Microbial communities in placentas from term normal pregnancy exhibit spatially variable profiles

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Supplementary Table Legends

Table S1: **Clinical and Demographic Features of Human Subjects**

Table S2: **Sequences of primer pairs used to generate 16S amplicons (V1 – V9)**

Table S3: **Read and Sample Summary of V1-V9 datasets with detectable OTUs**

Clinical characteristics	Mean ± SD
Age (yrs)	28.86 ± 0.67
BMI at first pregnancy visit	29.35 ± 1.21
BMI at delivery	33.90 ± 1.06
Infant weight (g)	3187.39 ± 88.16
Gestation Age (days)	273.19 ± 1.25

			Percent
Race	Asian/ Pacific Islander	2	3.51%
	Black	19	33.33%
	Native American	1	1.75%
	White	35	61.40%
Gestation Number	1	50	87.72%
	2	7	12.28%
	3	0	0.00%
Fetal Sex	Male	25	43.86%
	Female	32	56.14%

	Number (yes)	Percent (yes)
Vaginal Delivery	34	59.65%
Cesarean Section	23	40.35
Multiple Gestation	7	12.28%

Table S1: **Clinical and Demographic Features of Human Subjects**

Amplicon Size	Variable Region	Primer Name	Fluidigm Forward Adapter	Forward Sequence	Primer Name	Fluidigm Reverse Adapter	Reverse Sequence	Miseq 2x150 F/R overlap	Strand bias F,R	PMID	Forward Pubmed historic name	Reverse Pubmed historic name
113	V1	V1	ACACTGACGAC ATGGTTCTACA	AGAGTTTGA TCMTGGCTC AG	V1	TACGGTAGCA GAGACTTGGTC T	TTACTCACCCG TICGCCRCT	all	0	18047683		
261	V2	V2	ACACTGACGAC ATGGTTCTACA	AGYGGCGIA CGGGTGAGT AA	V2	TACGGTAGCA GAGACTTGGTC T	CYIACTGCTGC CTCCCGTAG	39	111,111	18047683		
170	V3	V3_2	ACACTGACGAC ATGGTTCTACA	CCTACGGGA GGCAGCAG	V3_2	TACGGTAGCA GAGACTTGGTC T	GTATTACCGCG GCTGCTGG	130	20,20	22853944, 21460107	F341	R518
250	V4	V4	ACACTGACGAC ATGGTTCTACA	GTGCCAGC MGCCGCGG TAA	V4	TACGGTAGCA GAGACTTGGTC T	GGACTACHVG GGTWTCTAAT	50	100, 100	22179717, 20534432	F515	R806
100	V5	V5_2	ACACTGACGAC ATGGTTCTACA	AGGATTAGA TACCCT	V5_2	TACGGTAGCA GAGACTTGGTC T	CRTACTHCHCA GGYG	all	0	22853944	784F	880R
167	V6	V6_1	ACACTGACGAC ATGGTTCTACA	AAACTCAAA KGAATTGAC GG	V6_1	TACGGTAGCA GAGACTTGGTC T	ACGAGCTGAC GACARCCATG	133	17,17	18047683		
300	V7-V8	V7-V8	ACACTGACGAC ATGGTTCTACA	GYAACGAGC GCAACCC	V7-V8	TACGGTAGCA GAGACTTGGTC T	GACGGGCGGT GWGTRC	0	all	20880993	1099	1407
116	V9	V9	ACACTGACGAC ATGGTTCTACA	GTACACACC GCCCGT	V9	TACGGTAGCA GAGACTTGGTC T	TACCTTGTTAC GACTT	all	0	18047683		

Table S2. **Sequences of primer pairs used to generate 16S amplicons (V1 – V9)**

Variable Region	Mean read number	St. error read number	Total read number	Max read number	Number of Samples
V1	1224.64	218.33	106544	10627	87
V2	4622.7	374.3	855200	21637	185
V3	1088.16	192.12	178458	18400	164
V4	2160.91	277.61	345746	23972	160
V5	1492.38	273.55	141776	17737	95
V6	13733.23	988.06	2609314	58301	190
V9	1201.23	201.09	108111	11342	90

Table S3: **Read and Sample Summary of V1-V9 datasets with detectable OTUs**

Supplementary Figure Legends

Fig. S1: **Detected microbial taxa within control samples.** Total number of OTUs (y-axis) detected within each phyla (x-axis) by variable region (row) for a given control (column). The dots within the bars represent unique samples within respective control group (E. coli, n = 8; Blank, n = 8; Water, n = 5). The height of each bar represents total OTUs detected in each control. Each colored bar represents relative abundance of a given phylum. The horizontal lines within each phylum represent a unique OTU.

