

SUPPLEMENTARY MATERIAL

Rumen bacterial community responses to DPA, EPA and DHA in cattle and sheep

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Supplementary Table S1. Multivariate analysis of variance (MANOVA) conducted with relative abundances of operational taxonomic units (OTU) detected after 24 h of in vitro incubation with rumen inocula of cattle and sheep¹.

Pairwise comparison	<i>P</i> -value ²	
	Sheep	Cattle
Control vs. DPA	0.232	0.030
Control vs. EPA	0.020	0.024
Control vs. DHA	0.012	0.012
DPA vs. EPA	0.048	0.014
DPA vs. DHA	0.014	0.016
EPA vs. DHA	0.367	0.333

¹The incubated substrate was a total mixed ration containing no additional PUFA (control) or supplemented with 2% dry matter of docosapentaenoic acid (DPA), eicosapentaenoic acid (EPA), or docosahexaenoic acid (DHA).

²Adjusted for multiple comparisons using Benjamini and Hochberg's method. The *P*-values for the fixed effects were as follows: ruminant species, 0.001; PUFA treatment, 0.096; and their interaction, 0.559.

Supplementary Table S2. Relative frequencies (% of total sequences, non-transformed values) of bacterial 16S rRNA gene sequences of relevant phyla, families and genera after 24 h of in vitro incubation with rumen inocula of cattle and sheep¹. Results from the statistical analysis (log-transformed values) are reported in Table 1.

Taxonomic identification	Species		PUFA treatment			
	Cattle	Sheep	Control	DPA	EPA	DHA
<i>Bacteroidetes</i>	52.3	56.7	55.6	55.8	51.2	55.6
Unclassified	35.0	37.8	35.2	37.2	33.9	39.4
<i>Prevotellaceae</i>	14.2	13.1	16.9	14.7	12.2	10.8
Unclassified	5.28	4.31	5.13	5.10	4.65	4.21
<i>Prevotella</i>	7.02	6.85	10.29	7.66	5.32	4.48
<i>Hallella</i>	0.40	1.77	0.94	1.05	1.17	1.19
<i>Paraprevotella</i>	1.50	0.13	0.52	0.78	1.05	0.90
<i>Porphyromonadaceae</i>	2.06	5.22	2.78	3.13	4.24	4.42
Unclassified	0.34	4.58	2.08	2.18	2.76	2.81
<i>Barnesiella</i>	1.69	0.57	0.63	0.89	1.43	1.57
<i>Tannerella</i>	0.03	0.08	0.08	0.05	0.05	0.04
<i>Bacteroidales inc. sed.</i>	0.89	0.27	0.42	0.53	0.64	0.73
<i>Phocaeicola</i>	0.89	0.27	0.42	0.53	0.64	0.73
<i>Firmicutes</i>	19.9	16.4	17.9	17.7	18.9	18.1
Unclassified	7.73	8.03	7.34	7.69	8.35	8.15
<i>Ruminococcaceae</i>	6.72	4.18	5.84	5.38	5.49	5.09
Unclassified	6.24	3.54	5.32	4.84	4.88	4.53
<i>Ruminococcus</i>	0.19	0.09	0.11	0.11	0.19	0.16
<i>Oscillibacter</i>	0.04	0.33	0.20	0.20	0.18	0.16
<i>Lachnospiraceae</i>	3.64	2.01	3.19	2.69	2.79	2.62
Unclassified	1.73	1.33	1.55	1.54	1.57	1.46
<i>Butyrivibrio</i>	1.32	0.30	1.07	0.71	0.74	0.71
<i>Pseudobutyrvibrio</i>	0.36	0.30	0.40	0.30	0.32	0.30
<i>Roseburia</i>	0.03	0.01	0.01	0.01	0.03	0.02
<i>Veillonellaceae</i>	0.35	0.54	0.35	0.45	0.53	0.44
Unclassified	0.05	0.51	0.25	0.33	0.30	0.25

