Partial restoration of the microbiota of cesarean-born infants via vaginal microbial transfer

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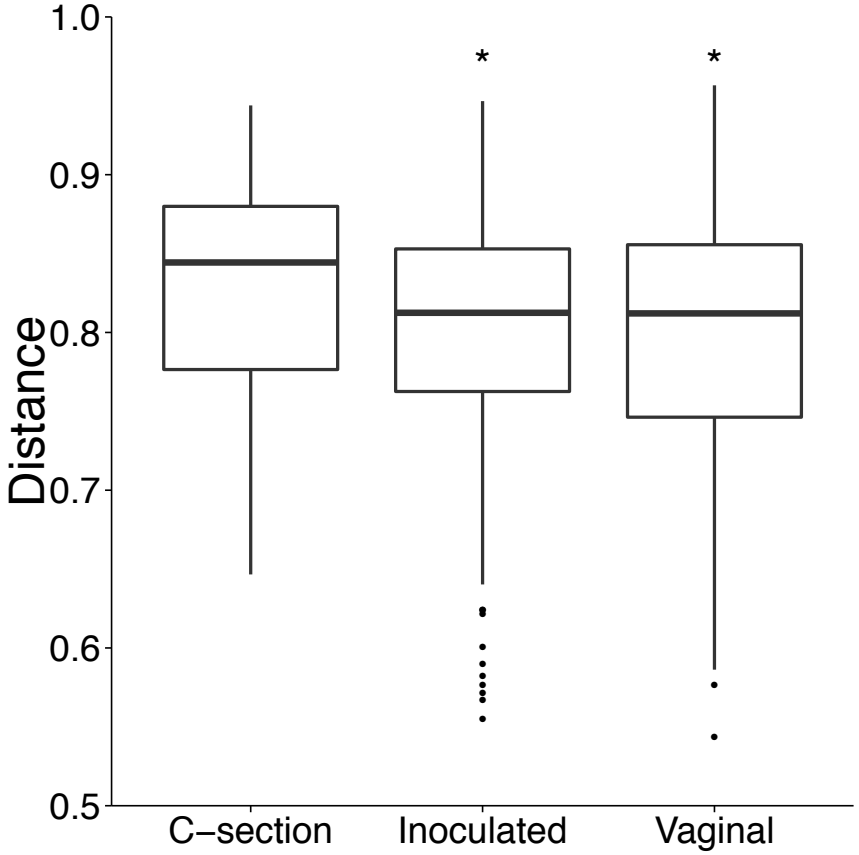
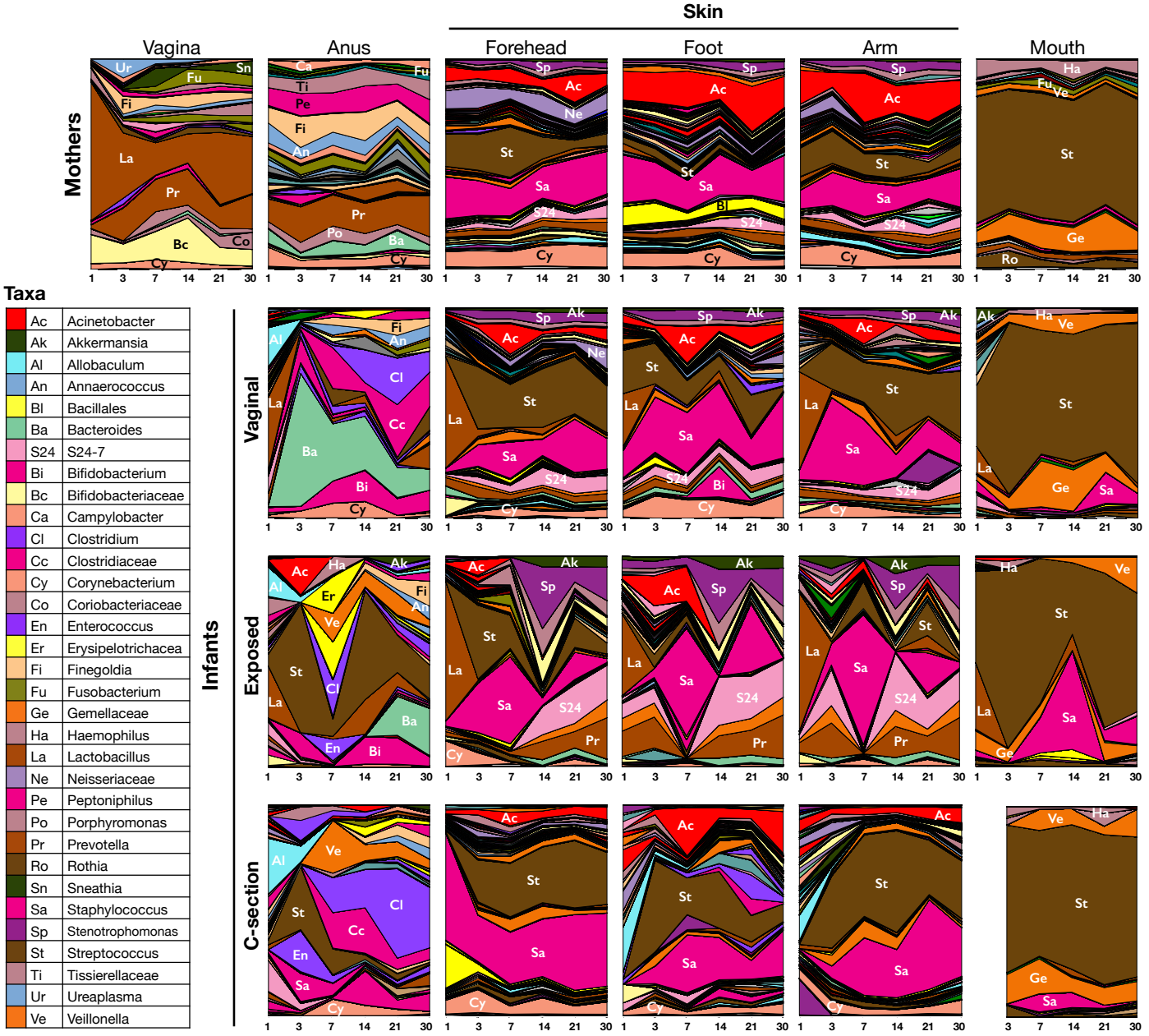
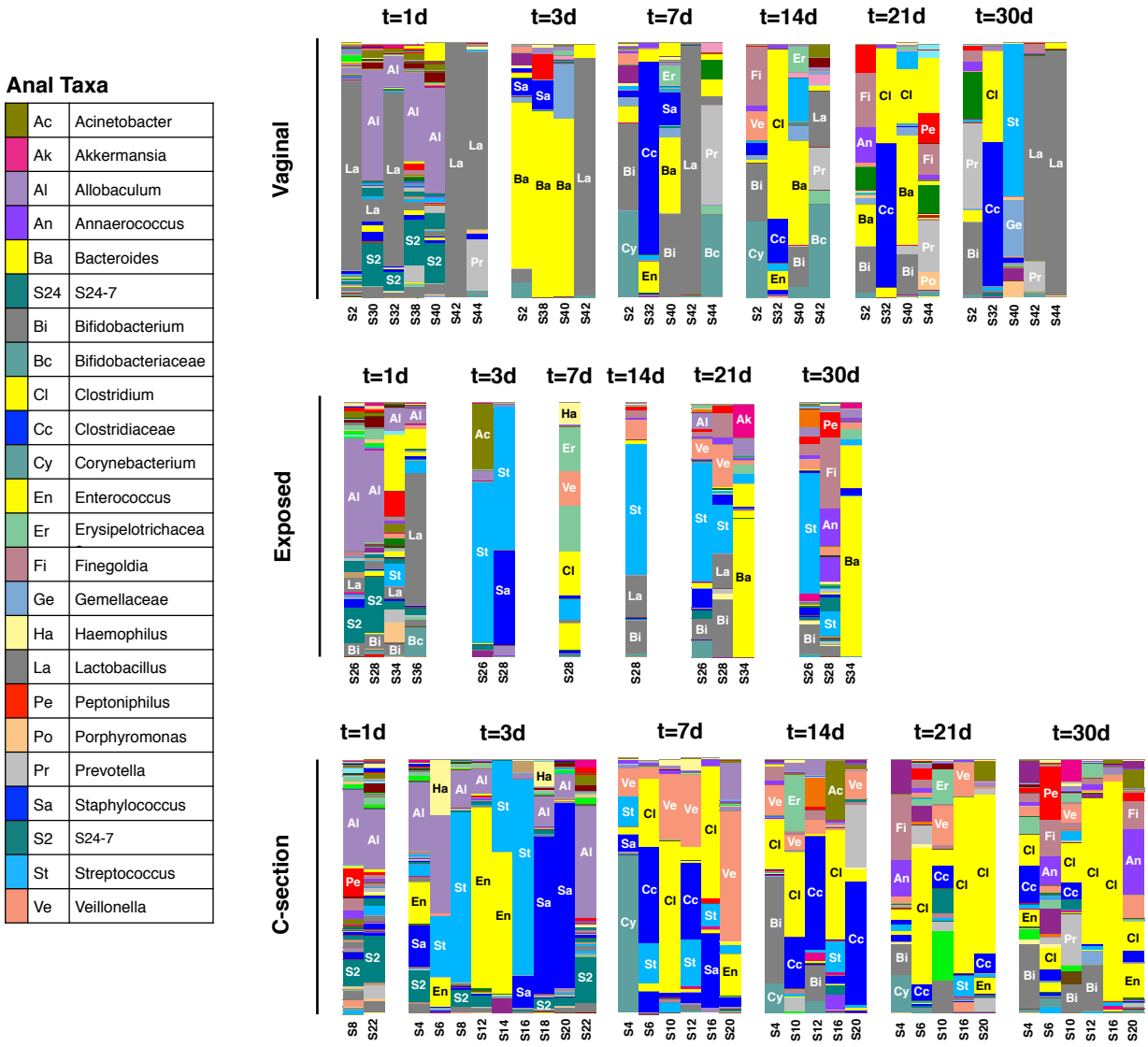
Supplementary Figure 1. Vaginally delivered and C-section delivered infants exposed to vaginal fluids are more similar to the maternal vaginal microbiome than unexposed C-section newborns. Unweighted UniFrac distances from samples at day 1 of C-section delivered (n = 7), C-section delivered and exposed to vaginal fluids (n = 18), and vaginally delivered (n = 34) infants to the maternal vaginal microbiome (ANOVA and Tukey's HSD test, $P < 0.01$ for vaginal and exposed).



