

Getting More from Forages



Targeted feeding strategies:

Accounting for variability

**Rumen microbes as agents of
production variation in dairy cattle**

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“We’re not feeding the cow, we’re feeding the bugs...”

The ruminal microflora do the heavy work of converting feed components to the VFA and microbial protein that supports the cow’s maintenance, growth and milk production.

We know that cows differ in performance and in their response to different feeding and management strategies.

But how much of these differences are due to differences in ruminal microflora?



Classical approach to rumen microbiology

IN VITRO MIXED CULTURE APPROACH

- Determine rates and extents of feed component utilization by mixed cultures removed directly from the rumen

DEFINED CULTURE APPROACH

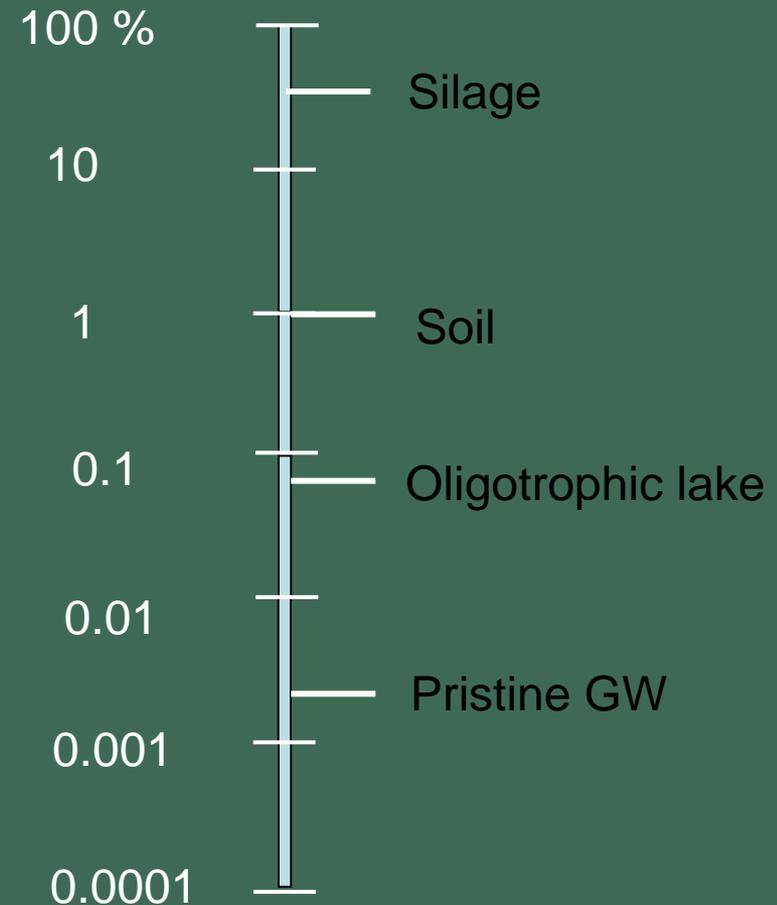
- Isolate bacterial species in pure culture.
 - Hungate's "22 species" plus a few others
- Characterize metabolic capabilities of these species in vitro.
 - Most of major metabolic types appear to have been accounted for
- Examine interactions between species in defined mixed cultures
 - identified the major interactions among ruminal species (competition for major feedstuff components; interspecies H₂ transfer; crossfeeding of nutrients, etc.)

KEY WORD IN BOTH CASES IS "CULTURE"



The tip of the iceberg

In most natural (and many engineered) microbial habitats, culturable species account for only a small percentage of the total number of microbes, as well as a small percentage of the microbial species.



Modern techniques allow us to examine the microbial community using “culture-independent” methods

Rely on detection of “chronometric macromolecules”, especially small-subunit ribosomal RNA

- present in all living cells**
- nucleotide sequences vary among species**
- used for reconstructing evolutionary history of taxa**

These 16S rRNA sequences also useful for designing probes and primers for quantifying individual taxa



Culture-independent methods

Quantitative methods

- Real-time PCR (qPCR)
 - FISH
- Allow quantitative measurement of specific taxa
- Exploit known sequences



Classical ruminal bacterial species are not abundant in the rumen

qPCR data from ruminal samples (combined liquid and solid phases) collected 6 h postfeeding

% of Bacterial 16S rRNA gene copy number

Target taxon	Cow 4884		Cow 4991		Mean \pm S.E.M.	P > F ^a	P > F ^a
	Day 30	Day 31	Day 30	Day 31		Cow	Day
<i>Butyrivibrio fibrisolvens</i>	0.0216	0.0273	0.0220	0.0243		0.584	0.256
<i>Eubacterium ruminantium</i>	0.1707	0.1581	0.1634	0.2130		0.584	0.658
<i>Fibrobacter succinogenes</i>	0.8384	0.8889	0.6152	0.9954		0.783	0.416
<i>Megasphaera elsdenii</i>	0.0011	0.0001	0.0003	0.0004		0.728	0.564
<i>Prevotella brevis</i>	0.1616	0.0988	0.1524	0.1282		0.693	0.266
<i>Prevotella bryantii</i>	1.226	0.7296	1.942	1.830		0.133	0.359
<i>Prevotella ruminicola</i>	1.600	1.5822	1.756	2.032		0.288	0.541
<i>Ruminobacter amylophilus</i>	0.1697	0.1406	0.3920	0.189		0.364	0.410
<i>Ruminococcus albus</i>	0.0030	0.0013	0.0044	0.0076		0.361	0.811
<i>Ruminococcus flavefaciens</i>	0.7573	0.3357	0.5580	0.7993		0.759	0.831
<i>Selenomonas ruminantium</i>	0.7061	0.3412	0.4681	0.6880		0.883	0.345
<i>Streptococcus bovis</i>	0.0077	0.0021	0.0025	0.0023		0.525	0.477
<i>Succinivibrio dextrinosolvens</i>	0.7148	0.6560	1.071	0.7988		0.257	0.365
Sum of individual species	6.186	4.920	6.900	7.213		0.308	0.654
Genus <i>Prevotella</i>	49.60	42.44	58.12	59.93		0.211	0.658



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Community Fingerprinting methods

- DGGE
- t-RFLP
- ARISA
- Allow profiling of the entire community
- No prior knowledge of specific taxa required



ARISA

Automated Ribosomal Intergenic Spacer Analysis

A culture-independent, “community fingerprinting” method that provides an indication of bacterial diversity within sample



