Early nasal microbiota and subsequent respiratory tract infections in infants with cystic fibrosis

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Materials and Corresponding: Requests regarding material and data beyond the publicly available dataset can be sent to insa.korten@insel.ch (corresponding author) or ruth.steinberg@insel.ch (first author).

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Abstract

Lower respiratory tract infections (LRTIs) are a driving factor for lung function decline in children with cystic fibrosis (CF). The respiratory microbiota is closely related to the pathogenesis of LRTIs in healthy infants, data in CF infants is scarce. We compared nasal microbiota development between CF and healthy infants and assessed associations between early-life nasal microbiota, LRTIs and antibiotic treatment in CF infants. We analyzed the microbiota after amplification of the V3-V4 region of the 16S rRNA gene from 1511 biweekly nasal samples from 50 CF and 30 healthy infants. Microbiota diversity differed between CF infants and healthy controls following LRTIs and/or antibiotic treatment. CF infants with a lower α-diversity presented with a subsequent higher number of LRTIs. Early nasal microbiota alterations may contribute to an increased susceptibility to LRTIs in CF infants and may further increase after LRTIs and antibiotic treatment. This emphasizes the possible preventive and therapeutic potential of targeting the nasal microbiota in CF-related LRTI management.

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Introduction

Several studies demonstrate a relationship between the dynamics of the upper respiratory microbiota in early life and susceptibility to respiratory tract infections in healthy individuals [1-7]. However, conclusive data studying the relationship between respiratory tract infections and the microbiota among infants with cystic fibrosis (CF) is scarce [8].

Pathogenic bacteria begin to colonize the airways of children with CF in early life, leading to recurrent, mostly polymicrobial lower respiratory tract infections (LRTIs) [9,10]. These may lead to pulmonary exacerbations that require antibiotic treatment and hospitalizations [11,12] and eventually drive persistent airway inflammation, structural damage, and lung function decline [9,13].

The respiratory microbiota profile is altered in infants with CF, independent of the presence of pathogens detected with culture-based techniques [14-17]. Early microbial development is characterized by early dominance and persistence of *Staphylococcus aureus* in infants with CF, shifting to *Corynebacterium spp.* and *Streptococcus spp.* dominance at age three months, compared with dominance of *Moraxella spp.,* higher abundance of *Dolosigranulum* and *Prevotella spp.* in healthy infants [17,18]. Antibiotic use in the first year of life is associated with lower bacterial density and increased colonization with gram-negative bacteria in infants with CF [18].

We have previously demonstrated that distinct nasal microbiota profiles are present in the first year of life in infants with CF compared to healthy controls [17]. LRTIs and antibiotic therapy are frequent even in early CF disease, hampering to disentangle the interrelations between the microbiota, respiratory infections and antibiotics. This is however of uttermost importance to be able to assess a possible preventive and therapeutic potential to target the

nasal microbiota in CF-related LRTI management. In this study, we thus aim to I. compare nasal microbiota development between infants with CF and healthy infants before and after the first LRTI and first antibiotic treatment, II. assess the interrelations between early-life nasal microbiota and number of LRTIs, before and after the first LRTI and first antibiotic treatment in infants with CF, and III. analyze in detail how the first antibiotic treatment or the first LRTI as "a first hit" influence the nasal microbiota in infants with CF.

Results

Studied cohort and samples

The characteristics of the study cohorts are displayed in table 1 and the study design in figure 1. The two cohorts were matched regarding sex, breastfeeding, delivery mode, and childcare. Mean number of reads was lower in infants with CF compared to healthy individuals, which could be due to higher exposure to antibiotics (Table 1).

I. Comparison of the nasal microbiota between infants with CF and healthy infants

β-diversity was higher in infants with CF compared to healthy individuals (PERMANOVA: R^2=0.016, p<0.001) (Table 2, Supplementary figure 1). This difference was not present if only antibiotic naïve swabs were considered or before the first LRTI was reported (Table 2), but could only be detected after the first antibiotic treatment (e.g. treatment of asymptomatic bacterial colonization in CF infants), first LRTI (prior first antibiotic treatment) or both (Table 2, Supplementary figure 2). Infants with CF had less stable bacterial communities than healthy individuals, reflected by higher subsequent within-subject dissimilarities (Figure 2, Supplementary table 1). Again, this difference was not present before the first antibiotic treatment or before the first respiratory symptoms were reported (Figure 2, Supplementary table 1). We did not assess within-subject dissimilarity in even smaller subgroups (in contrast

to the other diversity measures) because the number of consecutive taken samples of the same infant in subgroups was too low.

Infants with CF had a higher α-diversity compared to healthy individuals (Table 3). However, differences in α-diversity occurred first after the initial antibiotic treatment (with or without LRTI reported), and not after the first LRTI alone (Table 3).

In microbiota differential abundance analysis, results of the most abundant bacterial families are displayed in figure 3 and supplementary table 2. Taxa annotation on genus level was not possible for all samples and is thus reported in the supplemental data (Supplementary figure 3). In infants with CF, we detected overall more *Staphylococcaceae*, *Propionibacteriaceae* and more *Micrococcaceae*, less *Carnobacteriaceae* and less *Moraxellaceae* than in healthy infants (Figure 3 A and B, Supplementary table 2). In healthy individuals, we observed the typical shift towards a more *Moraxellaceae / Carnobacteriaceae (represented by Dolosigranulum)* dominated profile around age four to five months, while in the profile of infants with CF *Staphylococcaceae* dominated. When specifically examining swabs taken before the first antibiotic treatment (CF=539, healthy=559), consistent findings were observed for *Staphylococcaceae, Micrococcaceae* and *Carnobacteriaceae*. This emphasizes that these differences were not confounded by antibiotic treatment (Supplementary table 2). *Staphylococcaceae* and *Carnobacteriaceae* already differed before the first LRTI (CF=435, healthy=357 swabs) (Supplementary table 2, Figure 3 C and D). After the first antibiotic therapy alone (before the first LRTI) (CF=75, healthy= 357 swabs), we observed the same result as a trend. After the first LRTI but before the first antibiotic treatment, we detected more *Staphylococcaceae* in infants with CF (Supplementary table 2).

