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Postpartum Maternal Stress is Unrelated to the Infant Fecal Microbiome, but is Associated With the Human Milk Microbiome in Exclusively Breastfeeding Mother-Infant Dyads: The Mother-Infant Microbiomes, Behavior, and Ecology Study (MIMBES)

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ABSTRACT

Objectives: This study aimed to evaluate whether postpartum maternal stress is associated with infant gastrointestinal microbiome composition and diversity, and whether this relationship may be mediated by maternal caregiving and breastfeeding behaviors and human milk microbiome (HMM) composition.

Methods: Infant fecal and human milk samples were collected from 51 exclusively breastfeeding mother-infant dyads in the Pacific Northwest between 1 and 6 months postpartum. Infant fecal samples with sequencing read counts > 773 ($n = 48$) and milk samples with read counts > 200 ($n = 46$) were analyzed for bacterial alpha diversity (richness, Shannon diversity), beta diversity (Bray–Curtis dissimilarity), and genera differential abundances. Infant fecal microbiome (IFM) measures were tested for associations with mothers' self-reported Parenting Stress Index total and subscale scores in regression (richness, Shannon diversity), envfit (beta diversity), and MaAsLin2 (genera abundance) models. Potential mediators of the relationship between maternal stress and IFM were explored (observed total time breastfeeding; maternal–infant physical contact frequency; and HMM alpha diversity, beta diversity, and genera abundance).

Results: Maternal stress was not associated with IFM alpha or beta diversities. Two maternal stress subscales were associated with differential abundances of *Erysipelotrichaceae UCG-003* (positively) and *Eggerthella* (negatively) in infant feces. Maternal total stress and two stress subscales (Role Restriction, Attachment) were associated positively with HMM beta diversity ($q_{attachment} = 0.07$) and negatively with HMM richness ($q_{total} = 0.08$, $q_{role} = 0.03$).

Conclusions: Postpartum stress is not consistently associated with IFM composition during exclusive breastfeeding. However, postpartum maternal stress is associated with HMM diversity, suggesting that maternal stress might influence other developmental pathways in the breastfeeding infant.

1 | Introduction

1.1 | Postpartum Maternal Stress and the Developmental Origins of Health and Disease

Early life is a sensitive period for the impact of exposures and experiences on rapidly developing physiological systems, with health consequences in later life. This concept forms the basis of the framework of the Developmental Origins of Health and Disease (Gluckman and Hanson 2004; Kuzawa and Kim 2022; Low et al. 2012; McKerracher et al. 2020). A growing body of research has explored how psychosocial stress[1] may be an important exposure shaping lifelong health and wellness (Barrero-Castillero et al. 2019; Dar et al. 2019; Olvera Alvarez et al. 2018; Palma-Gudiel et al. 2020; Suglia et al. 2021; Trudel-Fitzgerald et al. 2017). For example, fetal exposure to maternal psychosocial stress during pregnancy has been previously associated with a variety of developmental trajectories such as differential methylation of the hypothalamic–pituitary–adrenal (HPA) axis gene *NR3C1* (Sosnowski et al. 2018), slower cognitive development (Delagneau et al. 2023), different developmental trajectories of the HPA axis (McGowan and Matthews 2018), and later health outcomes such as increased risk for cardiovascular disease (Eberle et al. 2021), infectious disease (Nielsen et al. 2011), depression, anxiety, attention deficit hyperactivity disorder (ADHD), and reduced telomere length (Lautarescu et al. 2020).

Maternal psychosocial stress may continue to be an important influence during the postnatal period, given an infant's rapid growth and development at this age and their continued reliance on caregivers for social–emotional interactions and nutrition, including breastfeeding. Indeed, maternal stress has been hypothesized to provide signals about the environment to shape an offspring's development, provoking developmentally plastic responses to better suit the environment in which an offspring will live (Sheriff and Love 2013). Based on this hypothesis, maternal stress would be expected to provoke changes in an infant's mechanisms of developmental plasticity, with downstream effects on energetic regulation (Thompson et al. 2015), behavior and temperament (Bianco et al. 2023; Fox et al. 2022; Sutin et al. 2022), response to stress (Rosin et al. 2021), and physical growth (Grier et al. 2017; Robertson et al. 2019, 2023; White et al. 2013). Indeed, research has provided evidence that various types of maternal postpartum stress are associated with infant health and development outcomes, including poor child social–emotional development (Garthus-Niegel et al. 2017), increased emotional reactivity (Bosquet Enlow et al. 2011), and less optimal temperament profiles (Bianco et al. 2023; Sutin et al. 2022).

Mechanisms whereby maternal postpartum stress may contribute to altered child developmental and health outcomes are not known, but some evidence suggests that one mediating factor may be through influences on the infant gastrointestinal microbiome (frequently proxied by the fecal microbiome). Preliminary research found an association between maternal postpartum stress and infant fecal microbiome composition. In a study of 46 women and their infants in Los Angeles, California, maternal stress at 5–7 and 11–13 months postpartum was associated with concurrent reduced phylogenetic diversity, Shannon diversity, and evenness in the infant fecal microbiome (Galley et al. 2023).

The infant gastrointestinal microbiome has been identified as an important mechanism for the developmental origins of health and disease because it is influenced by environmental and behavioral factors and is associated with a variety of health and disease outcomes in infancy, childhood, and potentially adulthood (Stinson 2020). Differences in the composition of the fecal microbiome have been associated with many infant health outcomes, including stress response reactivity (Dong and Gupta 2019; Rosin et al. 2021), temperament (Fox et al. 2022), asthma (Stokholm et al. 2018, 2020; van Nimwegen et al. 2011; Zimmermann et al. 2019), allergies (Azad et al. 2015; De Filippis et al. 2021; Lee et al. 2021; Peroni et al. 2020), and cognitive and neural development (Carlson et al. 2018; Gao et al. 2019; Streit et al. 2021; Tamana et al. 2021; Vaher et al. 2022).

1.2 | Pathways Connecting Postpartum Maternal Stress and the Infant Gastrointestinal Microbiome

There are multiple pathways by which postpartum maternal stress might influence the infant fecal microbiome (IFM), including stress-related changes to maternal caregiving behaviors, breastfeeding patterns, and alterations in human milk composition (e.g., the milk microbiome). Stress-related changes to maternal caregiving behavior might in turn provoke stress in the infant. Maternal mental and emotional state, such as depression, has been associated with inconsistent and less supportive caregiving behaviors (Luecken and Lemery 2004). Maternal caregiving behaviors have been associated with infant developmental outcomes observed in studies of maternal postpartum stress, such as infant stress response development (Luecken and Lemery 2004), including neural systems related to stress reactivity (Hane and Fox 2006), stress-induced cortisol responses (Grant et al. 2009), and DNA methylation (Holdsworth et al. 2023a; Provenzi et al. 2020). Caregiver behavior has also been found to moderate the association of socioeconomic risks on children's fecal microbiome composition (Flannery et al. 2020), providing preliminary evidence for the impact of maternal caregiving behavior on the infant fecal microbiome.

Postpartum maternal stress may also influence breastfeeding patterns, with potential downstream effects on the infant fecal microbiome. For example, post-delivery stress has been associated with less frequent breastfeeding (Doulougeri et al. 2013) and potentially shorter feedings due to impaired lactogenesis (Dewey 2001). Although whether breastfeeding patterns are associated with variation in the infant fecal microbiome is not known, previous research has provided evidence that some breastfeeding patterns are associated with infant length growth (Galler et al. 1998), more frequent bouts of breastfeeding at 1 month of age are associated with increased adiposity at 1 and 2 years of age (Agras et al. 1987), and schedule feeding is associated with more rapid weight gain compared to on-demand feeding (Mihrshahi et al. 2011). As other studies have found no association between on-demand or schedule feeding and infant

growth measures (Gubbels et al. 2011; Saxon et al. 2002), it is still unclear whether breastfeeding practices influence infant growth and development, including the composition of the fecal microbiome.

