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Intergenerational and early life associations of the gut microbiome and stress-related symptomatology among Black American mothers and children

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ABSTRACT

Recent evidence suggests that maternal childhood adversity may have an intergenerational impact, with children of adversity-exposed mothers exhibiting elevated symptoms of psychopathology. At the same time, many children demonstrate resilience to these intergenerational effects. Among the variety of factors that likely contribute to resilience, the composition of the gut microbiome may play a role in buffering the negative impacts of trauma and stress. The current prospective cohort study tested the novel hypothesis that offspring gut microbiome composition is a potential moderator in the relationship between maternal exposure to childhood adversity and offspring symptomatology (i.e., internalizing, externalizing, and posttraumatic stress symptoms). Maternal childhood adversity was self-reported during pregnancy via the Childhood Trauma Questionnaire and Adverse Childhood Experiences (ACEs) survey, and offspring symptomatology was assessed with the Child Behavior Checklist/1.5–5 when offspring were 2–4 years old. Offspring fecal samples were collected between these timepoints (i.e., during 6- to 24-month follow-up visits) for microbiome sequencing. Results indicated that maternal ACEs and the relative abundances of *Bifidobacterium*, *Lactobacillus*, and *Prevotella* were associated with offspring symptomatology. However, there was little evidence that microbial abundance moderated the association between maternal adversity and offspring symptoms. Overall, these findings further our understanding of how the gut microbiome associates with psychopathology, and informs future studies aimed at targeting modifiable factors that may buffer the intergenerational effects of childhood adversity.

1. Introduction

The link between stress and psychopathology is well established, with particularly pronounced effects stemming from early life stress exposure (Heim and Binder, 2012). Childhood adversity is one of the most robust and pervasive risk factors for later psychopathology, with studies demonstrating increased risk for posttraumatic stress disorder (PTSD), depression, anxiety, and a variety of other adverse outcomes (Cicchetti and Toth, 2005). Moreover, emerging evidence indicates that, among women in particular, the prolonged impact of childhood adversity may not be limited to one's own lifetime but extend to future generations as well. Maternal childhood trauma has been associated with increased offspring risk for psychopathology across multiple levels of analysis, ranging from emotional and behavioral symptomatology (Plant et al., 2018) to physiological alterations that characterize PTSD and stress-related disorders (e.g., Buss et al., 2017; Daskalakis et al., 2021). Similarly, maternal adverse childhood experiences (ACEs) have been significantly associated with both symptoms of psychopathology (e.g., negative emotionality, behavioral dysregulation, internalizing and externalizing symptoms) and physiological correlates of psychopathology (e.g., cortisol, inflammatory cytokines, HPA-axis functioning, and epigenetic aging) in offspring (Cooke et al., 2021; Zhang et al., 2022). At

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the same time, many offspring demonstrate resilience to these intergenerational effects, spurring efforts to identify modifiable factors that can be protective in the context of intergenerational risk.

While a range of biological and environmental factors likely contribute to resilience to adversity, emerging evidence points to one important factor that warrants further investigation: the gut microbiome (Leclercq et al., 2016). There is increasing support for a complex, bidirectional communication between the gut and the brain (commonly referred to as the *gut-brain axis*) that has been shown to play a role in the development of psychopathology (Foster et al., 2017). Moreover, early findings suggest that certain characteristics of the gut microbiome may be protective in the context of early childhood stress (Liang et al., 2015). The current study aims to examine whether and how variation in the gut microbiome may be protective against the impact of maternal childhood adversity, as a potential first step towards interrupting intergenerational risk for psychopathology.

1.1. The gut microbiome & stress-related symptomatology

The gut microbiome contains thousands of microbe species, primarily represented by bacteria but also featuring viruses, fungi, and other microorganisms (Turnbaugh et al., 2007). Empirical evidence suggests that the composition of the gut microbiome – for example, microbial diversity and/or the abundance of certain bacteria relative to others – directly and indirectly modulates brain functioning, which in turn influences risk for psychopathology (Cryan et al., 2019). This modulation has been shown to occur through a bidirectional, multisystemic *gut-brain axis* involving the afferent nervous system (e.g., vagus nerve), immune system (e.g., regulation of inflammatory cytokines), limbic system (e.g., secretion and regulation of neurotransmitters, such as GABA and serotonin), and hypothalamic-pituitary-adrenal (HPA) axis (Osadchiy et al., 2019). Indeed, both rodent and human studies have demonstrated that changes to gut microbiome composition can lead to brain alterations that have been associated with symptoms of depression, anxiety, and PTSD (Bravo et al., 2011; Carbia et al., 2020; Tillisch et al., 2013).

Although there is a dearth of studies examining the role of the gut microbiome in the context of intergenerational stress, early *intra*-generational studies have provided evidence that the gut microbiome could play a moderating role in the association between adversity and psychopathology. For example, correlational human studies have found that, among adults exposed to trauma, the relative abundance of certain bacteria can distinguish individuals who develop PTSD from those who do not (Hemmings et al., 2017). Moreover, experimental studies have demonstrated that modification of the gut microbiome can attenuate the relationship between adversity and symptomatology. For example, rodent studies found that the association between early life stress and the development of stress-related symptoms (e.g., memory impairment, anxiety- and depressive-like behaviors) was reduced in rats that ingested a probiotic containing *Lactobacillus*, compared to rats that did not (Liang et al., 2015; Karen et al., 2021). Similar findings have been demonstrated for both rodents and humans among stress-exposed adults (Lew et al., 2019; Takada et al., 2016). Together these results suggest that certain bacteria or microbial compositions may be protective against the impact of early adversity. However, no human study to date has examined these relationships, either intra- or intergenerationally.

1.2. Developmental & cultural factors

The human gut microbiome is established across the first few years of life (Matamoros et al., 2013; Palmer et al., 2007), and preliminary studies suggest that the brain may be particularly sensitive to variability in the microbiome during this developmental period. For example, an experimental study with germ-free mice (i.e., mice that lack any gut microbes) tested whether recolonizing the gut with protective bacteria such as *Bifidobacterium* could attenuate the impact of early life stress on the development of anxiety-like symptoms. Interestingly, results indicated that introducing these bacteria in *early* life successfully attenuated the development of symptoms, while introducing the bacteria in *later* life did not (Sudo et al., 2004). Combined with evidence that brain development is especially sensitive to environmental and physiological exposures during the first years of life (Heijtz, 2016), these findings suggest that the gut microbiome may be a particularly important moderator of the link between childhood adversity and increased symptomatology during early development.

