

1 **Title:** Ruminal bacterial community composition in dairy cows is dynamic over the course of  
2 two lactations and correlates with feed efficiency

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4 **Running title:** Dairy cow feed efficiency and ruminal bacteria

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18 cycles, efficiency

19 **TABLE S1** Feed ingredients and composition for total mixed ration (TMR) diet, averaged across  
 20 the lactation period.

<b>Ingredient</b> <sup>a</sup>	<b>First lactation % DM</b>	<b>Second lactation % DM</b>
Corn silage	32.9	33.7
Alfalfa haylage	26.2	23.3
High-moisture corn, finely ground	14.8	16.6
Dry corn	5.7	3.6
Roasted soybeans	7.4	8.1
Canola meal	6.3	7.7
Distillers dried grains	4.2	4.2
Vitamin/mineral mix	2.5	3.0
<b>Composition</b>		
Neutral detergent fiber	28.0	30.3
Acid detergent fiber	18.5	20.9
Non-fiber carbohydrates	43.6	43.3
Starch	27.0	16.5
Crude protein	17.1	17.1
Fat	5.2	4.9
Ash	4.8	4.6

21 <sup>a</sup> Diets also contained Rumensin (Elanco Animal Health, Greenfield, IN) at 12.2 mg/kg DM

22 **TABLE S2** Sequencing, richness, and diversity metrics for all samples.

<b>Cow ID</b>	<b>Cycle</b>	<b>Stage <sup>a</sup></b>	<b>Phase</b>	<b>Sequence counts</b>	<b>Coverage (Good's)</b>	<b>95 % OTUs</b>	<b>Richness (Chao1)</b>	<b>Diversity (Inverse Simpson)</b>
4255	First	Early	Liquid	1,035	0.80	362	707.02	71.37
4255	First	Early	Solid	1,523	0.86	409	797.55	65.74
4255	First	Middle	Liquid	1,296	0.83	417	742.96	117.04
4255	First	Middle	Solid	1,381	0.87	360	585.04	78.22
4255	First	Late	Liquid	778	0.77	322	575.40	157.51
4255	First	Late	Solid	1,558	0.85	442	825.96	109.19
4255	Second	Early	Liquid	1,210	0.88	289	461.08	51.32
4255	Second	Early	Solid	4,079	0.94	560	883.20	55.79
4255	Second	Middle	Liquid	1,252	0.81	423	909.00	127.48
4255	Second	Middle	Solid	3,473	0.93	590	883.04	92.20
4255	Second	Late	Liquid	1,160	0.84	375	602.51	151.09
4255	Second	Late	Solid	566	0.76	225	435.35	72.25
4260	First	Early	Liquid	1,164	0.83	370	673.09	102.37
4260	First	Early	Solid	1,693	0.88	430	708.67	75.20
4260	First	Middle	Liquid	1,795	0.84	512	966.19	100.08
4260	First	Middle	Solid	2,031	0.86	536	938.68	122.36
4260	First	Late	Liquid	1,422	0.84	424	751.08	130.23
4260	First	Late	Solid	1,931	0.88	437	815.42	67.08
4260	Second	Early	Liquid	1,226	0.86	324	576.17	78.66
4260	Second	Early	Solid	3,364	0.94	494	703.01	46.95
4260	Second	Middle	Liquid	1,212	0.82	396	812.27	101.56
4260	Second	Middle	Solid	2,922	0.91	566	908.18	74.66
4260	Second	Late	Liquid	1,360	0.84	412	776.01	90.99
4260	Second	Late	Solid	2,752	0.93	445	648.60	46.78
4261	First	Early	Liquid	2,858	0.92	501	868.97	72.53
4261	First	Early	Solid	1,209	0.86	317	570.61	52.24
4261	First	Middle	Liquid	2,631	0.90	567	972.01	115.16
4261	First	Middle	Solid	1,892	0.88	430	811.82	81.56
4261	First	Late	Liquid	1,643	0.87	435	759.01	101.51
4261	First	Late	Solid	1,711	0.88	410	706.13	105.12
4261	Second	Early	Liquid	1,685	0.86	454	775.16	118.82
4261	Second	Early	Solid	865	0.81	287	531.50	91.34
4261	Second	Middle	Liquid	1,347	0.83	417	757.50	116.21
4261	Second	Middle	Solid	1,241	0.84	355	693.35	79.05
4261	Second	Late	Liquid	1,226	0.82	411	708.69	124.76
4261	Second	Late	Solid	2,294	0.91	439	761.72	65.89
4262	First	Early	Liquid	1,967	0.85	535	1,058.14	122.98
4262	First	Early	Solid	1,600	0.88	399	645.33	92.80
4262	First	Middle	Liquid	1,659	0.86	454	811.00	97.75
4262	First	Middle	Solid	1,251	0.83	377	741.88	102.76
4262	First	Late	Liquid	1,786	0.88	435	760.54	78.99

