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Development of the Gut Microbiota throughout the First Year of Life and Its Association with Socio-Emotional Development into Childhood

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Abstract

Introduction: Early life is a critical window for the development of many bodily systems, including the gut microbiota and the central nervous system, that are interconnected through the gut-brain axis. These early life gut-brain axis connections are often studied through cross-sectional cohorts, limiting insights into temporal developmental trajectories. This longitudinal cohort study assessed whether gut microbial development over the first year of life is associated with socio-emotional development into childhood. ***Methods:*** The PRIDE (pregnancy and infant development) BIOME study (n = 81, n = 42 males) is a focus cohort within the larger prospective PRIDE study. Gut microbiome was measured 5 times throughout the first year of life (at 2, 4, 6, 9, and 12 months through V4 16S rRNA sequencing) and socio-emotional development 8 times over 4.5 years, between 6 months and 5 years through the Ages and Stages Questionnaire: Social-Emotional (ASQ-SE). We related the development of the gut microbiota of infants throughout their first year of life with their socio-emotional development into childhood, the latter modeled as a slope per individual (ASQ slope). We assessed effects of time, ASQ slope and its interaction with time on microbial community measures alpha and beta diversity, as well as taxonomy, using linear mixed-effects models and PERMANOVA, correcting for sex, birth weight, gestational age, and sequencing depth. ***Results:*** Expected developmental patterns on the gut microbiota over the first year of life were observed, including increased alpha diversity and clustering of beta diversity before and after solid food introduction. Interestingly, ASQ slope was a significant predictor of beta

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diversity ($F(1,394) = 25.90$, $p = 0.001$) and *Bifidobacterium* abundance across the first year of life ($b = -0.745$, SE = 0.24, pFDR = 0.023). Moreover, we observed a temporal association between ASQ slope and *Eggerthella* abundance (ASQ slope × timepoint interaction, $b = 0.709$, SE = 0.21, pFDR = 0.009). That is, *Eggerthella* abundance decreased across the group, but not in "late concern" infants, with concern about socio-emotional development at more recent timepoints. ***Discussion:*** This study shows that genera *Bifidobacterium* and *Eggerthella*, known to be altered in mental health conditions such as autism spectrum disorder and depression, are already linked to socio-emotional development during early life. Hence, this work contributes to the identification of gut microbial candidates relevant for preventive screening of healthy gut-brain development and microbiota-targeted interventions.

Introduction

The prenatal and early life period – referred to as the first 1,000 days – is a critical window for the development and maturation of the gut microbiota [1]. Drivers of bacterial colonization of the gut include maternal body mass index, delivery mode, gestational age, and feeding mode (breast or bottle) [2]. With the introduction of solid foods (around the sixth month of life), dietary variety increases, triggering an increase in bacterial diversity. For example, *Bifidobacterium*, feeding on human milk oligosaccharides, is dominant before weaning and decreases after the introduction of solid food, outcompeted by bacteria growing on dietary proteins and fibers, such as *Ruminococcus gnavus* [1].

Disturbances around this critical developmental window, such as preterm birth (pre, peri, and postpartum) malnutrition, and antibiotic use impact gut microbial colonization and result in higher risks for atopic diseases, gastrointestinal disorders, and obesity later in life [3, 4]. A recent study by [5] on the relationship between the timing of gut colonization and somatic health demonstrated that, for example, earlier acquisition of *R. gnavus* – driven by breastfeeding cessation before 3 months of age – predicted preschool asthma [5].

Beyond somatic health also neurodevelopment, encompassing socio-emotional behaviors such as emotion regulation and social interactions, coincides with gut microbial maturation [6, 7] linked through the vagal, immune, and metabolic routes of the gut-brain axis [8]. Healthy neurodevelopment depends on timely supply of gut-derived compounds supporting gut and brain immunity and neuronal growth, including peptidoglycans, short-chain fatty-acids, amino-acids/precursors such as tryptophan and histidine, but also bacteriophages [9–13]. Disturbances in early microbial colonization are linked with neurodevelopmental disorders. For example, Ahrens et al. [9] 2024 found early life exposure to antibiotics to be associated with an increased risk for a diagnosis of a neurodevelopmental disorder later in childhood (of 16,440 children, 1,197 were diagnosed for, e.g., autism spectrum disorder [ASD] = 1.6, 95% CI: 1.2–2.1), which in turn was associated with lower abundance of *Akkermansia muciniphila* and *Bifidobacterium* sp. at 1 year. In prospective studies, higher levels of genus *Prevotella_9* at 6 years of age were associated with more externalizing behavior at 10 years (in 196 children) [14]. Similarly, lower *Prevotella* abundance (coinciding with antibiotic use) at 12 months concurred with more internalizing behavioral problems at 2 years (in 200 children) [15].

Tamana et al. [16] (2021) is one of few studies taking a longitudinal approach, testing whether changes in cognitive performance between 2 timepoints relate with the microbiota. In a cohort of 405 children, those with gut microbial clusters dominant in *Bacteroides* and *Firmicutes* measured at 12 months displayed larger improvements in cognition and language between 1 and 2 years compared to infants with clusters dominant in *Proteobacteria*. The vast majority of these studies performed cross-sectional analyses, leaving the relation between temporal developmental trajectories of both microbiota and neurodevelopment uncovered.

The current PRIDE BIOME population cohort, a focus cohort of the PRIDE (pregnancy and infant development) study [17] allows to move beyond cross-sectional analyses, investigating gut microbiota diversity throughout the first year of life at 2, 4, 6, 9, and 12 months and age-appropriate socio-emotional development measured 8 times from 6 months to 5 years of age with the Ages and Stages Questionnaire: Socio-Emotional (ASQ-SE) [18]. We assessed gut microbial development over the first year of life and explored whether this associates with socio-emotional development into childhood, using early socio-emotional variation as a transdiagnostic indicator of later neurodevelopmental risk.

Methods

The PRIDE study (https://pridestudy.nl/) is a large ongoing pregnancy cohort study in the Netherlands, aiming to identify factors affecting the risk of pregnancy

complications, maternal and child health outcomes, and adverse developmental effects in offspring [17]. Multiple web-based questionnaires are administered during pregnancy and after delivery to assess sociodemographic factors, obstetric history, medical history, medication use, lifestyle factors, occupational exposures, and complications during pregnancy. Moreover, the mothers reported on the health and development of their child at 11 times and specifically on the ASQ-SE at 8 times between 2 months and 5 years at 6 months intervals (except the first questionnaire which is administered at 2 months of age). The PRIDE study is a population cohort using convenience sampling for which the only exclusion criteria are (1) mothers of age <18 years and (2) pregnancy of >16 weeks at enrolment.

The current PRIDE BIOME study was embedded in the PRIDE study, with stool sample collection running from December 2016 to September 2018. Stool samples were provided by 116 mother-infant pairs, for the infants at months 2, 4, 6, 9, and 12 of the first year of life (for the mothers, sampling was done between gestational weeks 19–22 and 34 – i.e., second and third trimester – as well as 2 months postpartum). The stool of the mothers was not used in the current analysis as our research question focused on the gut-brain connection in the infants.

Fecal samples were provided of 106 infants, 96 of which provided samples at all 5 microbiota collection timepoints, resulting a total of 518 infant samples. For 85 infants, ASQ-SE questionnaire data were available for all 8 timepoints (see Fig. 1).

Feces Collection, Sequencing of Bacterial DNA, and Quality Control

Fecal samples were collected by the mothers from the diapers of their infants in OMNIgene-GUT OM-200 collection kits containing a conserving buffer (DNA Genotek, Ottawa, CA, USA). The kits were shipped at room temperature within 2 weeks after collection (maximal deviation 60 days). Upon receipt at the Radboudumc, the samples were aliquoted into 1.5-mL Eppendorf tubes and frozen at −80°C. Samples were shipped to Wageningen University for sequencing. A total of 0.013 g of stool was weighed and bead-beaden. DNA extraction and purification was performed with the Maxwell kit AS1220. PCR amplification of the V4 region of the 16S rRNA gene was performed using the 515F modified [19] and 806R modified primers [20]. On average, 747.54 μL (SD ± 293.18 μL) of phosphate-buffered saline was added in 61 out of 400 stool samples (15%) when low amounts of DNA were present. Samples were randomized into 16 amplicon libraries that were sent to Novogene for sequencing on the Illumina NovaSeq 6000 platform using a 150-bp paired-end read protocol.

Raw sequences were imported in the Galaxy toolbox of the NG-Tax 2.0 pipeline [21] for amplicon sequence variants (ASVs) picking and taxonomic assignment using the SILVA 132 database [22]. Default settings were applied, apart from keeping 100 rather than 70 nucleotides of the paired-end sequences indicated by good Phred quality scores for this length. The ASV and taxonomy table were combined with the tree file in a BIOM file and imported into R Studio (version 4.3.1). Analyses were performed using the mia package [23]. Other packages are cited when used.

