Bacterial and Fungal Gut Community Dynamics Over the First Five Years of Life in Predominantly Rural Communities in Ghana

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Abstract

Background

Bacterial and fungal microbiotas are increasingly recognized as important in health and disease starting early in life. However, microbiota composition has not yet been investigated in most rural, low-resource settings, and in such settings, bacterial and fungal microbiotas have not been compared. Thus, we applied 16S and ITS2 amplicon sequencing, respectively, to investigate bacterial and fungal fecal microbiotas in rural Ghanaian children from birth to 5 years of age. Corresponding maternal fecal and breastmilk microbiotas were additionally investigated.

Results

While bacterial communities differed systematically across the age spectrum in composition and diversity, the same was not observed for the fungal microbiota. We also identified a novel and dramatic change in the maternal postpartum microbiota. This change included much higher abundance of *E. coli* and much lower abundance of *Prevotella* in the first versus fourth week postpartum. While infants shared more bacterial taxa with their mother's stool and breastmilk than with those of unrelated mothers, there were far fewer shared fungal taxa.

Conclusion

Given the known ability of commensal fungi to influence host health, the distinct pattern of their acquisition likely has important health consequences. Similarly, the dynamics of mothers' bacterial microbiotas around the time of birth may have important consequences for their children's health. Both topics require further study.

Introduction

The dynamics of the bacterial gut microbiota have been well studied, but mostly in resource-rich settings. These surveys have shown microbial diversity to increase with age and reach adult-like profiles by 2-3 years[1-6]. The breastmilk bacterial microbiota has also been studied using culture-based[7-9] and culture-independent[10, 11] approaches, which revealed its potential to seed the infant intestinal microbiota[12]. These findings together with disease-specific surveys have identified the bacterial microbiota as a key factor shaping health in early life[13].

In contrast, the fungal gut microbiota has been far less studied, despite emerging evidence that fungi are also key modulators of health and disease[14, 15]. Examples include recent mouse studies demonstrating that fungi effectively calibrate mucosal immune responses with an emphasis on Th17 immunity and neutrophil function[14] and that colonization with *Candida albicans* increases survival of systemic viral challenge[15]. Sequence-based surveys of infant fungal microbiotas have also revealed that a higher abundance of specific fungal taxa is associated with the development of allergic disease later in

childhood[16]. The human fungal microbiota in early life has been surveyed in very few studies, and in select populations: Europe (Norway, Florence and Luxembourg) and America (Puerto Rico and Ecuador) [16-20]. Based on diverse study designs, *Candida, Aspergillus*, and *Saccharomyces* feature prominently in newborns and older infants. There is, however, no clear age-dependent trajectory of fungal microbiota taxonomic composition[18-20] or diversity[19, 20] in the first two years of life, aside from lower diversity in 10-day-old newborns versus 3-month-olds or their mothers[18]. There were also no clear trajectories identified during the first month of life in a more granular assessment of the newborn fungal microbiota[17].

The breastmilk fungal microbiota has only recently been profiled using a culture-independent approach[21], revealing dominance by *Malassesia* spp. also commonly found on skin and compositional differences across diverse populations, which were confirmed in separate studies[22, 23]. Beyond this, the fungal composition of human breastmilk has not been investigated.

Despite the importance of both the bacterial and fungal gut microbiota in influencing the developmental trajectory towards health or disease, to our knowledge these two have never been jointly investigated in early life, which is likely the most important period of development. Equally surprising, despite the well-established fact that the maternal stool and breastmilk microbiotas shape the newborn's microbiota, no study so far has captured this interaction between mother and infant across the bacterial and fungal domains. We thus conducted a cross-sectional bacterial and fungal microbiota survey encompassing fecal microbiotas of children in the first 5 years of life plus fecal and breastmilk microbiotas of mothers of children 6 months and younger.

Methods

Study Participants

Study participant recruitment, Study participants were recruited in the Kintampo North Municipality, located in the former Brong Ahafo Region (now Bono-East Region) in the middle-belt of Ghana, and serving as a part of the Kintampo Health Research Centre (KHRC) study area. Prospective participants were selected from the database of the Kintampo Health and Demographic Surveillance System (KHDSS). Expectant mothers, or mothers of older children, were approached recruited into the study were approached during pregnancy. Inclusion criteria for this study were for participants to be (a) living in the study area, (b) healthy with no known congenital defects, (c) under 63 months, and (d) to have provided written informed consent. An exclusion criterion was refusal to provide informed consent. Study participant recruitment was stratified by the age groups outlined: the newborn period, (day of life; DOL 0-5; DOL 13-17; DOL 26-35); 3 months (DOL 83-115), 6 months (DOL 165-200), 1 year; month of life (MOL) 11-13; 2 years, MOL 22-26; 3 years, MOL 33-39; and 5 years, MOL 57-63.

Microbiota sample collection and analysis

Stool and Breastmilk Sample collection. Field workers supplied mothers with sterile containers and scoops to collect stool samples from soiled diapers. Stool samples were collected from the diapers of children with sterile plastic spoons transported to the clinical laboratory at the KHRC. Additionally, mothers of participants who were 0 to 1 or 4 to 5 weeks old and were exclusively breastfed were asked to provide stool and breast milk samples in supplied sterile containers. All samples collected in the study were kept in cold boxes with ice-packs and sent to the laboratory within 2 hours. At the clinical laboratory, stool and breastmilk samples were split into multiple 1.0-ml aliquots and stored at -80^{o}C until further analysis. Biospecimens were transported to the University of British Columbia on dry ice with temperature monitoring via World Courier Inc.

Stool DNA extraction for amplicon sequencing. DNA was extracted from swabs using the MagAttract PowerSoil DNA KF kit (Qiagen Cat. No. 27000-4-KF) using the manufacturer's protocol for the KingFisher Flex platform with the following modification using approximately 200 mg bulk stool loaded into each 96-well plate with sterile wooden picks.

