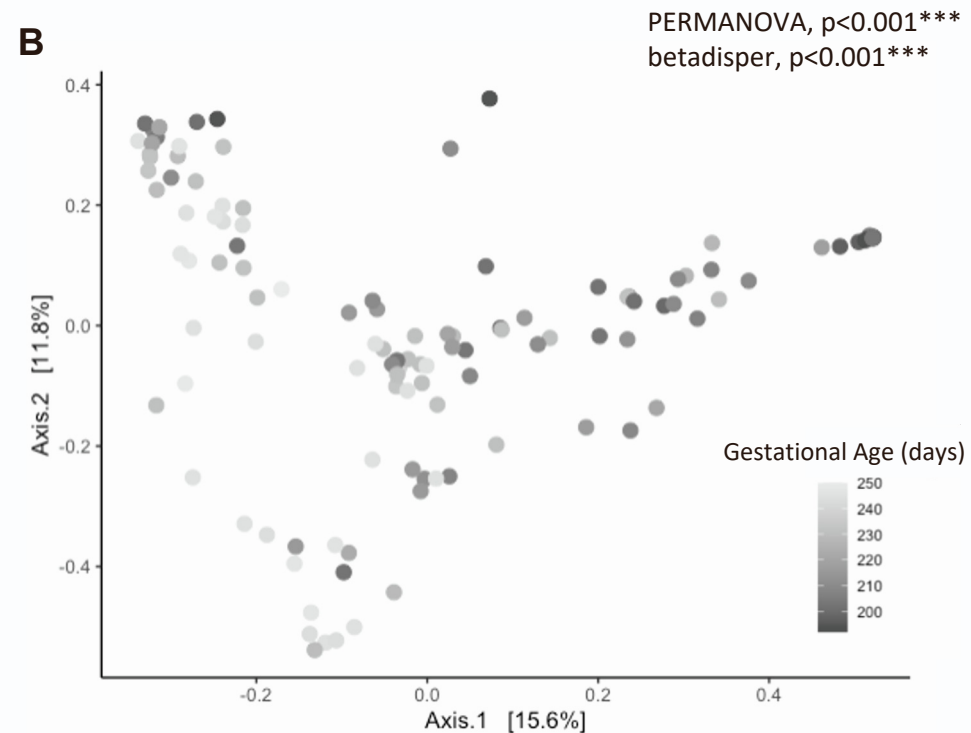
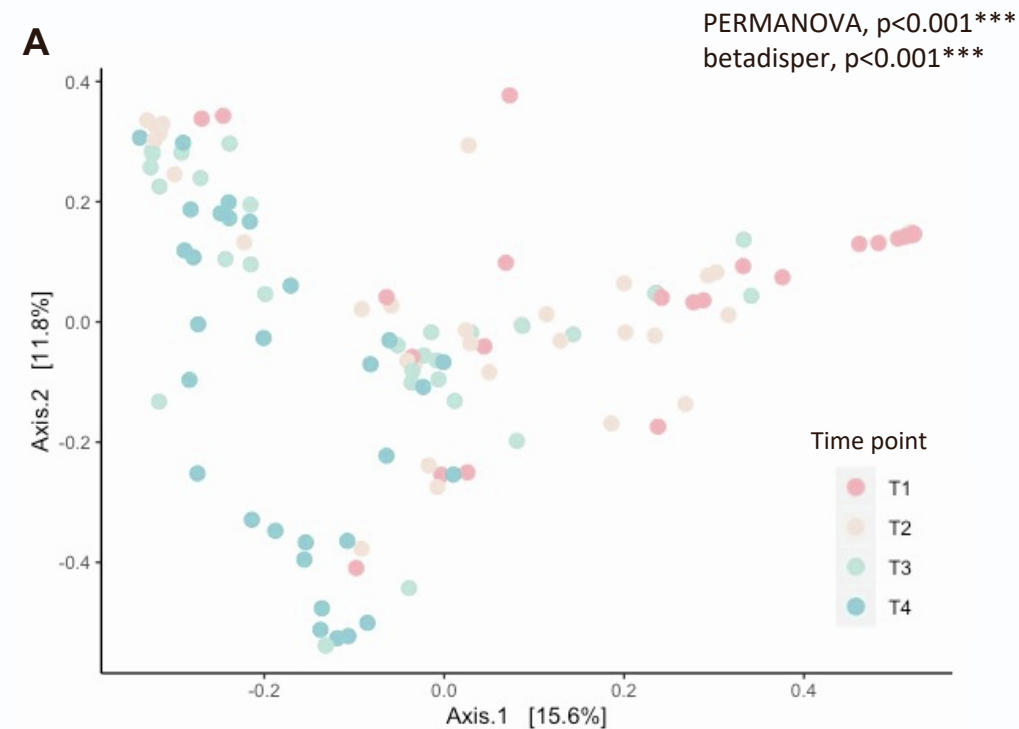