In addition to developmental timing, studies also suggest that the influence of the gut microbiome may vary according to racial and ethnic factors. The Human Microbiome Project found that racial/ethnic background was one of the strongest predictors of gut microbiome composition (Methé et al., 2012), and evidence suggests that a typical "healthy" gut microbiome composition is influenced by genetic, environmental, and dietary factors that vary across racial and cultural groups (Goodrich et al., 2014; Singh et al., 2017). Together, these findings highlight the importance of examining how gut microbiome composition may influence psychopathology within individuals of the same racial/ethnic background. Given consistent evidence that Black Americans are disproportionately exposed to childhood adversities and demonstrate elevated rates of posttraumatic stress symptomatology (Mersky and Janczewski, 2018; Roberts et al., 2011), the proposed study will specifically focus on this population to examine whether certain gut microbiome characteristics can ameliorate the intergenerational impact of childhood adversity.

1.3. The present study

Our prospective cohort study leveraged data from three related projects that comprise a sample of Black American mother-child dyads followed from pregnancy through three years postpartum: 1) The Pregnancy Study (Corwin et al., 2017), which examines the impact of social and environmental exposures on maternal and infant birth outcomes, 2) The Infant Microbiome Study (Brennan et al., 2019), which focuses on maternal stress and the infant gut-brain axis in the perinatal period; and 3) The Environmental Influences on Child Health Outcomes Study (ECHO; Gillman and Blaisdell, 2018), which examines how biological, behavioral, and social factors relate to developmental outcomes in early childhood. We hypothesized that:

Hypothesis 1. Maternal childhood adversity (i.e., childhood trauma and ACEs) would be positively associated with offspring symptomatology (i.e., internalizing, externalizing, and posttraumatic stress symptoms).

Hypothesis 2. Offspring gut microbiome composition, as defined by microbial diversity and relative abundance of particular taxa, would be associated with offspring symptomatology. Specifically, alpha diversity, beta diversity, and the relative abundances of *Bifidobacterium* and *Lactobacillus* would each be negatively associated with offspring symptoms.

Hypothesis 3. The association between maternal childhood adversity and offspring symptomatology would be moderated by offspring gut microbiome composition. Specifically, microbial diversity and higher relative abundances of *Bifidobacterium* and *Lactobacillus* would attenuate the association between maternal childhood adversity and offspring symptoms.

2. Methods

2.1. Participants

Pregnant Black American women (n=106) were initially recruited from prenatal clinics at a public and private hospital within a large metropolitan city in the southeastern United States. Mothers first

enrolled in the Pregnancy Study, where data was collected at two prenatal visits (typically during the second and third trimesters). After delivery, mothers and offspring were enrolled in the Infant Microbiome Study, which collected data at five time points across infants' first 18 months of life (ages 1-week and 3-, 6-, 12- and 18-months). When the child reached two years of age, participants were invited to enroll in the ECHO Study, which conducted annual follow-up visits from ages two to five years. A data collection timeline is illustrated in Fig. 1. Inclusion criteria for the three studies included: 1) Black/African American race (via self-report); 2) Maternal age of 18–35 years; 3) Singleton pregnancy (verified by clinical record); 4) Maternal comprehension of written and spoken English; and 5) Absence of infant congenital disorders. Additional inclusion criteria for the current study included: 6) completion of at least one Pregnancy Study visit, 7) availability of microbiome data from at least one collection timepoint between 6- to 24- months, and 8) completion of an ECHO Study visit at 2- or 3-years. Sample characteristics are provided in Table 1.

Table 1
Sample characteristics and descriptives.

	N = 106
Sociodemographic Characteristics	
Maternal Age	
Mean	25.66
Median [Min, Max]	25.00 [18, 35]
Maternal Education	
Some high school	6 (6%)
Graduated high school or GED	44 (42%)
Some college or technical school	36 (34%)
Graduated college	17 (16%)
Some graduate work or degree	3 (3%)
Maternal Marital Status	
Married/Cohabitating	17 (16%)
Single	89 (84%)
Delivery Mode	
Vaginal	74 (70%)
Cesarean	32 (30%)
Child Gestational Age at Birth	
Mean	38.74
Median [Min, Max]	39.10 [29.6, 41.4]
Child Sex	
Male	55 (52%)
Female	51 (48%)
Descriptives	
Maternal ACE total	
Mean	2.29
Median [Min, Max]	2.00 [0, 9]
Maternal CTQ total	
Mean	48.58
Median [Min, Max]	42 [28, 130]
CBCL Internalizing Score	
Mean	7.64
Median [Min, Max]	6 [0, 24.5]
CBCL Externalizing Score	
Mean	12.19
Median [Min, Max]	10.75 [0, 45.5]
CBCL Posttraumatic Stress Score	
Mean	3.74
Median [Min, Max]	3.00 [0, 10]
Ages at Data Collection	
Fecal Sample Collection Visit	
6 month	14 (13%)
12 month	6 (6%)
18 month	5 (5%)
24 month	81 (76%)
Age at Fecal Sample Collection (days)	
Mean	674.05
Median [Min, Max]	732 [179, 1016]

2.2. Procedure

Study procedures were approved by Emory University's Institutional Review Board and informed consent was obtained for each participant at enrollment in the Pregnancy Study, Infant Microbiome Study, and ECHO Study. Data collection was conducted by trained laboratory staff in participants' homes or a laboratory setting. At the pregnancy visit, mothers self-reported on childhood trauma and ACEs. At the infancy and toddlerhood visits, fecal samples were collected from offspring for gut microbiome sequencing. At the toddlerhood visits, mothers reported on offspring symptomatology. Covariates relevant to gut microbiome composition (e.g., delivery mode, recent antibiotic use) and adversity (e.g., SES) were collected at all time points.

2.3. Measures

2.3.1. Maternal exposure to trauma & stress

Childhood Trauma. Maternal childhood trauma was measured using the short form of the Childhood Trauma Questionnaire (CTQ; Bernstein et al., 2003). The CTQ has 28 questions regarding experiences of emotional abuse, physical abuse, sexual abuse, emotional neglect, and physical neglect. Responses are rated on a 5-point Likert scale ranging from "1—Never True" to "5—Very Often True." Higher scores are associated with more severe neglect and abuse. The CTQ has been well validated in non-clinical and Black American samples (Liebschutz et al., 2018). Internal consistency for the CTQ total score in the current sample was high (Cronbach's $\alpha = 0.86$).