Breastmilk DNA extraction for amplicon sequencing. Total DNA was extracted from 0.7 – 1.0 µL breastmilk using the Qiagen DNease PowerSoil DNA extraction kit (Qiagen Cat. 12830-50) with the following modifications: frozen breastmilk samples were thawed on ice and transferred to 2.0 mL screw-top tubes (VWR Cat. 211-0440) and spun for 10 min at 4^{o}C at 20,000 g to pellet all particles. The lipid portion of the milk remained on top of the aqueous phase after centrifugation. The lipid layer was retained, and the aqueous phase between the pellet and whey layer carefully removed with a 200 µL micropipette. The contents of one Qiagen 0.1 mm glass bead tube (Qiagen 13118-50) was then added to each sample with 500 µL Bead Solution and 200 µL phenol:chloroform:IAA pH 7-8 (Ambion Cat. AM 9730, pH adjusted with included Tris buffer). 60 µL of C1 was then added, and samples were homogenized in a Fasprep bead mill for 30s at 5.5 m/s for two cycles with a 5 min wait in between. The remainder of the protocol was carried out according to manufacturer's instructions and samples eluted in 2 x 30 µL (total of 60 µL).

16S amplicon sequencing

Samples were submitted to Microbiota Insights Inc. (Vancouver, BC, Canada) for PCR amplification and DNA sequencing. Library preparation was done using a previously published protocol[24] with the details and product information outlined below. 10 µl of the final product was used to normalize using the SepalPrep Normalization Prep Plate Kit (ThermoFisher Cat. A1051001) to 1-2 ng/µl and 5 µl of each normalized sample was pooled into a single library per 96-well plate. Library pools were further concentrated using the DNA Clean and Concentrator kit (Zymo Cat. D4013). A dilution series was performed for each of the pooled libraries for subsequent quality control steps. Each pool was analyzed using the Agilent Bioanalyzer using the High Sensitivity DS DNA assay (Agilent Cat. 5047-4626) to determine approximate fragment size, and to verify library integrity. Library pools with unintended amplicons were purified using the Qiagen QIAquick Gel Extraction Kit (Qiagen Cat. 28706). Pooled library concentrations were determined using the KAPA Library Quantification Kit for Illumina (KAPA Cat.

KK4824). The final libraries were loaded at 8 pM, with an additional PhiX spike-in of 20%. The amplicon library was sequenced on the MiSeq using the MiSeq 500 Cycle V2 Reagent Kit (Illumina Cat. MS-102-2003)

Paired-end reads were assembled using MOTHUR version 1.37.2, following the MiSeq SOP[25] (https://www.mothur.org/wiki/MiSeq_SOP; accessed Aug 2018). Operational Taxonomic Units (OTUs) were clustered at 97% identity and classified using the GreenGenes 13_8_99 database[26].

ITS2 amplicon sequencing

Processing for ITS2 amplicon sequencing were identical to those used for 16S amplicon sequencing, except with the following differences: Primers and PCR conditions were previously described[27]. Paired-end reads were trimmed at the non-overlapping ends, and high-quality reads classified using UNITE (v. 7.1)[28].

16S and ITS2 sequencing Quality control (QC).

The potential for contamination was addressed by co-sequencing DNA amplified from specimens and from four each of template-free PCR controls and extraction kit reagents processed the same way as the specimens (two for breastmilk specimens). Two positive controls, consisting of cloned SUP05 DNA, were also included (number of copies = 2*10^6). Breastmilk samples were extracted and PCR amplified separately from the stool samples but sequenced on the same lane. Given the different distribution of contaminating OTUs from breastmilk blanks compared to stool, QC was done for the breastmilk samples separately. Contaminating OTUs can come from two main sources: from PCR and extraction reagents, and carry-over from neighboring samples during the extraction and amplification process. OTUs were considered reagent contaminants and removed from the dataset if they were present in over 50% of blank samples and their count geometric mean plus one standard deviation was greater than that in the samples. Using this approach, OTUs with high counts in many samples (mainly including taxa commonly known to be highly abundant in these sample types) were not flagged for removal, despite their presence in blanks. For OTUs whose mean counts were high in the samples but also found in over 50% of blanks, the mean count plus standard deviation from the blanks was subtracted from each of the samples. Samples with total counts under 1000 were also removed. Once filtered, data from breastmilk and stool were recombined for further analysis.

Statistical Analysis

Alpha diversity. We used the *phyloseq* R package[29] to compute alpha and beta diversity. We estimated microbial diversity with abundance-dependent (Shannon) and independent (Observed richness) metrics, after subsampling the OTU table to account for unequal sampling depth. To test for relationships between age, we used the Wilcoxon test comparing each age bin to the Mothers of DOL 26-35 infants, as this was considered the adult microbiota group. P-values were adjusted using the Bonferroni correction and adjusted p-values under 0.05 considered significant.

Community composition. Beta diversity was computed using the Bray-Curtis index. Sample clustering was visualized using non-metric-multidimensional scaling (NMDS). To test whether child age (all age bins except maternal samples) explained community composition, we used the Adonis test from R package *vegan*[30] using age in days calculated between the time interval between participant's date of birth and visit date. To test if community composition between 5-year-olds (age bin MOL 57-63) and younger children differed, we computed the Bray-Curtis distance of individuals in each bin to their distance to all individuals in the MOL 57-63 bin. The Wilcoxon rank-sum test was then used to compare distances between each bin to the median distance among the 5-year-olds to others in the same bin. P-values were adjusted using the Bonferroni correction and adjusted p-values under 0.05 considered significant.

Differential Abundance. To identify OTUs differentially abundant between mothers of 0-5 day old vs. 26-35 day-old infants, we used the wald test implemented in the R package *DESeq2*[31]. Differential abundance was considered significant if the Benjamini-Hochberg adjusted p-value was less than 0.05. To further visualize select bacterial genera differentially abundant between maternal groups and their abundance across the age spectrum, we aggregated OTU counts by genus-level assignment and confirmed that the genera differed between groups using the Wilcoxon rank-sum test.

Shared OTUs between mother and infant. For shared OTU analysis, OTUs were considered present if their relative abundance was over 0.01%. For stool and breast milk separately, we calculated the number of shared OTUs between newborns and their mothers and newborns and unrelated mothers. Because the number of unrelated pairs was much greater than related pairs, we randomly sub-sampled the unrelated pairs, selecting the same number as related pairs in each analysis. The median number of shared OTUs between related and unrelated pairs and 0-5 and 25-36 days post-partum was then compared using the Wilcoxon rank-sum test.