II. Investigation of the interrelations between early-life nasal microbiota and number of LRTIs, first LRTI and first antibiotic treatment in infants with CF

β-diversity did not differ between CF infants with a higher or lower number of LRTIs in the first year of life, before the first antibiotic treatment, or before the first LRTI occurred (results not shown). Microbiota communities were more stable in infants with a higher number of LRTIs, reflected by lower within-subject dissimilarities between consecutive time-points (Table 4Table 4, Supplementary figure 4). This difference was already present in antibiotic naïve infants and before the first LRTI (Table 4).

Infants with CF with a higher number of LRTIs had a lower α-diversity compared to infants with a lower number of LRTIs. Again, this difference was present before the first antibiotic treatment and before the first LRTI occurred (Table 5, Figure 4).

In infants with a higher number of LRTIs, we observed reduced levels of *Neisseriaceae* (coefficient -1.959, q-value <0.05) and *Propionibacteriaceae* (coefficient -1.49, q-value <0.05), even prior to the occurrence of the first LRTI (*Neisseriaceae* (coefficient -2.34, q-value <0.05), *Propionibacteriaceae* (coefficient -2.12, q-value <0.05)). Before the first antibiotic treatment, we observed this as a trend: *Neisseriaceae* (coefficient -2.03, q-value <0.5), *Propionibacteriaceae* (coefficient -2.05, q-value <0.1).

III. Effect of a "first hit" on the nasal microbiota in infants with CF

Antibiotic treatment at nasal swab was associated with a higher α-diversity (Coef 0.286; 95% CI 0.059, 0.512, p=0.014). To investigate the effect of the first antibiotic treatment, infants with CF with at least one course of antibiotics (n = 33) were included. ß-diversity was higher after the first antibiotic treatment (PERMANOVA, R^2=0.005, p-adj=0.007). In addition, α-diversity increased after the first antibiotic treatment (Coef 0.396; 95% CI 0.140, 0.651;

p<0.01) (Figure 5), as observed in part I. We did not analyze within-subject dissimilarity and possible further changes in the microbiota after a second course of antibiotics due to low number of samples. Low abundant bacterial families ("others") (Coef 1.259, q-val <0.0001), as well as *Propionibacteriaceae* (Coef 1.051, q-val <0.05) and *Neisseriaceae* (Coef 1.259, q-val <0.05) increased after the first antibiotic treatment. There was no association between the number of antibiotic treatments in first year of life and ß-diversity, within sample dissimilarity, α-diversity or differential abundances (results not shown). However, there were only few infants with more than two courses of antibiotic therapy in our cohort.

After the first LRTI, β-diversity increased in infants with CF (PERMANOVA, p <0.001, R^2=0.009), reflecting a larger heterogeneity of the microbiota profiles in the CF group after the first infection. Within-subject dissimilarity did not change after the occurrence of the first LRTI. α-diversity also did not differ after the first LRTI in infants with CF (Coef 0.001; 95% CI -0.267, 0.269; p=0.994). In differential abundance analysis, we found an increase in abundance of *Neisseriaceae* (Coef 1.3, qval <0.05) after the first LRTI.

Discussion

In this prospective cohort study, we investigated the nasal respiratory microbiota over the first year of life in infants with CF and healthy controls and examined interrelations with antibiotic therapy and LRTIs.

Microbiota differences between infants with CF and healthy individuals in our study are in line with previous results from studies of upper and lower respiratory samples [8,17-21]. Importantly, differences in diversity indices first occurred after initial LRTI and/or antibiotic treatment. This suggests, that the microbiota community destabilizes in infants with CF following first antibiotic therapy and/or LRTI compared to healthy individuals. This can be

176 attributed to the event itself combined with a higher vulnerability of the CF microbiota
177 structure, which was observed in a metagenome network analysis [21]. Prevaes et al. report a
178 different β-diversity in the first three months of life in infants with CF compared to healthy
179 controls [18], too. Our study adds that this difference is not only an effect of progressing disease,
180 but due to antibiotic treatments.

181 Distinct bacterial families between young CF and healthy infants were present in antibiotic
182 naïve samples and prior respiratory infections also indicating a "CF specific microbiota", e.g.
183 we demonstrated a higher abundance of *Staphylococcaceae* and a lower abundance of
184 *Carnobacteriaceae*, in line with previous studies [8,17,18]. High and early abundance of
185 *Staphylococcus aureus* is associated with worse respiratory outcomes even in clinically stable
186 infants with CF, strain persistence and unbeneficial adaption [22-24]. Sequencing techniques with
187 a higher resolution (like whole metagenome sequencing) are required to distinguish whether
188 *Staphylococcaceae* dominance reflects early strain persistence of a potential pathogen
189 (*Staphylococcus aureus*) [25] or whether it reflects a different composition of commensal
190 bacteria among infants with CF in this cohort.

191 We report a lower α-diversity in CF infants with a higher number of LRTIs, and more stable
192 microbiota communities reflected by lower within-subject dissimilarities between
193 consecutive time-points. The latter seems counterintuitive, but could be explained by "a
194 bacterial overgrow" of certain (disadvantageous) bacterial species, which already persist in
195 the nasal cavity in infants with CF. This might differ to healthy individuals, Bosch et al. show
196 that bacterial community stability is lower in infants with higher number of RTIs within the
197 first year of life [6]. Ahmed et al. [19] report changes in β-diversity of the oropharyngeal
198 microbiota with increasing age in CF infants, but did not report respiratory symptoms, and

199 the increasing cumulative antibiotic dose in the first year of life, is likely to be associated with
200 changing β-diversity.