Quality checks were performed on the mock communities and negative controls across all sequencing batch (for results see online suppl. Figs. 1,2, 3; for all online suppl. material, see https://doi.org/10.1159/000552189). We then proceeded with the biological samples (infant), filtering out nonbacterial ASVs. The read depth of the infant samples ranged between 918 and 1090904 reads, with a total of 5,970 ASVs. A correlation matrix was calculated between sequencing depth, number of ASVs, and relevant early life covariates (see Covariate selection below) including library and timepoint (see online suppl. Fig. S4). The correlation between sequencing depth and library was low (rho = 0.08), indicating no batch effect in sequencing depth. Stronger correlations were observed between timepoint (i.e., infant age) and sequencing depth (rho = −0.29), with higher sequencing depth in the earlier compared to the later timepoints (median sequencing depth at 2 months: 255,312; 4 months: 156,068; 6 months: 168,114.5; 9 months: 139,304.5; 12 months: 96,142; see also online suppl. Fig. S5a; Table S1). The number of ASVs correlated with sequencing depth (rho = −0.33, see online suppl. Fig. S5b). As commonly observed, increased number of ASVs is driven by infant age (online suppl. Fig. S5c) [1, 24]. To account for variation in sequencing depth over time it was added as a covariate in the analyses.

Questionnaires

Mothers completed extensive web-based questionnaires about their own health and that of their infants, covering demographics, medical history, lifestyle factors, pregnancy, and early-life health events such as mode of delivery, feeding method, illnesses, and age of weaning (for a full list, see [17]). The current analyses focused on socio-emotional development using the ASQ-SE (2002 edition, official Dutch translation) [18, 25]. This

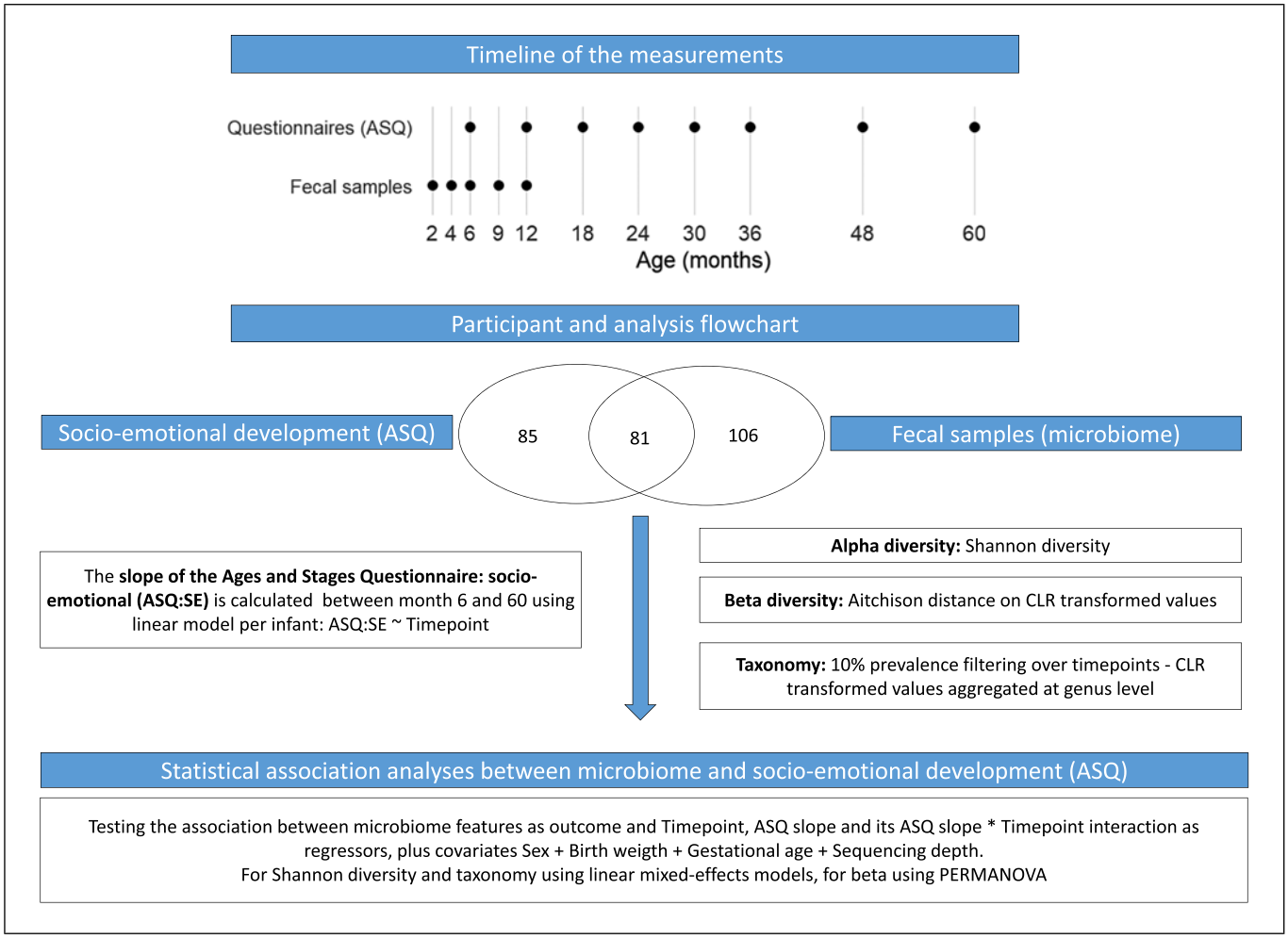

Fig. 1. Timeline of the measurements, participant, and analysis flowchart describing the overlap between ASQ-SE questionnaire and microbiota data, the calculation of the variable in each domain and the association analyses. ASQ-SE, Ages and Stages Questionnaire: Socio-Emotional; CLR, centered log ratio.

validated questionnaire assesses social and emotional behaviors across various domains, including self-regulation, compliance, social communication, adaptive functioning, autonomy, affect, and interactions with others.

The questions are age-specific, tailored to the developmental levels between 6 and 60 months, and are to be completed by parents or caregivers. Questions include: "Does your child make eye contact?" and "Does your child destroy or damage things on purpose?" Responses are rated on a three-point Likert scale (0, 5, or 10 points), with scores recorded where necessary so that 0 indicates no problematic behavior (never or rarely seen), while scores of 5 (sometimes) and 10 (most of the time) indicate some concern. An average score exceeding the age-dependent cut-off suggests the need for further testing. This quick, low-cost, paper-based questionnaire has been effective in identifying children at high risk for developing ASD through extensive clinical evaluation [26].

We calculated individual slopes across the 8 timepoints between 6 and 60 months, fitting linear models regressing timepoint on ASQ-SE mean scores. A negative ASQ slope (ASQ slope of <0) indicates more developmental concern during the earlier timepoints and fewer to no concerns at later timepoints, i.e., "early concern" socio-emotional development that is caught up over time. A positive ASQ slope (ASQ slope of >0) reflects fewer points of concern at the earlier timepoint but some/more points raising some concern during the later timepoints, i.e., a "late concern" socio-emotional development. Arguably a late concern (positive) ASQ

slope is more worrisome, as children with recent and persistent high ASQ-SE scores may receive a neurodevelopmental diagnosis later in childhood.

For the ASQ slope calculation, we excluded infants missing scores of either the first or last two timepoints or who were missing scores for ≥3 timepoints in total, resulting in ASQ scores for 85 infants. Finally, the association analyses were done on 81 infants with both fecal samples and ASQ slope availability (see Fig. 1).

Statistical Analyses: Association Analyses between ASQ Trajectory and Microbiota

Covariate Selection

All analyses on associations between ASQ, time, and microbiota measures included the covariates sex, birth weight, gestational age, and sequencing depth. These covariates were selected to account for their known impact on the (early life) microbiota (sex), as proxies for a healthy baby (being born at term, weighing >2,500 g) and as an additional control for technical variation (sequencing depth). Moreover, we estimated Spearman's correlation coefficients between these covariates and ASQ slope, which were all low (rho <0.1) (see online suppl. Fig. S4). The association between sex and ASQ slope was assessed with a *t*-test. Some known potential covariates were not included in the models because of the low prevalence (e.g., infants were mostly breastfed and antibiotic use was rare, see result section cohort demographics).

Microbiota Community Measures

Community analyses were performed without any prevalence filtering to prevent introducing a bias against less prevalent taxa. For alpha diversity, we assessed Shannon Diversity index, as this is a frequently used index. Moreover, correlation plots indicated that in this dataset, Shannon contained similar information compared to other alpha indices (see online suppl. Fig. S6). Using a linear mixed-effects model we assessed main effects of (1) time (infant age), (2) ASQ slope, and (3) their interaction on Shannon diversity, including covariates and a random participant intercept.