Results

Sample summary: amplicon sequencing

After quality filtering, we retained a total of 2.9 million reads, representing 4492 bacterial OTUs. These OTUs represented 435 unique taxa classified at the Genus level or above. Five samples yielded fewer than 1000 reads and were excluded from downstream analysis. The remainder yielded between 2.6 and 31.2 thousand reads per sample, with most fecal samples yielding greater than 10 thousand (Supl. Figure 1A).

After quality filtering, we retained a total of 2.5 million reads representing 1772 fungal OTUs, representing 375 unique taxa classified at the Genus level or above. Fifty-nine samples contained fewer than 1000 reads and were excluded from downstream analysis. The remainder contained between 1.2 and 96.5 thousand reads per sample (Supl. Figure 1B). Samples with no detectable fungi over our count threshold were found in both infant and adult stool samples alike (Supl. Figure 1C). The proportion of samples in each age stratification with no detectable fungi was highly variable, but usually below 50%.

Bacterial and fungal alpha diversity across the age spectrum. To measure alpha diversity, we used both observed richness, the number of distinct OTUs present, and the Shannon Diversity index, which reflects both richness and evenness. We compared diversity of fecal communities in each age bin to that of the one-month postpartum mothers, representing an adult microbiota. Alpha diversity of fecal bacterial communities increased with age. Observed bacterial richness was lower than that of adults until 3 years, and Shannon diversity, until 2 years (Figure 1A). Fungal fecal microbiota diversity of only a few age bins differed from adult diversity, otherwise there was no clear trend for fungal diversity versus age, except that it was lower during the first week of life (Figure 1B). Interestingly, the mothers of those newborns, also had lower fungal richness than one-month postpartum mothers. Most samples, especially from after 2 years of age contained fewer fungal OTUs (less than 60 OTUs in most samples), while between 100 and 300 bacterial OTUs were detected during this time.

Bacterial and fungal communities across the age spectrum. Beta diversity (community composition) of fecal bacterial communities differed across the age spectrum and, expectedly, was distinct from breastmilk bacterial communities (Figure 2A-B). There was no such trend for fungal fecal communities. While child age contributed to 18% of the variance of fecal bacterial communities (PERMANOVA $p = 0.001$), it accounted for only 2% of variance in fecal fungal communities (PERMANOVA $p = 0.015$). We did not identify one NMDS axis that drove an age gradient for either community. To further assess community composition across the age spectrum, we determined the similarity of communities in each age bin to those at 5 years. Fecal bacterial communities from all age bins, except 3 years, differed from those at 5 years (Figure 2C). Expectedly, fecal fungal communities did not follow this trend.

Relative abundances of the 25 most abundant Genera clearly revealed bacterial taxonomic shifts over the age spectrum (Figure 3A). In keeping with the literature on bacteria[4, 32], newborns were dominated by *Escherichia coli* in the first week of life, and it remained abundant up to 6 months. Then, *Bifidobacteria* were dominant from 1 month to 1 year. Subsequently, the diversity of genera increased and *Prevotella* became dominant, similar to the adult microbiota. *Bacteroides* was abundant during the first year, until the increase in *Prevotella.* Children were either dominated by *Bacteroides* or *Prevotella* at any given time (Supl. Figure 2). *Faecalibacterium* was abundant after the first year. The fecal fungal microbiota was dominated by *Candida* (primarily *Candida albicans* and *Candida tropicalis*) in children of all ages (Figure 3B). *Malassezia* featured prominently in the first 3 months of life, while *Aspergillus* was prominent after the first year.

Maternal bacterial microbiota. Our study revealed dramatic differences in bacterial stool microbiotas of mothers in the first postpartum week versus one month postpartum (Figure 3A). We further explored this difference by measuring the Bray-Curtis distance between mothers' microbiotas at both time points to their respective children at 1 week, finding that mothers in the first versus fourth post-partum week harbored a bacterial microbiota significantly more similar to that of their child (Figure 4A). We identified 68 OTUs differentially abundant between the two mother groups, representing 34 taxa at the Genus level or above (Figure 4B). Abundances of some taxa obviously contributed to the similarity of microbiotas of mothers 1 week postpartum and their children at 1 week. Thus, in both mothers and infants from one to

four weeks after birth, *Prevotella* had increasing abundance, while *Escherichia* had decreasing abundance (Figure 4C,D). Other taxa, such as *Faecalibacterium* and *Blautia,* were more abundant in mothers 1 week versus 4 weeks postpartum, yet were virtually absent in their infants at 1 week. We also found that, at 1 week, newborns share more bacterial but not fungal OTUs with their mother versus with unrelated mothers, but this was no longer the case at 1 month (Supl. Figure 3A,B). OTUs most commonly shared by mother-infant pairs include members of *Escherichia, Enterococcus,* and *Streptococcus,* all of which are of higher relative abundance in newborns than in their mothers (Supl. Figure 3C). Only two fungal OTUs were commonly shared between mother-infant pairs (Supl. Figure 3D).

Bacterial and fungal communities in mother's breastmilk. Breastmilk is a well-recognized modulator of the infant gut microbiota, serving both as a source of colonizing bacteria and bacterial growth substrates. The relationship of breastmilk and the fungal microbiota has not been well described. Thus, we compared the bacterial and fungal community composition of both breastmilk and fecal microbiotas. Breastmilk microbiotas were typically dominated by skin taxa of both bacteria (*Streptococcus,Staphylococcus,* or *Corynebacterium;* Figure 5A) and fungi (*Malassezia*, including *M. restricta, M. globosa, and M. furfur;* Figure 5B). Alpha diversity did not differ between breastmilk samples collected in the first or fourth post-partum week for either bacteria or fungi. Only minor differences in community composition were found between the first and fourth post-partum week, including only eight differentially abundant bacterial OTUs (Figure 5C) and no differentially abundant fungal OTUs. For bacterial communities, mother-infant pairs shared significantly more OTUs than unrelated pairs at both postpartum time points (Figure 5D). While fungal data was available for fewer pairs, we found a significant difference between related and unrelated pair sharing 4 weeks postpartum (Figure 5E). The most commonly shared bacterial OTUs belonged to typical skin commensal genera, *Streptococcus* and *Staphylococcus*, which had higher relative abundance in the breastmilk than in the newborn stool. Shared OTUs between mother and infant pairs also included gut commensals *Bifidobacterium* and *Bacteroides,* both of which were of higher relative abundance in the newborn's stool than in their mother's milk (Supl. Figure 4A). Shared fungal OTUs were much more limited, and did not have a clear pattern of differing abundance between mother and child (Supl. Figure 4B).