Another pathway whereby maternal stress might influence the infant fecal microbiome may be through alterations to milk composition itself, including the milk microbiome. The effect of postpartum maternal mood and stress on infant temperament was shown to be moderated by breastfeeding (Braithwaite et al. 2021), suggesting that milk composition is a potential pathway connecting postpartum stress to infant outcomes. Previous research has provided evidence that postpartum maternal distress is associated with reduced alpha diversity in the milk microbiome at 3 months postpartum (Browne et al. 2019) and with differences in beta-diversity and relative abundance of some genera at 10 and 24 days postpartum (Juncker et al. 2025). The milk microbiome is hypothesized to be partially seeded by the maternal gastrointestinal microbiome (Selvamani et al. 2021), variation in which has been associated with maternal stress (Hechler et al. 2019). However, whether these stress-associated differences in the human milk microbiome are associated with infant outcomes, including the infant fecal microbiome, is unknown.

1.3 | Aims and Hypotheses

The primary aim of this study was to explore how maternal postpartum stress might influence the infant fecal microbiome (IFM). Specifically, it tests two hypotheses (Figure 1): (1) *Maternal postpartum stress is associated with variation in the infant fecal microbiome and* (2) *Maternal caregiving behaviors, breastfeeding behaviors, and milk microbiome composition mediate the relationship between maternal postpartum stress and the infant fecal microbiome*.

2 | Methods

2.1 | Study Design and Participants

Fifty-one mother-infant dyads residing in rural eastern Washington and northeast Idaho were enrolled in the Mother-Infant Microbiomes, Behavior, and Ecology Study (MIMBES; Holdsworth et al. 2023b). All mothers reported they were exclusively breastfeeding or pumping at least 5 times daily to ensure adequate milk production, ≥ 18 years of age, 3 weeks to 5.5 months postpartum, they and their infants were healthy, and infants were exclusively consuming mother's own milk. Potential participants were excluded if they self-reported any indications of breast infection in the previous 7 days (i.e., fever, red streaks on breast, hard red portions on breast, any abnormal breast pain, discomfort, or lumps), infant or maternal signs of acute illness in the previous 7 days (i.e., fever, diarrhea, vomiting, severe cough, or rapid breathing in infants), or any antibiotics consumed by or administered to the mother or infant in the past 30 days.

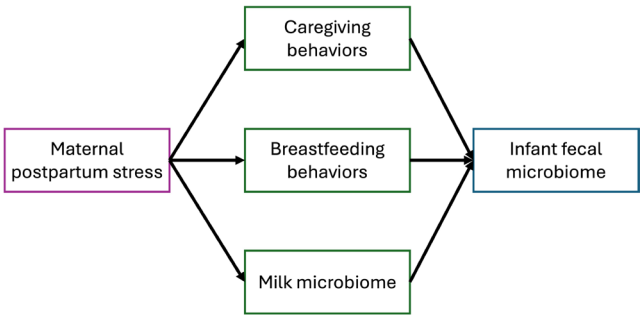

FIGURE 1 | Theoretical relationship of maternal postpartum stress and the infant fecal microbiome, potentially mediated by maternal caregiving and breastfeeding behaviors and the milk microbiome.

Mothers completed surveys, participated in behavioral observations of their infant, and provided a milk sample and their infant's fecal sample for microbiome analysis. The human milk microbiome and mothers' breastfeeding patterns data from this study have previously been described (Holdsworth et al. 2023b). This analysis only includes participants whose milk samples had sequencing read counts > 200 or infant fecal sample sequencing read counts > 773. As such, analyses of milk samples include 46 mother-infant dyads, analyses of infant fecal samples include 48 mother-infant dyads, and analyses incorporating both milk and infant fecal samples include 43 mother-infant dyads. Written informed consent to participate was provided by the mothers who also provided assent for infant participation. This study was approved by the Institutional Review Board of Washington State University (#15852).

2.2 | Parenting Stress Index

Mothers completed the parent domain items of the Parenting Stress Index (PSI; Abidin 1997) as part of the surveys completed at the start of the study. The PSI evaluates experiences of stress relating to aspects of parenting, including subscales of Competence, Attachment, Role Restriction, Depression, Relationship with Spouse, Health, and Isolation (Table 1). The parent domain is comprised of 54 items; however, two items in the Competence subscale were accidentally omitted from the questionnaire that mothers completed. As such, we calculated each participant's average response score on each subscale and across all items to generate a PSI Total score, as done in previous studies (Oddi et al. 2013). One participant had a missing value for one item, so we imputed the participant's average value for the subscale, following PSI scoring guidelines (Abidin 1995). Responses on each item ranged from 1 to 5, with higher values indicating more stress. Reliability of these scales with the two missing items was assessed with Cronbach's standardized alpha (Cronbach 1951).

2.3 | Infant Behavioral Observations

Infants were observed in their homes across 12 h in their regular lives for various behaviors and interactions relating to caregiving, interpersonal interactions, and breastfeeding. These observational data were used to derive infants' total time breastfeeding and how much time the mother was in physical contact with her infant during the observation period. More detailed descriptions of these observations are

TABLE 1 | Parenting Stress Index (PSI) subscales.

	Number of items	Description
Attachment	7	Parent does not feel a sense of emotional closeness to their child or they do not feel able to observe and understand their child's feelings and/or needs accurately.
Competence	11*	Parent does not feel competent in their ability to care for their child or feel overwhelmed by their child's needs and demands.
Depression	9	Parent is experiencing significant depression. Some items relate to guilt and unhappy feelings.
Health	5	Deterioration in health as a result of either parenting stress or an additional independent stress in the parent–child dyad.
Isolation	6	Social isolation from peers, relatives, and other emotional support systems.
Relationship with Spouse	7	Parent is lacking the emotional and active support of the other parent in the area of child management.
Role Restriction	7	Parent experiences the parental role as restricting their freedom and frustrating them in their attempts to maintain their own identity. Parent sees themself as being controlled and dominated by their children's demands and needs.

Note: Description of subscales is adapted from Abidin (1997).
*In the original Parenting Stress Index, the Competence subscale is comprised of 13 items. In our study, two items from this subscale were erroneously excluded from the questionnaire that participants completed, resulting in 11 items.

available in Holdsworth et al. (2023b). Briefly, research staff observed infants in their regular daily activities over 12 h split across 3 days (0700–1100, 1100–1500, and 1500–1900 h). Observations of select behaviors were recorded every 30 s. The observer took a 15-min break after 45 min of observation to avoid observer fatigue, resulting in 9 h of recorded observations.

Frequency of maternal physical contact was defined as any physical contact (touching or holding) of the infant by their mother in each observable interval (30 s). Total time breastfeeding and maternal physical contact frequency were transformed into minutes for ease of interpretation.

2.4 | Fecal and Milk Sample Collection and Sequencing

Milk samples were collected in a laboratory at Washington State University. Each participant provided a milk sample via full breast expression after having not nursed for at least 2 h on the designated "study breast" and after the period of behavioral observations described above. After cleaning the breast with a Castile soap wipe (Professional Disposable International, Woodcliff, New Jersey), milk was collected using a Medela Symphony electric breast pump and single-use sterile milk collection kit (Medela Inc.; McHenry, Illinois). Samples were swirled and immediately aliquoted into sterile vials and transferred to a freezer (−20°C).

At the time of milk sample collection, infants' diapers were removed and their posteriors cleaned with a Castile soap wipe and changed into a new, study-provided diaper. If the infant defecated during the study visit in the laboratory, the fecal sample was collected by research staff at that time. Otherwise, a fecal sample was collected by mothers at home within 24 h of the milk sample collection. Research staff or the mother put on gloves and scooped fecal material into the sterile storage tube (Sarstedt AG & Co., Nümbrecht, Germany) using the provided spatula. Samples were placed in the participant's home freezer and picked up by research personnel within a day, where they were transferred to the laboratory freezer (−20°C).

