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Stress and depression-associated shifts in gut microbiota: A pilot study of human pregnancy

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ABSTRACT

Background: Psychosocial stress and mood-related disorders, such as depression, are prevalent and vulnerability to these conditions is heightened during pregnancy. Psychosocial stress induces consequences via several mechanisms including the gut microbiota-brain axis and associated signaling pathways. Previous preclinical work indicates that prenatal stress alters maternal gut microbial composition and impairs offspring development. Importantly, although the fecal and vaginal microenvironments undergo alterations across pregnancy, we lack consensus regarding which shifts are adaptive or maladaptive in the presence of prenatal stress and depression. Clinical studies interrogating these relationships have identified unique taxa but have been limited in study design.

Methods: We conducted a prospective cohort study of pregnant individuals consisting of repeated administration of psychometrics (Perceived Stress Scale (PSS) and Center for Epidemiological Studies Depression Scale (CES-D)) and collection of fecal and vaginal microbiome samples. Fecal and vaginal microbial community composition across psychometric responses were interrogated using full-length 16S rRNA sequencing followed by α and β-diversity metrics and taxonomic abundance.

Results: Early pregnancy stress was associated with increased abundance of fecal taxa not previously identified in related studies, and stress from late pregnancy through postpartum was associated with increased abundance of typical vaginal taxa and opportunistic pathogens in the fecal microenvironment. Additionally, in late pregnancy, maternal stress and depression scores were associated with each other and with elevated maternal C–C motif chemokine ligand 2 (CCL2) concentrations. At delivery, concordant with previous literature, umbilical CCL2 concentration was negatively correlated with relative abundance of maternal fecal *Lactobacilli.* Lastly, participants with more severe depressive symptoms experienced steeper decreases in prenatal vaginal α-diversity.

Conclusion: These findings a) underscore previous preclinical and clinical research demonstrating the effects of prenatal stress on maternal microbiome composition, b) suggest distinct biological pathways for the consequences of stress versus depression and c) extend the literature by identifying several taxa which may serve critical roles in mediating this relationship. Thus, further interrogation of the role of specific maternal microbial taxa in relation to psychosocial stress and its sequelae is warranted.

Abbreviations: PSS, Perceived stress scale, specifically the 10-item version; CESD, Center for epidemiological studies depression scale.

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1. Introduction

Broadly, pregnancy is a period of many essential immunological, hormonal, and metabolic alterations (Sherer et al., 2018), and more recently, the maternal fecal and vaginal microbiomes have also been found to undergo compositional alterations across gestation (Nuriel-Ohayon et al., 2016). Prenatal stress, defined as psychosocial stress experienced during pregnancy, has been linked to increased risk of maternal anxiety and mood-related disorders (Lancaster et al., 2010), adverse obstetrical outcomes such as preterm birth and low birth weight (Littleton et al., 2010), and aberrant offspring neurodevelopment (Van den Bergh et al., 2020). Preclinical studies by our group and others suggest that the mechanisms underlying these consequences may be partially mediated by maternal gut and vaginal microbiome function as prenatal stress induces alterations to both maternal and offspring microbial composition and function (Antonson et al., 2020; Gur et al., 2017, 2019; Jašarević et al., 2017), and similar associations have been found in recent clinical studies (Galley et al., 2022; Zijlmans et al., 2015). Furthermore, our group and others have previously demonstrated that prenatal stress induces fetal inflammation and aberrant offspring development via maternal microbe-dependent mechanisms, in addition to altered immune and metabolic pathways (Chen et al., 2020; Galley et al., 2021; Jašarević et al., 2015, 2018). Indeed, emerging clinical evidence suggests that pregnant individuals with depression, a common supervening consequence of chronic stress exposure, may exhibit dysregulated immune responses, including altered cytokine production (Leff-Gelman et al., 2016; Osborne and Monk, 2013), which are hypothesized to be additional mediators of the relationship between prenatal stress and offspring outcomes (Hantsoo, Kornfield, et al., 2019). Specifically, while interleukin-6 (IL-6), interleukin-4 (IL-4), interleukin-1β (IL-1 β) and interleukin-10 (IL-10) are the most commonly measured cytokines of interest, IL-6 and IL-10 seem to most consistently demonstrate an association with susceptibility to mood disorders during pregnancy (Sherer et al., 2018). Additionally, the umbilical cord allows for nutrient and waste exchange between the growing fetus and the pregnant person, thus, it is a potential pathway through which influences from the maternal environment may be transmitted *in utero*. Indeed, maternal prenatal systemic inflammation and chronic stress have been associated with inflammatory markers (Ross et al., 2016), markers of hyperinsulinemia and insulin resistance (Valsamakis et al., 2020) in cord blood at delivery. Together, these findings demonstrate the intergenerational consequences of prenatal stress and its sequalae, in addition to their complex biological mechanisms, all of which are active areas of investigation.

During pregnancy, the vaginal and fecal microbiomes undergo alterations, though it remains unclear which shifts are adaptive or maladaptive. One of the earliest longitudinal studies of the pregnant vaginal microbiome found greater compositional stability and unique *Lactobacillus*-dominated community state type, as compared to the non-pregnant microbiome (Romero et al., 2014), and these findings were supported by a more recent study which found similar distinctions as early as the first trimester (Freitas et al., 2017). Additionally, one of the first characterizations of fecal microbial community composition across pregnancy found that, from first to third trimester, fecal microbiome composition had reduced within-sample diversity (α-diversity) (Koren et al., 2012), which was replicated by a recent study (Long et al., 2023). Koren and colleagues also reported increased between-sample diversity (β-diversity) regardless of participant health status (Koren et al., 2012), meaning that participants' microbiome communities became progressively dissimilar and less diverse through the progressing gestation. Thus, they and more recent groups argue that fecal microbiome composition during pregnancy is largely sensitive to pregnancy itself and individual-specific factors, such as immune or hormonal fluctuations (Koren et al., 2012; Yang et al., 2020). In contrast, a smaller study found that both fecal and vaginal α-diversity and β-diversity remained largely consistent across gestation, and a more recent study replicated these fecal α- and β-diversity findings (DiGiulio et al., 2015; Yang et al., 2020).

