



## Associations of air pollution and greenness with the nasal microbiota of healthy infants: A longitudinal study

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### ABSTRACT

**Background:** Air pollution and greenness are associated with short- and long-term respiratory health in children but the underlying mechanisms are only scarcely investigated. The nasal microbiota during the first year of life has been shown to be associated with respiratory tract infections and asthma development. Thus, an interplay between greenness, air pollution and the early nasal microbiota may contribute to short- and long-term respiratory health. We aimed to examine associations between fine particulate matter (PM<sub>2.5</sub>), nitrogen dioxide (NO<sub>2</sub>) and greenness with the nasal microbiota of healthy infants during the first year of life in a European context with low-to-moderate air pollution levels.

**Methods:** Microbiota characterization was performed using 16 S rRNA pyrosequencing of 846 nasal swabs collected fortnightly from 47 healthy infants of the prospective Basel-Bern Infant Lung Development (BILD) cohort. We investigated the association of satellite-based greenness and an 8-day-average exposure to air pollution (PM<sub>2.5</sub>, NO<sub>2</sub>) with the nasal microbiota during the first year of life. Exposures were individually estimated with novel spatial-temporal models incorporating satellite data. Generalized additive mixed models adjusted for known confounders and considering the autoregressive correlation structure of the data were used for analysis.

**Results:** Mean (SD) PM<sub>2.5</sub> level was 17.1 (3.8 µg/m<sup>3</sup>) and mean (SD) NO<sub>2</sub> level was 19.7 (7.9 µg/m<sup>3</sup>). Increased PM<sub>2.5</sub> and increased NO<sub>2</sub> were associated with reduced within-subject Ruzicka dissimilarity (PM<sub>2.5</sub>: per 1 µg/m<sup>3</sup> -0.004, 95% CI -0.008, -0.001; NO<sub>2</sub>: per 1 µg/m<sup>3</sup> -0.004, 95% CI -0.007, -0.001). Whole microbial community comparison with nonmetric multidimensional scaling revealed distinct microbiota profiles for different PM<sub>2.5</sub> exposure levels. Increased NO<sub>2</sub> was additionally associated with reduced abundance of *Corynebacteriaceae* (per 1 µg/m<sup>3</sup>: -0.027, 95% CI -0.053, -0.001). No associations were found between greenness and the nasal microbiota.

**Conclusion:** Air pollution was associated with Ruzicka dissimilarity and relative abundance of *Corynebacteriaceae*. This suggests that even low-to-moderate exposure to air pollution may impact the nasal microbiota during the first year of life. Our results will be useful for future studies assessing the clinical relevance of air-pollution-induced alterations of the nasal microbiota with subsequent respiratory disease development.

**Abbreviations:** GAMM, Generalized additive mixed model; SDI, Shannon diversity index; OT, Oligotype; NMDS, Non-metric multidimensional scaling; PCV, Pneumococcal conjugate vaccine; rRNA, ribosomal RNA; PCR, Polymerase chain reaction; NDVI, Normalized difference vegetation index; PM<sub>2.5</sub>, Particulate matter <2.5 µm; NO<sub>2</sub>, Nitrogen dioxide; BILD, Bern-Basel Infant Lung Development.

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## 1. Introduction

Respiratory infections and asthma constitute major health issues in infants and children (Kyu et al., 2016; Lozano et al., 1999; Zar and Ferkol, 2014). Several studies in these age groups have shown that air pollution and residential greenness are critical risk factors for acute and chronic respiratory diseases (Bowatte et al., 2015; Fuertes et al., 2020; Kyu et al., 2016; Lambert et al. 2017, 2019; Sbihi et al., 2017; Stern et al., 2013; To et al., 2020; Usemann et al., 2019; Zhang et al., 2019). The relevance of these environmental risk factors is amplified as, by their nature, they are omnipresent and able to affect all individuals (Kim et al., 2014; Mhuireach et al., 2016). In particular, infants belong to the most vulnerable group due to immaturity of their immune and respiratory system (Bowatte et al., 2015).

The underlying mechanisms for the association of air pollution and greenness with respiratory health are only scarcely investigated. However, the nasal microbiota of the first year of life has been identified as an important player in the progression of respiratory infections (Bosch et al., 2017; Toivonen et al., 2019) and asthma development (Bisgaard et al., 2007; Teo et al., 2015). Certain microbiota profiles have been linked to disease progression; others have been associated with improved respiratory health. Previous studies have found that the development of the nasal microbiota in the first year of life is driven by age of infant, mode of delivery, feeding type, childcare attendance, viruses and presence of siblings (Bosch et al. 2016, 2017; Hasegawa et al., 2017; Mika et al., 2015). In addition, first studies revealed an association of air pollution with the nasal microbiota profile in adults (Xue et al., 2020). However, evidence on the association of air pollution and greenness with the early nasal microbiota is missing. Since this time period is crucial for later respiratory health (Teo et al., 2015) and exposure to air pollution in particular is omnipresent, a better understanding of the association between air pollution and greenness and the infant's nasal microbiota is relevant to potentially improve respiratory health in the short- and long-term.

The aim of the present study was to examine the association of short-term exposure to particulate matter with an aerodynamic diameter  $\leq 2.5 \mu\text{m}$  (PM<sub>2.5</sub>), nitrogen dioxide (NO<sub>2</sub>) and residential greenness (normalized difference vegetation index (NDVI)) with the nasal microbiota of healthy infants during the first year of life. We focused on microbiota measures previously identified as clinically relevant (primary outcomes: bacterial diversity, bacterial density; secondary outcomes: relative abundance of bacterial families) (Acosta et al., 2018; Bisgaard et al., 2007; Hoggard et al., 2017; Teo et al., 2015; Valenzi et al., 2020). We took advantage of an available longitudinal dataset, including fortnightly obtained nasal swabs (Mika et al., 2015).

