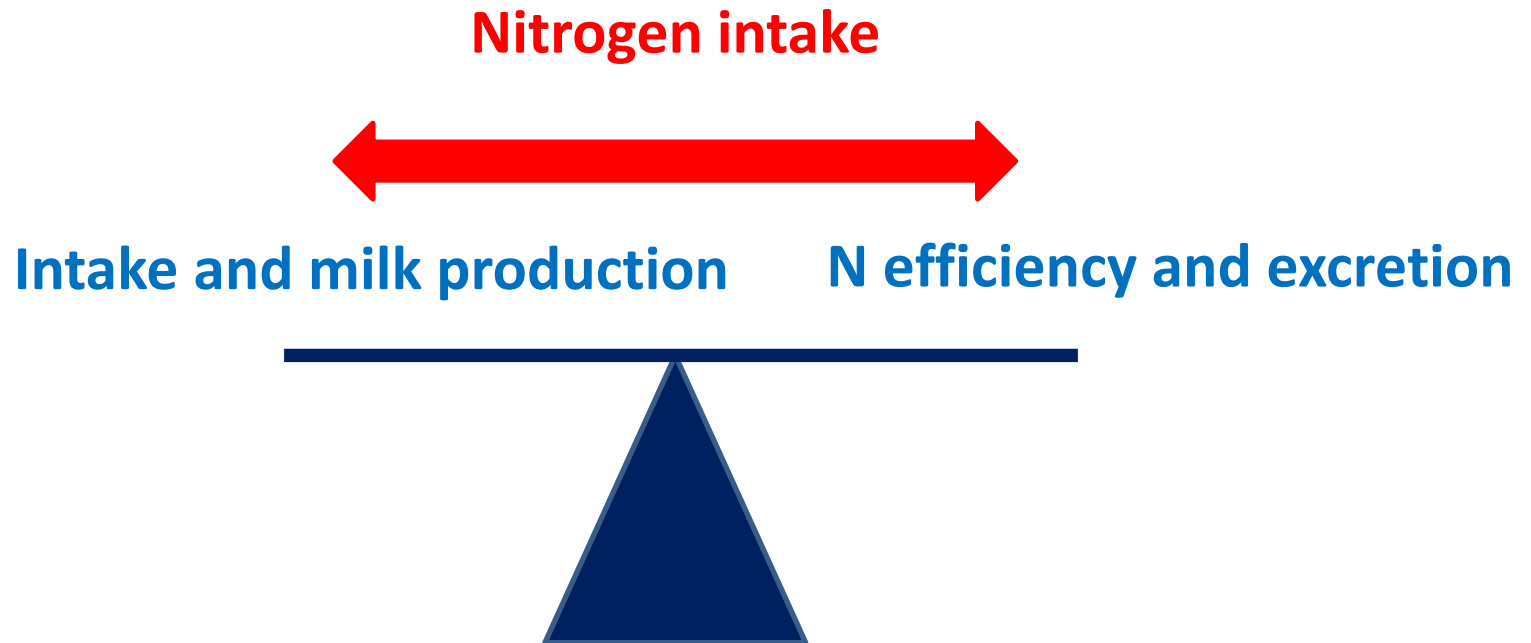
Dietary carbohydrate on emissions and the rumen microbiome in lactating cows

- Early-cut high D silage was gradually (0, 33, 67, 100%) replaced with low D silage + barley
- Diets formulated to produce same amount of milk
- 4 x 4 production study with intake, production and gas production (CH_4 and CO_2) with 20 cows
- 4 x 4 flow study using omasal (and reticular) sampling method, triple marker system and ^{15}N as microbial marker

Emissions and forage quality

- Through improvements in forage quality it was possible to decrease the amount of concentrate supplements without compromising production or the amount of CH₄ per unit of product.
- Taking into account the whole system green house gas emissions are likely to be less for grass based production systems

Fundamental conflict between performance and efficiency responses to dietary protein supplementation



Dietary protein content on emissions and the rumen microbiome in lactating cows

- 4 x 4 flow study using omasal (and reticular) sampling method, triple marker system and ^{15}N as microbial marker
- Barley replaced with heat-treated rapeseed meal
- Methane and CO_2 measurements made in a production study with 28 cows fed the same treatments with