Previous clinical studies interrogating the role of prenatal stress and its sequelae in altering maternal fecal and vaginal microbiota composition have found intriguing results across geographically and sociodemographically distinct populations, including sub-samples from larger studies in Los Angeles, CA; Philadelphia, PA; Chapel Hill, NC; and Arnhem-Nijmegen in the Netherlands (Galley et al., 2022; Hantsoo, Jašarević, et al., 2019; Hechler et al., 2019; Long et al., 2023). Long and colleagues found specific aspects of perceived stress to be associated with reduced microbial diversity, specifically alpha-diversity (Long et al., 2023), while maternal diversity metrics were not reported by the other studies. These studies focused on identifying compositional shifts in specific taxa in the maternal or infant fecal bacteriome. One study reported shifts in maternal fecal taxa associated with prenatal anxiety but not stress (Hechler et al., 2019). Additionally, two studies found altered maternal cytokine concentration associated with microbial taxa in the infant (Galley et al., 2022) and maternal bacteriome (Hantsoo, Jašarević, et al., 2019). However, few specific taxa have been consistently identified as differentially abundant in the human maternal microbiome and past studies have largely been limited to single or two timepoint cross-sectional studies across gestation.

Thus, there is a paucity of clinical studies characterizing prenatal stress-associated shifts in the maternal microbiome and maternal-fetal inflammation which may alter offspring risk of aberrant development; these findings could identify microbial and immune mediators of this relationship for further interrogation using preclinical models. Therefore, we aimed to test the hypotheses that more severe stress and depressive symptoms, measured via PSS and CES-D scores, respectively, would be associated with 1) steeper decreases in α-diversity and 2) increased abundance of pathogenic microbial taxa in both vaginal and fecal microenvironments across pregnancy. Additionally, we hypothesized that 3) umbilical cord inflammation (increased IL-6 and IL-10) would increase with relative abundance of microbial taxa known to induce inflammation or be otherwise pathogenic.

2. Materials and methods

2.1. Study design & participants

Pregnant individuals with singleton pregnancies were recruited from 2019 to 2021 through the Department of Obstetrics and Gynecology at The Ohio State University Wexner Medical Center. Participants were recruited through assessment of electronic medical records at participating clinics. Eligible participants provided verbal and written consent and were enrolled in the study during their first trimester of pregnancy (≤14 weeks gestation). Individuals with diagnosed and documented chronic health conditions such as diabetes, thyroid conditions, those with documented illicit substance or tobacco use, and those with histories of preterm birth or preeclampsia were excluded from this study. Study participation consisted of five timepoints across pregnancy and postpartum: once per trimester, within one week of delivery, and 4–8 weeks postpartum. Each timepoint included psychometric administration (Perceived Stress Scale and Center for Epidemiological Studies Depression Scale), biospecimen collection (maternal rectal and vaginal swabs, in addition to maternal blood at 3rd trimester and umbilical cord blood after delivery), and dietary intake (at each timepoint except delivery) (Fig. 1). This study was conducted with permission from the Ohio State University Institutional Review Board (2017H0362).

2.2. Logistical implications of COVID-19 pandemic

Participant enrollment and research activities were ongoing when the COVID-19 pandemic spread to the United States; a state of emergency was declared on March 9, 2020, in Ohio ("Executive Order 2020-01D," 2020). Stay-at-home and quarantine orders were initially

instituted for six weeks. Per medical center research guidelines, the study paused all in-person research activities from mid-March to June 2020. Additionally, maternal blood samples were not collected after the state of emergency was declared.

2.3. Demographic information, psychometric responses, and dietary intake

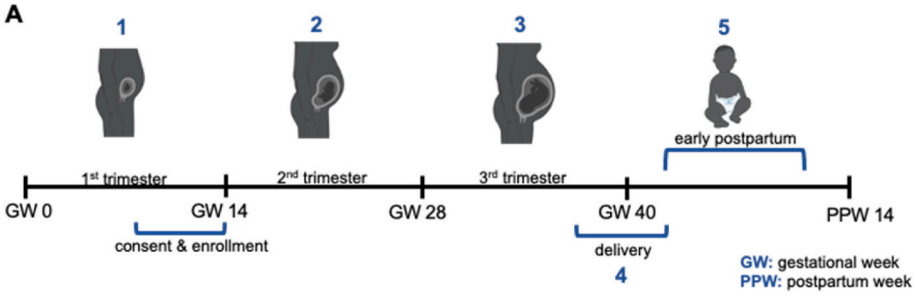
Key participant demographics, including age, race, educational attainment, legal marital status, health insurance status, and health history, including relevant obstetrical history, were abstracted from medical records. Additionally, participants were asked to report self-identified racial/ethnic background, marital status, and educational attainment. Self-report responses were used to verify and supplement medical record data. 10-item food frequency questionnaires were also administered during each prenatal visit and during the postpartum visit. Previously validated psychometrics were administered across the study to assess participant perceived stress and depressive symptoms (Fig. 1B). Surveys were administered via tablet at clinic visits until the pandemic, during which surveys were completed via securely emailed web-link. These data were collected and managed using Research Electronic Data Capture (REDCap), hosted by the Center for Clinical and Translational Science (CCTS) at The Ohio State University. REDCap is a secure, web-based application designed to support data capture for research studies (Harris et al., 2009).

The 10-item Perceived Stress Scale (PSS) was used to assess self-reported perception of stress in the past month. It is an adapted version of the original 14-item scale which has been tested for internal and test-retest validity and is widely used in research as a measure of global stress, including studies of maternal stress (Bann et al., 2017; Benediktsson et al., 2017; Cohen et al., 1983). Participants respond to questions assessing response to stressors using a 5-point Likert scale such that higher scores indicate worse or higher levels of perceived stress in the past month. Scores ranged from 0 to 34, and scale reliability was good across timepoints (Cronbach's α range: 0.73–0.87, Table S1). For categorical comparisons, PSS scores were initially categorized into three groups: 'low' (PSS score ≤13), 'medium' (14–26), and 'high' (≥27), in accordance with previous studies (Suárez-Rico et al., 2021; Tanpradit and Kaewkiattikun, 2020). However, distribution of scores and sample size necessitated collapsing the few 'high' PSS scores into the 'medium' category, yielding two final categories: 'low' (PSS score ≤13) and 'medium' (≥14). For the sake of brevity, we refer to 'perceived stress' as 'stress' for the duration of this paper.

The Center for Epidemiological Studies Depression Scale (CES-D) was used to assess depressive symptoms (Radloff, 1977). The CES-D has been used in similar studies of pregnant populations (Freedman et al., 2021; Gillespie et al., 2021). Higher scores indicate more severe depressive symptoms. Scores ranged from 0 to 43, and scale reliability was good (Cronbach's α range: 0.77–0.94, Table S1). For categorical comparisons, consistent with previous studies, CES-D scores ≥16 were considered as the clinically relevant cut-off and designated as "high depressive symptoms" (Freedman et al., 2021).

