ADVANCED BIOLOGY

Supporting Information

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Multiomic Analyses of Nascent Preterm Infant Microbiomes Differentiation Suggest Opportunities for Targeted Intervention

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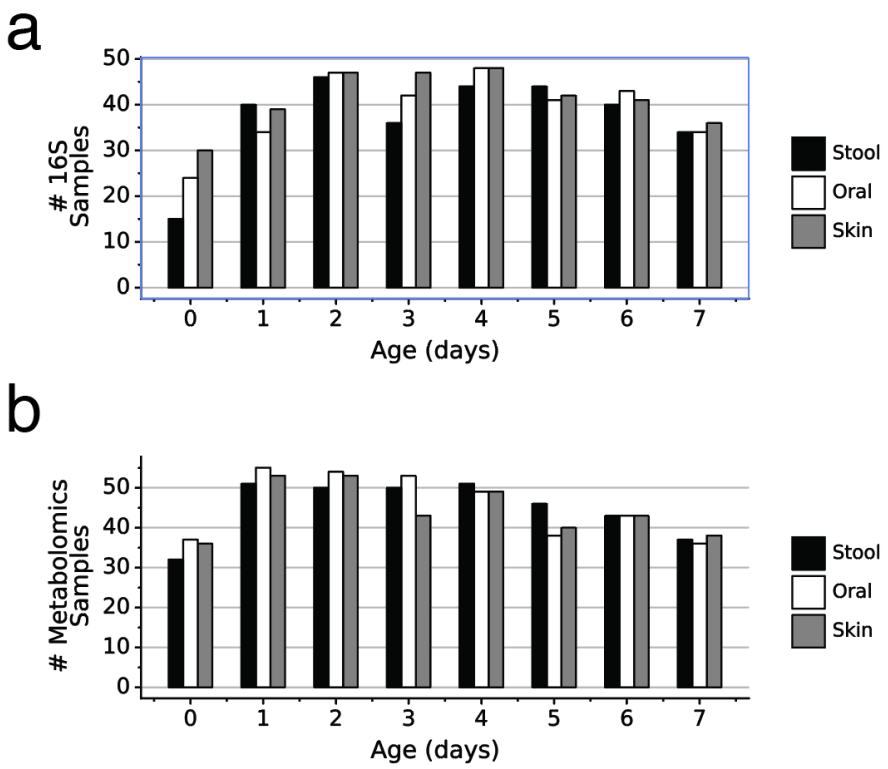
Extended Data

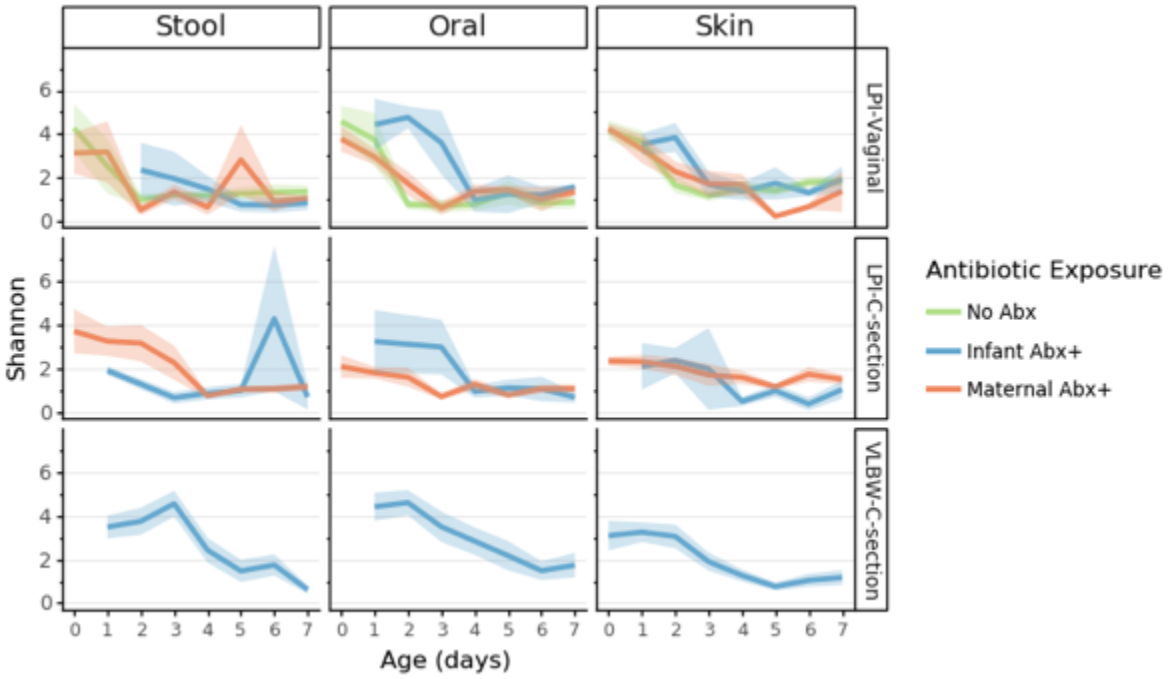
Extended Data Table 1. Stepwise RDA of metadata variables influencing microbial composition of all samples. Separate analyses run for samples for all body sites together and for each body site individually.