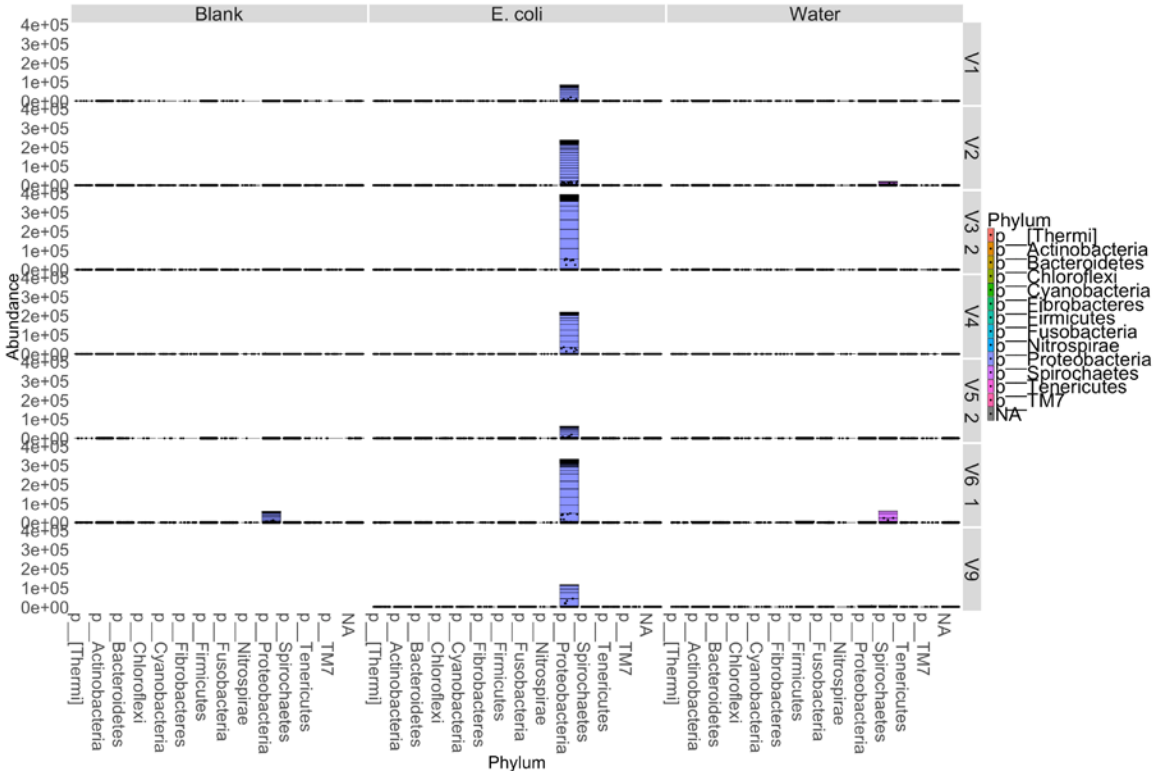
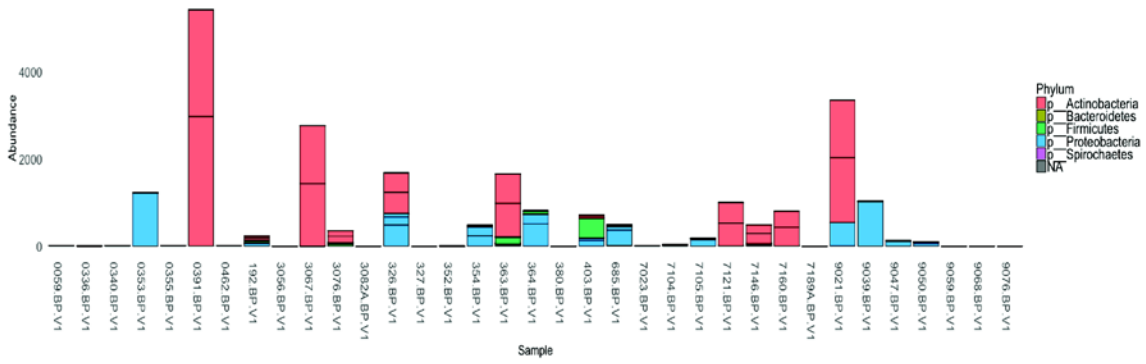


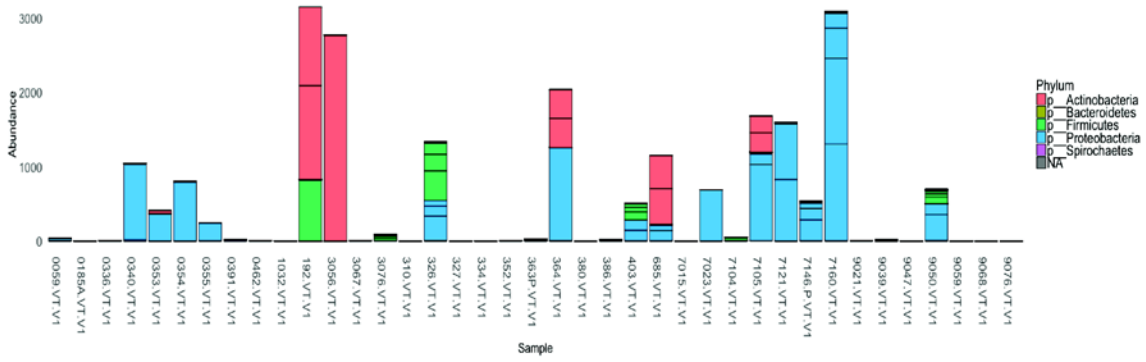
Fig. S2: **Relative abundance of each microbial phyla detected in samples per placental location:** (A) BP (B) PV and (C) FM using V1**,** V2**,** V3_2**,** V4**,** V5_2**,** V6_1**,** and V9 amplicons. Each bar represents total OTUs detected in the sample. Each color represents a different phylum and the horizontal lines within each phylum represent a unique OTU. The labels correspond to the raw data presented as Fasta files in NCBI GenBank.