- contains tRNA genes and noncoding sequences
- size varies with bacterial species (~130 to ~1500 nt)

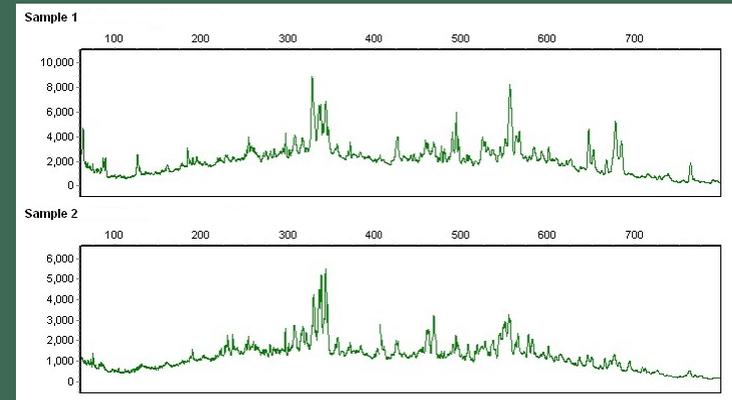
Each discrete “amplicon length” (AL) yields a separate peak on electrophoresis, and constitutes at least one individual species



ARISA methodology



Extract and purify DNA
 PCR-amplify ITS region
 between 16S and 23S
 rRNA genes

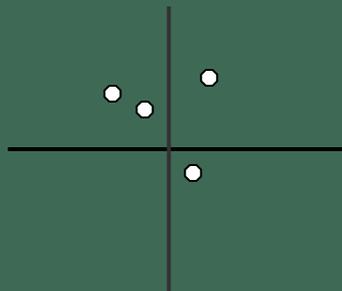


Separate by capillary
 electrophoresis

ITS length	samp 1	samp 2	samp 3	samp 4
461	0	0.6960	1.1559	1.3742
462	0	0	0	2.2501
468	0	0	2.0226	2.336
472	0	0	0	0
474	0.6882	0.5221	1.0031	1.2493
477	0.9635	1.0141	1.4755	1.8457
480	0.6669	1.4532	2.1095	2.6771
482	0	0	0	0
484	0.572	0.5045	0.9139	1.0761

~ 100 to 300 rows (ALs)