To calculate beta diversity, we transformed counts into centered log-ratio (CLR) transformed abundance values, with a pseudo count of 1 (half of the minimum positive value). Using PERMANOVA (vegan:adonis2, 999 permutations) we tested the main effects of (1) timepoint (infant age) and (2) ASQ slope including covariates (added by margin) on Aitchison distances as the outcome, using a restricted permutation scheme within the repeated measures. Third, the interaction between ASQ slope and timepoint (infant age) on Aitchison distance was tested in a separate PERMANOVA model, as including the interaction term will ignore the main effects.

To visualize effects, unsupervised ordination was done using principal coordinate analysis on Aitchison distance values (pco function from ecodist package [27]), plotting the first two unconstrained axes, colored by timepoint. Supervised ordination on ASQ slope was done using constrained analysis of principal coordinates (capscale function from vegan package [28]), plotting the constrained axis and the first unconstrained axis, colored by ASQ slope.

Taxonomy

For the compositional analyses, counts were agglomerated to genus level and genera unclassified at genus level were removed. We applied 10% prevalence filtering across all timepoints, as this allowed testing timepoint and ASQ slope together in one model, resulting in 43 genera. While we are aware that low prevalent taxa in earlier or later timepoints may remain untested in this approach, such taxa are unlikely to result in robust associations – not driven by zeros – with ASQ slope [29]. Counts were CLR transformed, with a pseudo count of 1 (using the default setting: half of the minimum positive value). Using the MaAsLin2 package [30], we ran linear mixed-effects models assessing the main (fixed) effects of (1) timepoint (infant age), (2) ASQ slope, and (3) their interaction, including covariates. To account for repeated measures by setting participant ID was added as a random effect. False discovery rate (FDR)-corrected (using Benjamini Hochberg procedure) results over all genera tested were reported. Subsequently, to interpret interactions between ASQ slope and timepoint, we tested effects of time split by ASQ slope group (early or late concern, ASQ slope scores of <0 or >0, respectively) and did post hoc linear regressions of ASQ slope on genus abundance as well as presence/absence per timepoint. These simple effects are presented descriptively and no comparison correction was applied.

Sensitivity Analyses

For significant results on ASQ slope, we performed sensitivity analyses. To evaluate robustness of these results to sequencing depth, we performed iterative rarefaction from 10k up to 120k reads in steps of 10k, balancing sequencing depth against sample retention. We capped the iterations at 120,000 reads per sample to ensure minimally 3 out of 5 timepoints available in at

Table 1. Characteristics of the 81 mother-infant pairs in the PRIDE BIOME cohort

Characteristics		
Mothers		*N* missing
Age, years	31.2 (3.6, 22–42)	0
Prepregnancy BMI	22.9 (4.1, 18.0–36.3)	4
Come around with income	Yes: 87.7% (71)	1
Educational attainment: low ≤5 – high ≥4	Low: 17.2% (14) – high: 81.5% (66)	1
Monthly household income, EUR	15-25k: 3.7% (3); 25-35k: 33% (27); 35-50k: 41.9% (34); >50k: 18.5% (15)	1
Infants		
Sex	42 males /39 females	0
Vaginal delivery (*n*)	86.4% (70)	2
Birth weight	3,554.6 g (422.7, 2,280–4,580)	1
Gestational age, weeks	39.2 (1.1, 36–41)	1
Breastfed – at least up to 2 months	81.5% (66)	3
At least up to 4 months	75.3% (61)	1
At least up to 6 months	54.3% (44)	1
Formula fed – at 2 months	27.2% (22)	3
6 months	64.2% (52)	1
Introduction solid food at 6 months	90.1% (73)	1
Antibiotic use in the first 12 months	17.3% (14)	3
Any medication use in the first 6 months	49.3% (40)	0

Values represent mean (SD, range), or percentages of total (*n*), calculated relative to the total sample.

least 75% of the participants. Moreover, where applicable the interaction between ASQ slope and sex on the microbiome variables was assessed.

Results

Cohort Characteristics

This is a cohort of infants born mostly at term with a healthy birth weight, see Table 1 for further cohort characteristics.

ASQ-SE Scores from 6 Months to 5 Years

The computed variable ASQ slope indicates whether children scored some point of concern in early childhood that subsided ("early concern") or whether their developmental concern builds up ("late concern"). To illustrate variation in ASQ slope, we split the group by ASQ slope score of 0 into an "early concern" group with negative ASQ slope values ($n = 25$) and a "late concern" group with positives ASQ slopes ($n = 56$) (see Fig. 2). Of these two groups, we view the late concern group, with more recent problematic behavior, as more concerning.

In our sample, the majority of infants scored below the clinical cut-off value. Specifically, only 16 children (14%) scored above the cut-off values on one or more timepoints, indicating the need for further clinical neurodevelopmental evaluation (see online suppl. Fig. S7; Table S2 for cut-off values per time point and flagged participants). Of these 16 children, 12 were in the late concern group, confirming our interpretation that the late concern group is most worrisome. Note that ASQ slope also captured infants with high mean ASQ scores across timepoints, as for most of them the high mean is driven by either earlier or later timepoints – resulting in a positive or negative slope (online suppl. Fig. S8). Lastly, ASQ slope was not different between boys and girls (*t* test: t = 1.51, $p = 0.13$).

Association between ASQ Trajectory and Microbiota

Microbiota Community Measures

Alpha Diversity. A linear mixed-effects model on Shannon diversity revealed a main effect of timepoint (infant age) ($b = 0.079$, SE = 0.007, $p = 2\times10^{-16}$), but no main effect of ASQ slope ($b = -4.42$, SE = 3.70, $p = 0.23$) or interaction with timepoint ($b = 0.792$, SE = 0.424, $p = 0.063$), see online supplementary Fig. S9 and Table S3.

Beta Diversity. PERMANOVA analyses revealed main effects of timepoint (infant age) and ASQ slope (see Table 2) but no ASQ slope × timepoint interaction (tested in a separate PERMANOVA: $F(1,381) = 0.904$, $p = 0.24$). Unsupervised visualization (using principal coordinate analysis) colored by timepoint (infant age) shows the changing community structure of the gut