Dietary intake was captured using a 10-item food frequency questionnaire (FFQ). Given the logistical constraints of the pilot study, our primary focus on assessing stress and mood-related outcomes during pregnancy, the typical length of FFQs and dietary recall instruments, and consideration of participant bandwidth for self-report measures, we strove to devise a short form FFQ which would be both informative and parsimonious. Consulting previous FFQs and our understanding of the most relevant nutrients to gut bacteriome community composition, we wrote a 10-item questionnaire (Survey A) and administered it at four of five study visits (each prenatal visit, postpartum visit).

Demographic, health, dietary intake, and psychometric data were examined. Pregnancy timepoint was treated as a categorical variable by trimester, delivery, and postpartum; distributions of gestational age by study visit are presented in Fig. S2. Fisher's exact tests were calculated for relevant comparisons, Wilcoxon rank-sum/Mann-Whitney U tests were used to compare medians across pre-pandemic and pandemic groups, and two sample Kolmogorov-Smirnov tests were used to compare cumulative distributions of psychometric responses across relevant binary health and obstetrical history variables using StataBE v.17 (StataCorp LLC, College Station, TX). Correlation between PSS and CES-D scores was calculated using Spearman's rank order correlation coefficients.



B

Study Visit	Psychometrics	Biospecimens	Surveys or Abstracted Information
1	PSS, CES-D	maternal rectal & vaginal swabs (RVS)	health history; demographics; dietary intake
2	PSS, CES-D	RVS	dietary intake
3	PSS, CES-D	RVS, maternal blood	dietary intake
4	PSS, CES-D	RVS, umbilical cord blood	obstetrical outcomes
5	PSS, CES-D	RVS	dietary intake

Fig. 1. Study Design. A total of 40 participants enrolled in the prospective cohort study. At each time point, psychometrics were administered and biospecimens were collected, in addition to dietary surveys and information abstracted from medical records (B). The psychometrics assessed global perceived stress and depressive symptoms: Perceived Stress Scale (PSS) and Center for Epidemiological Studies Depression Scale (CES-D). The current sample (n = 35) consists of participants who completed both psychometrics and biospecimen collection during at least one study visit which passed quality filtering after sequencing. Several participants were lost throughout the study (Fig. S1).

2.4. Microbiome sequencing

Microbiome samples were collected from the vaginal introitus and anal canal across the prenatal, delivery, and postpartum periods (Fig. 1B) using sterile foam-tipped applicators (Puritan Medical Products Co LLC, Guilford, ME). Samples were immediately chilled in a cooler with ice packs until transported, exteriors sanitized with 70 % ethanol, and stored at −80 °C until analysis. Fecal and vaginal RNA extractions and amplifications were performed using the Shoreline Complete StrainID Protocol 1 according to manufacturer's instructions. Next, amplicons were quantified, pooled, and cleaned. SMRT Cell sample libraries then were constructed per manufacturer's instructions (PacBio, Menlo Park, CA). Samples then underwent full-length 16S rRNA sequencing on a PacBio 8M SMRT Cell sequencer at Nationwide Children's Hospital Institute for Genomic Medicine (NCH IGM).

Using full length fastqs provided by NCH IGM, sequences were filtered and demultiplexed using Sbanalyzer. Next, following a workflow established by Shoreline Biome (Graf et al., 2021), amplicon sequence variants (ASVs) were inferred using the DADA2 pipeline (Callahan et al., 2016) on R-Studio, and these ASVs were further analyzed in Quantitative Insights into Microbial Ecology-2 (QIIME2). Rectal samples were rarefied to 5523 sequences per sample and vaginal samples were rarified to 2256 sequences per sample. All samples below this cutoff were removed from the study (Fig. S1). Rectal samples were representative of the fecal microbiota community.

To quantify α-diversity (within-sample diversity), Pielou's Evenness, Shannon's diversity index, and Faith's phylogenetic diversity were used. For β-diversity (between/across-sample diversity), unweighted Unifrac was used for distance matrices and PERMANOVA and Adonis statistics in QIIME2 were used to measure the effect of specific psychosocial variables on microbiome diversity. Additionally, Shannon's diversity index, Pielou's evenness metric, and PCoA plots of unweighted UniFrac distances were used to evaluate whether fecal and vaginal samples from participants who were prescribed antibiotics or psychotropics and experienced adverse obstetrical outcomes were clustering distinctly from the remaining participants. We found no such cases; thus, all samples remained in the subsequent analyses.

Comparisons of taxonomic abundance with psychometric outcomes were conducted using DESeq2, an R package (Love et al., 2014). Using the qiime2R package, QIIME output files (.qza) were imported into the phyloseq R package (McMurdie and Holmes, 2013). The phyloseq_to_deseq2 function was used to convert these files into DESeq2. ASVs at the species level were agglomerated using the tax_glom function in phyloseq. Species and genera were the phylogenetic levels of focus. Given the distribution of scores and the smaller sample sizes, PSS and CES-D scores were roughly bisected into 'low' and 'medium' (cutoff: PSS score ≥14) and 'low' and 'high' severity groups (cutoff: CES-D score ≥16), respectively. These groups were then assessed at each timepoint to identify patterns across pregnancy and postpartum. The Wald t-test was used for statistical analyses. Multiple corrections were applied using DESeq2, specifically using the Benjamini-Hochberg procedure, and a corrected alpha value of 0.01 was used for significance. A lower limit of >2 log2-fold-change was used.