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4262	First	Late	Solid	1,383	0.86	389	653.69	107.81
4262	Second	Early	Liquid	1,274	0.81	438	776.58	164.02
4262	Second	Early	Solid	3,764	0.92	651	1,108.53	99.10
4262	Second	Middle	Liquid	1,535	0.82	477	961.20	111.97
4262	Second	Middle	Solid	1,662	0.87	444	729.80	109.59
4262	Second	Late	Liquid	1,447	0.83	466	851.01	142.36
4262	Second	Late	Solid	2,341	0.91	459	674.97	82.18
4273	First	Early	Liquid	1,355	0.85	356	804.50	56.82
4273	First	Early	Solid	1,206	0.89	282	478.63	46.53
4273	First	Middle	Liquid	1,321	0.86	371	654.52	69.55
4273	First	Middle	Solid	1,975	0.90	425	713.77	68.18
4273	First	Late	Liquid	1,343	0.84	445	685.90	137.69
4273	First	Late	Solid	1,491	0.86	400	719.51	94.12
4273	Second	Early	Liquid	1,249	0.84	372	676.50	77.53
4273	Second	Early	Solid	1,619	0.86	397	789.10	50.14
4273	Second	Middle	Liquid	1,162	0.82	403	688.00	130.90
4273	Second	Middle	Solid	3,680	0.94	564	891.32	77.12
4273	Second	Late	Liquid	1,214	0.85	375	604.40	112.36
4273	Second	Late	Solid	3,231	0.93	490	865.39	59.91
4275	First	Early	Liquid	1,804	0.87	472	783.57	94.69
4275	First	Early	Solid	1,377	0.85	372	747.94	89.43
4275	First	Middle	Liquid	1,523	0.85	433	799.60	86.80
4275	First	Middle	Solid	1,170	0.83	350	728.87	98.53
4275	First	Late	Liquid	1,365	0.84	402	749.84	92.41
4275	First	Late	Solid	1,166	0.87	316	504.91	76.37
4275	Second	Early	Liquid	1,496	0.82	477	933.28	112.24
4275	Second	Early	Solid	2,711	0.92	517	798.28	101.46
4275	Second	Middle	Liquid	1,356	0.84	415	661.17	114.54
4275	Second	Middle	Solid	2,287	0.92	423	691.52	81.91
4275	Second	Late	Liquid	1,239	0.83	398	720.54	134.34
4275	Second	Late	Solid	2,962	0.92	491	812.16	78.36
4276	First	Early	Liquid	1,331	0.90	283	456.94	32.78
4276	First	Early	Solid	1,387	0.91	276	437.88	36.92
4276	First	Middle	Liquid	1,389	0.86	378	679.95	100.70
4276	First	Middle	Solid	1,012	0.84	284	610.15	74.34
4276	First	Late	Liquid	1,274	0.86	348	631.98	100.66
4276	First	Late	Solid	1,227	0.86	332	596.68	89.05
4276	Second	Early	Liquid	1,503	0.88	379	667.47	96.69
4276	Second	Early	Solid	2,319	0.93	391	611.23	42.13
4276	Second	Middle	Liquid	1,223	0.83	374	701.44	117.42
4276	Second	Middle	Solid	2,421	0.93	418	617.51	66.76
4276	Second	Late	Liquid	4,515	0.94	642	961.38	102.57
4276	Second	Late	Solid	2,294	0.92	409	676.99	73.22
4277	First	Early	Liquid	1,366	0.86	359	653.10	62.34
4277	First	Early	Solid	1,503	0.87	382	691.23	80.79
4277	First	Middle	Liquid	1,251	0.86	322	548.25	46.47