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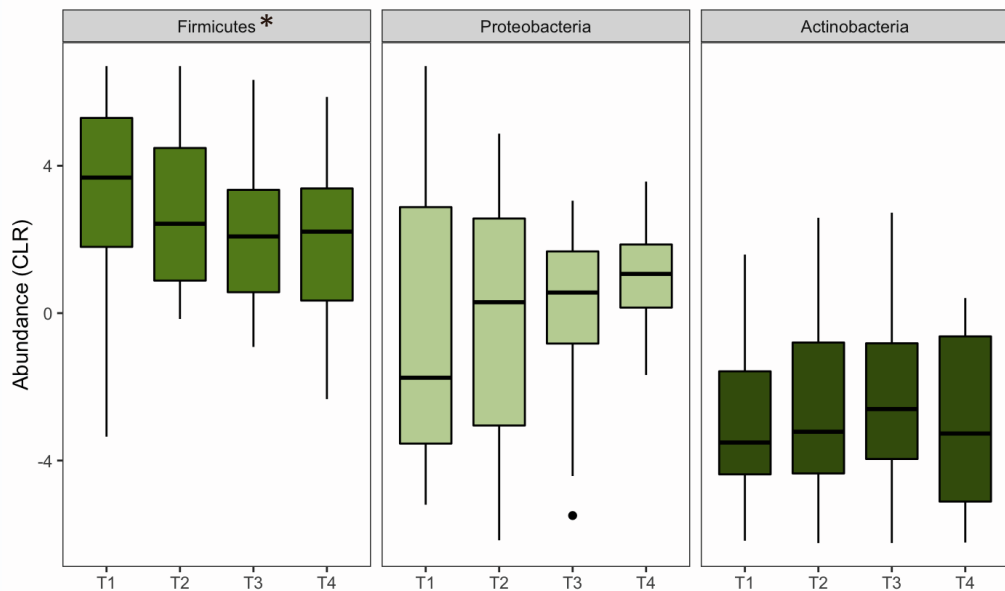
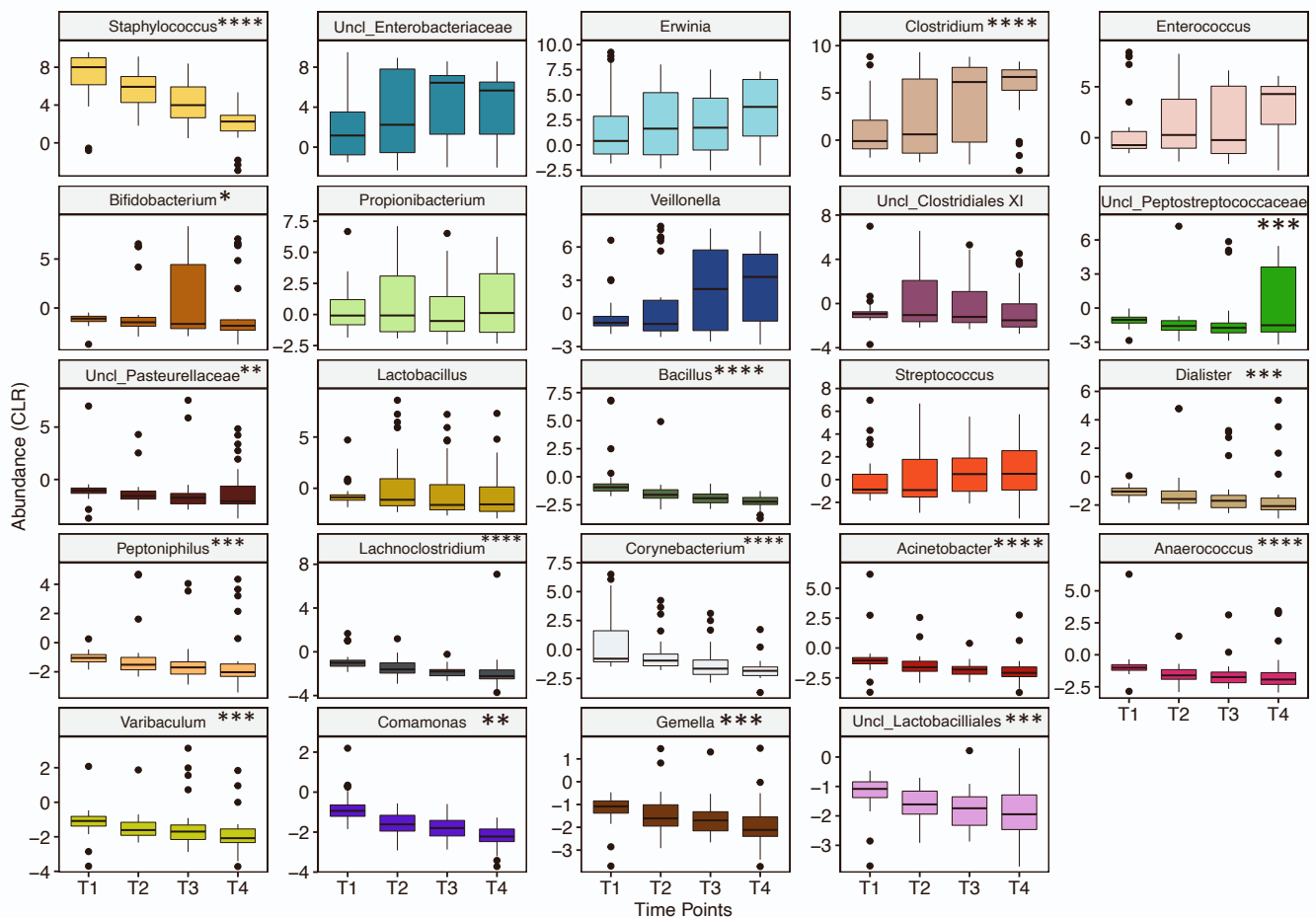
**Supplemental information**

**Source of human milk (mother or donor) is more  
important than fortifier type (human or bovine)  
in shaping the preterm infant microbiome**

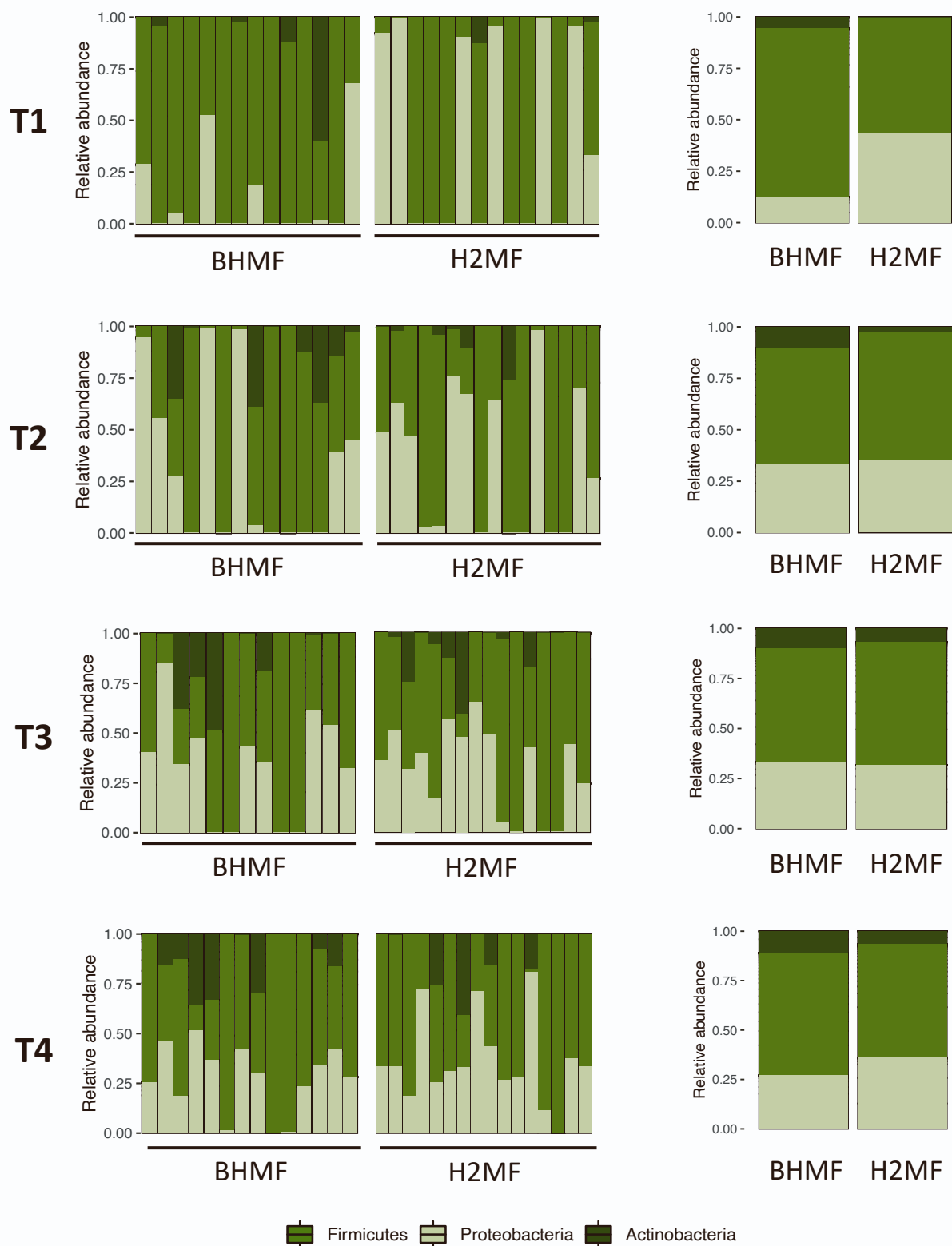
**Shreyas V. Kumbhare, William-Diehl Jones, Sharla Fast, Christine Bonner, Geert 't  
Jong, Gary Van Domselaar, Morag Graham, Michael Narvey, and Meghan B. Azad**