2.5 | DNA Extraction, Amplification, and Sequencing

At study completion, all samples were transferred to the laboratory at the University of Idaho where they were stored at −20°C until analysis. DNA was extracted from 200 mg fecal samples after thawing samples on ice. Thawed samples were vortexed with 0.5 mL of TE50 (10 mM Tris–HCl, 50 mM EDTA, pH 8) and DNA was extracted using the QIAamp Fast DNA Stool Mini Kit (Qiagen, Germantown, MD) as previously described (Lackey et al. 2019). DNA was eluted with 200 μL of ATE buffer and stored at −80°C until polymerase chain reaction (PCR) amplification.

DNA was extracted from 1 mL of each milk sample following a modified protocol of enzymatic lysis and bead-beating with the QIAamp DNA Mini Kit (Qiagen, Germantown, MD) described previously (Lackey et al. 2019). DNA was eluted with 50 μL nuclease-free water and stored at −80°C until further processing. Nuclease-free water was used for negative controls during DNA extraction and processed along with samples.

The V1-V3 hypervariable region of the 16S rRNA gene was amplified from the extracted DNA using a dual-barcoded two-step

30-cycle PCR as described previously (Lackey et al. 2019). PCR amplicons were cleaned and size-selected using paramagnetic beads from the HighPrep PCR Clean-up System (MagBio Genomics Inc., Gaithersburg, MD) following the manufacturer's instructions. Cleaned amplicons were quantified using the Accuclear Ultra High Sensitivity dsDNA Quantitation Kit (Biotium, Fremont, CA) and pooled to contain 50 ng DNA from each sample. Amplicon pools were cleaned using paramagnetic beads, quality checked on a Fragment Analyzer (Advanced Analytical Technologies, Ankeny, IA), and quantified using the KAPA Biosciences Illumina library quantification kit and the Applied Biosystems StepOne Plus real-time PCR system. Pools were sequenced using an Illumina MiSeq (San Diego, CA) v3 paired-end 300-bp protocol for 600 cycles at the University of Idaho Genomics and Bioinformatics Resources Core. Nuclease-free water was used for negative controls during PCR and processed along with samples.

Sequencing reads were demultiplexed using dbcAmplicons (https://github.com/msettles/dbcAmplicons) and processed using DADA2 v.1.26 (Callahan et al. 2016) in R v.4.3.2 (R Core Team 2023) as described by Pace et al. (2021). Additionally, to control for potential contaminating sequences, data from negative controls were used with the decontam package v.1.24 (Davis et al. 2018) to remove potential contaminants from sequenced data.

2.6 | Microbiome Alpha Diversity, Beta Diversity, and Genera Abundance Calculations

All statistical analyses were conducted in R v.4.3.2 (R Core Team 2023). Milk and fecal samples with low read counts (<200 and <773 reads, respectively) were removed from all analyses. Milk samples were rarefied to 200 read depth, and infant fecal samples were rarefied to 773 read depth prior to alpha and beta diversity calculations. Microbiome alpha diversity indices of richness and Shannon diversity were calculated with the vegan package v.2.6.6.1 in R (Oksanen et al. 2013). Beta diversity index of Bray–Curtis Dissimilarity Distance matrix was calculated for milk and fecal microbiome data using the vegdist function in the vegan package for R (Oksanen et al. 2013). Relative abundances and prevalence counts of bacterial taxa identified in infant fecal and milk samples were calculated from unrarefied count data aggregated at the genus level.

2.7 | Models of Hypothesis Testing

Given the small sample size, we constructed three models for each set of IFM outcome variables to test mediation via multiple regression (Baron and Kenny 1986); (1) predictor variables to outcome variables, (2) predictor variables to mediator variables, and (3) mediator variables to outcome variables. If the relationships between variables in steps #2 and #3 were significant, we planned to construct a mediation model and evaluate the strength of mediation.

To test for an association between maternal stress and IFM alpha diversity, separate regression models were conducted for each PSI subscale and total score, as well as potential mediators (maternal total time breastfeeding infant, maternal physical contact frequency with infant, HMM richness, and HMM Shannon diversity), with the outcome variables of IFM richness and Shannon diversity. Separate regression models also explored the association between PSI subscales and total scores on potential mediator variables as model outcomes (maternal total time breastfeeding infant, maternal physical contact frequency with infant, HMM richness, and HMM Shannon diversity). Each model controlled for infant age at sample collection and mode of delivery at birth (vaginal or cesarean-section). An exploratory post hoc analysis of the relationship between PSI and HMM alpha diversity metrics also controlled for maternal body mass index (BMI; derived from self-reported height and weight at milk sample collection) and maternal 7-day diet richness (self-reported number of food categories consumed in the previous 7 days). The stats::lm function was used in R for all regression models. *P*-values of the main predictor variables were corrected for multiple testing within the groups of outcome variables (IFM outcome models, HMM outcome models, maternal caregiving and breastfeeding outcome models) using the Benjamini-Hochberg procedure. Significance was declared at $q<0.10$.

To test for an association between maternal stress and IFM beta diversity, envfit (from vegan package) was run on the nonmetric multidimensional scaling (NMDS) of the IFM Bray–Curtis dissimilarity matrix calculated with metaMDS from the vegan package. The envfit function included factors of all PSI subscales and total score, potential mediators (maternal total time breastfeeding infant and maternal physical contact frequency with infant), delivery mode at birth, and infant age at sample collection, with 9999 permutations. Additionally, envfit was conducted with the NMDS of the HMM Bray–Curtis dissimilarity matrix to assess possible mediating effects of HMM beta diversity, including factors of each PSI subscale and total scores, delivery mode at birth, and infant age at sample collection, with 9999 permutations. All envfit models were corrected for multiple testing with the Benjamini-Hochberg procedure, and significance was declared at $q<0.10$. The potential mediating role of HMM beta diversity was also assessed with a Mantel test of the IFM and HMM Bray–Curtis dissimilarity matrices, which evaluated the Pearson product-moment correlation of the matrices (vegan package). Significance of the Mantel test was declared at $p<0.05$.

Associations of maternal stress and potential mediators with IFM genera abundance were evaluated using the Microbiome Multivariable Associations with Linear Models (MaAsLin2) package v.1.16.0 (Mallick et al. 2021) in R. This analysis considered separate models for each predictor variable. This analysis explored linear relationships with genera that were present in at least 10% of the samples, using the default transformation and normalization methods (i.e., log-transformation and total sum scaling normalization). To identify potential mediating effects of the HMM, separate MaAsLin2 models were created for each PSI subscale and total scores for the HMM samples with the same specifications. All models controlled for delivery mode at birth and infant age at sample collection as fixed effects. Each model was corrected for multiple testing with the Benjamini-Hochberg procedure, and variables were considered significantly associated with genus abundance if $q<0.10$.

We conducted an additional post hoc analysis of the models described above using binary cut-off values for the PSI scores. PSI binary variables were created for ≥75th percentile (High) and <75th percentile (Low), according to the reference percentiles for the Parenting Stress Index (Abidin 1995), as anything below this threshold is considered "normal" (Loyd and Abidin 1985). In the case of PSI Total and PSI Competence, an average 75th percentile score was calculated and used as the cut-off. The results from this post hoc analysis are described if they differ from the results of the main analyses.

3 | Results

3.1 | Analytic Sample, Sample Description, and IFM Genera

After removing samples with low read counts, 48 participants remained in the analytic sample assessing IFM measures only, 46 participants remained in the analytic sample assessing HMM measures only, and 43 participants remained in the analytic sample assessing IFM and HMM measures together. There were no notable differences in the means and distributions of variables or demographics depending on inclusion due to sample read count (Table 2).