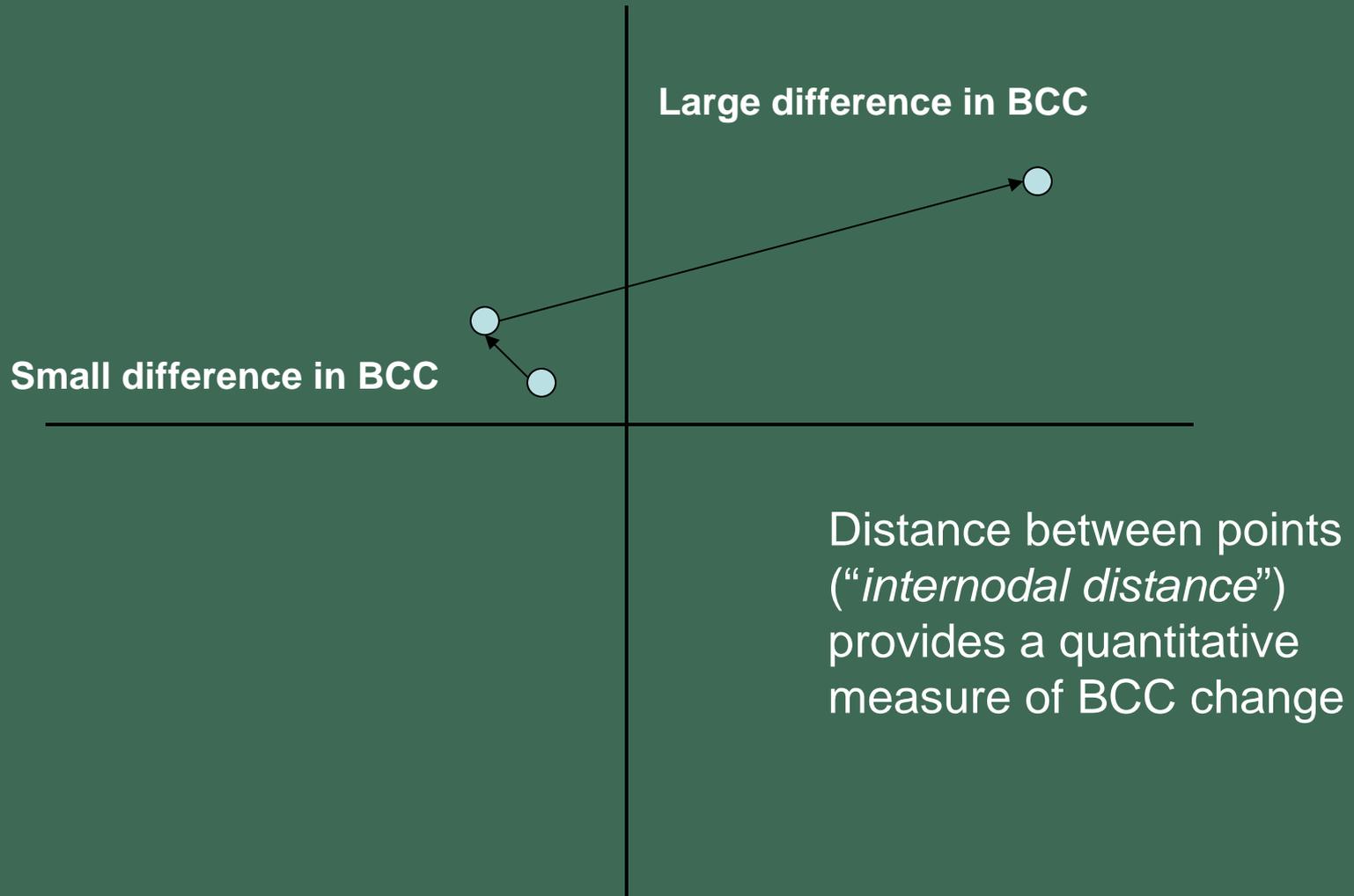
Construct
 data matrix



Multivariate Statistical
 Analysis



Ordination Bi-Plots

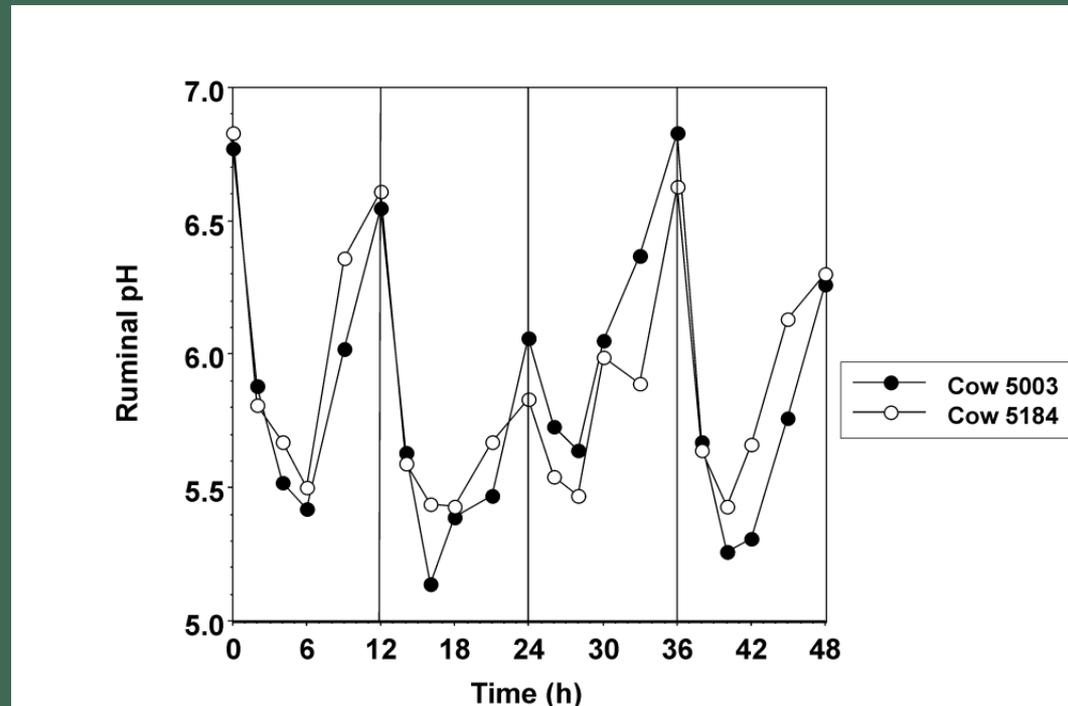


I. Changes in BCC during feeding cycle

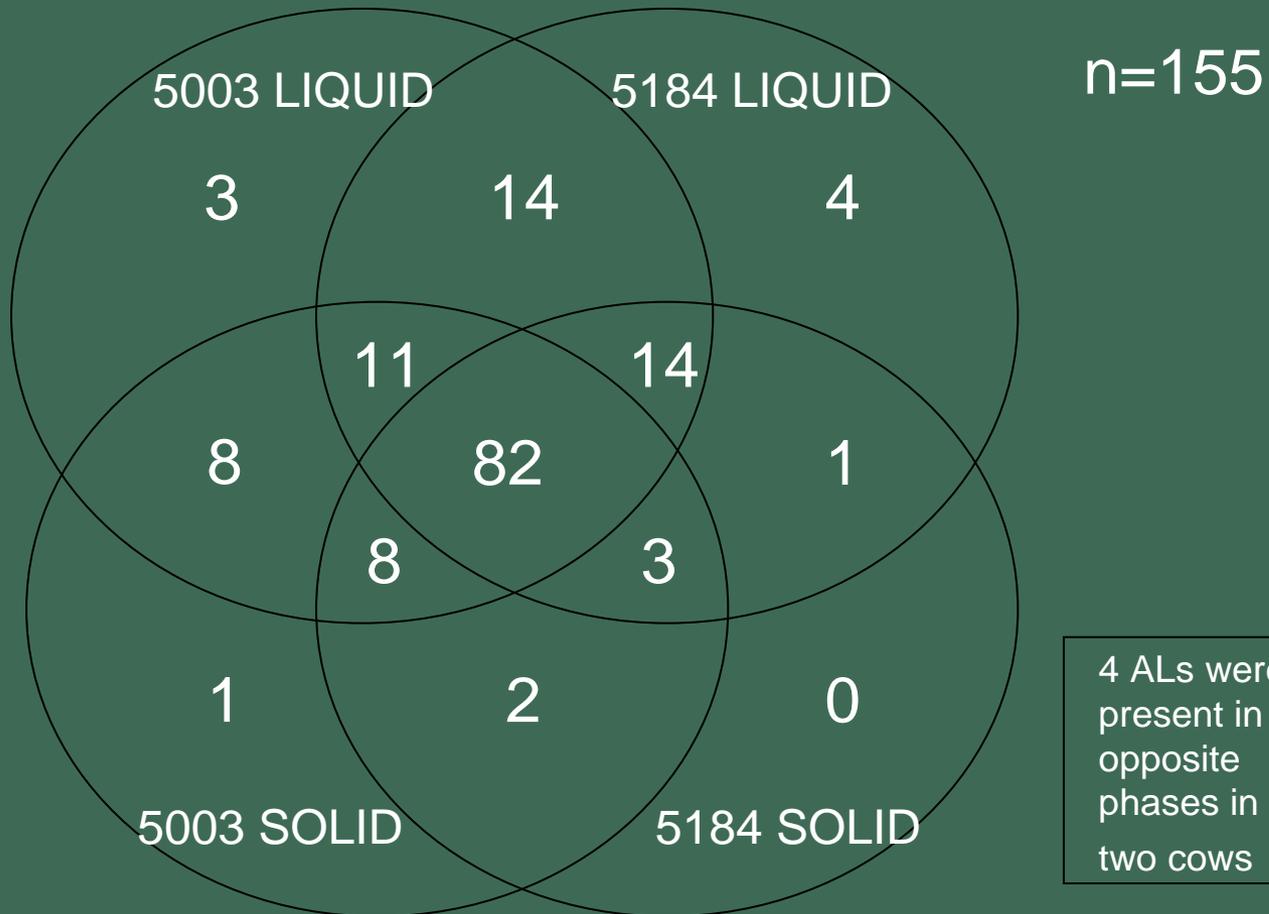
- 2 cows
- Fed same TMR at 12 h intervals
- Sample rumen contents at 2, 4, 6, 9, and 12 h post-feed