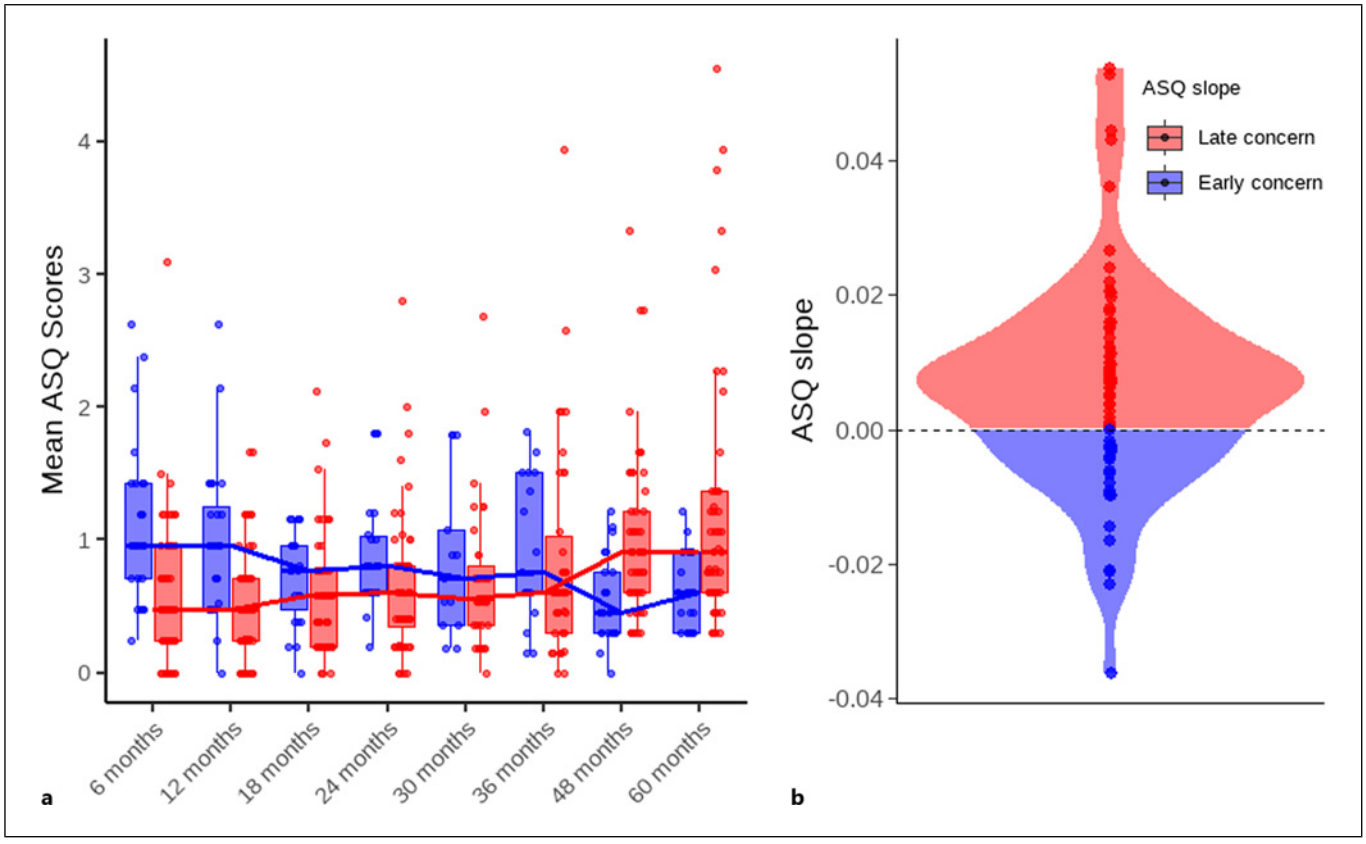

Fig. 2. a Mean ASQ scores per timepoint, measured 8 times between 6 and 60 months (a 5-year timespan), split by ASQ slope zero point. **b** ASQ slope, calculated per participant over the timepoints using linear models. The "early concern" group in blue has higher mean ASQ scores at earlier timepoints (ASQ slope of <0). The "late concern" group in red has higher mean ASQ scores at the later timepoints (ASQ slope of >0). Socio-emotional development in the early concern group has improved into childhood. In contrast, the late concern group is speculatively more worrisome as, in case the pattern continues, it may indicate impaired socio-emotional development into childhood.

Table 2. Main effect of timepoint and ASQ slope on Aitchison distance tested in PERMANOVA

	Df	Sum of squares	R^2	F	Pr (>F)
Timepoint	4	54,586	0.075	7.946	0.001
ASQ slope	1	4,466	0.006	2.601	0.001
Sex	1	3,726	0.005	2.170	0.010
Birth weight	1	2,479	0.003	1.444	0.001
Gestational age	1	3,580	0.005	2.085	0.002
Sequencing depth	1	4,867	0.007	2.834	0.003
Residual	385	6,61,173	0.901		
Total	394	7,33,742	1.000		

microbiota throughout the first year of life. There is a visual separation between the timepoints before and after 9 months (after the introduction of solid foods) (Fig. 3a). Supervised ordination using constrained analysis of principal coordinates shows the variation explained by ASQ slope (x-axis) (Fig. 3b).

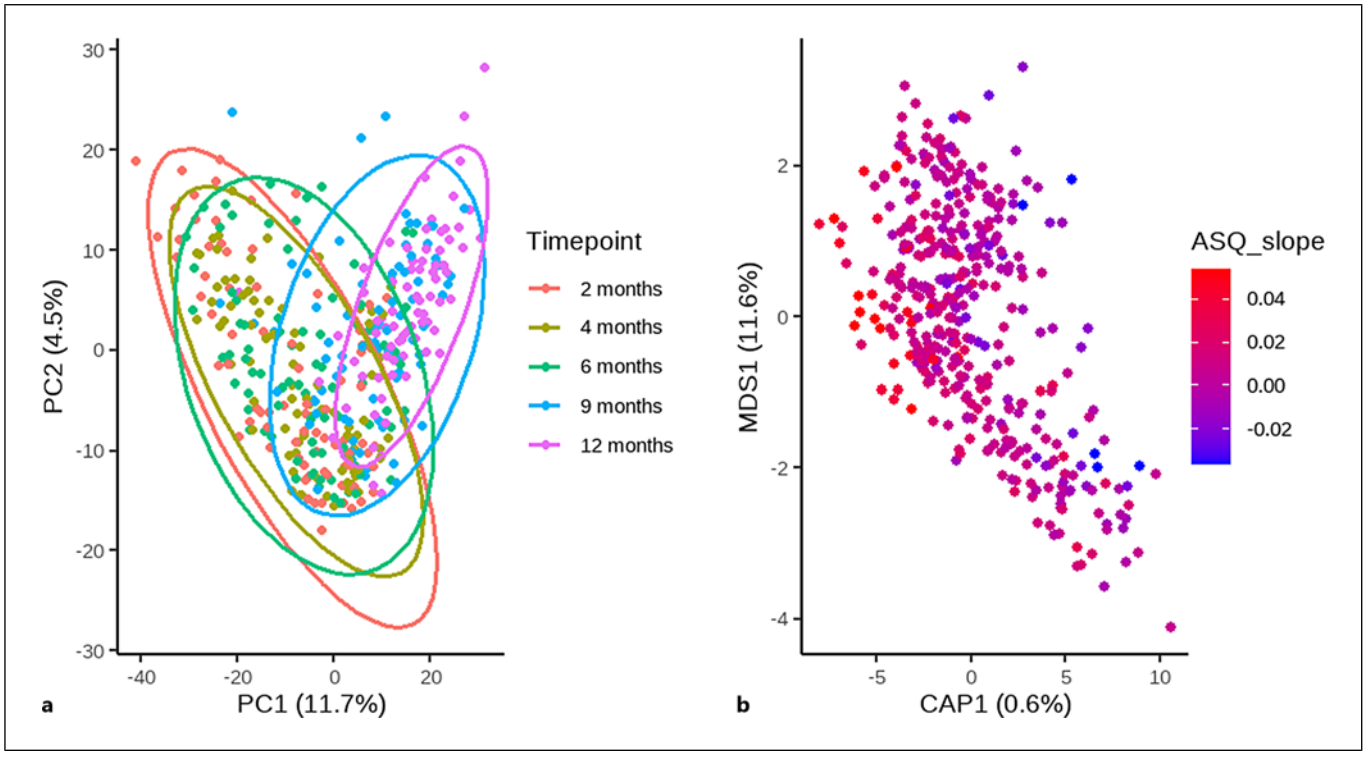

Fig. 3. a Unsupervised ordination (on Aitchison distance values) using PCoA, colored by timepoint to illustrate the maturation of the gut microbiota over the first year of life (PERMANOVA effect of timepoint: $R^2 = 0.075$, $p = 0.001$). **b** Supervised ordination using CAP, plotting variance explained by ASQ slope on Aitchison beta diversity (PERMANOVA effect of ASQ slope: $R^2 = 0.006$, $p = 0.001$).

Sensitivity Analyses

The effect of ASQ slope on beta diversity was robust when iteratively rarefying at sequencing depths between 10,000 and 1,20,000 (see online suppl. materials). As here, there was a main effect of sex on beta diversity, a sensitivity analysis assessed an interaction with ASQ slope and sex on beta diversity. We observed an interaction between ASQ slope and sex on beta diversity ($F(1,384) = 1.37$, $p = 0.001$). When subsequently stratifying the sample by sex shows that the effect ASQ slope on beta was present both in boys ($F(1,197) = 2.70$, $p = 0.001$) and girls ($F(1,180) = 1.29$, $p = 0.001$).

Taxonomy

In the following, we report the taxonomic results of interest: (1) main effect of timepoint (infant age), (2) ASQ slope, and (3) ASQ slope × timepoint interaction. See online supplementary information for detailed information: a summary heatmap created by MaAsLin2 (online suppl. Fig. S10), MaAsLin2 results for all models (genera) for each regressor (online suppl. Table S4), CLR values and prevalence per timepoint for significant genera on the regressors of interest (online suppl. Table S5; Table S6).

1. Effects of timepoint (infant age) on taxonomy

Repeated measures ANOVA in MaAsLin2 using genera as the outcome revealed main effects of timepoint (infant age) on 28 genera, of which 13 show a growth over time (Fig. 4a), largely after the introduction of solid foods after 6 months in the majority of infants (Table 1). Fifteen genera decrease in CLR-transformed abundance over time (Fig. 4b).

2. Main effect of socio-emotional development (ASQ) on *Bifidobacterium*

Out of all 43 genera tested, genus *Bifidobacterium* negatively associated with ASQ slope, see Figure 5 (b = −0.684, SE = 0.230, $p = 0.004$, pFDR = 0.030). Lower *Bifidobacterium* abundance throughout the first year of life was associated with more concern in socio-emotional development during the later timepoints and higher *Bifidobacterium* abundance with more concern during the earlier timepoints.

