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Contact with caregivers is associated with composition of the infant gastrointestinal microbiome in the first 6 months of life

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Abstract

Objectives: Little is known about how physical contact at birth and early caregiving environments influence the colonization of the infant gastrointestinal microbiome. We investigated how infant contact with caregivers at birth and within the first 2 weeks of life relates to the composition of the gastrointestinal microbiome in a sample of U.S. infants ($n = 60$).

Methods: Skin-to-skin and physical contact with caregivers at birth and early caregiving environments were surveyed at 2 weeks postpartum. Stool samples were collected from infants at 2 weeks, 2, 6, and 12 months of age and underwent 16S rRNA sequencing as a proxy for the gastrointestinal microbiome. Associations between early caregiving environments and alpha and beta diversity, and differential abundance of bacteria at the genus level were assessed using PERMANOVA, and negative binomial mixed models in DEseq2.

Results: Time in physical contact with caregivers explained 10% of variation in beta diversity at 2 weeks' age. The number of caregivers in the first few weeks of life explained 9% of variation in beta diversity at 2 weeks and the number of individuals in physical contact at birth explained 11% of variation in beta diversity at 6 months. Skin-to-skin contact on the day of birth was positively associated with the abundance of eight genera. Infants held for by more individuals had greater abundance of eight genera.

Discussion: Results reveal a potential mechanism (skin-to-skin and physical contact) by which caregivers influence the infant gastrointestinal microbiome. Our findings contribute to work exploring the social transmission of microbes.

KEYWORDS
allomothers, gastrointestinal microbiome, horizontal transmission, infancy, skin-to-skin contact

1 | INTRODUCTION

The human gastrointestinal microbiome is made up of trillions of microbial cells residing in the intestinal tract. This microbial community is largely comprised of bacteria, archaea, viruses, and eukaryotic taxa and plays an important role in shaping human health and physiology (Bordenstein & Theis, 2015; Peterson et al., 2009). Most research, including this paper, has focused on the bacterial component of the human gastrointestinal microbiome. Extensive bacterial colonization of newborns begins at birth (Dominguez-Bello et al., 2010). The gastrointestinal microbiome undergoes rapid changes through the first year of life and matures to adult status around 3–5 years of age, although some suggest that opportunities for significant change persist throughout childhood (Rodríguez et al., 2015; Yatsunenko et al., 2012). The adult microbiome is relatively stable, although minor shifts may occur due to life alterations, such as changes in diet or residence patterns, adoption of pets, or presence of children (Faith et al., 2013). However, alterations to commensal microbial communities during adulthood tend to be relatively minor compared to the opportunity for different community structures during early life when the microbiome is developing (Kurokawa et al., 2007). Gastrointestinal microbes have been implicated in regulating multiple aspects of host physiology, including metabolism (Yadav et al., 2018) and the immune system (Kau et al., 2011), among many aspects of health and disease risk (Durack & Lynch, 2019). As the gastrointestinal microbiome is hypothesized to play a role in shaping health and disease across the life course, identifying factors that shape the development of gastrointestinal microbial diversity and composition during sensitive periods may offer opportunities for intervention.

Despite broad recognition of the important roles of the gastrointestinal microbiome in human health and disease, the factors that contribute to its colonization and maturation remain poorly understood. Evidence suggests that experiences related to birth and early life contribute to the seeding of the infant gastrointestinal microbiome. In the earliest stages of postnatal life, the mode of delivery (Dominguez-Bello et al., 2010; Song, Dominguez-Bello, & Knight, 2013) and the initiation and maintenance of breastfeeding (Le Doare et al., 2018; Song, Dominguez-Bello, & Knight, 2013) contribute to the bacterial composition of the infant gastrointestinal tract. These are followed by increasing influences of environmental, behavioral, and cultural factors, such as diet (Palmer et al., 2007; Sprockett et al., 2020), the indoor physical environment, and exposure to animals, such as pets (Lax et al., 2014). These become the primary factors that shape interindividual variability in microbial composition as infants grow older (Rothschild et al., 2018; Yatsunenko et al., 2012). However, the influence of social exposures and interactions that may contribute to interindividual differences in the composition of the gastrointestinal microbiome remains understudied.

The most well-studied social transmission pathways for infants include physical contact with mothers and other members of the household. Mothers appear to be an important source of vertical transmission of strains such as *Bifidobacterium* and *Bacteroides*, particularly within the first year of life (Asnicar et al., 2017; Ferretti et al., 2018; Korpela et al., 2018). Household composition factors known to influence the infant gastrointestinal microbiome include overall household size, presence or absence of the biological father, siblings, or extended family (Azad et al., 2013; Lane et al., 2019; Laursen et al., 2017). Infants with older siblings tend to have less evenness and lower diversity of the intestinal microbiome (Azad et al., 2013), and accelerated acquisition of genera such as *Faecalibacterium* (Laursen et al., 2017). However, associations between household composition and the infant gastrointestinal microbiome are inconsistent. Others have found that number of siblings and household size are not associated with microbiome diversity measures (Lane et al., 2019), and cohabitation may not exert a strong enough effect to overcome age class effects in studies examining differences between parents and infants (Song, Lauber, et al., 2013). Infants may also come into contact with allomothers (caregivers other than the mother) who reside outside of the household and are thus not reflected in estimates of household size. For example, infants who attend out-of-home daycare have been shown to have greater diversity and species-richness compared to those not attending daycare (Thompson et al., 2015). Daycare attendance even may contribute to accelerated maturation toward an adult-like composition of the gastrointestinal microbiome (Amir et al., 2022). Thus, household composition metrics may not accurately reflect the amount of contact that infants have with other individuals and may mask the effect of social environments on the infant gastrointestinal microbiome.

Growing evidence suggests that social exposures also contribute to gastrointestinal microbial composition across the life course, although this literature is small. Studies of chimpanzees, baboons, howler monkeys, and white sifakas demonstrate that social group membership and social interaction predict the taxonomic structure of the gastrointestinal microbiome after adjusting for other established influences such as diet and genetic relatedness (Amato et al., 2017; Degnan et al., 2012; Perofsky et al., 2017; Tung et al., 2015). Shared residence patterns are associated with the gastrointestinal microbiome in humans across the life course, although these patterns may be partially explained by factors such as shared diet (Kinross & Nicholson, 2012; Song, Lauber, et al., 2013; Yatsunenko et al., 2012). In one of the largest human studies, Brito et al. (2019) found transmission within households between mother–child dyads and between spouses. Social network size is also associated with the diversity of the adult gastrointestinal microbiome, suggesting that social interactions impact bacterial composition (Johnson, 2020). However, the precise forms and sensitive periods by which social factors influence the horizontal transfer of microbiomes from allomothers to infants require further study.

Our study is motivated by the critical role of multiple caregivers in the evolution of human childrearing. Childcare provided by allomothers is part of an adaptive life history strategy that allows human mothers to handle multiple dependent offspring of different ages simultaneously (Hrdy, 2007). Assistance with childcare for mothers is considered to be a universal element of all human cultures, with allomothering often from fathers, grandmothers, older children, other kin, and non-kin (Helfrecht et al., 2020; Sear & Mace, 2008). Studies have

demonstrated health and development benefits for children who receive allomothering care, but the mechanism by which these benefits are conferred remains elusive (Hagen & Barrett, 2009; Sadruddin et al., 2019). Our study examines whether having more allomothers or more time spent in direct physical contact with mothers and allomothers could manifest in advantages to gastrointestinal microbiome diversity and composition.