Emissions and protein feeding

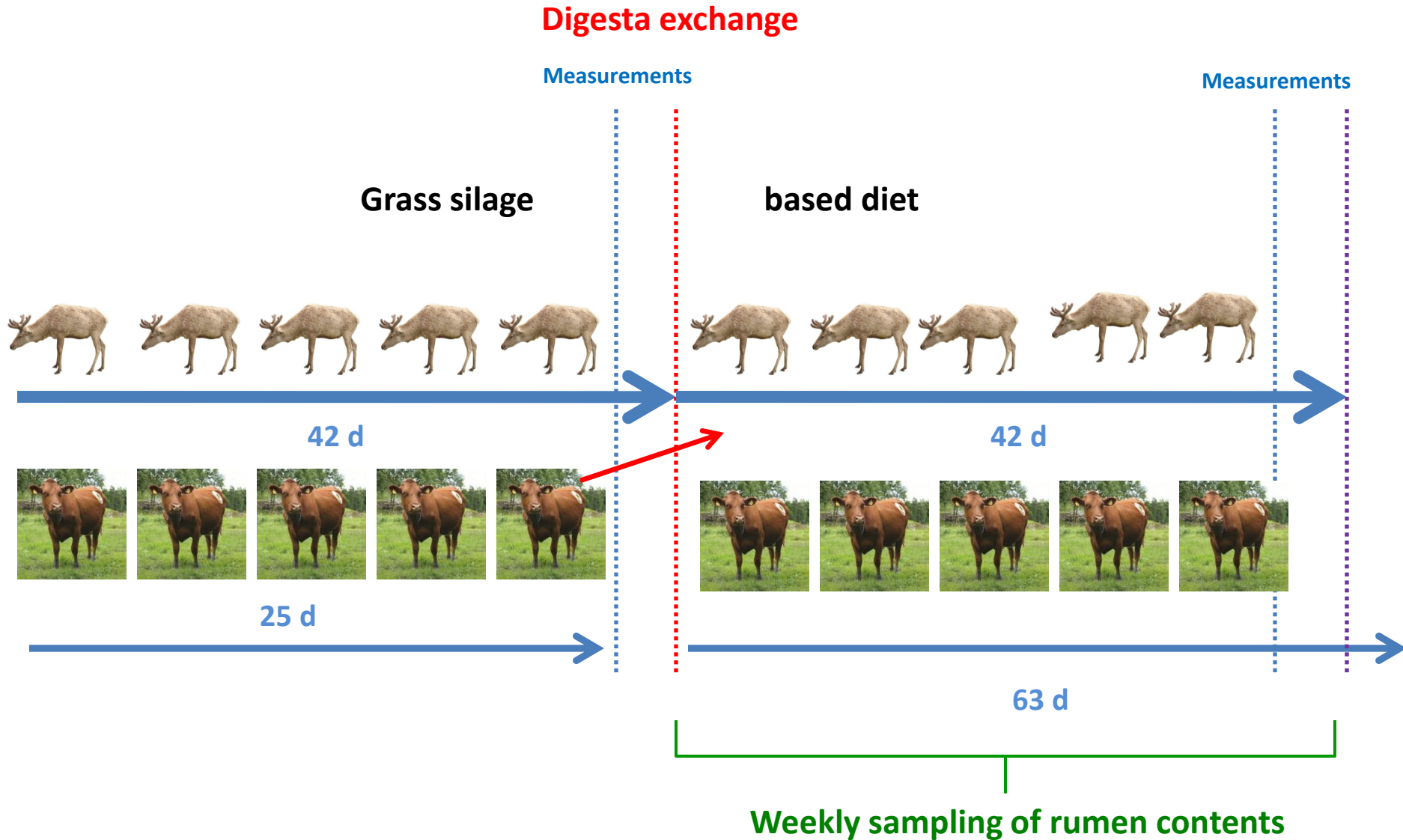
- Increasing the supply of rumen undegraded protein decreased the efficiency of rumen microbial protein synthesis
- Only about 65% of increased dietary undegraded protein was recovered as non-ammonia nitrogen at the omasum.
- These trade offs are not considered in metabolisable protein systems that has implications for optimising diets and lowering nitrogen losses into the environment.

Host animal genetics, emissions and the rumen microbiome

Experimental hypothesis

The host animal controls its own microbiome that influences rumen function, enteric methane production and nutrient digestion

Experimental design



Metagenomic analysis

- DNA extracted from rumen samples sequenced to assemble the rumen metagenome and predict genes and proteins
- Taxonomy based on GreenGenes and RIMDB databases to extract abundances
- MDS and heatmap plots generated to assess samples clustering of genes abundances and taxonomies

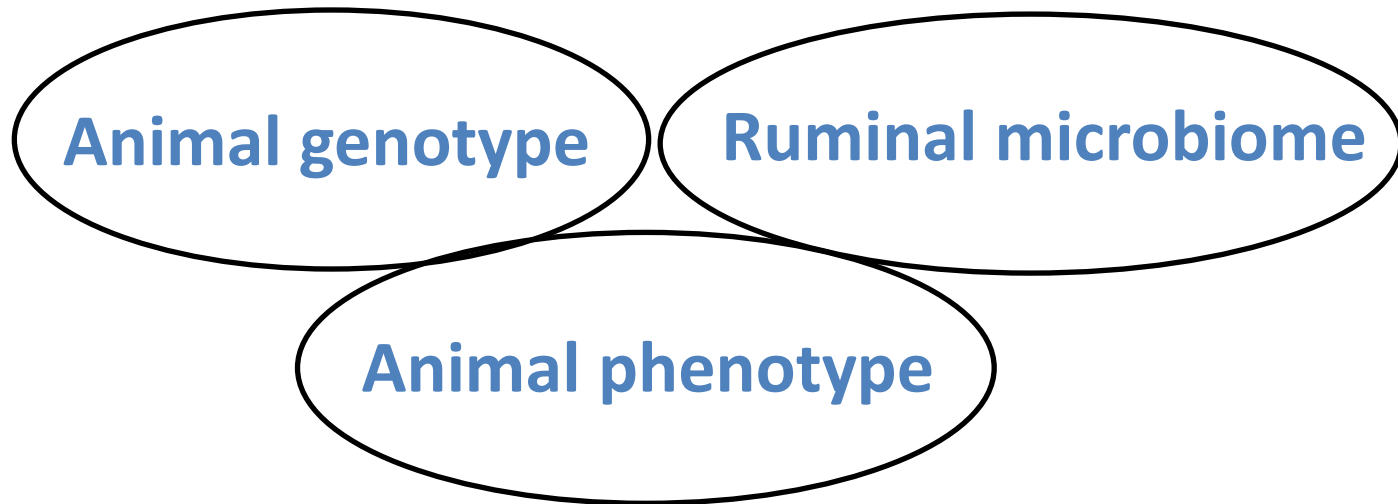
Emissions and ruminant species

- Rumen fermentation characteristics differed between ruminant species
- Reindeer produce less methane per unit of digestible organic matter intake than cows
- Reindeer excrete a higher proportion of dietary nitrogen in faeces and less in urine compared with cows