<i>Megasphaera</i>	0.11	0.00	0.01	0.03	0.11	0.07
<i>Selenomonas</i>	0.05	0.01	0.02	0.03	0.04	0.03
<i>Anaerovibrio</i>	0.08	0.02	0.04	0.05	0.06	0.05
<i>Acidaminococcaceae</i>	0.57	0.72	0.50	0.61	0.72	0.76
<i>Succiniclasticum</i>	0.57	0.72	0.49	0.61	0.72	0.76
<i>Clostridiales inc. sed. XIII</i>	0.64	0.41	0.39	0.48	0.64	0.59
<i>Anaerovorax</i>	0.46	0.27	0.29	0.32	0.44	0.41
<i>Proteobacteria</i>	10.4	4.27	5.66	5.82	10.2	7.56
Unclassified	5.17	1.68	3.04	3.15	4.06	3.45
<i>Succinivibrionaceae</i>	4.77	2.31	2.20	2.14	5.94	3.89
<i>Succinivibrio</i>	0.37	0.04	0.12	0.16	0.32	0.23
<i>Ruminobacter</i>	4.29	1.82	1.85	1.73	5.28	3.36
<i>Tenericutes</i> ²	4.56	5.43	7.81	5.31	4.46	2.38
<i>Anaeroplasma</i>	4.55	5.39	7.78	5.28	4.44	2.36
<i>Fibrobacteres</i> ³	2.03	2.24	1.15	1.79	2.63	2.97
<i>Spirochaetes</i>	2.00	2.18	1.78	2.02	2.19	2.38
<i>Spirochaetaceae</i>	1.96	2.04	1.67	1.92	2.10	2.32
<i>Sphaerochaeta</i>	1.30	1.55	1.07	1.35	1.54	1.74
<i>Synergistetes</i> ⁴	0.82	0.67	0.35	0.60	0.94	1.09
<i>Jonquetella</i>	0.80	0.63	0.34	0.58	0.91	1.04
Other phyla ⁵	3.04	1.24	2.36	2.21	1.84	2.15

¹The incubated substrate was a total mixed ration containing no additional PUFA (control) or supplemented with 2% dry matter of docosapentaenoic acid (DPA), eicosapentaenoic acid (EPA), or docosahexaenoic acid (DHA).

²The *Anaeroplasmataceae* family comprises > 99% of sequences within this phylum.

³The *Fibrobacter* genus comprises > 99% of sequences within this phylum.

⁴The *Synergistaceae* family comprises > 99% of sequences within this phylum.

⁵Sum of SR1, *Elusimicrobia*, *Lentisphaerae*, *Candidatus Saccharibacteria*, *Chloroflexi*, *Actinobacteria*, *Cyanobacteria/Chloroplast*, *Verrucomicrobia*, *Armatimonadetes* and *Fusobacteria*.

Supplementary Table S3. Relative frequencies (% of total sequences, non-transformed values) of bacterial 16S rRNA gene sequences of relevant phyla, families and genera in the rumen inocula of cattle and sheep (n = 3).