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4277	First	Middle	Solid	1,660	0.88	413	668.13	88.40
4277	First	Late	Liquid	2,776	0.91	557	873.89	85.02
4277	First	Late	Solid	1,875	0.90	392	677.05	73.76
4277	Second	Early	Liquid	20,434	0.98	1,226	1,692.67	90.24
4277	Second	Early	Solid	2,109	0.90	455	699.64	91.56
4277	Second	Middle	Liquid	2,684	0.92	468	754.00	81.64
4277	Second	Middle	Solid	2,985	0.94	452	653.66	74.52
4277	Second	Late	Liquid	3,398	0.93	536	918.51	63.19
4277	Second	Late	Solid	1,332	0.88	318	534.57	73.09
4278	First	Early	Liquid	1,074	0.83	301	623.20	40.43
4278	First	Early	Solid	1,121	0.87	277	493.20	55.28
4278	First	Middle	Liquid	2,908	0.91	505	875.91	36.01
4278	First	Middle	Solid	1,582	0.87	385	656.62	73.39
4278	First	Late	Liquid	1,182	0.87	270	561.10	33.42
4278	First	Late	Solid	1,239	0.87	309	537.11	62.28
4278	Second	Early	Liquid	2,501	0.92	454	686.65	66.10
4278	Second	Early	Solid	1,522	0.90	333	504.28	41.44
4278	Second	Middle	Liquid	1,502	0.87	373	659.59	57.18
4278	Second	Middle	Solid	2,680	0.92	474	795.04	89.71
4278	Second	Late	Liquid	2,448	0.91	456	770.50	52.61
4278	Second	Late	Solid	3,061	0.94	389	662.65	37.95
4281	First	Early	Liquid	1,148	0.87	280	555.65	58.21
4281	First	Early	Solid	1,289	0.84	358	701.10	79.35
4281	First	Middle	Liquid	1,020	0.82	338	629.61	104.23
4281	First	Middle	Solid	532	0.77	210	460.00	88.56
4281	First	Late	Liquid	1,287	0.84	370	716.55	77.18
4281	First	Late	Solid	1,162	0.85	320	545.33	77.86
4281	Second	Early	Liquid	3,287	0.92	578	914.49	83.24
4281	Second	Early	Solid	2,718	0.90	559	1,014.07	98.66
4281	Second	Middle	Liquid	4,988	0.95	727	970.16	127.21
4281	Second	Middle	Solid	2,792	0.92	483	770.73	80.39
4281	Second	Late	Liquid	6,342	0.95	798	1,142.88	105.97
4281	Second	Late	Solid	2,470	0.93	377	612.49	53.07
4282	First	Early	Liquid	1,036	0.84	311	515.44	89.18
4282	First	Early	Solid	1,465	0.86	412	664.74	97.85
4282	First	Middle	Liquid	1,230	0.86	328	590.89	59.01
4282	First	Middle	Solid	1,310	0.84	380	757.05	86.82
4282	First	Late	Liquid	1,009	0.85	290	505.33	58.07
4282	First	Late	Solid	1,456	0.88	377	613.91	93.27
4282	Second	Early	Liquid	2,306	0.90	524	813.20	108.92
4282	Second	Early	Solid	3,309	0.93	517	838.76	73.01
4282	Second	Middle	Liquid	1,829	0.88	441	724.11	95.35
4282	Second	Middle	Solid	626	0.79	231	423.00	88.16
4282	Second	Late	Liquid	3,543	0.93	600	890.19	100.64
4282	Second	Late	Solid	2,529	0.93	408	619.17	67.25
4288	First	Early	Liquid	922	0.85	259	464.50	56.82

