Supplementary Figures and Tables

Inter- and intra-domain functional redundancy in the rumen microbiome during plant biomass degradation

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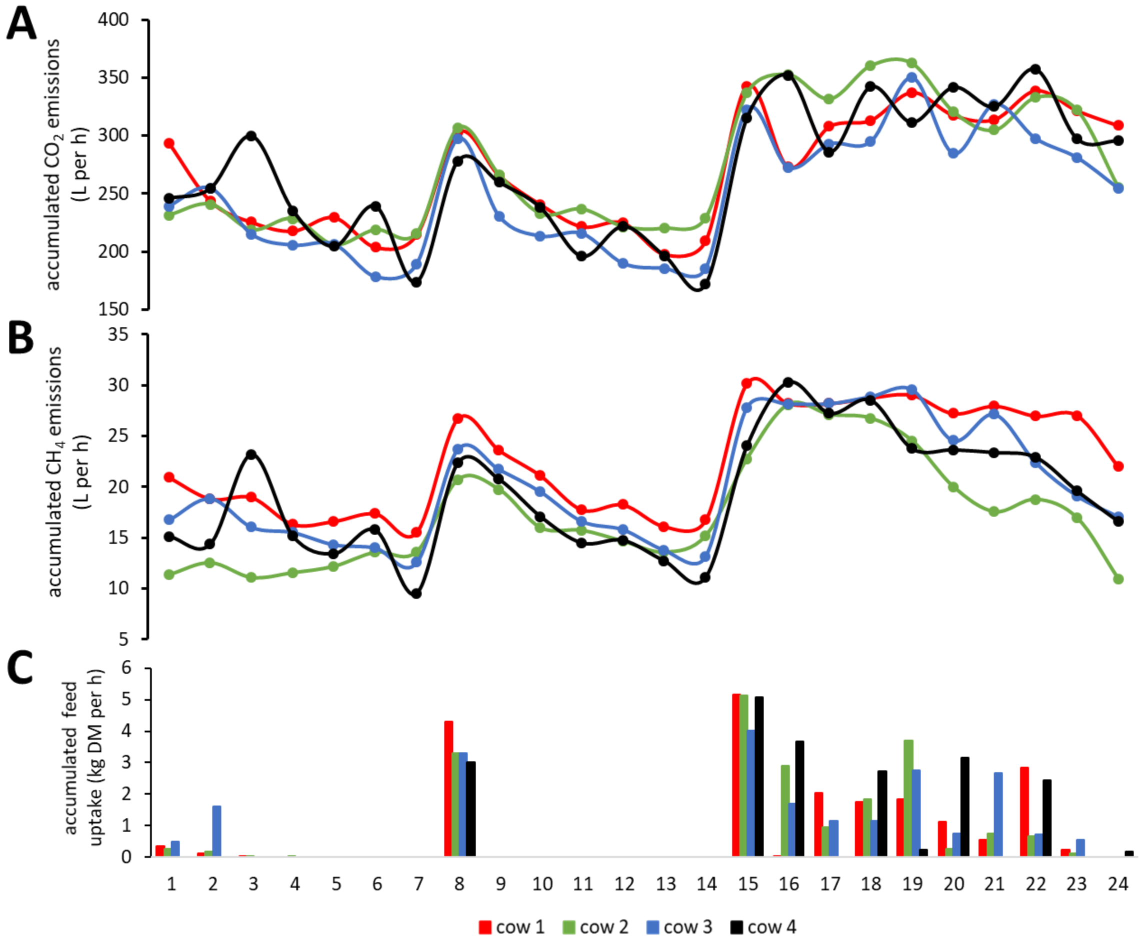
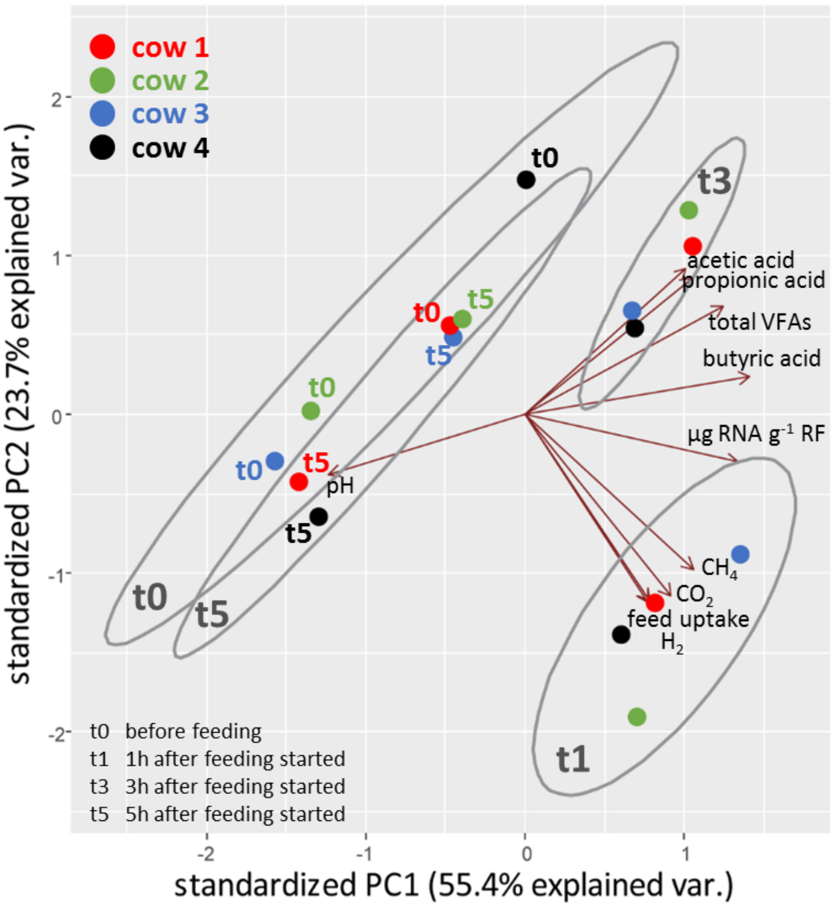
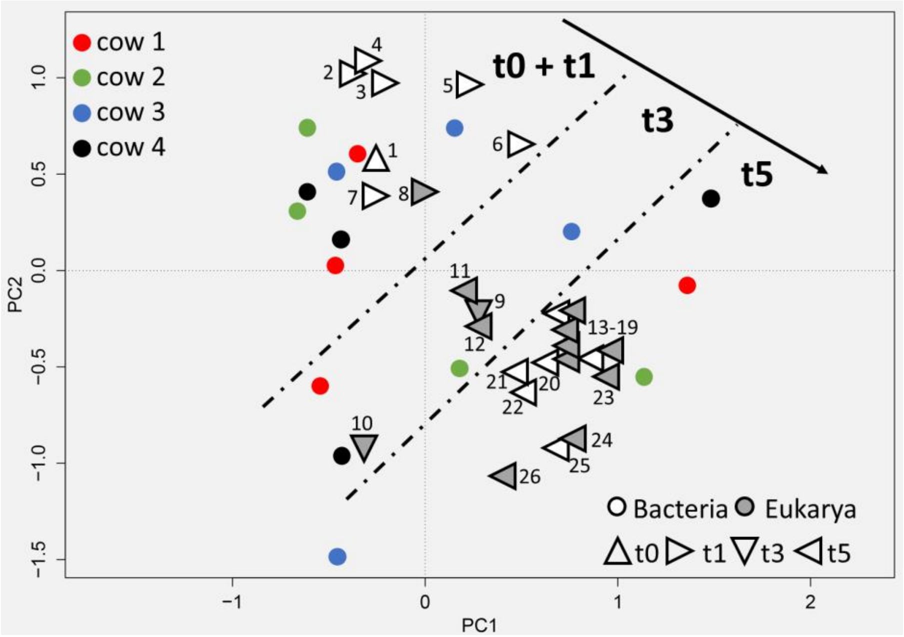


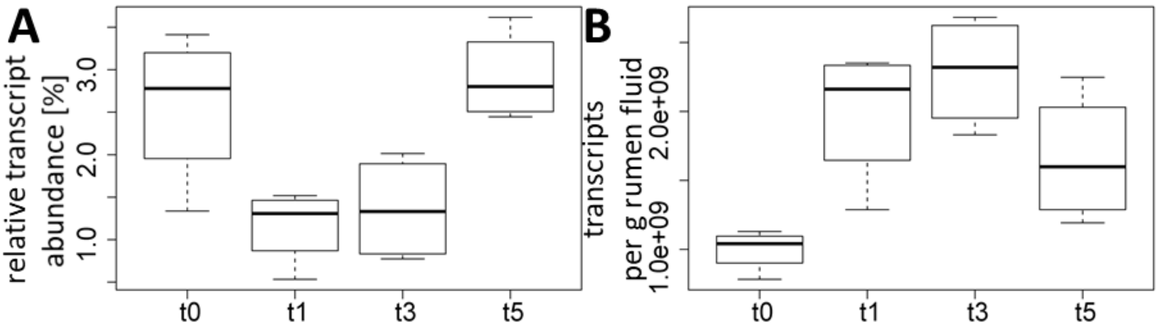
Supplementary Figure S1. Diurnal greenhouse gas emissions and feed intake of four lactating Holstein cows. The x-axis is indicating the time, 24 hours are shown (1 am to 12 pm). A and B show the accumulated CO_2 and CH_4 emissions over 24 hours, respectively. C shows the feed intake of the cows during gas emission measurements (day 4). The cows were fed in a semi-restrictive way, no feed was available from 4 am until the morning feeding (7 am to 8 am) and between 8 am and 2 pm. Between 2 pm and 4 am the cows were allowed to feed ad libitum. In the afternoon CH_4 and CO_2 emissions during the first hour of feeding increased similar to after the morning feeding. However, as cows were fed ad libitum and took up feed continuously, gas emissions stayed on high levels for several hours and eventually dropped at night.