Taxonomic identification	Cattle		Sheep	
	Mean	SEM ¹	Mean	SEM ¹
<i>Bacteroidetes</i>	59.6	3.59	61.3	0.99
Unclassified	42.8	0.79	33.0	1.50
<i>Prevotellaceae</i>	14.8	2.86	21.2	1.21
Unclassified	6.47	0.409	6.22	0.243
<i>Prevotella</i>	6.87	2.509	13.1	1.23
<i>Hallella</i>	1.08	0.034	0.11	0.022
<i>Paraprevotella</i>	0.34	0.058	1.73	0.170
<i>Porphyromonadaceae</i>	1.25	0.035	6.27	0.276
Unclassified	0.17	0.014	5.67	0.286
<i>Barnesiella</i>	1.05	0.058	0.52	0.076
<i>Tannerella</i>	0.03	0.025	0.06	0.012
<i>Bacteroidales inc. sed.</i>	0.48	0.099	0.30	0.029
<i>Phocaeicola</i>	0.48	0.099	0.30	0.029
<i>Firmicutes</i>	25.3	1.57	17.3	0.75
Unclassified	9.44	1.185	7.69	0.390
<i>Ruminococcaceae</i>	11.4	0.21	5.33	0.427
Unclassified	10.7	0.16	4.28	0.366
<i>Ruminococcus</i>	0.30	0.042	0.17	0.016
<i>Oscillibacter</i>	0.06	0.009	0.56	0.047
<i>Lachnospiraceae</i>	2.56	0.148	1.70	0.170
Unclassified	1.34	0.066	1.25	0.096
<i>Butyrivibrio</i>	0.79	0.087	0.17	0.046
<i>Pseudobutyrvibrio</i>	0.23	0.046	0.19	0.030
<i>Roseburia</i>	0.02	0.017	0.01	0.000
<i>Veillonellaceae</i>	0.67	0.271	0.72	0.066
Unclassified	0.08	0.028	0.65	0.074
<i>Megasphaera</i>	0.06	0.019	-	-
<i>Selenomonas</i>	0.21	0.115	0.04	0.012
<i>Anaerovibrio</i>	0.24	0.129	0.03	0.005
<i>Acidaminococcaceae</i>	0.30	0.114	0.81	0.078
<i>Succiniclasticum</i>	0.30	0.114	0.80	0.081
<i>Clostridiales inc. sed. XIII</i>	0.69	0.047	0.47	0.064
<i>Anaerovorax</i>	0.36	0.039	0.28	0.033
<i>Proteobacteria</i>	6.69	1.649	4.93	0.517
Unclassified	5.27	1.394	3.07	0.168
<i>Succinivibrionaceae</i>	0.78	0.130	1.48	0.353
<i>Succinivibrio</i>	0.33	0.060	0.37	0.361
<i>Ruminobacter</i>	0.41	0.183	0.44	0.299
<i>Tenericutes</i> ²	0.56	0.120	1.51	0.620
<i>Anaeroplasma</i>	0.55	0.118	1.47	0.614
<i>Fibrobacteres</i> ³	0.82	0.111	0.98	0.108
<i>Spirochaetes</i>	0.53	0.067	0.98	0.197
<i>Spirochaetaceae</i>	0.50	0.068	0.94	0.190
<i>Sphaerochaeta</i>	0.39	0.063	0.80	0.174

<i>Synergistetes</i> ⁴	0.12	0.003	0.30	0.048
<i>Jonquetella</i>	0.12	0.003	0.29	0.042
Other phyla ⁵	2.74	0.682	1.10	0.217