2.5. Multiplex assays

Approximately 8 mL umbilical cord blood was collected immediately after delivery using 10 mL BD Vacutainer sodium heparin collection tubes (BD Biosciences). Upon collection, tubes were inverted several times then stored at 4 °C for up to 24 h before being centrifuged at 1300 rpm for 10 min. The supernatant plasma was then aliquoted into microcentrifuge tubes and stored at −80 °C until analysis. Maternal blood was collected in the same manner at around 28 weeks of gestation. These samples were then assayed for several cytokines simultaneously using multiplex electrochemiluminescence immunoassay kits: V-PLEX Pro-Inflammatory Panel 1 (Human) and V-PLEX Human MCP-1 (Meso Scale Diagnostics (MSD), Rockville, MD). Samples were diluted according to manufacturer instructions and assayed in duplicate. Plates were read using the Meso QuickPlex SQ 120 instrument (MSD) and data were analyzed using GraphPad Prism v.9 (GraphPad Software, Boston, MA) for removal of outliers and summary statistics and R for taxonomic associations. Outliers were removed using ROUT method at Q = 1 % (α = 0.01). For these exploratory analyses, we focused on cytokines previously implicated with prenatal stress, anxiety and affective disorders, and maternal-fetal inflammation: CCL2 (MCP-1), interferon-gamma (IFN-λ), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) (Camacho-Arroyo et al., 2021; Chen et al., 2020; Christian et al., 2009; Stokkeland et al., 2019). Samples with concentrations below the lower limit of detection (LLD) of a given analyte were excluded from further analyses; this included 3 % of samples for IFN-λ, TNF-α, IL-10, and IL-6. CCL2 concentrations were above the LLD for all samples. Overall, intra-assay and inter-assay coefficients of variation were less than 9.5 % and 10.4 % for all analytes of interest, respectively. Correlations between psychometric scores and cytokine concentrations were calculated using Spearman's rank order correlation coefficients.

3. Results

3.1. Sample characteristics

The present study sample consisted of 35 pregnant individuals of an average age of 30 years, half of whom were experiencing their first pregnancy (50 %) and enrolled in the study prior to onset of COVID-19 pandemic-induced public health restrictions in the study's locale (Table 1). Briefly, most participants identified as white (60 %) and were married or cohabiting with a partner (85.7 %). Nearly half of all participants had private health insurance (48.6 %) and had earned a bachelor's degree or more (45.7 %) (Table S1). Regarding obstetrical outcomes, the median gestational age at birth was 39.3 weeks and 25.7 % of participants delivered via cesarean section (Table 1). Additionally, several participants experienced obstetrical complications such as low birth weight, preterm birth, testing positive for group B streptococcus, and chorioamnionitis (Table S1), but no participants had documented cases of SARS-CoV-2 infection for the duration of study participation.

3.2. Stress and depressive symptoms are not associated with differential fecal diversity

We found no significant differences in fecal α-diversity or β-diversity across pregnancy by stress or depressive symptoms, as captured by PSS and CESD scores (Table S3, Fig. S3).

3.3. Stress is associated with differential abundance of several fecal taxa across pregnancy and postpartum

Given that there were no significant differences in stress and depressive symptoms by pandemic timing (Table S2), responses were collapsed across pre-pandemic and during-pandemic to interrogate stress and depression-associated shifts in fecal taxa (i.e., found in the fecal microbiome). During the 1st trimester, participants reporting moderate levels of stress (PSS score ≥14) had a greater abundance of the genus *Faecalitalea* ($p < 0.0001$) and *Catenibacterium mitsuokai* ($p < 0.0001$), as compared to those reporting low stress (PSS score ≤13). During the 2nd trimester, the moderately stressed group had a greater abundance of the genus *Prevotella* ($p < 0.0001$) and *Streptococcus pasteurianus* ($p < 0.0001$) as compared to those reporting low stress. Additionally, high depressive symptoms (CES-D score ≥16) were associated with decreased abundance of the genus *Erysipelatoclostridium* ($p < 0.01$) (Table 2). During the 3rd trimester, the moderately stressed group had greater abundance of *Lagierella* ($p < 0.0001$) and *Lactobacillus* ($p < 0.0001$), especially *Lactobacillus iners* ($p < 0.001$). At delivery, moderate stress was associated with increased abundance of *Gardnerella*

Table 1
Brief Sample Characteristics. Demographic background, health and obstetrical history, and psychometric scores are presented for the current sample by timepoint. Additional sociodemographic and obstetrical outcomes are presented in Table S1.

	Overall	1st Tri.	2nd Tri.	3rd Tri.	Delivery	Postpartum
	N = 35	N = 30	N = 20	N = 27	N = 21	N = 14
Variable	N (%) or Median (IQR)					
Maternal Age (years)	31.0 (25.0–34.0)	31.0 (25.0–34.0)	32.0 (26.0–34.5)	31.0 (25.0–33.0)	32.0 (24.0–34.0)	32.0 (27.0–34.0)
Enrolled During Pandemic	11 (31.4 %)	10 (33.3 %)	5 (25.0 %)	8 (29.6 %)	7 (33.3 %)	6 (42.9 %)
Pre-pregnancy BMI (kg/m^2)	25.7 (23.3–31.1)	25.5 (23.3–31.1)	25.5 (23.2–29.7)	25.4 (23.2–28.5)	26.6 (24.0–31.1)	25.3 (23.3–28.3)
Health History						
Chronic health condition	6 (17.1 %)	6 (20.0 %)	1 (5.0 %)	2 (7.4 %)	3 (14.3 %)	1 (7.1 %)
Psychiatric condition	5 (14.3 %)	4 (13.3 %)	2 (10.0 %)	4 (14.8 %)	3 (14.3 %)	3 (21.4 %)
Gravidity						
Primigravida	16 (45.7 %)	14 (46.7 %)	8 (40.0 %)	11 (40.7 %)	9 (42.9 %)	6 (42.9 %)
Multigravida	19 (54.3 %)	16 (53.3 %)	12 (60.0 %)	16 (59.3 %)	12 (57.1 %)	8 (57.1 %)
Gestational Age at Birth (weeks)	39.3 (38.9–40.1)	39.3 (38.3–40.1)	39.5 (38.5–40.4)	39.4 (38.3–40.3)	39.6 (39.0–40.4)	39.9 (39.0–40.4)
Cesarean Section Delivery	9 (25.7 %)	8 (26.7 %)	6 (30.0 %)	6 (22.2 %)	6 (28.6 %)	2 (14.3 %)
Number of Adverse OB Outcomes						
0	22 (62.9 %)	19 (63.3 %)	13 (65.0 %)	16 (59.3 %)	11 (52.4 %)	9 (64.3 %)
1	12 (34.3 %)	10 (33.3 %)	7 (35.0 %)	10 (37.0 %)	9 (42.9 %)	4 (28.6 %)
2	1 (2.9 %)	1 (3.3 %)	0	1 (3.7 %)	1 (4.8 %)	1 (7.1 %)
10-item PSS Score	–	16.0 (8.0–21.0)	16.0 (12.5–20.0)	14.5 (10.0–19.0)	10.0 (7.0–19.5)	13.0 (8.0–18.5)
CES-D Score	–	10.0 (7.0–16.0)	12.5 (9.0–18.5)	11.0 (7.0–17.0)	7.5 (4.5–14.0)	6.5 (5.0–11.0)
Antibiotic Use						
During pregnancy	7 (20.0 %)	5 (16.7 %)	3 (15.0 %)	6 (22.2 %)	–	–
During labor	10 (28.6 %)	–	–	–	7 (33.3 %)	–
Postpartum	2 (5.7 %)	–	–	–	–	2 (14.3 %)
Psychotropic Use						
During pregnancy	4 (11.4 %)	4 (13.3 %)	2 (10.0 %)	4 (14.8 %)	4 (19.0 %)	–
During postpartum	2 (14.3 %)	–	–	–	–	2 (14.3 %)

IQR: interquartile range; BMI: body mass index; OB: obstetrical; PSS: Perceived Stress Scale; CES-D: Center for Epidemiological Studies Depression Scale; chronic health conditions include metabolic, endocrine, and gastrointestinal disorders; adverse OB outcomes include gestational diabetes, testing positive for group B *Streptococcus*, chorioamnionitis, low birthweight, and postpartum pre-eclampsia.

