



The effect of fat type and inclusion level on the rumen microbiome and methane in dairy cows

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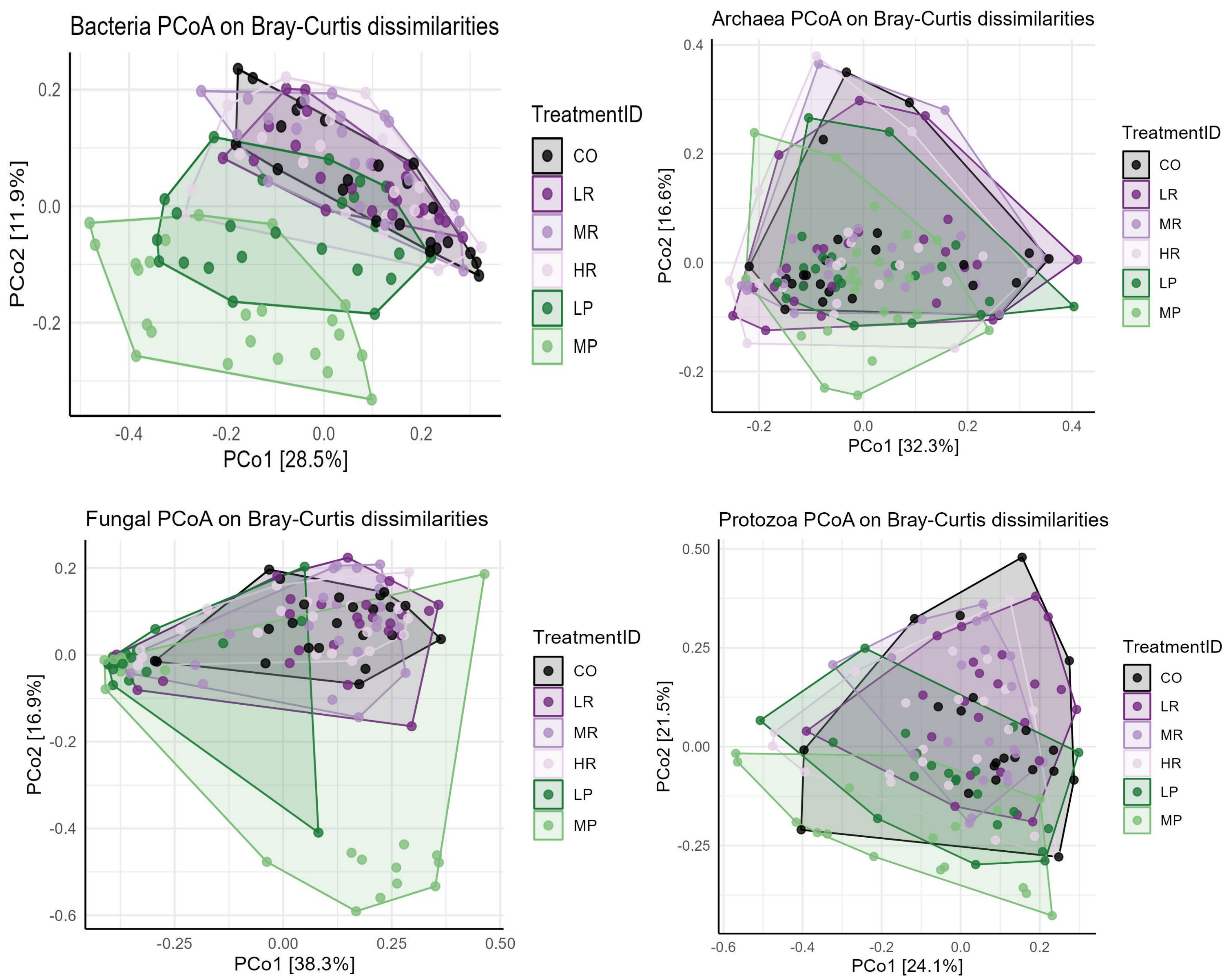
Background

Reducing anthropogenic greenhouse gas emissions is needed to mitigate climate change. Dairy production contributes substantially to agricultural greenhouse gas emissions, particularly through enteric methane (CH₄) produced during ruminal fermentation. Dietary fat supplementation has shown potential as a strategy to reduce enteric CH₄ emissions in dairy cows. Because fat sources differ in their fatty acid (FA) composition, their effects on rumen microbial communities may also vary. However, the impact of different fat types and inclusion levels on the rumen microbiome remains poorly understood. This study investigates the effects of two dietary fat sources fed at three inclusion levels on the rumen microbiome of dairy cows.