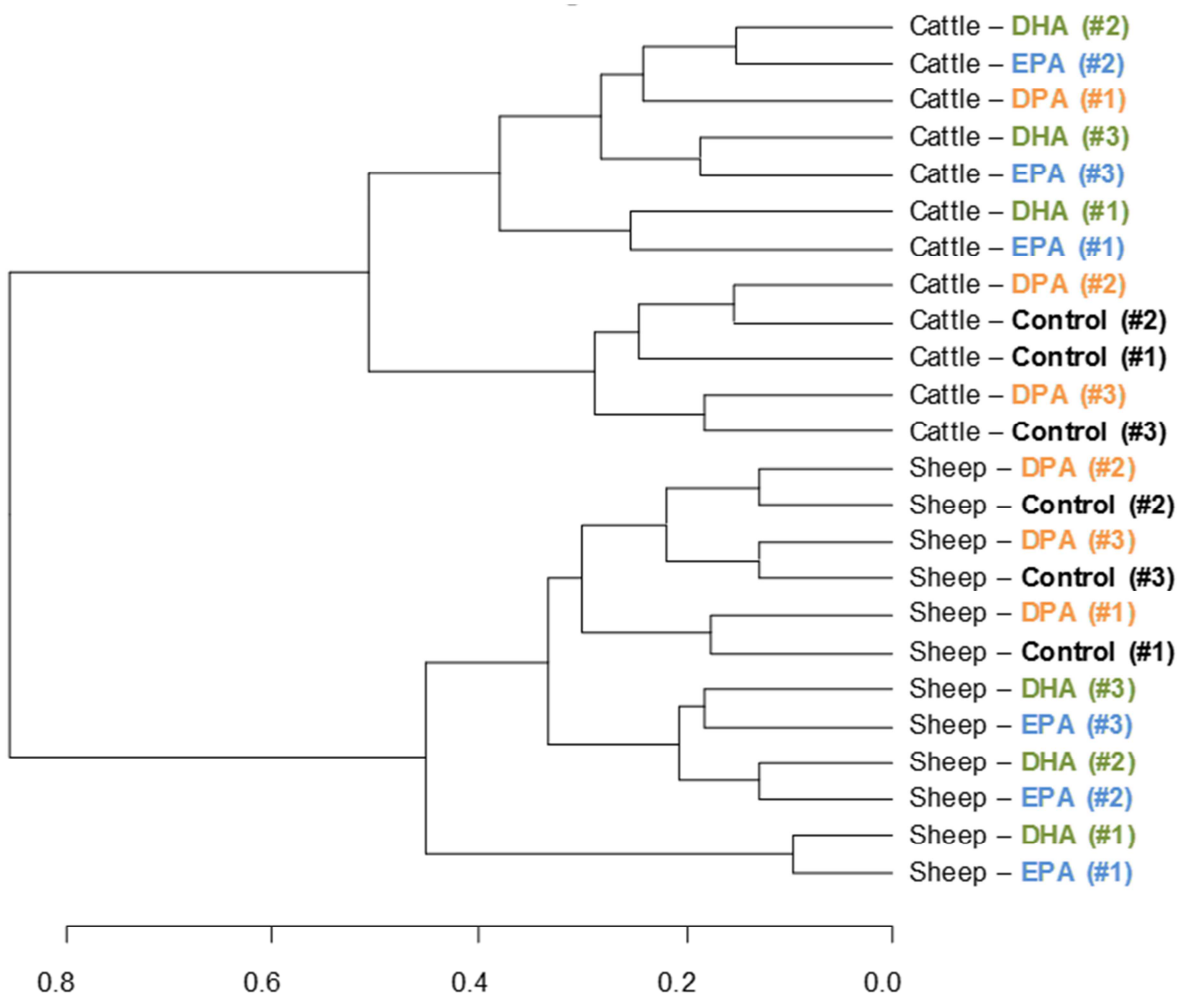
¹SEM = standard error of the mean.

²The *Anaeroplasmataceae* family comprises > 97% of sequences within this phylum.