201 Importantly, next to the (expected) microbial alterations through respiratory infections
202 and/or antibiotics, in our study, a lower α-diversity is associated with a higher number of
203 subsequent LRTIs in infants with CF independent from antibiotic therapy. Thus, certain
204 microbiota profiles in CF infants might predispose to respiratory disease. Studies in adult
205 patients with CF show an inverse relationship between microbiota diversity and disease
206 progression, in line with our finding [13,20]. However, the association between higher microbiota
207 biodiversity and beneficial health outcomes is not always consistent in humans [26-29]. In the
208 respiratory tract, acute infections like otitis media [30,31] or chronic rhinosinusitis [32] are
209 associated with decreased α-diversity. In other studies, an increased α-diversity has been
210 associated with disease (e.g. in elderly pneumonia patients even before antibiotic therapy) [33].
211 In CF infants, differing antibiotic treatment schemes throughout the world might further
212 explain different outcomes for α-diversity [34]. A recently published longitudinal study of
213 oropharyngeal samples of infants with CF [35] observes higher numbers of distinct communities
214 (leading to higher α-diversity) with higher cumulative antibiotic exposure in days at time of
215 sample collection in line with our data. In contrast, this study reports a mildly decreased α-
216 diversity in antibiotic treated infants compared to antibiotic naïve individuals at age nine
217 months [35]. Rare and transient (potentially environmental) species may increase after initial
218 antibiotic treatment in differential abundance analysis. An opening of microbial niche spaces
219 with elimination of single pathogens by antibiotics might explain our findings, e.g. a rise in
220 gram-negative bacteria after antibiotics in CF infants has been reported [18]. Thus, an increase
221 in α-diversity cannot necessarily be considered as an improvement. In line, we do not observe

an increase in commensal bacteria that play a key role for respiratory health (like *Carnobacteriaceae* or *Corynebacteriaceae*) [3,7].

A strength of our study is the prospective study design that allowed structured collection of high-quality longitudinal data. We provide one of the largest longitudinal datasets of nasal microbiota swabs and clinical data (including respiratory symptoms and antibiotic treatment) to date. We include healthy infants as control subjects, who followed the same study protocol. Our data allowed us to study microbiota dynamics before and after important respiratory events (LRTIs, antibiotic treatment), which is unique in infants with CF.

As limitation, we did not assess viral colonization [5]. Furthermore, as CF is not diagnosed at birth, information on the neonatal period was obtained retrospectively. In addition, LRTIs and antibiotic treatments occur together in infants with CF and thus, it is difficult to disentangle the effects of each individually. However, the last two points are general limitations for studies among patients with CF and cannot be improved by study design.

In the future, it might be promising to treat infants with CF with probiotics to prevent destabilization of the upper airway microbiota. Some studies show promising effects of enteric probiotic therapies on clinical outcomes in individuals with CF between 2-44 years of age [36], resulting in less frequent pulmonary exacerbations. Whether enteric probiotics influence the respiratory microbiota in CF has not been investigated so far. For a better understanding, large randomized controlled trials in infants with CF are required to study the effect of probiotic therapies on the respiratory microbiota and future LRTIs and exacerbations. Metagenome analyses could provide further insights into strain adaption and persistence processes in infants with CF and metabolomics might shed light into the

mechanisms that underlie the influence of respiratory microbiota alterations on respiratory health.

In conclusion, we could show that the nasal microbiota is already altered before the first LRTI or antibiotic treatment in infants with CF. It might predispose to a higher number of subsequent LRTIs and is not (only) a consequence of recurrent LRTIs or of antibiotic treatment in infants with CF. In the future, targeting the nasal microbiota might thus be an attractive option in CF-related LRTI management.

Methods

Additional methods information is available in the online supplement.

Study design

This study includes data of infants from two prospective birth cohort studies: the Swiss Cystic Fibrosis Infant Lung Development (SCILD) [17] and the Basel Bern Infant Lung Development (BILD) cohort [37]. Infants were followed throughout the first year of life [38]. Ethics Committee of the Canton of Bern, Switzerland approved the study.

Cohort

We included 50 infants with CF (30 of those were included in our previous study [17]) from nine centers around Switzerland (Supplementary table 3) and 30 matched healthy controls, all born between 2011 and 2019. We performed weekly structured interviews to obtain information about respiratory symptoms, antibiotic treatment, breastfeeding and childcare. Parents collected biweekly anterior nasal swabs and sent them to the coordinating study center in Bern.

Microbiota analysis

For transport and storage of nasal swabs UTM® system from Copan was used. DNA extraction was performed as described before [39]. The V3-V4 region of the 16S- ribosomal RNA gene was amplified, and PCR product purified. Paired-end sequencing was performed with Illumina NovaSeq6000 platform. The DADA2 pipeline was used to obtain amplicon sequence variants (ASVs) [40] using the Silva v138.2 database [5]. We removed ASVs not assigned to kingdom Bacteria or assigned to genus *Burkholderia*, identified as contaminating ASV with the decontam package in R [41]. We analyzed samples with at least 3000 reads, as suggested in comparable studies [5] and as a clear flattening of the rarefaction curves was observed after 3000 reads (Supplementary figure 5). We included 1511 samples after quality control (1557 before). α-diversity calculation (Shannon-Diversity Index (SDI)), was performed without further filtering. For other analyses, only ASVs with a prevalence > 0.01 % or abundance in at least five samples were preserved (43205 ASVs). Microbiota data was normalized using total sum scaling into relative abundance. Distance matrix to analyze β-diversity was calculated with Bray-Curtis dissimilarity.

Outcome Variables

<u>I. Comparison of the nasal microbiota between infants with CF and healthy infants</u>

We investigated 1. β–diversity: compositional microbiota differences between samples based on a dissimilarity matrix, 2. within-subject dissimilarity: β–diversity between consecutive taken swabs with less than three weeks difference of the same infant, 3. α-diversity: microbiota diversity within a sample, and 4. differential bacterial abundances (%). We assessed symptom scores as described previously [38] (Supplementary table 4). We included: a. all samples of all infants; b. samples taken before the first antibiotic treatment. c. samples

taken before the first LRTI. d. samples taken before the first LRTI, but after the first antibiotic treatment. e. samples taken before the first antibiotic treatment, but after the first LRTI (Figure 1).