V1

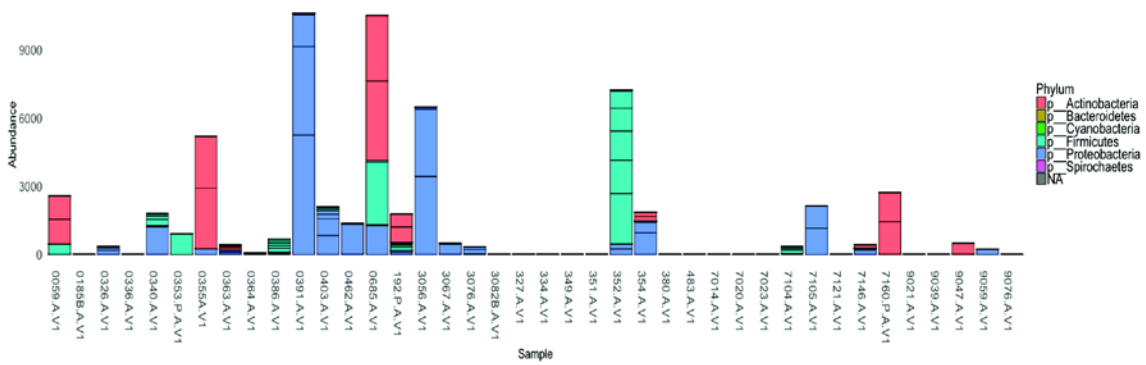
(A) Basal Plate



(B) Placental Villus

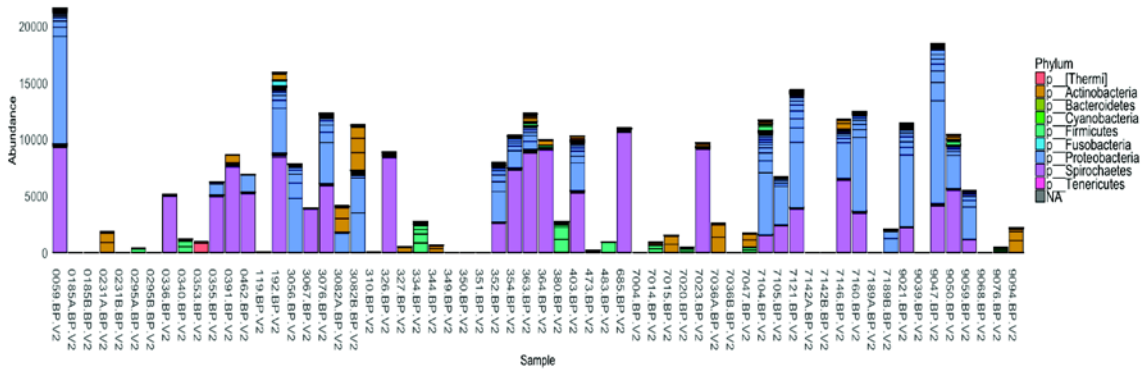


(C) Fetal Membrane

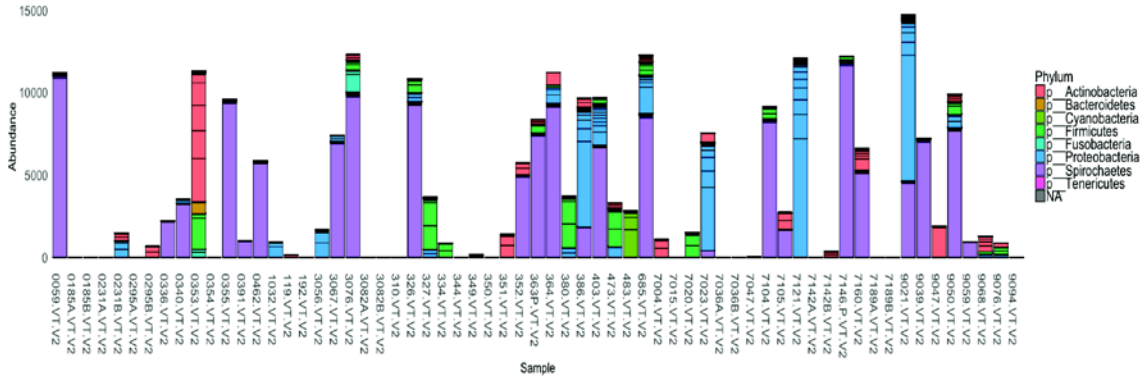


V2

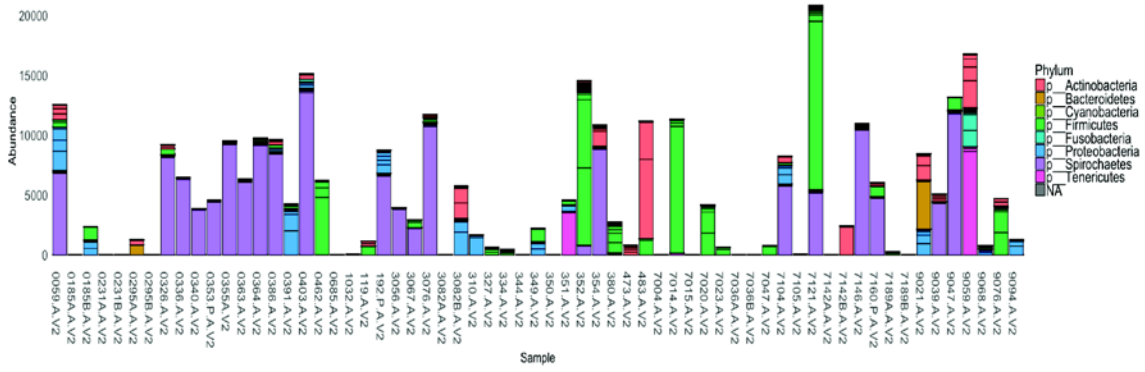
(A) Basal Plate



(B) Placental Villus

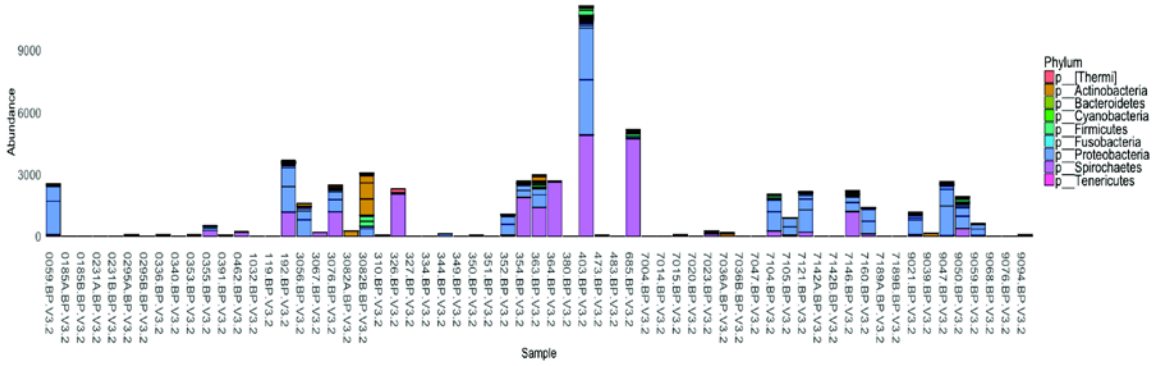