Extended Data Table 2. List of studies from which 16S data was used for Sourcetracker analysis.

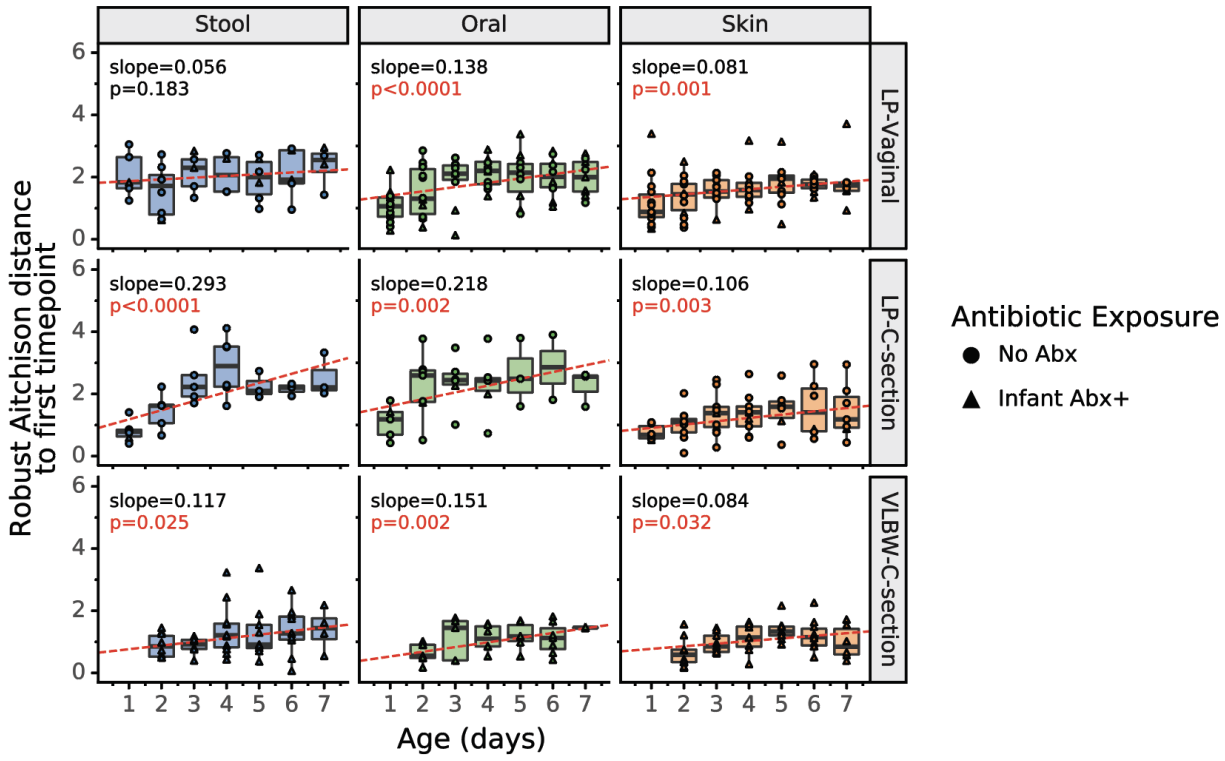
Extended Data Table 3. Sample counts for 16S and metabolomics data.