Cows were similar in

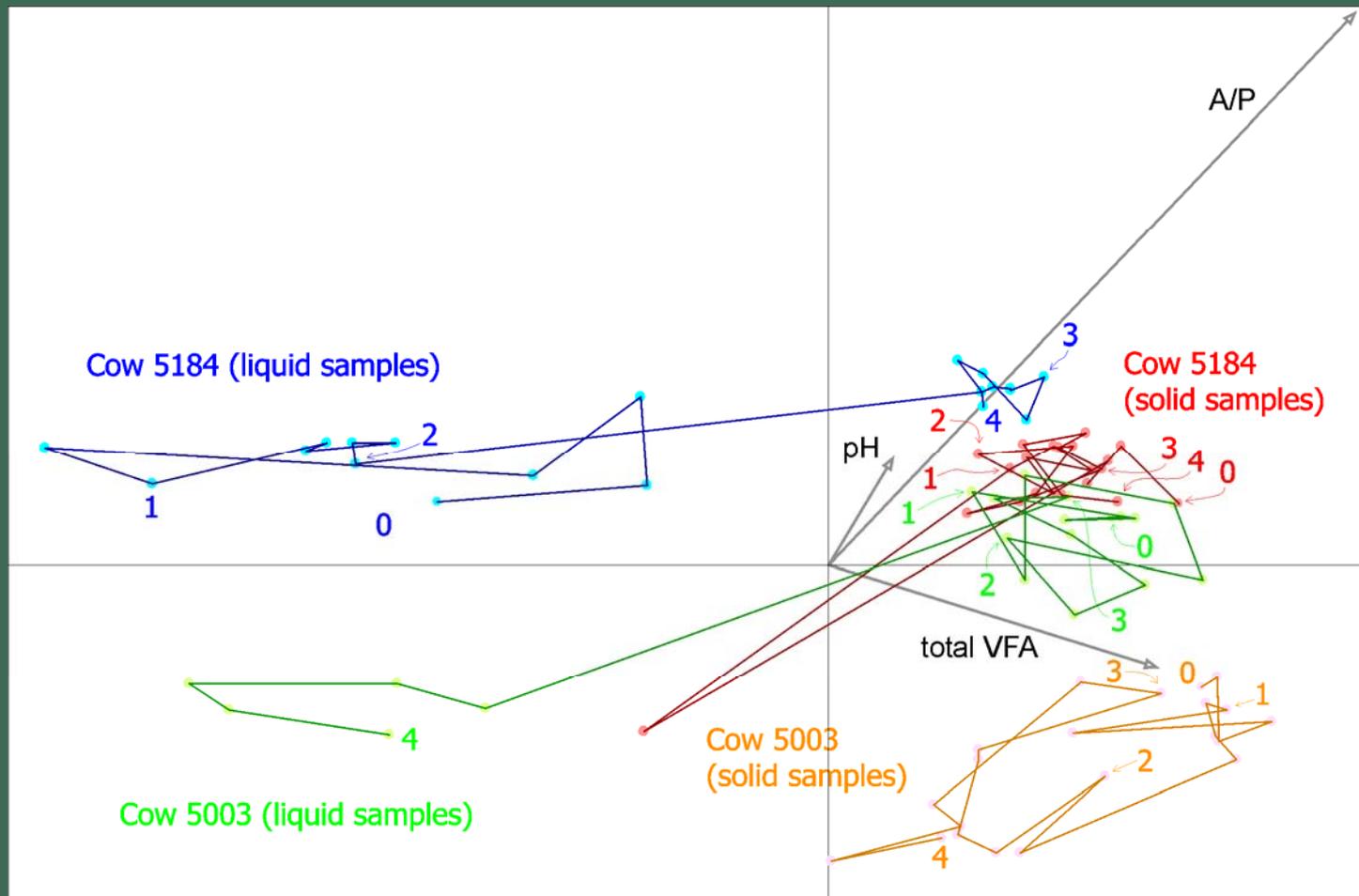
- ruminal pH
- ruminal concentrations of VFA
- milk and milk component yields



Distribution of Amplicon Lengths



Shifts in BCC during feeding cycles varied with cow and phase



So, in cows fed same diet:

- BCC changes during feeding cycle, and across feeding cycles.
- BCC in liquid phase differs from BCC in solid phase.
- BCC differs between cows having similar ruminal chemistry and similar milk yield and composition.

Differences in BCC do not necessarily translate to discernible differences in animal performance.

Suggests that there is substantial functional redundancy within the ruminal bacterial community.

Alternate approach: Identify cows with production differences, then compare BCCs.



II. Milk fat depression (*Elanco*)

- Long considered to have a microbial origin
- Several proposed mechanisms

Experimental strategy:

- Switch 18 cows through TMRs varying in starch source and monensin
 - Period 1: Starch primarily in form of corn silage (“SFS”)
 - Period 2: Starch primarily in form of ground high moisture corn (“RFS”)
 - Period 3: RFS + Monensin (“RFS/Mon”)
 - Period 4: RFS (monensin withdrawn: “RFS/Post”)
- Group cows by milk fat response, then examine BCC