Allomothers may contribute to a shared microbial environment with infants, potentially shaping the course of development of an infant's gastrointestinal microbiome, which may partially explain how allomothers contribute to infant development. Studies have begun to link the composition of the gastrointestinal microbiome to features of infant development. Evidence exists for greater microbiome diversity associated with both beneficial and deleterious infant and toddler developmental patterns, underscoring the need for further research in this area. Alpha diversity, or bacterial taxonomic richness within individuals, at two and a half months was negatively associated with fearful temperament at 6 months of age (Aatsinki et al., 2019). However, low alpha diversity in infancy has been shown to predict negative health outcomes, such as diabetes and asthma (Abrahamsson et al., 2014; Kostic et al., 2015). Beta diversity, reflecting differences in community composition between individuals, at 1-to-3 weeks' age accounted for a significant amount of variance in Surgency/Extroversion, a domain of temperament related to positive affect and sociable behaviors (Fox et al., 2021). In early toddlerhood, alpha diversity at 12 months of age was positively associated with concurrent fear behavior and cortisol reactivity (Carlson et al., 2021; Rosin et al., 2021). Phylogenetic alpha diversity at 1 year has also been negatively associated with cognitive and language abilities at 2 years of age (Carlson et al., 2018).

We hypothesize that caregivers contribute to the development of the infant gastrointestinal microbiome, particularly via differences in community composition, because beta diversity in early infancy has been associated with variation in positive temperamental traits, such as extroversion (Fox et al., 2021). If endorsed by data, it could suggest that social transmission of microbes via physical contact could be an avenue by which contact with allomothers confers health and developmental benefits to infants. To address gaps in the literature on the role of the non-household social environment in shaping the maturation of the infant gastrointestinal microbiome, we conducted this exploratory study to investigate associations between infants' early caregiving environments and the diversity and composition of the gastrointestinal microbiome across the first year of life. We investigated associations with alpha diversity, beta diversity, and differential abundance of bacterial taxa. We also investigated associations with household size, because this related construct has been assessed in previous similar studies. We took a longitudinal approach to examine how exposures during the first day of life, including the number of individuals and hours in skin-to-skin and physical contact with infants, and caregiving environments within the first 2 weeks of life predicted gastrointestinal microbiome composition at 2 weeks and 2, 6, and 12 months of age. The use of samples collected across infancy allowed us to examine if early social exposures were associated with concurrent or future diversity and composition of the infant gastrointestinal microbiome. To investigate factors beyond household composition (the metric used by previous studies), we asked mothers to provide information about all caregivers, including those from outside of the home. We hypothesized that infants with more (a) skin-to-skin and (b) physical contact on the first day of life and (c) more allomothers in early life would have more diverse gastrointestinal microbiomes with distinct bacterial composition across the first year of life after consideration of other factors known to influence the infant microbiome, including birth mode (Mitchell et al., 2020) and breast-feeding status (Ho et al., 2018). We predicted that infants with a greater number of caregivers and with more physical contact with caregivers would exhibit greater diversity in their gastrointestinal microbiome than those with fewer caregivers and less physical contact.

2 | METHODS

2.1 | Cohort

This project utilizes data from a larger, prospective, longitudinal cohort study of mother–child dyads in Southern California, the Pregnancy Experiences and Infant Development Study (PEIDS) (P50/MH096889). Briefly, women were offered voluntary participation in PEIDS and were recruited through their clinicians' offices, email, and print announcements in Southern California in their first trimester of pregnancy. Inclusion criteria included singleton intrauterine pregnancy, being 18 years of age or older, English-speaking, absence of tobacco, alcohol, or drug use during pregnancy, and being free of medical conditions impacting endocrine, cardiovascular, hepatic, or renal functioning. Written, informed consent was obtained from mothers for participation after full study procedures were described. PEIDS and our microbiome sub-study were approved by the Institutional Review Boards of the University of California, Los Angeles and Irvine and Chapman University. Our study adheres to the tenets of the Declaration of Helsinki. Because stool sample collection for microbiome analysis was implemented partway through the parent study, this study included a subset of the PEIDS cohort and involved four sessions: a home visit 2 weeks after birth and sessions at a clinical research site when the child was 2, 6, and 12-months old. The subset of infants included in this study was comprised of infants who produced stool during these sessions, resulting in 84 samples that were collected from 60 infants, across the different ages (2 weeks, 2, 6, and 12 months).

2.2 | Infant social environment

Mothers were interviewed at a home visit between 1 and 3 weeks postpartum, henceforth "2 weeks," using a questionnaire developed by the study team (Table S1) to interrogate their infant's social environment in the first weeks of life (factors that are assessed are described in Table 2). We chose a priori to investigate metrics that

involved skin-to-skin contact, physical contact, and the neonatal social environment (variables are presented in Table S2). Skin-to-skin contact was evaluated for the total number of people who ever had skin-to-skin contact on the day of birth—defined as the child being held either without any clothing, or in a diaper, against someone's bare chest, and mothers were asked to identify whether skin-to-skin contact was initiated immediately after birth, and for how long. Mothers were also asked to identify who had physical contact with the infant on the day of birth (DOB). Separate analyses were run for contact with hospital staff on the DOB. We chose to investigate skin-to-skin contact as it has previously been associated with variation in the gastrointestinal (Rozé et al., 2020) and oral microbiomes (Hendricks-Muñoz et al., 2015) of preterm infants. Contact with maternal skin, including areolar skin, may shape the vertical transfer of microbes from a mother to her infant's gastrointestinal tract (Pannaraj et al., 2017). The infant's oral microbiome may also provide microbes that initially seed the gastrointestinal microbiome.

We also investigated factors related to the caregiving environment in the first 2 weeks of life, including the number of individuals who regularly hold the baby, and the number of individuals whom the mother considered to be actively caring for the child (henceforth the "number of caregivers" and "number of active caregivers"). Household size was determined by asking the respondent the number of individuals living in the home. Caregiving and household environments may influence the diversity and composition of the infant gastrointestinal microbiome via pathways such as shared environments or direct physical contact with caregivers.

2.3 | Sample collection and processing

Each of the four postnatal study visits involved approximately two and a half hours of assessments both related and unrelated to the current project. The neonatal interview was conducted in participants' homes. For the collection of infant stools that were produced during this visit, study staff covered the stool with film to seal the sample inside the diaper during transport. The entire diaper was then wrapped up and sealed in a plastic bag and transported in a hard-sided cooler to the lab (maximum 45 min). Visits at 2, 6, and 12 months of age occurred at a clinical site with a laboratory. If the infant produced stool during the interview, the diaper was collected by the study staff. Study staff then covered the stool with film to seal the sample and bring to the onsite lab. Study staff transferred stool into OMNIgene Gut Collection Kits (OMR-200, DNA Genotek), aliquoted the mixture into cryovials and stored them at −80°C until sequencing. All samples were processed and sequenced by DNA Genotek.

2.4 | 16S ribosomal RNA bacterial gene sequencing

The details of bacterial DNA extraction from stool and sequencing have been previously described (Fox et al., 2021; Klindworth et al., 2013). In brief, bacterial DNA was extracted using a repeated bead-beating method (Yu & Morrison, 2004). The V3-V4 region of the 16S rRNA gene was amplified by polymerase chain reaction (Bact-0341 and Bact-0785) according to a previously published protocol (Klindworth et al., 2013). Illumina sequencing adapters and dual-index barcodes were added using the Illumina Nextera XT kit. The barcoded V3-V4 amplicons were combined into a library and MiSeq v3 300x2 paired-end sequencing was performed. Raw sequence data were processed, including joining of paired-end reads, in DADA2 to generate a count table of amplicon sequence variants (ASVs). After read processing as previously described, a sequence depth that ranged from 7295 to 72,989 with a mean of 29,653 was generated (Fox et al., 2021). Taxonomy was assigned for ASVs based on the SILVA database down to the level of family, genus, or species, depending on the depth of reliable classifier assignments (Quast et al., 2013). Chloroplast and mitochondria sequences represented 0.007% and 0.0007% of sequences, respectively. Chloroplast and mitochondria sequences were not filtered in preprocessing as they represented a very small portion of the data and were not expected to impact the results. Such sequences did not contribute to alpha diversity calculations.