Adverse Childhood Experiences. Maternal experiences of childhood adversity were measured using a shortened form of the Adverse Childhood Experiences questionnaire (ACEs; Felitti et al., 1998), which eliminates items that overlap with the CTQ. The shortened form consists of ten items assessing adversities related to family dysfunction (e.g.,

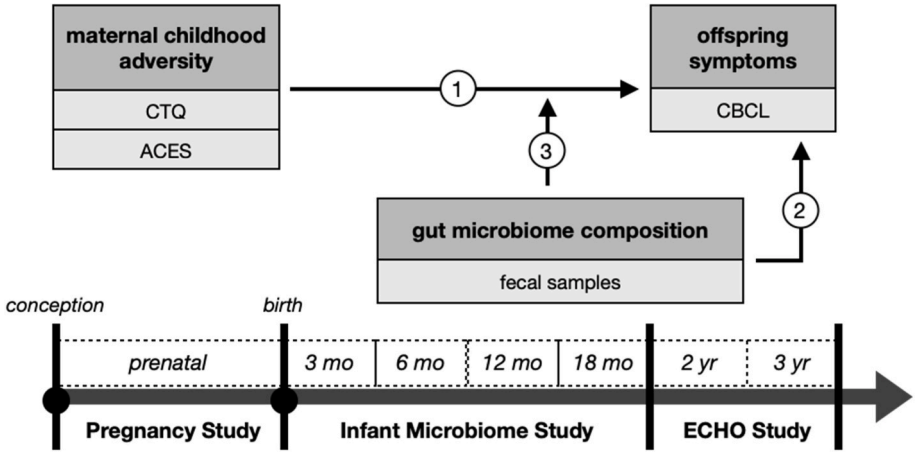

Fig. 1. Retrospective reports of maternal childhood adversity (via the CTQ and ACEs) were collected during mothers' pregnancy as part of the Pregnancy Study. Fecal samples were collected when offspring were 6, 12, 18, and/or 24 months old as part of the Infant Microbiome Study and ECHO Study. Maternal reports of offspring symptomatology (via the CBCL) were collected when offspring were two and/or three years old as part of the ECHO Study. Hypotheses are indicated with the numbered arrows: (1) Maternal childhood adversity would predict offspring symptoms; (2) Offspring gut microbiome composition would predict offspring symptoms; (3) Offspring gut microbiome composition would moderate the association between maternal childhood adversity and offspring symptoms.

mental illness, substance abuse, or suicidality within the household), parental loss (e.g., through divorce, imprisonment, death, or abandonment), and other childhood adversities (e.g., experiences of homelessness or foster care). Responses are rated in a yes/no format and items are coded as "0—Absent" or "1—Present." The total score is calculated by summing the items, with higher scores indicating more adverse experiences. Internal consistency in the current sample was adequate (Cronbach's α = 0.66).

2.3.2. Offspring symptomatology

Internalizing, externalizing, and posttraumatic stress symptoms were measured using the Child Behavior Checklist for Ages *1.5-5* (CBCL/1.5–5), a standardized form in which mothers report their children's behavioral and emotional symptoms (Achenbach and Ruffle, 2000). The CBCL/1.5–5 contains 100 items in which the mother indicates the option that best describes her child *now or within the past* 2 months with one of the following: 0 = *not true (as far as you know)*; 1 = *somewhat or sometimes true*; 2 = *very true or often true*. The internalizing symptoms score reflects the sum of 36 of these items, with possible scores ranging from 0 to 72, and the externalizing symptoms score reflects the sum of 24 items, with possible scores ranging from 0 to 48. The posttraumatic stress symptoms scale is based on a sum of 15 items (Supplemental Table 1; Dehon and Scheeringa, 2006), with scores ranging from 0 to 30. The CBCL is a well-established measure of child emotional and behavioral concerns and demonstrates strong test-retest in ethnically- and socioeconomically-diverse samples (e.g., Ivanova et al., 2010). The 15-item posttraumatic stress symptoms subscale demonstrated adequate internal consistency within the current sample (Cronbach's α = 0.62).

2.3.3. Gut microbiome composition

Mothers were instructed to collect offspring fecal samples according to protocols outlined by the Human Microbiome Project using a field-tested Stool Collection kit. Briefly, the kit contained three CatchAll swabs, MoBio tubes, and plastic biohazard bags. Mothers collected three CatchAll swabs of infant/toddler stool from a single diaper (plunging the swab into the stool in the diaper), placed each stool-coated swab in a plastic biohazard bag, and stored the samples in a home freezer. Mothers or lab staff transported the samples in an insulated bag to Emory, where lab staff transferred CatchAll swabs into pre-labelled MoBio tubes and placed all tubes in a −80 freezer for storage prior to assay. Laboratory

