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Infant gut microbiome shows resilience against mode of delivery but is susceptible to feed type

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SUMMARY As cesarean section and formula feeding become increasingly popular, it is important to examine how mode of delivery and diet influence the development and composition of the infant gut microbiome. Awareness of the factors that influence an infant's gut flora could help improve their short- and long-term health outcomes. With this in mind, here we investigate the influence of delivery and feeding method on the early infant gut microbiome; specifically, how vaginal versus cesarean section birth, and breastfeeding versus formula feeding impact gut flora. Additionally, we attempt to determine whether the type of feed and the method of delivery impact the vertical transmission of gut bacteria from mother to infant, and how the infant microbiome composition changes over time. We are able to show that breastfed and formula-fed infants had similar gut community richness but differed in microbial community composition at 6 months of age. Microbes belonging to the *Veillonellaceae* and *Pasteurellaceae* families were found to be more abundant in the gut microbiomes of breastfed infants, whereas microbes belonging to the *Lachnospiraceae* family were more abundant in formula-fed infants. Infants delivered via cesarean section and vaginal modes did not significantly differ in community richness and microbiome composition at 0.5 months of age. Microbes from the *Lachnospiraceae* family were found to be more abundant in the gut microbiomes of the cesarean section infants. All infant groups (cesarean section, vaginally delivered, breastfed and formula-fed infants) maintained stable community differences from their mothers over the 6-month period tested.

INTRODUCTION

The vast ensemble of microbes harboured in the human gut provide the host multiple metabolic capabilities. Without these microbes, extracting energy from indigestible dietary products such as polysaccharides would not be possible (1). In addition to aiding in digestion, microbial composition and diversity has been associated with other aspects of an individual's health status, such as one's metabolism and weight (2, 3). The importance of a balanced gut microbiota has been highlighted in a study by Turnbaug et al., which found that individuals who suffered from obesity had lower flora diversity compared to healthy, lean individuals (4). The microbiome has also been implicated in the development of immune response pathways in early life, such as the ability to produce appropriate levels of immune-related cytokines and growth factors (3). Dysbiosis of the gut flora has been associated with immune disorders such as celiac disease (5).

Diet is one of the major modulators of the gut microbiome (6). Previous studies have shown that different food products can be beneficial or detrimental to flora health. For instance, a high intake of dietary fiber has been shown to increase microbial diversity and promote expansion of beneficial *Bifidobacteria* (7). *Bifidobacterium* are known to provide resistance against pathogenic microbe colonization (8) and downregulate host inflammatory molecules (9). In contrast to fiber, the intake of fat has been shown to decrease gut microbiome diversity and richness (10). Interestingly, human milk has been associated with a reduced risk of obesity, by serving as a source of essential nutrition for infant growth and development (11). Milk oligosaccharides are believed to enrich beneficial microbiota, including *Bifidobacteria* (12), and are metabolized by certain microbes to produce short-chain fatty acids that decrease the pH of the intestinal lumen (11). In addition, human milk

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is known to reduce flora bacterial diversity and inhibit pathogen growth (11, 13).

Mode of delivery has also been determined to be critical for gut flora development (14). Caesarean section (c-section) is an alternative to vaginal delivery that is recommended by the World Health Organization in high-risk pregnancies (15). The usage of c-sections has increased worldwide, being used for 6.7% of births in 1990 and 19.1% in 2014 (15). It is thus important to understand how bypassing fecal and vaginal microbiota seeding affects infant gut flora (14). Infants delivered by c-section have been previously shown to have a high abundance of *Klebsiella* and *Enterococcus* strains and a low abundance of *Bifidobacterium* (14). A high *Klebsiella* to *Bifidobacterium* ratio in infants has been correlated with the development of allergic diseases later in life (9). These differences in microbiota composition disappear by 2 months of age as infant gut floras mature to form more adult-like profiles (14).

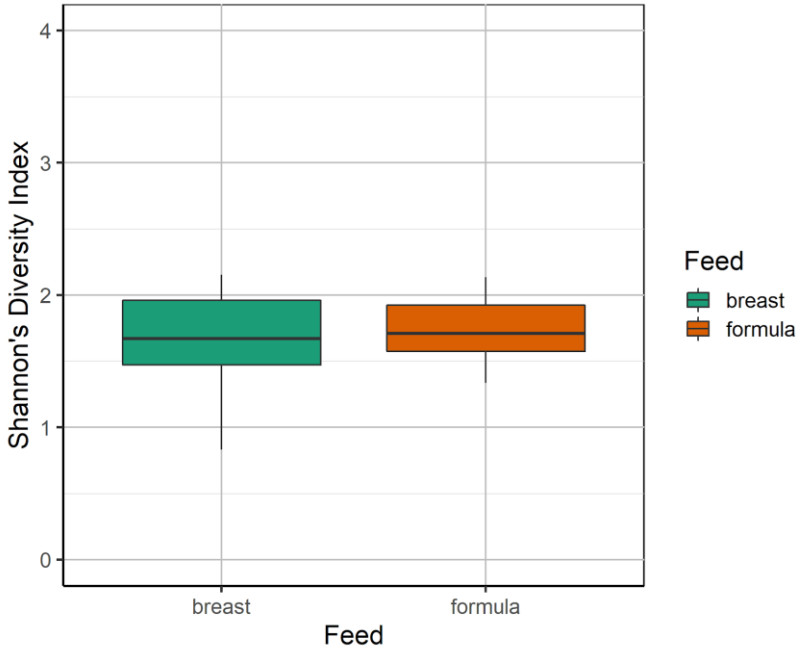

FIG. 1 Breastfed and formula-fed infants exhibited similar community richness. Shannon's diversity index was calculated and graphed using R for breastfed (green; n = 11) and formula-fed (red; n = 6) infants at 6 months of age. (p = 0.67).

With increased understanding of how the gut microbiome influences host health, there is a growing interest in factors that have previously demonstrated a vital role in shaping one's microbiome in early life. Diet and mode of delivery have been suggested as major modulators of the gut microbiome. We aim to determine whether these factors contribute to the shaping of the gut microbiome throughout the first 6 months of life, only immediately after birth, not until later in life, or not at all.