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4288	First	Early	Solid	1,449	0.86	389	647.24	92.22
4288	First	Middle	Liquid	1,094	0.82	331	717.12	64.14
4288	First	Middle	Solid	1,008	0.81	341	635.00	92.89
4288	First	Late	Liquid	737	0.80	240	530.38	44.29
4288	First	Late	Solid	1,097	0.81	373	661.12	94.00
4288	Second	Early	Liquid	2,629	0.90	565	855.51	111.27
4288	Second	Early	Solid	2,749	0.91	505	882.92	63.48
4288	Second	Middle	Liquid	1,189	0.85	355	612.36	96.42
4288	Second	Middle	Solid	2,495	0.91	456	772.97	70.60
4288	Second	Late	Liquid	1,986	0.90	449	681.10	114.91
4288	Second	Late	Solid	2,305	0.92	423	625.84	75.17
4294	First	Early	Liquid	856	0.81	283	552.45	82.83
4294	First	Early	Solid	1,328	0.87	368	608.63	106.12
4294	First	Middle	Liquid	865	0.79	310	660.22	111.02
4294	First	Middle	Solid	1,169	0.86	311	534.16	83.23
4294	First	Late	Liquid	828	0.81	266	541.62	71.49
4294	First	Late	Solid	1,115	0.83	336	595.02	89.71
4294	Second	Early	Liquid	2,236	0.89	476	885.45	74.80
4294	Second	Early	Solid	2,512	0.91	461	806.96	94.32
4294	Second	Middle	Liquid	2,850	0.91	574	872.81	101.59
4294	Second	Middle	Solid	2,066	0.91	401	675.66	64.48
4294	Second	Late	Liquid	3,521	0.93	545	962.24	79.46
4294	Second	Late	Solid	2,250	0.93	359	514.30	35.75
4297	First	Early	Liquid	927	0.83	289	518.69	75.11
4297	First	Early	Solid	744	0.78	272	590.07	81.44
4297	First	Middle	Liquid	6,803	0.94	907	1,425.77	80.68
4297	First	Middle	Solid	1,610	0.86	450	776.33	125.96
4297	First	Late	Liquid	1,380	0.85	385	721.38	74.33
4297	First	Late	Solid	1,388	0.85	394	700.01	105.27
4297	Second	Early	Liquid	1,195	0.81	430	782.58	146.13
4297	Second	Early	Solid	2,962	0.91	585	988.53	107.77
4297	Second	Middle	Liquid	2,089	0.91	450	631.01	98.18
4297	Second	Middle	Solid	2,056	0.89	479	752.39	95.87
4297	Second	Late	Liquid	2,312	0.90	499	838.00	109.53
4297	Second	Late	Solid	1,562	0.89	357	624.00	68.12

23 <sup>a</sup> Early = 76-82 days DIM, Middle = 151-157 DIM, and Late = 251-257 DIM.

24 **TABLE S3** Individual 95 % (genus-level) OTUs with total relative abundance > 0.1 % that  
 25 displayed significant ( $P < 0.05$ ) correlation of total relative abundance (y) with gross feed  
 26 efficiency (GFE, x) by linear regression analysis.

<b>Increases with increasing GFE<sup>a</sup></b>				
<b>From liquid phase (OTU)<sup>b</sup></b>	<b>y-intercept when <math>x = 0</math></b>	<b>Slope</b>	<b>R<sup>2</sup></b>	<b>P&gt;F</b>
<i>Prevotella</i>	-1.8250	1.5202	0.318	0.036
<i>Coprococcus</i>	-1.0176	0.9221	0.269	0.057
<i>Prevotella</i>	-3.5540	2.3774	0.263	0.061
<i>Prevotella</i>	-0.9785	0.8167	0.220	0.091
Lachnospiraceae	-1.2932	0.9703	0.310	0.039
<i>Prevotella</i>	-0.4728	0.4418	0.324	0.034
Mollicutes <sup>c</sup> RF39	-0.6971	0.5666	0.327	0.029
<i>Prevotella</i>	-0.5472	0.4668	0.241	0.075
<i>Prevotella</i>	-1.0330	0.7486	0.352	0.025
<b>From solid phase (OTU)</b>				
Succinivibrionaceae	-14.6080	10.7705	0.240	0.075
<i>Prevotella</i>	-0.9823	1.0964	0.299	0.043
<i>Prevotella</i>	-2.4911	2.1606	0.289	0.047
<i>Prevotella</i>	-1.8728	1.5035	0.299	0.043
Lachnospiraceae	-0.7556	0.7553	0.217	0.093
<i>Prevotella</i>	-0.5217	0.5289	0.330	0.032
<i>Prevotella</i>	-0.6601	0.5910	0.272	0.056
<i>Prevotella</i>	-1.3105	0.9645	0.279	0.052
Lachnospiraceae	-0.4428	0.4100	0.242	0.074
<i>Prevotella</i>	-2.0392	1.3273	0.262	0.061
<i>Prevotella</i>	-1.5400	1.0205	0.266	0.059
<b>Increases with decreasing GFE</b>				
<b>From liquid phase (OTU)</b>	<b>y-intercept when <math>x = 0</math></b>	<b>Slope</b>	<b>R<sup>2</sup></b>	<b>P&gt;F</b>
<i>Prevotella</i>	11.5485	-4.6026	0.234	0.079
<i>Prevotella</i>	7.1686	-3.1769	0.247	0.071
<i>Prevotella</i>	7.2300	-3.2709	0.271	0.056
<i>Butyrivibrio</i>	5.3399	-2.4969	0.403	0.015
<i>Prevotella</i>	4.0020	-1.9062	0.331	0.031
<i>Sharpea</i>	4.8951	-2.4570	0.326	0.033
<i>Succinivibrio</i>	2.9200	-1.4260	0.443	0.009
<i>Pseudobutyrvibrio</i>	2.0852	-0.9469	0.266	0.059
Bacteroidales	2.3023	-1.1252	0.238	0.077
<i>Prevotella</i>	1.5191	-0.6849	0.241	0.075
Prevotellaceae	1.5847	-0.7477	0.285	0.049
<i>Anaerovibrio</i>	1.3086	-0.6330	0.392	0.017