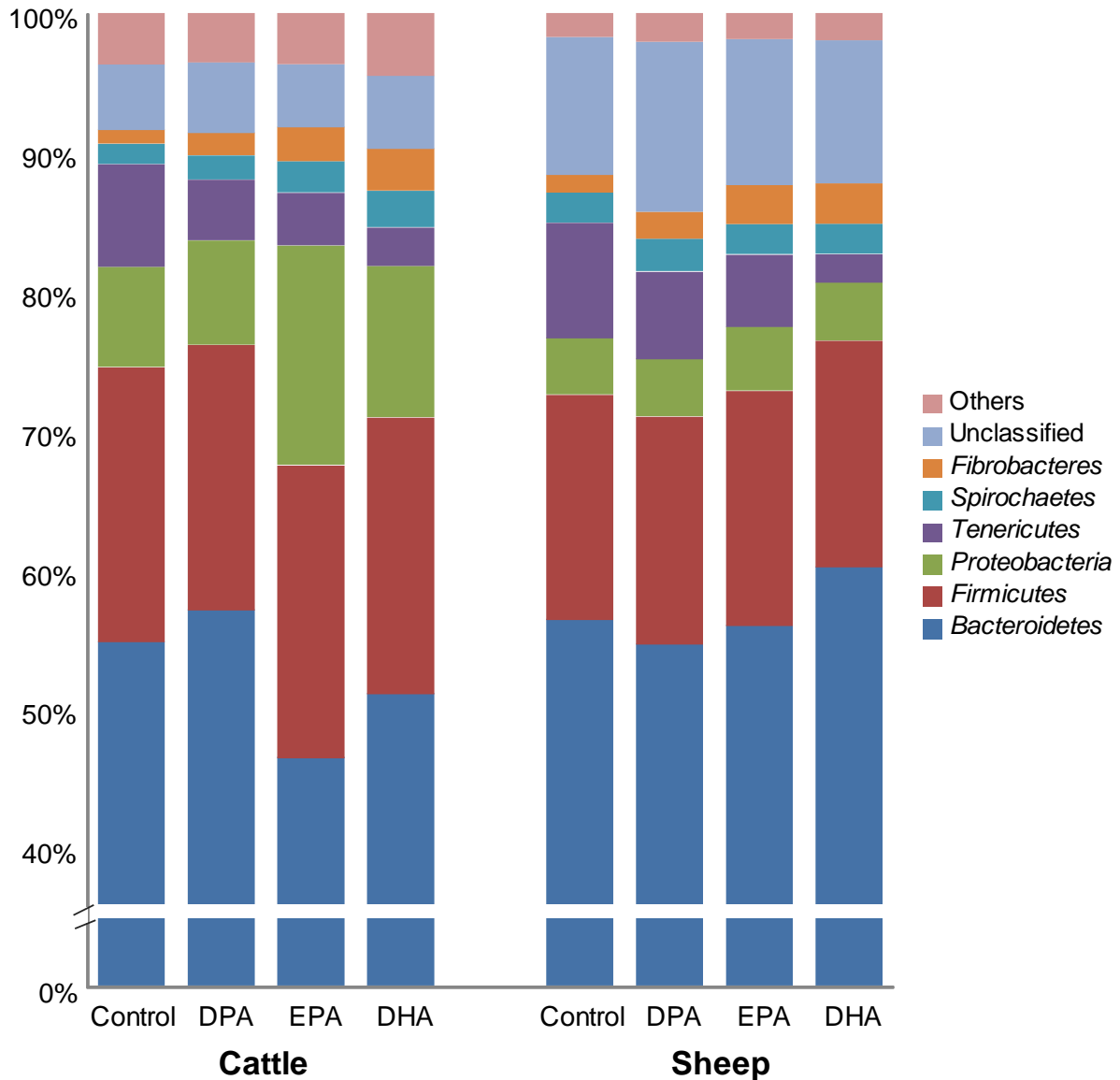
³The *Fibrobacter* genus comprises 100% of sequences within this phylum.

⁴The *Synergistaceae* family comprises 100% of sequences within this phylum.