3. Interaction effect of between socio-emotional developmental slope (ASQ slope) on *Eggerthella*

Out of all 43 genera tested, we observed an interaction between time and ASQ slope on genus *Eggerthella* ($b = 0.709$,

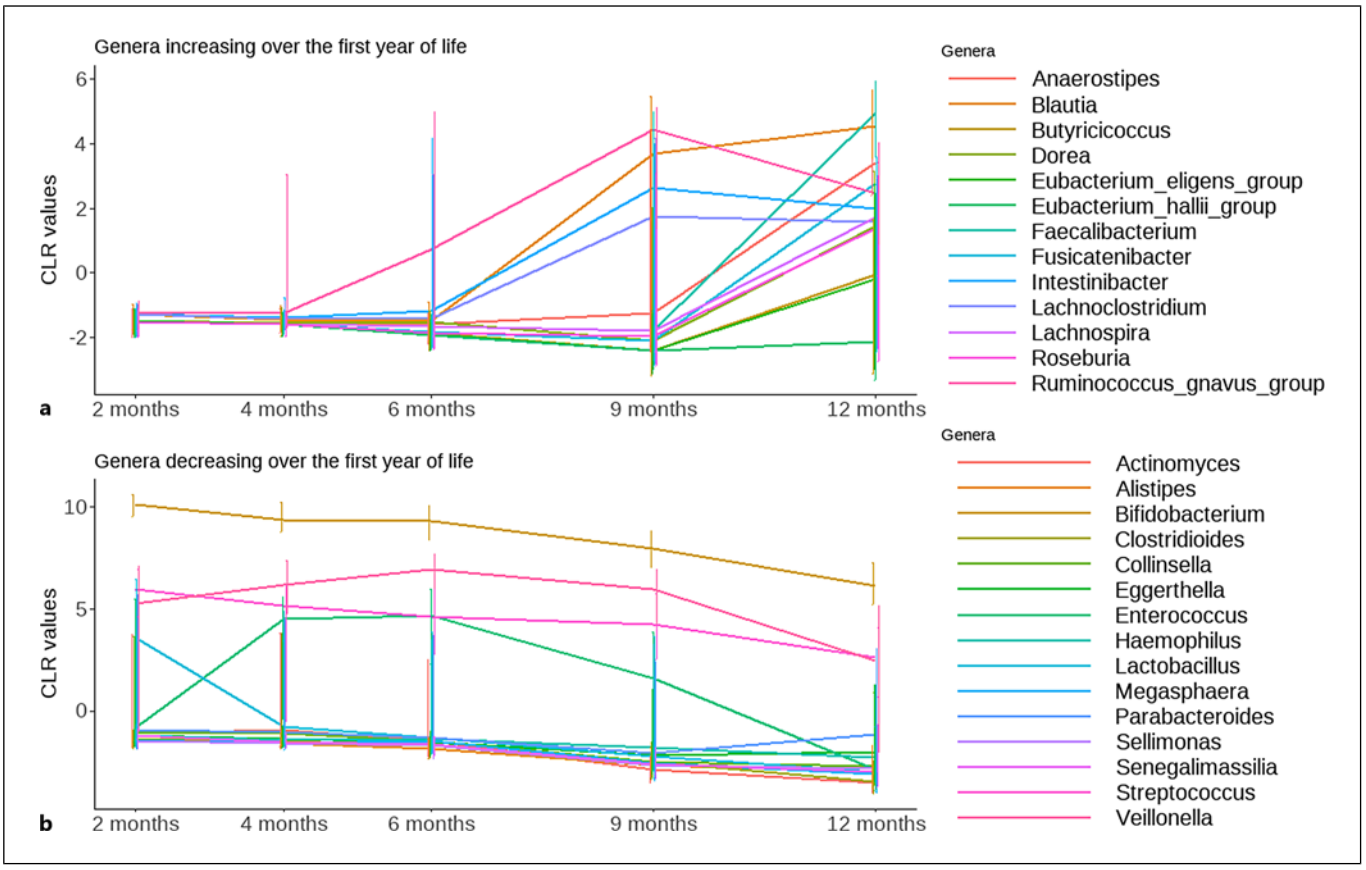

Fig. 4. Genera where FDR significantly changed in abundance over the first year of life, i.e., showing a main effect of timepoint in linear mixed-effects models in MaAslin2, plotted by (**a**) genera increasing in abundance and (**b**) genera decreasing in abundance.

SE = 0.210, $p = 9 \times 10^{-4}$, pFDR = 0.009). That is, while *Eggerthella* abundance decreased over the first year of life across the whole group (main effect of timepoint: b = −0.467, SE = 0.110, pFDR = 0.00035, Fig. 6a), this is driven by the early concern ASQ slope group (in blue) and not present in the late concern ASQ slope group (in red), see Figure 6b (early concern group: n = 25, b timepoint = −0.586, SE = 0.172, p = 1x 10^{-3}, late concern group: n = 56, b timepoint = −0.205, SE = 0.126, p = 0.106).

Further post hoc testing revealed that the interaction was not driven by any of the single microbial timepoints; *Eggerthella* abundance was not associated with ASQ slope in any of the five timepoints (post hoc linear regressions per timepoint on *Eggerthella* abundance by ASQ slope including covariates: 2 months $F(1,74) = 3.52$, $p = 0.06$; 4 months $F(1,73) = 0.56$, $p = 0.46$; 6 months $F(1,73) = 0.64$, $p = 0.43$; 9 months $F(1,73) = 3.52$, $p = 0.06$; 12 months $F(1,72) = 2.01$, $p = 0.16$; see online suppl. Fig. S11).

Presence/absence patterns are visible in *Eggerthella* abundance over time, especially in the first months (see online suppl. Fig. 6; S11). When plotting *Eggerthella* prevalence per timepoint (by ASQ slope, see online suppl. Fig. S12), we observed that *Eggerthella* is present at 2 months in only a few infants (P/A 16/64). In contrast, at 12 months, *Eggerthella* was present in a larger group of infants (P/A 34/45). We tested whether ASQ slope varied by presence/absence pattern per timepoint, which was only the case at 2 months (t = 2.70, p = 0.01), where ASQ slope was higher in infants without *Eggerthella*. ASQ slope did not differ with P/A patterns in any of the other timepoints (4 months: t = 0.84, p = 0.41; 6 months: t = −0.93, p = 0.36; 9 months: t = −1.29, p = 0.21; 12 months: t = −1.83, p = 0.071).

Sensitivity Analyses

All results on ASQ slope at taxonomic level were robust when iteratively rarefying at sequencing depths between 10,000 and 1,20,000 (see online suppl. materials). As no sex differences were observed for the genera *Bifidobacterium* and *Eggerthella*, we did not test sex by ASQ slope interactions.

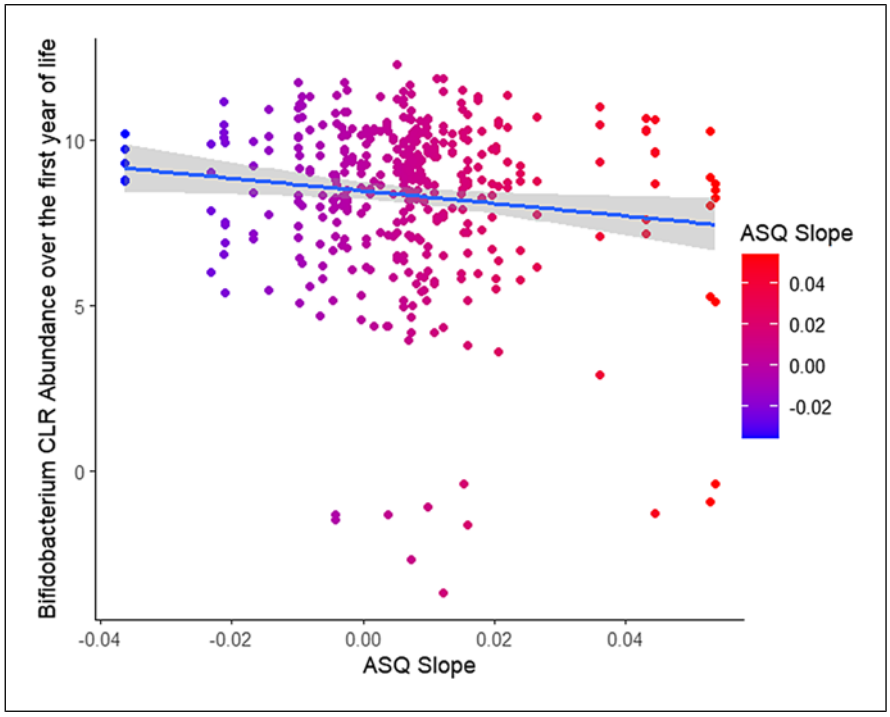

Fig. 5. Association between *Bifidobacterium* abundance across the first year of life and ASQ slope, i.e., showing a main effect of ASQ slope in a linear in mixed-effects models in MaAslin2 (b = −0.684, SE = 0.230, pFDR = 0.030).