## 2. Materials and methods

### 2.1. Study design

A total of 778 healthy infants were enrolled in the prospective Basel-Bern Infant Lung Development (BILD) cohort study (Fuchs et al., 2012) between April 1999 and July 2013 (Fig. S1). Pregnant women were recruited at maternity hospitals and obstetric practices. Due to relevant differences in lung parameters, infants with ethnicity other than Caucasian, preterm delivery (<37 weeks' gestation), major birth defects, disease or later diagnosis of airway malformation or specific chronic respiratory disease were excluded (Fuchs et al., 2012). Longitudinal microbiome data for the first year of life were collected and analysed in previous studies (Korten et al. 2016, 2019; Mika et al. 2015, 2017) for a subgroup of 48 infants of the BILD cohort born between February 2010 and July 2013. For the present study, this dataset was used and combined with the following exposure data calculated for the residential address of each infant and date of swab: PM<sub>2.5</sub>, NO<sub>2</sub> and NDVI. The study was approved by the Ethics Committee of the Canton of Bern and written consent was obtained at enrolment.

### 2.2. Microbiome data

Nasal swabs were collected fortnightly by the parents starting from the fifth week of life until completion of the first year of life. Study nurses instructed parents how to obtain the nasal swabs. Swabs were sent back to the study centre in transport tubes and medium (nasal swabs from Copan, Italy; UTM® tubes from Verridial E. Muller, Switzerland) on average within 3 days and stored in a  $-80^\circ\text{C}$  freezer until further processing. Nasal swabs obtained during respiratory tract infection or antibiotic therapy were excluded (Fig. S1).

Methods for PCR amplification and amplicon sequencing are described in detail elsewhere (Hilty et al., 2012; Mika et al., 2015). Briefly, V3 to V5 regions of the bacterial 16 S rRNA genes were amplified using the primer pair 341 F/907 R. After purification with the Wizard SV PCR cleanup system (Promega, Madison, WI), PCR mixtures were eluted in 40  $\mu\text{L}$  of double-distilled water. For a reliable threshold, 1 pg/ $\mu\text{L}$  bacterial DNA corresponding to a PCR product concentration of 1 ng/ $\mu\text{L}$  is recommended (Biesbroek et al., 2012). PCR products with a concentration below that threshold were excluded from the study. Furthermore, samples with less than 70 sequence reads and samples displaying >5% sequence reads identical to two negative control samples were excluded (Fig. S1). Taxa that were recently identified as contaminants (Salter et al., 2014) were not detected.

From each PCR product, 40 ng/ $\mu\text{L}$  was pooled and every multiplex identifier was used once. The resulting 8 pools were sequenced with the 454-sequencing platform. Reads were submitted to the National Center for Biotechnology Information Sequence Read Archive. Analysis of the sequencing products was performed with PyroTagger, which comprises the definition of operational taxonomic units (OTUs) based on 97% sequencing identity, estimation of chimeras and taxonomic assignments (Kunin and Hugenholtz, 2010).

Concentration of the 16 S rRNA PCR product (PCR) in ng/ $\mu\text{L}$  was used to estimate bacterial density. Alpha-diversity was assessed using the Shannon Diversity Index (SDI) and species richness (Xia et al., 2018). SDI was calculated in R (version 3.0.2) using the "vegan" package. The "Vegdist" function of the "vegan" package was used to assess the Manhattan-type Ruzicka dissimilarity (abundance-based) between 2 samples. Within-subject dissimilarity was calculated by comparison of two consecutive samples from one infant. As this dissimilarity depends on the length of the time interval ( $\Delta t$ ) between the two consecutive samples (Mika et al., 2015), we excluded dissimilarity values derived from samples with <10 days or >21 days distance. In addition, we calculated Ruzicka dissimilarities between all samples of the study population. These data were used as input matrix for the whole microbial community comparison by nonmetric multidimensional scaling (nMDS).

Relative abundances of bacterial families were defined through taxonomic assignment of PyroTagger. Mean and 95% CI were calculated for all bacterial families in each sample. The five most abundant bacterial families (*Pasteurellaceae*, *Corynebacteriaceae*, *Moraxellaceae*, *Staphylococcaceae*, *Streptococcaceae*) were analysed separately and remaining families were grouped to "others".

### 2.3. Air pollution exposure

Daily average concentrations of PM<sub>2.5</sub> and NO<sub>2</sub> for April 2010 to December 2013 were estimated using a 4-stage spatiotemporal modeling framework, as described previously (de Hoogh et al. 2018, 2019). Since air pollution data for later years were not available, we excluded nasal swabs obtained after December 2013 (Fig. S1). Briefly, data from the ozone monitoring instrument (OMI), Copernicus Atmosphere Monitoring Service, land use and meteorological variables were included to estimate daily NO<sub>2</sub> concentrations during the sampling period. A multistage framework with mixed-effect and random forest models were used to downscale satellite measurements and incorporate local sources. These models explain 73% of the overall variation in NO<sub>2</sub>

exposure measured at monitoring sites in Switzerland (de Hoogh et al., 2019). Daily ambient PM<sub>2.5</sub> concentration at a spatial resolution of 100 × 100 m was estimated with geostatistical hybrid models taking advantage of the multiangle implementation of atmospheric correction (MAIAC) spectral aerosol optical depth (AOD) data combined with other spatiotemporal predictor variables (de Hoogh et al., 2018). These models explain 89% (total), 95% (spatial) and 88% (temporal) of the local variation in PM<sub>2.5</sub> concentrations.