Discussion

Here, we report for the first time the bacterial and fungal microbiota dynamics during early childhood. In conducting a direct comparison between bacterial and fungal gut communities, we demonstrated how the two follow very distinct developmental trajectories, having contrasting associations with the mother's microbiota. We also found a previously unreported and surprising compositional change in maternal stool microbiotas during the postpartum period.

We found undetectable levels of fungi in a large subset of stool samples from children and mothers in a rural African setting. This finding is consistent with a previous study in Norway, an urban high-resource setting[18]. We additionally confirmed that the samples with undetectable fungi, including those from the youngest age groups, contained readily amplifiable bacterial genes providing further evidence that the

lack of detectable fungi in these samples reflected their absence or extremely low abundance, rather than sample processing artefacts. However, lysis methods that successfully lysed bacteria may have failed to lyse fungal cells. Interestingly, most breastmilk samples were positive for fungi, especially those from 4 weeks postpartum, while we did not detect fungi in half of the corresponding breastfeeding infants.

As in our study, increasing bacterial[4, 6], and unchanging fungal[19, 20] richness and diversity during infancy were previously reported, but never together in the same study. However, the timing of increases in bacterial richness and diversity in our study differed from those in other studies in both high- and low-resource regions. In our Ghanaian cohort, richness and diversity remained stable over the first year of life and dramatically increased thereafter. In studies of American and European cohorts, alpha diversity steadily increased from birth to two years[6] or 3 months to 5 years[4], respectively. Numbers of observed OTUs increased steadily from birth to 3 years among Amerindian and Malawian infants[1], and from birth to two years in an Indian setting[33]. The distinct timing in our study may reflect lifestyles, diet or an environment unique to this study cohort in Ghana.

The bacterial succession we observed, with sequential dominance by *E. coli*, *Bifidobacterium,* and *Prevotella,* as well as sequential high abundance of *Staphylococcus,Streptococcus,Bacteroides,* and *Faecalibacterium,* was consistent with previous studies of newborn and adult microbiotas[4, 32]. The Bacteroidetes phylum in older children and adults is typically dominated by either *Bacteroides* or *Prevotella*[1, 34, 35]. *Prevotella* was dominant in adults in rural Ghana, as demonstrated among the one month post-partum women in our study. We found that *Bacteroides* was abundant in the Ghanaian infants prior to 1 year, after which, *Prevotella* colonized the children and became dominant. The ability of *Bacteroides* species to utilize human milk oligosaccharides (HMOs)[36]as well as diet-derived polysaccharides[37] as an energy source, is thought to contribute to their unique ability to persist in both infant and adult guts. However, *Prevotella* appears to outcompete *Bacteroides* in adults with a fiber-rich diet [38]. This transition occurs early in life in this Ghanaian population, probably associated changes to the child's diet at this time.

We found dominance of the gut fungal microbiota of some children by *Candida sp.*, as has been found in some[17, 20] but not other[16, 19] studies, where additional fungal taxa, such as *Aspergillus* and *Penicillium,* featured prominently. We also detected an early increase in the relative abundance of *Malassezia* that was not seen in other studies of newborns[17, 18]. Given the high relative abundance of this taxon in breastmilk in our study and another[21], and evidence of its' transmission from mother to newborn[39], absence of this taxon in other studies is puzzling but may reflect regional differences in maternal breastmilk fungal microbiotas, which have not been well-studied.

We were surprised to find substantial differences between the gut bacterial microbiotas of mothers 1 week versus 4 weeks postpartum. These dramatic differences were not due to cross-contamination between maternal and infant samples, as mothers in both groups were sampled using the same protocol and time points, and samples from both groups were processed simultaneously. Given the cross-

sectional study design, we cannot definitively conclude that the microbiota changed in these women over this time. However, both the 3-month recruitment window and the greater microbiota similarity of mothers and their infants 1 week postpartum imply that such a change is the most likely interpretation of the data. Studies of the stool microbiota during pregnancy have yielded mixed results, with some studies showing no change in community composition during pregnancy[40, 41] and others showing substantial shifts[42, 43]. The latter two studies showed an increase in Proteobacteria during pregnancy, which is consistent dominance of Proteobacteria that we observed 1 week postpartum. However, our finding of a low relative abundance of *Prevotella* 1 week postpartum is novel, and was one of the greatest differences we observed in the postpartum period. One week postpartum, we also observed a high relative abundance of *Faecalibacterium,* associated with decreased inflammation and protection from inflammatory bowel diseases[44], and *Blautia*, negatively associated with type 1 diabetes[45] and gestational diabetes melittus (GDM) without prescribed dietary intervention [43]. Together, our findings suggest that differences in maternal microbiota around the time of birth reflect both increases in taxa common to newborns (*Escherichia*) and decreases in taxa absent in newborns (*Prevotella*), as well as increases in abundance of taxa not abundant in infants but with potential health benefits to the mother (*Faecaibacterim* and *Blautia*).

We did not identify fungal taxa that were differentially abundant in the gut microbiota 1 week versus 4 weeks postpartum. We also did not identify many commonly shared fungal OTUs between pairs. In contrast to the bacterial gut microbiota, the fungal gut microbiota of mothers does not appear to be substantially affected by postpartum time and does not appear to strongly influence colonization of the infant's gut.