($p < 0.01$) and *Sneathia* ($p < 0.01$)—specifically *Sneathia amnii* ($p < 0.0001$)—and decreased abundance of *Actinomyces* ($p < 0.0001$) (Table 2).

3.4. Depressive symptoms are associated with steeper decrease in vaginal alpha diversity

Pairwise distance comparisons of two measures of α-diversity revealed that participants with high depressive symptoms (CES-D score ≥16) experienced a steeper decrease in vaginal α-diversity as their pregnancy progressed from 1st to 3rd trimester (Shannon's entropy: $p = 0.009$; Pielou's evenness: $p = 0.017$) (Fig. 2). There were no significant differences in vaginal β-diversity between participants by varying levels of stress or depressive symptoms at each timepoint (Table S3). Lastly, we found no statistically significant differences in abundance of vaginal taxa by stress or depressive symptoms, as captured via PSS and CES-D scores, respectively.

3.5. During late pregnancy, maternal stress and depression are correlated with each other and with maternal CCL2

Our interrogation of inflammation focused on cytokines previously associated with the microbiome and prenatal stress. We found 3rd trimester PSS scores to be associated with higher CESD scores at both 3rd trimester (Spearman's $\rho = 0.774$, $p < 0.001$) and delivery ($\rho = 0.758$, $p < 0.001$), and the correlation between psychometric scores

Table 2
Differential abundance of fecal taxa by stress and depressive symptoms across intrapartum and postpartum periods.

Timepoint	Fecal Phylum	Fecal Genus or Species	Psychometric	n	Fold Change
1st Tri.	Firmicutes	Faecalitalea	PSS	25	23.101***
		Catenibacterium mitsuokai			22.905***
2nd Tri.	Bacteroidetes	Prevotella	PSS	20	5.160***
	Firmicutes	*Streptococcus pasteurianus*			20.580***
		Erysipelatoclostridium	CESD	20	−6.711*
3rd Tri.	Firmicutes	Lagierella	PSS	21	19.579***
		Lactobacillus			22.406***
		L. iners			19.695**
Delivery	Actinobacteria	Actinomyces	PSS	18	−19.823***
		Gardnerella			7.565*
	Fusobacteriota	Sneathia			10.745*
		S. amnii			23.684***
Postpartum	Actinobacteria	Schaalia	PSS	10	25.996***
		S. turicensis			28.210***
	Firmicutes	*Megasphaera elsdenii*			24.721***

Significance of adjusted p-values: *$p < 0.01$, **$p < 0.001$, ***$p < 0.0001$.
N = number of paired fecal samples & psychometric responses.
PSS: Perceived Stress Scale; CES-D: Center for Epidemiological Studies Depression Scale.

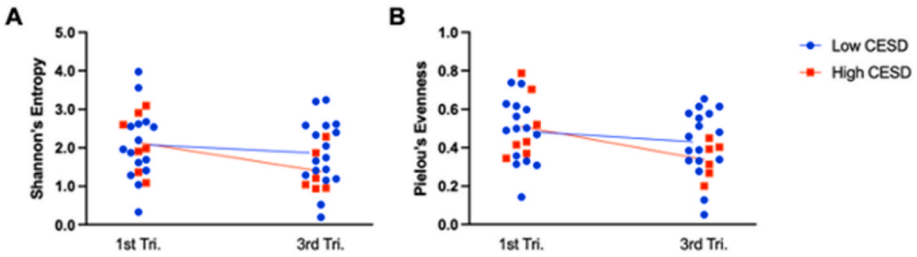


Fig. 2. Steeper decreases in vaginal α-diversity from early to late pregnancy among participants reporting more severe depressive symptoms, as measured by Shannon's Entropy (A) ($p = 0.009$) and Pielou's Evenness (B) ($p = 0.017$). Red indicates 'high' or more severe depressive symptoms (CES-D score ≥16); blue indicates 'low' or less severe depressive symptoms (CES-D score <16). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

continued at delivery (Table S4). Furthermore, concentration of maternal CCL2 during 3rd trimester was associated with higher PSS ($\rho = 0.587$, $p < 0.05$) and CESD ($\rho = 0.641$, $p < 0.05$) scores at the same timepoint (Table S4).

3.6. At delivery, umbilical cord CCL2 is inversely associated with abundance of maternal fecal Lactobacilli

In the fecal microbiome at delivery, relative abundance of *Lactobacillus* species, a known beneficial commensal genus, was negatively correlated with cord concentration of CCL2 ($n = 13$, $r = -0.724$, $p = 0.012$). We found no statistically significant associations between the remaining fecal taxa and cord and maternal cytokine concentrations queried (Table S4).

3.7. Dietary intake is largely similar across categorical comparisons of stress and depressive symptom groups

While limited sample size restricted the ability to statistically control for dietary intake in our psychometric score and fecal taxa comparisons, we compared frequency of consumption of key dietary components by stress and depressive symptom groups. Grouping by low vs. medium PSS scores, we found differences in frequency of consumption of non-whole grain foods at 2nd trimester ($p < 0.05$) and fruits during postpartum ($p < 0.05$) (Table 3). Grouping by low vs. high CESD scores, we found no differences in key dietary intake factors (Table 3).