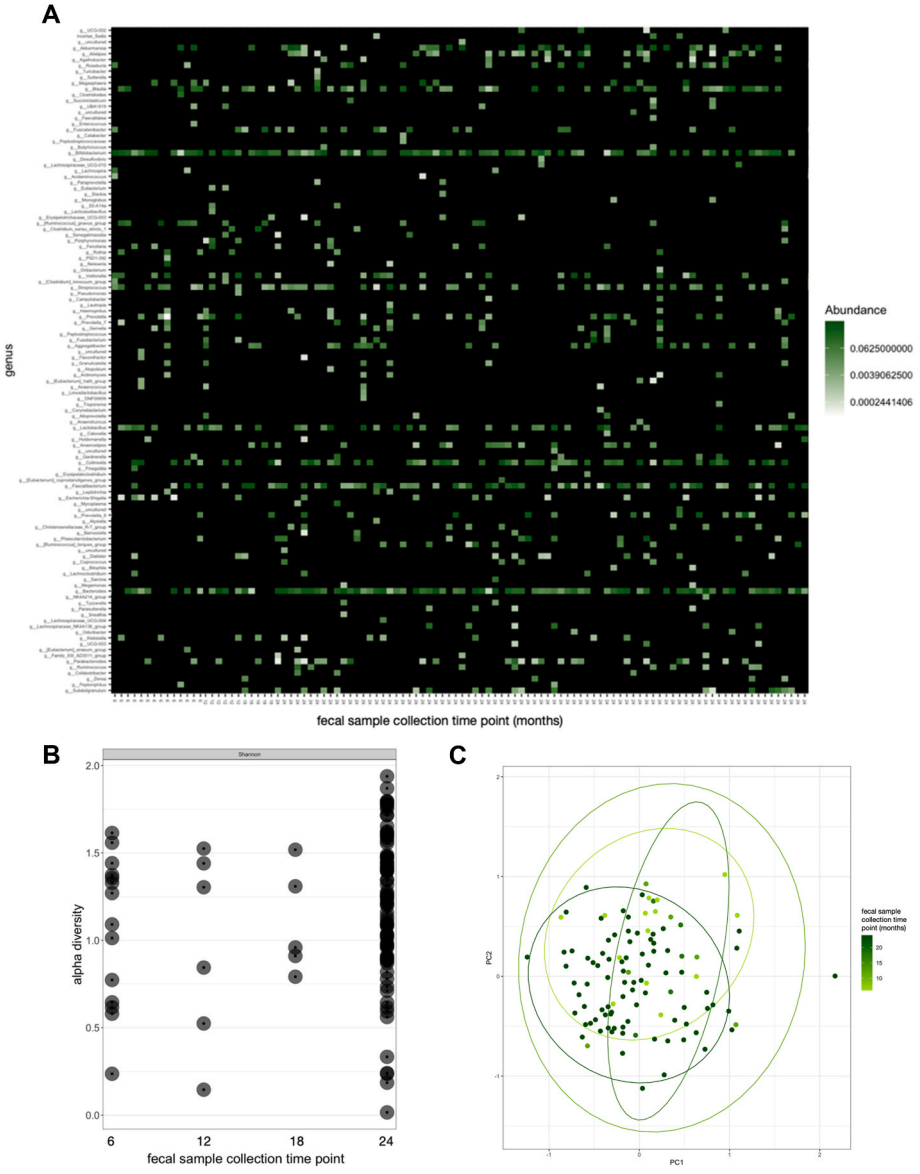

Fig. 2. Patterns of relative abundance (A), alpha diversity (B), and beta diversity (C) were largely similar across samples collected at 6-, 12-, 18-, and 24-month timepoints. As such, these four timepoints were collapsed to maximize the final sample size (n=106).

assay and data processing procedures are described in detail in the Supplementary Materials. After processing, 54 offspring (50.9% of the total sample) had viable microbiome data from only the 24-month visit. Based on prior evidence that the composition of the gut microbiome differs significantly between infants above and below 6-months of age (Mancabelli et al., 2020), we excluded samples from the 1-week and 3-month collection timepoints. Next, given that we did not find substantial differences in alpha diversity, beta diversity, or relative abundance patterns between the 6-, 12-, 18-, and 24-month samples (Fig. 2), we collapsed the 6- to 24-month timepoints. For offspring with viable microbiome data from multiple timepoints, the oldest available timepoint was selected to maximize developmental consistency across samples. We then removed samples with <300 read counts across all taxa, which resulted in a final sample of 106 offspring with data from 14 offspring at 6-months, six offspring at 12-months, five offspring at 18-months, and 81 offspring at 24-months (Table 1). Finally, we removed taxa with 0 read counts, which resulted in a total of 114 taxa represented across the 106 samples (Fig. 2a).

2.4. Statistical analysis

Analyses were performed in R version 4.1.3. All analyses adjusted for covariates that have been previously associated with the infant microbiome: maternal SES, maternal age, maternal prenatal BMI, mode of delivery, gestational age at birth, breastfeeding status (at three months), offspring sex, visit time point and offspring age at stool collection, antibiotic use in the preceding two weeks, and illness in the preceding two weeks. Complete data was available for all covariates except breastfeeding status, which was missing for 25 infants. This data was imputed using predictive mean matching via the MICE package (Van Buuren and Groothuis-Oudshoorn, 2011).

Within the Phyloseq package (version 1.38.0), we used the *tax_glom* function to merge taxa classified at the level of species to calculate the corresponding genus level abundances (Fig. 2). Alpha diversity – the diversity of genera represented in an individual's microbiome (i.e., *within*-subject diversity) – was analyzed using Shannon index. While other measures of alpha diversity exist, including the commonly used Simpson index, we selected Shannon index given its function in evaluating species richness, rather than evenness, as well as evidence that Shannon index has demonstrated more consistent and significant associations with psychopathology-related outcomes (Kuo and Chung, 2019). Beta diversity – the dissimilarity of an individuals' microbiome compared to others' (i.e., *between*-subject diversity) – was measured using two-dimensional principal coordinates (PC1 and PC2) obtained from the *ordinate* function with non-metric multidimensional scaling (NMDS) using the Bray-Curtis method. Robust linear regressions were used to test for associations between diversity variables (i.e., Shannon index, PC1, PC2) and offspring symptomatology, controlling for covariates.

Robust linear regressions were also used to test our *a priori* hypotheses that maternal adversity would be positively associated with offspring symptoms (Hypothesis 1), and that *Bifidobacterium* and *Lactobacillus* would be negatively associated with offspring symptoms (Hypothesis 2) and would buffer the impact of maternal adversity on offspring symptoms (Hypothesis 3). Power analyses indicated that the main effect analyses were adequately powered ($f^2 > 0.059$, power = 0.8, $p = 0.05$) and the interaction analyses were adequately powered to detect medium and large effects but potentially underpowered to detect small effects ($f^2 > 0.08$, power = 0.8, $p = 0.05$). Given that the expected interaction effect sizes are not well established, we chose to report both significant ($p < 0.05$) and non-significant trend ($p < 0.10$) interaction findings. Interactions were further probed to identify directionality of moderating effects.

Finally, we used the linear decomposition model (LDM; Hu and Satten, 2020) to explore whether the relative abundances of other genera (beyond *Bifidobacterium* and *Lactobacillus*) were associated with offspring symptomatology. LDM is a permutation-based analysis that can accommodate clustered data while maintaining validity for small sample sizes when it subjected to over-dispersion. In the LDM test, we attained FDR adjusted and unadjusted p-values <0.05 for taxa that were individually associated with the outcome of interest before and after controlling for false discovery rate (FDR) at the genus level. Given the exploratory nature of this analysis, we chose to report both significant ($p < 0.05$) and non-significant trend ($p < 0.10$) associations to inform more focused hypothesis-testing in future studies.