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Clostridiales	0.0654	-0.2735	0.232	0.081
<i>Prevotella</i>	0.8004	-0.3614	0.446	0.009
<b>From solid phase</b>				
<i>Prevotella</i>	8.9829	-3.9266	0.280	0.052
<i>Prevotella</i>	9.4350	-4.3057	0.462	0.020
<i>Pseudobutyrvibrio</i>	4.1622	-1.7607	0.336	0.030
<i>Prevotella</i>	3.4779	-1.3584	0.318	0.036
Ruminococcaceae	2.4365	-0.9748	0.217	0.062
Bacteroidetes S24-7	5.7987	-2.5629	0.377	0.111
Lachnospiraceae	3.1281	-1.4263	0.313	0.038
<i>Prevotella</i>	3.2554	-1.5287	0.330	0.032
Clostridiales	2.2319	-1.0048	0.465	0.007
Clostridia	2.6496	-1.3434	0.515	0.004
Lachnospiraceae	2.2191	-1.1066	0.473	0.007
Paraprevotellaceae CF231	1.4508	-0.6660	0.402	0.015
<i>Ruminococcus</i>	2.1965	-1.1140	0.384	0.018
<i>Succinivibrio</i>	1.5021	-0.7338	0.404	0.015
Paraprevotellaceae YRC22	0.6556	-0.2389	0.324	0.034
<i>Prevotella</i>	0.9567	-0.4215	0.231	0.082
<i>Clostridium</i>	1.1794	-0.5535	0.529	0.003
Lachnospiraceae	1.3632	-0.6784	0.384	0.018

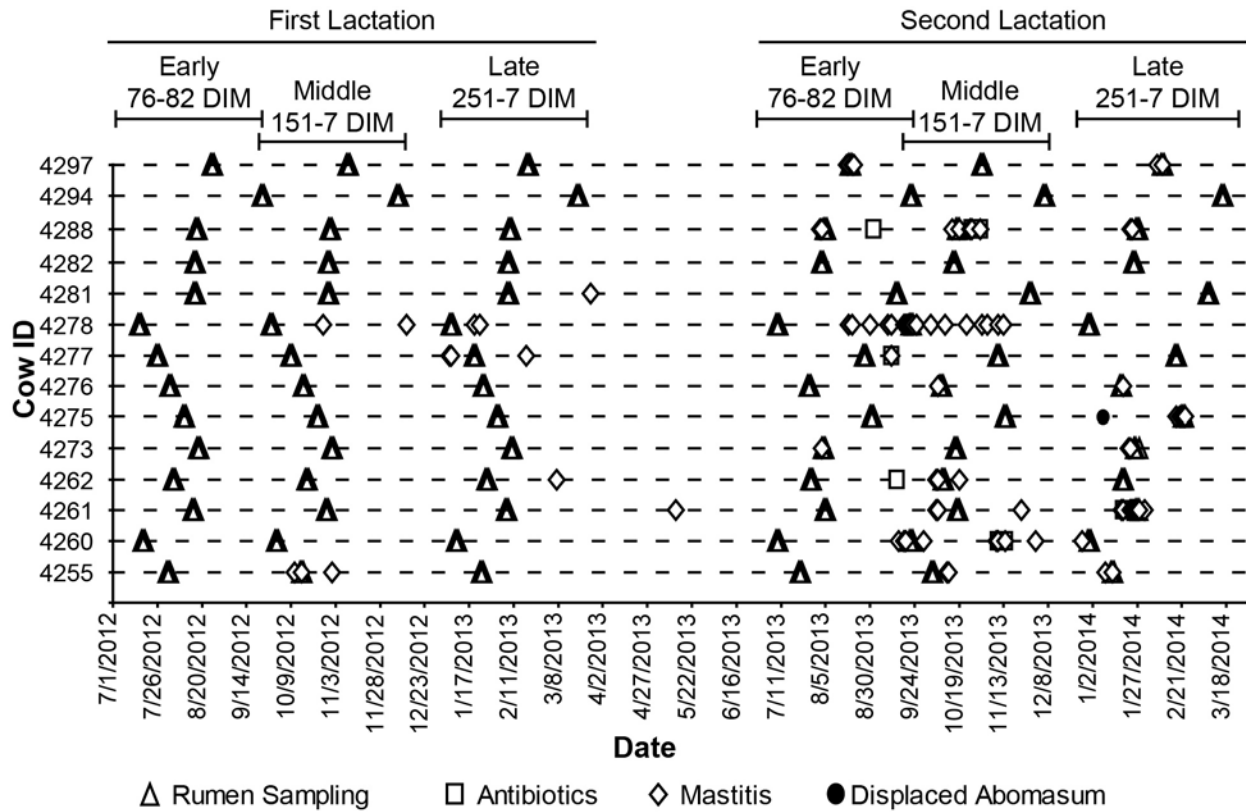
27 <sup>a</sup> GFE ranged from 0.95 to 2.43, excluding the GFE value for 4275 of 0.27 due to recovery from

