

Microbial succession in the gastrointestinal tract of dairy cows from 2 weeks to first lactation

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Running Head: Microbial succession in the gut of dairy cows

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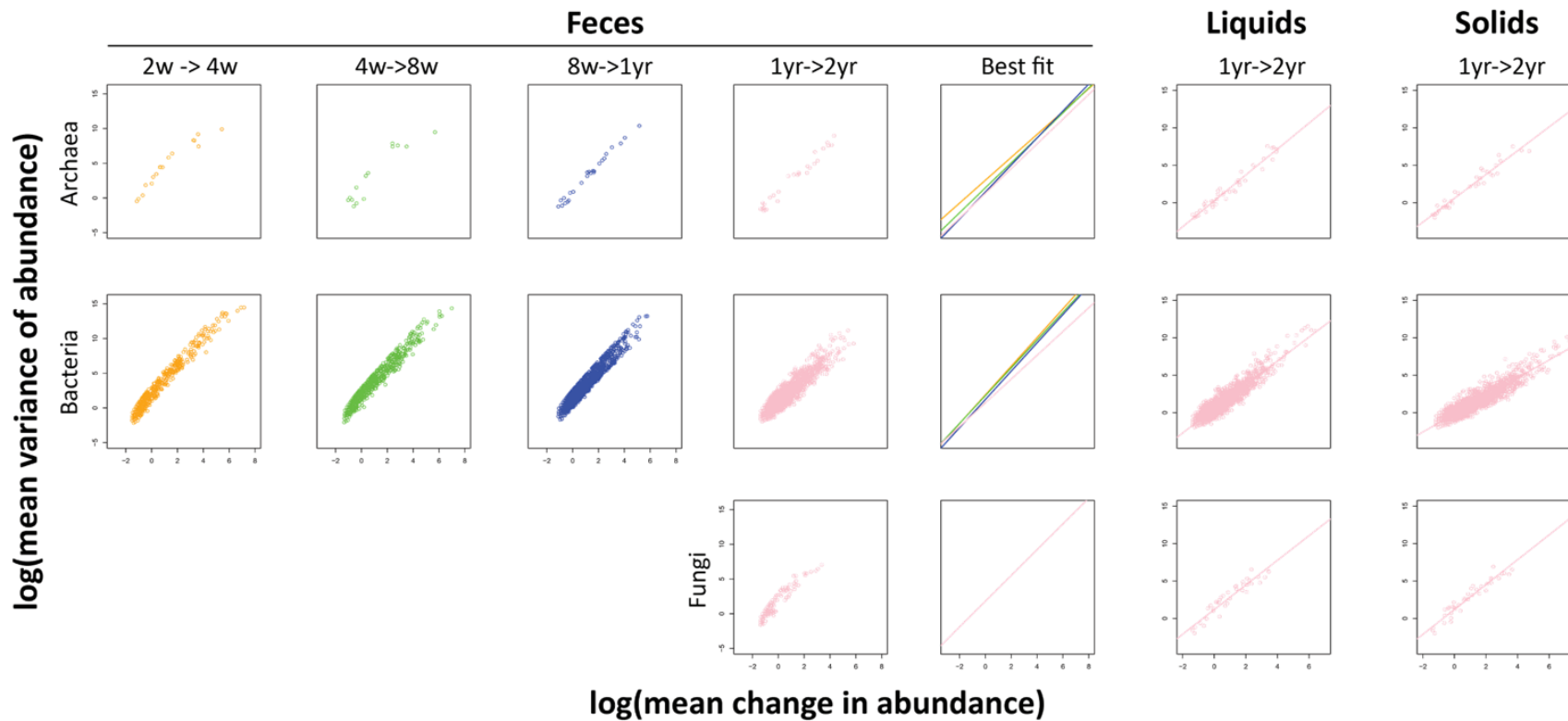


Fig. S1. Mean change in abundance versus mean variance of abundance of OTUs. Log OTU abundances were compared by animal, pairwise between age groups, and corrected for number of days elapsed between samplings. Only animals with both samples in the pairwise comparison and OTUs present in at least two samples were included. Rumen liquid and solid 8 week samples were not compared to 1-year samples, because they came from different animals.

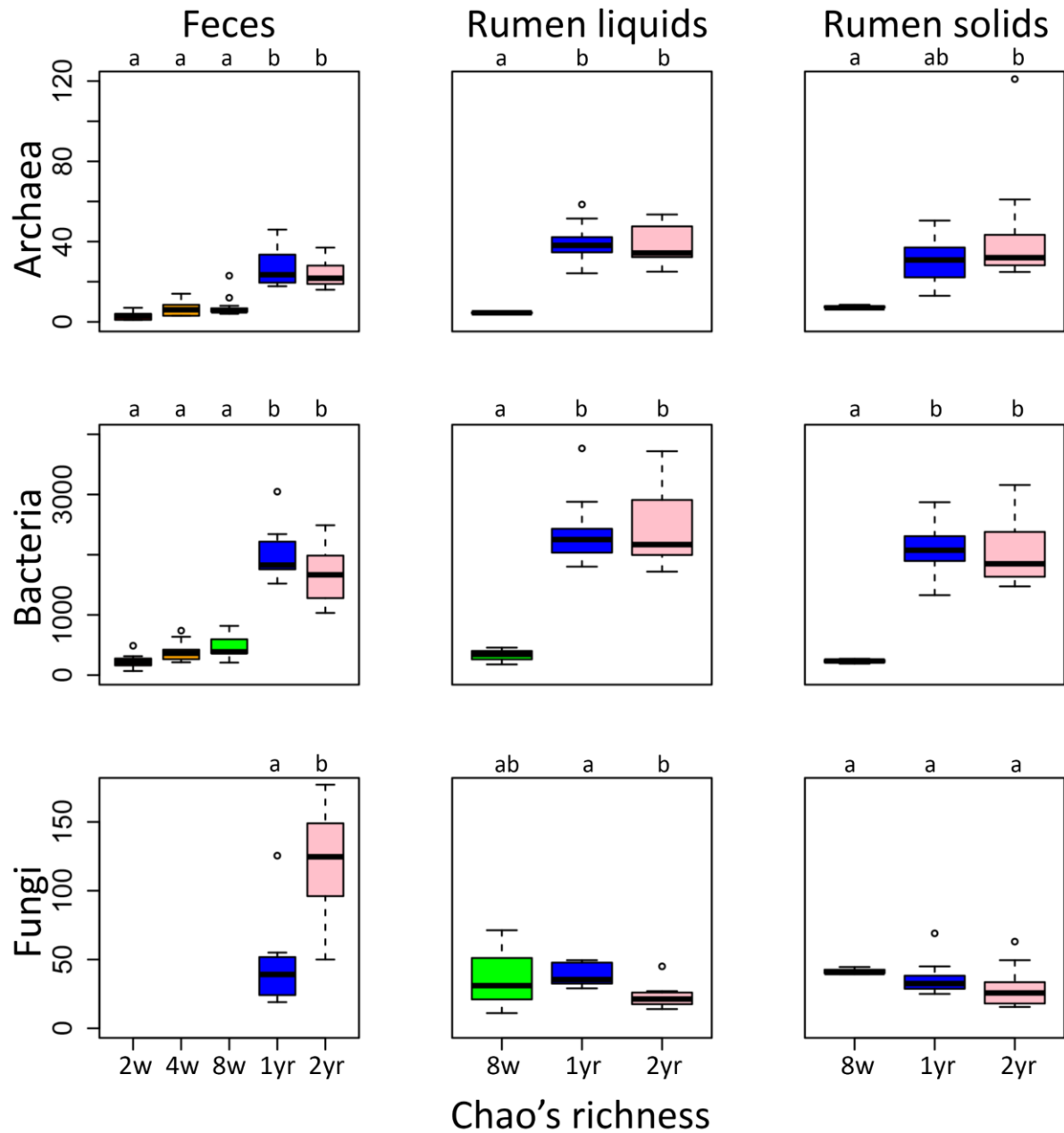


Fig. S2. Chao's richness estimate for archaeal, bacterial, and fungal communities in dairy cow feces as well as rumen liquids and solids. Data are expressed as boxplots with outliers shown as dots. Groups with different letters within the same plot are significantly different (Tukey's HSD $P < 0.05$). Boxes are colored by animal age group: 2-week (red), 4-week (orange), 8-week (green), 1-year (blue), and 2-year (pink).