2.5 | Statistical analysis

Microbiome data were analyzed for alpha diversity, beta diversity, and differential abundance of individual taxa. Bacterial alpha diversity was assessed in QIIME 1.9.1 using the Chao1 index (richness) and Shannon index (a diversity measure which increases with both rising richness—that is, increased numbers of ASVs—and evenness—that is, greater similarity of relative abundances across ASVs) (Caporaso et al., 2010). Data were rarefied to the lowest read count sample (7295) to equalize sequencing depth for alpha diversity analysis. Statistically significant differences in alpha diversity measures by age were determined using repeated one-way analysis of variance (ANOVA) adjusting for subject to control for effects of repeated sampling from the same individual. The statistical significance of associations of infant social environment metrics with alpha diversity measures at each timepoint was determined by multivariable linear regression.

Beta diversity of the unrarefied genus-level dataset, after removing genera that were present in less than 10% of the samples, was calculated using root square Jensen-Shannon divergence in R (calculated with a script made publicly available by Arumugam et al., 2011), then visualized with principal coordinates analysis (Arumugam et al., 2011; Martino et al., 2019). The statistical significance of differences in beta diversity was assessed using permutational multivariate analysis of variance (PERMANOVA, adonis function of the vegan package in R) (McArdle & Anderson, 2001). Statistical significance testing for the relationship of beta diversity with infant age was adjusted for subject. Metrics that did not follow a normal distribution were confirmed with the Shapiro–Wilk test. These data were then log-transformed for subsequent PERMANOVA analysis.

The differential abundance of bacterial genera inputted as unrarefied count data was determined using multivariate negative binomial

mixed models in DESeq2. This algorithm performs normalization by size factors (median value of all ratios for a given sample) (Anders & Huber, 2010). DESeq2 was chosen for differential abundance testing due to evidence that it displays high sensitivity for detecting differentially abundant taxa even with small sample sizes of 20 or fewer individuals (Weiss et al., 2017). Differential abundance testing was only performed for infant social environment metrics which showed $p < 0.1$ in beta diversity analysis by PERMANOVA. Results of differential abundance testing were adjusted for multiple hypotheses testing with a significance threshold of false discovery rate <0.1. Plots visualizing results of differential abundance testing show the normalized differential abundance of each genus, which was calculated by dividing the mean normalized counts for that genus calculated by DESeq2 by the mean sum of all normalized counts.

Covariates for multivariate models were chosen based on prior analysis of this data set, which identified infant sex, breastfeeding (encoded as a dichotomous variable identifying infants who received any breastmilk, regardless of supplementation with other foods, vs those who did not receive any breastmilk) at the age of sample collection, and mode of delivery as potential confounders (Fox et al., 2021). All three covariates were included in alpha and beta diversity and DEseq2 analyses. We did not adjust for antibiotic use as we previously showed that it was not associated with diversity or community composition in our sample (Fox et al., 2021).

3 | RESULTS

3.1 | Gastrointestinal bacterial diversity and composition change across infant age groups

Cohort demographics are presented in Table 1. We used ANOVA for continuous and chi-square goodness of fit to test for differences in subcohort demographics at each assessment. The timepoint-based subcohorts differed only by the number born by c-section, with slightly more c-section births represented at the 6-month assessment, and by breastfeeding as fewer infants were still receiving breastmilk after 6 months. Alpha diversity was higher in older infants according to the Chao1 ($p < 0.0001$) and Shannon ($p < 0.0001$) indices. Beta diversity analysis showed significant bacterial community differences between age groups after adjusting for participant to account for intraindividual similarity (Figure S1C, $p < 0.0001$). Household size was not correlated with the number of caregivers (p-value >0.05).

3.2 | Gastrointestinal microbiota diversity, richness, and composition associations with social environment

The amount of time spent in physical contact with others in the first 2 weeks of life demonstrated a significant association with bacterial alpha diversity at 6 months of age (Table S3) as measured by Chao1 ($\beta = 10.11$, $p = 0.038$) and Shannon diversity ($\beta = 0.056$, $p = 0.035$). However, the other predictors of interest were not significantly associated with alpha diversity at any age. Household size was not associated with alpha or beta diversity of the infant gastrointestinal microbiome at any time of assessment.

There were significant associations of caregiver factors with beta diversity at 2 weeks, 2 months, and 6 months of age; none were found to be significant at 12 months (Table 2). The amount of time that the infant experienced physical contact on the day of birth was significantly associated with gastrointestinal bacterial beta diversity at 2 weeks' age, explaining approximately 10% of the variation in community composition ($R^2 = 0.102$, $p = 0.027$) (Figure 1a). Additionally, the number of caregivers for the infant at 2 weeks' age was significantly associated with gastrointestinal bacterial beta diversity ($R^2 = 0.090$, $p = 0.050$) (Figure 1b), explaining 9% of the variation in composition. In addition, we observed a significant association between gastrointestinal bacterial beta diversity at 2 weeks of age and whether skin-to-skin contact was initiated after birth ($R^2 = 0.086$, $p = 0.039$) (Figure 1c), and a trend with the amount of time spent in skin-to-skin contact on the day of birth ($R^2 = 0.069$, $p = 0.096$) (Figure 1d), which explained approximately 8% and 7% of the variation in community composition, respectively. However, the amount of time in skin-to-skin initiated on the day of birth fell below conventional statistical significance thresholds. We note that time spent in skin-to-skin contact on the day of birth did not follow a normal distribution, and therefore was analyzed after performing a log transformation.

The number of people in physical contact with infants on DOB was significantly associated with bacterial beta diversity at 6 months of age (R^2 0.116, $p = 0.043$), and explained approximately 11% of the variation in community composition (Figure 2a). The amount of time the infant spends in physical contact during the first 2 weeks of life was marginally associated with bacterial beta diversity at 6 months of age, though this fell above the conventional statistical significance threshold ($R^2 = 0.105$, $p = 0.075$) (Figure 2b).

3.3 | Community composition and differential abundance of individual taxa associated with the social environment

For the relationships between microbiota beta diversity and infant social environment that emerged as statistically significant or approaching significance ($p < 0.10$), we then performed differential abundance testing to identify specific genera from the infant gastrointestinal microbiome that were associated with physical contact with caregivers. The $\log_2$ fold changes (LFC), p-values, and q-values for the differentially abundant taxa are provided in Tables S4–8. Notably, the number of hours per day the infant was held in physical contact was positively associated with the differential abundance of six taxa (total number of taxa tested = 27), including negative associations of five (*Bacteroides*, unclassified bacteria in the family Lachnospiraceae, *Lachnoclostridium*, *Parabacteroides*, and *Veillonella*) and positively associated with the differential abundance of the genus *Bifidobacterium*

TABLE 1 Participant characteristics ($N = 84$ samples from 60 infants).