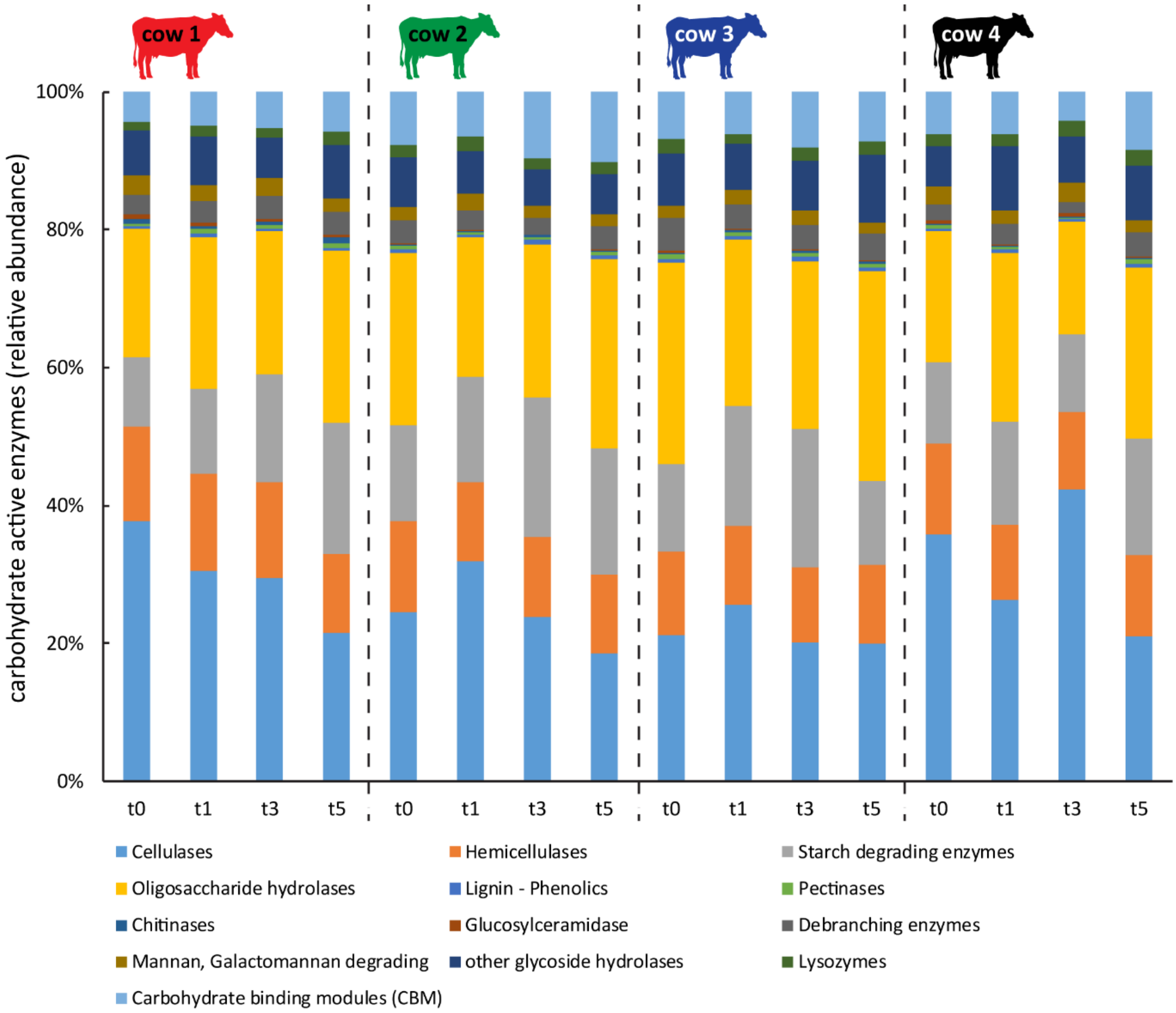
Supplementary Figure S2. Biplot showing principal component analysis (PCA) results on rumen fluid metadata. The metadata (i.e. gas emissions (CH_4, CO_2, H_2), feed and water intake, pH, volatile fatty acid (VFA) concentrations and total RNA content) obtained from the rumen fluid samples used for metatranscriptomics (t0, t1, t3, t5) were subject to PCA using R (prcomp, ggbiplot). VFAs not detected in the majority of the samples were excluded from the principal components analysis. Prior to the principal component analysis the dimensionally heterogeneous variables were standardised by applying z-scoring for each cow individually. The distance between the samples and their clustering indicates the differences in sample metadata (x axis: first dimension explaining 55.4 % of the variance; y axis: second dimension explaining 23.7 % of variance; ellipses: normal data ellipse for each group (time) with 95% probability). The length of the variable vectors (arrows) is equivalent to the contribution of particular metadata to the ordination. The contribution of a particular variable to PC1 and PC2 is given by projecting the arrow-tip of the vector onto PC1 and PC2. The direction of the variable vectors is indicating the sample orientation of its weight (e.g. gas emissions are highest at t1, one hour after the feeding started). Variables with minor contribution to the ordination as well as low abundant VFAs are not shown. Colour code indicates the four different cows; RF, rumen fluid.



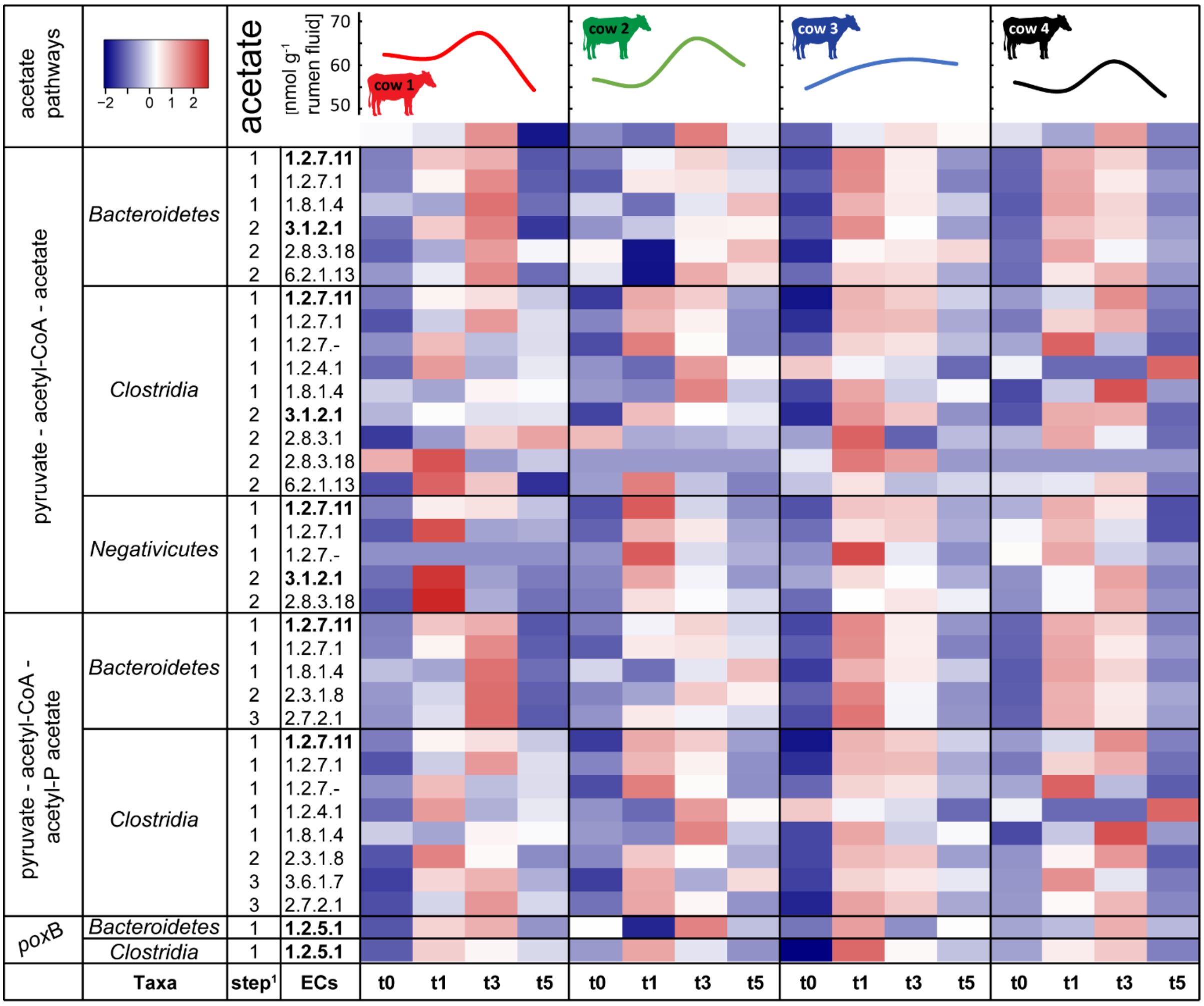
Supplementary Figure S3. Biplot showing temporal separation and succession of rumen microbiomes during plant biomass degradation. Rumen fluid microbial communities were subject to various analyses using R (i.e. principal component analysis (PCA, function: rda), indicator species analysis (function: signassoc), non-metric multidimensional scaling (NMDS, function: metaMDS), PERMANOVA (permutational multivariate analysis of variance using distance matrices, function: adonis), differential gene expression analysis (function: glmFit). PCA result is shown above. NMDS on a Bray-Curtis dissimilarity matrix (function: vegdist) led to a similar time wise separation but less pronounced. For both, the PCA and the NMDS, the taxon count matrices were normalized to the library sizes and transformed using cow separated z-scoring. Triangles indicate taxa identified by indicator species analysis to be significantly higher abundant at a certain time (species scores were plotted onto the PCA biplot). Before feeding (t0), one, three, five hours after feeding started (t1, t3, t5), *Succinivibrionaceae* (1), *Bacteroidaceae* (2), *Synergistaceae* (3), *Lactobacillaceae* (4), *Burkholderiales* (5), unassigned Bacteria (6), *Pseudomonadales* (7), *Ascomycota* (8), *Rhizobiales* (9), *Eremoplastron* (10), *Neocallimastigaceae* (11), unassigned *Fungi* (12), unassigned *Tubulinea* (13), *Rhizobiales* (14), *Chytridiomycota* (15), *Eccrinales* (16), Viridiplantae (17), *Victivallaceae* (18), *Mastigamoeba* (19), *Anaeroplasmataceae* (20), Candidate division TM6 (21), RFP12 gut group (22), unassigned *Amoebozoa* (23), unassigned *Spirotrichea* (24), *Rhodospirillaceae* (25), *Trichomonadidae* (26). Additionally, relative abundance of three eukaryotic taxa (i.e. *Epidinium*, *Eudiplodinium* and unassigned *Litostomata*) were identified by differential gene expression analysis to be significantly decreasing from t3 to t5. Besides these three taxa no other taxa significantly different over time could be detected via differential gene expression analysis.