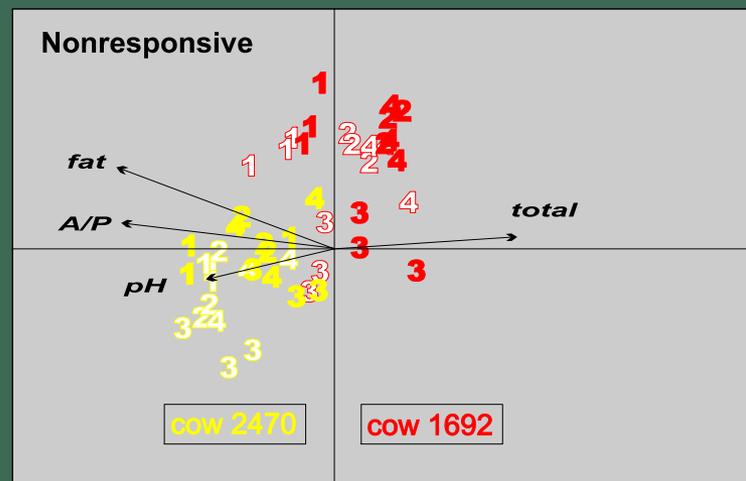
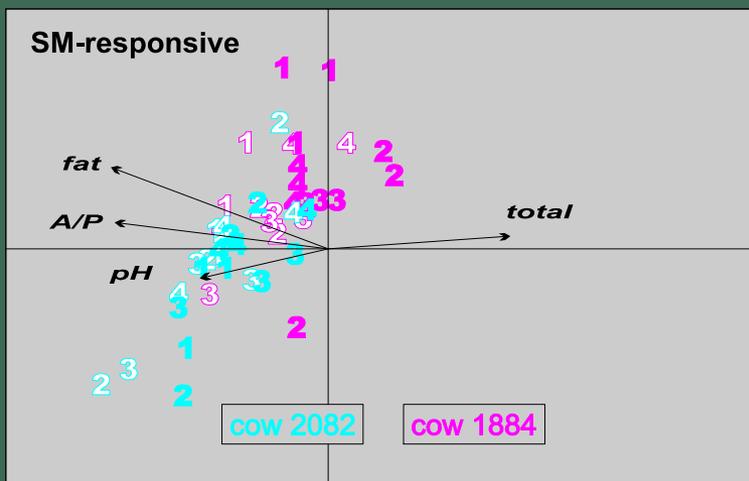
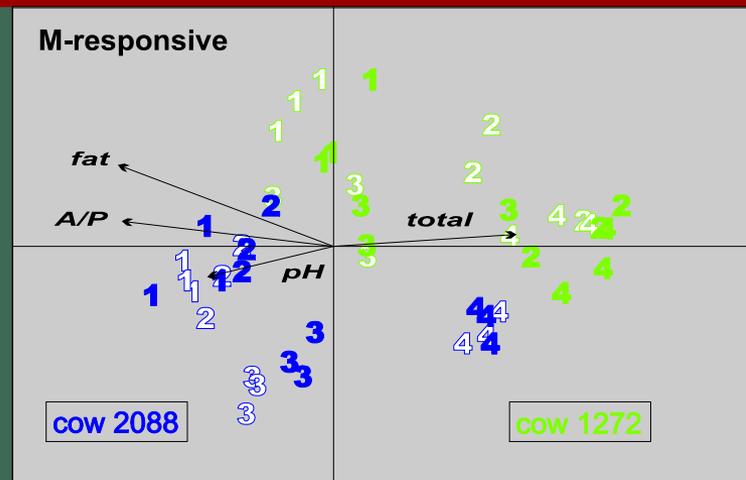
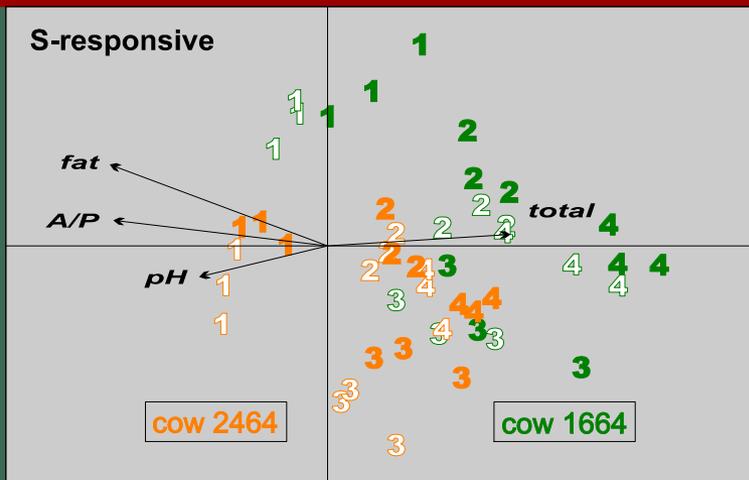


Milk fat response groups

Dietary Treatment	Cow	Group 1 (SM-responsive)		Group 2 (S-responsive)		Group 3 (M-responsive)		Group 4 (Non-responsive)	
		1884	2082	1664	2464	1272	2088	1692	2470
% Fat:									
SFS		4.25	4.27	3.43	3.68	3.61	3.49	3.53	3.76
RFS		3.92	3.80	2.98	2.98	3.65	3.68	3.38	3.47
RFS/Mon		3.51	3.80	2.43	2.80	2.59	2.58	3.59	3.57
RFS/Post		4.01	4.12	2.70	2.63	2.67	2.60	3.51	3.35
Fat yield (kg/d):									
SFS		1.89	1.24	1.72	1.36	1.80	1.47	1.69	1.42
RFS		1.12	0.99	1.04	1.09	1.51	1.01	1.43	1.25
RFS/Mon		1.23	0.86	1.11	1.01	1.03	0.83	1.53	1.32
RFS/Post		1.45	1.03	0.98	0.98	1.49	1.10	1.43	1.52



BCC shifts in response to starch form or monensin



So...

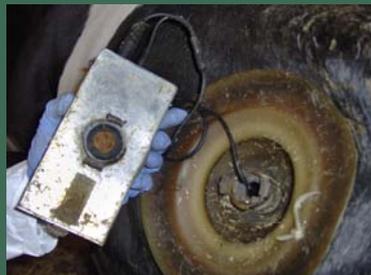
- **Cows displaying similar production response (diet-induced MFD) have different BCC**
- **Within cow, changes in performance are associated with shifts in BCC**

Do differences in BCC among cows hold when we examine cows with natural differences in ruminal chemistry?



III. pH Dynamics

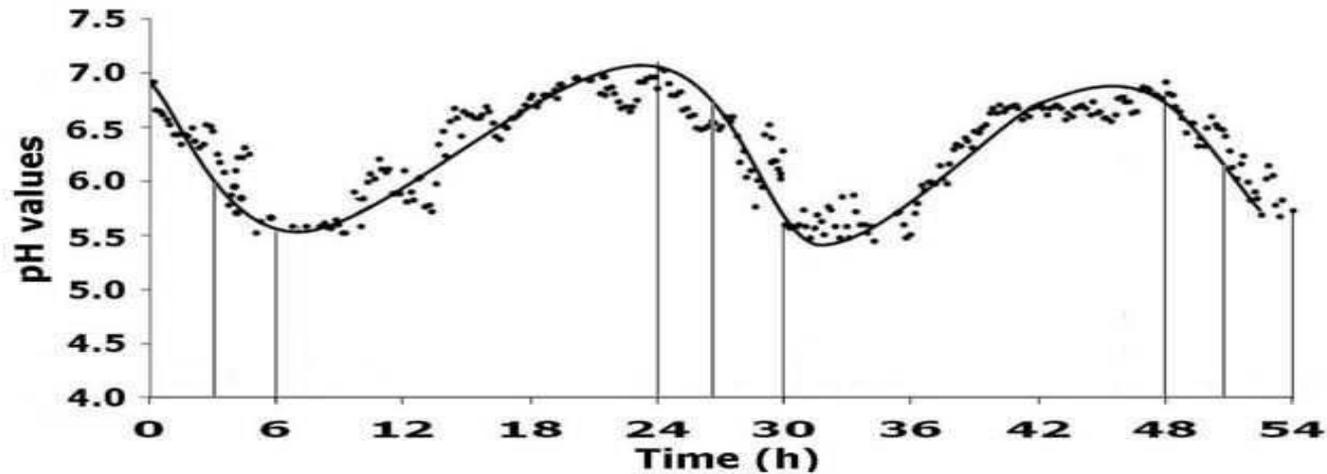
- 8 cows fed TMR once daily
 - corn silage / alfalfa haylage / HMC-based
 - 28.3% aNDF, 17.5% CP
- pH automatically recorded at 10 min intervals over 54 h period