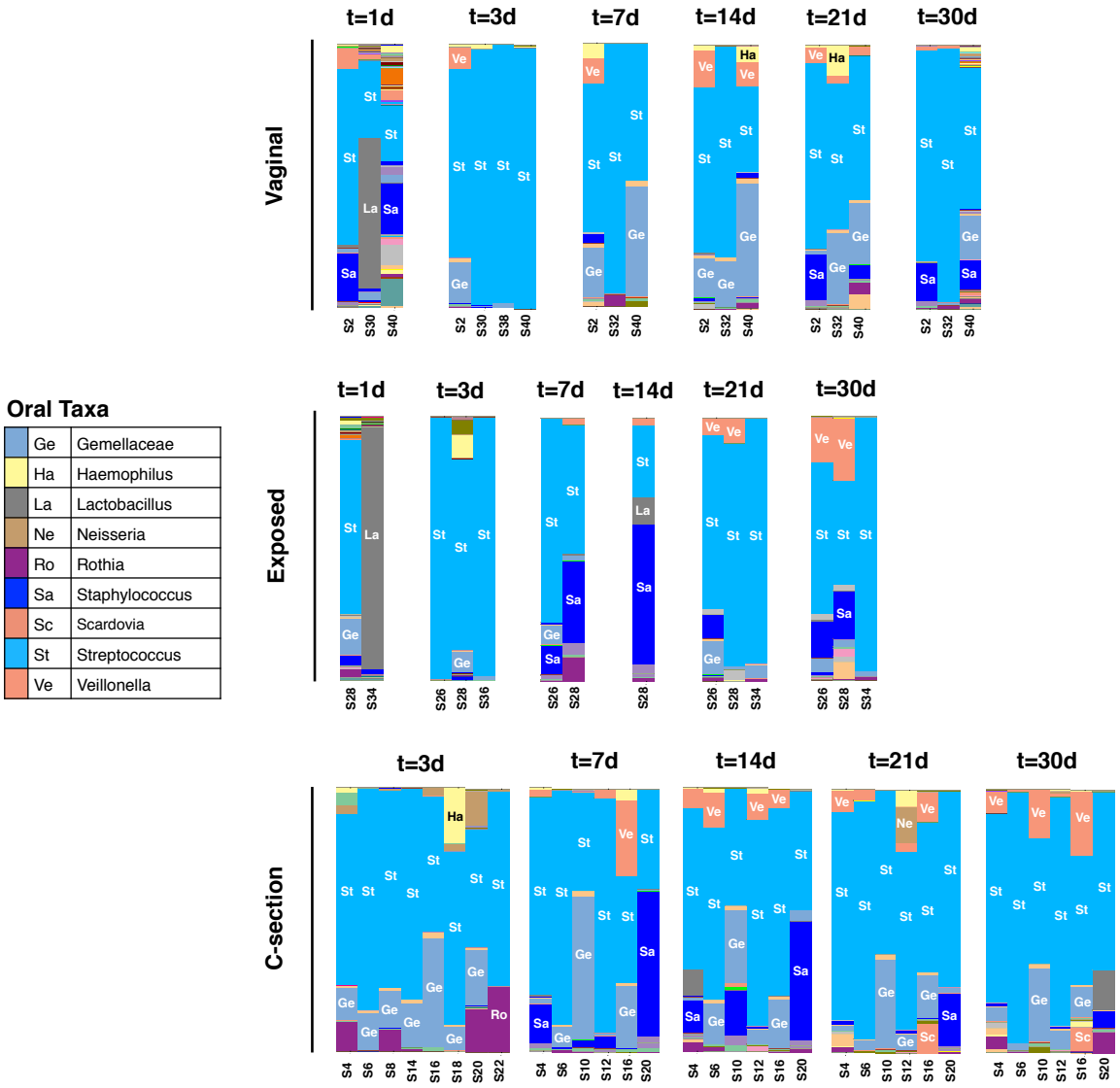
Supplementary Figure 2. Relative abundance of bacterial taxa in mothers and babies during the first month after birth. The horizontal axis in each panel indicates day after birth, while the vertical represents mean relative abundance of different taxa. Top panel row: maternal samples from vagina, anus, forehead, foot, volar arm, and oral cavity (n = 19 mothers). Second to fourth panel rows: infant samples from anus, forehead, foot, volar arm, and oral cavity (n = 7 vaginally delivered infants; n = 4 delivered by C-section exposed to vaginal fluids; n = 8 delivered by C-section, not exposed to vaginal fluids). Major bacterial groups are indicated at the most precise resolution achieved and color-coded as indicated in the table on the left of the figure. Despite the low number of infants, vaginal markers (*Lactobacillus* in all sites, *Bacteroides* and *Bifidobacterium* in anal site, S24–7 in the skin) were absent from infants who were not exposed to vaginal fluids.