(C) Fetal Membrane

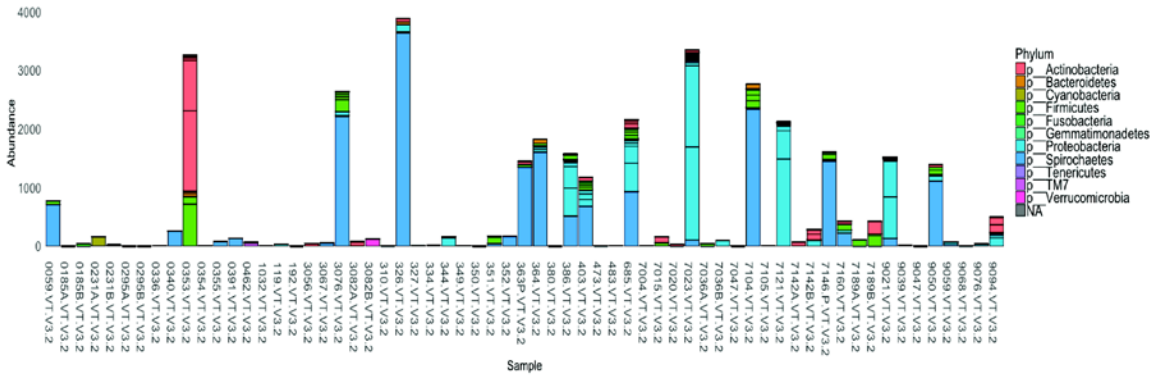


V3_2

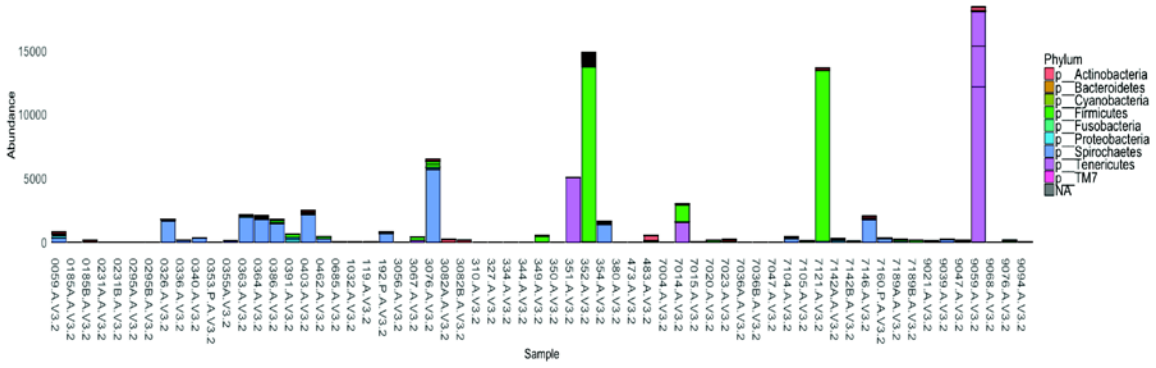
(A) Basal Plate



(B) Placental Villus

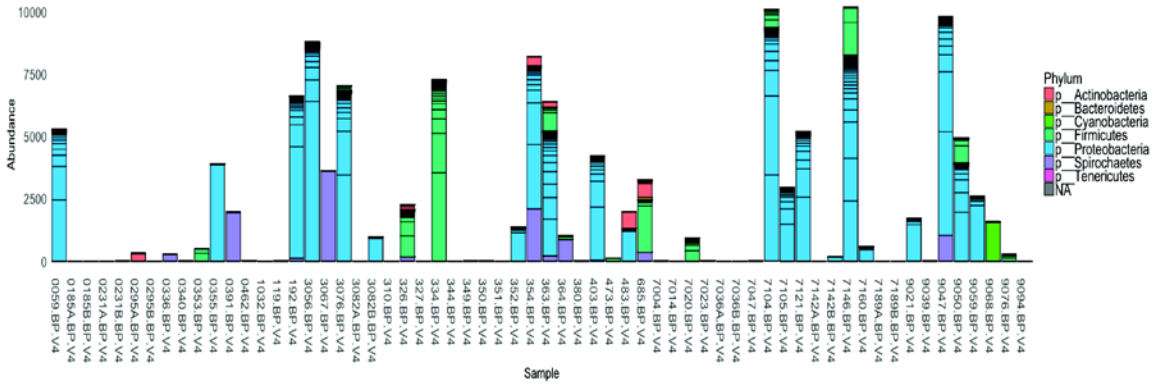


(C) Fetal Membrane

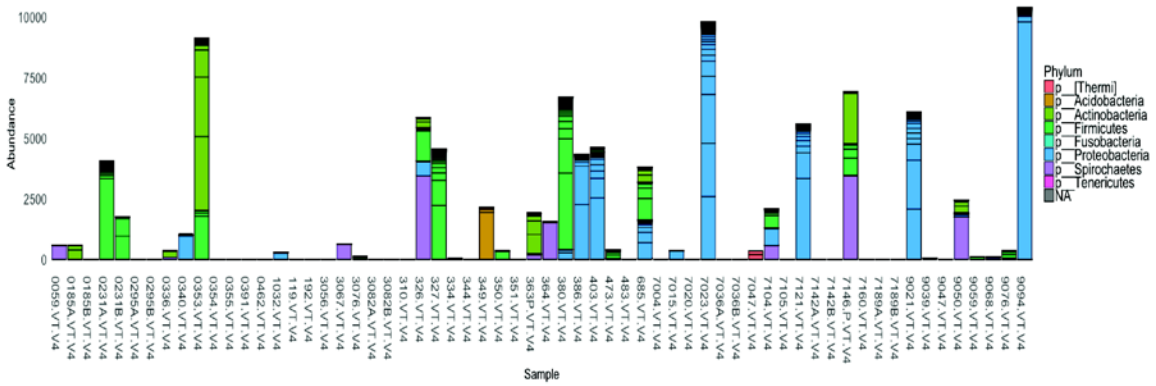


V4

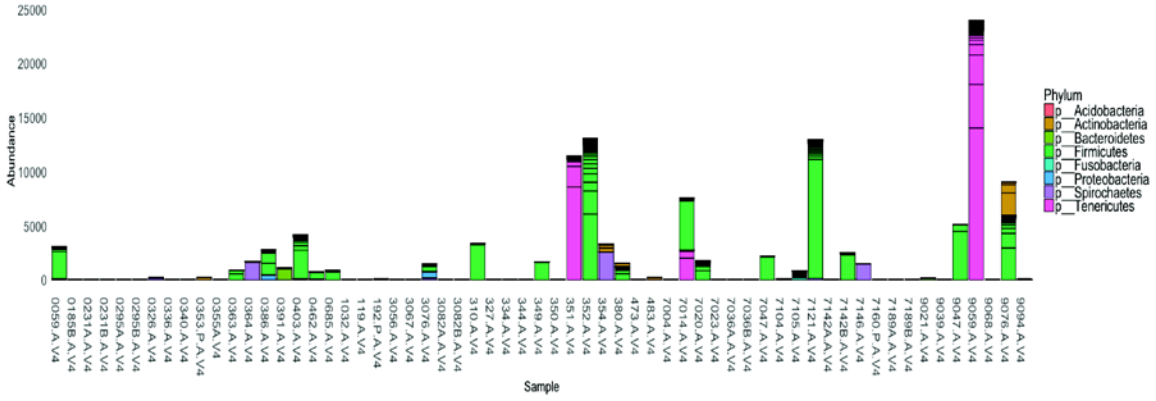
