The trade-off between enteric and manure methane emissions and their bacterial ecology in lactating cows fed diets varying in forage to concentrate ratio and rapeseed oil

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Supplementary material

Supplemental Table S1. Effects of forage to concentrate ratio and dietary rapeseed oil supplement on alpha diversity of ruminal and fecal bacteria

	Diversity estimate	Treatment[1]					*P*-value[2]		
		HF	HFO	LF	LFO	SEM	FC	RO	FC×RO
Rumen	Shannon	5.78	5.52	5.69	5.63	0.151	0.95	0.29	0.50
	Simpson	0.996	0.994	0.996	0.996	0.001	0.53	0.27	0.38
	Observed ASVs	439	374	400	379	378.8	0.69	0.34	0.61
Feces[3]	Shannon	5.55	5.41	5.65	5.61	0.085	0.05	0.20	0.47
	Simpson	0.995	0.994	0.996	0.996	0.001	0.09	0.25	0.41
	Observed ASVs	335	301	372	352	21.3	0.03	0.13	0.64
Feces, BMP	Shannon	5.38	5.32	5.41	5.39	0.051	0.30	0.40	0.58
	Simpson	0.994	0.993	0.994	0.994	0.001	0.27	0.11	0.27
	Observed ASVs	306	309	321	323	20.4	0.42	0.90	0.97
Feces, static	Shannon	5.27	5.24	5.59	5.32	0.086	0.05	0.12	0.20
	Simpson	0.993	0.993	0.996	0.994	0.001	0.04	0.28	0.27
	Observed ASVs	275	257	357	272	31.2	0.14	0.12	0.28

[1] Refers to diets based on high (0.65) or low (0.35) forage ratio supplemented with 0 (HF and LF, respectively) or 5.0% (HFO and LFO, respectively) of rapeseed oil on DM basis. Values are LS means and pooled SEM for n = 4.

[2] FC, effect of forage to concentrate ratio in the diet; RO, effect of rapeseed oil supplement; FC × RO, interaction of FC and RO.

[3] Feces refer to feces collected from animals; Feces, BMP, biochemical methane potential; Feces, static, *in vitro* incubation for 75 days without using inoculum, mixing and $NaHCO_3$ buffer under temperature of 25°C.

Supplemental Table S2. Effects of forage to concentrate ratio and dietary rapeseed oil supplement on abundance (%) of ruminal bacteria

	Treatment[1]					*P*-value[2]		
Bacterial taxa	HF	HFO	LF	LFO	SEM	FC	RO	FC×RO
Bacteroidales RF16 group	3.0	2.2	1.4	0.85	0.38	0.007	0.48	0.94
Paraprevotella	1.0	0.77	1.8	1.7	0.3	0.011	0.80	0.96
Prevotellaceae UCG-001	3.2	2.7	3.6	3.6	0.2	0.038	0.31	0.34
Fibrobacter	1.3	1.5	1.4	0.52	0.24	0.023	0.28	0.026
Christensenellaceae R-7 group	4.1	4.1	2.1	2.8	0.6	0.008	0.50	0.12
Saccharofermentans	2.0	1.9	1.6	1.0	0.1	0.003	0.37	0.31
Acetitomaculum	1.5	1.4	3.1	2.5	0.4	0.005	0.99	0.78
[Ruminococcus] gauvreauii group	0.72	0.74	1.3	1.4	0.18	0.004	0.07	0.66
Ruminococcaceae spp.	0.55	0.29	1.4	1.5	0.30	0.004	0.67	0.28
WCHB1-41	2.0	1.2	0.53	0.72	0.23	0.004	0.41	0.056
Succiniclasticum	1.0	1.3	1.4	1.4	0.1	0.037	0.011	0.50
Candidatus Saccharimonas	1.6	0.74	0.90	0.27	0.16	0.005	0.010	0.69
Bacteroidales g F082	1.4	1.6	1.3	1.9	0.3	0.44	0.002	0.29
Clostridia UCG-014	2.1	1.1	3.3	1.1	0.3	0.21	0.008	0.58

[1] Refers to diets based on high (0.65) or low (0.35) forage ratio supplemented with 0 (HF and LF, respectively) or 5.0% (HFO and LFO, respectively) of rapeseed oil on DM basis. Values are LS means and pooled SEM for n = 4

[2] FC, effect of forage to concentrate ratio in the diet; RO, effect of rapeseed oil supplement; FC × RO, interaction of FC and RO.