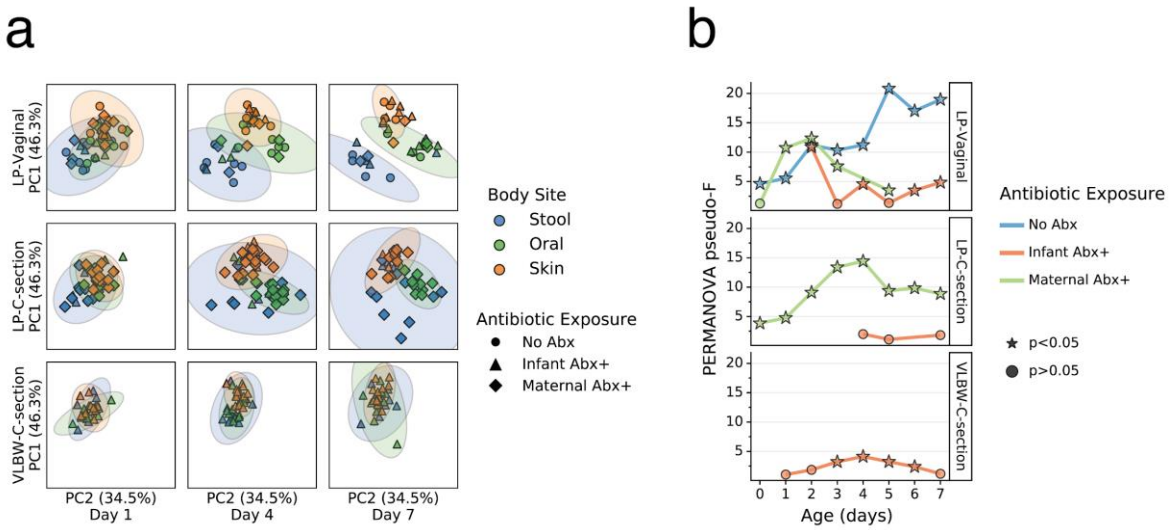
Extended Data Figure 1. Sample counts for preterm infants by day after birth. a) Number of 16S samples for each body site by infant day after birth. **b**) Number of metabolomics samples for each body site by infant day after birth. Stool (black), Oral (white), Skin (grey).



Extended Data Figure 2. Alpha diversity in preterm infants over the first week after birth. Lines represent the mean (+/- SEM) alpha diversity as measured by Shannon index for all samples from preterm infants within each cohort: late preterm (LP) or very low birth weight (VLBW) displayed by birth-mode (rows) and body site (column). Lines are colored based on reported exposure to antibiotics, none (green), direct exposure (blue) or potential exposure due to maternal transfer (orange). Refer to Extended Data Table 3 for sample sizes in each comparison.

