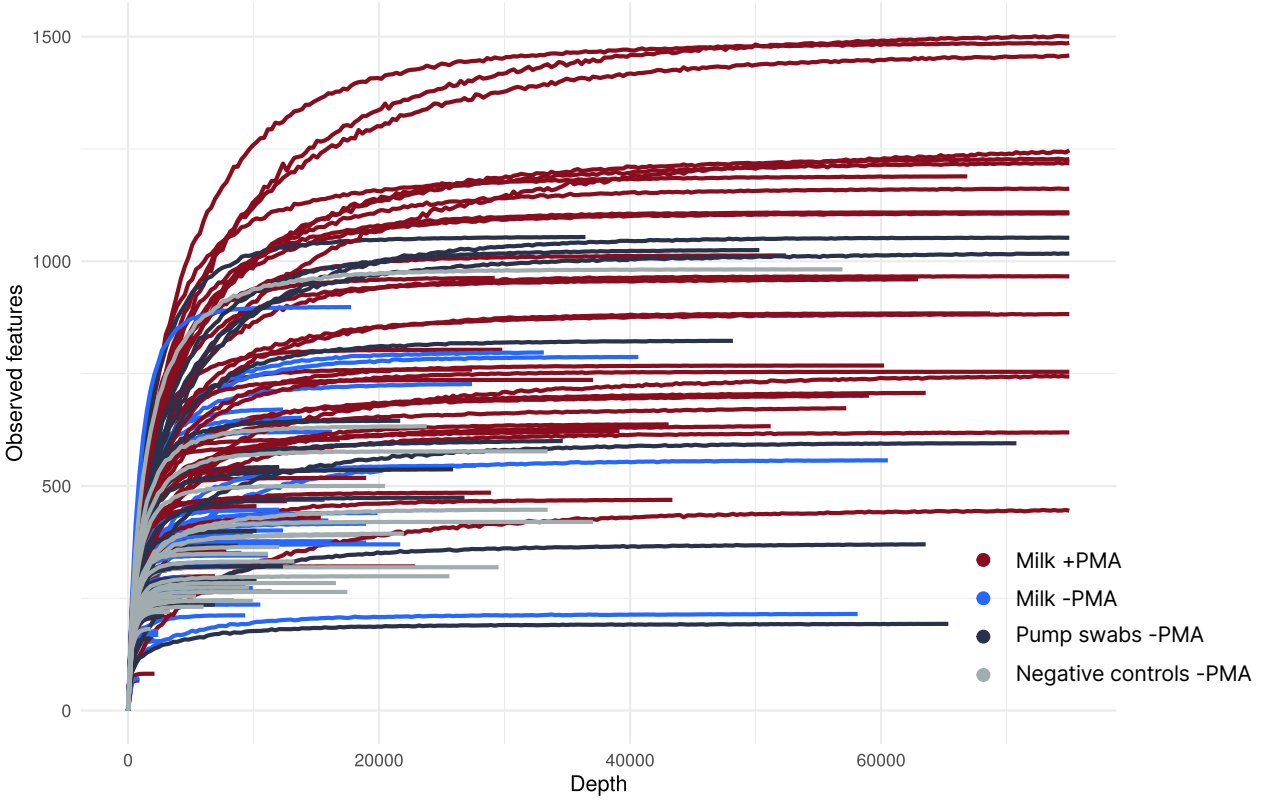
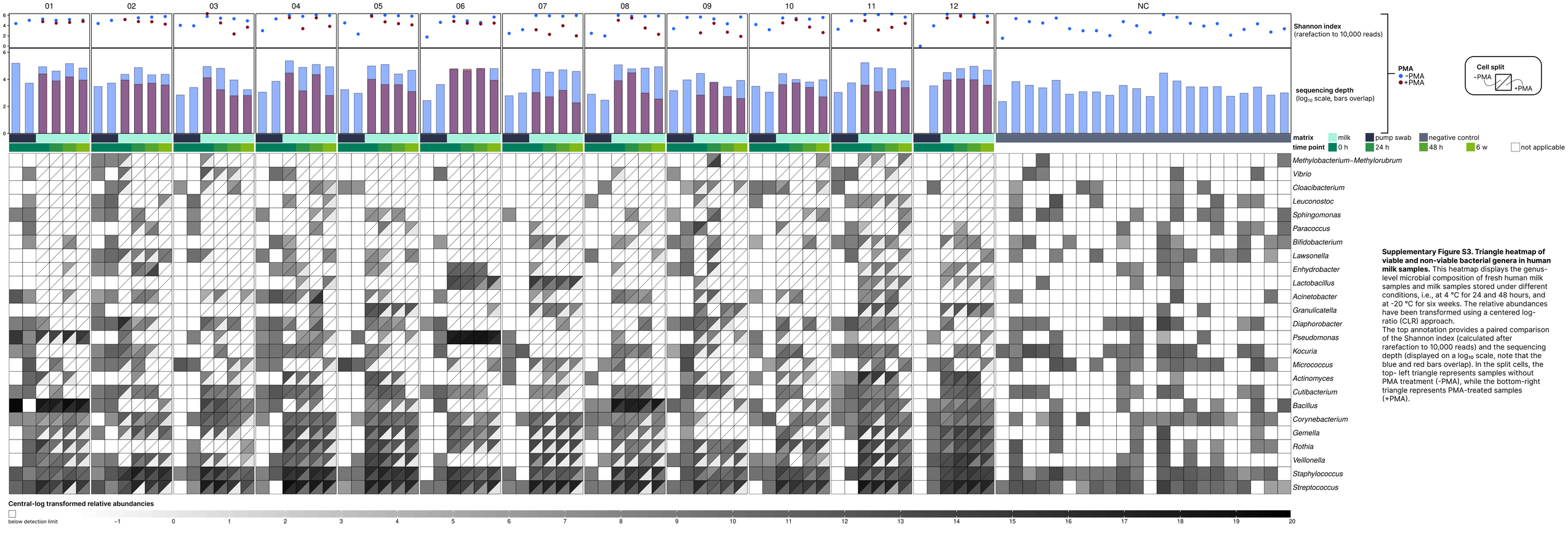



**Supplementary Figure S1. Sequencing depth with alpha rarefaction curves in all analyzed samples (n = 142).** Samples with PMA treatment (+PMA; only viable genera) as well as without PMA treatment (-PMA; both viable and non-viable bacterial genera) are shown.

| | | human milk | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 h | | 24 h | | 48 h | | 6 weeks | | pump swabs | negative controls | | |
| total<br>n = 142 | label | +PMA<br>n = 12 (8.5%) | -PMA<br>n = 12 (8.5%) | +PMA<br>n = 12 (8.5%) | -PMA<br>n = 12 (8.5%) | +PMA<br>n = 12 (8.5%) | -PMA<br>n = 12 (8.5%) | +PMA<br>n = 12 (8.5%) | -PMA<br>n = 12 (8.5%) | -PMA<br>n = 24 (16.9%) | -PMA<br>n = 22 (15.5%) | *p*-value |
| **sequencing depth** | | | | | | | | | | | | |
| [0 \| 1440 \| 5959.5 \| 34602 \| 236697] | five number summary | [687 \| 3923 \| 9003.5 \| 19490 \| 60027] | [23342 \| 29982 \| 72746 \| 109257.5 \| 236697] | [518 \| 1589.5 \| 4950.5 \| 9283.5 \| 58138] | [6369 \| 38159.5 \| 61510.5 \| 74114.5 \| 125347] | [557 \| 1249.5 \| 3333 \| 12312.5 \| 64698] | [2780 \| 15560.5 \| 56298 \| 80459 \| 148187] | [187 \| 448 \| 965.5 \| 3781.5 \| 8890] | [1753 \| 8956 \| 43313 \| 66746 \| 86622] | [0 \| 876 \| 1619 \| 4751.5 \| 157290] | [229 \| 803 \| 2242 \| 3548 \| 29572] | **<0.001** |
| 25658.81±40063.76 | mean±sd | 14562.92±16904.53 | 85814.17±65165.99 | 11022.08±16947.94 | 56355.5±32357.58 | 10766.67±18297.46 | 56371.08±47246.3 | 2636±3143.96 | 41003.75±30897.43 | 9055.79±31665.87 | 3810.27±6223.63 | **<0.001** |
| **Shannon index** | | | | | | | | | | | | |
| [0 \| 3.7 \| 4.75 \| 5.61 \| 6.29] | five number summary | [2.58 \| 4.63 \| 4.98 \| 5.49 \| 6.29] | [5.16 \| 5.56 \| 5.85 \| 5.97 \| 6.18] | [2.28 \| 3.91 \| 4.49 \| 4.95 \| 5.82] | [4.9 \| 5.32 \| 5.63 \| 5.95 \| 6.1] | [2.36 \| 3.58 \| 4.13 \| 4.67 \| 5.61] | [4.33 \| 5.2 \| 5.7 \| 5.97 \| 6.23] | [1.89 \| 2.47 \| 3.98 \| 4.44 \| 4.8] | [4.9 \| 5.59 \| 5.7 \| 5.83 \| 5.94] | [0 \| 2.74 \| 3.95 \| 4.56 \| 5.57] | [1.53 \| 2.98 \| 3.93 \| 4.75 \| 6.09] | **<0.001** |