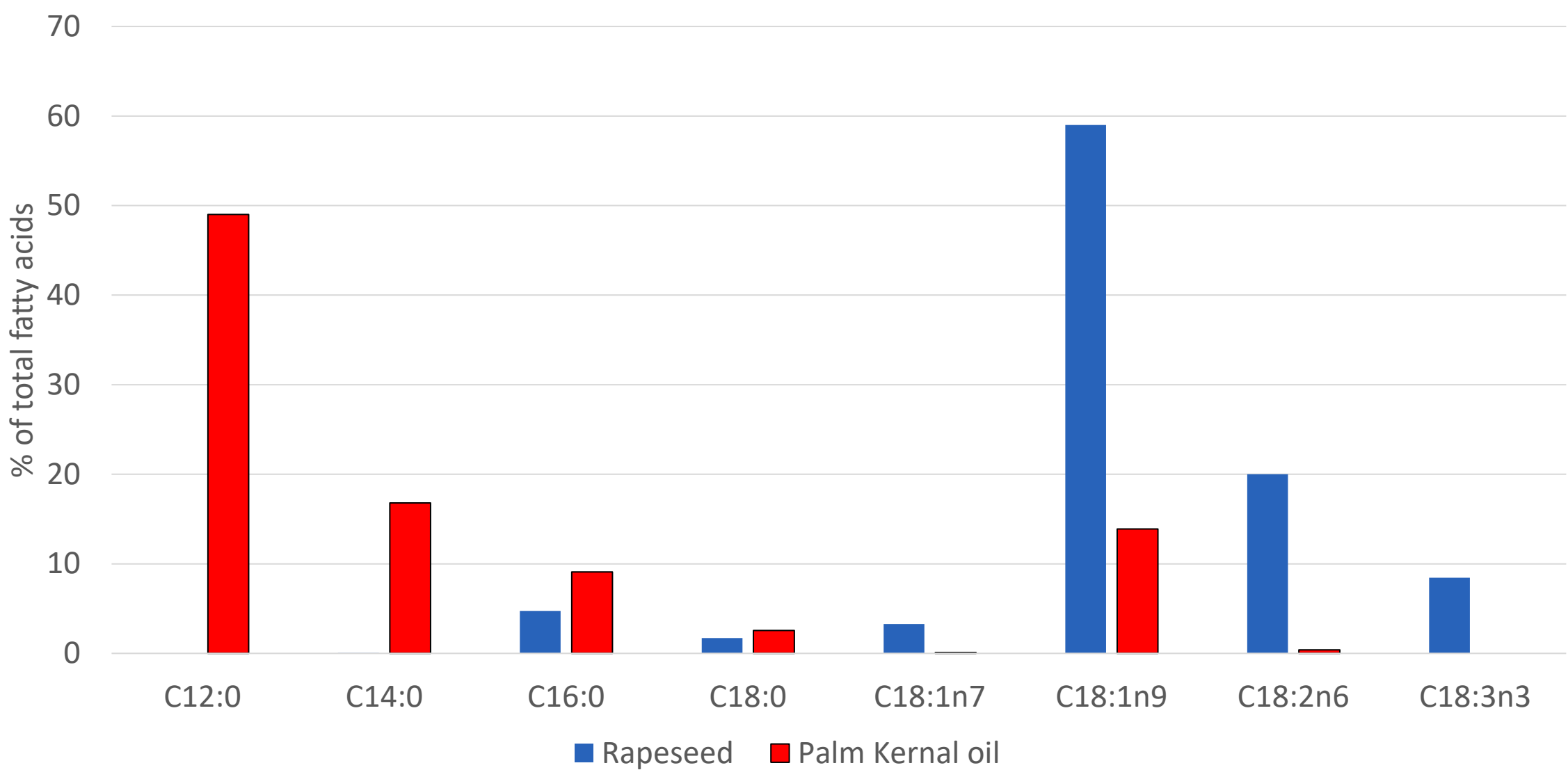
Methods

Using a 6x6 Latin square design, 24 multiparous Holstein cows (4 per treatment) were randomly allocated to 6 dietary treatments across 6 treatment periods of 21 days (N=144 observations). Treatments consisted of a control diet, three levels of rapeseed (**RS**) inclusion (low-**LR**, mid-**MR**, high-**HR**), and two levels of palm kernel oil (**PK**) inclusion (low-**LP**, mid-**MP**). Gas production and milk composition were determined. Rumen samples were collected via an oral sampling device at the end of each period for amplicon sequencing on four microbial target groups, namely; Bacteria, Archaea, Protozoa and Fungi using specific primer sets. Analysis for each microbial target group included microbial composition, diversity, spearman correlation with dietary intake of FA and differential abundance.