3. Results

3.1. Intergenerational association of maternal adversity and offspring symptomatology

First, we examined whether maternal experiences of childhood adversity were intergenerationally associated with offspring symptomatology, after adjusting for maternal SES, maternal age, offspring age, and offspring sex. Results indicated that maternal ACEs were significantly associated with offspring internalizing symptoms (t=2.31, p=0.02; Fig. 3a), externalizing symptoms (t=2.49, p=0.01; Fig. 3b), and posttraumatic stress symptoms (t=2.20, p=0.03; Fig. 3c). Maternal childhood trauma was not significantly associated with internalizing symptoms (t=1.86, p=0.065), externalizing symptoms (t=1.41, p=0.16), or posttraumatic stress symptoms (t=1.57, p=0.12).

3.2. Association of offspring gut microbiome composition and offspring symptomatology

Next, we examined the main effects of offspring gut microbiome composition on offspring symptomatology. Results indicated that offspring gut microbiome alpha diversity (i.e., Shannon index) was not significantly associated with internalizing (t=1.2, p=0.22), externalizing (t=0.44, p=0.66), or posttraumatic stress (t=1.08, p=0.28) symptoms. Beta diversity PC1 was also not associated with internalizing (t=0.75, p=0.45), externalizing (t=1.56, p=0.12), or posttraumatic stress (t=1.34, p=0.18) symptoms, and beta diversity PC2 was not associated with internalizing symptoms (t=−1.39, p=0.17) or posttraumatic stress symptoms (t=−1.83, p=0.07). However, beta diversity PC2 was significantly associated with externalizing symptoms (t=−2.08, p=0.04) such that lower PC2 values were associated with greater symptomatology. To better understand how beta diversity PC2 represents microbial composition, we assessed the associations between PC2 values and the relative abundance of each genus (Supplemental Table 2). Results indicated that the primary taxa associated with beta diversity PC2 was *Bifidobacterium* (t=8.31, p<0.001), such that lower levels of *Bifidobacterium* were associated with lower PC2 values. *Lactobacillus* was also significantly associated with beta diversity PC2, such that higher levels of *Lactobacillus* were associated with lower PC2 values. Together, these beta diversity results suggest that infant microbiomes characterized by a low relative abundance of *Bifidobacterium* and a high relative abundance of *Lactobacillus* are associated with greater externalizing symptoms.

Next, we examined whether the relative abundance of *Bifidobacterium* and *Lactobacillus* were directly associated with offspring symptomatology. Results indicated that a lower relative abundance of *Bifidobacterium* was associated with significantly greater externalizing symptoms (t=−2.14; p=0.02; Fig. 4a) and posttraumatic stress symptoms (t=−1.98; p=0.03; Fig. 4a). A greater relative abundance of *Lactobacillus* was significantly associated with greater externalizing symptoms (t=2.81; p=0.01; Fig. 4a). All other associations were nonsignificant (including two trend associations; Fig. 4c).

Using LDM, we then conducted exploratory analyses to identify genera whose relative abundance was significantly associated with offspring symptomatology. Although no relationships were significant after FDR correction, results indicated that a greater relative abundance

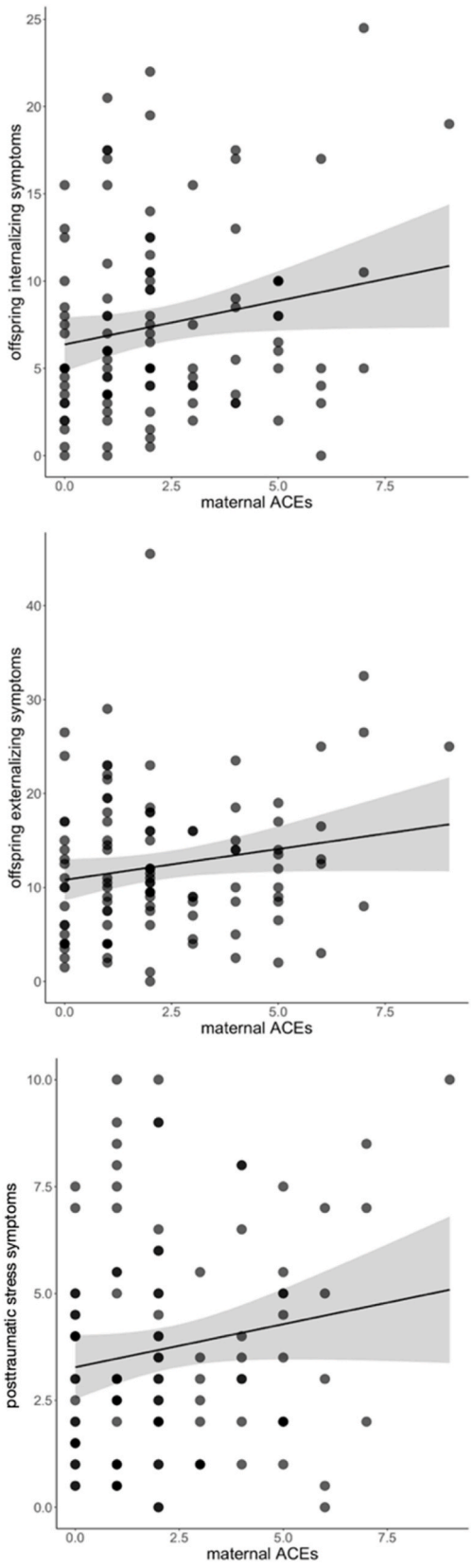

Fig. 3. Maternal ACEs were intergenerationally associated with offspring symptomatology.

of *Prevotella* was significantly associated with higher internalizing symptoms (p=0.03; Fig. 4b) and demonstrated a trend with higher posttraumatic stress symptoms (p=0.08; Fig. 4b), and a greater relative abundance of *Prevotella 7* demonstrated a trend with higher externalizing symptoms (p=0.06; Fig. 4b). Results also corroborated the associations between *Bifidobacterium* and *Lactobacillus* and offspring posttraumatic stress symptoms reported above. LDM findings are illustrated in Fig. 5.