| 4.53±1.28 | mean±sd | 4.83±1.05 | 5.76±0.33 | 4.38±1 | 5.59±0.41 | 4.08±0.98 | 5.51±0.6 | 3.58±1.08 | 5.63±0.33 | 3.62±1.32 | 3.84±1.24 | **<0.001** |
| ***Acinetobacter* (CLR transformation)** | | | | | | | | | | | | |
| [-1.27 \| -0.56 \| -0.2 \| 7.16 \| 15.4] | five number summary | [-0.88 \| -0.58 \| -0.2 \| 6.71 \| 9.78] | [-1.15 \| -0.56 \| 6.56 \| 8.79 \| 9.65] | [-0.91 \| -0.54 \| -0.32 \| 7.68 \| 10.29] | [-0.78 \| 3.03 \| 7.37 \| 8.09 \| 11.09] | [-0.7 \| -0.18 \| 6.53 \| 8.24 \| 15.4] | [-0.9 \| -0.6 \| -0.17 \| 8.03 \| 9.41] | [-0.67 \| -0.45 \| -0.21 \| -0.13 \| 0.09] | [-0.94 \| -0.8 \| -0.62 \| -0.39 \| 6.3] | [-1.13 \| -0.41 \| -0.21 \| 6.44 \| 12.14] | [-1.27 \| -0.56 \| -0.28 \| -0.04 \| 11.41] | 0.1 |
| 2.61±4.34 | mean±sd | 2.94±4.4 | 4.88±4.41 | 2.59±4.6 | 5.81±4.05 | 5.14±5.32 | 3.07±4.52 | -0.27±0.23 | -0.04±2.01 | 2.5±4.45 | 0.97±3.59 | **<0.001** |
| ***Anaerococcus* (CLR transformation)** | | | | | | | | | | | | |
| [-1.37 \| -0.58 \| -0.3 \| 0.21 \| 10.54] | five number summary | [-0.83 \| -0.57 \| -0.33 \| 7.04 \| 10.38] | [-1.13 \| -0.69 \| 6.5 \| 8.51 \| 10.51] | [-1 \| -0.55 \| -0.46 \| -0.28 \| 9.12] | [-0.92 \| -0.77 \| -0.53 \| 7.09 \| 8.27] | [-0.92 \| -0.74 \| -0.45 \| -0.24 \| 8.93] | [-0.99 \| -0.47 \| 3.41 \| 8.1 \| 10.31] | [-0.51 \| -0.19 \| -0.13 \| -0.03 \| 0.21] | [-1.29 \| -0.62 \| 2.74 \| 8.47 \| 10.54] | [-1 \| -0.73 \| -0.26 \| -0.11 \| 10.02] | [-1.37 \| -0.49 \| -0.35 \| -0.29 \| 8.06] | 0.519 |
| 1.57±3.74 | mean±sd | 2.92±4.41 | 4.42±4.69 | 0.77±3.12 | 2.1±4.12 | 0.26±2.74 | 3.88±4.66 | -0.13±0.19 | 3.63±4.71 | 0.43±2.87 | -0.07±1.85 | **0.007** |
| ***Bacillus* (CLR transformation)** | | | | | | | | | | | | |