Discussion

The PRIDE-BIOME cohort provided the unique opportunity to associate variation in gut microbial changes throughout the first year of life with socio-emotional development up to 5 years of age. Beta diversity in the first year was linked to socio-emotional development and associated negatively with the abundance of genus *Bifidobacterium*. In addition, socio-emotional development positively associated with *Eggerthella* development in the first year of life. That is, while across all infants *Eggerthella* abundance decreased over time, this was not the case in infants with more concern about socio-emotional development at later timepoints.

Development of the Gut Microbiota over the First Year of Life

We confirmed commonly observed gut microbial early life patterns, including an increase in alpha diversity over time and altered beta diversity after 6 months of age [1, 31]. At the taxonomic level we observed a group of genera with a steep increase in abundance after 6 months, such as *R. gnavus*, concurring with solid food introduction [1]. Other genera showed a gradual decrease in abundance over the first year of life, also often driven by weaning. For example, decreased *Bifidobacterium* and *Lactobacillus* abundance over the first year of life is common, due to increased competition with other nutrients than human milk oligosaccharides [1, 31]. Also, *Eggerthella* abundance decreased over the first year of life. In contrast, previous early life studies show *Eggerthella* abundance increases in the first year of life, for example measured between 1 and 4 months [14] and between 0 and 12 months [1]. A gradual decline was previously seen after, rather than before 12 months [1]. The difference between these cohorts may be partly driven by a lower percentage and faster decrease in breast-fed infants in Roswall et al. as breastfeeding is known to associate with lower *Eggerthella* abundance [24].

Association between Gut Microbiota and Socio-Emotional Development

Healthy neurodevelopment depends on timely supply of gut-derived compounds, supporting gut and brain immunity and neuronal growth through the routes of the gut-brain axis [9–13]. Specifically, *Bifidobacterium* abundance in infancy is widely regarded as a hallmark of normative microbial maturation [32]. As the most abundant early life colonizers, Bifidobacteria are a pivotal player in gut and brain immunity and central nervous system maturation processes, directly via passage through the blood-brain-barrier or indirectly via vagal signaling [32]. *Bifidobacteria*-derived metabolites such as short-chain fatty-acids [33] and mono-amine (precursors) including bile acids [34], indole-3-lactic acid [35], 4-hydroxyphenylacetate [9], serotonin [36]

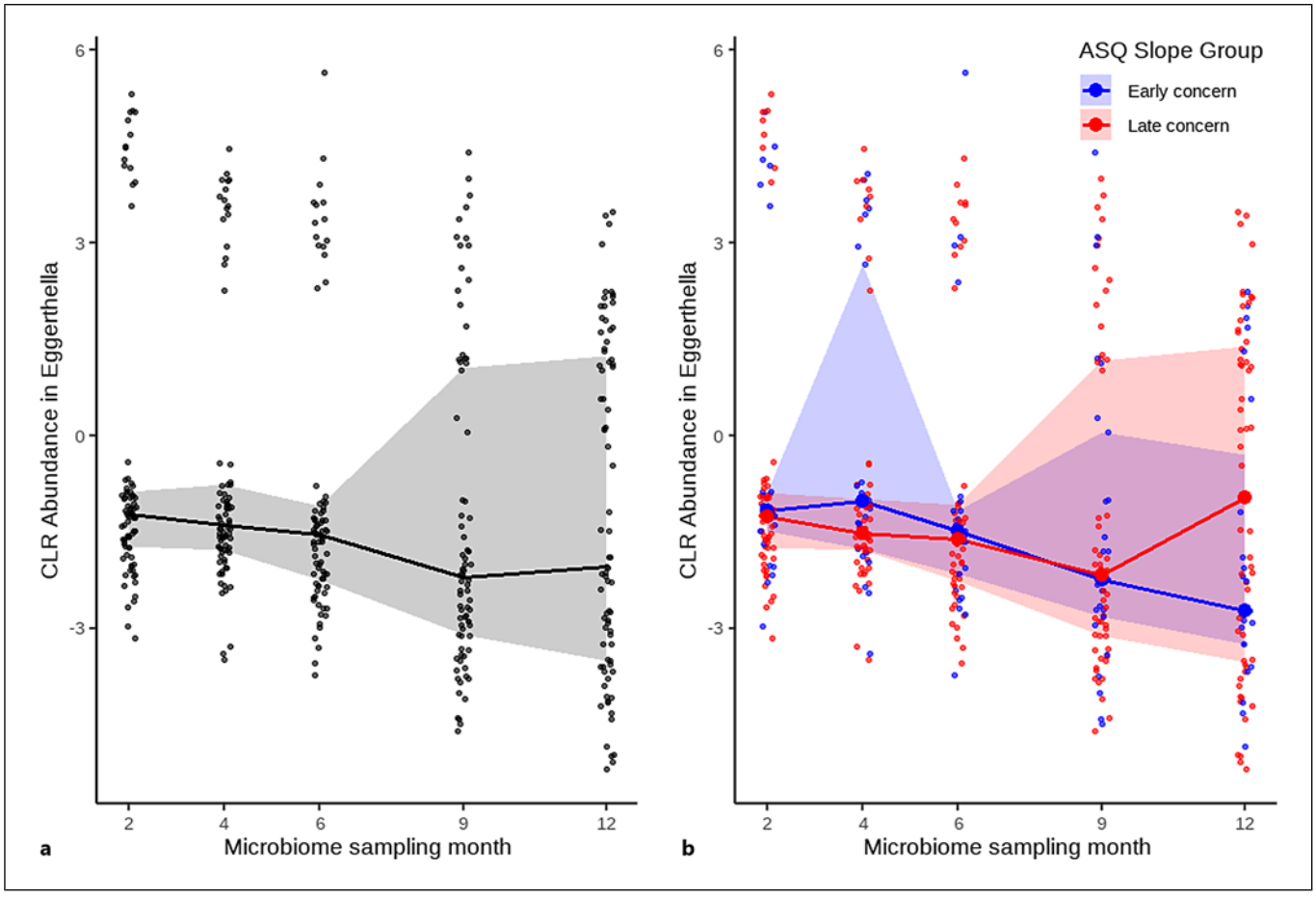

Fig. 6. Timepoint × ASQ slope interaction on *Eggerthella* (in a linear mixed-effects models in MaAslin2 [b = 0.709, SE = 0.210, pFDR = 0.009]). **a** Median abundance of *Eggerthella* with a shaded interquartile range and individual values plotted, across the whole sample (**b**) split by ASQ slope early vs. late concern groups.

promote neuronal growth and/or mediate immunity and behavior. Moreover, fecal γ-aminobutyric acid, which can be of *Bifidobacterium* origin [34], is linked to higher likelihood of ASD [37]. We hypothesize that *Bifidobacterium*-derived metabolites are implicated in the association between infant *Bifidobacterium* abundance and childhood socio-emotional development.

So far, associations between *Bifidobacterium* and socio-emotional outcomes in infants have been observed, though in different directions and lacking functional data. For example, in 2.5-month-old infants, higher *Bifidobacterium* abundance related with positive emotionality on the Infant Behavior Questionnaire, and *Bifidobacterium*-dominated community composition was related to better self-regulation at 6 months [38]. This is in line with the current findings, where a regression in socio-emotional development associated with lower *Bifidobacterium* abundance. In contrast, in the first month of life, higher abundance associated with worse emotion regulation on the same questionnaire [39] and at 2 years with poorer social behaviors on the Social Responsiveness Scale [40].

Beyond these conflicting results regarding socio-emotional development, *Bifidobacteria* are key species for early life development. The association we observe aligns with the most robust and replicated early life somatic health associations, e.g., higher abundance of *Bifidobacterium* – within the first 1,000 days of life – results in lower odds of overweight, diabetes and atopic diseases [41]. Hence, our findings underscore that *Bifidobacterium* is an important marker for overall infant development, with more research needed on the timing and directionality for neurodevelopmental outcomes.

While we studied younger and generally healthy – not (yet) diagnosed – infants, the pattern we observed indicates that a reduction in *Eggerthella* abundance into

childhood is associated with healthy socio-emotional development. This is an interesting, hypothesis-generating observation, in line with findings of enriched *Eggerthella (lenta)* in the microbiota of children (~4.5 years old) with (suspected) ASD [42]. Moreover, in adulthood, *Eggerthella* is repeatedly reported increased in psychiatric conditions [43]. At the same time, we observed higher ASQ slope in infants lacking *Eggerthella* at 2 months, potentially indicating delayed acquisition of *Eggerthella* in relation to more concern about socio-emotional development at later timepoints. This finding highlights that longitudinal microbial dynamics reveal relevant patterns for neurodevelopmental outcomes not observed in cross-sectional comparisons, that may occur through sustained modulation of gut-derived neuroactive compounds. Larger mother-infant cohorts including meconium samples may study how *Eggerthella* is acquired in early life as well as the effects of absence or altered timing of acquisition, considering maternal-infant transfer and breastfeeding.

