Investigating the development of the fecal microbiome in growing diary calves

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Background

- Since the gut of young mammals is devoid of microorganisms at birth, its colonization by symbionts and their arrangement into complex communities is a critical aspect of a young host's post-natal development
- Indeed, these microbial communities allow for increased health and nutrition utilization in young calves at the most vulnerable time in their lives.
- A better understanding of how the gut microbiome develops is therefore critical for improving calf health and optimizing their later productivity as mature animals

Objectives

- Determine the gut bacterial composition of 12 neonatal dairy calves using fecal samples

- Identify prominent known and uncharacterized gut bacterial species in growing dairy calves

Methods

- Fresh fecal samples were collected from 12 dairy calves. Samples were collected at weeks 0, 4, 8 and 12 after birth.
- Microbial DNA was extracted using a repeated bead beating plus column method.
- 16S rRNA gene sequence diversity was determined by high-throughput sequencing (MiSeq 2X300, Illumina) of PCR-generated amplicons
  - 27F – 519R primers (V1-V3 region)
- Operational Taxonomic Unit (OTU) clustering was performed using custom-written Perl scripts (96% cutoff)
- Taxonomic affiliation was determined using the online tools RDP classifier and BLAST
- Alpha diversity indices and Principal Coordinate Analysis (PCoA) were performed using the open source software MOTHUR (v.1.44.1)
- Kruskall-Wallis and Wilcox-pairwise tests were performed in R (Version 3.6.2)

Results

Table 1. Comparative analysis of main taxonomic groups between time points. Different superscripts indicate statistical difference ($P < 0.05$).