Results



Ordination of bacterial (top left) archaeal (top right) fungal (bottom left) and protozoal (bottom right) communities using principal coordinates analysis (PCoA) of Bray-Curtis dissimilarities. Sequence counts were normalized by multiple rarefaction to the minimum library size across samples (100 iterations). Samples are colored by TreatmentID, and the first two axes represent the major sources of variation in community composition.



The fatty acid composition of palm kernel oil (PK) and rapeseed (RS) feed additives

References

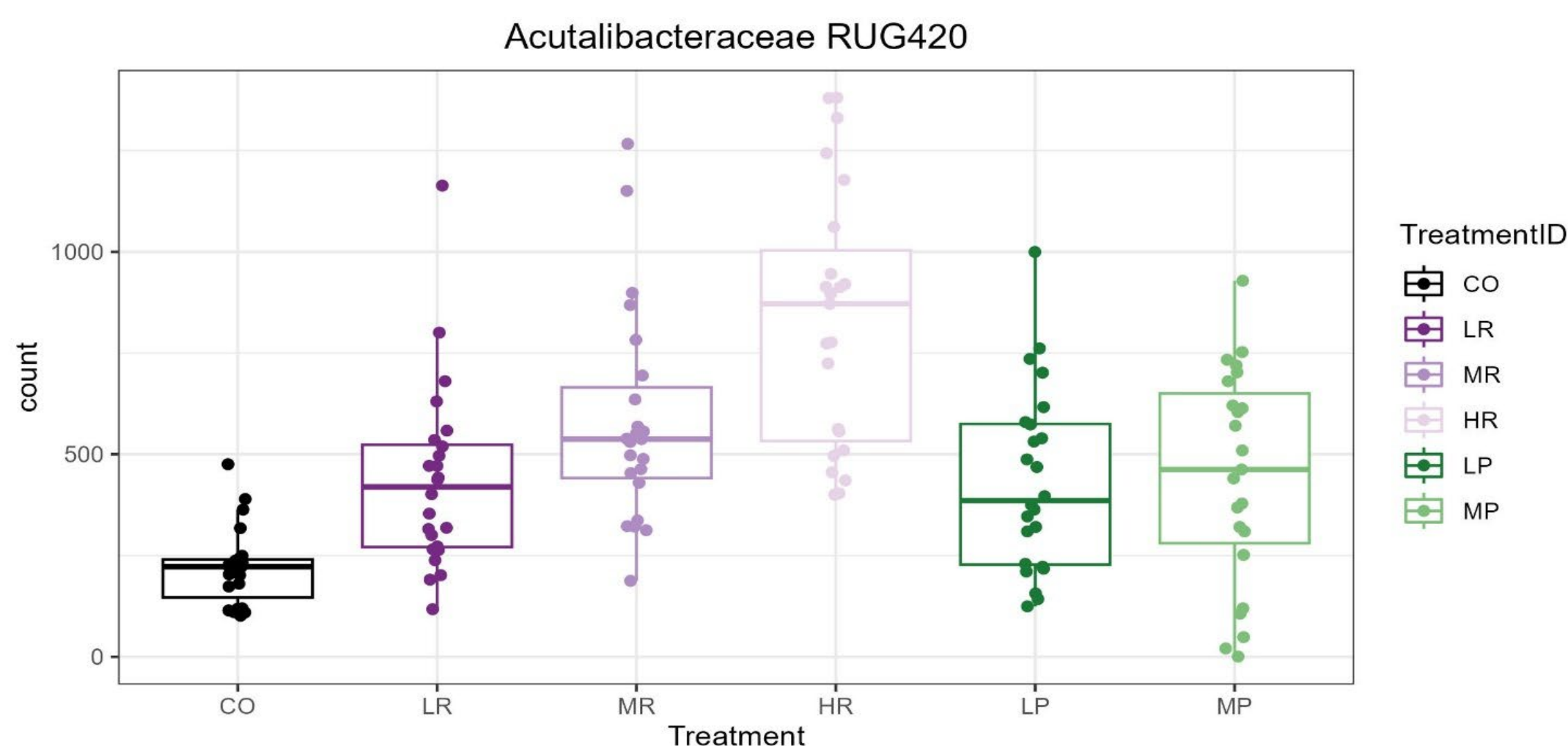
Giagnoni, G., P. Lund, M. Johansen and M. R. Weisbjerg (2025). "Effect of dietary fat source and concentration on feed intake, enteric methane, and milk production in dairy cows." *J Dairy Sci* **108**(1): 553-567.