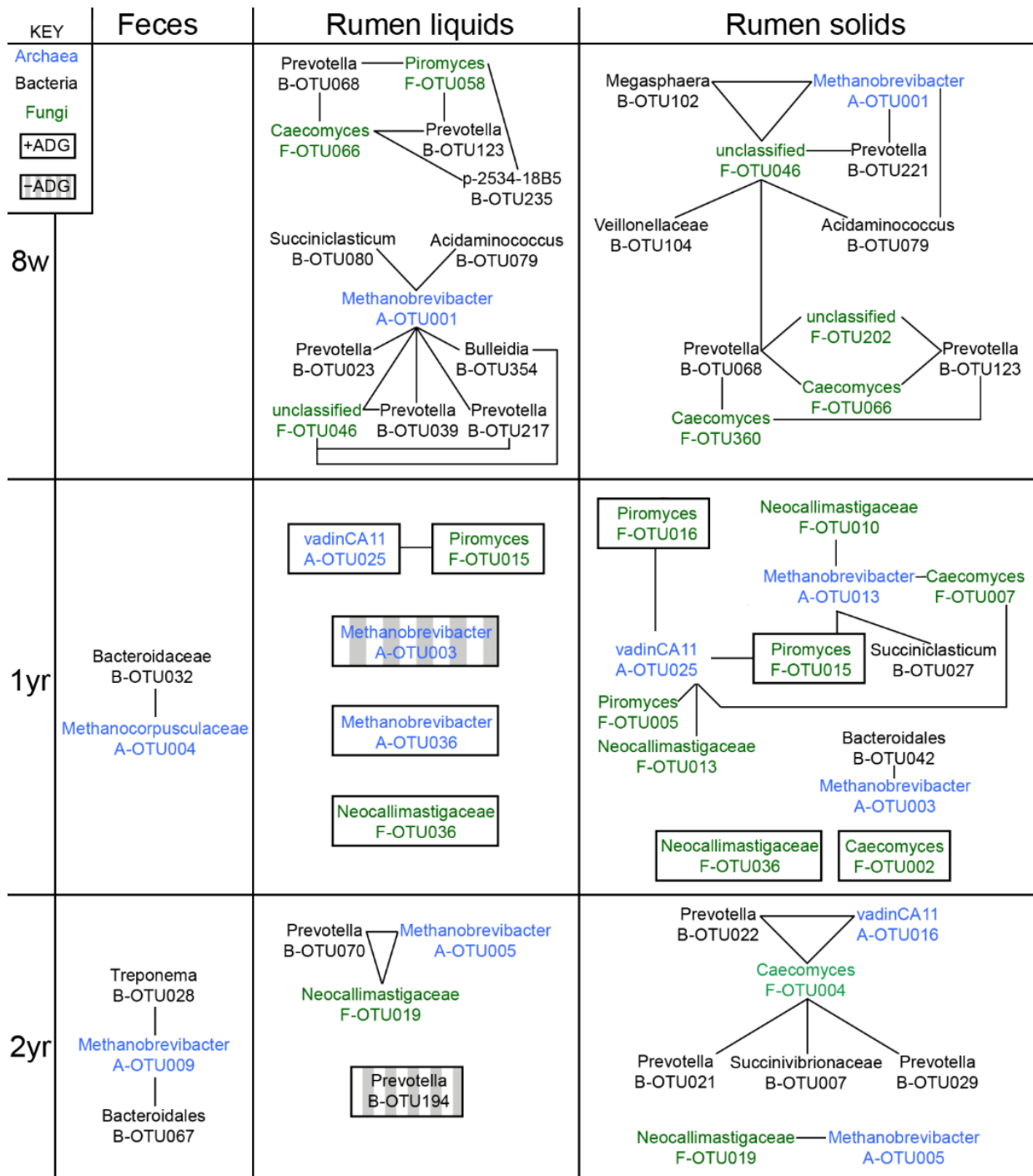


Fig. S3. Co-occurrence networks of OTUs significantly different by animal age and/or growth. OTUs at a relative abundance of 0.5% or greater in at least one sample, found to change with age (SIMPER), and co-occur (Kendall) are shown unboxed. OTUs above the abundance cutoff and also found to correlate to animal growth (ADG, Kendall) are shown in boxes. Open shapes indicate a positive correlation (Kendall > 0.70), while lines indicate a negative correlation (Kendall < -0.70). Solid lines between OTUs indicate strong co-occurrence (Kendall > 0.70). Archaea are blue, bacteria are black, and fungi are green.

Table S1a. Nutritional analysis of feed components by % dry matter (DM)

	CP	Starch	Sugar (ESC)	Fat (EE)	Ash	ADF	NDF	Ca	Mg	P	K	S	Lasalocid (antibiotic, g/ton)
Calf starter	22.68	42.30	1.75	2.82	6.52	5.18	11.06	1.02	0.22	0.59	1.13	0.28	60
Hay silage	22.5	3.1	-	2.8	-	30.1	37.5	1.3	-	0.40	2.83	-	0
Corn silage	7.2	33.3	-	3.4	-	23.9	39	0.23	-	0.23	0.7	-	0
Cracked corn	8.1	73.7	-	4.3	-	3.5	9.7	0.02	-	0.29	0.44	-	0
Heifer base rum	0	0.01	-	0	-	0	0	25.4	-	1.13	1.14	-	0
Grass silage base	14.5	3.6	-	3	-	37	52	0.83	-	0.36	2.9	-	0
Wheat straw	3.8	-	-	1.7	-	54.3	80.4	0.27	-	0.08	1.4	-	0
Heifer base	0	0.008	-	0	-	0	0	25.9	-	1.11	1.13	-	0
Corn, high moisture shell	8.2	69.4	-	2.9	-	2.6	7.0	0.03	-	0.33	0.44	-	0
Corn, grade shell	9.0	68.0	-	4.2	-	3.5	9.7	0.03	-	0.29	0.44	-	0

Values are averages across the sampling period as crops vary slightly by year. CP: crude protein, ESC: ethanol soluble carbohydrates, EE: ether extraction, ADF: acid detergent fiber, NDF: neutral detergent fiber

Table S1b. Feed components offered at different ages (%DM)

	0-8 weeks	2-3 months*	3-5 months	6-10 months	11-14 months	15+ months	Lactating
Calf starter	100	17.3 - 100	17.3	0	0	0	0
Hay silage	0	0 - 44.5	44.5	32.1	38.6	39.0	28.8
Corn silage	0	0 - 22.2	22.2	30.7	25.7	20.6	28.8
Cracked corn	0	0 - 14.8	14.8	8.9	0	0	0
Heifer base rum	0	0 - 1.2	1.2	1.5	1.4	0	0
Grass silage base	0	0	0	16.1	17.2	21.7	0
Wheat straw	0	0	0	0	14.3	17.4	0
Heifer base	0	0	0	0	0	1.3	0
Dry distillers grains	0	0	0	10.7	2.9	0	4.2
Corn, high moisture shell	0	0	0	0	0	0	16.3
Corn, grade shell	0	0	0	0	0	0	5.5
Soybeans	0	0	0	0	0	0	7.4
Canola meal	0	0	0	0	0	0	6.5
USDA minerals	0	0	0	0	0	0	2.5

*This time period was transitional with a gradual daily change from the 8 week to the 3 month diet

Table S2. Metadata on calf sex, age, growth, and health.

Calf	Age group	Sex	Age (dy)			Growth ADG (kg/dy)	Scour sample	Health (cumulative dy)				
			Fecal sampling	Rumen sampling	Weaning			Scours	Resp	Ketosis	Mastitis	All
4924	2w	M	22			1.54	N	0	0	0	0	0
4924	4w	M	29			1.16	N	0	0	0	0	0
4924	8w	M	54	58	58	1.59	N	0	0	0	0	0
4956	2w	M	12			1.45	N	0	0	0	0	0
4956	4w	M	25			0.89	N	0	0	0	0	0
4956	8w	M	54	55	55	1.36	N	0	0	0	0	0
4971	2w	M	12			0.36	Y	8	0	0	0	8
4971	4w	M	28			1.31	N	10	0	0	0	10
4971	8w	M	56	62	60	1.23	N	10	0	0	0	10
5017	2w	F	13			1.00	N	0	0	0	0	0
5017	4w	F	26			0.60	N	9	0	0	0	9
5017	8w	F	53		55	1.38	N	9	0	0	0	9
5017	1yr	F	369	369-371		0.88	N	9	0	0	0	9
5017	2yr	F	930	929-931		0.52	N	9	0	0	0	9
5020	2w	F	15			1.01	N	0	0	0	0	0
5020	4w	F	27			1.03	N	0	0	0	0	0
5020	8w	F	54		53	1.00	N	0	0	0	0	0
5020	1yr	F	365			1.07	N	0	0	0	0	0
5020	2yr	F	895			0.70	N	0	0	0	0	0
5026	2w	F	11			1.56	N	0	0	0	0	0
5026	4w	F	26			1.10	N	1	0	0	0	1
5026	8w	F	53		59	1.23	N	1	0	0	0	3
5026	1yr	F	365			0.98	N	1	0	0	0	8
5026	2yr	F	803			0.67	N	1	0	1	0	9

5031	2w	F	15		1.22	Y	6	0	0	0	6
5031	4w	F	27		0.71	N	11	0	0	0	11
5031	8w	F	53	56	1.37	N	11	0	0	0	11
5031	1yr	F	368	368-370	0.86	N	14	0	0	0	14
5031	2yr	F	843	842-844	0.60	N	14	0	0	9	23
5037	2w	F	11		0.35	N	5	0	0	0	5
5037	4w	F	25		0.18	N	11	6	0	0	17
5037	8w	F	54	58	0.95	N	15	6	0	0	21
5037	1yr	F	358		0.94	N	18	6	0	0	24
5037	2yr	F	841		0.63	N	18	6	0	0	24
5041	2w	F	11		1.11	Y	5	0	0	0	5
5041	4w	F	25		1.89	N	9	0	0	0	9
5041	8w	F	54	52	1.35	N	9	0	0	0	9
5041	1yr	F	364		0.93	N	9	0	0	0	17
5041	2yr	F	843		0.72	N	9	0	0	0	17
5045	2w	F	14		0.91	N	4	6	0	0	10
5045	4w	F	25		1.83	N	4	6	0	0	10
5045	8w	F	55	52	0.90	N	4	6	0	0	10
5045	1yr	F	367	367-369	0.91	N	4	6	0	0	18
5045	2yr	F	868	867-869	0.72	N	4	6	0	0	18
5046	2w	F	14		1.26	N	0	5	0	0	5
5046	4w	F	25		1.77	N	0	5	0	0	5
5046	8w	F	NS	58							
5046	1yr	F	367		0.85	N	0	5	0	0	24
5046	2yr	F	867		0.61	N	0	5	0	0	24
5049	2w	F	11		0.76	N	0	6	0	0	6
5049	4w	F	26		0.64	N	0	6	0	0	6
5049	8w	F	NS	59							
5049	1yr	F	359		0.86	N	0	6	0	0	26

5049	2yr	F	867		0.66	N	0	6	0	9	35
5053	2w	F	11		0.05	Y	2	0	0	0	2
5053	4w	F	27		1.42	N	6	0	0	0	6
5053	8w	F	55	53	0.88	N	18	12	0	0	30
5053	1yr	F	365	365-367	0.93	N	18	12	0	0	38
5053	2yr	F	827	826-828	0.60	N	18	12	0	0	44
5058	2w	F	11		0.76	Y	1	0	0	0	1
5058	4w	F	25		1.10	N	5	0	0	0	5
5058	8w	F	NS	54							
5058	1yr	F	364		0.94	N	5	0	0	0	14
5058	2yr	F	752		0.75	N	5	0	0	0	14
5063	2w	F	14		0.49	N	0	0	0	0	0
5063	4w	F	28		1.01	N	5	0	0	0	5
5063	8w	F	NS	54							
5063	1yr	F	360		0.86	N	5	0	0	0	12
5063	2yr	F	862		0.66	N	5	0	1	9	22

NS: no sample taken when one was planned.

ADG: Average daily gain

Table S3. Sequence coverage.