	Total samples ($N = 84$)	2 weeks ($N = 22$)	2 months ($N = 24$)	6 months ($N = 15$)	12 months ($N = 23$)	*p*-Value
Infant age (weeks or months), M (SD)	–	2.94 (0.79)	2.13 (0.28)	6.42 (0.43)	12.41 (0.36)	<0.001
Infant sex, female ($N = 60$)	29	11	9	8	16	0.183
Birth by C-section ($N = 60$)	16	4	4	9	6	0.007
Infants receiving any breastmilk at the time of sample collection, including mixed-feeding (*N*)	54	20	19	8	7	<0.001
Birthweight (kg), M (SD)	3.32 (0.48)	3.33 (0.47)	3.40 (0.34)	3.42 (0.62)	3.24 (0.39)	0.589
Birth Length (cm), M (SD)	50.01 (2.48)	50.18 (2.21)	50.27 (2.32)	50.65 (2.55)	49.75 (1.92)	0.683
Mother's self-identified race (*N*)						0.611
White	18	5	5	4	8	
African American	2	0	1	1	1	
Asian	8	5	4	0	4	
Multi-ethnic	6	1	3	4	2	
Mother's self-identified ethnicity						
Hispanic or Latino	26	11	11	6	8	
Mother's parity, M (SD)	0.83 (0.9)	0.68 (0.78)	0.71 (0.86)	0.93 (0.59)	1.09 (1.0)	0.309
Any physical contact, number of people, M (SD)	5.22 (2.09)	5.82 (2.46)	5.17 (1.97)	4.93 (2.31)	5.17 (2.12)	0.620
Time in physical contact (h/day), M (SD)	18.55 (9.84)	17.26 (5.13)	15.54 (4.89)	17.88 (8.72)	20.69 (14.04)	0.274
Number of people with skin-to-skin since DOB, M (SD)	0.97 (1.06)	1.14 (0.98)	0.83 (1.01)	0.83 (0.96)	0.96 (1.19)	0.644
Infants with skin-to-skin contact since birth (*N*)	59	22	24	14	22	0.442+
Time in skin-to-skin since birth (mean h/day), M (SD)	1.56 (2.42)	1.83 (2.95)	1.17 (1.83)	1.06 (1.67)	1.44 (1.98)	0.699
Number of people holding baby in a typical week, M (SD)	4.13 (1.47)	4.36 (1.60)	4.25 (1.73)	3.83 (1.55)	3.87 (1.51)	0.640
Number of active caregivers, M (SD)	2.37 (1.02)	2.64 (1.22)	2.38 (1.17)	2.00 (0.85)	2.17 (0.78)	0.269
Any physical contact on DOB, Number of People, M (SD)	7.5 (5.08)	8.18 (5.88)	6.75 (3.63)	8.07 (4.28)	8.43 (5.30)	0.643
Any physical contact DOB by hospital staff, number of people, M (SD)	5.98 (3.99)	5.98 (4.72)	4.77 (2.45)	5.87 (2.91)	6.13 (4.45)	0.605
Infants with any skin-to-skin on DOB (N)	50	19	20	11	19	0.780+
Time in skin-to-skin on DOB (minutes), M (SD)	71.43 (63.93)	65.70 (73.16)	61.79 (50.23)	75.67 (73.41)	72.09 (44.32)	0.881
Household size, M (SD)	4.48 (2.12)	4.86 (2.68)	4.50 (1.87)	4.00 (1.51)	4.39 (2.17)	0.682
Attending daycare (*N*)	6	0	2	2	4	0.121

Note: The *p*-value column reflects one-way ANOVA for continuous variables or chi-square test of goodness of fit for dichotomous variables compared across the four age groups. + Chi-square test.

(LFC = 0.42) at 2 weeks' age (Figure 3a; Table S4). In addition, skin-to-skin contact after birth was associated with the differential abundance of eight genera at 2 weeks' age (total number of taxa tested = 27), including negative associations with *Escherichia/Shigella* (LFC = −19.11) and *Enterococcus* (LFC = −21.7108). It was positively associated with *Streptococcus* (LFC = 7.98), *Bifidobacterium* (LFC = 20.10), and *Bacteroides* (LFC = 26.10), among others (*Enterobacter*, Unclassified bacteria in the family Enterobacteriaceae, and *Klebsiella*) in the two-week-old infants' microbiota (Figure 3b; Table S5). Although there was a trend toward an association between the length of time in skin-to-skin contact on the day of birth and beta diversity at 2 weeks' age (Table 2, $p = 0.096$), no individual taxa were associated with this metric. Time spent in physical contact on the day of birth and skin-to-skin contact initiated immediately after birth were both positively associated with the differential abundance of the genus *Bifidobacterium* and *Bacteroides* (Figure 3a,b; Tables S4-5). Lastly, there was a positive association between the total number of caregivers at 2 weeks of age and the differential abundance of

TABLE 2 Infant beta diversity association with caregiver contact using PERMANOVA, adjusted for infant sex, breastfeeding, and c-section delivery.

		2 weeks			2 months			6 months			12 months		
		F. Model	R^2	*p*-Value	F. Model	R^2	*p*-Value	F. Model	R^2	*p*-Value	F. Model	R^2	*p*-Value
Household size		0.858	0.038	0.529	1.14	0.044	0.306	0.803	0.051	0.601	0.536	0.025	0.930
In a typical week													
	Number of active caregivers in physical contact with infant	1.01	0.044	0.398	0.792	0.031	0.632	1.20	0.073	0.273	0.917	0.041	0.512
	Amount of time infant spends in physical contact with others	2.51	0.102	0.027*	0.684	0.027	0.743	1.80	0.105	0.075.	0.901	0.041	0.524
	Number of active caregivers	2.18	0.090	0.050*	1.37	0.053	0.180	133	0.081	0.215	0.850	0.038	0.596
In a typical day													
	Number of caregivers that hold the baby	0.832	0.037	0.545	0.731	0.029	0.693	1.56	0.093	0.126	0.731	0.033	0.737
On day of birth													
	Number of caregivers in physical contact with infant	0.944	0.042	0.455	0.769	0.031	0.650	2.03	0.116	0.043 *	0.826	0.037	0.635
	Number of hospital staff in physical contact with infant	1.17	0.051	0.311	0.891	0.035	0.519	1.46	0.088	0.170	1.14	0.051	0.294
Skin-to-skin contact													
	Any skin-to-skin contact since birth	–	–	–	–	–	–	0.960	0.060	0.408	0.558	0.026	0.881
	Skin-to-skin initiated immediately after birth	2.08	0.086	0.039*	1.01	0.040	0.415	0.674	0.043	0.761	0.853	0.034	0.588
	Length of time spent in skin-to-skin contact on day of birth[a]	1.63[a]	0.069[a]	0.096[a],†	0.999[a]	0.039[a]	0.424[a]	0.551[a]	0.036[a]	0.845[a]	1.13[a]	0.050[a]	0.316[a]
	Number of caregivers in skin-to-skin contact with infant in a typical week	0.648	0.030	0.707	0.955	0.038	0.458	0.765	0.049	0646	0.600	0.028	0.877
	Average number of hours infant spends in skin-to-skin contact in a typical week	1.14	0.050	0.323	0.915	0.036	0.501	0.653	0.042	0.776	0.802	0.036	0.660

Note: The breastfeeding covariate is a dichotomous variable reflecting whether the infant was receiving any breast milk at the time of sample collection.

[a]Data analyzed with log transformation.

†*p*-value <0.10.

*p-value <0.05.