We found that common skin taxa dominated breastmilk bacterial (*Streptococcus, Staphylococcus*) and fungal (*Malasezzia*) communities, as previously shown[11, 21, 46, 47]. When dominant in a mother's breastmilk, OTUs of these taxa tended to be present at lower relative abundance in her infant's gut microbiota, consistent with the breastmilk being a source for these organisms colonizing the infant gut. However, it is important to note that detection of their DNA in breastmilk and feces does not demonstrate that these OTUs colonize, or even survive these two environments. Further investigation is required to determine if these skin taxa have any function in breastmilk or the gut. OTUs of common gut taxa (*Bifidobacterium, Bacteroides, Lactobacillus* and *Rothia*) were also frequently shared between a mother's breastmilk and her infant's gut microbiota, as previously reported[46]. However, the gut commensals tended to have higher relative abundance in the gut than the breastmilk, suggesting that the infant's (or mother's) gut was a source for these organisms colonizing the breastmilk. Overall, our study identifies strong and novel associations between the microbiotas of breastmilk and the infant gut, particularly in the case of bacteria versus fungi.

With its cross-sectional design, this study cannot definitively prove that differences among age groups represent microbiota changes over time; although, such changes are the most compelling interpretation

of the results. Sample size, a lack of data on infant and mother health, and on mode of delivery, limited the analysis to effects of age and not other host factors that also may have influenced the microbiotas.

Conclusion

The goal of this study was to assess, for the first time, the bacterial and fungal gut microbiota composition of children in Ghana to help identify microbial signatures relevant to the health of women and their children. In doing so, we not only identified bacterial and fungal community dynamics shared with other study cohorts, but also identified characteristics not previously reported. Unique to the Ghanaian cohort, alpha diversity was low and stable during the first year of life, and *Prevotella* became the dominant bacterial genus between the first and second year. Also, the mother's bacterial microbiota changed drastically between 1 week and 4 weeks postpartum. We also contrasted fungal and bacterial community composition across early life in a consolidated study. While mothers are a major source of bacteria colonizing newborns, the sources of colonizing fungi are less apparent.

Future Directions

This small cross-sectional study broadly assessed ecology mother-newborn bacterial and fungal microbiotas, together with the microbiota in children over the first five years in rural Ghana. Crucial insights from this study inform future approaches to identify microbial signatures relevant to the health of women and their children. Based on our findings, longitudinal studies will address the following questions:

1. What demographic or environmental factors explain differences in maternal microbiotas after childbirth? And are these changes associated with maternal health or disease?
2. Are there host microbiota (bacterial and fungal) signatures associated with increased risk for, or protection from severe disease, especially in the newborn period?
3. Are commensal fungi identified in mother-infant pairs transient, or do they represent a stable community of commensal microbes co-inhabiting the gut with bacteria?

These studies will inform novel interventions targeting the microbiota, to improve the health of mothers and their children.

Declarations

Ethics approval and consent to participate

All work in this study was in accordance to ethical guidelines at the University of British Columbia and done under the approved ethics protocol number H11-01423. Separate ethical approval was obtained from the Kintampo Health Research Centre Institutional Ethics Committee (KHRCIEC_2016-14).

Consent for Publication

Not Applicable

Availability of data and material

The datasets supporting the conclusions of this article are available in the National Centre for Biotechnology Information Sequence Read Archive (NCBI SRA) accessible via the following URL: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA658595.

During the peer-review process, codes produced to analyze these data are available through Dropbox, https://www.dropbox.com/sh/iedyqnghu4ugdku/AAB2z69zvRxjM6pinyG7nju0a?dl=0.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

SOA, UG, NA, TRK, KPA, YE and PP contributed to the study design. UG, YE, SK, and DD facilitated participant recruitment and sample collection. NA and WWM generated the amplicon sequencing data. NA performed the statistical analyses and prepared the manuscript. WWM and SOA substantially edited the manuscript. All authors contributed to editing the manuscript.