3.3. Does infant gut microbiome composition buffer associations between maternal adversity and offspring symptomatology?

Finally, we examined whether the composition of the infant gut microbiome buffered the association between maternal childhood adversity and offspring symptomatology. Results indicated that a greater relative abundance of *Lactobacillus* may attenuate the impact of maternal ACEs on offspring internalizing symptoms, although the interaction was a nonsignificant trend (t=−1.66, p=0.1). Upon plotting the interaction, it was apparent that the positive association between maternal ACEs and offspring internalizing symptoms was significant at low levels of *Lactobacillus* but was attenuated at higher levels of *Lactobacillus* (see Fig. 6). All other interactions were nonsignificant.

4. Discussion

The current study is the first to show that variation in the gut microbiome during early life is associated with internalizing, externalizing, and posttraumatic stress symptoms in a community sample of Black American children. Moreover, we are the first to investigate whether greater microbial diversity or a higher relative abundance of protective bacteria may buffer the intergenerational effects of maternal trauma on offspring symptomatology. While we do not aim to establish causality in the present study, these findings offer an important first step towards identifying when and how the gut microbiome could serve as an early intervention target to reduce the impact of adversity on child psychopathology.

Our results indicated that alpha diversity (i.e., within-subject microbial richness) was not associated with offspring symptomatology. While the early assumption was that microbial richness is indicative of gut health, our findings add to the growing evidence that assessing the health of the gut microbiome is more complex than the relatively reductive measure of microbial richness (Carlson et al., 2018; Nikolova et al., 2021; Sanada et al., 2020; Sordillo et al., 2019). Our findings did, however, indicate that beta diversity (i.e., between-subject diversity) may differentiate children with and without elevated symptomatology. Children with lower beta diversity PC2 values demonstrated greater externalizing symptoms, and secondary analyses indicated that lower PC2 values were characterized by low levels of *Bifidobacterium* and high levels of *Lactobacillus*. These associations aligned with our findings that externalizing symptoms were negatively associated with the relative abundance of *Bifidobacterium* and positively associated with the relative abundance of *Lactobacillus*. These results may be interpreted in several ways. First, it is possible that high levels of *Bifidobacterium* and/or low levels of *Lactobacillus* cause (or serve as a proxy for) greater microbial diversity, which in turn is protective. Alternatively, it may be the case that beta diversity PC2 values serve as a proxy for the relative abundances of *Bifidobacterium* and *Lactobacillus,* which would be consistent with prior studies that have found the relative abundances of *Bifidobacterium* and *Lactobacillus* to predict symptomatology even when beta diversity was not a significant predictor (e.g., Pulikkan et al., 2018). In the case of *Bifidobacterium,* bacteria within this genus are largely regarded as “protective” bacteria (despite some inconsistencies; see Nikolova et al., 2021), with experimental animal studies, correlational human studies, and human clinical trials demonstrating that *Bifidobacterium* is negatively associated with psychopathology (e.g., major depression, anxiety, ASD) as well as symptoms and correlates of

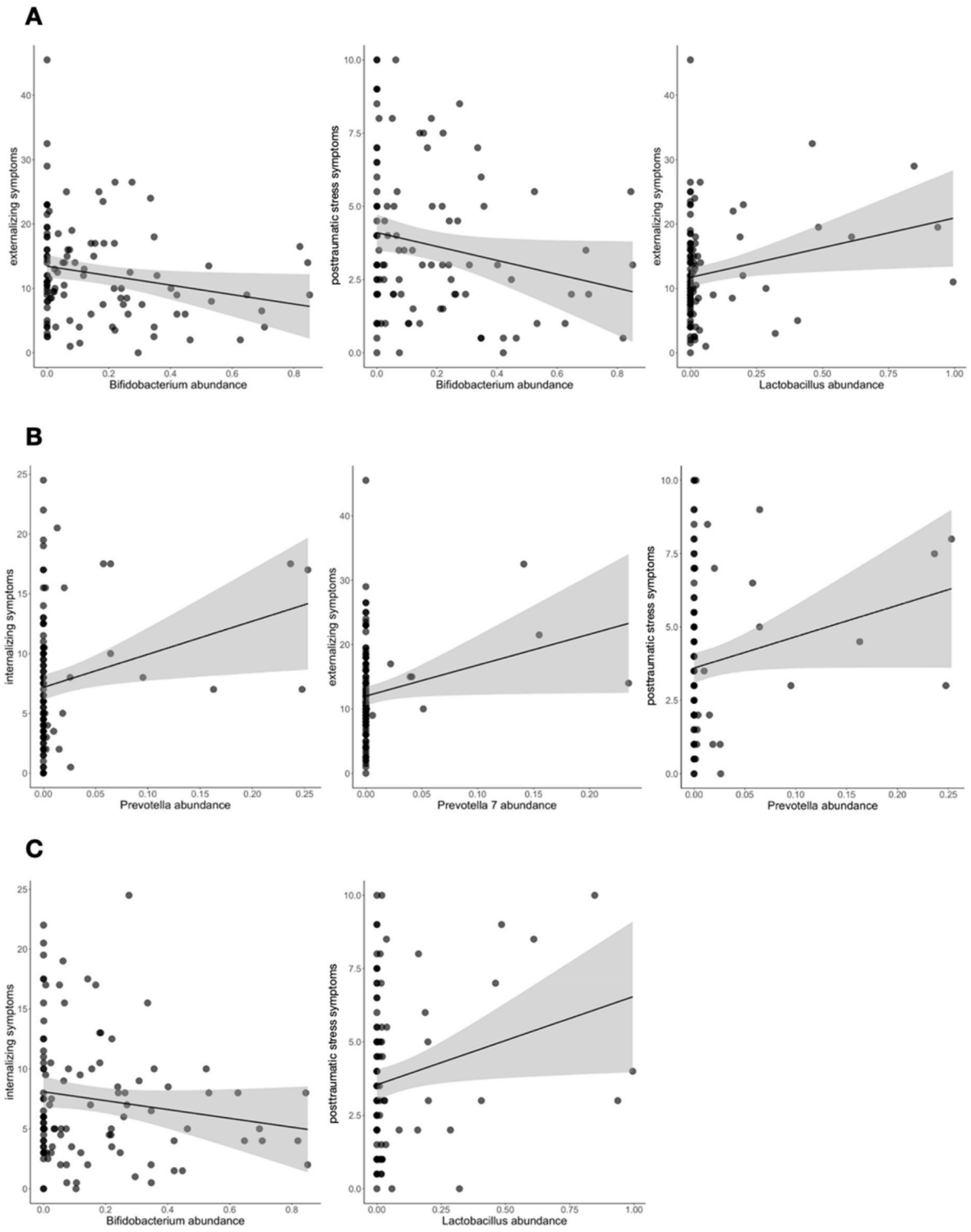

Fig. 4. Significant a priori hypothesis results are demonstrated in (A). Exploratory hypothesis results are demonstrated in (B). Non-significant trend findings ($p < 0.10$) from a priori hypotheses are demonstrated in (C).