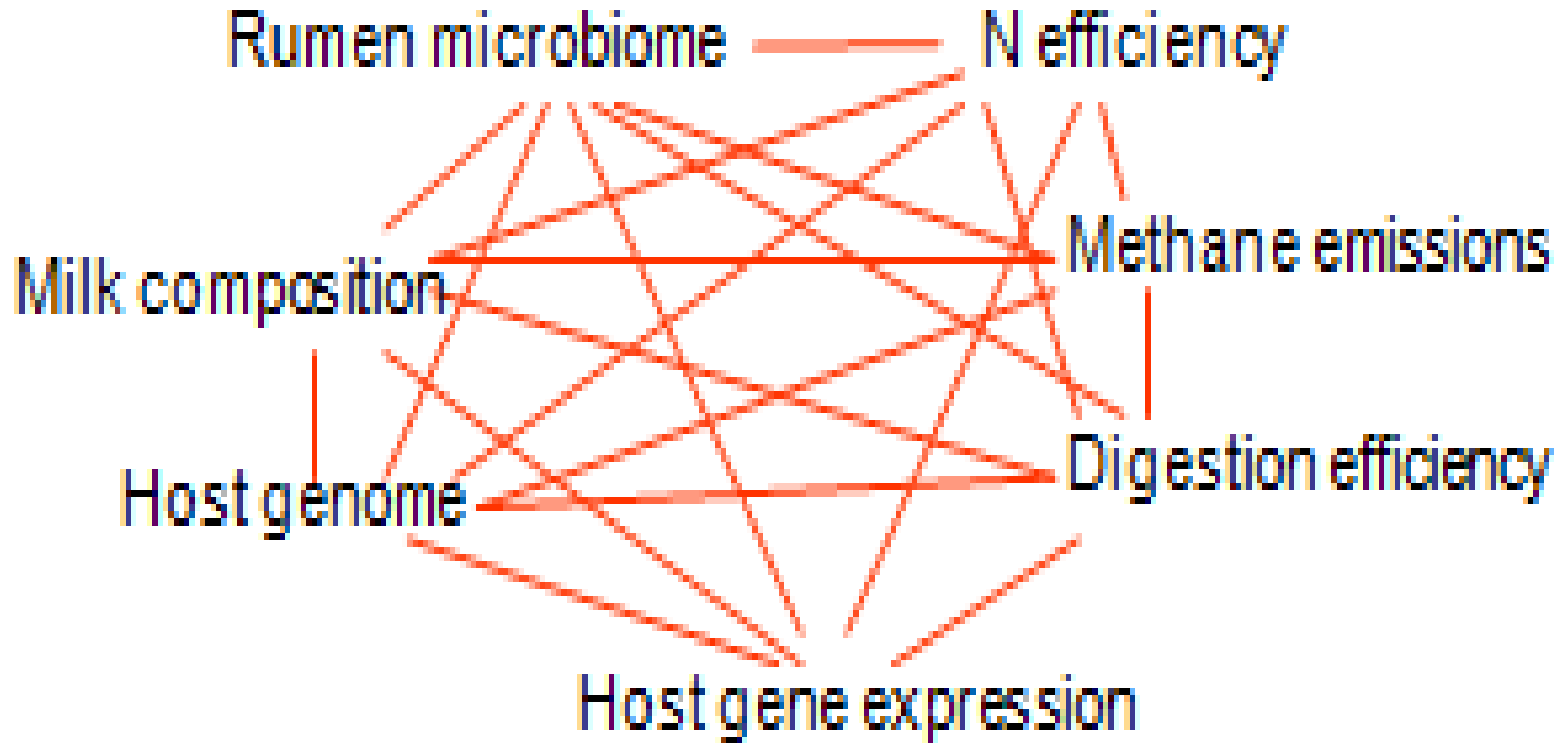
Host effects on the rumen microbial community

- Rumen microbial communities differed between cows and reindeer
- Most pronounced differences in anaerobic fungi and ciliate protozoa.
- Relatively small differences in ruminal archaeal and bacterial communities before and after digesta exchange and between reindeer and cows.
- After digesta exchange microbial communities in the rumen of reindeer were more similar to that of cows than the original populations.
- The microbial communities established in the reindeer after digesta exchange remained stable for a period of 9 weeks.

Connecting animal genotype-phenotype and the rumen microbiome



1000 cow study



Phenotypic data and samples of rumen fluid, faeces and blood for 1,000 cows

- **UK 407 Italy 410**
 - Holsteins
 - Maize + Grass silage/ hay diets
- **Sweden 100 Finland 100**
 - Red & White
 - Grass silage diets