The inclusion of the mother microbiome population in the selected dataset provided us an opportunity to gain insight on the effects of mode of delivery and type of feed on vertical transmission of microbes between infant-mother pairs. Using beta diversity analysis, we determined that infant-mother compositional differences were not influenced by mode of delivery or type of feed. This unique analysis not only provided insight into the maturation of the infant microbiome but may also suggest a lack of impact by the mother's breast milk and vaginal microbiota on the rate of gut flora maturation over the first 6 months.

METHODS AND MATERIALS

Data Collection and Description. A dataset was obtained from the European Nucleotide Archive concerning infant feeding behaviors and the gut microbiome (16). Collection of 193 infant and 114 mother stool samples was performed at infant ages of 0.5 months, 4 months, 6 months, and 9 months. Mode of delivery and type of feed was recorded for each

infant-mother pair. Data on infant weight and eating behaviors, such as their satiety responsiveness and working for food, were also collected at each time point.

QIIME2 Sample Filtration and Taxonomy Classification. Infant and mother microbiome 16s rRNA samples were demultiplexed and imported into QIIME2 v2020.08.0 (16). Data was quality filtered using the DADA2 plugin to detect and correct reads where possible. Reads were not trimmed due to high (> ~30) scores throughout. Samples were then rarefied to 13 000 sequences. A phylogenetic tree was generated using QIIME2. A Naive Bayes classifier pretrained on Silvia 138 99% OTUs full-length sequences was used to assign taxonomy to the ASVs. ASV and taxonomy information was exported for downstream R analysis (17–22). A full QIIME2 script of all commands used can be found in the supplement.

R Analysis of Diversity and Differential Abundance. Weighted UniFrac distances were calculated to compare microbial composition between different feed and delivery groups. Significance of microbial compositional differences was determined with permutation multivariate analysis of variance (PERMANOVA) using the Phyloseq package (23). Differential abundance of families was determined by performing a differential expression analysis using DESeq2 (24). Shannon's diversity index was calculated and a linear regression used to test for any changes in microbial alpha diversity between feed and delivery groups over time. To determine if there are any microbial compositional changes between infant-mother pairs, weighted UniFrac distances between infant-mother pairs were calculated. The earliest sample from each mother was used for these infant-mother comparisons. The full R script used can be found in the supplement.

RESULTS

Breastfed and formula-fed infants did not differ in alpha diversity. In order to determine the effect of feed type on the infant microbiome diversity, Shannon's diversity index was calculated for breastfed and formula-fed infants at 6 months of age. A similar level of richness was observed between breastfed and formula-fed infants (Fig. 1).

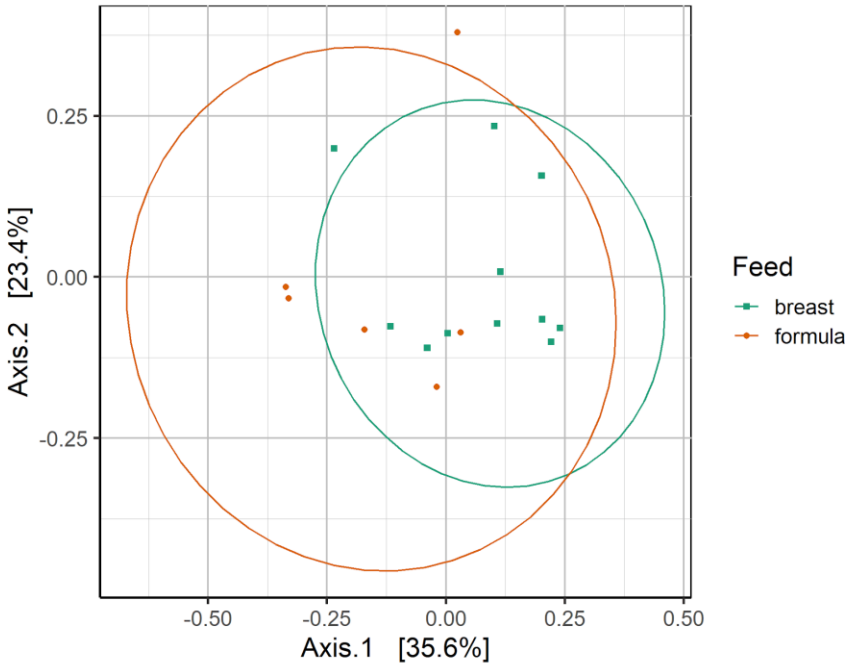

FIG. 2 Microbial community composition differed between breastfed and formula-fed infants. Weighted UniFrac distances were calculated and graphed using R for breastfed (green; n = 11) and formula-fed (red; n = 6) infants at 6 months of age (p = 0.01).

Breastfed and formula-fed infants differed in microbial community composition. To investigate whether type of feed is a determining factor in the composition of the infant microbiome, a principal coordinates analysis (PCoA) plot based on weighted UniFrac was used to compare the microbial communities of breastfed and formula-fed infants at 6 months of age. The two-dimensional (2D) weighted UniFrac PCoA plot provided a good representation of the data, as the two axes combined were able to explain 59% of the variation in the data (Fig. 2). Infants appeared to cluster based on feed type, suggesting that the microbiomes of breastfed and formula-fed infants were unique (Fig. 2, $p = 0.01$).

Three differentially expressed families were identified between breastfed and formula-fed infants. Differential abundance analysis was performed at the family level in order to identify potential taxonomic groups responsible for the difference in microbial composition between breastfed and formula-fed infants at 6 months of age. Three families were differentially expressed ($p < 0.05$): *Veillonellaceae* and *Pasteurellaceae* were more abundant in breastfed infants, whereas *Lachnospiraceae* were more abundant in formula-fed infants (Fig. 3).