Mothers were overall highly educated: 81% of the IFM analytic sample had at least a bachelor's degree. Infants were on average 2.6 months old, ranging from just under 1 month to just under 6 months of age. Parenting stress measures were relatively low in mean and maximum reported score (Table 2, Supplemental Figure 1). Possible scores could range from 1 (least stress) to 5 (most stress). All PSI subscales and total means were less than 3, the midpoint for the scales. When using the 75th percentile to indicate high stress, multiple mothers indicated high stress on the Isolation ($n=16$), Attachment ($n=15$), Health ($n=29$), Role Restriction ($n=18$), Depression ($n=11$), Relationship with Spouse ($n=14$), and Competence subscales ($n=7$), as well as Total Parenting Stress ($n=13$).

The accidental exclusion of two items from the Parenting Stress Index did not appear to impact the internal consistency of the scale. The standardized Cronbach's alpha for the total scale was 0.91. The standardized Cronbach's alpha for the Competence subscale was 0.71, similar to the 0.72 reported in the validation sample of the Parenting Stress Index (Abidin 1995).

One participant had a substantially greater amount of observed total time breastfeeding (313 min). To minimize the influence of this extreme outlier on the bivariate and multivariable analyses, this value was Winsorized to 1 min above the next highest value, resulting in a value of 179 min (Supplemental Figure 2).

Among the 48 infant fecal samples with sufficient read counts (Figure 2, Supplemental Table 1), 110 genera were identified. *Bifidobacterium* had the highest mean relative abundance across all samples (19%) and was present in 43 samples (89.6% prevalence). Although *Streptococcus* was the most prevalent genus (present in 46 samples, 95.8%), it was present in relatively low abundance (mean 2.2%). Other highly abundant genera were also present in most samples: *Veillonella* (mean relative abundance 16.0%, present in 43 samples), *Bacteroides* (mean relative abundance 15.2%, present in 40 samples), *Escherichia-Shigella* (mean relative abundance 11.5%, present in 38 samples), and *Clostridium sensu stricto 1* (mean relative abundance 6.4%, present in 30 samples).

Overall, 14 genera had >1% mean relative abundance. Some of these were not among the top 14 most prevalent genera, indicating that their mean relative abundance may be driven by greater abundance in fewer samples. This includes *Erysipelotrichaceae UCG-003* (present in 5 samples, mean relative abundance = 1.31%), *Parabacteroides* (present in 17 samples, mean relative abundance = 1.33%), *Citrobacter* (present in 17 samples, mean relative abundance = 2.77%), *Akkermansia* (present in 12 samples, mean relative abundance = 3.20%), and *Ruminococcus gnavus* group (present in 18 samples, mean relative abundance = 5.27%).

There were 83 genera identified in the 46 HMM samples with sufficient read counts (>200). HMM genera relative abundance (Supplemental Figure 3) in this study has been previously described in more detail (Holdsworth et al. 2023b). Only *Streptococcus* was present at >1% mean relative abundance in both HMM and IFM samples (Figure 2, Supplemental Figure 3) and it was highly prevalent in both (100% of milk samples and 95.8% of infant fecal samples). As expected for microbiomes of different body sites, many genera that were present in the IFM samples were not present in HMM samples, including *Erysipelotrichaceae UCG-003* and *Eggerthella*.

3.2 | IFM Alpha Diversity Associations With Maternal Stress and Mediators

In separate regression models controlling for infant age and mode of delivery, no measure of maternal parenting stress was significantly associated with IFM richness or Shannon diversity (Figure 3A,B). Coefficient estimates' 95% confidence intervals were wide and crossed 0, indicating that it is unclear whether PSI scores could be positively or negatively associated with IFM richness or Shannon diversity. Potential mediators (maternal physical contact frequency, total time breastfeeding, HMM richness, and HMM Shannon diversity) were also not significantly associated with IFM richness or Shannon diversity.

To test for potential mediating effects, we also evaluated the association of maternal parenting stress with the proposed mediator variables. After correcting for multiple testing, PSI Total ($B=-5.99$, $q=0.08$) and PSI Role Restriction ($B=-3.94$, $q=0.03$) scores were negatively associated with HMM richness, and no variables were associated with HMM Shannon diversity in separate regression models controlling for infant age and delivery mode (Figure 3C,D). Coefficient estimates of all stress subscale scores were negative in relation to HMM richness. Though not significant after correcting for multiple tests, PSI Health and PSI Competence had similar coefficient estimates to PSI Role Restriction, suggesting possible similar effects of these dimensions of stress on HMM richness. Each unit increase in PSI Total score was associated with 6 fewer observed unique amplicon sequence variants (ASVs) in the HMM, while each unit increase in PSI Role Restriction score was associated with

TABLE 2 | Participant characteristics and variable distribution.

	IFM Sample ($n = 48$)	HMM Sample ($n = 46$)	IFM and HMM Sample ($n = 43$)	Total Study Sample ($N = 51$)
Mother's age (years)				
Mean (SD)	29.2 (3.62)	29.1 (3.81)	29.2 (3.71)	29.0 (3.71)
Median [Min, Max]	28.0 [23.0, 38.0]	28.0 [22.0, 38.0]	28.0 [23.0, 38.0]	28.0 [22.0, 38.0]
Mother's ethnicity				
Caucasian/European American	39 (81.3%)	39 (84.8%)	36 (83.7%)	42 (82.4%)
Asian/Asian American	4 (8.3%)	3 (6.5%)	3 (7.0%)	4 (7.8%)
Hispanic/Latino	2 (4.2%)	1 (2.2%)	1 (2.3%)	2 (3.9%)
Other	3 (6.3%)	3 (6.5%)	3 (7.0%)	3 (5.9%)
Mother's educational level				
High school degree or equivalent	4 (8.3%)	5 (10.9%)	3 (7.0%)	6 (11.8%)
Vocational/technical school	1 (2.1%)	1 (2.2%)	1 (2.3%)	1 (2.0%)
Associate's degree	4 (8.3%)	5 (10.9%)	4 (9.3%)	5 (9.8%)
Bachelor's degree	26 (54.2%)	23 (50.0%)	23 (53.5%)	26 (51.0%)
Master's degree	10 (20.8%)	10 (21.7%)	10 (23.3%)	10 (19.6%)
PhD	3 (6.3%)	2 (4.3%)	2 (4.7%)	3 (5.9%)
Infant sex				
Female	24 (50.0%)	22 (47.8%)	21 (48.8%)	25 (49.0%)
Male	24 (50.0%)	24 (52.2%)	22 (51.2%)	26 (51.0%)
Infant's age (months)				
Mean (SD)	2.55 (1.39)	2.56 (1.41)	2.56 (1.42)	2.55 (1.39)
Median [Min, Max]	2.20 [0.970, 5.87]	2.12 [0.970, 5.87]	2.20 [0.970, 5.87]	2.20 [0.970, 5.87]
Delivery mode				
C-section	13 (27.1%)	11 (23.9%)	11 (25.6%)	13 (25.5%)
Vaginal	35 (72.9%)	35 (76.1%)	32 (74.4%)	38 (74.5%)
Mother's body mass index (BMI)				
Mean (SD)	27.25 (6.18)	27. 37 (6.31)	27.48 (6.42)	27.16 (6.09)
Median [Min, Max]	25.5 [20.0, 50.5]	25.6 [20.0, 50.5]	25.5 [20.0, 50.5]	25.5 [20.0, 50.5]
Mother's 7-day diet richness				
Mean (SD)	9.7 (1.3)	9.8 (1.3)	9.7 (1.3)	9.8 (1.3)
Median [Min, Max]	10 [6, 12]	10 [6, 12]	10 [6, 12]	10 [6, 12]
PSI Attachment				
Mean (SD)	1.72 (0.452)	1.69 (0.447)	1.72 (0.445)	1.69 (0.453)
Median [Min, Max]	1.64 [1.00, 3.00]	1.57 [1.00, 3.00]	1.71 [1.00, 3.00]	1.57 [1.00, 3.00]
PSI Role Restriction				
Mean (SD)	2.84 (0.654)	2.86 (0.664)	2.84 (0.669)	2.85 (0.650)
Median [Min, Max]	2.71 [1.71, 4.71]	2.71 [1.71, 4.71]	2.71 [1.71, 4.71]	2.71 [1.71, 4.71]