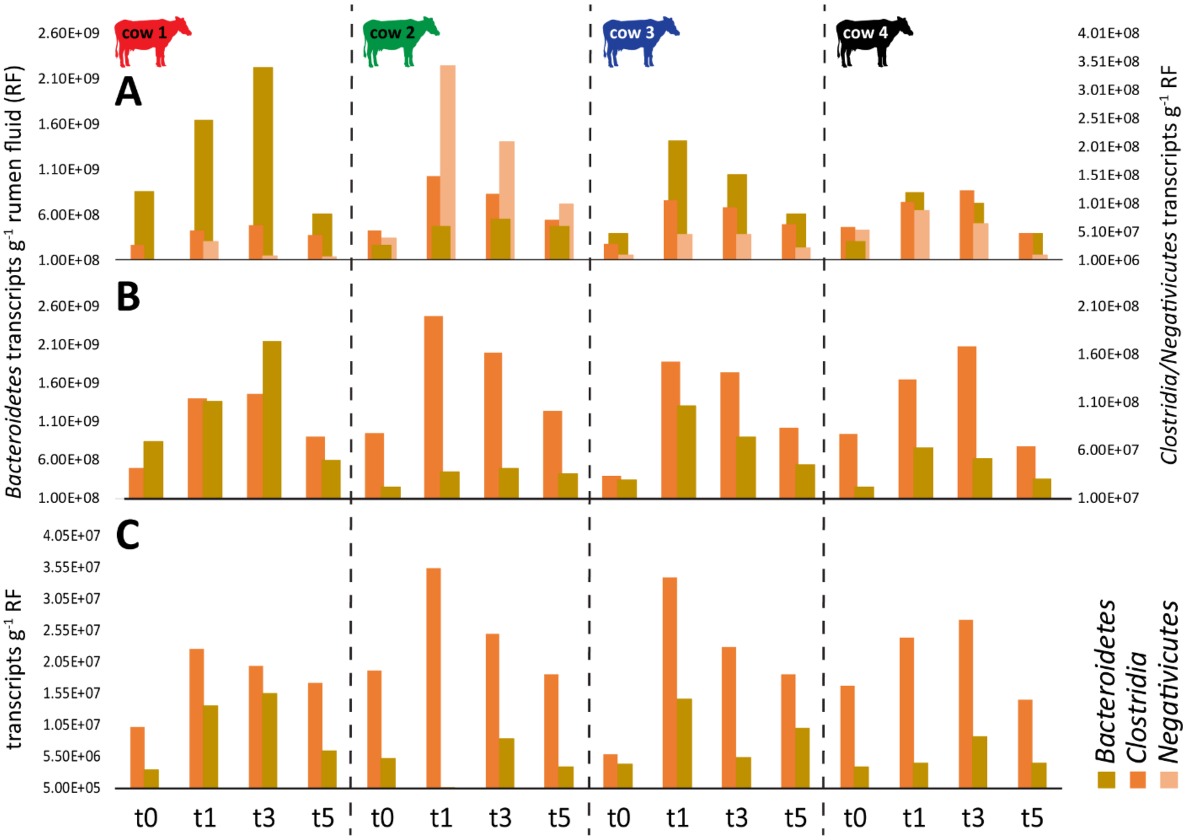
Supplementary Figure S4. DNA Replication - comparison of relative and quantified transcript abundance. Relative (A) and quantified (B) transcript abundance of reads assigned to the level two SEED subsystem DNA Replication. For details on the quantification see Methods section and equation 1; x-axis: before feeding (t0), one, three, five hours after feeding started (t1, t3, t5). Quantified transcripts followed the observed dynamics in total nucleic acid content extracted per g rumen fluid (average nucleic acid concentrations [µg g^{-1} rumen fluid]: 64.7 (t0), 99.0 (t1), 107.8 (t3), 58.0 (t5).



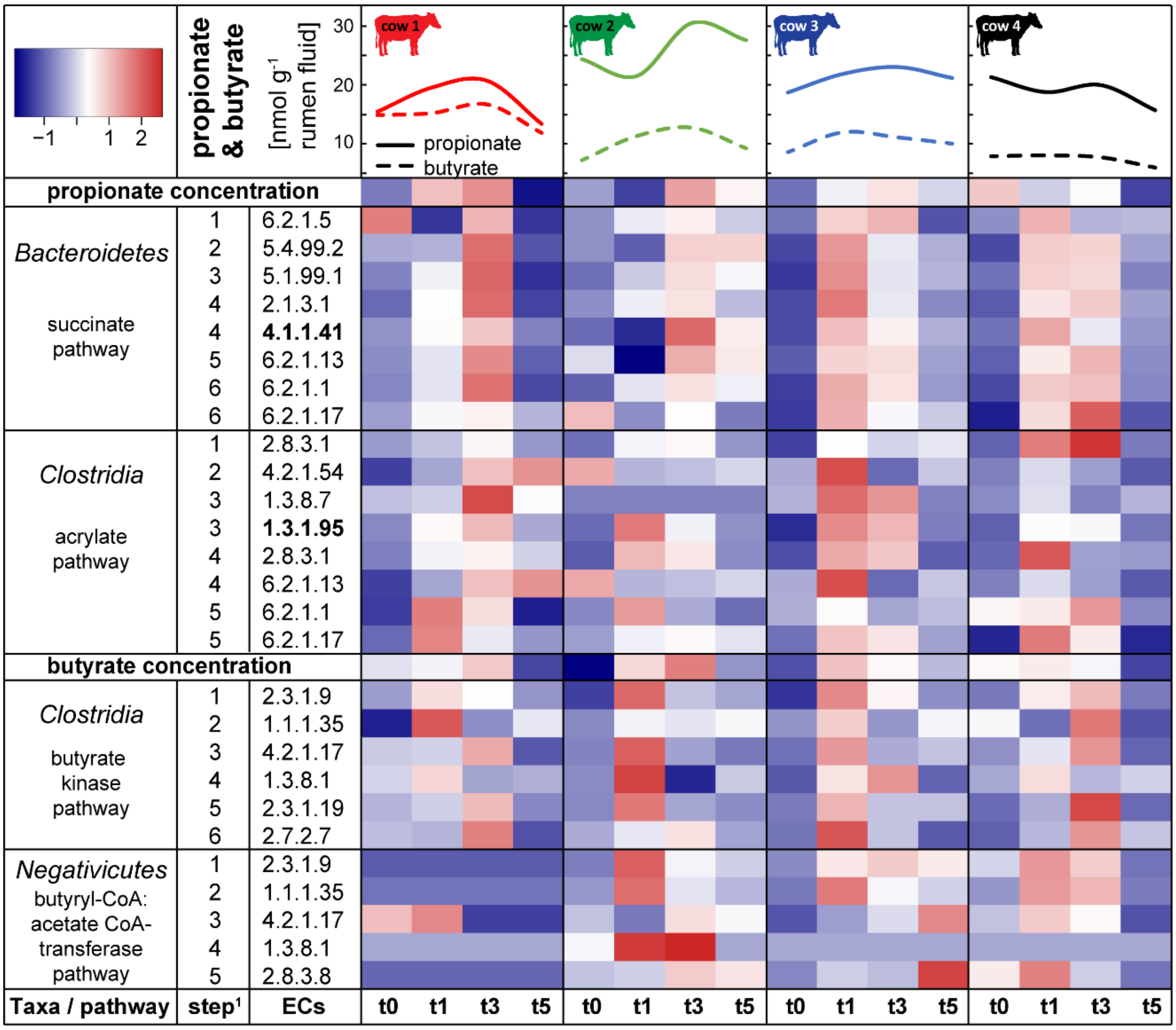
Supplementary Figure S5. Relative abundance of carbohydrate active enzymes (CAZymes) present in the cow rumen metatranscriptomes. CAZymes were summarized according to their substrate specificity/activity (see Supplementary Table S6 for details on the underlying pfam models). X-axis: before feeding (t0), one, three, five hours after feeding started (t1, t3, t5).



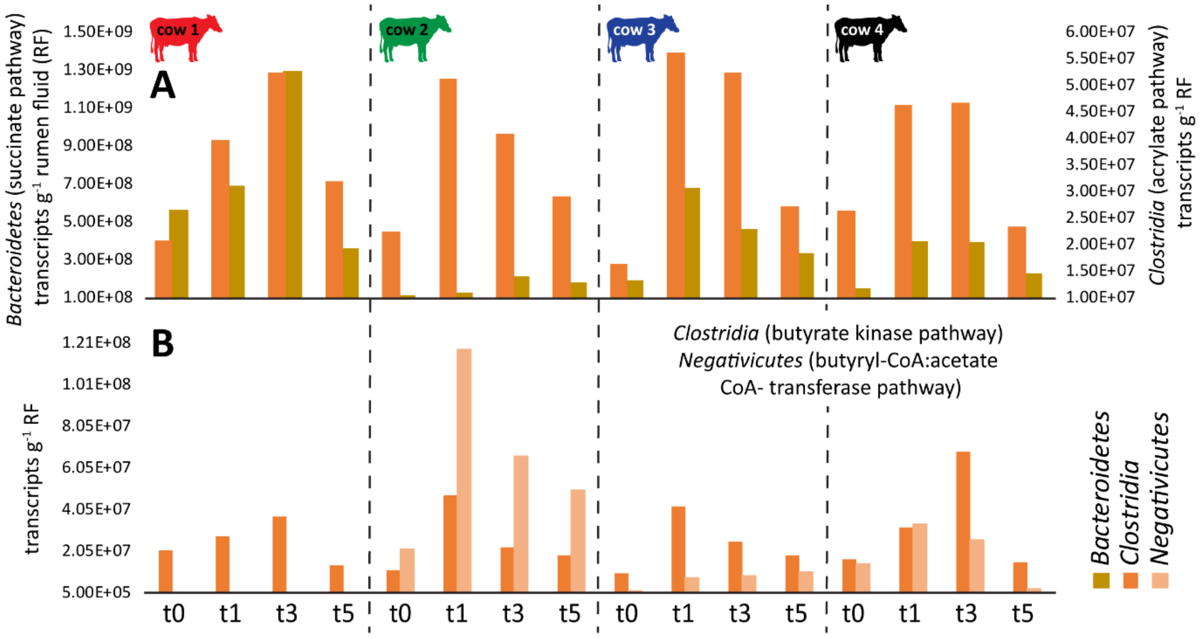
Supplementary Figure S6. Dynamics and distribution of transcripts involved in acetate production in the rumen. Heatmap depicting the dynamics of quantified transcripts (encoded by their EC numbers) involved in acetate production (turnover) from pyruvate via acetyl-CoA, acetyl-CoA and acetyl-P and direct production from pyruvate via *pox*B (pyruvate:ubiquinone oxidoreductase) by the rumen microbiome separated for the individual cows. ECs in bold indicate production specific transcripts. The first row of the heatmap depicts the acetate concentrations measured before feeding (t0) and one (t1), three (t3) and five (t5) hours after the feeding started. Additionally, the acetate concentrations (nmol g^{-1} rumen fluid) for each cow are depicted above the heatmap. [1]Numbers indicate in which step of the pathway the respective enzymes are involved.