II. Investigation of the interrelations between early-life nasal microbiota and number of LRTIs, first LRTI and first antibiotic treatment in infants with CF

We defined "higher number" of LRTIs above the 75th percentile of LRTI weeks of healthy infants. We divided infants in two groups: Infants with a "lower number" of LRTIs (0 - 3 weeks of LRTI, n = 35) and infants with a "higher number" of LRTIs (> 3 weeks of LRTI, n=15). We performed analyses analogous to 1.-4. and a-c of the first part (I.) of the study.

III. Effect of a "first hit" on the microbiota in infants with CF

We investigated the role of first antibiotic treatment and the role of number of antibiotic treatments in the first year of life analogous to 1.-4. of the first part (I.) of the study. Infants with CF were divided into three groups depending on the number of antibiotic treatments: "Never" (0 antibiotic treatments, n=17), "<75th Percentile" (1 antibiotic treatment, n=25), and ">=75th Percentile" (2 or more antibiotic treatments, n=8). We investigated the role of first LRTI in the first year of life analogous to 1.-4. of part I.

Statistical Analyses

Statistics were performed with statistical software R (version 4.1.2). For normal distributed microbiota exposures, generalized additive mixed models (gamm function, mgcv package) were performed with autoregressive structure (lag 1) to account for temporal correlation within subjects (random effect) and corrected for before named possible confounders (fixed effect) as described before [39]. The age of the infant in weeks was added in a smooth term. β-diversity was tested with permutational multivariate analysis of variance (PERMANOVA).

Terms were added with the option “marginal” to account for all predictor variables equally (adonis2 function, vegan package). Differential abundance analysis of bacterial families was calculated with MaAsLin2 after total sum scaling normalization as described previously [5,42]. We focused on the nine most abundant families for the main manuscript and summarized the rest (“others”) due to 16S rRNA sequencing resolution. Reported q-values were corrected for multiple testing with Benjamini-Hochberg procedure as recommended in MaAsLin2 default [42]. To control for confounding, we corrected for sweat chloride levels in infants with CF, mode of delivery, feeding type (breastfeeding vs formula fed), parental smoking, siblings, season, and antibiotic treatment. Due to only two infants with CF attending childcare in the first year of life, we matched healthy cases that did not attend childcare either without correcting in the further analyses. Results with $p < 0.05$, $q < 0.05$ respectively, were considered significant.

Data availability statement

Sequencing data generated during this study have been stored accessible for the public in the NCBI bioproject repository (https://www.ncbi.nlm.nih.gov/bioproject/ with the accession code “PRJNA1019921”). Study participant and further metadata is available upon reasonable request.

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Tables

Table 1 Characteristics of studied cohort

Characteristic	CF, N = 50*	Healthy, N = 30*	*P* value†
Data points	933	578	
Sex (female)	26 (52)	15 (50)	0.9
Gestational age (weeks)	39.29 (38.50, 40.25)	39.78 (38.89, 40.25)	0.5
Gestational weight (kg)	3.3 (3.0, 3.6)	3.3 (3.1, 3.5)	>0.9
Cesarean Section	14 (28)	9 (30)	0.8
Breastfeeding	38 (76)	23 (77)	>0.9
Childcare	2 (4)	3 (10)	0.4
Parental Smoking	4 (8)	0 (0)	0.3
Antibiotic treatment in first year	33 (66)	2 (6.7)	<0.001
Sweat Chloride	98 (79, 106)	-	
Swabs per patient	19 (16, 21)	22 (16, 23)	0.2
Mean reads per swab per patient	70,027 (50,071, 99,511)	90,396 (70,944, 105,308)	0.043
Any symptoms reported (weeks)	3 (2, 5)	3 (1, 4)	0.2
LRTI (weeks)	3 (1, 4)	1 (0, 3)	0.13
Age at first LRTI (weeks)	24 (16, 35)	31 (20, 40)	0.11
Antibiotic treatments	1 (0, 2)	0 (0, 0)	<0.001
Age at first antibiotic treatment (weeks)	26 (13, 34)	-	
Higher number of LRTIs	15 (30)	-	
Lower number of LRTIs	35 (70)	-	
Antibiotic treatment			
Never	17 (34)	-	
< 75th Percentile	25 (50)	-	
>= 75th Percentile	8 (16)	-	

*Median (IQR); n (%)

†Wilcoxon rank sum test; Pearson's Chi-squared test; Fisher's exact test

Abbreviations: CF= Cystic Fibrosis, LRTI= Lower Respiratory Tract Infection, IQR= Interquartile Range

Table 2 Comparison of β-diversity between CF and healthy infants overall, and stratified in groups regarding first LRTI and first antibiotic treatment

Antibiotic treatment	LRTI	CF N; swabs	Healthy N; swabs	R^2 value†	*P* value†
All	All	50; 933	30; 578	0.016	<0.001
Before	All	46; 539	30; 559	0.011	1.00
All	Before	48; 435	30; 357	0.099	0.733
After*	Before	13; 75	30; 357	0.020	<0.001
Before	After	20; 135	19; 162	0.015	0.023

Abbreviations: LRTI = lower respiratory tract infection; CF = Cystic Fibrosis; All = all samples were included irrespective of antibiotic treatment or LRTI; Before/After = only samples before or after the first antibiotic treatment or LRTI were included * Only samples of CF infants after antibiotic treatment and before LRTI were compared with healthy individuals before LRTI. † by PERMANOVA, additional terms that were added marginally: breastfeeding, siblings, mode of delivery, age in weeks and season as fixed effects, and subject as random effect (not shown)

Table 3 Comparison of α-diversity between CF and healthy infants overall, and stratified in groups regarding first LRTI and first antibiotic treatment

Antibiotic treatment	LRTI	CF N; swabs	Healthy N; swabs	Mean SDI CF / Healthy	Estimate (Healthy)†	95% CI†	*P* value†
All	All	50; 933	30; 578	2.124 / 1.927	-0.20	-0.320, -0.064	<0.005
Before	All	46; 539	30; 559	1.976 / 1.916	-0.08	-0.228, 0.065	0.277
All	Before	48; 435	30; 357	2.044 / 1.999	0.009	-0.169, 0.187	0.925
After *	Before	13; 75	30; 357	2.369 / 1.999	-0.405	-0.684, -0.125	0.005
Before	After	20; 135	19; 162	1.929 / 1.723	-0.168	-0.433, 0.097	0.220