psychopathology (Aizawa et al., 2016; Akkasheh et al., 2016; Groen et al., 2018; Pinto-Sanchez et al., 2017; Sarkar et al., 2016). Our findings extend this evidence by demonstrating that higher levels of *Bifidobacterium* predict lower symptoms of psychopathology during the sensitive period of early development. In the case of *Lactobacillus*, however, our findings are a bit more nuanced.

Results from our beta diversity and relative abundance analyses supported the *a priori* hypothesis that levels of *Lactobacillus* would predict offspring symptomatology. However, the direction of these associations was unexpected. Species within the *Lactobacillus* genus have generally been considered "protective" given evidence from intervention studies that probiotics containing strains of *Lactobacillus* can alleviate symptoms of depression, anxiety, and other forms of psychopathology (e.g., Foster and Neufeld, 2013; Messaoudi et al., 2011). However, our study found that *greater* levels of *Lactobacillus* were associated with greater externalizing and (as a trend) posttraumatic

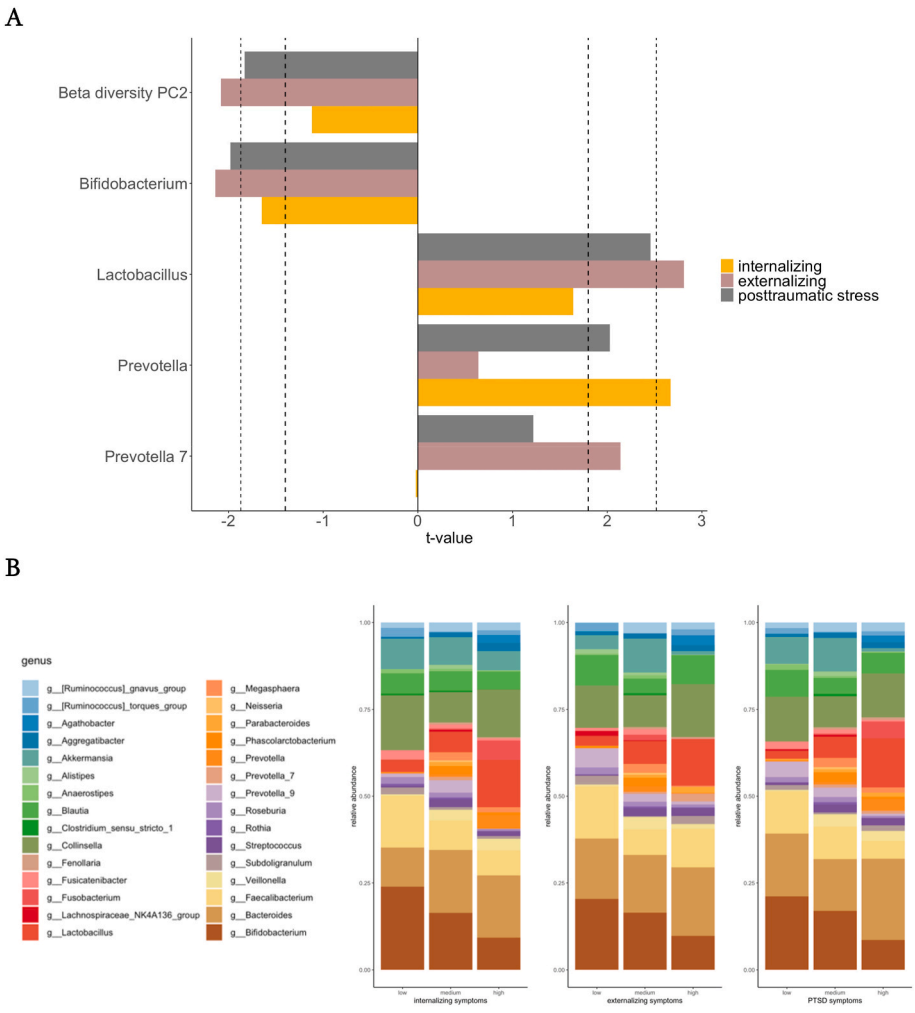

Fig. 5. (A) Associations between infant gut microbiome composition and symptomatology. Thick dotted lines indicate threshold for non-significant trends ($p < 0.10$), thin dotted lines indicate threshold for statistical significance ($p < 0.05$). (B) Relative abundances of top 30 genera across low, medium, and high levels of symptomatology.

stress symptoms. While these results conflict with the general conception, they are not the first to suggest that greater levels of *Lactobacillus* may be detrimental in certain contexts. In fact, a recent review and meta-analysis found a positive association between *Lactobacillus* and adult psychopathology (i.e., major depressive disorder, schizophrenia; Nikolova et al., 2021) and a case-comparison study of children ages three to 16 found that children with autism spectrum disorder (ASD) demonstrate greater levels of *Lactobacillus* compared to healthy controls (Pulikkan et al., 2018). Importantly, none of these studies examined relative abundance at the species level, and it is possible that different species within the Lactobacillacae family and *Lactobacillus* genus play differential roles such that some species are protective while others confer risk (Pulikkan et al., 2018). While we were not able to examine this in the present study, it is an important question for future studies that are able to differentiate bacteria at the species level.