(A) Basal Plate



(B) Placental Villus

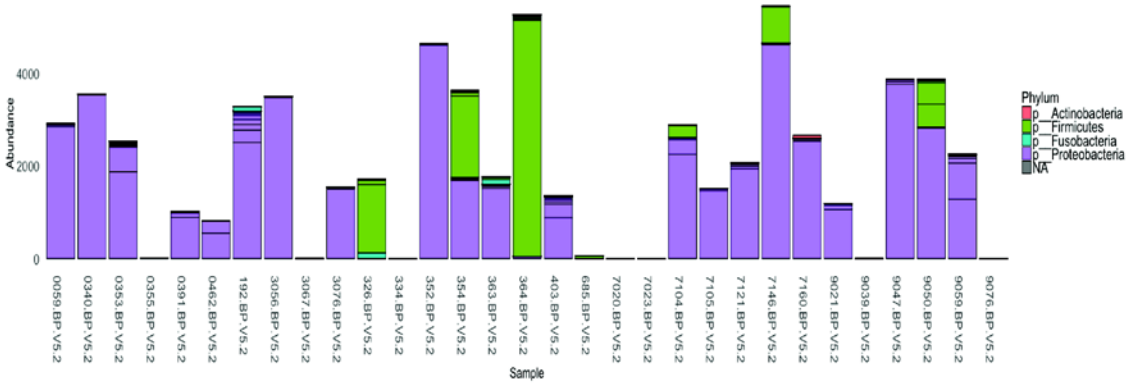


(C) Fetal Membrane

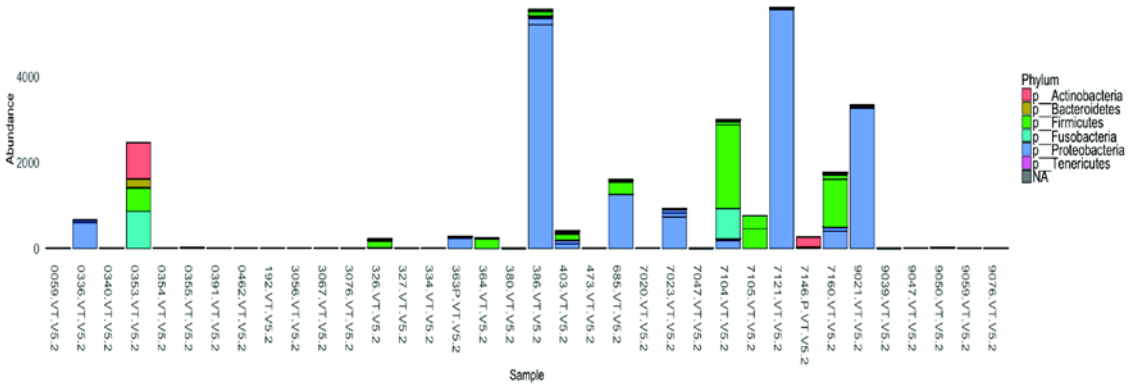


V5_2

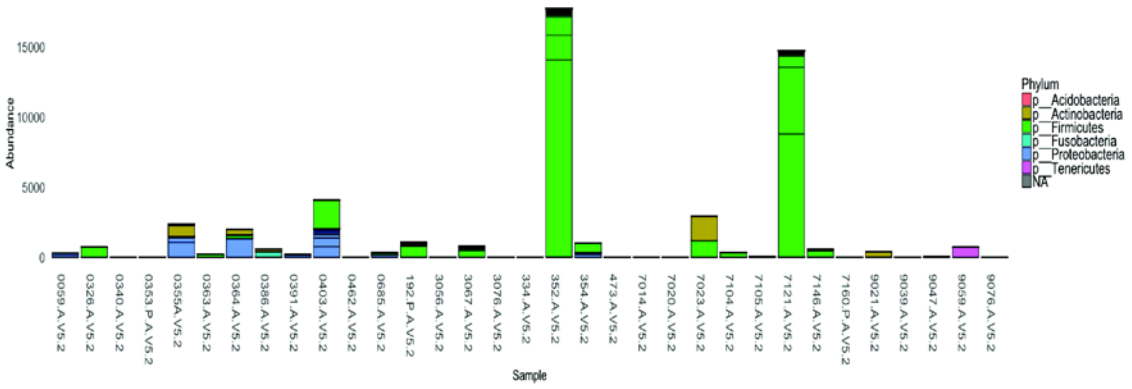
(A) Basal Plate



(B) Placental Villus

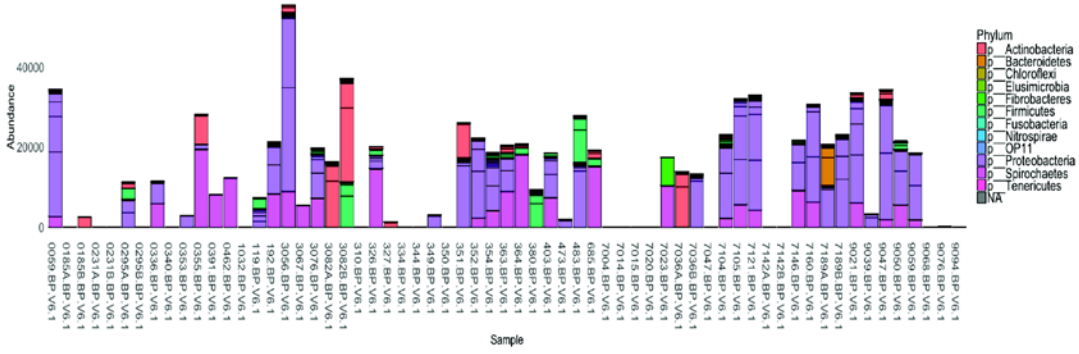


(C) Fetal Membrane