Measurements and samples collected

Cow data

Milk yield, live weight

Automatic

Milk composition

Routine

Blood samples

Routine

Methane

During milking/GreenFeed

Respiration Chambers

Rumen samples

Rumen sample probe

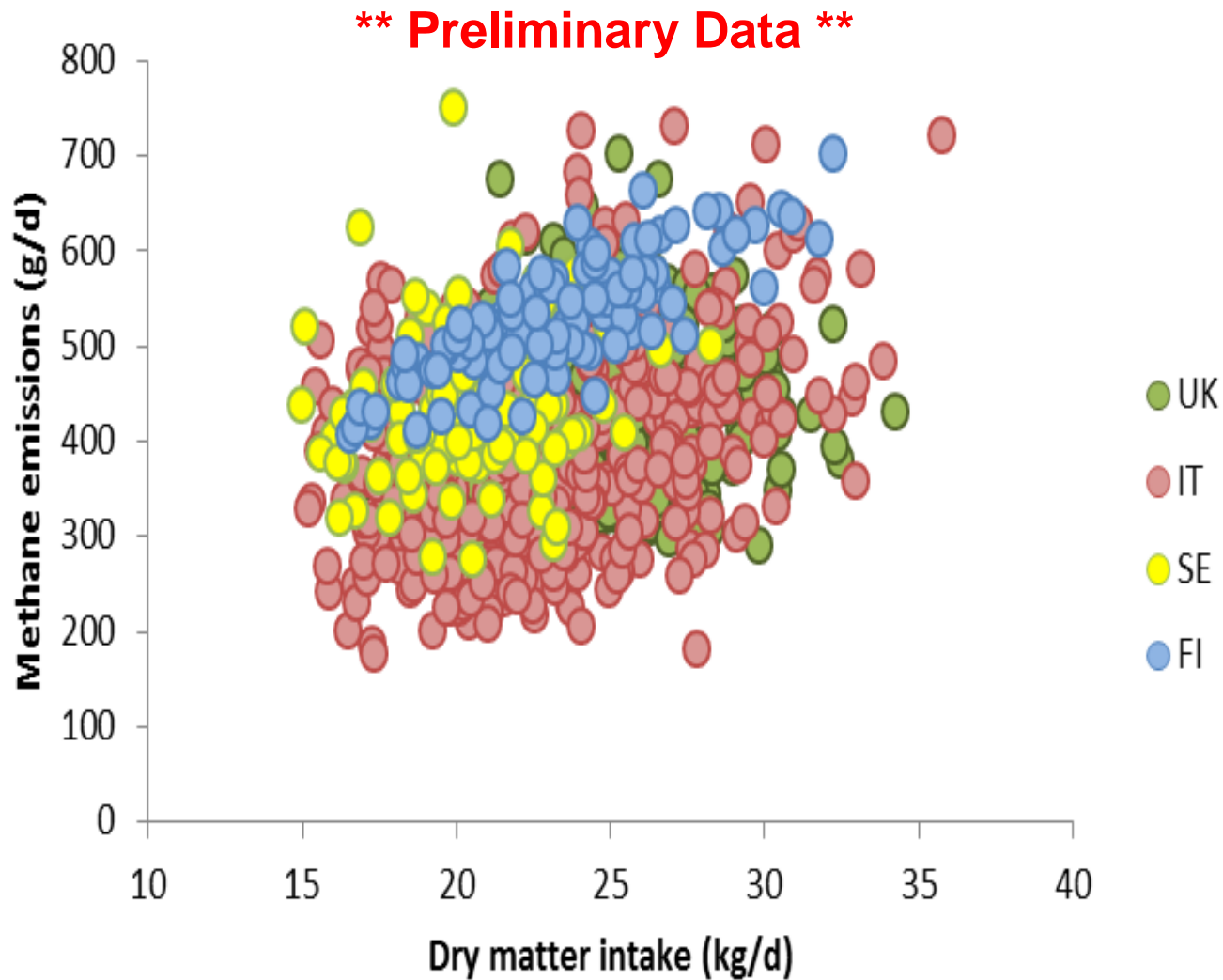
Feed intake

Direct measurement / Alkanes

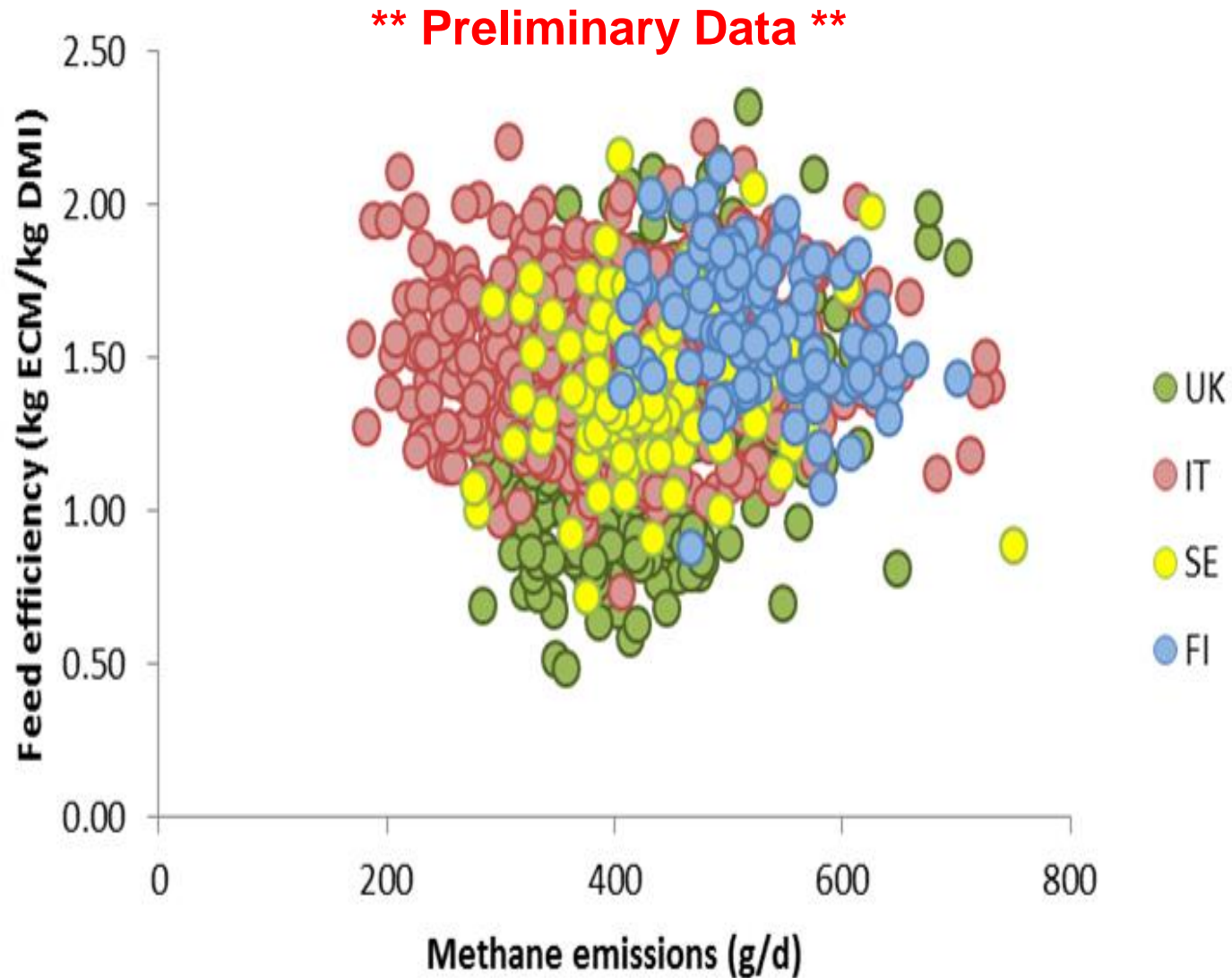
Digestibility

AIA / iNDF

Is methane related to intake on farms?



Are low methane emitters more efficient?