- Ruminal sampling just prior to feeding (0 h) and at 3 h and 6 h postfeeding on 3 successive days



pH Profiles

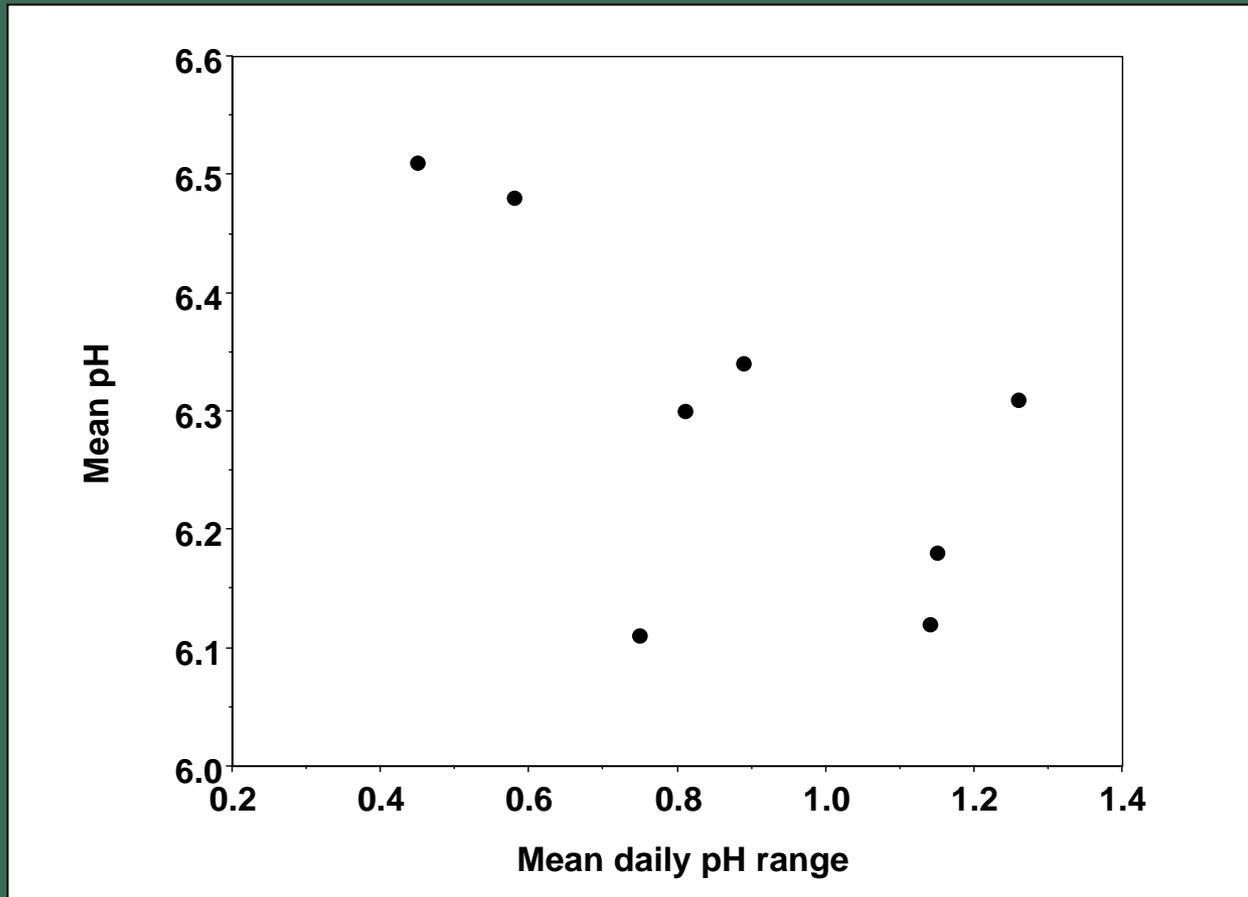


Profiles were used to calculate daily mean pH and daily pH range



Cows differed in pH dynamics

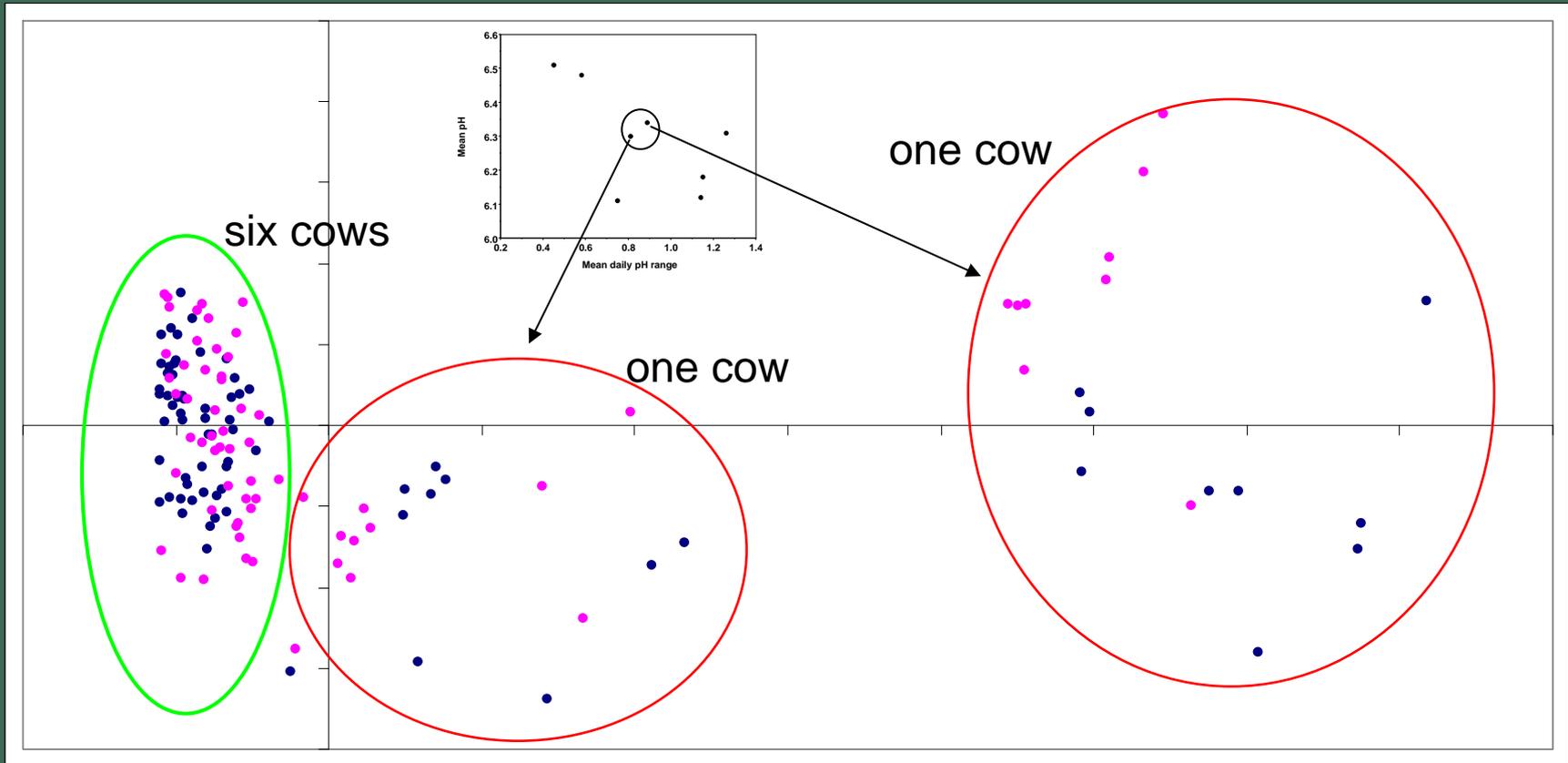
6.11
to
6.51



0.42 to 1.26



Cows differ in bacterial community composition



Differences in BCC associated with differences in milk composition

Yield (kg/d)	Fat (%)	Protein (%)	
40.6	2.8	2.8	
37.0	2.4	2.8	→ 2 cows with different BCC had low fat content
36.2	2.7	2.6	
23.3	3.5	2.8	
41.2	3.4	2.8	
46.1	2.2	2.4	
28.1	5.0	3.9	
<u>22.4</u>	<u>3.8</u>	<u>3.0</u>	
2.2	0.51	0.15	S.E.D.



Which populations differ with MFD status?