Calf	Age	Type	ARCHAEA							BACTERIA							FUNGI							
			Raw	Clean	Abund	OTUs	Coverage	Norm	Norm OTUs	Raw	Clean	Abund	OTUs	Coverage	Norm	Norm OTUs	Raw	Clean	Abund	OTUs	Coverage	Norm	Norm OTUs	
4924	2w	F	38753	406	366	7	100.0%	375	7	25092	19137	18994	211	99.7%	8971	159								
4924	4w	F	37175	813	730	11	99.7%	377	11	54693	44664	44472	257	99.9%	8970	143								
4924	8w	F	30399	5006	4595	11	100.0%	373	4	26262	17632	17538	281	99.7%	9040	281								
4924	8w	L	27667	4877	4554	7	100.0%	375	4	66983	49926	49771	243	99.9%	8988	164	8369	2193	1438	34	99.7%	247	19	
4924	8w	S	34793	1440	1306	9	99.8%	375	6	56733	46007	45836	235	99.9%	8985	136	29376	11002	7430	96	99.9%	244	21	
4956	2w	F	37485	21	15	3	93.3%	375	3	27150	12715	12616	73	99.9%	8998	73								
4956	4w	F	18227	216	182	3	99.5%	375	3	35626	27991	27830	265	99.8%	9009	222								
4956	8w	F	24558	5748	5343	7	100.0%	374	3	98423	68898	68562	655	99.9%	8965	406								
4956	8w	L	20795	4561	4184	8	100.0%	375	4	94976	58398	58206	254	99.9%	8969	130	32729	12604	10589	42	100.0%	248	10	
4956	8w	S	22610	2823	2576	8	100.0%	373	4	72470	44647	44479	215	99.9%	8982	144	33977	21169	17151	152	100.0%	241	25	
4971	2w	F	36883	1	0	0	0.0%	0	0	78382	37751	37591	191	99.9%	8965	105								
4971	4w	F	26135	750	675	5	99.4%	377	5	131637	106001	105723	488	99.9%	8966	222								
4971	8w	F	14099	1844	1714	8	99.9%	374	5	83489	56910	56635	623	99.9%	8938	408								
4971	8w	L	32448	5619	5236	8	100.0%	374	4	102314	49188	48893	328	99.9%	8980	201	18919	5908	4373	69	99.9%	247	33	
4971	8w	S	17192	537	486	7	99.4%	377	7	105803	55523	55285	349	99.9%	8947	170	12491	3830	2820	91	99.9%	240	42	
5017	2w	F	30989	4916	4577	9	100.0%	374	4	78629	53505	53322	290	99.9%	9002	186								
5017	4w	F	24383	1092	979	8	99.7%	376	5	40857	33419	33244	301	99.8%	9007	242								
5017	8w	F	43652	11397	10786	7	100.0%	375	4	42976	30682	30347	453	99.7%	9010	368								
5017	1yr	F	15263	2562	2284	30	99.8%	372	18	68486	41465	40743	1834	99.5%	8841	1160	86447	53219	40737	375	99.9%	238	18	
5017	1yr	L1	36150	2637	2335	43	99.7%	370	25	91567	55906	55197	2662	99.2%	8694	1439	70063	37070	29489	405	99.9%	237	27	
5017	1yr	L2	28270	3120	2727	40	99.8%	374	26	98193	63991	63177	2578	99.3%	8805	1386	38419	17704	13656	261	99.7%	238	27	
5017	1yr	L3	19761	2020	1757	34	99.9%	370	22	24097	15320	15012	1394	98.0%	9066	1394	66123	26394	19963	376	99.8%	236	28	
5017	1yr	S1	16769	680	591	25	99.3%	376	25	63086	37577	37086	2174	99.0%	8711	1339	72073	24044	18185	346	99.8%	235	26	
5017	1yr	S2	20235	518	442	21	99.5%	378	21	16038	9899	9637	1188	97.0%	9137	1188	70719	29207	22063	398	99.8%	236	27	
5017	1yr	S3	23609	1129	933	33	99.2%	372	26	79659	54209	53440	2475	99.2%	9026	1661	84079	35218	28482	342	99.9%	238	26	
5017	2yr	F	18382	1431	1253	23	99.7%	374	19	37498	24599	24262	1265	99.1%	8941	1039	68670	34791	25149	740	99.9%	202	59	
5017	2yr	L1	24718	4178	3619	54	99.9%	372	29	54153	31637	31140	1802	98.9%	9026	1474	32393	18220	13605	183	99.8%	239	14	
5017	2yr	L2	21520	2797	2435	48	99.6%	369	23	48154	29695	29274	1741	98.9%	9049	1407	37549	14618	10474	185	99.8%	238	16	
5017	2yr	L3	32408	3220	2808	44	99.8%	373	28	67361	43069	42345	2044	99.1%	8817	1285	69234	28634	20197	348	99.9%	236	17	
5017	2yr	S1	20759	942	835	27	99.8%	375	25	62079	41263	40573	1787	99.2%	8838	1114	46251	27966	21387	248	99.9%	239	13	
5017	2yr	S2	13379	452	396	29	98.7%	377	29	58685	36908	36403	1679	99.3%	8739	1072	41521	17945	13188	246	99.7%	234	14	
5017	2yr	S3	16131	880	762	30	99.1%	370	23	49978	30799	30368	1679	99.1%	9026	1395	79409	37883	27782	343	99.9%	239	16	
5020	2w	F	64498	6	4	1	100.0%	375	1	117917	84246	83968	314	99.9%	8972	127								
5020	4w	F	45606	102	87	3	98.9%	374	3	58209	47658	47405	298	99.9%	8992	186								
5020	8w	F	12726	1617	1462	8	100.0%	376	8	51296	39302	39120	389	99.9%	8965	259								
5020	1yr	F	22561	3766	3381	35	99.9%	371	18	88062	58330	57580	1881	99.6%	8839	1064	41719	25572	21403	210	99.9%	241	17	
5020	2yr	F	18336	2650	2315	30	99.7%	374	19	48062	30800	30442	1320	99.3%	9023	1110	18586	7984	5582	321	99.7%	208	42	
5026	2w	F	70435	31	24	2	100.0%	375	2	36807	30376	30207	135	99.9%	9001	106								
5026	4w	F	47052	10	6	3	83.3%	376	3	59378	42440	42295	261	99.9%	8970	156								
5026	8w	F	12514	202	167	5	98.8%	374	5	74621	43291	43123	192	99.9%	8979	117								
5026	1yr	F	13404	1635	1429	25	99.8%	376	22	39478	26135	25726	1284	99.3%	8963	1099	50137	33564	25251	324	99.9%	243	34	
5026	2yr	F	23101	1222	1091	26	99.8%	375	24	62225	44153	43559	1308	99.5%	8900	838	37535	21500	15797	832	99.8%	192	55	

5046	1yr	F	16284	1245	1132	21	99.7%	374	18	100939	66154	65256	1978	99.5%	8859	1109	37552	21644	17025	281	99.8%	240	23
5046	2yr	F	47681	3957	3421	29	99.9%	372	15	62433	40583	40154	1501	99.5%	8879	1037	33047	19716	15193	744	99.9%	193	61
5049	2w	F	67782	2	1	1	0.0%	375	1	25913	17707	17620	102	99.8%	9019	102							
5049	4w	F	20315	763	694	4	100.0%	375	4	47350	37169	36971	273	99.9%	8966	176							
5049	1yr	F	15130	2762	2315	47	99.9%	371	33	49716	29830	29076	1745	99.4%	9143	1560	49963	32931	25559	389	99.9%	241	27
5049	2yr	F	51016	6414	5792	42	99.9%	370	14	76438	50663	50129	1723	99.6%	8959	1192	72212	33147	24720	892	99.9%	195	46
5053	2w	F	52388	48	28	3	92.9%	374	3	24309	17900	17835	130	99.7%	9035	130							
5053	4w	F	10664	345	301	6	99.3%	374	6	41589	31268	31097	386	99.8%	9019	333							
5053	8w	F	17300	931	842	7	99.8%	373	5	73452	56218	55899	455	99.9%	8945	248							
5053	1yr	F	33165	4515	4117	40	99.8%	371	17	81913	53224	52444	1866	99.5%	8945	1239	45940	27742	22526	273	99.9%	243	25
5053	1yr	L1	28920	3197	2836	46	99.8%	373	35	37587	24165	23553	1743	98.5%	8992	1398	85305	35980	28322	440	99.8%	236	26
5053	1yr	L2	32212	4168	3683	44	99.9%	373	31	24347	14966	14639	1517	97.7%	9034	1517	38044	18301	13775	306	99.8%	233	26
5053	1yr	L3	21044	2322	2016	42	99.8%	374	31	25542	15463	15157	1458	97.9%	9096	1458	37283	20033	14852	264	99.8%	239	25
5053	1yr	S1	24846	1309	1109	26	99.8%	375	24	15466	9507	9205	1241	96.9%	9091	1241	40554	19260	14338	286	99.8%	236	27
5053	1yr	S2	17703	822	704	27	99.3%	377	27	39899	25445	24797	1839	98.5%	8916	1464	67289	32212	24140	377	99.9%	238	27
5053	1yr	S3	16405	838	703	29	99.0%	380	29	35359	23749	23228	1787	98.3%	8945	1399	84060	45842	34977	469	99.9%	234	22
5053	2yr	F	14231	2430	2151	29	99.7%	372	19	59109	37983	37534	1239	99.5%	8859	820	16798	7946	5616	204	99.6%	228	27
5053	2yr	L1	17673	2800	2386	39	100.0%	371	24	53884	32939	32440	1850	99.2%	9095	1579	55563	20496	15024	340	99.8%	228	17
5053	2yr	L2	16144	2222	1879	48	99.6%	370	28	113902	74259	73251	2497	99.5%	8704	1263	79947	32063	23573	405	99.9%	233	18
5053	2yr	L3	12930	659	413	26	99.3%	378	26	177596	115126	114241	2869	99.7%	8735	1321	47070	17438	12283	310	99.8%	232	19
5053	2yr	S1	12933	696	598	23	99.3%	376	23	187083	128311	127434	2666	99.8%	8764	1231	80892	35845	24103	475	99.9%	231	20
5053	2yr	S2	14938	1092	959	30	99.8%	378	28	58231	37487	37006	1833	99.4%	8743	1197	83817	37411	26684	523	99.9%	226	16
5053	2yr	S3	14339	980	870	28	99.7%	373	25	54019	34693	34229	1817	99.2%	9136	1540	101954	43446	32177	531	99.9%	233	22
5058	2w	F	71673	24	19	1	100.0%	375	1	25159	18367	18269	75	99.9%	8984	50							
5058	4w	F	62362	80	64	3	98.4%	375	3	27546	20504	20392	235	99.8%	8989	198							
5058	1yr	F	35524	8440	7759	33	100.0%	372	15	91916	62211	61291	1990	99.5%	8827	1113	57997	33538	26944	216	99.9%	243	23
5058	2yr	F	16938	2057	1801	31	99.8%	371	22	41371	27847	27456	1165	99.3%	9026	978	30778	16586	11670	684	99.9%	192	62
5063	2w	F	23719	312	283	4	100.0%	374	4	43038	35337	35117	169	99.9%	9007	132							
5063	4w	F	81657	15	9	3	88.9%	375	3	56178	42105	41916	224	99.9%	8984	151							
5063	1yr	F	27901	4285	3904	24	99.9%	371	13	44457	29980	29488	1671	99.2%	9093	1423	66270	41536	33526	292	99.9%	238	15
5063	2yr	F	25328	3811	3354	26	99.9%	374	18	79341	56762	55982	1500	99.6%	8844	866	33499	18325	13944	550	99.9%	210	47