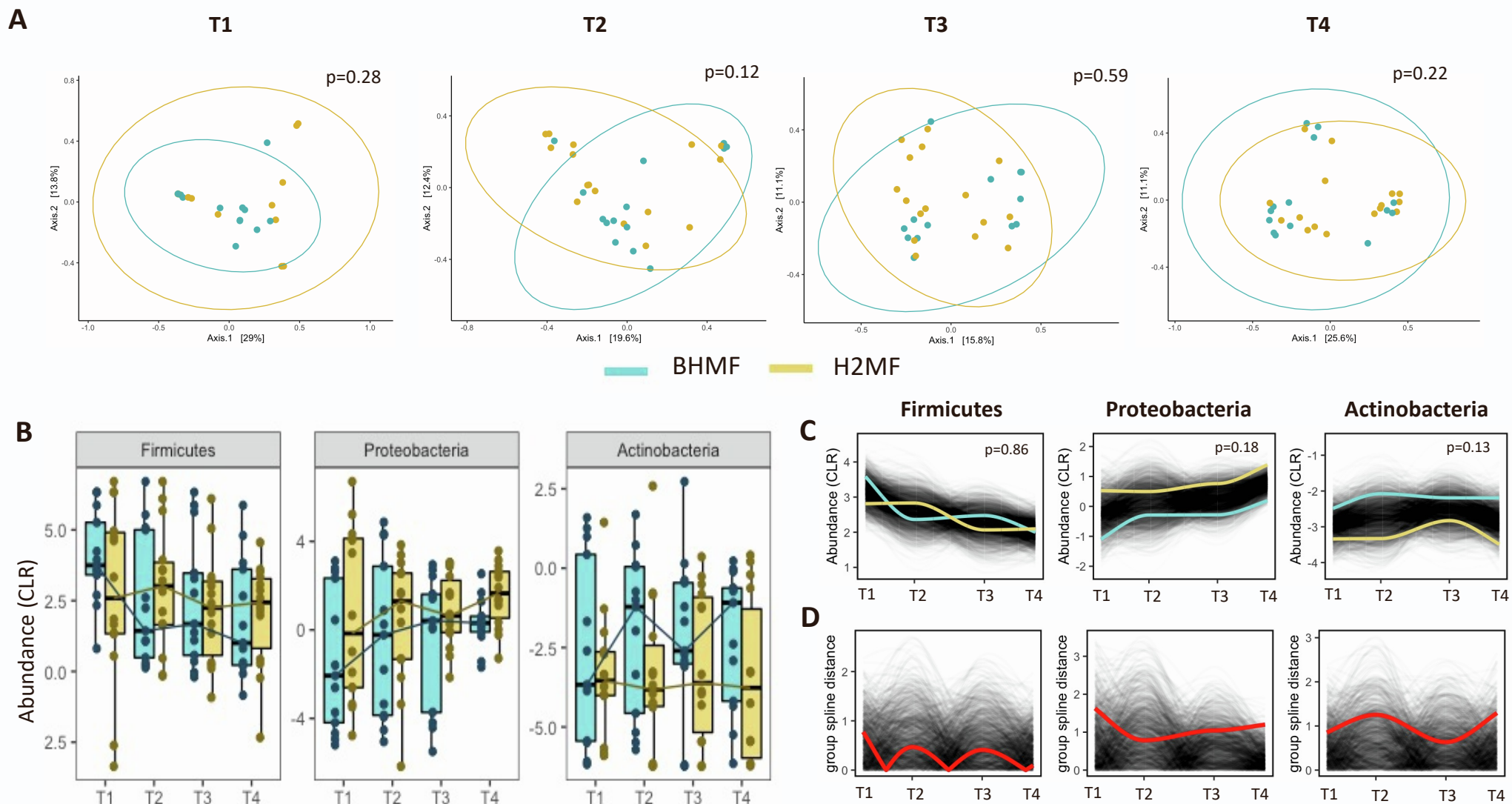
**Figure S1. Changes in gut bacterial diversity in VLBW infants over time.** Principal coordinate analysis based on Jaccard distances (Bray-Curtis distances in **Figure 1D**) showing diversity between samples (A) across 4 study time points and (B) with adjusted gestational age (in days). P-values from PERMANOVA for beta diversity and betadisper test (permutation test for homogeneity of multivariate dispersions; (permutations=99,999). Adjustments for repeated measures were done by setting strata (blocks) to infant IDs. T1 = Study Day 0 (before fortification), T2 = Study Day 7 (during fortification), T3 = Week 33 AGA (end of fortification) and T4 = Week 35 AGA (follow up after fortification). VLBW, very low birth infants.

**A****B**

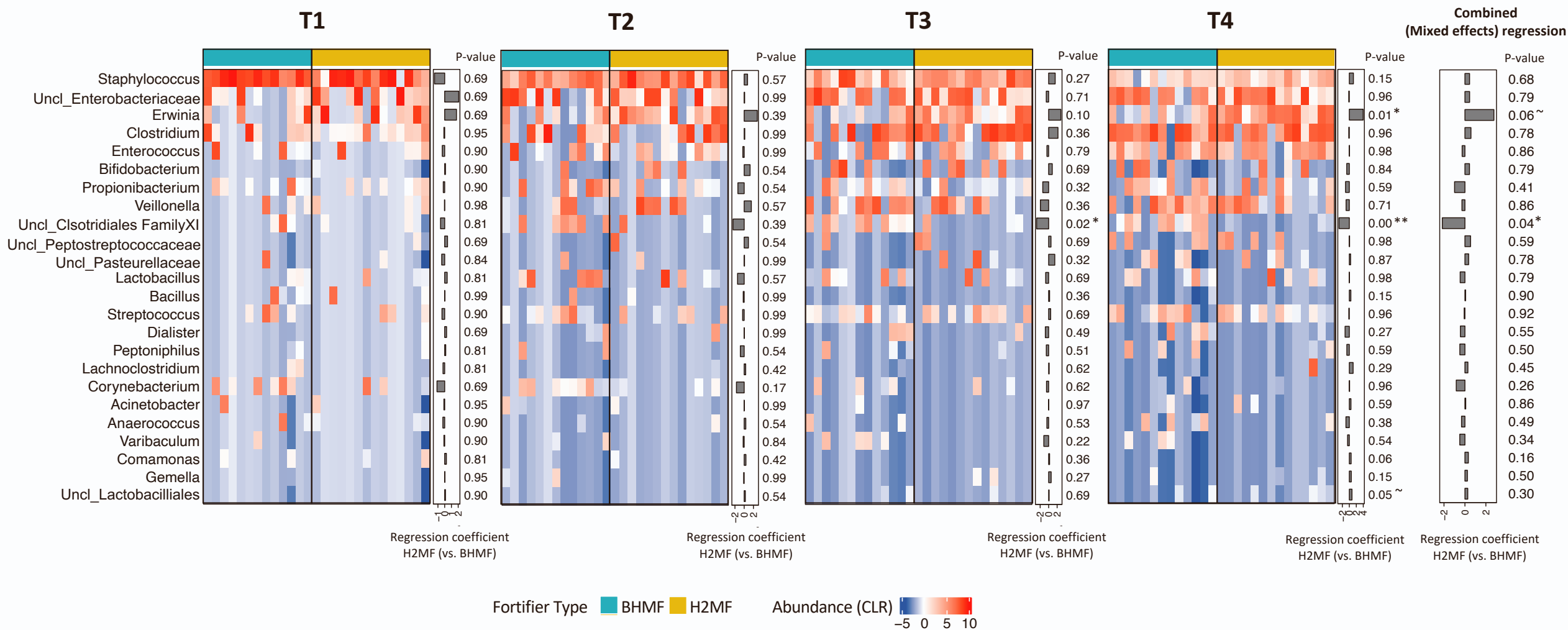
**Figure S2. Changes in gut bacterial relative abundance across study time points (related to Figure 1 C&D).** Relative abundance (CLR-transformed) of bacterial phyla (A) and genera (B) at study time points (P values from Kruskal–Wallis test: \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , \*\*\*\*  $p < 0.0001$ ). T1 = Study Day 0 (before fortification), T2 = Study Day 7 (during fortification), T3 = Week 33 AGA (end of fortification) and T4 = Week 35 AGA (follow up after fortification). The prefix ‘Uncl\_’ indicates an unclassified genus of the named bacterial family or order.