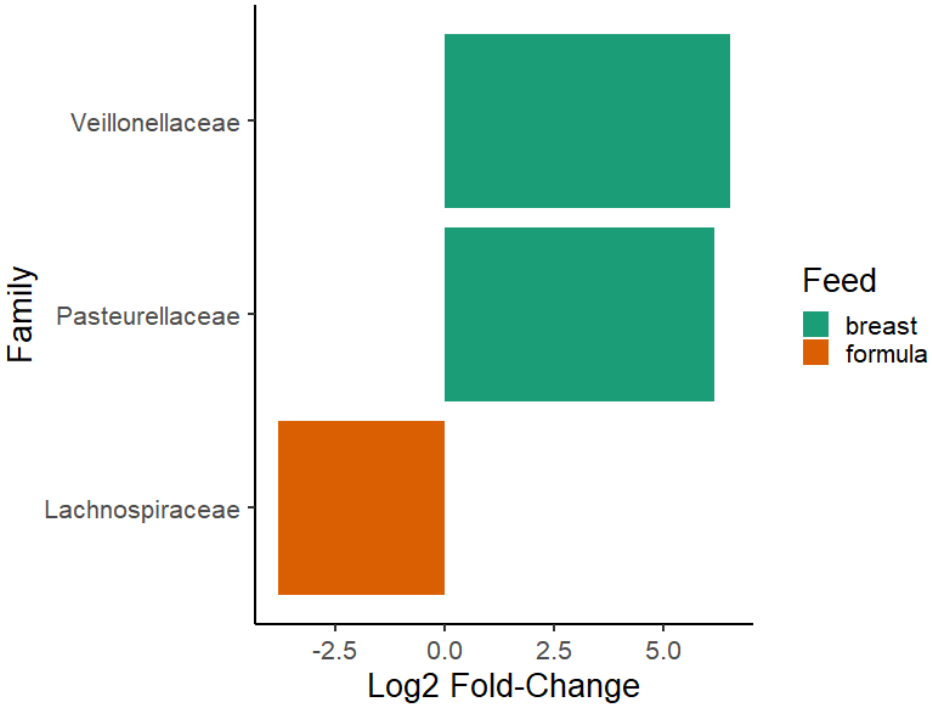

FIG. 3 Three families are differentially abundant between breast and formula fed infants. ASVs were classified to the family level using QIIME2 with Silva 138. Families with relative abundances above 0.1% were used to perform a differential abundance analysis with R. Families represented in green are relatively more abundant in breastfed (n=11) infants while those in red are more abundant in formula-fed (n=6) infants ($p < 0.05$).

Infants delivered via c-section and vaginal modes did not significantly differ in alpha diversity. Shannon's diversity index was calculated using R and compared between 0.5-month-old infants delivered vaginally or by c-section to determine the effect of mode of delivery on the infant microbiome diversity. No significant difference in community richness was observed between c-section and vaginally delivered infants (Fig. 4).

C-section and vaginally delivered infants had similar microbiome composition. To determine whether mode of delivery impacts the composition of the infant microbiome, a weighted UniFrac PCoA plot was used to compare the microbiomes of c-section and vaginally delivered infants at 0.5 months of age. With 71% of the variance explained in the two axes, the 2D plot appears to represent the data well (Fig. 5). No distinct clustering of infant samples was observed based on mode of delivery, indicating that c-section and vaginally delivered infants did not differ in the composition of their microbiome.

C-section infants were associated with a greater abundance of *Lachnospiraceae*. Although no significant difference in beta diversity was observed based on mode of

delivery, differential abundance analysis was performed to determine if individual microbial families were more abundant in c-section or vaginally delivered infants at 0.5 months of age. The *Lachnospiraceae* family was found to be significantly more abundant in infants delivered via c-section than those delivered vaginally (Log 2-fold change = 4.75, p = 0.002).

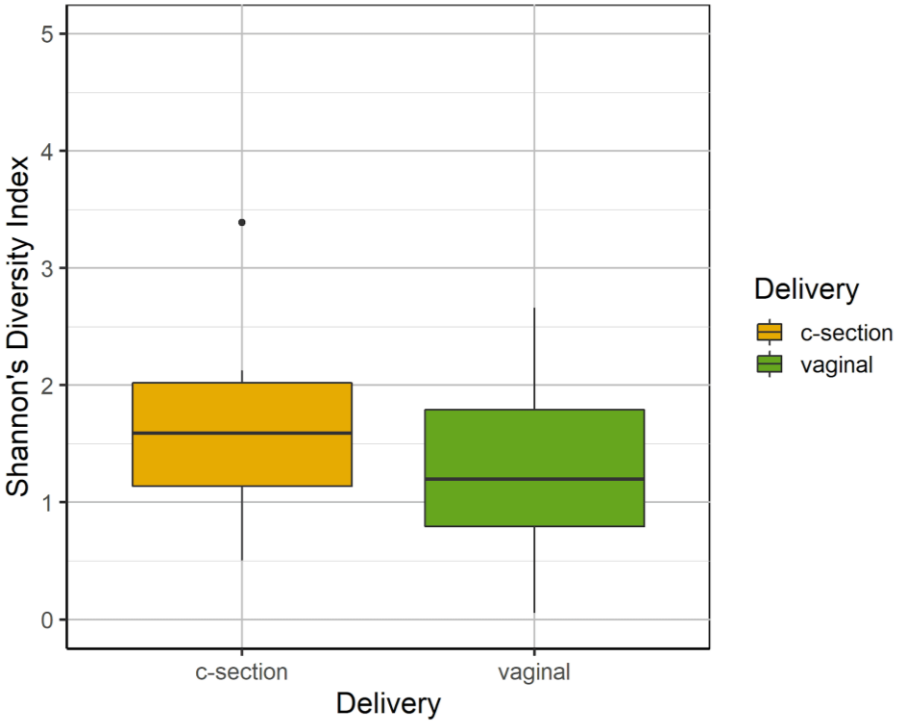

FIG. 4 Vaginally and c-section delivered infants did not differ in community richness. Shannon's diversity indexes were calculated and graphed using R for c-section (yellow; n = 11) and vaginally (green; n = 31) delivered infants at 0.5 years of age (p = 0.294).