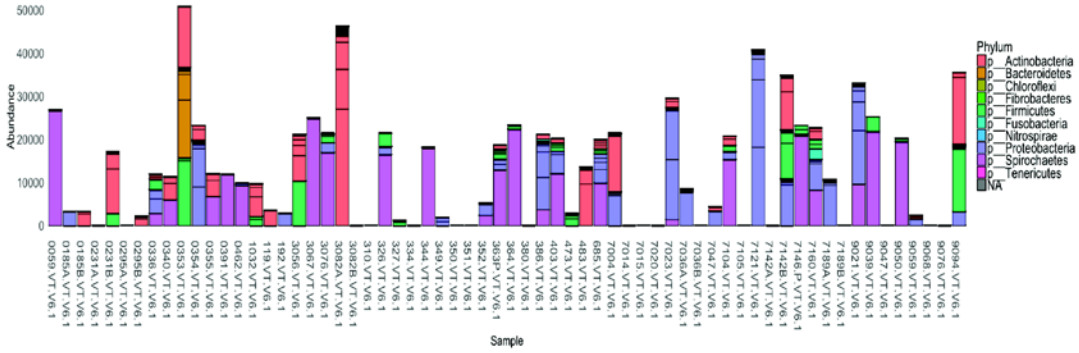


V6_1

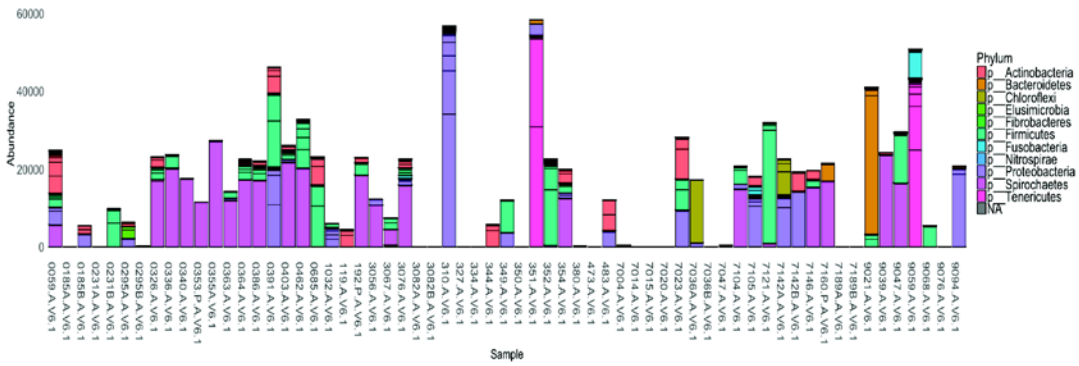
(A) Basal Plate



(B) Placental Villus

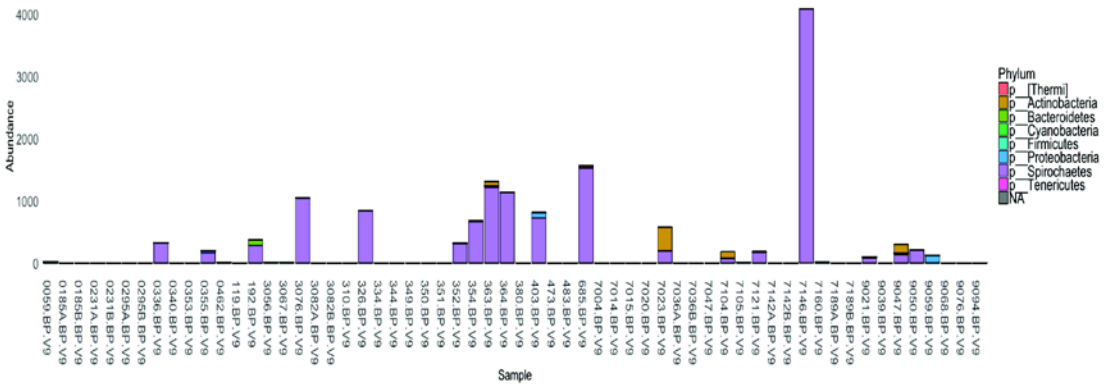


(C) Fetal Membrane

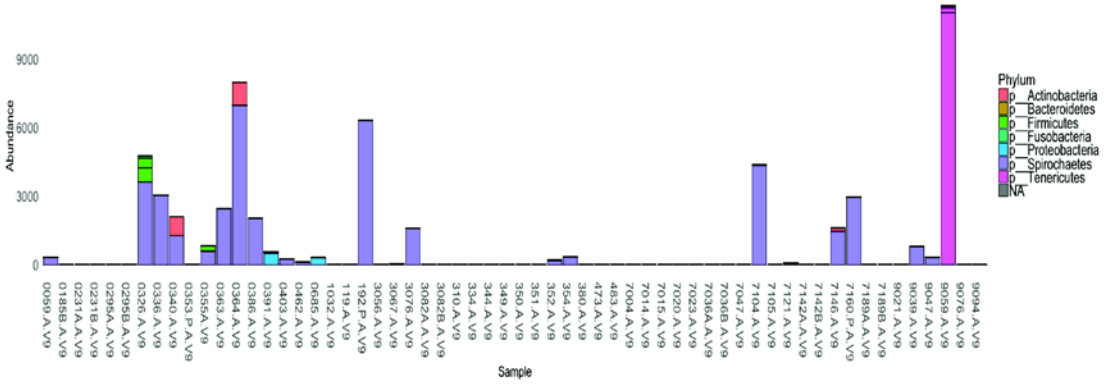


V9

(A) Basal Plate



(B) Placental Villus



(C) Fetal Membrane