Previous studies have shown that short-term air pollution exposure is associated with changes in the nasal microbiota occurring within one week (Mariani et al., 2018; Qin et al., 2019). Therefore, we focused on a 1-week exposure window and calculated mean PM<sub>2.5</sub> and NO<sub>2</sub> levels for the week prior to each nasal swab. To capture potential delayed effects, we additionally generated time lags by shifting back the exposure window 1–10 days (e.g., lag 1: exposure window starts 1 day prior to nasal swab, and so forth). In addition, we calculated yearly subject means for PM<sub>2.5</sub> and NO<sub>2</sub> to facilitate comparison with annual exposure limits suggested by the World Health Organization (World Health Organization (WHO) 2006).

#### 2.4. Greenness exposure

Greenness surrounding the child's home was determined using the Normalized Difference Vegetation Index (NDVI) obtained from the MODIS VI MOD13Q1 product (data generated every 16 days at 250 × 250 m resolution (Didan et al., 2015)). The NDVI is a common metric used to define residential greenness in epidemiological studies (Lambert et al., 2017). This index approximates greenness based on the visible (red) and near-infrared light reflected by the land surface. NDVI is measured on a scale from −1 to 1 (NDVI ≤ 0.1: water, barren areas, sand or snow; 0.2–0.3: shrub, grassland; > 0.3 indicate increasing greenness) (Weier and Herring, 2000). The post-processed dataset was adjusted for cloud coverage. On the basis that greenness changes slowly, to further reduce a potential bias from cloud coverage, we averaged the last two NDVI estimates prior to each nasal swab.

#### 2.5. Additional risk factors

Analyses were adjusted for known covariates based on previous findings (Mika et al. 2015, 2017). Information about presence of siblings (binary), childcare at date of swab (binary), any breastfeeding at date of swab (binary), parental smoking (binary) and delivery mode (vaginal delivery vs. caesarean section) was derived from hospital records, questionnaires and weekly phone interviews performed by trained study nurses. Pneumococcal conjugate vaccination (PVC) era was defined by the year of birth (7-valent PVC if born < 2011 = 0, 13-valent PVC if born ≥ 2011 = 1). In addition, we adjusted the regression models for the date of nasal swab to control for seasonal variation (Gorlanova et al., 2016; Stern et al., 2013).

#### 2.6. Exposure assignment

Environmental exposures (PM<sub>2.5</sub>, NO<sub>2</sub>, NDVI) were linked to each child's home address. Addresses were geocoded using the building registry of the Swiss Federal Statistical Office (Neuchâtel). Address changes within the study period were considered for the exposure assignment.

#### 2.7. Statistical analysis

Time-series data including repeated nasal swabs and environmental exposures were used. The associations of PM<sub>2.5</sub>, NO<sub>2</sub> and NDVI with bacterial diversity (SDI, species richness, within-subject Ruzička dissimilarity) and bacterial density (PCR concentration) were assessed using a generalized additive mixed model (GAMM) with Gaussian distribution and autoregressive structure (lag 1) to account for temporal

correlation within subjects. Log-transformation was performed for species richness and PCR concentration to approach normal distribution. Coefficient estimates were back transformed and shown as percentage change ((exp(β)−1)\*100). The associations of PM<sub>2.5</sub>, NO<sub>2</sub> and NDVI with the relative abundance of bacterial families were assessed using a GAMM with autoregressive structure (lag 1) and negative binomial distribution to account for the zero-inflated outcome data. Simple models were adjusted for date of swab. The following covariates were used in the adjusted models: age at swab, sex, siblings, parental smoking, childcare, breastfeeding, delivery mode, vaccination era and date of swab. Age and date of swab were introduced into the model as smoothing terms in case a non-linear relationship with the outcome was identified. PM<sub>2.5</sub> and NO<sub>2</sub> were analysed in separate models to avoid multicollinearity. NDVI was studied in the same model as PM<sub>2.5</sub>, respectively NO<sub>2</sub>, as correlation between NDVI and the air pollutants was moderate (Pearson correlation ≤ −0.6). For the main analysis, we focused on the short-term effect of PM<sub>2.5</sub> and NO<sub>2</sub> exposure without time lag (lag 0: 1-week exposure window starting at date of swab). We additionally performed a sensitivity analysis for time-lagged PM<sub>2.5</sub> and NO<sub>2</sub> exposures to assess delayed effects of air pollution on SDI, species richness, bacterial density and relative abundance of bacterial families. Therefore, exposure windows were shifted backwards by 1–10 days prior to the date of swab.

NMDS was applied for whole microbial community comparison, using Ruzička dissimilarities as input matrix and considering repeated measures. Phylogenetic variation among all samples with respect to air pollution and greenness levels was assessed using “metaMDS” of the “vegan” package in R. For the nMDS the population was divided into terciles by individual levels of PM<sub>2.5</sub>, NO<sub>2</sub> and NDVI (1st tercile: “low-exposed”, 2nd tercile: “mid-exposed”, 3rd tercile: “high-exposed”). The “adonis” function of the “vegan” package was used as PERMANOVA analysis for the clustering (p < 0.05 indicates significant clustering due to exposure group) and “envfit” was used to fit vectors which indicate the most rapid change (arrow direction).