Beta diversities of infant-mother pairs did not differ significantly over time for breastfed and formula-fed infants. Next, we wanted to determine whether the type of feed impacts the vertical transmission of flora from mother to infant, and how the composition of the infant microbiome changes over time. To investigate this, weighted UniFrac distances were calculated between infants and their mothers over a period of 6 months, as grouped by type of feed (Fig. 6A). In addition, linear regressions were performed to statistically evaluate potential changes in microbial composition over time for breastfed and formula-fed infants (Fig. 6B). At all timepoints studied, the weighted UniFrac distances between infants and their mothers did not differ between breastfed and formula-fed infants (Fig. 6A). Although the values appeared to differ at 0.5 and 6 months, these differences were not statistically significant due to the low number of formula-fed samples (Table S1). This indicates that breastfed and formula-fed infants had a comparable level of similarity with their mothers' microbiome. As expected, the weighted UniFrac distances between infants and their mothers at birth appeared to be similar between breastfed and formula-fed infants, according to the intercept of the linear regressions (Fig. 6B, Table S1, $p < 2\times10^{-16}$ and $p = 8\times10^{-9}$ respectively). Based on the slope of the linear regressions, when compared to the microbiomes of their mothers, the microbial composition of breastfed and formula-fed infants did not significantly change (p = 0.25 and p = 0.17 respectively) from 0.5 to 6 months of age (Fig. 6B, Table S1). These results indicate that the differences between infants' and their mother's microbiomes remained constant over time and do not differ significantly between breastfed and formula-fed infants.

Compositional differences between infant-mother pairs remained constant over time for c-section and vaginally delivered infants. We then determined how gut microbiome composition of vaginally and c-section delivered infants changed from their mothers over time. Weighted UniFrac distances between infant-mother pairs were calculated over 6 months and grouped by mode of delivery (Fig. 7A). Linear regressions were performed to determine whether compositional differences were significantly altered between infant-

mother pairs for each delivery group (Fig. 7B). C-section and vaginally delivered infants had similar compositional differences from mothers at all timepoints (Fig. 7A, Table S2). The intercepts of the linear regressions were similar between c-section and vaginally delivered infants ($p = 1.7\times10^{-11}$ and $p < 2\times10^{-16}$ respectively), suggesting both groups had a similar microbiome at birth when compared to their mothers (Fig. 7B, Table S2). These differences remained stable for both delivery groups over time (Fig. 7B, Table S2, $p = 0.88$ and $p = 0.13$ respectively). These results suggest infant-mother flora composition distance did not significantly differ between c-section and vaginally delivered infants, and that this distance remained constant over time.

DISCUSSION

Our study analyzed a dataset concerning infant feeding behaviors and the gut microbiome that contains 193 infant and 114 mother gut flora samples collected at several time points. We focused on method of delivery (c-section or vaginal) and type of feed (breastmilk or formula) to study the effects of these categories on microbial diversity and to determine the similarity between each group's and of infant-mother pairs' communities over time.

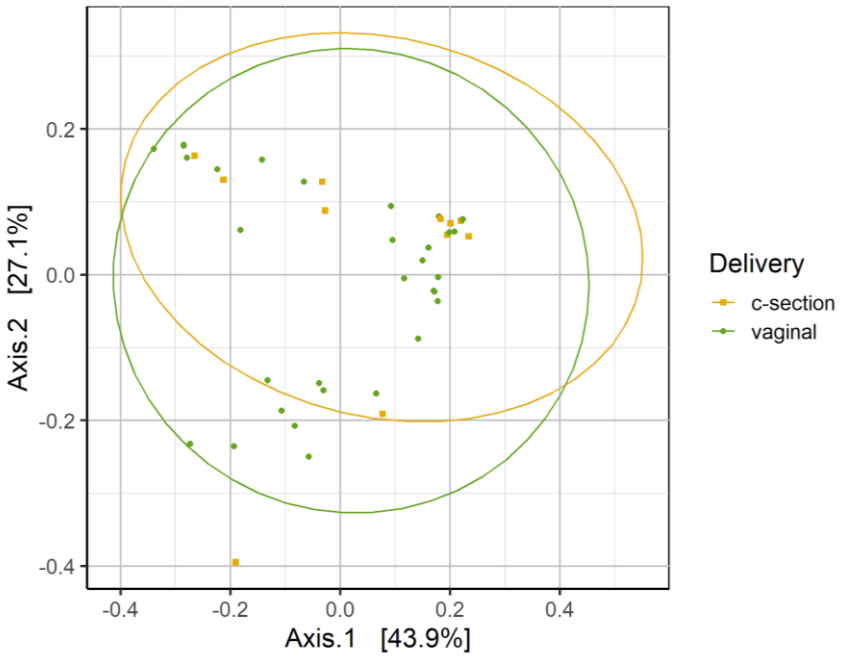

FIG. 5 Microbiome composition did not significantly differ between c-section and vaginally delivered infants. Weighted UniFrac distances were calculated and graphed using R for c-section (yellow; n = 11) and vaginally (green; n = 31) delivered infants at 0.5 months of age ($p = 0.294$).

Variance in breastmilk and formula composition may influence the difference in microbial community composition. Interestingly, infant microbial community composition displayed significant differences between breastfed and formula-fed infants at 6 months of age. Distinct clusters of the two feed types were observed when plotted on a PCoA plot based on weighted UniFrac. This difference in community composition suggests that human milk and formula have profound and different effects in modulating the gut flora. More specifically, we identified microbes belonging to the *Veillonellaceae* and *Pasteurellaceae* families to be more abundant in the gut microbiomes of breastfed infants when compared to formula-fed infants. In contrast, formula-fed infants displayed a large abundance of microbes belonging to the *Lachnospiraceae* family. To our surprise, we did not observe the previously reported characteristic differences in *Bifidobacteria* between breastfed and formula breastfed infants. Newer formulas have been supplemented with various prebiotics that promote *Bifidobacteria* expansion, which may have resulted in formula-fed infants achieving a similar abundance to breastfed infants (25).