Taxonomy	Week 0	Week 4	Week 8	Week 12
Firmicutes				
Clostridiaceae 1	$21.30^a \pm 5.30$	$1.51^b \pm 1.34$	$0.60^{bc} \pm 0.17$	$1.35^c \pm 0.40$
Ruminococcaceae	$5.31^a \pm 2.65$	$26.50^b \pm 2.92$	$35.48^c \pm 1.72$	$33.78^c \pm 1.51$
Lactobacillaceae	$9^a \pm 3.16$	$8.84^a \pm 2.37$	$< 0.01^b$	$0.01^b \pm < 0.01$
Lachnospiraceae	$2.25^a \pm 0.78$	$22.43^b \pm 2.13$	$22.19^b \pm 1.68$	$24.65^b \pm 1.94$
Enterococcaceae	$4.13^a \pm 1.43$	$0.09^b \pm 0.05$	$< 0.01^c$	$< 0.01^c$
Erysipelotrichaceae	$1.48^a \pm 1.29$	$4.38^b \pm 0.48$	$3.06^{bc} \pm 0.37$	$2.42^c \pm 0.21$
Streptococcaceae	$2.89^a \pm 0.82$	$0.16^b \pm 0.11$	$< 0.01^c$	0 ± 0^c
Other Firmicutes	0.53 ± 0.11	5.42 ± 1.16	9.51 ± 1.00	12.25 ± 1.15
Bacteriodetes				
Porphyromonadaceae	$0.44^a \pm 0.43$	$1.88^b \pm 0.38$	$3.15^c \pm 0.48$	$2.94^c \pm 0.28$
Bacteroidaceae	9.90 ± 3.65	10.10 ± 1.68	5.39 ± 1.04	4.63 ± 0.45