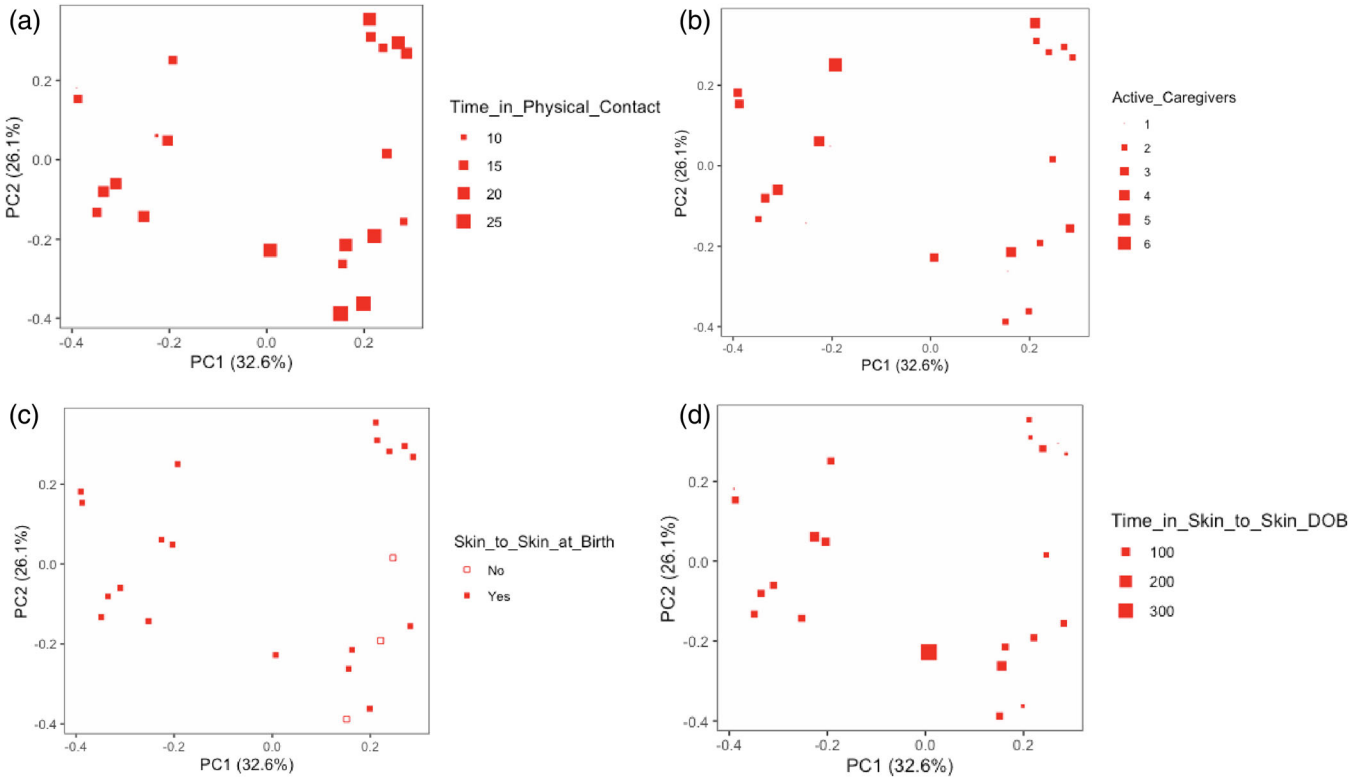

FIGURE 1 PCoA plots based upon root square Jensen-Shannon divergence of beta diversity of infant fecal microbiota at 2 weeks of age. Aspects of the infant social environment associated with beta diversity include (A) the amount of time in physical contact, (B) the total number of active caregivers, (C) skin-to-skin contact initiated immediately after birth, and (D) the amount of time spent in skin-to-skin contact immediately after birth.

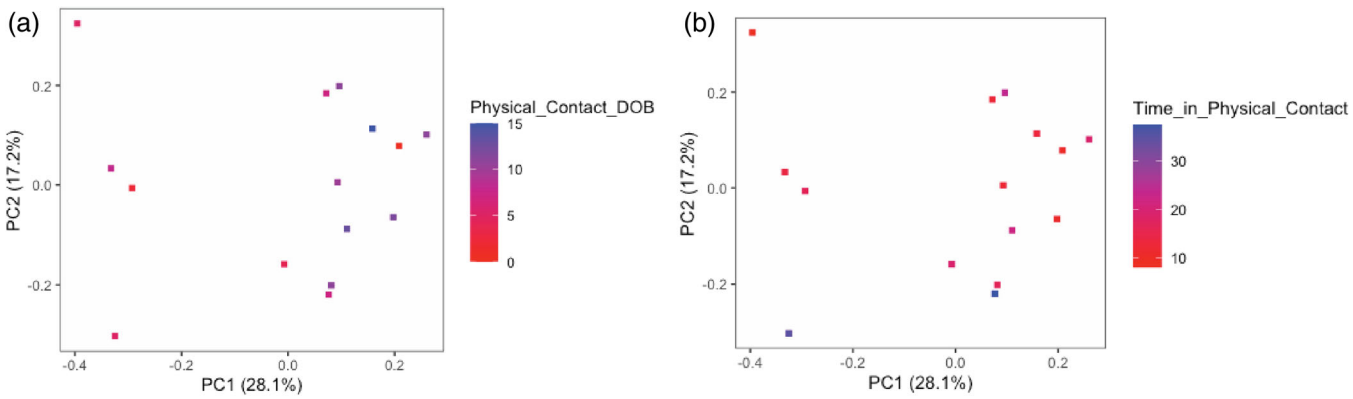

FIGURE 2 PCoA plots based upon root square Jensen–Shannon divergence of beta diversity of infant fecal microbiota at 6 months of age. Aspects of the infant social environment that are associated with beta diversity include (A) the total number of caregivers who held the newborn on the day of birth, and (B) the total number of people who held the baby on an average daily basis in the first 2 weeks of life.

Bacteroides (LFC = 2.20) and *Parabacteroides* (LFC = 2.46) (total number of taxa tested = 22, Figure 3c, Table S6).

The number of people who regularly hold the baby was associated with the differential abundance of eight genera at 6 months (total number of taxa tested = 45, Figure 3d, Table S7), including negative associations with three taxa (*Enterobacter*, unclassified bacteria in the family Enterobacteriaceae, and *Lactobacillus*) and positive associations with five taxa (unclassified bacteria in the family Erysipelotrichaceae, *Haemophilus*, *Megasphaera*, *Parabacteroides*, and *Sutterella*). The number of people in physical contact with the infant on the day of birth was associated with the differential abundances of 13 genera at 6 months (total number of taxa tested = 32, Figure 3e, Table S8), including negative associations with nine taxa (*Bacteroides*, *Enterobacter*, *Flavonifractor*, *Hungatella*, *Lactobacillus*, *Megasphaera*, *Parabacteroides*, *Sutterella*, and *UBA1819*) and positive associations with four (*Escherichia/Shigella*, *Intestinibacter*, *Tyzzerella_4*, and