Previously, and in concert with our results, researchers have shown that the increased abundance of *Veillonellaceae* and *Pasteurellaceae* have been associated with exclusive

breast-feeding (26, 27). While the role of *Pasteurellaceae* is unknown, *Veillonellaceae* is a known utilizer of lactose and human milk oligosaccharides found in breast milk, which may explain its greater abundance in breastfed infants. Also consistent with our results, most studies have found a depletion of *Lachnospiraceae* in exclusively breastfed infants (27, 28). Interestingly, a high abundance of *Lachnospiraceae* has been previously associated with obesity by impairing glucose metabolism which can also promote the onset of type 1 diabetes (29). Exclusively breastfed infants may be protected against obesity as observed by the depletion of *Lachnospiraceae*. These results suggest that the unique compositions of breastmilk and formula create differences in flora composition by promoting different bacterial families. Future research into how these compositional differences in feed shape the gut flora will allow for further understanding of the gut microbiome and the development of therapeutic prebiotics that can shift the microbiome composition in a beneficial manner.

No difference between breastfed and formula-fed infant microbial diversity. Previous research has shown that gut microbiome diversity was significantly greater in formula-fed infants (13). Our analysis suggests that type of feed does not significantly alter microbial community richness in infants at 6 months of age. Both breastfed and formula-fed infants displayed similar community richness when compared using Shannon's diversity indices, contradicting previous findings. As the differential abundance analysis showed a similar level of *Bifidobacteria* between breastfed and formula-fed infants, consumed formulas may be designed to promote this family of healthy bacteria, thus reducing the alpha diversity in formula-fed infants. Insight on the types of formulas fed to the infants could help clarify this proposal.

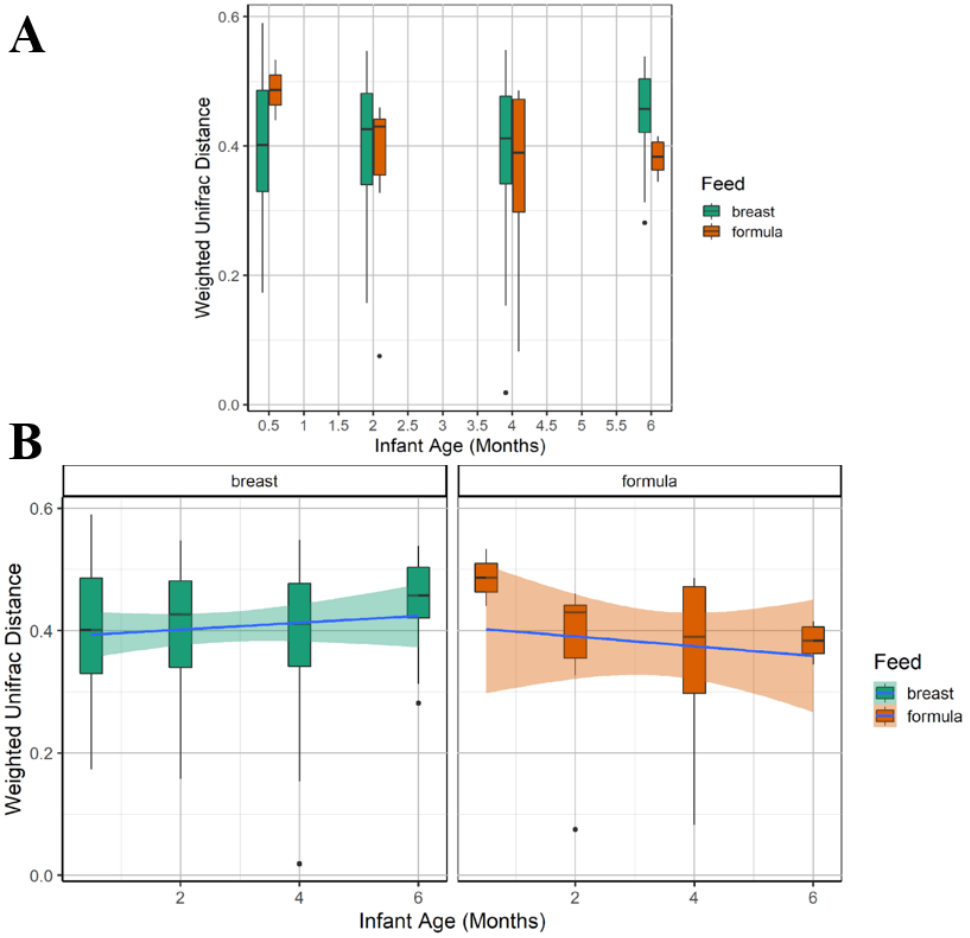

FIG. 6 Breastfed and formula fed infant groups maintained stable community differences from their mothers. Weighted UniFrac distances were calculated between infant-mother pairs and graphed by feed using R. Breastfed and formula-fed infants had similar community differences from mothers (A) which remained stable over time (B). Number of samples, linear regressions, and p-values are provided in Table S1.

Gut microbiome richness and composition did not differ between the two modes of delivery. Gut microbiome richness in infants born vaginally or via c-section displayed a

lack of difference in community richness at 0.5 months of age. In a systematic review by Rutayisire et al., infants delivered via c-section were shown to display lower gut microbiome diversity when compared to infants born vaginally from birth to 7 days of life and from 8 to 30 days of life (30). The 7 datasets analyzed by Rutayisire et al. employed varying sample collection methods including shotgun sequencing and cultured media, along with a focus on the diversity within specific taxa as opposed to overall alpha diversity. It is also unclear as to which alpha diversity metric was used. The majority of c-section mothers described by Rutayisire et al. were also said to have consumed antibiotics which have previously been shown to influence infant microbiome through placenta transfer (31). These factors may have contributed to an apparent reduction in the microbiome diversity of infants delivered by c-section that differed from our analysis.