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Figures

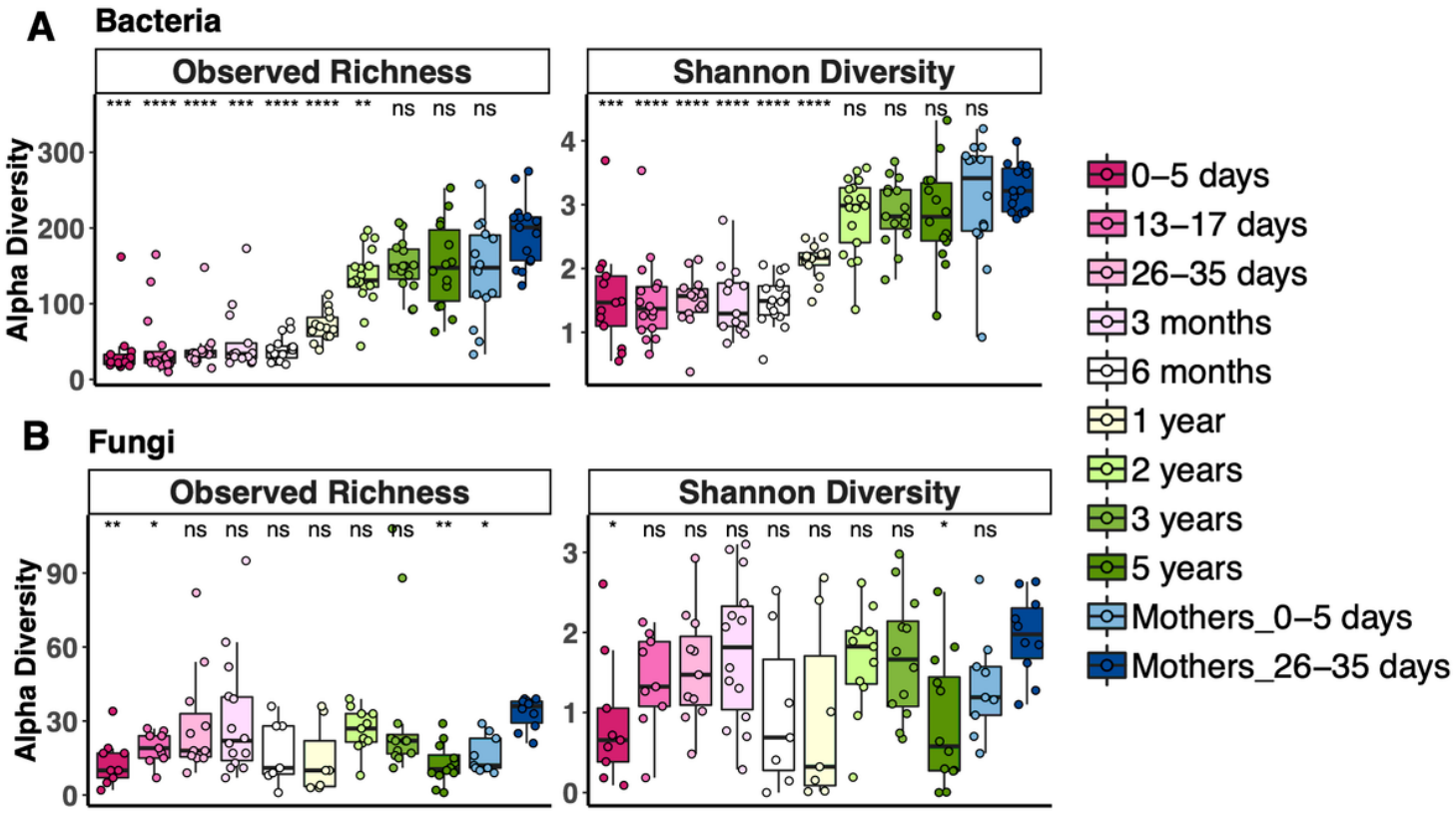

Figure 1

Alpha diversity across the age spectrum for bacterial fecal communities. (A) and fungal fecal communities (B). Coding indicates values significantly different from those of adult communities (Mothers of 26-35 day-old newborns): **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$; Wilcoxon Rank Sum test adjusted using the Bonferroni correction. Boxplots indicate medians with first and third quartiles (25% to 75%). Whiskers extend no further than 1.5*IQR from the hinge.

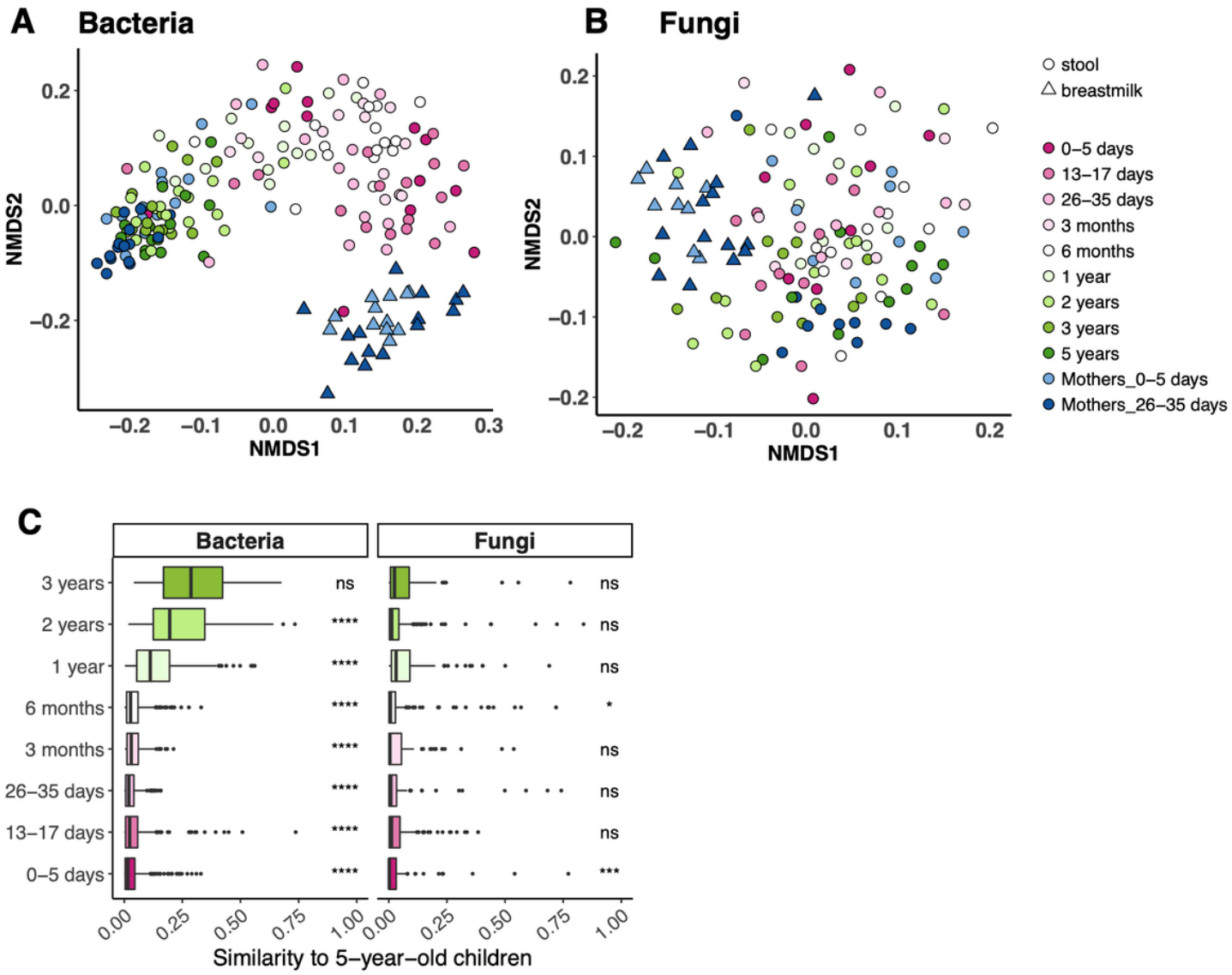

Figure 2

Bacterial and Fungal communities across the age spectrum. A-B. Ordinations of community composition based on Bray-Curtis distance for bacterial (A) and fungal (B) fecal communities. C. Bray-Curtis distance between each age bin and 5-years. **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, Wilcoxon Rank Sum test, adjusted using the Bonferroni correction.