Type: feces (F), rumen liquids (L), rumen solids (S), numbers indicate day during 3 consecutive days of sampling

Raw: number of contigs obtained off the MiSeq before any clean-up

Clean: number of contigs after clean-up but before singleton removal

Abund: number of contigs after clean-up and singleton removal

OTUs: number of OTUs among abund contigs

Coverage: Good's coverage calculated from abund contigs

Norm: number of contigs after normalization

Norm OTUs: number of OTUs among norm contigs

Table S5. P-values associated with statistical analyses of age groups, animal growth, health, and control variables.

Test	Community	Type	Metric	AgeGroup	Animal	Scours	Random	POST FDR-CORRECTION									
								2w-4w	4w-8w	8w-1yr	1yr-2yr	2w	4w	8w	1yr	2yr	
ANOSIM	Archaea	Feces	Bray-Curtis	2.67E-04	1.000	1.000	0.795										
		Feces	Jaccard	2.67E-04	1.000	1.000	0.795										
		Liquids	Bray-Curtis	2.67E-04			1.000										
		Liquids	Jaccard	2.67E-04			1.000										
		Solids	Bray-Curtis	2.67E-04			1.000										
		Solids	Jaccard	2.67E-04			1.000										
	Bacteria	Feces	Bray-Curtis	2.67E-04	1.000	0.009	1.000										
		Feces	Jaccard	2.67E-04	1.000	0.008	1.000										
		Feces.2week	Bray-Curtis				0.972										
		Feces.2week	Jaccard				0.972										
		Liquids	Bray-Curtis	2.67E-04			1.000										
		Liquids	Jaccard	2.67E-04			1.000										
		Solids	Bray-Curtis	2.67E-04			1.000										
		Solids	Jaccard	2.67E-04			1.000										
	Fungi	Feces	Bray-Curtis	2.67E-04	1.000	NA	1.000										
		Feces	Jaccard	2.67E-04	1.000	NA	1.000										
		Liquids	Bray-Curtis	2.67E-04			1.000										
		Liquids	Jaccard	2.67E-04			1.000										
		Solids	Bray-Curtis	2.67E-04			0.517										
		Solids	Jaccard	2.67E-04			0.517										
ANOVA	Archaea	Feces	Shannon	1.44E-15			0.979										
		Feces	Chao	1.44E-15			0.979										
		Liquids	Shannon	1.44E-15			0.979										
		Liquids	Chao	4.16E-05			0.979										
		Solids	Shannon	7.98E-14			0.979										
		Solids	Chao	0.074			0.891										
	Bacteria	Feces	Shannon	1.44E-15			0.987										
		Feces	Chao	1.44E-15			0.979										
		Liquids	Shannon	1.89E-13			0.979										
		Liquids	Chao	5.92E-05			0.979										
		Solids	Shannon	4.59E-12			0.979										
		Solids	Chao	6.69E-05			0.979										
Fungi	Feces	Shannon	0.003			0.673											
	Feces	Chao	5.92E-05			0.979											
	Liquids	Shannon	9.06E-05			0.979											
	Liquids	Chao	0.023			0.979											
	Solids	Shannon	0.040			0.979											
	Solids	Chao	0.438			0.891											
TukeyHSD	Archaea	Feces	Shannon				0.678	0.054	< 1E-08	0.605							
		Feces	Chao				0.498	0.990	< 1E-08	0.742							
		Liquids	Shannon						< 1E-08	0.096							
		Liquids	Chao						1.01E-05	0.926							
		Solids	Shannon						< 1E-08	0.004							
		Solids	Chao						0.180	0.351							
	Bacteria	Feces	Shannon				2.40E-04	2.06E-05	< 1E-08	0.328							
		Feces	Chao				0.582	0.957	< 1E-08	0.053							
		Liquids	Shannon						< 1E-08	0.966							
		Liquids	Chao						3.10E-05	0.926							
		Solids	Shannon						< 1E-08	0.001							
		Solids	Chao						2.41E-05	0.979							

		Feces	Shannon				NA	0.001						
		Feces	Chao				NA	1.81E-05						
	Fungi	Liquids	Shannon				5.89E-05	0.003						
		Liquids	Chao				0.996	0.010						
		Solids	Shannon				0.673	0.013						
		Solids	Chao				0.793	0.364						
	Archaea	Feces	Bray-Curtis	0.020	0.794	0.007	4.13E-05	0.170						
		Liquids	Bray-Curtis	3.33E-04			1.43E-08	1.74E-09						
		Solids	Bray-Curtis	0.013			0.019	0.063						
PERMDISP	Bacteria	Feces	Bray-Curtis	3.33E-04	0.017	0.104	9.08E-08	0.336						
		Liquids	Bray-Curtis	3.33E-04			0.027	8.18E-07						
		Solids	Bray-Curtis	0.003			0.016	4.21E-05						
	Fungi	Feces	Bray-Curtis	0.104			NA	0.104						
		Liquids	Bray-Curtis	9.00E-04			0.134	0.003						
		Solids	Bray-Curtis	0.029			0.209	0.098						
	Archaea	Feces	Bray-Curtis						0.298	0.941	0.727	0.941	0.941	
		Feces	Jaccard						0.226	0.938	0.727	0.967	0.934	
		Liquids	Bray-Curtis								0.346	0.012	0.087	
		Liquids	Jaccard								0.346	0.009	0.087	
		Solids	Bray-Curtis								0.870	0.163	0.080	
		Solids	Jaccard								0.870	0.080	0.080	
PERMANOVA, ADG weight	Bacteria	Feces	Bray-Curtis						0.941	0.080	0.980	0.941	0.938	
		Feces	Jaccard						0.941	0.080	0.980	0.941	0.938	
		Liquids	Bray-Curtis								0.789	0.087	0.012	
		Liquids	Jaccard								0.789	0.080	0.020	
		Solids	Bray-Curtis								0.789	0.072	0.080	
		Solids	Jaccard								0.789	0.060	0.080	
	Fungi	Feces	Bray-Curtis									0.107	0.870	
		Feces	Jaccard									0.087	0.870	
		Liquids	Bray-Curtis								0.870	0.053	0.380	
		Liquids	Jaccard								0.870	0.026	0.309	
		Solids	Bray-Curtis								0.870	0.012	0.726	
		Solids	Jaccard								0.870	0.009	0.619	
	Archaea	Feces	Bray-Curtis						0.822	0.513	0.513	NA	NA	
		Feces	Jaccard						0.714	0.513	0.513	NA	NA	
		Liquids	Bray-Curtis								0.714	NA	NA	
		Liquids	Jaccard								0.714	NA	NA	
		Solids	Bray-Curtis								0.513	NA	NA	
		Solids	Jaccard								0.513	NA	NA	
PERMANOVA, scours	Bacteria	Feces	Bray-Curtis						0.513	0.529	0.566	NA	NA	
		Feces	Jaccard						0.513	0.566	0.566	NA	NA	
		Liquids	Bray-Curtis								0.513	NA	NA	
		Liquids	Jaccard								0.513	NA	NA	
		Solids	Bray-Curtis								0.513	NA	NA	
		Solids	Jaccard								0.513	NA	NA	
	Fungi	Feces	Bray-Curtis						NS	NS	NS	NA	NA	
		Feces	Jaccard						NS	NS	NS	NA	NA	
		Liquids	Bray-Curtis								0.513	NA	NA	
		Liquids	Jaccard								0.513	NA	NA	
		Solids	Bray-Curtis								0.513	NA	NA	
		Solids	Jaccard								0.513	NA	NA	
		Feces	Bray-Curtis						0.255	0.255	0.361	NA	NA	
		Feces	Jaccard						0.255	0.261	0.361	NA	NA	

PERMANOVA, resp	Archaea	Liquids	Bray-Curtis				NV	NA	NA	
		Liquids	Jaccard				NV	NA	NA	
		Solids	Bray-Curtis				NV	NA	NA	
		Solids	Jaccard				NV	NA	NA	
	Bacteria	Feces	Bray-Curtis			0.255	0.255	0.788	NA	NA
		Feces	Jaccard			0.255	0.255	0.788	NA	NA
		Liquids	Bray-Curtis					NV	NA	NA
		Liquids	Jaccard					NV	NA	NA
	Fungi	Solids	Bray-Curtis					NV	NA	NA
		Solids	Jaccard					NV	NA	NA
		Feces	Bray-Curtis			NS	NS	NS	NA	NA
		Feces	Jaccard			NS	NS	NS	NA	NA
PERMANOVA, illness all	Archaea	Liquids	Bray-Curtis			0.624	0.624	0.624	0.819	0.819
		Liquids	Jaccard			0.624	0.624	0.624	0.819	0.819
		Solids	Bray-Curtis					0.817	0.624	0.819
		Solids	Jaccard					0.624	0.624	0.624
	Bacteria	Feces	Bray-Curtis			0.817	0.624	0.801	0.624	0.649
		Feces	Jaccard			0.819	0.624	0.801	0.624	0.649
		Liquids	Bray-Curtis					0.624	0.819	0.817
		Liquids	Jaccard					0.624	0.819	0.817
	Fungi	Solids	Bray-Curtis					0.624	0.705	0.695
		Solids	Jaccard					0.624	0.705	0.695
		Feces	Bray-Curtis						0.624	0.705
		Feces	Jaccard						0.624	0.716
Fungi	Liquids	Bray-Curtis					0.624	0.917	0.624	
	Liquids	Jaccard					0.624	0.917	0.624	
	Solids	Bray-Curtis					0.624	0.817	0.624	
	Solids	Jaccard					0.624	0.817	0.624	