Results

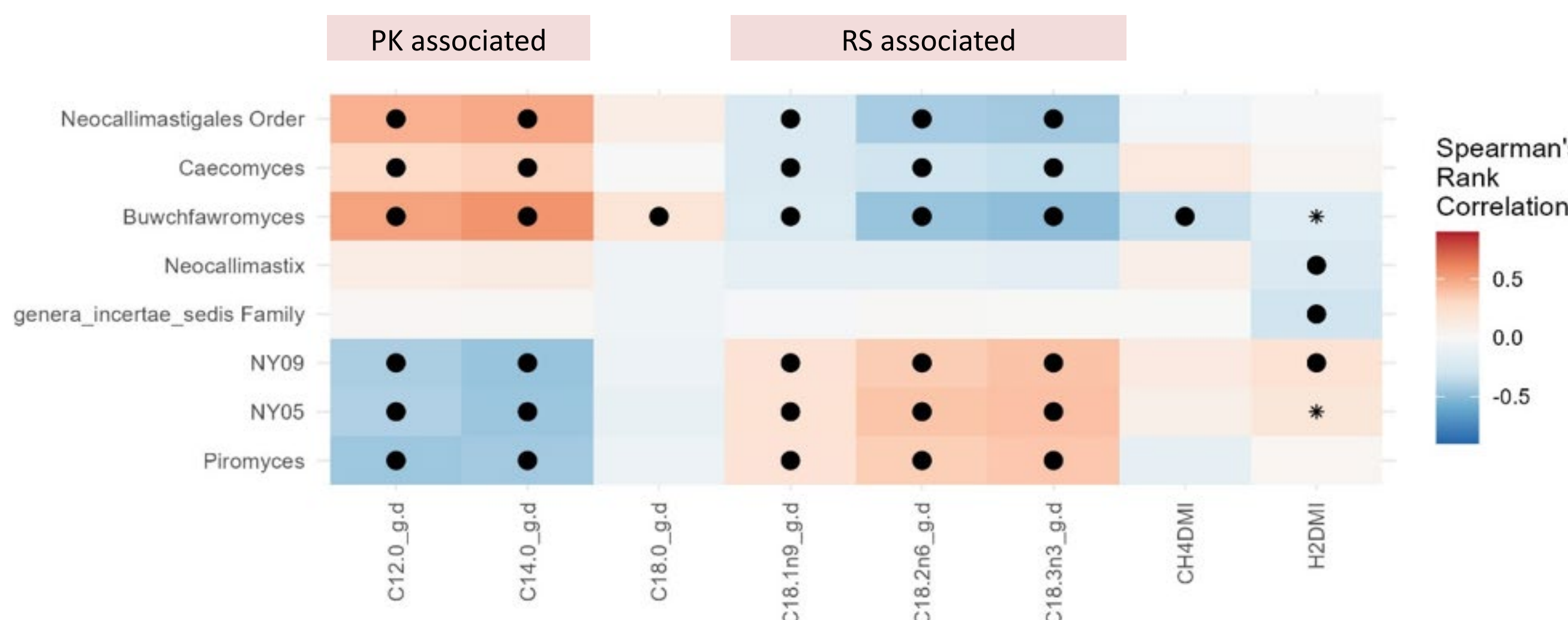
Intake (DMI), milk yield (ECM) and Gas production (g/d), yield (g/kg of DMI) and intensity (g/kg of ECM).