Our exploratory findings indicated that bacteria in the Prevotellacae family may also confer risk for psychopathology in early life. Specifically, higher levels of *Prevotella* were significantly associated with greater internalizing symptoms and demonstrated a trend with greater posttraumatic stress symptoms, and higher levels of *Prevotella 7* demonstrated a trend with greater externalizing symptoms. While *Prevotella* are generally regarded as commensal bacteria (i.e., protective against invasive species and beneficial to overall health), there is increasing evidence that *Prevotella*-rich microbiomes are associated with increased inflammation (Iljazovic et al., 2021) and neurological markers of psychopathology (Tillisch et al., 2017). It has been suggested that *Prevotella* may be generally protective at low levels, but an overabundance past a certain threshold may influence physiological systems in a way that confers risk for psychopathology (Iljazovic et al., 2021). It is also possible that, similar to with *Lactobacillus*, different species within the *Prevotella* and *Prevotella 7* genera may have differential functions such that certain bacteria are protective while others are detrimental.

Finally, we were interested in examining whether and how the infant gut microbiome may buffer the impact of early life stress on the development of psychopathology, with a particular focus on intergenerational stress. Our results indicated that maternal adverse childhood experiences (ACEs) were positively associated with offspring early life symptomatology across all domains (i.e., internalizing, externalizing, and posttraumatic stress symptoms). We also found a trend towards a buffering effect of *Lactobacillus* on the relationship between maternal ACEs and offspring internalizing symptoms. These findings are consistent with evidence from experimental studies in both rodents and humans which have found that probiotics containing strains of *Lactobacillus* can attenuate the impact of stress, including early life stress, on the development of symptomatology (Liang et al., 2015; Takada et al., 2016). This potential buffering effect for internalizing symptoms is interesting, however, given that the relative abundance of *Lactobacillus* was associated with *increased* risk for externalizing symptoms when examining main effects. As previously mentioned, these opposing directional effects may be due to species differences such that certain species within

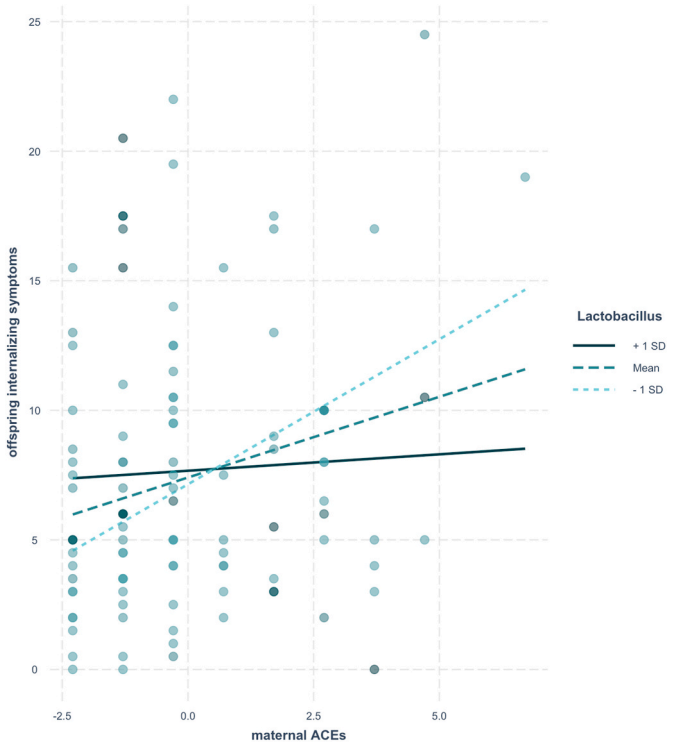

Fig. 6. The association between maternal ACEs and offspring internalizing symptoms was significant at low levels of *Lactobacillus* (light dotted line) but attenuated at higher levels of *Lactobacillus* (dark solid line).

the *Lactobacillus* genus are harmful, thus conferring risk for externalizing symptoms, while others are helpful, thus driving the protective effect for internalizing symptoms. Alternatively, it may be that *Lactobacillus* is not universally beneficial but is uniquely useful as prevention or intervention for high-risk individuals. Much of the evidence that has identified *Lactobacillus* as "good bacteria" has stemmed from clinical studies that target individuals with existing symptoms of depression, anxiety, or PTSD (Johnson et al., 2021), or from intervention studies that target individuals with heightened stress exposure (Liang et al., 2015; Takada et al., 2016). Each of these studies share a focus on individuals either with or at a heightened risk for developing symptoms of psychopathology, and our moderation results align with the results of these studies that *Lactobacillus* may serve a protective role in these contexts. However, the role of *Lactobacillus* in healthy, community, and/or low-risk samples may be less beneficial. Of course, these speculations require a more thorough examination from future studies that are better powered to detect small interaction effects. However, our findings provide a foundation and justification for future studies to examine when and for whom *Lactobacillus* may be protective versus harmful.

4.1. Limitations and future directions

Our findings must be considered in the context of several limitations. First, due to insufficient data at each fecal sample timepoint (i.e., 6, 12, 18, and 24 months), we chose to combine data across timepoints to maximize sample size. While this strengthened the statistical power of our study, it may have also introduced noise given that the composition of the gut microbiome is relatively dynamic during this developmental period (Sordillo et al., 2019). It should also be noted that, while our sample size was large compared to most human microbiome studies, we lacked statistical power to detect small effects, particularly interaction effects. Considering these limitations, we focused on the individual associations of specific taxa with offspring symptomatology. However, future studies would benefit from examining whether the influence of certain taxa may differ according to the presence or abundance of other taxa. In addition, the use of 16S rRNA sequencing limited our ability to determine the specificity and functional relevance of our findings. Future studies utilizing metagenomic sequencing will yield better insight into how species-level (rather than genus-level) differences may contribute to differences in early risk for psychopathology and will allow for examination of the functional pathways impacted by these taxonomic differences. Finally, we relied solely on maternal report to assess offspring posttraumatic stress symptoms. Future research would benefit from including additional measures of offspring posttraumatic stress symptoms such as a standardized diagnostic assessment or physiological measures (e.g., startle responsivity) to corroborate our intergenerational findings.

4.2. Conclusion

Our findings add to the growing literature that gut microbiome composition during the sensitive period of early life is associated with symptoms of psychopathology later in development. However, despite early evidence and theory that a "healthy" gut microbiome may protect against the impacts of early life stress, there was limited evidence that variation in the infant gut microbiome buffers the intergenerational impacts of maternal childhood adversity. Overall, these findings further our understanding of how the gut microbiome associates with the development of psychopathology, and informs future studies aimed at targeting modifiable factors that may buffer the intergenerational effects of childhood adversity.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The authors do not have permission to share data.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.bbih.2023.100651.

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