Statistical analyses were performed with STATA (version 16.1, STATA Corp, Texas) and R (version 3.0.2. and 4.0.3).

### 3. Results

#### 3.1. Study population

The final data set comprised 846 nasal swabs from the first year of life of 47 healthy infants. Nasal swabs were obtained between April 2010 and December 2013. The mean (SD) of included swabs per infant was 18 (3.35). Details on the study population are outlined in Table 1.

#### 3.2. Environmental exposures

We found seasonal fluctuations for air pollution levels and greenness (Fig. 1). PM<sub>2.5</sub> and NO<sub>2</sub> concentrations decreased during warmer months (4-year mean April–September: PM<sub>2.5</sub> 14.4 µg/m<sup>3</sup>, NO<sub>2</sub> 13.4 µg/m<sup>3</sup>) and increased during colder months (4-year mean October–March: PM<sub>2.5</sub> 19.2 µg/m<sup>3</sup>, NO<sub>2</sub> 25.8 µg/m<sup>3</sup>). In contrast, NDVI values increased during warmer months (4-year mean April–September: 0.6) and decreased during colder months (4-year mean October–March: 0.5).

For each infant we calculated the yearly subject mean of the individual air pollution and greenness levels. Mean (SD) PM<sub>2.5</sub> level was 17.1 (3.8 µg/m<sup>3</sup>) and mean (SD) NO<sub>2</sub> level was 19.7 (7.9 µg/m<sup>3</sup>). Across all subjects, the mean (SD) greenness was 0.5 (0.1) during the first year of life.

#### 3.3. Nasal microbiota composition

Within the 846 nasal swabs, SDI ranged from 0.0 to 3.5 with a mean (SD) of 1.2 (0.8), species richness ranged from 2.0 to 120.0 with a mean (SD) of 31.9 (20.6) and PCR concentration ranged from 1.0 to 154.6 ng/

**Table 1**  
Characteristics of the study population (N = 47).

Anthropometrics	
Boys, n (%)	24 (51)
Gestational age at birth, mean (SD) week	39.68 (1.65)
Birth weight, mean (SD) kg	3.40 (0.48)
Risk factors	
Cesarean section, n (%)	8 (17)
Breastfeeding <sup>a</sup> , n (%)	47 (100)
Siblings, n (%)	37 (79)
Childcare, n (%)	11 (23)
Parental smoking, n (%)	7 (15)
PCV13 vaccination era <sup>b</sup> , n (%)	22 (47)
Environmental exposures	
PM <sub>2.5</sub> tertiles <sup>c</sup>	
Low (mean, range: 10.0, 4.6–12.7 µg/m <sup>3</sup> ), n (%)	282 (33)
Moderate (mean, range: 15.4, 12.8–18.5 µg/m <sup>3</sup> ), n (%)	282 (33)
High (mean, range: 25.1, 18.6–45.8 µg/m <sup>3</sup> ), n (%)	282 (33)
NO <sub>2</sub> tertiles <sup>c</sup>	
Low (mean, range: 8.4, 1.2–13.0 µg/m <sup>3</sup> ), n (%)	282 (33)
Moderate (mean, range: 18.2, 13.1–23.6 µg/m <sup>3</sup> ), n (%)	282 (33)
High (mean, range: 32.6, 23.7–58.2 µg/m <sup>3</sup> ), n (%)	282 (33)
NDVI <sup>d</sup> tertiles	
Low (mean, range: 0.4, 0.1–0.5), n (%)	282 (33)
Moderate (mean, range: 0.5, 0.5–0.6), n (%)	282 (33)
High (mean, range: 0.7, 0.6–0.9), n (%)	282 (33)
Season of birth	
Spring, n (%)	12 (26)
Summer, n (%)	13 (28)
Fall, n (%)	11 (23)
Winter, n (%)	11 (23)
Number of nasal swabs per infant, mean (SD)	18 (3.35)

<sup>a</sup> Infants were breastfed for a mean ± SD duration of 9 ± 3 months.

<sup>b</sup> Pneumococcal conjugate vaccine era: PCV7 if born <2011, PCV13 born ≥2011.

<sup>c</sup> Mean, range of day 0–7 prior to swab (lag 0: exposure window of 1 week starts at day of swab).

<sup>d</sup> Mean, range of last two NDVI estimates prior to swab.

µL with a mean (SD) of 32.6 (28.7). Within-subject Ruzička dissimilarity between two consecutive swabs with time interval ≥10 and ≤21 days ranged from 0.0 to 1.0 with a mean (SD) of 0.6 (0.3). The distribution of relative abundances of the five bacterial families for each subject is shown in the supplement (Fig. S2).

### 3.4. Association of air pollution and greenness with the nasal microbiota

We found associations of air pollution with Ruzička dissimilarity and the relative abundance of *Corynebacteriaceae* in healthy infants during the first year of life. Results from simple and adjusted models are shown in Tables 2 and 3.