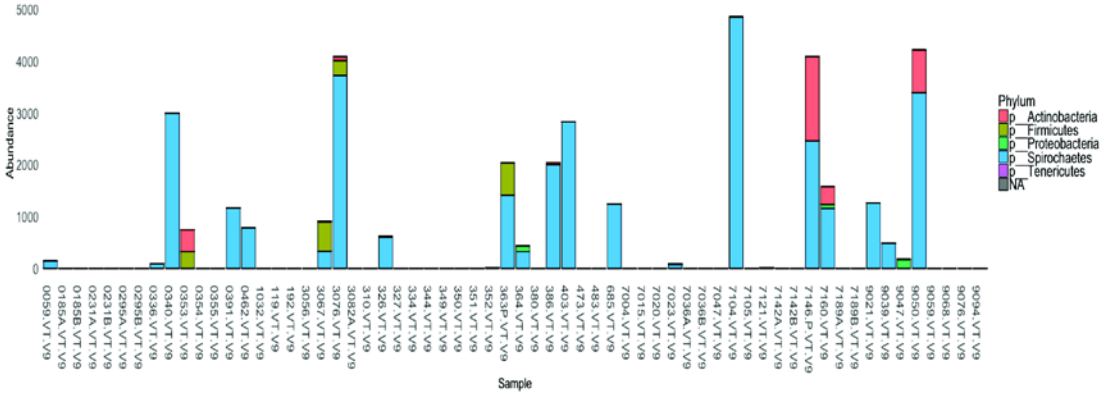


Fig. S3. **Multiple Rarefaction Curves of V4 samples.** The expected Shannon diversity (*H*), which assesses the even-ness and abundance of OTUs, per sample (each color) is shown at multiple sequencing depths (at a step-size of 10, starting from 10 to 300 reads).