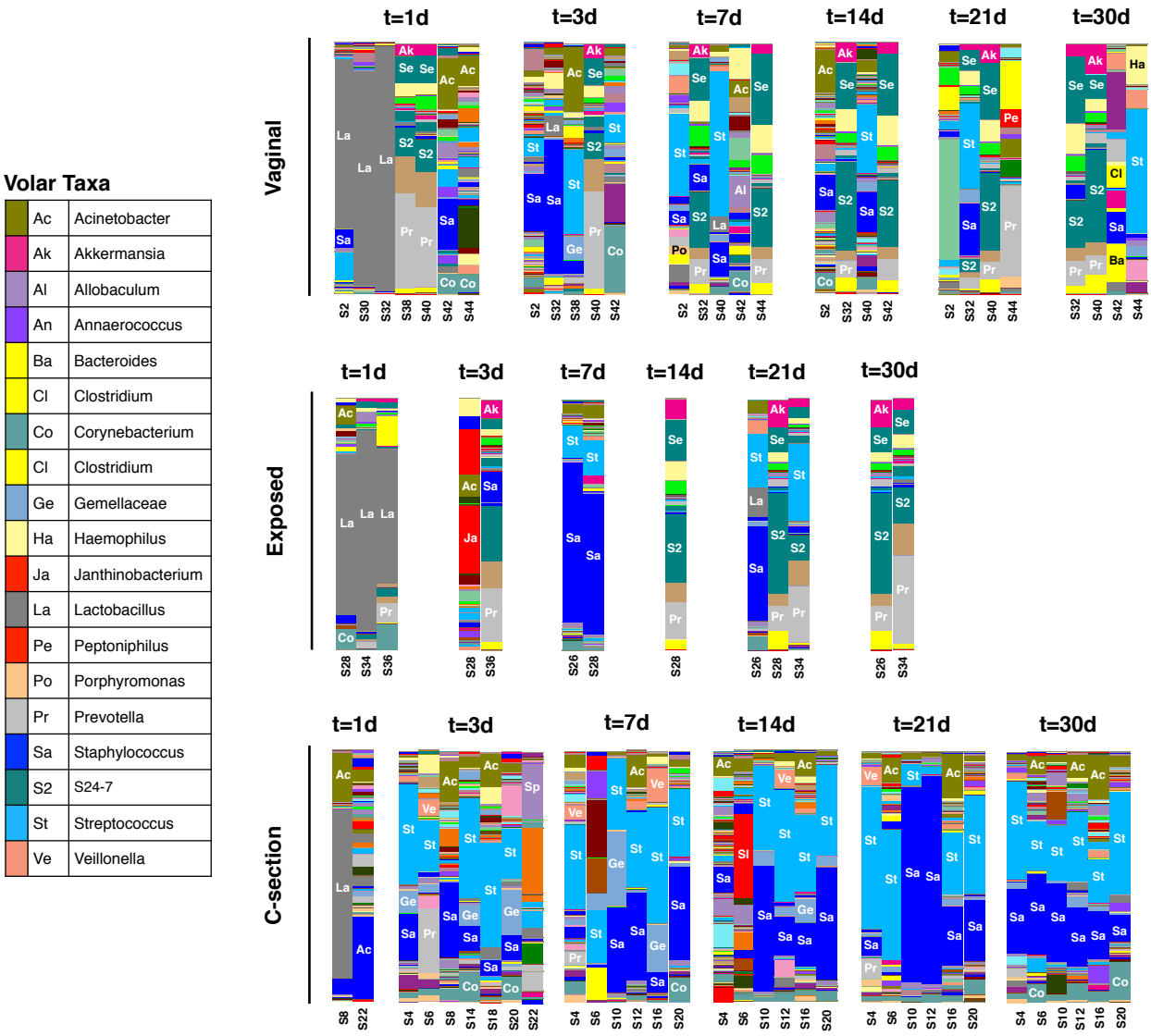
Supplementary Figure 3. Relative abundance of bacterial taxa in anal samples split per infant and time point. Each subplot represents the abundance of different anal taxa for a given time point, indicated in the subplot title, with the horizontal axis indicating the subject number. Top row: vaginally delivered infants. Middle row: C-section infants exposed to vaginal fluids. Bottom row: C-section infants unexposed to vaginal fluids.