Supplementary Figure S7. Quantified transcripts involved in acetate production/turnover. Bar charts showing quantified transcripts (g^{-1} rumen fluid) of *Bacteroidetes* (mainly *Prevotella*), *Clostridiales* (*Clostridia)* and *Negativicutes* possibly involved in acetate production from pyruvate in the rumen, i.e. (A) via acetyl-CoA, (B) via acetyl-CoA and acetyl-P, (C) direct production from pyruvate via *pox*B (pyruvate:ubiquinone oxidoreductase). Each bar summarizes all possibly involved enzymes (ECs) at a certain timepoint divided by the number of steps for each pathway (see Supplementary Figure S6). X-axis: before feeding (t0), one, three, five hours after feeding started (t1, t3, t5).



Supplementary Figure S8. Dynamics and distribution of transcripts involved in propionate and butyrate production in the rumen. Heatmap depicting the dynamics of quantified transcripts (encoded by their EC numbers) involved in propionate production (turnover) from succinate (succinate pathway) and lactate (acrylate pathway) and butyrate production (turnover) from acetyl-CoA by the rumen microbiome separated for the individual cows. ECs in bold indicate production specific transcripts. Propionate and butyrate concentrations measured before feeding (t0) and one (t1), three (t3) and five (t5) hours after the feeding started are depicted as heatmaps and total (nmol g^{-1} rumen fluid). [1]Numbers indicate in which step of the pathway the respective enzymes are involved. The butyryl-CoA:acetate CoA-transferase pathway (Negativicutes) was not detected in cow 1.



Supplementary Figure S9. Quantified transcripts involved in propionate and butyrate production/turnover. Bar charts showing quantified transcripts (g^{-1} rumen fluid) of *Bacteroidetes* (mainly *Prevotella*), *Clostridiales* (*Clostridia)* and *Negativicutes* possibly involved in propionate production (A) from succinate (succinate pathway) and lactate (acrylate pathway) and butyrate production (B) from acetyl-CoA in the rumen. Each bar summarizes all possibly involved enzymes (ECs) at a certain timepoint divided by the number of steps for each pathway (see Supplementary Figure S8). X-axis: before feeding (t0), one, three, five hours after feeding started (t1, t3, t5).

Supplementary Table S1. Composition of the diet fed to the rumen-cannulated Holstein dairy cows. Cows were fed twice a day in a semi-restrictive way (see Methods section, Fig. 1a, and Supplementary Figure S1).

Dietary composition[1]	
Spring barley	217.9
Rape seed cake (12% fat)	221.5
Clover grass silage	303.5
Corn silage	244.8
Sodium Chloride	1.6
Vitamins and minerals	10.2
Chemical composition[1]	
Dry matter content [g/kg][2]	446
Ash[3]	65
Crude protein[4]	157
Fat[5]	36
Neutral detergent fibre[6]	340

[1] Given in g/kg DM if nothing else is stated. [2] The dry matter concentration was determined by drying the samples for 48 hours at 60°C. [3] Ash was determined by combustion at 525°C for 6 hours. [4] Nitrogen was determined by the Dumas principle as described by Hansen [1] using a Vario MAX CN (Elementar Analysesysteme GmbH, Hanau, Germany), and crude protein was calculated as N x 6.25. [5] Crude fat was measured by Soxhlet extraction with petroleum ether (Soltex 2050, Foss Analytical, Hillerød, Denmark) after hydrolysis with HCl [2]. [6] Ash-free neutral detergent fibre was measured by FibertecTM M 6 system (Foss Analytical, Hillerød, Denmark) using heat stable amylase and sodium sulphite as described by Mertens [3].

Supplementary Table S2. Daily dry matter intake (kg/day) for the four cows on the four experimental days. The dry matter intake was calculated as feed given minus leftovers multiple with dry matter concentration of diet. The cows had a dry matter intake at 20.8 and 20.0 kg dry matter on the days with ad libitum and restricted feeding, respectively, and the intake did not differ between days with ad libitum and restricted feeding (t-test, P = 0.49). The cows had an intake of 14.5 ± 2.2 (mean ± standard deviation) and 9.8 ± 1.6 kg dry matter between 7 am and 6 pm on days with ad libitum feeding (day 1 and 3) and restricted feeding (day 2 and day 4), respectively, but the intake did not differ between day 1 and 3 and day 2 and 4 (t-test; day 1 versus day 3, P = 0.65; day 2 versus day 4, P = 0.95; ad libitum versus restricted, P = 0.0002). The cows had a dry matter intake at 2.7 ± 1.6 and 3.5 ± 0.6 kg between 7:00 and 8:00 on the days with rumen sampling and methane measurements, respectively, and the intake did not differ between the days (t-test, P = 0.43). For detail on the experimental setup see Methods section.

Time	Cow ID	Day 1	Day 2	Day 3	Day 4
24 h	Cow 1	19.2	15.4	19.9	20.1
	Cow 2	20.9	22.5	22.3	20.4
	Cow 3	19.4	18.2	19.1	20.1
	Cow 4	22.5	20.5	22.7	23.2
7 am – 6 pm	Cow 1	15.2	8.2	12.7	9.3
	Cow 2	18.2	12.1	17.3	10.4
	Cow 3	12.5	8.0	12.1	8.3
	Cow 4	14.0	10.7	14.5	11.4
7 am – 8 am	Cow 1		3.6		4.3
	Cow 2		4.4		3.3
	Cow 3		2.3		3.3
	Cow 4		0.6		3.0

Supplementary Table S3. Overview of sequencing and data processing results.

sample ID	raw sequences[1]	after pre-processing[2]		SortMeRNA results[3]		annotation of mRNA	
	no. of read pairs	no. of reads	average read length	rRNA reads	mRNA reads	DIAMOND	pfam[4]
c1_07h (t0)	65,317,599	60,466,622	214	52,334,976	8,131,646	3,365,969	410,419
c2_07h (t0)	59,186,503	51,812,887	214	42,488,514	9,324,373	3,365,969	290,817
c3_07h (t0)	75,330,840	53,433,658	215	43,639,108	9,794,550	3,365,969	368,829
c4_07h (t0)	53,259,101	62,901,199	217	52,540,980	10,360,219	3,365,969	342,208
c1_08h (t1)	66,549,413	59,656,114	209	51,068,193	8,587,921	3,365,969	491,989
c2_08h (t1)	67,146,045	59,149,252	215	50,666,579	8,482,673	3,365,969	424,483
c3_08h (t1)	63,379,266	53,417,765	198	44,106,590	9,311,175	3,365,969	417,605
c4_08h (t1)	63,533,899	36,342,383	191	29,672,369	6,670,014	3,365,969	304,716
c1_10h (t3)	64,634,701	58,801,041	219	50,430,178	8,370,863	3,365,969	542,244
c2_10h (t3)	66,938,760	42,031,443	223	35,888,902	6,142,541	3,365,969	399,755
c3_10h (t3)	67,312,364	58,695,012	207	48,385,273	10,309,739	3,365,969	384,594
c4_10h (t3)	65,740,801	48,605,118	217	42,611,059	5,994,059	3,365,969	478,864
c1_12h (t5)	62,897,615	58,136,430	224	52,364,413	5,772,017	3,365,969	467,908
c2_12h (t5)	66,954,185	62,273,447	214	54,042,405	8,231,042	3,365,969	387,441
c3_12h (t5)	71,966,923	50,534,927	221	44,197,682	6,337,245	3,365,969	370,793
c4_12h (t5)	62,238,171	57,829,771	220	49,765,881	8,063,890	3,365,969	395,907

[1]read length 125 bp; sequenced on a Illumina HiSeq 2500 125 bp; [2]after overlapping the reads, trimming and quality filtering; [3]SortMeRNA separated rRNA and putative mRNA reads based on the SILVA db; [4]number of reads annotated to a protein family from a subsample (2,000,000 reads) of total mRNA reads

Supplementary Table S4. Relative abundance[1] of eukaryotic, bacterial and archaeal SSU rRNA reads [%]. c1 - c4, cow 1 – 4.