Abbreviations: LRTI = lower respiratory tract infection; CF = Cystic Fibrosis; SDI = Shannon-Diversity Index; CI = confidence interval; All = all samples were included irrespective of antibiotic treatment or LRTI; Before/After = only samples before or after the first antibiotic treatment or LRTI were included· * CF infants after antibiotic

treatment and before LRTI were compared with healthy individuals before LRTI. † gamm model corrected for breastfeeding, siblings, mode of delivery, antibiotic treatment, season (fixed effects), age in weeks (smooth term) and subject (random effect)

Table 4 Comparison of within-subject dissimilarities between infants with CF with higher number of LRTIs and lower number of LRTIs

Antibiotic treatment	LRTI	Higher number of LRTIs N; consecutive swabs	Lower number of LRTIs N; consecutive swabs	Estimate (higher number)†	95% CI†	*P* value†
All	All	15; 243	35; 464	-0.064	-0.100, -0.027	<0.001
Before	All	13; 115	29; 282	-0.068	-0.136, -0.026	<0.0005
All	Before	14; 59	33; 276	-0.069	-0.133, -0.005	0.038

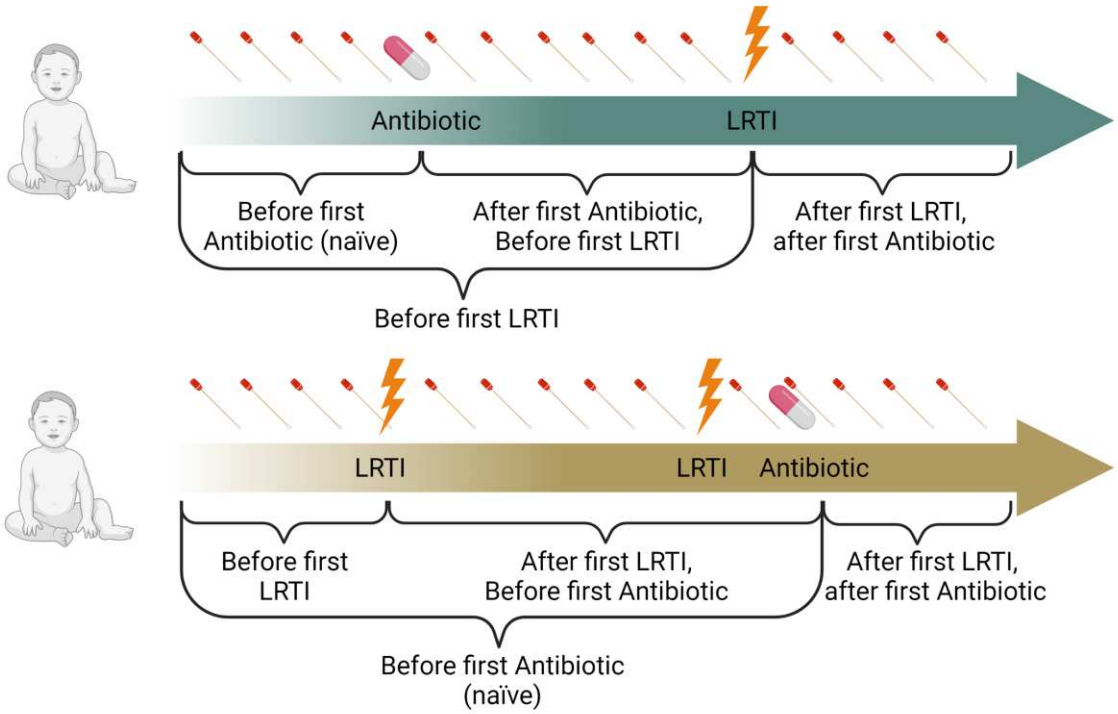
Abbreviations: LRTI = lower respiratory tract infection; CI = confidence interval; All = all samples were included irrespective of antibiotic treatment or LRTI; Before = only samples before the first antibiotic treatment or LRTI were included. † gamm model corrected for breastfeeding, siblings, mode of delivery, antibiotic therapy, season (fixed effects), sweat chloride, age in weeks (smooth term) and subject (random effect)

Table 5 Comparison of α-diversity between infants with CF with higher and lower number of LRTIs

Antibiotic treatment	LRTI	Mean SDI LRTI lower / higher number	Estimate † (Higher number of LRTIs)	95% CI†	*P* value†
All	All	2.260 / 1.838	-0.395	-0.589, -0.201	<0.0001
Before	All	2.128 / 1.564	-0.485	-0.759, -0.212	0.0006
All	Before	2.157 / 1.625	-0.473	-0.831, -0.116	0.01