In the adjusted model an increase in PM<sub>2.5</sub> and NO<sub>2</sub> was associated with reduced within-subject Ruzička dissimilarity (PM<sub>2.5</sub>: per 1 µg/m<sup>3</sup> -0.004, 95% CI -0.008, -0.001; NO<sub>2</sub>: per 1 µg/m<sup>3</sup> -0.004, 95% CI -0.007, -0.001) (Table 2, Fig. 2). In the whole community comparison among all samples using nMDS, we observed significant differences by PM<sub>2.5</sub> exposure for the “mid-exposed” group (PM<sub>2.5</sub> range: 12.8–18.5 µg/m<sup>3</sup>; “adonis” function: p = 0.001) and “high-exposed” group (PM<sub>2.5</sub> range: 18.6–45.8 µg/m<sup>3</sup>; “adonis” function: p = 0.032). No significant differences could be identified for the “low-exposed”, “mid-exposed” and “high-exposed” NO<sub>2</sub> exposure groups (Fig. S3).

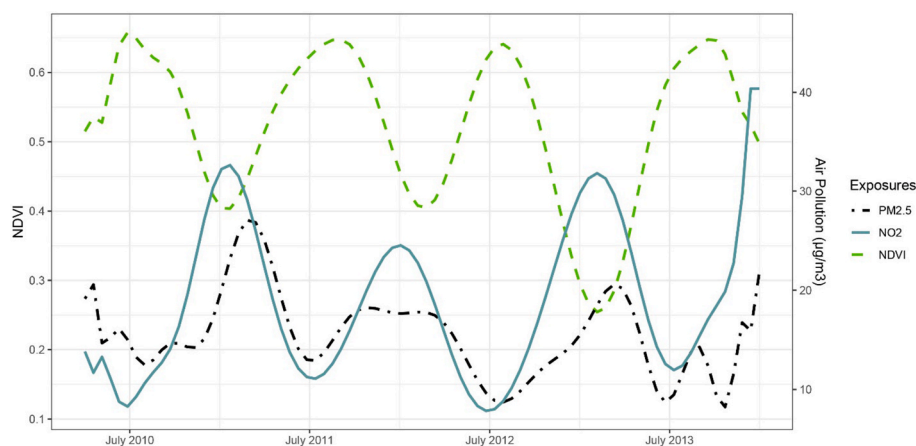
Our adjusted models allowed us to estimate the association of *Corynebacteriaceae*, *Moraxellaceae* and *Streptococcaceae* with air pollution and greenness. However, the models did not converge for *Pasteurellaceae* and *Staphylococcaceae*, indicating inadequate fit for these data. We found that time-lagged NO<sub>2</sub> exposure was associated with decreased abundance of *Corynebacteriaceae*. Associations were significant for lags of 3–10 days (NO<sub>2</sub> lag 3: per 1 µg/m<sup>3</sup> -0.027, 95% CI -0.053, -0.001) (Table 3, Fig. 2). No association could be identified for PM<sub>2.5</sub> and the abundance of *Corynebacteriaceae* (Table 3, Fig. 2). Results for lag 3 are shown in Table 3 and Fig. 2. Results for all time lags (0–10 days) are shown in Figure S6 and S7.

No associations could be found for PM<sub>2.5</sub> and NO<sub>2</sub> with SDI, bacterial density (PCR concentration), species richness and the relative abundance of *Moraxellaceae* and *Streptococcaceae* (Tables 2 and 3, Fig. 2). Also, the sensitivity analysis for time-lagged PM<sub>2.5</sub> and NO<sub>2</sub> exposure did not reveal any associations with these measures (Figs. S4–S7). Furthermore, no associations between greenness and any of the examined microbiota measures were identified in models mutually adjusted for air pollution (Tables 2 and 3, Fig. 2, Fig. S3).

## 4. Discussion

In this study we examined the association of air pollution (PM<sub>2.5</sub> and NO<sub>2</sub>) and greenness (NDVI) with the nasal microbiota of healthy infants using longitudinal data. This data structure enabled us to study the associations of air pollution and greenness with the nasal microbiota during the first year of life in detail.

We found that increasing PM<sub>2.5</sub> and NO<sub>2</sub> levels were associated with a decreased within-subject Ruzička dissimilarity comparing two consecutive swabs of one subject, indicating increased bacterial stability. There is evidence that decreased within-subject dissimilarity is associated with a reduced susceptibility to respiratory tract infections, but there is no evidence on the association between air pollution and within-subject Ruzička dissimilarity (Biesbroek et al., 2014; Bosch et al., 2017). As air pollution has been positively associated with respiratory symptoms and respiratory tract infections in infants (Horne et al., 2018;



**Fig. 1.** Seasonal fluctuations of greenness (normalized difference vegetation index, NDVI) and air pollution levels (PM<sub>2.5</sub> and NO<sub>2</sub>) from April 2010 to January 2014 (means per month across all subjects).

**Table 2**  
Association of air pollution (lag 0) and NDVI with  $\alpha$ - and  $\beta$ -diversity of the nasal microbiota.