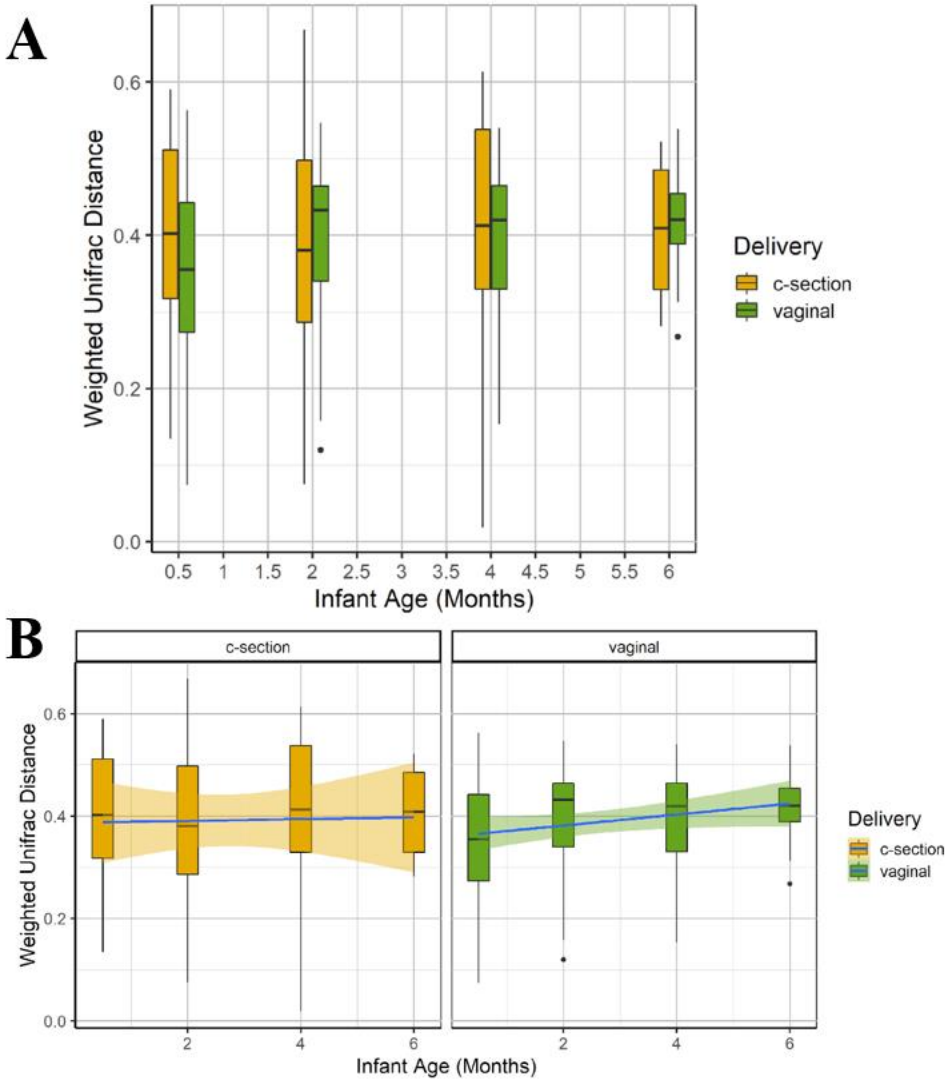

FIG. 7 C-section and vaginally delivered infant groups maintained stable community differences from their mothers. Weighted UniFrac distances were calculated between infant-mother pairs and graphed by mode of delivery using R. c-section and vaginally delivered infants had similar community differences from mothers (A) and remained stable over time (B). Number of samples, linear regressions, and p-values are provided in Table S2**.**

We also observed no significant difference between the gut microbiome composition of c-section and vaginally delivered infants at 0.5 months of age. This contrasts a study by Reyman et al. which found differences in microbial composition between infants delivered vaginally and via c-section until 2 months of age (14). As possible clustering in the PCoA plot was observed but statistically insignificant ($p = 0.294$), a low number of c-section infant samples at 0.5 months ($n = 11$ compared to $n = 31$ for vaginal) and the impact of other factors may have caused this inconsistency compared to previous findings. To confirm our present findings, future studies can incorporate a larger dataset.

Although no significant difference in microbiome composition was observed, our team found a greater abundance of *Lachnospiraceae* family bacteria in infants delivered via c-section. These results are consistent with past studies, in which increased abundance of *Lachnospiraceae* was observed in the c-section cohort (32, 33). Although it is unknown why *Lachnospiraceae* may be more abundant in c-section infants, *Lachnospiraceae* has been associated with obesity and impaired glucose metabolism (29). This may suggest a reason why infants delivered via c-section have a greater risk of obesity and type 1 diabetes compared to those who were delivered vaginally.

The maternal microbiomes may not represent the mature microbiome of the infants. To compare maternal and infant community composition in all four cohorts (breastfed, formula-fed, vaginally delivered and via c-section), we utilized linear regression and weighted UniFrac distances to study the changes in compositional differences over time. Our analysis showed that infant and maternal microbiomes of both the breastfed and formula-fed groups remained similar in terms of paired compositional differences throughout the course of 0.5 to 6 months of age (Fig. 6). Differences in infant-mother beta diversity were also constant over time for c-section and vaginally delivered infants (Fig. 7).

We had initially expected the infant microbiome to increasingly become more adult-like as the infants aged due to an acclimatization of gut flora to the external environment and intake of food. Past studies supported these expectations, with overall development of microbial community composition towards a profile characteristic of the adult gastrointestinal tract (14, 34). However, there was no observed decrease in the infant-mother beta diversities in all four cohorts of our study. It is possible that an alternative beta diversity metric such as Bray-Curtis, used by Reyman et al. (14), decreases over time but the weighted UniFrac dissimilarity between infants and mothers does not. Bray-Curtis and weighted UniFrac both factor in abundance in their calculation, but only weighted UniFrac incorporates phylogenetic distance. Phylogenetic distance may not have significantly changed during development of the microbiome.

Originally, we assumed that the microbiome of each infant's mother would be representative of the infants' eventual adult microbiomes. This represents a limitation of our infant-mother beta diversity analysis, as we did not directly test whether the mothers' microbiomes collectively represented an "adult-like" microbiome that could be used as a general reference for a mature microbiome. If type of feed or mode of delivery does impact infant microbiome development, but the mature microbiome of the infants is not very similar to their mothers, this may explain lack of an observed decrease in infant-mother beta diversities.