4. Discussion

4.1. Fecal diversity was not associated with stress or depression across pregnancy

Overall, contrary to our hypothesis, we found no significant differences in fecal α-diversity or β-diversity across pregnancy by stress or depressive symptoms (Table S3, Fig. S3), which can be seen as consistent with the largely non-statistically significant reductions in fecal α-diversity found across several studies of non-pregnant adults with depression, as detailed by a recent systematic review (Barandouzi et al., 2020). Contrary to our results, three cohort studies report significant shifts in either fecal β-diversity or both α- and β-diversity associated with depression (Bosch et al., 2022; Jiang et al., 2015; Zhernakova et al., 2016). Some pregnancy-focused studies have found significantly decreased fecal α-diversity and increased β-diversity across gestation as a feature of pregnancy (Koren et al., 2012) while others have reported no significant changes to either metric across gestation (DiGiulio et al., 2015). A recent study found increased α-diversity to be associated with decreased stress and, interestingly, increased ability to cope with adversity (Long et al., 2023).

Together, these findings suggest that perhaps the extent to which overall metrics of fecal diversity are impacted depends on the severity of the condition experienced (i.e., clinically diagnosed major depressive disorder vs. self-report depressive symptoms), exogenous influences on the host, and the specific α-metric used (i.e., Shannon's diversity vs. Simpson's), which speaks to the limitations of employing α- and β-diversity metrics to interrogate more subtle effects. For instance, a study of adolescents with anxiety and depression found that oral microbiome

Table 3
Associations between dietary intake and psychometric responses across pregnancy. P-values of Fisher's exact tests are presented.

	Low vs. Medium PSS				Low vs. High CESD			
	1st Tri	2nd Tri	3rd Tri	Postpartum	1st Tri	2nd Tri	3rd Tri	Postpartum
Dietary Restrictions	1.00	0.32	–	0.44	0.54	1.00	–	1.00
Prenatal Vitamins as Prescribed	0.32	0.56	0.27	–	1.00	0.26	0.24	–
Other Supplements	1.00	0.52	0.52	1.00	0.55	1.00	0.15	1.00
Caffeine in Any Form	0.67	0.09	0.35	0.17	1.00	1.00	0.64	1.00
Processed Meats	0.47	0.43	1.00	1.00	0.39	0.08	0.57	0.27
Unprocessed Meats	0.44	0.62	0.78	1.00	0.14	1.00	0.67	0.55
Oily Fish	0.81	0.75	0.66	1.00	0.59	0.28	1.00	0.27
Non-oily Fish	0.16	0.080	0.73	0.19	1.00	1.00	0.20	1.00
Whole-Grain Bread/Foods	0.58	0.11	0.64	1.00	0.92	0.60	0.86	0.09
Non-Whole Grain Breads/Foods	0.11	0.043*	0.24	1.00	0.93	0.84	0.60	0.18
Vegetables	0.68	0.93	0.21	1.00	0.37	0.93	0.12	1.00
Beans or Legumes	1.00	0.55	1.00	0.30	0.73	0.72	0.91	0.73
Fruits	0.83	0.28	1.00	0.048*	0.95	0.63	0.08	1.00
Any Form of Probiotics	1.00	0.54	0.94	1.00	0.76	0.81	0.53	0.55
Milk or Dairy Products	0.23	0.33	0.77	1.00	0.73	0.85	1.00	1.00
N	25	20	21	10	26	20	21	12

PSS: Perceived Stress Scale; CESD: Center for Epidemiological Studies Depression Scale; *$p < 0.05$.

diversity differed by taxonomic composition but not by overall diversity (Simpson et al., 2020).

4.2. Differential abundance of fecal taxa not previously associated with pregnancy or stress

During the 1st trimester, we found that abundance of fecal *Faecalitalea* increased with stress. *Faecalitalea* have been previously associated with prenatal alcohol exposure in rodents, obesity in adults, and autism spectrum disorder in children (Bodnar et al., 2022; Ding et al., 2020; Gong et al., 2022). Also during the 1st trimester, we found that abundance of fecal *Catenibacterium mitsuokai* increased with stress. *C. mitsuokai*, which ferments glucose to produce several acids—including essential short chain fatty acids, acetate and butyrate—was first isolated over twenty years ago (Kageyama and Benno, 2000) but has only been reported by one study since then (Dubé et al., 2018). Additionally, during the 2nd trimester, abundance of fecal *Streptococcus pasteurianus* was greater in the moderately stressed group. *S. pasteurianus* has been previously implicated with inflammatory conditions and infections (Chang et al., 2023; W.C. Chen et al., 2021; Corredoira et al., 2014; Nguyen et al., 2019). Recent work has also found *S. pasteurianus* to be depleted in a small sample of children with neurodevelopmental disorders (Bojović et al., 2020). During 3rd trimester, the abundance of *Lagierella* was greater among stressed participants. *Lagierella* is a recently proposed genus of bacteria within the Peptoniphilaceae family, based on species isolated from a two-year-old boy's stool sample (Traore et al., 2016, 2021). To our knowledge, this is the first study to identify *Faecalitalea, C. mitsuokai, S. pasteurianus,* and *Lagierella* as differentially abundant in a cohort of pregnant individuals and in the context of psychosocial stress.

4.3. Stress, but not depression, associated with abundance of fecal Prevotella in mid-pregnancy

During the 2nd trimester, *Prevotella* were more abundant among moderately stressed participants. This can be seen as consistent with two previous findings: a study of non-pregnant women found that greater abundance of *Prevotellacea*e was predictive of major depressive disorder (Y. Chen et al., 2021) and a study of pregnant women found a greater abundance of *Prevotella* among women with adverse childhood experiences (Hantsoo, Jašarević, et al., 2019). Contrary to our finding, studies of non-pregnant adults have found negative associations between relative abundance of *Prevotellaceae* or *Prevotella* and major depressive disorder and general anxiety disorder (Barandouzi et al., 2020; Jiang et al., 2015).

4.4. CCL2 associated with psychometric responses and lower abundance of Lactobacilli during late pregnancy and parturition

During the 3rd trimester, stress and depressive symptoms were associated with elevated maternal CCL2 concentration. At delivery, relative abundance of fecal *Lactobacillus* species was negatively associated with umbilical cord concentration of CCL2. CCL2 is a pro-inflammatory chemokine which, in tandem with microbes, has been demonstrated to mediate the consequences of prenatal stress on intrauterine inflammation and offspring development in murine models of stress (Chen et al., 2020). *Lactobacilli* are a commensal genus known to inhabit the gastrointestinal tract and generally thought to confer beneficial effects, as evidenced by depleted abundance among depressed non-pregnant adults (Barandouzi et al., 2020) and greater abundance in infants of mothers with lower prenatal anxiety and depression (Galley et al., 2022). Thus, this finding is concordant with existing literature and is an intriguing translational finding that dovetails our previous work.