(Continues)

TABLE 2 | (Continued)

	IFM Sample ($n = 48$)	HMM Sample ($n = 46$)	IFM and HMM Sample ($n = 43$)	Total Study Sample ($N = 51$)
PSI Depression				
Mean (SD)	2.08 (0.631)	2.08 (0.575)	2.07 (0.585)	2.08 (0.619)
Median [Min, Max]	2.06 [1.00, 3.56]	2.06 [1.00, 3.56]	2.00 [1.00, 3.56]	2.11 [1.00, 3.56]
PSI Spouse				
Mean (SD)	2.35 (0.671)	2.33 (0.635)	2.34 (0.653)	2.34 (0.655)
Median [Min, Max]	2.21 [1.14, 3.86]	2.14 [1.14, 3.86]	2.14 [1.14, 3.86]	2.14 [1.14, 3.86]
PSI Health				
Mean (SD)	2.86 (0.569)	2.86 (0.487)	2.87 (0.494)	2.85 (0.559)
Median [Min, Max]	2.80 [1.40, 4.00]	2.80 [2.20, 4.00]	2.80 [2.20, 4.00]	2.80 [1.40, 4.00]
PSI Isolation				
Mean (SD)	2.14 (0.682)	2.13 (0.663)	2.11 (0.665)	2.15 (0.679)
Median [Min, Max]	2.00 [1.00, 3.50]	2.00 [1.00, 3.50]	2.00 [1.00, 3.50]	2.00 [1.00, 3.50]
PSI Competence				
Mean (SD)	1.98 (0.419)	1.97 (0.386)	1.98 (0.398)	1.97 (0.408)
Median [Min, Max]	1.91 [1.09, 2.73]	1.91 [1.09, 2.73]	1.91 [1.09, 2.73]	1.91 [1.09, 2.73]
PSI Total				
Mean (SD)	2.23 (0.408)	2.22 (0.375)	2.23 (0.381)	2.23 (0.401)
Median [Min, Max]	2.15 [1.33, 3.12]	2.15 [1.33, 3.06]	2.15 [1.33, 3.06]	2.15 [1.33, 3.12]
Physical contact frequency (minutes)				
Mean (SD)	280 (108)	280 (108)	281 (110)	279 (106)
Median [Min, Max]	274 [48.5, 504]	265 [48.5, 504]	268 [48.5, 504]	268 [48.5, 504]
Total time breastfeeding (minutes)				
Mean (SD)	76.3 (40.6)	73.9 (41.5)	76.2 (41.6)	74.3 (40.6)
Median [Min, Max]	68.3 [17.0, 179]	63.3 [17.0, 179]	65.5 [17.0, 179]	65.5 [17.0, 179]
HMM Shannon				
Mean (SD)	2.44 (0.446)	2.45 (0.442)	2.44 (0.446)	2.45 (0.442)
Median [Min, Max]	2.51 [1.29, 3.11]	2.52 [1.29, 3.11]	2.51 [1.29, 3.11]	2.52 [1.29, 3.11]
Missing	5 (10.4%)	0 (0%)	0 (0%)	5 (9.8%)
HMM Richness				
Mean (SD)	26.3 (5.79)	26.3 (5.76)	26.3 (5.79)	26.3 (5.76)
Median [Min, Max]	26.0 [14.0, 41.0]	26.0 [14.0, 41.0]	26.0 [14.0, 41.0]	26.0 [14.0, 41.0]
Missing	5 (10.4%)	0 (0%)	0 (0%)	5 (9.8%)
IFM Richness				
Mean (SD)	34.0 (11.8)	34.3 (12.0)	34.3 (12.0)	34.0 (11.8)
Median [Min, Max]	31.5 [16.0, 72.0]	32.0 [16.0, 72.0]	32.0 [16.0, 72.0]	31.5 [16.0, 72.0]
Missing	0 (0%)	3 (6.5%)	0 (0%)	3 (5.9%)

(Continues)

TABLE 2 | (Continued)

	IFM Sample ($n=48$)	HMM Sample ($n=46$)	IFM and HMM Sample ($n=43$)	Total Study Sample ($N=51$)
IFM Shannon				
Mean (SD)	2.28 (0.425)	2.27 (0.446)	2.27 (0.446)	2.28 (0.425)
Median [Min, Max]	2.33 [1.18, 3.50]	2.31 [1.18, 3.50]	2.31 [1.18, 3.50]	2.33 [1.18, 3.50]
Missing	0 (0%)	3 (6.5%)	0 (0%)	3 (5.9%)

Note: Unless otherwise noted, rows indicate *n* (%).

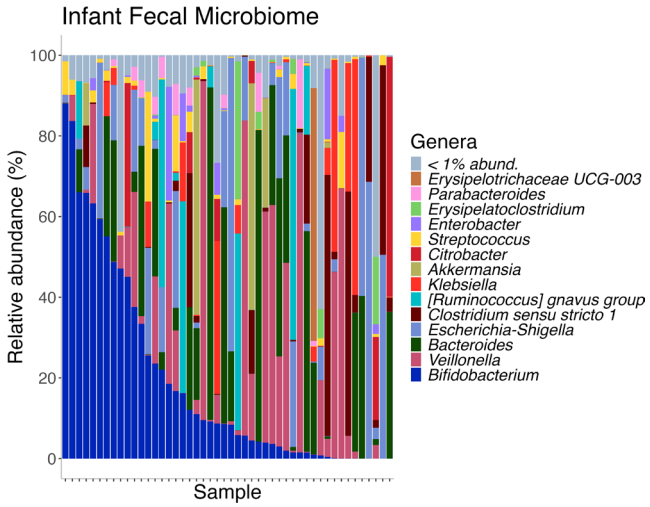

FIGURE 2 | Relative abundances of bacterial genera in the infant fecal samples with read counts > 773. Relative abundance was calculated on unrarefied count data. Genera are ordered by mean relative abundance across all samples, in ascending order. Samples are ordered by relative abundance of *Bifidobacterium*, in descending order.

4 fewer ASVs in the HMM. These results were similar when additionally controlling for maternal diet richness and BMI (Supplemental Figure 4). Maternal parenting stress variables were not associated with total time breastfeeding or maternal physical contact time (Figure 3E,F). Results from the post hoc analyses of binary PSI variables did not notably differ from these results (Supplemental Figure 5).

Thus, while there were significant associations between maternal parenting stress and HMM alpha diversity measures, the lack of any significant associations or large effect sizes with IFM alpha diversity indicates that maternal stress is not associated with IFM alpha diversity measures, and that this relationship is not mediated by any factors we explored.

3.3 | IFM Beta Diversity Associations With Maternal Stress and Mediators

Only delivery mode ($r^2=0.10$, $p<0.01$) was a significant factor in explaining the distribution of IFM Bray–Curtis dissimilarity (Figure 4A). Infant age ($r^2=0.11$) explained most of the variation in IFM beta diversity. Of all the PSI scores, Health explained the most variation in IFM beta diversity ($r^2=0.07$). Overall, PSI scores explained very little of the variation in IFM beta diversity. All maternal parenting stress variables, maternal mediator variables (total time breastfeeding and physical contact time), and infant age were not significant predictors.