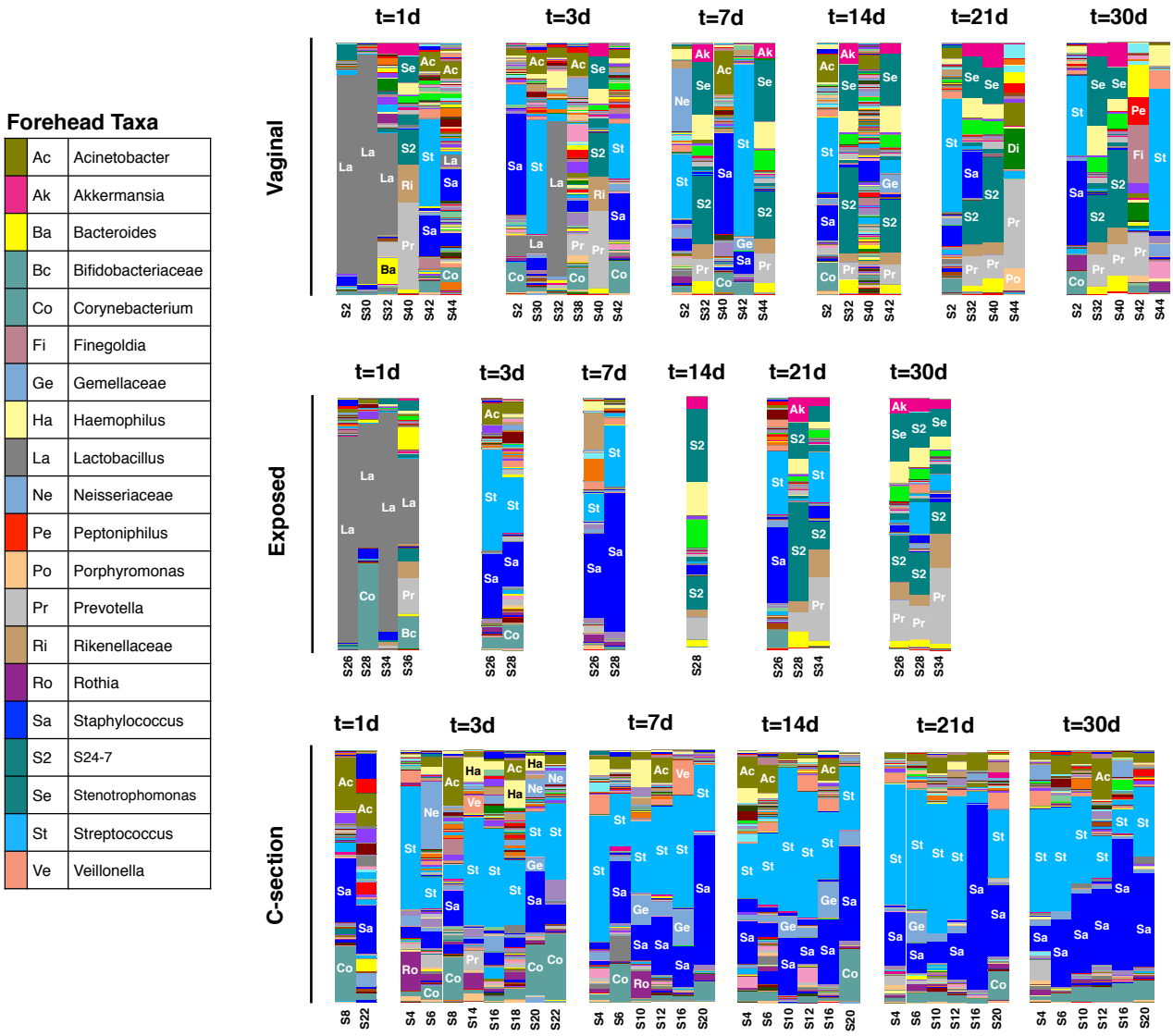
Supplementary Figure 4. Relative abundance of bacterial taxa in oral samples split per infant and time point. Each subplot represents the abundance of different oral taxa for a given time point, indicated in the subplot title, with the horizontal axis indicating the subject number. Top row: vaginally delivered infants. Middle row: C-section infants exposed to vaginal fluids. Bottom row: C-section infants unexposed to vaginal fluids.