4.5. Stress is associated with natively vaginal taxa and opportunistic pathogens from late pregnancy to postpartum

During the 3rd trimester, abundance of fecal *Lactobacillus* and specifically *L. iners* was greater among moderately stressed participants, as compared to low-stress participants. *Lactobacilli* are known commensals inhabiting the gastrointestinal tract and are generally thought to confer beneficial effects, including anti-inflammatory effects on stress responses, and may also be depleted in those with depression (Barandouzi et al., 2020). Thus, our finding contrasts with the existing literature. However, *Lactobacilli* species are also key dominant commensals of the vaginal microenvironment, helping to maintain a low vaginal pH via production of lactic acid, and become increasingly dominant during gestation (Romero et al., 2014). Interestingly, *L. iners,* among other taxa, have recently been identified as migrating to the fecal microbiome across pregnancy (Shin et al., 2023). Thus, one explanation for *L. iners* increased abundance among our more stressed participants is that stress potentiates this transmission of vaginal microbes to the fecal microenvironment.

At delivery, moderate stress was associated with increased abundance of fecal *Gardnerella* and *Sneathia,* especially *S. amnii*. Generally, *Sneathia* species are thought to be pathogenic; specifically, *S. amnii* is pathogenic in the female urogenital tract and is associated with several gestational complications and increased intra-amniotic inflammation (Theis et al., 2021). Similarly, increased relative abundance of *Gardnerella* species in people with more diverse vaginal microbiomes, specifically in the absence of *Lactobacilli*, are associated with increased risk of complications such as bacterial vaginosis and spontaneous preterm birth (DiGiulio et al., 2015; Hočevar et al., 2019). A recent murine model underscores these findings, concluding that colonization of *G. vaginalis* alone (i.e., without simultaneous absence of *Lactobacilli*) was insufficient to induce spontaneous preterm birth (Joseph et al., 2023). Interestingly, abundance of *Gardnerella* and *Sneathia* have previously been positively associated with each other and with bacterial vaginosis (Sparvoli et al., 2020). These findings are in accordance with our hypothesis.

Contrary to our hypothesis, moderate stress was negatively associated with abundance of fecal *Actinomyces* at delivery. *Actinomyces* are commensals in many human microbial communities but can also be opportunistic pathogens (Könönen and Wade, 2015). Interestingly, increased abundance of *Actinomyces* have been reported in those with depression, Crohn's disease, and SARS-CoV-2 infections (Barandouzi et al., 2020; Farsi et al., 2022; Pittayanon et al., 2020). During pregnancy, *Actinomyces* infections appear to be rare, but increased abundance of *Actinomyces* species is associated with increased risk of spontaneous preterm birth (Yu et al., 2023). Therefore, our results contrast with the extant literature reporting *Actinomyces*.

During the postpartum period, stress was associated with increased abundance of fecal *Schaalia,* specifically *S. turicensis,* and *Megasphaera elsdenii.* Much of the extant literature documents *Megasphaera* in the vaginal microenvironment: vaginal *Megasphaera* have been associated with bacterial vaginosis, preterm birth, and other pregnancy complications (Fettweis et al., 2019; Glascock et al., 2021). Isolated vaginal *M. elsdenii* has been demonstrated to induce immune activation, specifically dendritic cell maturation and production of several pro-inflammatory cytokines (van Teijlingen et al., 2020). Furthermore, recent preliminary studies report greater abundance of fecal *M. elsdenii* in gastrointestinal- and immune-associated chronic conditions including psoriatic arthritis, HIV infection, and type II diabetes with gastrointestinal autonomic neuropathy (Du et al., 2021; Fulcher et al., 2022; Lin et al., 2022). Additionally, *Schaalia* are heterogenous in function (Nouioui et al., 2018). *S. turicensis,* previously classified as *Actinomyces turicensis,* has been isolated from the female urogenital tract, including the vaginal canal, and from wounds and genital infections, suggesting its role as a commensal species in select microenvironments and an opportunistic pathogen in others (Könönen and Wade, 2015; Sabbe et al., 1999; Vandamme et al., 1998). A recent study of pregnant

individuals found *S. turicensis* to be increased in abundance among those with impaired glucose tolerance (Dreisbach et al., 2022).

Overall, we identified several conventionally vaginal-associated taxa enriched with stress in the fecal microbiome from late pregnancy to postpartum: *L. iners, S. amnii, Gardnerella, Actinomyces, S. turicensis,* and *M. elsdenii.* This is consistent with a recent study concluding that vaginal and fecal microbial communities become increasingly similar in composition across gestation and into postpartum (Shin et al., 2023), extending earlier work demonstrating similarities in these communities' composition in late pregnancy using culture-based methods (El Aila et al., 2009). We found most of these taxa to increase in abundance with stress; thus, it is intriguing to consider whether stress potentiates the progressive compositional homogeneity between fecal and vaginal microenvironments or conversely, whether these taxa contribute to increased stress. Taken together, these taxa present additional targets for further mechanistic interrogations of the relationships between perinatal stress and gut and vaginal microbial function.

4.6. At mid-pregnancy, depressive symptoms associated with depletion of diet-associated taxa

During the 2nd trimester, participants reporting moderate depressive symptoms had a lower abundance of *Erysipelatoclostridium*, as compared to those reporting low depressive symptoms. *Erysipelatoclostridium* are glucose, fructose, and sucrose-fermenting bacteria (Yutin and Galperin, 2013) which have mostly been reported with diet-associated alterations: lower relative abundance of *Erysipelatoclostridium* has been implicated with increased caffeine consumption as well as folate and vitamin B2 intake in non-pregnant adults (Dai et al., 2023; Gurwara et al., 2019). Of the few preliminary studies during pregnancy, most report on the infant fecal microbiome: *Erysipelatoclostridium* was more abundant in infants born to mothers with low fruit and vegetable consumption during pregnancy (Fan et al., 2021) and was depleted in infants born to mothers with gestational diabetes (Zhao et al., 2022). Thus, this is the first study to associate *Erysipelatoclostridium* with depressive symptoms. However, given that our analyses do not control for maternal diet, it is possible that dietary intake confounds this association such that, for example, participants reporting higher depressive symptoms also have a significantly different diet at 2nd trimester, as compared to those reporting lower depressive symptoms.