When evaluating HMM beta diversity for possible mediating effects, PSI Attachment score was the only significant factor that explained the distribution of HMM Bray–Curtis dissimilarity (Figure 4B), explaining 21% of the variation in HMM beta diversity ($r^2=0.21$, $p<0.01$). Similarly, a Mantel test on the correlation of the IFM and HMM Bray–Curtis dissimilarity matrices was non-significant ($r=-0.006$, $p=0.50$). Given the lack of significant association between PSI Attachment score and IFM Bray–Curtis dissimilarity, HMM beta diversity is unlikely to mediate the relationship between PSI variables and IFM beta diversity.

3.4 | IFM Genera Abundance Associations With Maternal Stress and Mediators

In separate MaAsLin 2 models controlling for infant age and delivery mode, only PSI Attachment score was associated with the abundance of *Erysipelotrichaceae UCG-003* (coefficient = 2.41, $q=0.04$), and PSI Spouse score was associated with the abundance of *Eggerthella* (coefficient = −0.88, $q=0.03$). Each model evaluated associations with the 44 different genera that were present in at least 10% of the participant samples. These relationships may be driven by the absence of *Erysipelotrichaceae UCG-003* and *Eggerthella* among lower PSI Attachment scores and higher PSI Spouse scores, respectively, as these genera had relatively low prevalence (5 samples and 8 samples out of 48, respectively). These associations also had notable outliers that may have influenced these estimates (Supplemental Figure 6). The association with *Erysipelotrichaceae UCG-003* is evidently driven by an outlier and is absent in all other samples (Supplemental Figure 6A). The association with *Eggerthella* may also be driven by an extreme data point, though it is also present in seven samples with mothers whose PSI Spouse scores are < 2.2 and only present in one sample with PSI Spouse score > 2.2 (Supplemental Figure 6B). In our post hoc analysis of High stress (≥ 75th percentile) and Low stress groups, these associations were no longer significant at $q<0.1$, further demonstrating that the significant findings were due to outlier influences.

No maternal parenting stress measure was associated with the abundance of any HMM genus (evaluating the 43 genera present in at least 10% of the participant samples). This indicates that HMM genera abundance is an unlikely mediator of the relationship between PSI scores and IFM genera abundance.

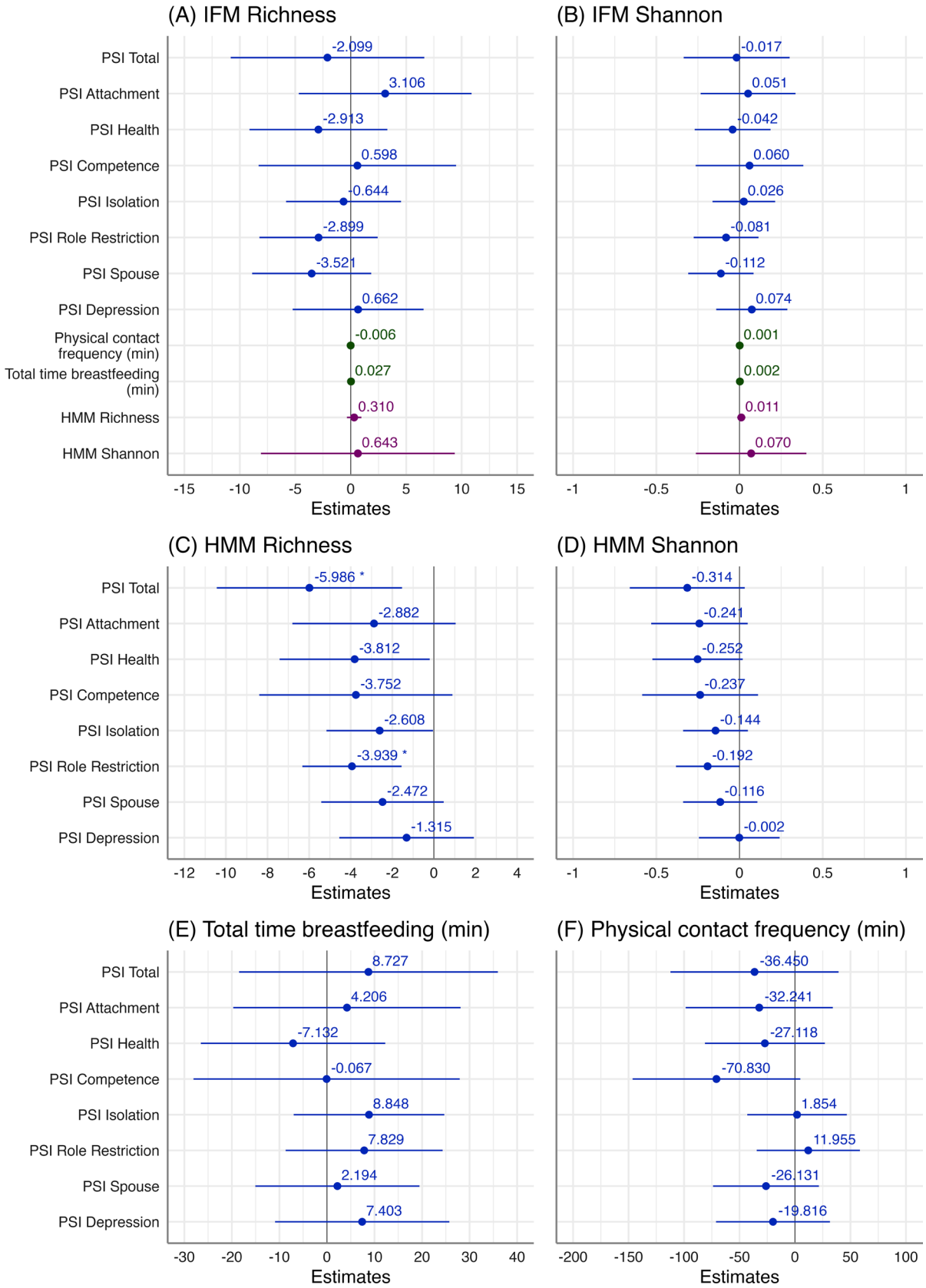

FIGURE 3 | Regression models of the associations between dependent variables (A) infant fecal microbiome (IFM) richness, (B) IFM Shannon diversity, (C) human milk microbiome (HMM) richness, (D) HMM Shannon diversity, (E) potential mediator total time breastfeeding, and (F) frequency of maternal physical contact with infant and Parenting Stress Index (PSI) total and subscale values. Points indicate the unstandardized coefficients, lines are 95% confidence intervals. Each coefficient represents a separate regression model. Every regression model controls for infant age and mode of delivery. Colors reflect groups of variables: Blue = PSI variables, green = maternal behavior mediators, purple = HMM mediators. *$q < 0.1$.