Prevotellaceae	$0.04^a \pm < 0.01$	$6.10^b \pm 1.65$	$7.10^b \pm 1.37$	$5.86^b \pm 1.01$
Other Bacteriodetes	$0.02 \pm < 0.01$	3 ± 0.83	5.86 ± 0.86	5.91 ± 0.98
Proteobacteria				
Enterobacteriaceae	$41.22^a \pm 3.68$	$2.35^b \pm 1.69$	$0.12^{bc} \pm 0.07$	$0.04^c \pm 0.03$
Other Proteobacteria	1.08 ± 0.63	1.44 ± 0.40	1.25 ± 0.30	0.76 ± 0.14
Verrucomicrobia				
Verrucomicrobiaceae	$0.24^a \pm 0.23$	$< 0.01^b$	$1.50^c \pm 0.72$	$2.24^{ac} \pm 0.94$
Actinobacteria				
Coriobacteriaceae	$6.21^a \pm 0.56$	$11.48^b \pm 0.93$	$7.93^c \pm 0.24$	$7.83^c \pm 0.22$
Spirochaetae				
Spirochaetae	$< 0.01^a$	$0.12^b \pm 0.08$	$2.30^c \pm 1.23$	$1.11^c \pm 0.31$
Other Bacteria	0.09 ± 0.04	0.34 ± 0.14	0.93 ± 0.35	0.74 ± 0.20
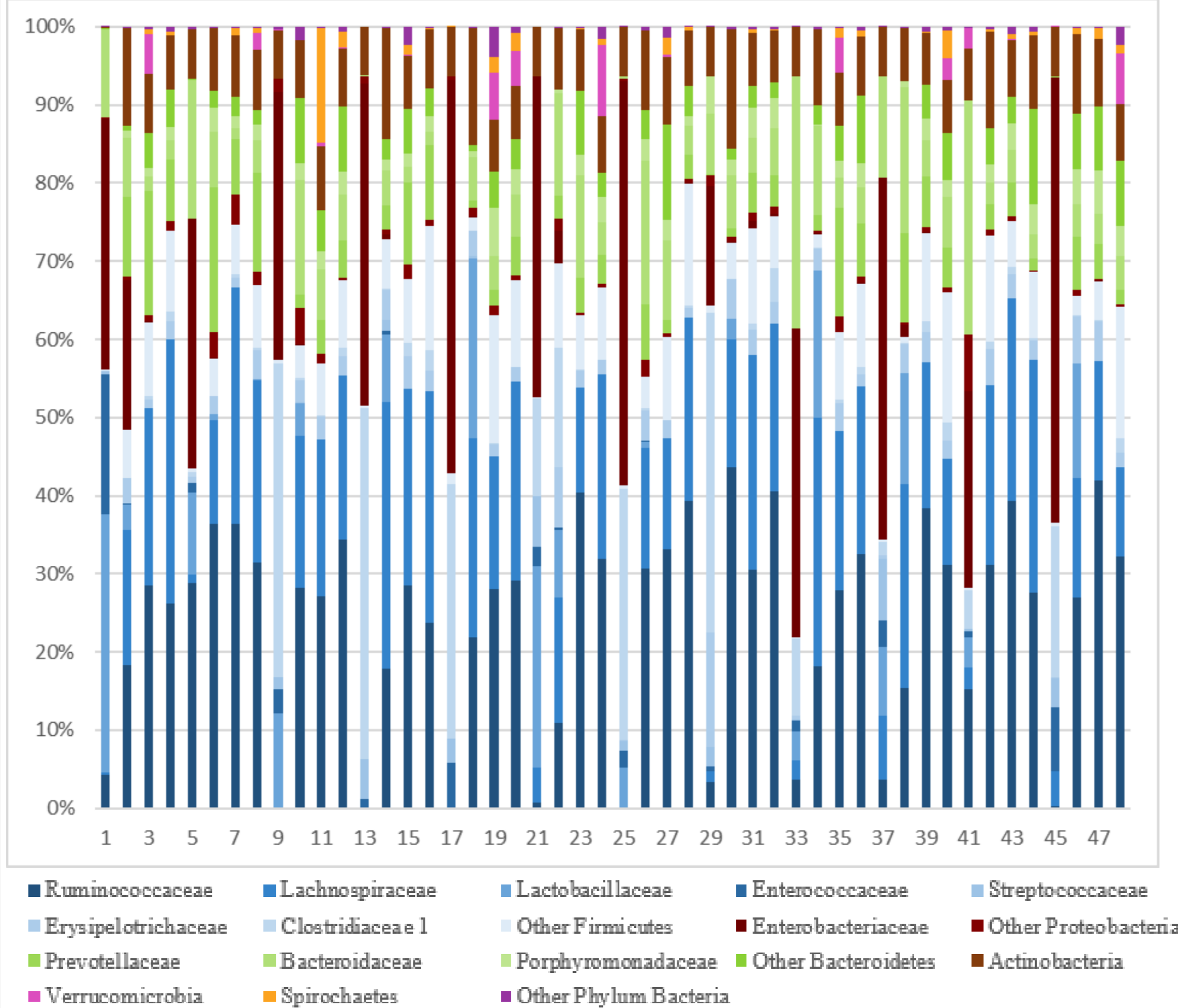
Table 2. Comparative analysis of α diversity indices between time points. Different superscripts indicate statistical difference ($P < 0.05$).