Emissions and the 1000 cow database

- Range in values for all phenotypes
- Within countries, and overall, data are normally distributed
- CH₄ emissions (g/d and g/kg DMI) vary widely between cows
- CH₄ is not necessarily related to efficiency, so genetic selection for low methane emissions may not be advisable

Emissions and genes in the rumen

Analysis of the rumen metagenome of 60 cows from selected from 4 partner countries

- **High, low and medium methane emitters**
- **Analysis of genes and biological pathways**
- **Key genes and pathways involved in methane production were found present at higher levels in high methane emitters**

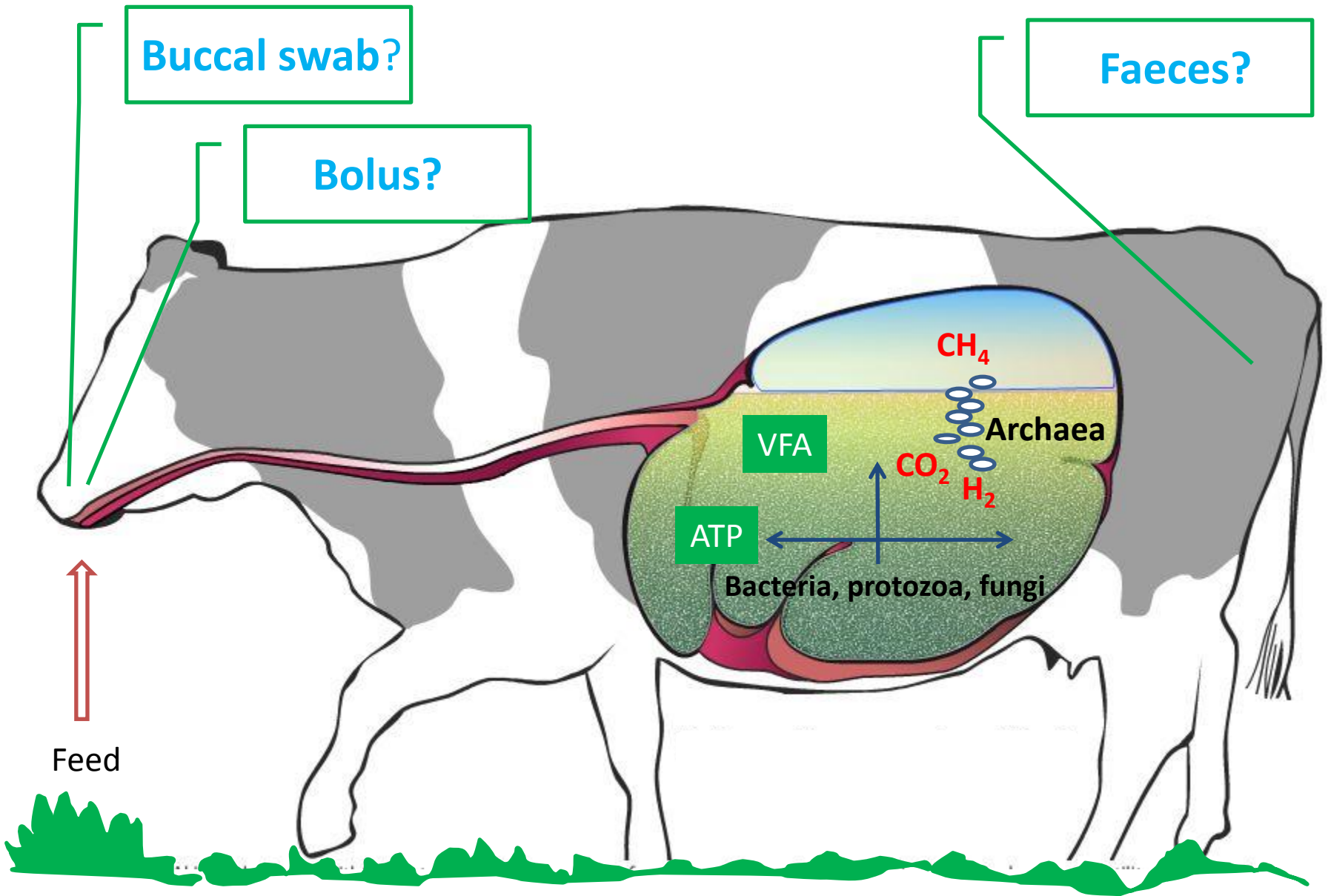
Emissions and animal genotype

Analysis of 1000 cow genotypes and phenotypes:

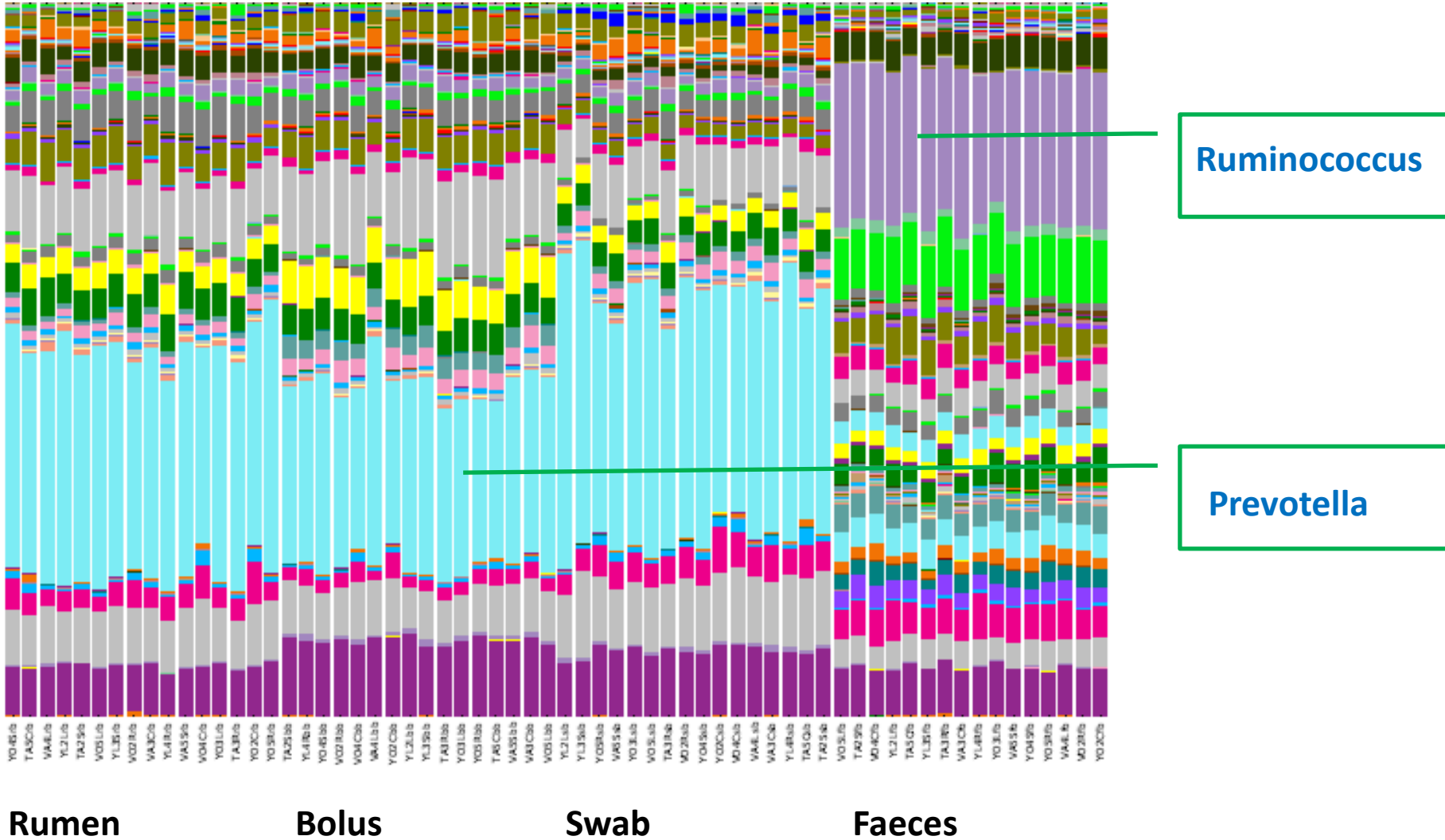
- **Bovine array 150K**
- **Preliminary genome wide association analysis suggests specific bovine genomic regions may be associated with methane emissions**