| [-1.42 \| -0.44 \| 6.93 \| 9.36 \| 19.53] | five number summary | [-0.59 \| -0.48 \| 6.67 \| 9.67 \| 16.72] | [-1.21 \| -0.74 \| 7.3 \| 12.86 \| 18.38] | [-0.58 \| -0.39 \| 7.92 \| 10.9 \| 17.07] | [-0.94 \| -0.58 \| 7.81 \| 11.51 \| 17.56] | [-0.89 \| -0.56 \| -0.17 \| 10.32 \| 16.17] | [-0.8 \| -0.21 \| 8.35 \| 10.22 \| 19.15] | [-0.36 \| -0.13 \| -0.03 \| 8.46 \| 15.34] | [-1.16 \| 3.14 \| 8.22 \| 9.23 \| 18.33] | [-0.71 \| -0.09 \| 7.88 \| 9.38 \| 19.53] | [-1.42 \| -0.47 \| -0.16 \| 7.56 \| 11.02] | 0.768 |
| 5.39±6.2 | mean±sd | 5.8±6.27 | 6.73±7.63 | 6.43±6.59 | 6.63±7.14 | 4.48±6.4 | 7.37±6.82 | 3.36±5.42 | 7.7±6.37 | 5.59±5.54 | 2.26±4.52 | 0.519 |
| ***Bacteroides* (CLR transformation)** | | | | | | | | | | | | |
| [-1.35 \| -0.76 \| -0.44 \| -0.11 \| 9.33] | five number summary | [-0.89 \| -0.78 \| -0.41 \| 2.87 \| 8.61] | [-1.13 \| -1.04 \| -0.72 \| -0.3 \| 8.93] | [-0.98 \| -0.67 \| -0.63 \| -0.5 \| -0.26] | [-1.35 \| -1.18 \| -0.78 \| -0.5 \| 0.03] | [-0.87 \| -0.45 \| -0.33 \| -0.26 \| 7.78] | [-1.22 \| -1.1 \| -0.77 \| -0.43 \| -0.06] | [-0.76 \| -0.32 \| -0.25 \| -0.08 \| 0.1] | [-0.98 \| -0.89 \| -0.43 \| -0.18 \| -0.11] | [-1.09 \| -0.6 \| -0.3 \| 3.52 \| 9.33] | [-1.08 \| -0.58 \| -0.22 \| 0 \| 8.97] | **0.007** |
| 0.45±2.73 | mean±sd | 1.3±3.48 | 0.64±3.32 | -0.6±0.18 | -0.78±0.47 | 0.28±2.37 | -0.74±0.39 | -0.24±0.24 | -0.5±0.34 | 1.71±3.87 | 1.38±3.49 | **0.001** |
| ***Cutibacterium* (CLR transformation)** | | | | | | | | | | | | |
| [-0.86 \| -0.26 \| 7.53 \| 9.65 \| 11.99] | five number summary | [-0.32 \| 7.59 \| 8.37 \| 9.29 \| 10.61] | [-0.41 \| 8.85 \| 10.43 \| 10.77 \| 11.59] | [7.21 \| 7.72 \| 8.23 \| 9.39 \| 11.41] | [6.34 \| 7.54 \| 9.16 \| 10.54 \| 11.99] | [-0.78 \| 5.82 \| 7.15 \| 9.45 \| 10.73] | [-0.54 \| 7.31 \| 9.4 \| 10.57 \| 11.5] | [-0.51 \| -0.28 \| -0.23 \| -0.15 \| 0.01] | [-0.86 \| -0.8 \| -0.62 \| -0.27 \| -0.06] | [-0.69 \| -0.45 \| -0.16 \| 9.48 \| 11.94] | [-0.73 \| -0.22 \| 0 \| 9.25 \| 10.68] | **<0.001** |
| 5.39±4.82 | mean±sd | 7.86±2.78 | 9.24±3.24 | 8.71±1.39 | 9.14±1.92 | 6.63±3.77 | 7.91±4.17 | -0.22±0.14 | -0.53±0.29 | 4.21±5.24 | 3.58±4.77 | **<0.001** |