taxon sting	c1_t0	c1_t1	c1_t3	c1_t5	c2_t0	c2_t1	c2_t3	c2_t5	c3_ t0	c3_t1	c3_t3	c3_t5	c4_ t0	c4_t1	c4_t3	c4_t5
unassigned Eukaryota	1.03	0.93	0.92	1.69	0.31	0.30	0.49	0.59	0.62	0.63	0.84	0.62	0.41	0.18	0.55	1.53
Metazoa	0.33	0.55	0.68	0.73	0.20	0.21	0.29	0.32	0.37	0.53	0.65	0.50	0.40	0.22	0.58	1.14
Alveolata; Apicomplexa; Aconoidasida; Piroplasmida	0.53	0.73	0.61	0.73	0.12	0.14	0.19	0.21	0.26	0.53	0.52	0.38	0.36	0.27	0.39	0.96
Alveolata; Ciliophora; Litostomatea; unassigned Litostomatea	0.12	0.14	0.19	0.18	0.02	0.02	0.03	0.01	0.06	0.06	0.11	0.05	0.07	0.06	0.14	0.12
Alveolata; Ciliophora; Litostomatea; other Litostomatea	0.03	0.06	0.04	0.04	0.00	0.00	0.00	0.00	0.02	0.01	0.02	0.02	0.01	0.01	0.04	0.02
Alveolata; Ciliophora; Litostomatea; unassigned Entodiniomorphida	3.85	2.42	3.83	1.70	0.43	0.67	0.34	0.17	0.96	0.94	1.17	0.32	1.10	0.16	1.49	1.01
Alveolata; Ciliophora; Litostomatea; unassigned Ophryoscolecidae	9.26	6.51	7.65	6.88	0.84	1.15	0.64	0.31	2.98	2.58	3.04	2.43	4.81	1.52	5.75	4.40
Alveolata; Ciliophora; Litostomatea; Entodinium	8.89	7.42	7.15	10.29	0.19	0.13	0.20	0.12	2.46	1.82	3.41	2.27	2.01	0.77	1.36	5.15
Alveolata; Ciliophora; Litostomatea; Epidinium	8.21	5.79	8.05	2.59	1.00	1.77	0.91	0.39	2.17	2.42	2.78	0.43	3.21	1.15	4.19	1.68
Alveolata; Ciliophora; Litostomatea; Eremoplastron	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.02	0.05	0.03	0.11	0.01
Alveolata; Ciliophora; Litostomatea; Eudiplodinium	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.02	0.02	0.09	0.01
Alveolata; Ciliophora; Litostomatea; Isotricha	4.07	8.04	5.71	3.50	1.19	1.73	1.01	0.95	5.39	4.95	4.33	9.79	2.03	9.16	18.69	5.32
Alveolata; Ciliophora; Litostomatea; Dasytricha	0.92	0.88	0.73	0.50	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.33	0.09	0.15	0.17	0.01
Alveolata; Ciliophora; Spirotrichea; unassigned Spirotrichea	0.05	0.07	0.07	0.11	0.03	0.04	0.04	0.06	0.09	0.10	0.14	0.18	0.12	0.05	0.16	0.19
Alveolata; Ciliophora; Colpodea; unassigned Colpodea	0.02	0.02	0.01	0.02	0.01	0.02	0.02	0.03	0.01	0.02	0.03	0.01	0.01	0.01	0.00	0.02
Alveolata; Ciliophora; Phyllopharyngea; Chonotrichia	0.14	0.13	0.13	0.11	0.03	0.05	0.03	0.04	0.23	0.32	0.18	0.09	0.12	0.06	0.13	0.52
Alveolata; low abundadnt Alveolata	0.05	0.04	0.05	0.10	0.05	0.03	0.02	0.03	0.04	0.04	0.05	0.02	0.03	0.02	0.04	0.05
Fungi; unassigned Fungi	0.03	0.04	0.06	0.06	0.01	0.00	0.00	0.01	0.02	0.02	0.01	0.02	0.02	0.00	0.01	0.02
Fungi; Dikarya; unassigned Dikarya	0.06	0.09	0.04	0.08	0.07	0.08	0.08	0.14	0.03	0.08	0.08	0.04	0.05	0.07	0.05	0.11
Fungi; Dikarya; Ascomycota	0.08	0.42	0.13	0.16	0.13	0.31	0.27	0.21	0.13	0.34	0.22	0.14	0.29	0.21	0.13	0.13
Fungi; Dikarya; Basidiomycota	0.05	0.05	0.03	0.07	0.03	0.03	0.02	0.05	0.11	0.09	0.08	0.07	0.08	0.03	0.07	0.18
Fungi; Chytridiomycota	0.02	0.03	0.02	0.04	0.02	0.01	0.01	0.03	0.01	0.01	0.01	0.02	0.02	0.01	0.01	0.03
Fungi; Neocallimastigomycota; Neocallimastigomycetes; Neocallimastigaceae	1.29	2.19	2.18	3.09	0.44	0.42	0.23	0.32	0.99	0.68	0.83	1.12	0.67	0.38	0.66	1.16
Fungi; low abundant Fungi	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01
Parabasalia; unassigned Parabasalia	0.00	0.00	0.00	0.01	0.81	0.55	0.89	1.52	0.11	0.08	0.13	1.44	0.41	0.25	0.58	0.29
Parabasalia; Trichomonadida; Trichomonadidae	0.05	0.03	0.06	0.11	4.92	4.38	5.27	11.16	0.79	0.59	1.04	13.14	2.05	2.49	4.90	2.68
Parabasalia; Hypotrichomonadida	0.07	0.04	0.02	0.08	0.01	0.01	0.00	0.02	0.13	0.14	0.15	0.01	0.01	0.00	0.01	0.28
Parabasalia; low abundant Parabasalia	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00
Stramenopiles	0.02	0.04	0.04	0.04	0.02	0.01	0.01	0.02	0.01	0.02	0.03	0.02	0.02	0.00	0.02	0.02
Viridiplantae	0.08	0.07	0.05	0.08	0.11	0.10	0.14	0.25	0.07	0.06	0.09	0.11	0.07	0.06	0.07	0.18
Rhizaria	0.08	0.07	0.12	0.12	0.03	0.02	0.03	0.04	0.09	0.14	0.29	0.11	0.06	0.04	0.10	0.30
Amoebozoa; unassigned Amoebozoa	0.01	0.01	0.01	0.02	0.02	0.02	0.02	0.04	0.01	0.01	0.02	0.02	0.00	0.01	0.02	0.04
Amoebozoa; Tubulinea; unassigned Tubulinea	0.02	0.02	0.02	0.02	0.02	0.01	0.03	0.04	0.01	0.01	0.02	0.01	0.01	0.02	0.01	0.03
Amoebozoa; Archamoebae; Mastigamoebidae; Mastigamoeba	0.50	0.33	0.39	0.64	0.47	0.48	0.61	0.81	0.26	0.38	0.39	0.37	0.24	0.14	0.36	0.84
Amoebozoa; Flabellinea; Vannellidae	0.08	0.07	0.07	0.13	0.01	0.01	0.02	0.02	0.03	0.02	0.07	0.03	0.01	0.03	0.02	0.08
Amoebozoa; low abundant Amoebozoa	0.00	0.00	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.00	0.00	0.01	0.01	0.00	0.01	0.00
Glaucocystophycea	0.04	0.04	0.04	0.06	0.01	0.01	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.01
Eccrinales	0.01	0.02	0.02	0.03	0.00	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.01	0.02
low abundant Eukaryota	0.03	0.03	0.04	0.08	0.02	0.02	0.02	0.04	0.02	0.03	0.04	0.02	0.01	0.00	0.02	0.09
unassigned Bacteria	0.04	0.09	0.04	0.11	0.10	0.12	0.11	0.09	0.16	0.22	0.20	0.16	0.11	0.17	0.12	0.18
Proteobacteria; unassigned Proteobacteria	0.00	0.02	0.02	0.03	0.01	0.08	0.24	0.16	0.02	0.21	0.35	0.01	0.05	0.29	0.04	0.08
Proteobacteria; Gammaproteobacteria; unassigned Gammaproteobacteria	0.00	0.02	0.01	0.02	0.03	0.05	0.12	0.04	0.01	0.05	0.07	0.01	0.03	0.03	0.02	0.02