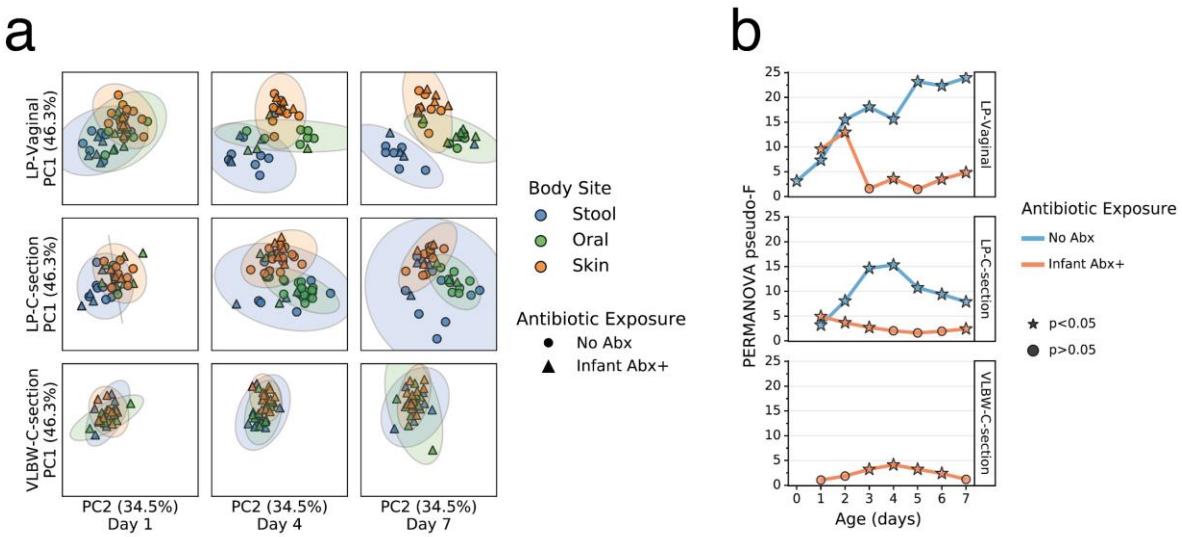


Extended Data Figure 3. 16S profile changes over the first week after birth in LP and VLBW infants. Within-infant 16S Robust Aitchison distance to first sample over time. For each infant, the Robust Aitchison distance to the first sample (day 0 for LPI infants, day 1 for VLBW infants) is plotted over time for each body site. Red lines show the fitted linear mixed effects model (individual as a random effect and age as a fixed effect); the corresponding p-value and slope is shown. All body sites show a trend of increasing distance over time, indicating that microbial profiles change over the first week after birth. Boxplots show median and interquartile range with whiskers extending to the furthest value within 1.5 times the edge of the interquartile range. Refer to Extended Data Table 3 for sample sizes in each comparison.



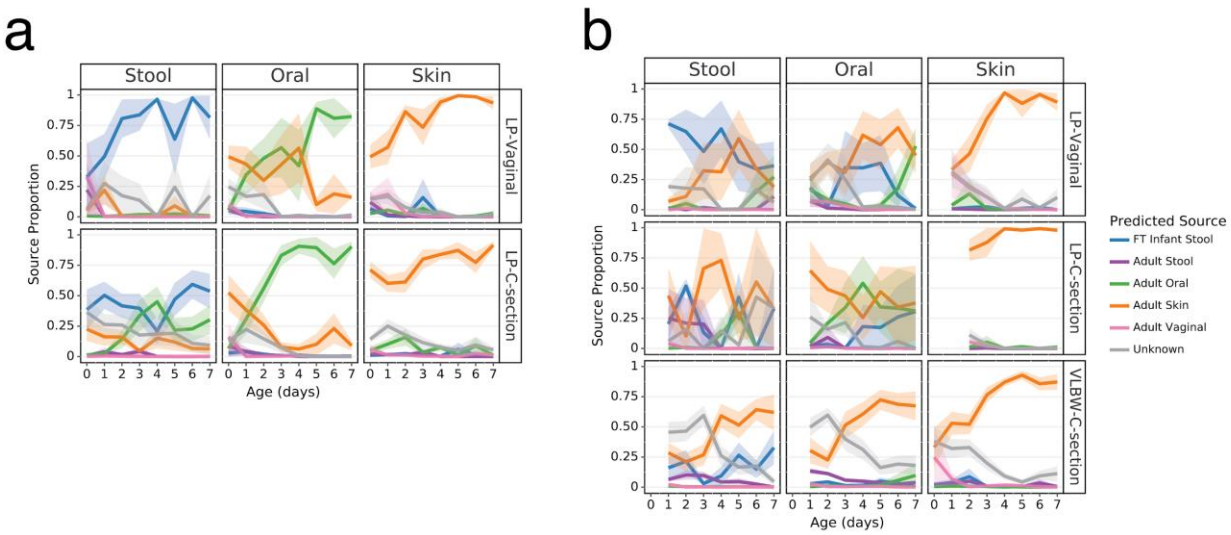
Extended Data Figure 4. Impact of maternal antibiotics exposure on the differentiation of the microbial community of preterm infants over the first week after birth.