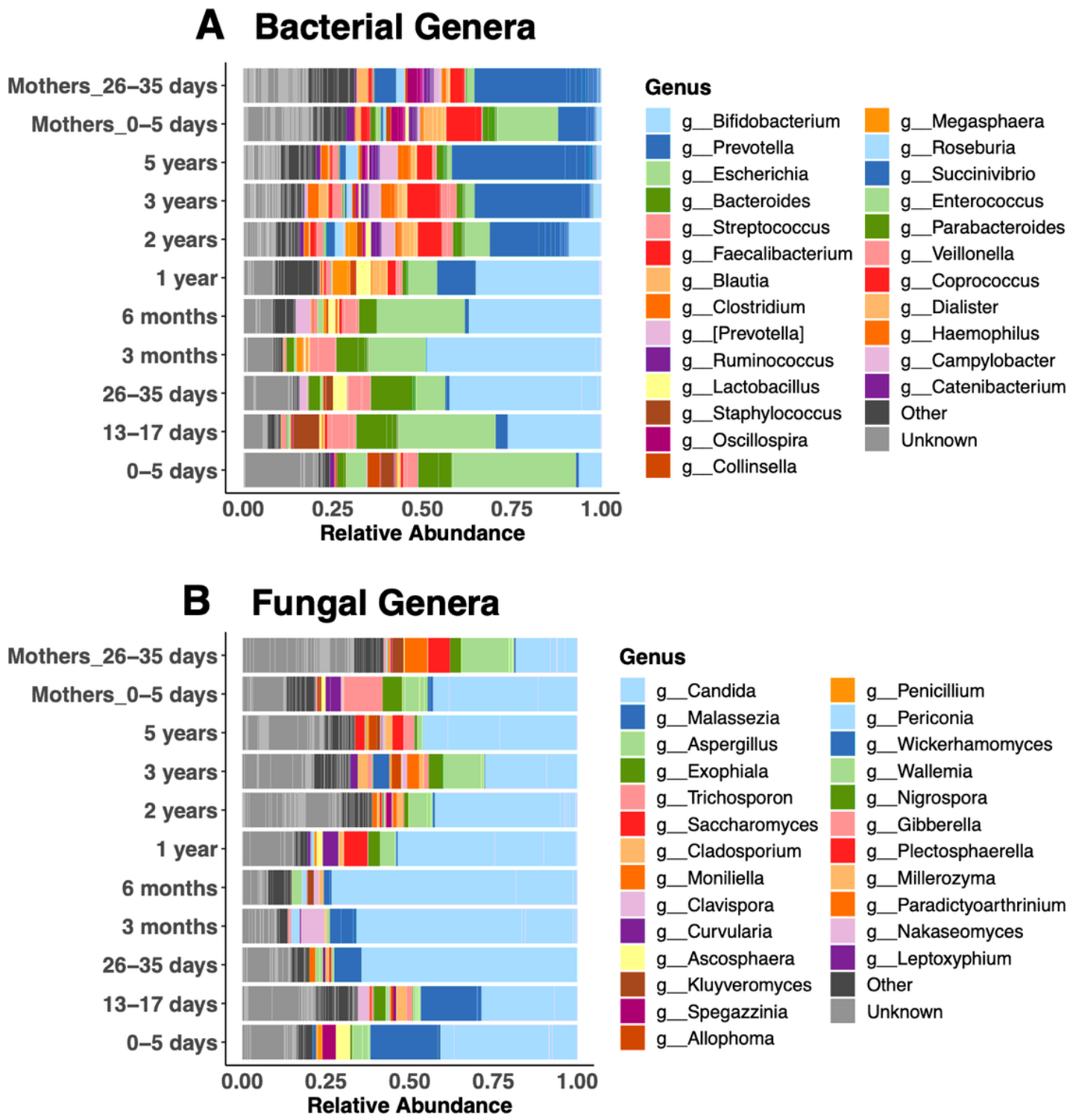

Figure 3

Relative abundance of the 25 most abundant genera. Bacterial (A) and fungal (B) community composition across the age spectrum.