Proteobacteria; Gammaproteobacteria; Pseudomonadales	0.01	0.05	0.01	0.02	0.01	0.02	0.01	0.01	0.00	0.01	0.00	0.01	0.02	0.02	0.00	0.01
Proteobacteria; Gammaproteobacteria; Aeromonadales; Succinivibrionaceae	1.56	0.97	0.96	1.73	27.84	18.39	17.91	18.53	18.09	11.34	12.80	9.53	23.46	27.10	16.41	10.93
Proteobacteria; Gammaproteobacteria; Cardiobacteriales	0.00	0.01	0.00	0.01	0.07	0.06	0.05	0.04	0.07	0.04	0.02	0.03	0.05	0.05	0.02	0.06
Proteobacteria; Alphaproteobacteria; unassigned Alphaproteobacteria	0.01	0.00	0.01	0.01	0.00	0.01	0.01	0.03	0.02	0.03	0.03	0.02	0.02	0.05	0.04	0.04
Proteobacteria; Alphaproteobacteria; Rhodospirillales; Rhodospirillaceae	0.03	0.03	0.03	0.06	0.24	0.27	0.29	0.46	0.91	0.96	1.10	2.14	1.22	1.25	1.79	1.77
Proteobacteria; Alphaproteobacteria; Rickettsiales	0.01	0.02	0.01	0.02	0.00	0.00	0.01	0.01	0.04	0.05	0.04	0.03	0.01	0.02	0.04	0.05
Proteobacteria; Alphaproteobacteria; Rhizobiales	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.01	0.01	0.03	0.01	0.01	0.01	0.03
Proteobacteria; Betaproteobacteria; Burkholderiales	0.02	0.04	0.01	0.01	0.03	0.03	0.01	0.02	0.00	0.03	0.02	0.00	0.01	0.01	0.01	0.01
Proteobacteria; Deltaproteobacteria; Desulfuromonadales	0.00	0.00	0.00	0.00	0.26	0.37	0.44	0.55	0.58	0.84	1.23	0.77	0.66	0.62	1.07	1.43
Proteobacteria; Deltaproteobacteria; Desulfobacterales	0.02	0.02	0.01	0.03	0.04	0.04	0.03	0.03	0.03	0.02	0.02	0.04	0.03	0.03	0.02	0.03
Proteobacteria; Deltaproteobacteria; Desulfovibrionales	0.02	0.06	0.02	0.05	0.06	0.06	0.03	0.03	0.03	0.05	0.04	0.03	0.04	0.03	0.03	0.02
Proteobacteria; low abundant Proteobacteria	0.02	0.08	0.02	0.04	0.05	0.04	0.06	0.07	0.07	0.07	0.06	0.07	0.04	0.06	0.06	0.07
Proteobacteria; Epsilonproteobacteria	0.00	0.00	0.00	0.00	0.03	0.01	0.01	0.02	0.02	0.01	0.01	0.01	0.01	0.02	0.01	0.02
Planctomycetes; Planctomycetacia; Planctomycetales; Planctomycetaceae	0.02	0.01	0.01	0.03	0.08	0.07	0.03	0.07	0.07	0.07	0.06	0.06	0.06	0.05	0.03	0.06
Planctomycetes; low abundant Planctomycetes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cyanobacteria; Melainabacteria	0.18	0.21	0.21	0.15	0.14	0.21	0.17	0.20	0.26	0.38	0.43	0.56	0.35	0.54	0.48	0.34
Bacteroidetes; unassigned Bacteroidetes	0.03	0.03	0.03	0.04	0.01	0.01	0.01	0.02	0.04	0.04	0.05	0.03	0.02	0.02	0.03	0.07
Bacteroidetes; Flavobacteria; Flavobacteriales	0.01	0.03	0.01	0.01	0.01	0.01	0.00	0.01	0.01	0.02	0.02	0.00	0.00	0.01	0.01	0.02
Bacteroidetes; Sphingobacteria; Sphingobacteriales	0.01	0.02	0.01	0.03	0.01	0.01	0.01	0.00	0.03	0.03	0.01	0.02	0.01	0.01	0.02	0.03
Bacteroidetes; Bacteroidia; Bacteroidales; S24-7	0.28	0.47	0.31	0.48	0.61	0.60	0.66	0.91	0.49	0.71	0.75	0.28	0.40	0.42	0.27	0.49
Bacteroidetes; Bacteroidia; Bacteroidales; Bacteroidaceae	0.09	0.21	0.11	0.10	0.06	0.06	0.05	0.06	0.11	0.12	0.09	0.07	0.07	0.09	0.06	0.06
Bacteroidetes; Bacteroidia; Bacteroidales; Prevotellaceae	35.31	36.53	39.02	32.44	20.17	19.11	18.33	16.61	28.81	30.24	25.04	17.32	19.91	20.85	13.35	22.70
Bacteroidetes; Bacteroidia; Bacteroidales; RF16	2.09	1.92	1.87	2.34	0.67	0.64	1.16	0.93	0.91	0.84	0.94	1.63	1.48	1.19	1.10	0.99
Bacteroidetes; Bacteroidia; Bacteroidales; Rikenellaceae	1.29	1.25	0.93	1.03	0.91	0.72	0.57	0.67	1.02	1.09	0.91	0.62	0.78	0.90	0.48	0.74
Bacteroidetes; Bacteroidia; Bacteroidales; Porphyromonadaceae	0.03	0.03	0.02	0.05	0.09	0.16	0.17	0.24	0.04	0.08	0.08	0.03	0.03	0.02	0.03	0.05
Bacteroidetes; Bacteroidia; Bacteroidales; BS11 gut group	0.89	1.00	0.75	0.77	0.74	0.69	0.70	0.74	0.80	1.02	0.95	0.65	0.78	1.17	0.54	0.71
Bacteroidetes; Bacteroidia; Bacteroidales; other Bacteroidales families	0.02	0.02	0.01	0.02	0.08	0.06	0.02	0.03	0.03	0.03	0.03	0.01	0.02	0.03	0.01	0.02
Bacteroidetes; Cytophagia; Cytophagales	0.05	0.06	0.06	0.06	0.01	0.00	0.00	0.01	0.05	0.07	0.07	0.07	0.04	0.05	0.06	0.10
Bacteroidetes; low abundant Bacteroidetes	0.84	0.76	0.68	0.86	0.43	0.45	0.49	0.54	0.97	1.05	0.95	0.61	0.74	0.87	0.63	0.75
Actinobacteria; Actinobacteria; Corynebacteriales	0.02	0.06	0.03	0.03	0.08	0.05	0.02	0.04	0.15	0.07	0.01	0.01	0.02	0.03	0.01	0.02
Actinobacteria; Actinobacteria; Bifidobacteriales; Bifidobacteriaceae	0.03	0.02	0.01	0.02	0.09	0.10	0.08	0.08	0.37	0.34	0.38	0.02	0.05	0.07	0.03	0.20
Actinobacteria; Coriobacteriia; Coriobacteriales; Coriobacteriaceae	0.12	0.31	0.12	0.20	0.12	0.11	0.09	0.08	0.17	0.18	0.18	0.12	0.15	0.13	0.06	0.12
Actinobacteria; low abundant Actinobacteria	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.01	0.02	0.01	0.01	0.01	0.00	0.01
Firmicutes; unassigned Firmicutes	0.00	0.01	0.00	0.00	0.03	0.02	0.03	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.00
Firmicutes; Bacilli; Lactobacillales; Streptococcaceae	0.00	0.00	0.00	0.00	0.10	0.05	0.05	0.06	0.14	0.12	0.14	0.01	0.01	0.01	0.01	0.12
Firmicutes; Bacilli; Lactobacillales; Lactobacillaceae	0.01	0.03	0.01	0.01	0.03	0.04	0.02	0.03	0.03	0.04	0.03	0.02	0.03	0.02	0.01	0.01
Firmicutes; Erysipelotrichi; Erysipelotrichales; Erysipelotrichaceae	1.30	1.22	1.17	2.08	4.08	4.05	4.22	3.78	1.98	2.15	2.08	1.96	1.90	1.62	1.05	1.94
Firmicutes; Clostridia; Clostridiales; unassigned Clostridiales	0.06	0.07	0.06	0.14	0.11	0.09	0.09	0.10	0.10	0.10	0.12	0.09	0.11	0.11	0.07	0.14
Firmicutes; Clostridia; Clostridiales; Lachnospiraceae	4.85	6.09	4.43	6.49	10.30	10.40	11.62	9.12	9.30	9.46	9.48	8.34	11.47	8.28	7.71	8.48
Firmicutes; Clostridia; Clostridiales; Veillonellaceae	2.38	2.36	1.81	2.11	3.10	3.27	2.69	2.44	1.02	1.01	0.81	1.16	1.83	1.44	1.05	0.72
Firmicutes; Clostridia; Clostridiales; Ruminococcaceae	4.49	3.55	3.28	6.16	7.06	6.94	7.55	7.76	6.00	6.08	7.13	5.50	6.51	5.19	4.80	6.81
Firmicutes; Clostridia; Clostridiales; Family XIII Incertae Sedis	0.17	0.14	0.15	0.32	0.30	0.24	0.25	0.22	0.31	0.28	0.25	0.18	0.22	0.22	0.15	0.25
Firmicutes; Clostridia; Clostridiales; Eubacteriaceae	0.03	0.02	0.03	0.03	0.46	0.34	0.32	0.31	0.03	0.02	0.02	0.05	0.05	0.04	0.04	0.05
Firmicutes; low abundant Firmicutes	0.01	0.03	0.02	0.01	0.03	0.03	0.01	0.02	0.02	0.03	0.04	0.01	0.01	0.03	0.02	0.04
Verrucomicrobia; Opitutae	0.00	0.00	0.00	0.00	0.02	0.03	0.03	0.03	0.11	0.16	0.14	0.10	0.03	0.08	0.04	0.16
Verrucomicrobia; low abundant Verrucomicrobia	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Tenericutes; Mollicutes; Anaeroplasmatales; Anaeroplasmataceae	0.60	0.43	0.61	1.16	0.18	0.22	0.29	0.28	0.52	0.50	0.45	0.53	0.35	0.25	0.24	0.56
Tenericutes; Mollicutes; other Mollicutes	0.02	0.03	0.03	0.11	0.28	0.21	0.23	0.21	0.28	0.25	0.26	0.36	0.45	0.28	0.29	0.32
Tenericutes; low abundant Tenericutes	0.02	0.02	0.01	0.03	0.01	0.01	0.01	0.03	0.02	0.02	0.03	0.01	0.02	0.01	0.01	0.03
RF3	0.04	0.02	0.02	0.06	0.01	0.00	0.02	0.02	0.04	0.04	0.03	0.02	0.02	0.00	0.00	0.03
Lentisphaerae; Lentisphaeria; Victivallales; Victivallaceae	0.01	0.02	0.01	0.03	0.13	0.18	0.18	0.26	0.13	0.16	0.18	0.23	0.08	0.09	0.07	0.18
Lentisphaerae; Lentisphaeria; RFP12 gut group	0.09	0.11	0.09	0.19	0.22	0.30	0.38	0.45	0.30	0.34	0.37	0.71	0.25	0.39	0.29	0.33
Lentisphaerae; low abundant Lentisphaerae	0.01	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.01	0.00	0.01	0.00	0.01	0.01	0.01
Spirochaetes; Spirochaetes (class); Spirochaetales; Spirochaetaceae	1.63	2.16	2.13	3.93	4.32	6.21	6.00	6.17	3.23	5.20	4.51	3.12	3.16	2.72	2.11	3.31
Spirochaetes; Spirochaetes (class); Spirochaetales; PL-11B10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.20	0.21	0.04	0.03	0.03	0.02	0.30
Spirochaetes; low abundant Spirochaetes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Candidate division TM7	0.02	0.02	0.02	0.01	0.15	0.10	0.11	0.10	0.05	0.05	0.05	0.07	0.09	0.07	0.07	0.04
Synergistetes; Synergista; Synergistales; Synergistaceae	0.06	0.09	0.05	0.05	0.03	0.06	0.02	0.02	0.06	0.06	0.06	0.02	0.03	0.06	0.02	0.03
Elusimicrobia; Elusimicrobia (class); Elusimicrobiales; Elusimicrobiaceae	0.01	0.00	0.00	0.01	0.19	0.21	0.21	0.17	0.08	0.08	0.13	0.23	0.20	0.16	0.12	0.14
Elusimicrobia; low abundant Elusimicrobia	0.01	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.00	0.00	0.01	0.01
Candidate division SR1	0.00	0.00	0.00	0.00	0.03	0.02	0.02	0.02	0.10	0.13	0.11	0.12	0.15	0.15	0.12	0.13
Fibrobacteres; Fibrobacteria; Fibrobacterales; Fibrobacteraceae	0.70	1.47	1.13	1.48	3.65	11.18	11.46	8.65	2.84	5.15	5.17	7.50	2.95	4.64	3.60	4.60
Fibrobacteres; low abundant Fibrobacteres	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Candidate division TM6	0.01	0.02	0.02	0.03	0.00	0.00	0.00	0.00	0.01	0.01	0.02	0.03	0.02	0.01	0.02	0.04
low abundant Bacteria	0.01	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.03	0.04	0.02	0.03	0.02	0.02	0.02	0.03
Euryarchaeota; Thermoplasmata; Methanomassiliicoccales; Methanomassiliicoccales GIT clade	0.16	0.23	0.20	0.36	0.20	0.22	0.23	0.24	0.13	0.14	0.12	0.11	0.19	0.12	0.10	0.14
Euryarchaeota; Thermoplasmata; Thermoplasmatales; unassigned Thermoplasmatales	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Euryarchaeota; Methanobacteria; Methanomacteriales; unassigned Methanobacteriaceae	0.03	0.03	0.02	0.06	0.02	0.01	0.01	0.01	0.03	0.03	0.03	0.02	0.01	0.02	0.01	0.03
Euryarchaeota; Methanobacteria; Methanomacteriales; Methanobrevibacter	0.10	0.08	0.06	0.17	0.19	0.13	0.10	0.17	0.09	0.10	0.12	0.18	0.21	0.15	0.09	0.11
Euryarchaeota; Methanobacteria; Methanomacteriales; Methanosphaera	0.01	0.01	0.01	0.01	0.02	0.02	0.01	0.01	0.00	0.00	0.01	0.01	0.01	0.01	0.00	0.02
Euryarchaeota; Methanomicrobia; Methanosarcinales; Methanosarcinaceae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

[1] Taxa which could not be assigned on family level and/or showed relative abundance ≤ 0.01 % level are shown on higher taxonomic levels

Supplementary Table S5. Significantly higher expressed functions one hour after ruminant feed intake.