NS: not sequenced; NV: no variation in variable of interest; NA: not applicable
Significant P-values (P < 0.05) are in red

RAW, NO FDR-CORRECTION

Test	Community	Type	Metric	AgeGroup	Animal	Scours	Random	2w-4w	4w-8w	8w-1yr	1yr-2yr	2w	4w	8w	1yr	2yr	
ANOSIM	Archaea	Feces	Bray-Curtis	1.00E-04	0.959	0.871	0.396										
		Feces	Jaccard	1.00E-04	0.956	0.877	0.398										
		Liquids	Bray-Curtis	1.00E-04			0.668										
		Liquids	Jaccard	1.00E-04			0.670										
		Solids	Bray-Curtis	1.00E-04			0.890										
		Solids	Jaccard	1.00E-04			0.895										
	Bacteria	Feces	Bray-Curtis	1.00E-04	1.000	0.004	0.914										
		Feces	Jaccard	1.00E-04	1.000	0.003	0.904										
		Feces.2week	Bray-Curtis				0.526										
		Feces.2week	Jaccard				0.525										
		Liquids	Bray-Curtis	1.00E-04			0.791										
		Liquids	Jaccard	1.00E-04			0.785										
		Solids	Bray-Curtis	1.00E-04			0.704										
		Solids	Jaccard	1.00E-04			0.701										
	Fungi	Feces	Bray-Curtis	1.00E-04	0.999	NA	0.900										
		Feces	Jaccard	1.00E-04	0.998	NA	0.901										
		Liquids	Bray-Curtis	1.00E-04			0.635										
		Liquids	Jaccard	1.00E-04			0.629										
		Solids	Bray-Curtis	1.00E-04			0.233										
		Solids	Jaccard	1.00E-04			0.237										
	ANOVA	Archaea	Feces	Shannon	< 2E-16			0.944									
Feces			Chao	< 2E-16			0.662										
Liquids			Shannon	< 2E-16			0.952										
Liquids			Chao	1.04E-05			0.714										
Solids			Shannon	1.33E-14			0.858										
Solids			Chao	0.035			0.520										
Bacteria		Feces	Shannon	< 2E-16			0.987										
		Feces	Chao	< 2E-16			0.850										
		Liquids	Shannon	3.67E-14			0.763										
		Liquids	Chao	1.78E-05			0.740										
		Solids	Shannon	1.02E-12			0.729										
		Solids	Chao	2.23E-05			0.946										
Fungi		Feces	Shannon	0.001			0.355										
		Feces	Chao	1.81E-05			0.778										
		Liquids	Shannon	3.27E-05			0.952										
		Liquids	Chao	0.010			0.714										
		Solids	Shannon	0.018			0.858										
		Solids	Chao	0.219			0.520										
TukeyHSD	Archaea	Feces	Shannon				0.678	0.054	< 1E-08	0.605							
		Feces	Chao				0.498	0.990	< 1E-08	0.742							
		Liquids	Shannon						< 1E-08	0.096							
		Liquids	Chao						1.01E-05	0.926							
		Solids	Shannon						< 1E-08	0.004							
		Solids	Chao						0.180	0.351							
	Bacteria	Feces	Shannon				2.40E-04	2.06E-05	< 1E-08	0.328							
		Feces	Chao				0.582	0.957	< 1E-08	0.053							
		Liquids	Shannon						< 1E-08	0.966							
		Liquids	Chao						3.10E-05	0.926							
		Solids	Shannon						< 1E-08	0.001							
		Solids	Chao						2.41E-05	0.979							

		Feces	Shannon			NA	0.001							
		Feces	Chao			NA	1.81E-05							
	Fungi	Liquids	Shannon			5.89E-05	0.003							
		Liquids	Chao			0.996	0.010							
		Solids	Shannon			0.673	0.013							
		Solids	Chao			0.793	0.364							
	Archaea	Feces	Bray-Curtis	0.012	0.794	0.003	6.88E-06	0.153						
		Liquids	Bray-Curtis	1.00E-04			9.55E-10	5.80E-11						
		Solids	Bray-Curtis	0.006			0.011	0.044						
PERMDISP	Bacteria	Feces	Bray-Curtis	1.00E-04	0.009	0.080	9.08E-09	0.325						
		Liquids	Bray-Curtis	1.00E-04			0.017	1.09E-07						
		Solids	Bray-Curtis	0.001			0.008	8.42E-06						
	Fungi	Feces	Bray-Curtis	0.087			NA	0.083						
		Liquids	Bray-Curtis	3.00E-04			0.116	0.001						
		Solids	Bray-Curtis	0.019			0.195	0.072						
	Archaea	Feces	Bray-Curtis						0.129	0.824	0.412	0.894	0.802	
		Feces	Jaccard						0.094	0.779	0.404	0.935	0.732	
		Liquids	Bray-Curtis								0.167	0.001	0.030	
		Liquids	Jaccard								0.167	0.000	0.032	
		Solids	Bray-Curtis								0.667	0.065	0.024	
		Solids	Jaccard								0.667	0.023	0.018	
PERMANOVA, ADG weight	Bacteria	Feces	Bray-Curtis						0.849	0.016	0.980	0.882	0.782	
		Feces	Jaccard						0.865	0.017	0.980	0.870	0.774	
		Liquids	Bray-Curtis								0.500	0.030	0.001	
		Liquids	Jaccard								0.500	0.022	0.002	
		Solids	Bray-Curtis								0.500	0.012	0.022	
		Solids	Jaccard								0.500	0.009	0.019	
	Fungi	Feces	Bray-Curtis						NS	NS	NS	0.041	0.639	
		Feces	Jaccard						NS	NS	NS	0.031	0.585	
		Liquids	Bray-Curtis								0.667	0.007	0.190	
		Liquids	Jaccard								0.667	0.003	0.139	
		Solids	Bray-Curtis								0.667	0.001	0.387	
		Solids	Jaccard								0.667	0.000	0.320	
	Archaea	Feces	Bray-Curtis						0.822	0.228	0.062	NA	NA	
		Feces	Jaccard						0.684	0.342	0.059	NA	NA	
		Liquids	Bray-Curtis								0.667	NA	NA	
		Liquids	Jaccard								0.667	NA	NA	
		Solids	Bray-Curtis								0.333	NA	NA	
		Solids	Jaccard								0.333	NA	NA	
PERMANOVA, scours	Bacteria	Feces	Bray-Curtis						0.310	0.375	0.463	NA	NA	
		Feces	Jaccard						0.327	0.471	0.472	NA	NA	
		Liquids	Bray-Curtis								0.333	NA	NA	
		Liquids	Jaccard								0.333	NA	NA	
		Solids	Bray-Curtis								0.333	NA	NA	
		Solids	Jaccard								0.333	NA	NA	
	Fungi	Feces	Bray-Curtis						NS	NS	NS	NA	NA	
		Feces	Jaccard						NS	NS	NS	NA	NA	
		Liquids	Bray-Curtis								0.333	NA	NA	
		Liquids	Jaccard								0.333	NA	NA	
		Solids	Bray-Curtis								0.333	NA	NA	
		Solids	Jaccard								0.333	NA	NA	
		Feces	Bray-Curtis						0.102	0.132	0.301	NA	NA	
		Feces	Jaccard						0.109	0.174	0.288	NA	NA	

PERMANOVA, resp	Archaea	Liquids	Bray-Curtis				NV	NA	NA	
		Liquids	Jaccard				NV	NA	NA	
		Solids	Bray-Curtis				NV	NA	NA	
		Solids	Jaccard				NV	NA	NA	
	Bacteria	Feces	Bray-Curtis			0.041	0.123	0.788	NA	NA
		Feces	Jaccard			0.047	0.149	0.773	NA	NA
		Liquids	Bray-Curtis					NV	NA	NA
		Liquids	Jaccard					NV	NA	NA
	Fungi	Solids	Bray-Curtis					NV	NA	NA
		Solids	Jaccard					NV	NA	NA
		Feces	Bray-Curtis			NS	NS	NS	NA	NA
		Feces	Jaccard			NS	NS	NS	NA	NA
PERMANOVA, illness all	Archaea	Liquids	Bray-Curtis			0.296	0.055	0.065	0.788	0.738
		Liquids	Jaccard			0.267	0.113	0.061	0.785	0.754
		Solids	Bray-Curtis					0.667	0.167	0.792
		Solids	Jaccard					0.667	0.167	0.792
	Bacteria	Liquids	Bray-Curtis					0.333	0.292	0.125
		Liquids	Jaccard					0.333	0.292	0.125
		Solids	Bray-Curtis			0.655	0.061	0.561	0.266	0.368
		Solids	Jaccard			0.683	0.099	0.553	0.276	0.360
	Fungi	Liquids	Bray-Curtis					0.333	0.792	0.625
		Liquids	Jaccard					0.333	0.792	0.625
		Solids	Bray-Curtis					0.333	0.458	0.417
		Solids	Jaccard					0.333	0.458	0.417
Fungi	Feces	Bray-Curtis						0.310	0.437	
	Feces	Jaccard						0.330	0.477	
	Liquids	Bray-Curtis					0.333	0.917	0.208	
	Liquids	Jaccard					0.333	0.917	0.208	
Fungi	Solids	Bray-Curtis					0.333	0.667	0.042	
	Solids	Jaccard					0.333	0.667	0.042	

Table S6. OTUs which contribute to differences between age groups (SIMPER) and/or have strong correlations (Kendall) to animal growth or health.