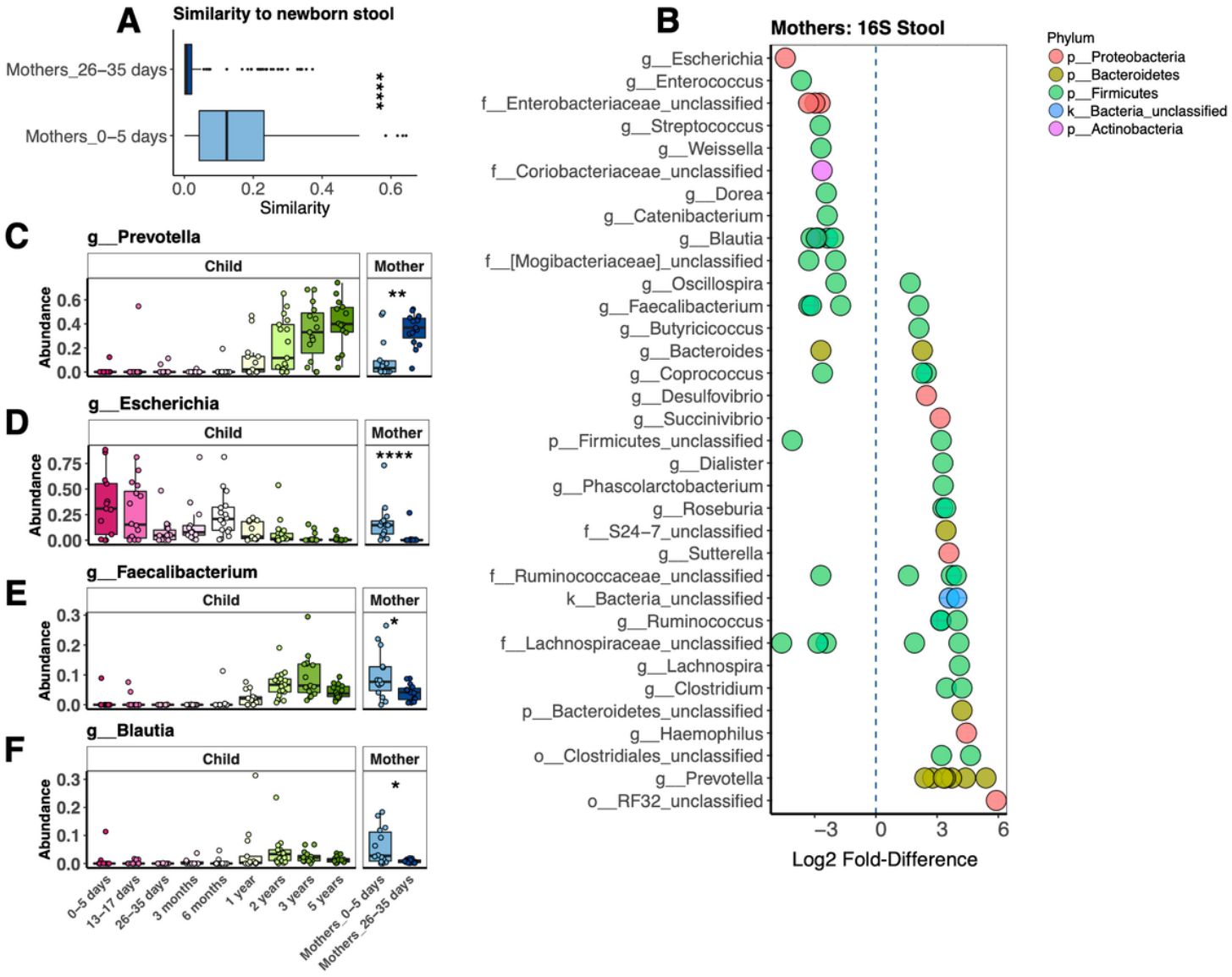

Figure 4

Comparison of fecal bacterial microbiotas of mothers 1 and 4 weeks postpartum and children at 1 week. A. Average pairwise similarity of the microbiotas of mothers versus those of their children. B. OTUs identified by DESeq2 differentially-abundant between mothers 1 week versus 4 weeks postpartum. Negative fold-difference, indicates greater abundance 1 week postpartum. C-F. Relative abundance of selected taxa that were differentially abundant between mothers 1 week versus 4 weeks postpartum. **** $p < 0.0001$, ** $p < 0.01$, * $p < 0.05$, Wilcoxon Rank Sum test, adjusted using the Bonferroni correction.

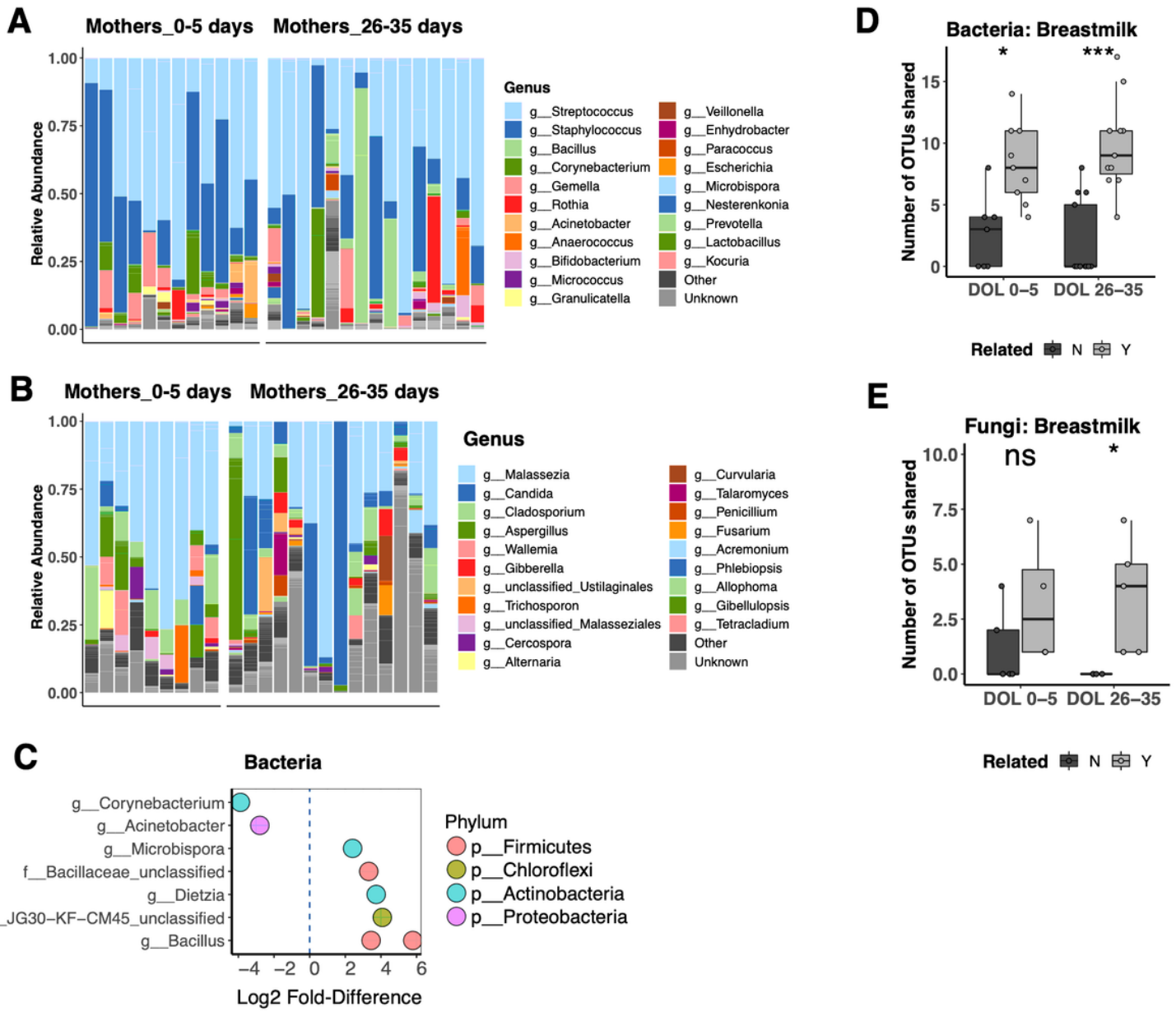

Figure 5

Breastmilk Bacterial and Fungal community composition. A-B. Top 25 most abundant bacterial (A) and fungal (B) genera across all samples reveal that both communities are dominated by common skin taxa, but also contain a diverse repertoire of microbes at lower abundances. C. Differentially abundant taxa between mothers in the first compared to the fourth post-partum week. D-E. Number of shared bacterial (D) and fungal (E) OTUs between mother-infant pairs in the first and fourth post-partum weeks. Statistics: G. * $p < 0.05$, Wilcoxon Rank Sum test, adjusted using the Bonferroni correction.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

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