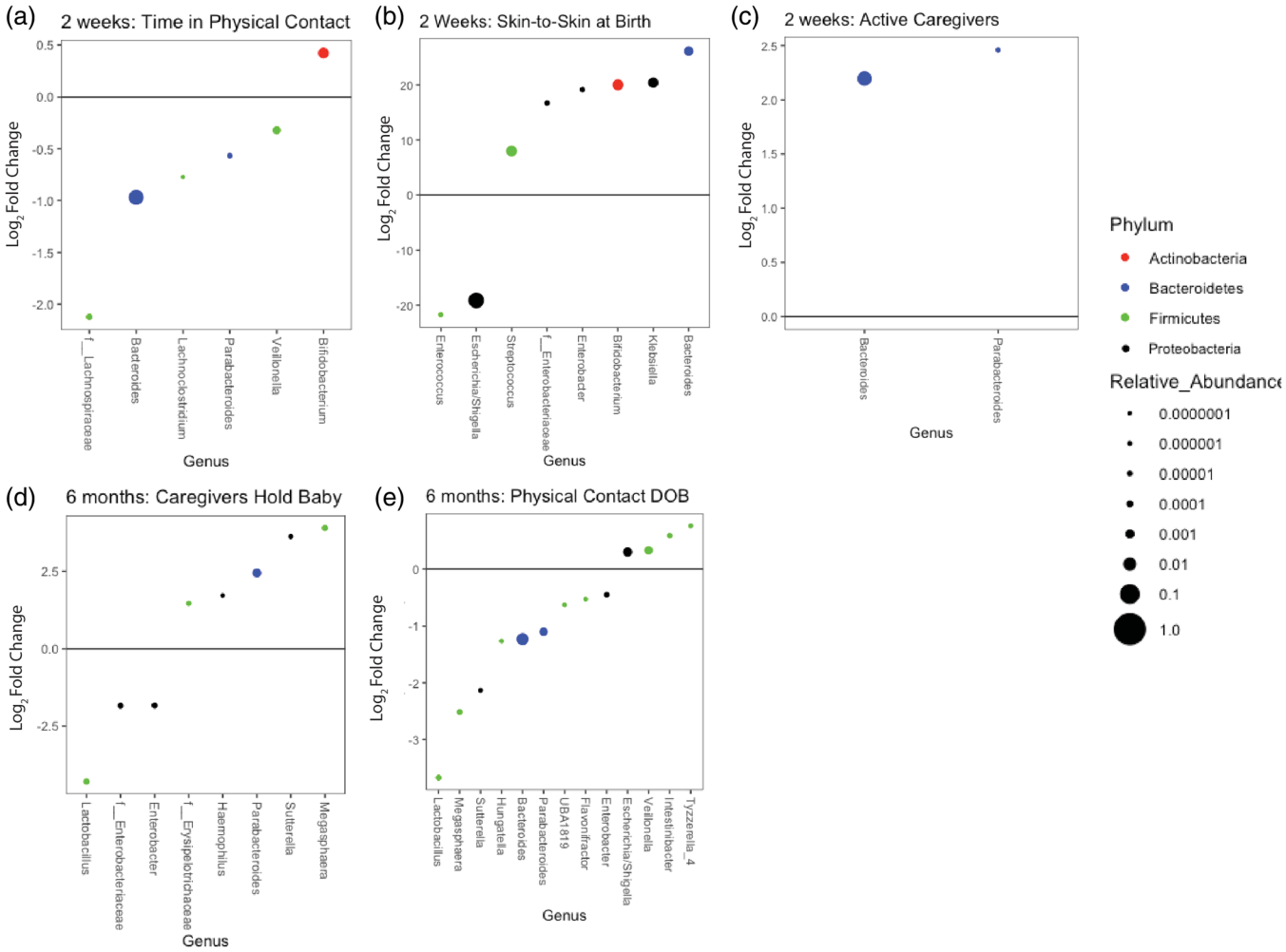

FIGURE 3 Specific genera associated with infant social environments. Significant genera ($q < 0.1$) associated with (A) time spent in physical contact at 2 weeks, (B) skin-to-skin contact initiated immediately after birth at 2 weeks, (C) total number of caregivers actively caring for the infant at 2 weeks, (D) the number of caregivers that regularly hold the infant at 6 months, and (E) the total number of caregivers who held the newborn on the day-of-birth at 6 months using DESeq2 with infant sex, breastfeeding, and delivery mode as covariates. Log_2 fold change is used to show the effect size and direction of these associations. The impact of each unit of a predictor variable is calculated as $2^{\text{Log2 value of the predictor}}$. A log_2 fold change of 2.2 for *Bacteroides* at 2 weeks of age by the number of active caregivers means that an infant with one caregiver has a four-and-a-half-fold greater relative abundance of *Bacteroides* relative to an infant with no caregivers. Dot size is proportional to the mean relative abundance of the genus and color corresponds to phylum. The notation "f__" denotes an unclassified bacteria in the specified family.

Veillonella). Both variables showed negative associations with the differential abundance of *Lactobacillus* (LFC = -4.29, LFC = −3.67, respectively) and *Enterobacter* (LFC = -1.83, LFC = −0.45, respectively) at 6 months' age. The differential abundance of the genera *Haemophilus* (LFC = 1.72) and *Sutterella* (LFC = 3.62) were positively associated with the number of people who regularly held the baby (Figure 3d, Table S7). The number of people who had physical contact with the baby on the DOB was negatively associated with *Hungatella* (LFC = −1.26) and *Sutterella* (LFC = −2.13, Figure 3e, Table S8).

4 | DISCUSSION

This study examined longitudinal associations between infants' early social and caregiving environments and the composition of their gastrointestinal microbiomes across the first year of life. We hypothesized that more a) skin-to-skin and b) physical contact on the DOB, and c) care from more allomothers in the first few weeks of life would be positively associated with gastrointestinal microbiome diversity and intraindividual differences in community composition across the first year of life. Different aspects of the caregiving environment were associated with gastrointestinal community composition (beta diversity) at 2 weeks', 2 months', and 6 months', but not 12 months' ages, and with the differential abundance of some bacterial genera at 2 weeks', 2 months' and 6 months' ages. The majority of observed associations were in early life. Newborn gastrointestinal microbiome beta diversity at 2 weeks' age was associated with the number of individuals and time spent in physical contact on the DOB, which explained 10% of the variation in beta diversity. Skin-to-skin contact initiated immediately after birth and the number of active caregivers

were also associated with beta diversity at 2 weeks' age, and each explained 9% of the variation in beta diversity. Skin-to-skin contact initiated after birth was associated with the differential abundance of eight taxa, including positively with *Bacteroides*, *Bifidobacterium*, *and Streptococcus*. The amount of time in physical contact in early life was negatively associated with the differential abundance of specific genera in newborns, including *Bacteroides*, *Parabacteroides*, and *Lachnoclostridium* at 2 weeks of age. However, we found no association between the amount of skin-to-skin contact on the DOB and alpha diversity of the newborn gastrointestinal microbiome, with the exception of the average amount of time in physical contact in early life and alpha diversity at 6 months. Furthermore, we found no evidence that contact with medical personnel on the DOB was associated with variation in the infant gastrointestinal microbiome. The number of caregivers in the first 2 weeks of life explained 9% of variation in bacterial community composition and was positively associated with differential abundance of *Bacteroides* and *Parabacteroides* at 2 weeks' age. The number of caregivers in physical contact with the infants on the DOB was also associated with community composition at 6 months' age, and explained approximately 14% of the variation in beta diversity. Finally, we also found that the number of individuals that regularly hold the baby at 2 weeks' age and the amount of physical contact on the DOB were negatively associated with differential abundance of *Lactobacillus* and *Enterobacter* at 6 months' age. Contact with allomothers may be an important factor for the development of the infant gastrointestinal microbiome.

We found that differences in the caregiving environments in the neonatal period are associated with differences in community composition in the gastrointestinal microbiome at 2 weeks, 2 months, and 6 months of life, although the strength of these associations varied by specific predictors. For example, the number of active caregivers was associated with community composition at 2 weeks and explained approximately 9% of the variation in community composition. However, these associations seem to be primarily driven by two genera within one phylum, such as a greater differential abundance of *Bacteroides* and *Parabacteroides*. In comparison, time spent in physical contact in the first 2 weeks of life explained more than 19% of the variation in community composition, with differences driven by six genera across three phyla, including less differential abundance of *Bacteroides*, *Parabacteroides*, *Lachnoclostridium*, *Lachnospiraceae*, and *Veillonella* and greater differential abundance of *Bifidobacterium*. In contrast, skin-to-skin contact at birth explained roughly 9% of the variation in community composition at 2 weeks, but this was driven by large differences in the differential abundance of eight taxa. This suggests that not all social environmental factors exert the same level of influence on the infant gastrointestinal microbiome.

Skin-to-skin contact seemed to have the greatest influence on the community composition of the infant gut microbiome as it was associated with the largest effect sizes on the differential abundance of specific taxa. For the six taxa that were positively associated with skin-to-skin contact at birth, the $\log_2$ fold changes ranged from 7.98 to 26.10, while the two taxa that were negatively associated had $\log_2$ fold changes that ranged from −19.11 to −21.71. These were far greater than differential abundances for other predictor variables. This may be due to the influence of skin-to-skin contact on the infant oral microbiome, which is an important seed for the developing gastrointestinal microbiome (Costello et al., 2013). For example, skin-to-skin contact has been associated with a greater abundance of *Streptococcus* in the oral microbiome of preterm infants (Hendricks-Muñoz et al., 2015). Together, this suggests that contact with caregivers' skin may help seed the gut microbiome through the oral cavity.

The numbers of differentially abundant genera did not always map onto the significant beta diversity associations. This may indicate that in some cases there are individual taxa that are strong drivers of compositional differences detected by beta diversity analysis whereas in other cases significant differences in beta diversity may be driven by a larger number of taxa each contributing to a small extent. Additionally, many of the genera that were identified in differential abundance were present in low relative abundance, and the differences due to our variables of interest may not be biologically meaningful. As the field is still young, it is still unclear how to interpret differential abundance in terms of biological significance, and future studies are needed to identify the thresholds at which different taxa may significantly impact infant physiology, behavior, or development.

Our results suggest that sustained physical contact over a typical week may exert slightly more influence than the number of individuals caring for the infant as it had a slightly larger effect size for beta diversity ($R^2 = 0.102$ vs. $R^2 = 0.900$) and was associated with six differentially abundant taxa as opposed to two. However, the two differentially abundant taxa for the number of individuals caring for the infant had greater $\log_2$ fold changes, ranging from 2.2 to 2.5. Unique caregivers may spend time with infants differently, resulting in both qualitative and quantitative differences in interaction with infants. In our study, *Bifidobacterium* was positively associated with physical contact at 2 weeks of age with an effect size of approximately 0.42 $\log_2$ fold change. This corresponds to a 34% increase in *Bifidobacterium* for an infant with 1 h of physical contact per day compared to an infant with no physical contact. However, physical contact at this age was negatively associated with the differential abundance of *Bacteroides*, with an effect size of $\log_2$ fold change of 0.97, representing 51% less abundance of this genus for an infant with 1 h of physical contact compared to one with no contact. The majority (five of the six) of the differentially abundant genera had negative associations with physical contact. While it is currently difficult to estimate the biological significance of differential abundance of statistically significant taxa in microbiome studies, effect sizes may provide some assistance for interpreting these associations (Valeggia & Fernández-Duque, 2022).