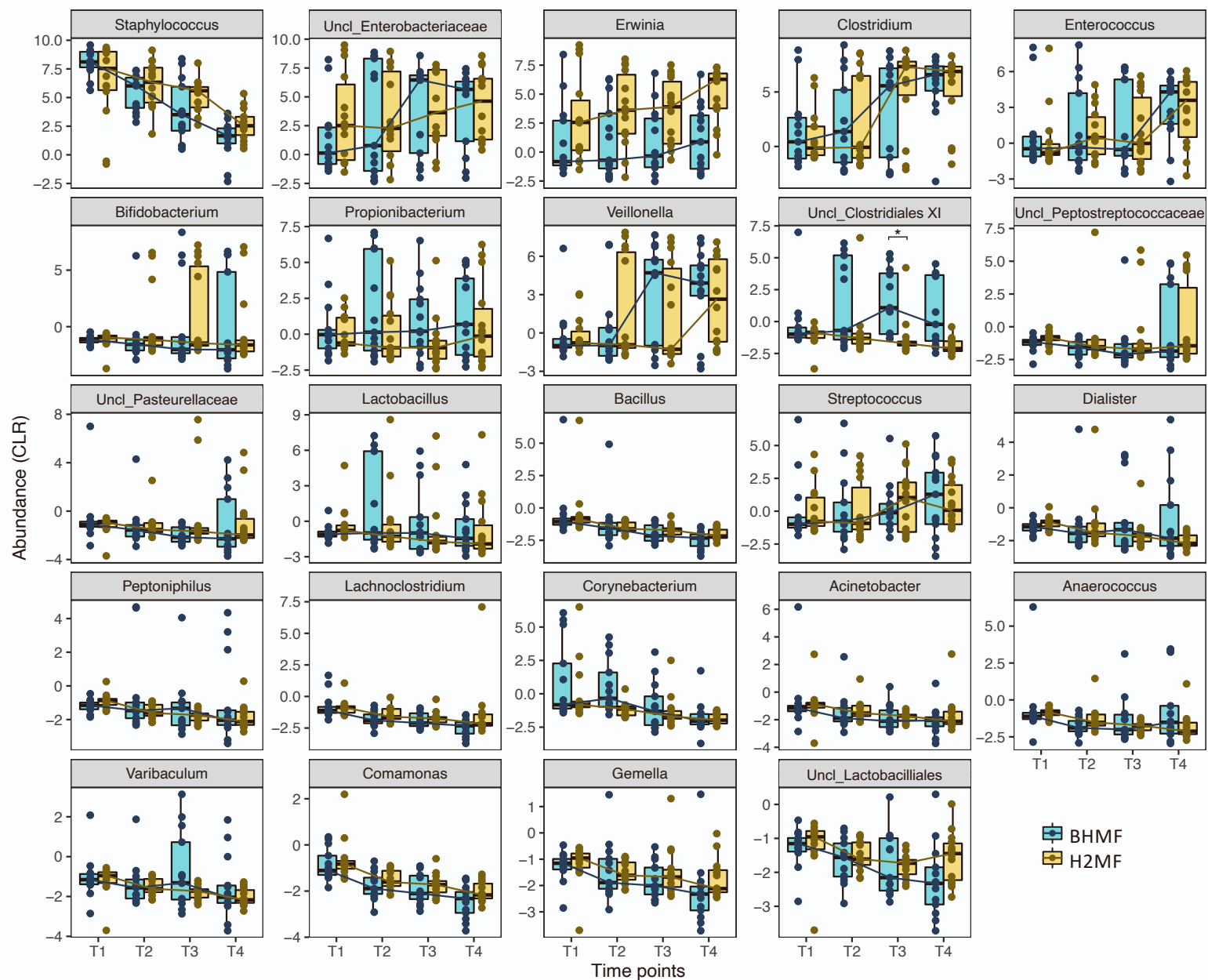
**Figure S3. Impact of human milk fortifier type (bovine (BHMF) or human (H2MF)) on gut bacterial composition in VLBW infants (related to Figure 2, which illustrates group-wise comparison at genus level). Bar plots depict the relative abundance of gut bacterial phyla (Left: individual infants, Right: group means). T1 = Study Day 0 (before fortification), T2 = Study Day 7 (during fortification), T3 = Week 33 AGA (end of fortification) and T4 = Week 35 AGA (follow up after fortification). BHMF, bovine-derived human milk fortifier; H2MF, human-derived human milk fortifier; VLBW, very low birthweight. **Figure 3** shows results at the genus level.**



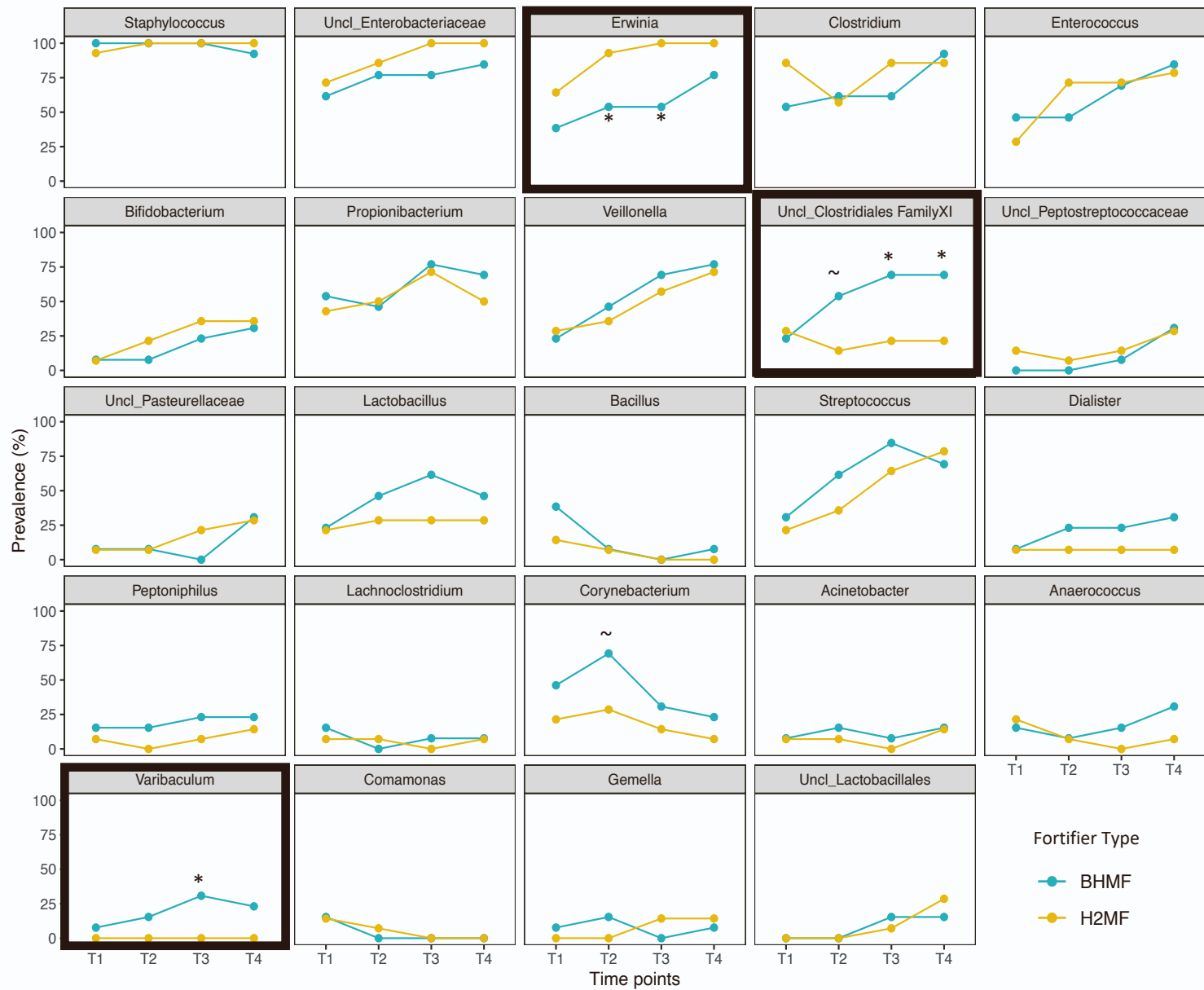
**Figure S4. Impact of human milk fortifier type on gut microbial diversity and trajectories in VLBW infants (related to Figure 3C).** (A) PCoA depicts between-sample diversity based on Jaccard distances (Bray-Curtis distances in **Figure 3C**) in BHMf and H2Mf group infants over time, p-values from PERMANOVA (permutations=99,999). See **Table S1A** for betadisper test results (permutation test for homogeneity of multivariate dispersions). (B and C) Trajectories of bacterial phyla relative abundances (CLR transformed) in each fortifier group over time. P-values from splinectomeR (permutations=999) (D) Red line indicates the spline distance between groups over time. T1 = Study Day 0 (before fortification), T2 = Study Day 7 (during fortification), T3 = Week 33 AGA (end of fortification) and T4 = Week 35 AGA (follow up after fortification). BHMf, bovine-derived human milk fortifier; H2Mf, human-derived human milk fortifier; VLBW, very low birthweight.