	SDI			PCR Concentration			Species Richness			Within-Subject Ruzicka Dissimilarity		
	Coef	95% CI	p-Value	$\Delta\%$ <sup>c</sup>	95% CI <sup>c</sup>	p-Value	$\Delta\%$ <sup>c</sup>	95% CI <sup>c</sup>	p-Value	Coef	95% CI	p-Value
Simple model												
PM <sub>2.5</sub> $\mu\text{g}/\text{m}^3$ <sup>a</sup>	-0.005	-0.014, 0.003	0.229	0.148	-1.102, 1.415	0.817	-0.099	-0.841, 0.649	0.794	-0.004	-0.007, 0.000	0.043
NO <sub>2</sub> $\mu\text{g}/\text{m}^3$ <sup>a</sup>	-0.001	-0.008, 0.006	0.788	-0.148	-1.174, 0.888	0.778	-0.320	-0.883, 0.246	0.267	-0.003	-0.005, 0.000	0.051
NDVI <sup>b</sup>	0.009	-0.051, 0.069	0.771	-2.831	-11.546, 6.743	0.549	0.149	-4.727, 5.274	0.953	0.007	-0.016, 0.030	0.545
Adjusted Model												
PM <sub>2.5</sub> $\mu\text{g}/\text{m}^3$ <sup>a</sup>	-0.007	-0.015, 0.002	0.143	0.034	-1.243, 1.327	0.959	-0.224	-0.998, 0.555	0.571	-0.004	-0.008, -0.001	0.018
NO <sub>2</sub> $\mu\text{g}/\text{m}^3$ <sup>a</sup>	-0.003	-0.011, 0.005	0.443	-0.449	-1.597, 0.712	0.446	-0.472	-1.141, 0.202	0.170	-0.004	-0.007, -0.001	0.017
NDVI <sup>b</sup>												
PM <sub>2.5</sub> adjusted	-0.006	-0.064, 0.051	0.828	-4.621	-12.570, 4.050	0.286	-0.517	-5.323, 4.532	0.837	-0.002	-0.026, 0.022	0.847
NO <sub>2</sub> adjusted	-0.007	-0.073, 0.060	0.844	-6.798	-15.683, 3.024	0.168	-2.361	-7.766, 3.361	0.411	-0.012	-0.040, 0.015	0.389

Simple models were adjusted for date of swab (seasonal component).

Adjusted models were adjusted for date of swab, siblings, gender, childcare at swab, age at swab, feeding type at swab, delivery mode, vaccination era and parental smoking.

<sup>a</sup> Average values of day 0–7 prior to swab (lag 0: exposure window of 1 week starts at day of swab, coefficients for all lags are shown in Figures S4 and S5).

<sup>b</sup> Average values of last two NDVI estimates prior to swab.

<sup>c</sup>  $\Delta\%$  represents the percent increase per  $1 \mu\text{g}/\text{m}^3$  increase in PM<sub>2.5</sub> and NO<sub>2</sub>, and per 0.1 increase in NDVI: PCR concentration and species richness:  $(\exp(\beta)-1)*100$ .

**Table 3**  
Association of air pollution (lag 3) and NDVI with relative abundance of bacterial families.

	Corynebacteriaceae			Moraxellaceae			Streptococcaceae		
	Coef <sup>c</sup>	95% CI <sup>c</sup>	p-Value	Coef <sup>c</sup>	95% CI <sup>c</sup>	p-Value	Coef <sup>c</sup>	95% CI <sup>c</sup>	p-Value
Simple Model									
PM <sub>2.5</sub> $\mu\text{g}/\text{m}^3$ <sup>a</sup>	-0.001	-0.033, 0.031	0.965	-0.003	-0.014, 0.007	0.561	-0.001	-0.013, 0.012	0.918
NO <sub>2</sub> $\mu\text{g}/\text{m}^3$ <sup>a</sup>	-0.003	-0.027, 0.021	0.800	-0.003	-0.013, 0.006	0.497	-0.003	-0.013, 0.006	0.497
NDVI <sup>b</sup>	-0.039	-0.239, 0.161	0.699	-0.003	-0.092, 0.086	0.947	0.042	-0.038, 0.121	0.302
Adjusted Model									
PM <sub>2.5</sub> $\mu\text{g}/\text{m}^3$ <sup>a</sup>	-0.005	-0.034, 0.024	0.749	-0.003	-0.014, 0.009	0.673	0.003	-0.010, 0.016	0.621
NO <sub>2</sub> $\mu\text{g}/\text{m}^3$ <sup>a</sup>	-0.027	-0.053, -0.001	0.041	-0.002	-0.015, 0.011	0.762	0.003	-0.009, 0.015	0.624
NDVI <sup>b</sup>									
PM <sub>2.5</sub> adjusted	0.018	-0.156, 0.192	0.839	-0.017	-0.120, 0.087	0.754	0.066	-0.020, 0.153	0.134
NO <sub>2</sub> adjusted	-0.103	-0.310, 0.103	0.326	-0.015	-0.134, 0.103	0.797	0.074	-0.027, 0.176	0.151