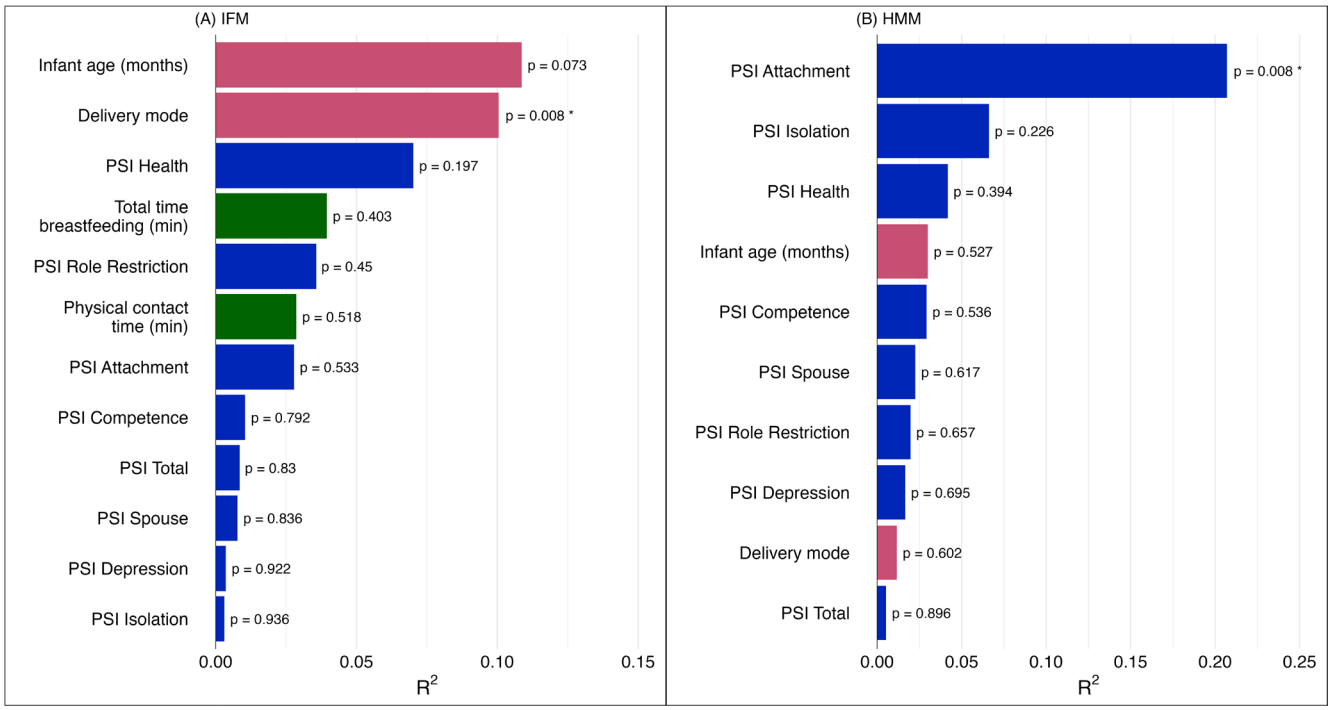

FIGURE 4 | Envfit predictors' R^2 and *p*-values in models of the non-metric dimensional scaling ordination of the Bray–Curtis dissimilarity matrix of (A) the infant fecal microbiome (IFM) and (B) the milk microbiome (HMM). Parenting Stress Index (PSI) variables are noted in blue, potential mediator variables are noted in green, and covariates are noted in pink. *$q < 0.10$.

4 | Discussion

Overall, we found little evidence in support of Hypothesis 1. There were no significant relationships with any measure of maternal parenting stress and infant fecal microbiome alpha diversity (richness and Shannon diversity) or beta diversity (Bray-Curtis). Although there were significant associations between two parenting stress measures and the abundances of two IFM genera, these associations might be due to outliers. As such, these results are not strongly supportive of the hypothesis given the number of genera and parenting stress measures evaluated.

We also found no evidence to support Hypothesis 2 related to maternal caregiving and breastfeeding behavior. Maternal caregiving behavior (physical contact frequency with infant) and breastfeeding behavior (total time breastfeeding infant) were not significantly associated with maternal parenting stress measures, nor with IFM alpha diversity measures, IFM beta diversity, or any IFM genera differential abundances. This indicates that maternal caregiving and breastfeeding behaviors are not mediators of any relationship between maternal parenting stress and the infant fecal microbiome.

We found minimal support for Hypothesis 2 regarding HMM as a mediator, as HMM measures were consistently unassociated with IFM measures. However, we did find significant associations between parenting stress measures and the HMM. More parenting stress overall and in the Role Restriction subscale were associated with reduced HMM richness. Notably, PSI Attachment was the only stress measure associated with both HMM composition (beta diversity) and IFM composition (abundance of *Erysipelotrichaceae UCG-003*).

Although this study does not indicate a consistent relationship between maternal postpartum stress and IFM in this population, it did indicate a relationship between maternal postpartum stress and the HMM. These findings are in line with Browne et al.'s (2019) study demonstrating reduced HMM alpha diversity with more maternal postnatal distress. While these results also broadly align with Juncker et al.'s (2025) findings of significant differences in HMM composition by maternal stress, the aspects of the HMM that were associated with stress were different from our own findings. Juncker and colleagues did not find differences in HMM alpha diversity by maternal stress, contrary to our findings. Similarly, they found differences in the relative abundance of specific genera by maternal stress, while we found no differences. These findings may point to different impacts of maternal stress on HMM by both the type of stress experienced and temporal changes in human milk composition. In assessing the effects of stress on the HMM, Juncker et al. (2025) compared differences between mothers whose infants were hospitalized for at least 2 days after birth (high stress group) and those who did not experience this (control group), as well as differences by Perceived Stress Scale scores. These measures may capture more severe and acute stress than our measure of Parenting Stress, as well as other stressors besides parenting. They also measured the HMM at 10 and 24 days postpartum, much earlier than our study. Future research should explore these relationships longitudinally, to better test whether temporal changes in stress are associated with changes in HMM composition, and whether effect sizes vary by time postpartum. However, the results from this study and others (Browne et al. 2019; Juncker et al. 2025)

indicates that maternal postpartum parenting stress may have a negative impact on HMM richness among exclusively breastfeeding mother-infant dyads.

Total PSI score was associated with reduced HMM richness, with an estimated 6 fewer ASVs for each unit increase of total PSI. This means that for the highest (3.12) to lowest (1.33) reported scores, it is estimated that the number of unique bacteria would decrease by approximately 12 ASVs. While the biological significance of this effect size is unknown, it is likely important given that the HMM samples in the study only had a maximum richness of 41 ASVs, meaning a decrease of 12 ASVs could be a reduction of up to 29% of the number of distinct bacteria present in milk.

The Role Restriction PSI subscale was also associated with reduced HMM richness, but the possible mechanistic pathway for this relationship is unclear. The Role Restriction subscale assesses whether a parent experiences the parental role as restricting their freedom and frustrates them in their attempts to maintain their own identity (Table 1). There may be fewer microbial exposures from other people with more restriction due to the parenting role. Previous research among horticulturalists and hunter-gatherers in the Central African Republic found that a more expansive maternal social network was associated with greater HMM alpha diversity and evenness (Meehan et al. 2018). This may be due to more skin microbial exposures through social contact that transfer to breast skin and then transfer into the HMM, or through the infant oral microbiome's influence on retrograde flow during breastfeeding (Consales et al. 2022). The Role Restriction subscale may reflect mothers' decreased microbial exposures from other people (e.g., "Since having this child, I have been unable to do new and different things.") but it also includes emotional responses to these feelings of restriction (e.g., "I feel trapped by my responsibilities as a parent."). As such, it is not clear whether these patterns may be due to reduced microbial exposures from other people or to other factors more related to the emotional components of these scales. PSI Attachment subscale was associated with HMM community composition (Bray–Curtis dissimilarity), indicating that mothers' reports of their emotional attachment to their child captured the greatest differences in overall HMM composition between participants. Notably, PSI Attachment was more strongly associated with HMM beta diversity than infant age and delivery mode.