As our data were obtained through 16S sequencing, we cannot assess which *Eggerthella* species our cohort contained. A biologically plausible species would be *Eggerthella lenta*, found to be enriched in ASD and correlating with neurotransmitter (precursors) tyrosine and glutamate by Wang et al. [32], known for their role in neuronal signaling supporting cognition and social interactions [44, 45]. Excessive glutamate levels in gut and specifically brain are implicated in excitatory-inhibitory imbalance linked synaptic plasticity and several neurodevelopmental and psychiatric diagnoses [46, 47]. Speculatively, the absence of decline in *Eggerthella* abundance across the first year of life in the late concern group may result in prolonged microbial activity potentially influencing tyrosine and glutamate metabolism during a sensitive developmental window.

Future Directions and Clinical Implications

The observed trajectories of gut microbial acquisition and development over time in relation to socio-emotional behavior need to be replicated, preferably in larger, longitudinal cohorts or meta-analyzed over independent cohorts, including broader gut-omics measures. In terms of clinical implications, we captured neurodevelopmental variation in a relatively homogeneous and healthy sample that may never transition into a clinical neurodevelopmental diagnosis. Hence, the predictive value of these gut microbial signals for a clinical diagnosis needs to be further tested. However, specifically deviations in the late concern group require attention as most deviating ASQ-SE scores were in this group and such scores are indicative of, for e.g., ASD diagnosis [26]. So far, increased odds for ASD have only been indirectly linked to the microbiome through for example early life antibiotic exposure (Ahrens et al. 2024). For somatic childhood health outcomes in early life microbial variability has been observed; a recent example shows an association between early acquisition of *R. gnavus* (present before 3 months) and preschool asthma [5]. If replicated, such microbial patterns may inform future studies on early-life risk stratification and hypothesis-driven microbiota-targeted interventions.

Limitations

This is an observational study that does not allow causal or mechanistic conclusions. Moreover, the limited sample size of this population cohort prevented stratifying analyses into meaningful subgroups by events disrupting early life colonization (as the majority of infants were born vaginally, at term, with a normal birth weight, and breast-fed). The association between *Eggerthella* prevalence and abundance per timepoint and ASQ slope also suffered from low study power, meaning the interaction of ASQ slope over time could not be convincingly attributed to one of the timepoints. Another point of attention is the low variance explained by ASQ slope on beta diversity over the first year of life, which was even comparable to the R2 for sequencing depth. This is not uncommon, see [48] on technical variation being the largest source of variation in the early life microbiota. Lastly, time-lags between defecation by the infants and collection by the caregivers were not logged and hence quantifying and accounting for this was not possible. Nevertheless, previous research shows a very limited effect of short term (2 h) exposure to room temperature on microbial composition of feces in infant feces [49], a timespan unlikely surpassed in these infants in their first year of life.

Conclusion

This study observed that variation in socio-emotional development up to 5 years of age related with altered trajectories in the gut microbiota already in the first year of life. The genera involved are known to be altered in ASD in childhood and mental diagnoses in adulthood, and may serve, if replicated, as screening candidates for potential future neurodevelopmental deviations.

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Statement of Ethics

The PRIDE study has been approved by the Regional Committee on Research involving Human Subjects (CMO 2009/305). Written informed consent was obtained from participants and their legal guardians to participate in the study.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

M.B.: conceptualization, software, visualization, methodology, formal analysis, data curation, writing – original draft. D.M.: writing – review and editing, software, formal analysis. J.G.: software, visualization, methodology, formal analysis, data curation. I.H.: data curation, project administration, resources. A.I. and C.B.: supervision, writing – review and editing. E.E.N.: formal analysis, writing – review and editing. S.E.T. and A.A.: writing – review and editing. M.G.: conceptualization, funding acquisition, investigation, writing – review and editing. A.A.V.: conceptualization, funding acquisition, supervision, writing – review and editing.

Data Availability Statement

The data that support the findings of this study are not publicly available due to privacy reasons but are available from the corresponding author upon reasonable request. The R script for the analyses can be found here: https://rpubs.com/MirjamBloemendaal/.

References

1 Roswall J, Olsson LM, Kovatcheva-Datchary P, Nilsson S, Tremaroli V, Simon MC, et al. Developmental trajectory of the healthy human gut microbiota during the first 5 years of life. Cell Host Microbe. 2021;29(5):765–76.e3. https://doi.org/10.1016/j.chom.2021.02.021

2 Roager HM, Stanton C, Hall LJ. Microbial metabolites as modulators of the infant gut microbiome and host-microbial interactions in early life. Gut Microbes. 2023;15(1):2192151. https://doi.org/10.1080/19490976.2023.2192151

3 Ahearn-Ford S, Berrington JE, Stewart CJ. Development of the gut microbiome in early life. Exp Physiol. 2022;107(5):415–21. https://doi.org/10.1113/EP089919

4 Arrieta MC, Stiemsma LT, Amenyogbe N, Brown EM, Finlay B. The intestinal microbiome in early life: health and disease. Front Immunol. 2014;5:427. https://doi.org/10.3389/fimmu.2014.00427

5 Shenhav L, Fehr K, Reyna ME, Petersen C, Dai DLY, Dai R, et al. Microbial colonization programs are structured by breastfeeding and guide healthy respiratory development. Cell. 2024;187(19):5431–52.e20. https://doi.org/10.1016/j.cell.2024.07.022

6 Coley EJL, Hsiao EY. Malnutrition and the microbiome as modifiers of early neurodevelopment. Trends Neurosci. 2021;44(9):753–64. https://doi.org/10.1016/j.tins.2021.06.004

7 Naspolini NF, Schüroff PA, Figueiredo MJ, Sbardellotto GE, Ferreira FR, Fatori D, et al. The gut microbiome in the first one thousand days of neurodevelopment: a systematic review from the microbiome perspective. Microorganisms. 2024;12(3):424. https://doi.org/10.3390/microorganisms12030424

8 Cryan JF, O'Riordan KJ, Cowan CSM, Sandhu KV, Bastiaanssen TFS, Boehme M, et al. The microbiota-gut-brain axis. Physiol Rev. 2019;99(4):1877–2013. https://doi.org/10.1152/physrev.00018.2018

9 Ahrens AP, Hyötyläinen T, Petrone JR, Igelström K, George CD, Garrett TJ, et al. Infant microbes and metabolites point to childhood neurodevelopmental disorders. Cell. 2024;187(8):1853–73.e15. https://doi.org/10.1016/j.cell.2024.02.035

10 Tisza MJ, Lloyd RE, Hoffman K, Smith DP, Rewers M, Javornik Cregeen SJ, et al. Longitudinal phage–bacteria dynamics in the early life gut microbiome. Nat Microbiol. 2025;10(2):420–30. https://doi.org/10.1038/s41564-024-01906-4

11 Mayneris-Perxachs J, Castells-Nobau A, Arnoriaga-Rodríguez M, Garre-Olmo J, Puig J, Ramos R, et al. Caudovirales bacteriophages are associated with improved executive function and memory in flies, mice, and humans. Cell Host Microbe. 2022;30(3):340–56.e8. https://doi.org/10.1016/j.chom.2022.01.013

12 Gonzalez-Santana A, Diaz Heijtz R. Bacterial peptidoglycans from Microbiota in neurodevelopment and behavior. TICS. 2020;26(8):729–43. https://doi.org/10.1016/j.molmed.2020.05.003

13 Cerdó T, Ruiz-Rodríguez A, Acuña I, Torres-Espínola FJ, Menchén-Márquez S, Gámiz F, et al. Infant gut microbiota contributes to cognitive performance in mice. Cell Host Microbe. 2023;31(12):1974–88.e4. https://doi.org/10.1016/j.chom.2023.11.004

14 Ou Y, Belzer C, Smidt H, De Weerth C. Development of the gut microbiota in healthy children in the first ten years of life: associations with internalizing and externalizing behavior. Gut Microbes. 2022;14(1):2038853. https://doi.org/10.1080/19490976.2022.2038853

15 Loughman A, Ponsonby AL, O'Hely M, Symeonides C, Collier F, Tang MLK, et al. Gut microbiota composition during infancy and subsequent behavioural outcomes. EBioMedicine. 2020;52:102640. https://doi.org/10.1016/j.ebiom.2020.102640

16 Tamana SK, Tun HM, Konya T, Chari RS, Field CJ, Guttman DS, et al. Bacteroides-dominant gut microbiome of late infancy is associated with enhanced neurodevelopment. Gut Microbes. 2021;13(1):1–17. https://doi.org/10.1080/19490976.2021.1930875

17 Van Gelder MMHJ, Merkus PJFM, Van Drongelen J, Swarts JW, Van De Belt TH, Roeleveld N. The PRIDE Study: evaluation of online methods of data collection. Paediatric Perinatal Epid. 2020;34(5):484–94. https://doi.org/10.1111/ppe.12618

18 Squires J, Bricker D, Twombly E. The ASQ:SE user's guide: for the Ages & Stages Questionnaires: Social-emotional. Paul H. Brookes Publishing Co; 2002. Report No.