a) Robust Aitchison Principal Components Analysis (PCA) based on 16S V4 amplicon sequence variants (ASVs) of the gut (blue), oral (green), and skin (orange) microbiomes collected at day 1, 4 and 7 after birth from late preterm infants (LPs) born vaginally (LP-Vaginal) or by Cesarean section (LP-C-section) and very-low birth weight (VLBW) preterm infants (VLBW-C-section), with samples from children exposed to antibiotics as reported by clinical metadata (Infant Abx$^{(+)}$; triangles), from children whose mothers were exposed to antibiotics (Maternal Abx; diamonds), and those without exposure to antibiotics (No Abx$^{(-)}$; circles) differentiated. **b)** PERMANOVA for differences in sample type (stool, oral, skin) based on Aitchison distances between all within-group samples for LP-Vaginal, LP-C-section, and VLBW-C-section preterm infants daily over the first week after birth, separated by maternal Abx exposure based on clinical metadata. Refer to Extended Data Table 3 for sample sizes in each comparison.

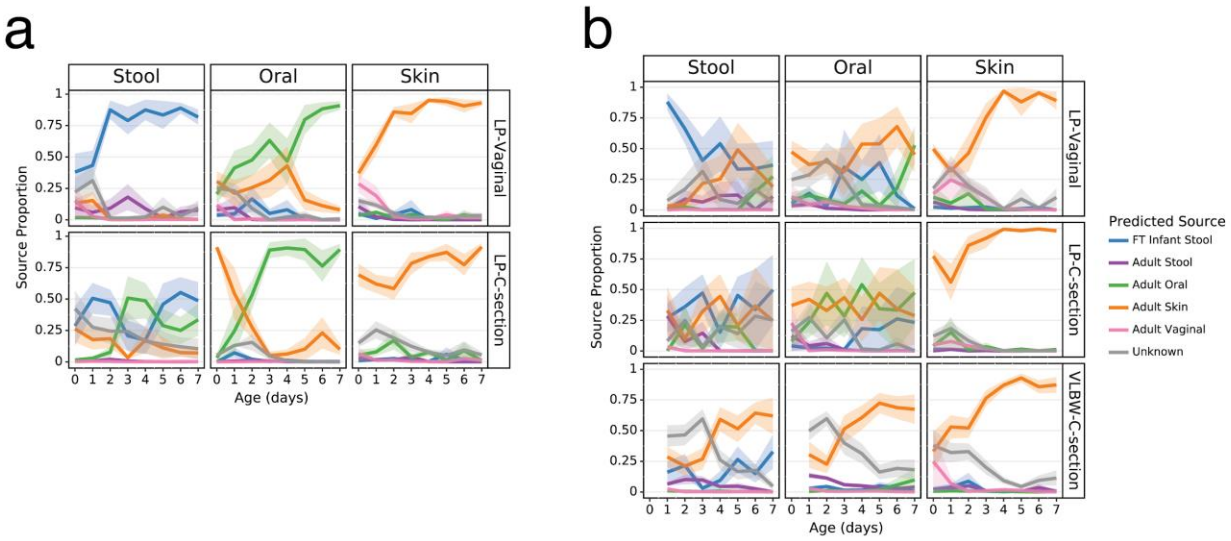


Extended Data Figure 5. 16S profile stratified by metabolome-informed antibiotic exposure