**Figure S5. Effect of human milk fortifier type on gut bacterial relative abundances in VLBW infants (related to Figure 3).** Heatmap illustrating relative abundance (CLR transformed) of bacterial genera in individual infants, stratified by the fortifier group. Regression coefficients and FDR-corrected p-values from MaAsLin2, cross-sectionally (at discrete time points) and longitudinally (combined across all time points): \* $p < 0.05$ , \*\* $p < 0.01$ . T1 = Study Day 0 (before fortification), T2 = Study Day 7 (during fortification), T3 = Week 33 AGA (end of fortification) and T4 = Week 35 AGA (follow up after fortification). BHMF, bovine-derived human milk fortifier; H2MF, human-derived human milk fortifier. The prefix ‘Uncl\_’ indicates an unclassified genus of the named bacterial family or order.

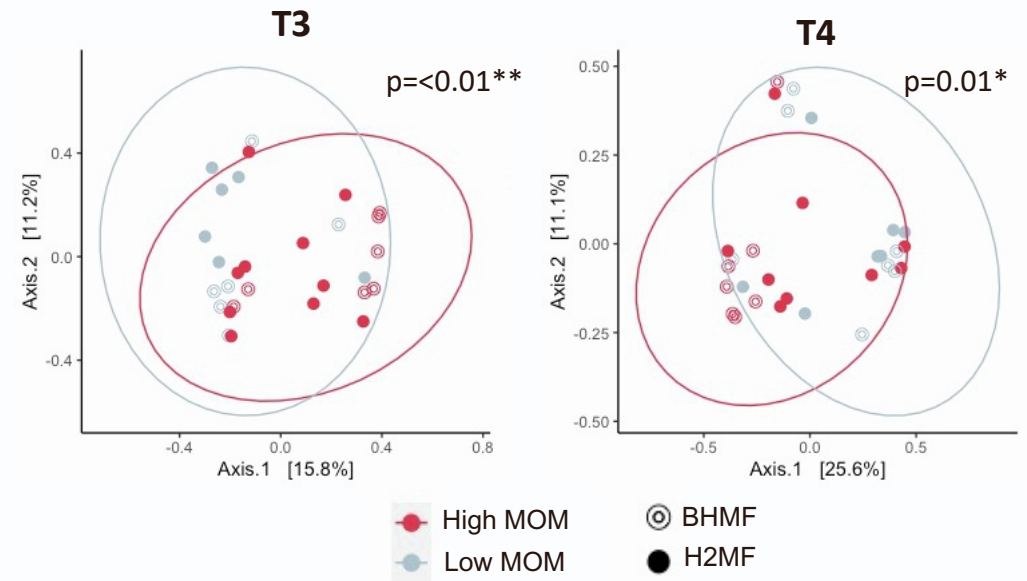
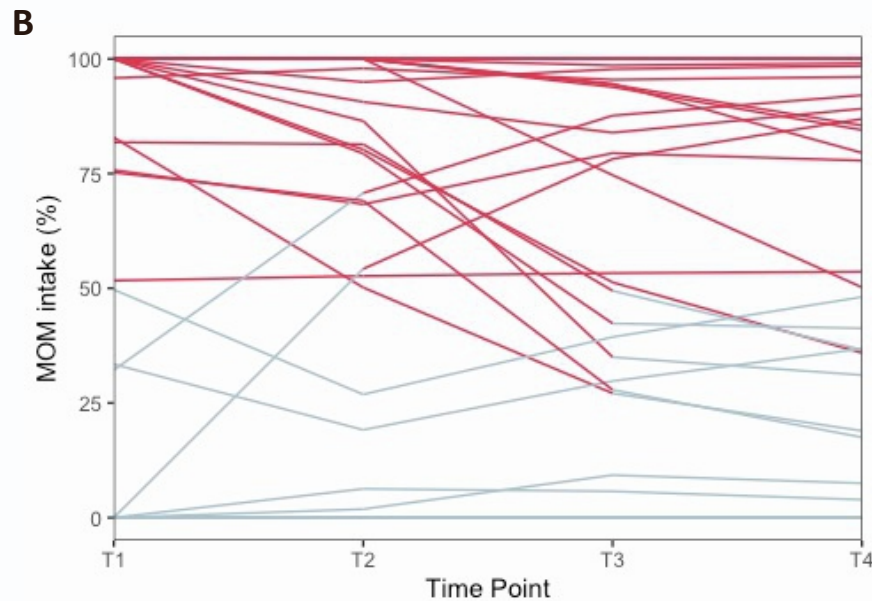
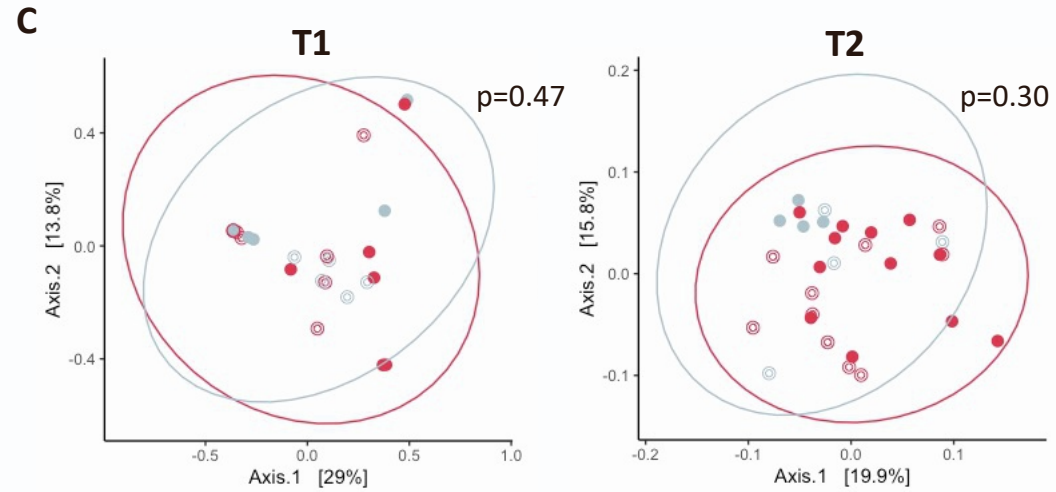
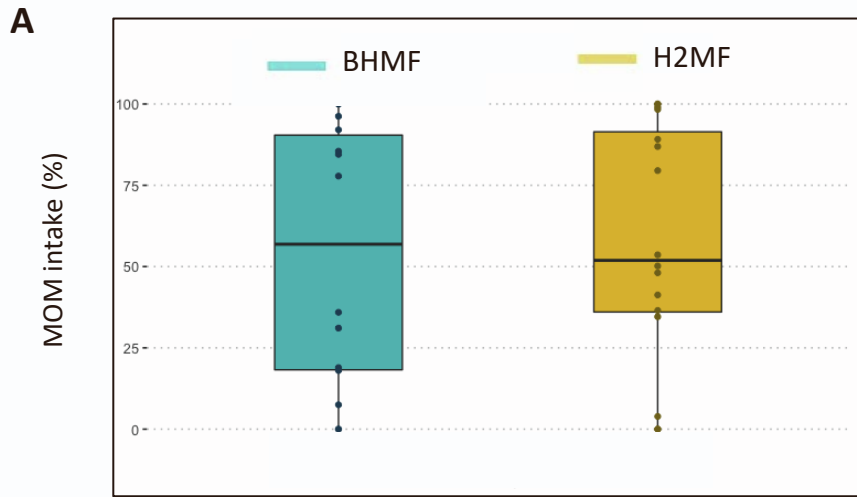