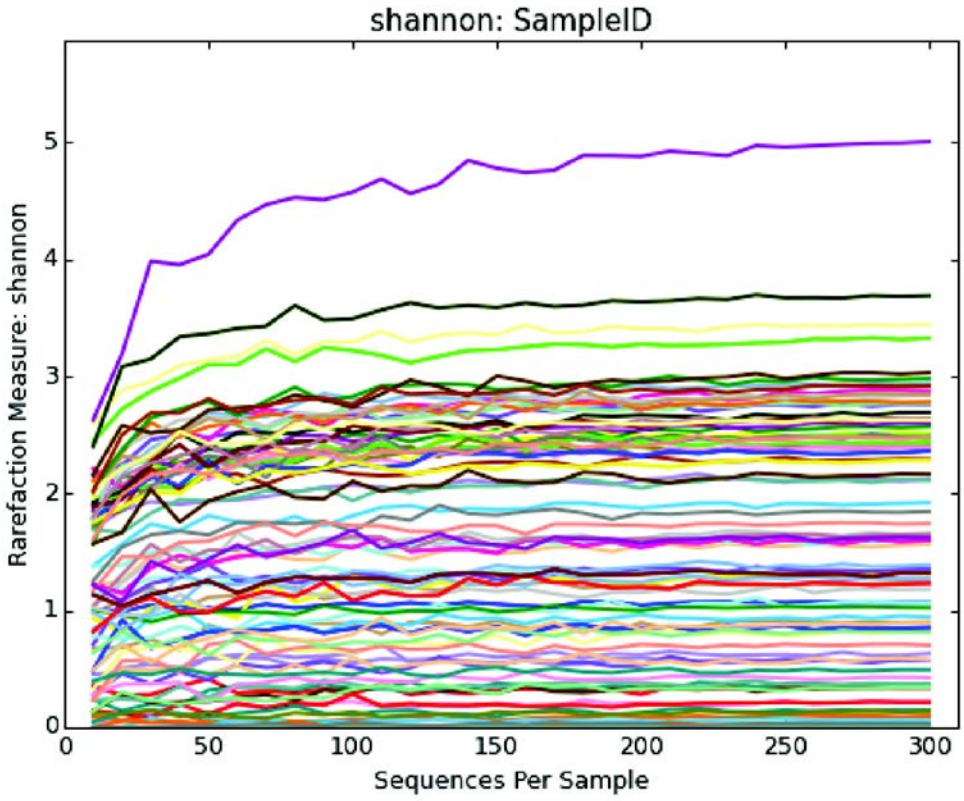


Fig. S4**: qPCR analysis of placental sample and controls for a given run:** Using the logarithmic standard curve generated from one round of qPCR, copy number/µl of placental samples meeting stringent filtering criteria (rarefied to 300 OTUs, no singletons, >34 copies/µL copy number) are highly significantly different from negative controls (p = 0.005 by Mann-Whitney test). p=* <0.05; ** <0.005

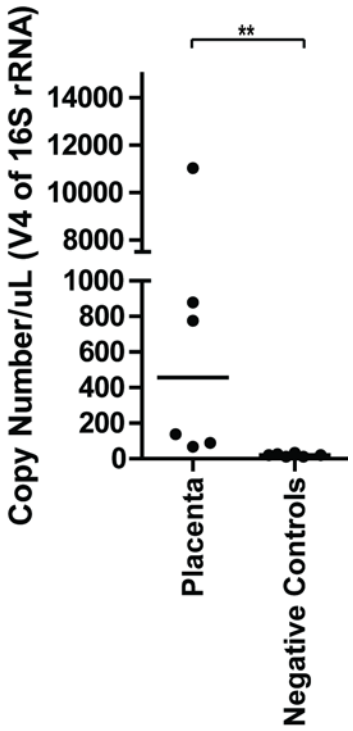


Fig. S5: **Multidimensional scaling (MDS) plot showing relationships between microbial profiles at placental locations using random_tree in the *R* Phyloseq package.** Samples within the BP, PV, and FM were analyzed. Distance measurements are based on Bray-Curtis, unweighted UniFrac, and weighted UniFrac distance metrics (PERMANOVA, Bray: P = 0.001, weighted UniFrac: P = 0.001, weighted UniFrac: P = 0.001).

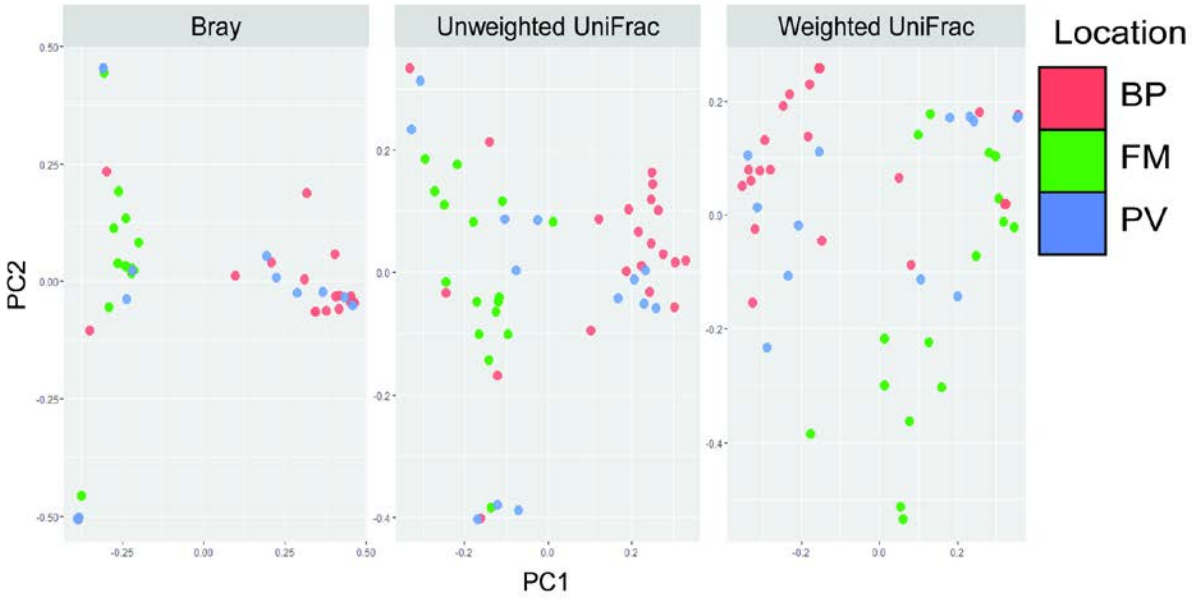


Fig. S6: **qPCR validation of *Ralstonia Insidiosa presence* in placentas using *R.i*-specific primers**

qPCR using *R.i*-specific primers confirmed *R.i* amplification in Basal Plate (BP) samples positive for *R.i* based on species-specific analysis. Each bar represents mean ± SEM Cq value of each sample run in triplicate. Negative controls [Blanks (N=3), molecular biology grade water (N=1)], and samples that did not detect this particular taxa during sequencing; positive control [*E. coli*)], did not amplify *R.i.*