We investigated associations between the infant gastrointestinal microbiome and allomother contact in two different timeframes: on the day of birth as well as in a typical week. We also investigated associations with allomother contact with two different metrics: time and number of people. As previous research on the early social and caregiving environmental factors that influence the infant gastrointestinal microbiome is small and limited, it is unclear how associations may vary by exposure as a dichotomous factor compared to the

intensity of exposure. Our community composition results suggest that influences on the newborn gastrointestinal microbiome may be driven by day of birth associations, that is, the amount of skin-to-skin contact on DOB, while associations in later ages (2 weeks and 6 months) may be due to typical week factors, such as the number of caregivers that care for infants. This suggests that the DOB associations may occur primarily through time-driven exposures while the typical week effects in the newborn period may be driven by count-based exposures- in this case, a greater number of caregivers. Additional research is needed to replicate these associations and further investigate how these unique aspects of the infant caregiving environment shape the maturation of the gastrointestinal microbiome, as well as sensitive periods in which such exposures are particularly impactful.

This is the first study, to our knowledge, to examine associations between skin-to-skin and physical contact at birth and infant gastrointestinal microbiome composition outside of the maternal–infant dyad. Additionally, this is one of very few studies to assess the composition of the infant gastrointestinal microbiome in the first month of life (Kelsey et al., 2021; Lane et al., 2019; Loughman, Ponsonby, et al., 2020; Rosin et al., 2021; Thompson et al., 2015). This study also provides some of the first evidence of the role of physical contact, other than skin-to-skin, with allomothers at birth in shaping infant gastrointestinal microbiome composition. We contrasted allomother effects with physical contact with hospital staff on the DOB, which was not associated with infant gastrointestinal microbiome diversity or composition, as expected given standard hospital practices to prevent bacterial transmission. This study contributes to research investigating how social exposures, even at birth, may influence the development and composition of the gastrointestinal microbiome in ways that may shape infant health. Differences in diversity, evenness, and community composition have been associated with infant outcomes, such as temperament (Aatsinki et al., 2019; Fox et al., 2021), cortisol reactivity (Rosin et al., 2021), cognitive development (Carlson et al., 2018), and brain connectivity (Kelsey et al., 2021), highlighting the role of the gut-brain axis in infant health and development (Cowan et al., 2020).

Social interactions may contribute to the maturation of the infant gastrointestinal microbiome through the vertical or horizontal transfer of skin or other microbiomes from contact with mothers or allomothers, respectively. Research on the vertical transfer of bacteria from mothers to infants has traditionally focused on birth mode (Dominguez-Bello et al., 2010) and the maternal milk and skin microbiomes (Lackey et al., 2019; Williams et al., 2019). Our study suggests that physical contact on the day of birth and in the neonatal period may be an important factor that shapes the early development of the gastrointestinal microbiome. Infants are likely exposed to bacteria through physical contact with mothers and allomothers and early exposures may have enduring effects on the gastrointestinal microbiome. For example, we found that skin-to-skin contact initiated after birth may be protective as it was associated with less differential abundance of *Escherichia/Shigella* at 2 weeks' of age, taxa that are known to cause diarrheal illnesses (Cohen, 1991). Physical contact with mothers and allomothers may also introduce pathobiont bacterial taxa that have the potential for pathogenic activity. For instance, we observed that the number of individuals who regularly hold the baby was positively associated with the differential abundance of *Haemophilus* and *Sutterella* at 6 months, genera that are believed to be pathogenetic and have been implicated in pediatric gastrointestinal diseases, including irritable bowel syndrome (Saulnier et al., 2011) and ulcerative colitis (Hyams et al., 2019). Members of the *Haemophilus* genus are typically not observed in fecal samples captured later in infancy (Ferretti et al., 2018), suggesting that physical contact may contribute to the persistence of some bacterial genera.

A small number of studies have investigated associations between contact with allomothers and the infant gastrointestinal microbiome. A recent study of associations between allocare and the infant gastrointestinal microbiome reported, in contrast to our results, a positive association between the number of alloparents and alloparental co-sleeping with Shannon (alpha) diversity in a sample of 27 U.S. infants (Manus, Sardaro et al., 2023). In a large, multi-country study, Lane et al. (2019) did not detect associations between household composition and gastrointestinal microbiome diversity, although household size and the presence of extended family were nonlinearly associated with the differential abundance of specific taxa, including *Enterobacter* and *Lactobacillus*. Manus et al. (2023) reported that prenatal household size predicted Shannon (alpha) diversity in infancy, although the association was negative at 2 weeks of age and positive at 6 months of age. However, Thompson et al. (2015) found that infants, aged 2–14 months, enrolled in out-of-home daycare had higher diversity and species richness compared to infants cared for at home, as well as significantly greater *Lactobacillus* and *Sutterella*. Infants with more alloparents may have a broader range of sources of colonizing gastrointestinal microbes through physical contact with allomothers or other infants from in or outside of the household. As the number of caregivers was not correlated with household size, our results suggest that the broader caregiving environment may be as important as household size or composition in shaping the infant gastrointestinal microbiome. Such differences may also be due to differing methodologies. For example, Lane et al. (2019) sequenced the V1-V3 region of the 16S rRNA gene, used unrarefied data to generate alpha diversity, used Observed Richness instead of Chao1, and conducted differential abundance tests with the Analysis of Compositions of Microbiomes (ANCOM) rather than DESeq2. Manus et al. (2023) sequenced the V4-V5 region and also used ANCOM. Thompson et al. (2015) sequenced the V1-V2 region of the 16 s rRNA gene and used rarefied data but also used Observed Richness. Such differences in sequencing and analysis methods may contribute to heterogeneity in study findings.

Studies of the milk and infant skin microbiomes also suggest that the number of allomothers is associated with greater bacterial diversity (Manus et al., 2020; Meehan et al., 2018). Meehan et al. (2018) reported that the size of the mother and infants' social network and the frequency of allomother care was associated with higher evenness, but not richness, of the milk microbiome. Manus et al. (2020) found that household size and the number of allomothers were

associated with community composition differences (beta diversity) of the infant skin microbiome. Our work suggests that the social environment influences the infant gastrointestinal microbiome. However, the mechanisms linking these two findings are unclear. It is possible that caregiving and physical contact directly influence an infant's gastrointestinal microbiome through infant suckling on caregivers' skin. These findings may also be mediated by the milk microbiome, as it is an important source of bacteria for the infant gastrointestinal microbiome. It is also possible that the gastrointestinal microbiome may influence the infant oral microbiome and ultimately the milk microbiome via retrograde flow that occurs during infant suckling and nursing (Fernández et al., 2013). Additional studies are needed to explore the links between social and caregiving environments, the gastrointestinal microbiome, and the milk microbiome during infancy.

We observed that neonatal social exposures were associated with bacterial composition through 6 but not 12 months of age. This observation does not undermine the importance of our findings, as critical developmental progress across the brain and many physiological systems occurs during the first 6 months of life (Grotheer et al., 2022). These overlapping windows of development provide gastrointestinal microbes that are influenced by the social environment an opportunity to alter the development of other tissues and physiological systems with potentially long-term effects. For instance, we recently reported that early-infancy gastrointestinal bacterial composition is associated with infant temperament as late as 12 months of age (Fox et al., 2021). It is possible that larger sample sizes will observe persisting effects of neonatal social interactions on microbiome composition beyond 6 months. It is also possible that neonatal caregivers may not be correlated with bacterial signatures beyond 6 months given the dramatic changes in infant diet and environmental exposures across the first year of life. For example, the caregiving environment may not exert effects over and above those due to changes in diet occurring as infants shift from breastfeeding to other foods. Future studies are needed to address this question.