Fig. 3 ID	Differential gene expression analysis (edgeR)			SEED subsystems		
	SEED functions (level 4)	**logFC**	**FDR[1]**	**level 1**	**level 2**	**level 3**
1	Fumarate reductase subunit C	4.70	***	Respiration	Electron donating reactions	Succinate_dehydrogenase
2	Putative phosphatase YqaB	3.76	**	DNA Metabolism	DNA repair	2-phosphoglycolate_salvage
3	Lipid A biosynthesis (KDO) 2-(lauroyl)-lipid IVA acyltransferase	2.68	*	Cell Wall and Capsule	Gram-Negative cell wall components	KDO2-Lipid_A_biosynthesis
4	DNA-directed RNA polymerase subunit B (EC 2.7.7.6)	2.18	**	RNA Metabolism	Transcription	RNA_polymerase_archaeal
5	DNA-directed RNA polymerase subunit N (EC 2.7.7.6)	2.01	***	RNA Metabolism	Transcription	RNA_polymerase_archaeal
6	LSU ribosomal protein L7Ae	1.49	**	Protein Metabolism	Protein biosynthesis	Ribosome_LSU_bacterial
7	3-hydroxybutyryl-CoA dehydratase (EC 4.2.1.55)	1.34	*	Carbohydrates	Fermentation	Acetyl-CoA_fermentation_to_Butyrate
8	Phosphoenolpyruvate carboxykinase [ATP] (EC 4.1.1.49)	1.18	**	Carbohydrates	Central carbohydrate metabolism	Pyruvate_metabolism_I:_anaplerotic_reactions,_PEP
9	Na()-translocating NADH-quinone reductase subunit F	1.17	*	Respiration	Electron donating reactions	Na()-translocating_NADH-quinone_oxidoreductase[2]
10	LSU ribosomal protein L14p (L23e)	1.11	***	Protein Metabolism	Protein biosynthesis	Ribosome_LSU_bacterial
11	Na()-translocating NADH-quinone reductase subunit C	1.03	**	Respiration	Electron donating reactions	Na()-translocating_NADH-quinone_oxidoreductase[2]
12	NADH-ubiquinone oxidoreductase chain L (EC 1.6.5.3)	1.01	**	Respiration	Electron donating reactions	Respiratory_Complex_I
13	Choline binding protein A	1.00	**	Stress Response	Osmotic stress	Choline_and_Betaine_Uptake_and_Betaine_Biosynthesis
14	Cysteine desulfurase (EC 2.8.1.7), NifS subfamily	1.00	*	Amino Acids and Derivatives	Alanine, serine, and glycine	Alanine_biosynthesis
15	6-phospho-beta-glucosidase (EC 3.2.1.86)	0.92	*	Carbohydrates	Di- and oligosaccharides	Beta-Glucoside_Metabolism
16	LSU ribosomal protein L24p (L26e)	0.89	***	Protein Metabolism	Protein biosynthesis	Ribosome_LSU_bacterial
17	LSU ribosomal protein L10p (P0)	0.87	**	Protein Metabolism	Protein biosynthesis	Ribosome_LSU_bacterial
18	LSU ribosomal protein L31p	0.84	**	Protein Metabolism	Protein biosynthesis	Ribosome_LSU_bacterial
19	LSU ribosomal protein L27p	0.82	***	Protein Metabolism	Protein biosynthesis	Ribosome_LSU_bacterial
20	SSU ribosomal protein S8p (S15Ae)	0.80	***	Protein Metabolism	Protein biosynthesis	Ribosome_SSU_bacterial
21	SSU ribosomal protein S4p (S9e)	0.79	***	Protein Metabolism	Protein biosynthesis	Ribosome_SSU_bacterial
22	LSU ribosomal protein L18p (L5e)	0.78	**	Protein Metabolism	Protein biosynthesis	Ribosome_LSU_bacterial
23	SSU ribosomal protein S5p (S2e)	0.77	***	Protein Metabolism	Protein biosynthesis	Ribosome_SSU_bacterial
24	SSU ribosomal protein S3p (S3e)	0.76	***	Protein Metabolism	Protein biosynthesis	Ribosome_SSU_bacterial
25	SSU ribosomal protein S16p	0.75	**	Protein Metabolism	Protein biosynthesis	Ribosome_SSU_bacterial
26	Biosynthetic arginine decarboxylase (EC 4.1.1.19)	0.75	*	Stress Response	Acid stress	Acid_resistance_mechanisms
27	SSU ribosomal protein S11p (S14e)	0.73	**	Protein Metabolism	Protein biosynthesis	Ribosome_SSU_bacterial
28	Ketol-acid reductoisomerase (EC 1.1.1.86)	0.71	*	Amino Acids and Derivatives	Branched-chain amino acids	Branched-Chain_Amino_Acid_Biosynthesis
29	LSU ribosomal protein L17p	0.70	**	Protein Metabolism	Protein biosynthesis	Ribosome_LSU_bacterial
30	SSU ribosomal protein S13p (S18e)	0.69	**	Protein Metabolism	Protein biosynthesis	Ribosome_SSU_bacterial
31	Translation elongation factor G	0.68	**	Protein Metabolism	Protein biosynthesis	Universal_GTPases
32	SSU ribosomal protein S14p (S29e)	0.68	**	Protein Metabolism	Protein biosynthesis	Ribosome_SSU_bacterial
33	LSU ribosomal protein L11p (L12e)	0.68	*	Protein Metabolism	Protein biosynthesis	Ribosome_LSU_bacterial
34	LSU ribosomal protein L1p (L10Ae)	0.67	**	Protein Metabolism	Protein biosynthesis	Ribosome_LSU_bacterial
35	LSU ribosomal protein L3p (L3e)	0.67	**	Protein Metabolism	Protein biosynthesis	Ribosome_LSU_bacterial
36	LSU ribosomal protein L5p (L11e)	0.66	**	Protein Metabolism	Protein biosynthesis	Ribosome_LSU_bacterial
37	SSU ribosomal protein S18p	0.65	*	Protein Metabolism	Protein biosynthesis	Ribosome_SSU_bacterial
38	LSU ribosomal protein L30p (L7e)	0.65	*	Protein Metabolism	Protein biosynthesis	Ribosome_LSU_bacterial
39	LSU ribosomal protein L16p (L10e)	0.62	**	Protein Metabolism	Protein biosynthesis	Ribosome_LSU_bacterial
40	LSU ribosomal protein L33p	0.62	*	Protein Metabolism	Protein biosynthesis	Ribosome_LSU_bacterial
41	SSU ribosomal protein S1p	0.54	*	Protein Metabolism	Protein biosynthesis	Ribosome_SSU_bacterial
42	SSU ribosomal protein S17p (S11e)	0.52	*	Protein Metabolism	Protein biosynthesis	Ribosome_SSU_bacterial
43	SSU ribosomal protein S9p (S16e)	0.50	*	Protein Metabolism	Protein biosynthesis	Ribosome_SSU_bacterial

[1] FDR, corrected p-value, *** ≤ 0.001, ** ≤ 0.01, * ≤ 0.05 [2] Na()-translocating_NADH-quinone_oxidoreductase_and_rnf-like_group_of_electron_transport_complexes

Supplementary Table S6. Raw count data of carbohydrate active enzymes detected in the rumen metatranscriptomes. c1 - c4, cow 1 - 4; 7h (t0), 8h (t1), 10h (t3), 12h (t5)

Categorie	pfam model	name	c1 7h	c2 7h	c3 7h	c4 7h	c1 8h	c2 8h	c3 8h	c4 8h	c1 10h	c2 10h	c3 10h	c4 10h	c1 12h	c2 12h	c3 12h	c4 12h
Lignin - phenolics	PF02578.10	Cu-oxidase_4	47	27	49	30	61	42	54	36	65	43	57	32	54	41	47	45
	PF00775.16	Dioxygenase_C	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Cellulases	PF12876.2	Cellulase_like	1	1	2	1	1	6	0	0	1	4	2	0	0	3	1	1
	PF00150.13	Cellulase	1419	404	537	834	901	747	732	441	1239	473	387	1544	692	351	522	525
	PF01341.12	Glyco_hydro_6	3	1	2	1	2	3	1	2	4	1	4	0	8	2	2	5
	PF00759.14	Glyco_hydro_9	2370	546	655	1123	1232	1033	922	725	1641	644	562	2273	804	489	570	629
	PF02011.10	Glyco_hydro_48	68	276	125	208	55	267	209	183	58	372	242	223	119	338	212	236
	PF12891.2	Glyco_hydro_44	0	14	9	12	2	12	7	5	1	16	16	9	7	24	7	12
	PF02015.11	Glyco_hydro_45	2390	301	575	1224	1491	861	732	566	2096	246	392	2731	1174	151	560	439
Hemicellulases	PF01270.12	Glyco_hydro_8	14	39	48	63	16	52	41	32	17	68	49	51	41	75	100	74
	PF00331.15	Glyco_hydro_10	1197	450	522	593	968	517	557	373	1319	412	393	816	747	381	478	454
	PF00457.12	Glyco_hydro_11	395	113	120	216	274	129	143	128	297	99	88	367	201	123	147	146
	PF02156.10	Glyco_hydro_26	444	119	149	258	262	219	207	144	451	130	159	450	250	120	155	153
	PF00295.12	Glyco_hydro_28	79	35	56	38	84	34	54	29	93	35	46	37	79	35	51	53
	PF07745.8	Glyco_hydro_53	143	77	183	98	104	96	151	95	179	112	127	84	184	105	137	154
Pectinases	PF00295	Glyco_hydro_28	79	35	56	38	84	34	54	29	93	35	46	37	79	35	51	53
Chitinases	PF00704	Glyco_hydro_18	111	9	26	23	49	18	25	7	82	15	27	43	110	23	24	26
Glucosylceramidase	PF02055	Glyco_hydro_30	100	17	29	52	63	16	18	14	70	8	10	78	44	10	25	18
	PF06964.7	Alpha-L-AF_C	178	78	190	88	163	122	144	85	236	73	103	77	165	110	131	125
	PF09206.6	ArabFuran-catal	2	6	2	2	0	4	2	1	0	5	3	1	3	7	5	1
	PF07477.7	Glyco_hydro_67C	48	32	29	27	41	27	33	41	57	11	33	26	65	24	42	22
	PF07488.7	Glyco_hydro_67M	74	22	57	31	55	34	54	27	97	14	46	27	70	23	55	51
	PF03648.9	Glyco_hydro_67N	2	1	3	0	3	2	1	0	3	3	3	1	3	0	2	3
	PF05592.6	Bac_rhamnosid	127	41	104	54	85	55	98	49	124	55	77	89	103	53	81	79
	PF08531.5	Bac_rhamnosid_N	45	28	38	16	28	14	31	19	46	22	17	22	38	26	33	22
Starch degrading enzymes	PF00128	Alpha-amylase	792	390	477	492	696	519	665	515	1097	524	674	882	1167	543	596	702
	PF03065	Glyco_hydro_57	221	77	156	69	123	83	122	85	275	86	112	85	233	88	119	133
	PF02446	Glyco_hydro_77	651	413	513	549	651	802	975	497	1307	888	817	831	1075	717	423	650
Mannan, Galactomannan degrading	PF02156	Glyco_hydro_26	444	119	149	258	262	219	207	144	451	130	159	450	250	120	155	153
	PF03663	Glyco_hydro_76	10	3	6	0	7	1	0	2	7	1	4	5	6	2	6	5
Oligosaccharide hydrolases	PF00232.13	Glyco_hydro_1	58	56	78	51	98	98	74	46	95	55	42	47	158	83	88	81
	PF00703.16	Glyco_hydro_2	96	43	94	56	49	53	60	41	110	46	65	72	82	61	91	60
	PF02836.12	Glyco_hydro_2_C	327	163	252	169	225	205	261	175	368	186	223	227	316	235	308	248
	PF02837.13	Glyco_hydro_2_N	298	158	284	183	204	193	264	148	351	194	201	195	309	211	294	227
	PF00933.16	Glyco_hydro_3	644	440	559	414	583	382	564	509	717	393	470	828	758	483	721	491
	PF01915.17	Glyco_hydro_3_C	343	184	305	215	325	205	297	240	418	200	186	403	412	226	381	256
	PF01120.12	Alpha_L_fucos	86	39	74	41	58	40	65	49	68	36	50	35	78	66	71	67
	PF01301.14	Glyco_hydro_35	73	29	66	35	45	39	67	56	75	36	58	39	67	42	60	60
	PF01074.17	Glyco_hydro_38	9	6	12	9	8	12	9	6	17	5	8	8	8	8	15	14