4.7. Across gestation, depressive symptoms are associated with steeper decrease in vaginal diversity, but not specific taxa

In the vaginal microbiome, participants reporting worse depressive symptoms experienced a steeper decrease in vaginal α-diversity from early to late pregnancy (1st vs. 3rd trimester). While this finding seems to agree with the broad notion that decreased microbial diversity may be associated with disease, in contrast to the fecal microbiome, greater diversity, as measured via community state type, in the vaginal microbiome has been associated with spontaneous preterm birth (DiGiulio et al., 2015; Dunlop et al., 2021). Thus, our finding contrasts with the vaginal microbiome literature thus far, however, few studies have focused on shifts in the vaginal microbiome related to stress and depression. Overall, the lack of significant changes in vaginal microbiome composition associated with stress or depressive symptoms can be seen as consistent with literature demonstrating that the vaginal microbiome is compositionally distinct and especially stable during pregnancy and, after delivery, transitions to a different community state type less dominated by *Lactobacilli* (DiGiulio et al., 2015; Romero et al., 2014).

4.8. Differential abundance of fecal taxa across pregnancy by stress, but (largely) not depressive symptoms suggests distinct biological pathways

Intriguingly, although participants' stress and depressive symptom scores were positively correlated across most timepoints (Table S1), only stress was associated with differential abundance of fecal taxa at all timepoints except the 2nd trimester. This may be due to the subjective and highly transient nature of 'perceived stress' versus the transitory presence or absence of depressive symptoms. A mechanistic explanation is that this discordance hints at the distinct microbe-associated mechanisms underlying perceived stress versus depression. Lastly, a logistical explanation is the timing of psychometric administration: following delivery—a significant life event—it is possible that participants feel relieved and, in some ways, much less stressed as compared to immediately prior to delivery so that their perceived stress score reflects their post-delivery psychological state while their microbiome samples and umbilical cord blood samples reflect their pre-/intrapartum biological state.

4.9. Limitations and future directions

One strength of the present study is that we aimed to minimize the possibility of cross-contamination during sample collection by having samples collected by trained clinicians who regularly conduct physical exams during prenatal visits (i.e., usually the patient's physician or midwife). Simultaneously, it is important to consider these findings with the context of several limitations. Firstly, we prioritized recruiting an ethno-racially and socioeconomically diverse sample, intentionally recruiting participants from two clinics serving generally distinct patient populations. However, the demographic makeup of our sample varied across the study due to several factors including existing patterns in participant attrition and non-clinical barriers to health care access, which were exacerbated by the onset of the COVID-19 pandemic (Louis et al., 2022; Whipps et al., 2021; Young et al., 2006). Additionally, due to this being a pilot study and some psychometrics having been validated only in English, our study excluded participants who required the use of an interpreter. Also, we recruited participants in their first trimester of pregnancy; some pregnant individuals do not receive prenatal care until further along in pregnancy owing to a variety of non-clinical factors. Furthermore, we excluded patients with diagnosed and documented chronic health conditions such as diabetes, thyroid conditions, those with documented illicit substance or tobacco use, and those with histories of preterm birth or preeclampsia. These conditions may also be related to maternal psychosocial stress and the maternal microbiome and should be meaningfully included in future studies. Additionally, the study included multiple time points across gestation, but the limited number of participants may lower the study's statistical power while possibly increasing the likelihood of Type I, false positive, and Type II, false negative, errors. Relatedly, while we captured dietary intake across the intrapartum and postpartum periods, limited sample size prohibited statistically controlling for dietary intake when examining the relationships between psychometric responses and fecal microbiome composition. Similar future studies should endeavor to include meaningful measures of nutritional intake. Another limitation is that we neglected to capture participants' coping resources, which are likely to be mediators of the relationships between stress and affective disorders, especially during intrapartum and postpartum (Guardino and Schetter, 2014; Williamson et al., 2023). Logistics-wise, participants responded to psychometrics after delivery whereas the rectal and vaginal swabs were collected prior to delivery. It is possible that the psychosocial stressors they felt in the month or week prior to parturition are perceived differently (and therefore, reported differently) following parturition. Lastly, to minimize participant exposure risk and comply with medical center guidelines at the onset of the COVID-19 pandemic, we modified our study design to administer surveys via emailed link and paused maternal blood collection. During the first several months of 2020, some participants aged out of the study or did not respond to attempts to continue study participation.

Further studies could generate functional/metabolic profiles of fecal microbial communities across pregnancy to further interrogate the role

of specific taxa and their metabolites in mediating the relationship between maternal psychological state and microbial activity. Additionally, a larger sample size would facilitate controlling for additional possible covariates such as diet, physical activity, and non-clinical protective factors such as socioeconomic status, social support network, and health behaviors. Lastly, future studies may also consider characterizing sources, types, and duration of perinatal stress to elucidate the unique microbial mechanisms underlying different types of stress exposure.

5. Conclusions

In summary, we conducted a prospective cohort study of pregnant individuals, collecting fecal and vaginal microbiome samples and assessing stress and depression across gestation and postpartum. We found stress during early pregnancy to be associated with increased abundance of several fecal taxa not previously identified in studies of pregnancy or psychosocial stress and intrapartum and postpartum stress to be associated with increased abundance of largely vaginal-associated taxa and opportunistic pathogens. Additionally, concordant with previous literature, we found umbilical CCL2 concentration to be negatively correlated with relative abundance of fecal *Lactobacilli* at delivery. Lastly, we found that depressed participants experienced steeper decreases in prenatal vaginal alpha-diversity. Together, these findings underscore previous preclinical and clinical work demonstrating the effects of prenatal stress on the maternal microbiome, suggest distinct biological pathways for the consequences of stress versus depression, and extend the literature by offering several taxa which may serve a critical role in mediating this relationship.

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CRediT authorship contribution statement

Therese A. Rajasekera: Writing – review & editing, Writing – original draft, Visualization, Project administration, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Jeffrey D. Galley:** Writing – review & editing, Visualization, Investigation, Formal analysis, Data curation, Conceptualization. **Amy R. Mackos:** Writing – review & editing, Investigation. **Helen J. Chen:** Writing – review & editing, Investigation. **Justin G. Mitchell:** Investigation. **Joshua J. Kleinman:** Investigation. **Paige Cappelucci:** Investigation. **Lauren Mashburn-Warren:** Investigation. **Christian L. Lauber:** Methodology. **Michael T. Bailey:** Writing – review & editing, Supervision. **Brett L. Worly:** Writing – review & editing, Supervision, Investigation, Funding acquisition, Conceptualization. **Tamar L. Gur:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization.

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

Data will be made available on request.

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Appendix A. Supplementary data

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