| ***Diaphorobacter* (CLR transformation)** | | | | | | | | | | | | |
| [-1.29 \| -0.31 \| 6.28 \| 8.96 \| 15.82] | five number summary | [6.73 \| 8.23 \| 8.92 \| 9.59 \| 10.92] | [-0.72 \| 2.89 \| 7.92 \| 9.97 \| 15.82] | [-0.58 \| -0.19 \| 8.16 \| 8.67 \| 11.7] | [-1.29 \| -0.62 \| -0.24 \| 6.89 \| 10.78] | [-0.49 \| 7.09 \| 8.22 \| 9.6 \| 11.51] | [-1.12 \| 2.59 \| 7.81 \| 8.8 \| 9.32] | [-0.61 \| -0.39 \| -0.16 \| 0 \| 0.21] | [-1.13 \| -0.67 \| -0.57 \| -0.29 \| -0.08] | [-0.99 \| -0.23 \| 3.59 \| 9.59 \| 11.99] | [-0.89 \| -0.31 \| -0.01 \| 9.4 \| 13.53] | **<0.001** |
| 4.54±4.86 | mean±sd | 8.91±1.13 | 6.94±5.11 | 5.75±4.63 | 2.71±4.34 | 7.24±3.79 | 5.78±4.17 | -0.18±0.25 | -0.54±0.32 | 4.63±5.14 | 4.27±5.3 | **<0.001** |
| ***Gemella* (CLR transformation)** | | | | | | | | | | | | |
| [-1.37 \| -0.38 \| 0.11 \| 9.91 \| 14.47] | five number summary | [-0.82 \| -0.51 \| 3.67 \| 9.04 \| 11.07] | [-0.6 \| 8.98 \| 11.64 \| 12.98 \| 14.09] | [-0.6 \| -0.5 \| -0.28 \| 6.34 \| 11.32] | [-0.5 \| 9.24 \| 11.48 \| 12.85 \| 14.04] | [-0.59 \| -0.39 \| -0.23 \| 2.81 \| 10.29] | [-0.45 \| 8.62 \| 10.5 \| 12.23 \| 14.47] | [-0.53 \| -0.16 \| -0.03 \| 8.42 \| 9.1] | [-0.58 \| 7.77 \| 10.55 \| 12 \| 13.6] | [-0.92 \| -0.57 \| -0.25 \| 2.97 \| 8.92] | [-1.37 \| -0.66 \| -0.23 \| 0 \| 12.62] | **<0.001** |
| 4.75±5.54 | mean±sd | 4.37±5.13 | 10.44±3.96 | 2.51±4.54 | 10.54±3.91 | 1.59±3.61 | 9.21±4.74 | 2.81±4.4 | 8.9±4.76 | 1.63±3.67 | 1.38±4.11 | **<0.001** |
| ***Rothia* (CLR transformation)** | | | | | | | | | | | | |
| [-1.16 \| -0.29 \| 7.07 \| 10.33 \| 15.87] | five number summary | [-0.68 \| -0.39 \| 7.72 \| 9.22 \| 10.94] | [-0.77 \| 10.89 \| 12.63 \| 13.96 \| 15.87] | [-1.12 \| -0.61 \| -0.18 \| 8.28 \| 11.32] | [-1.16 \| 9.46 \| 11.11 \| 13.02 \| 13.76] | [-0.47 \| -0.3 \| -0.2 \| 8.05 \| 10.33] | [-0.66 \| 9.06 \| 11.76 \| 13.45 \| 14.17] | [-0.63 \| -0.19 \| -0.03 \| 6.64 \| 8.8] | [-0.16 \| 9.71 \| 11.9 \| 12.44 \| 13.12] | [-0.99 \| -0.5 \| -0.28 \| 0.04 \| 10.58] | [-1.04 \| -0.3 \| 0.02 \| 8.17 \| 11.96] | **<0.001** |