28 surgically correcting a displaced abomasum (Second, Late).

29 <sup>b</sup> Each OTU is reported with the nearest classifiable taxonomic level.

30 <sup>c</sup> Additional taxonomic information is given for clarity.





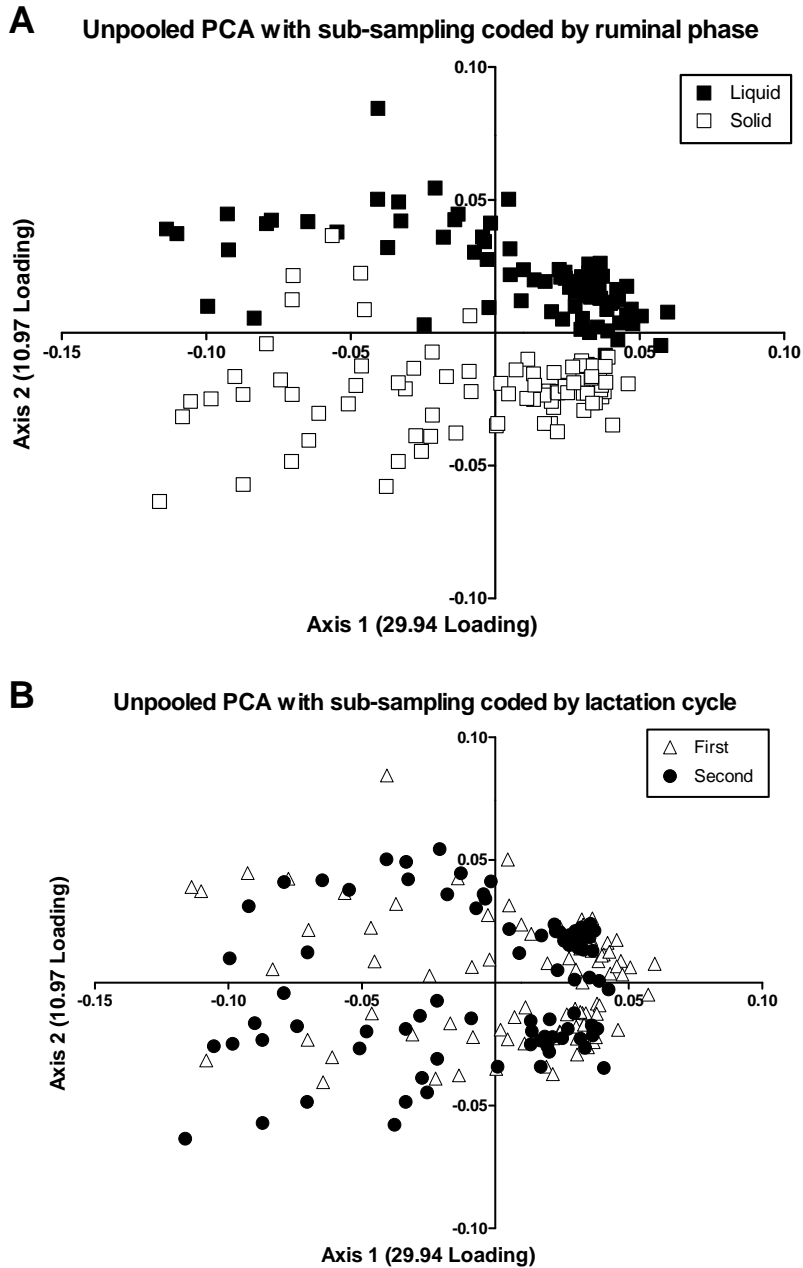
31                      △ Rumen Sampling      □ Antibiotics      ◇ Mastitis      ● Displaced Abomasum