The possible mechanistic pathways by which maternal parenting stress may be associated with HMM composition are not entirely clear and were not conclusively addressed in this study. It is possible that maternal stress and reduced HMM richness have a common cause, such as delivery-related complications (Cabrera-Rubio et al. 2012). Alternatively, increased stress may result in changes to the gastrointestinal microbiome (e.g., Bailey et al. 2011; Dinan and Cryan 2012; Galley and Bailey 2014; Hechler et al. 2019) which then translocate to the mammary gland through the entero-mammary pathways (De Andrés et al. 2017; Rodríguez 2014). Another potential pathway may be that psychosocial stress results in differences in milk composition, such as cortisol concentrations (Aparicio et al. 2020), immune components (Thibeau et al. 2016; Ziomkiewicz et al. 2021), the metabolome (Kortesniemi et al. 2021), or fat content (Keith et al. 2012). These changes to milk composition could create environments that are conducive to the proliferation of different bacteria. For example, previous research has found that concentrations of various milk components including fatty acids, immune factors, lactose, protein, and human milk oligosaccharides (HMOs) are associated with different HMM profiles (Castro et al. 2022; Keith et al. 2012; Kumar et al. 2016; LoCascio et al. 2007; Pace et al. 2021; Ward et al. 2006). Additionally, increased cortisol concentrations in milk have been associated with variation in lipid profiles in milk (Linderborg et al. 2020). Though one recent study found no associations between cortisol concentrations in milk, maternal stress, and HMM composition (Juncker et al. 2025), more research is needed to test this possible pathway by which maternal postpartum stress may influence HMM composition.

It is notable that we did not find any significant associations between the variation in HMM and IFM diversity measures in mother-infant dyads. Previous research has found similar limited associations between HMM and IFM alpha diversity and beta diversity measures in mother-infant dyads. In a large, multi-site study, Lackey et al. (2019) found only one significant correlation of HMM diversity indices with IFM diversity indices in a single study cohort. Similarly, they found that within cohorts, beta diversity measures of the HMM and IFM including the Bray-Curtis distance matrix were not more similar within a mother-infant dyad than any other mother-infant combination. This is similar to our study's results, which found no associations between HMM and IFM alpha and beta diversity indices. However, despite limited associations of HMM and IFM alpha and beta diversity indices, Lackey et al. (2019) study did find strong associations between mother-infant dyads' HMM and IFM holistic composition through a canonical correlations analysis. Future analyses of the relationship between the HMM and IFM in this study may yield similar findings.

While we found significant associations between PSI Attachment and IFM *Erysipelotrichaceae UCG-003* abundance, and PSI Spouse and IFM *Eggerthella* abundance, we are skeptical regarding these findings, given the probable influence of outliers. More research is needed to test possible relationships with *Eggerthella*, as *Eggerthella* abundance in the IFM has been associated with child temperament (positively associated with negative emotionality and stress response, negatively associated with positive emotionality and reward-seeking; Ueda et al. 2024), indicating that there might be a psychosocial or social–emotional component to its abundance in the IFM.

Based on these results, we conclude that maternal parenting stress does not appear to be an environmental signal that provokes developmentally plastic responses (Sheriff and Love 2013) in the taxonomic composition of the IFM in this sample. It is possible that maternal stress could contribute to developmental plasticity in the IFM in ways we were not able to measure, such as through changes to microbial metabolic activity, functions, or interactive effects with other physiological systems. Similarly, it is possible that the effects of maternal postpartum stress may not be evident until later in infancy, as previously found in a study of maternal postpartum stress and the IFM at 5–13 months postnatal (Galley et al. 2023). The significant associations between maternal postpartum stress and the HMM composition indicate that maternal stress may still be a relevant signal for developing infants through milk, but that infant developmental responses

to this signal are not occurring in the gastrointestinal microbiome. HMM composition has previously been found to be associated with variation in infant adiposity, fat-free mass, and length (Cheema et al. 2022), head circumference (Ajeeb et al. 2022), and in growth faltering (Ajeeb et al. 2024), suggesting that the HMM may prompt developmentally plastic responses in infant growth. Future research should explore the relationship between maternal postpartum stress and other key mechanisms of infant developmental plasticity. For example, previous research has found significant associations between maternal prenatal stress and mechanisms of developmental plasticity such as the epigenome (Kotsakis Ruehlmann et al. 2023; Quinn et al. 2023; Sosnowski et al. 2018) and the metabolome in a rat model (Li et al. 2023).

Aspects of this study may limit generalizability and bias results toward the null hypotheses. Our measure of stress only assesses stress relating to parenting. While the measure encompasses multiple dimensions of parenting stress, there are many other possible sources of stress that we did not measure. Participants were drawn from a community sample and overall had relatively low reported stress scores. As such, this study may underestimate effects of maternal stress, making null results more likely. Additionally, we are unable to test whether observed relationships with parenting stress were uniquely due to parenting stress or due to other factors causing or exacerbating parenting stress (e.g., pre-existing depression, stressors unrelated to parenting). It is possible that this study did not observe significant relationships due to the timing of measures; there may be a critical developmental window wherein the IFM is more sensitive to stress exposures that occur outside the age range in this study (1–6 months of age). This study may not have had enough samples to detect significant effect sizes less than 0.14 in the regression models. Additionally, results based on this study population may not be generalizable to other populations. This is an exclusively breastfeeding population, with mothers who are generally highly educated and over-representative of mothers who identified as Caucasian/European American. The relationship between stress and the IFM may be different for mother-infant dyads who are not exclusively breastfeeding or of different socioeconomic status.

However, this study has multiple strengths in assessing the potential impact of postpartum maternal stress on the IFM. For example, it used observations of maternal caregiving and breastfeeding behaviors to evaluate possible mediating factors, which eliminated any possible self-report bias that may arise from survey data. This analysis also uses multiple measures of the IFM to triangulate results across different methods of analyzing the microbiome. For example, rarefied data were used for alpha and beta diversity measures, while the MaAsLin2 approach used total sum scaling normalization on unrarefied genera count data to correct for differences in sampling depth. As different methods of correcting for sampling depth can yield variable results (Schloss 2024), it is recommended to incorporate multiple analytic approaches to validate results (Nearing et al. 2022).

5 | Conclusion

Maternal postpartum parenting stress is not strongly associated with variation in the infant fecal microbiome. However, maternal postpartum parenting stress is associated with the beta diversity and negatively associated with the richness of the human milk microbiome. In particular, total stress and the stress subscales of Role Restriction and Attachment were significantly associated with the composition of the human milk microbiome. This suggests that while maternal postpartum stress may not impact the infant gastrointestinal microbiome, it may still serve as a signal to the developing, breastfeeding infant through changes to milk microbial composition. Future research should identify whether maternal postpartum stress jointly impacts both the milk microbiome and other milk components, as well as clarify the relationship of the milk microbiome to other mechanisms of infant developmental plasticity.

Author Contributions

Conceptualization – Elizabeth A. Holdsworth, Courtney L. Meehan; Data Curation – Janet E. Williams, Elizabeth A. Holdsworth; Formal Analysis – Elizabeth A. Holdsworth, Janet E. Williams, Ryan M. Pace; Funding Acquisition – Courtney L. Meehan, Michelle K. McGuire, Maria Gartstein, Mark A. McGuire; Investigation – Courtney L. Meehan, Janet E. Williams, Beatrice Caffé; Methodology – Elizabeth A. Holdsworth; Project Administration – Courtney L. Meehan, Michelle K. McGuire; Resources – Mark A. McGuire, Michelle K. McGuire, Courtney L. Meehan; Software – Elizabeth A. Holdsworth, Janet E. Williams, Ryan M. Pace; Supervision – Courtney L. Meehan; Validation – Elizabeth A. Holdsworth, Courtney L. Meehan, Visualization – Elizabeth A. Holdsworth; Writing Original Draft Preparation – Elizabeth A. Holdsworth; Writing Review and Editing – Elizabeth A. Holdsworth, Janet E. Williams, Ryan M. Pace, Beatrice Caffé, Maria Gartstein, Mark A. McGuire, Michelle K. McGuire, Courtney L. Meehan.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Replicable data and analytic code are available upon request to the corresponding authors.

Endnotes

[1] Psychosocial stress refers to distress throughout this paper, as the majority of research on the effects of psychosocial stress tend to use "distress" and "stress" synonymously. We do not discuss eustress in this paper.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.