Amplicon	Type	OTU	Contribution to SIMPER (%)									Kendall correlation		Classification		
			2w-4w	2w-8w	2w-1yr	2w-2yr	4w-8w	4w-1yr	4w-2yr	8w-1yr	8w-2yr	1yr-2yr	1yr-ADG	2yr-ADG	Phyla	Lowest
Archaea	Feces	OTU01	39.7%	47.6%	34.2%	34.6%	48.0%	33.2%	35.2%	47.5%	47.5%			Euryarchaeota	<i>Methanobrevibacter</i>	
		OTU02		11.8%	7.6%	12.4%		6.1%	12.6%		11.7%	12.0%		Euryarchaeota	<i>Methanobrevibacter</i>	
		OTU03	16.6%	19.5%	14.5%	9.3%		11.5%		10.3%		11.6%		Euryarchaeota	<i>Methanobrevibacter</i>	
		OTU04			18.8%			18.3%		17.7%		23.8%		Euryarchaeota	Methanocorpusculaceae	
		OTU05	11.4%			12.1%	16.0%		12.7%		11.8%	16.9%		Euryarchaeota	<i>Methanobrevibacter</i>	
		OTU09				8.8%		9.0%				11.3%		Euryarchaeota	<i>Methanobrevibacter</i>	
		OTU17	11.6%				15.8%	6.5%	6.9%					Euryarchaeota	<i>Methanosphaera</i>	
	Feces	cumulative	79.4%	78.9%	75.1%	77.2%	79.8%	75.5%	76.3%	75.6%	71.0%	75.5%				
	Liquids	OTU01								49.6%	49.5%			Euryarchaeota	<i>Methanobrevibacter</i>	
		OTU02									10.3%	13.4%		Euryarchaeota	<i>Methanobrevibacter</i>	
		OTU03							7.0%			6.3%	-0.78	Euryarchaeota	<i>Methanobrevibacter</i>	
		OTU05										5.7%		Euryarchaeota	<i>Methanobrevibacter</i>	
		OTU06								8.9%		13.1%		Euryarchaeota	<i>Methanobrevibacter</i>	
		OTU07							6.3%	7.4%		8.7%		Euryarchaeota	<i>Methanobrevibacter</i>	
		OTU08										10.0%		Euryarchaeota	<i>Methanobrevibacter</i>	
		OTU09									9.7%	16.9%		Euryarchaeota	<i>Methanobrevibacter</i>	
		OTU25											0.79	Euryarchaeota	<i>vadinCA11</i>	
		OTU36											0.80	Euryarchaeota	<i>Methanobrevibacter</i>	
	Liquids	cumulative								71.7%	76.9%	74.0%				
	Solids	OTU01								47.8%	47.9%			Euryarchaeota	<i>Methanobrevibacter</i>	
		OTU02								10.7%		11.7%		Euryarchaeota	<i>Methanobrevibacter</i>	
		OTU03								17.5%	12.8%	12.3%		Euryarchaeota	<i>Methanobrevibacter</i>	
		OTU05									10.6%	22.4%		Euryarchaeota	<i>Methanobrevibacter</i>	
		OTU08										6.0%		Euryarchaeota	<i>Methanobrevibacter</i>	
		OTU11										7.8%		Euryarchaeota	<i>Methanobrevibacter</i>	
		OTU13										3.7%		Euryarchaeota	<i>Methanobrevibacter</i>	
OTU16											5.8%		Euryarchaeota	<i>vadinCA11</i>		
OTU25										3.1%		Euryarchaeota	<i>vadinCA11</i>			
Solids	cumulative								76.0%	71.3%	72.9%					
		OTU001	10.8%	5.4%	4.3%	4.3%	10.9%	10.0%	10.0%	2.6%	2.7%			Firmicutes	<i>Faecalibacterium</i>	
		OTU003	9.2%	6.2%	5.6%	5.6%	6.5%	5.6%	5.6%					Firmicutes	<i>Faecalibacterium</i>	
		OTU006	7.2%	6.4%	6.0%	6.0%	2.9%	2.5%	2.6%					Actinobacteria	<i>Collinsella</i>	
		OTU008	4.3%	3.9%	3.6%	3.6%								Actinobacteria	<i>Bifidobacterium</i>	
		OTU009	1.8%	1.7%			2.0%	1.5%	1.5%	1.6%	1.7%			Bacteroidetes	<i>Bacteroides</i>	
		OTU010	3.1%	3.9%	2.6%	2.6%	1.7%			1.4%	1.4%			Firmicutes	<i>Clostridium</i>	
		OTU012			1.7%	2.1%		1.7%	2.1%	1.5%	1.9%	1.1%		Firmicutes	Ruminococcaceae	
		OTU013	1.0%	4.0%			4.5%			3.7%	3.8%			Bacteroidetes	S24-7	
		OTU015	3.7%	3.6%	3.4%	3.4%	1.2%							Firmicutes	Ruminococcaceae	
		OTU016	1.8%	2.2%	1.5%	1.5%	1.3%			1.1%	1.1%			Proteobacteria	Enterobacteriaceae	
		OTU017	2.9%	2.8%			4.1%	2.0%	2.0%	2.3%	2.3%			Bacteroidetes	<i>Bacteroides</i>	
		OTU018	4.0%	1.1%			4.4%	3.3%	3.3%	1.0%	1.1%			Bacteroidetes	<i>Bacteroides</i>	
		OTU020	2.2%	1.1%			1.6%	1.3%	1.3%					Bacteroidetes	<i>Bacteroides</i>	
		OTU024			1.5%	1.1%		1.5%	1.1%	1.6%	1.1%	1.1%		Bacteroidetes	5-7N15	
		OTU025	3.7%	3.4%	3.2%	3.2%								Firmicutes	<i>Dorea</i>	
		OTU026	3.1%	2.8%	2.6%	2.6%								Firmicutes	<i>Lactobacillus</i>	

	OTU028				2.3%			2.3%		2.4%	3.8%	Spirochaetes	<i>Treponema</i>
Feces	OTU030	1.4%				1.3%	1.2%	1.2%				Bacteroidetes	<i>Bacteroides</i>
	OTU032		1.4%				1.4%		1.4%		1.7%	Bacteroidetes	Bacteroidaceae
	OTU034	1.3%	1.0%			1.1%						Firmicutes	Ruminococcaceae
	OTU035	1.6%	1.4%	1.0%	1.0%							Firmicutes	<i>Blautia</i>
	OTU038				1.0%			1.0%		1.1%		Bacteroidetes	<i>CF231</i>
	OTU043				1.3%			1.3%		1.4%	1.9%	Bacteroidetes	<i>S24-7</i>
	OTU046				1.3%			1.6%		1.3%	2.2%	Actinobacteria	<i>Bifidobacterium</i>
	OTU061										1.5%	Bacteroidetes	<i>CF231</i>
	OTU064	1.2%	1.1%									Bacteroidetes	<i>Prevotella</i>
	OTU067										1.0%	Bacteroidetes	Bacteroidales
	OTU075	1.5%				1.5%	1.3%	1.3%				Firmicutes	<i>Blautia</i>
	OTU091										1.5%	Bacteroidetes	<i>CF231</i>
	OTU097										1.1%	Proteobacteria	<i>Succinivibrio</i>
	OTU101										1.1%	Bacteroidetes	<i>CF231</i>
	OTU106	1.1%										Firmicutes	<i>Blautia</i>
	OTU122					1.1%						Spirochaetes	<i>Treponema</i>
	OTU152	2.1%	1.9%	1.8%	1.8%							Firmicutes	<i>Peptostreptococcus</i>
	OTU157					1.0%						Bacteroidetes	Prevotellaceae
	OTU170		1.3%			1.5%			1.3%	1.3%		Actinobacteria	Coriobacteriaceae
Feces	cumulative	69.2%	55.1%	40.2%	44.8%	48.6%	33.3%	38.4%	19.6%	24.4%	18.1%		
	OTU002								2.7%	2.7%	2.1%	Proteobacteria	Succinivibrionaceae
	OTU004								3.1%	2.1%	2.9%	Bacteroidetes	<i>Prevotella</i>
	OTU005								3.4%	3.4%	2.6%	Firmicutes	<i>Succiniclasticum</i>
	OTU007								1.1%	3.2%	4.3%	Proteobacteria	Succinivibrionaceae
	OTU014								1.7%	2.0%	1.0%	Bacteroidetes	<i>Prevotella</i>
Bacteria	OTU019								2.7%		3.7%	Bacteroidetes	<i>Prevotella</i>
	OTU021									1.5%	2.2%	Bacteroidetes	<i>Prevotella</i>
	OTU022									1.4%	1.9%	Bacteroidetes	<i>Prevotella</i>
	OTU023								7.5%	7.6%		Bacteroidetes	<i>Prevotella</i>
	OTU031								2.0%		3.0%	Bacteroidetes	<i>Prevotella</i>
Liquids	OTU039								1.1%	1.1%		Bacteroidetes	<i>Prevotella</i>
	OTU057								5.5%	5.6%		Bacteroidetes	<i>Prevotella</i>
	OTU068								1.2%	1.2%		Bacteroidetes	<i>Prevotella</i>
	OTU070										1.0%	Bacteroidetes	<i>Prevotella</i>
	OTU079								1.4%	1.4%		Firmicutes	<i>Acidaminococcus</i>
	OTU080								3.6%	3.7%		Firmicutes	<i>Succiniclasticum</i>
	OTU089								3.3%	3.4%		Firmicutes	<i>Ruminococcus</i>
	OTU123								2.2%	2.2%		Bacteroidetes	<i>Prevotella</i>
	OTU194											Bacteroidetes	<i>Prevotella</i>
	OTU217								1.1%	1.2%		Bacteroidetes	<i>Prevotella</i>
	OTU235								1.3%	1.4%		Bacteroidetes	p-2534-18B5
	OTU354								1.0%	1.0%		Firmicutes	<i>Bulleidia</i>
Liquids	cumulative								46.1%	46.0%	24.7%		
	OTU002								2.2%	4.2%	8.6%	Proteobacteria	Succinivibrionaceae
	OTU004								1.2%	1.6%		Bacteroidetes	<i>Prevotella</i>
	OTU005								2.3%	3.0%	1.5%	Firmicutes	<i>Succiniclasticum</i>
	OTU007									3.2%	4.4%	Proteobacteria	Succinivibrionaceae
	OTU014									1.5%		Bacteroidetes	<i>Prevotella</i>