Index	Week 0	Week 4	Week 8	Week 12
Ace	$197.50^a \pm 33.81$	$445.20^b \pm 44.21$	$770.73^c \pm 26.40$	$960.77^d \pm 52.13$
Chao	$117.39^a \pm 16.18$	$372.50^b \pm 36.64$	$682.15^c \pm 20.45$	$850.26^d \pm 34.80$
Shannon	$1.83^a \pm 0.06$	$3.89^b \pm 0.12$	$4.76^c \pm .082$	$5.05^d \pm 0.05$
Simpson	$0.27^a \pm 0.02$	$0.05^b \pm 0.01$	$0.02^c \pm 0.003$	$0.02^c \pm 0.001$
Sobs	$64.42^a \pm 4.08$	$250.33^b \pm 24.61$	$451.58^c \pm 15.31$	$550^d \pm 13.98$

Table 3. Comparative analysis between time points of the most abundant OTUs. Different superscripts indicate statistical difference ($P < 0.05$).

OTUs	Week 0	Week 4	Week 8	Week 12	Closest valid taxon (id%)
Firmicutes					
JA_17-00190	$1.6^a \pm 4.6$	$.90^b \pm .90$	$< 0.01^b$	$< 0.01^b$	*Cl perfringens* (98.73%)
JA_90-20062	$1.0^a \pm 1.0$	$7.3^b \pm 2.6$	$.72^c \pm 2.2$	$0.23^a \pm 0.01$	*F. prausnitzii* (96.57%)
JA_13-22430	$3.3^a \pm 1.8$	$4.0^a \pm 1.3$	$< 0.01^b$	$< 0.01^b$	*Lactobacillus johnsonii* (99.63%)
JA_70-18057	$< 0.01^a$	$3.1^b \pm 0.64$	$1.7^b \pm 0.39$	$0.64^c \pm 0.15$	*Blautia wexlerae* (96.59%)
JA_46-21334	$0.76^a \pm 0.66$	$4.5^b \pm 1.22$	$< 0.01^c$	$< 0.01^c$	*Lactobacillus reuteri* (99.26%)
JA_81-39081	$4.1^a \pm 1.81$	$6.0^a \pm 0.22$	$< 0.01^b$	$0^b \pm 0$	*Butyriciococcus pullicaecorum* (95.19%)
JA_18-34620	$0.01^{ab} \pm < 0.01$	$4.5^b \pm 1.7$	$0.03^a \pm 0.02$	$0.01^a \pm < 0.01$	*Blautia stercori* (96.18%)
JA_16-68527	$< 0.01^a$	$0.03^a \pm 0.03$	$2.4^b \pm 0.92$	$1.7^b \pm 0.489$	*Oscillibacter ruminantium* (90%)
JA_5-29412	$4.0^a \pm 2.12$	$0.04^b \pm 0.027$	$< 0.01^b$	$< 0.01^b$	*Lactobacillus murinus* (100%)
JA_46-37837	$< 0.01^a$	$2.76^b \pm 1.03$	$0.89^b \pm 0.25$	$0.26^c \pm 0.12$	*Blautia caecimuris* (97.09%)
JA_33-48728	$2.95^a \pm 1.40$	$< 0.01^b$	$< 0.01^c$	$< 0.01^c$	*Enterococcus lactis* (99.36%)
JA_36-17512	$< 0.01^a$	$0.04^a \pm 0.04$	$1.22^b \pm 0.40$	$1.41^b \pm 0.41$	*Oscillibacter valericigenes* (90.28%)
JA_27-17537	$< 0.01^a$	$0.20^a \pm 0.20$	$1.00^b \pm 0.30$	$1.14^b \pm 0.27$	*Oscillibacter ruminantium* (89.02%)
JA_84-17601	$< 0.01^{ab}$	$0.20^a \pm 0.20$	$0.64^b \pm 0.51$	$1.4^c \pm 0.33$	*Oscillibacter ruminantium* (89.21%)
JA-17-04639	$1.68^a \pm 0.69$	$0.01^b \pm 0.01$	$0.09^b \pm 0.04$	$< 0.01^b$	*Clostridium paraputrificum* (99.80%)
JA_5-10150	$1.60^a \pm 0.62$	$0.14^b \pm 0.11$	$< 0.01^c$	$0^c \pm 0$	*Streptococcus macedonicus* (99.25%)
Bacteriodetes					
JA_18-16727	$< 0.01^a$	$1.7^b \pm 0.65$	$4.7^c \pm 0.86$	$4.6^c \pm 0.87$	*Muribaculum intestinale* (89.39%)
JA_35-04049	$< 0.01^a$	$3.6^b \pm 1.3$	$0.24^c \pm 0.09$	$0.47^{bc} \pm 0.11$	*Bacteroides coprophilus* (96.77%)
JA_7-08334	$< 0.01^a$	$0.78^b \pm 0.51$	$1.91^b \pm 0.72$	$1.07^b \pm 0.34$	*Prevotella copri* (99.24%)
JA_54-41198	$0.03^a \pm 0.03$	$0.88^b \pm 0.33$	$1.06^b \pm 0.29$	$0.84^b \pm 0.24$	*Bacteroides uniformis* (99.81%)
JA_16-69458	$< 0.01^a$	$0.37^{ab} \pm 0.27$	$0.94^b \pm 0.34$	$1.44^b \pm 0.35$	*Prevotella shahii* (90.53%)
JA_5-13995	$< 0.01^a$	$1.22^b \pm 0.55$	$0.22^b \pm 0.07$	$0.42^b \pm 0.20$	*Prevotella stercorea* (99.05%)
Proteobacteria					
JA_89-27339	$39.33^a \pm 3.6$	$2.3^b \pm 1.7$	$0.12^{bc} \pm 0.07$	$0.05^c \pm 0.03$	*Shigella sonnei* (99.81%)
Verrucomicrobia					
JA_36-61905	$0.01^a \pm < 0.01$	$< 0.01^b$	$1.47^c \pm 0.72$	$2.23^c \pm 0.94$	*Methylacidimicrobium fagopyrum* (91.43%)
Actinobacteria					
JA_6-16768	$< 0.01^a$	$3.34^b \pm 0.91$	$< 0.01^a$	$< 0.01^a$	*Collinsella aerofaciens* (98.38%)