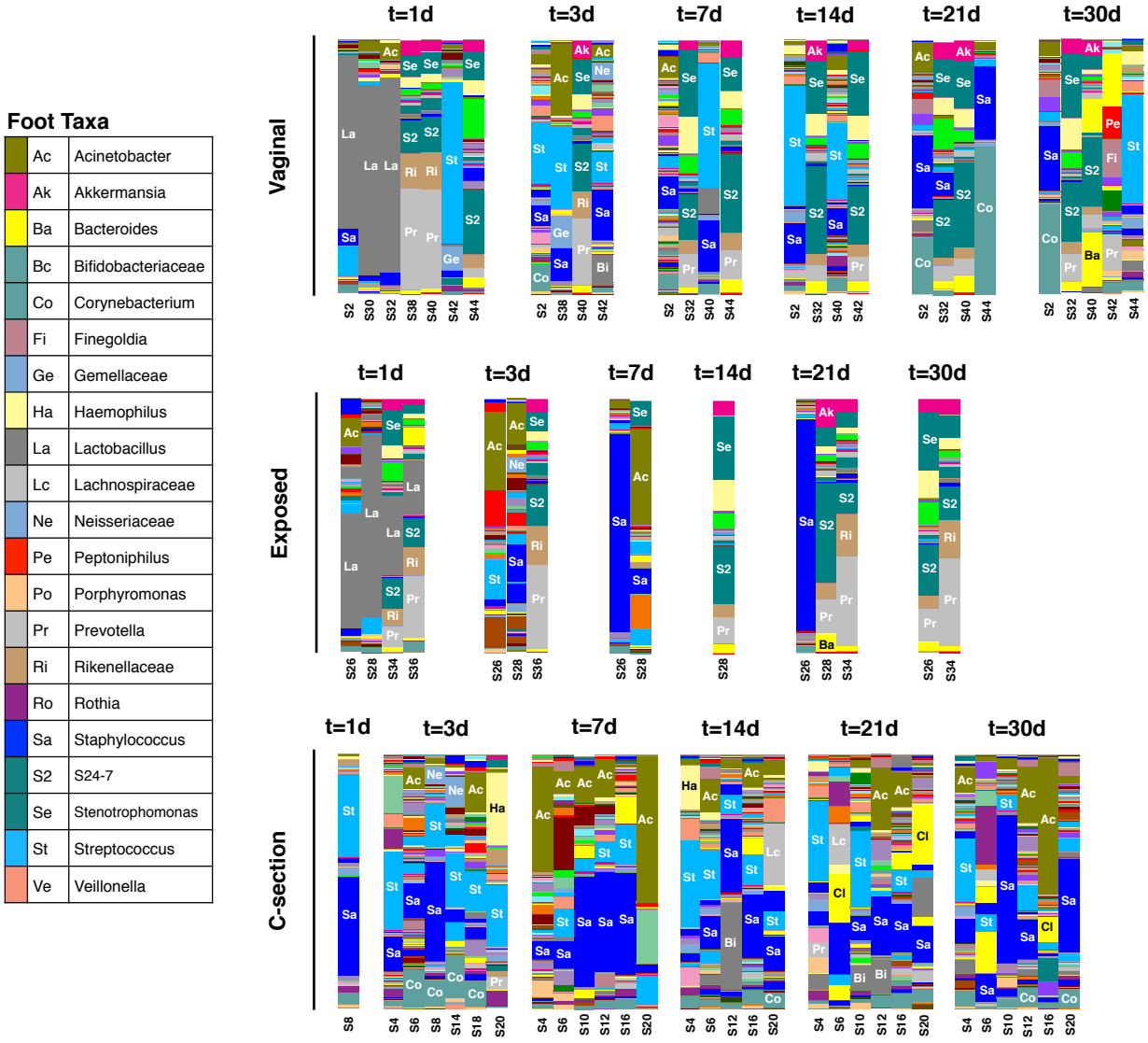
Supplementary Figure 5. Relative abundance of bacterial taxa in volar samples split per infant and time point. Each subplot represents the abundance of different volar taxa for a given time point, indicated in the subplot title, with the horizontal axis indicating the subject number. Top row: vaginally delivered infants. Middle row: C-section infants exposed to vaginal fluids. Bottom row: C-section infants unexposed to vaginal fluids.



