SUPPLEMENTARY METHODS

Polymerase chain reaction (PCR) amplification and purification by Diversigen^{SM}

DNA from extracted samples was amplified using Invitrogen's AccuPrime High Fidelity kit, catalogue #12346094. Each PCR reaction was prepared by combining: 16 µl of the master mix (13.85 µl water + 2 µl 10 Å~ reaction buffer + 1.5 µl Taq DNA polymerase), 2ul template DNA, and 2 µl forward (515F) and reverse (806R) primers. PCR primers used for amplification incorporated adapters enabling DNA sequencing of the amplified product using an Illumina MiSeq. Primer sequences are shown below:

515F 5'-

AATGATACGGCGACCACCGAGATCTACACTATGGTAATTGTGTGCCAGCMGCCGCGGTAA-3'

806R 5'-

CAAGCAGAAGACGGCATACGAGATTCCCTTGTCTCCAGTCAGTCAGCCGGACTACHVGGGT

WTCTAAT-3'

The DNA samples were amplified using the following thermocycler conditions: Initial denaturation at 95°C for 2 min followed by 33 amplification cycles of 20 s at 95°C, 45 s at 50°C, and 90 s at 72°C followed by final extension at 72°C for 10 min. The PCR product was purified using the QIAquick PCR purification kit (Catalogue# 28104) and the yield quantified using Invitrogen's Quant-iT Picogreen dsDNA assay kit (Catalogue # P7589). Amplicons were pooled, barcoded, and cleaned by Invitrogen charge switch kit for 16S rRNA sequencing.

16S rRNA gene sequencing and compositional analysis by Diversigen^{SM}

The 16S rDNA V4 region amplified by PCR was sequenced in the MiSeq platform (Illumina, San Diego, CA) using the 2x250 bp paired-end protocol yielding pair-end reads that overlap almost completely. The primers used for PCR amplification contain adapters for MiSeq sequencing and single-end barcodes allowing pooling and direct sequencing of PCR products (1).

After sequencing, the read pairs were demultiplexed based on the unique molecular barcodes, and reads were merged using USEARCH v7.0.1090 (2), allowing zero mismatches and a minimum overlap of 50 bases. Merged reads were trimmed at the first base with Q5. In addition, a quality filter was applied to the resulting merged reads; reads containing over 0.05 expected errors were discarded.

16S rRNA gene sequences were clustered into Operational Taxonomic Units (OTUs) at a similarity cutoff value of 97% using the UPARSE algorithm. OTUs were mapped to an optimized version of the SILVA Database (3,4) containing only the 16S V4 region to determine taxonomies. Abundances were recovered by mapping the demultiplexed reads to the UPARSE OTUs. A custom script constructed a rarefied OTU table from the output files generated in the previous two steps for downstream analyses of alpha-diversity, beta diversity (5), and phylogenetic trends.

REFERENCES

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4. Quast C, Pruesse E, Yilmaz P, et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res 2012;41:D590–6.

5. Lozupone C, Knight R. UniFrac: a new phylogenetic method for comparing microbial communities. Appl Environ Microbiol 2005;71:8228–35.

Supplemental Table 1. Mean Abundance of the Top 10 Most Abundant Genera in TPN and Control Groups by Week

TPN (n = 25)

Week 1

Genus	Abundance (%)
Enterobacter (Proteobacteria)	25.17
Escherichia_Shigella (Proteobacteria)	15.99
Clostridium_sensu_stricto_1 (Firmicutes)	11.03
Streptococcus (Firmicutes)	9.27
Enterococcus (Firmicutes)	9.15
Staphylococcus (Firmicutes)	7.76
Veillonella (Firmicutes)	5.17
Akkermansia (Verrucomicrobia)	4.28
Lactobacillus (Firmicutes)	3.63
Pseudomonas (Proteobacteria)	1.54

Week 2

Genus	Abundance (%)
Enterobacter (Proteobacteria)	37.80
Escherichia_Shigella (Proteobacteria)	25.38
Streptococcus (Firmicutes)	9.86
Veillonella (Firmicutes)	5.27
Staphylococcus (Firmicutes)	4.90
Enterococcus (Firmicutes)	4.75
Clostridium_sensu_stricto_1 (Firmicutes)	4.25
Lactobacillus (Firmicutes)	2.59
Bifidobacterium (Actinobacteria)	2.24
Peptoclostridium (Firmicutes)	1.28