**Figure S6. Impact of fortifier type on gut bacterial relative abundance over time in VLBW infants (related to Figure 3).** Relative abundance (CLR transformed) of bacterial genera at study time points. Significance symbols from differential abundance testing using Wilcoxon sum-rank test; \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ . T1 = Study Day 0 (before fortification), T2 = Study Day 7 (during fortification), T3 = Week 33 AGA (end of fortification) and T4 = Week 35 AGA (follow up after fortification). The prefix 'Uncl\_' indicates an unclassified genus of the named bacterial family or order.

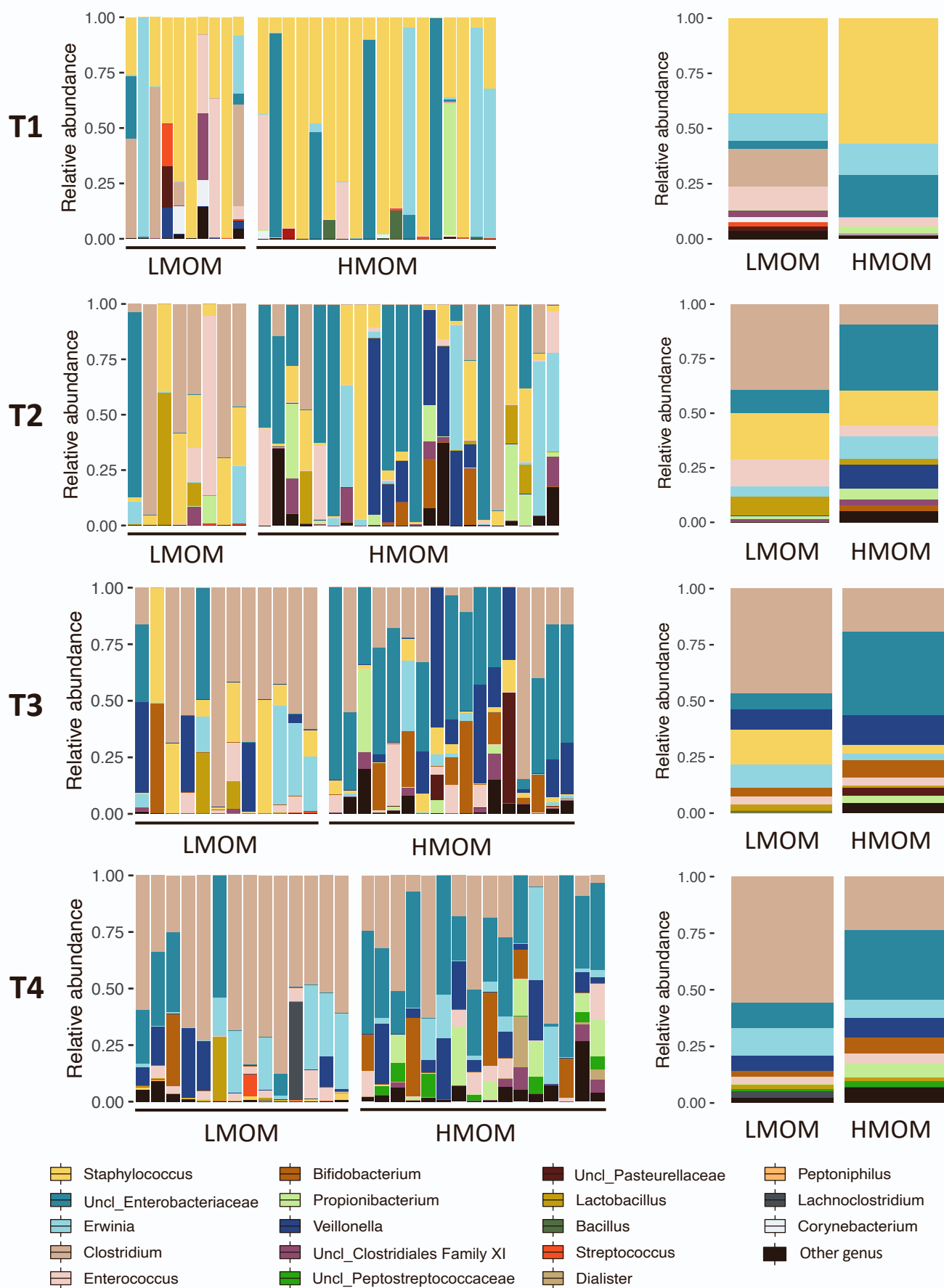


**Figure S7. Impact of human milk fortifier type on the prevalence of gut bacterial genera over time in VLBW infants (related to Figure 3).** Prevalence (%) of each genus across study time points, compared between fortifier groups (Fisher test:  $\sim p < 0.10$ ;  $*p < 0.05$ ). Facets highlighted (black border) indicate significant differences ( $p < 0.05$ ) for at least one time point (see **Table S1B-D** for complete data). T1 = Study Day 0 (before fortification), T2 = Study Day 7 (during fortification), T3 = Week 33 AGA (end of fortification) and T4 = Week 35 AGA (follow up after fortification). BHMf, bovine-derived human milk fortifier; H2MF, human-derived human milk fortifier. The prefix 'Uncl\_' indicates an unclassified genus of the named bacterial family or order.