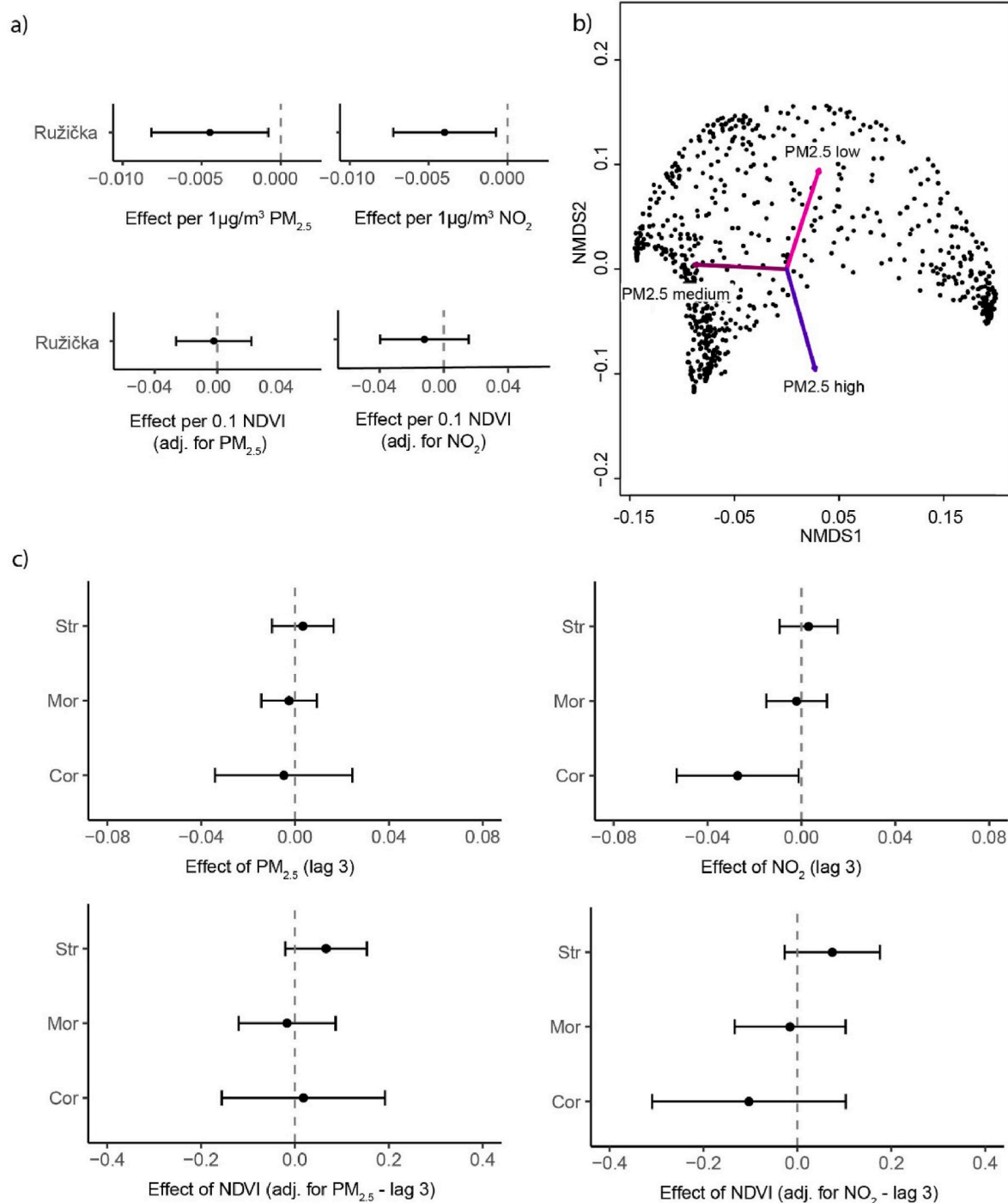
Simple models were adjusted for date of swab (seasonal component).

Adjusted models were adjusted for date of swab, siblings, gender, childcare at swab, age at swab, feeding type at swab, delivery mode, vaccination era and parental smoking.

<sup>a</sup> Average values of day 0–7 prior to swab (lag 3: exposure window of 1 week starts 3 days prior to swab, coefficients for all lags are shown in Figures S6 and S7).

<sup>b</sup> Average values of last two NDVI estimates prior to swab.

<sup>c</sup> Coef represent the difference in the logs of relative abundance per  $1 \mu\text{g}/\text{m}^3$  increase in PM<sub>2.5</sub> and NO<sub>2</sub>, and per 0.1 increase in NDVI, while holding the other variables in the model constant.



**Fig. 2.** Associations of air pollution and greenness with the nasal microbiota during the first year of life. a) Effect of  $PM_{2.5}$ ,  $NO_2$  and NDVI on within-subject Ružička dissimilarity shown as  $\beta$  coefficients with 95% CI derived from the adjusted models (Table 2) (per  $1 \mu\text{g}/\text{m}^3$  change in  $PM_{2.5}$  and  $NO_2$ , per 0.1 change in NDVI). b) Assessment of phylogenetic variation among all samples using nMDS showed significant difference by  $PM_{2.5}$  exposure for the “mid-exposed” group ( $12.8\text{--}18.5 \mu\text{g}/\text{m}^3$ , “adonis” function:  $p = 0.001$ ) and “high-exposed” group ( $18.6\text{--}45.8 \mu\text{g}/\text{m}^3$ , “adonis” function:  $p = 0.032$ ). Each dot represents a sample. Vectors indicate the most rapid change (arrow direction). c) Associations of 3-days-lagged air pollution exposure and NDVI with the abundance of bacterial families (Str: *Streptococaceae*, Mor: *Moraxellaceae*, Cor: *Corynebacteriaceae*) shown as  $\beta$  coefficients with 95% CI derived from the adjusted models (Table 3).

Stern et al., 2013; Zhang et al., 2019), we expect lower bacterial stability for increased exposure levels. The negative association between air pollution and within-subject Ružička dissimilarity identified in the present study is therefore counterintuitive. We speculate that the exposure to a constantly dominating external factor, such as higher levels of air pollution, may trigger a typical bacterial profile. The clinical relevance of reduced within-subject dissimilarity for respiratory health remains

unclear and has to be investigated in longitudinal studies assessing the relationship between environmental factors and the nasal microbiome in parallel to the association with respiratory disease development.

In the whole microbial community comparison among all samples we observed significant differences in the phylogenetic variation by  $PM_{2.5}$  exposure for groups exposed to medium and high levels of  $PM_{2.5}$ , suggesting distinct microbiota profiles. To our knowledge, this is the

first study investigating the association of PM<sub>2.5</sub> and NO<sub>2</sub> with Ruzička dissimilarity of the nasal microbiota in infants. However, our results are supported by other studies which found that the exposure to PM<sub>2.5</sub> was related with a distinct composition of the pharyngeal microbiome in adults (Qin et al., 2019) and a distinct composition of the lung microbiome in mice (Li et al., 2020).