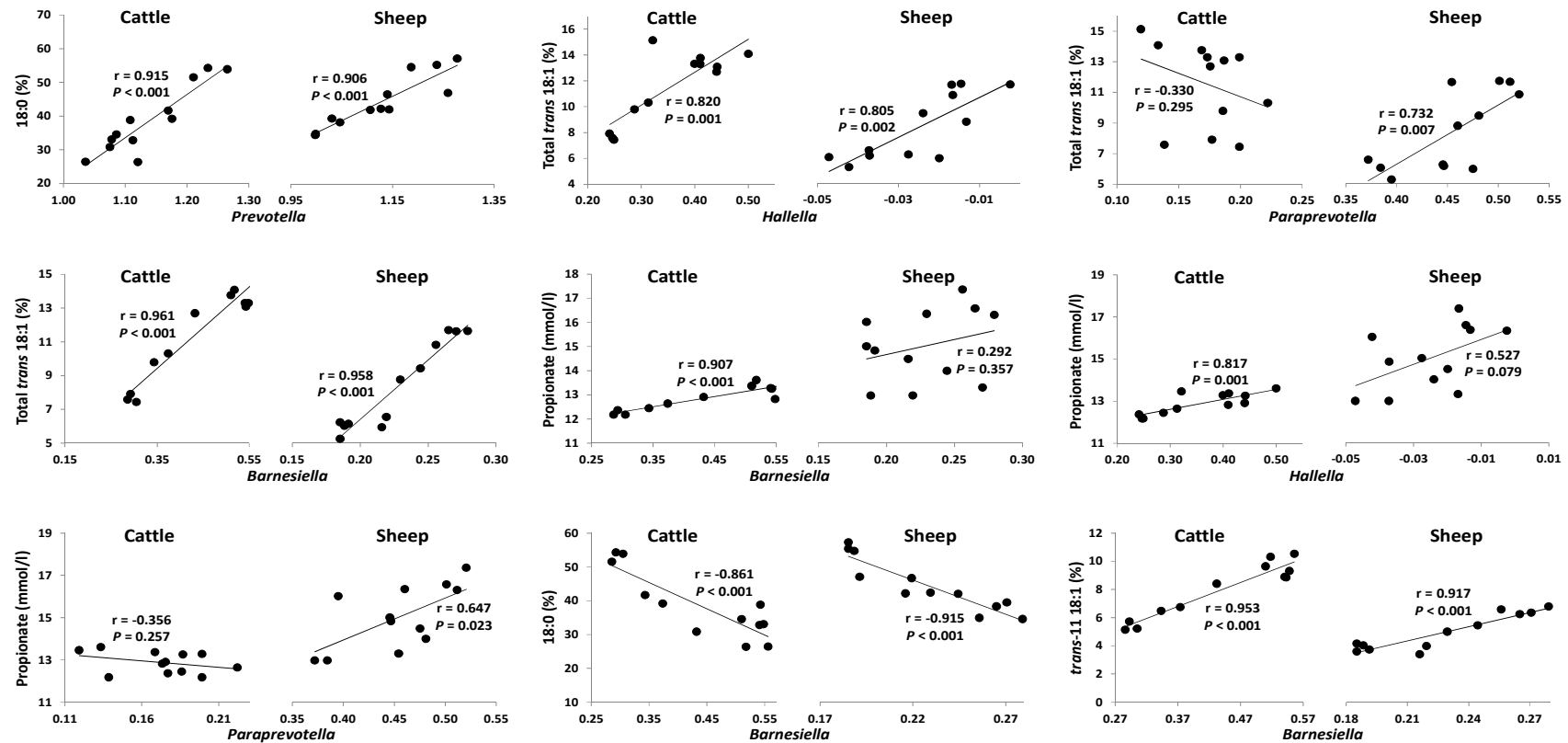
⁵Sum of SR1, *Elusimicrobia*, *Lentisphaerae*, *Candidatus Saccharibacteria*, *Chloroflexi*, *Actinobacteria*, *Cyanobacteria/Chloroplast*, *Verrucomicrobia*, *Armatimonadetes* and *Fusobacteria*.