Limitations Limitations in our ability to assess infant-mother beta diversity results stemmed from a lack of any analyses demonstrating the collective mothers' gut microbiome to be representative of a mature microflora. A future study should be conducted to show that the mothers' microbiome is representative of the adult microbiome composition, which may explain the results of our infant-mother beta diversity analysis. One approach would be to perform a beta diversity analysis and PCoA plot to visualize the maternal microbiomes alongside the infant microbiomes at a particular age. If the maternal microbiomes form a tight cluster that is separate from the microbiomes of the infants, it suggests that the maternal microbiomes do indeed represent an "adult-like" microbiome. Alternatively, another experimental approach would be to investigate the infants' own microbiomes at a much later time point. This would address the potential issue of infants developing a mature, "adult-like" microbiome, but it being unique from the microbiome of their mothers. However, this would require a follow-up study to acquire new microbiome data.

Conclusions Our study investigated the influence of type of feed and mode of delivery on the infant gut microbiota diversity and composition as well as its development to an adult-like profile. Contrary to our hypothesis, type of feed did not influence microbiome community richness at 6 months of age. However, the composition of the infant microbiome does depend on feed type. Neither richness nor composition were found to be impacted by mode of delivery at 0.5 months of age, which was inconsistent with previous

findings. Additionally, the weighted UniFrac distances showed that there were no significant shifts towards increasing or decreasing similarity to the maternal microbiome over time in all infant groups (cesarean section, vaginally delivered, breastfed and formula-fed infants). The groups maintained a stable community difference from their mothers over the 6-month period.

Future Directions We found that at 6 months of age, breastfed and formula-fed infants had similar gut community richness, but differed in microbial community composition, with the *Veillonellaceae* and *Pasteurellaceae* families being more abundant in the gut microbiome of breastfed infants, and the *Lachnospiraceae* family being more abundant in formula-fed infants. With the knowledge of the differing taxonomies based on the two different feeding methods, future studies may continue to look at whether or not these compositional differences may correlate with certain short- or long-term diseases associated with the varying abundances of a particular taxonomy. Additionally, with the understanding that there are differentially abundant bacteria found in the infant gut microbiome based on type of feed, it would be useful to conduct future research into how these differentially abundant taxa contribute to gut health in breastfed and formula-fed infants, especially as the role of *Pasteurellaceae* is unknown. This would allow for investigation of therapeutic prebiotics that shift the microbiome composition in a beneficial manner, inhibiting microbiome-related diseases that may arise later in life.

Furthermore, a future study should be conducted to demonstrate that the mothers' microbiome is representative of the adult microbiome composition, which may explain the results of our infant-mother beta diversity analysis. One approach would be to perform a beta diversity analysis and PCoA plot to visualize the maternal microbiomes alongside the infant microbiomes at a particular age. If the maternal microbiomes form a tight cluster that is separate from the microbiomes of the infants, it would suggest that the maternal microbiomes do indeed represent an "adult-like" microbiome. Alternatively, another experimental approach would be to investigate the infants' own microbiomes at a much later time point. This would address the potential issue of infants developing a mature, "adult-like" microbiome, but it being unique from the microbiome of their mothers. However, this would require a follow-up study to acquire new microbiome data.

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CONTRIBUTIONS

Xu and Lee devised the project idea. Xu helped develop the future directions with Wong and wrote the abstract. Lee wrote the discussion with Wong. Wong analyzed the data with Khan and provided the supplemental material. Khan carried out the experiments, described the methods used, and recognized the project's supporters. All authors contributed to the paper's introduction, conclusions, references, and editing.

REFERENCES

1. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. 2006. An obesity-associated gut microbiome with increased capacity for energy harvest. 7122. Nature 444:1027–1031.
2. Castaner O, Goday A, Park Y-M, Lee S-H, Magkos F, Shiow S-ATE, Schröder H. 2018. The Gut Microbiome Profile in Obesity: A Systematic Review. Int J Endocrinol 2018.