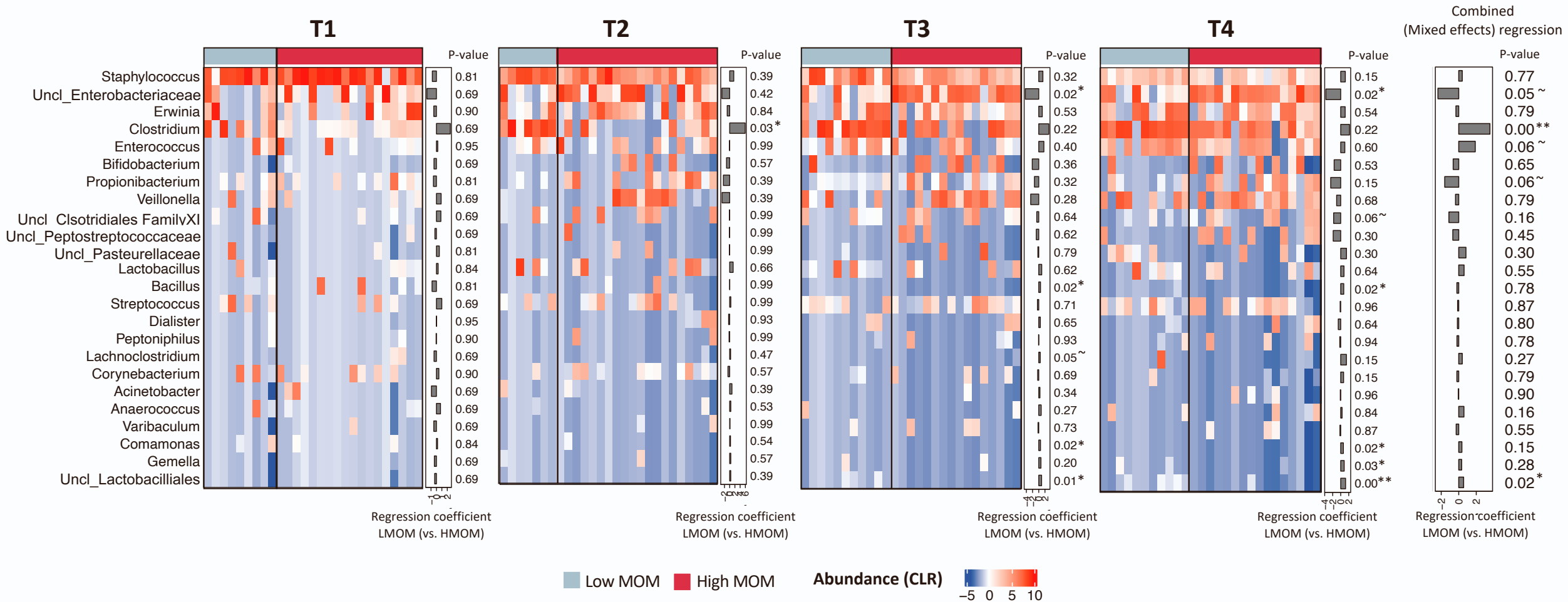




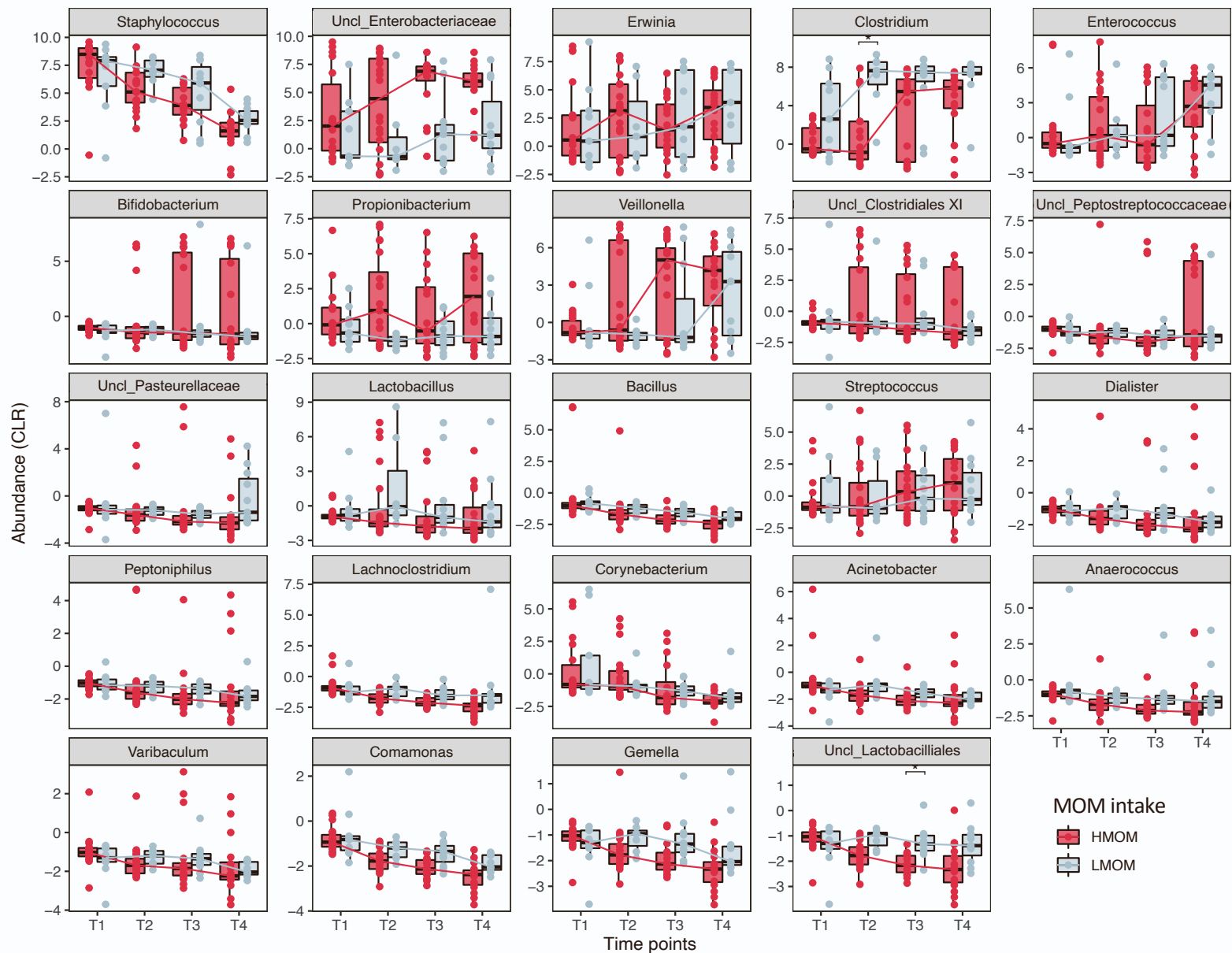
**Figure S8. Distribution and trajectory of MOM intake and its association with gut microbiome composition in VLBW infants (related to STAR methods section: ‘Mother’s Own Milk (MOM) intake’).** (A) MOM proportion used to prepare feeds prior to fortification (average until a particular study time point) received by infants during the total study duration. Infants from both fortifier groups were stratified into two groups: High MOM group: Infants who received %MOM proportion in TEV  $\geq 50\%$  and Low MOM (LMOM): infants who received %MOM proportion in TEV  $< 50\%$ . (B) The trajectory of MOM proportion in feeds in infants receiving high or low MOM proportions at each study time point. (C) PCoA depicts beta diversity between MOM groups (Low MOM and High MOM infants) over time based on Jaccard distances (Bray-Curtis distances in **Figure 4B**), P-values modeled from PERMANOVA for MOM groups (permutations=99,999). See **Table S1A** for betadisper test results (permutation test for homogeneity of multivariate dispersions). T1 = Study Day 0 (before fortification), T2 = Study Day 7 (during fortification), T3 = Week 33 AGA (end of fortification) and T4 = Week 35 AGA (follow up after fortification). BHMf, bovine-derived human milk fortifier; H2MF, human-derived human milk fortifier; MOM, mother’s own milk; VLBW, very low birth weight.