Figure1. Taxonomy-based composition analysis of fecal bacterial communities in individual samples collected from neonatal dairy calves.

Legend: Ruminococcaceae, Lachnospiraceae, Lactobacillaceae, Enterococcaceae, Streptococcaceae, Erysipelotrichaceae, Clostridiaceae 1, Other Firmicutes, Enterobacteriaceae, Other Proteobacteria, Prevotellaceae, Bacteroidaceae, Porphyromonadaceae, Other Bacteroidetes, Actinobacteria, Verrucomicrobia, Spirochaetes, Other Phylum Bacteria

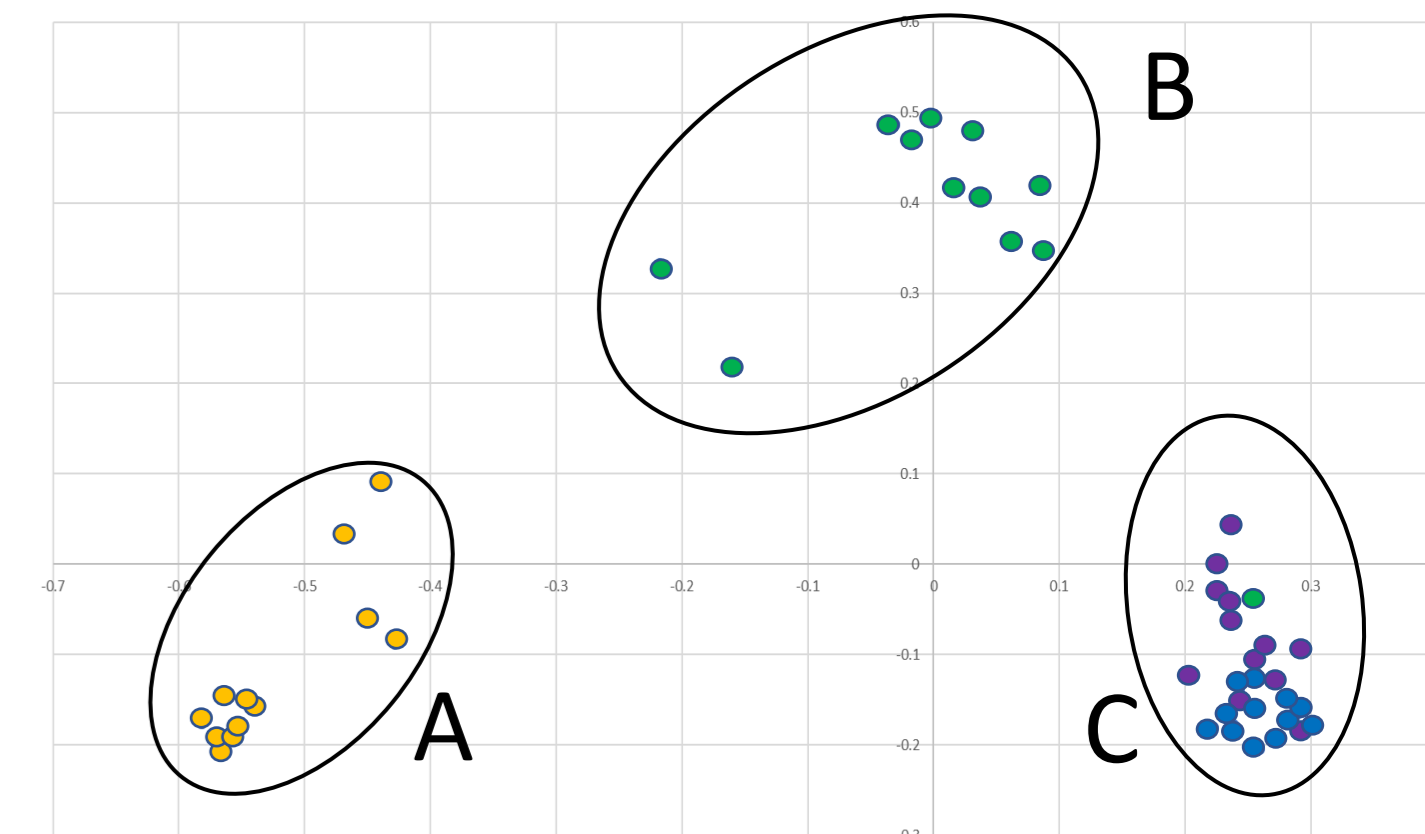**Figure 2.** β diversity analysis by PCoA. Each sample is represented by a circle, with distance between points indicating the relative similarity in bacterial community structure between them. Samples were found to form three main groups (A, B, C), each clustered in a fashion consistent with their time of collection.
Cluster A - Week 0 (yellow)
Cluster B - Week 4 (green)
Cluster C - Week 8 (purple) ;and Week 12 (blue)

Figure 1 Legend.
Samples (1-48) with corresponding week
Week 0 - 1,5,9,13,17,21, 25, 29,33,37,41,45
Week 4 – 2,6,10,14,18,22,26,30,34,38,42,46
Week 8 – 3,7,11,15,19,23,27,31,35,39,43,47
Week 12- 4,8,12,16,20,24, 28,32,36,40,44,48

Conclusions

- A gradual increase in species diversity was observed during the trial, with 3 distinctive bacterial community compositions observed at week 0, week 4 and weeks 8-12, respectively.
- Changes in composition were observed at both the levels of taxonomy and OTUs.
- The respective abundance of 25 distinct OTUs, representing a combination of known and uncharacterized bacterial species, was found to fluctuate during the time period of study.
- Together, these results are indicative of microbial succession taking place in the gut of neonatal dairy calves, with the period of week 0 to week 8 representing the time when gut bacterial communities are most fluid in composition.