Furthermore, we found that a 3-to-10-day time-lagged exposure to NO<sub>2</sub> was negatively associated with the relative abundance of *Corynebacteriaceae*. Our finding is supported by Mariani et al., (2018), who identified a negative association between time-lagged PM<sub>2.5</sub> exposure and the abundance of *Corynebacteriaceae* (% change per 1 µg/m<sup>3</sup>: -2.52). However, comparability is limited, as they assessed single-day exposures to PM<sub>2.5</sub>, while we studied the effect of averaged NO<sub>2</sub> (mean of 8 days). Higher abundance of *Corynebacteriaceae* has been previously related to respiratory health in infants and children (Biesbroek et al., 2014; McCauley et al., 2019; Neumann et al., 2018; Rawls and Ellis, 2019; Teo et al., 2015), which suggests that the association between increasing air pollution exposure and reduced abundance of this commensal bacterial family might be of clinical relevance.

The association of PM<sub>2.5</sub> and NO<sub>2</sub> with Ruzička dissimilarity and bacterial composition identified herein supports our hypothesis that there may be an interplay between the nasal microbiota and air pollution with potential consequences for short- and long-term respiratory health. To put the air pollution levels in our study in context, and compare with the WHO annual guideline limits, the mean annual exposure for all infants was 17 µg/m<sup>3</sup> PM<sub>2.5</sub> (guideline 10 µg/m<sup>3</sup>) and 20 µg/m<sup>3</sup> NO<sub>2</sub> (guideline 40 µg/m<sup>3</sup>). Our results indicate that low-to-moderate air pollution levels may still impact the nasal microbiota profile and thus affect respiratory health (World Health Organization (WHO) 2006).

Our study has considerable strengths. Most relevant is the high-quality exposure data that has been estimated for each nasal swab included in the analysis. We have used novel modeling techniques which allowed us to capture fine-scale spatial and temporal variation of PM<sub>2.5</sub> and NO<sub>2</sub>. The daily air pollution data has enabled us to study time-lagged air pollution exposures. Further, we used fortnightly data as a basis for the NDVI exposure. Another major strength of our study is the dense longitudinal sampling of nasal swabs. Samples were collected every two weeks in 47 infants, resulting in 846 samples available for the analysis. Previous studies investigating the association of air pollutant with the nasal microbiota, included one (Mariani et al., 2018) to two (Qin et al., 2019) swabs per subject, which limits the conclusion on effects. Our longitudinal dataset with several repeated measures per subject increases the power to detect potential associations between the dynamic nasal microbiota and highly variable environmental exposures.

Certain limitations should be addressed. First, variation in our exposure data was small. More variation in exposure data and a larger sample size might be necessary to detect additional associations of air pollution and greenness with the nasal microbiota that are rather weak compared to the associations with other previously described factors such as age or presence of siblings (Bosch et al. 2016, 2017; Hasegawa et al., 2017; Mika et al., 2015). In addition, more distinct microbiota profiles could be obtained using novel metagenome sequencing techniques which would enable more insights on the association between environmental exposures and the microbiota. Furthermore, associations with air pollution and greenness could be examined only for *Corynebacteriaceae*, *Moraxellaceae* and *Streptococcaceae*. The GAMM did not converge for *Pasteurellaceae* and *Staphylococcaceae*, most likely due to zero inflation and low abundance of these bacterial families (Wood 2021). This indicates that the GAMM model, even when accounting for negative binomial distribution, does not provide the ideal fit for zero-inflated microbiome data. It is well-known that the analysis of this type of data is challenging, and is even more complex for longitudinal data (Zhang et al., 2020). Nonetheless, the GAMM is a good fit for investigating our primary outcomes (bacterial diversity, bacterial density) under consideration of the longitudinal data structure and was

therefore viewed as a reasonable compromise.

## 5. Conclusion

This study shows that exposure to PM<sub>2.5</sub> and NO<sub>2</sub> is related to the early nasal microbiota. The association of air pollution with Ruzička dissimilarity and the abundance of *Corynebacteriaceae* suggests that even low-to-moderate exposure levels may impact the nasal microbiota during the first year of life, which has been associated with respiratory infections and asthma development. Our results can be useful for future studies assessing the clinical relevance of air-pollution-induced alterations of the nasal microbiota and subsequent disease development. However, further longitudinal studies with more distinct exposure levels are needed to extend upon our findings.

## Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: PL reports personal fees from OM Pharma, Polyphor, Santhera, Vertex and Vifor, outside the submitted work. JU reports personal fees from Vertex outside the submitted work. MH reports personal fees and grants from Pfizer, outside the submitted work. The remaining authors declare no conflicts of interest.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envres.2021.111633>.

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## Author contributions

PL, UF, IK and JU designed the study. IK, JU, FD, OG, AS assisted in collection of the clinical and metadata. MH was responsible for sample amplicon sequencing and bioinformatics analyses. AG performed the data analysis and wrote the main manuscript with input from the co-authors. JU, PL, IK, OG and UF contributed to the statistical interpretation of results. DV and KdH provided the environmental exposure data and helped in interpretation of the data. UF is the principal investigator of the BILD cohort. All authors read and approved the final manuscript.

## Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Canton of Bern, Bern, Switzerland.

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