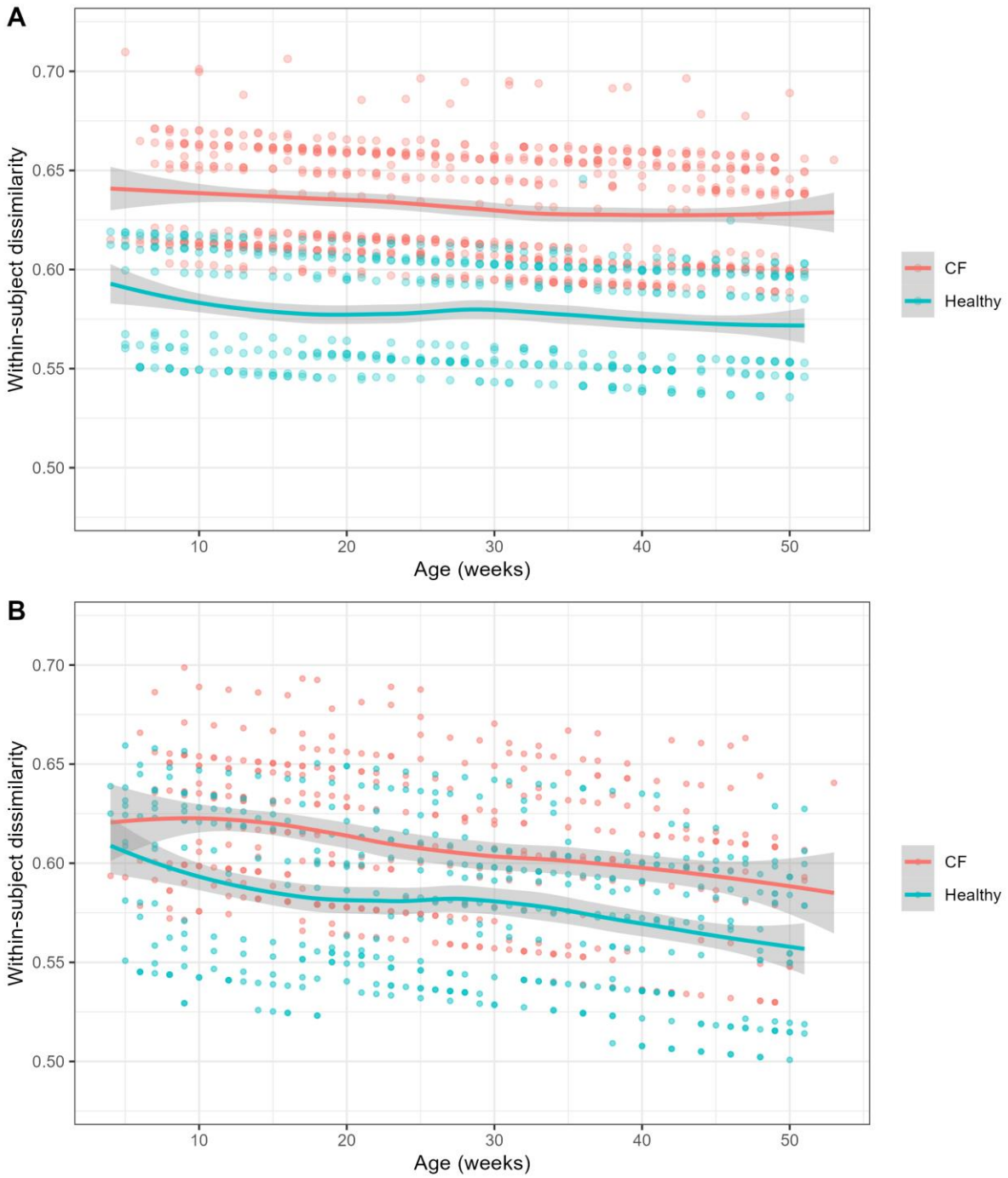
Abbreviations: LRTI = lower respiratory tract infection; SDI = Shannon-Diversity Index; CI = confidence interval; All = all samples were included irrespective of antibiotic treatment or LRTI; Before = only samples before the first antibiotic treatment or LRTI were included. † gamm model corrected for breastfeeding, siblings, mode of delivery, antibiotic treatment, season (fixed effects), sweat chloride, age in weeks (smooth term) and subject (random effect)

460 **Figures**



461 **Figure 1: Collection of biweekly nasal swabs and longitudinal event courses.** This figure
462 displays two example longitudinal courses of events in infants. In the upper part (green
463 arrow), the rare case of antibiotic treatment before the first LRTI is displayed (e.g., due to
464 Otitis media). The lower part (ochre arrow) shows an infant with two LRTIs in the first year of
465 life, one of which was treated with antibiotics. To disentangle the interrelations between
466 nasal microbiota, lower respiratory tract infections (LRTIs), and antibiotic treatment, we
467 calculated statistics overall and before/after the first antibiotic treatment or before/after the
468 first LRTI in the first year of life.

469



470

471 **Figure 2**: **Comparison of within-subject dissimilarity in infants with CF and healthy controls.**

472 The x-axes display the age of infants in weeks and y-axes display model fitted values of the

473 median within-subject dissimilarity of (A) all swabs differed between infants with CF and

474 healthy controls ($p<0.001$) but not if we compared (B) only swabs before the first antibiotic

475 treatment ($p=0.145$).