Categorie	pfam model	name	c1 7h	c2 7h	c3 7h	c4 7h	c1 8h	c2 8h	c3 8h	c4 8h	c1 10h	c2 10h	c3 10h	c4 10h	c1 12h	c2 12h	c3 12h	c4 12h
Oligosaccharide hydrolases	PF07748.8	Glyco_hydro_38_C	2	4	6	1	3	2	0	3	5	3	2	0	2	1	5	4
	PF01229.12	Glyco_hydro_39	4	3	6	3	4	3	6	0	2	4	1	5	3	4	6	7
	PF02449.10	Glyco_hydro_42	9	12	14	14	10	16	17	15	8	15	17	3	14	14	18	15
	PF08533.5	Glyco_hydro_42_C	0	3	0	0	0	2	0	0	0	1	1	2	0	1	2	2
	PF04616.9	Glyco_hydro_43	749	265	486	410	658	342	444	278	845	273	363	487	674	331	516	407
	PF07470	Glyco_hydro_88	236	70	228	108	183	109	175	116	280	95	128	143	213	100	170	132
	PF03632	Glyco_hydro_65m	8	3	9	4	3	3	8	4	2	5	15	5	4	6	4	6
	PF02065	Melibiase	158	98	138	105	211	131	135	105	193	96	95	125	146	136	116	111
	PF01204	Trehalase	2	5	2	4	0	3	5	0	1	1	1	1	4	3	6	4
Other glycoside hydrolases	PF02056	Glyco_hydro_4	6	5	2	9	13	10	4	4	6	5	10	5	9	5	10	6
	PF00722	Glyco_hydro_16	129	50	58	52	121	85	66	57	93	57	55	78	187	75	101	91
	PF00728	Glyco_hydro_20	88	29	42	33	46	22	42	34	56	22	46	46	49	28	56	52
	PF01055	Glyco_hydro_31	465	172	347	241	428	248	339	251	615	186	268	341	503	196	317	284
	PF00251	Glyco_hydro_32N	374	133	197	167	212	161	182	289	185	90	149	567	217	94	391	203
	PF02012	BNR	2	5	0	2	0	5	2	0	0	5	1	1	1	0	2	1
	PF03200	Glyco_hydro_63	0	0	0	2	1	0	0	1	1	1	3	3	1	1	0	3
	PF03639	Glyco_hydro_81	0	0	1	0	1	1	1	1	2	0	1	0	1	1	1	2
	PF05089	NAGLU	37	55	33	37	39	37	43	38	37	15	46	22	38	37	33	46
	PF03562	MltA	0	1	0	0	0	0	0	1	1	2	1	2	0	0	2	3
Lysozymes	PF01183	Glyco_hydro_25	187	112	186	171	178	180	142	131	223	113	151	361	245	125	182	196
Cellulose binding	PF00553	CBM_2	2	0	2	1	1	1	3	1	1	3	3	2	4	2	1	1
	PF00942	CBM_3	31	5	9	15	15	14	6	5	25	4	2	19	10	11	6	9
	PF02018	CBM_4_9	76	100	71	78	64	112	80	51	91	118	60	99	89	120	104	93
	PF03425	CBM_11	1	19	6	10	4	9	10	13	2	38	18	18	7	33	24	11
Chitin binding	PF02839	CBM_5_12	40	0	18	16	18	7	21	16	43	6	13	39	20	2	2	14
Starch binding	PF00686	CBM_20	96	20	58	41	66	67	81	40	174	74	64	60	142	55	42	65
	PF02903	Alpha-amylase_N	3	5	1	8	19	8	9	8	18	14	7	3	10	6	4	5
	PF03423	CBM_25	0	0	3	0	0	1	0	0	0	1	0	0	0	2	1	1
Oligosaccaride binding	PF03422	CBM_6	212	164	143	170	164	171	145	128	219	201	147	218	176	207	206	173
Other carbohydrate binding modules (CBM)	PF00652	Ricin_B_lectin	24	11	5	11	10	15	3	5	8	12	5	18	7	12	13	6
	PF00754	F5_F8_type_C	35	24	37	20	27	12	19	11	37	21	12	19	30	30	17	30
	PF08305	NPCBM	3	4	6	12	3	10	5	2	8	6	9	9	3	9	11	8
Bacterial cell wall degradation (CBM)	PF01476	LysM	213	138	257	202	213	176	249	169	283	222	301	181	261	259	253	333

Supplementary Table S7. Volatile fatty acid (VFA) concentrations [nmol g^{-1} rumen fluid] of all rumen fluid samples taken between 4 am and 4 pm. Between t0 (before the feeding) and t5 (five hours after the feeding started) a strong negative linear correlation between pH and total VFA was observed (r (14) = -0.84, p = < 0.0001). Although pH measurements were within a narrow range (pH 6-7).

Cow	Time	Formic acid	Acetic acid	Propionic acid	Iso-butyric acid	Butyric acid	Iso-valeric acid	Valeric acid	Iso-caproic acid	Caproic acid	Heptanoic acid	Sorbic acid	Benzoic acid	Lactic acid	Succinic acid	Hippuric acid	Total SCFAs
cow 1	04 am	0.0	63.4	16.8	0.8	16.2	0.5	1.8	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	99.7
	07 am (t0)	0.0	62.4	15.4	0.9	14.9	0.6	1.6	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	95.9
	08 am (t1)	0.0	61.6	19.6	0.8	15.2	0.6	1.5	0.0	0.2	0.0	0.0	0.0	9.1	0.1	0.0	108.8
	10 am (t3)	0.0	67.2	20.7	1.0	16.7	0.6	2.2	0.0	0.2	0.0	0.0	0.0	0.3	0.0	0.0	108.8
	12 am (t5)	0.0	54.3	13.4	0.8	11.9	0.4	1.3	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	82.1
	02 pm	0.0	55.9	13.0	0.8	11.0	0.4	1.0	0.0	0.1	0.0	0.0	0.0	1.5	0.0	0.0	83.8
	04 pm	0.0	59.3	22.6	0.8	15.9	0.7	1.7	0.0	0.2	0.0	0.0	0.0	0.9	0.0	0.0	102.1
cow 2	04 am	0.0	49.7	23.3	0.8	7.3	0.4	3.0	0.0	0.5	0.1	0.0	0.0	0.0	0.0	0.0	85.1
	07 am (t0)	0.0	56.7	24.4	0.9	7.3	0.4	2.7	0.0	0.5	0.1	0.0	0.0	0.0	0.0	0.0	93.1
	08 am (t1)	0.0	55.7	21.6	0.9	11.4	0.5	4.2	0.0	1.0	0.1	0.0	0.0	0.9	0.0	0.0	96.2
	10 am (t3)	0.0	66.1	30.6	1.0	12.8	0.6	4.9	0.0	1.2	0.2	0.0	0.0	0.0	0.0	0.0	117.3
	12 am (t5)	0.0	60.1	27.7	1.0	9.3	0.5	3.4	0.0	0.8	0.1	0.0	0.0	0.0	0.0	0.0	102.8
	02 pm	0.0	59.7	24.4	0.9	8.6	0.5	2.9	0.0	0.6	0.1	0.0	0.0	6.8	0.2	0.0	104.7
	04 pm	0.3	54.2	23.0	0.7	15.3	0.5	4.9	0.0	1.4	0.1	0.0	0.0	16.2	0.2	0.0	116.7
cow 3	04 am	0.0	59.9	24.8	0.7	10.6	0.4	2.1	0.0	0.5	0.1	0.0	0.0	0.0	0.0	0.0	99.0
	07 am (t0)	0.0	54.7	18.7	0.8	8.6	0.3	1.3	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	84.8
	08 am (t1)	0.0	59.4	21.8	0.9	12.0	0.5	2.0	0.0	0.6	0.0	0.0	0.0	0.0	0.0	0.0	97.2
	10 am (t3)	0.0	61.3	23.0	0.8	11.1	0.4	1.7	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	98.9
	12 am (t5)	0.0	60.3	21.1	0.9	10.0	0.5	1.4	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	94.6
	02 pm	0.0	62.6	20.9	0.9	11.3	0.5	1.3	0.0	0.4	0.0	0.0	0.0	8.3	0.1	0.0	106.2
	04 pm	0.0	65.5	29.1	1.0	17.6	0.8	2.4	0.0	0.8	0.0	0.0	0.0	1.7	0.0	0.0	118.9
cow 4	04 am	0.0	70.2	32.3	0.8	12.4	0.5	3.2	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	119.9
	07 am (t0)	0.0	56.1	21.4	0.7	8.0	0.3	1.7	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	88.4
	08 am (t1)	0.0	54.2	18.9	0.8	8.1	0.3	1.5	0.0	0.2	0.0	0.0	0.0	0.0	0.1	0.0	84.0
	10 am (t3)	0.0	60.8	20.0	0.9	7.8	0.4	1.3	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	91.4
	12 am (t5)	0.0	53.0	15.8	0.9	6.1	0.4	0.8	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	77.0
	02 pm	0.0	53.5	15.5	0.9	8.7	0.4	1.2	0.0	0.2	0.0	0.0	0.0	4.9	0.0	0.0	85.2
	04 pm	0.0	64.5	24.8	0.9	21.6	0.7	3.5	0.0	0.6	0.0	0.0	0.0	10.7	0.0	0.0	127.3

Supplementary Table S8. Additional information on the cows.

Cow ID	Parity	Days in milk	Live weight (kg)	Milk yield (kg)	Days in pregnancy
Cow 1	3	242	629	25.4	97
Cow 2	4	164	597	36.5	24
Cow 3	3	95	600	34.6	-
Cow 4	2	357	581	37.4	174

Supplementary references

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