| 5.38±5.72 | mean±sd | 5.69±4.72 | 10.86±5.56 | 3.35±4.92 | 9.57±5.14 | 3.47±4.74 | 9.73±5.09 | 2.38±3.85 | 10.23±3.84 | 1.22±3.7 | 3.26±4.9 | **<0.001** |
| ***Staphylococcus* (CLR transformation)** | | | | | | | | | | | | |
| [-0.85 \| 9.38 \| 11.58 \| 13.4 \| 17.51] | five number summary | [8.57 \| 9.7 \| 11.99 \| 13 \| 15.06] | [10.73 \| 13.16 \| 14.36 \| 15.85 \| 16.5] | [8.87 \| 9.57 \| 11.34 \| 12.88 \| 13.93] | [7.09 \| 12.48 \| 14.13 \| 15.9 \| 17.51] | [-0.85 \| 9.47 \| 11.1 \| 13.04 \| 15.09] | [9.77 \| 11.71 \| 13.43 \| 15.17 \| 16.43] | [6.57 \| 9.71 \| 11.18 \| 13.03 \| 14.47] | [10.64 \| 12.29 \| 13.29 \| 16.02 \| 16.31] | [-0.41 \| 3.72 \| 9.21 \| 10.3 \| 12.52] | [-0.42 \| 8.42 \| 9.07 \| 11.34 \| 13.52] | **<0.001** |
| 11±3.96 | mean±sd | 11.62±2.24 | 14.31±1.75 | 11.35±1.76 | 13.84±2.89 | 10.52±4.13 | 13.41±2.11 | 11.18±2.26 | 13.66±2.1 | 7.36±4.62 | 8.51±3.9 | **<0.001** |
| ***Streptococcus* (CLR transformation)** | | | | | | | | | | | | |
| [-1.2 \| 8.81 \| 10.85 \| 15.13 \| 19.2] | five number summary | [9.52 \| 10.7 \| 12.66 \| 13.61 \| 16.13] | [11.64 \| 16.05 \| 16.51 \| 17.58 \| 19.2] | [-1.2 \| 9.28 \| 9.93 \| 11.22 \| 13.5] | [11.55 \| 14.82 \| 16.73 \| 17.23 \| 17.9] | [-0.26 \| 2.3 \| 8.51 \| 9.84 \| 12.71] | [13.38 \| 14.87 \| 15.68 \| 17.23 \| 18.07] | [-0.28 \| 0.1 \| 7.75 \| 9.25 \| 11.53] | [12.9 \| 14.68 \| 15.62 \| 16.7 \| 17.58] | [-0.69 \| 2.94 \| 8.51 \| 9.45 \| 12.05] | [-0.57 \| 6.13 \| 9.8 \| 11.28 \| 14.15] | **<0.001** |
| 10.76±5.3 | mean±sd | 12.33±1.93 | 16.53±1.89 | 9.61±3.72 | 15.96±1.88 | 6.59±4.56 | 15.79±1.51 | 5.86±4.51 | 15.61±1.41 | 6.69±4.42 | 8.55±4.24 | **<0.001** |
| ***Veillonella* (CLR transformation)** | | | | | | | | | | | | |