Relative ARISA peak area of 2 of the ALs that differed among cows in response to MFD (starch/monensin study) also differed in the pH dynamics study

- **AL246 elevated in low-milkfat cows**
- **AL383 depressed in low-milkfat cows**
- Partial sequencing of 16S rRNA gene of AL246 cloned into an *E. coli* vector. Sequencing of this OTU246 revealed a >98% similarity to a sequence from *Megasphaera* sp.
 - *Megasphaera elsdenii* is one of a few species that has been suggested (controversially) from in vitro studies to be involved in metabolism of long chain fatty acids known to regulate mammary lipogenesis.

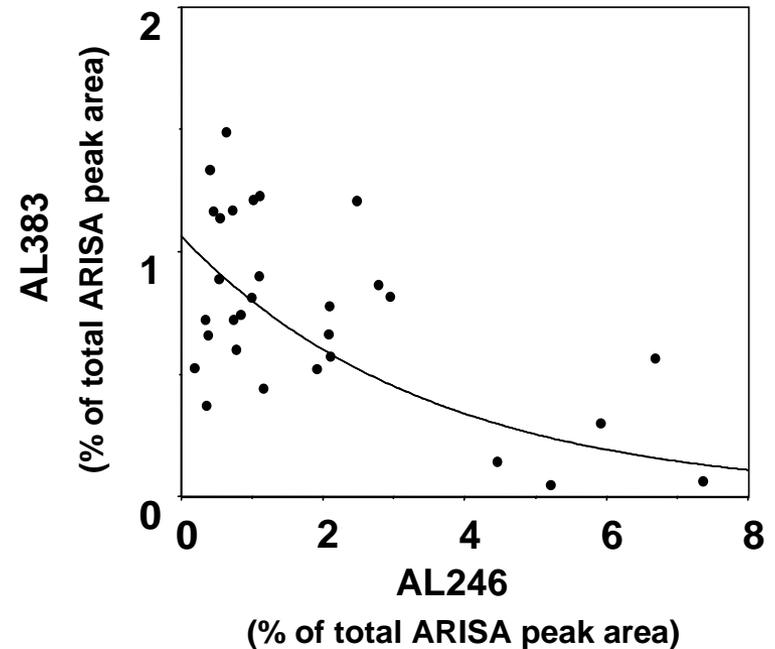
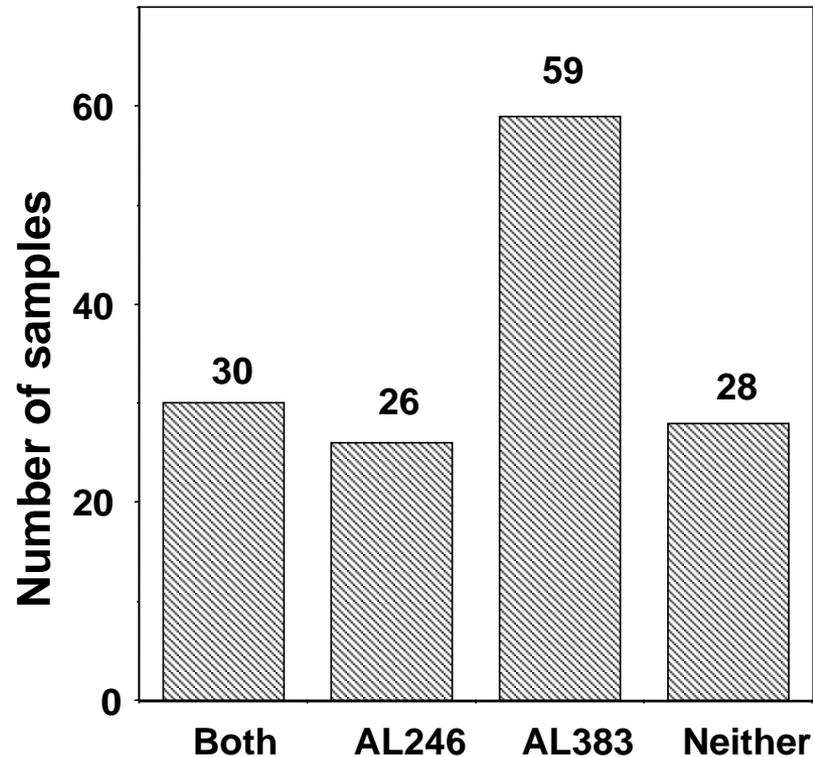