3. O'Sullivan A, He X, McNiven EMS, Haggarty NW, Lönnerdal B, Slupsky CM. 2013. Early Diet Impacts Infant Rhesus Gut Microbiome, Immunity, and Metabolism. J Proteome Res 12:2833–2845.
4. Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, Sogin ML, Jones WJ, Roe BA, Affourtit JP, Egholm M, Henrissat B, Heath AC, Knight R, Gordon JI. 2009. A core gut microbiome in obese and lean twins. Nature 457:480–484.
5. Mårild K, Stephansson O, Montgomery S, Murray JA, Ludvigsson JF. 2012. Pregnancy outcome and risk of celiac disease in offspring: A nationwide case-control study. Gastroenterology 142:39-45.e3.
6. Conlon M, Bird A. 2014. The Impact of Diet and Lifestyle on Gut Microbiota and Human Health. Nutrients 7:17–44.
7. Yang, Liang, Balakrishnan, Belobrajdic, Feng, Zhang. 2020. Role of Dietary Nutrients in the Modulation of Gut Microbiota: A Narrative Review. Nutrients 12:381.
8. Kondepudi KK, Ambalam P, Nilsson I, Wadström T, Ljungh Å. 2012. Prebiotic-non-digestible oligosaccharides preference of probiotic bifidobacteria and antimicrobial activity against Clostridium difficile. Anaerobe 18:489–497.
9. Low JSY, Soh S-E, Lee YK, Kwek KYC, Holbrook JD, Van der Beek EM, Shek LP, Goh AEN, Teoh OH, Godfrey KM, Chong Y-S, Knol J, Lay C. 2017. Ratio of Klebsiella/Bifidobacterium in early life correlates with later development of paediatric allergy. Benef Microbes 8:681–695.
10. Mokkala K, Houttu N, Cansev T, Laitinen K. 2020. Interactions of dietary fat with the gut microbiota: Evaluation of mechanisms and metabolic consequences. Clin Nutr 39:994–1018.
11. Koleva P, Bridgman S, Kozyrskyj A. 2015. The Infant Gut Microbiome: Evidence for Obesity Risk and Dietary Intervention. Nutrients 7:2237–2260.
12. Zivkovic AM, German JB, Lebrilla CB, Mills DA. 2011. Human milk glycobiome and its impact on the infant gastrointestinal microbiota. Proc Natl Acad Sci 108:4653–4658.
13. Ho NT, Li F, Lee-Sarwar KA, Tun HM, Brown BP, Pannaraj PS, Bender JM, Azad MB, Thompson AL, Weiss ST, Azcarate-Peril MA, Litonjua AA, Kozyrskyj AL, Jaspan HB, Aldrovandi GM, Kuhn L. 2018. Meta-analysis of effects of exclusive breastfeeding on infant gut microbiota across populations. Nat Commun 9.
14. Reyman M, van Houten MA, van Baarle D, Bosch AATM, Man WH, Chu MLJN, Arp K, Watson RL, Sanders EAM, Fuentes S, Bogaert D. 2019. Impact of delivery mode-associated gut microbiota dynamics on health in the first year of life. 1. Nat Commun 10:4997.
15. Magne F, Puchi Silva A, Carvajal B, Gotteland M. 2017. The Elevated Rate of Cesarean Section and Its Contribution to Non-Communicable Chronic Diseases in Latin America: The Growing Involvement of the Microbiota. Front Pediatr 5.
16. Rhee KE. Project: PRJEB39437.
17. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO. 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res 41:D590–D596.
18. Yilmaz P, Parfrey LW, Yarza P, Gerken J, Pruesse E, Quast C, Schweer T, Peplies J, Ludwig W, Glöckner FO. 2014. The SILVA and "All-species Living Tree Project (LTP)" taxonomic frameworks. Nucleic Acids Res 42:D643–D648.
19. Glöckner FO, Yilmaz P, Quast C, Gerken J, Beccati A, Ciuprina A, Bruns G, Yarza P, Peplies J, Westram R, Ludwig W. 2017. 25 years of serving the community with ribosomal RNA gene reference databases and tools. J Biotechnol 261:169–176.
20. Yarza P, Yilmaz P, Pruesse E, Glöckner FO, Ludwig W, Schleifer K-H, Whitman WB, Euzéby J, Amann R, Rosselló-Móra R. 2014. Uniting the classification of cultured and uncultured bacteria and archaea using 16S rRNA gene sequences. 9. Nat Rev Microbiol 12:635–645.
21. Nicholas Bokulich, Mike Robeson, Matthew Dillon, Ben Kaehler, Michal Ziemski, Devon O'Rourke. 2020. bokulich-lab/RESCRIPt: 2020.11. Zenodo.
22. Bokulich NA, Kaehler BD, Rideout JR, Dillon M, Bolyen E, Knight R, Huttley GA, Gregory Caporaso J. 2018. Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. Microbiome 6:90.
23. McMurdie PJ, Holmes S. 2013. phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. PLOS ONE 8:e61217.
24. Love MI, Huber W, Anders S. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol 15:550.
25. Borewicz K, Suarez-Diez M, Hechler C, Beijers R, de Weerth C, Arts I, Penders J, Thijs C, Nauta A, Lindner C, Van Leusen E, Vaughan EE, Smidt H. 2019. The effect of prebiotic fortified infant formulas on microbiota composition and dynamics in early life. 1. Sci Rep 9:2434.
26. Laursen MF, Andersen LBB, Michaelsen KF, Mølgaard C, Trolle E, Bahl MI, Licht TR. 2016. Infant Gut Microbiota Development Is Driven by Transition to Family Foods Independent of Maternal Obesity. mSphere 1:e00069-15.
27. Forbes JD, Azad MB, Vehling L, Tun HM, Konya TB, Guttman DS, Field CJ, Lefebvre D, Sears MR, Becker AB, Mandhane PJ, Turvey SE, Moraes TJ, Subbarao P, Scott JA, Kozyrskyj AL. 2018. Association of Exposure to Formula in the Hospital and Subsequent Infant Feeding Practices With Gut Microbiota and Risk of Overweight in the First Year of Life. JAMA Pediatr 172:e181161.
28. Ma J, Li Z, Zhang W, Zhang C, Zhang Y, Mei H, Zhuo N, Wang H, Wang L, Wu D. 2020. Comparison of gut microbiota in exclusively breast-fed and formula-fed babies: a study of 91 term infants. Sci Rep 10.
29. Vacca M, Celano G, Calabrese FM, Portincasa P, Gobbetti M, De Angelis M. 2020. The Controversial Role of Human Gut Lachnospiraceae. Microorganisms 8:573.

30. Rutayisire E, Huang K, Liu Y, Tao F. 2016. The mode of delivery affects the diversity and colonization pattern of the gut microbiota during the first year of infants' life: a systematic review. BMC Gastroenterol 16.
31. Šumilo D, Nirantharakumar K, Willis BH, Rudge G, Martin J, Gokhale K, Thayakaran R, Adderley NJ, Chandan JS, Okoth K, Hewston R, Skrybant M, Deeks JJ, Brocklehurst P. 2019. Long-term impact of giving antibiotics before skin incision versus after cord clamping on children born by caesarean section: protocol for a longitudinal study based on UK electronic health records. BMJ Open 9:e033013.
32. Azad MB, Konya T, Guttman DS, Field CJ, Chari RS, Sears MR, Becker AB, Scott JA, Kozyrskyj AL. 2014. Impact of cesarean section delivery and breastfeeding on infant gut microbiota at one year of age 10:A24.
33. Tun HM, Bridgman SL, Chari R, Field CJ, Guttman DS, Becker AB, Mandhane PJ, Turvey SE, Subbarao P, Sears MR, Scott JA, Kozyrskyj AL. 2018. Roles of Birth Mode and Infant Gut Microbiota in Intergenerational Transmission of Overweight and Obesity From Mother to Offspring. JAMA Pediatr 172:368.
34. Neu J, Rushing J. 2011. Cesarean Versus Vaginal Delivery: Long-term Infant Outcomes and the Hygiene Hypothesis. Clin Perinatol 38:321–331.