19 Parada AE, Needham DM, Fuhrman JA. Every base matters: assessing small subunit primers for marine microbiomes with mock communities, time series and global field samples. Environ Microbiol. 2016;18(5):1403–14. https://doi.org/10.1111/1462-2920.13023

20 Apprill A, McNally S, Parsons R, Weber L. Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. Aquat Microb Ecol. 2015;75(2):129–37. https://doi.org/10.3354/ame01753

21 Poncheewin W, Hermes GDA, Van Dam JCJ, Koehorst JJ, Smidt H, Schaap PJ. NG-Tax 2.0: a semantic framework for high-throughput amplicon analysis. Front Genet. 2019;10:1366. https://doi.org/10.3389/fgene.2019.01366
22 Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 2013;41(Database issue):D590–6. https://doi.org/10.1093/nar/gks1219
23 Lathi L, Shetty SA, Ernst FM. Orchestrating microbiome analysis with bioconductor. 2021. [Internet] Available from: microbiome.github.io/oma/
24 Stewart CJ, Ajami NJ, O'Brien JL, Hutchinson DS, Smith DP, Wong MC, et al. Temporal development of the gut microbiome in early childhood from the TEDDY study. Nature. 2018;562(7728):583–8. https://doi.org/10.1038/s41586-018-0617-x
25 Krijnen LJG, Verhoeven M, Van Baar AL. Assessing social-emotional development in infants and toddlers using parent-reports: comparing the ASQ-SE-NL to the Social-Emotional Scale of the Bayley-III-NL. Early Hum Dev. 2021;161:105439. https://doi.org/10.1016/j.earlhumdev.2021.105439
26 Dolata JK, Sanford-Keller H, Squires J. Modifying a general social-emotional measure for early autism screening. Int J Dev Disabil. 2019;66(4):296–303. https://doi.org/10.1080/20473869.2019.1577024
27 Goslee SC, Urban DL. The ecodist package for dissimilarity-based analysis of ecological data. J Stat Soft. 2007;22(7). https://doi.org/10.18637/jss.v022.i07
28 Anderson MJ, Willis TJ. Canonical analysis OF principal coordinates: a USEFUL method OF constrained ordination for ECOLOGY. Ecology. 2003;84(2):511–25. https://doi.org/10.1890/0012-9658(2003)084[0511:caopca]2.0.co;2
29 Cao Q, Sun X, Rajesh K, Chalasani N, Gelow K, Katz B, et al. Effects of rare microbiome taxa filtering on statistical analysis. Front Microbiol. 2020;11:607325. https://doi.org/10.3389/fmicb.2020.607325
30 Mallick H, Rahnavard A, McIver LJ, Ma S, Zhang Y, Nguyen LH, et al. Multivariable association discovery in population-scale meta-omics studies. Coelho LP. PLoS Comput Biol. 2021;17(11):e1009442. https://doi.org/10.1371/journal.pcbi.1009442
31 Sinha T, Brushett S, Fernández-Pato A, Garmaeva S, Andreu-Sánchez S, Spreckels J, et al. Pregnancy and early life gut microbiome: influencing factors and health implications. 2024 [Internet] [cited 2024 Nov 10]. Available from: https://www.researchsquare.com/article/rs-5334252/v1
32 Sharon G, Sampson TR, Geschwind DH, Mazmanian SK. The central nervous System and the gut microbiome. Cell. 2016;167(4):915–32. https://doi.org/10.1016/j.cell.2016.10.027
33 Sorboni SG, Moghaddam HS, Jafarzadeh-Esfehani R, Soleimanpour S. A comprehensive review on the role of the gut microbiome in human neurological disorders. Clin Microbiol Rev. 2022;35(1):e0033820–20. https://doi.org/10.1128/CMR.00338-20
34 Aatsinki AK, Lamichhane S, Isokääntä H, Sen P, Kråkström M, Alves MA, et al. Dynamics of gut metabolome and microbiota maturation during early life. iScience. 2025;28(11):113596. https://doi.org/10.1016/j.isci.2025.113596
35 Wong CB, Tanaka A, Kuhara T, Xiao J. Potential effects of Indole-3-Lactic acid, a metabolite of Human Bifidobacteria, on NGF-Induced neurite outgrowth in PC12 cells. Microorganisms. 2020;8(3):398. https://doi.org/10.3390/microorganisms8030398
36 Engevik MA, Luck B, Visuthranukul C, Ihekweazu FD, Engevik AC, Shi Z, et al. Human-Derived Bifidobacterium dentium modulates the Mammalian serotonergic System and gut–brain axis. Cell Mol Gastroenterol Hepatol. 2021;11(1):221–48. https://doi.org/10.1016/j.jcmgh.2020.08.002
37 Zuffa S, Schimmel P, Gonzalez-Santana A, Belzer C, Knol J, Bölte S, et al. Early-life differences in the gut microbiota composition and functionality of infants at elevated likelihood of developing autism spectrum disorder. Transl Psychiatry. 2023;13(1):257. https://doi.org/10.1038/s41398-023-02556-6
38 Aatsinki AK, Lahti L, Uusitupa HM, Munukka E, Keskitalo A, Nolvi S, et al. Gut microbiota composition is associated with temperament traits in infants. Brain Behav Immun. 2019;80:849–58. https://doi.org/10.1016/j.bbi.2019.05.035
39 Kelsey CM, Prescott S, McCulloch JA, Trinchieri G, Valladares TL, Dreisbach C, et al. Gut microbiota composition is associated with newborn functional brain connectivity and behavioral temperament. Brain Behav Immun. 2021;91:472–86. https://doi.org/10.1016/j.bbi.2020.11.003
40 Laue HE, Korrick SA, Baker ER, Karagas MR, Madan JC. Prospective associations of the infant gut microbiome and microbial function with social behaviors related to autism at age 3 years. Sci Rep. 2020;10(1):15515. https://doi.org/10.1038/s41598-020-72386-9
41 Saturio S, Nogacka AM, Alvarado-Jasso GM, Salazar N, De Los Reyes-Gavilán CG, Gueimonde M, et al. Role of bifidobacteria on infant health. Microorganisms. 2021;9(12):2415. https://doi.org/10.3390/microorganisms9122415
42 Wang M, Wan J, Rong H, He F, Wang H, Zhou J, et al. Alterations in gut glutamate metabolism associated with changes in gut Microbiota composition in children with autism spectrum disorder. mSystems. 2019;4(1):e00321-18. https://doi.org/10.1128/msystems.00321-18
43 Nikolova VL, Smith MRB, Hall LJ, Cleare AJ, Stone JM, Young AH. Perturbations in gut Microbiota composition in psychiatric disorders: a review and meta-analysis. JAMA Psychiatry. 2021;78(12):1343–54. https://doi.org/10.1001/jamapsychiatry.2021.2573
44 Bloemendaal M, Froböse MI, Wegman J, Zandbelt BB, Van De Rest O, Cools R, et al. Neuro-Cognitive effects of acute Tyrosine administration on reactive and proactive response inhibition in healthy older adults. eNeuro. 2018;5(2):ENEURO.0035–17. https://doi.org/10.1523/ENEURO.0035-17.2018
45 Park G, Jeon SJ, Ko IO, Park JH, Lee KC, Kim MS, et al. Decreased in vivo glutamate/GABA ratio correlates with the social behavior deficit in a mouse model of autism spectrum disorder. Mol Brain. 2022;15(1):19. https://doi.org/10.1186/s13041-022-00904-z
46 Wang D, Jiang Y, Jiang J, Pan Y, Yang Y, Fang X, et al. Gut microbial GABA imbalance emerges as a metabolic signature in mild autism spectrum disorder linked to overrepresented Escherichia. Cell Rep Med. 2025;6(1):101919. https://doi.org/10.1016/j.xcrm.2024.101919
47 Mutluay SU, Karataş H. A review of glutamate and its receptors: their roles in brain physiology and pathology. Acta Med. 2022;53(2):99–109. https://doi.org/10.32552/2022.ActaMedica.650
48 Jokela R, Ponsero AJ, Dikareva E, Wei X, Kolho KL, Korpela K, et al. Sources of gut microbiota variation in a large longitudinal Finnish infant cohort. EBioMedicine. 2023;94:104695. https://doi.org/10.1016/j.ebiom.2023.104695
49 Guo Y, Li SH, Kuang YS, He JR, Lu JH, Luo BJ, et al. Effect of short-term room temperature storage on the microbial community in infant fecal samples. Sci Rep. 2016;6(1):26648. https://doi.org/10.1038/srep26648