The results of this study point to a new potential mechanism by which skin-to-skin contact benefits infants. Skin-to-skin contact immediately after birth, particularly for preterm infants, has been shown to result in better outcomes related to emotionality, stress physiology, cognitive development, and executive function in infancy and later childhood (Feldman et al., 2014; Feldman & Eidelman, 2003; Hardin et al., 2020; Selman et al., 2020). Several studies of the infant gastrointestinal microbiome suggest that it could serve as a mechanism by which skin-to-skin contact benefits infants and have associated the infant gastrointestinal microbiome with temperament, cognitive development, and colic (Aatsinki et al., 2019; Carlson et al., 2018; Fox et al., 2021; Loughman, Ponsonby, et al., 2020; Loughman, Quinn, et al., 2020). The initial seeding of the gastrointestinal microbiome may be largely stochastic as newborns have been shown to have substantial inter-individual variation in diversity and community composition of the gastrointestinal microbiome (Ferretti et al., 2018). Skin-to-skin and oral-to-skin contact (via suckling on skin or caregivers kissing the infant) after birth and in the first weeks of life may facilitate the maturation of the infant microbiome through the transfer of maternal and caregiver skin microbiomes to the infant gastrointestinal tract through the oral cavity. Physical contact with, or infant suckling on, caregivers' skin, including maternal areolar skin during breastfeeding, may also shape the vertical or horizontal transfer of microbes to the infant's gastrointestinal tract (Pannaraj et al., 2017). One study of preterm infants found that skin-to-skin contact at birth was associated with a greater probability of gastrointestinal microbiomes being characterized by taxa indicating a more mature developmental state (Rozé et al., 2020). However, subsequent colonization and development of the infant gastrointestinal microbiome may be a selective process driven by the survival of taxa that favor anaerobic environments (Jost et al., 2012) or availability of nutrients driven by breastfeeding or cessation of breastfeeding (Bäckhed et al., 2015). This may explain why we did not detect significant associations between early caregiving environments and infant gastrointestinal microbiome diversity or composition at 12 months.

In our study, initiation of skin-to-skin contact at birth was positively associated with the differential abundance of *Streptococcus and Enterobacter* at 2 weeks' age, genera that are common in skin and gastrointestinal flora in early infancy (Dominguez-Bello et al., 2010; Manus et al., 2020; Pantoja-Feliciano et al., 2013). Maternal skin bacteria may colonize the gastrointestinal tract by inoculation at birth, particularly for infants delivered by cesarean section, as well as through contact with maternal areolar skin during breastfeeding and presence in breast milk (Dominguez-Bello et al., 2010; Ferretti et al., 2018; Pannaraj et al., 2017). Increased skin-to-skin contact also may enhance infant exposure to other maternal or allomaternal skin microbes that seed the infant gastrointestinal microbiome and displace early pathobiont colonizers. For example, we found that skin-to-skin contact was inversely associated with the differential abundance of *Escherichia/Shigella*. This parallels the results of recent studies of infants that found that skin-to-skin contact at birth was associated with a reduced abundance of *Staphylococcus*, another frequently pathogenic taxon, in the gastrointestinal and nasal microbiomes (Lamy Filho et al., 2015; Rozé et al., 2020). While these are imperfect comparisons, they provide examples of how skin-to-skin contact may facilitate the decolonization processes of pathobiontic microbes. This likely occurs via bacterial ecological processes, such as resource competition and niche partitioning (Miller et al., 2018) and selective pressures as the gastrointestinal environment shifts from an aerobic environment to an anaerobic one and infants transition away from breastfeeding to other food sources (Bäckhed et al., 2015). This process may be beneficial for the infant as *Escherichia/Shigella* and *Staphylococcus* are often associated with intestinal problems in infancy (Cohen, 1991; Stewart et al., 2012).

A strength of this study is the assessments of the amount of time that infants were in contact with caregivers and the number of allomothers caring for infants. Previous studies using household composition as the primary predictor of features of the infant microbiome may be less informative because household composition or size may not accurately reflect how many individuals care for or are in physical contact with infants (Lane et al., 2019). It is possible that the number of caregivers may not correlate with household size, particularly in

early infancy when infants and parents may require more support than is available from individuals living in the household. For example, grandparents may serve as frequent allomothers for newborns but may not be living in the home and thus may not be captured in household composition metrics. Household composition was not correlated with the number of active caregivers in our study. Future studies may benefit from naturalistic or direct observation of neonatal social interactions and caregiving contexts.

Our findings should be considered in light of several limitations. First, this study utilizes a relatively small sample size. While such sample sizes are common in microbiome research, future studies with larger samples are needed to replicate these associations, detect associations with smaller effects, and allow for the inclusion of more potentially relevant covariates, such as more detailed data on dietary composition and antibiotic use that are known to influence the infant gastrointestinal microbiome. For example, we did not ask if infants received any supplementation with formula while in the hospital. Notably, even minimal supplementation during this time may impact the composition and maturation of the gastrointestinal microbiome (Forbes et al., 2018). However, the effect of breastfeeding on the infant gastrointestinal microbiome may operate in a dose-dependent fashion with mixed feeding resulting in an intermediate phenotype between exclusively breastfed and exclusively formula-fed infants (Fehr et al., 2020), justifying our inclusion and prioritization of breastfeeding as a covariate. Second, this analysis relied on maternal reports of physical contact and the number of caregivers that were assessed via retrospective interviews at 2 weeks postpartum and did not use direct observational data to confirm these numbers. Questions about social experiences on the DOB were also retrospective over the one-to-three-week neonatal interview period. We are unable to determine whether concurrent social environments are driving the observed associations, rather than neonatal social exposures. Third, we did not characterize caregiver gastrointestinal microbiome composition. This would have allowed us to examine relative similarity and better support the hypothesis of the social transfer of microbiomes from allomothers to infants. Fourth, 16S rRNA V3-V4 gene sequencing does not provide sufficient resolution for species-level taxonomic identification, which would require other approaches such as shotgun metagenomics that could reveal compositional relationships at the species and strain levels which were not detectable here (Jovel et al., 2016). Primer selection may also potentially bias results (Pruesse et al., 2007). Finally, we did not adjust p-values for multiple comparisons in alpha and beta diversity analyses due to the small sample size of this pilot study (FDR thresholds were used for differential abundance testing) and acknowledge that our findings await replication in larger studies.

5 | CONCLUSIONS

The results of our study contribute to the growing literature on the influence of caregivers on the development of the infant gastrointestinal microbiome. Our results suggest that infants' social environments and exposures on the day of birth are associated with community composition and differential abundance of bacteria of the gastrointestinal microbiome composition as early as the first few weeks of neonatal life and up to 6 months of age. Initiation of skin-to-skin contact immediately after birth, time in physical contact on the day of birth, and the number of caregivers that hold the baby in early infancy were associated with community composition and the differential abundance of specific microbes in the first 6 months of infancy. Future studies are needed to identify mechanisms of social transmission of microbes from mothers and allomothers to infants and how variation in the infant gastrointestinal microbiome is associated with variation in later health and development outcomes.

AUTHOR CONTRIBUTIONS

Kyle S. Wiley: Conceptualization (equal); writing – original draft (lead); writing – review and editing (equal). **Andrew M. Gregg:** Formal analysis (lead); visualization (lead); writing – original draft (supporting). **Molly Fox:** Conceptualization (equal); funding acquisition (lead); project administration (equal); supervision (equal); writing – review and editing (equal). **Venu Lagishetty:** Methodology (equal); writing – review and editing (equal). **Curt A. Sandman:** Funding acquisition (equal); project administration (equal); writing – review and editing (equal). **Jonathan P. Jacobs:** Formal analysis (equal); methodology (equal); supervision (equal); writing – review and editing (equal). **Laura M. Glynn:** Conceptualization (equal); data curation (equal); funding acquisition (equal); project administration (equal); supervision (equal); writing – review and editing (equal).

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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