-0.74

	OTU019		2.1%		2.5%	Bacteroidetes	<i>Prevotella</i>
	OTU021			1.0%	1.5%	Bacteroidetes	<i>Prevotella</i>
	OTU022				1.4%	Bacteroidetes	<i>Prevotella</i>
	OTU023		9.0%	9.3%		Bacteroidetes	<i>Prevotella</i>
	OTU027		1.6%		1.9%	Firmicutes	<i>Succiniclasticum</i>
	OTU029				1.2%	Bacteroidetes	<i>Prevotella</i>
Solids	OTU039		1.0%	1.1%		Bacteroidetes	<i>Prevotella</i>
	OTU042		1.6%		2.3%	Bacteroidetes	Bacteroidales
	OTU057		4.0%	4.1%		Bacteroidetes	<i>Prevotella</i>
	OTU062		1.3%	1.3%		Bacteroidetes	<i>Prevotella</i>
	OTU068		2.5%	2.6%		Bacteroidetes	<i>Prevotella</i>
	OTU079		1.7%	1.8%		Firmicutes	<i>Acidaminococcus</i>
	OTU080		3.0%	3.1%		Firmicutes	<i>Succiniclasticum</i>
	OTU102		1.2%	1.2%		Firmicutes	<i>Megasphaera</i>
	OTU104		1.6%	1.6%		Firmicutes	Veillonellaceae
	OTU114				1.6%	Bacteroidetes	Bacteroidales
	OTU123		2.6%	2.7%		Bacteroidetes	<i>Prevotella</i>
	OTU221		1.1%	1.1%		Bacteroidetes	<i>Prevotella</i>
	OTU275		1.0%	1.1%		Firmicutes	<i>Roseburia</i>
	OTU277		1.1%	1.1%		Firmicutes	<i>Acidaminococcus</i>
Solids cumulative			41.9%	46.9%	27.0%		
Feces	OTU001				11.5%	Chytridiomycota	<i>Caecomyces</i>
	OTU002				7.5%	Chytridiomycota	<i>Caecomyces</i>
	OTU003				1.0%	Chytridiomycota	Neocallimastigaceae
	OTU004				3.2%	Chytridiomycota	<i>Caecomyces</i>
	OTU005				1.1%	Chytridiomycota	<i>Piromyces</i>
	OTU006				3.3%	Chytridiomycota	Neocallimastigaceae
	OTU007				6.2%	Chytridiomycota	<i>Caecomyces</i>
	OTU008				3.7%	Chytridiomycota	<i>Orpinomyces</i>
	OTU009				1.6%	Chytridiomycota	<i>Cyllamyces</i>
	OTU010				1.7%	Chytridiomycota	Neocallimastigaceae
	OTU013				2.3%	Chytridiomycota	Neocallimastigaceae
	OTU017				8.6%	unclassified	
	OTU018				1.9%	Chytridiomycota	<i>Orpinomyces</i>
	OTU019				1.1%	Chytridiomycota	Neocallimastigaceae
	OTU022				1.6%	Chytridiomycota	<i>Orpinomyces</i>
	OTU023				1.0%	Chytridiomycota	<i>Orpinomyces</i>
	OTU027				1.6%	Chytridiomycota	<i>Cyllamyces</i>
	OTU039				1.9%	unclassified	
	OTU041				1.5%	unclassified	
	OTU044				1.1%	unclassified	
Feces cumulative					63.6%		
	OTU001		4.3%	8.8%	8.4%	Chytridiomycota	<i>Caecomyces</i>
	OTU002		3.3%	7.1%	6.1%	Chytridiomycota	<i>Caecomyces</i>
	OTU003		3.8%	9.3%	9.4%	Chytridiomycota	Neocallimastigaceae
	OTU004			6.3%	8.5%	Chytridiomycota	<i>Caecomyces</i>
	OTU005		3.8%		5.5%	Chytridiomycota	<i>Piromyces</i>
	OTU006		2.7%	5.0%	4.3%	Chytridiomycota	Neocallimastigaceae
	OTU007		4.6%		3.8%	Chytridiomycota	<i>Caecomyces</i>

		OTU008		4.5%		6.4%			Chytridiomycota	<i>Orpinomyces</i>
	Liquids	OTU011				4.8%			Chytridiomycota	Neocallimastigaceae
		OTU014		2.6%		3.7%			Chytridiomycota	Neocallimastigaceae
	Fungi	OTU015		3.4%		4.8%	0.88		Chytridiomycota	<i>Piromyces</i>
		OTU019				4.9%			Chytridiomycota	Neocallimastigomycetes
		OTU036					0.81		Chytridiomycota	Neocallimastigomycetes
		OTU037		13.6%	13.3%				unclassified	
		OTU046		10.5%	10.3%				unclassified	
		OTU058		4.0%	3.9%				Chytridiomycota	<i>Piromyces</i>
		OTU066		4.5%	4.4%				Chytridiomycota	<i>Caecomyces</i>
		OTU076		4.7%	4.6%				unclassified	
	Liquids	cumulative		70.2%	73.0%	70.6%				
		OTU001		5.6%	11.0%	9.7%			Chytridiomycota	<i>Caecomyces</i>
		OTU002		4.1%	7.9%	7.6%	0.81		Chytridiomycota	<i>Caecomyces</i>
		OTU003		5.0%	5.2%	6.7%			Chytridiomycota	Neocallimastigaceae
		OTU004			8.0%	10.2%			Chytridiomycota	<i>Caecomyces</i>
		OTU005		4.3%		6.1%			Chytridiomycota	<i>Piromyces</i>
		OTU006		2.2%	5.1%	5.3%			Chytridiomycota	Neocallimastigaceae
		OTU007		4.3%		6.1%			Chytridiomycota	<i>Caecomyces</i>
		OTU008		5.0%		7.2%			Chytridiomycota	<i>Orpinomyces</i>
		OTU010		2.1%					Chytridiomycota	Neocallimastigaceae
		OTU011			4.1%	5.2%			Chytridiomycota	Neocallimastigaceae
		OTU012		6.8%	7.2%				Chytridiomycota	<i>Caecomyces</i>
	Solids	OTU013		1.7%					Chytridiomycota	Neocallimastigaceae
		OTU015		2.3%		3.3%	0.83		Chytridiomycota	<i>Piromyces</i>
		OTU016					0.74		Chytridiomycota	<i>Piromyces</i>
		OTU019			3.4%	4.8%			Chytridiomycota	Neocallimastigaceae
		OTU036					0.89		Chytridiomycota	Neocallimastigaceae
		OTU037		5.5%	5.6%				unclassified	
		OTU040							Chytridiomycota	Neocallimastigaceae
		OTU046		7.7%	7.8%				unclassified	
		OTU066		2.3%					Chytridiomycota	<i>Caecomyces</i>
		OTU076		3.4%	3.4%				unclassified	
		OTU103		3.7%	3.8%				Chytridiomycota	<i>Caecomyces</i>
		OTU202		2.4%					unclassified	
		OTU360		2.2%					Chytridiomycota	<i>Caecomyces</i>
	Solids	cumulative		70.6%	72.4%	72.2%				

Only OTUs at 0.5% or greater in at least one sample are shown. Only SIMPER contributions > 1% and strong correlations (Kendall < -0.70 or > 0.70) are show

Text S1. Sequence clean-up and analysis in mothur v1.36.1 and analysis in R v3.2.3 (R markdown format)

mothur v1.36.1

Archaea: A = 13862
B = 23444
C = silva.nr_v119.align
D = silva.nr_v119.tax
E = unknown;-Bacteria;-Eukaryota;
F = 375
G = gg_2013.fasta
H = gg_2013.gg.tax
I = Example.final.gg.wang.taxonomy

Bacteria: A = 28439
B = 42539
C = silva.nr_v119.align
D = silva.nr_v119.tax
E = unknown;-Archaea;-Eukaryota;-Bacteria;Cyanobacteria;-
Bacteria;Proteobacteria;Alphaproteobacteria;Rickettsiales;mitochondria;
F = 9000
G = gg_2013.fasta
H = gg_2013.gg.tax
I = Example.final.gg.wang.taxonomy

Fungi: skip align.seqs, screen.seqs, filter.seqs, and unique.seqs
C,G = UNITEv6_sh_dynamic_s.fasta
D,H = UNITEv6_sh_dynamic_s.tax
E = unknown;-Archaea;-Bacteria;
F = 250
I = Example.final.UNITEv6_sh_dynamic_s.wang.taxonomy

mothur clean-up

```
make.file(inputdir=/Example)
make.contigs(file=Example.file)
screen.seqs(fasta=Example.trim.contigs.fasta, group=Example.contigs.groups, maxambig=0,
            maxhomop=8, minlength=200, maxlength=600)
unique.seqs(fasta=Example.trim.contigs.good.fasta)
count.seqs(name=Example.trim.contigs.good.names, group=Example.contigs.good.groups)
summary.seqs(fasta=Example.trim.contigs.good.unique.fasta,
             count=Example.trim.contigs.good.count_table)
align.seqs(fasta=Example.trim.contigs.good.unique.fasta, reference=silva.nr_v119.align, flip=T)
screen.seqs(fasta=Example.trim.contigs.good.unique.align,
            count=Example.trim.contigs.good.count_table,
            summary=Example.trim.contigs.good.unique.summary, start=A, end=B)
filter.seqs(fasta=Example.trim.contigs.good.unique.good.align, vertical=T, trump=.)
unique.seqs(fasta=Example.trim.contigs.good.unique.good.filter.fasta,
            count=Example.trim.contigs.good.good.count_table)
pre.cluster(fasta=Example.trim.contigs.good.unique.good.filter.unique.fasta,
            count=Example.trim.contigs.good.unique.good.filter.count_table, diffs=2)
```

```

chimera.uchime(fasta=Example.trim.contigs.good.unique.good.filter.unique.precluster.fasta,
               count=Example.trim.contigs.good.unique.good.filter.unique.precluster.count_table)
remove.seqs(fasta=Example.trim.contigs.good.unique.good.filter.unique.precluster.fasta,
            count=Example.trim.contigs.good.unique.good.filter.unique.precluster.count_table,
            accnos=Example.trim.contigs.good.unique.good.filter.unique.precluster.denovo.uchime.accnos)
classify.seqs(fasta=Example.trim.contigs.good.unique.good.filter.unique.precluster.pick.fasta,
              count=Example.trim.contigs.good.unique.good.filter.unique.precluster.pick.count_table,
              reference=C, taxonomy=D, cutoff=60)
remove.lineage(fasta=Example.trim.contigs.good.unique.good.filter.unique.precluster.pick.fasta,
               count=Example.trim.contigs.good.unique.good.filter.unique.precluster.pick.count_table,
               taxonomy=Example.trim.contigs.good.unique.good.filter.unique.precluster.pick.nr_v119.wang.taxonomy, taxon=E)
count.groups(count=Example.trim.contigs.good.unique.good.filter.unique.precluster.pick.count_table)
split.abund(fasta=Example.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.fasta,
            count=Example.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.count_table,
            cutoff=1)
count.groups(count=Example.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.abund.count_table)
system(cp Example.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.abund.fasta
        Example.final.fasta)
system(cp Example.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.abund.count_table
        Example.final.count_table)