Tools

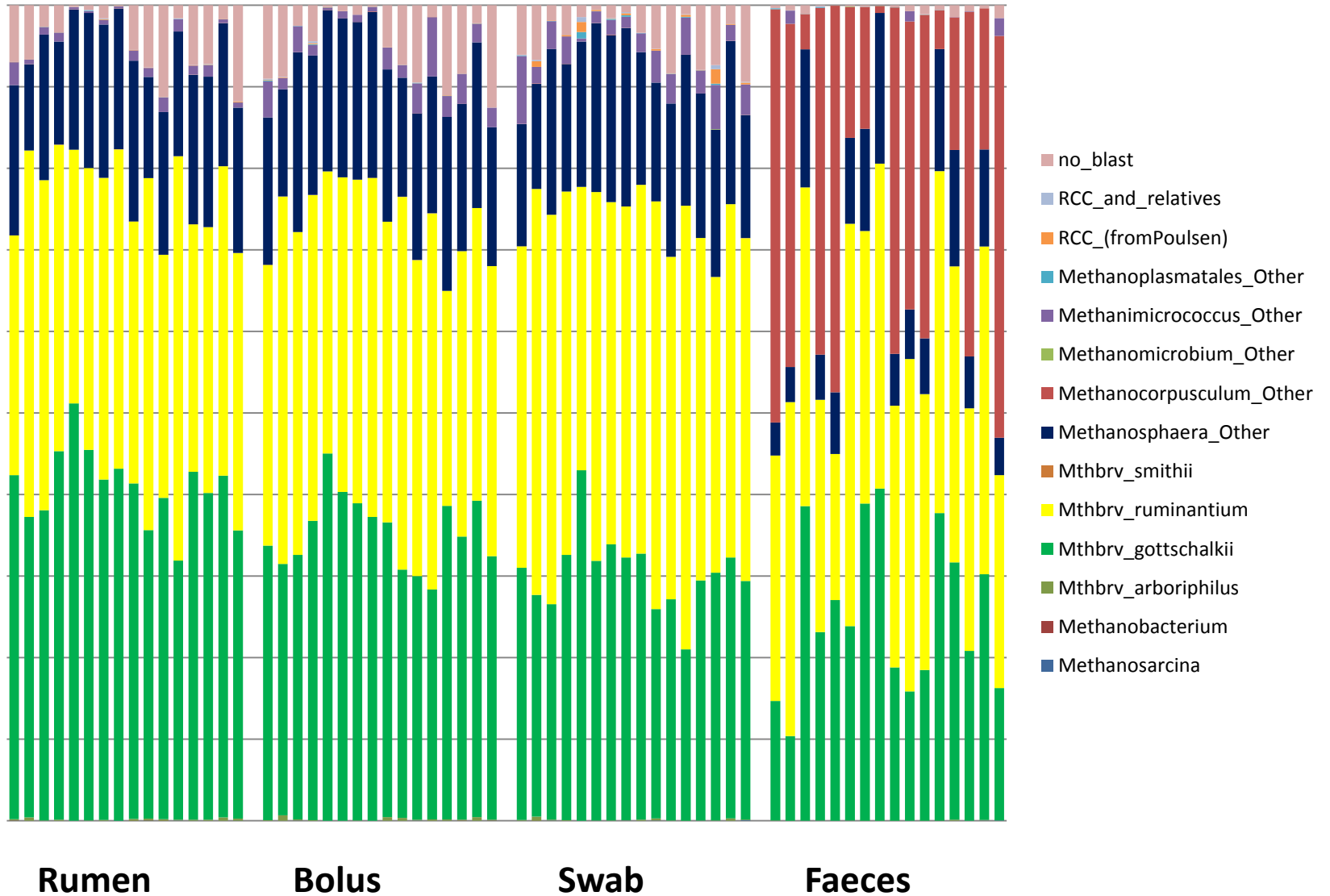
**Can we find an alternative to sampling
rumen contents?**