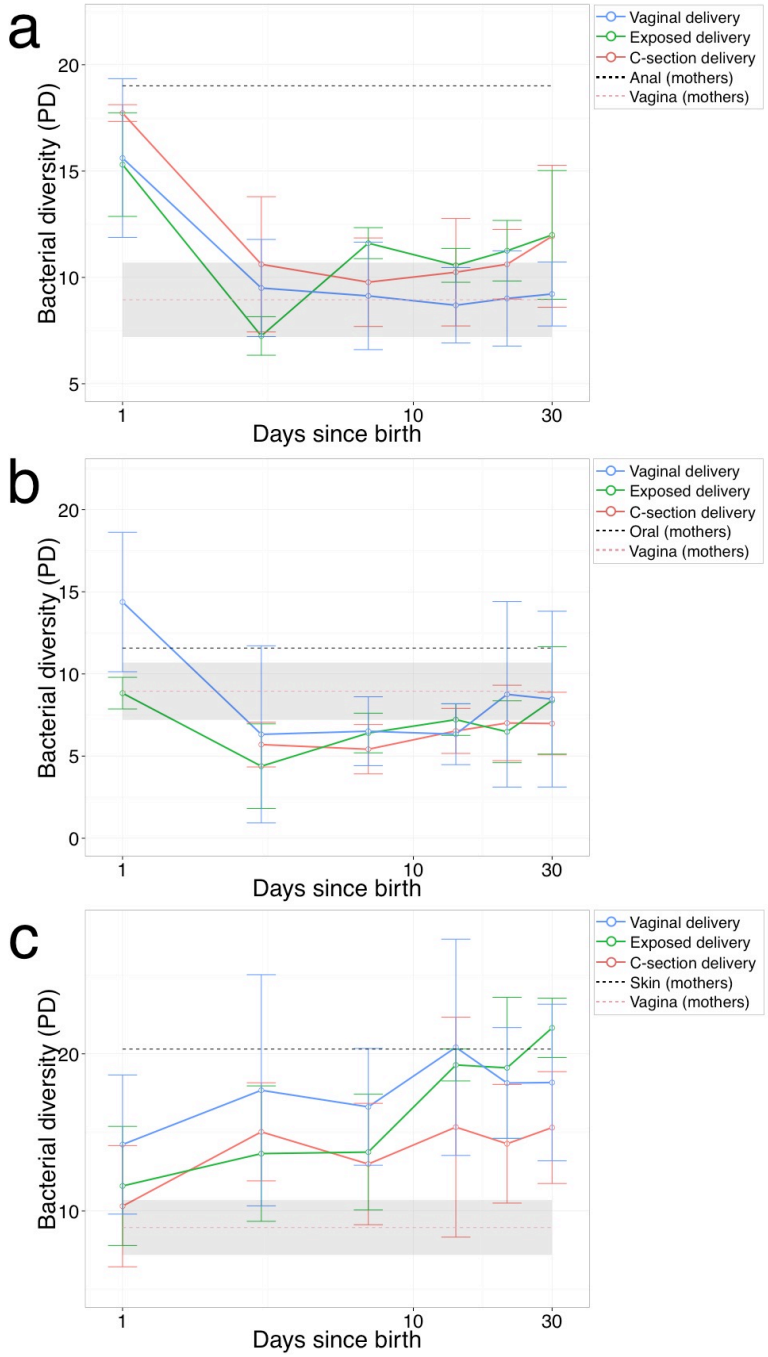
Supplementary Figure 6. Relative abundance of bacterial taxa in forehead samples split per infant and time point. Each subplot represents the abundance of different forehead taxa for a given time point, indicated in the subplot title, with the horizontal axis indicating the subject number. Top row: vaginally delivered infants. Middle row: C-section infants exposed to vaginal fluids. Bottom row: C-section infants unexposed to vaginal fluids.