OTU246 populations match those of *Megasphaera elsdenii*

<u>Milk fat status</u>	<u>Cow</u>	<u>% of bacterial 16S rRNA gene copy number</u>	
		<u><i>M. elsdenii</i></u>	<u>OTU246</u>
Fat-depressed	1699	0.320 ± 0.059	0.289 ± 0.054
	2097	1.946 ± 0.759	1.582 ± 0.435
Not fat-depressed	1272	0.018 ± 0.176	0.014 ± 0.012
	2088	0.032 ± 0.024	0.028 ± 0.019



AL246 and AL383 track in opposite directions



IV. Severe perturbation of BCC

Can animal performance be improved by introduction of desirable species into the rumen?

Demonstrated successes all involve filling an empty niche in the rumen.

Any introduced strain would encounter a well-adapted microbial community that is likely to be characteristic of the individual cow.

Can we demonstrate the assumed tight connection between a cow and her microflora?



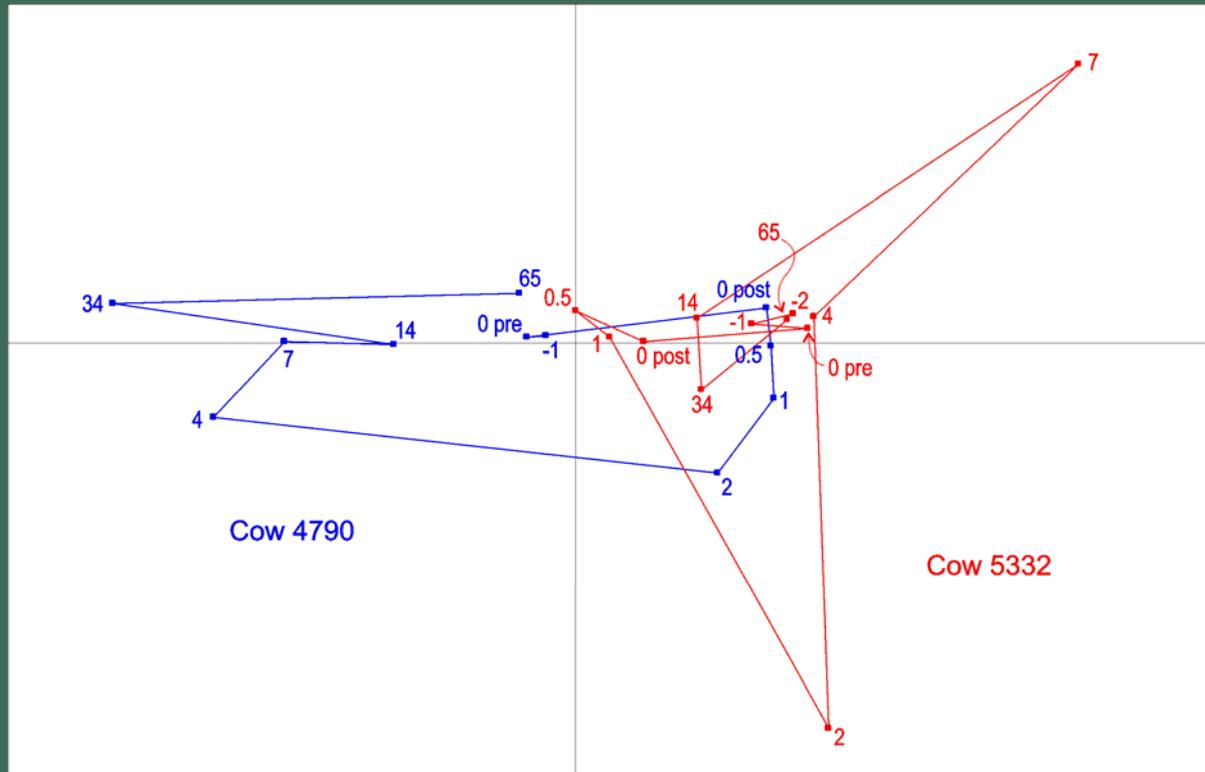
Switching ruminal contents

- 2 cows selected on basis of different ARISA profiles
- Feed CS/AH-based TMR once daily
- Removed >95% of the rumen contents from each cow, exchanged into other cow
- Sampled at intervals to assess changes in BCC

Ruminal pH		
<u>Days</u>	<u>Cow 4790</u>	<u>Cow 5332</u>
-2	6.74	5.73
-1	6.75	6.29
0 Pre	7.05	6.39
0 Post	6.53	7.12
0.5	6.04	5.64
1	6.82	6.14
2	6.91	6.36
4	6.69	6.04
7	6.92	6.54
14	6.85	5.96
34	6.95	6.18
65	6.99	6.47
Mean Pre	6.85	6.14
Mean Post	6.88	6.24



Response of ruminal microflora to nearly complete switch of ruminal contents



Individual cows retain their own unique ruminal bacterial community that resists displacement even by ruminal bacteria that have successfully adapted to other cows.



Our story thus far...

- Cultured species account for only a small fraction of the ruminal bacterial community, and the understanding of the ruminal microbial community will require consideration of the vast uncultured population.
- Individual cows harbor their own unique ruminal bacterial communities.
- Some communities have very similar outputs, suggesting functional redundancy and niche replacement.
- Different production responses can have discernible differences in community composition.
- In some cases we can identify specific taxa associated with specific production responses.

Understanding of differences among these communities may one day provide keys to more targeted feeding of herd members.