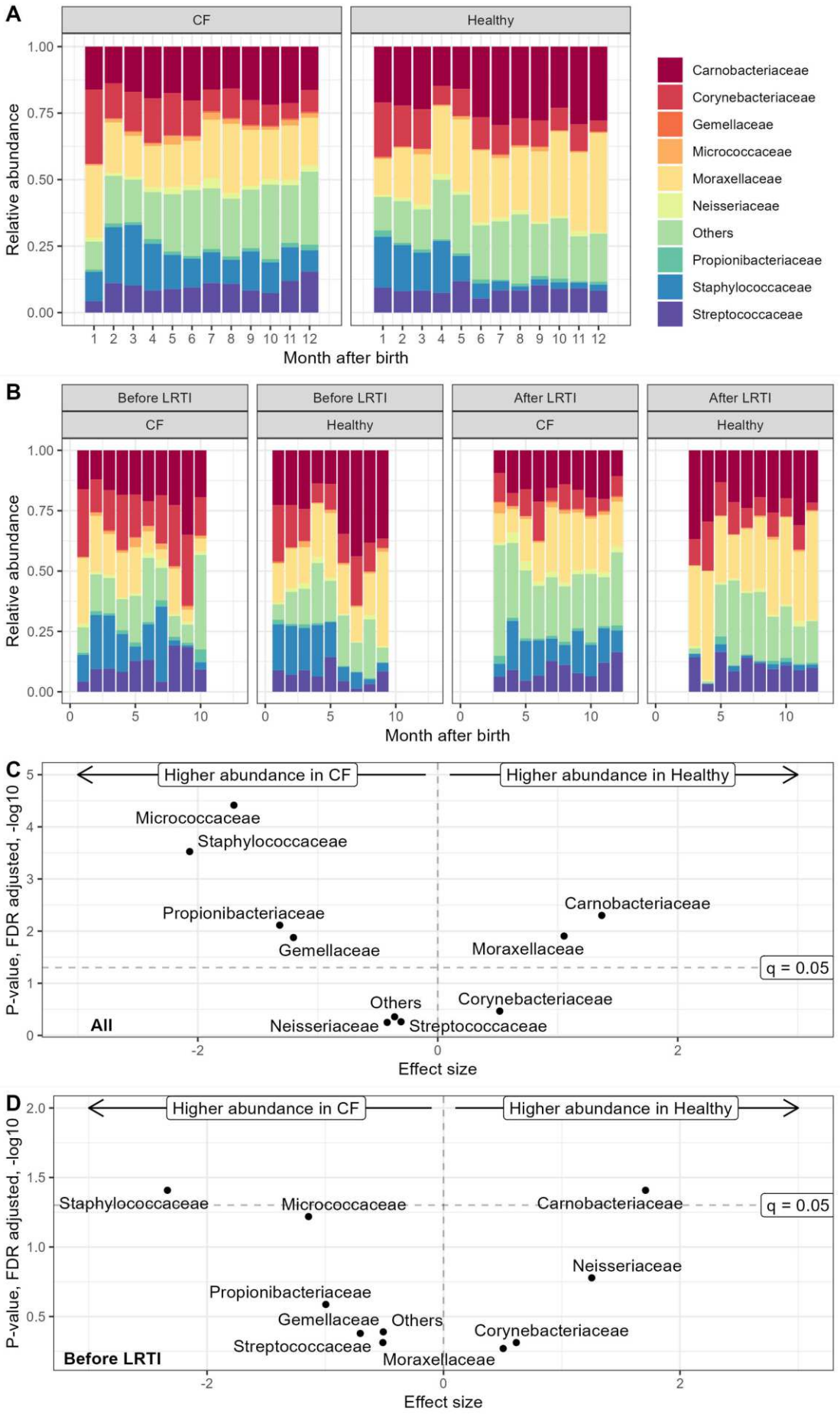

A
CF
Healthy
Relative abundance
1.00
0.75
0.50
0.25
0.00
1 2 3 4 5 6 7 8 9 10 11 12
Month after birth
Carnobacteriaceae
Corynebacteriaceae
Gemellaceae
Micrococcaceae
Moraxellaceae
Neisseriaceae
Others
Propionibacteriaceae
Staphylococcaceae
Streptococcaceae
B
Before LRTI
After LRTI
CF
Healthy
Relative abundance
0 5 10
Month after birth
C
Higher abundance in CF
Higher abundance in Healthy
P-value, FDR adjusted, -log10
Micrococcaceae
Staphylococcaceae
Propionibacteriaceae
Gemellaceae
Carnobacteriaceae
Moraxellaceae
q = 0.05
Others
Neisseriaceae
Corynebacteriaceae
Streptococcaceae
All
-2
0
2
Effect size
D
Higher abundance in CF
Higher abundance in Healthy
P-value, FDR adjusted, -log10
Staphylococcaceae
Micrococcaceae
Carnobacteriaceae
q = 0.05
Neisseriaceae
Propionibacteriaceae
Gemellaceae
Others
Corynebacteriaceae
Streptococcaceae
Moraxellaceae
Before LRTI
-2
0
2
Effect size

Figure 3: **Comparison of differential abundance of bacterial families between infants with CF and healthy infants in first year of life.** (A) and (B): Relative abundance of the 9 most abundant families and "others" are displayed in alphabetical order and by age (months). (A) for infants with CF and healthy controls and (B) divided in swabs taken before and after the first LRTI. (C) and (D): Results obtained from differential abundance analysis with MaAslin2 are plotted with effect size on the x-axes and FDR adjusted p-values (q-values) on the y-axes. The dashed horizontal line shows significance threshold (q=0.05). We display differential abundance analysis (C) for all swabs and (D) for swabs only before the first LRTI occurred.