Control (n = 22)

Week 1

Genus	Abundance (%)
Enterobacter (Proteobacteria)	19.31
Escherichia_Shigella (Proteobacteria)	18.88
Streptococcus (Firmicutes)	11.75
Enterococcus (Firmicutes)	10.30
Bacteroides (Bacteroidetes)	7.06
Veillonella (Firmicutes)	6.42
Haemophilus (Proteobacteria)	5.91
Clostridium_sensu_stricto_1 (Firmicutes)	5.42
Erysipelatoclostridium (Firmicutes)	2.89
Staphylococcus (Firmicutes)	1.83

Week 2

Genus	Abundance (%)
Enterobacter (Proteobacteria)	38.99
Staphylococcus (Firmicutes)	16.34
Bacteroides (Bacteroidetes)	14.46
Bifidobacterium (Actinobacteria)	9.17
Escherichia_Shigella (Proteobacteria)	4.97
Streptococcus (Firmicutes)	3.55
Haemophilus (Proteobacteria)	2.76
Clostridium_sensu_stricto_1 (Firmicutes)	2.40
Peptoclostridium (Firmicutes)	2.03
Enterococcus (Firmicutes)	1.71

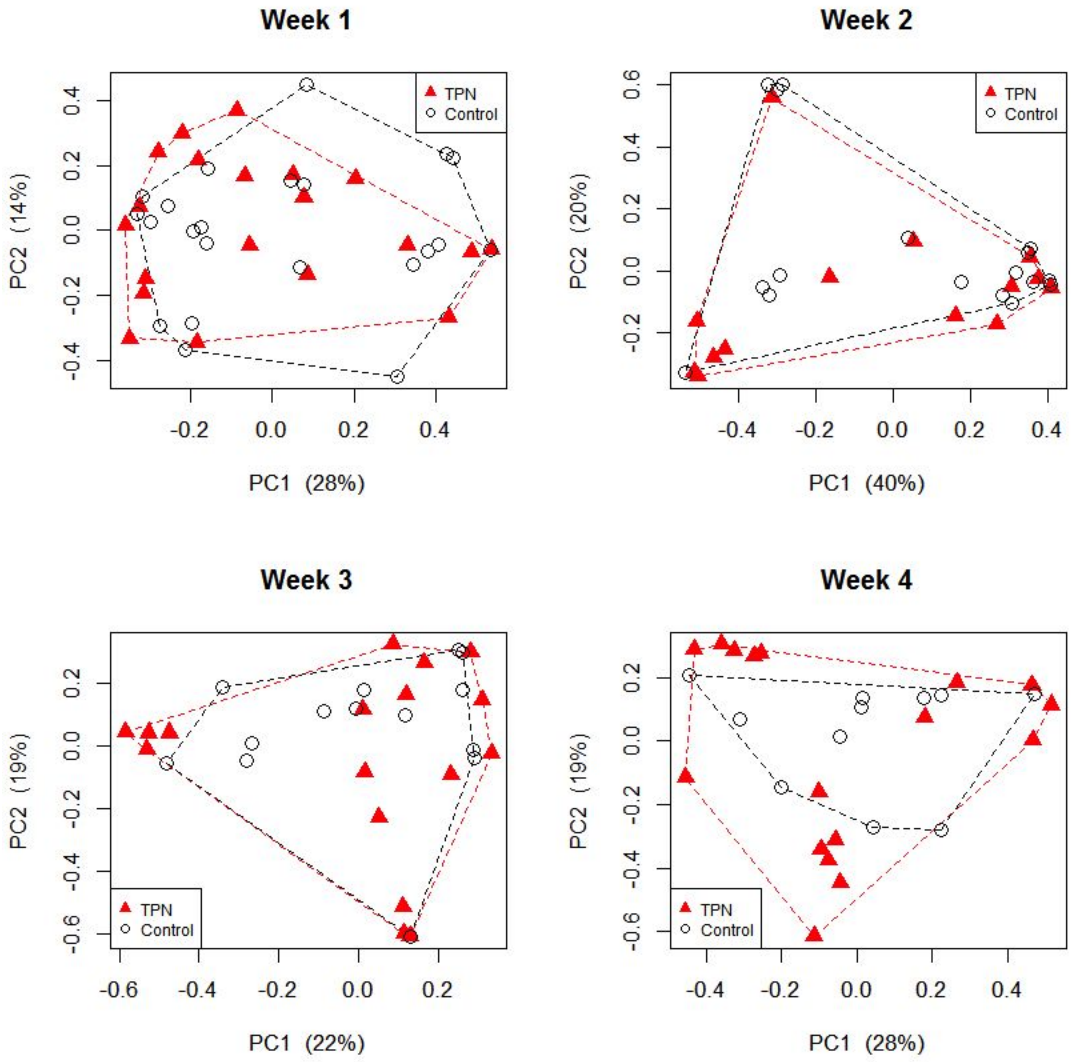
Week 3	
Genus	**Abundance (%)**
Streptococcus (Firmicutes)	17.90
Staphylococcus (Firmicutes)	17.39
Enterobacter (Proteobacteria)	13.92
Clostridium_sensu_stricto_1 (Firmicutes)	13.78
Escherichia_Shigella (Proteobacteria)	12.53
Haemophilus (Proteobacteria)	7.06
Veillonella (Firmicutes)	5.70
Akkermansia (Verrucomicrobia)	3.45
Paenibacillus (Firmicutes)	3.11
Enterococcus (Firmicutes)	1.97