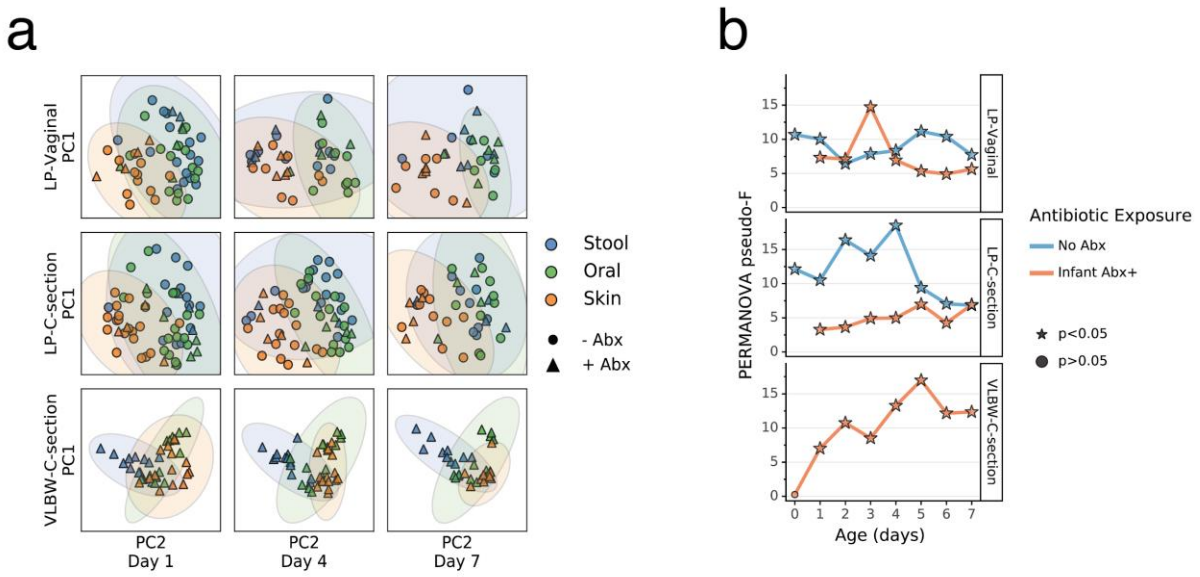
a) Robust Aitchison Principal Components Analysis (PCA) based on 16S V4 amplicon sequence variants (ASVs) of the gut (blue), oral (green), skin (orange) microbiomes collected at day 1, 4 and 7 after birth from late preterm infants (LPs) born vaginally (LP-Vaginal) or by Cesarean section (LPI-C-section) and very-low birth weight (VLBW) preterm infants (VLBW-C-section), with samples from children separated by Abx exposure based on metabolically-informed metadata. **b)** PERMANOVA for differences in sample type (stool, oral, skin) based on Aitchison distances between all within-group samples for LP-Vaginal, LP-C-section, and VLBW-C-section preterm infants daily over the first week after birth, separated by Abx exposure based on metabolically-informed metadata. Refer to Extended Data Table 3 for sample sizes in each comparison.