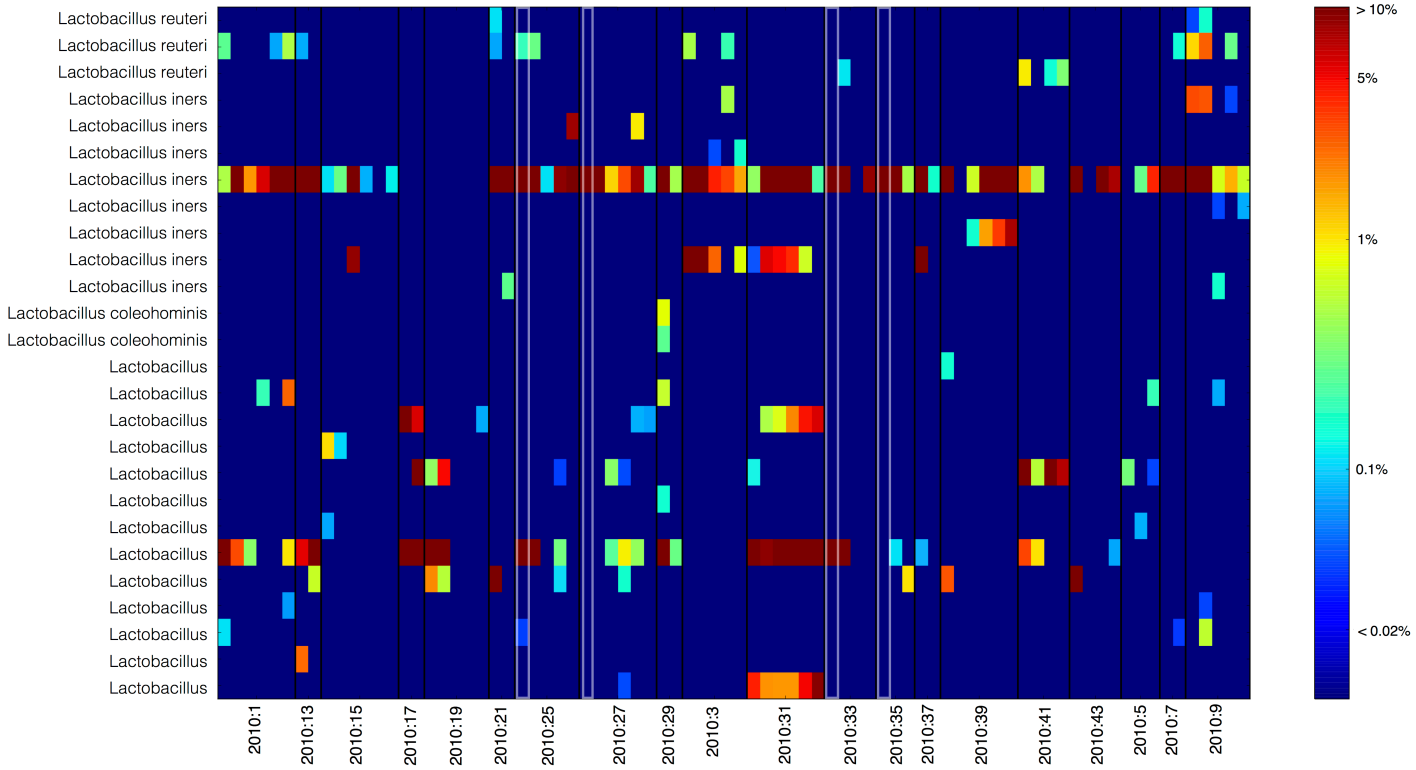
Supplementary Figure 7. Relative abundance of bacterial taxa in foot samples split per infant and time point. Each subplot represents the abundance of different foot taxa for a given time point, indicated in the subplot title, with the horizontal axis indicating the subject number. Top row: vaginally delivered infants. Middle row: C-section infants exposed to vaginal fluids. Bottom row: C-section infants unexposed to vaginal fluids.



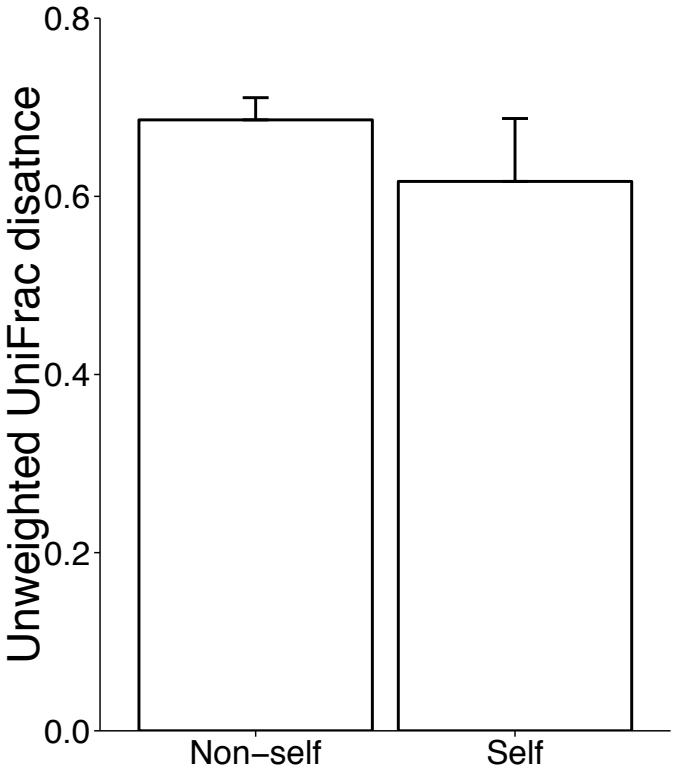
Supplementary Figure 8. Higher microbial diversity at birth in anal and oral samples. (**a**) Alpha diversity of infant anal samples (*n* = 146 over 6 time points), in relation to maternal sites, during the first 30 days of life. (**b**) Alpha diversity of infant oral samples (*n* = 193), in relation to maternal sites, during the first 30 days of life. (**c**) Alpha diversity of skin (forehead, arm and foot) samples (*n* = 443), in relation to maternal sites, during the first 30 days of life. At birth, the bacterial diversity in baby's anus and mouth (but not in the skin) is higher than the maternal vagina diversity (gray area), but during the first week, it decreases to values below or similar to vaginal diversity, remaining low at least throughout the first month of life. Bars indicate standard deviation from the mean.



Supplementary Figure 9. Abundance of Lactobacilli in gauze and maternal vaginal samples. Deblurred sequences (see Methods Online) were assigned taxonomy using the RDP classifier v2.2 through the assign_taxonomy.py script in QIIME. All sequences classified as Lactobacillus are shown in the figure. Each row corresponds to a unique sequence and each column is a single sample, grouped by participant ID. Gauze samples are indicated with a white rectangle around them.



Supplementary Figure 10. Gauzes partially recapture the microbiome of their paired maternal vaginal sample. Unweighted UniFrac distances of gauze samples (n = 4) to vaginal samples (n = 18), either that of the mother in which the gauze was incubated (self) or that of other mothers (non-self). Bars indicate standard deviation from the mean.



Supplementary Table 1. Characteristics of participating infants and mothers. Delivery mode (C-section, C-section exposed to vaginal fluids, or vaginal delivery), gender, days after birth at which samples were collected, feeding mode (breastfed supplemented with formula or exclusive breastfeeding), antibiotics usage (perinatal and postnala), maternal group B strep infection status, and episiotomies.