Week 3	
Genus	**Abundance (%)**
Enterobacter (Proteobacteria)	18.71
Clostridium_sensu_stricto_1 (Firmicutes)	13.25
Escherichia_Shigella (Proteobacteria)	12.45
Staphylococcus (Firmicutes)	12.15
Enterococcus (Firmicutes)	10.65
Streptococcus (Firmicutes)	9.70
Bifidobacterium (Actinobacteria)	5.86
Bacteroides (Bacteroidetes)	3.42
Erysipelatoclostridium (Firmicutes)	2.49
Peptoclostridium (Firmicutes)	2.10

Week 4	
Genus	**Abundance (%)**
Enterobacter (Proteobacteria)	28.02
Escherichia_Shigella (Proteobacteria)	19.76
Streptococcus (Firmicutes)	13.33
Staphylococcus (Firmicutes)	11.10
Haemophilus (Proteobacteria)	7.60
Enterococcus (Firmicutes)	6.77
Clostridium_sensu_stricto_1 (Firmicutes)	5.71
Veillonella (Firmicutes)	4.04
Actinomyces (Actinobacteria)	1.06
Akkermansia (Verrucomicrobia)	0.95

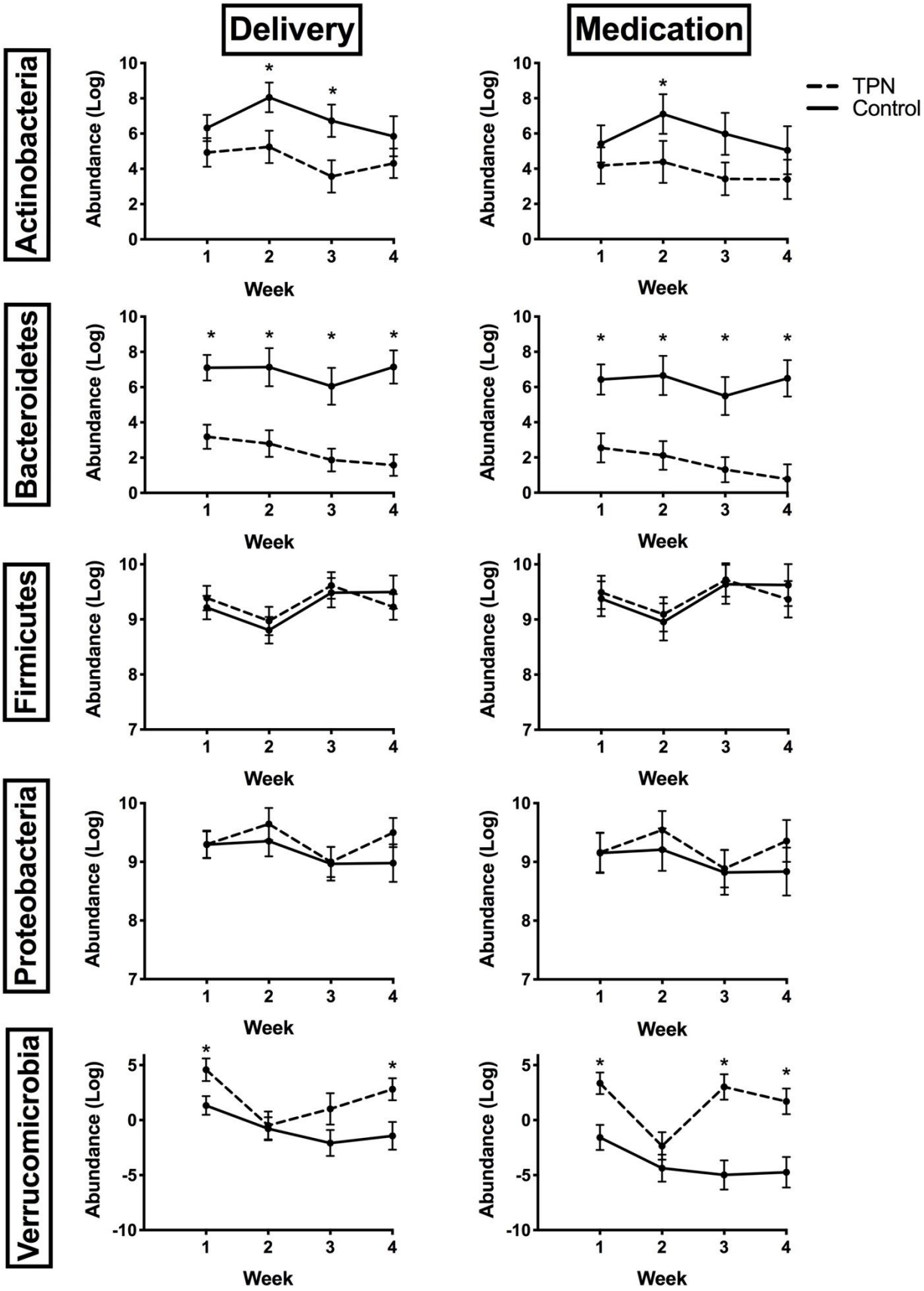
Week 4	
Genus	**Abundance (%)**
Enterobacter (Proteobacteria)	20.39
Clostridium_sensu_stricto_1 (Firmicutes)	14.01
Escherichia_Shigella (Proteobacteria)	11.73
Streptococcus (Firmicutes)	10.09
Erysipelatoclostridium (Firmicutes)	8.90
Bacteroides (Bacteroidetes)	8.28
Veillonella (Firmicutes)	6.09
Lachnoclostridium (Firmicutes)	5.69
Staphylococcus (Firmicutes)	3.99
Enterococcus (Firmicutes)	3.36

Data are presented as percents (%).
Genus name in italics, and corresponding phylum name in parenthesis.