Extended Data Figure 6. Sourcetracker2 analysis stratified by maternal antibiotic exposure. SourceTracker2 analysis showing proportion of LP infant microbial communities attributed to adult or full term (FT) infant microbiomes based on 11 public studies in Qiita and a cohort of 87 FT infants not exposed to antibiotics aged 0.5-4 months (Methods). Lines represent the mean (+/- SEM) proportion of the microbial profile attributed to a specific source for all samples from preterm infants within each cohort displayed by birth-mode (rows) and body site (column). **a)** LP infant samples with no infant or maternal antibiotic exposure. **b)** LP infant samples with maternal antibiotic exposure but without infant antibiotic exposure. Refer to Extended Data Table 3 for sample sizes at each time point.

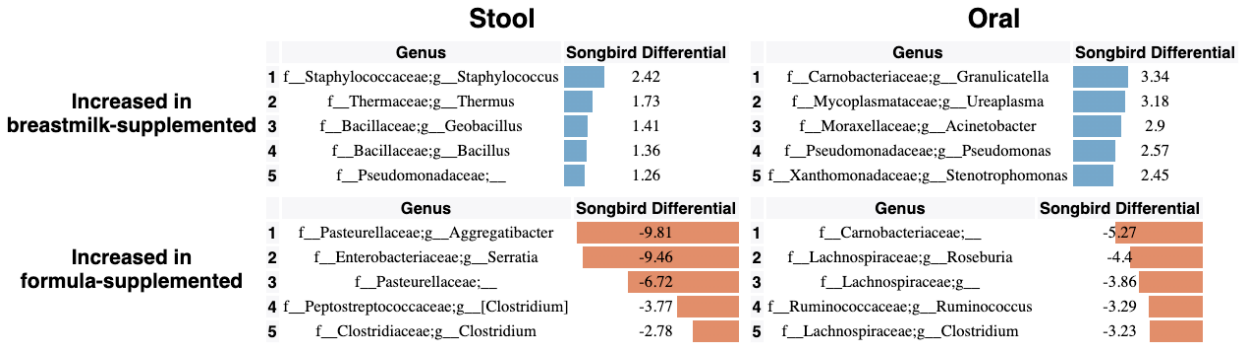


Extended Data Figure 7. Sourcetracker2 analysis stratified by metabolomics-informed antibiotic exposure. SourceTracker2 analysis showing proportion of LP infant microbial communities attributed to adult or full term (FT) infant microbiomes based on 11 public studies in Qiita and a cohort of 87 FT infants not exposed to antibiotics aged 0.5-4 months (Methods). Antibiotic exposure is defined by infants exposed to antibiotics by clinical metadata or antibiotics detected by metabolomics. Lines represent the mean (+/- SEM) proportion of the microbial profile attributed to a specific source for all samples from preterm infants within each cohort displayed by birth-mode (rows) and body site (column). **a)** LP infant samples with no antibiotic exposure. **b)** LP infant samples with antibiotic exposure. Refer to Extended Data Table 3 for sample sizes at each time point.