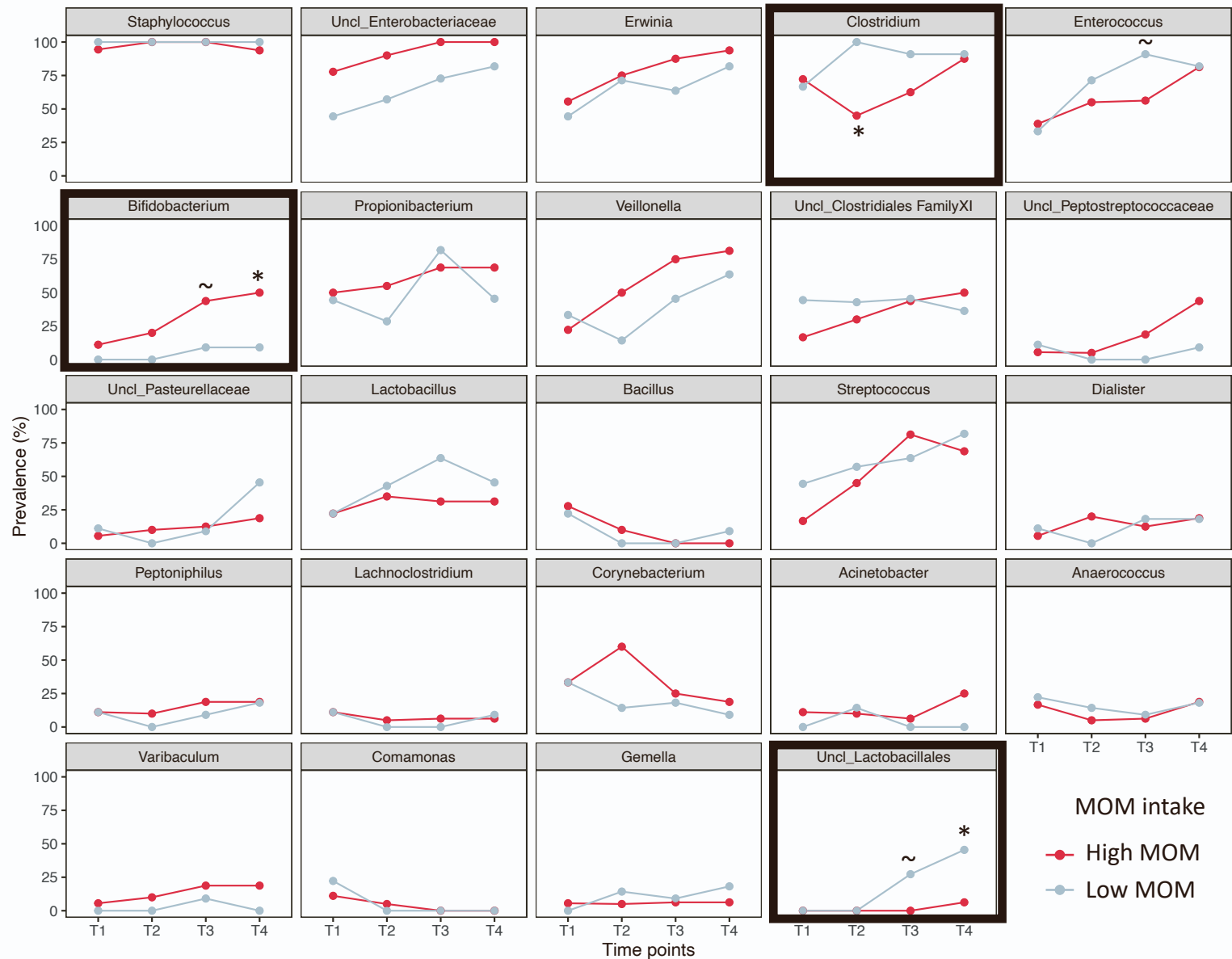
**Figure S9. Association of MOM intake with gut bacterial composition in VLBW infants (related to Figure 5A).** Bar plots depict the relative abundance of gut bacterial genera (Left: individual infants, Right: group means). T1 = Study Day 0 (before fortification), T2 = Study Day 7 (during fortification), T3 = Week 33 AGA (end of fortification) and T4 = Week 35 AGA (follow up after fortification). MOM, Mother's Own Milk; LMOM, Low MOM intake group; HMOM, High MOM intake group; VLBW, very low birthweight. The prefix 'Uncl\_' indicates an unclassified genus of the named bacterial family or order. MOM groups were determined based on %MOM volumes that represent the proportion of MOM used to prepare feeds prior to fortification.



**Figure S10. Association of MOM intake with gut bacterial relative abundances in VLBW infants (related to Figure 5A).** (A) Heatmap illustrates relative abundance (CLR transformed) of bacterial genera with the multivariable association, both cross-sectional (at discrete time points) and longitudinal (combined) with MOM intake using MaAsLin2. Regression coefficient from MaAsLin2, P-values (corrected) indicating the significance of association (\*  $p < 0.05$ , \*\*  $p < 0.01$ ). T1 = Study Day 0 (before fortification), T2 = Study Day 7 (during fortification), T3 = Week 33 AGA (end of fortification) and T4 = Week 35 AGA (follow up after fortification). The prefix ‘Uncl\_’ indicates an unclassified genus of the named bacterial family or order. MOM groups were determined based on %MOM volumes that represent the proportion of MOM used to prepare feeds prior to fortification.



**Figure S11. Association of MOM intake and gut bacterial relative abundance over time in VLBW infants (related to Figure 5A).** Relative abundance (CLR transformed) of bacterial genera at study time points (Significance symbols from differential abundance testing using Wilcoxon sum-rank test; \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ ). Y-axis represents CLR transformed abundances of the respective taxa in each facet. T1 = Study Day 0 (before fortification), T2 = Study Day 7 (during fortification), T3 = Week 33 AGA (end of fortification) and T4 = Week 35 AGA (follow up after fortification). VLBW, very low birthweight. The prefix 'Uncl\_' indicates an unclassified genus of the named bacterial family or order. MOM groups were determined based on %MOM volumes representing the proportion of MOM used to prepare feeds prior to fortification.



**Figure S12. Association of MOM intake and prevalence of gut microbiota genera over time in VLBW infants (related to Figure 5A).** Prevalence (%) of each genus across study time points, compared between MOM intake groups (Fisher test:  $\sim p < 0.10$ ;  $*p < 0.05$ ). Facets highlighted (black border) indicate significant differences ( $p < 0.05$ ) for at least one time point (see **Table S1E-G** for complete data). T1 = Study Day 0 (before fortification), T2 = Study Day 7 (during fortification), T3 = Week 33 AGA (end of fortification) and T4 = Week 35 AGA (follow up after fortification). BHMF, bovine-derived human milk fortifier; H2MF, human-derived human milk fortifier; MOM, mother's own milk used to prepare feeds prior to fortification. The prefix 'Uncl\_' indicates an unclassified genus of the named bacterial family or order.