| [-1.13 \| -0.26 \| 7.57 \| 10.56 \| 15.58] | five number summary | [-0.79 \| -0.39 \| 8.43 \| 10.49 \| 13.16] | [-0.76 \| 8.79 \| 11.33 \| 12.83 \| 14.71] | [-0.65 \| -0.3 \| 3.62 \| 9.19 \| 13.76] | [-0.55 \| 8.89 \| 11.02 \| 13.85 \| 15] | [-0.62 \| -0.43 \| -0.21 \| 7 \| 9.25] | [-0.22 \| 8.47 \| 10.93 \| 13.45 \| 15.58] | [-0.68 \| -0.33 \| -0.14 \| -0.03 \| 9.15] | [-0.4 \| 7.85 \| 11.09 \| 12.22 \| 14.17] | [-0.78 \| -0.26 \| 6.74 \| 8.62 \| 11.41] | [-1.13 \| -0.37 \| -0.08 \| 8.23 \| 11.33] | **<0.001** |
| 5.69±5.62 | mean±sd | 6.48±5.49 | 9.71±5.17 | 4.68±5.37 | 9.84±5.28 | 2.4±4.09 | 9.72±5.14 | 1.27±3.55 | 9.68±4.33 | 4.6±4.75 | 2.39±4.49 | **<0.001** |

Five-number summary stands for [minimum | 1st quartile | median | 3rd quartile | maximum].
To test difference in means an ANOVA test was used.
To test difference in medians a non-parametric ANOVA (Kruskal-Wallis test) was used.
All *p*-values were adjusted using Holm's method.

**Supplementary Figure S2. Descriptive statistics for sequencing depth, Shannon diversity index, and centered log-ratio (CLR)–transformed relative abundances of selected genera, stratified by sample matrix, time point, and PMA treatment group.** All values are rounded to two decimals.




**Supplementary Figure S3. Triangle heatmap of viable and non-viable bacterial genera in human milk samples.** This heatmap displays the genus-level microbial composition of fresh human milk samples and milk samples stored under different conditions, i.e., at 4 °C for 24 and 48 hours, and at -20 °C for six weeks. The relative abundances have been transformed using a centered log-ratio (CLR) approach.
The top annotation provides a paired comparison of the Shannon index (calculated after rarefaction to 10,000 reads) and the sequencing depth (displayed on a $\log_{10}$ scale, note that the blue and red bars overlap). In the split cells, the top- left triangle represents samples without PMA treatment (-PMA), while the bottom-right triangle represents PMA-treated samples (+PMA).