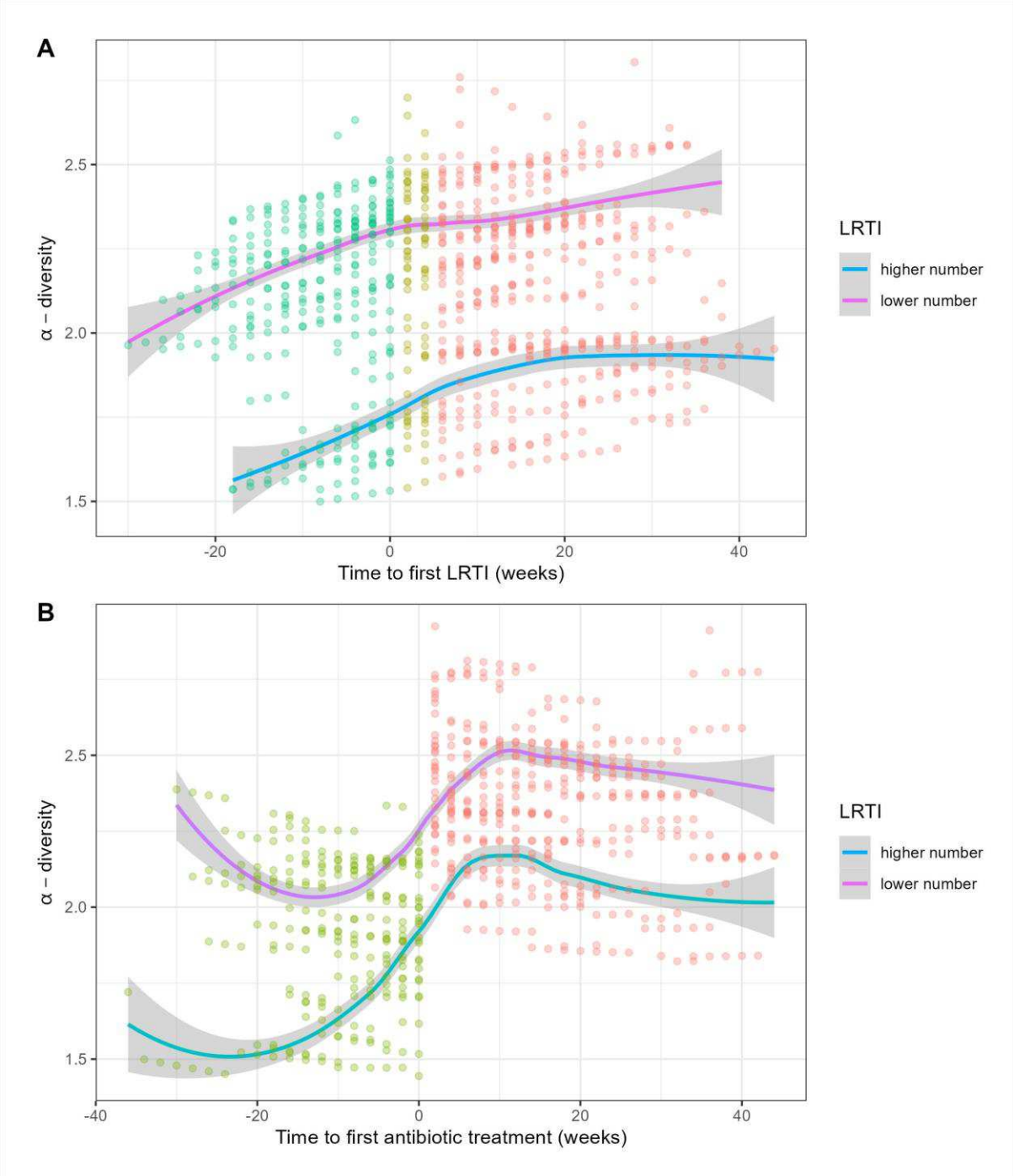


Figure 4: α-diversity in infants with CF with higher and lower number of LRTIs. Displayed are the model fitted SDI values on the y-axis and (A) time to first LRTI or (B) time to first antibiotic treatment on the x-axis (each step corresponds to two weeks due to biweekly sampling). Swabs taken before the first LRTI (A) or antibiotic treatment (B) are displayed in green, and

swabs taken after in red. The blue line shows infants with a lower number of LRTIs and magenta shows infants with a higher number of LRTIs in first year of life.

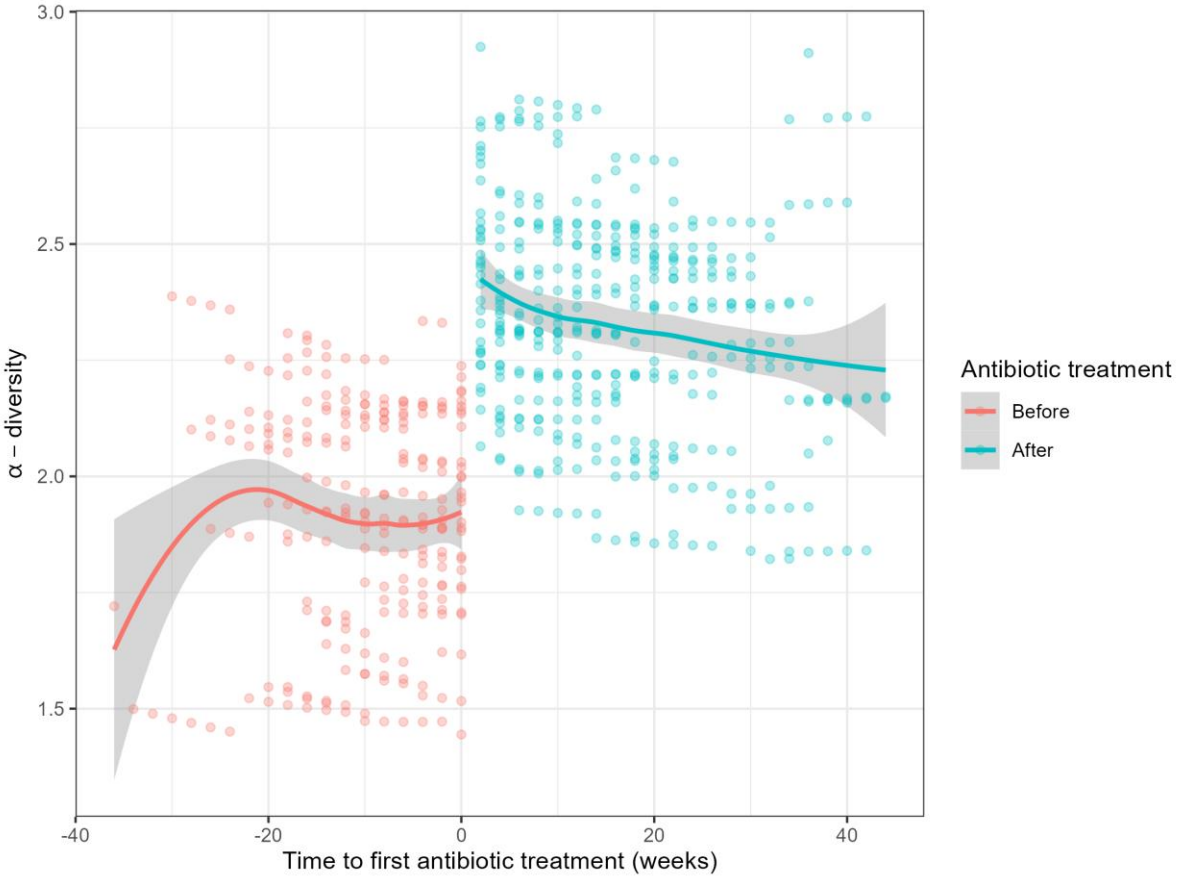


Figure 5: α-diversity before and after the first antibiotic treatment in infants with CF. Displayed are the model fitted SDI values on the y-axis and the time to first antibiotic treatment on the x-axis. Swabs taken before the first antibiotic treatment are displayed in red and after in green.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- 20240227earlymicrobiotaOLS.pdf