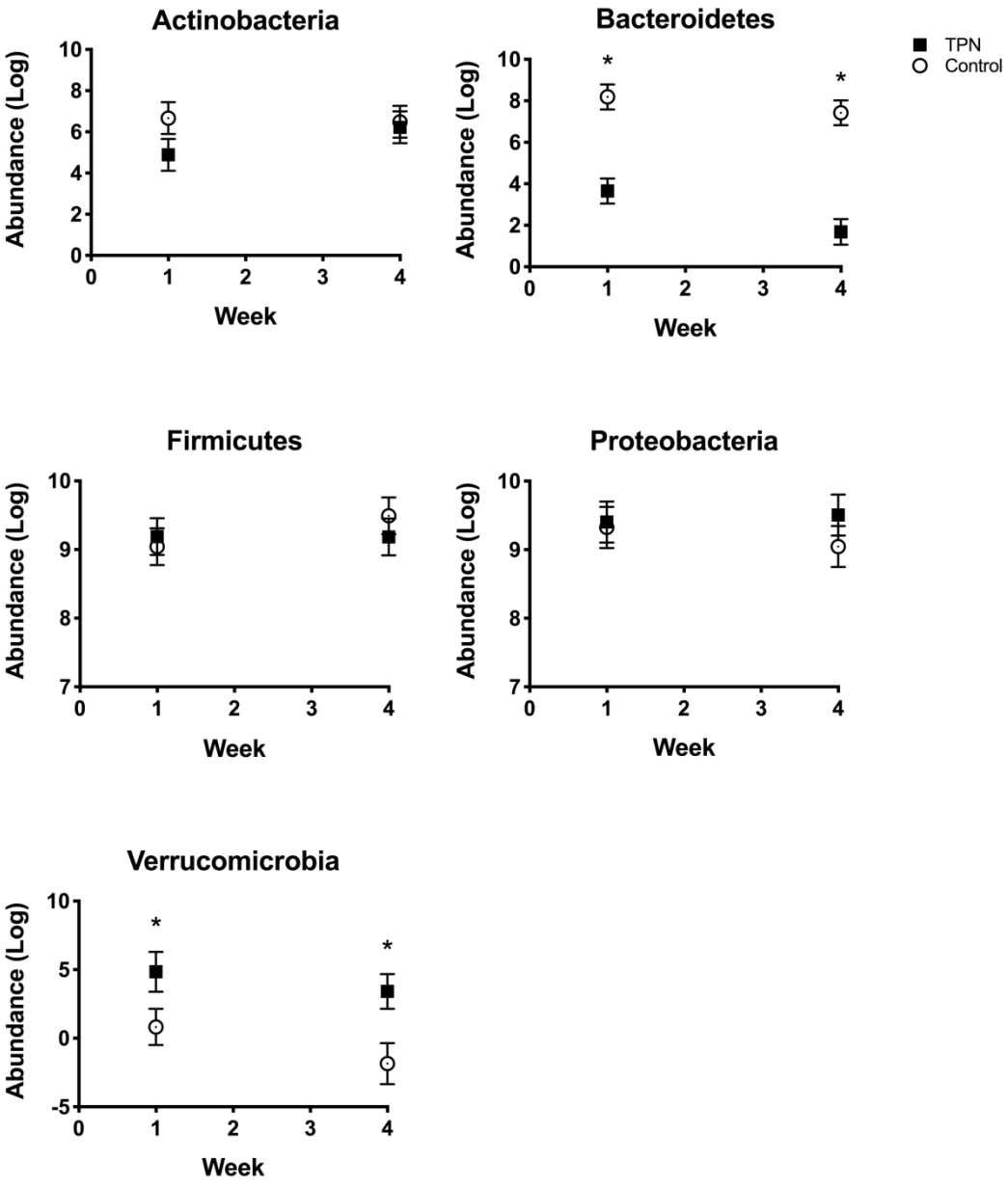


Supplemental Figure S1. Bacterial community structure comparisons between TPN and control groups. Principal coordinate analysis (PCoA) on Bray-Curtis distance measurements of beta diversity, or the differences in microbial community composition among samples, for TPN and control infants in each week (A-D) for the first 4 weeks of life. PC1 and PC2 are plotted on the x-axis and y-axis, respectively. Each point represents

a single sample. Red triangles indicate TPN group and empty circles indicate control group. PERMANOVA p-value > 0.1 in all weeks.