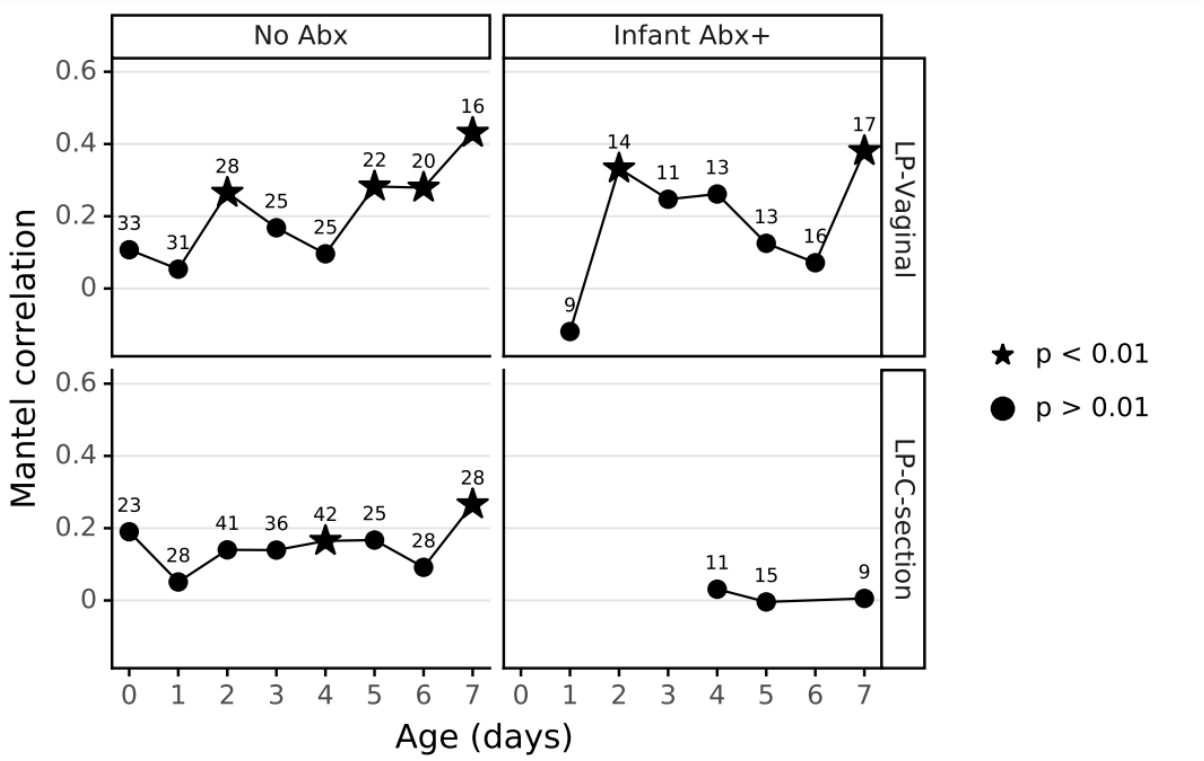


Extended Data Figure 8. Impact of antibiotics exposure on the differentiation of the metabolome of preterm infants over the first week after birth. a) Principal Coordinates Analysis (PCoA) based on Jaccard distances of metabolomics profile of the gut (blue), oral (green), and skin (orange) at day 1, 4, and 7 after birth from late preterm infants (LPs) born vaginally (LP-Vaginal) or by Cesarean section (LP-C-section) and very-low birth weight (VLBW) preterm infants (VLBW-C-section), with samples from children exposed to antibiotics as reported by clinical metadata (Infant Abx$^{(+)}$; triangles) and those without exposure to antibiotics (No Abx$^{(-)}$; circles) differentiated. Jaccard distances and PCoA for VLBW infants calculated separately from LP-infants because samples were run on different instruments. Principal Components (PCs) differ for LP-infants and VLBW-infants (LP-infant PC1 variance= 54.5%, PC2 variance= 30.6%; VLBW PC1 variance= 70.3%, PC2 variance= 25.1%). **b)** PERMANOVA for differences in sample type (stool, oral, skin) based on Jaccard distances between all within-group samples for LP-Vaginal, LP-C-section, and VLBW-C-section preterm infants daily over the first week after

birth, separated by Abx exposure. Refer to Extended Data Table 3 for sample sizes in each comparison.



Extended Data Figure 9. Microbial genera associated with supplemental formula or breastmilk. Songbird differentials for top 5 genera positively and negatively associated with supplemental breastmilk or supplemental formula in the stool and oral samples from LP infants. The songbird model fit for stool (Q^2=0.072) and oral (Q^2=0.014).



Extended Data Figure 10. Mantel correlation between metabolome and 16S profile over time. Stool, oral, and skin samples with matched 16S and metabolomics data were divided by delivery mode, and antibiotic exposure, then Mantel tests were run for each day to measure the relation between the two data types over time. Significant Mantel correlations are depicted as stars. Sample size for each comparison shows as the number above each point.