```

mothur analysis

```

dist.seqs(fasta=Example.final.fasta)
cluster.split(column=Example.final.dist, count=Example.final.count_table, method=furthest, cutoff=0.05)
make.shared(list=Example.final.fn.unique_list.list, count=Example.final.count_table, label=0.03)
normalize.shared(shared=Example.final.fn.unique_list.shared, method=totalgroup, norm=F)

summary.single(shared=Example.final.fn.unique_list.shared, label=0.03, calc=nseqs-sobs-coverage)
summary.single(shared=Example.final.fn.unique_list.0.03.norm.shared, label=0.03, calc=chao-shannon)

classify.seqs(fasta=Example.final.fasta, count=Example.final.count_table, template=G, taxonomy=H,
              cutoff=80)
classify.otu(list=Example.final.fn.unique_list.list, taxonomy=I, count=Example.final.count_table,
             label=0.03, cutoff=80, basis=otu, probs=F)

```

R analysis

```

```{r Load data}
#Normalized OTU table
#rows = samples IDs, columns = OTUs, data=normalized counts
OTU = read.table("Normalized.OTUs.txt", header=TRUE, row.names=1, sep="/t")

#Variables and metrics of interest
#rows = samples IDs, columns = variables/metrics i.e. AgeGroup, Shannon's diversity, etc., data=varied
var = read.table("Variables.txt", header=TRUE, row.names=1, sep="/t")
```

```{r Community diversity and richness: ANOVA, TukeyHSD}
#EXAMPLE: test Shannon's diversity metric between age groups

```

```

#ANOVA test for differences
anova.test = aov(shannon~AgeGroup, data=var)
summary(anova.test)

#Tukey's honest significant difference (HSD) from multiple pairwise comparisons between age groups
TukeyHSD(anova.test)

#Boxplot of Shannon's by age group
plot(var$shannon~MetFA$AgeGroup, boxwex=0.75)
```



```

```{r Non-metric multidimensional scaling plots}
#Load packages
library(vegan)
library(plotrix)
library(TeachingDemos)

#Calculate Bray-Curtis distance matrix for OTU table
nmds=metaMDS(OTU, distance="bray", k=2, trymax=1000)

#Create color vector for continuous rainbow coloring by exact age in days
#Use the natural log of exact age (Daysln) to scale the colors properly
colvec=colorRampPalette(c('darkred', 'red', 'orangered', 'orange', 'yellow', 'lightgreen', 'green', 'darkgreen',
'cyan', 'light blue', 'blue', 'darkblue', 'purple', 'pink'))
var$col=colvec(200)[as.numeric(cut(var$Daysln, breaks=200))]

#EXAMPLE: basic plot
#Plot the nmds with rainbow colors
plot(nmds, type="n")
points(nmds, pch=20, cex=2.5, display="sites", col=var$col)

#EXAMPLE: split x-axis plot
#Pull out x and y values for the nmds
nmds.xy=scores(nmds, display="sites")

#Specify which values are above or below the desired x-axis gap and scale them to reduce the gap size
#In this example, the first 3 samples are very different from the rest
xgap=ifelse(nmds.xy[,1]>nmds.xy[4,1]-1, (nmds.xy[,1]+(nmds.xy[3,1]-nmds.xy[4,1]+1.5)), nmds.xy[,1])

#Plot the nmds with new scaled x-axis values
plot(xgap, nmds.xy[,2], pch=20, cex=2.5, col=var$col, xaxt="n")

#Label with x-axis with the scaled values
xat = pretty(xgap)
xat = xat[xat!=nmds.xy[3,1]+0.6]
xlab = ifelse(xat>nmds.xy[3,1]+0.6, xat-(nmds.xy[3,1]-nmds.xy[4,1]+1.5), xat)
axis(1, at=xat, labels=xlab)

#Add slash to designate axis break
axis.break(1, nmds.xy[3,1]+0.6, brw=0.05, style="slash")

```


```



```

#EXAMPLE: plot with smaller inset plot in upper right
#Basic plot
plot(nmds, type="n")
points(nmds, pch=20, cex=2.5, display="sites", col=var$col)

#Define smaller area for inset plot
par(fig=c(3/6,2/3,1/6,1/3), new=TRUE)

#Basic inset plot
#EXAMPLE: only use samples 4 through 27
plot(nmds.xy[4:27,1], nmds.xy[4:27,2], pch=20, cex=2.5, col=var$col[4:27])
```



```

```{r Assess differences in dispersion in nMDS plots: PERMDISP}
#Load package
library(vegan)

#Calculate Bray-Curtis dissimilarity
dist = vegdist(OTU, method="bray")

#Calculate dispersions of age groups
disp = betadisper(dist, var$AgeGroup)

#Test for differences between dispersions of groups (PERMDISP)
permutest(disp, pairwise=TRUE, permutations=10000)
```

```{r Model change in log abundances and log variances}
#Load packages
library(vegan)
library(lme4)
library(car)

#Define data frames and variables. num=number of animals in the comparison
OTU = OTU
var = var
time1 = "1yr"
time2 = "2yr"
num=15

#Make data frame for only samples from time 1. Add a number smaller than the min non-zero abundance
(which is 1)
t1 = OTU[Met$AgeGroup == time1, ]
t1[t1 == 0] = .1

#Repeat for time 2.
t2 = OTU[Met$AgeGroup == time2, ]
t2[t2 == 0] = .1

#Remove any samples that do not have both time 1 and 2 samples, if necessary

```


```

```

t1 = t1[!rownames(t1) %in% c(),]
t2 = t2[!rownames(t2) %in% c(),]
Met=Met[!rownames(Met) %in% c(),]

#Make a matrix of log ratios between time 1 and 2
lr1.2 = as.matrix(log(t2/t1))
hist(lr1.2[, 1:100])

#Make a matrix that corrects the change in abundance by the number of days elapsed
days.elapsed1.2 = Met[Met$AgeGroup == time2,]$AgeExact - Met[Met$AgeGroup == time1,]$AgeExact
lr1.2c = sweep(lr1.2, 1, days.elapsed1.2, "/")
hist(lr1.2c[, 1:100])

#create data frame for samples from time 1 and time 2
t1.2 = rbind(t1, t2)
plot(log(apply(t1.2, 2, mean)), log(apply(t1.2, 2, var)))

#Find which OTUs in the merged data frame are above the cutoff equal of presence in at least 2 samples
t1.2.cutoff = (0.1*(num-2)+2)/num
which.t1.2.cutoff = which(apply(t1.2, 2, mean) > t1.2.cutoff)
t1.2.cut = t1.2[, which.t1.2.cutoff]
t1.cut = t1[, which.t1.2.cutoff]
t2.cut = t2[, which.t1.2.cutoff]
lr.t1.2c.cut = lr1.2c[, which.t1.2.cutoff]

#Use the general rule that the log variance scales with log mean abundance to get a measurement of
variability of OTUs
plot(log(apply(t1.2.cut, 2, mean)), log(apply(t1.2.cut, 2, var)))
tay.lm.t1.2 = summary(lm(log(apply(t1.2.cut, 2, var)) ~ log(apply(t1.2.cut, 2, mean))))
abline(tay.lm.t1.2, lwd = 2)
```



```

```{r Test for differences in community structure (Bray-Curtis) and composition (Jaccard) between
groups: ANOSIM}
#Load package
library(vegan)

#Calculate Bray-Curtis distance matrix
dist = vegdist(OTU, method="bray")

#Perform ANOSIM for age groups
anosim(dist, var$AgeGroup, permutations=10000)
```



```

```{r Identify OTUs important in significant ANOSIMs: SIMPER}
#Load package
library(vegan)

#Run SIMPER
simper(OTU, var$AgeGroup, permutations = 10000)

```


```


```

```
```
```

```
```{r Determine co-occurrences of OTUs of different amplicons: Mantel}
```

```
#Load package
```

```
library(vegan)
```

```
#Calculate distance matrices for OTU tables. For example, 1 = archaea, 2 = bacteria, 3 = fungi
```

```
dist1 = vegdist(OTU)
```

```
dist2 = vegdist(OTU2)
```

```
dist3 = vegdist(OTU3)
```

```
#Run co-variance (Mantel)
```

```
#For 2 tables
```

```
mantel(dist1, dist2, permutations=10000)
```

```
#For 3 tables
```

```
mantel.partial(dist1, dist2, dist3, permutations=10000)
```

```
```
```

```
```{r Assess differences in community structure (Bray-Curtis) and composition (Jaccard) across a continuous variable: PERMANOVA}
```

```
#All such tests occurred within age groups
```

```
#Subset the OTU table to only 2 week samples
```

```
OTU2w = OTU[var$AgeGroup == "2w",]
```

```
#Run PERMANOVA between 2 week OTU table and ADG
```

```
adonis(OTU2w~ADG, var[var$AgeGroup=="2w",], permutations = 10000, method="bray")
```

```
```
```

```
```{r Calculate correlations between OTUs or between OTUs and variables: Kendall}
```

```
#Run Kendall correlation between two sets of OTUs
```

```
cor.OTU1.OTU2 = cor(OTU1, OTU2, method="kendall")
```

```
#Run Kendall correlations between OTUs and a continuous variable
```

```
cor.OTU.ADG = cor(OTUs, var$ADG, method="kendall")
```

```
#Print results to a text file
```

```
write.table(cor.OTU.ADG, file="cor.OTU.ADG.txt", sep="\t")
```

```
```
```