Supplemental Table S3. Association between relative abundance of rumen bacteria at genus level and enteric methane production calculated as methane intensity (g/kg milk or ECM)[1]

Bacterial taxa	CH_4/milk (g/kg)	CH_4/ECM (g/kg)
Bacteroidales RF16 group	0.64	0.67
Rikenellaceae RC9 gut group	0.52	0.45
Saccharofermentans	0.62	0.54
Succiniclasticum	-0.70	-0.62
Candidatus Saccharimonas	0.75	0.67
Succinivibrionaceae UCG-002	-0.69	-0.66

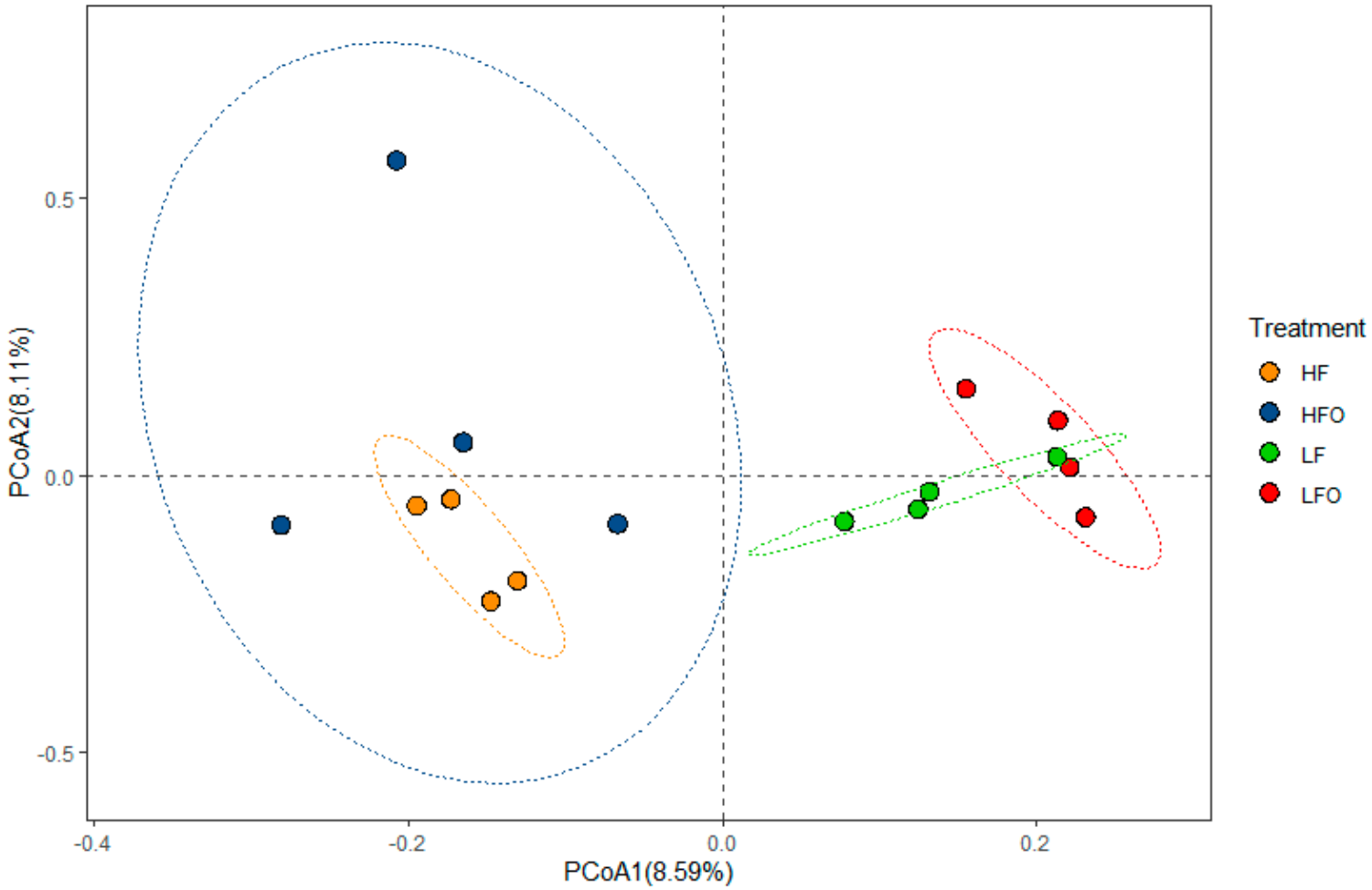
[1]The values in the table show Spearman's correlation coefficients. Only significant ($P < 0.05$) correlations are presented.