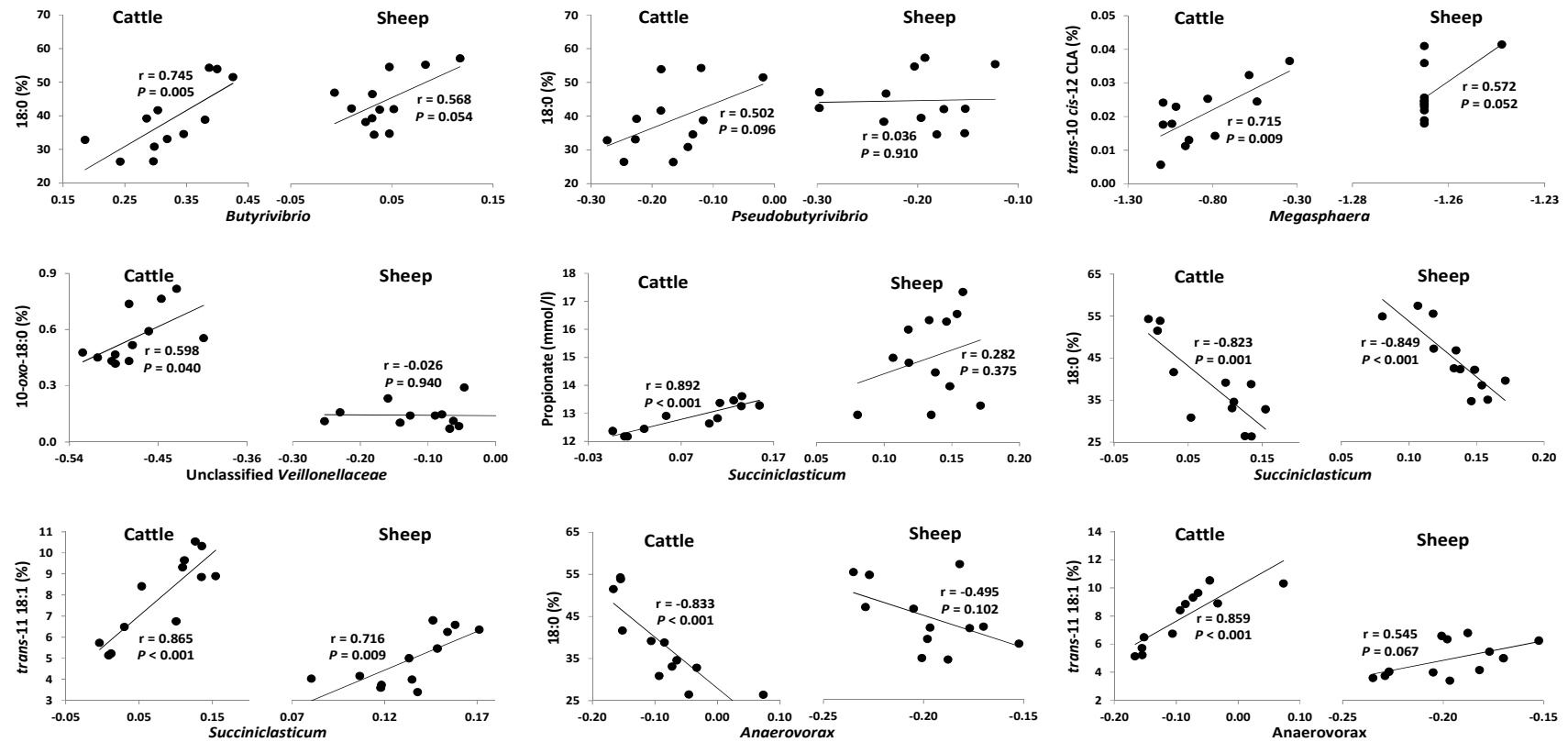
Supplementary Figure S1. Cluster analysis of sequencing profiles based on the method of maximum distance or minimum similarity (complete-linkage) and the Bray-Curtis distances, of bacterial 16S rRNA gene sequences after 24 h of in vitro incubation with rumen inocula of cattle and sheep. The incubated substrate was a total mixed ration containing no additional PUFA (control; black) or supplemented with 2% dry matter of docosapentaenoic acid (DPA; orange), eicosapentaenoic acid (EPA; blue), or docosahexaenoic acid (DHA; green). Values in parenthesis indicate the statistical replicate.



Supplementary Figure S2. Relative abundances of bacterial phyla (% of total sequences, non-transformed values) after 24 h of in vitro incubation with rumen inocula of cattle and sheep. The incubated substrate was a total mixed ration containing no additional PUFA (control) or supplemented with 2% dry matter of docosapentaenoic acid (DPA), eicosapentaenoic acid (EPA), or docosahexaenoic acid (DHA). The group "other phyla" includes SR1, *Elusimicrobia*, *Lentisphaerae*, *Candidatus Saccharibacteria*, *Actinobacteria*, *Cyanobacteria/Chloroplast*, *Chloroflexi*, *Verrucomicrobia*, *Armatimonadetes* and *Fusobacteria*.



Supplementary Figure S3. Relationships between relative abundances (log-transformed data) of relevant genera of *Bacteroidetes* phylum and ruminal C18 FA concentrations and fermentation parameters, after 24 h of in vitro incubation with rumen inocula of cattle and sheep. The incubated substrate was a total mixed ration containing no additional PUFA (control) or supplemented with 2% dry matter of docosapentaenoic acid (DPA), eicosapentaenoic acid (EPA), or docosahexaenoic acid (DHA). Pearson correlation coefficients (r) and P -values are reported in each panel.



Supplementary Figure S4. Relationships between relative abundances (log-transformed data) of relevant genera of the *Firmicutes* phylum and ruminal C18 FA concentrations and fermentation parameters, after 24 h of in vitro incubation with rumen inocula of cattle and sheep. The incubated substrate was a total mixed ration containing no additional PUFA (control) or supplemented with 2% dry matter of docosapentaenoic acid (DPA), eicosapentaenoic acid (EPA), or docosahexaenoic acid (DHA). Pearson correlation coefficients (r) and P -values are reported in each panel.