Family #	Delivery mode	Infant gender	Sampling times (days)	Lactation	Perinatal anbitiotics	Postnatal antibiotics	Maternal GBS status	Episiotomy
4	C-section	Male	1,2	Breastfed + formula	Cephalosporin	None	Negative	No
5	C-section	Male	1,2,8,14,21,29	Breastfed + formula	Cephalosporin	None	Negative	No
7	C-section	Female	1,2	Breastfed + formula	Cephalosporin	None	Negative	No
8	C-section	Male	1,2,9,15,21,29	Breastfed + formula	Cephalosporin	None	Negative	No
9	C-section	Female	1,2	Breastfed + formula	Cephalosporin	None	Negative	No
10	C-section	Male	1,2,8,17,25,40	Breastfed + formula	Cephalosporin	None	Positive	No
11	C-section	Male	1,2	Breastfed + formula	Cephalosporin	None	Positive	No
13	C-section + vaginal	Male	1,2,10,24,31	Breastfed + formula	Cephalosporin	None	Negative	No
14	C-section + vaginal	Female	1,2,7,13,22,36	Breastfed + formula	Cephalosporin	None	Negative	No
17	C-section + vaginal	Male	1,26,29	Breastfed + formula	Cephalosporin	None	Negative	No
18	C-section + vaginal	Male	1,2	Breastfed + formula	Cephalosporin	None	Negative	No
1	Vaginal	Male	1,2,7,15,21,28	Breastfed	None	None	Negative	Yes
15	Vaginal	Male	1,2	Breastfed + formula	Penicilin	None	Positivie	Yes
16	Vaginal	Male	1,2,9,14,22,28	Breastfed + formula	None	None	Negative	Yes
19	Vaginal	Male	1,2	Breastfed + formula	None	None	Negative	Yes
20	Vaginal	Female	1,3,614,22,30	Breastfed	None	None	Negative	Yes
21	Vaginal	Male	1,3,10,14,31	Breastfed	None	None	Negative	Yes
22	Vaginal	Female	1,5,20,28	Breastfed	None	None	Negative	Yes

Supplementary Table 2. Number of collected samples from mothers and infants during the first month of the infant's life. Samples from infants were collected at six time points: day 1, 3, 7, 14, 21, and 30.

		Cesarean		Cesarean +Exposure		Vaginal	
Body site	**N swabs**	**Mother**	**Infant**	**Mother**	**Infant**	**Mother**	**Infant**
Vaginal gauze	7	0	-	7	-	0	-
Vaginal swab	126	49	-	28	-	49	-
Aureole	126	49	-	28	-	49	-
Skin	756	147	147	84	84	147	147
Anal	252	49	49	28	28	49	49
Oral	252	49	49	28	28	49	49
Total	1519	343	245	203	140	343	245

Supplementary Table 3. The microbiome of C-section infants exposed to the vaginal gauze resembles that of vaginally delivered infants. Confusion matrix of anal (n = 40 samples), oral (i = 51), and skin (n = 119) samples during the first week of life by birth mode and exposure. The confusion matrix is based on a Random Forest classifier, and results are averaged (mean ± s.d.) over 10 rarefactions of the full OTU tables. The highest values for each classification are shown in bolded text.

	Predicted site		
True site	Vaginal	Inoculated	C-section
Anal samples			
Vaginal	**0.79 ± 0.08**	0.00 ± 0.00	0.21 ± 0.08
Inoculated	0.38 ± 0.06	0.00 ± 0.00	**0.62 ± 0.06**
C-section	0.21 ± 0.06	0.00 ± 0.00	**0.79 ± 0.06**
Oral samples			
Vaginal	**0.54 ± 0.05**	0.05 ± 0.03	0.41 ± 0.04
Inoculated	**0.64 ± 0.04**	0.00 ± 0.00	0.36 ± 0.04
C-section	0.24 ± 0.05	0.00 ± 0.00	**0.76 ± 0.05**
Skin samples			
Vaginal	**0.70 ± 0.02**	0.01 ± 0.02	0.29 ± 0.01
Inoculated	**0.50 ± 0.06**	0.05 ± 0.05	0.46 ± 0.02
C-section	0.06 ± 0.02	0.02 ± 0.00	**0.91 ± 0.02**

Supplementary Table 4. The predicted metagenome of inoculated infants is distinct to that of C-section infants and resembles that of vaginally delivered newborns. Confusion matrix of anal (*n* = 40 samples), oral (*n* = 51), and skin (*n* = 119) samples during the first week of life classified into vaginally delivered, C-section exposed to vaginal fluids, or C-section unexposed infants using a Random Forest classifier based on PICRUSt predicted metagenomes. Rows indicate true classes and columns predicted classes. Results are averaged (mean ± s.d.) over 10 rarefactions of the full OTU tables. The highest values for each classification are shown in bolded text.

Anal samples	Vaginal	Inoculated	C-section
Vaginal	**0.92 ± 0.00**	0.00 ± 0.00	0.07 ± 0.00
Inoculated	0.26 ± 0.11	0.00 ± 0.00	**0.73 ± 0.11**
C-section	0.13 ± 0.00	0.06 ± 0.00	**0.80 ± 0.00**
Oral samples			
Vaginal	**0.55 ± 0.00**	0.16 ± 0.00	0.27 ± 0.00
Inoculated	**0.62 ± 0.00**	0.00 ± 0.00	0.37 ± 0.00
C-section	0.17 ± 0.03	0.05 ± 0.00	**0.77 ± 0.03**
Skin samples			
Vaginal	**0.58 ± 0.05**	0.09 ± 0.04	0.32 ± 0.01
Inoculated	**0.51 ± 0.02**	0.10 ± 0.02	0.37 ± 0.02
C-section	0.15 ± 0.04	0.01 ± 0.01	**0.82 ± 0.05**