32 **FIG S1** Sampling periods and health-impacting events for the 14 cows retained through both

33 lactation cycles. Triangles mark ruminal sampling days (3 successive days), rectangles mark

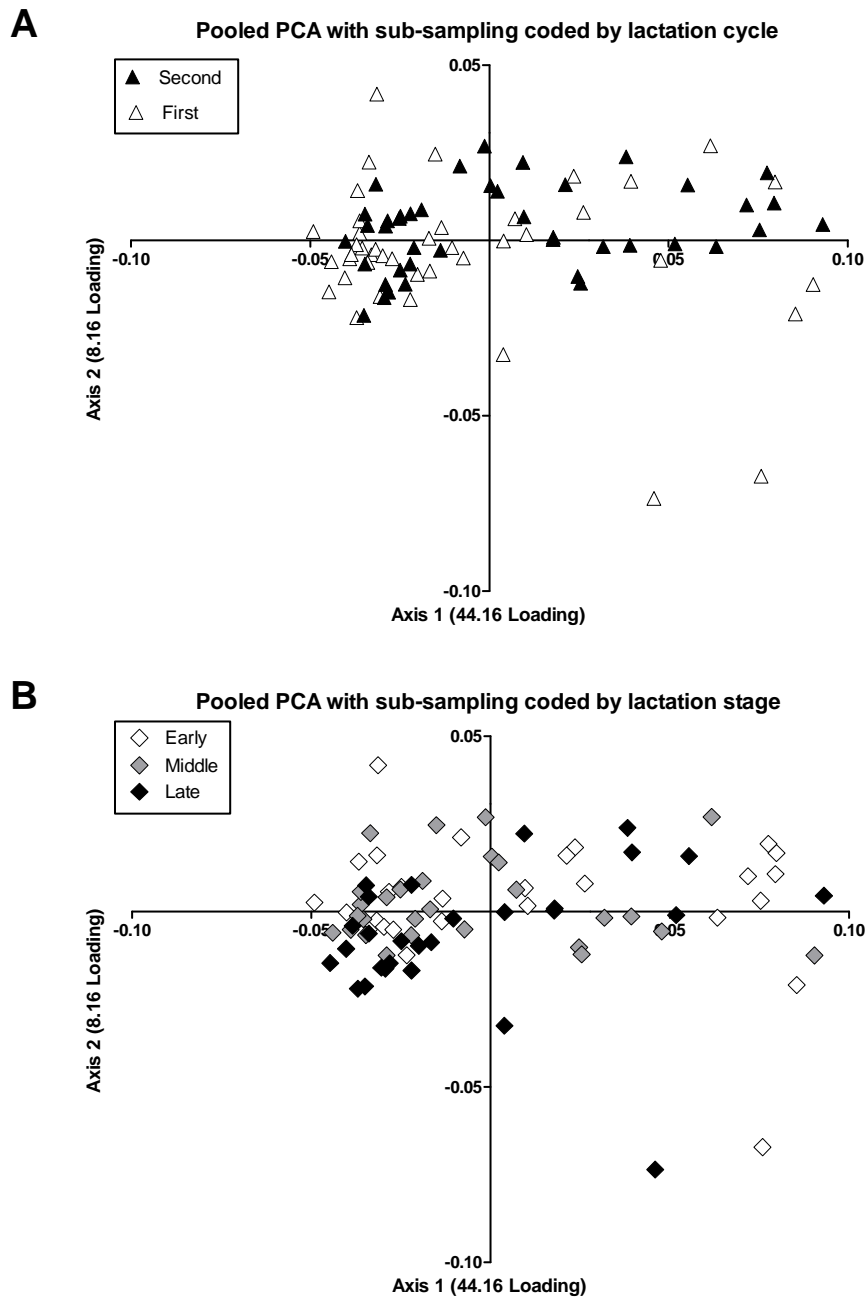
34 topical or localized antibiotic treatments, diamonds indicate mastitis detection (culture- or SCC-

35 based definition), and the single oval indicates a displaced abomasum for cow 4275.