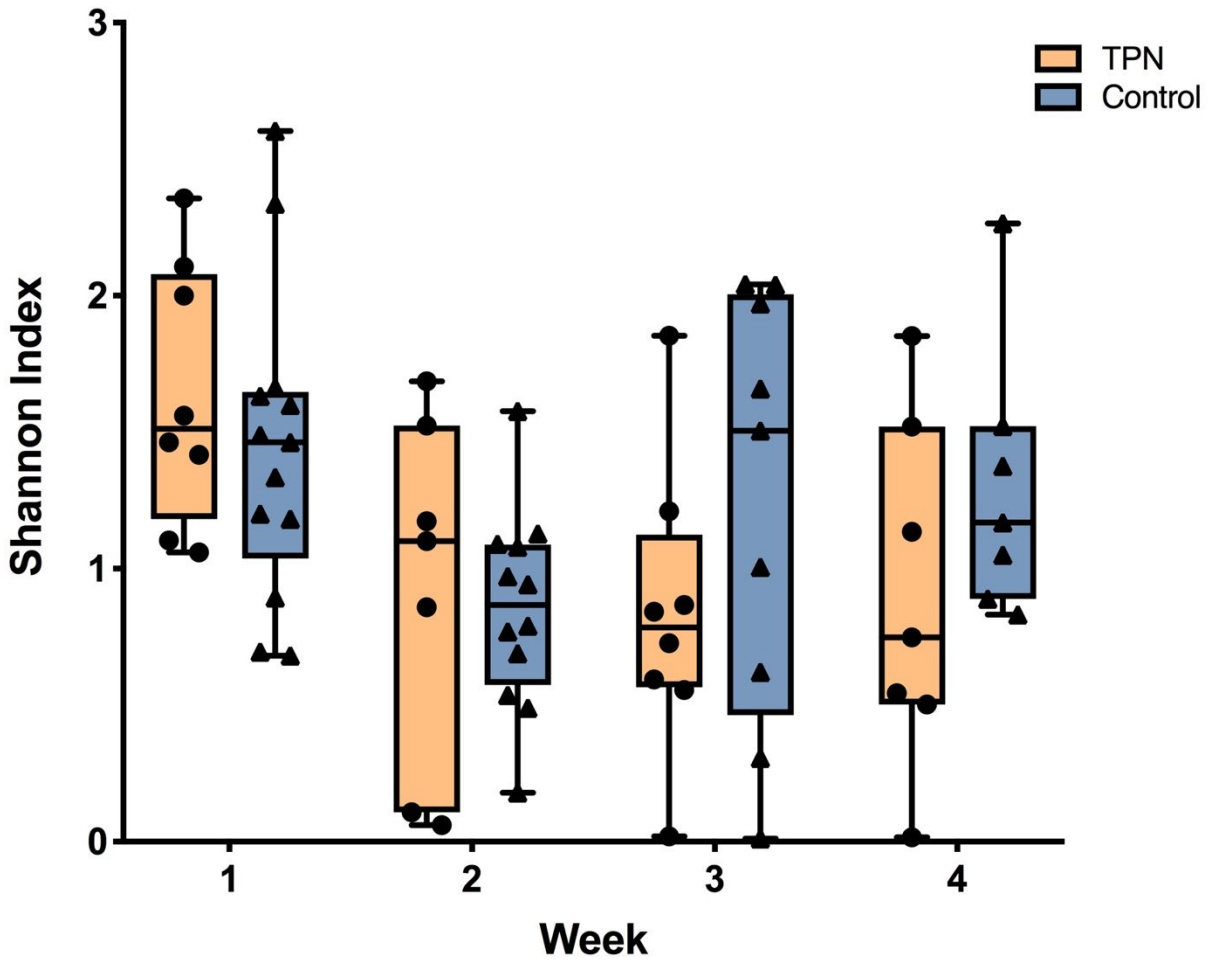

Delivery
Medication
TPN
Control
Actinobacteria
Bacteroidetes
Firmicutes
Proteobacteria
Verrucomicrobia
Abundance (Log)
Week

Supplemental Figure S2. Differences in the abundance of the dominant phyla over time in TPN and control groups when mode of delivery and treatment with proton pump inhibitor (PPI) or histamine-2 receptor antagonist (H2 blocker) medication were included in the generalized linear mixed model along with TPN exposure. Graphs are grouped vertically by the covariate that was included in the model, and horizontally by phyla. Model estimates for log of the abundance are shown by the circles and standard error as the error bars. *P-value < 0.05.



Supplemental Figure S3. Differences in the abundance of the dominant phyla over time in 10 TPN infants and 10 control infants who all had fecal samples in weeks 1 and 4. All TPN infants received TPN in week 1 but had weaned off of TPN and were receiving

full enteral feeds in week 4. Model estimates for log of the abundance are shown by the symbols and standard error as the error bars. *P-value < 0.05.



Supplemental Figure S4. Comparison of changes in alpha diversity in 10 TPN infants and 13 control infants who all received a short course of intravenous antibiotics for $\leq$ 3 days immediately after birth. Shannon diversity index is shown on the y-axis and individual weeks are shown on the x-axis. Orange boxes indicate TPN group and blue boxes indicate control group. Box represents the 25th and 75th percentiles; line inside the box represents the median; error bars represent the minimum and maximum.