Response	Treatment ^a						P-value ^b			
	CO	LR	MR	HR	LP	MP	RS.L	RS.Q	PK.L	PK.Q
CH ₄ production (g/d)	396 ^d	388 ^d	364 ^c	343 ^b	358 ^{bc}	279 ^a	<0.001	0.21	<0.001	0.001
CH ₄ yield (g/kg of DMI)	16.8 ^c	16.1 ^{bc}	15.7 ^{bc}	15.3 ^{ab}	16.1 ^{bc}	14.1 ^a	<0.001	0.65	<0.001	0.04
CH ₄ intensity (g/kg of ECM)	10.8 ^c	10.4 ^c	9.51 ^{ab}	9.33 ^a	10.1 ^{bc}	9.52 ^{ab}	<0.001	0.70	<0.001	0.90
CO ₂ (g/d)	13808 ^c	13775 ^c	13562 ^{bc}	13257 ^b	13408 ^{bc}	12393 ^a	<0.001	0.24	<0.001	0.02
H ₂ (g/d)	2.32b ^c	2.53 ^c	2.37 ^c	2.32 ^{bc}	1.87 ^{ab}	1.68 ^a	0.73	0.25	<0.001	0.37
ECM yield	37.3 ^{cd}	38.6 ^d	38.9 ^d	37.3 ^{cd}	35.9 ^c	30.6 ^b	0.76	0.003	<0.001	<0.001
DMI	23.7 ^d	24.1 ^d	23.5 ^{cd}	22.5 ^{bc}	22.3 ^b	20.1 ^a	<0.001	0.006	<0.001	0.13

^a CO = control, LR = low rapeseed, MR = medium rapeseed, HR = high rapeseed, LP = low palm kernel, MP = medium palm kernel.
^b RS.L = linear effect of rapeseed supplementation, RS.Q = quadratic effect of rapeseed supplementation, PK.L = linear effect of palm kernel supplementation, PK.Q = quadratic effect of palm kernel supplementation.
(Giagnoni *et.al.* 2025)



Abundance of differentially abundant bacterial genus RUG420 in rumen content. CO = control, LR = low rapeseed, MR = medium rapeseed, HR = high rapeseed, LP = low palm kernel, MP = medium palm kernel. This was the only differential abundant bacterial genus with all levels of RS diets. Low and mid PK had 14 and 41 differentially abundant bacterial genera respectively.



Fungal genus-level Spearman correlations taxa and gas emissions and dietary fatty acid intake variables. Estimates are shown after centered log-ratio transformation of abundance data. Asterisks indicate nominal significance (p < 0.05), while filled circles denote correlations significant after false discovery rate (FDR) correction (p < 0.05). Taxa are ordered by hierarchical clustering of correlation profiles.

Conclusions

- Bacterial and fungal communities responded strongly to fat type, protozoa community had modest shift where archaeal community had a minor shift due to fat type
- Correlations between microbial taxa and dietary FA intake clustered into PK and RS associated FA groups (PK has predominately C12 and C14 FA and RS had predominately C18:1n9 and C18:2n6 FA).
- Mid PK resulted in a greater disruption of the microbiome and the largest drop in CH₄ production but was accompanied by a drop in DMI and ECM resulting in a lower CH₄ intensity reduction than HR.
- Low RS had minimal effects on the rumen microbiome, DMI and ECM but also CH₄.
- Mid and high levels of RS resulted in less changes to the rumen microbiome than PK and reduced CH₄ measures linearly.