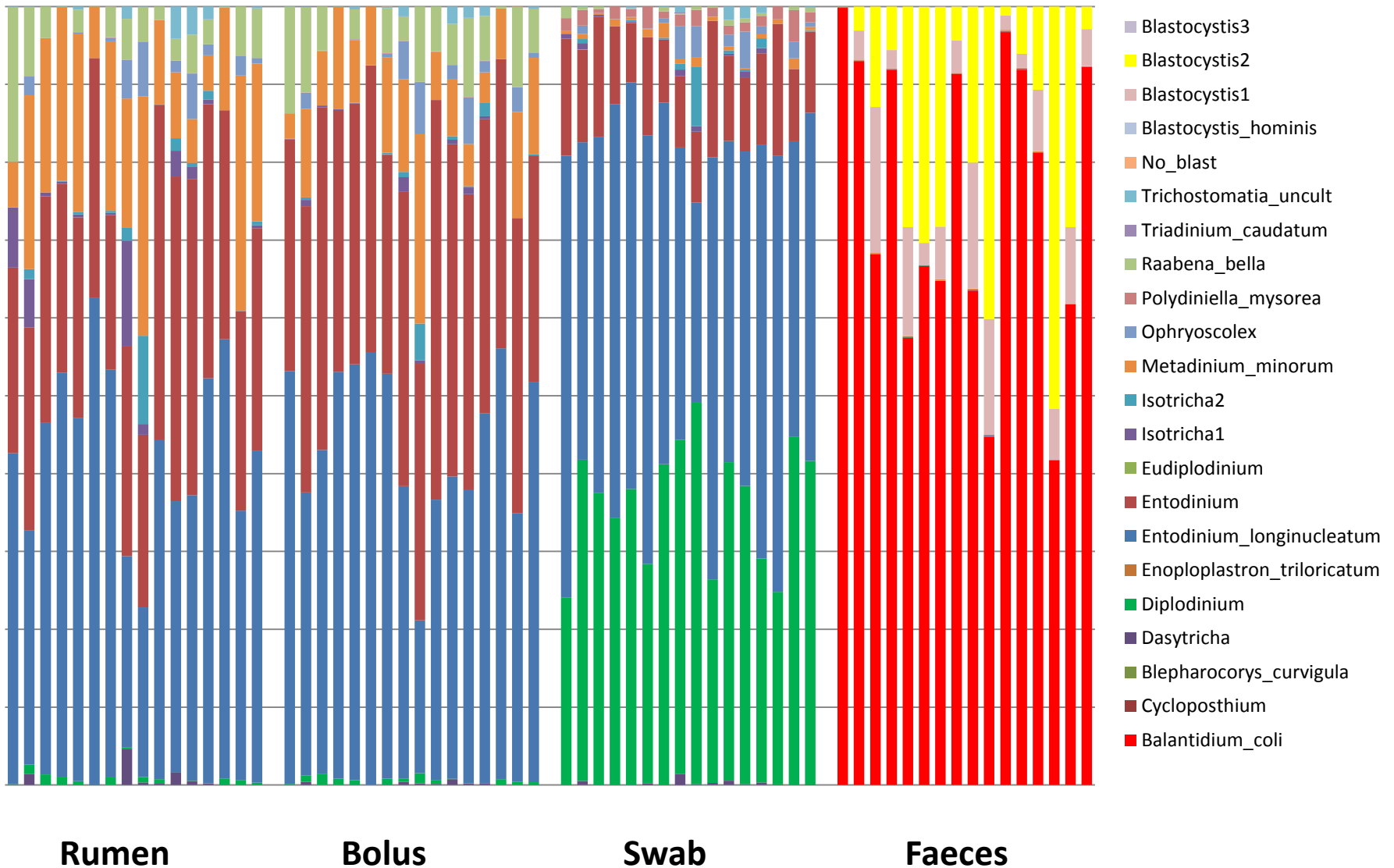
Bacteria relative abundance



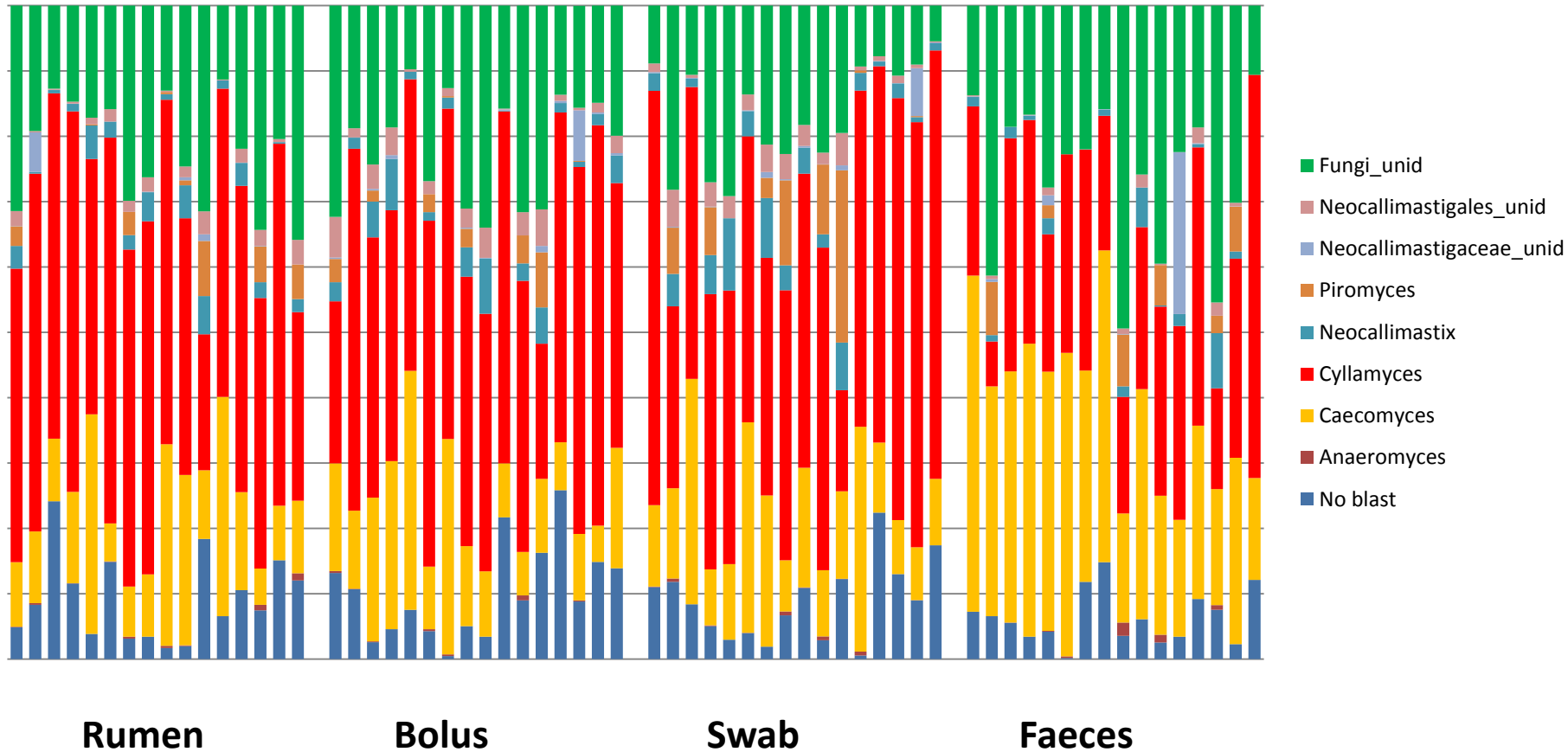
Archaea relative abundance



Ciliate protozoa relative abundance



Anaerobic fungi relative abundance



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

Analysis of the rumen microbiome

DNA extraction methods and sample preservation

- Comparison of the bacterial profile of intracellular (iDNA) and extracellular DNA (eDNA) isolated from rumen content stored under different conditions
- Rumen fluid treatment
 - Physical: cheesecloth squeezed, centrifuged, filtered
 - Storage temperature (RT, -80 C)
 - Cryoprotectants (PBS-glycerol, ethanol)
- Quality and quantity parameters of extracted DNA evaluated by bacterial DGGE analysis, real-time PCR quantification and metabarcoding

Recommended methodology

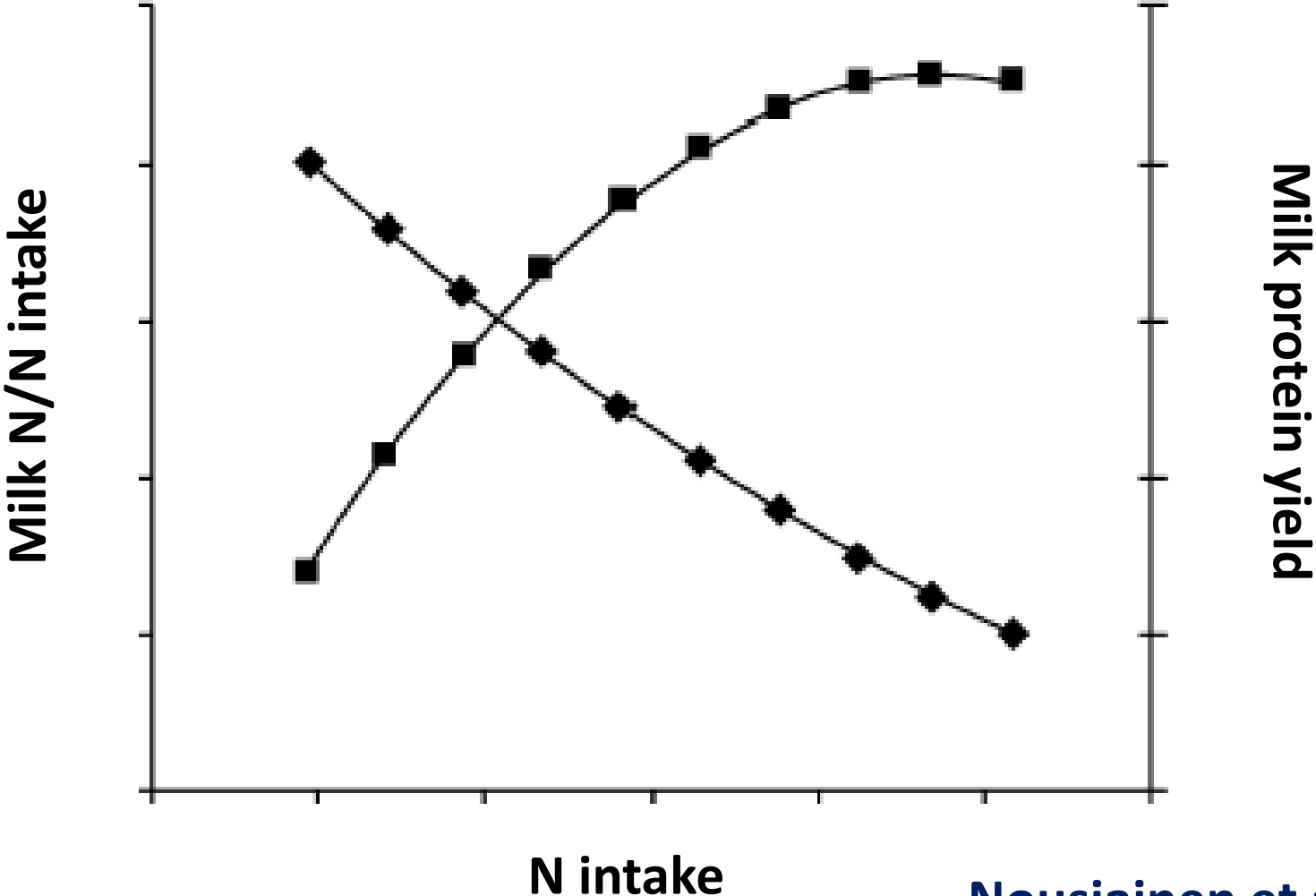
- **Samples clustered according to the type of extracted DNA due to considerable differences between intracellular DNA and extracellular DNA bacterial profiles**
- **Storage temperature and cryoprotectants had little effect on sample clustering**
- **Intracellular DNA extraction using bead-beating method from cheesecloth sieved rumen content mixed with PBS-glycerol and stored at -80 C is optimal for characterising the rumen bacterial community**

New tools for measuring nutrient flow

- **Compare techniques for determining nutrient flow**
- **Collection of digesta from the rumen, reticulum and omasum in lactating cows**
- **Nutrient flow was calculated a triple marker system**
- **Small difference in DM flow based on reticular or omasal sampling (+0.13 kg/d)**
- **Sampling digesta from the reticulum but not the rumen has potential to estimate nutrient supply**

Can we use milk urea nitrogen as a trait for breeding more nitrogen efficient cows?

Nitrogen intake, production and nitrogen use efficiency



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**

Emissions and milk urea nitrogen

- **Measurements of milk urea nitrogen is useful for optimising protein feeding in the dairy cow**
- **Smaller effects due to between-animal variation than dietary crude protein content**
- **Not a useful measurements for ranking animals for nitrogen use efficiency**

Thank you for your attention