Supplemental Table S4. Association between relative abundance of faecal bacteria at genus level and enteric methane production from static manure incubation experiment. Methane output was calculated as g/d. [1]

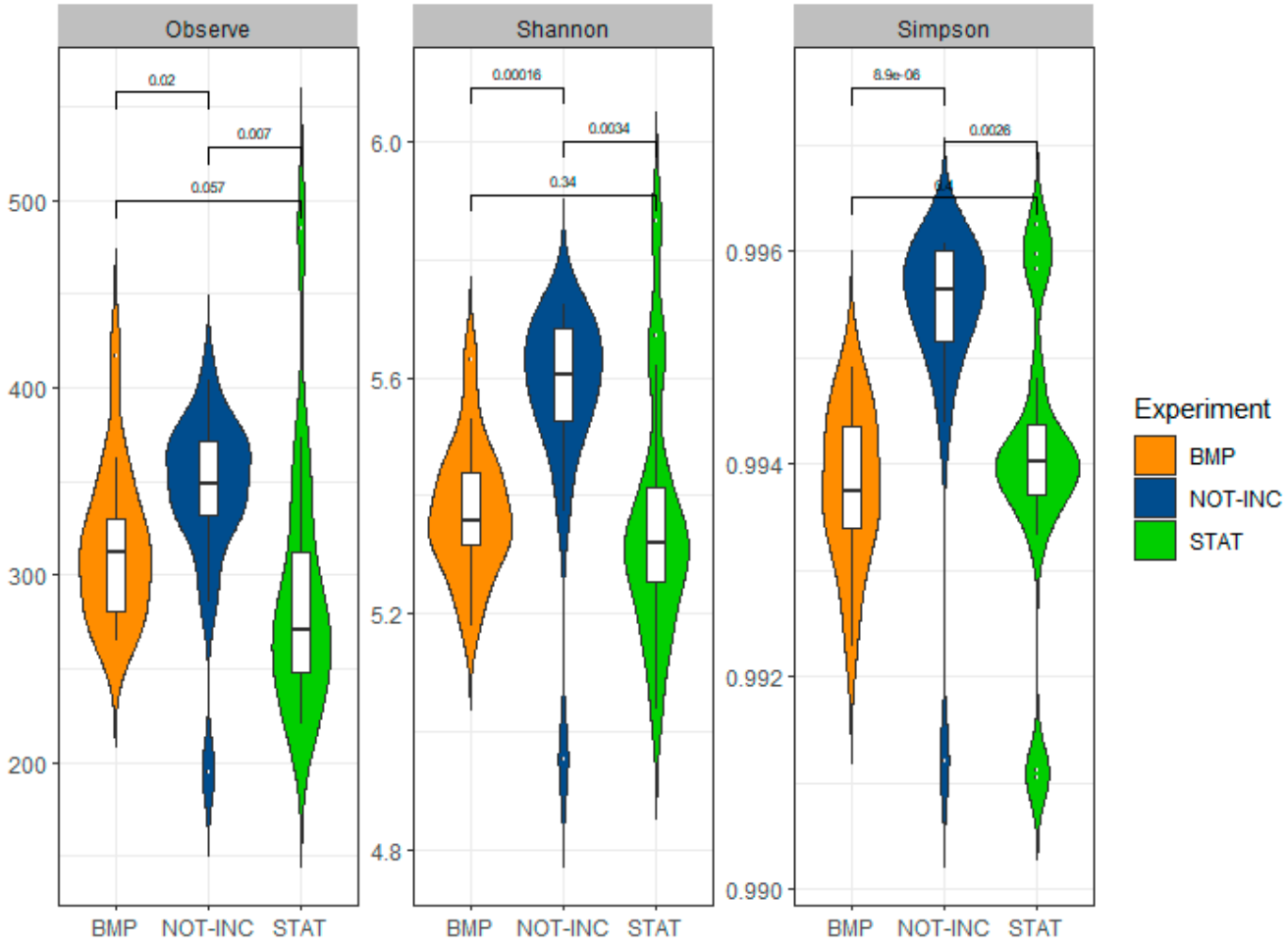
Bacterial taxa	CH_4 (g/d)
Methanosarcina	0.91
Muribaculaceae	-0.59
Prevotellaceae UCG-004	-0.58
Alistipes	-0.67
Rikenellaceae RC9 gut group	-0.54
Izemoplasmatales	0.78
Clostridia vadinBB60 group	0.83
Clostridium sensu stricto 1	-0.31
Oscillospiraceae genus UCG-005	-0.63
Romboutsia	-0.77
Treponema	-0.64

[1]The values in the table show Spearman's correlation coefficients. Only significant ($P < 0.05$) correlations are presented.