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37 **FIG S2** The ruminal communities separate by phase but not by lactation cycle. (A) Black  
 38 squares = liquid-phase communities. White squares = solid-phase communities. (B) White  
 39 triangles = first-lactation communities. Black circles = second-lactation communities. Both parts  
 40 are graphed from the same sub-sampled PCA of all samples.



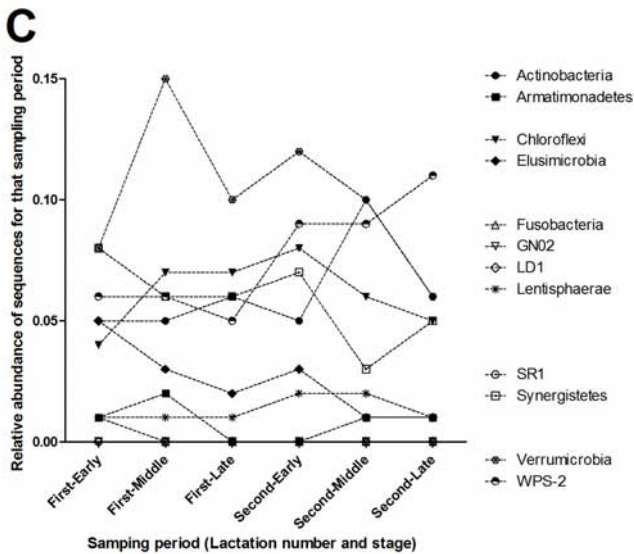
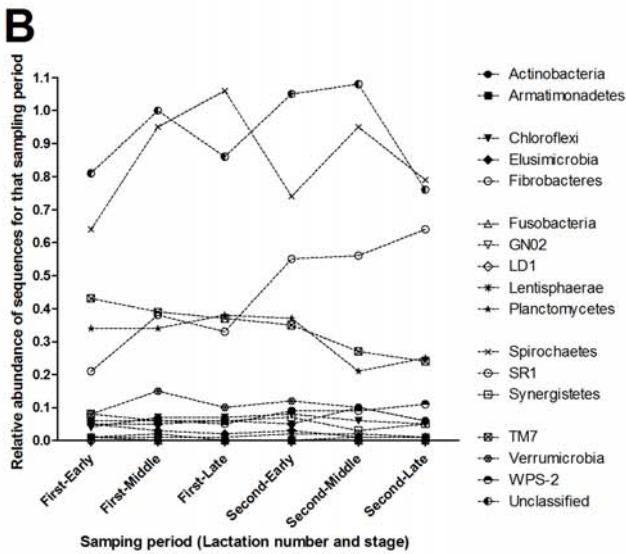
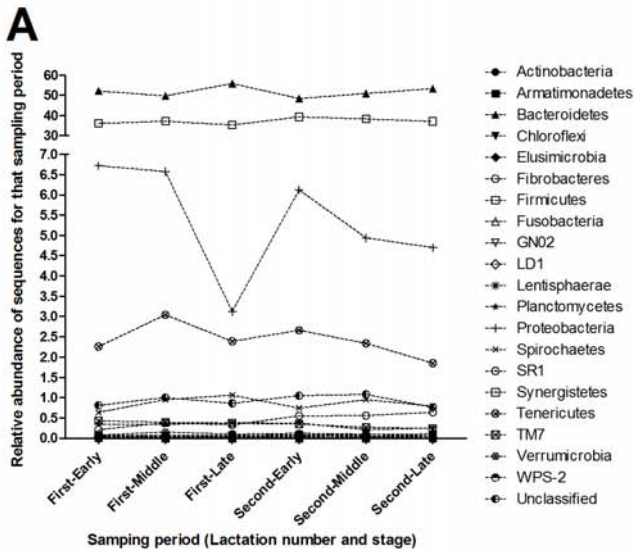
41

42 **FIG S3** The pooled (solid and liquid) ruminal communities overlap by PCA with sub-sampling.

43 (A) PCA coded by first (empty triangle) or second (filled triangle) lactation cycle. (B) PCA

44 coded by Early (76 - 82 DIM, empty diamond), Middle (151 - 157 DIM, gray diamond), and

45 Late (251 - 257 DIM, black diamond) stages within both lactation cycles.



47 **FIG S4** The changes in the relative sequence abundance for each phylum at each sampling  
48 period for both lactation cycles. The sequences were pooled (solid and liquid phase and all cows)  
49 for each sampling period. (A) All values are shown, (B) all values with a maximum of 1.1%  
50 relative sequence abundance are shown, and (C) all values with a maximum of 0.15% relative  
51 sequence abundance are shown.