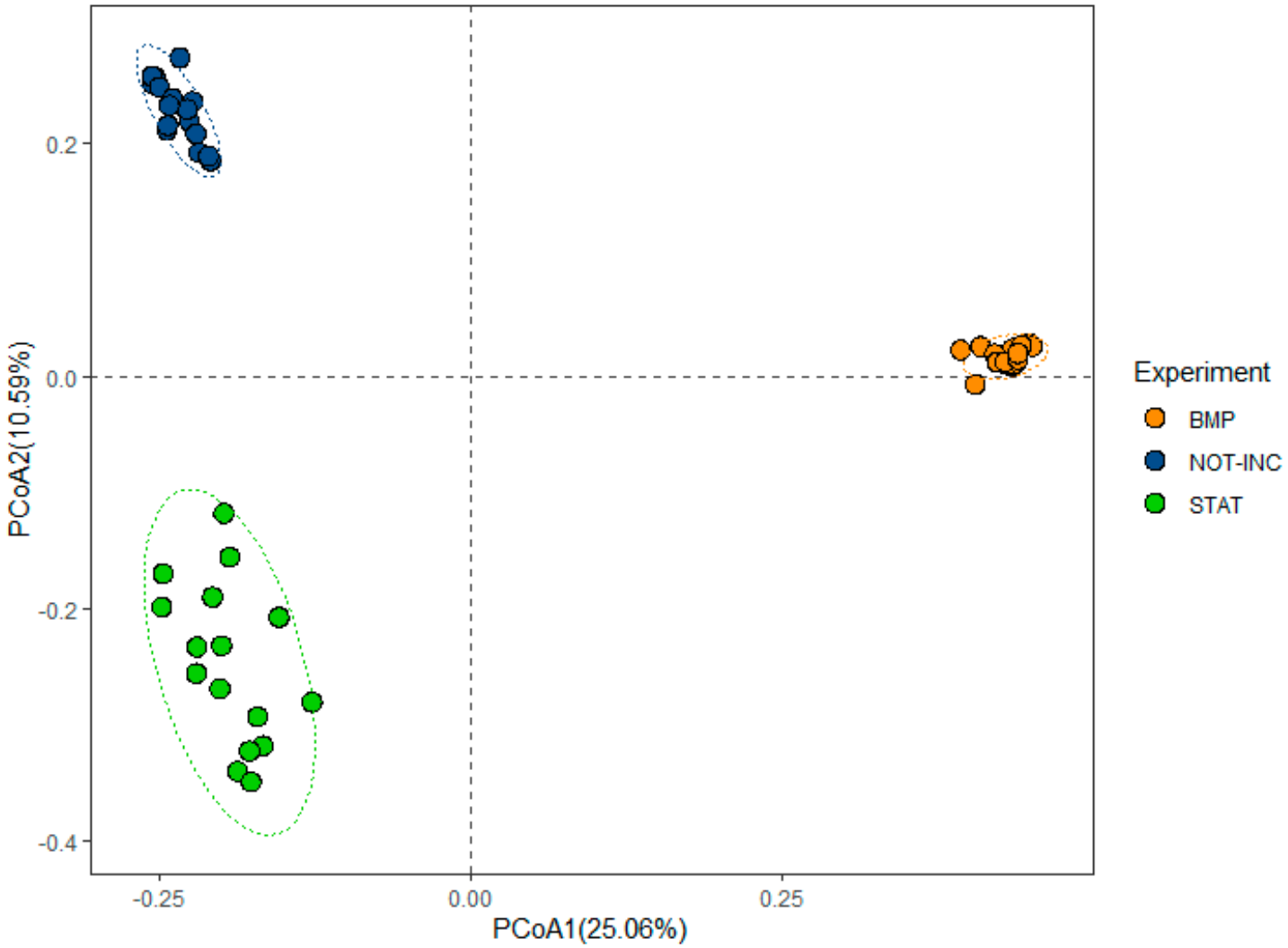
Supplemental Figure S1. Effects of forage to concentrate ratio and dietary rapeseed oil supplement on beta diversity of ruminal bacteria. Treatments refer to diets based on high (0.65) or low (0.35) forage ratio supplemented with 0 (HF and LF, respectively) or 5.0% (HFO and LFO, respectively) of rapeseed oil on DM basis.



Supplemental Figure S2. Alpha diversity estimates for fecal samples before incubation experiment (NOT-INC) and after manure BMP (BMP), and static (STAT) incubation trials.



Supplemental Figure S3. Beta diversity for fecal samples before incubation experiment (NOT-INC) and after manure BMP (BMP), and static (STAT) incubation trials.



Supplemental Figure S4. Effects of forage to concentrate ratio and dietary rapeseed oil supplement on beta diversity of fecal bacteria before incubation (A), after BMP (B) and static manure incubation experiments (C). Treatments refer to diets based on high (0.65) or low (0.35) forage ratio supplemented with 0 (HF and LF, respectively) or 5.0% (HFO and LFO, respectively) of rapeseed oil on DM basis